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Synthesis and antibacterial activity of substituted flavones, 4-thioflavones and 4-iminoflavones

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Abstract—Synthesis of flavones, 4-thioflavones and 4-iminoflavones was carried out with the substitution of variable halogens, methyl, methoxy and nitro groups in the A, B and AB rings of the respective compounds and we also report here their antibacterial activity. Most of the synthesized compounds were found to be active against *Escherichia coli*, *Bacillus subtilis*, *Shigella flexnari*, *Salmonella aureus*, *Salmonella typhi* and *Pseudomonas aeruginosa*. Activity of 4-thioflavones and 4-iminoflavones was found to be higher than that of their corresponding flavone analogues. Investigated compounds having substituents like F, OMe and NO_2 at 4′-position in ring-B exhibited enhanced activity and the presence of electronegative groups in the studied compounds showed a direct relationship to the antibacterial activity.

1. Introduction

Flavonoids are a group of natural products present in a wide variety of plants. They are found in seeds, citrus fruits, olive oil, tea and red wine and are commonly consumed with the human diet.[1,2] Flavonoids exhibit a broad range of biological activities, including antiviral, antiinflammatory, antioxidant, antiallergic, hepatoprotective, antithrombotic and antitumoral actions.[3–5] Furthermore, these compounds are used in bacteriology, pharmacology and medicine due to their bactericidal activities.[6] Moreover, amino group substituted flavone derivatives exhibit strong antitumour activity in breast cancer cells.[7]

Although, a little work has been carried out on sulfur containing flavones; thioflavones have been reported to show considerable antimicrobial activities, while flavone imines show antimicrobial and antimalarial activities.[8,9]

It is well known that halogenated compounds are also strongly biologically active[10] but to our knowledge no natural flavonoids have been reported with halogens as substituents. Additionally, we were also interested to check the effect of sulfur and nitrogen atoms at 4-position of flavones 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 flavones, 4-thioflavones and 4-iminoflavones and in vitro evaluation of the new and known flavones, 4-thioflavones and 4-iminoflavones against a number of bacteria.

2. Chemistry

In view of the importance of bioactive flavones, 4-thioflavones and 4-iminoflavones in recent years as described above, it was thought to replace the oxygen atom of the keto group of flavone with a sulfur atom and with a substituted amino group to check their effect as well as the effect of various substituents on the biological activities of flavones.

Flavones (**6** series) were synthesized using the Baker–Venkataraman rearrangement[11,12] as shown in Scheme 1.

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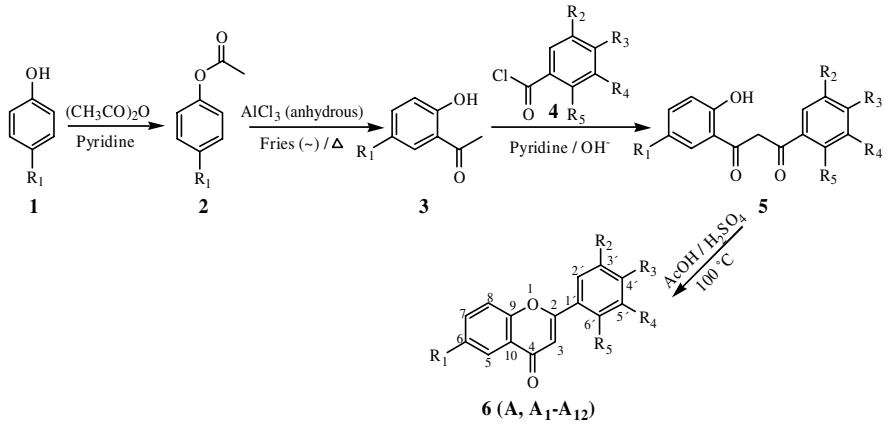


Scheme 1. Synthesis of A, B-ring substituted flavones.

Acetylation of substituted/unsubstituted phenols (**1**) was carried out in the presence of acetic anhydride to yield intermediates **2** which on refluxing in the presence of anhydrous aluminium chloride underwent Fries rearrangement to afford **3** in good yields (~80%). Substituted/unsubstituted 2-hydroxyacetophenones (**3**) were further allowed to react with substituted/unsubstituted benzoyl chlorides (**4**) in the presence of anhydrous pyridine, and subsequent heating the mixture in the presence of KOH yielded substituted/unsubstituted-2-hydroxydibenzoyl methane (**5**). Intermediates **5** were further cyclized in the presence of glacial acetic acid and concd H_2SO_4 at 100 °C to afford the desired flavone series **6** (**A**, $\mathbf{A_1}$–$\mathbf{A_{12}}$) again in good yield (~80%). The substitution pattern in all of the synthesized compounds (**6** series) is given in Table 1 (Scheme 2).

4-Thioflavones, that is, series **7** (**B**, $\mathbf{B_1}$–$\mathbf{B_{12}}$) were synthesized from the previously synthesized flavones, that is, series **6** (**A**, $\mathbf{A_1}$–$\mathbf{A_{12}}$) by refluxing them with toluene (dry) in the presence of Lawesson's reagent.[13] The substitution pattern in all of the synthesized 4-thioflavones (**7** series) is given in Table 2.

A parallel series of compounds 4-iminoflavones, that is, **8** (**C**, $\mathbf{C_1}$–$\mathbf{C_{12}}$) was synthesized by refluxing the flavone series **6** (**A**, $\mathbf{A_1}$–$\mathbf{A_{12}}$) with 2,4-dinitrophenylhydrazine[14] in the presence of concd H_2SO_4 in EtOH. The substitution pattern in all of the synthesized 4-iminoflavones (**8** series) is given in Table 3.

All of the compounds from different series (**6**–**8**) were fully characterized through spectroscopic and other analytical techniques, details of which are given separately in Section 6.

Table 1. Substituent values for flavones **A**, $\mathbf{A_1}$–$\mathbf{A_{12}}$

Compound	R1	R2	R3	R4	R5
A	H	H	H	H	H
$\mathbf{A_1}$	H	H	H	OCH_3	H
$\mathbf{A_2}$	H	H	CH_3	H	H
$\mathbf{A_3}$	H	H	NO_2	H	H
$\mathbf{A_4}$	H	H	OCH_3	H	H
$\mathbf{A_5}$	H	NO_2	H	H	NO_2
$\mathbf{A_6}$	H	H	H	NO_2	H
$\mathbf{A_7}$	F	H	H	H	H
$\mathbf{A_8}$	Cl	H	H	H	I
$\mathbf{A_9}$	Br	H	H	H	I
$\mathbf{A_{10}}$	F	H	H	H	I
$\mathbf{A_{11}}$	H	H	F	H	H
$\mathbf{A_{12}}$	H	H	OCH_3	OCH_3	H

3. Biological activity

Synthesized compounds were tested for their antibacterial activity by adopting agar well diffusion[15] method. The following bacterial cultures were used.

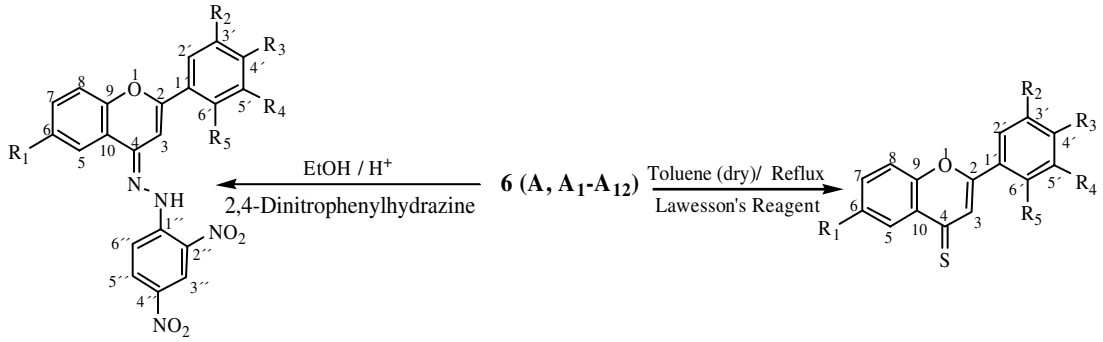
(i) *Escherichia coli* (ii) *Bacillus subtilis* (iii) *Shigella flexnari* (iv) *Staphylococcus aureus* (v) *Salmonella typhi* and (vi) *Pseudomonas aeruginosa*

Imipenem was used as standard drug and 24 h-old culture containing approximately 10^4–10^6 colony forming unit (CFU) was spread on the surface of Muller Hinton Agar (MHA) plates. Wells were created in the medium with the help of a sterile metallic borer at appropriate distances. Test samples of 100 μL (1 mg of test compounds) were poured into each well and the plates were incubated at 37 °C for 24 h. The results, in terms of inhibition zones, were noted and compared with standard drug. As a negative control, DMSO was used. The results of these experiments are summarized in Table 4.

4. Results and discussion

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

Antibacterial activity analysis of B-series flavones showed moderate activity against *E. coli* and *S. typhi* but exhibited very low activity against *B. subtilis*, *S. flexneri*, *S. aureus* and *P. aruginosa*. However, 4-thioflavones and 4-iminoflavones of the same series exhibited significant activity against *E. coli*, *S. flexneri*, *P. aeruginosa* and *S. typhi*, but did not show significant activity against *B. subtilis* and *S. aureus*.



Scheme 2. Synthesis of A, B-ring substituted 4-thioflavones (**7**) and 4-iminoflavones (**8**).

Table 2. Substituent values for 4-thioflavones **B**, $\mathbf{B_1}$–$\mathbf{B_{12}}$

Compound	R1	R2	R3	R4	R5
B	H	H	H	H	H
$\mathbf{B_1}$	H	H	H	OCH_3	H
$\mathbf{B_2}$	H	H	CH_3	H	H
$\mathbf{B_3}$	H	H	NO_2	H	H
$\mathbf{B_4}$	H	H	OCH_3	H	H
$\mathbf{B_5}$	H	NO_2	H	H	NO_2
$\mathbf{B_6}$	H	H	H	NO_2	H
$\mathbf{B_7}$	F	H	H	H	H
$\mathbf{B_8}$	Cl	H	H	H	I
$\mathbf{B_9}$	Br	H	H	H	I
$\mathbf{B_{10}}$	F	H	H	H	I
$\mathbf{B_{11}}$	H	H	F	H	H
$\mathbf{B_{12}}$	H	H	OCH_3	OCH_3	H

Table 3. Substituent values for 4-iminoflavones **C**, $\mathbf{C_1}$–$\mathbf{C_{12}}$

Compound	R1	R2	R3	R4	R5
C	H	H	H	H	H
$\mathbf{C_1}$	H	H	H	OCH_3	H
$\mathbf{C_2}$	H	H	CH_3	H	H
$\mathbf{C_3}$	H	H	NO_2	H	H
$\mathbf{C_4}$	H	H	OCH_3	H	H
$\mathbf{C_5}$	H	NO_2	H	H	NO_2
$\mathbf{C_6}$	H	H	H	NO_2	H
$\mathbf{C_7}$	F	H	H	H	H
$\mathbf{C_8}$	Cl	H	H	H	I
$\mathbf{C_9}$	Br	H	H	H	I
$\mathbf{C_{10}}$	F	H	H	H	I
$\mathbf{C_{11}}$	H	H	F	H	H
$\mathbf{C_{12}}$	H	H	OCH_3	OCH_3	H

4.1. Structure–activity relationship

Considering the varied structure–activity relationships of different series of compounds, it cannot be inferred that the biological behaviour of a drug is determined by the influence of a single parameter or variable. Furthermore, 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. However, and taking the necessary precautions, the information in Table 4 may allow us to make some general remarks on the structure and antibacterial activity of the analyzed flavones, 4-thioflavones and 4-iminoflavones.

From Table 4, it is clear that antibacterial activity of flavones increases accordingly on replacing oxygen atom with sulfur and nitrogen atoms at 4-position. Thioanalogues of flavones have more antibacterial activity as compared to flavones. Similarly, 4-iminoflavones are much more active against bacteria than 4-thioflavones.

Furthermore, when flavones, 4-thioflavones and 4-iminoflavones have methoxy group in the B-ring at position 3′, the linear growth inhibition against *E. coli* and *S. typhi* was 12 and 13 ($\mathbf{A_1}$), respectively, for flavones, while 15 and 12 ($\mathbf{B_1}$) for 4-thioflavones and 15 and 9 ($\mathbf{C_1}$) for 4-iminoflavones, whereas standard drug Imipenem showed 30 and 25, respectively. However, when a methoxy group was present at 4′-position in ring-B, the activity increased accordingly in flavones, 4-thioflavones and 4-iminoflavones. The same pattern of activity is found with the replacement of –OMe group in ring-B with $-NO_2$ group but the percentage of inhibition is decreased to some extent. Thus increase in activity is probably due to the resonance effect of –OMe group that is more prominent at 4′-position than 3′-position, whereas in case of a nitro group, it operates in opposite direction giving the results otherwise.

Moreover, when a methoxy group was present at both positions like 3′ and 4′, then both groups enhanced the activity mutually against *E. coli*, *P. aeruginosa* and *S. typhi*. Linear growth inhibition against these three bacteria, in this case ($\mathbf{A_{12}}$), was 19, 16 and 16, respectively, whereas in case of $\mathbf{B_{12}}$ inhibition was enhanced to 19, 16 and 17, respectively. The 4-imino analogue, that is, $\mathbf{C_{12}}$ exhibited further enhanced inhibition, that is, 21, 20 and 19 against these bacterial species.

In the presence of electron-withdrawing groups like $-NO_2$ at 2′-and 5-′ positions in ring-B, the linear growth inhibition decreased to lower percentage. Flavones, 4-thioflavones and 4-iminoflavones having F atom at position 4′ in ring-B showed a very significant inhibition against *E. coli*, *B. subtilis*, *S. flexneri*, *S. typhi* and *P. aeruginosa* but were inactive against *S. aureus*. This increase in activity is probably due to the presence of

Table 4. Antibacterial activities of series of compounds, relative to the standard drug Imipenem

Compound	Bacteria					
	E. coli	*B.subtilis*	*S. flexneri*	*S. aureus*	*P.aeruginosa*	*S. typhi*
A	9	—	—	—	—	10
B	11	—	10	—	—	12
C	—	—	10	—	21	15
$\mathbf{A_1}$	12	—	—	—	—	13
$\mathbf{B_1}$	15	—	—	—	15	12
$\mathbf{C_1}$	15	—	19	—	11	9
$\mathbf{A_2}$	—	—	—	—	—	—
$\mathbf{B_2}$	10	—	—	—	—	9
$\mathbf{C_2}$	—	—	12	—	10	16
$\mathbf{A_3}$	12	—	—	—	—	10
$\mathbf{B_3}$	16	10	—	—	10	12
$\mathbf{C_3}$	15	—	15	—	12	20
$\mathbf{A_4}$	15	—	16	—	16	17
$\mathbf{B_4}$	15	16	16	—	15	16
$\mathbf{C_4}$	16	—	18	14	—	19
$\mathbf{A_5}$	10	—	—	—	—	13
$\mathbf{B_5}$	15	—	13	—	—	14
$\mathbf{C_5}$	16	—	14	—	15	15
$\mathbf{A_6}$	10	—	—	—	—	10
$\mathbf{B_6}$	12	—	—	10	—	14
$\mathbf{C_6}$	14	—	—	—	—	—
$\mathbf{A_7}$	10	—	—	—	—	15
$\mathbf{B_7}$	—	—	12	10	14	—
$\mathbf{C_7}$	15	—	11	—	11	17
$\mathbf{A_8}$	11	—	—	—	—	15
$\mathbf{B_8}$	—	—	16	—	15	15
$\mathbf{C_8}$	18	—	11	—	16	13
$\mathbf{A_9}$	14	—	—	—	—	16
$\mathbf{B_9}$	20	—	14	—	—	21
$\mathbf{C_9}$	16	—	—	—	15	15
$\mathbf{A_{10}}$	14	—	16	—	14	—
$\mathbf{B_{10}}$	12	—	14	—	15	—
$\mathbf{C_{10}}$	—	—	—	—	13	15
$\mathbf{A_{11}}$	22	17	16	—	16	21
$\mathbf{B_{11}}$	25	19	15	—	17	20
$\mathbf{C_{11}}$	25	21	16	—	20	21
$\mathbf{A_{12}}$	19	—	15	10	16	16
$\mathbf{B_{12}}$	19	10	—	—	16	17
$\mathbf{C_{12}}$	21	—	—	16	20	19
Imipenem	30	33	27	33	24	25

Inhibition zones are given in millimeter.

fluorine at 4′-position which is the most electronegative atom. However, compounds with a methyl group as a substituent on B-ring were found to be inactive against each bacterial species.

Above results show that electron-donating and electronegative groups are responsible for the antibacterial activity of flavones, 4-thioflavones and 4-iminoflavones provided these groups are at 4′-position in ring-B rather than any other position. Additionally, increase in inhibition of thio-analogues is probably due to the larger size of the sulfur atom.

Antibacterial analysis of C-series compounds revealed that all flavones show good or low activity against *E. coli* and *S. typhi* but were inactive against *B. subtilis*, *S. flexneri*, *S. aureus* and *P. aeruginosa*, whereas their respective sulfur and nitrogen analogues exhibited significant activity against *E. coli*, *S. flexneri*, *P. aeruginosa* and *S. typhi*, however, they were found to be inactive against *B. subtilis* and *S. aureus*.

The flavones like $\mathbf{A_7}$, $\mathbf{A_8}$, $\mathbf{A_9}$ and $\mathbf{A_{10}}$ and their respective sulfur and nitrogen analogues ($\mathbf{B_7}$, $\mathbf{B_8}$, $\mathbf{B_9}$ and $\mathbf{B_{10}}$), ($\mathbf{C_7}$, $\mathbf{C_8}$, $\mathbf{C_9}$ and $\mathbf{C_{10}}$) have similar structures except the nature of halogen atom present at 5-position. A comparison of the antibacterial activities of the series of compounds (Table 4) shows that the flavone $\mathbf{A_9}$ having a bromine atom at 6-position in ring-A shows linear growth inhibition of 14 and 16 against *E. coli* and *S. typhi* and its respective thio- and imino- analogues exhibit 20, 21 and 16, 15, respectively, against the same bacterial species. However, when the bromine atom in ring-B is replaced by chlorine ($\mathbf{A_8}$) and fluorine ($\mathbf{A_{10}}$), inhibition is changed to 11 and 14, respectively, whereas standard drug Imipenem shows 30 inhibition. The same results are found in case of their respective thio-($\mathbf{B_8}$, $\mathbf{B_{10}}$) and imino-($\mathbf{C_8}$, $\mathbf{C_{10}}$) analogues.

Antibacterial analysis of unsubstituted flavones and their derivatives showed significant activity against *E. coli*, *S. flexneri*, *P. aeruginosa* and *S. typhi* but were inactive against *B. subtilis* and *S. aureus* species.

Compound **C** showed linear growth inhibition of 21, 15 and 10 against *P. aeruginosa*, *S. typhi* and *S. flexneri*, respectively, whereas standard drug Imipenem showed 24, 27 and 25, respectively. However, compounds **A** and **B** exhibited very low activity against *E. coli* and *S. typhi* and no activity against other bacterial species. Therefore, it is concluded that a 2,4-dinitrophenyl hydrazine moiety in compound **C** is responsible for its enhanced antibacterial activity, while unsubstituted flavones and 4-thioflavone remain inactive or less active.

5. Conclusion

Synthesis of flavones, 4-thioflavones and 4-iminoflavones was carried out with the substitution of various halogens, methyl, methoxy and nitro groups in the A, B and AB-rings of the respective compounds. The synthesized flavones, 4-thioflavones and 4-iminoflavones were checked for their antibacterial activity. It is concluded from the results that substituents on the flavones' skeleton are responsible for the enhancement of the antibacterial activity. It can further be concluded that the percentage inhibition increases as the electronegativity of the halogen atom on A-ring increases. Similarly, sulfur and nitrogen atoms, in addition to substituents at certain positions, enhance the antibacterial activity of flavone derivatives. Activities exhibited by thioflavones as well as iminoflavones could be due to the combined effect of the heteroatom as well as substituents present in the system.

6. Experimental

Chemicals used in the present study were purchased from Merck and Fluka (Germany). Solvents used were dried with specific drying reagent under nitrogen. R_f values were calculated by using precoated silica gel aluminium packed plates Kiesel gel $60F_{254}$ Merck (Germany). Melting points were determined in open capillaries using Gallenkamp melting point apparatus and are uncorrected. FTIR spectra were recorded on Bio-Rad Merlin Spectrophotometer using KBr discs. ^{1}H NMR spectra were recorded on Bruker (400 MHz) AM-250 in DMSO-d_6 solution using TMS as internal standard. EIMS was recorded on VG-70-SE Mass Spectrometer. Purity of each compound was monitored by TLC. Chemical analyses were carried out on Carlo-Erba-1108 instrument.

The procedure for the synthesis of a representative compound from each series (**A**–**C**) is given below, while the syntheses of rest of the members of each series are based on similar procedures with the variation in substitution pattern in reactants for different targets.

6.1. Synthesis of 6 series (A, A_1–A_{12})

All of the compounds in the **6** series were synthesized according to the standard procedures[16,17] as outlined in Scheme 1. Spectroscopic as well as other physical data of the known compounds in **6** series (**A**, A_1–A_7, A_{10}–A_{12}) are given in the cited literature, while those of the new compounds (A_8, A_9) are given below.

6.1.1. 2-(2′-Iodophenyl)-6-chloro-4*H*-1-benzopyran-4-one (A_8). Yield: 80%; mp 130–131 °C; MS (EI): *m*/*z* (%) = 384 [M+2] (12), 382.5 [M^+] (100), 255 [M^+−X] (57), 354 [M^+−CO] (12), 318.5 [M^+−CO–X] (2); IR (film): $\tilde{\nu} = 1645\ cm^{-1}$ (s, C=O), 1587 (m, C=C), 725 (C–X); ^{1}H NMR (DMSO-d_6, 500 MHz): δ (ppm) 6.59 (s, 1H, H3), 8.06 (d, J = 2.18 Hz, 1H, H5), 7.91 (dd, J = 8.96/2.27 Hz, 1H, H7), 7.86 (d, J = 7.06 Hz, 1H, H8), 8.03 (dd, J = 7.70/2.17 Hz, 1H, H3′), 7.34 (m, 1H, H4′), 7.59 (m, 1H, H5′), 7.78 (dd, J = 8.91/2.30 Hz, 1H, H6′); Anal. Calcd for $C_{15}H_8ICIO_2$ (382.58): C, 47.09; H, 2.11. Found: C, 46.91; H, 2.31.

6.1.2. 2-(2′-Iodophenyl)-6-bromo-4*H*-1-benzopyran-4-one (A_9). Yield: 85; mp 133–134 °C; MS (EI): *m*/*z* (%) = 429 [M+2] (50), 427 [M^+] (100), 300 [M^+–X] (37), 399 [M^+−CO] (15), 363 [M^+−CO–X] (7); IR (film): $\tilde{\nu} = 1650\ cm^{-1}$ (s, C=O), 1591 (m, C=C), 740 (C–X); ^{1}H NMR(DMSO-d_6, 500 MHz): δ (ppm) 6.63 (s, 1H, H3), 8.14 (d, J = 2.31 Hz, 1H, H5), 7.99 (dd, J = 8.52/1.19 Hz, 1H, H7), 7.73 (d, J = 7.16 Hz, 1H, H8), 8.09 (dd, J = 7.83/2.13 Hz, 1H, H3′), 7.40 (m, 1H, H4′), 7.60 (m, 1H, H5′), 7.81 (dd, J = 8.38/2.37 Hz, 1H, H6′); Anal. Calcd for $C_{15}H_8IBrO_2$ (427.04): C, 42.19; H, 1.89. Found: C, 42.03; H, 1.95.

6.2. General procedure for the synthesis of 7 series (B, B_1–B_{12})

A mixture of a substituted/unsubstituted flavone of the described **6** series (5.0 mmol each), Lawesson's reagent (3.0 mmol) and anhydrous toluene (30 mL) was refluxed under dry conditions for 3 h. The solvent was evaporated under reduced pressure and the residue was crystallized from ethanol to afford substituted/unsubstituted 4-thioflavone (**B**, B_1–B_{12}) in good to excellent yields. Spectroscopic as well as other physical data of the known compounds in **7** series (**B**, B_1–B_4, B_6, B_7, B_{11} and B_{12}) are given in the literature[13,18], while those of the new compounds (B_5, B_8–B_{10}) are given below.

6.2.1. 2-(2′,5′-Dinitrophenyl)-4*H*-1-benzopyran-4-thione (B_5). Yield: 60%; mp 232–233 °C; MS (EI): *m*/*z* (%) = 328 [M^+] (100), 237 [M^+−X] (17), 284 [M^+−CS] (27), 193 [M^+−CS–X] (20); IR (film): $\tilde{\nu} = 1175\ cm^{-1}$ (s, C=S), 1603 (m, C=C), 1502 (C–NO_2); ^{1}H NMR (DMSO-d_6, 500 MHz): δ (ppm) 6.98, (s, 1H, H3), 8.15 (d, 1H, J = 7.03 Hz, H5), 7.81, (dd, 1H, J = 7.75, 7.40, 1.5 Hz, H6), 7.79 (dd, 1H, J = 8.31, 2.01 Hz, H7), 7.52 (d, 1H, J = 8.01 Hz, H8), 8.75 (d, 1H, J = 8.00, H3′), 8.73 (d, 1H, J = 7.59 Hz, H4′), 8.81 (s, 1H, H6′); Anal. Calcd for $C_{15}H_8O_5N_2S$ (328.30): C, 54.88; H, 2.46; N, 8.53. Found: C, 54.81; H, 2.42; N, 8.58.

6.2.2. 2-(2′-Iodophenyl)-6-chloro-4*H*-1-benzopyran-4-thione (B_8). Yield: 78%; mp 158–160 °C; MS (EI): *m*/*z* (%) = 400 [M+2] (66), 398 [M^+] (100), 271 [M^+−X] (92), 354 [M^+−CS] (18), 319 [M^+−CS–X] (40); IR (film): $\tilde{\nu} = 1176\ cm^{-1}$ (s, C=S), 1607 (m, C=C), 805 (C–N); ^{1}H NMR (DMSO-d_6, 500 MHz): δ (ppm) 7.19 (s, 1H, H3),

8.37 (d, J = 2.23 Hz, 1H, H5), 7.97 (dd, J = 7.96/2.36 Hz, 1H, H7), 7.76 (d, J = 7.48 Hz, 1H, H8), 8.08 (dd, J = 7.89/2.12 Hz, 1H, H3′), 7.36 (m, 1H, H4′), 7.61 (m, 1H, H5′), 7.83 (dd, J = 8.90/2.33 Hz, 1H, H6′); Anal. Calcd for $C_{15}H_8ICIOS$ (398.65): C, 45.19; H, 2.02. Found: C, 45.39; H, 2.12.

6.2.3. 2-(2′-Iodophenyl)-6-bromo-4*H*-1-benzopyran-4-thione (B_9). Yield: 80%; mp 146–147 °C; MS (EI): m/z (%) = 445 [M+2] (50), 443 [M^+] (100), 316 [M^+-X] (95), 399 [M^+-CS] (27), 319 [M^+-CS-X] (30); IR (film): $\tilde{\nu}$ = 1171 cm^{-1} (s, C=S), 1598 (m, C=C), 770 (C–X); ^{1}H NMR (DMSO-d_6, 500 MHz): δ (ppm) 7.20 (s, 1H, H3), 8.40 (d, J = 2.50 Hz, 1H, H5), 7.98 (dd, J = 7.45/2.15 Hz, 1H, H7), 7.78 (d, J = 7.50 Hz, 1H, H8), 8.09 (dd, J = 7.90/2.23 Hz, 1H, H3′), 7.40 (m, 1H, H4′), 7.69 (m, 1H, H5′), 7.89 (dd, J = 8.51/2.43 Hz, 1H, H6′); Anal. Calcd for $C_{15}H_8BrIOS$ (443.10): C, 40.66; H, 1.82. Found: C, 40.69; H, 1.72.

6.2.4. 2-(2′-Iodophenyl)-6-fluoro-4*H*-1-benzopyran-4-thione (B_{10}). Yield: 78%; mp 162–163 °C; MS (EI): m/z (%) = 382 [M^+] (100), 255 [M^+-X] (36), 338 [M^+-CS] (2), 319 [M^+-CS-X] (28); IR (film): $\tilde{\nu}$ = 1182 cm^{-1} (s, C=S), 1603 (m, C=C), 780 (C–X); ^{1}H NMR (DMSO-d_6, 500 MHz): δ (ppm) 7.35 (s, 1H, H3), 8.11 (d, J = 2.66 Hz, 1H, H5), 8.09 (dd, J = 7.92/2.89 Hz, 1H, H7), 7.75 (d, J = 7.42 Hz, 1H, H8), 7.83 (dd, J = 7.88/4.79 Hz, 1H, H3′), 7.45 (m, 1H, H4′), 7.59 (m, 1H, H5′), 7.86 (dd, J = 7.88/4.78 Hz, 1H, H6′); Anal. Calcd for $C_{15}H_8FIOS$ (382.19): C, 47.14; H, 2.11. Found: C, 47.10; H, 2.17.

6.3. General procedure for the synthesis of 8 series (C, C_1–C_{12})

A fresh solution of 5.05 mmol of 2,4-dinitrophenylhydrazine in concd H_2SO_4 (2 mL), 95% alcohol (15 mL) and 5.05 mmol of substituted/unsubstituted flavones, that is, **6** series were refluxed and allowed to stand in a stoppered flask for 24 h in order to precipitate the product completely. The solution was diluted with 1 M solution of concd H_2SO_4 in water and the precipitate washed with dilute acid and crystallized from dioxane. Spectroscopic as well as other physical data of the known compound in **8** series (**C**) are given in the literature[19], while those of the new compounds (C_1–C_{12}) are given below.

6.3.1. 2-(2,4-Dinitrophenyl)-1-(2-(3′-methoxyphenyl)-4*H*-chromen-4-ylidene)hydrazine (C_1). Yield: 83%; mp 230–231 °C; MS (EI): m/z (%) = 432 [M^+] (100), 401 [M^+-X] (36), 415 [M^+-OH] (20), 356 [$M^+-C_6H_4$] (33), 310 [$M^+-C_6H_4-NO_2$] (51); IR (film): $\tilde{\nu}$ = 3425 cm^{-1} (NH), 1616 (s, C=N), 1601 (m, C=C), 1097 (C–OMe); ^{1}H NMR (DMSO-d_6, 500 MHz): δ (ppm) 3.87 (s, 3H, OMe), 7.12 (s, 1H, H3), 7.69 (m, 1H, H5), 7.51 (m, 1H, H6), 7.66 (m, 1H, H7), 7.42 (m, 1H, H8), 8.07 (d, J = 2.38 Hz, 1H, H2′), 7.45 (m, 1H, H4′), 7.21 (m, 1H, H5′), 7.75 (m, 1H, H6′), 8.89 (d, J = 2.52 Hz, IH, H3″), 8.38 (dd, J = 7.50/2.46 Hz, IH, H5″), 8.29 (d, J = 7.83 Hz, 1H, H6″); Anal. Calcd for $C_{22}H_{16}O_6N_4$ (432.39): C, 61.11; H, 3.73; N, 12.96. Found: C, 61.01; H, 3.75; N, 12.99.

6.3.2. 2-(2,4-Dinitrophenyl)-1-(2-(4′-methylphenyl)-4*H*-chromen-4-ylidene)hydrazine (C_2). Yield: 81%; mp 241–242 °C; MS (EI): m/z (%) = 416 [M^+] (100), 401 [M^+-X] (4), 399 [M^+-OH] (36), 340 [$M^+-C_6H_4$] (4), 294 [$M^+-C_6H_4-NO_2$] (56); IR (film): $\tilde{\nu}$ = 3460 cm^{-1} (NH), 1614 (s, C=N), 1665 (m, C=C), 1333 (C–Me); ^{1}H NMR (DMSO-d_6, 500 MHz): δ (ppm) 2.40 (s, 3H, Me), 7.07 (s, 1H, H3), 7.66 (d, J = 7.05 Hz, 1H, H5), 7.44 (m, 1H, H6), 7.46 (m, 1H, H7), 7.60 (d, J = 7.43 Hz, 1H, H8), 8.06 (d, J = 9.63 Hz, 2H, H2′/6′), 7.95 (d, J = 8.19 Hz, 1H, H3′/5′), 8.88 (d, J = 2.52 Hz, IH, H3″), 8.37 (dd, J = 7.01/2.50 Hz, IH, H5″), 8.28 (d, J = 7.25 Hz, 1H, H6″); Anal. Calcd for $C_{22}H_{16}O_5N_4$ (416.39): C, 63.46; H, 3.87; N, 13.46. Found: C, 63.41; H, 3.90; N, 13.44.

6.3.3. 2-(2,4-Dinitrophenyl)-1-(2-(4′-nitrophenyl)-4*H*-chromen-4-ylidene)hydrazine (C_3). Yield: 72%; mp 297–298 °C; MS (EI): m/z (%) = 447 [M^+] (100), 401 [M^+-X] (19), 430 [M^+-OH] (25), 371 [$M^+-C_6H_4$] (28), 325 [$M^+-C_6H_4-NO_2$] (48); IR (film): $\tilde{\nu}$ = 3440 cm^{-1} (NH), 1611 (s, C=N), 1590 (m, C=C), 1475 (C–NO_2); ^{1}H NMR (DMSO-d_6, 500 MHz): δ (ppm) 7.02 (s, 1H, H3), 7.68 (d, J = 7.11 Hz, 1H, H5), 7.49 (m, 1H, H6), 7.52 (m, 1H, H7), 7.38 (d, J = 7.40 Hz, 1H, H8), 8.21 (d, J = 8.03 Hz, 2H, H2′/6′), 8.30 (d, J = 7.81 Hz, 2H, H3′/5′), 8.89 (d, J = 2.31 Hz, IH, H3″), 8.51 (dd, J = 7.51/2.50 Hz, IH, H5″), 8.28 (d, J = 7.81 Hz, 1H, H6″); Anal. Calcd for $C_{21}H_{13}O_7N_5$ (447.36): C, 56.38; H, 2.93; N, 15.65. Found: C, 56.33; H, 2.97; N, 15.60.

6.3.4. 2-(2,4-Dinitrophenyl)-1-(2-(4′-methoxyphenyl)-4*H*-chromen-4-ylidene)hydrazine (C_4). Yield: 69%; mp 150–152 °C; MS (EI): m/z (%) = 432 [M^+] (100), 401 [M^+-X] (38), 415 [M^+-OH] (27), 356 [$M^+-C_6H_4$] (39), 310 [$M^+-C_6H_4-NO_2$] (58); IR (film): $\tilde{\nu}$ = 3390 cm^{-1} (NH), 1619 (s, C=N), 1600 (m, C=C), 1030 (C–OMe); ^{1}H NMR (DMSO-d_6, 500 MHz): δ (ppm) 3.65 (s, 3H, –OMe), 7.01 (s, 1H, H3), 7.60 (d, J = 7.05 Hz, 1H, H5), 7.52 (m, 1H, H6), 7.50 (m, 1H, H7), 7.70 (d, J = 7.43 Hz, 1H, H8), 8.16 (d, J = 7.66 Hz, 2H, H2′/6′), 8.22 (d, J = 7.01 Hz, 2H, H3′/5′), 8.69 (d, J = 2.53 Hz, IH, H3″), 8.52 (dd, J = 7.50/2.03 Hz, IH, H5″), 8.41 (d, J = 8.00 Hz, 1H, H6″); Anal. Calcd for $C_{22}H_{16}O_6N_4$ (432.39): C, 61.11; H, 3.73; N, 12.96. Found: C, 61.17; H, 3.69; N, 12.98.

6.3.5. 2-(2,4-Dinitrophenyl)-1-(2-(2′,5′-dinitrophenyl)-4*H*-chromen-4-ylidene)hydrazine (C_5). Yield: 60%; mp 185–186 °C; MS (EI): m/z (%) = 492 [M^+] (100), 400 [M^+-X] (19), 475 [M^+-OH] (21), 416 [$M^+-C_6H_4$] (48), 370 [$M^+-C_6H_4-NO_2$] (66); IR (film): $\tilde{\nu}$ = 3445 cm^{-1} (NH), 1614 (s, C=N), 1595 (m, C=C), 1495 (C–NO_2); ^{1}H NMR: (DMSO-d_6, 500 MHz): δ (ppm) 7.18 (s, 1H, H3), 7.91 (d, J = 8.01Hz, 1H, H5), 7.47 (m, 1H, H6), 7.80 (m, 1H, H7), 7.40 (d, J = 7.79 Hz, 1H, H8), 8.03 (d, J = 7.98 Hz, 2H, H3′), 8.10 (s, 2H, H6′), 8.90 (d, J = 2.33 Hz, IH, H3″), 8.86 (dd, J = 8.30/2.50 Hz, IH, H5″), 8.81 (d, J = 7.65 Hz, 1H, H6″); Anal. Calcd for $C_{21}H_{12}O_9N_6$ (492.36): C, 51.23; H, 2.46; N, 17.07. Found: C, 51.21; H, 2.49; N, 17.03.

6.3.6. 2-(2,4-Dinitrophenyl)-1-(2-(3′-nitrophenyl)-4*H*-chromen-4-ylidene)hydrazine (C_6). Yield: 81%; mp 225–227 °C; MS (EI): *m/z* (%) = 447 [M^+] (100), 401 [M^+-X] (9), 430 [M^+-OH] (35), 371 [$M^+-C_6H_4$] (78), 325 [$M^+-C_6H_4-NO_2$] (68); IR (film): $\tilde{\nu} = 3480\ cm^{-1}$ (NH), 1610 (s, C=N), 1593 (m, C=C), 1479 (C–NO_2); ^1H NMR (DMSO-d_6, 500 MHz): δ (ppm) 7.26 (s, 1H, H3), 8.08 (d, $J = 8.55$ Hz, 1H, H5), 7.87 (m, 1H, H6), 7.92 (m, 1H, H7), 7.69 (d, Hz, 1H, H8), 8.46 (s, 1H, 2′-H), 7.93 (m, 1H, H4′) 7.45 (m, 1H, H5′), 8.43 (d, $J = 8.25$ Hz, 1H, H6′), 8.89 (d, $J = 1.47$ Hz, IH, H3″), 8.84 (dd, $J = 7.95/1.47$ Hz, IH, H5″), 8.57 (d, $J = 7.85$ Hz, 1H, H6″); Anal. Calcd for $C_{21}H_{13}O_7N_5$ (447.36): C, 56.38; H, 2.93; N, 15.65. Found: C, 56.31; H, 2.97; N, 15.67.

6.3.7. 2-(2,4-Dinitrophenyl)-1-(2-phenyl-6-fluoro-4*H*-chromen-4-ylidene)hydrazine (C_7). Yield: 85%; mp 270–271 °C; MS (EI): *m/z* (%) = 420 [M^+] (100), 328 [M^+-X] (24), 403 [M^+-OH] (80), 344 [$M^+-C_6H_4$] (53), 298 [$M^+-C_6H_4-NO_2$] (95); IR (film): $\tilde{\nu} = 3495\ cm^{-1}$ (NH), 1611 (s, C=N), 1585 (m, C=C), 1329 (C–NO_2), 1115 (C–F); ^1H NMR (DMSO-d_6, 500 MHz): δ (ppm) 7.10 (s, 1H, H3), 11.34 (s, 1H, N–H), 8.87 (s, 1H, H3″), 8.34 (d, $J = 8.35$ Hz, 1H, H5″), 8.15 (d, $J = 9.3$ Hz, 1H, H6″), 8.03 (m, 3H, H5/7/8), 7.53–7.67 (m, 5H, H2′/3′/4′/5′/6′); Anal. Calcd for $C_{21}H_{13}O_5FN_4$ (420.36): C, 60.00; H, 3.12; N, 13.33. Found: C, 59.89; H, 3.17; N, 13.30.

6.3.8. 2-(2,4-Dinitrophenyl)-1-(2-(2′-iodophenyl)-6-chloro-4*H*-chromen-4-ylidene)hydrazine (C_8). Yield: 82%; mp 250–251 °C; MS (EI): *m/z* (%) = 564 [M+2] (70), 562 [M^+] (100), 435 [M^+-X] (7), 545 [M^+-OH] (6), 486 [$M^+-C_6H_4$] (31), 440 [$M^+-C_6H_4-NO_2$] (22); IR (film): $\tilde{\nu} = 3425\ cm^{-1}$ (NH), 1616 (s, C=N), 1601 (m, C=C), 1097 (C–X); ^1H NMR (DMSO-d_6, 500 MHz): δ (ppm) 6.59 (s, 1H, H3), 8.84 (d, $J = 1.75$ Hz, 1H, H5), 7.78 (dd, $J = 8.90/1.96$ Hz, 1H, H7), 7.34 (d, $J = 7.75$ Hz, 1H, H8), 7.91 (dd, $J = 8.35/2.61$ Hz, 1H, H3′), 6.72 (m, 1H, H4′), 7.69 (m, 1H, H5′), 7.58 (dd, $J = 7.09/4.12$ Hz, 1H, H6′), 8.28 (s, IH, H3″), 8.06 (dd, $J = 8.20/2.45$ Hz, IH, H5″), 8.03 (d, $J = 8.60$ Hz, 1H, H6″), 11.24 (s, 1H, N–H); Anal. Calcd for $C_{21}H_{12}ClO_5N_4$ (562.71): C, 44.82; H, 2.15; N, 9.96. Found: C, 44.88; H, 2.11; N, 9.99.

6.3.9. 2-(2,4-Dinitrophenyl)-1-(2-(2′-iodophenyl)-6-bromo-4*H*-chromen-4-ylidene)hydrazine (C_9). Yield: 82%; mp 152–153 °C; MS (EI): *m/z* (%) = 609 [M+2] (54), 607 [M^+] (100), 480 [M^+-X] (10), 590 [M^+-OH] (26), 531 [$M^+-C_6H_4$] (36), 485 [$M^+-C_6H_4-NO_2$] (70); IR (film): $\tilde{\nu} = 3310\ cm^{-1}$ (NH), 1612 (s, C=N), 1596 (m, C=C), 757 (C–X); ^1H NMR (DMSO-d_6, 500 MHz): δ (ppm) 6.53 (s, 1H, H3), 8.83 (d, $J = 2.50$ Hz, 1H, H5), 7.76 (dd, $J = 8.30/2.01$ Hz, 1H, H7), 7.40 (d, $J = 7.50$ Hz, 1H, H8), 7.98 (dd, $J = 8.35/2.68$ Hz, 1H, H3′), 6.74 (m, 1H, H4′), 7.59 (m, 1H, H5′), 7.50 (dd, $J = 7.35/3.98$ Hz, 1H, H6′), 8.24 (s, IH, H3″), 8.21 (dd, $J = 8.01/2.47$ Hz, IH, H5″), 8.05 (d, $J = 8.36$ Hz, 1H, H6″), 11.24 (s, 1H, N–H); Anal. Calcd for $C_{21}H_{12}BrIO_5N_4$ (607.16): C, 41.54; H, 1.99; N, 9.23. Found: C, 41.58; H, 2.02; N, 9.20.

6.3.10. 2-(2,4-Dinitrophenyl)-1-(2-(2′-iodophenyl)-6-fluoro-4*H*-chromen-4-ylidene)hydrazine (C_{10}). Yield: 83%; mp 263–264 °C; MS (EI): *m/z* (%) = 546 [M^+] (100), 419 [M^+-X] (4), 529 [M^+-OH] (3), 470 [$M^+-C_6H_4$] (5), 424 [$M^+-C_6H_4-NO_2$] (28); IR (film): $\tilde{\nu} = 3292\ cm^{-1}$ (NH), 1614 (s, C=N), 1584 (m, C=C), 775 (C–X); ^1H NMR(DMSO-d_6, 500 MHz): δ (ppm) 6.56 (s, 1H, H3), 8.84 (d, $J = 2.40$ Hz, 1H, H5), 7.71 (dd, $J = 6.65/0.9$ Hz, 1H, H7), 7.62 (d, $J = 7.45$ Hz, 1H, H8), 8.05 (dd, $J = 8.01/2.80$ Hz, 1H, H3′), 6.69 (m, 1H, H4′), 7.33 (m, 1H, H5′), 7.55 (dd, $J = 5.25/4.85$ Hz, 1H, H6′), 8.17 (s, IH, H3″), 8.35 (dd, $J = 8.55/2.45$ Hz, IH, H5″), 8.07 (d, $J = 8.0$ Hz, 1H, H6″), 11.22 (s, 1H, N–H); Anal. Calcd for $C_{21}H_{12}FIO_5N_4$ (546.25): C, 46.17; H, 2.21; N, 10.26. Found: C, 46.20; H, 2.18; N, 10.22.

6.3.11. 2-(2,4-Dinitrophenyl)-1-(2-(4′-fluorophenyl)-4*H*-chromen-4-ylidene)hydrazine (C_{11}). Yield: 85%; mp 180–182 °C; MS (EI): *m/z* (%) = 420 [M^+] (100), 401 [M^+-X] (2), 403 [M^+-OH] (69), 344 [$M^+-C_6H_4$] (47), 298 [$M^+-C_6H_4-NO_2$] (95); IR (film): $\tilde{\nu} = 3470\ cm^{-1}$ (NH), 1622 (s, C=N), 1603 (m, C=C), 1139 (C–F); ^1H NMR (DMSO-d_6, 500 MHz): δ (ppm) 6.98 (s, 1H, H3), 7.70 (d, $J = 7.90$ Hz, 1H, H5), 7.60 (m, 1H, H6), 7.67 (m, 1H, H7), 7.21 (d, $J = 8.03$ Hz, 1H, H8), 8.28 (d, $J = 7.98$ Hz, 2H, H2′/6′), 8.38 (d, $J = 8.02$ Hz, 2H, H3′/5′), 8.75 (d, $J = 1.98$ Hz, IH, H3″), 8.62 (dd, $J = 7.78/2.02$ Hz, IH, H5″), 8.50 (d, $J = 7.49$ Hz, 1H, H6″); Anal. Calcd for $C_{21}H_{13}FO_5N_4$ (420.36): C, 60.00; H, 3.12; N, 13.33. Found: C, 60.07; H, 3.10; N, 13.39.

6.3.12. 2-(2,4-Dinitrophenyl)-1-(2-(3′,4′-dimethoxyphenyl)-4*H*-chromen-4-ylidene)hydrazine (C_{12}). Yield: 82%; mp 268–270 °C; MS (EI): *m/z* (%) = 462 [M^+] (100), 400 [M^+-X] (7), 445 [M^+-OH] (52), 386 [$M^+-C_6H_4$] (42), 340 [$M^+-C_6H_4-NO_2$] (87); IR (film): $\tilde{\nu} = 3410\ cm^{-1}$ (NH), 1618 (s, C=N), 1608 (m, C=C), 1090 (C–OMe); ^1H NMR (DMSO-d_6, 500 MHz): δ (ppm) 3.90 (s, 6H, 2-OMe), 7.08 (s, 1H, H3), 7.92 (dd, $J = 7.50/1.49$ Hz, 1H, H5), 7.51 (m, 1H, H6), 7.87 (dd, $J = 7.06/1.47$ Hz, 1H, H7), 7.41 (dd, $J = 8.0/2.14$ Hz, 1H, H8), 8.32 (s, 1H, H2′), 8.15 (d, $J = 8.30$ Hz, 1H, H5′), 8.01 (d, $J = 8.03$ Hz, 1H, H6′), 8.88 (d, $J = 2.0$ Hz, IH, H3″), 8.84 (dd, $J = 7.75/2.01$ Hz, IH, H5″), 8.81 (d, $J = 7.77$ Hz, 1H, H6″); Anal. Calcd for $C_{23}H_{18}O_7N_4$ (462.42): C, 59.74; H, 3.92; N, 12.12. Found: C, 59.78; H, 3.88; N, 12.19.

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