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SYNTHESIS OF SOME NEW HETEROCYCLIC COMPOUNDS DERIVED FROM 3-FORMYLCHROMONES AND THEIR ANTIMICROBIAL EVALUATION

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The synthesis and antimicrobial evaluation of a series of chromone-linked substituted heterocyclic derivatives is described. The condensation of 3-formylchromone with acetoacetamide under Knoevenagel–Cope reaction conditions was also explored, and the condensation with 4-hydroxy-6-methyl-2H-pyran-2-one constitutes a facile route to pyranopyrone fused systems. Most of the compounds exhibit good antimicrobial properties.

Keywords: 3-formylchromone, pyranopyrones, antimicrobial activity, enzyme inhibitors, Knoevenagel–Cope reaction, pharmacophore, urease inhibitory.

3-Formylchromone is considered a versatile synthetic intermediate for the preparation of a variety of heterocyclic systems due to the presence of an unsaturated system, a conjugated carbonyl group and, more importantly, an electron deficient center [1]. This center is very reactive and serves as Michael acceptor resulting in the formation of fused polycyclic heterocycles by reaction with bifunctional nucleophiles. Moreover, from the biological viewpoint, chromone is an important component of pharmacophores of various biologically active natural products [2-5]. In addition, the core structure of many flavanoids is based on the chromone moiety and has been found to possess several therapeutically interesting properties [6-9]. Several formylchromone derivatives have been examined for their antitumor [10], urease inhibitory [11-12], anti-HIV [13], and antimicrobial activities [14-15]. It is also known that 3-formylchromone derivatives promote induction of chloroplast-free mutants in *Euglena gracilis* [16], have antiproliferative activity [17], and acts as tyrosine kinase inhibitors [18]. On the other hand, the biological activity of isatin and substituted thiosemicarbazones has been known for long time, both as anticancer and antiviral compounds [19]. These isatin thiosemicarbazones undergo cyclization reactions at sulfur and nitrogen afforded oxindoles [20]. Oxindole derivatives have been used as important intermediates in synthetic approaches to obtain various heterocyclic systems [21];

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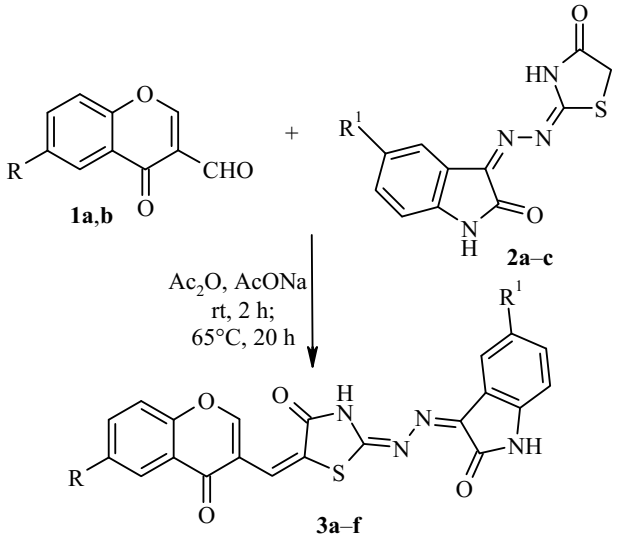
moreover, the methylene group of the thiazolidinone ring could be utilized for further transformations. It was therefore thought worthwhile to link these oxindoles to the chromone nucleus *via* condensation. In the last few decades, much interest has been shown towards systematic studies on condensation reactions of 3-formylchromones with heterocycles and substituted binucleophiles to generate various fused heterocyclic derivatives possessing different types of biological activities associated with them [22].

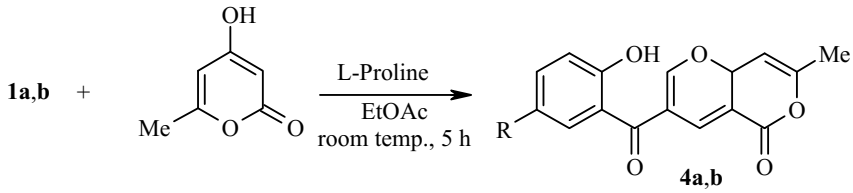
Additionally, condensations of 3-formylchromones with active methylene and methyl compounds generated great interest in the earlier research owing to their interesting biological properties [23-24]. This paper presents the synthetic capability and the exploitation of the above-mentioned types of condensation, achieved using the Knoevenagel–Cope method, as a very convenient rate-enhancing approach. Several types of subsequent reactions of the active methylene molecules illustrate the ability of chromone derivatives to serve as excellent precursors for the synthesis of a wide variety of heterocyclic systems [25].



1 a R = H, **b** R = Cl; **2 a** R^1 = H, **b** R^1 = Cl, **c** R^1 = F;
3 a–c R = H, **d–f** R = Cl; **a**, **d** R^1 = H, **b**, **e** R^1 = Cl, **c**, **f** R^1 = F

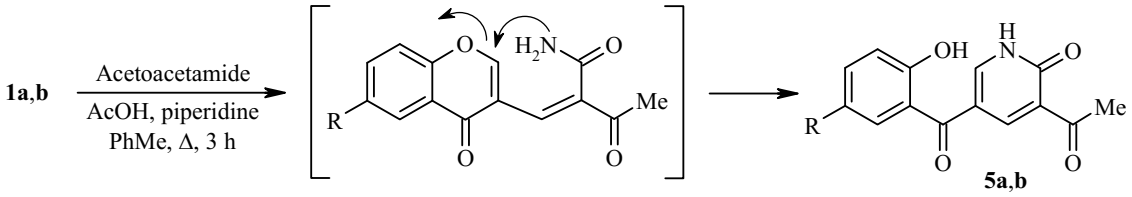
Compounds **1a**,**b** were prepared by the literature procedure [26], and the substituted pseudothiohydantoin derivatives **2a-c** were obtained by the condensation of isatin with thiosemicarbazides, which were cyclized with monochloroacetic acid in glacial AcOH to yield the oxindole products **2a-c** as described in the literature [27]. The reaction of 3-formylchromone **1a**,**b** with substituted oxindoles gave compounds **3a-f** in moderate to good yields when heated at 65°C for 20 h. Further heating of the mixture at higher temperature resulted in the formation of various side products and lower yields. Compounds **3a-f** were characterized by IR, ^{1}H and ^{13}C NMR spectroscopy, mass spectrometry, and elemental analysis. In the IR spectrum, absorption bands due to the stretching vibrations of the NH group of indole and the thiazolidine ring appear in the 3050-3140 cm^{-1} range. The spectra of compounds **3a-f** display also characteristic bands representing C=O groups (intense bands at 1650-1732 cm^{-1}). In the ^{1}H NMR spectrum of compounds **3a-f** the NH protons of thiazolidinone and isatin moieties appear as two broad singlets at 11.18-11.37 and 12.10-12.28 ppm, while the disappearance of the aldehyde proton signal and appearance of a singlet signal of the methyne proton at ~7.35 ppm suggest the formation of the desired product. All the compounds **3a-f** were isolated as (*E*)-isomers, further confirmed by gated-decoupling (GD) measurements in which a strong vicinal resonance was calculated for vinylic proton. Peaks at 160-166 ppm in the ^{13}C NMR spectra of compounds **3a-f** confirm the presence of three carbonyl groups. Signals of aromatic protons appeared in their respective regions. The elemental analysis data are also in accord with the proposed structures.

The condensation of formylchromones **1a**,**b** with 4-hydroxy-6-methyl-2*H*-pyran-2-one in the presence of 0.5 equiv. of L-proline as catalyst was performed in ethyl acetate at room temperature to get the desired compounds **4a**,**b**. The same reaction conditions were reported for the condensation of carbohydrate enals with pyrones and L-proline in the presence of $TiCl_4$ catalysts [28]. However, L-proline provides a cleaner reaction, consumes less time, and the products are required to be only purified by column chromatography. It was observed that the reaction proceeds with higher yields at room temperature only, whereas at 0°C or at refluxing temperature of the solvent using $TiCl_4$ as catalyst gave lower yields and the compounds required rigorous purification. The reaction is thought to proceed with the *in situ* formation of the imine salt of L-proline; moreover, the carbinol amine formed during the reaction, releases a water molecule to equilibrate with the iminium salt. Regeneration of catalyst and the ring transformation involving 6 π-electrons lead to formation of the pyranopyrone fused system. The IR spectrum of compounds **4a**,**b** showed bands at 1670 and 1730 cm^{-1} (for compound **4a**) and at 1680 and 1725 cm^{-1} (for compound **4b**) corresponding to C=O groups. Additionally, absorption bands at ~3290 cm^{-1} confirm the presence of the OH group. In the ^1H NMR spectrum of compounds **4a**,**b**, two pyran ring protons appeared as two doublets and exhibit characteristic *W* coupling, having coupling constants $J = 2.1$ Hz; this confirms the opening of the pyrone ring and the formation of a new ring. The ^{13}C NMR spectra of the obtained compounds also indicated the presence of two C=O groups at 162.2 and ~189.6 ppm, which supports the postulated structure. The mass spectra are also in accordance with the proposed structure of compounds **4a**,**b**, indicating the molecular ion peaks $[M+H]^+$ at *m*/*z* 285 and 319, as well as base peaks of fragment $[M-C_{10}H_4O_2]^+$ at *m*/*z* 128 and 162, respectively.



a R = H, **b** R = Cl

Compounds **5a**,**b** were synthesized by reacting compounds **1a**,**b** with acetoacetamide under Knoevenagel–Cope conditions. Synthesis of compound **5a** by a tedious two-step process in the presence of liquid ammonia was reported previously [29-30]. Under similar conditions, various side products were formed with low overall yield. We herein report the current reaction in refluxing toluene, which was used in strictly anhydrous conditions using a Dean–Stark trap. The optimum temperature and conditions for the completion of this reaction with good yield was determined by a series of reactions of compound **1a** with acetoacetamide for 3 h in boiling toluene. The reaction proceeds by the deprotonation of the active methylene group. Piperidinium acetate formed *in situ* is used as an active catalyst in this reaction.



a R = H, **b** R = Cl

The attack at the formyl carbon atom followed by the attack of the NH_2 group at the C-1 atom of the chromone moiety results in the concomitant opening of the ring to give the desired compounds **5a**,**b** in good to excellent yields. Yellow crystals of compound **5a** suitable for X-ray crystallography were obtained by crystallization from AcOEt–PhMe mixture.

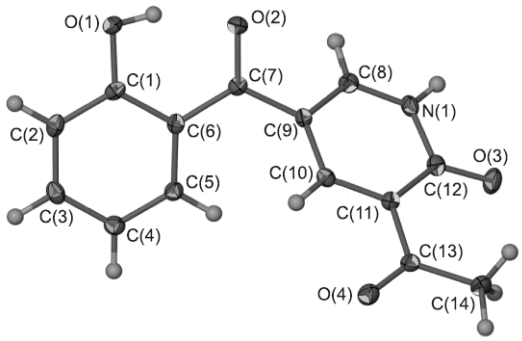


Fig. 1. Crystal structure of compound **5a**.
Thermal ellipsoids are shown at the 50% probability level.

The crystal structure of compound **5a** is shown in the Figure 1, and the crystal data are listed in Table 1. In the ^{1}H NMR spectra of compounds **5a**,**b** two protons of the pyridine ring appeared as two doublets with a characteristic *W* coupling, having coupling constant J = 2.5 Hz. The NH signal was observed in the downfield region of the ^{1}H NMR spectrum as a characteristic broad singlet. The ^{13}C NMR spectra of compounds **5a**,**b** contain signals characteristic for C=O groups. Additionally, in the IR spectrum of compounds **5a**,**b**, strong absorption bands of C=O, NH, and OH groups are found at 1710-1730, 3050, and 3270 cm^{-1} (for compound **5a**) and at 1710-1727, 3030, and 3275 cm^{-1} (for compound **5b**), respectively.

TABLE 1. The Main Crystallografic Parameters for Compound **5a**

Parameters	**5a**	Parameters	**5a**
Empirical formula	$C_{14}H_{11}NO_4$	d_{calc}, g·cm^{-3}	1.557
Molecular weight	257.25	μ, cm^{-1}	0.97
Color, shape	Yellow prism	F(000)	536
Crystal measurement, mm	0.40×0.30×0.20	Range for θ, deg	6.7-76.5
T, K	100	Range for indices	-12 < h < 12 -20 < k < 27 -8 < l < 8
Crystal system	Monoclinic	Number of measured reflections	5543
Space group	*Cc*	Number of independent reflections (R_{int})	2079 (0.017)
Z	4	Number of reflections with $I > 2\sigma(I)$	2070
a, Å	9.6930(2)	Number of refined parameters	181
b, Å	21.8024(4)	R1	0.029
c, Å	6.9173(1)	wR2	0.077
β, deg	131.367(2)	*GOOF*	1.05
V, Å^3	1097.10(3)	Residual electron density, e·Å^{-3}(d_{min}/d_{max})	0.29 / -0.25

The results of antibacterial activities of compounds **3a**-**f**, **4a**,**b**, and **5a**,**b** against gram-positive bacteria (*S. aureus*, *S. mutans*, *B. subtilis*) and gram-negative bacteria (*E. coli*, *S. typhi*, *P. aeruginosa*) are summarized in Table 2. It has been observed that all compounds exhibited interesting antibacterial activities except compound **3e**. Compound **3f** showed very high activity against most of the tested bacteria, while compounds

TABLE 2. Antibacterial Activity* of Compounds **3a**-**f**, **4a**,**b**, **5a**,**b** against Gram-positive and Gram-negative Bacteria

Compound	Gram-positive bacteria			Gram-negative bacteria		
	S. aureus	*S. mutans*	*B. subtilis*	*E. coli*	*S. typhi*	*P. aeruginosa*
3a	—	22	21	22	13	—
3b	32	—	–	22	15	—
3c	47	14	24	–	—	—
3d	40	—	24	–	—	—
3e	—	—	–	–	19	—
3f	37	19	20	24	14	17
4a	46	24	33	–	—	—
4b	46	23	37	28	—	—
5a	37	21	23	—	—	—
5b	41	—	—	—	—	—
Ciprofloxacin	45	30	35	18	—	11
Naldixic Acid	—	—	—	34	28	23

*Values are in millimeter (mm).
"–" indicates either no inhibition or inhibition zone diameter lower than 10 mm.
Values are expressed as mean (n = 3).

4a,**b** and **5a** showed promising results against gram-positive bacteria. On the other hand, it was found that compounds **3c**,**d** are less active against gram-negative bacteria. Considering the results obtained from antibacterial tests, it is noteworthy to mention that the tested compounds exhibited more activity towards gram-positive bacteria than towards gram-negative bacteria.

The results of antifungal activities of all synthesized compounds are evaluated in Table 3. Most of the studied compounds showed a significant level of activity in comparison with the standard used. Almost all compounds exhibited medium activity against *C. albicans*, however, for the species *A. niger* and *A. flavus* compounds **3f**, **4a**,**b** and **5a** exhibited more potent activity than other compounds.

TABLE 3. Antifungal activity* of compounds **3a**-**f, 4a**,**b**, and **5a**,**b** against *C. albicans*, *A. niger*, and *A. flavus*

Compound	*C. albicans*	*A. niger*	*A. flavus*
3a	24	21	—
3b	19	—	17
3c	—	17	21
3d	23	—	—
3e	—	—	—
3f	20	22	25
4a	39	31	33
4b	37	33	35
5a	27	14	—
5b	—	—	—
Amphotericin B	33	35	30

*Values are in millimeter (mm).
"–" indicates either no inhibition or inhibition zone diameter less than 10 mm.
Values are expressed as mean (n = 3).

In conclusion, we have devised an effective and environmentally benign method for the synthesis of chromone, pyrano[4,3-*b*]pyran-5(8a*H*)-one, and pyridin-2(1*H*)-one derivatives. The notable merits of the present methods are short reaction times, simple work-up procedure, and moderate to good yield of products. Many of the obtained products exhibit significant antibacterial and antifungal activity.

EXPERIMENTAL

IR spectra were recorded with a Perkin-Elmer spectrum BX FT-IR spectrometer using KBr pellets. ^{1}H and ^{13}C NMR spectra were recorded on a Bruker instrument (500 and 125 MHz, respectively) in DMSO-d_6 (compounds **3a-f**) and $CDCl_3$ (compounds **4a**,**b**, **5a**,**b**), TMS was used as internal standard. Mass spectra were obtained on a JEOL JMS-700 mass spectrometer, ionization method was EI (70 eV). Microanalysis was performed on a Perkin-Elmer CHNS-2440 analyzer and Thermo Scientific Orion ion selective electrodes. Melting points were measured with a Thermo Scientific 9100 apparatus and are uncorrected. Thin-layer chromatography (TLC) was performed with fluorescent silica gel HF_{254} plates (Merck) and visualized under UV 254 and 265 lights and charring with the EtOH–H_2SO_4 system. Merck silica gel 60 (230-400 mesh) was used for column chromatography. Organic solvents used were dried by standard methods. Acetoacetamide, 4-hydroxy-6-methyl-2*H*-pyran-2-one, and piperidine were purchased from Aldrich Chemical Co. and were used as obtained. Chromones **1a**,**b** were prepared by the literature procedure [26], and the substituted pseudothiohydantoin derivatives **2a-c** were obtained by the method described in [27].

Synthesis of Compounds 3a-f (General Method). A mixture of chromones **1a**,**b** (3.0 mmol), compounds **2a-c** (3.1 mmol), and freshly fused NaOAc (0.25 g, 3.0 mmol) in Ac_2O (20 ml) was stirred under N_2 atmosphere at room temperature for 2 h, and then at 65°C for 20 h. The obtained residue was purified by column chromatography on silica gel, eluting with AcOEt–hexane, 2:3.

5-[(4-Oxo-4*H*-chromen-3-yl)methylene]-2-[(*Z*)-(2-oxoindolin-3-ylidene)hydrazono]thiazolidin-4-one (3a). Yield 56%, yellow solid, mp 250-254°C. IR spectrum, ν, cm^{-1}: 1672 (C=O), 1710 (C=O amide), 1732 (C=O amide), 3055 (NH), 3110 (NH). ^{1}H NMR spectrum, δ, ppm (*J*, Hz): 7.36 (1H, s, =CH–); 7.40 (1H, s, H Ar); 7.53-7.57 (2H, m, H Ar); 7.65 (1H, t, *J* = 8.8, H Ar); 7.78 (1H, t, *J* = 8.7, H Ar); 8.33-8.39 (2H, m, H Ar); 8.55 (1H, t, *J* = 8.1, H Ar); 8.68 (1H, s, H-2 chromone); 11.37 (1H, br. s, NH); 12.20 (1H, br. s, NH). ^{13}C NMR spectrum, δ, ppm: 114.8; 117.0; 118.5; 121.3; 122.1; 123.1; 123.2; 124.7; 128.9; 131.1; 132.4; 149.1; 150.5; 152.1; 153.5; 153.6; 155.7; 155.9; 160.3; 166.1; 166.3. Mass spectrum, *m*/*z* (I_{rel}, %): 417 $[M+H]^+$ (100), 403 (77), 389 (44), 259 (21). Found, %: C 60.55; H 2.86; N 13.38; S 7.66. $C_{21}H_{12}N_4O_4S$. Calculated, %: C 60.57; H 2.90; N 13.45; S 7.70.

2-[(*Z*)-(5-Chloro-2-oxoindolin-3-ylidene)hydrazono]-5-[(4-oxo-4*H*-chromen-3-yl)methylene]thiazolidin-4-one (3b). Yield 48%, yellow solid, mp 269-273°C. IR spectrum, ν, cm^{-1}: 1660 (C=O), 1710 (C=O amide), 1728 (C=O amide), 3070 (NH), 3100 (NH). ^{1}H NMR spectrum, δ, ppm (*J*, Hz): 7.32 (1H, s, =CH–); 7.40 (1H, s, H Ar); 7.54-7.58 (2H, m, H Ar); 7.65 (1H, t, *J* = 8.8, H Ar); 7.77 (1H, t, *J* = 8.8, H Ar); 8.32-8.35 (1H, m, H Ar); 8.53 (1H, t, *J* = 8.5, H Ar); 8.66 (1H, s, H-2 chromone); 11.20 (1H, br. s, NH); 12.28 (1H, br. s, NH). ^{13}C NMR spectrum, δ, ppm: 115.1; 117.0; 119.4; 120.8; 123.1; 123.2; 124.1; 124.5; 129.1; 132.2; 134.9; 150.0; 150.7; 153.3; 153.4; 155.5; 158.6; 156.3; 161.2; 165.6; 166.4. Mass spectrum, *m*/*z* (I_{rel}, %): 451 $[M+H]^+$ (73), 437 (34), 283 (17), 260 (11). Found, %: C 55.91; H 2.43; Cl 7.83; N 12.40; S 7.09. $C_{21}H_{11}ClN_4O_4S$. Calculated, %: C 55.94; H 2.46; Cl 7.86; N 12.43; S 7.11.

2-[(*Z*)-(5-Fluoro-2-oxoindolin-3-ylidene)hydrazono]-5-[(4-oxo-4*H*-chromen-3-yl)methylene]thiazolidin-4-one (3c). Yield 52%, yellow solid, mp 267-272°C. IR spectrum, ν, cm^{-1}: 1655 (C=O), 1710 (C=O amide), 1732 (C=O amide), 3050 (NH), 3110 (NH). ^{1}H NMR spectrum, δ, ppm (*J*, Hz): 7.33 (1H, s, =CH–); 7.38 (1H, s, H Ar); 7.55-7.58 (2H, m, H Ar); 7.66 (1H, t, *J* = 8.8, H Ar); 7.77 (1H, t, *J* = 8.2, H Ar); 8.33-8.36 (1H, m, H Ar); 8.55 (1H, t, *J* = 8.9, H Ar); 8.65 (1H, s, H-2 chromone); 11.21 (1H, br. s, NH); 12.24 (1H, br. s, NH). ^{13}C NMR spectrum, δ, ppm: 115.2; 116.9; 119.2; 121.2; 123.2; 123.3; 124.0; 124.6; 129.1; 132.2; 135.1; 150.2;

150.8; 153.5; 153.6; 155.5; 156.5; 158.7; 160.7; 165.7; 166.2. Mass spectrum, *m*/*z* (I_{rel}, %): 435 $[M+H]^+$ (47), 421 (65), 403 (33), 288 (91). Found, %: C 58.03; H 2.52; F 4.36; N 12.88; S 7.35. $C_{21}H_{11}FN_4O_4S$. Calculated, %: C 58.06; H 2.55; F 4.37; N 12.90; S 7.38.

5-[(6-Chloro-4-oxo-4*H*-chromen-3-yl)methylene]-2-[(*Z*)-(2-oxoindolin-3-ylidene)hydrazono]thiazolidin-4-one (3d). Yield 52%, yellow solid, mp 263-272°C. IR spectrum, ν, cm^{-1}: 1650 (C=O), 1715 (C=O amide), 1720 (C=O amide), 3090 (NH), 3120 (NH). ^{1}H NMR spectrum, δ, ppm (*J*, Hz): 7.34 (1H, s, =CH–); 7.41 (1H, s, H Ar); 7.55-7.59 (2H, m, H Ar); 7.62 (1H, t, *J* = 8.7, H Ar); 7.78 (1H, t, *J* = 8.7, H Ar); 8.32-8.36 (1H, m, H Ar); 8.55 (1H, t, *J* = 9.0, H Ar); 8.66 (1H, s, H-2 chromone); 11.18 (1H, br. s, NH); 12.21 (1H, br. s, NH). ^{13}C NMR spectrum, δ, ppm: 114.8; 117.0; 118.5; 121.0; 122.1; 123.1; 123.2; 124.7; 128.9; 131.0; 132.2; 149.1; 150.5; 152.1; 153.4; 153.5; 155.6; 155.9; 159.8; 166.1; 166.3. Mass spectrum, *m*/*z* (I_{rel}, %): 452 $[M+H]^+$ (41), 437 (51), 416 (33), 389 (21), 261 (77). Found, %: C 55.91; H 2.43; Cl 7.85; N 12.40; S 7.10. $C_{21}H_{11}ClN_4O_4S$. Calculated, %: C 55.94; H 2.46; Cl 7.86; N 12.43; S 7.11.

2-[(*Z*)-(5-Chloro-2-oxoindolin-3-ylidene)hydrazono]-5-[(6-chloro-4-oxo-4*H*-chromen-3-yl)methylene]-thiazolidin-4-one (3e). Yield 55%, yellow solid, mp 282-286°C. IR spectrum, ν, cm^{-1}: 1652 (C=O), 1725 (C=O amide), 1730 (C=O amide), 3060 (NH), 3140 (NH). ^{1}H NMR spectrum, δ, ppm (*J*, Hz): 7.35 (1H, s, =CH–); 7.42-7.45 (1H, m, H Ar); 7.54-7.57 (2H, m, H Ar); 7.77 (1H, t, *J* = 8.8, H Ar); 8.35-8.38 (1H, m, H Ar); 8.54 (1H, t, *J* = 8.9, H Ar); 8.66 (1H, s, H-2 chromone); 11.20 (1H, br. s, NH); 12.10 (1H, br. s, NH). ^{13}C NMR spectrum, δ, ppm: 115.3; 117.0; 119.0; 121.2; 122.9; 123.3; 123.9; 124.5; 129.2; 132.2; 135.0; 149.6; 151.1; 152.5; 153.7; 155.6; 155.7; 156.5; 159.9; 166.1; 166.5. Mass spectrum, *m*/*z* (I_{rel}, %) 486 $[M+H]^+$ (100), 472 (59), 416 (21), 323 (19), 153 (11). Found, %: C 51.94; H 2.05; Cl 14.58; N 11.51; S 6.59. $C_{21}H_{10}Cl_2N_4O_4S$. Calculated, %: C 51.97; H 2.08; Cl 14.61; N 11.54; S 6.61.

5-[(6-Chloro-4-oxo-4*H*-chromen-3-yl)methylene]-2-[(*Z*)-(5-fluoro-2-oxoindolin-3-ylidene)hydrazono]-thiazolidin-4-one (3f). Yield 48%, yellow solid, mp 293-297°C. IR spectrum, ν, cm^{-1}: 1662 (C=O), 1710 (C=O amide), 1730 (C=O amide), 3070 (NH), 3140 (NH). ^{1}H NMR spectrum, δ, ppm (*J*, Hz): 7.35 (1H, s, =CH–); 7.42 (1H, s, H Ar); 7.50-7.60 (3H, m, H Ar); 8.36 (1H, t, *J* = 8.7, H Ar); 8.59-8.62 (1H, m, H Ar); 8.63 (1H, s, H-2 chromone); 11.21 (1H, br. s, NH); 12.24 (1H, br. s, NH). ^{13}C NMR spectrum, δ, ppm: 115.2; 117.2; 119.1; 120.8; 122.7; 123.2; 124.4; 124.5; 126.2; 129.2; 134.8; 135.2; 150.2; 150.7; 153.2; 153.4; 155.5; 156.5; 160.6; 165.2; 166.5. Mass spectrum, *m*/*z* (I_{rel}, %): 469 $[M+H]^+$ (33), 451 (51), 403 (100), 279 (65). Found, %: C 53.76; H 2.12; Cl 7.54; F 4.02; N 11.91; S 6.82. $C_{21}H_{10}ClFN_4O_4S$. Calculated, %: C 53.80; H 2.15; Cl 7.56; F 4.05; N 11.95; S 6.84.

Synthesis of Compounds 4a,b (General Method). 4-Hydroxy-6-methyl-2*H*-pyran-2-one (3.2 mmol) was added to a stirred solution of compounds **1a**,**b** (3.0 mmol) and L-proline (1.5 mmol) in dry EtOAc at room temperature. The reaction mixture was stirred for a further 5 h; then saturated aq. $NaHCO_3$ was added. The mixture was extracted with EtOAc (2×100 ml). The organic layer was washed with brine and dried over Na_2SO_4. After removal of the solvent in vacuum, the crude product is purified by column chromatography (EtOAc–PhMe, 1:1).

3-(2-Hydroxybenzoyl)-7-methylpyrano[4,3-*b*]pyran-5(8a*H*)-one (4a). Yield 44%, white solid, mp 195-198°C. IR spectrum, ν, cm^{-1}: 1670 (C=O lactone), 1730 (C=O), 3295 (OH). ^{1}H NMR spectrum, δ, ppm (*J*, Hz): 1.96 (3H, s, CH_3); 5.54 (1H, d, *J* = 9.0, 8a-CH); 5.98 (1H, d, *J* = 9.0, H-8); 6.80-6.83 (1H, m, H Ar); 7.48 (1H, t, *J* = 8.6, H Ar); 7.58-7.63 (2H, m, H Ar); 7.82 (1H, d, *J* = 2.1, H-4); 8.07 (1H, d, *J* = 2.1, H-2); 11.22 (1H, br. s, OH). ^{13}C NMR spectrum, δ, ppm: 20.7; 76.2; 98.3; 99.1; 107.1; 118.1; 119.2; 123.3; 123.9; 127.3; 132.4; 142.2; 155.3; 162.2; 165.2; 189.5. Mass spectrum, *m*/*z* (I_{rel}, %): 285 $[M+H]^+$ (51), 271 (27), 243 (19), 128 (100). Found, %: C 67.58; H 4.22. $C_{16}H_{12}O_5$. Calculated, %: C 67.60; H 4.25.

3-(5-Chloro-2-hydroxybenzoyl)-7-methylpyrano[4,3-*b*]pyran-5(8a*H*)-one (4b). Yield 52%, colorless crystals, mp 210-215°C. IR spectrum, ν, cm^{-1}: 1680 (C=O lactone), 1725 (C=O), 3290 (OH). ^{1}H NMR spectrum, δ, ppm (*J*, Hz): 2.10 (3H, s, CH_3); 5.53 (1H, d, *J* = 9.0, 8a-CH); 5.95 (1H, d, *J* = 9.0, H-8); 6.82 (1H, s, H Ar); 7.44 (1H, t, *J* = 8.8, H Ar); 7.55-7.57 (1H, m, H Ar); 7.80 (1H, d, *J* = 2.1, H-4); 8.12 (1H, d, *J* = 2.1, H-2); 11.19 (1H, br. s, OH). ^{13}C NMR spectrum, δ, ppm: 20.7; 76.6; 98.7; 99.2; 107.1; 118.1; 119.2; 123.3;

123.9; 132.4; 133.3; 142.4; 155.3; 162.2; 165.3; 188.7. Mass spectrum, *m*/*z* (I_{rel}, %): 319 $[M+H]^+$ (91), 271 (43), 243 (13), 196 (100). Found, %: C 60.27; H 3.43; Cl 11.10. $C_{16}H_{11}ClO_5$. Calculated, %: C 60.30; H 3.48; Cl 11.12.

Synthesis of Compounds 5a,b (General Method). A mixture of compounds **1a**,**b** (1.00 mmol), acetoacetamide (1.50 mmol), AcOH (0.49 mmol), and piperidine (0.15 mmol) in PhMe (25 ml) was heated at reflux using the Dean–Stark trap for 3 h. After removal of the solvent, the residue was purified by column chromatography (eluent EtOAc–hexane, 3:1).

3-Acetyl-5-(2-hydroxybenzoyl)pyridin-2(1*H*)-one (5a). Yield 78%, yellow crystals, mp 183-188°C. IR spectrum, ν, cm^{-1}: 1710 (C=O acetyl), 1717 (C=O amide), 1730 (C=O), 3050 (NH), 3270 (OH). ^{1}H NMR spectrum, δ, ppm (*J*, Hz): 2.66 (3H, s, CH_3); 6.88-6.91 (1H, m, H Ar); 7.03 (1H, d, *J* = 2.5, H-4); 7.50-7.55 (2H, m, H Ar); 8.19 (1H, t, *J* = 9.0, H Ar); 8.31 (1H, d, *J* = 2.5, H-6); 11.70 (1H, br. s, OH). ^{13}C NMR spectrum, δ, ppm: 30.2; 118.1; 118.4; 118.5; 119.5; 119.9; 130.7; 132.5; 136.8; 137.4; 145.1; 162.7; 162.9; 194.2. Mass spectrum, *m*/*z* (I_{rel}, %): 258 $[M+H]^+$ (100), 242 (66), 216 (31), 200 (23). Found, %: C 65.35; H 4.29; N 5.42. $C_{14}H_{11}NO_4$. Calculated, %: C 65.37; H 4.31; N 5.44.

3-Acetyl-5-(5-chloro-2-hydroxybenzoyl)pyridin-2(1*H*)-one (5b). Yield 68%, colorless crystals, mp 185-190°C. IR spectrum, ν, cm^{-1}: 1710 (C=O acetyl), 1720 (C=O amide), 1727 (C=O), 3030 (NH), 3275 (OH). ^{1}H NMR spectrum, δ, ppm (*J*, Hz): 2.68 (3H, s, CH_3); 6.95-6.98 (1H, m, H Ar); 7.05 (1H, d, *J* = 2.1, H-4); 7.53-7.57 (1H, m, H Ar); 8.21 (1H, t, *J* = 8.8, H Ar); 8.38 (1H, d, *J* = 2.5, H-6); 11.63 (1H, br. s, OH). ^{13}C NMR spectrum, δ, ppm: 30.3; 118.2; 118.4; 119.1; 119.4; 126.4; 130.2; 132.1; 137.4; 144.7; 145.9; 162.5; 163.4; 194.1. Mass spectrum, *m*/*z* (I_{rel}, %): 292 $[M+H]^+$ (71), 277 (61), 235 (41), 200 (11). Found, %: C 57.62; H 3.43; Cl 12.12; N 4.76. $C_{14}H_{10}ClNO_4$. Calculated, %: C 57.65; H 3.46; Cl 12.15; N 4.80.

X-ray Crystallographic Study of Compound 5a. Crystals of compound **5a** were obtained by crystallization from EtOAc–PhMe (1:4) by allowing slow solvent evaporation. X-ray structure determination was performed on an Agilent Technologies SuperNova Dual diffractometer with an Atlas detector. Graphite-monochromated CuKα radiation (1.54184 Å) was used. The structure was solved by the Patterson heavy atom method and successive cycles of full-matrix refinement and Fourier difference syntheses. All non-hydrogen atoms were refined anisotropically by the full-matrix least-squares method. Carbon-bound hydrogen atoms were placed in calculated positions (C–H 0.95 to 0.98 Å, U_{iso}(H) 1.2 to 1.5U_{eq}(C)) and were included in the refinement in the rider model approximation (Table 1). The hydrogen atoms of hydroxy and amino groups were freely refined. Molecular graphics were performed from X-SEED software. Crystallographic data for the structure of compound **5** have been deposited at the Cambridge Crystallographic Data Center (deposit CCDC 918116).

Antimicrobial testing. All synthesized compounds were screened for their *in vitro* growth inhibitory activity. For antifungal test, *Aspergillus niger* MTCC 282 and *Apergillus flavus* AIIMS and *Candida albicans* ATCC 2091 were chosen. Amphotericin B was used for comparison. The bacterial strains used were *Staphylococcus aureus* ATCC 6538P, *Bacillus subtilis* ATCC 6633, and *Streptococcus Mutans* MTCC 890 (all gram-positive) and *Escherichia coli* ATCC 25922, *Salmonella typhi* MTCC 733, and *Pseudomonas aeruginosa* MTCC 741 (all gram-negative).

The plates were incubated at 35°C for 24 and 48 h, respectively, for bacteria and fungi. The inhibition zones of microbial growth were measured by the paper disc method [31] in which 6-mm paper discs containing specified amounts of the synthesized compounds (250 μg) were placed on an agar plate inoculated with a standardized suspension of the microorganisms tested. Ciprofloxacin (15 μg) for gram-positive bacteria, nalidixic acid (25 μg) for gram-negative bacteria and amphotericin B (25 μg) for fungi were used as standards. The experiments were repeated thrice, and the diameters of inhibition zones produced by various synthesized compounds were measured in millimeters.

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