

Research Article

Synthesis and Evaluation of Substituted 4,4a-Dihydro-3H,10H-pyrano[4,3-b][1]benzopyran-10-one as Antimicrobial Agent

Amanpreet Kaur,¹ Vishal Sharma,¹ Abhishek Budhiraja,² Harpreet Kaur,³ Vivek Gupta,⁴ Rajni Kant,⁴ and Mohan Paul S. Ishar¹

¹ Bio-Organic and Photochemistry Laboratory, Department of Pharmaceutical Sciences, Guru Nanak Dev University, Amritsar, Punjab 143 005, India

² ISF College of Pharmacy, Moga, Punjab 143008, India

³ Department of Microbiology, Guru Nanak Dev University, Amritsar, Punjab 143 005, India

⁴ Post-Graduate Department of Physics, University of Jammu, Jammu-Tawi 180 006, India

Correspondence should be addressed to Mohan Paul S. Ishar; mpsishar@yahoo.com

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A series of pyrano[4,3-b][1]benzopyranones (**7a-t**) were synthesized through hetero-Diels-Alder reaction of substituted 3-formylchromones (**5**) with enol ethers (**6**), characterized by IR, ¹H NMR, ¹³C NMR, and mass spectral techniques. All the compounds were evaluated for antimicrobial activity against various bacterial and fungal strains, found to possess significant inhibitory potential, particularly, compounds bearing electron withdrawing group -fluoro such as **7i** and **7h**. Compounds were also tested and displayed a significant inhibitory potential against methicillin-resistant *Staphylococcus aureus* (MRSA).

1. Introduction

Despite decades of extensive progress in treatment and prevention, infectious diseases remain a major cause of death and are responsible for worsening the living conditions of many millions of people around the world [1]. Additionally, resistance to known antibiotics is also a serious problem and presents a challenge for the medicinal chemists to develop new effective molecular entities against pathogenic microorganism resistant to available current treatments [2]. Chromones are an important class of heterocyclic molecules naturally occurring, and synthetic analogs are found to display a wide range of pharmacological activities such as antimicrobial, anticancer, neuroprotective, HIV-inhibitory, antifungal activities, and antioxidant [3–9]. Natural products such as aposhaerin A (**1**), isolated from *Aposhaeria* sp. possess remarkable antibacterial activity [10]. Recently, we have

reported that 3-(5-phenyl-3H-[1,2,4]dithiazol-3-yl-) chromen-4-ones (**2**) possess significant antibacterial activity against *Shigella flexneri* (Figure 1) [11].

Similarly, pyran moiety is widely present in animal and plant kingdom; it exhibits diverse pharmacological activities such as antimicrobial, antiviral, antiproliferative, antitumor, antiinflammatory [12–16]. Pyrano[3,2-c]chromene derivatives (**3a-c**), bearing a 2-thiophenoxyquinoline nucleus, have been found to display excellent antibacterial activity against *B. subtilis*, *E. coli*, and *P. aeruginosa*, respectively, [17]. 2-Amino-3-cyano-6-(3,5-dibromo-4-methoxyphenyl)-4-arylpyrans (**4**) have been found to exhibit potent antimicrobial and antimycobacterial activity (Figure 2) [18].

Taking cognizance of high antimicrobial activity of both chromone and pyran derivatives, it was decided to synthesize chromone fused pyrans and evaluate against various pathogenic bacterial and fungal strains.

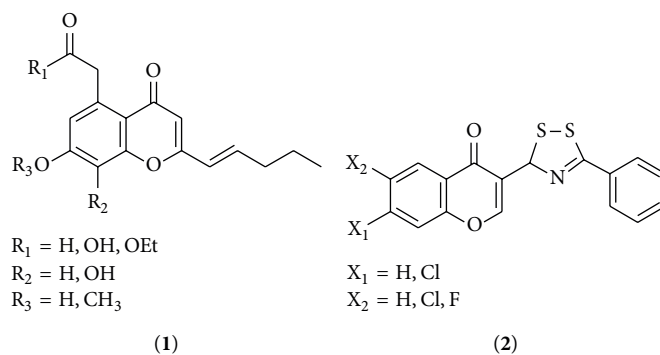


FIGURE 1: Chromone based compounds as antimicrobial agents.

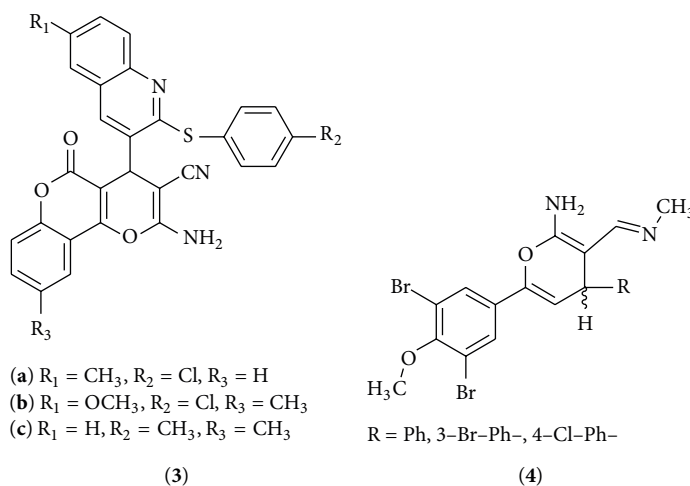


FIGURE 2: Pyran based compounds as antimicrobial agents.

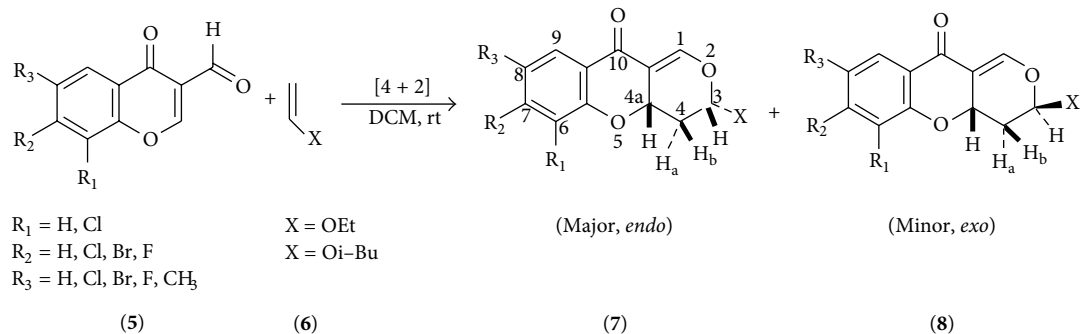
2. Results and Discussion

2.1. Chemistry. Substituted pyrano[4,3-b][1]benzopyranones were synthesized by the hetero-Diels-Alder reaction of substituted 3-formylchromones (**5a-j**) with excess of enol ethers (**6**) in dichloromethane at room temperature [19–22]. All the purified products were characterized by rigorous spectroscopic techniques (IR, ^1H NMR, ^{13}C NMR, and mass) and elemental analysis (Scheme 1, Table 1). Finally, the structure of compound **7k** was confirmed by X-ray crystallography (Figure 3) [23].

^1H NMR spectrum of **7k** displayed doublet of C9-H at δ 7.91 with $J = 6$ Hz, and C1-H showed up as a doublet at δ 7.51 with $J = 1.2$ Hz. Resonances of C3-H and C4a-H appeared as a multiplet at δ 5.16–5.10. C4-Ha resonance appeared at δ 2.53 as dd with $J_{\text{gem}} = 12.9$ Hz and $J = 6.9$ Hz; C4-Hb showed up as a dt with $J_{\text{gem}} = 12.9$ Hz and $J = 9.9$ Hz at δ 2.30. ^{13}C NMR revealed a quaternary carbon resonance at 181.8 ppm attributed to carbonyl carbon (C10), which was further corroborated by a strong characteristic band at 1668 cm^{-1} in the IR spectrum. Further, the mass spectrum of **7k** (ESI) showed the highest ion peak at m/z 247 ($M^+ + 1$). The stereochemistry of product **7k** (*endo*) was assigned on the basis of NMR spectral evidence. C4-Hb showed vicinal coupling constants of 6.9 and 2.4 Hz which can be attributed to axial-equatorial

relationships with C4a-H and C3-H, whereas C4-Ha showed vicinal coupling of ~ 10.0 Hz with both C4a-H and C3-H indicating its diaxial relationship with both neighbouring protons, and this alludes to *cis*-relationship between C4a-H and C3-H; this *trans*-diaxial relationship was further confirmed by X-ray crystallographic structure determination of **7k** (Figure 3) [20–22]. The corresponding *exo*-isomers (**8a-t**, traces) were detected by ^1H NMR of some column fractions, and the ratio of *endo/exo* was determined from NMR of crude reaction mixture (4:1 approximately). The *endo* and *exo* approaches leading to compounds **7** and **8** are shown in Figure 4.

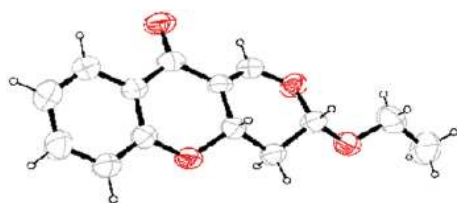
2.2. Antibacterial Activity. All the synthesized compounds (**7a-t**) were screened for their antibacterial potential in triplicate against two Gram-positive bacteria, *Staphylococcus aureus* (MTCC96), *Bacillus subtilis* (MTCC2451), and three gram-negative bacteria, *Escherichia coli* (MTCC 82), *Pseudomonas aeruginosa* (MTCC 2642), and *Salmonella typhimurium* (MTCC 1251), by using disc diffusion method [24]. The activity of compounds was determined in comparison to standard antibiotic discs of amoxicillin ($5\ \mu\text{g}$) and ciprofloxacin ($10\ \mu\text{g}$). Minimum inhibitory concentration (MIC) in $\mu\text{g/mL}$ of compounds exhibiting activity (Table 2) was determined by using serial tube dilution method [25].



SCHEME 1: Synthesis of substituted pyrano[4,3-b][1]benzopyranones.

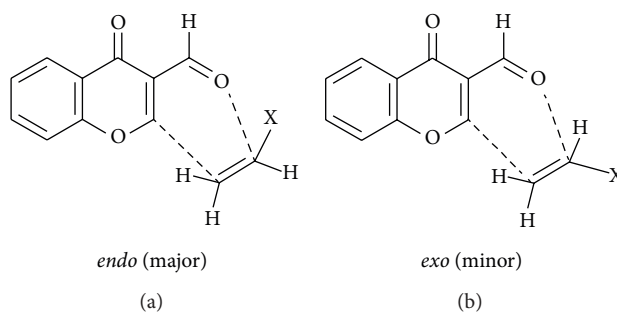
TABLE I: Reaction time (h) and yield (%) of various purified products.

Sr. number	R_1	R_2	R_3	X	Reaction time (h)	Products yield (%)	
						<i>endo</i>	<i>exo</i>
1	H	H	H	O- ⁱ Bu	72	7a (77)	8a (traces)
2	H	H	CH ₃	O- ⁱ Bu	72	7b (70)	8b (traces)
3	Cl	H	Cl	O- ⁱ Bu	36	7c (74)	8c (traces)
4	H	H	Cl	O- ⁱ Bu	24	7d (72)	8d (traces)
5	H	Cl	F	O- ⁱ Bu	36	7e (78)	8e (traces)
6	H	H	Br	O- ⁱ Bu	24	7f (71)	8f (traces)
7	H	Br	H	O- ⁱ Bu	48	7g (73)	8g (traces)
8	H	F	H	O- ⁱ Bu	48	7h (70)	8h (traces)
9	H	H	F	O- ⁱ Bu	72	7i (72)	8i (traces)
10	H	Cl	H	O- ⁱ Bu	48	7j (73)	8j (traces)
11	H	H	H	OEt	168	7k (75)	8k (traces)
12	H	H	CH ₃	OEt	96	7l (72)	8l (traces)
13	Cl	H	Cl	OEt	216	7m (73)	8m (traces)
14	H	H	Cl	OEt	192	7n (76)	8n (traces)
15	H	H	F	OEt	72	7o (72)	8o (traces)
16	H	H	Br	OEt	48	7p (74)	8p (traces)
17	H	Br	H	OEt	96	7q (75)	8q (traces)
18	H	F	H	OEt	120	7r (76)	8r (traces)
19	H	Cl	F	OEt	96	7s (74)	8s (traces)
20	H	Cl	H	OEt	72	7t (72)	8t (traces)

FIGURE 3: ORTEP view of **7k**.

All tested compounds were found to exert prominent antibacterial activity against both gram-positive and gram-negative bacterial strains. Compound **7i** showed comparable potent inhibitory activity with positive controls against various bacterial strains such as MIC 0.48 against both *S. aureus* and *E. coli*, whereas MIC 1.12 against both *B. subtilis* and *P. aeruginosa*. Compound **7d** showed high inhibitory potential

against gram-negative bacterial strain *E. coli* with MIC 1.56, followed by MIC 1.82 against both *B. subtilis* and *S. aureus*, and MIC 12.5 against *P. aeruginosa* and *S. typhi*. Compounds **7e** and **7n** showed good activity against *B. subtilis* with MIC 6.25, whereas compound **7j** showed activity against *E. coli* and *S. aureus* with MIC 6.25 and 12.5, respectively. Compounds **7m** and **7q** showed significant activity against *S. aureus* and *B. subtilis* with MIC 6.25 and 12.5, respectively. Compounds **7n** and **7o** showed promising inhibitory activity against both *S. aureus* and *E. coli*, whereas compounds **7j** and **7q** showed inhibitory potential against *P. aeruginosa* with MIC 12.5. The literature reports reveal that these types of tricyclic compounds have been isolated from a strain of *Chaetomium funicola*, which act as potent broad-spectrum metallo- β -lactamase inhibitors [26]. 3-Formylchromones use as a starting reactant in the synthesis of these pyrano[4,3-b][1]benzopyranones has also shown good antibacterial activity against various bacterial strains [27, 28].

FIGURE 4: Modes of addition: *endo* and *exo*.TABLE 2: MIC ($\mu\text{g/mL}$) of compounds 7a–t against different bacterial strains.

Comp. number	<i>B. subtilis</i>	<i>S. aureus</i>	<i>E. coli</i>	<i>P. aeruginosa</i>	<i>S. typhi</i>
7a	—	—	12.5	25	—
7b	—	12.5	—	—	—
7c	12.5	12.5	25	—	—
7d	1.82	1.82	1.56	12.5	12.5
7e	6.25	12.5	3.12	12.5	—
7f	12.5	25	25	—	—
7g	15.5	—	—	12.5	—
7h	12.5	32.5	—	—	—
7i	1.12	0.48	0.48	1.12	25
7j	25	12.5	6.25	12.5	—
7k	—	15.5	13.3	—	25
7l	25	12.5	25	—	—
7m	12.5	6.25	25	25	—
7n	6.25	12.5	12.5	25	—
7o	25	12.5	12.5	—	50
7p	—	15.5	—	30.5	—
7q	12.5	6.25	25	12.5	25
7r	—	25.5	—	—	—
7s	—	25	—	—	—
7t	12.5	—	30.5	—	—
Amoxicillin	0.5	0.5	0.12	1.0	0.9
Ciprofloxacin	0.75	1.2	0.9	1.8	—

TABLE 3: MIC (mg/mL) of compounds against methicillin-resistant *Staphylococcus aureus* (MRSA).

Comp. number	7a	7b	7c	7d	7e	7f	7g	7h	7i	7j	7k	7l	7m	7n	7p	7t
MRSA	0.7	0.5	0.7	0.5	0.5	0.7	0.7	0.5	0.6	0.1	0.5	0.7	0.4	0.5	0.6	0.5

Active compounds were further evaluated against bacterial resistant strains such as methicillin resistant *Staphylococcus aureus* (MRSA), a clinically isolate obtained from PGIMER, Chandigarh, and *Klebsiella pneumoniae* (MTCC 530) by using disk-diffusion assay. Compounds were found to be active against MRSA and completely inactive against *Klebsiella pneumoniae*. Minimum inhibitory concentration (MIC) in mg/mL of compounds exhibiting activity (Table 3) was determined by using serial tube dilution method [25]. All the compounds were found to be active against resistant bacterial strain MRSA, whereas compound 7j showed a maximum activity in comparison to other compounds.

2.3. Antifungal Activity. All synthesized compounds 7a–t were tested against five reference fungal strains: *Aspergillus niger* (MTCC 1344), *Saccharomyces cerevisiae* (MTCC 172), *Candida albicans* (MTCC 3018), *Cryptococcus gastricus* (MTCC 1715), and *Microsporum gypseum* (MTCC 4490) by using disc diffusion method [24]. Moreover, the compounds were found to exert prominent antifungal activity against various fungal strains, specially, against *A. niger*, *S. cerevisiae*, and *C. albicans* (Table 4). Compound 7h showed significant inhibitory activity with MIC 2.4, whereas, compounds 7d, 7g, 7j, and 7m exhibit good inhibitory potential against *A. niger* with MIC < 15. Compound 7j possesses maximum inhibitory

TABLE 4: MIC ($\mu\text{g/mL}$) of compounds **7a–t** against different fungal strains.

Compound no.	<i>A. niger</i>	<i>S. cerevisiae</i>	<i>C. albicans</i>	<i>C. gastricus</i>	<i>M. gypseum</i>
7a	55.4	31.5	>100	>100	88.4
7b	30	14.1	65.3	>100	>100
7c	25.2	18.6	32.5	>100	>100
7d	12.4	>100	70	48.5	90.2
7e	19.5	30.5	16.5	75.4	>100
7f	28.2	18.2	11.8	80.4	94
7g	13.8	32.1	55.5	>100	55.4
7h	2.4	11.7	16.6	>100	48.5
7i	16.6	10.5	17.8	58.4	>100
7j	11.2	2.9	61.2	>100	88.5
7k	29.9	71.4	54.5	>100	>100
7l	73.5	56.4	72.4	>100	>100
7m	13.2	40.5	76	>100	53.4
7n	34	>100	14.7	32.5	>100
7o	18.5	21.4	20.5	>100	93
7p	35	20	>100	43.5	>100
7q	21.0	32.1	>100	20.5	31.5
7r	15.3	>100	12.4	>100	53
7s	45.4	20	>100	63.5	>100
7t	21.0	12.1	>100	40.5	>100
Fluconazole	1.9	1.9	3.9	31.2	1.9

potential against *S. cerevisiae* with MIC 2.9, whereas compounds **7b**, **7h**, **7i**, and **7t** displayed good inhibitory potential with MIC < 15. Compounds **7f**, **7n**, and **7r** showed promising activity against *C. albicans* with MIC < 15. Compound **7q** is found to display high antifungal activity as compared to standard drug with MIC 20.5 against *C. gastricus*.

Compounds bearing electron withdrawing groups such as -fluoro and -chloro at chromone ring were found to display high activity against both bacterial and fungal strains, whereas substitution with electron donating group led to a decrease in activity. According to Craig's plot, these -fluoro, -chloro, and -bromo groups are lipophilic in nature, having high π -values; from the literature, it was found that lipophilicity is essential for the compound permeability across the microbes cell membrane [27]. Therefore, compounds having these lipophilic groups exhibit valuable inhibitory potential; similarly, compounds having bulkier or lipophilic group such as Oi-Bu- at position 3 of the fused pyran ring were found to be more active than -OEt against various pathogenic bacterial strains. Disubstitution with electron withdrawing groups such as -fluoro and -chloro on chromone ring showed moderate inhibitory activity against both bacterial and fungal strains.

3. Conclusion

Variously substituted pyrano[4,3-b][1]benzopyrans (**7a–t**) were synthesized through the hetero-Diels-Alder reaction [4 + 2] of substituted 3-formylchromones (**5**) with excess of enol ethers (**6**) in dichloromethane at room temperature. Compounds bearing electron withdrawing groups such as -fluoro and -chloro at chromone ring were found to display high activity against both bacterial and fungal strains such as compound **7i** which showed excellent antibacterial activity and

compound **7h** which displayed promising antifungal activity. All active compounds were also evaluated against bacterial resistant strain MRSA and found to possess good inhibitory potential, particularly, compound **7j**. These "lead" compounds can be taken under consideration for further antimicrobial development and their mode of action.

4. Experimental

4.1. General. Starting materials and reagents were purchased from commercial suppliers and used after further purification (crystallization/distillation). Bruker (400 MHz), JEOL AL-300 FT (300 MHz), and NMR spectrometer were used to record ^1H NMR (300 MHz and 400 MHz) and ^{13}C NMR (75 MHz and 100 MHz) spectra, and chemical shifts (δ) are reported as downfield displacements from tetramethylsilane (TMS) used as an internal standard, and coupling constants (J) are reported in Hz. IR spectrum was recorded with Shimadzu FT-IR-8400S and Bruker spectrophotometers on KBr pellets. Mass spectrum, EI, and ESI methods were recorded on Shimadzu GCMS-QP-2000A and Bruker Daltonics Esquire 300 mass spectrometer, respectively. Elemental analyses were carried out on a Thermoelectron EA-112 elemental analyzer and are reported in percent abundance.

4.2. Synthesis of Substituted Pyrano[4,3-b][1]benzopyrans. Substituted pyrano[4,3-b][1]benzopyrans (**7a–t**) were synthesized by the [4 + 2] cycloaddition of substituted 3-formylchromones (**5a–j**, 300 mg) with excess of alkoxy-ethenes (**6**) in dichloromethane at room temperature [19–22]. The progress of the reaction was determined by thin layer chromatography (TLC). After completion of reaction, the residue obtained on removal of solvent under vacuum was purified by

column chromatography, using neutral (pH~7) silica gel 60–120 mesh, (Loba Chemie, 30 g, packed in hexane), and eluted with 1%–2% ethyl acetate in hexane. All the purified products were characterized by rigorous spectroscopic techniques such as IR, ^1H and ^{13}C NMR, and mass and elemental analysis. The spectroscopic data of purified compounds are as follows.

4.2.1. 3-Isobutoxy-4,4a-dihydro-3H,10H-pyrano[4,3-b][1]benzopyran-10-one (7a). Light-yellow amorphous solid (231 mg, 77%), mp 135–144°C; IR ν_{max} (CHCl_3): 2960, 2875, 1668, 1610, and 1461 cm^{-1} ; ^1H NMR (CDCl_3 , 300 MHz): δ 7.91 (d, 1H, $J = 7.8$ Hz, C_9H), 7.52 (s, 1H, C_1H), 7.45–7.01 (m, 2H, Ar-Hs), 6.90 (d, 1H, $J = 8.1$ Hz, C_6H), 5.18 (m, 2H, C_{4a}H and C_3H), 3.75 (dd, 1H, $J_{\text{gem}} = 9.3$ Hz and $J = 6.9$ Hz, $-\text{OCH}_2$), 3.33 (dd, 1H, $J_{\text{gem}} = 9$ Hz and $J = 6.9$ Hz, $-\text{OCH}_2$), 2.57 (unresolved dd, 1H, $J_{\text{gem}} = 12.9$ Hz and $J = 6.9$ Hz, C_4H_a), 2.33 (dt, 1H, $J_{\text{gem}} = 12.9$ Hz and $J = 9.9$ Hz, C_4H_b), 1.98–1.89 (m, 1H, $-\text{CH}$), 0.96 (d, 6H, $J = 6.9$ Hz, $2 \times \text{CH}_3$); ^{13}C NMR (CDCl_3 , 75 MHz): δ 180.1 (C=O), 160.4 (q-arom.), 151.1 (olefinic-CH), 137.4 (Ar-CH), 127.1 (Ar-CH), 121.6 (q-arom.), 117.3 (Ar-CH), 100.5 (C_{4a} and C_3), 70.18 ($-\text{OCH}_2$), 33.2 ($-\text{CH}$), 28.1 (C_4), 18.9 ($2 \times -\text{CH}_3$); mass (ESI) m/z : 274 (M^+), 275 ($\text{M}^+ + 1$); analysis: calculated for ($\text{C}_{16}\text{H}_{18}\text{O}_4$), C 70.06 H 6.61% and found C 70.02 H 6.55%.

4.2.2. 3-Isobutoxy-8-methyl-4,4a-dihydro-3H,10H-pyrano[4,3-b][1]benzopyran-10-one (7b). Yellowish amorphous solid (210 mg, 70%), mp 138–147°C; IR ν_{max} (CHCl_3): 2954, 2887, 1668, 1618, and 1465 cm^{-1} ; ^1H NMR (CDCl_3 , 300 MHz): δ 7.70 (brs, 1H, C_9H), 7.50 (s, 1H, C_1H), 7.28–7.12 (m, 1H, C_7H), 6.85 (d, 1H, $J = 8.4$ Hz, C_6H), 5.16–5.10 (m, 2H, C_{4a}H and C_3H), 3.77–3.72 (m, 1H, $-\text{OCH}_2$), 3.50–3.41 (m, 1H, $-\text{OCH}_2$), 2.54–2.51 (m, 1H, C_4H_a), 2.33–2.21 (m, 1H, C_4H_b), 1.95–1.90 (m, 1H, $-\text{CH}$), 2.30 (s, 3H, $-\text{CH}_3$) 0.95 (d, 6H, $J = 6.6$ Hz, $2 \times \text{CH}_3$); ^{13}C NMR (CDCl_3 , 75 MHz): δ 181.0 (C=O), 157.1 (q-arom.), 153.1 (olefinic-CH), 138.2 (Ar-CH), 129.1 (Ar-CH), 120.6 (q-arom.), 117.1 (Ar-CH), 100.2 (C_{4a} and C_3), 70.18 ($-\text{OCH}_2$), 33.2 ($-\text{CH}$), 28.1 (C_4), 25.3 ($-\text{CH}_3$), and 18.9 ($2 \times -\text{CH}_3$); mass (ESI) m/z : 288 (M^+), 289 ($\text{M}^+ + 1$); analysis: calculated for ($\text{C}_{17}\text{H}_{20}\text{O}_4$), C 70.81 H 6.99% and found C 70.74 H 6.94%.

4.2.3. 6,8-Dichloro-3-isobutoxy-4,4a-dihydro-3H,10H-pyrano[4,3-b][1]benzopyran-10-one (7c). Brownish amorphous solid (222 mg, 74%), mp 144–154°C; IR ν_{max} (CHCl_3): 2960, 2873, 1670, 1606, and 1456 cm^{-1} ; ^1H NMR (CDCl_3 , 300 MHz): δ 7.79 (d, 1H, $J = 2.4$ Hz, C_9H), 7.56 (s, 1H, C_1H), 7.47 (d, 1H, $J = 2.7$ Hz, C_7H), 5.21 (m, 2H, $J = 9.9$ Hz and $J = 1.2$ Hz, C_{4a}H and C_3H), 3.73 (dd, 1H, $J_{\text{gem}} = 9$ Hz and $J = 6.6$ Hz, $-\text{OCH}_2$), 3.33 (dd, 1H, $J_{\text{gem}} = 9.0$ Hz and $J = 6.9$ Hz, $-\text{OCH}_2$), 2.68 (ddd, 1H, $J_{\text{gem}} = 13.5$ Hz and $J = 6.6$, 1.8 Hz, C_4H_a), 2.40 (dt, 1H, $J_{\text{gem}} = 13.5$ Hz and $J = 9.9$ Hz, C_4H_b), 1.99–1.87 (m, 1H, $-\text{CH}$), 0.94 (d, 6H, $J = 6.6$ Hz, $2 \times \text{CH}_3$); ^{13}C NMR (CDCl_3 , 100 MHz): δ 180.2 (C=O), 153.0 (olefinic-CH), 134.7 (Ar-CH), 125.5 (Ar-CH), 124.0 (q-arom.), 110.2 (Ar-CH), 101.1 (C_{4a} and C_3), 71.42 ($-\text{OCH}_2$), 33.2 ($-\text{CH}$), 28.4 (C_4), 19.3 ($2 \times -\text{CH}_3$); mass (ESI) m/z : 343 (M^+); analysis: calculated for ($\text{C}_{16}\text{H}_{16}\text{Cl}_2\text{O}_4$), C 55.99 H 4.70% and found C 55.93 H 4.64%.

4.2.4. 8-Chloro-3-isobutoxy-4,4a-dihydro-3H,10H-pyrano[4,3-b][1]benzopyran-10-one (7d). Yellowish-brown amorphous solid (216 mg, 72%), mp 141–150°C; IR ν_{max} (CHCl_3): 2962, 2869, 1664, 1616, 1471 cm^{-1} ; ^1H NMR (CDCl_3 , 300 MHz): δ 7.86 (d, 1H, $J = 2.4$ Hz, C_9H), 7.53 (s, 1H, C_1H), 7.36 (dd, 1H, $J_{\text{gem}} = 8.7$ Hz and $J = 2.7$ Hz, C_7H), 6.87 (d, 1H, $J = 8.7$ Hz, C_6H), 5.14 (m, 2H, $J = 9.6$ Hz and $J = 2.8$ Hz, C_{4a}H and C_3H), 3.74 (dd, 1H, $J_{\text{gem}} = 9$ Hz and $J = 6.9$ Hz, $-\text{OCH}_2$), 3.32 (dd, 1H, $J_{\text{gem}} = 9$ Hz and $J = 6.6$ Hz, $-\text{OCH}_2$), 2.56 (ddd, 1H, $J_{\text{gem}} = 13.5$ Hz and $J = 6.3$, 2.1 Hz, C_4H_a), 2.30 (dt, 1H, $J_{\text{gem}} = 13.5$ Hz and $J = 9.6$ Hz, C_4H_b), 1.96–1.87 (m, 1H, $-\text{CH}$), 0.94 (d, 6H, $J = 6.6$ Hz, $2 \times \text{CH}_3$); ^{13}C NMR (CDCl_3 , 75 MHz): δ 180.03 (C=O), 159.1 (q-arom.), 153.3 (olefinic-CH), 139.0 (Ar-CH), 127.4 (q-arom.), 126.7 (Ar-CH), 123.6 (q-arom.), 119.4 (Ar-CH), 110.9 (q-arom.), 100.9 (C_{4a} and C_3), 70.6 ($-\text{OCH}_2$), 33.2 ($-\text{CH}$), 28.4 (C_4), 19.1 ($2 \times -\text{CH}_3$); mass (ESI) m/z : 308.5 (M^+), 309 ($\text{M}^+ + 1$); analysis: calculated for ($\text{C}_{16}\text{H}_{17}\text{ClO}_4$), C 62.24 H 5.55% and found C 62.18 H 5.48%.

4.2.5. 8-Fluoro-3-isobutoxy-4,4a-dihydro-3H,10H-pyrano[4,3-b][1]benzopyran-10-one (7e). Yellowish-brown amorphous solid (234 mg, 78%), mp 137–146°C; IR ν_{max} (CHCl_3): 2960, 2885, 1668, 1610, 1458 cm^{-1} ; ^1H NMR (CDCl_3 , 300 MHz): δ 7.58–7.52 (m, 1H, Ar-H), 7.53 (d, 1H, $J = 1.8$ Hz, C_1H), 7.17–6.87 (m, 2H, Ar-Hs), 5.13 (m, 2H, $J = 9.9$ Hz and $J = 2.4$ Hz, C_{4a}H and C_3H), 3.74 (dd, 1H, $J_{\text{gem}} = 9.3$ Hz and $J = 6.6$ Hz, $-\text{OCH}_2$), 3.32 (dd, 1H, $J_{\text{gem}} = 9.3$ Hz and $J = 6.9$ Hz, $-\text{OCH}_2$), 2.55 (ddd, 1H, $J_{\text{gem}} = 12.9$ Hz and $J = 6.9$, 2.1 Hz, C_4H_a), 2.30 (dt, 1H, $J_{\text{gem}} = 12.9$ Hz and $J = 9.9$ Hz, C_4H_b), 1.96–1.87 (m, 1H, $-\text{CH}$), 0.94 (d, 6H, $J = 6.6$ Hz, $2 \times \text{CH}_3$); ^{13}C NMR (CDCl_3 , 100 MHz): δ 180.1 (C=O), 160.8 (q-arom.), 151.5 (olefinic-CH), 140.4 (q-arom.), 127.8 (Ar-CH), 122.5 (Ar-CH), 121.1 (q-arom.), 116.8 (Ar-CH), 111.0 (q-arom.), 100.8 (C_{4a} and C_3), 70.7 ($-\text{OCH}_2$), 33.4 ($-\text{CH}$), 28.5 (C_4), 19.3 ($2 \times -\text{CH}_3$); mass (ESI) m/z : 292 (M^+), 293 ($\text{M}^+ + 1$); analysis: calculated for ($\text{C}_{16}\text{H}_{17}\text{FO}_4$), C 65.74 H 5.86% and found C 65.70 H 5.81%.

4.2.6. 8-Bromo-3-isobutoxy-4,4a-dihydro-3H,10H-pyrano[4,3-b][1]benzopyran-10-one (7f). Yellowish-brown amorphous solid (213 mg, 71%), mp 143–153°C; IR ν_{max} (CHCl_3): 2960, 2867, 1664, 1616, 1457 cm^{-1} ; ^1H NMR (CDCl_3 , 300 MHz): δ 8.04–8.01 (m, 1H, C_9H), 7.54 (s, 1H, C_1H), 7.28–7.24 (m, 1H, C_7H), 6.86–6.83 (m, 1H, C_6H), 5.20–5.13 (m, 2H, C_{4a}H and C_3H), 3.76–3.63 (m, 1H, $-\text{OCH}_2$), 3.38–3.32 (m, 1H, $-\text{OCH}_2$), 2.59–2.53 (m, 1H, C_4H_a), 2.39–2.32 (m, 1H, C_4H_b), 1.98–1.90 (m, 1H, $-\text{CH}$), 0.98–0.91 (m, 6H, $2 \times \text{CH}_3$); ^{13}C NMR (CDCl_3 , 75 MHz): δ 179.2 (C=O), 159.7 (q-arom.), 152.5 (olefinic-CH), 136.4 (Ar-CH), 130.8 (Ar-CH), 123.1 (q-arom.), 118.7 (Ar-CH), 113.1 (q-arom.), 110.2 (q-arom.), 100.5 (C_{4a} and C_3), 70.1 ($-\text{OCH}_2$), 32.4 ($-\text{CH}$), 25.1 (C_4), 18.7 ($2 \times -\text{CH}_3$); mass (ESI) m/z : 353 (M^+); analysis: calculated for ($\text{C}_{16}\text{H}_{17}\text{BrO}_4$), C 54.41 H 4.85% and found C 54.34 H 4.80%.

4.2.7. 7-Bromo-3-isobutoxy-4,4a-dihydro-3H,10H-pyrano[4,3-b][1]benzopyran-10-one (7g). Yellowish-brown amorphous solid (219 mg, 73%), mp 142–151°C; IR ν_{max} (CHCl_3): 2974,

2887, 1670, 1591, 1465 cm^{-1} ; $^1\text{H NMR}$ (CDCl_3 , 300 MHz): δ 7.76 (d, 1H, $J = 8.4$ Hz, C_9H), 7.50 (d, 1H, $J = 1.5$ Hz, C_1H), 7.17 (dd, 1H, $J_{\text{gem}} = 8.4$ Hz and $J = 1.5$ Hz, C_8H), 7.12 (d, 1H, $J = 1.8$ Hz, C_6H), 5.17 (m, 2H, $J = 9.6$ Hz and $J = 2.4$ Hz, C_{4a}H and C_3H), 3.73 (dd, 1H, $J_{\text{gem}} = 9.3$ Hz and $J = 6.6$ Hz, $-\text{OCH}_2$), 3.32 (dd, 1H, $J_{\text{gem}} = 9.3$ Hz and $J = 6.9$ Hz, $-\text{OCH}_2$), 2.57 (ddd, 1H, $J_{\text{gem}} = 13.2$ Hz and $J = 6.6, 1.8$ Hz, C_4H_a), 2.30 (dt, 1H, $J_{\text{gem}} = 13.2$ Hz and $J = 9.9$ Hz, C_4H_b), 1.98–1.85 (m, 1H, $-\text{CH}$), 0.94 (d, 6H, $J = 6.9$ Hz, $2 \times \text{CH}_3$); $^{13}\text{C NMR}$ (CDCl_3 , 75 MHz): 179.8 (C=O), 158.6 (q-arom.), 153.1 (olefinic-CH), 135.3 (Ar-CH), 131.7 (Ar-CH), 122.1 (q-arom.), 118.3 (Ar-CH), 113.7 (q-arom.), 110.9 (q-arom.), 100.1 (C_{4a} and C_3), 70.1 ($-\text{OCH}_2$), 32.7 ($-\text{CH}$), 25.4 (C_4), 18.2 ($2 \times -\text{CH}_3$); mass (ESI) m/z : 353 (M^+), 355 ($\text{M}^+ + 2$); analysis: calculated for ($\text{C}_{16}\text{H}_{17}\text{BrO}_4$), C 54.41 H 4.85% and found C 54.34 H 4.80%.

4.2.8. 7-Fluoro-3-isobutoxy-4,4a-dihydro-3H,10H-pyrano[4,3-b][1]benzopyran-10-one (7h). Yellowish-brown amorphous solid (210 mg, 70%), mp 136–144°C; IR ν_{max} (CHCl_3): 2954, 2885, 1670, 1618, 1444 cm^{-1} ; $^1\text{H NMR}$ (CDCl_3 , 300 MHz): δ 7.96–7.91 (m, 1H, C_9H), 7.50 (s, 1H, C_1H), 6.78–6.59 (m, 2H, Ar-Hs), 5.16 (m, 2H, $J = 9.6$ Hz and $J = 6.3$ Hz, C_{4a}H and C_3H), 3.73 (dd, 1H, $J_{\text{gem}} = 9$ Hz and $J = 6.6$ Hz, $-\text{OCH}_2$), 3.31 (dd, 1H, $J_{\text{gem}} = 9$ Hz and $J = 6.6$ Hz, $-\text{OCH}_2$), 2.55 (ddd, 1H, $J_{\text{gem}} = 13.5$ Hz and $J = 6.9$ and 2.1 Hz, C_4H_a), 2.31 (dt, 1H, $J_{\text{gem}} = 13.5$ Hz and $J = 9.9$ Hz, C_4H_b), 1.98–1.87 (m, 1H, $-\text{CH}$), 0.94 (d, 6H, $J = 6.9$ Hz, $2 \times \text{CH}_3$); $^{13}\text{C NMR}$ (CDCl_3 , 100 MHz): δ 180.0 (C=O), 151.9 (olefinic-CH), 129.8 (q-arom.), 110.9 (Ar-CH), 110.0 (Ar-CH), 104.8 (Ar-CH), 100.8 (C_{4a} and C_3), 71.06 ($-\text{OCH}_2$), 33.3 ($-\text{CH}$), 29.9 (C_4), 19.1 ($2 \times -\text{CH}_3$); mass (ESI) m/z : 292 (M^+), 293 ($\text{M}^+ + 1$); analysis: calculated for ($\text{C}_{16}\text{H}_{17}\text{FO}_4$), C 65.74 H 5.86% and found C 65.70 H 5.81%.

4.2.9. 8-Fluoro-7-chloro-3-isobutoxy-4,4a-dihydro-3H,10H-pyrano[4,3-b][1]benzopyran-10-one (7i). Yellowish-brown amorphous solid (216 mg, 72%), mp 140–149°C; IR ν_{max} (CHCl_3): 2960, 2885, 1663, 1610, 1461 cm^{-1} ; $^1\text{H NMR}$ (CDCl_3 , 300 MHz): δ 7.70 (d, 1H, $J = 7.4$ Hz, C_9H), 7.52 (s, 1H, C_1H), 6.89 (s, 1H, C_6H), 5.23 (m, 2H, $J = 9.6$ Hz and $J = 1.8$ Hz, C_{4a}H and C_3H), 3.75 (dd, 1H, $J_{\text{gem}} = 9.3$ Hz and $J = 6.6$ Hz, $-\text{OCH}_2$), 3.33 (dd, 1H, $J_{\text{gem}} = 9.3$ Hz and $J = 6.9$ Hz, $-\text{OCH}_2$), 2.67 (dd, 1H, $J_{\text{gem}} = 12.9$ Hz and $J = 6.9$, C_4H_a), 2.39 (dt, 1H, $J_{\text{gem}} = 12.9$ Hz and $J = 6.6$ Hz, C_4H_b), 1.96–1.85 (m, 1H, $-\text{CH}$), 0.94 (d, 6H, $J = 6.9$ Hz, $2 \times \text{CH}_3$); $^{13}\text{C NMR}$ (CDCl_3 , 75 MHz): δ 180.1 (C=O), 159.7 (q-arom.), 151.1 (olefinic-CH), 129.1 (Ar-CH), 119.5 (q-arom.), 110.5 (Ar-CH), 104.3 (Ar-H), 100.2 (C_{4a}), 71.4 (C_3), 65.5 ($-\text{OCH}_2$), 32.4 (C_4), 15.1 ($-\text{CH}_3$); mass (ESI) m/z : 298.5 (M^+), 299 ($\text{M}^+ + 1$); analysis: calculated for ($\text{C}_{16}\text{H}_{17}\text{ClFO}_4$), C 58.81 H 4.94% and found C 58.75 H 4.87%.

4.2.10. 7-Chloro-3-isobutoxy-4,4a-dihydro-3H,10H-pyrano[4,3-b][1]benzopyran-10-one (7j). Yellowish-brown amorphous solid (219 mg, 73%), mp 139–147°C; IR ν_{max} (CHCl_3): 2937, 2887, 1670, 1614, 1423 cm^{-1} ; $^1\text{H NMR}$ (CDCl_3 , 300 MHz): δ 7.89 (d, 1H, $J = 8.4$ Hz, C_9H), 7.51 (s, 1H, C_1H), 7.01 (dd, 1H,

$J_{\text{gem}} = 8.1$ Hz and $J = 1.8$ Hz, C_8H), 6.94 (d, 1H, $J = 1.8$ Hz, C_6H), 5.17 (m, 2H, $J = 10.8$ Hz and $J = 1.2$ Hz, C_{4a}H and C_3H), 3.77 (dd, 1H, $J_{\text{gem}} = 9.0$ Hz and $J = 6.9$ Hz, $-\text{OCH}_2$), 3.36 (dd, 1H, $J_{\text{gem}} = 9.0$ Hz and $J = 6.9$ Hz, $-\text{OCH}_2$), 2.57 (ddd, 1H, $J_{\text{gem}} = 13.2$ Hz and $J = 6.6, 2.1$ Hz, C_4H_a), 2.30 (dt, 1H, $J_{\text{gem}} = 13.2$ Hz and $J = 9.6$ Hz, C_4H_b), 1.96–1.85 (m, 1H, $-\text{CH}$), 0.94 (d, 6H, $J = 6.6$ Hz, $2 \times \text{CH}_3$); $^{13}\text{C NMR}$ (CDCl_3 , 100 MHz): δ 180.2 (C=O), 161.1 (q-arom.), 152.1 (olefinic-CH), 141.03 (q-arom.), 128.5 (Ar-CH), 122.7 (Ar-CH), 121.2 (q-arom.), 117.9 (Ar-CH), 111.03 (q-arom.), 100.9 (C_{4a} and C_3), 70.93 ($-\text{OCH}_2$), 33.2 ($-\text{CH}$), 29.61 (C_4), 19.1 ($2 \times -\text{CH}_3$); mass (ESI) m/z : 308.5 (M^+), 309 ($\text{M}^+ + 1$); analysis: calculated for ($\text{C}_{16}\text{H}_{17}\text{ClO}_4$), C 62.24 H 5.55% and found C 62.18 H 5.48%.

4.2.11. 3-Ethoxy-4,4a-dihydro-3H,10H-pyrano[4,3-b][1]benzopyran-10-one (7k). White amorphous solid (225 mg, 75%), mp 115–121°C; IR ν_{max} (CHCl_3): 2929, 2887, 1668, 1614, 1473 cm^{-1} ; $^1\text{H NMR}$ (CDCl_3 , 300 MHz): δ 7.91 (d, 1H, $J = 6$ Hz, C_9H), 7.51 (d, 1H, $J = 1.2$ Hz, C_1H), 7.45–7.01 (m, 2H, Ar-Hs), 6.91 (d, 1H, $J = 8.7$ Hz, C_6H), 5.16–5.10 (m, 2H, C_{4a}H and C_3H), 4.02 (dq, 1H, $J_{\text{gem}} = 8.4$ Hz and $J = 6.9$ Hz, $-\text{OCH}_2$), 3.64 (dq, 1H, $J_{\text{gem}} = 7.5$ Hz and $J = 6.9$ Hz, $-\text{OCH}_2$), 2.53 (dd, 1H, $J_{\text{gem}} = 12.9$ Hz and $J = 6.9$ Hz, C_4H_a), 2.30 (dt, 1H, $J_{\text{gem}} = 12.9$ Hz and $J = 9.9$ Hz, C_4H_b), 1.29 (t, 3H, $J = 6.9$ Hz, $-\text{CH}_3$); $^{13}\text{C NMR}$ (CDCl_3 , 100 MHz): δ 181.8 (C=O), 160 (q-arom.), 151.6 (olefinic-CH), 135.2 (Ar-CH), 127.3 (Ar-CH), 122.7 (q-arom.), 122.0 (Ar-CH), 111.6 (Ar-CH), 100.5 (C_{4a}), 70.4 (C_3), 65.6 ($-\text{OCH}_2$), 33.5 (C_4), 15.01 ($-\text{CH}_3$); mass (ESI) m/z : 246 (M^+), 247 ($\text{M}^+ + 1$); analysis: calculated for ($\text{C}_{14}\text{H}_{14}\text{O}_4$), C 68.28 H 5.73% and found C 68.20 H 5.68%.

4.2.12. 3-Ethoxy-8-methyl-4,4a-dihydro-3H,10H-pyrano[4,3-b][1]benzopyran-10-one (7l). Light-yellow amorphous solid (216 mg, 72%), mp 118–125°C; IR ν_{max} (CHCl_3): 2918, 2896, 1668, 1618, 1498 cm^{-1} ; $^1\text{H NMR}$ (CDCl_3 , 300 MHz): δ 7.69 (d, 1H, $J = 3.6$ Hz, C_9H), 7.45 (d, 1H, $J = 1.5$ Hz, C_1H), 7.20 (dd, 1H, $J = 8.4$ Hz and 2.1 Hz, C_7H), 6.78 (d, 1H, $J = 8.4$ Hz, C_6H), 5.17 (m, 2H, $J = 10.2$ Hz and $J = 1.5$ Hz, C_{4a}H and C_3H), 3.81 (dq, 1H, $J_{\text{gem}} = 8.1$ Hz and $J = 7.2$ Hz, $-\text{OCH}_2$), 3.57 (dq, 1H, $J_{\text{gem}} = 8.1$ Hz and $J = 7.2$ Hz, $-\text{OCH}_2$), 2.49 (ddd, 1H, $J_{\text{gem}} = 12.3$ Hz and $J = 6.3, 2.1$ Hz, C_4H_a), 2.20 (dt, 1H, $J_{\text{gem}} = 12.3$ Hz and $J = 10.7$ Hz, C_4H_b), 1.17 (t, 3H, $J = 6.9$ Hz, $-\text{CH}_2\text{CH}_3$), 0.05 (s, 3H, $-\text{CH}_3$); $^{13}\text{C NMR}$ (CDCl_3 , 75 MHz): δ 180.4 (C=O), 158.5 (q-arom.), 152.9 (olefinic-CH), 137.5 (Ar-CH), 129.6 (Ar-CH), 120.3 (q-arom.), 117.7 (Ar-CH), 100.1 (C_{4a}), 70.05 (C_3), 65.1 ($-\text{OCH}_2$), 31.6 (C_4), 15.5 ($-\text{CH}_3$); mass (ESI) m/z : 262 (M^+), 261 ($\text{M}^+ - 1$); analysis: calculated for ($\text{C}_{15}\text{H}_{17}\text{O}_4$), C 69.22 H 6.20% and found C 69.15 H 6.15%.

4.2.13. 6,8-Dichloro-3-ethoxy-4,4a-dihydro-3H,10H-pyrano[4,3-b][1]benzopyran-10-one (7m). Brownish amorphous solid (219 mg, 73%), mp 125–131°C; IR ν_{max} (CHCl_3): 2975, 2898, 1674, 1610, 1456 cm^{-1} ; $^1\text{H NMR}$ (CDCl_3 , 300 MHz): δ 7.79 (d, 1H, $J = 2.4$ Hz, C_9H), 7.57 (d, 1H, $J = 1.2$ Hz, C_1H), 7.49 (d, 1H, $J = 2.7$ Hz, C_7H), 5.22 (m, 2H, $J = 9.9$ Hz and $J = 6.9$, 1.8 Hz, C_{4a}H and C_3H), 4.02 (dq, 1H, $J_{\text{gem}} = 9.6$ Hz and $J = 7.2$ Hz, $-\text{OCH}_2$), 3.67 (dq, 1H, $J_{\text{gem}} = 9.6$ Hz and $J = 7.5$ Hz,

-OCH₂), 2.66 (ddd, 1H, $J_{\text{gem}} = 12.3$ Hz and $J = 6.6, 1.8$ Hz, C₄H_a), 2.38 (dt, 1H, $J_{\text{gem}} = 12.3$ Hz and $J = 9.6$ Hz, C₄H_b), 1.29 (t, 3H, $J = 6.9$ Hz, -CH₃); ¹³C NMR (CDCl₃, 75 MHz): δ 180.1 (C=O), 153.5 (olefinic-CH), 133.2 (Ar-CH), 125.1 (Ar-CH), 124.5 (q-arom.), 110.6 (Ar-CH), 101.3 (C_{4a}), 70.08 (C₃), 65.5 (-OCH₂), 31.1 (C₄), 15.6 (-CH₃); mass (ESI) m/z : 315 (M⁺); analysis: calculated for (C₁₄H₁₂Cl₂O₄), C 53.36 H 3.84% and found C 53.30 H 3.79%.

4.2.14. 8-Chloro-3-ethoxy-4,4a-dihydro-3H,10H-pyrano[4,3-b][1]benzopyran-10-one (7n). Yellowish amorphous solid (228 mg, 76%), mp 121–129°C; IR ν_{max} (CHCl₃): 2979, 2896, 1672, 1610, 1475 cm⁻¹; ¹H NMR (CDCl₃, 300 MHz): δ 7.86 (d, 1H, $J = 2.4$ Hz, C₉H), 7.52 (s, 1H, C₁H), 7.36 (dd, 1H, $J = 9$ Hz and 2.7 Hz, C₇H), 6.86 (d, 1H, $J = 8.7$ Hz, C₆H), 5.18 (m, 2H, $J = 9.9$ Hz and $J = 1.8$ Hz, C_{4a}H and C₃H), 4.04 (dq, 1H, $J_{\text{gem}} = 8.4$ Hz and $J = 7.2$ Hz, -OCH₂), 3.68 (dq, 1H, $J_{\text{gem}} = 9.3$ Hz and $J = 7.2$ Hz, -OCH₂), 2.55 (ddd, 1H, $J_{\text{gem}} = 13.2$ Hz and $J = 6.9, 2.1$ Hz, C₄H_a), 2.29 (dt, 1H, $J_{\text{gem}} = 13.2$ Hz and $J = 9.9$ Hz, C₄H_b), 1.29 (t, 3H, $J = 6.9$ Hz, -CH₃); ¹³C NMR (CDCl₃, 100 MHz): δ 180.3 (C=O), 161.1 (q-arom.), 151.9 (olefinic-CH), 142.2 (q-arom.), 127.2 (Ar-CH), 121.7 (Ar-CH), 120.2 (Ar-CH), 117.1 (Ar-CH), 110.0 (q-arom.), 100.1 (C_{4a}), 70.02 (C₃), 65.1 (-OCH₂), 31.9 (C₄), 15.2 (-CH₃); mass (ESI) m/z : 280.5 (M⁺), 281 (M⁺ + 1); analysis: calculated for (C₁₄H₁₃ClO₄), C 59.90 H 4.67% and found C 59.82 H 4.62%.

4.2.15. 8-Fluoro-3-ethoxy-4,4a-dihydro-3H,10H-pyrano[4,3-b][1]benzopyran-10-one (7o). Orange-brownish amorphous solid (216 mg, 72%), mp 120–127°C; IR ν_{max} (CHCl₃): 2992, 2904, 1662, 1598, 1483 cm⁻¹; ¹H NMR (CDCl₃, 300 MHz): δ 7.67–7.61 (m, 1H, Ar-H), 7.33 (d, 1H, $J = 1.8$ Hz, C₁H), 7.22–6.96 (m, 2H, Ar-Hs), 5.26 (m, 2H, $J = 9.6$ Hz and $J = 1.8$ Hz, C_{4a}H and C₃H), 4.04 (dq, 1H, $J_{\text{gem}} = 8.7$ Hz and $J = 7.2$ Hz, -OCH₂), 3.65 (dq, 1H, $J_{\text{gem}} = 8.7$ Hz and $J = 7.2$ Hz, -OCH₂), 2.54 (dt, 1H, $J_{\text{gem}} = 12.9$ Hz and $J = 6.6$ Hz, C₄H_a), 2.31 (dist.dd, 1H, $J_{\text{gem}} = 12.9$ Hz and $J = 9.9$ Hz, C₄H_b), 1.29 (t, 3H, $J = 6.9$ Hz, -CH₃); ¹³C NMR (CDCl₃, 75 MHz): δ 180.1 (C=O), 159.8 (q-arom.), 151.4 (olefinic-CH), 129.3 (Ar-CH), 118.9 (q-arom.), 110.1 (Ar-CH), 104.2 (Ar-H), 100.7 (C_{4a}), 71.07 (C₃), 65.1 (-OCH₂), 33.2 (C₄), 15.03 (-CH₃); mass (ESI) m/z : 264 (M⁺), 265 (M⁺ + 1); analysis: calculated for (C₁₄H₁₃FO₄), C 63.63 H 4.96% and found C 63.58 H 4.91%.

4.2.16. 8-Bromo-3-ethoxy-4,4a-dihydro-3H,10H-pyrano[4,3-b][1]benzopyran-10-one (7p). Orange-brownish amorphous solid (222 mg, 74%), mp 125–133°C; IR ν_{max} (CHCl₃): 2975, 2877, 1666, 1600, 1456 cm⁻¹; ¹H NMR (CDCl₃, 300 MHz): δ 8.01 (d, 1H, $J = 2.4$ Hz, C₉H), 7.53 (s, 1H, C₁H), 7.49 (dd, 1H, $J = 8.7$ Hz and 2.4 Hz, C₇H), 6.82 (d, 1H, $J = 8.7$ Hz, C₆H), 5.16 (m, 2H, $J = 9.6$ Hz and $J = 1.8$ Hz, C_{4a}H and C₃H), 4.04 (dq, 1H, $J_{\text{gem}} = 9.6$ Hz and $J = 7.2$ Hz, -OCH₂), 3.65 (dq, 1H, $J_{\text{gem}} = 9.6$ Hz and $J = 7.2$ Hz, -OCH₂), 2.55 (dt, 1H, $J_{\text{gem}} = 12.6$ Hz and $J = 6$ Hz, C₄H_a), 2.29 (dist.dd, 1H, $J_{\text{gem}} = 12.6$ Hz and $J = 9.9$ Hz, C₄H_b), 1.29 (t, 3H, $J = 6.9$ Hz, -CH₃); ¹³C NMR (CDCl₃, 100 MHz): δ 179.3 (C=O), 159.5 (q-arom.), 152.1 (olefinic-CH), 137.7 (Ar-CH), 130.0 (Ar-CH), 124.2 (q-arom.),

119.7 (Ar-CH), 114.8 (q-arom.), 110.9 (q-arom.), 100.5 (C_{4a}), 70.7 (C₃), 65.5 (-OCH₂), 33.5 (C₄), 15.1 (2 × -CH₃); mass (ESI) m/z : 325 (M⁺); analysis: calculated for (C₁₄H₁₃BrO₄), C 51.71 H 4.03% and found C 51.65 H 3.97%.

4.2.17. 7-Bromo-3-ethoxy-4,4a-dihydro-3H,10H-pyrano[4,3-b][1]benzopyran-10-one (7q). Yellowish-brown amorphous solid (225 mg, 75%), mp 123–129°C; IR ν_{max} (CHCl₃): 2985, 2896, 1683, 1616, 1419 cm⁻¹; ¹H NMR (CDCl₃, 300 MHz): δ 7.76 (d, 1H, $J = 8.1$ Hz, C₉H), 7.50 (d, 1H, $J = 1.5$ Hz, C₁H), 7.31 (s, 1H, C₆H), 7.16 (d, 1H, $J = 7.8$ Hz, C₈H), 5.17 (m, 2H, $J = 9.3$ Hz and $J = 1.5$ Hz, C_{4a}H_x and C₃H_y), 4.01 (dq, 1H, $J_{\text{gem}} = 9.9$ Hz and $J = 7.5$ Hz, -OCH₂), 3.64 (dq, 1H, $J_{\text{gem}} = 9.6$ Hz and $J = 7.5$ Hz, -OCH₂), 2.54 (dist.dd, 1H, $J_{\text{gem}} = 12.3$ Hz and $J = 6.9$ Hz, C₄H_a), 2.31 (dt, 1H, $J_{\text{gem}} = 12.3$ Hz and $J = 10.6$ Hz, C₄H_b), 1.27 (t, 3H, $J = 7.2$ Hz, -CH₃); ¹³C NMR (CDCl₃, 100 MHz): δ 180.3 (C=O), 161.8 (q-arom.), 152.8 (olefinic-CH), 129.5 (q-arom.), 128.5 (Ar-CH), 125.5 (Ar-CH), 121.0 (Ar-CH), 111.0 (q-arom.), 100.5 (C_{4a}), 71.2 (C₃), 65.7 (-OCH₂), 33.3 (C₄), 15.03 (-CH₃); mass (ESI) m/z : 325 (M⁺); analysis: calculated for (C₁₄H₁₃BrO₄), C 51.71 H 4.03% and found C 51.65 H 3.97%.

4.2.18. 7-Fluoro-3-ethoxy-4,4a-dihydro-3H,10H-pyrano[4,3-b][1]benzopyran-10-one (7r). Orange-brownish amorphous solid (228 mg, 76%), mp 119–124°C; IR ν_{max} (CHCl₃): 2985, 2904, 1670, 1622, 1436 cm⁻¹; ¹H NMR (CDCl₃, 300 MHz): δ 7.97–7.91 (m, 1H, Ar-H), 7.51 (d, 1H, $J = 1.2$ Hz, C₁H), 7.22–6.59 (m, 2H, Ar-Hs), 5.18 (m, 2H, $J = 9.6$ Hz and $J = 6.6, 1.8$ Hz, C_{4a}H and C₃H), 4.02 (dq, 1H, $J_{\text{gem}} = 9.6$ Hz and $J = 7.2$ Hz, -OCH₂), 3.65 (dq, 1H, $J_{\text{gem}} = 9.3$ Hz and $J = 7.2$ Hz, -OCH₂), 2.55 (ddd, 1H, $J_{\text{gem}} = 12.9$ Hz and $J = 6.9, 1.8$ Hz, C₄H_a), 2.31 (dt, 1H, $J_{\text{gem}} = 12.9$ Hz and $J = 9.6$ Hz, C₄H_b), 1.29 (t, 3H, $J = 6.9$ Hz, -CH₃); ¹³C NMR (CDCl₃, 100 MHz): δ 179.9 (C=O), 160.8 (q-arom.), 151.9 (olefinic-CH), 129.8 (Ar-CH), 119.4 (q-arom.), 110.2 (Ar-CH), 104.6 (Ar-H), 100.4 (C_{4a}), 71.03 (C₃), 65.6 (-OCH₂), 33.3 (C₄), 15.01 (-CH₃); mass (ESI) m/z : 264 (M⁺), 265 (M⁺ + 1); analysis: calculated for (C₁₄H₁₃FO₄), C 63.63 H 4.96% and found C 63.58 H 4.91%.

4.2.19. 8-Fluoro-7-chloro-3-ethoxy-4,4a-dihydro-3H,10H-pyrano[4,3-b][1]benzopyran-10-one (7s). Orange-brownish amorphous solid (222 mg, 74%), mp 124–132°C; IR ν_{max} (CHCl₃): 2974, 2877, 1670, 1600, 1461 cm⁻¹; ¹H NMR (CDCl₃, 300 MHz): δ 7.69 (s, 1H, C₉H), 7.52 (s, 1H, C₁H), 6.81 (s, 1H, C₆H), 5.21 (m, 2H, $J = 9.6$ Hz and $J = 1.5$ Hz, C_{4a}H and C₃H), 4.01 (dq, 1H, $J_{\text{gem}} = 9.9$ Hz and $J = 7.2$ Hz, -OCH₂), 3.66 (dq, 1H, $J_{\text{gem}} = 9.6$ Hz and $J = 7.2$ Hz, -OCH₂), 2.60 (unresolved dd, 1H, $J_{\text{gem}} = 12.0$ Hz and $J = 6.9, 1.8$ Hz, C₄H_a), 2.36 (dt, 1H, $J_{\text{gem}} = 12.3$ Hz and $J = 9.9$ Hz, C₄H_b), 1.29 (t, 3H, $J = 6.6$ Hz, -CH₃); ¹³C NMR (CDCl₃, 75 MHz): δ 179.7 (C=O), 159.9 (q-arom.), 151.5 (olefinic-CH), 129.3 (Ar-CH), 119.2 (q-arom.), 110.1 (Ar-CH), 104.9 (Ar-H), 100.4 (C_{4a}), 71.0 (C₃), 65.2 (-OCH₂), 33.1 (C₄), 15.6 (-CH₃); mass (ESI) m/z : 298.5 (M⁺), 299 (M⁺ + 1); analysis: calculated for (C₁₄H₁₂ClFO₄), C 56.29 H 4.05% and found C 56.22 H 3.98%.

4.2.20. 7-Chloro-3-ethoxy-4,4a-dihydro-3H,10H-pyrano[4,3-b][1]benzopyran-10-one (**7t**). Orange-brownish amorphous solid (216 mg, 72%), mp 121–129°C; IR ν_{\max} (CHCl₃): 2360, 2331, 1670, 1616, 1458 cm⁻¹; ¹H NMR (CDCl₃, 300 MHz): δ 7.85 (d, 1H, J = 8.4 Hz, C₉H), 7.52 (s, 1H, C₁H), 7.01 (dd, 1H, J = 8.4 Hz and 2.1 Hz, C₈H), 6.93 (d, 1H, J = 2.1 Hz, C₆H), 5.17 (m, 2H, J = 9.6 Hz and J = 2.1 Hz, C_{4a}H and C₃H), 4.01 (dq, 1H, J_{gem} = 9.3 Hz and J = 7.2 Hz, -OCH₂), 3.66 (dq, 1H, J_{gem} = 9.3 Hz and J = 7.2 Hz, -OCH₂), 2.55 (ddd, 1H, J_{gem} = 12.9 Hz and J = 6.6, 2.1 Hz, C₄H_a), 2.31 (dt, 1H, J_{gem} = 12.9 Hz and J = 9.9 Hz, C₄H_b), 1.29 (t, 3H, J = 6.9 Hz, -CH₃); ¹³C NMR (CDCl₃, 100 MHz): δ 180.1 (C=O), 161.0 (q-arom.), 152.0 (olefinic-CH), 141.05 (q-arom.), 128.5 (Ar-CH), 122.7 (Ar-CH), 121.2 (Ar-CH), 117.9 (Ar-CH), 111.05 (q-arom.), 100.4 (C_{4a}), 70.9 (C₃), 66.6 (-OCH₂), 33.3 (C₄), 15.01 (-CH₃); mass (ESI) m/z : 280.5 (M⁺), 281 (M⁺ + 1); analysis: calculated for (C₁₄H₁₃ClO₄), C 59.90 H 4.67% and found C 59.82 H 4.62%.

4.3. X-Ray Data of Compound **7k**

CCDC No.: 885423
 Crystal description: block
 Crystal colour: white
 Crystal size: 0.30 × 0.20 × 0.20 mm
 Empirical formula: C₁₄H₁₄O₄
 Formula weight: 246.25
 Radiation, wavelength: Mo K α , 0.71073 Å
 Unit cell dimensions: a = 10.6891(10), b = 20.5792(14), c = 5.4621(4) Å and β = 94.924(7)°
 Crystal system: monoclinic
 Space group: P2₁/c
 Unit cell volume: 1197.08(16)
 No. of molecules per unit cell, Z: 4
 Temperature: 293(2)
 Absorption coefficient: 0.100 mm⁻¹
 $F(000)$: 520
 Scan mode: ω scan
 θ range for entire data collection: 3.53 < θ < 26.00°
 Range of indices: h = -13 to 13, k = -25 to 25, and l = -6 to 6
 Reflections collected/unique: 39266/2343
 Reflections observed ($I > 2\sigma(I)$): 998
 Structure determination: direct methods
 Refinement: full-matrix least-squares on F^2
 No. of parameters refined: 164
 Final R: 0.0798
 wR (F^2): 0.1831
 Weight: $1/[\sigma^2(F_o^2) + (0.1084 P)^2 + 0.0188 P]$, where $P = [F_o^2 + 2F_c^2]/3$

Goodness-of-fit: 0.997

(Δ/σ)_{max}: 0.002

Final residual electron density: -0.234 < $\Delta\rho$ < 0.321 eÅ⁻³

Measurement: Xcalibur system—Oxford diffraction make, UK

Software for structure solution: SHELXS97 (Sheldrick, 2008)

Software for refinement: SHELXL97 (Sheldrick, 2008)

Software for molecular plotting: ORTEP-3 (Farrugia, 1997) and PLATON (Spek, 2009)

Software for geometrical calculation: PLATON (Spek, 2009) and PARST (Nardelli, 1995).

4.4. Microbiological Evaluation

4.4.1. *Antibacterial Activity.* All the synthesized compounds (**7a–t**) were screened for their antibacterial potential in triplicate against two gram-positive bacteria, *Staphylococcus aureus* (MTCC96), *Bacillus subtilis* (MTCC2451), and three gram-negative bacteria, *Escherichia coli* (MTCC 82), *Pseudomonas aeruginosa* (MTCC 2642), and *Salmonella typhimurium* (MTCC 1251) by using disc diffusion method [24]. The activity of compounds was determined with comparison to standard antibiotic discs of amoxicillin (5 μ g) and ciprofloxacin (10 μ g). Prewarmed Mueller-Hinton agar plates were inoculated with 10⁶ CFU/mL of test bacteria. Each compound was dissolved in DMSO (1 mg/mL), and then 30 μ L of each was pipetted onto sterile paper discs (6 mm diameter) placed on the surface of inoculated agar plates. Plates were incubated at 37°C for 24 h. Activity was expressed as the diameter of the inhibition zone (mm) produced by the compounds (Table 2). DMSO was used as negative control. MIC of compounds exhibiting considerable activity was evaluated by turbidimetry method [25]. The initial optical density (OD) of the medium was measured by spectrophotometer at 600 nm. The test strains were incubated in nutrient broth until the OD reached 0.4–0.6. Then, the different concentrations of compounds (0.78, 1.56, 3.12, 6.25, 12.5, 25, and 50 μ g/mL) were tested for the inhibition of growth of these microbes, in separate tubes. The 10 mL tubes, each containing 5 mL nutrient broth and 1 mL of different concentrations of compounds, were incubated for 24 hrs with shaking at 180 rpm using a rotary shaker. Each tube corresponding to different concentrations was observed, and the concentration showing apparently no turbidity was considered to be the MIC of respective compound.

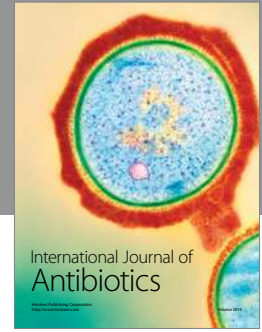
4.4.2. *Antifungal Activity.* All synthesized compounds **7a–t** were tested against five reference fungal strains, *Aspergillus niger* (MTCC 1344), *Saccharomyces cerevisiae* (MTCC 172), *Candida albicans* (MTCC 3018), *Cryptococcus gastricus* (MTCC 1715), and *Microsporium gypseum* (MTCC 4490) by using disc diffusion method [20–22]. The antifungal activity of synthesized compounds was determined by observing the zone of inhibition in comparison to the standard antifungal

discs (fluconazole and griseofulvin). Test compounds were dissolved in DMSO to make a stock solution of 1 mg/mL. The fresh subculture of strains in normal saline was added to the sterile assay medium (Sabouraud Dextrose agar with chloramphenicol) at 40–45°C and mixed well. The medium was poured into each of the petri dishes. Sterile discs of diameter 6 mm were placed on the medium. 20 μ L of each test solution was added to the previously marked discs and the media were allowed to stand for 5 min. The petri dishes were kept aside for 1 h, and then incubated at 28°C for 48 h. Zone of inhibition was measured, and the average of the three readings was calculated; DMSO was also used as negative control. The MIC of active compounds (zone of inhibition) was determined by serial tube dilution method [25]. Different dilutions of test compounds (1.9 μ g/mL–500 μ g/mL) were made from stock solution, 1 mL nutrient broth was taken in each test tube, and 20 μ L of standard strains was added to previously marked test tubes.

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