Scientia Iranica C (2015) 22(6), 2241–2248

Sharif University of Technology
Scientia Iranica
Transactions C: Chemistry and Chemical Engineering
www.scientiairanica.com

Synthesis, characterization and biological screening of different carbamates derived from 7-hydroxy-4-methyl-2*H*-1-benzopyran-2-one

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Received 23 November 2013; received in revised form 26 May 2014; accepted 13 April 2015

KEYWORDS
7-hydroxy-4-methylcoumarin;
Carbamates;
Substituted aliphatic amine;
Antibacterial activity;
Spectral analysis.

Abstract. A facile and environmentally benign series of carbamates derived from coumarin were synthesized. The synthesis of 7-hydroxy-4-methyl-2*H*-1-benzopyran-2-one (**3**) was carried out by gearing up resorcinol (**1**) and ethyl acetoacetate (**2**) in a strong acidic media. Carbamates **7** and **8** were synthesized by reacting phenylchloroformate (**4**) with substituted aliphatic amines **5** and **6** under dynamic pH control in aqueous media in the presence of Na_2CO_3 solution. The molecules **3**, **7**, and **8** were brominated to **3a**, **7a** and **8a**. The molecules **9**, **9a**, **10**, and **11** were synthesized by further reaction of **3** and **3a** with the electrophilic carbamates **7**, **7a**, and **8** in a polar aprotic solvent using LiH as an activator. Finally, the compound **12** was synthesized by the nitration of **10**. Structures of all the compounds were determined by IR, ^{1}H-NMR, ^{13}C-NMR and EI-MS spectroscopic techniques. The bioactivity results of all the synthesized compounds against four gram-negative and two gram-positive bacteria showed that the synthesized coumarin derivatives exhibited moderate to good %age inhibition activity.

1. Introduction

Coumarins, a group of compounds bearing 1-benzopyran-2-one ring, are found in many plants, particularly in the tonka bean (Dipteryxodorata), vanilla grass (Anthoxanthumodoratum), woodruff (Galiumodoratum), mullein (Verbascumspp), and sweet grass (Hierochloeodorata) with high concentration [1]. Coumarin, having fused benzene and α-pyrone rings, is an aromatic compound giving a typical odor to hay. A number of possible permutations, offered by substitution and conjugation, readily describe the natural occurrence of such molecules [2]. 7-hydroxycoumarin (7-HC) is a foremost human metabolite and plays a role as dietary antioxidant in the human food like fruits and vegetables [3]. It is acknowledged that coumarin and their derivatives exhibit different biological and pharmacological activities, such as anticoagulant, antibacterial, anti-tubercular, anti-malarial, antioxidant, anticancer, anti-HIV, antiviral, antioxidants, and anti-inflammatory activities. Hence, a great deal of efforts has been devoted to design and synthesize the functional coumarin derivatives [4-7].

Our prime objective of the presented research was to synthesize new compounds bearing great potential as antimicrobial agent, helping out the drug development program in the pharmacological industries. In continuation of our previous work on *O*-substituted compounds [8-10], the undertaken research was executed

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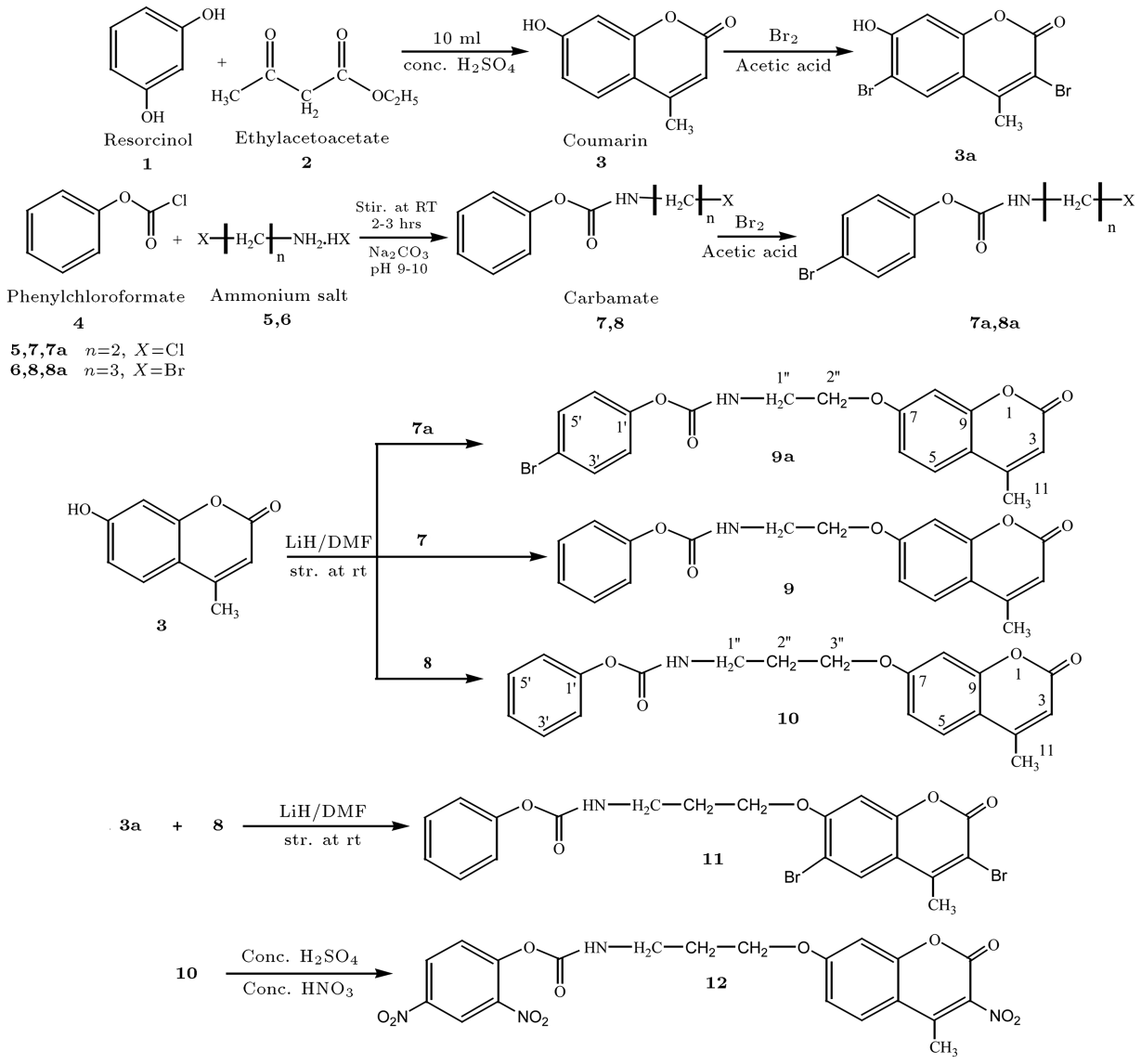
to inaugurate a series of new potent compounds with antibacterial activity exhibiting diverse and improved pharmacological potential. We have synthesized the *O*-substituted derivatives of coumarin with an objective to search new contenders of drug having significantly enhanced activity and could be helpful in controlling many degenerative diseases.

2. Results and discussion

New *O*-substituted carbamate derivatives of 7-hydroxy-4-methyl-2*H*-1-benzopyran-2-one were adeptly synthesized according to the protocol sketched in Scheme 1. The proposed structures of synthesized compounds were corroborated through spectral analysis and then processed further for the antibacterial activity evaluation using four bacterial strains of gram-negative and two of gram-positive bacteria. The synthesized compounds were yielded in awesome amounts with moderate antibacterial activity. The general reaction conditions and the structure characterization are described in experimental section.

2.1. Chemistry

The parent compound 7-hydroxy-4-methyl-2*H*-1-benzopyran-2-one (**3**) was synthesized in good yield by coupling of resorcinol (**1**) and ethyl acetoacetate (**2**) in strong acidic conditions. Synthesis of *O*-substituted derivatives of **3** was brought about by treating parent compound with different electrophilic carbamates in the presence of LiH as base and an activator, and DMF as a solvent, by simple stirring for 3-4 hours. Precipitates were quenched by adding cold water and recrystallized from methanol. The different molecules were synthesized from **3** by the Scheme 1 and were well characterized by ^{1}H-NMR, ^{13}C-NMR, IR, and



Scheme 1. *O*-substituted derivatives of 7-hydroxy-4-methyl-2*H*-1-benzopyran-2-one (**3**)

Table 1. %age inhibition of antibacterial activity of synthesized compounds.

Compound	%age inhibition					
	Gram negative bacteria			Gram positive bacteria		
	S. typhi	*E. coli*	*K. pneumonae*	*P. aeroginosa*	*B. subtilis*	*S. aerus*
3a	73.57±3.43	79.31±1.19	75.90±1.46	83.01±0.81	71.13±3.53	77 .40±3.38
7	55.86±1.86	63.06±3.19	54.17±3.03	63.31±2.99	57.60±4.20	51.88±1.75
7a	54.64±4.01	66.75±3.47	58.68±4.51	79.26±3.39	61.33±3.33	51.04±3.66
8	61.64±2.07	65.38±1.00	59.17±0.83	83.46±3.75	63.40±1.27	60.84±2.53
8a	62.43±1.57	68.13±0.13	69.38±3.26	84.12±3.82	71.20±3.47	66.43±0.58
9	60.07±3.93	66.38±1.25	57.43±2.01	66.10±1.99	54.53±2.53	60.58±1.49
9a	54.57±2.00	57.69±3.44	54.38±0.49	60.59±3.09	51.87±2.67	40.97±4.00
11	51.43±2.57	55.75±4.13	64.44±2.08	71.18±2.86	63.73±2.53	61.88±4.13
12	62.57±3.71	67.81±3.44	67.99±4.13	84.78±1.40	61.93±4.47	63.96±2.98
Ciprofloxacin	**88.95±1.43**	**91.02±0.25**	**89.00±1.74**	**92.12±1.00**	**90.27±0.33**	**88.15±2.14**

EI-MS. The only *p*-substituted product [11] during bromination of **7** and **8** was attributed because of steric hindrance effect as we know the well-known reaction that is, bromination of acetanilide of such type.

The compound **9** was obtained as white sticky solid in 73% yield. Its molecular formula was determined through EI-MS showing molecular ion peak at m/z 339 corresponding to $C_{19}H_{17}NO_5$. Other characteristic peaks appeared at m/z 218, 176, 148, and 120 while base peak appeared at m/z 94 corresponding to phenoxy group. ^{1}H-NMR spectrum revealed signals of aromatic protons at δ/ppm 7.69 (d, J = 9.0 Hz, 1H, H-5), 6.68 (s, 1H, H-8), 6.62 (d, J = 8.7 Hz, 1H, H-6), and 6.18 (s, 1H, H-3) for coumarin ring, while signals of phenyl ring of carbamate appeared at 7.40 (t, J = 9.0 Hz, 2H, H-3′ & H-5′), 7.31 (d, J = 9.3 Hz, 2H, H-2′ & H-6′), and 7.24 (t, J = 7.5 Hz, 1H, H-4′). Aliphatic protons appeared at 3.52 (t, J = 6.0 Hz, 2H, H-1″), 3.25 (t, J = 6.6 Hz, 2H, H-2″), and 2.83 (s, 3H, CH_3-11). ^{13}C-NMR (BB and DEPT) spectrum recorded in CD_3OD at 75 MHz revealed signals for seven quaternary carbons at 168.2 (C-2), 160.1 (C-7), 157.2 (C-4), 156.2 (C-1′), 154.2 (C-9), 153.3 (C-7′), and 115.1 (C-10); for seven methine carbons at 129.2 (C-3′ & C-5′), 126.5 (C-5), 121.4 (C-4′), 115.2 (C-2′ & C-6′), 112.1 (C-6), 109.3 (C-3), and 101.2 (C-8); and for two methylene and one methyl carbon at 65.6 (C-2″), 39.7 (C-1″), and 18.7 (CH_3-11). On the basis of these evidences, the structure was allocated as phenyl *N*-(2-(4-methyl-2-oxo-2*H*-1-benzopyran-7-yloxy)ethyl) carbamate. In the NMR spectrum for compound **9a**, the signal for H-4′ disappeared because of substitution of a bromine atom. The compound **10** also showed similar pattern except an additional signal as multiplet for H-2″ in the range of 2.14-2.08. The spectra of **10** and **11** only had a difference of two singlets for H-3 and H-6 which were absent in **11**. The molecule **12** was deprived off further three signals originating for H-3, H-2″ and H-4″. Similarly the structures of other synthesized derivatives of 3 were elucidated as described in experimental section and outlined in Scheme 1.

2.2. Antibacterial activity

All the synthesized compounds were screened for antibacterial activity against four gram-negative and two gram-positive bacteria. The %age inhibition (Table 1) and MIC values (Table 2) were calculated for all the synthesized compounds. The outcomes were evaluated with those of reference standard, ciprofloxacin.

The compound **3a** was more active and showed relatively good inhibition of *S. typhi, E. coli, K. pneumonae* and *S. aureus* with %age inhibition of 73.57±3.43, 79.31±1.19, 75.90±1.46, and 77.40±3.38, respectively, against ciprofloxacin, the reference standard, having %age inhibition of 88.95±1.43, 91.02±0.25, 89.00±1.74, and 88.15±2.14, respectively. The preliminary screening showed that some compounds executed better activity against gram-positive bacteria and some other against gram-negative bacteria as compared to reference drug ciprofloxacin. Minimum Inhibitory Concentration (MIC) analysis was also checked out for all these compounds. Overall, the compounds **3a**, **7**, **7a**, **8**, **8a**, **9**, and **12** remained active against all the bacterial strains. The compounds **3a** and **12** showed excellent MIC values of 8.51±3.41 and 8.22±3.64, respectively, against *P. aeroginosa*, relative to the reference standard, ciprofloxacin with MIC value of 8.03±1.42 μM.

3. Conclusion

All the synthesized compounds were afforded in good yields and also were well supported by the results of IR, ^{1}H-NMR, ^{13}C-NMR, and EIMS spectral data. These

Table 2. MIC values of antibacterial activity of synthesized compounds.

Compound	MIC values					
	Gram negative bacteria			Gram positive bacteria		
	S. typhi	*E. coli*	*K. pneumonae*	*P. aeroginosa*	*B. subtilis*	*S. aerus*
3a	12.53±0.51	15.05 ±2.12	13.34±2.66	8.51±3.41	10.91±0.98	11.62±0.51
7	17.95±0.32	17.46±2.61	19.13±2.63	16.35±2.91	15.14±2.71	19.48±0.32
7a	18.06±0.21	16.64±2.83	18.36±1.87	10.91±4.32	12.11±2.89	19.43±0.21
8	15.49±0.96	17.51±4.06	18.37±1.71	13.94±3.64	10.73±1.34	16.90±0.96
8a	16.22±0.53	16.82±0.19	16.69±1.40	14.00±1.05	12.54±4.91	15.31±0.53
9	17.45±3.26	15.23±2.97	18.62±0.26	16.23±3.70	17.68±1.92	17.56±3.26
9a	18.45±2.36	18.40±4.72	19.02±2.68	17.41±3.30	18.11±3.39	—
11	19.59±2.30	18.58±0.85	16.54±1.46	16.29±3.07	15.30±3.54	17.85±2.85
12	16.21±3.85	17.10±1.73	16.90±2.38	8.22±3.64	14.24±1.34	16.16±3.85
Ciprofloxacin	**9.34±1.50**	**8.26±2.00**	**9.17±1.84**	**8.03±1.42**	**8.72±2.73**	**9.73±1.00**

molecules were evaluated for their antimicrobial activity against gram-negative and gram-positive bacterial strains. The results rendered these compounds moderately good inhibitors of all the bacterial strains taken into account. These molecules can be further subjected to in vivo study and so may be considerable by the pharmacists for further process in drug development program.

4. Experimental

4.1. General

All the chemicals employed for synthesis were purchased from Alfa Aesar. All the solvents used were of analytical grade. Reaction progress and product purity was checked by Thin Layer Chromatography (TLC) with different solvent systems using EtOAc and *n*-hexane as mobile phase on aluminum sheets pre-coated with silica gel giving single spot. Visualization of the TLC plates was carried out under G-25-UV_{254}. Melting points of all synthesized compounds were recorded on a Gallon kamp melting point apparatus by open capillary tube and were uncorrected. ^{1}H-NMR spectra were recorded in CD_3OD on Bruker spectrometers (at 300 MHz). The chemical shift values are given in ppm (δ) units and the coupling constants (J) are in Hz. Mass spectra (EIMS) were recorded on JMS-HX-110 spectrometer.

4.2. Procedure for the synthesis of 7-hydroxy-4-methyl-2H-1-benzopyran-2-one (3)

Resorcinol (**1**, 0.2 mol) was taken in a conical flask. Ethyl acetoacetate (**2**, 0.2 mol) was added and the mixture was slightly heated to completely dissolve the compound. Concentrated H_2SO_4 (10 ml) was added to reaction mixture drop by drop with continuous stirring and temperature was maintained at 10°C. The mixture was shaken well and left for overnight. The crushed ice and excess of distilled water was poured into the reaction mixture with continuous stirring. The precipitated product was filtered by using Buchner funnel, washed by distilled water and recrystallized from ethanol [12]. Dark yellow amorphous solid; Yield: 92%; M. P.: 188-190°C; Molecular formula: $C_{10}H_8O_3$; Mol. Weight: 176 gmol^{-1}; IR (KBr, υ_{max}/cm^{-1}): 3550 (O-H), 3418 (Ar C-H), 1714 (ester C=O), 1629 (Ar C=C); ^{1}H-NMR (CD_3OD, 300 MHz): δ/ppm 7.58 (d, J = 8.7 Hz, 1H, H-5), 6.82 (dd, J = 8.7, 2.4 Hz, 1H, H-6), 6.70 (d, J = 2.4 Hz, 1H, H-8), 6.09 (s, 1H, H-3), 2.41 (d, J = 0.9 Hz, 3H, CH_3-11); EIMS (m/z): 176 [M]$^+$ (5%), 159 (19%), 144 (27%), 108 (33%), 51 (100%).

4.3. General procedure for the synthesis of substituted carbamates, 7 and 8

Substituted aliphatic ammonium salts, **5-6** (0.1 mol), were taken in a clean, dried and stoppered 250 mL conical flask containing 10 ml distilled water. The pH of the solution was kept at 9-10 by adding aqueous Na_2CO_3 and was maintained during the whole reaction at Room Temperature (RT). Phenyl chloroformate was added in equimolar ratio along with vigorous shaking. To remove the liberated gases, stopper was removed time by time to avoid explosion. The flask was set to stirring at RT for 1-2 hrs. Product formation was confirmed by TLC (*n*-hexane: EtOAc; 70:30) visualized by UV lamp. The white precipitates were collected by filtration, washed with distilled water and dried to acquire the compounds, **7-8** [11].

Phenyl *N*-(2-chloroethyl)carbamate (**7**): White amorphous solid; Yield: 78%; M. P.: 50-52°C; Molecular formula: $C_9H_{10}ClNO_2$; Mol. Weight: 199.5 gmol^{-1}; IR (KBr, υ_{max}/cm^{-1}): 3062 (Ar C-H), 1680 (amide C=O), 1585 (Ar C=C), 703 (C-Cl); ^{1}H-NMR (CD_3OD, 300 MHz): δ/ppm 7.35 (t, J = 7.8 Hz, 2H, H-3′ & H-5′), 7.19 (t, J = 7.2 Hz, 1H, H-4′), 7.09 (d, J

= 7.8 Hz, 2H, H-2′ & H-6′), 3.64 (t, J = 6.0 Hz, 2H, H-1″), 3.48 (t, J = 6.0 Hz, 2H, H-2″); ^{13}C-NMR (CD_3OD, 75 MHz): δ/ppm 157.2 (C-1′), 153.4 (C-7′), 127.7 (C-3′ & C-5′), 124.8 (C-4′), 121.1 (C-2′ & C-6′), 43.5 (C-1″), 42.9 (C-2″); EIMS (m/z): 201 $[M+2]^+$ (3%), 199 $[M^+]$ (10%), 122 (23%), 93 (100%), 78 (41%), 65 (25), 63 (12%).

Phenyl *N*-(3-bromopropyl)carbamate (**8**): White amorphous solid; Yield: 83%; M. P.: 55-58°C; Molecular formula: $C_{10}H_{12}BrNO_2$; Mol. Weight: 258 gmol^{-1}; IR (KBr, v_{max}/cm^{-1}): 2880 (Ar C-H), 1685 (amide C=O), 1582 (Ar C=C), 671 (C-Br); ^{1}H-NMR (CD_3OD, 300 MHz): δ/ppm 7.43 (t, J = 7.2 Hz, 2H, H-3′ & H-5′), 7.18 (t, J = 7.5 Hz, 1H, H-4′), 7.08 (d, J = 7.8 Hz, 2H, H-2′ & H-6′), 3.50 (t, J = 6.6 Hz, 2H, H-3″), 3.29 (t, J = 6.9 Hz, 2H, H-1″), 2.12-2.04 (m, 2H, H-2″); ^{13}C-NMR (CD_3OD, 75 MHz): δ/ppm 150.3 (C-7′), 149.3 (C-1′), 130.1 (C-3′ & C-5′), 123.6 (C-2′ & C-6′), 117.0 (C-4′), 30.9 (C-1″), 24.3 (C-2″), 21.6 (C-3″); EIMS (m/z): 259 $[M+2]^+$ (6%), 257 $[M^+]$ (6%), 177 (13%), 149 (2%), 137 (14%), 121 (5%), 94 (100%), 65 (23%), 57 (12%).

4.4. General procedure for the bromination of **3**, **7** *and* **8**

Calculated amount of **3**, **7** or **8** (0.1 mol) was completely dissolved in glacial acetic acid (5-10 mL) in a dried Round Bottomed (RB) flask (50 mL) and then liquid bromine was added in equimolar ratio. The reaction mixture was stirred at RT and monitored with TLC (*n*-hexane: EtOAc; 70:30) for the completion of reaction. Distilled water was added to reaction flask to quench the product. Precipitated product was filtered, washed with distilled water and dried [11].

3,6-Dibromo-7-hydroxy-4-methyl-2*H*-1-benzopyran-2-one (**3a**): Bright yellow amorphous solid; Yield: 76%; M.P.: 225-227°C; Molecular formula: $C_{10}H_6Br_2O_3$; Mol. Weight: 334 gmol^{-1}; IR (KBr, v_{max}/cm^{-1}): 2980 (Ar C-H), 1710 (ester C=O), 1600 (Ar C=C), 1200 (C-O), 550 (C-Br); ^{1}H-NMR (CD_3OD, 300 MHz): δ/ppm 8.56 (zs, 1H, H-5), 7.98 (s, 1H, H-8), 2.60 (s, 3H, CH_3-11); ^{13}C-NMR (CD_3OD, 75 MHz): δ/ppm 162.9 (C-2), 153.4 (C-7), 152.9 (C-4), 150.5 (C-9), 132.7 (C-5), 116.9 (C-10), 106.5 (C-6), 106.0 (C-3), 103.4 (C-8), 18.9 (CH_3-11); EIMS (m/z): 336 $[M+4]^+$ (2%), 334 $[M+2]^+$ (2%), 332 $[M^+]$ (2%), 316 (9%), 301 (15%), 222 (30%), 142 (35%), 114 (17%), 51 (100%).

4-Bromophenyl *N*-(2-chloroethyl)carbamate(**7a**): Light yellow amorphous solid; Yield: 81%; M. P.: 57-60°C; Molecular formula: $C_9H_9BrClNO_2$; Mol. Weight: 278.5 gmol^{-1}; IR (KBr, v_{max}/cm^{-1}): 3054 (Ar C-H), 1689 (amide C=O), 1590 (Ar C=C), 701 (C-Cl), 545 (C-Br); ^{1}H-NMR (CD_3OD, 300 MHz): δ/ppm 7.49 (d, J = 8.7 Hz, 2H, H-3′ & H-5′), 7.04 (d, J = 8.7 Hz, 2H, H-2′ & H-6′), 3.63 (t, J = 6.0 Hz, 2H, H-2″), 3.48 (t, J = 6.0 Hz, 2H, H-1″); ^{13}C-NMR (CD_3OD, 75 MHz): δ/ppm 153.2 (C-7′), 150.2 (C-1′), 131.6 (C-3′ & C-5′), 121.9 (C-2′ & C-6′), 118.5 (C-4′), 42.9 (C-1″), 42.0 (C-2″); EIMS (m/z): 281 $[M+4]^+$ (2%), 279 $[M+2]^+$ (7%), 277 $[M^+]$ (5%), 200 (17%), 171 (8%), 92 (100%), 78 (45%).

4-Bromophenyl *N*-(3-bromopropyl)carbamate (**8a**): Off-white amorphous solid; Yield: 85%; M. P.: 65-67°C; Molecular formula: $C_{10}H_{11}Br_2NO_2$; Mol. Weight: 337 gmol^{-1}; IR (KBr, v_{max}/cm^{-1}): 2999 (Ar C-H), 1677 (amide C=O), 1610 (Ar C=C), 560 (C-Br); ^{1}H-NMR (CD_3OD, 300 MHz): δ/ppm 7.35 (d, J = 7.5 Hz, 2H, H-3′ & H-5′), 7.15 (d, J = 7.5 Hz, 2H, H-2′ & H-6′), 3.45 (t, J = 6.6 Hz, 2H, H-3″), 3.21 (t, J = 6.3 Hz, 2H, H-1″), 2.08-2.03 (m, 2H, H-2″); ^{13}C-NMR (CD_3OD, 75 MHz): δ/ppm 150.3 (C-7′), 149.6 (C-1′), 130.8 (C-3′ & C-5′), 123.2 (C-2′ & C-6′), 118.4 (C-4′), 38.9 (C-1″), 32.2 (C-2″), 30.5 (C-3″); EIMS (m/z): 339 $[M+4]^+$ (6%), 337 $[M+2]^+$ (6%), 335 $[M^+]$ (6%), 229 (9%), 200 (21%), 172 (100%), 137 (31%), 122 (12%), 93 (25%), 77 (58%).

4.5. General procedure for the synthesis of O-substituted derivatives of **3** *and* **3a**

Compound **3** or **3a** (0.1 mmol) was taken in a dried RB flask (50 mL). Dimethylformamide (DMF) was added to dissolve it followed by the addition of lithium hydride LiH (0.002 g) to the reaction contents. The mixture was stirred for 45 minutes at Room Temperature (RT) and then equimolar electrophilic reagents **7**, **8** and **7a** (0.1 mmol) were added to the mixture. The reaction contents were further stirred for 3-4 hours. The progress of reaction was monitored by TLC (20% Ethyl acetate: 80% *n*-Hexane). On completion, 1-2 drops of aqueous NaOH were added to reaction mixture. The products were precipitated by adding cold distilled water which were filtered, washed with distilled water and dried [12,13].

Phenyl *N*-[2-(4-methyl-2-oxo-2*H*-1-benzopyran-7-yloxy)ethyl]carbamate (**9**): White amorphous solid; Yield: 73%; M. P.: 67-69°C; Molecular formula: $C_{19}H_{17}NO_5$; Mol. Weight: 339 gmol^{-1}; IR (KBr, v_{max}/cm^{-1}): 2975 (Ar C-H), 1715 (ester C=O), 1672 (amide C=O), 1602 (Ar C=C), 1208 (C-O); ^{1}H-NMR (CD_3OD, 300 MHz): δ/ppm 7.69 (d, J = 9.0 Hz, 1H, H-5), 7.40 (t, J = 9.0 Hz, 2H, H-3′ & H-5′), 7.31 (d, J = 9.3 Hz, 2H, H-2′ & H-6′), 7.24 (t, J = 7.5 Hz, 1H, H-4′), 6.68 (s, 1H, H-8), 6.62 (d, J = 8.7 Hz, 1H, H-6), 6.18 (s, 1H, H-3), 3.52 (t, J = 6.0 Hz, 2H, H-1″), 3.25 (t, J = 6.6 Hz, 2H, H-2″), 2.83 (s, 3H, CH_3-11); ^{13}C-NMR (CD_3OD, 75 MHz): δ/ppm 168.2 (C-2), 160.1 (C-7), 157.2 (C-4), 156.2 (C-1′), 154.2 (C-9), 153.3 (C-7′), 129.2 (C-3′ & C-5′), 126.5 (C-5), 121.4 (C-4′), 115.2 (C-2′ & C-6′), 115.1 (C-10), 112.1 (C-6), 109.3 (C-3), 101.2 (C-8), 65.6 (C-2″), 39.7 (C-1″), 18.7 (CH_3-11); EIMS (m/z): 339 $[M^+]$ (1%), 218 (5%), 176 (71%), 148

(85%), 147 (55%), 120 (15%), 94 (100%), 77 (32%), 65 (43%).

4-Bromophenyl *N*-[2-(4-methyl-2-oxo-2*H*-1-benzopyran-7-yloxy)ethyl]carbamate (**9a**): White amorphous solid; Yield: 75%; M. P.: 72-74°C; Molecular formula: $C_{19}H_{16}BrNO_5$; Mol. Weight: 418 gmol^{-1}; IR (KBr, υ_{max}/cm^{-1}): 3066 (Ar C-H), 1705 (ester C=O), 1695 (amide C=O), 1584 (Ar C=C), 1204 (C-O), 558 (C-Br); ^{1}H-NMR (CD_3OD, 300 MHz): δ/ppm 7.56 (d, J = 9.0 Hz, 2H, H-3′ & H-5′), 7.49 (d, J = 8.7 Hz, 1H, H-5), 7.29 (s, 1H, H-8), 7.24 (d, J = 9.0 Hz, 2H, H-2′ & H-6′), 7.04 (d, J = 8.7 Hz, 1H, H-6), 6.22 (s, 1H, H-3), 3.80 (s, 3H, CH_3-11), 3.63 (t, J = 6.0 Hz, 2H, H-2″), 3.48 (t, J = 6.0 Hz, 2H, H-1″); ^{13}C-NMR (CD_3OD, 75 MHz): δ/ppm 166.9 (C-2), 159.4 (C-7), 157.9 (C-4), 154.5 (C-9), 153.7 (C-7′), 149.2 (C-1′), 130.6 (C-3′ & C-5′), 125.7 (C-5), 122.9 (C-2′ & C-6′), 118.1 (C-4′), 115.6 (C-10), 112.3 (C-6), 109.4 (C-3), 101.4 (C-8), 65.9 (C-2″), 39.4 (C-1″), 18.5 (CH_3-11); EIMS (m/z): 419 [M+2]$^+$ (2%), 417 [M$^+$] (2%), 338 (5%), 218 (16%), 203 (12%), 176 (7%), 163 (11%), 135 (19%), 120 (21%).

Phenyl *N*-[3-(4-methyl-2-oxo-2*H*-1-benzopyran-7-yloxy)propyl]carbamate (**10**): White amorphous solid; Yield: 79%; M. P.: 71-73°C; Molecular formula: $C_{20}H_{19}NO_5$; Mol. Weight: 353 gmol^{-1}; IR (KBr, υ_{max}/cm^{-1}): 2884 (Ar C-H), 1703 (amide C=O), 1692 (ester C=O), 1609 (Ar C=C), 1217 (C-O); ^{1}H-NMR (CD_3OD, 300 MHz): δ/ppm 7.67 (d, J = 8.7 Hz, 1H, H-5), 7.36 (t, J = 9.0 Hz, 2H, H-3′ & H-5′), 7.29 (d, J = 8.7 Hz, 2H, H-2′ & H-6′), 7.22 (t, J = 7.8 Hz, 1H, H-4′), 6.65 (s, 1H, H-8), 6.60 (d, J = 8.4 Hz, 1H, H-6), 6.23 (s, 1H, H-3), 3.76 (t, J = 6.6 Hz, 2H, H-1″), 3.21 (t, J = 6.9 Hz, 2H, H-3″), 2.69 (s, 3H, CH_3-11), 2.14-2.08 (m, 2H, H-2″); ^{13}C-NMR (CD_3OD, 75 MHz): δ/ppm 168.5 (C-2), 160.3 (C-7), 157.6 (C-4), 156.3 (C-1′), 154.5 (C-9), 153.6 (C-7′), 128.8 (C-3′ & C-5′), 126.7 (C-5), 121.2 (C-4′), 115.7 (C-2′ & C-6′), 115.3 (C-10), 112.9 (C-6), 109.1 (C-3), 101.4 (C-8), 65.8 (C-3″), 39.6 (C-1″), 29.3 (C-2″), 18.9 (CH_3-11); EIMS (m/z): 353 [M$^+$] (2%), 218 (5%), 176 (71%), 148 (85%), 147 (55%), 120 (15%), 94 (100%), 77 (32%), 65 (43%).

Phenyl *N*-[3-(3,6-dibromo-4-methyl-2-oxo-2*H*-1-benzopyran-7-yloxy)propyl]carba mate (**11**): White amorphous solid; Yield: 76%; M. P.: 72-75°C; Molecular formula: $C_{20}H_{17}Br_2NO_5$; Mol. Weight: 511 gmol^{-1}; IR (KBr, υ_{max}/cm^{-1}): 2880 (Ar C-H), 1700 (amide C=O), 1698 (ester C=O), 1605 (Ar C=C), 1210 (C-O), 555 (C-Br); ^{1}H-NMR (CD_3OD, 300 MHz): δ/ppm 7.99 (s, 1H, H-5), 7.39 (t, J = 9.6 Hz, 2H, H-3′ & H-5′), 7.24 (d, J = 7.4 Hz, 2H, H-2′ & H-6′), 7.19 (t, J = 7.2 Hz, 1H, H-4′), 6.90 (s, 1H, H-8), 3.98 (t, J = 6.9 Hz, 2H, H-1″), 3.20 (t, J = 6.3 Hz, 2H, H-3″), 2.51 (s, 3H, CH_3-11), 2.02-1.98 (m, 2H, H-2″); ^{13}C-NMR (CD_3OD, 75 MHz): δ/ppm 158.5 (C-7), 156.8 (C-1′), 155.8 (C-2), 153.1 (C-7′), 150.5 (C-9), 146.9 (C-4), 133.8 (C-5), 128.9 (C-3′ & C-5′), 121.5 (C-4′), 114.2 (C-2′ & C-6′), 109.5 (C-10), 107.5 (C-6), 106.0 (C-3), 104.4 (C-8), 69.7 (C-3″), 37.7 (C-1″), 29.0 (C-2″), 19.2 (CH_3-11); EIMS (m/z): 513 [M+4]$^+$ (0.5%), 511 [M+2]$^+$ (0.5%), 509 [M$^+$] (0.5%), 390 (5%), 332 (7%), 178 (18%), 136 (23%), 121 (20%).

4.6. Procedure for the nitration of compound 10

The calculated amount of compound **10** (0.1 mol) was taken in a 50 mL RB flask and 5-10 mL concentrated H_2SO_4 was added to dissolve the compound. The mixture was stirred for 15-20 minutes at RT and then equimolar nitric acid was added to the mixture drop wise at 10°C temperature. The reaction mixture was stirred for 4 hrs and was monitored by TLC (*n*-hexane: EtOAc; 70:30). On reaction completion, ice cold water was added to reaction flask to acquire the precipitates. The precipitated product was collected by filtration followed by washing with distilled water and dried to afford the title compound.

2,4-Dinitrophenyl *N*-[3-(3-nitro-4-methyl-2-oxo-2*H*-1-benzopyran-7-yloxy)propy l] carbamate (**12**): Pale yellow amorphous solid; Yield: 79%; M. P.: 91-93°C; Molecular formula: $C_{20}H_{16}N_4O_{11}$; Mol. Weight: 488 gmol^{-1}; IR (KBr, υ_{max}/cm^{-1}): 3000 (Ar C-H), 1717 (ester C=O), 1694 (amide C=O), 1596 (Ar C=C), 1495 (N=O), 1195 (C-O), 850 (C-N); ^{1}H-NMR (CD_3OD, 300 MHz): δ/ppm 8.94 (d, J = 2.7 Hz, 1H, H-3′), 8.87 (d, J = 3.0 Hz, 1H, H-8), 8.61 (dd, J = 9.3, 2.7 Hz, 1H, H-5′), 8.41 (dd, J = 9.0, 2.7 Hz, 1H, H-6), 7.74 (d, J = 9.0 Hz, 1H, H-5), 7.28 (d, J = 9.3 Hz, H-6′), 3.95 (s, 3H, CH_3-11), 3.50 (t, J = 6.6 Hz, 2H, H-3″), 3.30 (t, J = 6.6 Hz, 2H, H-1″), 2.11-2.05 (m, 2H, H-2″); ^{13}C-NMR (CD_3OD, 75 MHz): δ/ppm 157.4 (C-7), 155.5 (C-9), 154.1 (C-1′), 152.9 (C-2), 149.7 (C-7′), 143.1 (C-4′), 142.8 (C-3), 142.4 (C-4), 135.5 (C-2′), 131.3 (C-5′), 128.5 (C-5), 122.4 (C-3′), 118.5 (C-6′), 114.2 (C-10), 111.3 (C-6), 101.2 (C-8), 72.6 (C-3″), 37.4 (C-1″), 30.4 (C-2″), 18.4 (CH_3-11); EIMS (m/z): 488 [M$^+$] (1%), 442 (2%), 396 (1.5%), 353 (7%), 231 (5%), 217 (11%), 148 (85%), 132 (4%), 119 (17%).

4.7. Antibacterial activity assay

Screening of all the synthesized compounds was carried out against Gram-bacteria for their antibacterial activity. The antibacterial activity was accomplished in sterile 96-wells microplates under aseptic atmospheres. The principle of this technique is that microbial cell number increases as the microbial growth proceeds in a log phase of growth which results in increased absorbance of broth medium [14,15]. Four gram-negative (*Klebsiella pneumonae*, *Escherichia coli*, *Pseudomonas aeroginosa* and *Salmonella typhi*) and two gram-positive bacteria (*Bacillus subtilis* and *Staphylococcus aureus*) were included in the study. The organisms were retained on stock culture agar medium. The

test samples with suitable solvents and dilutions were pipette into wells (20 μg/well). Overnight maintained fresh bacterial culture after suitable dilution with fresh nutrient broth was poured into wells (180 μL). The initial absorbance of the culture was strictly maintained between 0.12-0.19 at 540 nm. The total volume in each well was kept to 200 μL. The incubation was done at 37°C for 16-24 hours with lid on the micro-plate. The absorbance was measured at 540 nm using micro-plate reader, before and after incubation and the difference was noted as an index of bacterial growth. The percent inhibition was calculated using the formula:

$$\text{Inhibition}(\%) = \frac{X - Y}{Y} \times 100,$$

where X is absorbance in control with bacterial culture, and Y is absorbance in test sample.

Results are mean of triplicate ($n = 3$, $\pm$ sem). Ciprofloxacin was taken as reference standard. Minimum Inhibitory Concentration (MIC) was also computed with suitable dilutions (5-30 μg/well) and results were calculated using EZ-Fitz Perrella Scientific Inc. Amherst USA software.

4.7.1. Statistical analysis

All the measurements were done in triplicate and statistical analysis was performed by Microsoft Excel 2010. Results are presented as mean $\pm$ sem.

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