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MEDICINAL CHEMISTRY RESEARCH

ORIGINAL RESEARCH

Quaternary ammonium and amido derivatives of pyranochromenones and chromenones: synthesis and antimicrobial activity evaluation

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Abstract Infectious diseases and the development of their resistance to antimicrobial agents are alarming issues worldwide and consistent efforts are being made to develop efficient antimicrobial agents. In this perspective, a series of novel ammonium and amide derivatives of pyranocoumarin and coumarin were synthesized and evaluated for their antimicrobial activity. Among them, six compounds exhibited significant activity against gram-positive bacteria *Bacillus cereus* (MTCC 430) and *Bacillus subtilis* (MTCC 121) and a gram-negative bacterium *Pseudomonas aeruginosa* (MTCC 741). The compound *N,N,N*-triethyl-10-(4,8,8-trimethyl-2-oxo-2,6,7,8-tetrahydropyrano[3,2-*g*]chromen-10-yloxy)decan-1-aminium bromide (**16**) exhibited the highest antibacterial activity with MIC value of 5 μg/ml in MDA. Compounds **17** and **18** exhibited modest antifungal activity with MIC of 6.25 μg/ml against *Trichophyton rubrum* (clinical isolate) in MDA. Haemolytic assay results demonstrated that three out of six compounds were safe at their respective MIC values. These results provide insights for further optimization of the scaffolds for designing the next generation of compounds as lead antimicrobial agents.

Keywords Antimicrobial activity · Chromen-2-ones · Haemolytic assay · Pyranochromen-2-ones · Quaternary ammonium compounds

Introduction

Infectious diseases, a clinically evident transmissible or communicable diseases caused by a pathogenic biological agent (bacterium, fungus, virus or parasite) that enters the body of a host organism and multiplies, have resulted in an increase in morbidity and mortality, and thus have emerged as a global health issue (Khloya *et al*., 2013). They have plagued humans throughout history and are the second leading cause of death worldwide following heart disease (www.bcm.edu/molvir/id 2013). Though, the development of antimicrobial drugs represent one of the most important advances in therapeutics that has improved the quality of life and advances in many other areas of medicine such as cancer chemotherapy, organ transplantation and major surgery etc., (CDC, 2013), infectious diseases still remain a very serious threat. The development of drugs for the control and eradication of infectious disease poses unique challenges as the very use of potent antibiotics creates newer resistant strains and fosters their spread. The loss of effective antibiotic treatments due to drug resistance has not only crippled the ability to fight routine infectious diseases, but has also vulnerably increased the complications of immunosuppressive therapy such as joint replacements, organ transplants, cancer therapy, asthma and rheumatoid arthritis (Projan and Bradford, 2007). According to the recent studies, a highly potent antimicrobial agent when applied with appropriate dosage regime not only abrogates bacterial growth but also minimises the probability of bacterial resistance formation (Bonde and Gaikwad, 2004).

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Infections by pathogenic fungal species too have been recognised as an emerging challenge despite recent advances in antifungal chemotherapeutic regimen (Andes *et al*., 2009; Saral, 1991; McNeil *et al*., 2001). Amphotericin B has been the drug of choice but at the same time, it suffers from severe drawbacks such as toxicity, immunosuppression and non-availability of an absorbable oral form (Peacock *et al*., 1993; Fischer *et al*., 1989). Also some synthetic antifungals have been reported to induce serious side effects in human beings which limit their use as drugs (Goa and Barradell, 1995; Gearhart, 1994; Lazar and Wilkner, 1990). Further, literature reports reveal the development of resistance in fungi to most available drugs (Guinet *et al*., 1983; Ghannoum and Rice, 1999; Cuenca-Estrella *et al*., 2000). Thus, the continued commitment to the arduous tasks involved in the discovery of novel antimicrobials with activity against resistant strains remains critically important in order to meet a perpetual change.

Quaternary ammonium compounds (QACs), known bioactive substances, have been widely used as antiseptics and disinfectants (Demberelnyamba *et al.*, 2004). These compounds have been reported to possess antibacterial activity along with activity against some pathogenic species of fungi and protozoa (Thorsteinsson *et al.*, 2003). The antimicrobial action of QACs involves perturbation of cytoplasmic and outer membrane lipid bilayers through interaction of the positively charged quaternary nitrogen with the polar head groups of acidic phospholipids and that of hydrophobic tail with the hydrophobic membrane core causing the rearrangement of the membrane and the subsequent leakage of intracellular constituents and ultimately cell death (Ioannou *et al.*, 2007). Earlier, our research group has synthesized ammonium derivatives of chromenones and quinolinones as promising antimicrobial agents (Gupta *et al.*, 2012; Singh *et al.*, 2012). Pyranocoumarins, a widely distributed class of chromen-2-ones across the plant kingdom, contains a pyran scaffold annelated to the benzopyran-2-one moiety. It displays myriad pharmacological activities such as antifungal (Magiatis *et al.*, 1998), anticancer (Magiatis *et al.*, 1998), anti-HIV (McKee *et al.*, 1996), anti-inflammatory (Peng-Jiu *et al.*, 2012), antibacterial (Melliou *et al.*, 2005) and photoactive drugs for skin disorders (Mali *et al.*, 1995). In this context and in an attempt to explore chemical diversity space around pyranocoumarin, we tried to blend the two pharmacophores (pyranocoumarin and QACs) in one unit and screened them for antimicrobial activity.

Coumarins, a pharmacophore of considerable biological importance have been reported to exert notably antibacterial as well as antifungal activity (Muratovic *et al.*, 2013; Appendino *et al.*, 2004). On the other hand, amide derivatives found ubiquitously in nature have become a research hot spot in the development of biologically active molecules because of their high efficiency active features and broad spectrum bioactivities, such as antifungal (Kim *et al.*, 2010; Fu *et al.*, 2010; Kushwaha *et al.*, 2011), antituberculosis, antimicrobial, anticonvulsant, analgesic, anti-inflammatory, insecticidal and antitumor properties (Kushwaha *et al.*, 2011; Cheptea *et al.*, 2013). Taking the cognizance of anticipated antimicrobial activities of coumarins and amides and also in search of novel antimicrobials with higher therapeutic index, we tried to incorporate amide groups in the coumarin scaffold and evaluated them for their antimicrobial efficacy.

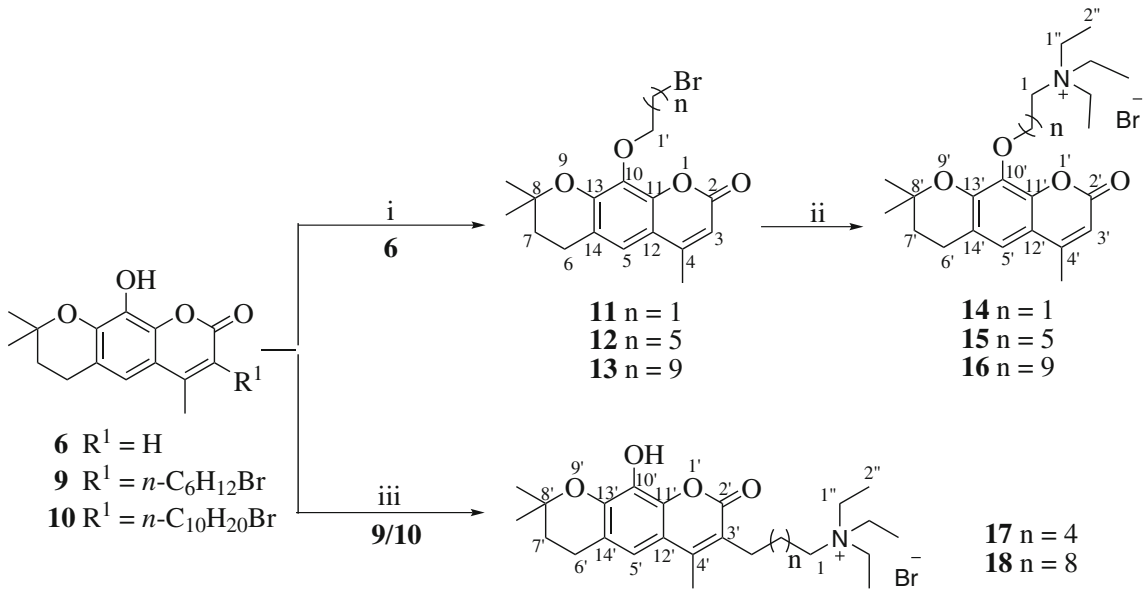
Results and discussion

Chemistry

A series of novel ammonium alkoxy and alkyl derivatives of pyranochromen-2-ones (**14–18**) were synthesized by reaction of corresponding bromo alkoxy and bromo alkyl pyranochromenones (**9–13**) with excess of triethylamine in anhydrous acetonitrile under refluxing conditions (Scheme 1) (Gupta *et al.*, 2012).

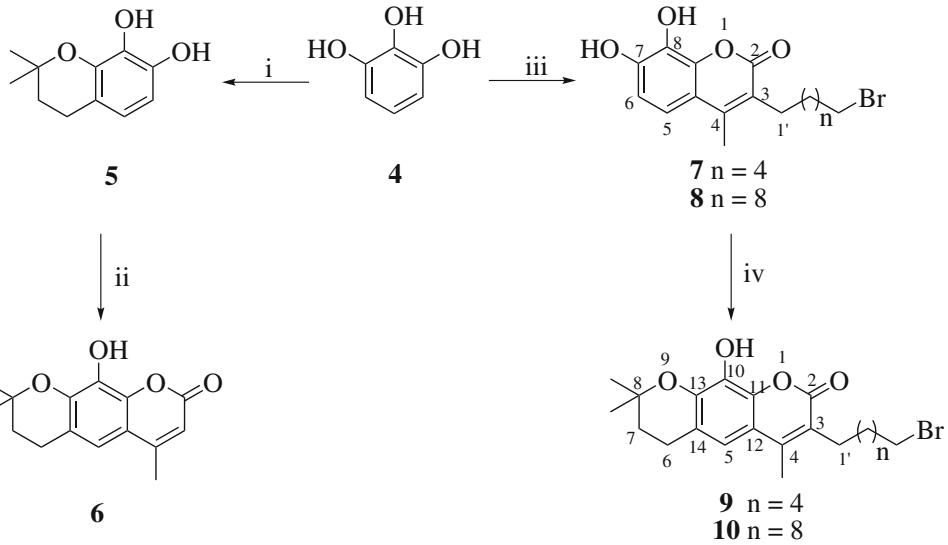
The desired precursors of quaternary ammonium compounds i.e. compounds **9–13** were obtained from 3-(ω-bromoalkyl)-7,8-dihydroxy-4-methyl-chromen-2-ones (**7/8**) and 10-hydroxy-4,8,8-trimethyl-chromen-2-ones (**6**), which in turn were synthesized from pyrogallol (**4**). In the case of bromo alkoxy pyranochromen-2-ones (**11–13**), firstly, pyrogallol (**4**) was made to undergo condensation with 2-methylbuta-l,3-diene (isoprene) in the presence of orthophosphoric acid and using xylene as a solvent to get 2,2-dimethylchroman-7,8-diol (**5**) (Ahluwalia *et al.*, 1982). The self-condensation of isoprene, the chief difficulty encountered while working with dienes, was minimised by slow addition of isoprene to a stirred solution of pyrogallol in xylene and orthophosphoric acid. Condensation of isoprene with phenols catalysed by acids may be regarded as the chemical equivalent of proposed biogenetic pathways (Ahluwalia *et al.*, 1982). 10-hydoxy-4,8,8-trimethyl-7,8-dihydropyrano[3,2-*g*]chromen-2(6*H*)-one (**6**) was synthesized in quantitative yields by Pechmann condensation of **5** with ethyl acetoacetate in the presence of sulphuric acid (Ahluwalia and Jolly 1982). The reaction of terminal dibromoalkanes with **6** yielded the corresponding 10-(ω-bromoalkoxy)-4,8,8-trimethyl-7,8-dihydropyrano[3,2-*g*]chromen-2(6*H*)-ones (**11–13**) using potassium carbonate as base and DMF as solvent (Schemes 1 and 2) (Gupta *et al.*, 2012).

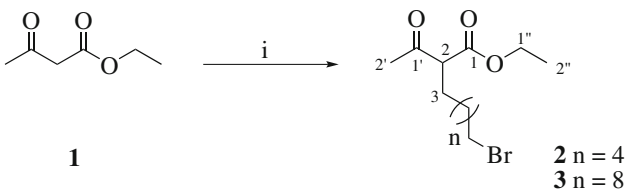
However, in order to form bromo alkyl pyranochromenones (**9/10**), a different protocol was employed wherein pyrogallol (**4**) was initially condensed with ethyl 2-acetyl-



Scheme 1 Synthesis of quaternary ammonium pyranocoumarin derivatives; Reagents and conditions: *i* K_2CO_3, $Br(CH_2)_nBr$, DMF, RT, 15 h; *ii* NEt_3 (5.0 equivalent), CH_3CN, 60 °C, 96 h; *iii* NEt_3 (5.0 equivalent), CH_3CN, 60 °C, 96 h

Scheme 2 Synthesis of pyranocoumarin derivatives; Reagents and conditions: *i* H_3PO_4, isoprene, xylene, RT, 12 h; *ii* Ethyl acetoacetate, conc. H_2SO_4, RT, 6 h; *iii* Ethyl 2-acetyl-ω-bromoalkanoates, conc. H_2SO_4, RT, 6 h; *iv* 2-Methyl-3-buten-2-ol, *p*-TSA, toluene, reflux, 30 h

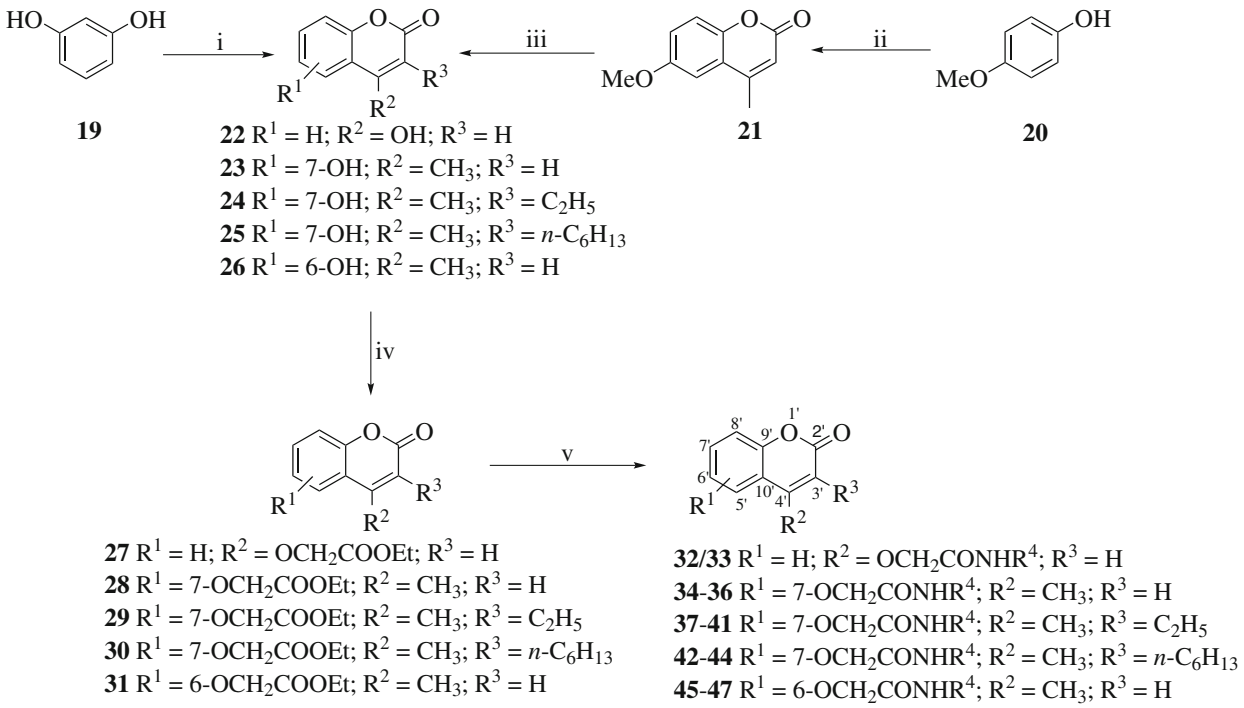


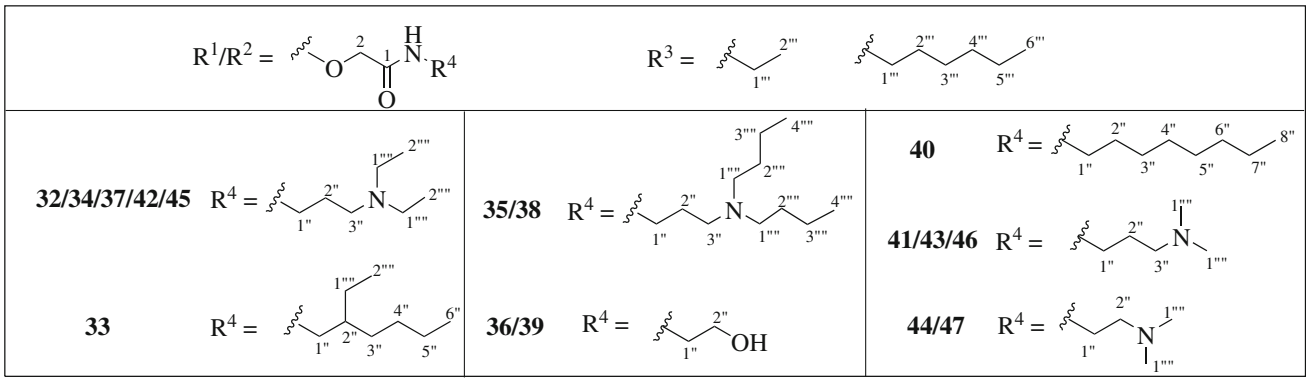


Scheme 3 Synthesis of ethyl 2-acetyl-ω-bromoalkanoates; Reagents and conditions: *i* NaH, $Br(CH_2)_nBr$, THF, reflux, 30 h

ω-bromoalkanoates (**2/3**) under Pechmann reaction conditions to yield 3-(ω-bromoalkyl)-7,8-dihydroxy-4-methylchromen-2-ones (**7/8**) (Scheme 2) (Kathuria *et al.*, 2011). Ethyl 2-acetyl-ω-bromoalkanoates (**2/3**) used in the reaction was in turn synthesized from ethyl acetoacetate (**1**) and dibromoalkanes under reflux conditions (Scheme 3). Nuclear prenylation of **7** and **8** was achieved using 2-methyl-3-buten-2-ol in the presence of *p*-toluene sulphonic acid, which undergoes in situ cyclization to afford corresponding pyranochromenones (**9/10**) (Scheme 2) (Kathuria *et al.*, 2011).

The synthesis of (coumarinyloxy)acetamide derivatives (**32–47**) is outlined in Scheme 4. 7-Hydroxy-4-methylcoumarins (**23–25**) were prepared via Pechmann condensation of resorcinol (**19**) and ethyl acetoacetate as well as alkylated ethyl acetoacetate with drop wise addition of





Scheme 4 Synthesis of (coumarinyloxy)acetamide derivatives; Reagents and conditions: *i* Ethyl 2-alkylacetoacetates, Conc. H_2SO_4, RT, 12 h; *ii* Ethyl acetoacetate, H_2SO_4–C_2H_5OH (7:3), RT, 24 h; *iii* HBr–AcOH (7:3), 110 °C, 24 h; *iv* Ethyl bromoacetate, K_2CO_3, DMF, RT, 12 h; *v* R^5NH_2, *n*-butanol, RT, 48 h

sulphuric acid acting as catalyst (Pechmann and Duisberg, 1883; Kathuria *et al.*, 2009). On the other hand, 4-methoxyphenol (**20**) was used as the starting material in order to obtain 6-hydroxy-4-methyl-2*H*-chromen-2-one (**26**) (Kathuria *et al.*, 2011). Synthesis of alkylated β-ketoester was achieved according to the earlier published procedure from our group (Kathuria *et al.*, 2009). Furthermore, to have positional variations in the synthesized molecules, commercially available 4-hydroxy-2*H*-chromen-2-one (**22**) was used. The key intermediates (**27**–**31**) were obtained from corresponding hydroxycoumarins (**22**–**26**) on treatment with ethyl bromoacetate (Fonseca *et al.*, 2012), which further on reaction with alkylamines, *N*,*N*-dialkylaminoalkanes and ethanolamine and using *n*-butanol as solvent afforded the desired coumarin amides in almost quantitative yields.

The structural characterization of all the synthesized compounds was achieved using various spectroscopic techniques. The structures of known compounds were confirmed by comparison of their melting point and spectral data with those reported in the literature.

Biology

Antimicrobial activity

The antibacterial screening of all the synthesized quaternary ammonium pyranocoumarins (**14**–**18**) and (coumarinyloxy)

Table 1 Zone of inhibition and minimum inhibitory concentration of active compounds **16–18**, **38**, **42**, **43** and their precursors **9**, **10**, **13**, **29**, **30** against pathogenic microbial test strains using gentamicin and amphotericin B as positive controls

Compounds	*B. cereus*		*B. subtilis*		*P. aeruginosa*		*T. rubrum*	
	ZI[a] (mm)	MIC[b] (μg/ml)	ZI[a] (mm)	MIC[b] (μg/ml)	ZI[a] (mm)	MIC[b] (μg/ml)	ZI[a] (mm)	MIC[b] (μg/ml)
9	–[c]	200	–[c]	200	–[c]	200	–[c]	550
10	–[c]	200	–[c]	200	–[c]	200	–[c]	475
13	–[c]	200	–[c]	200	–[c]	200	–[c]	450
16	24	5	24	5	26	5	14	50
17	15	20	14	10	13	40	21	6.25
18	20	5	16	10	18	10	21	6.25
29	–[c]	200	–[c]	200	–[c]	200	–[c]	475
30	–[c]	200	–[c]	200	–[c]	200	–[c]	550
38	16	80	11	100	14	80	12	100
42	19	20	17	20	16	40	11	500
43	15	40	14	40	14	40	11	500

[a] Diameter of zone of inhibition (mm) including disc diameter of 6 mm; Concentration of compound = 250 μg/disc

[b] Minimum inhibitory concentration

[c] No activity at the tested concentration. All the experiments were carried out in triplicate

acetamides (**32–47**) was carried out against a broad range of pathogenic microbial test strains using disc diffusion assay with some modifications (Pathak *et al.*, 2013; Yadav *et al.*, 2014). To evaluate the antimicrobial activities against six pathogenic bacterial strains namely *Bacillus cereus*, *Bacillus subtilis*, *Pseudomonas aeruginosa*, *Escherichia coli*, *Salmonella typhimurium* and *Klebsiella pneumoniae*, disc diffusion assay was performed at a concentration of 250 μg/disc (Table S1 (supporting information)). The screening results revealed that six compounds (**16–18/38/42/43**) exhibited excellent to moderate activity against two gram-positive bacteria (*B. cereus* and *B. subtilis*) and a gram-negative bacterium (*P. aeruginosa*). All of these compounds developed a zone of inhibition equal to or greater than 12 mm, the cut-off value for a molecule to be considered active. However, none of the compounds were found to be active against the other three tested gram-negative bacteria viz. *E. coli*, *S. typhimurium* and *K. pneumoniae*. Quaternary ammonium derivatives were found to possess excellent activity against *B. cereus*, *B. subtilis* and *P. aeruginosa* wherein three out of five quaternary ammonium derivatives inhibited the growth of bacteria up to a diameter of 12 mm or more. *N,N,N*-Triethyl-10-(4,8,8-trimethyl-2-oxo-2,6,7,8-tetrahydropyrano[3,2-*g*]chromen-10-yloxy)decan-1-aminium bromide (**16**) developed the maximum zone of inhibition of 24, 24 and 26 mm against *B. cereus*, *B. subtilis* and *P. aeruginosa*, respectively, followed by compound **18** bearing quaternary ammonium group with a ten carbon spacer at C-3 position of pyranocoumarin. It led to the inhibition of bacterial growth up to a diameter of 20 mm in case of *B. cereus*. On the other hand, a slight decrease in the activity was observed for *P. aeruginosa* and *B. subtilis* wherein the inhibition zone decreased to 18 and 16 mm, respectively. However, on decreasing the carbon chain length from ten to six in case of both the ammonium alkoxy (**15**) and alkyl (**17**) derivatives of pyranochromen-2-ones, a downward trend in the activity profile was observed. Though ammonium alkyl derivative (**17**) showed a downfall of zone of inhibition from 20 to 15 mm, 16 to 14 mm and 18 to 13 mm for *B. cereus*, *B. subtilis* and *P. aeruginosa*, respectively; however, this decrease in the activity for the corresponding alkoxy derivative (**15**) was much more abrupt i.e. from 24 to 11 mm and 26 to 11 mm for *B. cereus* and *P. aeruginosa*, respectively. Furthermore, the activity was completely lost against *B. subtilis* on reducing the size of alkyl chain from ten carbons (**16**) to six carbons (**15**) in case of ammonium alkoxy derivatives (Table 1 and Table S1).

In order to further study the antimicrobial activity of newer derivatives of coumarins, we synthesized a series of novel coumarin derivatives by incorporating acetamido functionality on coumarin skeleton and screened them for their antimicrobial efficacy. From the antibacterial activity screening results, it could be established that the substitution at C-3 position of coumarin with long alkyl chain along with the use of longer *N,N*-diaminoalkanes for amidation was important for a molecule to possess activity against pathogenic bacteria. This fact was substantiated by the observation that compounds **38**, **42** and **43** led to the inhibition of growth of *B. cereus*; *B. subtilis*; *P. aeruginosa* by creating a zone of 16, 19 and 15 mm; 11, 17 and 14 mm; 14, 16 and 14 mm, respectively (Table 1 and Table S1, supporting information).

The precursors (**9/10/13/29/30**) of the shortlisted active molecules (**16–18/38/42/43**) were also screened against the bacterial strains *B. cereus*, *B. subtilis* and *P. aeruginosa*. However, none of the precursors were found to be active against the bacterial pathogens at the tested concentration (250 μg/disc). In order to have further insights on the antimicrobial action of these compounds, the antifungal activity screening was carried out using *Trichophyton rubrum* as a model pathogen. Compounds **17** and **18** bearing quaternary ammonium group at C-3 position of the pyran ring and a hydroxyl group at C-10 position exhibited excellent antifungal activity by inhibiting the growth of the fungus up to a diameter of 21 mm. Also compound **38** having an ethyl substituent at C-3 position of the pyran ring had a zone of inhibition (13 mm) higher than that for compounds **42** and **43** (11 mm) bearing a hexyl chain at the C-3 position of the pyran ring. Thus, it could be concluded that in contrast to antibacterial activity results, the size of alkyl chain is not an important criteria for a molecule to exhibit activity against pathogenic fungi. However, presence of hydroxyl group along with quaternary ammonium functionality has been found to be critical for a compound to possess significant antifungal activity (Table 1).

Furthermore, in order to have an in-depth understanding of factors that affect the growth inhibition process of various bacterial strains and fungi, minimum inhibitory concentration i.e. MIC was evaluated using microbroth dilution assay (Gupta *et al.*, 2012; Pathak *et al.*, 2013; Yadav *et al.*, 2014). Table 1 shows the MIC values of the active compounds along with their precursors. Compound **16** which developed the maximum zone of inhibition against *Bacillus cereus*, *Bacillus subtilis* and *Pseudomonas aeruginosa* inhibited the growth of these pathogenic bacteria at a concentration of 5 μg/ml. *N*,*N*,*N*-Triethyl-10-(10-hydroxy-4,8,8-trimethyl-2-oxo-2,6,7,8-tetrahydropyrano[3,2-*g*]chromen-3-yl)decan-1-aminium bromide (**18**) exhibited MIC of 5 μg/ml against *B. cereus*, 10 μg/ml against *B. subtilis* and *P. aeruginosa*. However, on decreasing the size of alkyl chain from ten carbons (**18**) to six carbons (**17**), MIC value increased and a concentration of 20, 10 and 40 μg/ml was sufficient to inhibit the growth of *B. cereus*, *B. subtilis* and *P. aeruginosa*, respectively.

In the case of (coumarinyloxy)acetamides (**38/42/43**), compound **42** bearing a hexyl chain and *N,N*-diethylamino group was found to possess the lowest MIC value of 20, 20 and 40 μg/ml against *B. cereus*, *B. subtilis* and *P. aeruginosa*, respectively. Antifungal activity results revealed that the compounds **17** and **18** (bearing hydroxyl groups), which developed the maximum zone of inhibition had the lowest MIC value of 6.25 μg/ml amongst the various tested compounds. Furthermore, the precursors of all the shortlisted active compounds exhibited an MIC value much higher than that of the active compounds itself (Table 1).

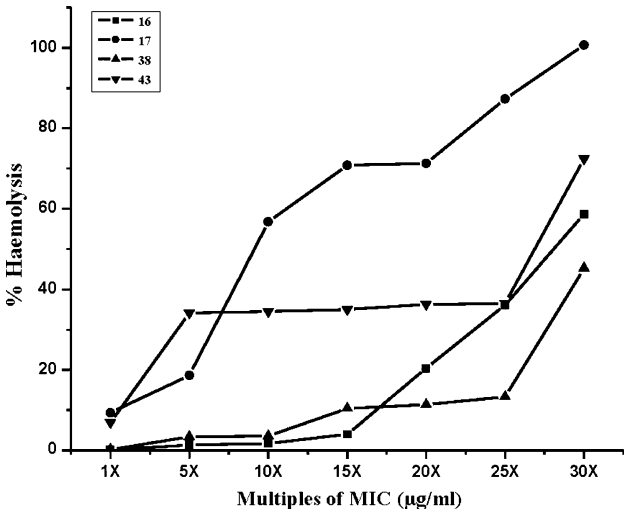


Fig. 1 Haemolytic assay of compounds **16**, **17**, **38** and **43** against human erythrocytes

Haemolytic study

In order to shed light on toxicity, the promising compounds (**16–18/38/42/43**) were investigated for the viability of human erythrocytes by haemolytic assay (Ciornei *et al.*, 2005; Dobrovolskaia *et al.*, 2008) at various multiples of their MIC values. Compound **16**, the most potent antibacterial compound was found to be non-haemolytic with marginal toxicity of 20 % even up to concentration 20 times of its MIC value (5 μg/ml, the MIC in case of bacterial pathogens). Also it could be established that **16** was non-toxic at the MIC for the tested fungal strain *T. rubrum*. However, the least active antibacterial compound (**38**) was found to be much safer as it exhibited 13 and 11 % haemolysis at 25 X MIC, the MIC for *B. subtilis*, *T. rubrum* and *B. cereus*, *P. aeruginosa*, respectively. The haemolysis results for compound **17** provided a vivid picture that the human RBCs were negligibly lysed at various MICs of **17**. RBCs were lysed by less than 18 % at the MIC for *P. aeruginosa*, while a lysis of 9 % and less was observed at the MIC for *B. cereus*, *B. subtilis* and *T. rubrum*. In addition to this, *N*-(3-(dimethylamino)propyl)-2-((3-hexyl-4-methyl-2-oxo-2*H*-chromen-7-yl)oxy) acetamide (**43**) exhibited haemolysis of 7 % at the MICs for pathogenic bacterial strains, while 34–35 % cells were lysed at the MIC for *T. rubrum*. Compound **18** was found to be the most toxic to the erythrocytes. It caused 80 % lysis of the cells at its MIC for *B. subtilis* and *P. aeruginosa*. On the other hand, 42 % cells were lysed at the MIC for

P. aeruginosa in case of compound **42** (Fig. 1 and Table S2 (supporting information)).

Conclusions

In summary, a total of twenty one compounds including five quaternary ammonium derivatives and sixteen (coumarinyloxy)acetamides were synthesized and fully characterized by ^{1}H NMR, ^{13}C NMR, UV, FTIR and high resolution mass spectroscopy (HRMS). Of these, twenty compounds i.e. **14–18**, **32–35** and **37–47** are novel. Although compound **36** is known in literature (Wang *et al.*, 2011), its complete spectral data were not reported. Herein, we have reported the spectral data for all the compounds in the experimental section. All of the compounds synthesized were evaluated for their antibacterial activity against both gram-positive (*B. cereus* and *B. subtilis*) and gram-negative bacteria (*P. aeruginosa*, *E. coli*, *S. typhimurium* and *K. pneumoniae*). The antibacterial activity results showed that six compounds (**16–18/38/42/43**) exhibited significant activity against both, the gram-positive bacteria *B. cereus* and *B. subtilis* and a gram-negative bacterium *P. aeruginosa*. These compounds were further screened against a fungal strain *T. rubrum* along with the investigation of their MIC values. Out of the various compounds screened, compound **16**, a C_{10} alkyl chain containing quaternary ammonium derivative was found to be the most potent against bacterial growth with an MIC value of 5 μg/ml, while compounds **17** and **18** (quaternary ammonium compounds with hydroxyl groups) exhibited modest anti-*Trichopyhton* activity (MIC: 6.25 μg/ml). The haemolytic assay results revealed that out of six compounds i.e. **16–18/38/42/43** shortlisted for potent antibacterial activity, three compounds (**16/17/38**) were found to be reasonably safe at their respective MIC values. Compound **43** too had negligible toxicity at the MIC for the bacterial strains and a lysis of 34–35 % was observed at the MIC for the fungus. These results can be used for the design and synthesis of newer pharmacophores with improved antimicrobial activity.

Experimental section

Chemistry

Materials and methods

The organic solvents were dried and distilled prior to their use. Reactions were monitored by precoated TLC plates (Merck silica gel 60F_{254}); the spots were visualized either by UV light or by spraying with 5 % alcoholic $FeCl_3$ solution. Silica gel (100–200 mesh) was used for column chromatography. All of the chemicals and reagents were procured from Spectrochem Pvt. Ltd., India and Sigma-Aldrich Chemicals Pvt. Ltd., USA. Melting points were measured on a Buchi M-560 equipment and are uncorrected. Infrared spectra were recorded on Perkin-Elmer SPECTRUM BX FTIR and SPECTRUM XIFT IR spectrophotometer. The ^{1}H and ^{13}C NMR spectra were recorded on Jeol-400 (400 MHz, 100.5 MHz) NMR spectrometer using tetramethylsilane as internal standard. The chemical shift values are on a δ scale and the coupling constant values (*J*) are in Hertz. The UV data were recorded on Cary 300 UV–Vis spectrophotometer, Agilent Technologies. The HRMS data were recorded on Waters (Micromass) LCT 1 using ESI TOF MS technique.

*General procedure for the synthesis of ammonium alkoxy derivatives of pyranochromen-2-ones (**14–16**)*

To a stirred solution of 10-hydroxy-4,8,8-trimethyl-7,8-dihydropyrano[3,2-*g*]chromen-2(6*H*)-one (**6**) (1 g, 3.84 mmol) in anhydrous DMF (50 ml), fused potassium carbonate (2.2 equivalent) was added and the solution stirred at room temperature for 30 min followed by slow addition of 1,2-dibromoethane/1,6-dibromohexane/1,10-dibromodecane (1.1 equivalent). The resulting reaction mixture was stirred at room temperature for 24 h. The progress of the reaction was monitored on TLC. On completion of the reaction, the solvent was removed under reduced pressure and the solid so obtained was dissolved in chloroform (50 ml). The chloroform layer was washed with water (2 × 30 ml) and then dried over anhydrous sodium sulphate. The solvent was then removed *in vacuo* in a rotary evaporator and the resulting crude product subjected to column chromatography to give corresponding bromo alkoxy derivative of pyranochromen-2-one (**11–13**). The bromo compound (**11–13**) was then converted to quaternary ammonium derivative (**14–16**) by dissolving it in acetonitrile (1 g in 50 ml) followed by the addition of triethylamine (5 equivalent) and stirring the reaction mixture under nitrogen at 60 °C for 96 h. The progress of reaction was monitored using TLC. After completion of the reaction, the solvent was evaporated under reduced pressure and the resultant product was then subjected to column chromatography to give the pure quaternary ammonium compound (**14–16**).

*10-(2-Bromoethoxy)-4,8,8-trimethyl-7,8-dihydropyrano[3,2-g]chromen-2(6H)-one (**11**)* The reaction of 10-hydroxy-4,8,8-tr imethyl-7,8-dihydropyrano[3,2-*g*]chromen-2(6*H*)-one (**6**) (1 g, 3.84 mmol) with 1,2-dibromoethane (0.79 g,

4.22 mmol) gave the title compound **11** as a light brown solid (1.27 g, 90 %) by following the general procedure; m.p.: 117.9–118.7 °C; R_f: 0.30 (ethyl acetate:petroleum ether: 3:7); UV (MeOH) λ_{max}: 259 and 327 nm; IR (KBr) ν_{max}: 2976.44, 2944.92, 1716.10 (C=O), 1609.35, 1568.01, 1395.70, 1105.82, 1056.20, 843.74, 754.67, 665.33 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): δ 1.41 (s, 6H, 2× C-8 CH_3), 1.86 (t, 2H, $J = 6.59$ Hz, H-7), 2.36 (s, 3H, C-4 CH_3), 2.85 (t, 2H, $J = 6.59$ Hz, H-6), 3.65 (t, 2H, $J = 7.32$ Hz, H-2′), 4.36 (t, 2H, $J = 7.32$ Hz, H-1′), 6.11 (s, 1H, H-3), 7.06 (s, 1H, H-5) ppm; ^{13}C NMR (100.5 MHz $CDCl_3$): δ 18.94 (C-4 *C*H_3), 22.49 (C-6), 27.07 (2× C-8 *C*H_3), 29.71 (C-2′), 32.54 (C-7), 73.13 (C-1′), 76.28 (C-8), 112.24 (C-3), 113.41 (C-12), 118.62 (C-14), 119.70 (C-5), 134.10 (C-10), 146.25 (C-11), 150.62 (C-13), 152.69 (C-4), 160.99 (C-2) ppm; HRMS: *m/z* $[M + Na]^+$ Calculated for $C_{17}H_{19}BrO_4$: 389.0364; found: 389.0373.

*10-((6-Bromohexyl)oxy)-4,8,8-trimethyl-7,8-dihydropyrano[3,2-g]chromen-2(6H)-one (**12**)* The reaction of 10-hydroxy-4,8,8-trimethyl-7,8-dihydropyrano[3,2-*g*]chromen-2(6*H*)-one (**6**) (1 g, 3.84 mmol) with 1,6-dibromohexane (1.03 g, 4.22 mmol) gave the title compound **12** as a light brown solid (1.44 g, 89 %) by following the general procedure; m. p.: 61.0–61.9 °C; R_f: 0.40 (ethyl acetate: petroleum ether: 3:7); UV (MeOH) λ_{max}: 260 and 329 nm; IR (KBr) ν_{max}: 2974.00, 2935.02, 1724.94 (C=O), 1610.76, 1568.49, 1399.08, 1109.93, 1057.34, 865.80, 771.70, 709.02 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): δ 1.39 (s, 6H, 2× C-8 CH_3), 1.47–1.65 (m, 4H, H-3′ and H-4′), 1.75–1.93 (m, 6H, H-2′, H-5′ and H-7), 2.35 (s, 3H, C-4 CH_3), 2.84 (t, 2H, $J = 6.59$ Hz, H-6), 3.43 (t, 2H, $J = 6.59$ Hz, H-6′), 4.06 (t, 2H, $J = 6.59$ Hz, H-1′), 6.08 (s, 1H, H-3), 7.02 (s, 1H, H-5) ppm; ^{13}C NMR (100.5 MHz $CDCl_3$): δ 18.90 (C-4 *C*H_3), 22.50 (C-6), 25.33 (C-3′/C-4′), 27.06 (2× C-8 *C*H_3), 28.10 (C-3′/C-4′), 30.06 (C-2′), 32.54 (C-5′), 32.94 (C-7), 34.22 (C-6′), 73.72 (C-1′), 75.86 (C-8), 112.08 (C-3), 113.39 (C-12), 118.51 (C-14), 119.06 (C-5), 134.97 (C-10), 146.48 (C-11), 150.87 (C-13), 152.73 (C-4), 161.21 (C-2) ppm; HRMS: *m/z* $[M + Na]^+$ Calculated for $C_{21}H_{27}BrO_4$: 445.0990; found: 445.1006.

*10-((10-Bromodecyl)oxy)-4,8,8-trimethyl-7,8-dihydropyrano[3,2-g]chromen-2(6H)-one (**13**)* The reaction of 10-hydroxy-4,8,8-trimethyl-7,8-dihydropyrano[3,2-*g*]chromen-2(6*H*)-one (**6**) (1 g, 3.84 mmol) with 1,10-dibromodecane (1.26 g, 4.22 mmol) gave the title compound **13** as a dark brown solid (1.65 g, 90 %) by following the general procedure; m. p.: 45.5–46.0 °C; R_f: 0.45 (ethyl acetate: petroleum ether: 3:7); UV (MeOH) λ_{max}: 260 and 328 nm; IR (KBr) ν_{max}: 2928.31, 2854.32, 1725.14 (C=O), 1610.18, 1569.04, 1398.99, 1109.01, 1058.51, 865.10, 771.51, 709.74 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): δ 1.24–1.43 (m, 16H, H-4′–H-8′ and 2× C-8 CH_3), 1.46–1.55 (m, 2H, H-3′), 1.73–1.88 (m, 6H, H-7, H-2′ and H-9′), 2.35 (s, 3H, C-4 CH_3), 2.84 (t, 2H, $J = 6.59$ Hz, H-6), 3.40 (t, 2H, $J = 6.59$ Hz, H-10′), 4.06 (t, 2H, $J = 6.59$ Hz, H-1′), 6.09 (s, 1H, H-3), 7.02 (s, 1H, H-5) ppm; ^{13}C NMR (100.5 MHz $CDCl_3$): δ 18.93 (C-4 *C*H_3), 22.55 (C-6), 27.08 (2× C-8 *C*H_3), 26.12, 28.34, 28.93, 29.57, 29.68, 30.28, 32.58 (C-2′–C-9′), 33.01 (C-7), 34.28 (C-10′), 74.12 (C-1′), 75.82 (C-8), 112.13 (C-3), 113.42 (C-12), 118.45 (C-14), 118.97 (C-5), 135.12 (C-10), 146.56 (C-11), 150.94 (C-13), 152.68 (C-4), 161.29 (C-2) ppm; HRMS: *m/z* $[M + Na]^+$ Calculated for $C_{25}H_{35}BrO_4$: 501.1617; found: 501.1604.

*N,N,N-Triethyl-2-((4,8,8-trimethyl-2-oxo-2,6,7,8-tetrahydropyrano[3,2-g]chromen-10-yl)oxy)ethanaminium bromide (**14**)* The reaction of 10-(2-bromoethoxy)-4,8,8-trimethyl-7,8-dihydropyrano[3,2-*g*]chromen-2(6*H*)-one (**11**) (1 g, 2.72 mmol) with triethylamine (1.18 g, 13.60 mmol) gave the title compound **14** as an off-white solid (0.89 g, 70 %) by following the general procedure; m. p.: 193.8–195.3 °C; R_f: 0.58 ($MeOH:CHCl_3$: 1:4); UV (MeOH) λ_{max}: 258 and 327 nm; IR (KBr) ν_{max}: 3410.62, 2973.07, 2926.76, 1720.42 (C=O), 1610.65, 1569.44, 1388.62, 1367.85, 1105.26, 1061.05, 999.46, 893.14, 834.17, 782.60, 583.60 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): δ 1.38 (s, 6H, 2× C-8′ CH_3), 1.45 (t, 9H, $J = 6.59$ Hz, H-2″), 1.84 (t, 2H, $J = 6.59$ Hz, H-7′), 2.35 (s, 3H, C-4′ CH_3), 2.82 (t, 2H, $J = 6.59$ Hz, H-6′), 3.77 (q, 6H, $J = 7.32$ Hz, H-1″), 4.15 (t, 2H, $J = 4.39$ Hz, H-1), 4.40 (t, 2H, $J = 4.39$ Hz, H-2), 6.06 (s, 1H, H-3′), 7.09 (s, 1H, H-5′) ppm; ^{13}C NMR (100.5 MHz $CDCl_3$): δ 8.44 (C-2″), 18.93 (C-4′ *C*H_3), 22.29 (C-6′), 27.10 (2× C-8′ *C*H_3), 32.31 (C-7′), 54.57 (C-1″), 57.48 (C-1), 67.03 (C-2), 77.40 (C-8′), 111.91 (C-3′), 113.32 (C-12′), 119.14 (C-14′), 120.65 (C-5′), 132.92 (C-10′), 145.91 (C-11′), 150.64 (C-13′), 153.19 (C-4′), 160.43 (C-2′) ppm; HRMS: *m/z* $[M]^+$ Calculated for $C_{23}H_{34}NO_4^+$: 388.2488; found: 388.2478.

*N,N,N-Triethyl-6-((4,8,8-trimethyl-2-oxo-2,6,7,8-tetrahydropyrano[3,2-g]chromen-10-yl)oxy)hexan-1-aminium bromide (**15**)* The reaction of 10-((6-bromohexyl)oxy)-4,8,8-trimethyl-7,8-dihydropyrano[3,2-*g*]chromen-2(6*H*)-one (**12**) (1 g, 2.36 mmol) with triethylamine (1.20 g, 11.80 mmol) gave the title compound **15** as an off-white solid (0.93 g, 75 %) by following the general procedure; m. p.: 148.0–149.3 °C; R_f: 0.58 ($MeOH:CHCl_3$: 1:4); UV (MeOH) λ_{max}: 259 and 329 nm; IR (KBr) ν_{max}: 3468.46, 3407.60, 2930.43, 2919.42, 1722.35 (C=O), 1607.89, 1452.32, 1395.06, 1370.45, 1107.23, 1061.77, 926.06, 865.70, 847.21, 564.86 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): δ 1.32–1.42 (m, 15H, 2× C-8′ CH_3 and 3× H-2″), 1.52–1.61

(m, 2H, H-3), 1.70–1.88 (m, 8H, H-2, H-4, H-5 and H-7′), 2.36 (s, 3H, C-4′ CH_3), 2.84 (t, 2H, $J = 6.59$ Hz, H-6′), 3.32–3.39 (m, 2H, H-1), 3.49 (q, 6H, $J = 7.32$ Hz, 3× H-1″), 4.02–4.06 (m, 2H, H-6), 6.03 (s, 1H, H-3′), 7.04 (s, 1H, H-5′) ppm; ^{13}C NMR (100.5 MHz $CDCl_3$): δ 8.10 (C-2″), 18.93 (C-4′ *C*H_3), 22.03, 22.43, 25.51, 25.77, 29.67 (C-2–C-5 and C-6′), 27.02 (2× C-8′ *C*H_3), 32.45 (C-7′), 53.49 (C-1″), 57.42 (C-1), 73.10 (C-6), 76.07 (C-8′), 111.69 (C-3′), 113.29 (C-12′), 118.82 (C-14′), 119.31 (C-5′), 134.75 (C-10′), 146.21 (C-11′), 150.97 (C-13′), 153.31 (C-4′), 161.31 (C-2′) ppm; HRMS: *m/z* $[M]^+$ Calculated for $C_{27}H_{42}NO_4^+$: 444.3114; found: 444.3115.

*N,N,N-Triethyl-10-((4,8,8-trimethyl-2-oxo-2,6,7,8-tetrahydropyrano[3,2-g]chromen-10-yl)oxy)decan-1-aminium bromide (**16**)* The reaction of 10-((10-bromodecyl)oxy)-4,8,8-trimethyl-7,8-dihydropyrano[3,2-*g*]chromen-2(6*H*)-one **(13)** (1 g, 2.09 mmol) with triethylamine (1.06 g, 10.45 mmol) gave the title compound **16** as a light brown low melting solid (0.91 g, 75 %) by following the general procedure; R_f: 0.41 (MeOH:$CHCl_3$: 1:4); UV (MeOH) λ_{max}: 260 and 328 nm; IR (KBr) ν_{max}: 3412.46, 2933.91, 1718.29 (C=O), 1609.88, 1568.95, 1399.37, 1108.57, 1054.36, 937.64, 887.63, 792.37, 585.05 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.30–1.43 (m, 23H, H-4–H-7, 2× C-8′ CH_3 and 3× H-2″), 1.46–1.57 (m, 2H, H-3), 1.67–1.87 (m, 8H, H-2, H-8, H-9 and H-7′), 2.36 (s, 3H, C-4′ CH_3), 2.84 (t, 2H, $J = 6.59$ Hz, H-6′), 3.21–3.28 (m, 2H, H-1), 3.50 (q, 6H, $J = 7.32$ Hz, 3× H-1″), 4.05 (t, 2H, $J = 6.59$ Hz, H-10′), 6.07 (s, 1H, H-3′), 7.03 (s, 1H, H-5′) ppm; ^{13}C NMR (100.5 MHz $CDCl_3$): 8.18 (C-2″), 18.93 (C-4′ *C*H_3), 27.03 (2× C-8′ *C*H_3), 22.01, 22.47, 25.93, 26.42, 29.04, 29.12, 29.28, 29.30, 30.17 (C-2–C-9 and C-6′), 32.48 (C-7′), 53.60 (C-1″), 57.64 (C-1), 73.97 (C-10), 75.92 (C-8′), 111.87 (C-3′), 113.32 (C-12′), 118.62 (C-14′), 119.11 (C-5′), 134.93 (C-10′), 146.38 (C-11′), 150.92 (C-13′), 153.03 (C-4′), 161.36 (C-2′) ppm; HRMS: *m/z* $[M]^+$ Calculated for $C_{31}H_{50}NO_4^+$: 500.3740; found: 500.3753.

*General procedure for the synthesis of ammonium alkyl derivatives of pyranochromen-2-ones (**17/18**)*

*Synthesis of ethyl 2-acetyl-ω-bromoalkanoates (**2/3**)* Sodium hydride (1.7 equivalent) was added to a cold solution (0 °C) of ethyl 3-oxobutanoate (**1**) (5 g, 38.42 mmol) in THF (15 ml) and the reaction mixture was heated at 60 °C for 2 h. The contents of the flask were then allowed to attain room temperature, further, addition of 1,6-dibromohexane/1,10-dibromodecane (1.1 equivalent) dissolved in THF was carried out under ice cold conditions. The resultant mixture thus obtained was heated at 80 °C for 30 h. The reaction was monitored by TLC (10 % ethyl acetate–petroleum ether). On completion of the reaction, ethyl acetate was added and the solution was then filtered. The filtrate was washed with water, the organic layer dried over anhydrous sodium sulphate and the solvent evaporated. The crude product so obtained was subjected to column chromatography to get the desired product (**2/3**) using ethyl acetate–petroleum ether (1:49) as eluent.

*Ethyl 2-acetyl-8-bromooctanoate (**2**)* The reaction of ethyl 3-oxobutanoate (**1**) (5 g, 38.42 mmol) with 1,6-dibromohexane (10.31 g, 42.26 mmol) gave the title compound **2** as a pale yellow coloured oil (6.19 g, 55 %) by following the general procedure; R_f: 0.40 (ethyl acetate: petroleum ether: 1:9); IR (KBr) ν_{max}: 2927.81, 2855.37, 1741.54 (*CO*O–), 1717.59 (C=O), 1465.15, 1357.82, 1241.75, 1150.40, 1025.29, 859.31, 722.35, 644.01 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.21–1.43 (m, 9H, H-4–H-6 and H-2″), 1.73–1.85 (m, 4H, H-3 and H-7), 2.18 (s, 3H, H-2′), 3.36 (t, 3H, $J = 6.96$ Hz, H-2 and H-8), 4.16 (q, 2H, $J = 7.32$ Hz, H-1″) ppm; ^{13}C NMR (100.5 MHz $CDCl_3$): δ 14.24 (C-2″), 27.30, 27.94, 28.11, 28.58, 28.90, 32.72 (C-3–C-7 and C-2′), 33.91 (C-8), 59.93 (C-2), 61.44 (C-1″), 169.97 (C-1), 203.32 (C-1′) ppm.

*Ethyl 2-acetyl-12-bromododecanoate (**3**)* The reaction of ethyl 3-oxobutanoate (**1**) (5 g, 38.42 mmol) with 1,10-dibromodecane (12.68 g, 42.26 mmol) gave the title compound **3** as a pale yellow coloured oil (8.05 g, 60 %) by following the general procedure; R_f: 0.45 (ethyl acetate: petroleum ether: 1:9); UV (MeOH) λ_{max}: 259 nm; IR (KBr) ν_{max}: 2927.70, 2855.22, 1742.93 (*CO*O–), 1717.80 (C=O), 1459.09, 1358.00, 1241.22, 1150.28, 1026.33, 858.78, 772.26, 643.73 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.20–1.30 (m, 15H, H-5–H-10 and H-2″), 1.35-1.43 (m, 2H, H-4), 1.78–1.87 (m, 4H, H-3 and H-11), 2.20 (s, 3H, H-2′), 3.34–3.41 (m, 3H, H-2 and H-12), 4.18 (q, 2H, $J = 7.32$ Hz, H-1″) ppm; ^{13}C NMR (100.5 MHz $CDCl_3$): δ 14.25 (C-2″), 27.50, 28.28, 28.32, 28.85, 29.37, 29.43, 29.48, 32.95 (C-3–C-11 and C-2′), 34.13 (C-12), 60.08 (C-2), 61.39 (C-1″), 170.08 (C-1), 203.47 (C-1′) ppm; HRMS: *m/z* $[M + Na]^+$ Calculated for $C_{16}H_{29}BrO_3$: 371.1198; found: 371.1198.

*Synthesis of 3-(ω-bromoalkyl)-7,8-dihydroxy-4-methyl-2H-chromen-2-ones (**7/8**)* A mixture of pyrogallol (**4**) (3 g, 23.78 mmol) and **2/3** (1.1 equivalent) was taken in a round bottom flask and the mixture was cooled to 0 °C. Conc. H_2SO_4 (10 ml) was then added dropwise and the temperature of the reaction mixture was maintained at 0 °C. The reaction mixture was then stirred at room temperature for 6 h. On completion of the reaction, the contents of the flask

were poured over ice. The solid so obtained (**7/8**) was filtered, washed with water, dried under vacuum and purified by column chromatography.

*3-(6-Bromohexyl)-7,8-dihydroxy-4-methyl-2H-chromen-2-one (**7**)* The reaction of pyrogallol (**4**) (3 g, 23.78 mmol) with ethyl 2-acetyl-8-bromooctanoate (**2**) (7.67 g, 26. 16 mmol) gave the title compound **7** as a white solid (6. 59 g, 78 %) by following the general procedure; m. p.: 138.0–138.7 °C; R_f: 0.30 (MeOH:$CHCl_3$: 1:19); UV (MeOH) λ_{max}: 262 and 323 nm; IR (KBr) ν_{max}: 3422.44 (OH), 2929.20, 1671.08 (C=O), 1610.19, 1583.60, 1379. 83, 1306.96, 1090.04, 1053.73, 955.29, 814.43, 776. 52 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.38–1.58 (m, 6H, H-2′–H-4′), 1.82-1.91 (m, 2H, H-5′), 2.38 (s, 3H, C-4 CH_3), 2.63 (t, 2H, $J = 7.63$ Hz, H-1′), 3.40 (t, 2H, $J = 7.$ 02 Hz, H-6′), 6.90 (d, 1H, $J = 8.54$ Hz, H-6), 7.09 (d, 1H, $J = 8.54$ Hz, H-5) ppm; ^{13}C NMR (100.5 MHz $CDCl_3$): δ 15.21 (C-4 *C*H_3), 27.61, 28.17, 28.74, 28.94, 29.57, 29.61, 29.80 (C-1′–C-4′), 32.90 (C-5′), 34.08 (C-6′), 112.03 (C-6), 114.47 (C-10), 116.28 (C-5), 123.08 (C-3), 130.37 (C-8), 141.01 (C-4), 146.52 (C-7), 147.75 (C-9), 161.70 (C-2) ppm; HRMS: *m/z* $[M + Na]^+$ Calculated for $C_{16}H_{19}BrO_4$: 377.0364; found: 377.0371.

*3-(10-Bromodecyl)-7,8-dihydroxy-4-methyl-2H-chromen-2-one (**8**)* The reaction of pyrogallol (**4**) (3 g, 23. 78 mmol) with ethyl 2-acetyl-12-bromododecanoate (**3**) (9. 14 g, 26.16 mmol) gave the title compound **8** as an off-white solid (7.73 g, 79 %) by following the general procedure; m. p.: 112.9–113.2 °C; R_f: 0.35 (MeOH:$CHCl_3$: 1: 19); UV (MeOH) λ_{max}: 262 and 324 nm; IR (KBr) ν_{max}: 3431.60 (OH), 2927.00, 1670.58 (C=O), 1610.35, 1581.83, 1379.22, 1307.47, 1089.07, 1055.91, 954.26, 811.76, 776. 15, 526.67 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.23–1. 54 (m, 14 H, H-2′–H-8′), 1.82–1.89 (m, 2H, H-9′), 2.38 (s, 3H, C-4 CH_3), 2.62 (t, 2H, $J = 8.05$ Hz, H-1′), 3.40 (t, 2H, $J = 6.59$ Hz, H-10′), 6.90 (d, 1H, $J = 8.05$ Hz, H-6), 7.09 (d, 1H, $J = 8.05$ Hz, H-5) ppm; ^{13}C NMR (100.5 MHz $CDCl_3$): δ 15.12 (C-4 *C*H_3), 27.60, 28.20, 28.87, 29.54, 29. 57, 29.61, 29.80 (C-1′–C-8′), 32.95 (C-9′), 34.21 (C-10′), 112.23 (C-6), 114.40 (C-10), 115.95 (C-5), 122.89 (C-3), 130.71 (C-8), 141.19 (C-4), 146.97 (C-7), 148.12 (C-9), 162.50 (C-2) ppm; HRMS: *m/z* $[M + Na]^+$ Calculated for $C_{20}H_{27}BrO_4$: 433.0990; found: 433.0980.

*Synthesis of 3-(ω-bromoalkyl)-10-hydroxy-4,8,8-trimethyl-7,8-dihydropyrano[3,2-g]chromen-2(6H)-ones (**9/10**)* To a stirred solution of 3-(ω-bromoalkyl)-7,8-dihydroxy-4-methyl-2*H*-chromen-2-ones (**7/8**) (1.0 g) and *p*-toluenesulfonic acid monohydrate (1 equivalent) in toluene was added 1.5 equivalent of 2-methyl-3-buten-2-ol and the reaction mixture refluxed for 30 h. The progress of the reaction was monitored using TLC. On completion of the reaction, the solvent was removed in *vacuo* and the solid obtained was dissolved in chloroform (50 ml). The chloroform layer was washed first with sodium hydroxide solution (1 N, 2 × 50 ml) and then with brine solution (20 ml). The organic layer was then dried over anhydrous sodium sulphate. The solvent was removed under reduced pressure and purified by column chromatography to give the desired product (**9/10**).

*3-(6-Bromohexyl)-10-hydroxy-4,8,8-trimethyl-7,8-dihydropyrano[3,2-g]chromen-2(6H)-one (**9**)* The reaction of 3-(6-bromohexyl)-7,8-dihydroxy-4-methyl-2*H*-chromen-2-one (**7**) (2 g, 5.63 mmol) with 2-methyl-3-buten-2-ol (0. 73 g, 8.45 mmol) gave the title compound **9** as an off-white solid (1.55 g, 65 %) by following the general procedure; m. p.: 123.8–124.2 °C; R_f: 0.30 (ethyl acetate: petroleum ether: 3:7); UV (MeOH) λ_{max}: 265 and 327 nm; IR (KBr) ν_{max}: 3380.20 (OH), 2924.18, 1675.32 (C=O), 1458.46, 1388.78, 1121.13, 1032.89, 930.27, 781.64 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.36–1.57 (m, 12H, H-2′–H-4′ and 2× C-8 CH_3), 1.82-1.90 (m, 4H, H-5′ and H-7), 2. 35 (s, 3H, C-4 CH_3), 2.63 (t, 2H, $J = 7.93$ Hz, H-1′), 2.84 (t, 2H, $J = 6.71$ Hz, H-6), 3.40 (t, 2H, $J = 6.71$ Hz, H-6′), 5.60 (*brs*, 1H, OH), 6.87 (s, 1H, H-5) ppm; ^{13}C NMR (100. 5 MHz $CDCl_3$): δ 15.08 (C-4 *C*H_3), 22.32 (C-6), 27.01 (2× C-8 *C*H_3), 27.64 (C-1′), 28.18, 28.78, 28.91 (C-2′–C-4′), 32.91 (C-5′ and C-7), 34.13 (C-6′), 76.39 (C-8), 114.06 (C-12), 114.90 (C-5), 117.65 (C-14), 123.62 (C-3), 131.98 (C-10), 139.00 (C-11), 143.47 (C-4), 146.55 (C-13), 161.55 (C-2) ppm; HRMS: *m/z* $[M + Na]^+$ Calculated for $C_{21}H_{27}BrO_4$: 445.0990; found: 445.1001.

*3-(10-Bromodecyl)-10-hydroxy-4,8,8-trimethyl-7,8-dihydropyrano[3,2-g]chromen-2(6H)-one (**10**)* The reaction of 3-(10-bromodecyl)-7,8-dihydroxy-4-methyl-2*H*-chromen-2-one (**8**) (2 g, 4.86 mmol) with 2-methyl-3-buten-2-ol (0.63 g, 7.29 mmol) gave the title compound **10** as an off-white solid (1.46 g, 63 %) by following the general procedure; m. p.: 87.7–88.1 °C; R_f: 0.40 (ethyl acetate: petroleum ether: 3:7); UV (MeOH) λ_{max}: 266 and 329 nm; IR (KBr) ν_{max}: 3385.74 (OH), 2927.15, 2854.00, 1704.54 (C=O), 1622.97, 1580.91, 1466.46, 1390.52, 1121.57, 1054.72, 937.24, 783.79 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.24-1.55 (m, 20H, H-2′–H-8′ and 2× C-8 CH_3), 1.80–1.90 (m, 4H, H-7 and H-9′), 2.34 (s, 3H, C-4 CH_3), 2. 61 (t, 2H, $J = 8.05$ Hz, H-1′), 2.84 (t, 2H, $J = 6.59$ Hz, H-6), 3.40 (t, 2H, $J = 6.59$ Hz, H-10′), 6.86 (s, 1H, H-5) ppm, ^{13}C NMR (100.5 MHz $CDCl_3$): δ 14.98 (C-4 *C*H_3), 22.23 (C-6), 26.93 (2× C-8 *C*H_3), 27.69 (C-1′), 28.22, 28. 81, 28.93, 29.46, 29.54, 29.71 (C-2′–C-8′), 32.79 (C-9′), 32.90 (C-7), 34.18 (C-10′), 76.28 (C-8), 114.00 (C-12), 114.81 (C-5), 117.56 (C-14), 123.74 (C-3), 131.87 (C-10),

138.88 (C-11), 143.34 (C-4), 146.40 (C-13), 161.48 (C-2) ppm; HRMS: *m/z* $[M + Na]^+$ Calculated for $C_{25}H_{35}BrO_4$: 501.1617; found: 501.1611.

*Synthesis of N,N,N-triethyl-ω-(10-hydroxy-4,8,8-trimethyl-2-oxo-2,6,7,8-tetrahydropyrano[3,2-g]chromen-3-yl)alkan-1-aminium bromide (**17/18**)* Triethylamine (5 equivalents) was added to a solution of **9** (1 g, 2.36 mmol)/**10** (1 g, 2.08 mmol) in anhydrous acetonitrile (50 ml) and the reaction mixture was stirred under nitrogen at 60 °C for 96 h. The progress of reaction was monitored using TLC. After completion of the reaction, the solvent was evaporated under reduced pressure, followed by column chromatography of the resultant product to give the pure quaternary ammonium compound (**17/18**) in moderate yields.

*N,N,N-Triethyl-6-(10-hydroxy-4,8,8-trimethyl-2-oxo-2,6,7,8-tetrahydropyrano[3,2-g]chromen-3-yl)hexan-1-aminium bromide (**17**)* The reaction of 3-(6-bromohexyl)-10-hydroxy-4,8,8-trimethyl-7,8-dihydropyrano[3,2-*g*]chromen-2(6*H*)-one (**9**) (1 g, 2.36 mmol) with triethylamine (1.20 g, 11.80 mmol) gave the title compound **17** as a brown low melting solid (0.73 g, 70 %) by following the general procedure; R_f: 0.38 (MeOH:$CHCl_3$: 1:4); UV (MeOH) λ_{max}: 264 and 329 nm; IR (KBr) ν_{max}: 3387.59, 2927.26, 2854.88, 1698.02 (C=O), 1619.60, 1579.03, 1467.63, 1390.63, 1370.34, 1122.01, 1054.68, 938.90, 753.43, 660.97 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): δ 1.16.1.44 (m, 23H, H-2–H-5, 2× C-8 CH_3 and 3× H-2″), 1.80 (t, 2H, $J = 6.59$ Hz, H-7′), 2.27 (s, 3H, C-4′ CH_3), 2.51 (t, 2H, $J = 7.69$ Hz, H- 6), 2.78 (t, 2H, $J = 6.59$ Hz, H- 6′), 3.21-3.30 (m, 2H, H-1) 3.40–3.50 (m, 6H, H-1″), 6.81 (s, 1H, H-5′) ppm; ^{13}C NMR (100.5 MHz $CDCl_3$): δ 8.15 (C-2″), 15.16 (C-4′ *C*H_3), 21.74 (C-6′), 22.27 (C-6), 27.01 (2 x C-8 *C*H_3), 25.98, 27.09, 28.17, 28.63 (C-2– C-5), 32.87 (C-7′), 53.56 (C-1″), 57.65 (C-1), 76.52 (C-8′), 114.04 (C-12′), 115.04 (C-5′), 117.76 (C-14′), 123.07 (C-3′), 132.06 (C-10′), 138.98 (C-11′), 143.66 (C-4′), 147.23 (C-13′), 161.85 (C-2′) ppm; HRMS: *m/z* $[M]^+$ Calculated for $C_{27}H_{42}NO_4^+$: 444.3114; found: 444.3118.

*N,N,N-Triethyl-10-(10-hydroxy-4,8,8-trimethyl-2-oxo-2,6,7,8-tetrahydropyrano[3,2-g]chromen-3-yl)decan-1-aminium bromide (**18**)* The reaction of 3-(10-Bromodecyl)-10-hydroxy-4,8,8-trimethyl-7,8-dihydropyrano[3,2-*g*]chromen-2(6*H*)-one (**10**) (1 g, 2.09 mmol) with triethylamine (1.06 g, 10.45 mmol) gave the title compound **18** as a brown solid (0.83 g, 69 %) by following the general procedure; m. p.: 83.6–84.9 °C; R_f: 0.40 (MeOH:$CHCl_3$: 1:4); UV (MeOH) λ_{max}: 266 and 328 nm; IR (KBr) ν_{max}: 3385.86, 2929.96, 2857.37, 1701.41 (C=O), 1617.97, 1578.18, 1466.10, 1389.38, 1369.68, 1121.52, 1056.00, 937.94, 783.36, 752.11 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): δ 1.20–1.50 (m, 29H, H-3–H-9, 2 x C-8 CH_3 and 3× H-2″), 1.63-1.72 (m, 2H, H-2), 1.83 (t, 2H, $J = 6.59$ Hz, H-7′), 2.32 (s, 3H, C-4′ CH_3), 2.57 (t, 2H, $J = 8.05$ Hz, H-10), 2.81 (t, 2H, $J = 6.59$ Hz, H-6′), 3.20–3.30 (m, 2H, H-1), 3.45–3.55 (m, 6H, H-1″), 6.00 (*brs*, 1H, OH), 6.85 (s, 1H, H-5′) ppm; ^{13}C NMR (100.5 MHz $CDCl_3$): δ 8.15 (C-2″), 15.13 (C-4′ *C*H_3), 22.06 (C-6′), 22.30 (C-10), 27.01 (2 x C-8 *C*H_3), 26.40, 27.43, 28.72, 28.92, 29.01, 29.23, 29.84, (C-2–C-9), 32.87 (C-7′), 53.60 (C-1″), 57.64 (C-1), 76.44 (C-8′), 114.11 (C-12′), 114.87 (C-5′), 117.67 (C-14′), 123.60 (C-3′), 132.09 (C-10′), 139.01 (C-11′), 143.60 (C-4′), 146.84 (C-13′), 161.86 (C-2′) ppm; HRMS: *m/z* $[M]^+$ Calculated for $C_{31}H_{50}NO_4^+$: 500.3740; found: 500.3753.

*General procedure for the synthesis of (coumarinyloxy)acetamide derivatives (**32–47**)*

To a stirred solution of (coumarinyloxy)acetate (**27–31**) (1 g) in *n*-butanol (20 ml) was added aminoalkane/*N*,*N*-diaminoalkane (4 equivalent) and the reaction was stirred at room temperature for 48 h. The progress of reaction was monitored on TLC and on completion of the reaction, the solvent was removed *in vacuo*. The solid so obtained was washed with hexane (3 × 20 ml) and crystallised from ethanol/ethyl acetate–petroleum ether to give (coumarinyloxy)acetamides as off-white/white/brown solid.

*N-(3-(Diethylamino)propyl)-2-((2-oxo-2H-chromen-4-yl)oxy)acetamide (**32**)* The reaction of ethyl 2-((2-oxo-2*H*-chromen-4-yl)oxy)acetate (**27**) (1 g, 4.03 mmol) with N^1,N^1-diethylpropane-1,3-diamine (2.09 g, 16.12 mmol) gave the title compound **32** as a light brown solid (0.92 g, 69 %) by following the general procedure; m. p.: 105.4–106.1 °C; R_f: 0.42 (MeOH:$CHCl_3$: 1:49); UV (MeOH) λ_{max}: 266 nm; IR (KBr) ν_{max}: 3293.20 (N–H str), 2966.72, 1717.49 (C=O), 1654.36 (NH*CO*–), 1623.56, 1549.98, 1248.95, 1112.80, 1018.36, 934.79, 855.76, 769.16, 605.22 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): δ 0.93 (t, 6H, $J =$ 7.32 Hz, H-2‴), 1.67–1.73 (m, 2H, H-2″), 2.42 (q, 4H, $J = 7.32$, Hz, H-1‴), 2.53 (t, 2H, $J = 5.86$ Hz, H-3″), 3.51–3.47 (m, 2H, H-1″), 4.65 (s, 2H, H-2), 5.70 (s, 1H, H-3′), 7.28–7.36 (m, 2H, H-6′ and H-8′), 7.58–7.62 (m, 1H, H-7′), 7.85–7.87 (m, 1H, H-5′), 8.30 (*brs*, 1H, NH) ppm; ^{13}C NMR (100.5 MHz, $CDCl_3$): δ 11.28 (C-2‴), 25.20 (C-2″), 40.12 (C-1″), 47.14 (C-1‴), 52.00 (C-3″), 67.80 (C-2), 91.83 (C-3′), 114.99 (C-6′), 116.94 (C-8′), 122.77 (C-10′), 123.80 (C-7′), 132.84 (C-5′), 153.31 (C-9′), 162.10, 164.26 and 165.25 (C-2′, C-1 and C-4′) ppm; HRMS: *m/z* $[M + Na]^+$ Calculated for $C_{18}H_{24}N_2O_4$: 355.1634; found: 355.1634.

*N-(2-Ethylhexyl)-2-((2-oxo-2H-chromen-4-yl)oxy)acetamide (**33**)* The reaction of ethyl 2-((2-oxo-2*H*-chromen-4-yl)oxy)acetate (**27**) (1 g, 4.03 mmol) with 2-ethylhexan-1-amine (2.08 g, 16.12 mmol) gave the title compound **33** as a white solid (1 g, 75 %) by following the general procedure; m. p.: 123.6–124.3 °C; R_f: 0.32 (MeOH:$CHCl_3$: 1:49); UV (MeOH) λ_{max}: 266 nm; IR (KBr) ν_{max}: 3349.29 (N–H str), 3087.88, 2929.24, 1718.03 (C=O), 1654.64 (NH*CO*–), 1624.09, 1545.03, 1248.32, 1113.84, 1025.39, 937.60, 859.31, 771.85, 594.68 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$): δ 0.89 (t, 3H, $J = 5.86$ Hz, H-6″), 0.91 (t, 3H, $J = 8.05$ Hz, H-2‴″), 1.28–1.38 (m, 8H, H-3″– H-5″ and H-1‴″), 1.48–1.54 (m, 1H, H-2″), 3.32–3.35 (m, 2H, H-1″), 4.64 (s, 2H, H-2), 5.70 (s, 1H, H-3′), 6.32 (*brs*, 1H, NH), 7.30–7.36 (m, 2H, H-6′ and H-8′), 7.58–7.61 (m, 1H, H-7′), 7.75–7.77 (m, 1H, H-5′) ppm; ^{13}C NMR (100.5 MHz, $CDCl_3$): δ 10.85 (C-6″), 13.98 (C-2‴″), 22.95 (C-5″), 24.30 (C-1‴″), 28.77 (C-4″), 31.02 (C-3″), 39.25 (C-2″), 42.06 (C-1″), 67.57 (C-2), 92.00 (C-3′), 114.86 (C-8′), 117.13 (C-6′), 122.26 (C-10′), 124.15 (C-7′), 132.93 (C-5′), 153.34 (C-9′), 161.92, 163.83 and 165.41 (C-2′, C-1 and C-4′) ppm; HRMS: *m/z* $[M + Na]^+$ Calculated for $C_{19}H_{25}NO_4$: 354.1681; found: 354.1688.

*N-(3-(Diethylamino)propyl)-2-((4-methyl-2-oxo-2H-chromen-7-yl)oxy)acetamide (**34**)* The reaction of ethyl 2-((4-methyl-2-oxo-2*H*-chromen-7-yl)oxy)acetate (**28**) (1 g, 3.81 mmol) with N^1,N^1-diethylpropane-1,3-diamine (1.98 g, 15.24 mmol) gave the title compound **34** as a white solid (1.08 g, 82 %) by following the general procedure; m. p.: 106.2–107.7 °C; R_f: 0.40 (MeOH:$CHCl_3$: 1:49); UV (MeOH) λ_{max}: 292 and 318 nm; IR (KBr) ν_{max}: 3255.61 (N–H str), 2956.53, 1730.14 (C=O), 1679.02 (NH*CO*–), 1617.18, 1527.43, 1276.70, 1158.08, 1073.82, 846.35, 735.40, 589.81 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.00 (t, 6H, $J = 7.32$ Hz, H-2‴″), 1.65–1.73 (m, 2H, H-2″), 2.40 (d, 3H, $J = 1.48$ Hz, C-4′ CH_3), 2.48–2.56 (m, 6H, H-1‴″ and H-3″), 3.43–3.49 (m, 2H, H-1″), 4.52 (s, 2H, H-2), 6.16 (d, 1H, $J = 1.48$ Hz, H-3′), 6.83 (d, 1H, $J = 2.20$ Hz, H-8′), 6.87 (dd, 1H, $J = 2.92$ and 8.80 Hz, H-6′), 7.54 (d, 1H, $J = 8.76$ Hz, H-5′), 8.47 (*brs*, 1H, NH) ppm; ^{13}C NMR (100.5 MHz, $CDCl_3$): δ 11.34 (C-2‴″), 18.64 (C-4′ *C*H_3), 25.11 (C-2″), 39.66 (C-1″), 46.92 (C-1‴″), 52.21 (C-3″), 67.58 (C-2), 102.58 (C-8′), 111.44 (C-6′), 112.66 (C-3′), 114.57 (C-10′), 125.94 (C-5′), 152.23 (C-4′), 155.04 and 160.16 (C-9′ and C-7′), 160.93 and 166.89 (C-2′ and C-1) ppm; HRMS: *m/z* $[M + Na]^+$ Calculated for $C_{19}H_{26}N_2O_4$: 369.1790; found: 369.1789.

*N-(3-(Dibutylamino)propyl)-2-((4-methyl-2-oxo-2H-chromen-7-yl)oxy)acetamide (**35**)* The reaction of ethyl 2-((4-methyl-2-oxo-2*H*-chromen-7-yl)oxy)acetate (**28**) (1 g, 3.81 mmol) with N^1,N^1-dibutylpropane-1,3-diamine (2.83 g, 15.24 mmol) gave the title compound **35** as a white solid (1.21 g, 79 %) by following the general procedure; m. p.: 129.5–130.8 °C; R_f: 0.41 (MeOH:$CHCl_3$: 1:49); UV (MeOH) λ_{max}: 290 and 317 nm; IR (KBr) ν_{max}: 3383.32 (N–H str), 2954.60, 1708.46 (C=O), 1666.97 (NH*CO*–), 1629.14, 1545.91, 1153.54, 1081.08, 845.86, 595.42 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$): δ 0.79–0.85 (m, 6H, H-4‴″), 1.16–1.27 (m, 4H, H-3‴″), 1.31–1.40 (m, 4H, H-2‴″), 1.61–1.69 (m, 2H, H-2″), 2.31–2.37 (m, 4H, H-1‴″), 2.39 (s, 3H, C-4′ CH_3), 2.46–2.52 (m, 2H, H-3″), 3.40–3.47 (m, 2H, H-1″), 4.51 (s, 2H, H-2), 6.15 (s, 1H, H-3′), 6.81–6.88 (m, 2H, H-8′ and H-6′), 7.51–7.55 (m, 1H, H-5′), 8.27 (*brs*, 1H, NH) ppm; ^{13}C NMR (100.5 MHz, $CDCl_3$): δ 14.20 (C-4‴″), 18.80 (C-4′ *C*H_3), 20.88 (C-3‴″), 25.60 (C-2″), 29.05 (C-2‴″), 40.02 (C-1″), 53.70 (C-3″), 54.29 (C-1‴″), 67.86 (C-2), 102.78 (C-8′), 111.64 (C-6′), 112.86 (C-3′), 114.74 (C-10′), 126.05 (C-5′), 152.36 (C-4′), 155.23 and 160.39 (C-9′ and C-7′), 161.04 and 167.01 (C-2′ and C-1) ppm; HRMS: *m/z* $[M + Na]^+$ Calculated for $C_{23}H_{34}N_2O_4$: 425.2416; found: 425.2402.

*N-(2-Hydroxyethyl)-2-((4-methyl-2-oxo-2H-chromen-7-yl)oxy)acetamide (**36**) (Wang et al., 2011)* The reaction of ethyl 2-((4-methyl-2-oxo-2*H*-chromen-7-yl)oxy)acetate (**28**) (1 g, 3.81 mmol) with 2-aminoethanol (0.97 g, 15.24 mmol) gave the title compound **36** as a white solid (0.68 g, 65 %) by following the general procedure; m. p.: 188.2–188.8 °C; R_f: 0.25 (MeOH:$CHCl_3$: 1:49); UV (MeOH) λ_{max}: 291 and 318 nm; IR (KBr) ν_{max}: 3396.13 (O–H str), 3309.01 (N–H str), 2922.84, 1700.11 (C=O), 1659.28 (NHCO–), 1615.46, 1515.50, 1285.30, 1146.79, 991.46, 853.63, 754.94, 505.12 cm^{-1}; ^{1}H NMR (400 MHz, DMSO-d_6): δ 2.38 (s, 3H, C-4′ CH_3), 3.18-3.23 (m, 2H, H-1″), 3.40–3.46 (m, 2H, H-2″), 4.60 (s, 2H, H-2), 4.70–4.75 (m, 1H, OH), 6.21 (s, 1H, H-3′), 6.95 (d, 1H, $J = 2.92$ Hz, H-8′), 6.99 (dd, 1H, $J = 2.96$ and 8.80 Hz, H-6′), 7.69 (d, 1H, $J = 8.80$ Hz, H-5′), 8.11 (*brs*, 1H, NH) ppm; ^{13}C NMR (100.5 MHz, DMSO-d_6): δ 18.13 (C-4′ *C*H_3), 41.23 (C-1″), 59.60 (C-2″), 67.14 (C-2), 101.65 (C-8′), 111.43 (C-6′), 112.44 (C-3′), 113.60 (C-10′), 126.51 (C-5′), 153.37 (C-4′), 154.50 and 160.07 (C-9′ and C-7′), 160.66 and 167.01 (C-2′ and C-1) ppm; HRMS: *m/z* $[M + Na]^+$ Calculated for $C_{14}H_{15}NO_5$: 300.0848; found: 300.0852.

*N-(3-(Diethylamino)propyl)-2-((3-ethyl-4-methyl-2-oxo-2H-chromen-7-yl)oxy)acetamide (**37**)* The reaction of ethyl 2-((3-ethyl-4-methyl-2-oxo-2*H*-chromen-7-yl)oxy)acetate (**29**) (1 g, 3.44 mmol) with N^1,N^1-diethylpropane-1,3-diamine (1.79 g, 13.76 mmol) gave the title compound **37** as a white solid (1.04 g, 81 %) by following the general procedure; m. p.: 122.1–122.9 °C; R_f: 0.40 (MeOH:$CHCl_3$: 1:49); UV (MeOH) λ_{max}: 291 and 318 nm; IR (KBr) ν_{max}: 3375.70 (N–H str), 2964.89, 1712.57 (C=O), 1661.90

(NH*CO*–), 1618.23, 1545.43, 1161.61, 1096.47, 872.25, 778.04, 596.54 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): δ 1.00 (t, 6H, $J = 7.32$ Hz, H-2‴′), 1.13 (t, 3H, $J = 7.32$ Hz, H-2‴), 1.65-1.72 (m, 2H, H-2″), 2.38 (s, 3H, C-4′ CH_3), 2.48–2.55 (m, 6H, H-1‴′ and H-3″), 2.66 (q, 2H, $J = 7.32$ Hz, H-1‴), 3.43–3.47 (m, 2H, H-1″), 4.50 (s, 2H, H-2), 6.80 (d, 1H, $J = 2.20$ Hz, H-8′), 6.84 (dd, 1H, $J = 2.20$ and 8.80 Hz, H-6′), 7.53 (d, 1H, $J = 8.80$ Hz, H-5′), 8.44 (*brs*, 1H, NH) ppm; ^{13}C NMR (100.5 MHz, $CDCl_3$): δ 11.61 (C-2‴′), 13.26 (C-2‴), 14.73 (C-4′ *C*H_3), 21.02 (C-1‴), 25.38 (C-2″), 39.88 (C-1″), 47.13 (C-1‴′), 52.45 (C-3″), 67.77 (C-2), 102.47 (C-8′), 111.35 (C-6′), 115.52 (C-10′), 125.91 (C-3′), 125.95 (C-5′), 145.62 (C-4′), 153.64 and 159.29 (C-9′ and C-7′), 161.87 and 167.23 (C-2′ and C-1) ppm; HRMS: *m/z* $[M + Na]^+$ Calculated for $C_{21}H_{30}N_2O_4$: 397.2103; found: 397.2093.

*N-(3-(Dibutylamino)propyl)-2-((3-ethyl-4-methyl-2-oxo-2H-chromen-7-yl)oxy)acetamide (**38**)* The reaction of ethyl 2-((3-ethyl-4-methyl-2-oxo-2*H*-chromen-7-yl)oxy) acetate (**29**) (1 g, 3.44 mmol) with N^1,N^1-dibutylpropane-1,3-diamine (2.56 g, 13.76 mmol) gave the title compound **38** as a white solid (1.23 g, 83 %) by following the general procedure; m. p.: 121.3–130.0 °C; R_f: 0.42 (MeOH:$CHCl_3$: 1:49); UV (MeOH) λ_{max}: 292 and 318 nm; IR (KBr) ν_{max}: 3371.69 (N–H str), 2957.40, 1712.48 (C=O), 1661.03 (NH*CO*–), 1618.49, 1546.92, 1160.04, 1095.98, 870.93, 777.91, 599.61 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): δ 0.82 (t, 6H, $J = 7.32$ Hz, H-4‴′), 1.12 (t, 3H, $J = 7.36$ Hz, H-2‴), 1.16–1.26 (m, 4H, H-3‴′), 1.32–1.39 (m, 4H, H-2‴′), 1.63–1.69 (m, 2H, H-2″), 2.34 (t, 4H, $J = 8.08$ Hz, H-1‴′), 2.37 (s, 3H, C-4′ CH_3), 2.50 (t, 2H, $J = 6.24$ Hz, H-3″), 2.65 (q, 2H, $J = 7.32$ Hz, H-1‴), 3.41–3.47 (m, 2H, H-1″), 4.51 (s, 2H, H-2), 6.80 (d, 1H, $J = 2.20$ Hz, H-8′), 6.84 (dd, 1H, $J = 2.92$ and 8.76 Hz, H-6′), 7.52 (d, 1H, $J = 8.80$ Hz, H-5′), 8.26 (*brs*, 1H, NH) ppm; ^{13}C NMR (100.5 MHz, $CDCl_3$): δ 13.22 (C-2‴), 14.20 (C-4‴′), 14.70 (C-4′ *C*H_3), 20.87 (C-3‴′), 21.00 (C-1‴), 25.60 (C-2″), 28.91 (C-2‴′), 39.91 (C-1″), 53.58 (C-3″), 54.24 (C-1‴′), 67.82 (C-2), 102.43 (C-8′), 111.44 (C-6′), 115.51 (C-10′), 125.87 (C-3′), 125.92 (C-5′), 145.57 (C-4′), 153.64 and 159.33 (C-9′ and C-7′), 161.82 and 167.27 (C-2′ and C-1) ppm; HRMS: *m/z* $[M + Na]^+$ Calculated for $C_{25}H_{38}N_2O_4$: 453.2729; found: 453.2729.

*2-((3-Ethyl-4-methyl-2-oxo-2H-chromen-7-yl)oxy)-N-(2-hydroxyethyl)acetamide (**39**)* The reaction of ethyl 2-((3-ethyl-4-methyl-2-oxo-2*H*-chromen-7-yl)oxy)acetate (**29**) (1 g, 3.44 mmol) with 2-aminoethanol (0.84 g, 13.76 mmol) gave the title compound **39** as an off-white solid (0.65 g, 62 %) by following the general procedure; m. p.: 192.3–193.1 °C; R_f: 0.25 (MeOH:$CHCl_3$: 1:49); UV (MeOH) λ_{max}: 292 and 318 nm; IR (KBr) ν_{max}: 3402.94 (O–H str), 3302.37 (N–H str), 2959.90, 2914.37, 1692.92 (C=O), 1649.99 (NH*CO*–), 1609.72, 1553.78, 1258.79, 1181.98, 1095.72, 863.67, 779.60, 600.59 cm^{-1}; ^1H NMR (400 MHz, DMSO-d_6): δ 1.02 (t, 3H, $J = 7.32$ Hz, H-2‴), 2.36 (s, 3H, C-4′ CH_3), 2.54 (q, 2H, $J = 7.32$ Hz, H-1‴), 3.18–3.22 (m, 2H, H-1″), 3.42 (*brm*, 2H, H-2″), 4.58 (s, 2H, H-2), 4.74 (*brs*, 1H, OH), 6.92 (d, 1H, $J = 2.92$ Hz, H-8′), 6.97 (dd, 1H, $J = 2.20$ and 8.80 Hz, H-6′), 7.70 (d, 1H, $J = 8.76$ Hz, H-5′), 8.11 (*brs*, 1H, NH) ppm; ^{13}C NMR (100.5 MHz, DMSO-d_6): δ 12.95 (C-2‴), 14.35 (C-4′ *C*H_3), 20.22 (C-1‴), 41.23 (C-1″), 59.61 (C-2″), 67.13 (C-2), 101.35 (C-8′), 112.33 (C-6′), 114.16 (C-10′), 124.02 (C-3′), 126.36 (C-5′), 146.42 (C-4′), 152.88 and 159.68 (C-9′ and C-7′), 160.72 and 167.09 (C-2′ and C-1) ppm; HRMS: *m/z* $[M + Na]^+$ Calculated for $C_{16}H_{19}NO_5$: 328.1161; found: 328.1151.

*2-((3-Ethyl-4-methyl-2-oxo-2H-chromen-7-yl)oxy)-N-octylacetamide (**40**)* The reaction of ethyl 2-((3-ethyl-4-methyl-2-oxo-2*H*-chromen-7-yl)oxy)acetate (**29**) (1 g, 3.44 mmol) with octan-1-amine (1.77 g, 13.76 mmol) gave the title compound **40** as a white solid (1.02 g, 79 %) by following the general procedure; m. p.: 140.2–141.0 °C; R_f: 0.41 (MeOH:$CHCl_3$: 1:49); UV (MeOH) λ_{max}: 293 and 318 nm; IR (KBr) ν_{max}: 3372.19 (N–H str), 2927.59, 1709.18 (C=O), 1664.71 (NH*CO*–), 1618.08, 1559.72, 1154.82, 1096.29, 872.47, 715.80, 601.06 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): δ 0.86 (t, 3H, $J = 6.6$ Hz, H-8″), 1.13 (t, 3H, $J = 7.36$ Hz, H-2‴), 1.21–1.32 (m, 10H, H-7″–H-3″), 1.51-1.58 (m, 2H, H-2″), 2.38 (s, 3H, C-4′ CH_3), 2.66 (q, 2H, $J = 7.32$ Hz, H-1‴), 3.31–3.38 (m, 2H, H-1″), 4.52 (s, 2H, H-2), 6.54 (*brs*, 1H, NH), 6.83 (d, 1H, $J = 2.96$ Hz, H-8′), 6.86 (dd, 1H, $J = 2.16$ and 8.80 Hz, H-6′), 7.54 (d, 1H, $J = 8.80$ Hz, H-5′) ppm; ^{13}C NMR (100.5 MHz, $CDCl_3$): δ 13.25 (C-8″), 14.26 (C-2‴), 14.74 (C-4′ *C*H_3), 21.04 (C-1‴), 22.79 (C-7″), 27.02 (C-6″), 29.34 and 29.36 (C-5″ and C-4″), 29.68 (C-3″), 31.92 (C-2″), 39.37 (C-1″), 67.62 (C-2), 102.49 (C-8′), 111.46 (C-6′), 115.72 (C-10′), 125.99 (C-3′), 126.07 (C-5′), 145.58 (C-4′), 153.62 and 158.91 (C-9′ and C-7′), 161.81 and 167.31 (C-2′ and C-1) ppm; HRMS: *m/z* $[M + Na]^+$ Calculated for $C_{22}H_{31}NO_4$: 396.2151; found: 396.2162.

*N-(3-(Dimethylamino)propyl)-2-((3-ethyl-4-methyl-2-oxo-2H-chromen-7-yl)oxy)acetamide (**41**)* The reaction of ethyl 2-((3-ethyl-4-methyl-2-oxo-2*H*-chromen-7-yl)oxy) acetate (**29**) (1 g, 3.44 mmol) with N^1,N^1-dimethylpropane-1,3-diamine (1.40 g, 13.76 mmol) gave the title compound **41** as a white solid (0.95 g, 80 %) by following the general procedure; m. p.: 136.6–137.5 °C; R_f: 0.35 (MeOH:$CHCl_3$.: 1:49); UV (MeOH) λ_{max}: 290 and 317 nm; IR (KBr) ν_{max}:

3375.80 (N–H str), 2966.80, 1713.47 (C=O), 1666.42 (NH*CO*–), 1618.60, 1549.02, 1159.70, 1095.12, 871.43, 777.79, 599.98 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): δ 1.12 (t, 3H, $J = 7.32$ Hz, H-2‴), 1.65–1.71 (m, 2H, H-2″), 2.18 (s, 6H, H-1⁗), 2.38 (s, 3H, C-4′ CH_3), 2.40 (t, 2H, $J = 5.84$ Hz, H-3″), 2.65 (q, 2H, $J = 7.32$ Hz, H-1‴), 3.41–3.47 (m, 2H, H-1″), 4.50 (s, 2H, H-2), 6.79 (d, 1H, $J = 2.92$ Hz, H-8′), 6.83 (dd, 1H, $J = 2.92$ and 8.80 Hz, H-6′), 7.53 (d, 1H, $J = 8.80$ Hz, H-5′), 8.31 (*brs*, 1H, NH) ppm; ^{13}C NMR (100.5 MHz, $CDCl_3$): δ 13.24 (C-2‴), 14.72 (C-4′ *C*H_3), 21.01 (C-1‴), 25.59 (C-2″), 39.55 (C-1″), 45.48 (C-1⁗), 58.99 (C-3″), 67.66 (C-2), 102.36 (C-8′), 111.34 (C-6′), 115.50 (C-10′), 125.89 (C-3′), 125.95 (C-5′), 145.61 (C-4′), 153.64 and 159.29 (C-9′ and C-7′), 161.82 and 167.31 (C-2′ and C-1) ppm; HRMS: *m/z* $[M + Na]^+$ Calculated for $C_{19}H_{26}N_2O_4$: 369.1790; found: 369.1789.

*N-(3-(Diethylamino)propyl)-2-((3-hexyl-4-methyl-2-oxo-2H-chromen-7-yl)oxy)acetamide (**42**)* The reaction of ethyl 2-((3-hexyl-4-methyl-2-oxo-2*H*-chromen-7-yl)oxy) acetate (**30**) (1 g, 2.88 mmol) with N^1,N^1-diethylpropane-1,3-diamine (1.50 g, 11.52 mmol) gave the title compound **42** as a white solid (1 g, 81 %) by following the general procedure; m. p.: 115.6–117.2 °C; R_f: 0.48 (MeOH:$CHCl_3$: 1:49); UV (MeOH) λ_{max}: 319 nm; IR (KBr) ν_{max}: 3370.84 (N–H str), 2922.59, 1710.76 (C=O), 1662.03 (NH*CO*–), 1618.44, 1549.66, 1426.54, 1286.31, 1160.00, 1088.95, 781.66, 599.06 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): δ 0.86 (t, 3H, $J = 6.87$ Hz, H-6‴), 0.98 (t, 6H, $J = 7.33$ Hz, H-2⁗), 1.27–1.31 (m, 4H, H-4‴ and H-5‴), 1.33–1.40 (m, 2H, H-3‴), 1.45–1.53 (m, 2H, H-2‴), 1.63–1.69 (m, 2H, H-2″), 2.36 (s, 3H, C-4′ CH_3), 2.47 (q, 4H, $J = 7.33$ Hz, H-1⁗), 2.51 (t, 2H, $J = 5.95$ Hz, H-3″), 2.61 (t, 2H, $J = 7.33$ Hz, H-1‴), 3.42–3.46 (m, 2H, H-1″), 4.49 (s, 2H, H-2), 6.79 (d, 1H, $J = 2.29$ Hz, H-8′), 6.68 (dd, 1H, $J = 2.29$ and 8.70 Hz, H-6′), 7.52 (d, 1H, $J = 8.70$ Hz, H-5′), 8.47 (*brs*, 1H, NH) ppm; ^{13}C NMR (100.5 MHz, $CDCl_3$): δ 11.52 (C-2⁗), 14.04 (C-6‴), 14.80 (C-4′ *C*H_3), 22.57 (C-5‴), 25.19 (C-2″), 27.53 (C-4‴), 28.71 (C-3‴), 29.31 (C-2‴), 31.65 (C-1‴), 39.82 (C-1″), 46.95 (C-1⁗), 52.32 (C-3″), 67.55 (C-2), 102.26 (C-8′), 111.04 (C-6′), 115.30 (C-10′), 124.56 (C-3′), 125.73 (C-5′), 145.59 (C-4′), 153.41 and 159.03 (C-9′ and C-7′), 161.79 and 166.96 (C-2′ and C-1) ppm; HRMS: *m/z* $[M + Na]^+$ Calculated for $C_{25}H_{38}N_2O_4$: 453.2729; found: 453.2729.

*N-(3-(Dimethylamino)propyl)-2-((3-hexyl-4-methyl-2-oxo-2H-chromen-7-yl)oxy)acetamide (**43**)* The reaction of ethyl 2-((3-hexyl-4-methyl-2-oxo-2*H*-chromen-7-yl)oxy) acetate (**30**) (1 g, 2.88 mmol) with N^1,N^1-dimethylpropane-1,3-diamine (1.17 g, 11.52 mmol) gave the title compound **43** as a white solid (0.93 g, 80 %) by following the general procedure; m. p.: 136.0–137.1 °C; R_f: 0.47 (MeOH:$CHCl_3$: 1:49); UV (MeOH) λ_{max}: 318 nm; IR (KBr) ν_{max}: 3373.96 (N–H str), 2923.56, 1709.94 (C=O), 1662.74 (NH*CO*–), 1618.85, 1550.06, 1430.12, 1285.53, 1162.34, 1090.23, 871.91, 780.88, 601.50 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): δ 0.88 (t, 3H, $J = 6.96$ Hz, H-6‴), 1.29–1.31 (m, 4H, H-4‴ and H-5‴), 1.36–1.41 (m, 2H, H-3‴), 1.47–1.54 (m, 2H, H-2‴), 1.66–1.70 (m, 2H, H-2″), 2.18 (s, 6H, H-1⁗), 2.38 (s, 3H, C-4′ CH_3), 2.39 (t, 2H, $J = 5.86$ Hz, H-3″), 2.62 (t, 2H, $J = 8.05$ Hz, H-1‴), 3.42–3.47 (m, 2H, H-1″), 4.51 (s, 2H, H-2), 6.80 (d, 1H, $J = 2.20$ Hz, H-8′), 6.83 (dd, 1H, $J = 2.20$ and 8.79 Hz, H-6′), 7.53 (d, 1H, $J = 8.79$ Hz, H-5′), 8.33 (*brs*, 1H, NH) ppm; ^{13}C NMR (100.5 MHz, $CDCl_3$): δ 14.02 (C-6‴), 14.79 (C-4′ *C*H_3), 22.56 (C-5‴), 25.43 (C-2″), 27.53 (C-4‴), 28.70 (C-3‴), 29.31 (C-2‴), 31.64 (C-1‴), 39.43 (C-1″), 45.34 (C-1⁗), 58.86 (C-3″), 67.46 (C-2), 102.15 (C-8′), 111.06 (C-6′), 115.30 (C-10′), 124.57 (C-3′), 125.73 (C-5′), 145.56 (C-4′), 153.43 and 159.06 (C-9′ and C-7′), 161.74 and 167.08 (C-2′ and C-1) ppm; HRMS: *m/z* $[M + Na]^+$ Calculated for $C_{23}H_{34}N_2O_4$: 425.2416; found: 425.2402.

*N-(2-(Dimethylamino)ethyl)-2-((3-hexyl-4-methyl-2-oxo-2H-chromen-7-yl)oxy)acetamide (**44**)* The reaction of ethyl 2-((3-hexyl-4-methyl-2-oxo-2*H*-chromen-7-yl)oxy) acetate (**30**) (1 g, 2.88 mmol) with N^1,N^1-dimethylethane-1,2-diamine (1.01 g, 11.52 mmol) gave the title compound **44** as a white solid (0.89 g, 79 %) by following the general procedure; m. p.: 133.4–135.0 °C; R_f: 0.48 (MeOH:$CHCl_3$: 1:49); UV (MeOH) λ_{max}: 319 nm; IR (KBr) ν_{max}: 3372.39 (N–H str), 2923.11, 1709.01 (C=O), 1661.57 (NH*CO*–), 1619.11, 1552.59, 1430.03, 1287.14, 1160.80, 1089.68, 869.87, 781.53, 602.82, 533.51 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): δ 0.86 (t, 3H, $J = 7.32$ Hz, H-6‴), 1.28–1.31 (m, 4H, H-4‴ and H-5‴), 1.35–1.46 (m, 2H, H-3‴), 1.47–1.54 (m, 2H, H-2‴), 2.22 (s, 6H, H-1⁗), 2.37 (s, 3H, C-4′ CH_3), 2.43 (t, 2H, $J = 5.86$ Hz, H-2″), 2.62 (t, 2H, $J = 8.05$ Hz, H-1‴), 3.39–3.43 (m, 2H, H-1″), 4.53 (s, 2H, H-2), 6.83 (d, 1H, $J = 2.20$ Hz, H-8′), 6.87 (dd, 1H, $J = 2.20$ and 8.75 Hz, H-6′), 7.04 (*brs*, 1H, NH), 7.52 (d, 1H, $J = 8.75$ Hz, H-5′) ppm; ^{13}C NMR (100.5 MHz, $CDCl_3$): δ 14.10 (C-6‴), 14.78 (C-4′ *C*H_3), 22.55 (C-5‴), 27.52 (C-4‴), 28.68 (C-3‴), 29.30 (C-2‴), 31.63 (C-1‴), 36.46 (C-1″), 45.14 (C-1⁗), 57.69 (C-2″), 67.49 (C-2), 102.21 (C-8′), 111.49 (C-6′), 115.39 (C-10′), 124.63 (C-3′), 125.66 (C-5′), 145.54 (C-4′), 153.39 and 158.85 (C-9′ and C-7′), 161.70 and 167.21 (C-2′ and C-1) ppm; HRMS: *m/z* $[M + Na]^+$ Calculated for $C_{22}H_{32}N_2O_4$: 411.2260; found: 411.2260.

*N-(3-(Diethylamino)propyl)-2-((4-methyl-2-oxo-2H-chromen-6-yl)oxy)acetamide(**45**)* The reaction of ethyl 2-((4-methyl-2-oxo-2*H*-chromen-6-yl)oxy)acetate (**31**) (1 g, 3.81 mmol) with N^1,N^1-diethylpropane-1,3-diamine (1.98 g,

15.24 mmol) gave the title compound **45** as a white solid (1 g, 76 %) by following the general procedure; m. p.: 107. 2–108.9 °C; R_f: 0.44 (MeOH:$CHCl_3$: 1:49); UV (MeOH) λ_{max}: 318 nm; IR (KBr) ν_{max}: 3249.59 (N–H str), 2956.25, 1728.41 (C=O), 1678.24 (NH*CO*–), 1615.95, 1531.30, 1393.28, 1275.85, 1157.55, 1073.27, 846.08, 589.64 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$): δ 0.98 (t, 6H, J = 7.33 Hz, H-2‴′), 1.63–1.69 (m, 2H, H-2″), 2.39 (d, 3H, J = 0. 92 Hz, C-4′ CH_3), 2.48 (q, 4H, J = 7.33 Hz, H-1‴′), 2.51 (t, 2H, J = 5.95 Hz, H-3″), 3.42–3.47 (m, 2H, H-1″), 4.51 (s, 2H, H-2), 6.16 (d, 1H, J = 0.92 Hz, H-3′), 6.28 (d, 1H, J = 2.75 Hz, H-5′), 6.85 (dd, 1H, J = 2.75 Hz and 8. 70 Hz, H-7′), 7.53 (d, 1H, J = 8.70 Hz, H-8′), 8.46 (*brs*, 1H, NH) ppm; ^{13}C NMR (100.5 MHz, $CDCl_3$): δ 11.53 (C-2‴′), 18.62 (C-4′ *C*H_3), 25.19 (C-2″) 39.8 (C-1″), 46.98 (C-1‴′), 52.40 (C-3″), 67.60 (C-2), 102.62 (C-5′), 111.33 (C-3′), 112.57 (C-7′), 125.93 (C-8′ and C-10′), 152.20, 155.04 and 160.16 (C-9′, C-6′ and C-4′), 160.90 and 166.79 (C-2′ and C-1) ppm; HRMS: *m/z* $[M + Na]^+$ Calculated for $C_{19}H_{26}N_2O_4$: 369.1790; found: 369.1789.

*N-(3-(Dimethylamino)propyl)-2-((4-methyl-2-oxo-2H-chromen-6-yl)oxy)acetamide (**46**)* The reaction of ethyl 2-((4-methyl-2-oxo-2*H*-chromen-6-yl)oxy)acetate (**31**) (1 g, 3. 81 mmol) with N^1,N^1-dimethylpropane-1,3-diamine (1. 55 g, 15.24 mmol) gave the title compound **46** as a white solid (0.89 g, 73 %) by following the general procedure; m. p.: 119.7–118.8 °C; R_f: 0.46 (MeOH:$CHCl_3$: 1:49); UV (MeOH) λ_{max}: 272 nm; IR (KBr) ν_{max}: 3467.99 (N–H str), 2815.93, 1715.96 (C=O), 1648.52 (NH*CO*–), 1574.59, 1432. 04, 1245.18, 1173.60, 1058.26, 929.85, 838.82, 610. 28 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.63–1.69 (m, 2H, H-2″), 2.15 (s, 6H, H-1‴′), 2.23 (t, 2H, J = 6.40 Hz, H-3″), 2.39 (d, 3H, J = 0.92 Hz, C-4′ CH_3), 3.39–3.44 (m, 2H, H-1″), 4.49 (s, 2H, H-2), 6.29 (d, 1H, J = 0.92 Hz, H-3′), 7. 00 (d, 1H, J = 2.75 Hz, H-5′), 7.08 (dd, 1H, J = 2.75 and 9. 16 Hz, H-7′), 7.27 (d, 1H, J = 9.16 Hz, H-8′) 8.15 (*brs*, 1H, NH) ppm; ^{13}C NMR (100.5 MHz, $CDCl_3$): δ 18.63 (C-4′ *C*H_3), 25.66 (C-2″) 39.13 (C-1″), 45.33 (C-1‴′), 58.55 (C-3″), 67.86 (C-2), 108.28 (C-5′), 115.77 (C-3′), 118.31 (C-7′), 119.21 (C-8′), 120.55 (C-10′), 148.43, 151.62 and 153.76 (C-9′, C-6′ and C-4′), 160.61 and 167.47 (C-2′ and C-1) ppm; HRMS: *m/z* $[M + Na]^+$ Calculated for $C_{17}H_{22}N_2O_4$: 341. 1477; found: 341.1490.

*N-(2-(Dimethylamino)ethyl)-2-((4-methyl-2-oxo-2H-chromen-6-yl)oxy)acetamide (**47**)* The reaction of ethyl 2-((4-methyl-2-oxo-2*H*-chromen-6-yl)oxy)acetate (**31**) (1 g, 3. 81 mmol) with N^1,N^1-dimethylethane-1,2-diamine (1.37 g, 15.24 mmol) gave the title compound **47** as a white solid (0. 87 g, 75 %) by following the general procedure; m. p.: 116. 5–117.4 °C; R_f: 0.41 (MeOH:$CHCl_3$: 1:49); UV (MeOH) λ_{max}: 272 nm; IR (KBr) ν_{max}: 3483.31 (N–H str), 3266.03, 2831.81, 1711.00 (C=O), 1652.01 (NH*CO*–), 1573.45, 1552. 15, 1431.37, 1246.74, 1168.79, 1060.91, 930.85, 837.95, 607.85, 548.75 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$): δ 2.20 (s, 6H, H-1‴′), 2.39 (s, 3H, C-4′ CH_3), 2.43 (t, 2H, J = 5. 95 Hz, H-2″), 3.38–3.43 (m, 2H, H-1″), 4.52 (s, 2H, H-2), 6. 29 (s, 1H, H-3′), 7.04 (d, 1H, J = 2.75 Hz, H-5′), 7.08 (*brs*, 1H, NH), 7.14 (dd, 1H, J = 2.75 and 9.16 Hz, H-7′), 7.27 (d, 1H, J = 9.16 Hz, H-8′) ppm; ^{13}C NMR (100.5 MHz, $CDCl_3$): δ 18.64 (C-4′ *C*H_3), 36.45 (C-1″), 45.15 (C-1‴′), 57. 78 (C-2″), 68.02 (C-2), 108.77 (C-5′), 115.78 (C-3′), 118.27 (C-7′), 119.46 (C-8′), 120.55 (C-10′), 148.54, 151.69 and 153.61 (C-9′, C-6′ and C-4′), 160.63 and 167.62 (C-2′ and C-1) ppm; HRMS: *m/z* $[M + Na]^+$ Calculated for $C_{16}H_{20}N_2O_4$: 327.1321; found: 327.1331.

Biology

Pathogens

Various pathogenic strains of bacteria namely, *Bacillus cereus* (MTCC 430), *Bacillus subtilis* (MTCC 121), *Escherichia coli* (MTCC 1586), *Pseudomonas aeruginosa* (MTCC 741), *Klebsiella pneumoniae* (MTCC 3384), *Salmonella enterica typhimurium* (MTCC 98) were procured from Institute of Microbial Technology, Chandigarh (India). *Trichophyton rubrum* was a clinical isolate obtained from Sir Ganga Ram Hospital, Delhi (India).

Materials

Mueller-Hinton agar, Nutrient broth, Sabouraud dextrose agar, Sabouraud dextrose broth, sterile filter paper discs (6 mm) were procured from HiMedia, Mumbai, India. Gentamicin, Iodonitrotetrazolium chloride (INT), Amphotericin B, Triton X-100 and DMSO were purchased from Sigma-Aldrich Chemicals Pvt. Ltd., USA.

Antibacterial activity assay

The inhibitory potency of bacterial growth by the synthesized compounds was determined using Kirby-Bauer disc susceptibility test with some minor modifications (Pathak *et al.*, 2013; Yadav *et al.*, 2014). The samples at a concentration of 250 µg/disc were impregnated on a 6 mm sterile filter paper discs placed in the respective grid of each Mueller-Hinton agar plates already seeded with the target test organisms and then kept at 37 °C for overnight incubation. Gentamicin was used in the assay as a positive control. The zone of inhibition was recorded using Himedia Hiantibiotic Zone Scale. All the assays were carried out in triplicates.

Quantitative evaluation of antibacterial activity was performed by estimating the minimum inhibitory concentration (MIC) of the compounds using micro-well serial dilution method with some minor modifications as described previously by Pathak and co-workers (Pathak *et al.*, 2013). The bacterial strains were cultured overnight at 37 °C in nutrient broth and used as an inoculum. The wells of 96-well flat-bottom culture plates with various dilutions of the compounds (2–0.0001 mg/ml) were treated with 50 µl of mid-log phase inoculums ($OD_{600} = 0.4$–0.6) of the relevant culture and incubated at 37 °C for overnight using gentamicin as a positive control. Absorbance at 600 nm was measured. An additional control well containing an equivalent amount of solvent without any sample was used as a negative control. As an indicator of bacterial growth, 50 µl of 0.25 mg/ml iodonitrotetrazolium chloride (INT) was added to the wells and incubated at 37 °C for 30 min. The pervasiveness of live bacterial cells was indicated by significant intensity of red colour formation in microtiter plate, while clear solution signified the suppression of bacterial growth. All the assays were performed in triplicate.

Haemolytic activity assay

In order to have an insight of the in vitro human RBC toxicity, the active compounds were subjected to haemolytic assay as described by Ciornei et al., 2005 and Dobrovolskaia et al., 2008 with some minor modifications. Fresh human erythrocytes washed and centrifuged with phosphate buffer saline (PBS) thrice, were suspended in PBS (pH 7.4) to make 4 % (v/v) solution. A 100 µl of cell suspension, added to each well of 96-well flat-bottom tissue culture plate along with 100 µl of compounds of different concentrations, were incubated for 1 h at 37 °C and centrifuged at 1000 g for 5 min. Aliquots (100 µl) of supernatant were transferred to fresh 96-well microtitre plate and OD_{540} was recorded using ELISA plate reader (Bio-Teck Instruments, Inc). The absorbance of erythrocyte suspension treated with Triton X-100 was taken as 100 % haemolysis and used as positive control. On the other hand, cell suspension treated with PBS was used as a negative control. Percentage of haemolysis was calculated using the following formula:

$$\%\ \text{Haemolysis} = \left(\frac{(OD_{540\,nm}\ \text{of Sample} - OD_{540\,nm}\ \text{of PBS})}{(OD_{540\,nm}\ \text{of 0.1 \% Triton X} - 100 - OD_{540\,nm}\ \text{of PBS})}\right) \times 100.$$

Antifungal activity assay

The in vitro antifungal screening of compounds was achieved using *Trichophyton rubrum* as a model pathogen. The antifungal activity was assessed by disc diffusion assay (DDA) and percent growth inhibition assay (PGIA) with some minor modifications (Gupta *et al.*, 2012). For disc diffusion, sterile filter paper discs, 6 mm in diameter, placed on the surface of Sabouraud dextrose agar already seeded with the isolated conidia of target test organism, were impregnated with the samples at a concentration of 250 µg/disc. The zone of inhibition was examined after 72 h of incubation at 30 °C. In percent growth inhibition assay, the wells in microtitre plate, treated with the dilutions of compounds (2-0.0001 mg/ml) and inoculated with 1×10^6 of conidia isolated from *T. rubrum*, were incubated at 30 °C for 72 h and OD_{650} was measured to determine cell growth. Amphotericin B and an equivalent amount of solvent were used as positive and negative controls, respectively.

$$\text{PGI} = 100 - (\text{OD in drug treated well}/\text{OD in control well}) \times 100.$$

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