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Synthesis and Characterization of Piperine Analogs as Potent *Staphylococcus aureus* NorA Efflux Pump Inhibitors

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ABSTRACT

The efficient synthesis of novel 2,2-dimethyl-chroman-6-yl pentadienoic acid amides (**7a-e**) as synthetic piperine analogs has been established by the condensation of 5-(2,2-dimethyl-chroman-6-yl)-4-methyl-penta-2,4-dienoic acid **6** with various aromatic amines. All the synthesized piperine analogs were bioevaluated for their potential as inhibitors of multidrug efflux pump NorA overexpressing *Staphylococcus aureus* SA 1199B. Out of all the prepared analogs, 5-(2,2-dimethyl-chroman-6-yl)-4-methyl-penta-2,4-dienoic acid ethyl ester **5** and 5-(2,2-Dimethyl-chroman-6-yl)-4-methyl-2E,4E-pentadienoic acid pyrrolidide **7d** were found promising. The active compounds were also evaluated for their synergistic effect with ciprofloxacin, whose results substantially increase the activity of ciprofloxacin against both Nora overexpressing and wild type *Staphylococcus aureus* isolates. Structures of the synthesized compounds have been elucidated on the basis of spectral data (IR, ^{1}H NMR and Mass analysis).

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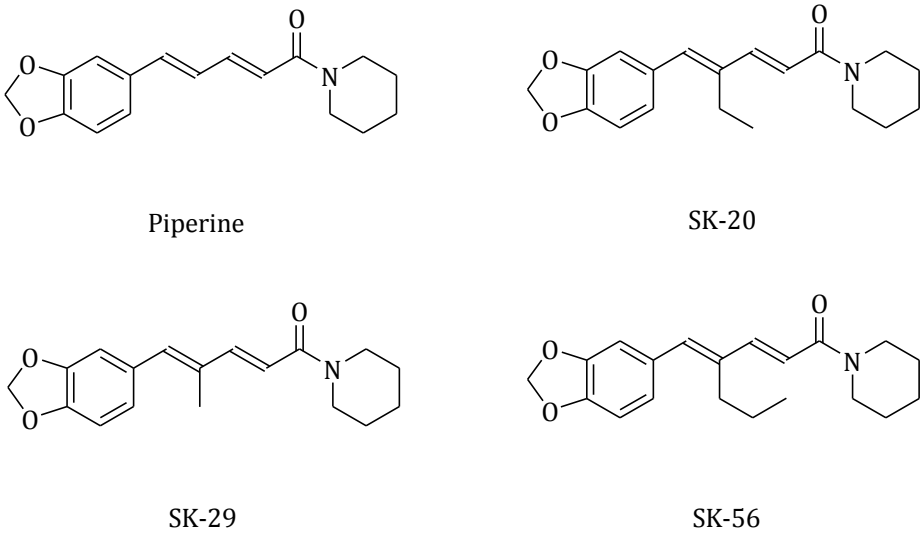
Graphical Abstract



Piperine analogs as potent efflux pump inhibitors

Introduction

Resistance of microorganisms to many classes of antibiotics and other drugs has become a serious problem of public health. Multidrug resistance (MDR) is increasingly prevalent, especially in gram-positive pathogens such as *Staphylococcus aureus, Streptococcus pneumoniae* and *Enterococcus* spp. [1]. According to the world wide surveillance studies, multidrug resistance phenomenon is found in many species of bacteria, fungi and tumor cells mediated through three mechanisms namely target modification [2-3], enzymatic inactivation of the antibiotic [4] or default of its accumulation within the cell (lack of entry or efflux systems), whereas in some cases resistance to given antibacterial drugs is intrinsic to bacteria and has arisen due to the antibiotic pressure [5], it has been acquired by many organisms. Efforts are made in order to identify original bacterial targets [6] and development of new generations of known antibiotics, such as ketolides [7]. However, only one class of new antibacterials, namely the oxazolidinones [8], has recently reached the market. Moreover, bacterial multidrug efflux pumps were found to be the major contributors of microbial resistance to several classes of antibiotics [9, 10]. Efflux of an antibiotic confers an environment of greater selection of resistant mutants having mutations in drug targets [11-13]. *Khan* et al., have previously described the role of piperine, a major constituent of *Piper nigrum*, as a putative bacterial EPI [14]. Based on these findings, new piperine analogues (named as SK series) were

synthesized and out of them SK-20, SK-29 and SK-56, proved to be much more potent efflux pump inhibitors than piperine and its analogs [15]. These molecules may prove to be useful in augmenting the antibacterial activities of fluoroquinolones in a clinical setting [16]. Accordingly, the present work provides a process for the preparation of novel piperine analogs 2,2-dimethyl-chroman-6-yl-2E, 4E-pentadienoic acid amides as efflux pump inhibitors.

Experimental

Material and methods

General

Melting points were determined by the open capillary tube method and are uncorrected. FTIR spectra were recorded on Perkin Elmer RX1 spectrophotometer using KBr pellets and are expressed in cm^{-1}. The ^1H NMR spectra were recorded on Brucker 200 MHz spectrometer in ($CDCl_3$) using TMS as an internal reference and chemical shifts were measured in δ ppm. Mass spectra were recorded on waters QTOF micro LC-MS/MS in 50% acetonitrile (HPLC grade), 50% water (HPLC grade) and 0.1% formic acid. The progress of the reaction was monitored by TLC using 0.2 mm thickness aluminium sheet precoated with Merck Silica gel 60 F_{254} and visualization was done using iodine/UV lamp or ceric sulfate solution for detection of the spots.

Chemicals and reagents

All reagents for the chemical synthesis were commercially procured from Sigma Aldrich. Structurally diverse 2,2-dimethyl-chroman-6-yl derivatives were synthesized. Ciprofloxacin was purchased from Sigma Chemical Co. (St Louis, MO, USA). Tetrahydrofuran (THF) was dried in the presence of sodium; and benzophenone and diethyl ether were dried using calcium chloride. All other used organic solvents were of LR grade, dried over anhydrous sodium sulfate and used as received.

Bacterial strains and media

NorA-overproducing *Staphylococcus aureus* strain 1199B was generously provided as a gift by Dr G. W. Kaatz (Wayne State University). Mueller Hinton broth (MHB; Becton–Dickinson, Cockeysville, MD, USA) supplemented with calcium (25 mg/L) and magnesium (12.5 mg/L) was used for MIC determination. Trypticase soy agar (TSA; Becton–Dickinson) was used for culturing bacteria.

Screening for EPIs

To evaluate the efficacy of the prepared analogs as EPIs for NorA inhibition, *Staphylococcus aureus* SA 1199B, a Nora-overexpressing mutant, was employed. The efflux of ethidium bromide, a well

known substrate for NorA, was used as a marker. Assays were performed in 96 well microtitre plates (Nunc, Denmark). The stock solutions of compounds were prepared in 100% dimethyl sulphoxide (DMSO); the highest final concentration of DMSO used in assays (1%, v/v) caused no inhibition of bacterial growth (data not shown). Test compounds (EPIs) were added to a final concentration of 50 mg/L. Ethidium bromide was added to a final concentration of 12.5 mg/L (1/4 MIC for *Staphylococcus aureus* SA 1199B). Bacteria at logarithmic growth phase were suspended in normal saline (0.85%) to an optical density of ≈0.1 at 625 nm corresponding to 1.5×10^8 cfu/mL. This inoculum was further diluted and a final inoculum of 1×10^5 cfu/mL in cation adjusted MHB was added to the plate. Plates were incubated for 18 h at 37 °C and observed visually for growth. Compounds that inhibited the growth were subsequently tested at a 2-fold serially diluted concentration range of 50–0.8 mg/L to obtain the optimal concentration of the compound that altered the MIC.

Determination of biological evaluation of the EPIs

The MIC of ciprofloxacin was determined for *Staphylococcus aureus* 1199B in MHB in the presence of increasing amounts of EPIs by a broth chequerboard synergy method in 96 well microtitre plates using 2-fold serial dilutions. Each candidate EPI was tested at five different concentrations (50 to 3.12 µg/mL) and ciprofloxacin was tested at 10 different concentrations (16 to 0.03 mg/L). The plates were incubated for 18 h at 37 ºC and the wells were assessed visually for growth. The MEC (Minimum effective concentration) was determined to be the minimal concentration of EPI that produced the maximal reduction in MIC of ciprofloxacin. No further decrease in MIC was observed at EPI concentrations greater than the MEC.

Synthetic methods

Synthesis of 2,2-dimethyl-chroman-6-carbaldehyde 2: Orthophosphoric acid (20 mL) was added drop wise to a stirred solution of *p*-hydroxybenzaldehyde **1** (25 g) in hexane (200 mL). To this mixture, isoprene (25 mL) was added drop wise and the reaction mixture was stirred for 12 hrs. After completion of the reaction, the reaction mixture was poured in to water and extracted with ethyl acetate (100 mL) thrice a time, separating the organic layer. The combined organic layer was washed with water (2×20 mL), dried over anhydrous sodium sulfate and concentrated in vacuum and the gummy residue purified by column chromatography using ethyl acetate: hexane (9:1) over silica gel to give **2**, green semisolid mass, yield 30%, ^{1}H NMR ($CDCl_3$): 1.36 (3H, s, -$C(CH_3)_2$), 1.82 (2H, t, -CH_2), 2.83 (2H, t, -CH_2), 6.86 (1H, d, J=8.93, olefinic and Ar-H), 7.63 (1H, s, -CH=C), 9.81 (1H, s, =C-CHO), 7.61 (1H, d, J=6.8, olefinic and Ar-H).

Synthesis of 1-(2,2-dimethyl-chroman-6-yl)-propan-1-ol 3: Solution of compound **2** (5 g) in dry diethylether was added drop wise to the ethereal solution of grignard reagent prepared from magnesium metal (0.631 g) and iodoethane (0.41 g) and the reaction mixture was additionally stirred for another l hour at room temperature. The reaction was further processed by adding saturated aqueous solution of ammonium chloride (10 mL) followed by dilution with water (100 mL). The compound **3** was then extracted from the aqueous layer using ether (2×100 mL) as solvent. The combined organic layer washed with water (2×20 mL) dried over anhydrous sodium sulfate and concentrated in vacuum to give **3**, brown semisolid, yield 90%, IR (KBr): 2972.42 (C-H stretch), 1586.44 (C=C stretch), 3423 (O-H stretch) cm^{-1}. ^1H NMR ($CDCl_3$): 0.92 (3H, t, $-CH_2CH_3$), 1.32 (3H each, s, -C $(CH_3)_2$), 1.76 (2H, m, 2H, $-CH_2CH_3$); 2.7 (2H, t, $-CH_2$), 1.81 (2H, t, $-CH_2$), 4.76 (t, 1H, -CHOH), 6.76(1H, s, =C-CHO), 7.05 (2H, d, J=5.95, olefinic and Ar-H), 6.72 (2H,d, J= 3.98, olefinic and Ar-H), EI-MS *m/z* 233 $[M^+]$.

Synthesis of 3-(2, 2-dimethyl-chroman-6-yl)-2-methyl-propenal 4: A solution of **3** (4 g) in dimethylformamide (10 mL) was added drop wise to the solution of phosphorus oxychloride (8 mL) in DMF (12 mL) with stirring while maintaining the temperature at 0 °C. The reaction mixture was further stirred for 2 hour, then, allowed to attain room temperature followed by heating at 40 °C on an oil bath for 36 hour. After the completion of the reaction as monitored by TLC, contents of the reaction mixture are poured into ice cold water (500 mL), neutralized with dilute alkali solution and saturated by adding sodium chloride. The aqueous layer was, then, extracted with ethyl acetate (3×100 mL), and the combined organic layer was washed with water (2×20 mL), dried over anhydrous sodium sulphate and stripped off the solvent under reduced pressure to furnish crude product **4**, brown semi-solid, yield 76%, IR (KBr): 1672 (C=O stretch), 2930 (C-H stretch), 1568 (C=C stretch) cm^{-1}. ^1H NMR ($CDCl_3$): 7.14 (1H, s, -C=CH), 7.37 (1H, s, =CH), 7.28 (1H, d, J=9.27, olefinic and Ar-H); 6.84(1H, d, J=8.36, olefinic and Ar-H); 9.51 (1H, s, -CHO), 1.84 (2H, t, $-CH_2$); 2.08 (3H, s, $=C-CH_3$); 2.82 (2H, t, $-CH_2$); 1.36 (3H each, s, -C $(CH_3)_2$), EI-MS *m/z* 231 $[M^+]$.

Synthesis of 5-(2,2-dimethyl-chroman-6-yl)-4-methyl-penta-2,4-dienoic acid ethyl ester 5: A mixture of triphenylphosphine (4.7 g) and ethyl bromoacetate (20 mL) in anhydrous dimethoxyethane was refluxed for 2 hour. After refluxing, a solution of sodium hydride (0.5 g) in dry ether was added at 0-5 °C with stirring to obtain intermediate ylide. Then, the ethereal solution of **4** (2.5 g) was added to ylide solution. The reaction mixture was continuously stirred for 72 hour at 40 °C. On cooling, the contents are diluted with ethyl acetate (100 mL) to quench unused sodium hydride. The organic layer was then washed with water (2×30 mL) and concentrated under

reduced pressure to give **5**, yellow gummy mass, yield 74%, IR (KBr): 1682.04 (C=O stretch), 2927.63 (C-H stretch), 1591 (C=C stretch) cm^{-1}. ^1H NMR ($CDCl_3$): 4.19 (2H, q, J=7.1, $-CH_2CH_3$), 7.48 (1H, d, J=15.5, -CH=CH), 7.10 (1H, d, J=4.8, olefinic and Ar-H), 6.77 (1H, d, J=8.62, olefinic and Ar-H), 5.91 (1H, d, J=15.5, -CH=CH), 2.78 (2H, t, $-CH_2$), 6.81 (s, 1H ,-C=CH), 7.15 (1H, s, -C=CH), 1.82 (2H, t, $-CH_2$), 2.05 (3H, s, $=C-CH_3$), 1.34 (3H, s, $-C(CH_3)_2$), 1.28 (3H, t, $-CH_2CH_3$), EI-MS *m/z* 301 [M^+].

Synthesis of 5-(2,2-dimethyl-chroman-6-yl)-4-methyl-penta-2,4-dienoic acid 6: Compound **5** obtained from previous step was further hydrolyzed directly without purification with 10% methanolic potassium hydroxide solution on a water bath for 3 hour, the reaction mixture was then concentrated and diluted with water (120 mL). The aqueous solution was acidified with 2N hydrochloric acid solution. The resulting precipitate was filtered, washed with water and air dried, and the residue was crystallized from ethyl acetate: benzene (19:1) to furnish compound **6**, yellow colored solid, yield 69%, m.p. 148-150 °C. IR (KBr): 1682 (C=O stretch), 2927.62 (C-H stretch), 1591 (C=C stretch) cm^{-1}. ^1H NMR ($CDCl_3$): 7.5 (1H, d, J=15.4, -CH=CH), 7.36 (1H, s, -C=CH), 7.15 (1H, d, J=8.0, olefinic and Ar-H), 6.78 (1H, d, J=8, olefinic and Ar-H), 7.1 (1H, s, -C=CH), 5.90 (1H, d, J=15.4, -CH=CH), 2.79 (2H, t, $-CH_2$), 1.82 (2H, t, $-CH_2$), 2.09 (3H, s, $=C-CH_3$), 1.30 (3H each, s, $-C(CH_3)_2$), EI-MS *m/z* 272 [M^+].

General procedure for the synthesis of compounds 7a-e: Freshly distilled thionyl chloride (0.5 mL) was added to a stirred solution of **6** (0.6 g) in dichloromethane (50 mL). The reaction mixture was refluxed on a water bath for 1 hour. Solvent was removed from the acid chloride under reduced pressure along with excess of thionyl chloride. A solution of appropriate amine (2.2 mmol) in dichloromethane was added drop wise to the acid chloride prepared above and the reaction mixture was further stirred for another 1 hour and the reaction progress was monitored by TLC. After completion of the reaction, the organic layer was made free from excess of aromatic amine using dilute hydrochloric acid solution. The organic layer was washed with water, dried and concentrated under vacuum. The resulting residue was collected and crystallized from ethyl acetate: hexane (9:1) to give the title compounds.

Analytical data of 5-(2,2-dimethyl-chroman-6-yl)-4-methyl-2E,4E-pentadienoic acid butylamide 7a: pale yellow liquid, yield 86%. IR (KBr): 3280, 1493.68 (N-H stretch), 1645.78 (C=O stretch), 3073.55 (C-H stretch), 1573 (C=C stretch), 1121.10(C-N stretch) cm^{-1}. ^1H NMR ($CDCl_3$): 0.94 (3H, t, $-CH_2CH_3$), 1.30 (3H each, s, $-C(CH_3)_2$), 1.74 (2H, t, $-CH_2$), 3.33 (2H, m, $-CH_2$), 2.08 (3H, s, $=C-CH_3$), 7.33 (1H, d, J=15.2, -CH=CH), 5.8 (1H, d, J=15.1 , -CH=CH); 1.45 (2H, m, 2H, $-CH_2CH_3$), 2.7

(2H, t, 2H, -CH_2), 6.99 (1H, d, J=8.4, olefinic and Ar-H), 6.69 (1H, d, J=8.45, olefinic and Ar-H), 7.19 (s,1H, -C=CH), 1.55 (2H, m, -CH_2CH_2), 6.64 (1H, s, =CH), EI-MS *m/z* 328 [M^+].

Analytical data of 5-(2,2-dimethyl-chroman-6-yl)-4-methyl-2E,4E-pentadienoic acid (3-cyano-phenyl)-amide 7b: brown colored solid, yield 83%, m.p. 60-62 °C. IR (KBr): 3327, 1588 (N-H stretch), 1662.68 (C=O stretch), 2975.33, 3018.90 (C-H stretch), 1547.52 (C=C stretch), 1120.38, 2231.55 (C-N stretch) cm^{-1}. ^{1}H NMR ($CDCl_3$): 8.0 (1H, s, -NH), 7.83 (1H, d, J=2.01, -Ar″H), 7.79 (1H, d, J=2.13,-Ar″H), 7.57 (1H, d, J=15.0, -CH'=CH'), 5.99 (1H, d, J=15.0, -CH'=CH'), 1.82 (2H, t, -CH_2), 7.48 (1H, m, -C=CH″), 7.36 (1H, s, =CH), 7.20 (1H, d, J=8.0, olefinic and Ar-H), 1.33 (3H each, s, -$C(CH_3)_2$), 2.04 (3H, s, =C-CH_3), 2.79 (2H, t, -CH_2), 6.79 (1H, d, J=9.0, olefinic and Ar-H); 7.13 (1H, s, -CH=C), 7.61(1H, s, -C=CH″), EI-MS *m/z* 373 [M^+].

Analytical data of 5-(2,2-dimethyl-chroman-6-yl)-4-methyl-2E,4E-pentadienoic acid benzothiazol-6-ylamide 7c: brown color solid, yield 83%, , m.p. 62-64 °C. IR (KBr): 3386.52, 1493.36 (N-H stretch), 1600.83 (C=O stretch), 2973.07 (C-H stretch), 1573.33 (C=C stretch), 1121.25 (C-N stretch) cm^{-1}. ^{1}H NMR ($CDCl_3$): 2.79 (2H, t, -CH_2), 1.82 (2H, t, -CH_2), 1.35 (3H, s, -$C(CH_3)_2$), 1.26 (s,3H, =C-CH_3), 6.19 (1H,d, J=15.3, -C=CH'), 6.85 (1H, s, =CH), 5.99 (1H, d, J=15.4, =CH'CO), 7.37 (1H, d, J=6.03, -CH'=CH'), 7.62 (1H, d, J=8.8, -ArH″), 7.76 (1H, s, =CH″), 7.71 (1H, s, -CH″=C), 6.80 (1H, d, J=8.49, olefinic and Ar-H), 6.77 (1H, d, J=5.03, olefinic and Ar-H), EI-MS *m/z* 405 [M^+].

Analytical data of 5-(2,2-Dimethyl-chroman-6-yl)-4-methyl-2E,4E-pentadienoic acid pyrrolidide 7d: brown color solid, yield 86%, m.p.140-142 °C. IR (KBr): 3399 (N-H stretch), 1638.18 (C=O stretch), 2972.05 (C-H stretch), 1492.97 (C=C stretch), 1120.35 (C-N stretch) cm^{-1}. ^{1}H NMR ($CDCl_3$): 2.78 (2H, t, -CH_2), 3.58 (2H each, t, -NCH_2″H), 7.49 (1H, d, J=15.0, -C=CH'); 7.11 (1H, d, J=7.36, olefinic and Ar-H), 1.30 (3H each, s, -$C(CH_3)_2$), 6.77 (1H, d, J=8.43, olefinic and Ar-H), 7.26 (1H, s, -C=CH), 1.93 (3H, s, =C-CH_3), 6.10 (1H, d, J=15.0, =CH'CO), 6.5 (1H, s, -C=CH'), 1.7 (2H each, m, -CH_2″), 2.0 (2H, t, -CH_2), EI-MS *m/z* 326 [M^+].

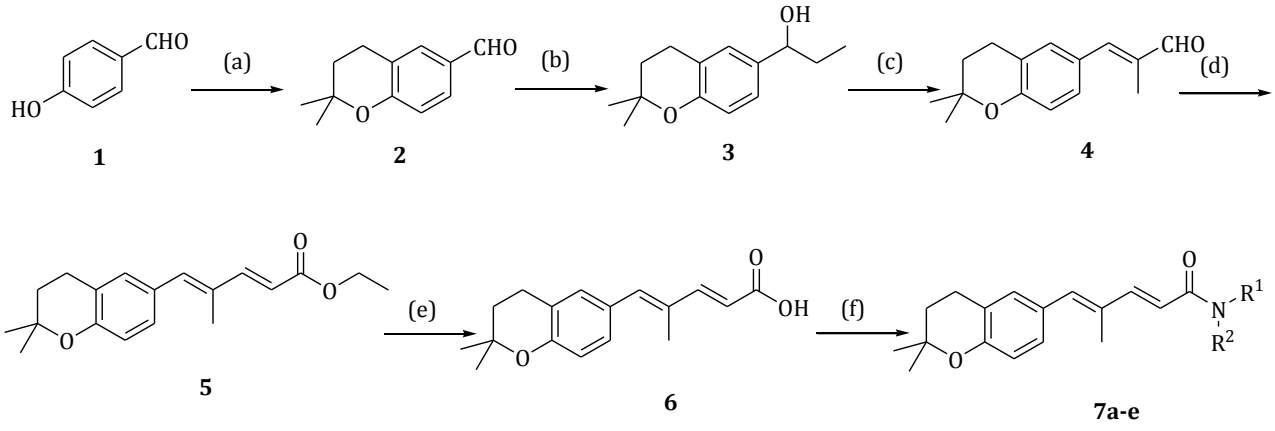
Analytical data of 5-(2,2-Dimethyl-chroman-6-yl)-4-methyl-penta-2E,4E-dienoic acid (4-methoxy-phenyl)-amide 7e: grey color solid, yield 72%, m.p.178-180 °C. IR (KBr): 3383 (N-H stretch), 1650.50 (C=O stretch), 2925.99 (C-H stretch), 1573.30 (C=C stretch), 1121.22 (C-N stretch) cm^{-1}. ^{1}H NMR ($CDCl_3$): 3.73 (3H, s, -OCH_3), 7.54 (2H, d, J=7.54, olefinic and Ar″H), 7.42 (1H, d, J=14.6 Hz, -CH'=CH), 1.30 (3H each, s, $C(CH_3)_2$), 7.26 (1H, s, =CH),7.13 (1H, d, J=8.8, olefinic and Ar-H), 6.88 (1H each, d, J=7.7, olefinic and Ar-H″), 2.78 (2H, t, -CH_2), 5.99 (1H, d, J=14.6, -CO=CH'),

6.79 (1H, d, J=9.9, olefinic and Ar-H″), 1.82 (2H, t, -CH_2), 2.09 (3H, s, =CH-CH_3), 6.76 (1H, s, =CH), EI-MS *m/z* 378 [M^+].

Result and Discussion

Chemistry

Piperine analogs were prepared as described in Scheme 1. The starting material *p*-hydroxybenzaldehyde **1** on the reaction with isoprene in presence of orthophosphoric acid in hexane produces 2,2-dimethyl-chroman-6-carbaldehyde **2** which, on treatment with ethereal solution of ethylmagnesium iodide gives 1-(2,2-dimethyl-chroman-6-yl)-propan-1-ol **3**. The compound **3** were treated with phosphorus oxychloride in dimethylformamide through Vilsmeier Haack reaction produced 3-(2,2-dimethyl-chroman-6-yl)-2-methyl-propenal **4** which on reaction with triphenylphosphine and ethyl bromoacetate in presence of sodium hydride afforded 5-(2,2-dimethyl-chroman-6-yl)-4-methyl-penta-2,4-dienoic acid ethyl ester **5** through Wittig reaction. The compound **5** was further hydrolysed with 10% methanolic potassium hydroxide solution and yielded 5-(2,2-dimethyl-chroman-6-yl)-4-methyl-penta-2,4-dienoic acid **6**. The target 2, 2-dimethyl-chroman-6-yl pentadienoic acid amides analogs **7a-e** was prepared by the condensation of compound **6** with various selected amines. The purity of the compounds was monitored by TLC using ethyl acetate: hexane (9:1) and the structures of all the newly synthesized compounds were elucidated with ^{1}H-NMR, FT-IR and Mass spectroscopy. Physical data of the synthesized compounds is reported in Table 1.



Scheme1. Showing preparation of piperine analogs**;** (a) isoprene, orthophosphoric acid; (b) C_2H_5I, Mg, Et_2O; (c) DMF, $POCl_3$; (d) $Br(PPh_3)CH_2COOEt$, C_6H_6, NaH; (e) NaOH/MeOH, HCl; (f) $SOCl_2$, C_6H_6, NHR^1R^2

Table 1. Physical data of the compounds **5, 6,** and **7a-e**

Compounds	R^1	R^2	R^1+R^2	Yield	M.P. (°C)	Mol. formula
5	-	-	-	74	-	$C_{19}H_{24}O_3$
6	-	-	-	69	148-150	$C_{17}H_{20}O_3$
7a	H	butylamine	-	86	-	$C_{21}H_{29}O_2N$
7b	-	-	3-amino benzonitrile	83	60-62	$C_{24}H_{24}O_2N_2$
7c	-	-	6-amino benzothiazole	83	62-64	$C_{24}H_{24}O_2N_2S$
7d	-	-	pyrrolidine	86	140-142	$C_{21}H_{27}O_2N$
7e	H	*o*-anisidine	-	72	178-180	$C_{24}H_{27}O_3N$

Biological activities

The prepared analogs **5, 6** and **7a-e** were bio evaluated for their potentiation of the anti infective activity of ciprofloxacin in *Staphylococcus aureus* 1199B over expressing the targeted Nor A efflux pump inhibitors. The results of the potentiating anti infective screening of the tested compounds are summarized in Tables 2. The MIC of ciprofloxacin alone was found to be 8 µg/mL. Out of all synthesized compounds only two 5-(2,2-dimethyl-chroman-6-yl)-4-methyl-penta-2,4-dienoic acid ethyl ester (**5**) and 5-(2,2-Dimethyl-chroman-6-yl)-4-methyl-2E,4E-pentadienoic acid pyrrolidide (**7d**) at an MEC of 50 µg/mL and 25 µg/mL reduced the MIC of ciprofloxacin by 2 fold.

Table 2. Potentiation of the anti-infective effect of ciprofloxacin by EPI's **5, 6,** and **7a-e**

Organism: *Staphylococcus aureus* 1199B (Nora over expressed)

Sr. No.	Compound	MIC (µg/ml)						
		Ciprofloxacin alone	Cipro with EPI expressed in µg/ml					Reduction (n fold) in MIC of ciprofloxacin
			+50	+25	+12.5	+6.25	+3.12	
1	5	8	4	4	8	8	8	2
2	6	8	8	8	8	8	8	-
3	7 a	8	8	8	8	8	8	-
4	7 b	8	8	8	8	8	8	-
5	7 c	8	8	8	8	8	8	-
6	7 d	8	4	4	8	8	8	2
7	7 e	8	8	8	8	8	8	-

Conclusion

The synthesis of series of novel 2,2-dimethyl-chroman-6-yl pentadienoic acid amides as synthetic analogs of piperine was achieved using synthetic strategy. Out of all the prepared analogs, the two compounds namely 5-(2,2-dimethyl-chroman-6-yl)-4-methyl-penta-2,4-dienoic acid ethyl ester **5** and 5-(2,2-Dimethyl-chroman-6-yl)-4-methyl-2E,4E-pentadienoic acid pyrrolidide **7d** were found to be effective against Nor A overexpressing *Staphylococcus aureus* SA 1199B as multidrug efflux pump inhibitors. In addition, the active compounds also show synergistic and potentiating effect with ciprofloxacin. In the amide group, pyrrolidide derivative was found to be the most active one and also as a replacement for pyrrolidide moiety by anilinyl or substituted anilinyl moiety shows no synergistic effect with ciprofloxacin and therefore didn't prove to be successful EPIs.

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