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Piperine analogs as potent *Staphylococcus aureus* NorA efflux pump inhibitors

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ABSTRACT

Based on our recent findings that piperine is a potent *Staphylococcus aureus* NorA efflux pump inhibitor (EPI), 38 piperine analogs were synthesized and bioevaluated for their EPI activity. Twenty-five of them were found active with potentiating activity equivalent or more than known EPIs like reserpine, carsonic acid and verapamil. The inhibitory mechanism of the compounds was confirmed by efflux inhibition assay using ethidium bromide as NorA substrate. The present communication describes the synthesis, bioevaluation and structure related activity of these efflux pump inhibitors.

1. Introduction

Emergence of multi drug resistant (MDR) pathogenic bacteria is proving a menace for the management of the bacterial infections and this MDR phenomenon[1,2] that occur (independently or synergistically) through several mechanisms[3–6] is responsible for reducing or preventing the accessibility of the drug to the target by decreasing the transport of the antibiotic into the cell or by enhancing the efflux of the drug from the cell to the outside medium resulting in a low or ineffective concentration of the drug found in many species of bacteria, fungi, and tumor cell.[7–10] This MDR mechanism also recognizes many structurally unrelated compounds.[11–15] Molecular properties of bacterial multidrug transporters of unlike specific drug resistance transporters and their presence may be there as a part of the detoxifying mechanisms in xenobiotics.[16] Among several MDR transporters encompassing Gram-positive and Gram-negative bacteria, MDR pumps such as NorA transporter[17] (member of the major facilitator family-MF family), considered to be one of the major contributors towards drug effluxing, contributes to the resistance of *Staphylococcus aureus* to wide range of structurally unrelated compounds such as ethidium bromide, acriflavin, quaternary amine compounds, fluoroquinolones, rhodamine-6-G, puromycin, and chloramphenicol by promoting their active extrusion from the cell.[18] However, attempts are being made to evolve an alternative approach wherein natural and synthetic molecules are being identified which when used in combination with anti-infective can restore the activity of the drug molecule/s.[19] Combination of anti-infective amoxicillin with anti-infective resistant inhibitor clavulanic acid is a classical example of that sort.[20] Therefore, development of clinically useful inhibitors that decrease the effectiveness of efflux pumps would represent a significant advance to provide successful treatment of multi drug resistant conditions.

In continuation of our research interest[21–29] towards drug development, we have earlier demonstrated several applications of piperine and piperine analogs such as inhibitors of cytochrome P450 when co-administered with different drugs,[28] besides their property as potent pungent/thermogenic agents,[27] and have, recently, reported the application of piperine as the inhibitor of bacterial NorA efflux pump,[21,29] capable of reducing the minimum inhibitory concentration (MIC) of ciprofloxacin resistant when tested against the strain of *S. aureus* or Methicillin resistant *S. aureus* [MRSA].[21] Based on piperine molecule, further studies towards the development of more potent EPIs have been carried out and in this communication, we report the preparation and identification of piperine analogs as potent EPIs and also discuss the structure–activity relationship of these analogs.

2. Results and discussion

One of the natural products that forms major constituent of Piper nigrum and Piper longum is 5-(3,4-methylenedioxyphenyl)-2*E*,4*E*-pentadienoic acid piperidine amide[27] commonly known as piperine and like many other amides both from natural and synthetic sources which possess many biological activities,[30–34] this

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amide also exhibits several biological activities such as P-gp inhibitor activity,[35] as melanocyte replication stimulant[36] and bioavailability enhancer activity (reported from our institute).[37] Of late, an important property that has been recognized with aromatic amides is that of they being efflux pump inhibitors of bacteria to check the emergence of multi drug resistance offered by the microorganisms,[38,39] which have also been demonstrated for piperine molecule in the recent studies carried out by us.[21,23] The piperine molecule reduced the MIC of antibiotic ciprofloxacin by twofold when used in combination against *S. aureus* 1199. In order to develop more potent efflux pump inhibitor/s than piperine, we have been exploring different strategies cum modifications of this molecule.[23,24,26,29] Piperine, is made up of a 1,3-benzodioxol group (represented as compartment A), a pentadienoic acid side chain (represented as compartment B), and piperidine as the amine moiety (represented as compartment C) as shown in Figure 1.

Modification of compartment B of piperine molecule (**1**) by way of introduction of an alkyl substituent at C_4 position, or replacement of 1,3-benzodioxol by other substituted phenyl moiety has been recently carried out by us. The modification incurred generated a new series of compounds[29] (Fig. 2) and on bioevaluation, several of these molecules exhibited potent efflux pump inhibitory activity showing fourfold reduction in MIC of ciprofloxacin against *S. aureus* 1199 compared to piperine which showed only twofold reduction.[23,29] The QSAR studies of these molecules have also been documented by us.[25]

In the present study, we have carried out the modifications in the compartment C in the form of replacement of the piperidine moiety by aliphatic and aromatic amines and have studied the influence of these substituent's on the overall efflux pump inhibitory activities of the synthesized molecules co-administered with ciprofloxacin and tested against *S. aureus* 1199 and NorA overexpressing *S. aureus* 1199B.

Piperic acid (**2**) used as the starting material for the preparation of piperine analogs was obtained by the saponification of naturally occurring piperine (**1**) isolated from the methanolic extract of *P. nigrum*. Conversion of piperic acid (**2**) into its acid chloride followed by the condensation of the latter with an appropriate amine (Scheme 1) afforded piperine analogs (**3–32**) in overall yields of 60–93%.

The MIC of the synthesized molecules was determined so as to use these molecules at concentration devoid of antibacterial activity, a prerequisite of any compound to be used as safe EPIs. The amide molecules (including the saturated molecules **33–38**) were used in combination with antibiotic ciprofloxacin and bioevaluated against *S. aureus* 1199 and NorA overexpressing *S. aureus* 1199B.[40–42] Along with these synthetic molecules, piperine and three known efflux pump inhibitors namely carsonic acid,[43] reserpine,[44] and verapamil[45] were also used for the comparative studies. Ciprofloxacin alone showed MIC at 0.25 μg/mL and 8 μg/mL against *S. aureus* 1199 and NorA overexpressing *S. aureus* 1199B, respectively. Among the library of 38 molecules used in combination with ciprofloxacin and tested against *S. aureus* 1199, only compounds **8**, **13**, **22**, **26–28**, **31**, and **36** could reduce the MIC of the drug by twofold (as shown in Table 1) and the rest

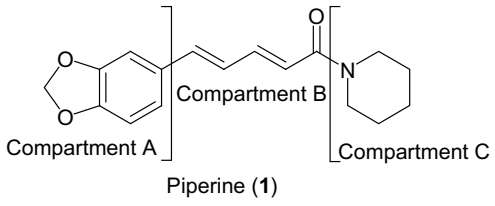


Figure 1.

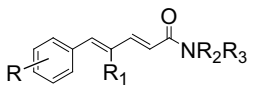


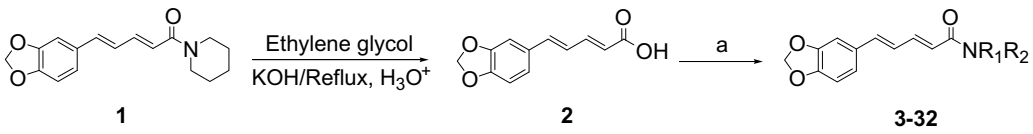
Figure 2. R = substituents; $R_1 = C_1–C_{10}$ aliphatic chain; NR_2R_3 = alkyl, aryl, alky aryl or aryl alkyl amine, etc.

of the molecules failed to potentiate the antibacterial activity of the drug.

However, the combination when tested against NorA overexpressing *S. aureus* 1199B showed encouraging results with molecules **22**, **26–28**, and **31** showing the reduction of MIC of ciprofloxacin by fourfold while twofold reduction was observed for molecules **4**, **6**, **7**, **8**, **10**, **12**, **13**, **17–21**, **23**, **25**, **29**, **30, 32, 33, 35**, and **36**, respectively. In the present studies, the concentration of the synthesized molecules was evaluated at dose dependent concentration that ranged from 0.8 to 50 μg/mL and used in combination with the anti-infective against *S. aureus* 1199B (Table 2). Since a large number of molecules (25 out of 38) exhibited efflux pump inhibitory activity against NorA overexpressing *S. aureus* 1199B, compared to wild type strain *S. aureus* 1199 where only a few were found active, this could be attributed due to the over expression of the target protein (NorA) by *S. aureus* 1199B strain.

In SAR studies, combination of drug with EPIs showed that the nature of the basic moiety is an important factor for its response towards drug potentiation, that is, exhibition of high, low or no potentiation of the drug activity when tested against *S. aureus* 1199B (NorA overexpressing). In comparison to piperine bearing piperidinyl as the basic moiety, the replacement of it by small alkyl chain substituents like isopropyl (**4**), isobutyl (**6**) or diisopropyl (**8**) proved slightly better potentiators than straight chain like *n*-propyl (**3**) or *n*-butyl substituents (**5**) as shown in Table 1. Replacement of piperidinyl moiety by anilinyl moiety proved to be a good substituent (**13**), but introduction of electron releasing group in the anilinyl moiety annihilated the effect and no potentiation of the drug could be observed for the molecules such as toluidinyl (**14**), anisidinyl (**15**) or 3,4-methylenedioxyanilinyl moieties (**16**). However, introduction of substituent such as 2-hydroxymethyl group in the anilinyl moiety (**19- 21**) (irrespective of the positional isomers *ortho*, *meta*, and *para*) potentiated ciprofloxacin activity. Introduction of electron withdrawing substituent (nitrile group) in the anilinyl moiety (**22** and **23**) showed potentiation of ciprofloxacin (with *m*-isomer proving to be better potentiator than *p*-isomer). Among *o*- and *p*-cyanomethyl anilinyl compounds **24** and **25**, *p*-isomer exhibited better potentiating activity than *o*-isomer. *Ortho*, *meta*, and *para* phenylacetamide moieties as replacement for piperidinyl moiety exhibited the best potentiating effect for the drug and fourfold reduction in the MIC of ciprofloxacin was observed for all the three isomers (**26–28**). Benzamidyl or 2-thiazolyl or 6-thiozolinyl moiety did not prove to be good potentiator of ciprofloxacin (**18**, **29**, and **30**). Three known NorA efflux pump inhibitors namely verapamil, carsonic acid, and reserpine were taken for comparative studies and from the results obtained, several molecules were identified which exhibited better or equivalent potentiating activity to those of known inhibitors as shown in Table 1.

Compounds **1**, **6**, **7**, **22**, **26**, and **31** were subjected to hydrogenation (Scheme 2) and saturated molecules **33–38** thus obtained were bioevaluated. The saturation of the double bonds resulted in lowering of potentiation of ciprofloxacin, for example, lowering or loss of EPI activity was observed for compound **33**, and **36–38**. These results are in agreement with our earlier results obtained for other different set of compounds.[22–24] The above studies



Scheme 1. Reagents: (a) $SOCl_2/C_6H_6$; NHR_1R_2. NR_1R_2 = *n*-propyl amine (**3**), isopropyl amine (**4**), *n*-butyl amine (**5**), isobutyl amine (**6**), *N*,*N*-diethyl amine (**7**), diisopropyl amine (**8**), D-2-amino butanol (**9**), L-2-amino butanol (**10**), morpholine (**11**), *N*-methyl piperazine (**12**), aniline (**13**), *o*-toluidine (**14**), *p*-anisidine (**15**), 3,4-methylenedioxyphenyl amine (**16**), 2-amino-1-phenyl ethanol (**17**), 4-benzamide amine (**18**), *o*-hydroxymethylphenyl amine (**19**), *m*-hydroxymethylphenyl amine (**20**), *p*-hydroxymethylphenyl amine (**21**), *m*-cyanophenyl amine (**22**), *p*-cyanophenyl amine (**23**), *o*-cyanomethylphenyl amine (**24**), *p*-cyanomethylphenyl amine (**25**), *o*-acetylaminophenyl amine (**26**), *m*-acetylaminophenyl amine (**27**), *p*-acetylaminophenyl amine (**28**), benzothiazol-2-yl amine (**29**), benzothiazol-6-yl amine (**30**), 4-benzanilide amine (**31**), and 4′-benzanilide (**32**).

Table 1
Piperine analogs as potent inhibitor of multi drug efflux pump in *S. aureus* 1199B and *S. aureus* 1199 and potentiation of ciprofloxacin MIC

Entry	MEC[a] of EPI	*S. aureus* 1199B MIC[b] of cipro. (μg/mL)			MIC[b] of cipro. (μg/mL)		
		Without EPI	With EPI	Fold reduction	Without EPI	With EPI	Fold reduction
3	>100	8	8	0	0.25	0.25	0
4	25	8	4	2	0.25	0.25	0
5	100	8	8	0	0.25	0.25	0
6	25	8	4	2	0.25	0.25	0
7	25	8	4	2	0.25	0.25	0
8	25	8	4	2	0.25	0.12	2
9	>100	8	8	0	0.25	0.25	0
10	25	8	4	2	0.25	0.25	0
11	>100	8	8	0	0.25	0.25	0
12	25	8	4	2	0.25	0.25	0
13	12.5	8	4	2	0.25	0.12	2
14	>100	8	8	0	0.25	0.25	0
15	>100	8	8	0	0.25	0.25	0
16	>100	8	8	0	0.25	0.25	0
17	12.5	8	4	2	0.25	0.25	0
18	25	8	4	2	0.25	0.25	0
19	25	8	4	2	0.25	0.25	0
20	25	8	4	2	0.25	0.25	0
21	6.25	8	4	2	0.25	0.25	0
22	6.25	8	2	4	0.25	0.12	0
	1.5	8	4	2	0.25	0.25	0
23	25	8	4	2	0.25	0.25	0
24	>100	8	8	0	0.25	0.25	0
25	25	8	4	2	0.25	0.25	0
26	25	8	2	4	0.25	0.12	2
	6.25	8	4	2	0.25	0.25	0
27	12.5	8	2	4	0.25	0.12	2
	6.25	8	4	2	0.25	0.25	0
28	25	8	2	4	0.25	0.12	2
	6.25	8	4	2	0.25	0.25	0
29	6.25	8	4	2	0.25	0.25	2
30	3.12	8	4	2	0.25	0.25	0
31	25	8	2	4	0.25	0.12	0
	3.12	8	4	2	0.25	0.25	0
32	3.12	8	4	2	0.25	0.25	0
33	50	8	4	2	0.25	0.25	0
34	>100	8	8	0	0.25	0.25	0
35	50	8	4	2	0.25	0.25	0
36	>100	8	8	0	0.25	0.25	0
37	50	8	4	2	0.25	0.12	2
38	>100	8	8	0	0.25	0.25	0
Piperine	50	8	4	2	0.25	0.12	2
Reserpine	25	8	2	4	0.25	0.12	2
Carsonic acid	25	8	4	2	0.25	0.12	2
Verapamil	50	8	4	2	0.25	0.12	2

No antibacterial activity of EPIs was observed at 100 μg/mL that was the highest concentration tested.
For determining potentiation of ciprofloxacin, EPI were tested at concentration range of 50–0.8 μg/mL.
[a] MEC, minimum effective concentration.
[b] MIC, minimum inhibitory concentration.

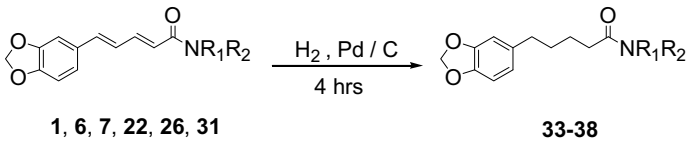
showed that unsaturation apparently does seem to be an important factor responsible for drug potentiation.

The MIC of ciprofloxacin was determined against *S. aureus* 1199 and NorA overexpressing *S. aureus* 1199B in Muller–Hinton Broth in the presence of increasing amounts of efflux pump inhibitors by broth checkerboard synergy method in a microtitre plates using twofold serial dilutions.[46]

The inhibitory mechanism of the compounds was confirmed by efflux inhibition assay using ethidium bromide as substrate of NorA and compounds **22**, and **26** which emerged as the most potent inhibitors among the piperine analogs bioevaluated along with the inactive EPI **5** and reserpine (known EPI). The ethidium bromide fluorescences only when it is bound to nucleic acids inside the cells. The NorA mediated ethidium bromide efflux from the

Table 2
Dose dependent MIC of ciprofloxacin in presence of EPI's against *S. aureus* 1199B

Entry	Cipro. alone	MIC (μg/mL) of ciprofloxacin In presence of EPI's (μg/mL)						
		+50	+25	+12.5	+6.25	+3.12	+1.56	+0.8
3	8	8	8	8	8	8	8	8
4	8	4	4	8	8	8	8	8
5	8	8	8	8	8	8	8	8
6	8	4	4	8	8	8	8	8
7	8	4	4	8	8	8	8	8
8	8	4	4	8	8	8	8	8
9	8	8	8	8	8	8	8	8
10	8	4	4	8	8	8	8	8
11	8	8	8	8	8	8	8	8
12	8	4	4	8	8	8	8	8
13	8	2	4	4	8	8	8	8
14	8	8	8	8	8	8	8	8
15	8	8	8	8	8	8	8	8
16	8	8	8	8	8	8	8	8
17	8	2	4	4	8	8	8	8
18	8	4	4	8	8	8	8	8
19	8	4	4	8	8	8	8	8
20	8	4	4	8	8	8	8	8
21	8	4	4	4	4	8	8	8
22	8	1	2	2	2	4	4	8
23	8	4	4	8	8	8	8	8
24	8	8	8	8	8	8	8	8
25	8	4	4	8	8	8	8	8
26	8	2	2	4	4	8	8	8
27	8	2	2	2	4	8	8	8
28	8	2	2	4	4	8	8	8
29	8	4	4	4	4	8	8	8
30	8	4	4	4	4	4	8	8
31	8	2	2	4	4	4	8	8
32	8	4	4	4	4	4	8	8
33	8	4	8	8	8	8	8	8
34	8	8	8	8	8	8	8	8
35	8	4	8	8	8	8	8	8
36	8	8	8	8	8	8	8	8
37	8	4	8	8	8	8	8	8
38	8	8	8	8	8	8	8	8
Piperine	8	4	4	8	8	8	8	8
Reserpine	8	2	2	4	8	8	8	8
Carsonic acid	8	4	4	8	8	8	8	8
Verapamil	8	4	8	8	8	8	8	8



1, **6**, **7**, **22**, **26**, **31** **33-38**

Scheme 2. Reagents: NR_1R_2 = piperidine (**33**), isobutyl amine (**34**), *N*,*N*-diethyl amine (**35**), *m*-cyanophenyl amine (**36**), *o*-acetylaminophenyl amine (**37**), and 4-benzanilide amine (**38**).

cells resulted in a rapid decrease in fluorescence. Results presented in Figure 3 are the averages from triplicate measurements. As shown in Figure 3, only the control cells without EPIs or in presence of inactive EPI **5** extruded ethidium bromide, resulting in a significant decrease in fluorescence over the time of the assay. Potent inhibitors such as compound **22** and **26** effectively blocked the extrusion of ethidium bromide, which resulted in the decreased fluorescence.

3. Conclusion

Preparation of piperine analogs and their bioevaluation as potent EPIs is described. Five out of 38 synthesized molecules are shown responsible for fourfold lowering of MIC of ciprofloxacin against overexpressing NorA *S. aureus* 1199B which is also observed for known EPI reserpine showing thereby the equipotency of the two set of molecules. In case of other known inhibitors namely carsonic acid, and verapamil, twofold reduction of the MIC is observed. The mechanism of action of these compounds has been established by ethidium bromide fluorescence experiment. In SAR studies, the nature of basic moiety and its effect on the inhibitory property of efflux pump NorA besides the role of unsaturation has also been described. The potential clinical utility of these potent EPIs molecules warrants further investigations.

4. Experimental

4.1. General methods

All reagents for chemical synthesis were purchased from Sigma–Aldrich and used as received. Piperine and carsonic acid were isolated from the methanol extract of *P. nigrum* and *Rosmarinus*

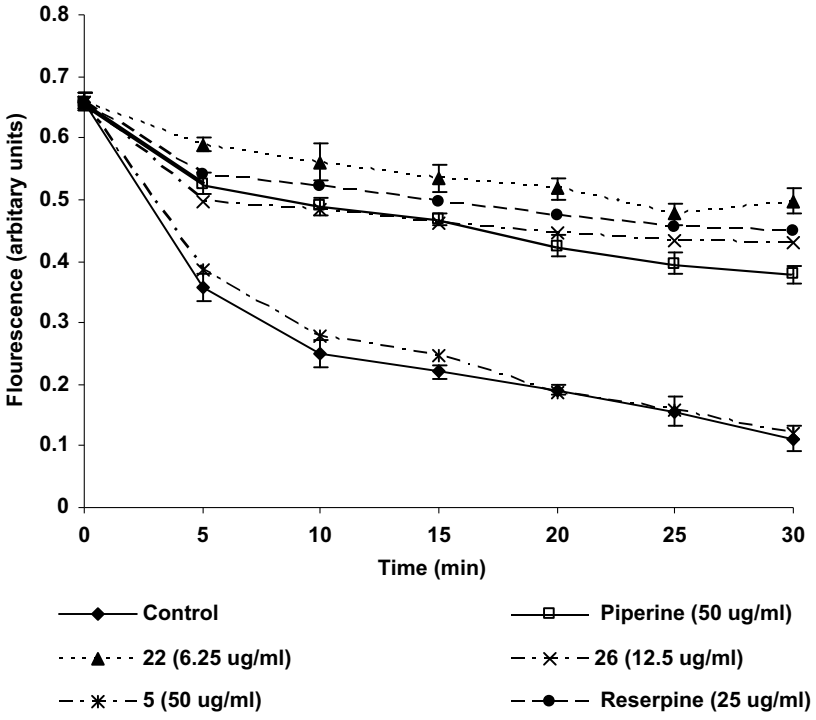


Figure 3. Ethidium bromide efflux inhibition assay from *S. aureus* 1199B cells. The cells were loaded with ethidium bromide and the efflux was allowed to occur in the absence of EPI (control) or in the presence of EPIs. Each time point represents the mean $\log_{10} \pm$ SD of three readings.

officinalis plants, respectively, in our lab and characterized by spectroscopic techniques. All the solvents used in reactions were distilled and dried before use. All reactions were monitored by TLC on 0.25 mm silica gel 60 F_{254} plates coated on aluminum sheet (E. Merck). ^{1}H NMR and ^{13}C NMR spectra were recorded on Brucker Avance DPX-200 instrument at 200 MHz and 50 MHz, respectively, using $CDCl_3$ as solvent with TMS as internal standard. Chemical shift is expressed in δ (ppm) and coupling constant in Hertz. Mass spectra were recorded on Jeol MSD-300 instrument and IR spectra on Perkin-Elmer FT-IR spectrometer as KBr Pellet or neat sample.

4.2. Preparation of substituted aryl pentadienoic acid amides

The amides were prepared from piperic acid (**2**) through acid chloride formation and subsequent condensation with appropriate amines. The structure analyses were carried out mainly through IR, NMR, and MS studies.

4.2.1. Preparation of 5-(3,4-methylenedioxyphenyl)-2*E*,4*E*-pentadienoic acid (piperic acid) (2)

A mixture of piperine **1** (28.5 g, 100 mmol, mp 132 °C) dissolved in ethylene glycol (200 mL) and potassium hydroxide (25 g) was refluxed at 180 °C. Completion of reaction was monitored on TLC and the reaction worked up by diluting with excess of ice-water (500 mL) followed by acidification with 2 N HCl. The resulting precipitate filtered, washed with water, and dried to give the crude product, which on crystallization from ethanol gave **2** as pale yellow crystals (17.4 g, 80%) mp 217 °C (lit. mp 217 °C).[28]

4.2.2. Preparation of 5-(3,4-methylenedioxyphenyl)-2*E*,4*E*-pentadienoic acid *n*-propyl amide (3)

To piperic acid **2** (2.18 g, 10 mmol) in dry dichloromethane (50 mL) was added freshly distilled thionyl chloride (1.0 mL) and the contents were refluxed for 1 h, excess of thionyl chloride was removed on rotavapour under reduced pressure and thereafter the reconstituted acid chloride in dichloromethane (20 mL) was allowed to condense with *n*-propyl amine (0.59 g, 10 mmol) dissolved in dichloromethane. The contents stirred for 1hr, diluted with water and the organic layer was separated out and washed with water (2× 25 mL), dried over anhydrous sodium sulfate and concentrated to give crude product which on crystallization from ethyl acetate/petroleum ether (1:4) gave a colorless crystalline compound **3** (2.38 g, yield 91%), mp 151–153 °C. Anal. Calcd for $C_{15}H_{17}NO_3$: C, 69.48; H, 6.607; N, 5.40%. Found: C, 69.81; H, 6.603; N, 5.46%. MS (%) M^+ at *m/z* 259 (20), 201 (100), 172 (32), 143 (53), 115 (45). IR: 3268, 2917, 2849, 1642, 1605, 1542, 1255, 1041, 991, 755 cm^{-1}. ^{1}H NMR: δ 0.98 (3H, t, J = 6.8, –CH_2CH_3), 1.58 (2H, m, –CH_2CH_3), 3.38 (2H, m, –NHCH_2), 5.96 (2H, s, –OCH_2O–), 6.01 (1H, d, J = 15.02 Hz, –CH=*CH*CO), 6.67–7.01 (5H, m, olefinic and Ar-*H*), 7.38 (2H, dd, J = 15.02, 9.2 Hz, –*CH*=CHCO). ^{13}C NMR: δ 12.1, 25.2, 46.6, 99.5, 107.2, 108.2, 120.2, 124.3, 126.5, 129.5, 129.7, 143.5, 147.1, 147.3, and 167.9.

4.2.3. Preparation of 5-(3,4-methylenedioxyphenyl)-2*E*,4*E*-pentadienoic acid isopropyl amide (4)

The isopropyl amide **4** was prepared from piperic acid **2** (2.2 g, 10 mmol) and isopropyl amine (0.59 g, 10 mmol) by the method as described for **3** to give crude product which on crystallization from ethyl acetate furnished a crystalline colorless solid **4** (2.38 g, yield 92%) mp 172–173 °C [lit.[47,48] mp 171–173 °C]. Anal. Calcd for $C_{15}H_{17}NO_3$: C, 69.48; H, 6.607; N, 5.40%. Found: C, 69.58; H, 6.613; N, 5.71%. MS (%) M^+ at *m/z* 259 (14), 244 (12), 216 (100), 201 (75), 172 (15), 143 (30), 116 (25). IR: 3278, 2972, 2917, 2849, 1644, 1604, 1503, 1252, 1039, 993 cm^{-1}. ^{1}H NMR: δ 1.19 (6H, d, J = 6.54 Hz, –CH(CH_3)$_2$), 4.17–4.21 (1H, m, –NHC*H*–), 5.89 (1H, d, J = 14.85 Hz, –CH=*CH*CO), 5.98 (2H, s, –OCH_2O–), 6.67–6.98 (5H, m, olefinic and Ar-*H*), 7.34 (1H, dd, J = 14.85, 9.80 Hz, –*CH*=CHCO). ^{13}C NMR: δ 19.5, 21.1, 37.4, 101.7, 107.7, 108.5, 118.6, 119.3, 120.5, 126.9, 134.8, 136.8, 146.9, 147.1, and 163.1.

4.2.4. Preparation of 5-(3,4-methylenedioxyphenyl)-2*E*,4*E*-pentadienoic acid *n*-butyl amide (5)

Compound **5** was prepared from piperic acid **2** (1.1 g, 5 mmol) and *n*-butyl amine (0.37 g, 5 mmol) by the method as described for **3** to give crude product as gummy mass which on crystallization in ethyl acetate compound **5** (1.26 g, yield 92%) obtained as colorless solid mp 142–143 °C [lit.[28,48] mp 144–145 °C]. Anal. Calcd for $C_{16}H_{19}NO_3$: C, 70.30; H, 7.006; N, 5.12%. Found: C, 70.78; H, 7.014; N, 5.32%. MS (%) M^+ at *m/z* 273 (24), 258 (22), 201 (100), 172 (23), 143 (52), 116 (35). IR: 3284, 2958, 2916, 1640, 1615, 1445, 1255, 990 cm^{-1}. ^1H NMR: δ 0.93 (3H, t, J = 6.5 Hz, $-CH_2CH_3$), 1.34 (4H, m, $(CH_2)_2CH_3$), 3.75 (2H, m, $-NHCH_2$), 5.90 (1H, d, J = 14.90 Hz, –CH=CHCO), 5.98 (2H, s, $-OCH_2O-$), 6.65–6.98 (5H, m, olefinic and Ar-*H*), 7.37 (1H, dd, J = 14.90, 9.80 Hz, –CH=CHCO). ^{13}C NMR: δ 20.2, 27.3, 28.4, 46.8, 101.6, 107.8, 108.9, 118.7, 120.5, 121.6, 126.8, 136.9, 137.8, 147.7, 148.0, and 167.4.

4.2.5. Preparation of 5-(3,4-methylenedioxyphenyl)-2*E*,4*E*-pentadienoic acid isobutyl amide (6)

Compound **6** was prepared from piperic acid **2** (2.2 g, 10 mmol) and isobutyl amine (0.73 g, 10 mmol) by the method as described for **3** to give crude product which on crystallization from ethyl acetate furnished a colorless crystalline solid **6** (2.53 g, yield 93%), mp 165–167 °C [lit.[48] mp 167–169 °C]. Anal. Calcd for $C_{16}H_{19}NO_3$: C, 70.30; H, 7.006; N, 5.12%. Found: C, 70.81; H, 7.011; N, 5.32%. MS (%) M^+ at *m/z* 273 (28), 258 (12), 243 (15), 201 (100), 172 (18), 143 (38), 116 (25). IR: 3283, 2959, 2917, 1643, 1613, 1446, 1256, 989 cm^{-1}. ^1H NMR: δ 0.98 (6H, d, J = 6.5 Hz, $-CH(CH_3)_2$), 2.35 (1H, m, $CH(CH_3)_2$), 3.74 (2H, m, $-NHCH_2$), 5.91 (1H, d, J = 14.95 Hz, –CH=CHCO), 5.99 (2H, s, $-OCH_2O-$), 6.64–6.97 (5H, m, olefinic and Ar-*H*), 7.36 (1H, dd, J = 14.95, 9.82 Hz, –CH=CHCO). ^{13}C NMR: δ *20.5* × *2,* 28.3, 47.4, 101.9, 107.6, 108.7, 118.9, 120.3, 121.7, 126.7, 136.6, 137.3, 147.9, 148.2, and 167.2.

4.2.6. Preparation of 5-(3,4-methylenedioxyphenyl)-2*E*,4*E*-pentadienoic acid *N*,*N*-diethyl amide (7)

Compound **7** was prepared from piperic acid **2** (1.1 g, 5 mmol) and *N*,*N*-diethyl amine (0.37 g, 5 mmol) by the method as described for **3** to give a solid **7** (1.25 g, yield 92%) mp 86 °C [lit.[47] mp 85–87 °C]. Anal. Calcd for $C_{16}H_{19}NO_3$: C, 70.30; H, 7.006; N, 5.12%. Found: C, 70.81; H, 7.016; N, 5.41%. MS (%) M^{+1} at *m/z* 274 (72), 259 (8), 244 (12), 201 (100), 173 (23), 135 (35), 105 (5). IR: 3164, 2977, 2929, 1737, 1641, 1444, 1404, 1250, 1038, 805 cm^{-1}. ^1H NMR: δ 1.08 and 1.15 (3H each, t, J = 6.8 Hz, 2× CH_2CH_3), 3.36 (4H, m, 2× $-CH_2CH_3$), 5.89 (2H, s, $-OCH_2O-$), 6.28 (1H, d, J = 14.50 Hz, –CH=CHCO), 6.67–6.91 (5H, m, olefinic and Ar-*H*), 7.37 (1H, dd, J = 14.50, 9.82 Hz, –CH=CHCO). ^{13}C NMR: δ 12.3, 14.0, 40.3, 41.2, 100.3, 104.7, 107.5, 119.3, 121.5, 124.4, 130.0, 137.4, 141.5, 147.2, 147.3, and 164.9.

4.2.7. Preparation of 5-(3,4-methylenedioxyphenyl)-2*E*,4*E*-pentadienoic acid *N*,*N*-diisopropyl amide (8)

Compound **8** was prepared from piperic acid **2** (1.1 g, 5 mmol) and diisopropyl amine (0.51 g, 5 mmol) by the method as described for **3** to give a colorless solid **8** (1.32 g, yield 88%) mp 83 °C [lit.[47] mp 81–83 °C]. Anal. Calcd for $C_{18}H_{23}NO_3$: C, 71.73; H, 7.691; N, 4.64%. Found: C, 72.04; H, 6.699; N, 4.92%. MS (%) M^{+1} at *m/z* 302 (86), 286 (15), 271 (12), 201 (100), 173 (13), 135 (10), 105 (15). IR: 3194, 2960, 2927, 1738, 1651, 1404, 1041, 761 cm $^{-1}$. ^1H NMR: δ 1.33 (12H, br s, 4× CH_3), 4.02 (2H, m, 2× –NCH), 5.98 (2H, s, $-OCH_2O-$), 6.35 (1H, d, J = 14.61 Hz, –CH=CHCO), 6.72–6.99 (5H, m, olefinic and Ar-*H*), 7.33 (1H, dd, J = 14.61, 9.52 Hz, –CH=CHCO). ^{13}C NMR: δ 19.8, 19.9, 20.6, 20.7, 44.8, 46.4, 100.3, 104.7, 107.5, 121.4, 122.1, 124.6, 130.2, 136.8, 140.3, 147.1, 147.2, and 165.7.

4.2.8. Preparation of 5-(3,4-methylenedioxyphenyl)-2*E*,4*E*-pentadienoic acid D-2-amino-butanol amide (9)

Compound **9** was prepared from piperic acid **2** (1.1 g, 10 mmol) and D-2-amino butanol (0.45 g, 5 mmol) by the method as described for **3** to give crude product which on crystallization from ethyl acetate furnished a colorless crystalline solid **9** (1.10 g, yield 76%), mp 206–207 °C. Anal. Calcd for $C_{16}H_{19}NO_4$: C, 66.42; H, 6.618; N, 4.84%. Found: C, 66.92; H, 6.621; N, 5.02%. MS (%) M^+ at *m/z* 289 (45), 271 (24), 242 (100), 201 (53), 172 (12), 143 (30), 116 (25). IR: 3410, 2925, 2854, 1725, 1665, 1458, 1378, 1050, 678 cm^{-1}. ^1H NMR: δ 0.90 (3H, t, J = 8.5 Hz, $-CH_2CH_3$), 1.17–1.58 (2H, m, $-CH_2CH_3$), 3.68 (2H, br s, CH_2OH), 3.95 (1H, br s, –NHCH), 5.91 (1H, d, J = 14.78 Hz, –CH=CHCO), 5.96 (2H, s, $-OCH_2O-$), 6.72–6.97 (5H, m, olefinic and Ar-*H*), 7.35 (1H, dd, J = 14.78, 9.81 Hz, –CH=CHCO). ^{13}C NMR: δ 10.5, 27.3, 52.5, 67.7, 100.5, 107.9, 108.7, 120.8, 124.7, 126.3, 129.3, 130.5, 143.4, 147.8, 147.9, and 165.3.

4.2.9. Preparation of 5-(3,4-methylenedioxyphenyl)-2*E*,4*E*-pentadienoic acid L-2-amino-butanol amide (10)

Compound **10** was prepared from piperic acid **2** (1.1 g, 5 mmol) and L-2-amino butanol (0.45 g, 5 mmol) by the method as described for **3** to give crude product which on crystallization from ethyl acetate furnished a white crystalline solid **10** (1.13 g, yield 78%), mp 210–211 °C. Anal. Calcd for $C_{16}H_{19}NO_4$: C, 66.42; H, 6.618; N, 4.84%. Found: C, 66.73; H, 6.625; N, 4.99%. MS (%) M^+ at *m/z* 289 (68), 271 (56), 242 (75), 201 (53), 172 (12), 143 (30), 116 (25). IR: 3410, 2925, 2854, 1725, 1665, 1458, 1378, 1050, 678 cm^{-1}. ^1H NMR: δ 0.93 (3H, t, J = 8.5 Hz, CH_2CH_3), 1.18–1.52 (2H, m, $-CH_2CH_3$), 3.71 (2H, br s, CH_2OH), 3.98 (1H, br s, –NHCH), 5.89 (1H, d, J = 14.81 Hz, –CH=CHCO), 5.94 (2H, s, $-OCH_2O-$), 6.76–6.96 (5H, m, olefinic and Ar-*H*), 7.39 (1H, dd, J = 14.81, 9.85 Hz, –CH=CHCO). ^{13}C NMR: δ 11.5, 26.4, 54.4, 66.8, 100.1, 107.8, 108.9, 121.3, 123.5, 126.8, 128.9, 131.1, 142.5, 146.9, 147.1, and 164. 7.

4.2.10. Preparation of 5-(3,4-methylenedioxyphenyl)-2*E*,4*E*-pentadienoic acid morpholide (11)

Compound **11** was prepared from piperic acid **2** (1.1 g, 5 mmol) and morpholine (0.4 g, 5 mmol) by the method as described for **3** to give white crystalline compound **11** (1.29 g, yield 93%) mp 162–163 °C [lit.[48,49] mp 167–168 °C]. Anal. Calcd for $C_{16}H_{17}NO_4$: C, 66.88; H, 5.963; N, 4.87%. Found: C, 67.04; H, 5.969; N, 5.13%. MS (%) M^{+1} at *m/z* 287 (25), 269 (14), 201 (100), 156 (11), 135 (28), 105 (10). IR: 3362, 2942, 2855, 1636, 1489, 1445, 1403, 1251, 1114, 1038, 759 cm^{-1}. ^1H NMR: δ 3.71 (8H, br s, 2× $-CH_2CH_2O-$), 5.98 (2H, s, $-OCH_2O-$), 6.38 (1H, d, J = 14.60 Hz, –CH=CHCO), 6.68–6.99 (5H, m, olefinic and Ar-*H*), 7.41 (1H, dd, J = 14.60, 9.40 Hz, –CH=CHCO). ^{13}C NMR: δ *67.0, 101.6,* 105.9, 108.7, 118.6, 123.1, 125.1, 131.0, 139.9, 144.2, 148.5, 148.7, and 166.6.

4.2.11. Preparation of 5-(3,4-methylenedioxyphenyl)-2*E*,4*E*-pentadienoic acid *N*-methyl piperazine amide (12)

Compound **12** was prepared from piperic acid **2** (1.1 g, 5 mmol) and *N*-methyl piperazine (0.5 g, 5 mmol) by the same method as described for **3** to give a white crystalline compound **12** (1.36 g, yield 88%) mp 185–186 °C [lit.[49] mp 167–168 °C]. Anal. Calcd for $C_{17}H_{20}N_2O_3$: C, 67.98; H, 6.711; N, 9.32%. Found: C, 68.37; H, 6.717; N, 9.63%. MS (%) M^{+1} at *m/z* 301 (58), 285 (12), 201 (100), 173 (41), 135 (18), 105 (13). IR: 3357, 2956, 2927, 1732, 1641, 1455, 1405, 1040, 748 cm^{-1}. ^1H NMR: δ 2.94 (3H, s, N–CH_3), 3.19 (4H, m, $-N(CH_2)_2$), 3.51 (4H, m, $-N(CH_2)_2$), 5.97 (2H, s, $-OCH_2O-$), 6.38 (1H, d, J = 14.62 Hz, –CH=CHCO), 6.74–7.02 (5H, m, olefinic and Ar-*H*), 7.35 (1H, dd, J = 14.62, 9.45 Hz, –CH=CHCO). ^{13}C NMR: δ 39.2, 50.2, 55.4, 101.3, 104.9, 107.8, 120.6, 122.8, 125.8, 131.7, 136.8, 141.3, 147.3, 147.4, and 165.8.

4.2.12. Preparation of 5-(3,4-methylenedioxyphenyl)-2*E*,4*E*-pentadienoic acid anilide (13)

The amide **13** was prepared from piperic acid **2** (1.1 g, 5 mmol) and aniline (0.47 g, 5 mmol) by the method as described for **3** to give crude product which on crystallization from ethyl acetate furnished a colorless crystalline solid **13** (1.2 g, yield 82%), mp 174–175 °C. Anal. Calcd for $C_{18}H_{15}NO_3$: C, 73.70; H, 5.154; N, 4.77%. Found: C, 73.91; H, 5.152; N, 4.98%. MS (%) M^+ at *m/z* 293 (74), 215 (28), 201 (14), 172 (100), 143 (15), 116 (12). IR: 3259, 2917, 2849, 1599, 1489, 1442, 1252, 1038, 753 cm^{-1}. ^1H NMR: δ 5.99 (2H, s, –OC*H*$_2$O–), 6.01 (1H, d, *J* = 14.15 Hz, –CH=C*H*CO), 6.66–6.98 (5H, m, olefinic and Ar-*H*), 7.01–7.26 (5H, m, Ar-*H*′), 7.32 (1H, dd, *J* = 14.15, 9.31 Hz, –C*H*=CHCO). ^{13}C NMR: δ 101.4, 107.5, 108.4, 120.5, 121.23, 121.24, 124.1, 124.6, 126.4, 128.7, 129.4, 130.1 × 2, 139.4, 142.8, 147.1, 147.2, and 165.2.

4.2.13. Preparation of 5-(3,4-methylenedioxyphenyl)-2*E*,4*E*-pentadienoic acid *o*-toluidide (14)

Compound **14** was prepared from piperic acid **2** (1.1 g, 5 mmol) and *o*-toluidine (0.54 g, 5 mmol) by the method as described for **3** to give crude product which on column chromatography over silica gel and eluted with petroleum ether and ethyl acetate (4:1) furnished a colorless crystalline compound **14** (1.41 g, yield 91%), mp 194–195 °C. Anal. Calcd for $C_{19}H_{17}NO_3$: C, 74.25; H, 5.574; N, 4.55%. Found: C, 74.70; H, 5.579; N, 4.76%. MS (%) M^+ at *m/z* 307 (18), 292 (100), 201 (35), 172 (41), 143 (63) 16 (25). IR: 3359, 2933, 2853, 2360, 1742, 1654, 1403, 1250, 1094, 1042, 760 cm^{-1}. ^1H NMR: δ 2.24 (3H, s, –C*H*$_3$), 5.98 (1H, d, *J* = 14.78 Hz, –CH=C*H*CO), 6.02 (2H, s, –OC*H*$_2$O), 6.60–6.67 (2H, m, olefinic-*H*), 6.98–7.18 (7H, m, Ar-*H,* and Ar-*H*′), 7.36 (1H, d, *J* = 14.78, 9.23 Hz, –C*H*=CHCO). ^{13}C NMR: δ 21.4, 101.8, 107.8, 108.8, 120.4, 121.4, 124.6, 124.9, 125.9, 126.7, 128.9, 129.7, 129.9, 130.5, 139.7, 143.2, 147.4, 147.5, and 165.0.

4.2.14. Preparation of 5-(3,4-methylenedioxyphenyl)-2*E*,4*E*-pentadienoic acid *p*-anisidide (15)

The compound **15** was prepared from piperic acid **2** (1.1 g, 5 mmol) and *p*-anisidine (0.62 g, 5 mmol) by the method as described for **3** to give crude product which on column chromatography over silica gel and eluted with 15% EtOAc in pet ether afforded a colorless crystalline solid **15** (1.17 g, yield 75%), mp 167–168 °C. Anal. Calcd for $C_{19}H_{17}NO_4$: C, 70.57; H, 5.298; N, 4.33%. Found: C, 70.97; H, 5.302; N, 4.52%. MS (%) M^+ at *m/z* 323 (5), 293 (12), 201 (35), 172 (100), 144 (43), 116 (52). IR: 3239, 2917, 2849, 1604, 1509, 1248, 1034, 757 cm^{-1}. ^1H NMR: δ 3.89 (3H, s, –OC*H*$_3$), 5.99 (2H, s, –OC*H*$_2$O–), 6.09 (1H, d, *J* = 15.0 Hz, –CH=C*H*CO), 6.72 (2H, d, *J* = 8.2 Hz, Ar-*H*-3′ and 5′), 6.82–7.35 (6H, m, olefinic and Ar-*H*), 7.53 (2H, d, *J* = 8.2 Hz, Ar-*H*-2′ and 6′). ^{13}C NMR: δ 58.3, 99.8, 107.8, 108.9, 115.1, 120.1, 123.3, 124.2, 126.6, 128.8, 129.1, 131.2, 143.5, 148.1, 148.2, 158.2, and 165.2.

4.2.15. Preparation of 5-(3,4-methylenedioxyphenyl)-2*E*,4*E*-pentadienoic acid 3,4-methylenedioxyphenyl amide (16)

The compound **16** was prepared from piperic acid **2** (2.2 g, 10 mmol) and 3,4-methylenedioxy phenyl amine (1.37 g, 10 mmol) by the method as described for **3** to give crude product which on crystallization from ethyl acetate and hexane yielded a colorless crystalline solid **16** (2.90 g, yield 86%), mp 202–203 °C. Anal. Calcd for $C_{19}H_{15}NO_5$: C, 67.65; H, 4.481; N, 4.15%. Found: C, 68.11; H, 4.484; N, 4.33%. MS (%) M^+ at *m/z* 337 (12), 201 (100), 172 (22), 141 (38), 115 (45). IR: 3346, 2924, 2853, 1641, 1403, 1259, 1125, 1039, 761 cm^{-1}. ^1H NMR: δ 5.94 and 5.97 (2H each, s, 2× –OC*H*$_2$O–), 6.13 (1H, d, *J* = 14.84 Hz, –CH=C*H*CO), 6.73–7.01 (8H, m, olefinic, Ar-*H,* and Ar-*H*′), 7.43 (1H, dd, *J* = 14.84, 9.23 Hz, C*H*=CHCO). ^{13}C NMR: δ 101.4, 101.5, 106.7, 107.9, 108.7, 108.8, 109.5, 120.5, 124.3, 126.4, 128.9, 129.3, 132.3, 143.1, 144.2, 146.6, 147.2, 147.4, and 165.5.

4.2.16. Preparation of 5-(3,4-methylenedioxyphenyl)-2*E*,4*E*-pentadienoic acid 2-amino-1-phenyl ethanol amide (17)

Compound **17** was prepared from piperic acid **2** (2.2 g, 10 mmol) and 2-amino-1-phenyl ethanol (1.37 g, 10 mmol) by the method as described for **3** to give crude product which on column chromatography on silica eluted in pet ether/ethyl acetate (3:2) furnished colorless crystalline solid **17** (2.76 g, yield 82%), mp 202–203 °C. Anal. Calcd for $C_{20}H_{19}NO_4$; C, 71.20; H, 5.676; N, 4.15%. Found: C, 71.66; H, 5.672; N, 4.27%. MS (%) M^+ at *m/z* 337 (14), 319 (25), 241 (70), 201 (100), 172 (35), 144 (35), 115 (25). IR: 3313, 2923, 2852, 2360, 1649, 1502, 1447, 1252, 1038, 758, 700 cm^{-1}. ^1H NMR: δ 4.02 (2H, m, –NHC*H*$_2$–), 4.80 (1H, m, –C*H*OH), 5.96 (2H, s, –OC*H*$_2$O–), 6.05 (1H, d, *J* = 14.95 Hz, –CH=C*H*CO), 6.68–6.96 (5H, m, olefinic and Ar-*H*), 7.23–7.32 (5H, m, Ar-*H*′), 7.35 (1H, dd, *J* = 14.95, 9.25 Hz, –C*H*=CHCO). ^{13}C NMR: δ 53.5, 77.2, 101.6, 107.8, 108.7, 119.9, 124.1, 125.6, 125.7, 125.71, 125.8, 128.4, 128.45, 128.49, 139.4, 141.2, 142.3, 147.2, 147.3, and 167.8.

4.2.17. Preparation of 5-(3,4-methylenedioxyphenyl)-2*E*,4*E*-pentadienoic acid 4-benzamide (18)

This compound was prepared by condensing piperic acid **2** (2.2 g, 10 mmol) with 4-amino benzamide (1.36 g, 10 mmol) as per the method described for **3** to furnish a crude product which on CC over SiO_2 and elution with ethyl acetate/pet ether gave a brown crystalline compound **18** (2.06 g, yield 60%), mp 190–192 °C. Anal. Calcd for $C_{19}H_{16}N_2O_4$: C, 67.84; H, 4.794; N, 8.32%. Found: C, 68.02; H, 4.793; N, 8.49%. MS (%) M^+ at *m/z* 336 (25), 292 (100), 201 (10), 173 (14), 143 (5), 115 (24). IR: 3395, 2916, 2848, 1673, 1603, 1446, 1251, 1037 cm^{-1}. ^1H NMR: δ 5.94 (1H, d, *J* = 15.0 Hz, –CH=C*H*CO), 5.99 (2H, s, –OC*H*$_2$O–), 6.72–7.10 (5H, m, olefinic, Ar-*H*), 7.36 (1H, dd, *J* = 15.0 and 9.5 Hz, –C*H*=CHCO), 7.66–7.97 (4H, m, Ar-*H*′). ^{13}C NMR: δ 101.1, 107.8, 108.9, 119.5, 121.9, 121.91, 123.9, 125.9, 127.9, 128.0, 128.01, 128.6, 130.1, 141.1, 142.6, 147.1, 147.5, 164.1, and 168.5.

4.2.18. Preparation of 5-(3,4-methylenedioxyphenyl)-2*E*,4*E*-pentadienoic acid 2-hydroxymethylphenyl amide (19)

Compound **19** was prepared from piperic acid **2** (2.18 g, 10 mmol) using thionyl chloride (1.0 mL) and 2-amino benzyl alcohol (1.23 g, 10 mmol) by the procedure as described for **3** to give crude product which on crystallization from methanol furnished white crystalline solid **19** (2.32 g, yield 72%), mp 162–163 °C. Anal. Calcd for $C_{19}H_{17}NO_4$: C, 70.57; H, 5.299; N 4.33%. Found: C, 70.85; H, 5.294; N, 4.41%. MS (%) M^+ at *m/z* 323 (49), 291 (100), 202 (51), 173 (36) 142 (9). IR: 3347, 2917, 2849, 2360, 1605, 1449, 1253, 1038, 755 cm^{-1}. ^1H NMR: δ 4.74 (2H, s, –C*H*$_2$OH), 5.99 (2H, s, –OC*H*$_2$O–), 6.11 (1H, d, *J* = 15.0 Hz, –CH=C*H*CO), 6.72–6.98 (5H, m, olefinic and Ar-*H*), 7.01–7.19 (2H, m, Ar- *H*-4′ and 5′), 7.26–7.56 (2H, m, –C*H*=CHCO and Ar-*H*-3′), 7.67 (1H, d, *J* = 8.4 Hz, Ar-*H*-6′). ^{13}C NMR: δ 57.6, 100.1, 107.4, 108.6, 119.4, 121.6, 122.4, 125.1, 126.5, 127.9, 128.1, 128.6, 128.7, 134.0, 141.4, 142.6, 147.1, 147.6, and 164.1.

4.2.19. Preparation of 5-(3,4-methylenedioxyphenyl)-2*E*,4*E*-pentadienoic acid 3-hydroxymethylphenyl amide (20)

This compound was prepared by condensing piperic acid **2** (2.20 g, 10 mmol) with 3-amino benzyl alcohol (1.23 g, 10 mmol) as per the method described for **3** to furnish a crude product which on CC over SiO_2 and elution with ethyl acetate/pet ether (1:4) gave a pale yellow crystalline compound **20** (1.93 g, yield 60%), mp 189–190 °C. Anal. Calcd for $C_{19}H_{17}NO_4$: C, 70.57; H, 5.298; N, 4.33%. Found: C, 70.01; H, 5.293; N, 4.50%. MS (%) M^+ at *m/z* 323 (15), 305 (70), 290 (100), 270 (80), 201 (15), 173 (8), 121 (7). IR: 3406, 2916, 2848, 1598, 1442, 1250, 1036 cm^{-1}. ^1H NMR: δ 4.66 (2H, br s, –C*H*$_2$OH), 5.98 (2H, s, –OC*H*$_2$O–), 6.14 (1H, d, *J* = 15.0 Hz, –CH=C*H*CO), 6.76–7.15 (7H, m, olefinic, Ar-*H* and Ar-*H*-4′ and 5′),

7.35 (1H, m, –C*H*=CHCO), 7.65 (2H, m, Ar-*H*-2′ and 6′). ^{13}C NMR: δ 61.0, 99.4, 107.6, 108.8, 119.6, 122.0, 122.1, 122.9, 125.3, 126.8, 128.1, 128.6, 128.9, 138.6, 141.5, 142.8, 147.1, 147.5, and 164.4.

4.2.20. Preparation of 5-(3,4-methylenedioxyphenyl)-2*E*,4*E*-pentadienoic acid 4-hydroxymethylphenyl amide (21)

This compound was prepared by condensing piperic acid **2** (1.1 g, 5 mmol) with 4-amino benzyl alcohol (0.62 g, 5 mmol) as per the method described for **3** to furnish a crude product which on crystallization with ethyl acetate: pet ether gave a white crystalline compound **21** (1.3 g, yield 80%), mp 140–142 °C. Anal. Calcd for $C_{19}H_{17}NO_4$: C, 70.57; H, 5.298; N, 4.33%. Found: C, 70.93; H, 5.295; N, 4.47%. MS (%) M^+ at *m/z* 323 (50), 306 (35), 292 (90), 271 (100), 201 (10), 166 (48), 143 (24), 115 (12). IR: 3395, 2917, 2849, 1653, 1602, 1502, 1488, 1448, 1252, 1038, 756 cm^{-1}. ^{1}H NMR: δ 4.67 (2H, br s, –CH_2OH), 6.00 (2H, s, –OCH_2O–), 6.25 (1H, d, *J* = 15.0 Hz, –CH=C*H*CO), 6.76–6.98 (5H, m, olefinic and Ar-*H*), 7.24 (2H, d, *J* = 8.4 Hz, Ar-*H*-3′ and 5′), 7.40 (1H, m, –C*H*=CHCO), 7.65 (2H, d, *J* = 8.4 Hz, Ar-*H*-2′ and 6′). ^{13}C NMR: δ 65.5, 99.3, 108.4, 109.6, 119.4, 122.5, 122.51, 125.1, 126.6, 128.19, 128.2, 128.4, 128.6, 137.0, 141.7, 142.6, 147.9, 148.0, and 164.3.

4.2.21. Preparation of 5-(3,4-methylenedioxyphenyl)-2*E*,4*E*-pentadienoic acid 3-cyanophenyl amide (22)

Compound **22** was prepared by condensing piperic acid **2** (2.2 g, 10 mmol) with 3-amino benzonitrile (1.20 g, 10 mmol) by the method as described for **3** to give a light brown crystalline compound **22** (2.54 g, yield 80%) after crystallization from ethyl acetate/pet ether (1:4), mp 175–176 °C. Anal. Calcd for $C_{19}H_{14}N_2O_3$: C, 71.68; H, 4.432; N, 8.80%. Found: C, 71.98: H, 4.431; N, 8.93%. MS (%) M^+ at *m/z* 318 (25), 294 (100), 201 (19), 175 (26) 143 (15), 117 (40). IR: 3376, 2917, 2849, 2230, 1602, 1543, 1486, 1252, 1038, 756 cm^{-1}. ^{1}H NMR: δ 5.90 (2H, s, –OCH_2O–), 6.02 (1H, d, *J* = 15.0 Hz, –CH=C*H*CO), 6.70–7.00 (5H, m, olefinic and Ar-*H*), 7.26 (1H, dd, *J* = 15.0 and 9.2 Hz, –C*H*=CHCO), 7.31–7.40 (2H, m, Ar-*H*-4′ and 5′), 7.85 (1H, s, Ar-*H*-2′), 7.91 (1H, d, *J* = 8.5 Hz, Ar-*H*-6′). ^{13}C NMR: δ 101.2, 107.7, 108.8, 116.1, 117.5, 121.2, 123.0, 123.6, 124.7, 125.8, 127.6, 128.0, 128.5, 129.4, 138.9, 142.7, 146.8, 147.5, and 163.8.

4.2.22. Preparation of 5-(3,4-methylenedioxyphenyl)-2*E*,4*E*-pentadienoic acid 4-cyanophenyl amide (23)

Compound **23** was prepared by condensing piperic acid **2** (2.2 g, 10 mmol) with 4-amino benzonitrile (1.20 g, 10 mmol) by the method as described for **3** to furnish a gummy mass **23** (1.90 g, yield 60%). Anal. Calcd for $C_{19}H_{14}N_2O_3$: C, 71.68; H, 4.432; N, 8.80%. Found: C, 72.04; H, 4.439; N, 8.94%. MS (%) M^+ at *m/z* 318 (15), 294 (100), 201 (9), 175 (76) 143 (75), 117 (30). IR: 3370, 2916, 2848, 2214, 1627, 1604, 1515, 1316, 1252, 1170, 1037, 830 cm^{-1}. ^{1}H NMR: δ 5.94 (2H, s, –OCH_2O–), 6.20 (1H, d, *J* = 15.0 Hz, –CH=C*H*CO), 6.72–6.98 (5H, m, olefinic and Ar-*H*), 7.52–7.60 (3H, m, –C*H*=CHCO and Ar-*H*-3′ and 5′), 7.81 (2H, d, *J* = 8.5 Hz, Ar-*H*-2′ and 6′). ^{13}C NMR: δ 101.3, 107.8, 108.2, 110.3, 117.8, 120.4, 121.2, 123.7, 125.1, 125.9, 127.4, 127.6, 133.8, 135.0, 139.0, 141.2, 147.2, 147.6, and 163.5.

4.2.23. Preparation of 5-(3,4-methylenedioxyphenyl)-2*E*,4*E*-pentadienoic acid 2-cyanomethylphenyl amide (24)

The compound **24** was prepared by condensing piperic acid **2** (2.2 g, 10 mmol) with 2-amino benzyl cyanide (1.30 g, 10 mmol) by the method as described for **3** to give a crude gummy mass which was purified on column chromatography over silica and elution with pet ether/ethyl acetate to give a semi solid compound **24** (2.65 g, yield 80%). Anal. Calcd for $C_{20}H_{16}N_2O_3$: C, 72.27; H, 4.852; N, 8.42%. Found: C, 72.63; H, 4.858; N, 8.60%. MS (%) M^+ at *m/z* 332 (100), 294 (10), 201 (15), 173 (75) 143 (5), 131 (12), 115 (8). IR: 3373, 3230, 2916, 2849, 1645, 1607, 1501, 1490, 1455, 1290, 1035, 997, 761 cm^{-1}. ^{1}H NMR: δ 3.85 (2H, s, –CH_2CN), 5.98 (2H, s, –OCH_2O–), 6.08 (1H, d, *J* = 15.0 Hz, –CH=C*H*CO), 6.74–6.98 (5H, m, olefinic and Ar-*H*), 7.05 (1H, d, *J* = 8.2 Hz, Ar-*H*-3′), 7.14–7.50 (4H, m, –C*H*=CHCO and Ar-*H*′). ^{13}C NMR: δ 20.7, 101.1, 107.9, 108.5, 115.0, 119.3, 121.9, 122.6, 124.1, 124.2, 125.9, 128.0, 128.1, 128.6, 129.2, 141.6, 142.6, 147.2, 147.6, and 164.3.

4.2.24. Preparation of 5-(3,4-methylenedioxyphenyl)-2*E*,4*E*-pentadienoic acid 4-cynomethylphenyl amide (25)

The compound **25** was prepared by condensing piperic acid **2** (2.18 g, 10 mmol) with 4-amino benzyl cyanide (1.32 g, 10 mmol) by the method as described for **3** to give a crude product which on crystallization from ethyl acetate/petroleum ether (1:4) gave a light brown crystalline compound **25** (2.65 g, yield 80%), mp 186 °C. Anal. Calcd for $C_{20}H_{16}N_2O_3$: C, 72.27; H, 4.850; N 8.42%. Found: C, 72.49; H, 4.849; N, 8.47%. MS (%) M^+ at *m/z* 332 (40), 294 (100), 201 (59), 172 (66), 131 (20). IR: 3346, 2917, 2849, 1605, 1516, 1502, 1252, 1038, 755 cm^{-1}. ^{1}H NMR: δ 3.73 (2H, s, –CH_2CN), 5.98 (2H, s, –OCH_2O–), 6.05 (1H, d, *J* = 15.0 Hz, CH=C*H*CO), 6.72–7.01 (5H, m, olefinic and Ar-*H*), 7.19 (2H, d, *J* = 8.6 Hz, Ar-*H*-3′ and 5′), 7.31–7.56 (1H, m, C*H*=CHCO), 7.62 (2H, d, *J* = 8.6 Hz, Ar-*H*-2′ and 6′). ^{13}C NMR: δ 24.9, 101.5, 106.1, 108.9, 115.2, 119.6, 121.0, 121.1, 123.0, 124.1, 126.1, 128.1, 128.9, 131.0, 131.0, 141.2, 142.8, 147.9, 148.0, and 164.2.

4.2.25. Preparation of 5-(3,4-methylenedioxyphenyl)-2*E*,4*E*-pentadienoic acid 2-acetylaminophenyl amide (26)

Compound **26** was prepared by condensing piperic acid **2** (2.2 g, 10 mmol) with 2′-amino acetanilide (1.50 g, 10 mmol) by the method as described for **3** to give a brown crystalline compound **26** (2.81 g, yield 80%) after crystallization from ethyl acetate/pet ether (1:4), mp 189–190 °C. Anal. Calcd for $C_{20}H_{18}N_2O_4$: C, 68.56; H, 5.173; N, 7.99%. Found: C, 68.85; H, 5.171; N, 8.03%. MS (%) M^+ at *m/z* 350 (25), 305 (12), 291 (100), 201 (23), 173 (16), 149 (15), 115 (20). IR: 3282, 2917, 2850, 1653, 1602, 1505, 1486, 1252, 1038, 754 cm^{-1}. ^{1}H NMR: δ 2.14 (3H, s, –COCH_3), 5.99 (2H, s, –OCH_2O–), 6.09 (1H, d, *J* = 14.70 Hz, –CH=C*H*CO), 6.75–7.00 (5H, m, olefinic and Ar-*H*), 7.10–7.49 (5H, m, –C*H*=CHCO and Ar-*H*′). ^{13}C NMR: δ 18.1, 101.5, 107.3, 108.6, 120.0, 120.1, 121.5, 123.5, 123.8, 124.3, 126.1, 128.3, 128.8, 135.1, 136.5, 142.6, 147.6, 147.7, 163.9, and 168.6.

4.2.26. Preparation of 5-(3,4-methylenedioxyphenyl)-2*E*,4*E*-pentadienoic acid 3-acetylaminophenyl amide (27)

Compound **27** was prepared by condensing piperic acid **2** (2.2 g, 10 mmol) with 3′-amino acetanilide (1.50 g, 10 mmol) by the method as described for **3** to give a light brown crystalline compound **27** (2.99 g, yield 85%) after crystallization from ethyl acetate/pet ether (1:4), mp 186–187 °C. Anal. Calcd for $C_{20}H_{18}N_2O_4$: C, 68.56; H, 5.173; N, 7.99%. Found: C, 68.79; H, 5.172; N, 8.13. MS (%) M^+ at *m/z* 350 (15), 305 (10), 291 (100), 201 (13), 173 (25), 149 (35), 115 (25). IR: 3327, 2916, 2849, 1655, 1605, 1545, 1502, 1253, 1038, 802 cm^{-1}. ^{1}H NMR: δ 2.12 (3H, s, COCH_3), 5.90 (2H, s, –OCH_2O–), 6.03 (1H, d, *J* = 15.0 Hz, –CH=C*H*CO), 6.72–7.01 (5H, m, olefinic and Ar-*H*), 7.05–7.48 (4H, m, –C*H*=CHCO and Ar-*H*′), 7.87 (1H, s, Ar-*H*-2′). ^{13}C NMR: δ 17.6, 101.3, 107.1, 108.2, 115.2, 117.0, 117.2, 119.8, 123.6, 125.9, 127.2, 128.2, 128.5, 140.5, 142.1, 142.4, 147.2, 147.5, 163.5, and 168.2.

4.2.27. Preparation of 5-(3,4-methylenedioxyphenyl)-2*E*,4*E*-pentadienoic acid 4-acetylaminophenyl amide (28)

Compound **28** was prepared by condensing piperic acid **2** (2.2 g, 10 mmol) with 4′-amino acetanilide (1.50 g, 10 mmol) by the method as described for **3** to give a brown crystalline compound **28** (2.91 g, yield 83%) after crystallization from ethyl acetate/pet

ether (1:4), mp 169–170 °C. Anal. Calcd for $C_{20}H_{18}N_2O_4$: C, 68.56; H, 5.173; N, 7.99%. Found: C, 68.92; H, 5.175; N, 8.08%. MS (%) M^+ at *m/z* 350 (5), 305 (15), 291 (100), 201 (28), 171 (43), 149 (30), 117 (20). IR: 3327, 2917, 2849, 1670, 1607, 1514, 1448, 1253, 1039, 756 cm^{-1}. ^1H NMR: δ 2.15 (3H, s, $COCH_3$), 5.95 (2H, s, $-OCH_2O-$), 6.04 (1H, d, J = 15.0 Hz, –CH=*CH*CO), 6.76–6.98 (5H, m, olefinic and Ar-*H*), 7.47–7.62 (5H, m, –*CH*=CHCO and Ar-*H*′). ^{13}C NMR: δ 17.9, 101.5, 107.5, 108.6, 119.8, 119.81, 120.1, 120.4, 120.41, 123.8, 126.1, 128.4, 128.8, 140.1, 141.3, 142.6, 147.4, 147.8, 163.8, and 168.5.

4.2.28. Preparation of 5-(3,4-methylenedioxyphenyl)-2*E*,4*E*-pentadienoic acid benzothiazol-2yl amide (29)

To piperic acid **2** (1.1 g, 5 mmol) dissolved in dry dichloromethane (30 mL) was added thionyl chloride (0.5 mL) and the resulting acid chloride was condensed with 2-amino benzothiazole (0.75 g, 5 mmol) and worked up as described for **3** to furnish a crude product which on crystallization with ethyl acetate/pet ether gave a brown crystalline compound **29** (1.23 g, yield 70%), mp 179–180 °C. Anal. Calcd for $C_{19}H_{14}N_2O_3S$: C, 65.12; H, 4.026; N, 7.99%. Found: C, 65.64; H, 4.023; N, 8.04%. MS (%) M^+ at *m/z* 350 (28), 279 (39), 262 (100), 201 (15), 173 (5), 143 (10), 116 (20). IR: 3374, 2917, 2849, 1670, 1602, 1502, 1447, 1254, 1130, 1038, 754 cm^{-1}. ^1H NMR: δ 5.99 (2H, s, $-OCH_2O-$), 6.37 (1H, d, J = 15.0 Hz, –CH=*CH*CO), 6.77–6.97 (5H, m, olefinic and Ar-*H*), 7.28–7.46 (3H, m, –*CH*=CHCO and Ar-*H*′), 7.79 (1H, d, J = 8.3 Hz, Ar-*H*′), 7.88 (1H, d, J = 8.3 Hz, Ar-*H*′). ^{13}C NMR: δ 101.6, 107.9, 108.2, 120.7, 122.0, 122.1, 122.3, 122.5, 123.3, 123.9, 125.6, 128.3, 128.6, 142.8, 146.1, 147.9, 148.1, 164.1, and 176.2.

4.2.29. Preparation of 5-(3,4-methylenedioxyphenyl)-2*E*,4*E*-pentadienoic acid benzothiazol-6yl amide (30)

To piperic acid **2** (1.1 g, 5 mmol) dissolved in dry dichloromethane (30 mL) was added thionyl chloride (0.5 mL) and the resulting acid chloride was condensed with 6-amino benzothiazole (0.75 g, 5 mmol) and worked up as described for **3** to furnish a crude product which on crystallization with ethyl acetate: pet ether gave a brown crystalline compound **30** (1.4 g, yield 80%), mp 174–176 °C. Anal. Calcd for $C_{19}H_{14}N_2O_3S$: C, 65.12; H, 4.026; N, 7.99%. Found: C, 65.81; H, 4.023; N, 8.06%. MS (%) M^+ at *m/z* 350 (24), 279 (29), 262 (100), 201 (4), 143 (12), 116 (12). IR: 3296, 2916, 2849, 1655, 1603, 1575, 1488, 1252, 1140, 1038, 755 cm^{-1}. ^1H NMR: δ 5.99 (2H, s, $-OCH_2O-$), 6.10 (1H, d, J = 15.0 Hz, –CH=*CH*CO), 6.71–6.83 (4H, m, olefinic and Ar-*H*), 6.93 (1H, d, J = 8.2 Hz, Ar-*H*), 7.29–7.35 (2H, m, –*CH*=CHCO and Ar-*H*-6′), 8.01 (1H, d, J = 8.4 Hz, Ar-*H*-5′), 8.05 (1H, s, Ar-*H*-2′), 8.92 (1H, s, N=*CH*–S). ^{13}C NMR: δ 101.3, 107.0, 108.1, 116.3, 119.2, 120.5, 123.5, 123.8, 125.9, 128.5, 128.8, 135.2, 139.6, 142.7, 146.2, 146.8, 147.0, 157.3, and 163.8.

4.2.30. Preparation of 5-(3,4-methylenedioxyphenyl)-2*E*,4*E*-pentadienoic acid 4-benzanilide amide (31)

The compound **31** was prepared from piperic acid **2** (1.1 g, 5 mmol) and 4-amino benzanilide (1.06 g, 5 mmol) by the procedure as described for **3** to give crude product which on column chromatography over silica gel and elution with pet ether/ethyl acetate furnished a light brown crystalline compound **31** (1.23 g, yield 60%), mp 232–233 °C. Anal. Calcd for $C_{25}H_{20}N_2O_4$: C, 72.80; H, 4.887; N 6.79%. Found: C, 72.99; H, 4.883; N, 6.88%. MS (%) M^+ at *m/z* 412 (15), 381 (30), 264 (25), 202 (100), 173 (24), 143 (5), 116 (11), 92 (24). IR: 3376, 2917, 2849, 2230, 1602, 1543, 1486, 1252, 1038, 756 cm^{-1}. ^1H NMR: δ 5.97 (2H, s, $-OCH_2O-$), 6.45 (1H, d, J = 15.0 Hz, –CH=*CH*CO), (6.79–7.15 (5H, m, olefinic and Ar-*H*), 7.18–7.27 (3H, m, Ar-*H*″), 7.38 (1H, dd, J = 15.0, 9.2 Hz, –*CH*=CHCO), 7.52 (2H, d, J = 8.4 Hz, Ar-*H*-2″ and 6″), 7.63 (2H, d, J = 8.4 Hz, Ar-*H*-2′ and 6′), 7.81 (2H, d, J = 8.4 Hz, Ar-*H*-3′ and 5′). ^{13}C NMR: δ 100.3, 107.8, 108.0, 119.5, 120.4, 120.41, 120.5, 120.51, 123.8, 124.1, 125.9, 127.5, 127.51, 128.5, 128.7, 128.71, 128.8, 129.0, 138.0, 141.0, 142.7, 146.8, 147.5, 163.8, and 165.5.

4.2.31. Preparation of 5-(3,4-methylenedioxyphenyl)-2*E*,4*E*-pentadienoic acid 4′-benzanilide amide (32)

Compound **32** was prepared from piperic acid **2** (2.2 g, 10 mmol) and 4′-amino benzanilide (2.12 g, 10 mmol) by the method as described for **3** to give crude product which on crystallization from ethyl acetate furnished a light brown crystalline compound **32** (3.66 g, yield 90%), mp 210–211 °C. Anal. Calcd for $C_{25}H_{20}N_2O_4$: C, 73.80; H, 4.887; N, 6.79%. Found: C, 73.11; H, 4.884; N, 6.97%. MS (%) M^+ at *m/z* 412 (45), 382 (24), 292 (100), 201 (53), 172 (12), 143 (30), 116 (25). IR: 3390, 2918, 2850, 1651, 1608, 1512, 1252, 1038 cm^{-1}. ^1H NMR: δ 5.80 (2H, s, $-OCH_2O-$), 6.03 (1H, d, J = 15.0 Hz, –CH=*CH*CO), 6.73–7.02 (5H, m, olefinic and Ar-*H*), 7.10–7.35 (3H, m, –*CH*=CHCO and Ar-*H*-3″ and 5″), 7.50–7.67 (5H, m, Ar-*H*-2′, 3′, 5′, 6′ and Ar-*H*-4″), 7.88 (2H, d, J = 8.2 Hz, Ar-*H*-2″ and 6″). ^{13}C NMR: δ 101.6, 107.1, 108.3, 119.8, 120.5, 120.51, 120.6, 120.61, 124.1, 126.1, 128.7, 129.5, 129.15, 130.0, 130.9, 131.5, 131.5, 131.6, 135.8, 135.9, 142.9, 147.7, 147.8, 164.0, and 165.5.

4.2.32. Preparation of 5-(3,4-methylenedioxyphenyl) pentanoic acid piperidine amide (tetra hydro piperine) (33)

A mixture of methanolic solution of Piperine **1** (142 mg) and 5% Pd/C (30 mg) was hydrogenated at 40 psi for 4 h and the contents filtered, washed with methanol (5× 15 mL), and concentrated on a rotavapour to give crude product that on column chromatography over silica gel and elution with pet ether/ethyl acetate (4:1) afforded compound **33** (141.8 mg, yield 98.5%) as a gummy mass [the spectral data was in agreement with data reported in the lit.[28,50]]. Anal. Calcd for $C_{17}H_{23}NO_3$: C, 70.56; H, 8.010; N, 4.84%. Found: C, 70.75; H, 8.017; N, 4.98%. MS (%) M^{+1} at *m/z* 290 (42), 205 (28), 177 (41), 156 (100), 119 (32), 85 (18). IR 3162, 2929, 1734, 1637, 1481, 1405, 1241, 1037, 932 cm^{-1}. ^1H NMR: δ *1.22–1.58* (10H, m, $-N-CH_2(CH_2)_3$ and $-C-CH_2)_2$), 2.28 (2H, t, J = 7.0 Hz, $COCH_2$), 2.50 (2H, t, J = 7.0 Hz, Ar-CH_2), 3.38 (4H, m, $-N-(CH_2)_2$), 5.90 (2H, s, $-OCH_2O-$), 6.62 (3H, m, Ar-*H*). ^{13}C NMR: δ 24.1, 24.2, 24.6, 30.8, 31.1, 32.9, 34.9, 42.3, 46.4, 100.4, 107.7, 108.5, 120.7, 134.9, 144.5, 146.5, and 170.3.

4.2.33. Preparation of 5-(3,4-methylenedioxyphenyl) pentanoic acid isobutyl amide (34)

The compound **34** was prepared in 96% yield as a colorless solid from compound **6** by the same procedure as described for compound **33** mp 62 °C. [Lit.[50,51] mp 61–63 °C]. Anal. Calcd for $C_{16}H_{23}NO_3$: C, 69.29; H, 8.357; N, 5.05%. Found: C, 69.82; H, 8.365; N, 5.31%. MS (%) M^+ at *m/z* 278 (100), 205 (35), 177 (58), 144 (32), 119 (13). IR: 3292, 2932, 1642, 1551, 1489, 1251, 1039, 856 cm^{-1}. ^1H NMR: δ 0.97 (6H, d, J = 6.5 Hz, $-CH(CH_3)_2$), 1.65 (4H, m, $(CH_2)_2$), 2.16 (2H, m, $CO-CH_2$), 2.36 (1H, m, $-CH(CH_3)_2$), 2.53 (2H, t, J = 6.5 Hz, Ar-CH_2), 3.72 (2H, m, $-NHCH_2$), 5.89 (2H, s, $-OCH_2O-$), 6.66 (3H, m, Ar-*H*). ^{13}C NMR: δ 12.8, 19.1, 24.3, 30.4, 30.7, 34.4, 35.7, 38.2, 99.7, 107.1, 107.8, 120.1, 135.1, 144.5, 146.5, and 171.9.

4.2.34. Preparation of 5-(3,4-methylenedioxyphenyl) pentanoic acid *N*,*N*-diethyl amide (35)

Compound **35** was prepared in 98% yield as a gummy mass from compound **7** by the procedure as described for compound **33** [the spectral data was in agreement with data reported in the lit.[28]]. Anal. Calcd for $C_{16}H_{23}NO_3$: C, 69.28; H, 8.357; N, 5.05%. Found: C, 69.98; H, 8.366; N, 5.29%. MS (%) M^{+1} at *m/z* 278 (75), 205 (13), 175 (23), 134 (35), 127 (100), 114 (45), 99 (23), 71 (5). IR: 3381, 2928, 1638, 1485, 1403, 1244, 1039, 932 cm^{-1}. ^{1}H NMR: δ 1.10

and 1.15 (3H each, t, J = 7.2 Hz, 2× –CH_3), 1.64 (4H, m, (CH_2)$_2$), 2.30 (2H, t, J = 7.0 Hz, –COCH_2), 2.55 (2H, t, J = 6.7 Hz, Ar-CH_2), 3.30 (4H, q, J = 7.2 Hz, –NH(CH_2)$_2$), 5.91 (2H, s, –OCH_2O–), 6.59–6.74 (3H, m, Ar-H). ^{13}C NMR: δ 13.5, 14.7, 25.4, 31.9, 33.3, 35.9, 40.4, 42.3, 101.1, 108.4, 109.2, 121.4, 136.6, 145.9, 147.9, and 172.4.

4.2.35. Preparation of 5-(3,4-methylenedioxyphenyl) pentanoic acid 3-cyano-phenyl amide (36)

This was prepared in 84% yield as a gummy mass from compound **22** by the procedure as described for compound **33**. Anal. Calcd for $C_{19}H_{18}N_2O_3$: C, 70.79; H, 5.627; N, 8.69%. Found: C, 71.13; H, 5.633; N, 8.93%. MS (%) M^{+1} at m/z 321(85), 296 (18), 220 (39), 205 (100), 143 (12), 117 (24). IR: 3326, 2926, 2855, 2231, 1669, 1604, 1585, 1487, 1252, 1039, 795 cm^{-1}. ^{1}H NMR: δ 1.67 (4H, m, (CH_2)$_2$), 2.33 (2H, t, J = 7.0 Hz, –COCH_2), 2.55 (2H, t, J = 6.7 Hz, Ar-CH_2), 5.96 (2H, s, –OCH_2O–), 6.67–6.93 (3H, m, Ar-H), 7.21–7.43 (2H, m, Ar-H'), 7.82 (1H, s, Ar-H-2′), 8.01 (1H, d, J = 8.2 Hz, Ar-H-6′). ^{13}C NMR: δ 24.4, 30.8, 34.1, 35.2, 100.3, 107.3, 108.1, 113.7, 118.5, 120.4, 122.1, 123.4, 126.6, 129.3, 134.7, 144.7, 146.6, 149.5, and 172.1.

4.2.36. Preparation of 5-(3,4-methylenedioxyphenyl)-pentanoic acid 2-acetylamino-phenyl amide (37)

This was prepared in 48% yield as a gummy mass from compound **26** by the procedure as described for compound **33**. Anal. Calcd for $C_{20}H_{22}N_2O_4$: C, 67.71; H, 6.256; N, 7.90%. Found: C, 68.01; H, 6.265; N, 8.04%. MS (%) M^+ at m/z 354 (21), 309 (72), 295 (30), 205 (100), 173 (18), 149 (34), 115 (12). IR: 3162, 2926, 1667, 1601, 1506, 1485, 1404, 1247, 1038, 756 cm^{-1}. ^{1}H NMR: δ 1.67 (4H, m, –(CH_2)$_2$), 2.11 (3H, s, –COCH_3), 2.32 (2H, t, J = 7.0 Hz, –COCH_2), 2.56 (2H, t, J = 7.0 Hz, Ar-CH_2), 5.93 (2H, s, –OCH_2O–), 6.68–6.96 (3H, m, Ar-H), 7.14–7.46 (4H, m, Ar-H). ^{13}C NMR (50 MHz): δ 22.4, 25.3, 29.7, 35.3, 36.5, 100.9, 107.9, 108.4, 121.2, 123.2, 123.4, 125.9, 126.0, 134.8, 134.9, 135.1, 146.9, 147.6, 166.7, and 172.3.

4.2.37. Preparation of 5-(3,4-methylenedioxyphenyl)-pentanoic acid 4-benzanilide amide (38)

This was prepared in 85% yield as a gummy mass from compound **31** by the procedure as described for compound **33**. Anal. Calcd for $C_{25}H_{24}N_2O_4$: C, 72.09; H, 5.808; N, 6.72%. Found: C, 72.68; H, 5.816; N, 6.93%. MS (%) M^{+1} at m/z 415 (95), 339 (38), 268 (25), 205 (100), 177 (28), 134 (15), 116 (31), 92 (20). IR: 3180, 2925, 1655, 1599, 1404, 1320, 1245, 1038, 758 cm^{-1}. ^{1}H NMR: δ 1.58 (4H, m, (CH_2)$_2$), 2.32 (2H, t, J = 7.0 Hz, –COCH_2), 2.54 (2H, t, J = 6.7 Hz, Ar-CH_2), 5.86 (2H, s, –OCH_2O–), 6.62–6.84 (3H, m, Ar-H), 7.08–7.26 (3H, m, Ar-H''), 7.56 (2H, d, J = 8.2 Hz, Ar-H-2″ and 6″), 7.69 (2H, d, J = 8.2 Hz, Ar-H-2′ and 6′), 7.86 (2H, d, J = 8.4 Hz, Ar-H-3′ and 5′). ^{13}C NMR: δ 23.9, 30.5, 32.3, 33.9, 99.6, 107.2, 108.4, 111.3, 118.6, 120.3, 120.4, 120.6, 123.5, 127.9, 128.0, 128.3, 128.7, 134.9, 136.8, 137.8, 144.6, 146.7, 151.6, 165.2, and 173.7.

4.3. Determination of minimum effective concentration (MEC) of the EPIs

The MIC of ciprofloxacin was determined against *S. aureus* 1199 and *S. aureus* 1199B in Muller–Hinton Broth in the presence of increasing amounts of efflux pump inhibitors by broth checkerboard synergy method in a microtitre plates using twofold serial dilutions.[46] Each candidate EPI was tested at seven concentrations (100–1.56 μg/mL) and ciprofloxacin was tested at 10 concentrations (16–0.03 μg/mL). The plates were incubated for 18 h at 37 °C and the wells were assessed visually for growth. The minimal effective concentration (MEC) was determined to be the minimal concentration of EPI that produced the maximal reduction in substrate MIC. No further decrease in substrate MIC was observed at EPI concentrations greater than the MEC.[52]

4.4. Efflux studies

Ethidium bromide accumulation and its efflux were determined from the active cells by reported method.[53] *S. aureus* 1199B was grown overnight on Trypticase Soya Agar. Bacterial suspension (0.2 OD_{550}) was prepared in uptake buffer (NaCl, 110 mM, KCl, 7 mM; NH_4Cl, 50 mM; Na_2HPO_4, 0.4 mM; Tris base, 52 mM; glucose, 0.2%, adjusted the solution to pH 7.5 with HCl). The suspensions were exposed to 2 μg/mL ethidium bromide in presence of the most active EPIs (**22** and **26** of 6.25 and 12.5 μg/mL concentration, respectively), inactive EPI **5** (100 μg/mL), piperine (50 μg/mL) and standard EPI reserpine (25 μg/mL) for 30 min at 37 °C. The cells were pellet down by centrifugation and re-suspended in fresh buffer. The loss of fluorescence was recorded for 30 min at 5 min interval at excitation and emission wavelengths of 530 and 600 nm in a spectrophotometer (Perkin-Elmer model LS50).

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