Available online at www.sciencedirect.com
SCIENCE DIRECT®
ELSEVIER
Bioorganic & Medicinal Chemistry 12 (2004) 1905–1920
Bioorganic & Medicinal Chemistry

Effects of piperine analogues on stimulation of melanocyte proliferation and melanocyte differentiation

Radhakrishnan Venkatasamy,[a] Laura Faas,[a] Antony R. Young,[b] Amala Raman[a] and Robert C. Hider[a,*]

[a]*Department of Pharmacy, King's College London, Franklin-Wilkins Building, 150 Stamford Street, London SE1 9NN, UK*
[b]*St John's Institute of Dermatology, King's College London, Lambeth Palace Road, London SE1 7EH. UK*

Received 11 November 2003; accepted 23 January 2004

Abstract—A wide range of piperine analogues has been synthesised in order to undertake a structure–activity study of their ability to stimulate melanocyte proliferation. Results demonstrate that an aromatic ring containing at least one ether function and a carbonyl group containing side chain is essential for this activity. A number of highly active piperine analogues have been identified, for instance 1-(3,4-methylenedioxyphenyl)-penta-2*E*,4*E*-dienoic acid methyl ester (**5a**), 1-*E,E*-piperinoyl-isobutylamine (**4f**) and 1-(3,4-methylenedioxyphenyl)-pentanoic acid cyclohexyl amide (**20**). A selection of analogues has also been evaluated for their effect on melanocyte morphology and melanogenesis. The piperine analogues altered cell morphology by increasing dendrite formation leading to bi-, tri- and quadripolar cells. These same analogues were found to increase total melanin in cell cultures, although melanin content per cell was not significantly altered from control in the presence of these compounds.

1. Introduction

Vitiligo is a common skin pigment disorder affecting 1% of the world population, the incidence varying between 0.1–9% in different countries.[1] Vitiligo is defined as a 'circumscribed, acquired, idiopathic, progressive hypomelanotic skin disorder, which is characterised by the development of patchy depigmented macules due to progressive loss of melanocytes'. From this definition vitiligo can be differentiated from other hypomelanotic skin disorders such as chemically induced depigmentation, depigmentaton associated with melanoma, and depigmentation secondary to various other dermatoses.[1] In ancient times various herbal remedies were used in Ayurvedic, Unani and Chinese medicine to treat vitiligo. Most of the formulations had psoralen as a main constituent but a few did not.[2]

In 1999, twenty-eight herbs were screened for potential stimulants of proliferation using a microtitre colorimetric assay.[3] In this study aqueous extracts of seven herbs were found to be active melanocyte proliferants, black pepper (*Piper nigrum* L.) being the most potent herb screened,[3,4] Piperine, the main pepper alkaloid, was also found very effective at micromolar concentrations.[4] This melanocyte proliferant activity of piperine had not previously been reported. Since *Piper nigrum* extract demonstrated high melanocyte proliferant activity it was assumed that not only piperine possesses this activity but also possibly other structural analogues may share the effect. In order to investigate this possibility, we have synthesized various piperine analogues in order to monitor their melanocyte proliferation stimulation activity.

A number of technical problems persist with culturing human melanocytes, for instance human melanocytes can not be passaged for more than three cycles and will not survive during routine frozen storage,[5] hence melanocyte cell lines from mouse skin were adopted for

Keywords: Piperine analogues; Melanocytes; Dendricity; Melanogenesis; Vitiligo.
Abbreviations: TPA, 12-*O*-Tetradecanoyl phorbol-13-acetate; CT, Cholera toxin; SRB, Sulphorhodamine B; DCM, Dicholormethane; DMSO, Dimethyl sulfoxide; $D_{7.4}$, Distribution coefficient at pH 7.4; TCA, Trichloroacetic acid; OD, Optical density; PBS, Phosphate buffer saline.
* Corresponding author. Tel.: +44-20-7848-4882; fax: +44-20-7848-4800; e-mail: robert.hider@kcl.ac.uk

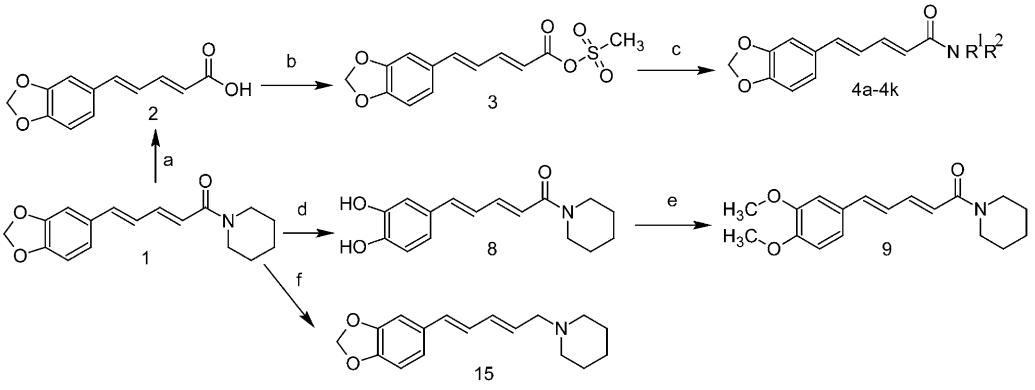
0968-0896/$ - see front matter © 2004 Elsevier Ltd. All rights reserved.
doi:10.1016/j.bmc.2004.01.036

bioassay. One such cell line is melan-a which is an immortal line of pigmented mouse cells cultured from epidermal melanoblasts obtained from embryos of inbred C57BL mice.[5] This cell line is non-tumorigenic and 12-*O*-tetradecanoyl phorbol-13-acetate (TPA) is essential for the proliferation of these cells. Cells can be readily cultured and survive well in routine frozen storage.[5]

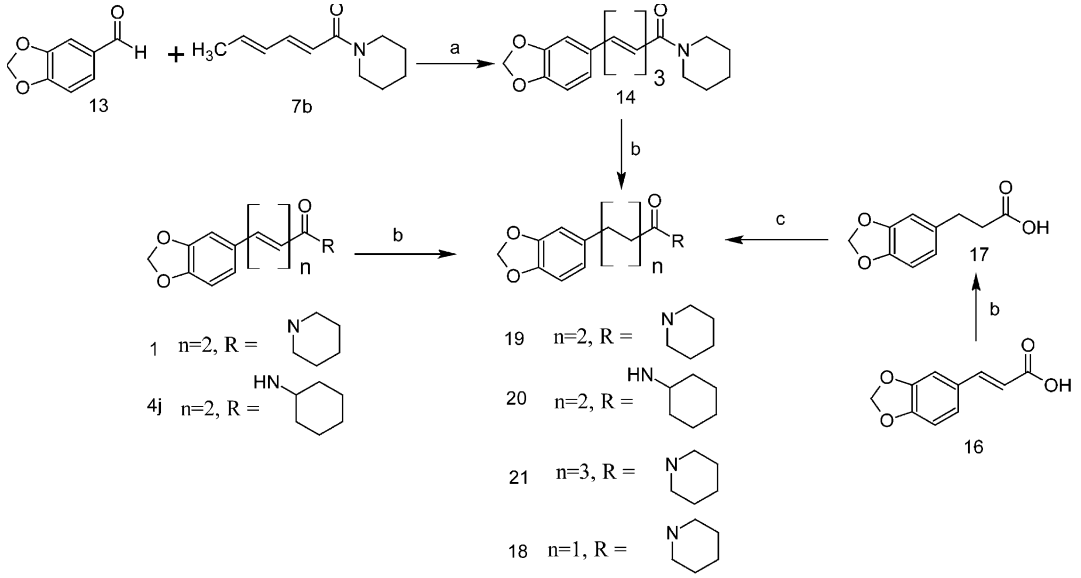
2. Chemistry

Other workers have previously prepared a wide range of piperine analogues, for instance Koul et al.[6] investigating inducible cytochrome P450 activities and Paula et al.[7] investigating insecticidal activity. In order to investigate the chemical structure responsible for the proliferant activity, four different series of compounds were prepared by using standard synthetic routes. All modifications were structural analogues of piperine. In order to determine the role of the amide function in the proliferation activity, a series of aliphatic amines (C_1–C_6), aromatic amines and heterocyclic amines were used to synthesise a series of amide analogues. Piperinic acid **2**, obtained by alkaline hydrolysis of piperine **1**, was converted to an ester intermediate **3** by reacting with methane sulfonyl chloride and triethylamine. By reacting this intermediate in situ with different amines, the corresponding amides **4a–k** were synthesised (Scheme 1).[8] The amide function of piperine was also replaced by a range of ester groups, yielding the corresponding analogues **5a–d**.

The methylenedioxy phenyl group was substituted with either methyl or phenyl groups, the corresponding amide derivatives **7a–b** were also obtained. The dimethoxyphenyl containing analogue **9** was also investigated. In order to achieve the synthesis of this latter molecule, Piperine **1** was sequentially treated with BBr_3 and methyl iodide[9] (Scheme 1). In addition to these structural modifications, the effect of length of aliphatic chain and degree of saturation on proliferation activity was investigated. The chain length was reduced to two carbons and the amide groups were systematically varied and also substituted by an ester group The methylenedioxy phenyl function was also substituted and these analogues **10a–d**, **11a–c**, and **12** were synthesised from the corresponding acids. Piperettine **14**, which contains a six carbon aliphatic chain was synthesised by modifying the method of Dehmlow and Shamout[10] (Scheme 2).

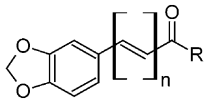


Scheme 1. (a) KOH (b) $MeSO_2Cl$, Et_3N, CH_2Cl_2, 0 °C 45 min (c) R^1R^2NH, 0 °C 1 h, rt 2 h (d) BBr_3 in CH_2Cl_2 rt 48 h (e) DMSO/KOH, CH_3I, nitrogen atomosphere, 30 min (f) $NaBH_4/I_2$, THF, 0 °C nitrogen atomosphere, 48 h.



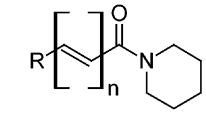
Scheme 2. (a) Aliquat 336, K_2CO_3/NaH, toluene, 90 °C 6 h (b) H_2, Pd-C (c) $MeSO_2Cl$, Et_3N, CH_2Cl_2, 0 °C 45 min, RNH, 0 °C 1 h, rt 1 h.

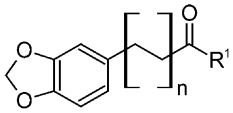
Table 1. Structures of piperine analogues



Code	*n*	R	Code	*n*	R
1	2	piperidinyl	**4k**	2	aminobutyl
4a	2	pyrrolidinyl	**5a**	2	methoxy
4b	2	morpholinyl	**5b**	2	ethoxy
4c	2	aminobenzyl	**5c**	2	propyloxy
4d	2	amino-5-methyl	**5d**	2	butyloxy
4e	2	aminohexyl	**11a**	1	piperidinyl
4f	2	aminoisobutyl	**11b**	1	pyrrollidinyl
4g	2	aminomethyl	**11c**	1	morpholinyl
4h	2	aminoethyl	**12**	1	methoxy
4i	2	aminoisopropyl	**14**	3	piperidinyl
4j	2	aminocyclohexyl			

Table 2. Structures of piperine analogues





Code	*n*	R	Code	*n*	R^1
7a	2	phenyl	**18**	1	piperidinyl
7b	2	methyl	**19**	2	piperidinyl
9	2	3,4-dimethoxyphenyl	**20**	2	cyclohexylaminyl
10a	1	2methoxyphenyl	**21**	3	piperidinyl
10b	1	3-methoxyphenyl			
10c	1	4-methoxyphenyl			
10d	1	3,4-dimethoxyphenyl			

The degree of saturation of the aliphatic chain was also modified. 5-(3,4-methylenedioxyphenyl)-penta-2*E*,4*E*-dienyl piperidine **15** (Scheme 2) was isolated during an attempt to reduce a single double bond of piperine according to the method of Das et al.[11] No trace of double bond reduction was observed despite systematic variation of reaction conditions.[11] 3,4-Methylenedioxycinnamic acid was hydrogenated in ethanol over 5% Pd-C under hydrogen to yield 5-(3,4-methlyenedioxyphenyl)-propanoic acid and this saturated acid was converted to the amide derivative **18** by condensation with piperidine (Scheme 2). Catalytic hydrogenation was used to reduce amides **1**, **4j**, and **14** to the corresponding saturated analogues **19**, **20**, and **21** (Scheme 2). Systematic presentation of the complete series of molecules synthesised in this work is given in Tables 1 and 2.

3. Partition coefficient measurement

Since the majority of the molecules investigated in this study are neutral over the pH range 4–9 and their degree of ionisation is diminishing small at pH 7.4, their distribution coefficients at pH 7.4 are expected to be identical to their partition coefficients. When designing a dosage form for transdermal application, compounds possessing Log P values around 3–4 are considered to be optimum.[12] CLog P values were calculated for the entire series[13] and Log P values were also measured by the shake-flask method for a small selection of compounds. These determined log P values were compared with the corresponding CLog P values (Fig. 1). The dotted line is indicative of the theoretically perfect correlation between ClogP and the experimentally determined log P. There was broad agreement between these two values for **1**, **5a**, **4j**, **19** and **20** but the compound **11c** deviated to an appreciable degree; this is likely to be associated with the presence of the morpholine group.

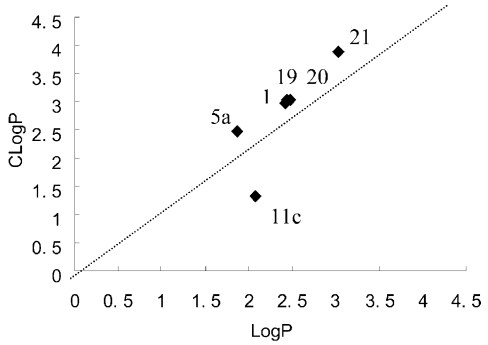


Figure 1. Comparison of practical LogP value and calculated logP value of piperine and its analogues.

4. Results and discussion

4.1. Melanocyte proliferation activity

Due to non-availability of a suitable medium capable of maintaining culture of normal melanocytes, melanoma cells have frequently been used to investigate the effect of growth factors on melanocyte growth in vitro.[14] However normal melanocytes are essential for the study of the effect of biological agents on melanocyte proliferation, as normal melanocytes possess critical properties[14]. Eisinger and Mako (1982)[14] reported that 12-*O*-tetradecanoyl phorbol-13-acetate (TPA) and cholera toxin (CT) are effective at supporting normal human melanocyte proliferation. These two compounds therefore provide a useful medium for the support of mouse melanocyte growth. The microplate assay is an efficient way of screening compounds for cytotoxic and cell proliferation activity and the sulphorhodamine B (SRB) staining procedure [3,4] was adopted to evaluate these activities. Melan-a cell proliferation activity was also established using the microplate assay.

(i) Effect of amide moiety

The amide containing compounds **4a**–**k** were found to possess proliferation activity with the exception of **4c** (Table 3). Compound **4a** stimulated melanocyte proliferation at 1–50 μM having weak activity at 1 μM

and peak activity at 10 μM. **4b** was found to be active in the range of 1–100μM with peak activity at 10 μM. Compound **4d** has peak activity at 50 μM and **4e** at 100 μM. Compound **4f** showed similar response at 1–100 μM. Compound **4g–i** showed melanocyte proliferation activity at 1–10 μM while being cytotoxic at 50 and 100 μM. Replacement of piperidine in piperine by cyclohexylamine and *n*-butylamine (**4j** and **4k**) showed most promising melanocyte proliferation effect at 1–50 μM. but no effect at 100 μM.

(ii) Effect of ester moiety

All piperine ester analogues **5a–d** were found to be effective at both 10 and 50 μM (Table 4). Compound **5a** showed melanocyte proliferation stimulatory activity at 1–100 μM. **5b** was cytotoxic at 100 μM. Compound **5c** showed greatest melanocyte proliferative stimulatory activity at 10 μM but no effect at 100 μM. **5d** possesses activity at 10–100 μM with no effect at 1 μM.

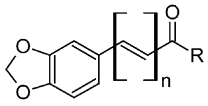
(iii) Effect of aromatic ring

The aromatic-substituted compounds **7a** and **b** were not found to be active at 1–100 μM concentrations. Compound **9** was found be moderately active over the range 10–100 μM, however this compound was ineffective at 1μM. This indicates that an etherified phenyl group is essential for activity (Table 5).

(iv) Effect of aliphatic chain length

None of the short chain piperine analogues **10a–d**, **11a–c**, and **12** possessed activity in the range of 1–100 μM, with the single exception of **11a**. The compound **11a** was not effective at 1 μM but it showed melanocyte proliferative activity at the other concentration studied. However, introduction of one more double bond in the piperine molecule, i.e., **14** showed melanocyte proliferation activity at 1–10 μM and 100 μM. From this observation it is possible to deduce that both the

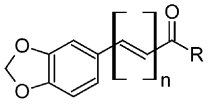
Table 3. Piperine amide analogues



Code	*n*	R	ClogP	Biological activity		Melanocyte proliferation stimulatory activity of piperine amide analogues			
				Stimulant activity	Dendricity	Proliferation stimulatory activity† at			
						1 μM	10 μM	50 μM	100 μM
1	2	piperidinyl	2.963	Strong activity	+ + +	192±19**	211±18**	181±14**	99±10
4a	2	pyrrolidinyl	2.354	Strong activity	+ + +	126±9**	185±3**	158±11**	92±13
4b	2	morpholinyl	1.783	Strong activity	+ + +	129±9**	171±9**	165±7**	130±14**
4c	2	aminobenzyl	3.883	No effect	–	93±6	99±9	ND	104±12
4d	2	piperinolaminyl	3.448	Positive	+	101±12	138±10**	150±16**	71±10
4e	2	aminohexyl	4.760	Strong activity	+ + +	93±6	152±11**	181±18**	197±18**
4f	2	aminoisobutyl	3.572	Strong activity	+ + +	145±9**	172±6**	160±14**	156±11**
4g	2	aminomethyl	1.824	Strong activity	+ +	140±12**	170±24**	71±5	46±2
4h	2	aminoethyl	1.597	Strong activity	+ + +	139±27**	200±14**	81±12	62±13
4i	2	aminoisopropyl	2.662	Strong activity	+ + +	187±15**	224±19**	85±5	42±6
4j	2	aminocyclohexyl	3.855	Strong activity	+ + +	301±20**	308±29**	155±22**	100±13
4k	2	aminobutyl	3.441	Strong activity	+ + +	189±6**	264±21**	158±20**	84±6

*$p<0.05$, **$p<0.01$ when compared to vehicle treatment (One way Anova followed by Dunnett's *t*-test) †Expressed as percentage cell growth±SEM compared to control (medium only) – no effect, + weakly dendritic, + + moderately dendritic, + + + highly dendritic.

Table 4. Piperine ester analogues



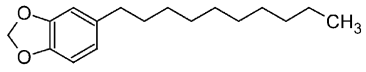
Compd	*n*	R	ClogP	Biological activity		Melanocyte proliferation stimulatory activity of ester analogues			
				Stimulant activity	Dendricity	Proliferation stimulatory activity† at			
						1 μM	10 μM	50 μM	100 μM
5a	2	methoxy	2.484	Strong activity	+	125±14**	147±17**	187±13**	171±8**
5b	2	ethoxy	2.783	Strong activity	+ +	149±7**	207±10**	169±7**	52±13
5c	2	propyloxy	3.542	Strong activity	+ +	175±6**	224±12**	148±19**	90±7
5d	2	butyloxy	4.071	Strong activity	+ +	103±5	148±18**	190±11**	128±17**

*$p<0.05$, **$p<0.01$ when compared to vehicle treatment (One way Anova followed by Dunnett's *t*-test) †Expressed as percentage cell growth±SEM compared to control (medium only) – no effect, + weakly dendritic, + + moderately dendritic, + + + highly dendritic.

methylenedioxyphenyl group and aliphatic chain length are important for activity (Table 6).

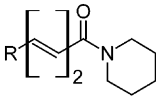
Saturated analogues **19**, **20**, and **21** all possessed strong activity except short chain saturated analogue **18**. Compound **19** showed the peak activity at 10 μM. It also showed very weak activity at 1 μM and was highly toxic to the cells at 100 μM. **20** showed consistent activity throughout the concentration range of 10–100 μM having weaker activity at 1 μM. **21** showed greatest melanocyte proliferative stimulatory activity at 50 μM. This compound was also found to be active at 10 μM (Table 7). However **15**, where the carbonyl group is reduced, did not show melanocyte proliferative stimulatory activity over the range 1–100 μM (Table 6), indicating that the carbonyl group is essential for maximum activity. Thus the length of connection chain between the two pharmacophores is apparently more important than the hybridization state of this carbon chain.

From these results it is clear that the nature of the amide residue has an important role for the activity of the piperine analogues. Thus with the introduction of benzylamine **4c** results in total loss of activity, whereas a wide range of acylic and cyclic aliphatic amines, were found to all possess activity (Table 3). Interestingly the compounds with smaller residues (**4g** and **i**) lose potency at high concentration (100 μM), in contrast to the more lipophilic analogue **4e** which maintains activity over a wide range (1–100 μM) (Fig. 2). When converted to the free acid **2** of the corresponding amine **1** activity is completely lost. In contrast simple aliphatic esters possess activity, albeit somewhat weaker than the corresponding amides (Table 4). The finding that a simple alkyl analogue **22** of identical length to **4k** totally lacks activity (data not shown) confirms that the concept the carbonyl function of the amide and ester is essential for receptor binding.



22

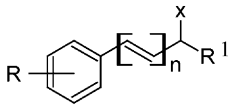
Table 5. Effect of aromatic moiety



Code	R	ClogP	Biological activity		Melanocyte proliferation stimulatory activity of aromatic substituted analogues			
			Stimulant activity	Dendricity	Proliferation stimulatory activity† at			
					1 μM	10 μM	50 μM	100 μM
7a	methyl	2.484	No effect	—	83±10	95±6	ND	72±10
7b	phenyl	2.783	No effect	—	96±8	92±10	ND	98±13
9	3,4-dimethoxyphenyl	3.542	Positive	—	104±7	127±11**	165±23**	161±5**

*$p<0.05$, **$p<0.01$ when compared to vehicle treatment (One way Anova followed by Dunnett's *t*-test) ND:Not done †expressed as percentage cell growth±SEM compared to control (medium only) — no effect.

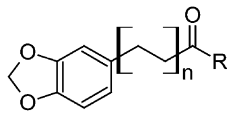
Table 6. Effect of aliphatic chain length



Code	R	R^1	x	*n*	ClogP	Biological activity		Melanocyte proliferation stimulatory activity of piperine analogues with modified connecting chain and connecting chain length			
						Stimulant activity	Dendricity	Proliferation stimulatory activity† at			
								1 μM	10 μM	50 μM	100 μM
10a	2-methoxy	piperidinyl	O	1	2.813	No effect	—	99±8	105±8	103±6	119±9
10b	3-methoxy	piperidinyl	O	1	2.813	No effect	—	99±5	95±18	110±11	127±9
10c	4-methoxy	piperidinyl	O	1	2.813	Weak	—	90±10	108±20	111±10	133±15*
10d	3,4-dimethoxy	piperidinyl	O	1	2.552	Weak	—	95±11	100±9	114±8	123±7
11a	3,4-methylenedioxy	piperidinyl	O	1	2.459	Strong Activity	+ +	119±10	180±8**	197±12**	195±13**
11b	3,4-methylenedioxy	pyrrolidinyl	O	1	1.900	Positive	—	106±11	140±14	ND	98±9
11c	3,4-methylenedioxy	morpholinyl	O	1	1.329	No effect	—	85±8	103±12	ND	134±12
12	3,4-methylenedioxy	methoxy	O	1	2.030	No effect	—	100±10	129±15	ND	110±14
14	3,4-methylenedioxy	piperidinyl	O	3	4.072	Strong Activity	+ + +	199±13**	217±15**	59±6	122±11*
15	3,4-methylenedioxy	piperidinyl	H_2	2	2.813	No effect	—	99±5	95±18	110±11	127±9

*$p<0.05$, **$p<0.01$ when compared to vehicle treatment (One way Anova followed by Dunnett's *t*-test) ND: Not done †Expressed as percentage cell growth±SEM compared to control (medium only) - no effect, + + moderately dendritic.

Table 7. Effect of aliphatic chain-saturated analogues



Code	n	R	ClogP	Biological activity		Melanocyte proliferation stimulatory activity of saturated piperine analogues			
				Stimulant activity	Dendricity	Proliferation stimulatory activity† at			
						1 μM	10 μM	50 μM	100 μM
18	1	piperidinyl	1.643	No effect	—	90±10	108±20	111±10	133±15**
19	2	piperidinyl	2.551	Strong Activity	+++	118±12	189±16**	160±15**	80±2
20	2	aminocyclohexyl	3.046	Strong Activity	+++	129±6**	193±6**	192±10**	191±13**
21	3	piperidinyl	3.459	Strong Activity	++	118±9	144±6**	160±16**	111±20

*$p<0.05$, **$p<0.01$ when compared to vehicle treatment (One way Anova followed by Dunnett's t-test) ND:Not done †Expressed as percentage cell growth±SEM compared to control (medium only) – no effect, ++ moderately dendritic.

When the linking chain length between the aromatic nucleus and the amide function is reduced to 2 carbons the activity remains virtually unchanged for the piperine analogue **11a** whereas compounds **11b**, **c**, and **12** (Table 6) are weaker than their 4 carbon chain analogues **4a**, **b**, and **5a** respectively (Tables 4 and 5).

One analogue series was used to investigate the influence of changes on the aromatic nucleus. Conversion to the dimethyl analogue **10d** and to various monomethyl analogues **10a**, **b**, and **c** leads to a reduced potency but all were found to possess some activity. In contrast when no methoxy or methylenedioxy group is present in the ring, the activity is totally abolished, for example with **7b**. In contrast, substitution of the methylenedioxy function in the parent compound (**1**) by two methoxy functions **9** failed to have a major influence on the biological activity compared to piperine (Table 5). Compound **9** did not alter the morphology of cells. Clearly at least one ether function is required for the melanocyte proliferation inducing-activity. Surprisingly when the aromatic and amide residues are conserved but the length of linking unsaturated chain is varied, activity is maintained. The length of the chain can range between 4.3 Å (**11a**) and 9.9 Å (**14**) and yet virtually full activity is conserved. Significantly when the linking chain is saturated, the activity is also conserved in many of the analogues (Tables 6). Interestingly the peak activity for **11a** ($n=1$) was at 50 μM (197%) and this effect was maintained at 100μM (195%) but not at 1 μM. In contrast with piperine (**1**) ($n=2$) peak activity was observed at 10 μM and the compound being ineffective at 100 μM. **14** ($n=3$) showed peak activity at 10 μM but was toxic at 50μM. This phenomenon could be due to different modes of action at lower and higher concentrations and reveal that the number of carbons might influence the cells in different ways Thus although the structure of both the aromatic residue and the amide portion of the molecules are essential for activity, the distance and type of linkage between these two functionalities is apparently less critical.

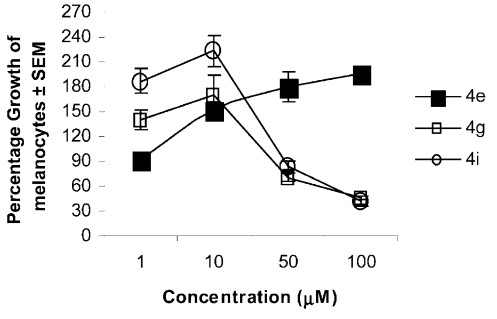


Figure 2. Influence of **4e**, **g**, **i** on the growth of melan-a cells. Cells were incubated at 37 °C for 4 days ($n=6$, mean±SEM). Percentage growth is expressed as percentage cell number compared to control (medium only).

5. Dendricity

Vitiliginous skin melanocytes lose their dendrites before disappearing from the epidermis.[15] In order to achieve pigmentation in vitiligo patients, it is important that treatments should aim not only to increase melanocyte proliferation but also increase melanocyte dendricity and melanogenesis.

Effect on dendricity of melan-a cells by the test compounds was given a rating based on observation under microscope. Very long dendrites and more number of dendrites were designed as highly dendritic. Medium length and bipolar dendrites were considered as moderate and bipolar dendrites with smaller length were designed as weakly dendritic. The results showed in Table 3–7 indicated that piperine amide analogues stimulate more dendricity when compared to piperine ester analogues. The inactive compounds did not show any morphological changes in melanocytes.

The detailed quantitative analysis of dendricity has been carried out. Melanocyte (melan-a) cultures were maintained for 72 h in the medium containing piperine or other piperine analogues (10 μM). More than 90% of these melanocytes displayed lengthy dendritic processes, varying from a bipolar to a quadruple polar nature.

Dendrites were analysed with the aid of a computer-aided image analyzer. Each compound was found to increase both the average dendrite length and dendrite

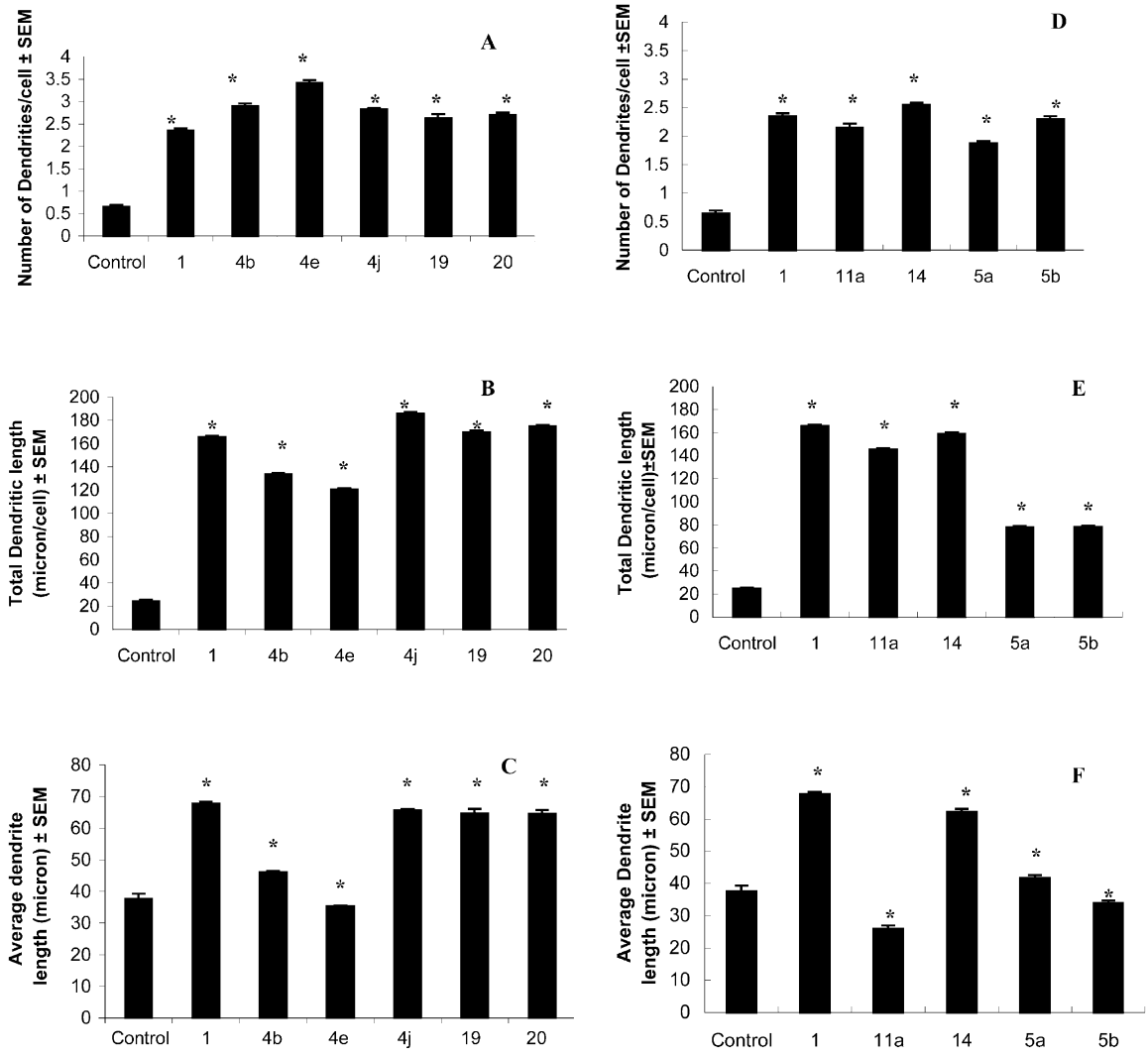


Figure 3. Effect of piperine and its analogues on dendricity.

numbers per cell. Compounds **1**, **4b**, **e**, **j**, **14**, **19**, and **20** showed a marked increase in dendricity of melanocytes (Fig. 3A) when compared to the control. This group of compounds increased the dendricity mainly to a bipolar state with a few cells possessing a tripolar and quadruple polar nature. In contrast, in the presence of compound **5a**, **b** and **11a**, melanocytes were limited to a bipolar nature (Fig. 3D). Having increased the number of dendrites per melanocyte all these compounds **1**, **4b**, **e**, **j**, **11a**, **14**, **19**, and **20** were also found to increase the total length of the dendrites whereas **5a**, **b** did not increase the total length of dendrites (Fig. 3B and E). The typical highly dendritic appearance of melanocytes treated with **1** and **4j** and **19** and **20** is shown in Figure 4.

The phenomenon of increasing dendricity appears to be close to an 'all or nothing' effect. With the possible exception of **5a**, **b** and **11a**, the total dendritic length of the cells and the length of individual dendrites were found to be remarkably constant.

Piperine analogues (10 μM) were added to the melanocyte cell culture ($n=4$). The cells were incubated at 37 °C for 3 days. The piperine amide analogues **4b**, **4e**,

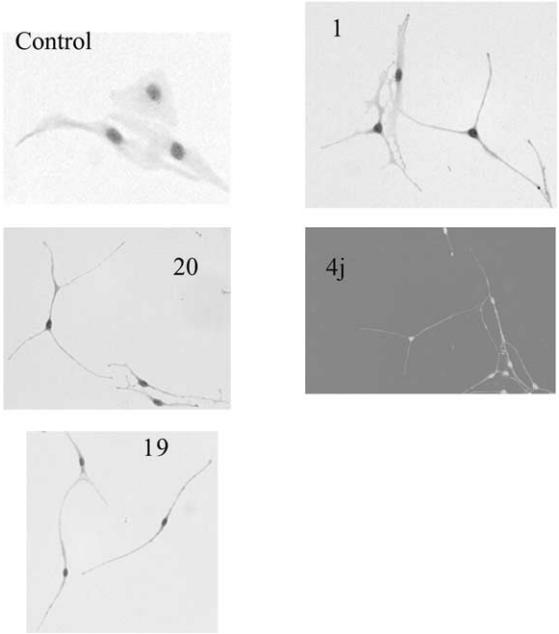


Figure 4. Effect of piperine (**1**) and its analogues **4j**, **19** and **20** on dendricity.

Table 8. Effect of piperine and its analogues on melanogenesis

Compd	Total melanin content per dish (μg/mL)	Melanin content/cell (ng/mL)
Control	16.4±0.7	0.29±. 0.03
1	24.5±1.7**	0.28±0.04
19	19.6±3.5	0.24±0.03
4j	22.3±4.1*	0.30±0.06
21	21.6±2.2	0.28±0.02

*$p<0.05$, **$p<0.01$ when compared to vehicle treatment saturated (One way Anova followed by Dunnett's *t*-test).

4j and its saturated analogues **19**, **20** showed increase in number of dendrites (**A**) increase in dendrite length (**B**) and increase in average dendrite length (**C**). Short chain piperine amide analogue **11a**, piperine ester analogues **5a**, **b** showed similar in dendrite numbers (**D**) but decrease in dendrite length (**E**) and average dendrite length (**F**) when compared to piperine whereas piperettine **14** showed the similar response to piperine. *$p<0.05$ when compared to vehicle treatment (One way Anova followed by Dunnett's *t*-test).

6. Melanogenesis

Pigmentation in the skin depends on the amount, size and type of melanins produced by melanocytes. Melanin quantification is necessary to investigate the ability of the compounds to influence melanogenesis. After 3 days incubation piperine and three analogues **4j**, **19**, and **21** showed evidence of increased total melanin although melanin content per cell was the same. This indicates that the compounds have neither a stimulatory nor significant inhibitory action on melanogenesis (Table 8).

7. Conclusion

The work reported in this communication confirms the previous observation that piperine induces melanocyte proliferation and enhanced dendricity (Lin et al.).[4] Furthermore, it has now been demonstrated that a range of closely related analogues also possess this property. Most of the compounds showed maximum activity at 10 μM. Variation in peak activity was observed with **4d**, **5a**, **d**, **11a**, **20**, **9** (50 μM), and **4e** (100 μM), Toxic effects were observed with **4d**, **g**, **h**, **i**, **5b**, and **19** at 100 μM.

Many of the active compounds retain their activity over a relatively wide concentration range (1–100 μM) for instance **4b**, **e**, **5a**, and **20**. In contrast others including **1**, **4a**, **g**, **h**, **i**, **5b**, **c**, and **19** were found to possess reduced potency at higher concentrations, indicating a saturation of the stimulus. The melanocyte proliferation effect is inhibited by RO-31-8220, a selective protein kinase C inhibitor strongly suggests that protein kinase C signaling is central to this activity (Lin et al.).[4] With the piperine analogues **19**, **4j** melanocyte proliferation activity was also abolished by protein kinase C inhibitors (unpublished data). Protein kinase C is a Ca^{2+}-activated phospholipid-dependent protein kinase, which consists of two major domains, a membrane-bound regulatory domain and a cytoplasmic catalytic domain.[16] Both domains possess multiple ligand binding sites and it is tempting to propose that because the distance between the two parts of the pharmacophore in active molecules, namely the aromatic centre and the aliphatic amide function, can be separated by a wide range of distances and yet retain full activity, that they interact simultaneously with two binding sites, one on each of the two receptor domains. Work is in progress to investigate this possibility.

The major observations resulting from these structure–activity studies are that the logP values of the molecules are not critical, but probably should exceed unity; the aliphatic chain can be either unsaturated or fully saturated, but must include a carbonyl function for optimal activity and that the aromatic function must include at least one ether group. Some of these analogues, for instance, **20** and **21**, by virtue of their saturated nature, are more stable towards autooxidation than the parent molecule piperine and hence will have advantages as pharmaceutical agents.

All the active compounds investigated in this study appear to induce identical changes to melanocytes, namely transformation to bipolar cells each possessing dendrites of similar length. The virtually identical response produced by these piperine analogues indicates that at a certain threshold level they activate a switching mechanism. Piperine and some of the analogues identified in this manuscript are under preclinical development for the treatment of vitiligo.

8. Experimental

8.1. General

Melting points were recorded using an Electrothermal IA9100 digital melting point apparatus and are corrected. ^{1}H and ^{13}C NMR spectra were recorded using a Bruker (360 MHz) NMR spectrophotometer unless specified. Mass spectra (EI) were recorded on a Joel AX505W. Elemental analysis was performed by Micro-Analytical Laboratories, Department of Chemistry, The University of Manchester, Manchester M13 9PL UK.

8.2. (2*E*,4*E*)-Piperinic acid (2)

Methanolic KOH (20%, 100 mL) was added to piperine (**1**) (2 g, 0.7 mmol, 1 equiv), and refluxed for 2 days. After completion of the hydrolysis, methanol was removed under reduced pressure and a yellow coloured oily solid was obtained. This residue was dissolved in water (50 mL) and acidified with 6N HCl to pH < 1 yielding a yellowish precipitate of (2*E*,4*E*)-piperinic acid (**2**). Recrystallization from methanol gave yellow needles (0.9 g, 60% yield). mp 206–208 °C.

8.3. Compounds (4a–k)

A mixture of (2*E*,4*E*)-piperinic acid (**2**) (200 mg, 0.9 mmol, 1 equiv) and triethylamine (0.25 mL, 1.8 mmol, 2

equiv) in dichloromethane (50 mL) was stirred for 15 min at 0 °C. To this mixture methane sulfonylchloride (0.1 mL, 1.3 mmol, 1.5 equiv) was added and stirred for further 30 min at 0 °C. The amine (1.5 equiv) was added to the mixture and stirred for 1 h at 0 °C and 2 h at room temperature. Dichloromethane (50 mL) was added to the mixture, which was then washed with 5% HCl (3×100 mL), saturated aqueous $NaHCO_3$ (3×100 mL) and water (3×100 mL). The organic fraction was dried over anhydrous sodium sulphate, filtered and rotary evaporated to yield a solid residue. Recrystallisation from ethylacetate and petroleum spirit gave crystals of amide (**4a–k**).

8.4. 1-*E,E*-piperinoyl-pyrrolidine (4a)

^{1}H NMR ($CDCl_3$): δ 6.26 (d, 1H, J = 14.7, CH=CH–CH=**CH**), 7.43 (dd, 1H, J = 9.5, 14.7, CH=CH–**CH**=CH), 6.73 (dd, 1H, J = 15.3, 9.5, CH=**CH**–CH=CH), 6.78 (d, 1H, J = 15.3, **CH**=CH–CH=CH), 6.98 (d, 1H J = 1.6, Ar-**H**), 6.77 (d, 1H J = 8.0, Ar-**H**), 6.89 (dd, 1H J = 1.6, 8.0, Ar-**H**), 5.97 (s, 2H, **O–CH$_2$–O**), 3.57 (t, 2H, J = 4.0, N–**CH$_2$** (pyrrolidine)), 3.54 (t, 2H, J = 4.0, N–**CH$_2$** (pyrrolidine)), 1.90 (m, 2H, CH_2–**CH$_2$** (pyrrolidine)), 1.87 (m, 2H, **CH$_2$**–CH_2 (pyrrolidine)); ^{13}C NMR ($CDCl_3$): δ 24.3 (**CH$_2$**), 26.1 (**CH$_2$**), 45.9 (**CH$_2$**), 46.4 (**CH$_2$**), 101.2 (**CH$_2$**), 105.7 (**CH**), 108.4 (**CH**), 121.4 (**CH**), 122.5 (**CH**), 125.2 (**CH**), 130.9 (**C**), 138.7 (**CH**), 141.7 (**CH**), 148.1 (**C**), 148.2 (**C**), 164.9 (**C**); MS m/z (%): 271 (M^+ 78), 201 (100), 173 (30), 172 (15), 171 (13), 143 (13), 115 (27); IR (KBr): ν_{max} (carbonyl group) cm^{-1} 1637. Yield: 186 mg (49.2%). Calcd for $C_{16}H_{17}NO_3$: C 70.83%, H 6.31%, N 5.16%; found: C 70.90%, H 6.99%, N 4.58%

8.5. 1-*E,E*-piperinoyl-morpholine (4b)

^{1}H NMR ($CDCl_3$): δ 6.37 (d, 1H, J = 14.6, CH=CH–CH=**CH**), 7.45 (dd, 1H, J = 10.2, 14.6, CH=CH–**CH**=CH), 6.72 (dd, 1H, J = 15.5, 10.2, CH=**CH**–CH=CH), 6.79 (d, 1H, J = 15.5 **CH**=CH–CH=CH), 6.98 (d, 1H J = 1.5, Ar-**H**), 6.80 (d, 1H J = 8.0, Ar-**H**), 6.89 (dd, 1H J = 1.5, 8.0 Ar-**H**), 5.98 (s, 2H, **O–CH$_2$–O**), 3.70 (t, 4H, J = 4.0 **CH$_2$**–N–**CH$_2$** (morpholine)), 3.60 (t, 4H, J = 4.0 **CH$_2$**–*O*–**CH$_2$** (morpholine)); ^{13}C NMR ($CDCl_3$): δ 42.3 (**CH$_2$**), 46.1 (**CH$_2$**), 66 (**CH$_2$**), 66 (**CH$_2$**), 101.3 (**CH$_2$**), 106.5 (**CH**), 108.5 (**CH**), 118.7 (**CH**), 122.7 (**CH**), 124.9 (**CH**), 130.8 (**C**), 139.1 (**CH**), 143.4 (**CH**), 148.2 (**C**), 148.3 (**C**), 165.6 (**C**); MS m/z (%): 287 (M^+ 57), 201 (100), 173 (25), 171 (10), 143 (10), 115 (30); IR (KBr): ν_{max} (carbonyl group) cm^{-1} 1641; Yield: 113 mg (44.1%); mp 161.8–162.5 °C (lit. mp 167–168 °C). Calcd for $C_{16}H_{17}NO_4$: C 66.88%, H 5.96%, N 4.84%; found: C 66.47%, H 5.78%, N 4.79%.

8.6. 1-*E,E*-piperinoyl-benzylamine (4c)

^{1}H NMR ($CDCl_3$): δ 6.15 (d, 1H, J = 15.0, CH=CH–CH=**CH**), 7.19 (dd, 1H, J = 10.2, 15.0, CH=CH–**CH**=CH), 6.92 (dd, 1H, J = 15.5, 10.2, CH=**CH**–CH=CH), 6.85 (d, 1H, J = 15.5 **CH**=CH–CH=CH), 7.22 (d, 1H J = 1.4, Ar-**H**), 6.89 (d, 1H J = 8.0, Ar-**H**), 6.98 (dd, 1H J = 1.4, 8.0 Ar-**H**), 6.03 (s, 2H, **O–CH$_2$–O**), 4.36 (d, 2H, J = 5.96, Ph-**CH$_2$**), 7.2–7.3 (m, 5H, **Ar**), 8.55 (t, 1H, J = 5.96 **NH**); ^{13}C NMR ($CDCl_3$): δ 43.8 (**CH$_2$**), 101.8 (**CH$_2$**), 106.0 (**CH**), 108.8 (**CH**), 123.1 (**CH**), 123.2 (**CH**), 125.1 (**CH**), 127.6 (**CH**), 128.0 (**CH**), 128.9 (**CH**), 139.6 (**CH**), 141.7 (**CH**), 148.7 (**C**), 148.8 (**C**), 167.6 (**C**); MS m/z (%): 307 (M^+ 100), 216 (13), 202 (28), 201 (26), 174 (36), 173 (49), 172 (16), 144 (10), 143 (13), 115 (26), 91 (25); IR (KBr): ν_{max} (carbonyl group) cm^{-1} 1637. Calcd for $C_{19}H_{17}NO_3$: C 74.23%, H 5.58%, N 4.56%; found: C 74.18%, H 5.35%, N 4.53%. Yield: 214 mg (50.1%); mp 177–178 °C.

8.7. 1-*E,E*-piperinoyl-3,4-methylenedioxyphenyl methylamine (4d)

^{1}H NMR ($CDCl_3$): δ 5.98 (d, 1H, J = 14.9, CH=CH–CH=**CH**), 7.34 (dd, 1H, J = 10.7, 14.9, CH=CH–**CH**=CH), 6.73 (dd, 1H, J = 15.5, 10.7, CH=**CH**–CH=CH), 6.79 (d, 1H, J = 15.5 **CH**=CH–CH=CH), 6.98 (d, 2H J = 1.5, Ar-**H**), 6.78 (d, 2H J = 8.0, Ar-**H**), 6.89 (dd, 2H J = 1.6, 8.0 Ar-**H**), 5.98 (s, 2H, **O–CH$_2$–O**), 5.93 (s, 2H, **O–CH$_2$–O**), 4.40 (d, 2H, **CH$_2$** N), 3.57 (br, 1H, N**H**); ^{13}C NMR ($CDCl_3$): δ 43.4 (**CH$_2$**), 101.1 (**CH$_2$**), 101.4 (**CH$_2$**), 105.8 (**CH**), 108.3 (**CH**), 108.5 (**CH**), 108.6 (**CH**), 121.2 (**CH**), 122.8 (**CH**), 124.7 (**CH**), 130.9 (**CH**), 132.2 (**CH**), 139.9 (**CH**), 141.6 (**C**), 147.0 (**C**), 147.9 (**C**)148.3 (**C**), 148.4 (**C**), 166.9 (**C**); MS m/z (%): 351 (M^+ 81), 216 (15), 203 (12), 202 (53), 201 (29), 174 (31), 173 (22), 150 (23), 144 (11), 143 (10), 135 (100), 116 (12), 115 (29). Calcd for $C_{20}H_{17}NO_5$: C 68.37%, H 4.88%, N 3.99%; found: C 68.27%, H 4.66%, N 3.86%. Yield: 157 mg (50.1%); mp 190.5–191.7 °C.

8.8. 1-*E,E*-piperinoyl-hexylamine (4e)

^{1}H NMR ($CDCl_3$): δ 5.90 (d, 1H, J = 14.8, CH=CH–CH=**CH**), 7.35 (dd, 1H, J = 10.6, 14.8, CH=CH–**CH**=CH), 6.66 (dd, 1H, J = 15.4, 10.6, CH=**CH**–CH=CH), 6.76 (d, 1H, J = 15.4 **CH**=CH–CH=CH), 6.97 (d, 1H J = 1.4, Ar-**H**), 6.77 (d, 1H J = 8.0, Ar-**H**), 6.88 (dd, 1H J = 1.5, 8.0 Ar-**H**), 5.97 (s, 2H, **O–CH$_2$–O**), 3.34 (q, 2H, **CH$_2$**–CH_2–CH_2–CH_2–CH_2), 1.54 (m, 2H, CH_2–**CH$_2$**–CH_2–CH_2–CH_2), 1.32 (m, 6H, CH_2–CH_2–**CH$_2$–CH$_2$–CH$_2$**), 0.88 (t, 3H, **CH$_3$**), 5.54 (br, **NH**); ^{13}C NMR ($CDCl_3$): δ 14.3 (**CH$_3$**), 22.5 (**CH$_2$**), 26.6 (**CH$_2$**), 29.6 (**CH$_2$**), 31.5 (**CH$_2$**), 39.7 (**CH$_2$**), 101.3 (**CH$_2$**), 105.7 (**CH**), 108.5 (**CH**), 122.5 (**CH**), 123.2 (**CH**), 124.6 (**CH**), 130.8 (**C**), 138.7 (**CH**), 140.9 (**CH**), 148.2 (**C**), 148.2 (**C**), 166.0 (**C**); MS m/z (%): 301 (M^+ 94), 202 (18), 201 (73), 174 (40), 173 (100), 172 (31), 171 (15), 143 (24), 115 (63); IR (KBr): ν_{max} (carbonyl group) cm^{-1} 1641. Yield: 168 mg, (40.1%); mp 149.5–149.8 °C (lit. mp 139–141 °C). Calcd for $C_{18}H_{23}NO_3$: C 71.72, H 7.69%, N 4.64%; found: C 71.09%, H 7.81%, N 4.56%.

8.9. 1-*E,E*-piperinoyl-isobutylamine (4f)

^{1}H NMR ($CDCl_3$): δ 5.96 (d, 1H, J = 14.8, CH=CH–CH=**CH**), 7.36 (dd, 1H, J = 10.5, 14.8, CH=CH–**CH**=CH), 6.66 (dd, 1H, J = 15.4, 10.5, CH=**CH**–CH=CH), 6.76 (d, 1H, J = 15.4 **CH**=CH–CH=CH), 6.96 (d, 1H J = 1.6, Ar-**H**), 6.76 (d, 1H J = 8.0, Ar-**H**), 6.87 (dd, 1H

J = 1.6, 8.0 Ar-**H**), 5.97 (s, 2H, **O–CH$_2$–O**), 3.18 (t, 2H, J = 6.5 **CH$_2$**–CH), 1.83 (m, 1H, J = 6.5 CH$_2$–**CH**), 0.94 (d, 6H, J = 6.5, (**CH$_3$**)$_2$), 5.82 (t, 1H, **NH** J = 5.3); ^{13}C NMR ($CDCl_3$): δ 20.4 (**CH$_3$**), 29.4 (**CH**), 47.3 (**CH$_2$**), 102.2 (**CH$_2$**), 106.2 (**CH**), 109.1 (**CH**), 123.3 (**CH**), 125.5 (**CH**), 126.0 (**CH**), 132.0 (**C**), 138.0 (**CH**), 140.4 (**CH**), 148.9 (**C**), 149.2 (**C**), 166.2 (**C**); MS m/z (%): 273 (M^+ 98), 216 (20), 201 (100), 174 (25), 173 (65), 172 (23), 171 (17), 143 (20), 115 (40), 96 (11); IR (KBr): ν_{max} (carbonyl group) cm^{-1} 1644. Yield: 120 mg, (32%); mp 161.2–161.7 °C (lit. mp 156–160 °C). Calcd for $C_{16}H_{19}NO_3$: C 70.29%, H 7.01%, N 5.12%; found: C 69.79%, H 7.02%, N 5.01%.

8.10. 1-*E*,*E*-piperinoyl-methylamine (4g)

^{1}H NMR ($CDCl_3$): δ 5.91 (d, 1H, J = 14.8, CH=CH–CH=**CH**), 7.36 (dd, 1H, J = 10.7, 14.8, CH=CH–**CH**=CH), 6.66 (dd, 1H, J = 15.4, 10.6, CH=**CH**–CH=CH), 6.77 (d, 1H, J = 15.4 **CH**=CH–CH=CH), 6.97 (d, 1H J = 1.5, Ar-**H**), 6.77 (d, 1H J = 8.0, Ar-**H**), 6.88 (dd, 1H J = 1.6, 8.0 Ar-**H**), 5.97 (s, 2H, **O–CH$_2$–O**), 2.91 (t, 3H, **CH$_3$**), 5.61 (br, **NH**); ^{13}C NMR ($CDCl_3$): δ 26.9 (**CH$_3$**), 101.7 (**CH$_2$**), 106.1 (**CH**), 108.9 (**CH**), 123.0 (**CH**), 123.3 (**CH**), 125.0 (**CH**), 131.2 (**C**), 139.2 (**CH**), 141.4 (**CH**), 148.6 (**C**), 148.6 (**C**), 167.2 (**C**); MS m/z (%): 231 (M^+ 89), 201 (42), 173 (67), 172 (32), 171 (17), 143 (27), 116 (21)115 (100), 89 (12). Yield: 155 mg (48.2%); mp 181.1–182.4 °C (lit. mp 186 °C). Calcd for $C_{13}H_{13}NO_3$: C 67.50%, H 5.66%, N 6.01%; found: C 66.96%, H 5.61%, N 5.90%.

8.11. 1-*E*,*E*-piperinoyl-ethylamine (4h)

^{1}H NMR (CD_3OD): δ 6.14 (d, 1H, J = 15.0, CH=CH–CH=**CH**), 7.37 (dd, 1H, J = 10.2, 15.0, CH=CH–**CH**=CH), 6.93 (dd, 1H, J = 15.7, 10.6, CH=**CH**–CH=CH), 6.87 (d, 1H, J = 15.7 **CH**=CH–CH=CH), 6.97 (d, 1H J = 1.5, Ar-**H**), 6.77 (d, 1H J = 8.0, Ar-**H**), 6.88 (dd, 1H J = 1.6, 8.0 Ar-**H**), 5.97 (s, 2H, **O–CH$_2$–O**), 3.39 (m, 2H, J = 6.2, **CH$_2$**), 1.22 (t, 3H, J = 6.1, **CH$_3$**), ^{13}C NMR ($CDCl_3$): δ 14.7 (**CH$_3$**), 36.9 (**CH$_2$**), 103.2 (**CH$_2$**), 107.2 (**CH**), 109.8 (**CH**), 121.2 (**CH**), 124.9 (**CH**), 125.9 (**CH**), 132.4 (**C**), 142.9 (**CH**), 145.2 (**CH**), 150.2 (**C**), 150.6 (**C**), 170 (**C**); MS m/z (%): 245 (M^+ 78), 218 (34), 201 (71), 200 (49), 174 (64), 173 (80), 172 (76), 171 (65), 143 (75), 116 (68), 115 (100). Yield: 156 mg (45.6%); mp 158.5–159.9 °C (lit. mp 162–164 °C).

8.12. 1-*E*,*E*-piperinoyl-isopropylamine (4i)

^{1}H NMR ($CDCl_3$): δ 5.87 (d, 1H, J = 14.8, CH=CH–CH=**CH**), 7.36 (dd, 1H, J = 10.7, 14.8, CH=CH–**CH**=CH), 6.66 (dd, 1H, J = 15.4, 10.6, CH=**CH**–CH=CH), 6.76 (d, 1H, J = 15.2 **CH**=CH–CH=CH), 6.97 (d, 1H J = 1.6, Ar-**H**), 6.77 (d, 1H J = 8.0, Ar-**H**), 6.88 (dd, 1H J = 1.6, 8.0 Ar-**H**), 5.97 (s, 2H, **O–CH$_2$–O**), 4.15 (m, 1H, J = 6.6, **CH**), 5.36 (d, 1H, J = 7.3 **NH**), 1.19 (d, 6H, J = 6.6, (**CH$_3$**)$_2$); ^{13}C NMR ($CDCl_3$): δ 23.2 (**CH$_3$**)$_2$, 41.9 (**CH**), 101.9 (**CH$_2$**), 106.4 (**CH**), 108.9 (**CH**), 123.0 (**CH**), 123.8 (**CH**), 124.1 (**CH**), 131.3 (**C**), 140.2 (**CH**), 141.2 (**CH**), 148.8 (**C**), 148.6 (**C**)165.6 (**C**); MS m/z (%): 259 (M^+ 80), 201 (62), 174 (34), 173 (74), 172 (31), 171 (15), 143 (30), 116 (16), 115 (100). Yield: 188 mg (52%); mp 169–169.4 °C (lit. mp 171–173 °C). Calcd for $C_{15}H_{17}NO_3$: C 69.46%, H 6.61%, N 5.40%; found: C 69.13%, H 6.34%, N 5.24%.

8.13. 1-*E*,*E*-piperinoyl-cyclohexylamine (4j)

^{1}H NMR ($CDCl_3$): δ 5.93 (d, 1H, J = 14.8, CH=CH–CH=**CH**), 7.35 (dd, 1H, J = 10.6, 14.8, CH=CH–**CH**=CH), 6.66 (dd, 1H, J = 15.3, 10.6, CH=**CH**–CH=CH), 6.76 (d, 1H, J = 15.4 **CH**=CH–CH=CH), 6.96 (d, 1H J = 1.6, Ar-**H**), 6.76 (d, 1H J = 8.0, Ar-**H**), 6.87 (dd, 1H J = 1.6, 8.0 Ar-**H**), 5.97 (s, 2H, **O–CH$_2$–O**), 3.87 (m, 1H, **CH** (cyclohexyl)), 1.99 (m, 2H, **CH$_2$** (cyclohexyl)), 1.65 (m, 4H, **CH$_2$–CH$_2$** (cyclohexyl)), 1.39 (m, 2H, **CH$_2$** (cyclohexyl)), 1.18 (m, 2H, **CH$_2$** (cyclohexyl)), 5.48 (d, J = 8.0 **NH**); ^{13}C NMR ($CDCl_3$): δ 25.3 ((**CH$_2$**)$_2$), 25.9 (**CH$_2$**), 33.6 ((**CH$_2$**)$_2$), 48.6 (**CH**), 101.7 (**CH$_2$**), 101.7 (**CH**), 106.1 (**CH**), 108.9 (**CH**), 123.0 (**CH**), 124.0 (**CH**), 125.1 (**CH**), 131.3 (**C**), 139.0 (**CH**), 141.2 (**CH**), 148.5 (**C**), 148.5 (**C**), 165.5 (**C**); MS m/z (%): 299 (M^+ 56), 259 (48), 216 (33), 201 (60), 174 (33), 173 (61), 172 (18), 171 (16), 143 (17), 115 (100). Yield: 239 mg (57.4%); mp 196.4–197.3 °C (lit. mp 199–200 °C). Calcd for $C_{18}H_{21}NO_3$: C 72.20%, H 7.07%, N 4.68%; found: C 72.14%, H 6.90%, N 4.61%.

8.14. 1-*E*,*E*-piperinoyl-butylamine (4k)

^{1}H NMR ($CDCl_3$): δ 5.97 (d, 1H, J = 14.8, CH=CH–CH=**CH**), 7.35 (dd, 1H, J = 10.7, 14.8, CH=CH–**CH**=CH), 6.66 (dd, 1H, J = 15.4, 10.6, CH=**CH**–CH=CH), 6.76 (d, 1H, J = 15.4 **CH**=CH–CH=CH), 6.97 (d, 1H J = 1.6, Ar-**H**), 6.77 (d, 1H J = 8.0, Ar-**H**), 6.89 (dd, 1H J = 1.5, 8.0 Ar-**H**), 5.97 (s, 2H, **O–CH$_2$–O**), 3.36 (q, 2H, **CH$_2$**–CH$_2$–CH$_2$–), 1.54 (m, 2H, CH$_2$–**CH$_2$**–CH$_2$), 1.39 (m, 6H, CH$_2$–CH$_2$–**CH$_2$**), 0.93 (t, 3H, **CH$_3$**), 5.47 (br, **NH**); ^{13}C NMR ($CDCl_3$): δ 14.2 (**CH$_3$**), 20.5 (**CH$_2$**), 32.2 (**CH$_2$**), 39.8 (**CH$_2$**), 101.7 (**CH$_2$**), 106.1 (**CH**), 108.9 (**CH**), 123.0 (**CH**), 123.6 (**CH**), 125.0 (**CH**), 131.3 (**C**), 139.2 (**CH**), 141.3 (**CH**), 148.6 (**C**), 148.6 (**C**), 166.4 (**C**). Yield: 146 mg (38.4%); mp 144.2–145.6 °C (lit. mp 144–145 °C). Calcd for $C_{16}H_{19}NO_3$: C 70.29%, H 7.01%, N 5.12%; found: C 70.16%, H 6.56%, N 4.78%.

8.15. Compounds (5a–d)

A mixture of (2*E*,4*E*)-piperinic acid (**2**) (200 mg, 0.9 mmol, 1 eq) and triethylamine (0.25 mL, 1.8 mmol, 2 eq) in dichloromethane (50 mL) was stirred for 15 min at 0 °C. To this mixture methane sulfonylcholride (0.1 mL, 1.3 mmol, 1.5 equiv) was added and stirred for further 30 min at 0 °C. The alcohol (10 mL) was added to the mixture and stirred for 1 h at 0 °C and 2 h at room temperature. Dichloromethane (50 mL) was added to the mixture, which was then washed with water (3×100 mL), 5% $NaHCO_3$ (3×100 mL) and water (3×100 mL). The organic fraction was dried over anhydrous sodium sulphate, filtered and rotary evaporated to yield a brownish solid residue. Recrystallisation from ethylacetate and petroleum spirit gave piperinic acid esters (**5a–d**).

8.16. 5-(3,4-Methylenedioxyphenyl)-penta-2*E*,4*E*-dienoic acid methyl ester (5a)

^{1}H NMR ($CDCl_3$): δ 5.94 (d, 1H, J = 15.2, CH=CH–CH=**CH**), 7.41 (dd, 1H, J = 10.8, 15.2, CH=CH–**CH**=CH), 6.70 (dd, 1H, J = 15.4, 10.8, CH=**CH**–CH=CH), 6.81 (d, 1H, J = 15.7 **CH**=CH–CH=CH), 6.99 (d, 1H J = 1.6, Ar-**H**), 6.79 (d, 1H J = 8.1, Ar-**H**), 6.91 (dd, 1H J = 1.5, 8.1 Ar-**H**), 5.98 (s, 2H, **O–CH$_2$–O**), 3.57 (t, 3H, br, **OCH$_3$** J = 4.7); ^{13}C NMR ($CDCl_3$): δ 51.5 (**CH$_3$**), 101.8 (**CH$_2$**), 106.2 (**CH**), 108.9 (**CH**), 120.0 (**CH**), 123.4 (**CH**), 124.7 (**CH**), 130.8 (**CH**), 140.9 (**C**), 145.5 (**CH**), 148.6 (**C**), 148.9 (**C**), 168.9 (**C**); MS m/z (%): 232 (M^+ 69), 201 (19), 174 (12), 173 (100), 172 (39), 171 (12), 143 (33), 116 (11), 115 (53), 101 (15), 100 (12). Yield: 117 mg (56.2%); mp 142.9–143 °C (lit. mp 140 °C). Calcd for $C_{13}H_{12}O_4$: C 67.22%, H 5.21%; found: C 66.91%, H 5.21%.

8.16.1. 5-(3,4-Methylenedioxyphenyl)-penta-2*E*,4*E*-dienoic acid ethyl ester (5b).

^{1}H NMR ($CDCl_3$): δ 5.94 (d, 1H, J = 15.2, CH=CH–CH=**CH**), 7.41 (dd, 1H, J = 10.8, 15.3, CH=CH–**CH**=CH), 6.70 (dd, 1H, J = 15.4, 10.8, CH=**CH**–CH=CH), 6.81 (d, 1H, J = 15.5 **CH**=CH–CH=CH), 6.99 (d, 1H J = 1.6, Ar-**H**), 6.78 (d, 1H J = 8.1, Ar-**H**), 6.91 (dd, 1H J = 1.6, 8.1 Ar-**H**), 5.98 (s, 2H, **O–CH$_2$–O**), 4.22 (q, 2H, **OCH$_2$** J = 7.2), 1.31 (t, 3H, **CH$_3$** J = 7.2); ^{13}C NMR ($CDCl_3$): δ 14.7 (**CH$_3$**), 60.7 (**CH$_2$**), 101.6 (**CH$_2$**), 106.3 (**CH**), 108.9 (**CH**), 120.8 (**CH**), 123.3 (**CH**), 124.9 (**CH**), 131.0 (**C**), 140.5 (**CH**), 145.1 (**CH**), 148.7 (**C**), 148.9 (**C**), 167.6 (**C**); MS m/z (%): 246 (M^+ 83), 201 (26), 174 (14), 173 (100), 143 (15), 115 (53). Calcd for $C_{14}H_{14}O_4$: C 68.27%, H 5.73%; found C 68.61%, H 5.54%. Yield: 120 mg (55%) mp 43.1–44 °C (lit. mp 45 °C).

8.17. 5-(3,4-Methylenedioxyphenyl)-penta-2*E*,4*E*-dienoic acid propyl ester (5c)

^{1}H NMR ($CDCl_3$): δ 5.94 (d, 1H, J = 15.2, CH=CH–CH=**CH**), 7.41 (dd, 1H, J = 10.7, 15.2, CH=CH–**CH**=CH), 6.70 (dd, 1H, J = 15.4, 10.8, CH=**CH**–CH=CH), 6.76 (d, 1H, J = 15.4 **CH**=CH–CH=CH), 6.99 (d, 1H J = 1.6, Ar-**H**), 6.78 (d, 1H J = 8.1, Ar-**H**), 6.91 (dd, 1H J = 1.5, 8.0 Ar-**H**), 5.98 (s, 2H, **O–CH$_2$–O**), 4.12 (t, 2H, **OCH$_2$** J = 6.7), 1.69 (m, 2H, **CH$_2$** J = 7.3), 0.97 (t, 3H, **CH$_3$** J = 7.4); ^{13}C NMR ($CDCl_3$): δ 10.9 (**CH$_3$**), 22.5 (**CH$_2$**), 66.3 (**CH$_2$**), 101.8 (**CH$_2$**), 106.2 (**CH**), 108.9 (**CH**), 120.9 (**CH**), 123.3 (**CH**), 124.9 (**CH**), 131.0 (**C**), 140.5 (**CH**), 145.1 (**CH**), 148.7 (**C**), 148.9 (**C**), 167.7 (**C**); MS m/z (%): 260 (M^+ 59), 201 (26), 174 (18), 173 (100), 172 (39), 171 (14), 143 (34), 116 (16), 115 (73), 100 (12). Yield: 84 mg 34% mp 119–120 °C. Calcd for $C_{15}H_{16}O_4$: C 69.20%, H 6.19%; found: C 69.04%, H 6.49%.

8.18. 5-(3,4-Methylenedioxyphenyl)-penta-2*E*,4*E*-dienoic acid butyl ester (5d)

^{1}H NMR ($CDCl_3$): δ 5.94 (d, 1H, J = 15.2, CH=CH–CH=**CH**), 7.40 (dd, 1H, J = 10.7, 15.3, CH=CH–**CH**=CH), 6.70 (dd, 1H, J = 15.4, 10.8, CH=**CH**–CH=CH), 6.76 (d, 1H, J = 15.4 **CH**=CH–CH=CH), 6.99 (d, 1H J = 1.6, Ar-**H**), 6.78 (d, 1H J = 8.0, Ar-**H**), 6.91 (dd, 1H J = 1.5, 8.0 Ar-**H**), 5.98 (s, 2H, **O–CH$_2$–O**), 4.12 (t, 2H, **OCH$_2$** J = 6.7), 1.69 (m, 2H, **CH$_2$** J = 7.3), 1.69 (m, 2H, **CH$_2$** J = 7.6), 0.95 (t, 3H, **CH$_3$** J = 7.5); MS m/z (%): 274 (M^+ 50), 201 (15), 174 (14), 173 (100), 172 (30), 171 (14), 143 (21), 115 (55). Yield: 102 mg 41.4%. Calcd for $C_{16}H_{18}O_4$: C 70.04%, H 6.61%; found: C 69.56%, H 6.27%.

8.19. 5-(Phenyl)-penta-2*E*,4*E*-dienoic acid (6a)

Cinnamaldehyde (1 mL, 0.0075 mol, 1 equiv) and malonic acid (1.56 g, 0.015 mol, 2 eq) was stirred in pyridine (15 mL) for 10 min and refluxed for overnight. Mixture is boiled for 15 min. Mixture was cooled and acidified with 1N HCl yielding a yellowish precipitate of mixture of 1-(phenyl)-penta-2,4-dienoic acid. Recrystallisation from chloroform gave 5-(phenyl)-penta-2*E*,4*E*-dienoic acid (**6a**) (56.2% yield). mp 164.5–165 °C. (lit. mp 164–165 °C).

8.20. 5-(Phenyl)-penta-2*E*,4*E*-dienoyl piperidine (7a)

A mixture of 5-(phenyl)-penta-2*E*,4*E*-dienoic acid (200 mg, 0.0011 mol, 1 equiv) and triethylamine (0.3 mL, 0.0022 mol, 2 eq) in dichloromethane (50 mL) was stirred for 15 min at 0 °C. To this mixture methane sulfonyl chloride (0.12 mL, 0.0016 mol, 1.5 equiv) was added and stirred for further 30 min at 0 °C. Piperidine (0.16 mL, 0.0016 mol, 1.5 equiv) was added to the mixture and stirred for 1 h at 0 °C and 1 h at room temperature. Dichloromethane (50 mL) was added to the mixture which was then washed with 5% HCl (3×100 mL), saturated aqueous $NaHCO_3$ (3×100 mL) and water (3×100 mL). The organic fraction was dried over anhydrous sodium sulphate, filtered and rotary evaporated to yield a yellowish solid residue. Recrystallisation from ethylacetate/ether yielded white crystals of 5-(phenyl)-penta-2*E*,4*E*-dienoyl piperidine. ^{1}H NMR ($CDCl_3$) (60 mHZ): δ 7.1–7.4 (1H ArH), 7.25 (1H ArH), 6.78 (2H ArH), 6.45 (1H, ArH), 3.4–3.6 (br, 4H, CH_2–N–CH_2 (piperidine)), 1.5–1.8 (m, 6H, CH_2–CH_2 CH_2 (piperidine)) (36% yield). mp 73–75 °C (lit. mp 77 °C).

8.21. 1-[(2*E*,4*E*)-Hexadienoyl]-piperidine (7b)

A mixture of 2,4-hexadienoic acid (300 mg, 0.0026 mol, 1 eq) and triethylamine (0.7 mL, 0.0052 mol, 2 equiv) in dichloromethane (50 mL) was stirred for 15 min at 0 °C. To this mixture methane sulfonyl chloride (0.3 mL, 0.0039 mol, 1.5 equiv) was added and stirred for a further 30 min at 0 °C. Piperidine (0.23 mL, 0.0039 mol, 1.5 equiv) was added to the mixture and stirred for 1 h at 0 °C and 1 h at room temperature. Dichloromethane (50 mL) was added to the mixture which was then washed with 5% HCl (3×100 mL), saturated aqueous $NaHCO_3$ (3×100 mL) and water (3×100 mL). The organic fraction was dried over anhydrous sodium sulphate, filtered and rotary evaporated to yield a yellowish solid residue. Recrystallisation from ethylacetate/ether yielding colourless crystals of 2,4-hexadienoyl piperidine (58.3% yield). mp 79.2–81.2 °C (lit. mp 83–84 °C).

^{1}H NMR ($CDCl_3$): δ 6.23 (d, 1H, J = 15.7, CH=CH–CH=**CH**), 7.25 (dd, 1H, J = 10.7, 15.7, CH=CH–**CH**=CH), 6.73 (dd, 1H, J = 15.3, 9.5, CH=**CH**–CH=CH), 6.78 (d, 1H, J = 15.3 **CH**=CH–CH=CH), 3.61 (br, 2H, N–**CH_2** (piperidine)), 3.48 (br, 2H, N–**CH_2** (piperidine)), 1.65 (m, 2H, **CH_2**-CH_2 CH_2 (piperidine)), 1.57 (m, 4H, **CH_2**–**CH_2** CH_2 (piperidine)), 1.83 (d, 3H, J = 6.6, **CH_3**); ^{13}C NMR ($CDCl_3$): δ 18.5 (**CH_3**), 24.6 (**CH_2**), 25.6 (**CH_2**), 26.7 (**CH_2**), 43.1 (**CH_2**), 46.8 (**CH_2**), 118.3 (**CH**), 130.0 (**CH**), 137.0 (**CH**), 142.6 (**CH**), 165.7 (**C**); MS m/z (%): 179 (M^+ 5), 168 (100), 167 (14), 138 (12), 112 (11), 85 (18), 83 (31), 69 (10), 47 (11). Calcd for $C_{11}H_{17}ON$: C 73.70%, H 9.55%, N 7.81%; found: C 72.95%, H 9.34%, N7.81%.

8.22. Piperidine-5-(3,4-dihydroxyphenyl)penta-2*E*,4*E*-dienoic acid amide (8)

Piperine (**1**) (500 mg, 1.75 mmol, 1 eq) was dissolved in dichloromethane (DCM, 10 mL) and BBr3 in dichloromethane (0.7 mL, 8.7 mmol, 5 equiv) was added and stirred at room temperature for 2 days. After the completion of reaction, DCM was removed under reduced pressure and a yellow solid was obtained. The yellow solid was washed with DCM and filtered to give a product (354 mg, 76.2%). ^{1}H NMR (MeOD): δ 6.54 (d, 1H, J = 14.6, CH=CH–CH=**CH**), 7.32 (dd, 1H, J = 9.5, 14.6, CH= CH–**CH**=CH), 6.78 (dd, 1H, J = 13.2, 9.5, CH=**CH**–CH=CH), 6.75 (d, 1H, J = 13.2 **CH**=CH–CH=CH), 6.98 (d, 1H J = 1.9, Ar-**H**), 6.74 (d, 1H J = 8.2, Ar-**H**), 6.85 (dd, 1H J = 1.9, 8.2 Ar-**H**), 3.59 (br, 4H, **CH_2**–N–**CH_2** (piperidine)), 1.59 (m, 6H, **CH_2**–**CH_2**–**CH_2** (piperidine)); ^{13}C NMR (MeOD): 25.9 (**CH_2**), 27.3 (**CH_2**), 28.2 (**CH_2**), 45.1 (**CH_2**), 48.6 (**CH_2**), 114.8 (**CH**), 116.9 (**CH**), 119.7 (**CH**), 121.7 (**CH**), 125.5 (**CH**), 141.7 (**CH**), 145.7 (**CH**), 147.0 (**C**), 148.3 (**C**), 148.3 (**C**), 168.3 (**C**).

8.23. Piperidine-5-(3,4-dimethoxyphenyl)penta-2*E*,4*E*-dienoic acid amide (9)

Potassium hydroxide (328 mg) was added to dimethylsulfoxide (5 mL, DMSO) and stirred under nitrogen atmosphere for 5mins at room temperature. The compound **8** (200 mg) was added to the mixture and followed by iodomethane (0.3 mL) and stirred for another 30 min at same temperature. The mixture was poured into water (50 mL) and extracted with dichoromethane (3×30 mL). The combined extracts were washed with water (50 mL) and dried over sodium sulphate, filtered and rotary evaporated to yield a solid. Recrystallisation using petroleum spirit and ethylacetate yielded pure greenish crystals (122 mg, 58%). ^{1}H NMR ($CDCl_3$): δ 6.46 (d, 1H, J = 14.6, CH=CH–CH=**CH**), 7.25–7.46 (m, 1H, CH=CH–**CH**=CH), 6.78 (1H, CH=**CH**–CH=CH), 6.80 (d, 1H, J = 14.6 **CH**=CH–CH=CH), 6.98 (d, 1H J = 1.8, Ar-**H**), 6.83 (d, 1H J = 8.1, Ar-**H**), 7.0 (dd, 1H J = 1.6, 8.1 Ar-**H**), 3.89 and 3.91 (2s, 6H, (**O**–**CH_3**–)$_2$), 3.63 (br, 2H, N–**CH_2** (piperidine)), 3.52 (b, 2H, N–**CH_2** (piperidine), 1.64 (m, 2H, CH_2-**CH_2** (piperidine)), 1.87 (m, 4H, **CH_2**–**CH_2**–CH_2 (piperidine)); ^{13}C NMR ($CDCl_3$): δ 23.3 (**CH_2**), 24.2 (**CH_2**), 25.3 (**CH_2**), 41.8 (**CH_2**), 45.5 (**CH_2**), 54.4 (**OCH_3**), 54.5 (**OCH_3**), 107.5 (**CH**), 109.7 (**CH**), 118.4 (**CH**), 123.8 (**CH**), 137.0 (**CH**), 141.2 (**CH**), 147.6 (**C**), 148.2 (**C**), 148.2 (**C**), 164.0 (**C**); MS m/z (%): 301 (M^+ 71), 218 (27), 217 (81), 188 (52), 182 (34), 148 (17), 140 (52), 127 (100), 115 (22), 84 (40).

8.24. Compounds (10a–d)

A mixture of monomethoxycinnamic acid (200 mg, 0.89 mmol, 1 equiv) and triethylamine (2.4 mL, 1.78 mmol, 2 eq) in dichloromethane (50 mL) was stirred for 15 min at 0 °C. To this mixture methane sulfonyl chloride (1.02 mL, 1.33 mmol, 1.5 equiv) was added and stirred for further 30 min at 0 °C. Piperidine (0.23 mL, 1.33 mmol, 1.5 eq) was added to the mixture and stirred for 1 h at 0 °C and 1 h at room temperature. Then dichloromethane (50 mL) was added to the mixture, which was then washed with 5% HCl (3×100 mL), saturated aqueous $NaHCO_3$ (3×100 mL) and water (3×100 mL). The organic fraction was dried over anhydrous sodium sulphate, filtered and rotary evaporated to yield an oil. This oil is purified by chromatography on silica gel using ethylacetate/petroleum spirit (2:8) as an eluent (**10a**–**c**). The piperidine amide of 3,4-dimethoxycinnamic acid was prepared in the same way utilising 200 mg of the acid (**10d**).

8.25. 1-(2-methoxycinnamoyl)-piperidine (10a)

^{1}H NMR ($CDCl_3$): δ 7.56 (d, 1H, CH=CH), 7.29 (d, 1H, J = 7.8 Ar-H), 7.12 (d, 1H, J = 7.6 Ar-H), 7.0 (dd 1H, J = 1.8 Ar-H), 6.86–6.90 (m, Ar-H), 6.88 (d, 1H, J = 15.4 CH=CH), 3.58–3.66 (br, 4H, CH_2–N–CH_2 (piperidine)), 1.56–1.71 (m, 6H, CH_2–CH_2 CH_2 (piperidine)), 3.83 (s, 3H, OCH_3); ^{13}C NMR ($CDCl_3$): δ 25.7 (CH_2), 26.0 (CH_2), 27.1 (CH_2), 43.7 (CH_2), 47.4 (CH_2), 55.7 (CH_3), 113.4 (CH), 115.3 (CH), 118.5 (CH), 120.6 (CH), 130.1 (CH), 142.4 (CH), 137.3 (C), 160.2 (C), 165.6 (C); MS m/z (%): 245 (M^+ 28), 162 (22), 161 (100), 133 (20), 118 (24), 113 (14), 84 (51). Yield: 55 mg (25.5%), mp 68–70 °C.

8.26. 1-(3-methoxycinnamoyl)-piperidine (10b)

^{1}H NMR ($CDCl_3$): δ 7.60 (d, 1H, J = 15.4, CH=CH), 7.29 (d, 1H, J = 7.8 ArH), 7.12 (d, 1H, J = 7.6 Ar-H), 7.0 (dd 1H, J = 1.8 Ar-H), 6.86–6.90 (m, Ar-H), 6.88 (d, 1H, J = 15.4 CH=CH), 3.58–3.66 (br, 4H, CH_2–N–CH_2 (piperidine)), 1.56–1.71 (m, 6H, CH_2–CH_2–CH_2 (piperidine)), 3.83 (s, 3H, OCH_3); ^{13}C NMR ($CDCl_3$): δ 25.7 (CH_2), 26.0 (CH_2), 27.1 (CH_2), 43.7 (CH_2), 47.4 (CH_2), 55.7 (CH_3), 113.4 (CH), 115.3 (CH), 118.5 (CH), 120.6 (CH), 130.1 (CH), 142.4 (CH), 137.3 (C), 160.2 (C), 165.6 (C); MS m/z (%): 245 (M^+ 77), 162 (65), 161 (100), 133 (20), 118 (24), 113 (14), 84 (51); mp 68–70 °C. Yield: 68 mg, (31.4%). Calcd for $C_{15}H_{19}NO_2$: C 73.42%, H 7.81%, N 5.71%; found: C 73.37%, H 7.96%, N 5.86%.

8.27. 1-(4-methoxycinnamoyl)-piperidine (10c)

^{1}H NMR ($CDCl_3$): δ 7.61 (d, 1H, J = 15.4, CH=CH), 7.47 (d, 2H, J = 7.8 Ar-H), 6.87–6.90 (m, 2H, Ar-H), 6.77 (d, 1H, J = 15.4 CH=CH), 3.58–3.65 (br, 4H, CH_2–N–CH_2 (piperidine)), 1.52–1.69 (m, 6H, CH_2–CH_2–CH_2

(piperidine)), 3.82 (s, 3H, OCH_3); ^{13}C NMR ($CDCl_3$): δ 25.6 (CH_2), 26.0 (CH_2), 26.4 (CH_2), 43.7 (CH_2), 47.4 (CH_2), 55.7 (CH_3), 114.5 (CH), 115.6 (CH), 118.5 (CH), 121.9 (CH), 129.6 (CH), 142.2 (CH), 132.8 (C), 161.0 (C), 166.0 (C); MS *m*/*z* (%): 245 (M^+ 71), 162 (17), 161 (100), 133 (26), 118 (12), 113 (14), 84 (24), 77 (36). Yield: 79.1 mg (36.3%). Calcd for $C_{15}H_{19}NO_2$: C 73.44%, H 7.80%, N 5.71%; found: C 73.49%, H 7.63%, N 5.89%.

8.28. 1-(3,4-dimethoxycinnamoyl)-piperidine (10d)

^1H NMR ($CDCl_3$), 60 MHz: δ: 7.61 (1H, CH=CH), 7.23 (1H, Ar-H), 6.98 (1H, Ar-H), 6.82 (1H, *J* = 1.8 Ar-H), 6.68 (1H, CH=CH), 3.58–3.65 (br, 4H, CH_2–N–CH_2 (piperidine)), 1.5–1.8 (6H, CH_2–CH_2 CH_2 (piperidine)), 3.91 (s, 6H, $OCH_3)_2$); MS *m*/*z* (%): 275 (M^+ 62), 192 (48), 191 (100), 161 (18), 118 (11), 84 (26), 77 (12). Yield: 111 mg (42.3%).

8.29. Compounds (11a–c)

A mixture of 3,4-(methylenedioxy)-cinnamic acid (500 mg, 0.0026 mol, 1 equiv) and triethylamine (0.54 mL, 3.9 mmol, 1.5 eq) in dichloromethane (50 mL) was stirred for 15 min at 0 °C. To this mixture methane sulfonylcholride (0.3 mL, 3.9 mmol, 1.5 equiv) was added and stirred for further 30 min at 0 °C. The amine (1.5 equiv) was added to the mixture and stirred for 2 h at 0 °C and 1 h at room temperature. Dichloromethane (50 mL) was added to the mixture, which was then washed with water (3×100 mL), 5% $NaHCO_3$ (3×100 mL) and water (3×100 mL). The organic fraction was dried over anhydrous sodium sulphate, filtered and rotary evaporated to yield a brownish solid residue. Recrystallisation from ethylacetate and petroleum spirit gave 3,4-(methylenedioxy)-cinnamic acid amides (**11a–c**).

8.30. 1-(3,4-Methylenedioxy-cinnamoyl)-piperidine (11a)

^1H NMR ($CDCl_3$): δ 7.56 (d, 1H, *J* = 15.3, CH=**CH**), 6.73 (d, 1H, *J* = 15.3, **CH**=CH), 7.03 (d, 1H *J* = 1.5, Ar-**H**), 6.79 (d, 1H *J* = 8.0, Ar-**H**), 6.99 (dd, 1H *J* = 1.6, 8.0 Ar-**H**), 5.98 (s, 2H, **O–CH_2–O**), 3.57 (br, 2H, CH_2–N–**CH_2**), 3.65 (br, 2H, **CH_2**–N– CH_2 (piperidine)), 1.65 (m, 6H, **CH_2–CH_2–CH_2-** (piperidine)); ^{13}C NMR ($CDCl_3$): δ 24.8 (**CH_2**), 25.6 (**CH_2**), 26.7 (**CH_2**), 43.3 (**CH_2**), 46.9 (**CH_2**), 101.3 (**CH_2**), 106.7 (**CH**), 108.4 (**CH**), 115.6 (**CH**), 123.5 (**CH**), 129.9 (**C**), 141.9 (**CH**), 148.1 (**C**), 148.8 (**C**), 165.4 (**C**). Yield :328 mg (58.2%); mp 80.1–82 °C (lit. mp 80–82 °C). Calcd for $C_{15}H_{17}O_3N$: C 69.47%, H 6.60%, N 5.40%; found: C 69.56%, H 6.58%, N 5.39%.

8.31. 1-(3,4-Methylenedioxy-cinnamoyl)-pyrrolidine (11b)

^1H NMR ($CDCl_3$): δ 7.60 (d, 1H, *J* = 15.2, CH=**CH**), 6.73 (d, 1H, *J* = 15.3, **CH**=CH), 7.04 (d, 1H *J* = 1.5, Ar-**H**), 6.80 (d, 1H *J* = 8.0, Ar-**H**), 7.01 (dd, 1H *J* = 1.5, 8.0 Ar-**H**), 5.99 (s, 2H, **O–CH_2–O**), 3.61 (br, 2H, CH_2–N–**CH_2** (pyrrolidine)), 3.57 (br, 2H, **CH_2**–N–CH_2 (pyrrolidine)), 1.99 (4H, **CH_2–CH_2** (pyrrolidine)), ^{13}C NMR ($CDCl_3$): δ 24.3 (**CH_2**), 26.1 (**CH_2**), 46.0 (**CH_2**), 46.5 (**CH_2**), 101.4 (**CH_2**), 106.4 (**CH**), 108.5 (**CH**), 116.8 (**CH**), 123.8 (**CH**), 129.7 (**C**), 141.0 (**CH**), 148.1 (**C**), 148.9 (**C**), 164.8 (**C**); MS *m*/*z* (%): 245 (M^+ 62), 176 (41), 175 (100), 145 (36), 117 (11), 89 (14). Yield: 280 mg (44.1%); mp 152.5–153 °C. Calcd for $C_{14}H_{15}NO_3$: C 68.54%, H 6.16%, N 5.71%; found: C 68.02%, H 6.26%, N 5.74%.

8.32. 1-(3,4-Methylenedioxy-cinnamoyl)-morpholine (11c)

^1H NMR ($CDCl_3$): δ 7.61 (d, 1H, *J* = 15.3, CH=**CH**), 6.73 (d, 1H, *J* = 15.3, **CH**=CH), 7.03 (d, 1H *J* = 1.4, Ar-**H**), 6.80 (d, 1H *J* = 8.0, Ar-**H**), 7.01 (dd, 1H *J* = 1.4, 8.0 Ar-**H**), 5.99 (s, 2H, **O–CH_2–O**), 3.72 (br, 4H, **CH_2**–N–**CH_2** (morpholine)), 3.67 (br, 4H, **CH_2**-*O*-CH_2 (morpholine)), ^{13}C NMR ($CDCl_3$): δ 42.6 (**CH_2**), 46.2 (**CH_2**), 66.8 (**CH_2**), 46.5 (**CH_2**), 101.4 (**CH_2**), 106.3 (**CH**), 108.5 (**CH**), 114.4 (**CH**), 123.9 (**CH**), 129.5 (**CH**), 143.0 (**CH**), 148.2 (**C**), 148.9 (**C**), 149.1 (**C**), 165.6 (**C**); MS *m*/*z* (%): 261 (M^+ 60), 176 (24), 175 (100), 145 (30), 117 (10), 89 (11). Yield: 339 mg (50.1%); mp 160–160.3 °C. Calcd for $C_{14}H_{15}NO_4$: C 64.34%, H 5.78%, N 5.36%; found: C 64.20%, H 5.88%, N 5.38%.

8.33. 3,4-(Methylenedioxy)-cinnamic acid methyl ester (12)

Methanol (4 mL, 10 equiv) was added to 3,4-(methylenedioxy)-cinnamic acid (2 g, 0.01 mol, 1 equiv). 0.2 mL of sulphuric acid was added to the methanolic solution and refluxed overnight. The solvent was rotary evaporated to yield solid residue. This residue was dissolved in ether and washed with water (2×100 mL) and 5% $NaHCO_3$ (3×100 mL) and with water (2×100 mL). The organic fraction was dried over anhydrous sodium sulphate and rotary evaporated to yield white solid. Recrystallisation from ethylacetate/petroleum spirit yielded crystals (1.42 g, 69.4% yield); mp 133.7–134.2 °C (lit. mp 134 °C). ^1H NMR ($CDCl_3$): δ 7.59 (d, 1H, *J* = 15.9, CH=**CH**), 6.26 (d, 1H, *J* = 15.9, **CH**=CH), 7.03 (d, 1H *J* = 1.5, Ar-**H**), 6.81 (d, 1H *J* = 8.0, Ar-**H**), 7.01 (dd, 1H *J* = 1.5, 8.0 Ar-**H**), 6.00 (s, 2H, **O–CH_2–O**), 3.79 (s, 3H, **OCH_3**); ^{13}C NMR ($CDCl_3$): δ 51.6 (**CH_3**), 101.5 (t **CH_2**), 106.5 (**CH**), 108.5 (**CH**), 115.7 (**CH**), 124.4 (**CH**), 128.8 (**CH**), 144.5 (**CH**), 148.3 (**C**), 148.6 (**C**), 148.2 (**C**), 167.6 (**C**); MS *m*/*z* (%): 206 (M^+ 100), 175 (68), 175 (100), 145 (27), 117 (10), 89 (11).

8.34. 7-(3,4-methylenedioxyphenyl)-2*E*,4*E*,6*E*-heptatrienoic acid piperidine amide (14)

1-[(2*E*,4*E*)-Hexadienoyl]-piperidine (**7b**) (500 mg, 2.79 mmol) piperonal (**13**) (419 mg, 2.69 mmol) and aliquat 336 (50 mg) were dissolved in toluene and anhydrous potassium carbonate (100 mg) and sodium hydride (20 mg) was added and refluxed under nitroger for 6 h at 90 °C. The reaction mixture was poured into water and extracted with dichloromethane (3×100 mL). The organic layer was dried over anhydrous sulphate, filtered and rotary evaporated to yield a solid residue. The mixture was purified by column chromatography using hexane:ethylacetate (1:9) as an eluent to give yellow solid ^1H NMR ($CDCl_3$): δ 6.95 (d, 1H *J* = 1.6, Ar-H), 6.76 (d, 1H *J* = 8.0, Ar-H), 6.85 (dd, 1H *J* = 1.6, 8.0 Ar-H),

6.37 (d, 1H $J=14.6$, CH=CH–CH=CH–CH=CH), 6.42 (dd, 1H $J=11.4$, 14.0, CH=CH–CH=CH–CH=CH), 7.35 (dd, 1H $J=11.4$, 14.6, CH=CH–CH=CH–CH=CH), 6.59 (d, 1H $J=15.0$, CH=CH–CH=CH–CH=CH), 6.66 (dd, 1H $J=15.0$, 10.0, CH=CH–CH=CH–CH=CH), 6.63 (dt, 1H $J=10.0$, 14.0, CH=CH–CH=CH–CH=CH), 5.96 (s, 2H, O–CH_2–O), 3.51 (br s, 2H, CH_2-N–CH_2), 3.62 (br s, 2H, $J=5.7$, (CH_2–N–CH_2), 1.54-1.59 (m, 4H, CH_2–CH_2–CH_2 (piperidine)), 1.60–1.68 (m, 2H, CH_2–CH_2–CH_2 (piperidine)); ^{13}C NMR ($CDCl_3$): δ 25.0 (CH), 26.0 (CH), 27.1 (CH), 43.6 (CH), 47.3 (CH), 101.6 (CH_2), 105.8 (CH), 108.8 (CH), 120.4 (CH), 122.4 (CH), 127.0 (CH), 130.8 (CH), 135.8 (CH), 139.5 (CH), 142.7 (CH), 135.9 (C), 148.1 (C), 148.5 (C), 165.8 (C); MS *m*/*z* (%): 311 (M^+ 99), 301 (18), 227 (27), 217 (21), 199 (42), 182 (30), 169 (59), 149 (29), 141 (100), 127 (86), 115 (43), 84 (81), 69 (43). Yield 45.6%; mp 144.3–145.6 °C.

8.35. 5-(3,4-methylenedioxyphenyl)-penta-2*E*,4*E*-dienyl piperidine (15)

Piperine (**1**) (1 g, 3.5 mmol) and $NaBH_4$ (0.35 g, 8.75 mmol) were stirred in THF (20 mL) at 0 °C under nitrogen atmosphere. Iodine (0.65 g, 2.62 mmol) was added dropwise to the mixture and left for 2 days. The reaction was quenched with methanol until the effervescence ceased. The solvent was rotary evaporated to yield a residue (0.9 g). This residue was purified by column chromatography on silicagel (31 cm/10 mm) using petroleum spirit/ethylacetate as an eluent. Recrystallisation of the fraction from methanol gave white crystals (**16**) (120 mg, 13%). mp 126.7–127.3 °C. ^{1}H NMR ($CDCl_3$): δ 3.48 (d, 2H, $J=7.4$ CH=CH–CH=CH–CH_2), 6.1 (m, 1H, $J=7.5$, 15.1, CH=CH–CH=CH), 6.35 (dd, 1H, $J=15.1$, 10.2, CH=CH–CH=CH), 6.67 (dd, 1H, $J=10.2$, 15.4, CH=CH–CH=CH), 6.48 (d, 1H, $J=15.6$ CH=CH–CH=CH), 6.94 (d, 1H $J=1.6$, Ar-H), 6.76 (d, 1H $J=8.0$, Ar-H), 6.82 (dd, 1H $J=1.5$, 8.0 Ar-H), 5.97 (s, 2H, O–CH_2–O), 3.57 (br, 2H, CH_2–N–CH_2), 3.65 (br, 2H, CH_2–N–CH_2 (piperidine)), 1.65 (m, 6H, CH_2–CH_2–CH_2– (piperidine)); ^{13}C NMR ($CDCl_3$): δ 20.5 (CH_2), 20.5 (CH_2), 22.7 (CH_2), 53.0 (CH_2), 57.8 (CH_2), 101.2 (CH_2), 124.1 (CH), 108.4 (CH), 147.6 (C), 148.1 (C), 105.5 (CH), 131.3 (C), 133.5 (CH), 126.2 (CH), 137.1 (CH), 121.6 (CH), 57.78 (CH_2); MS (*m*/*z*) 271 (M^+) 187, 157, 136, 110, 98, 84, 55. Calcd for $C_{17}H_{21}NO_2.H_2O$: C 70.23%, H 8.01%, N 4.84%; found: C 69.79%, H 7.72%, N 4.47%.

8.36. 5-(3,4-methylenedioxyphenyl)-propanoic acid (17)

3,4-methylenedioxycinnamic acid (**2g**) was hydrogenated in ethanol (50 mL) over 5% Pd-C under a pressure of hydrogen at 10 PSI for 40 min to give 5-(3,4-methlyenedioxyphenyl)-propanoic acid (1.67 g, 80% yield) as a solid. mp 86.1–88.3 °C (lit. mp 87–88 °C).

8.37. 5-(3,4-methylenedioxyphenyl)-propanoyl piperidine (18)

The method was adapted from that reported for piperlonguminine[8] but utilising 1-(3,4-methlyenedioxyphenyl)-propanoic acid and piperidine as the acid and amine components respectively. A mixture of 3-benzo[1,3]-dioxol-5-yl-propionic acid (200 mg, 0.0026 mole, 1 equiv) and triethylamine (0.27 mL, 0.002 mol, 2 eq) in dichloromethane (50 mL) was stirred for 15 min at 0 °C. To this mixture methane sulfonyl chloride (0.11 mL, 0.0015 mol, 1.5 equiv) was added and stirred for further 30 min at 0 °C. Piperidine (0.15 mL, 0.0015 mole, 1.5 eq) was added to the mixture and stirred for 1 h at 0 °C and 1 h at room temperature. Dichloromethane (50 mL) was added to the mixture which was then washed with 5% HCl (3×100 mL), saturated aqueous $NaHCO_3$ (3×100 mL) and water (3×100 mL). The organic fraction was dried over anhydrous sodium sulphate, filtered and rotary evaporated to yield a brown oil (203 mg, 65% yield). ^{1}H NMR ($CDCl_3$): δ 2.87 (t, 2H, $J=7.3$ **CH_2**), 2.57 (t, 2H, $J=7.0$ CH_2–**CH_2**), 6.70 (d, 1H $J=1.5$, Ar-**7H**), 6.72 (d, 1H $J=8.0$, Ar-**10H**), 6.66 (dd, 1H $J=1.2$, 8.0 Ar-**11H**), 5.90 (s, 2H, **O–CH_2–O**), 3.55 (t, 2H, N–**CH_2** (piperidine)), 3.34 (t, 2H, N–**CH_2** (piperidine)), 1.62 (m, 2H, **CH_2–CH_2**-CH_2 (piperidine)), 1.49 (m, 2H, CH_2–CH_2–**CH_2** (piperidine)); ^{13}C NMR ($CDCl_3$): δ 25.7 (**CH_2**), 25.9 (**CH_2**), 26.6 (**CH_2**), 31.7 (**CH_2**), 35.8 (**CH_2**), 43.1 (**CH_2**), 47.1 (**CH_2**), 101.2 (**CH_2**), 109.2 (**CH**), 109.3 (**CH**), 121.5 (**CH**), 135.6 (**C**), 146.2 (**C**), 148.0 (**C**), 170.8 (**C**).

8.38. Tetrahydropiperine (19)

Piperine (**1**) (2 g, 7 mmol) was hydrogenated in ethanol (50 mL) over 5% Pd-C under a pressure of hydrogen at 10 PSI for 30 min to give tetrahydropiperine (1.59g, 78%) as an oil. ^{1}H NMR ($CDCl_3$): δ 2.55 (t, 4H, $J=7.0$ CH_2–CH_2–CH_2–CH_2), 2.32 (t, 4H, $J=7.0$ CH_2–CH_2–CH_2–CH_2), 6.66 (d, 1H $J=1.3$, Ar-H), 6.70 (d, 1H $J=8.0$, Ar-H), 6.61 (dd, 1H $J=1.2$, 8.0 Ar-H), 5.89 (s, 2H, O–CH_2–O), 3.53 (t, 2H, N–CH_2 (piperidine)), 3.35 (t, 2H, N–CH_2 (piperidine)), 1.63 (m, 2H, CH_2–CH_2–CH_2 (piperidine)), 1.54 (m, 2H, CH_2–CH_2–CH_2 (piperidine)); ^{13}C NMR ($CDCl_3$): δ 24.5 (CH_2), 24.9 (CH_2), 25.5 (CH_2), 26.5 (CH_2), 31.4 (CH_2), 33.2 (CH_2), 35.4 (CH_2), 42.5 (CH_2), 46.6 (CH_2), 100.7 (CH_2), 108.0 (CH), 108.8 (CH), 109.0 (CH), 121.0 (C), 145.4 (C), 147.4 (C), 171.1 (C); MS *m*/*z* (%): 289 (M^+ 71), 204 (31), 154 (23), 148 (22), 141 (23), 140 (38), 135 (28), 127 (100), 112 (23), 86 (12), 84 (24), 70 (10), 36 (11). Calcd for $C_{17}H_{23}NO_3$: C 70.54%, H 8.01%, N 4.84%; found: C 70.70%, H 8.38%, N 4.85%.

8.39. 5-(3,4-Methylenedioxyphenyl)-pentanoicacid cyclohexyl amide (20)

5% Pd/C (30 mg) was added to 5-(3,4-methylenedioxy phenyl)-2*E*,4*E*-pentadienoic acid cyclohexyl amide **4j** (300 mg) and hydrogenated at 30 psi for 1 h. The solution was filtered and rotary evaporated to yield a white solid. Recrystallisation from ethylacetate and petroleum spirit yielded pure white crystals (255 mg, yield 84%). mp 145.4–146.3 °C. ^{1}H NMR ($CDCl_3$): δ 6.65 (d, 1H $J=1.6$, Ar-H), 6.71 (d, 1H $J=7.8$, Ar-H), 6.60 (dd, 1H $J=1.6$, 8.0 Ar-H), 5.90 (s, 2H, O–CH_2–O), 5.43 (s, 1H, NH), 3.87 (m, 1H, CH (cyclohexyl amide)), 2.53 (t, 2H, $J=7.7$ (CH_2–CH_2–CH_2CH_2)), 2.14 (t, 2H, $J=7.7$

((CH_2–CH_2–CH_2–CH_2)), 1.62–1.91 (m, 10H, CH_2–CH_2–CH_2–CH_2, CH_2–CH_2–CH_2 (cyclohexyl amide), 1.07–1.30 (m, 4H, CH_2–CH–CH_2 (cyclohexylamide)); ^{13}C NMR ($CDCl_3$): δ 25.3 ((CH_2)$_2$), 25.7 (CH_2), 25.9 (CH_2), 31.3 (CH_2), 31.7 (CH_2), 33.6 (CH_2), 35.8 (CH_2), 37.3 (CH_2), 48.4 (CH), 101.1 (CH_2), 108.4 (CH), 109.2 (CH), 121.4 (CH), 136.4 (C), 145.8 (C), 147.8 (C), 172.2 (C); MS *m*/*z* (%): 303 (M^+ 98), 204 (72), 176 (13), 168 (16), 162 (12), 161 (14), 154 (27), 148 (66), 141 (61), 135 (100), 74 (24), 60 (60). Calcd for $C_{20}H_{17}NO_5$: C 71.24%, H 8.31%, N4.62%; found C 71.22%, H 8.66%, N 4.61%.

8.40. 7-(3,4-Methylenedioxyphenyl)-heptanoic acid piperidine amide (21)

7-(3,4-Methylenedioxyphenyl)-hepta-2*E*,4*E*,6*E*-trienoyl piperidine **14** (150 mg) was dissolved in 30 mL of ethanol. 5% Pd/C (15 mg) was added and the contents hydrogenated at 30 psi. The solution was filtered and rotary evaporated to yield oil (83 mg, 43.6%). ^{1}H NMR ($CDCl_3$): δ 6.66 (d, 1H J = 1.5, Ar-H), 6.71 (d, 1H J = 7.8, Ar-H), 6.60 (d, d, 1H J = 1.6, 8.0 Ar-H), 5.90 (s, 2H, O–CH_2–O), 3.53 (t, 2H, J = 5.4 CH_2-N–CH_2), 3.37 (t, 2H, J = 5.7, (CH_2–N–CH_2), 2.51 (t, 2H, J = 7.7 (CH_2–CH_2–CH_2–CH_2–CH_2–CH_2)), 2.33 (t, 2H, J = 7.7 ((CH_2–CH_2–CH_2–CH_2–CH_2–CH_2)), 1.52-1.65 (m, 10H, CH_2–CH_2–CH_2–CH_2–CH_2–CH_2, CH_2–CH_2–CH_2 (piperidine)), 1.34 (m, 4H, CH_2–CH_2–CH_2–CH_2–CH_2–CH_2); ^{13}C NMR ($CDCl_3$): δ 24.9 (CH_2), 25.8 (CH_2), 25.9 (CH_2), 26.9 (CH_2), 29.3 (CH_2), 29.7 (CH_2), 31.3 (CH_2), 31.9 (CH_2), 33.8 (CH_2), 42.9 (CH_2), 47.1 (CH_2), 101.8 (CH_2), 108.4 (CH), 109.2 (CH), 121.4 (CH), 137.0 (C), 145.7 (C), 147.8 (C), 171.8 (C); MS *m*/*z* (%): 317 (M^+ 78), 232 (11), 204 (10), 183 (30), 182 (15), 154 (21), 148 (43), 141 (41), 127 (100), 112 (43), 85 (49).

8.41. Determination of distribution coefficients

Distribution coefficients between *n*-octanol and phosphate buffer (pH 7.4) were determined using the shake flask method. The aqueous and organic phases were presaturated with respect to each other before use. Compounds were dissolved in octanol (at 0.25 mM to 0.5 mM) and UV absorbance was measured before mixing with buffer. A 10 mL aliquot of octanol was taken and 100 mL of phosphate buffer was added to the solution and shaken well for 4 h. The solution was left overnight at 25 °C. After equilibrium, the octanol phase was collected very carefully and UV absorbance was obtained. The distribution coefficient ($D_{7.4}$) was calculated using the following equation. This experiment was repeated for four times and the average value taken.

$$D_{7,4} = \frac{A_1}{A_0 - A_1}\frac{V_w}{V_o}$$

A_0 — Absorbance of n-octanol before addition of buffer
A_1 — Absorbance of n-octanol layer after equilibrium
V_w — Volume of aqueous layer
V_o — Volume of octanol

8.42. Cell proliferation

Melan-a cells were diluted with RPMI 1640 medium. Aliquots (100 μL) containing 0.6×10^4 cells were inoculated into 96 well microlitre plates using a Finn Pipette. A 50 μL volume of each compound solution was added into 6 replicates of 100 μL of cells in 96-well microtitre plates. The final concentration of each compound in the well was 1–100 μM. After adding compounds into the melan-a cells (96 well plate), the plates were incubated at 37 °C in a 10% CO_2, 90% air humidified atmosphere incubator for 4 days.

After the stated length time of incubation, cells were attached to the substratum of the plate. These cells were fixed by addition of 50% of cold trichloroacetic acid (TCA, 40 μL) solution (20% v/v) on top of the cells and growth medium already present in each well. The plate was incubated at 4×C for 1 h. After the incubation period, each well was gently washed with tap water to remove TCA, growth medium and dead cells and allowed to dry in air.

To the dried cells, 50 μL volume of SRB (Sulphorhodamine B) dissolved in 1% acetic acid in water was added to each well and left for 30 min. At the end of the staining period, unbound SRB was removed by washing 5 times with 1% acetic acid. The plate was air-dried and 150 μL of Tris base tris (hydroxymethyl) aminomethane was added into each well to solubilize the cell bound dye. The plate was shaken for 15–30 min on a gyratory shaker followed by reading the optical density (OD) at 550nm in a microplate spectrophotometer.

8.43. Measurement of dendricity

Three sterile, round cover slips were placed in 60-mm culture dishes. Aliquots (2.9 mL) containing 1.5×10^4/mL cells were plated in the 60-mm culture dishes. A 100 μL volume of each compound solution was added into 3 replicates of 2.9 mL of cells in the culture dishes. The final concentration of each compound in the culture dish was 10 μM. After adding compounds and media into the melan-a cells (culture dish), the dishes were incubated at 37 °C in a 10% CO_2, 90% air humidified atmosphere incubator for 3 days.

After incubation, viable cells were found to be attached to the cover slips. The growth medium and dead cells were removed by aspiration and the attached cells gently washed with sterile phosphate buffer saline (PBS) twice. These cells were fixed by addition of 2 mL of methanol: acetone (1:1) to the culture dish. The dish was incubated at −20 °C for 10 min. Each dish was then gently washed with PBS to remove methanol: acetone and followed by addition of 2 mL of PBS and culture dishes were stored at 4 °C.

Staining procedures were carried out in order to view the cells and dendrites effectively. Eosin 1% (Raymond Alamb Ltd) was added to the culture dish and left for 1 min. After washing with tap water, 0.1% haematoxylin (Sigma Aldrich) was added to the dish and left for 5

min. The cover slips in the dish were then washed with tap water. After staining the cells within the dish, cover slips were mounted in D.P.X mounting medium (Raymond Alamb Ltd) on a glass slide and representative fields were taken through a phase contrast microscope (Nikon). These were used to assess melanocyte morphology and dendrite formation. At least a total of 30 cells from 3 cover slips were evaluated for each compound. Cell diameter and dendrite length was measured with a computer aided image analyzer (PC_IMAGE for Windows version 5.3).

Number of dendrites per cell was calculated as: total number of dendrites / number of cells

Total average dendrite length per cell was calculated on the basis of:

total dendrite length / number of cells

Average dendrite length was calculated by using:

total dendrite length / number of dendrites

8.44. Melanin assay

Melan-a cells (2.9 mL) were plated in a 60 mm culture dish at a density of 2×10^4 cells/mL. Piperine and its analogues were prepared by dissolving in methanol and diluting in medium. A 100 μL aliquot of piperine or piperine analogues were added to 4 replicates of each dish to get a final concentration of 10 μM. After adding the compounds the dishes were incubated at 37 °C in a 10% CO_2, 90% air humidified atmosphere incubator for 3 days. The concentration of methanol in the final volume of culture was 0.33% and this concentration was found to be non-toxic to the cells (data not shown).

After incubation, the medium was removed and the cells were washed with sterile PBSA and trypsinized using 250 μg/mL trypsin/EDTA at 37 °C for 5–7 min. Cells were collected and centrifuged at 1100 rpm for 7 min. A pellet was formed and the media was removed.

Fresh medium (2 mL) was added to the pellet. After dispersing the cells, 1 mL of melan-a cells were centrifuged again and the medium was removed. The remaining pellet was dissolved in 1.0M NaOH and vortexed for 10 min. The melanin content was calculated on the basis of absorbance at 475 nm and compared with standard curve of synthetic melanin. Synthetic melanin was analysed spectrophotometrically over the range of 3.25–100 μg/mL.

Acknowledgements

Mr. R. Venkatasamy was funded by Overseas Research Students Award Scheme (ORS) and the project was funded by British Technology Group (BTG), UK. The authors also wish to thank Prof. D. C. Bennett of St. George Medical School, UK for the gift of the melan-a cell line.

References and notes

1. Ortonne, J. P., Mosher, D. B., Fitzpatrick, D. B., Eds. *Vitiligo and Other Hypomelanosis of Hair and Skin*; Plenum: New York/London, 1983; pp 129–310.
2. Donata, K.; Austin, M.; Rajagopalan, K. *Ancient Science of Life* **1990**, *9*, 202.
3. Lin, Z.; Hoult, R.; Raman, A. *J. Ethnopharmacol.* **1999**, *66*, 141.
4. Lin, Z.; Hoult, R.; Bennet, D.; Raman, A. *Planta Med.* **1999**, *65*, 600.
5. Bennett, D. C.; Cooper, P. J.; Hart, I. R. *Int. J. Cancer* **1987**, *39*, 414.
6. Koul, S.; Koul, J. L.; Taneja, S. C.; Dhar, K. L.; Jamwal, D. S.; Singh, K.; Reen, R. K.; Singh, J. *Bioorg. Med. Chem.* **2000**, *8*, 251.
7. Paula, V. F.de.; Barbosa, A.; Luiz, C.de.; Demuner, A. J.; Pilo-Velosa, D.; Picanco, M. C. *Pest. Manag. Sci.* **2000**, *56* (2), 168.
8. Chatterjee, A.; Dutta, C. P. *Tetrahedron* **1967**, *23*, 1769.
9. Johnstone, R. A. W.; Rose, M. E. *Tetrahedron* **1979**, *35*, 2169.
10. Dehmlow, E. V.; Shamout, A. R. *J. Chem. Res.-S.* **1981**, 106.
11. Das, B.; Kasinathan, A.; Madhusudhan, P. *Tetrahedron Lett.* **1998**, *39*, 677.
12. http://www.raell.demon.co.uk/chem./logp/logppka.html.
13. http://www.daylight.com.
14. Eisenger, M.; Marko, O. *P. Natl. Acad. Bio-Sci.* **1982**, *79*, 2018.
15. Cui, J.; Shen, L.; Wang, G. *J. Invest. Dermatol.* **1991**, *97* (3), 410.
16. Burgoyone, R. D.; Peterson, O. H.; Portland Press: London, 1997; pp 139–151.