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Amides from *Piper nigrum* L. with dissimilar effects on melanocyte proliferation in-vitro

Zhixiu Lin, Yonghong Liao, Radhakrishnan Venkatasamy, Robert C. Hider and Amala Soumyanath

Abstract

Melanocyte proliferation stimulants are of interest as potential treatments for the depigmentary skin disorder, vitiligo. *Piper nigrum* L. (Piperaceae) fruit (black pepper) water extract and its main alkaloid, piperine (**1**), promote melanocyte proliferation in-vitro. A crude chloroform extract of *P. nigrum* containing piperine was more stimulatory than an equivalent concentration of the pure compound, suggesting the presence of other active components. Piperine (**1**), guineensine (**2**), pipericide (**3**), N-feruloyltyramine (**4**) and N-isobutyl-2*E*, 4*E*-dodecadienamide (**5**) were isolated from the chloroform extract. Their activity was compared with piperine and with commercial piperlongumine (**6**) and safrole (**7**), and synthetically prepared piperettine (**8**), piperlonguminine (**9**) and 1-(3, 4-methylenedioxyphenyl)-decane (**10**). Compounds **6–10** either occur in *P. nigrum* or are structurally related. Compounds **1, 2, 3, 8** and **9** stimulated melanocyte proliferation, whereas **4, 5, 6, 7** and **10** did not. Comparison of structures suggests that the methylenedioxyphenyl function is essential for melanocyte stimulatory activity. Only those compounds also possessing an amide group were active, although the amino component of the amide group and chain linking it to the methylenedioxyphenyl group can vary. *P. nigrum*, therefore, contains several amides with the ability to stimulate melanocyte proliferation. This finding supports the traditional use of *P. nigrum* extracts in vitiligo and provides new lead compounds for drug development for this disease.

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Introduction

Stimulants of melanocyte (pigment cell) proliferation are potential treatments for the skin disorder, vitiligo, a condition characterized by lesions of depigmentation where melanocytes are largely absent (Ortonne et al 1983). The aetiology of vitiligo remains relatively obscure and four hypotheses prevail to explain its onset and pathogenesis. The autoimmune hypothesis suggests that there is an antigen–antibody reaction that results in, or from, dysfunction of melanocytes and ultimately leads to their destruction (Ortonne et al 1983). The neural hypothesis, which suggests the involvement of neurotransmitters, is based on clinical observations that segmental vitiliginous patches often appear in the areas corresponding to one or more dermatomes (Lerner 1959). The self-destruct hypothesis postulates that normal, protective, free radical scavenging mechanisms for removing toxic melanin precursors and metabolites are somehow disrupted in vitiligo, causing accumulation of toxic substances and ultimate demise of melanocytes (Riley 1970; Lerner 1971). Finally, the melanocyte growth factor deficiency theory originates from the fact that the in-vitro growth and passage capacities of melanocytes derived from uninvolved vitiligo skin are usually defective, but that this defect can be partially corrected by addition of growth factors (Puri et al 1987). Although each of the above hypotheses has its supporting evidence, none of them alone satisfactorily explain the aetiopathogenesis of vitiligo, which may vary in different individuals.

Clinically, there are several therapeutic approaches available worldwide for vitiligo management, which include phototherapy with psoralen plus UVA irradiation (PUVA) (Ortonne 1989), corticosteroid therapy (Le Poole & Boissy 1997), pseudocatalase plus calcium therapy (Schallreuter et al 1995) and surgical grafting techniques (Falabella 1983; Olsson & Juhlin 1997). However, current treatments have limited success rates and a recent Cochrane collaboration review (Whitton et al 2006) highlights the urgent need for new

therapeutic options. While screening a range of traditional plant remedies used for vitiligo, Lin et al (1999a, b) found that a hot-water extract of *Piper nigrum* L. (Piperaceae) fruit (black pepper) promoted melanocyte proliferation in-vitro, as did piperine (**1**, Figure 1), the main alkaloid of black pepper. Piperine appears to work in-vitro by stimulation of protein kinase C (Lin et al 1999b). The ability of piperine to stimulate pigmentation in-vivo has recently been reported (Soumyanath et al 2006), validating the in-vitro melanocyte proliferation screen (Lin et al 1999a, b).

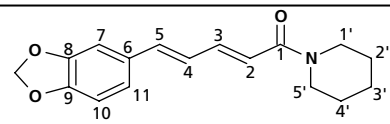
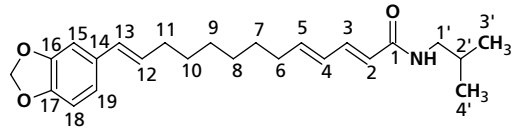
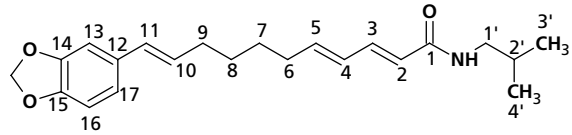
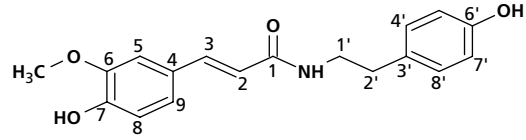
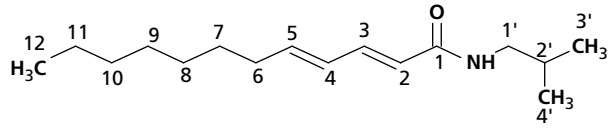
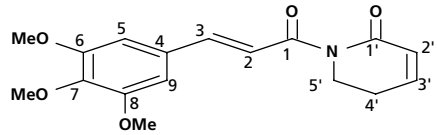
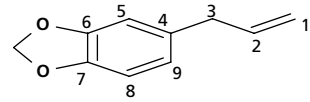
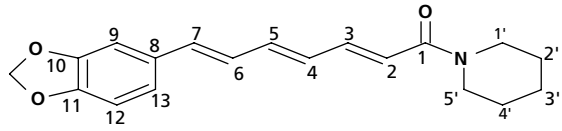
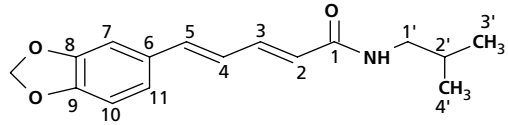
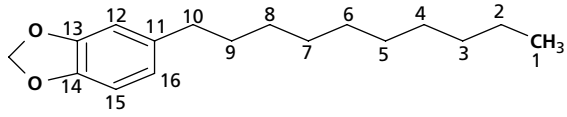
Compound	Chemical structure
Piperine (**1**)	
Guineensine (**2**)	
Pipericide (**3**)	
(2*E*)-*N*-Feruloyltyramine (**4**)	
N-Isobutyl-2*E*, 4*E*-dodecadienamide (**5**)	
Piperlongumine (**6**)	
Safrole (**7**)	
Piperettine (**8**)	
Piperlonguminine (**9**)	
1-(3,4-Methylenedioxyphenyl)-decane (**10**)	

Figure 1 Chemical structures of compounds isolated from *P. nigrum* (**1–5**), obtained from commercial sources (**6, 7**) or by synthesis (**8–10**).

P. nigrum fruit is an ingredient of Trikatu (together with *Piper longum* and *Zingiber officinale*), which is routinely prescribed in Ayurvedic medicine as a digestive cleanser for many conditions, including skin diseases (Johri & Zutshi 1992). In an Indian study, *P. nigrum* was used successfully in combination with other herbs to treat vitiligo (Donata et al 1990). *P. nigrum* contains approximately 5–9% alkaloids structurally related to piperine, including piperidine, piperettine, piperlonguminine, guineensine and pipericide (Epstein et al 1993; Parmar et al 1997), as well as 2–4% volatile oils containing safrole (Epstein et al 1993; Parmar et al 1997). A number of synthetic analogues of piperine share its melanocyte stimulatory activity (Venkatasamy et al 2004).

In this study, a chloroform extract of *P. nigrum* showed greater activity than an equivalent amount of piperine, suggesting the presence of other stimulatory compounds in this extract. This paper reports the isolation of 5 amides (**1–5**, Figure 1) from this extract and an investigation of their melanocyte stimulatory activity. This biological activity is reported for the first time for four of these compounds. The activities of five related compounds obtained from commercial sources or by chemical synthesis were determined to study their role in the activity of *P. nigrum* or investigate structural requirements for their activity (**6–10**, Figure 1).

Materials and Methods

Chemicals and reagents

Unless otherwise specified, chemicals and reagents used were obtained from Sigma Chemical Co. (Sigma-Aldrich Co. Ltd, Irvine, UK).

General methods for physical, elemental and spectroscopic analysis of compounds

Melting points were determined using an Electrothermal IA9100 digital melting point apparatus. UV spectra were recorded on a Perkin-Elmer UV spectrophotometer (model UV/VIS Lambda 2). IR spectra were obtained in KBr on a Perkin-Elmer spectrophotometer (model 1605 FTIR). ^{1}H and ^{13}C NMR spectra (including DEPT and 2D NMR (COSY and NOESY) experiments) were recorded in $CDCl_3$ for **1–3, 5** and **8–10** and in $CDCl_3 + CD_3OD$ for **4**, using tetramethylsilane (TMS) as internal standard at 400 MHz (^{1}H) and 100 MHz (^{13}C) on a Bruker AC-300 instrument equipped with the standard Bruker software. Electron impact mass spectra (EI MS) were recorded at 70 eV on a Perkin-Elmer SCIEX API-1 mass spectrometer or on a Joel AZ505W instrument. Fast atom bombardment mass spectra (FABMS; matrix: thioglycerol) were recorded on AutoSpec FAB$^+$ and FABMS (matrix: MNOBA + Na) and high resolution FABMS (HR FABMS) were recorded on a Zab-SE VG Analytical mass spectrometer (School of Pharmacy, University of London). Elemental analysis was performed by Microanalytical laboratories, Department of Chemistry, University of Manchester, UK. High-performance liquid chromatography (HPLC) analysis of piperine was conducted using an LDC Analytical CM4 solvent delivery system equipped with a Varian 9065 Polychrom photodiode array detector.

Plant material

P. nigrum L. fruit (black pepper; dried), from India, was purchased from the Food Centre (70 Turnpike Lane, London, UK). Identity was confirmed by comparison with the authenticated specimens stored in the Herbarium of the Pharmacy Department at King's College London, where a voucher herbarium specimen (Pi 108 L 22) of the material used in this study has been deposited.

Extraction and HPLC analysis

P. nigrum seeds (1000 g) were crushed in an electrical blender. The plant material was sequentially extracted with n-hexane, chloroform, methanol and distilled water in a Soxhlet extractor (12 h each). Organic solvents were removed under vacuum and water was removed by freeze-drying to yield dry extracts from hexane (29.7 g), chloroform (22.1 g), methanol (49.4 g) and water (37.0 g). HPLC analysis of piperine content was performed on an Adsorbosphere (4.6 mm × 25 cm, 5 μm) C18 column eluting with methanol–water (80:20; flow rate 2 mL min^{-1}). Piperine eluted at 6.3 min. Detector response (344 nm) was linear over the range 0.1–10 μg piperine.

Isolation of compounds 1–5 from the chloroform extract

The chloroform extract was fractionated (Figure 2) using silica gel 60, particle size 0.035–0.070; 200–440 mesh (ASTM; Fluka, Chemika, Switzerland) and lipophilic Sephadex LH-20, bead size 20–100 μm (Sigma, St Louis, MO). Extract (10 g) was initially chromatographed on a silica-gel column (GCC1, 6.3 × 20 cm), eluting with a chloroform, acetone and methanol gradient. GCC1 Fraction 5 (1.2–1.5 L; chloroform–acetone, 95:5) was dried and recrystallized from diethylether to yield **1** (653 mg). Fractions 20 and 21 (6.3–7.3 L; chloroform–acetone, 80:20) were combined and recrystallized from ether to yield **2** (89.1 mg). The mother liquor from the recrystallization of compound **1** was combined with GCC1 Fraction 4 (0.9–1.2 L; chloroform–acetone, 95:5) and dried. The resultant residue (3 g) was chromatographed on silica gel (GCC2; 2.5 × 20 cm) using a petroleum ether–ethylacetate gradient (80:20 to 75:25), finally washing with chloroform–methanol 50:50 to give 29 fractions. GCC2 Fractions 8 and 9 (700–900 mL; petroleum ether–ethylacetate, 77:23) were combined, dried and recrystallized from ether to yield compound **4** (15.2 mg). GCC2 Fractions 10–16 (0.9–1.6 L; petroleum ether–ethylacetate, 77:23 to 75:25) were combined and chromatographed on a Sephadex LH-20 column (1.5 × 30 cm) eluting with chloroform–methanol (50:50). Fractions 4–10 (0.6–2 L) were combined, dried and the residue (0.3 g) chromatographed on silica gel (GCC3; 2.5 × 20 cm) eluting with a petroleum ether–ethylacetate gradient (85:25 to 75:25). GCC3 Fractions 14 and 15 (0.9–1.0 L; petroleum ether–ethylacetate, 79:21) were dried and recrystallized from ether to yield compound **3** (8.7 mg). GCC2 Fraction 3 (200–300 mL; petroleum ether–ethylacetate, 80:20) was

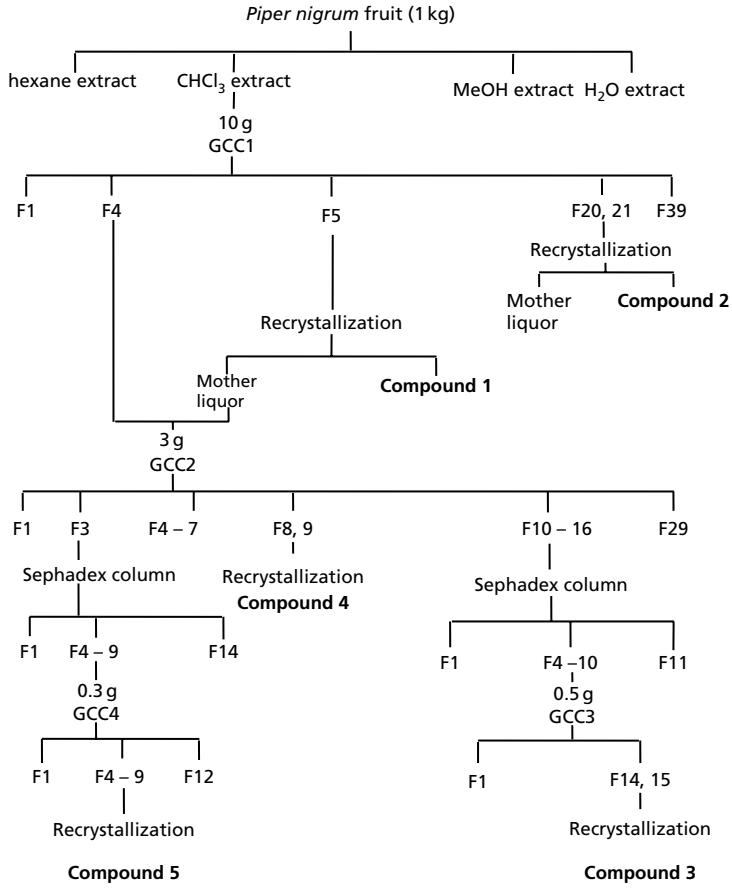


Figure 2 Scheme for the isolation of amides **1–5** from *P. nigrum* chloroform extract.

chromatographed on a Sephadex LH-20 column (1.5 × 30 cm) eluting with chloroform–methanol (50:50). Fractions 4–9 (0.3–1 L) were combined and rechromatographed on silica gel (GCC4; 2.5 × 20 cm) eluting with petroleum ether–acetone (90:10 to 85:15). Recrystallization from ether of the dried residue from GCC4 Fractions 4–9 (150–450 mL; petroleum ether–acetone 90:10) yielded compound **5** (9.3 mg). Compounds **1–5** have all been previously reported in the literature.

Compound **1** was identified as piperine by comparison with reported spectral data (Grewe et al 1970; Kiuchi et al 1988). Compound **2** was identified as guineensine by comparison with spectral data reported by Okogun & Ekong (1974). Compound **3** was identified as pipericide by comparison with published spectral data (Tabuneng et al 1983; Koul et al 1988). Compound **4** was determined as (*E*)-*N*-feruloyltyramine by comparison with reported spectral data (Fukuda et al 1993; Singh et al 1996). Compound **5** was identified as N-isobutyl-2E,4E-dodecadienamide by comparing with spectral data published by Dhar & Raina (1973).

Synthesis of compounds 8–10

Piperettine (**8**) and piperlonguminine (**9**) were synthesized using reported methods (Dehmlow & Shamout 1981; Nakamura et al 1988) with modifications (Venkatasamy et al 2004), and their identities were confirmed based on comparative studies with published spectral data (Venkatasamy et al 2004). The synthesis of 1-(3,4-methylenedioxyphenyl)-decane (**10**) has not previously been reported. This compound was obtained by initially refluxing piperinyl alcohol (2 g) and triphenylphosphine hydrobromide (4.96 g) in anhydrous THF at 160 °C for 2 h. After cooling, the salt was filtered (3 g) and recrystallised from chloroform to give white crystals of 3,4-methylenedioxyphenyl-triphenylphosphine bromine salt (2 g). This brominium salt (1.5 g) and nonaldehyde (0.5 mL) were stirred in dimethyl sulfoxide (DMSO) (5 mL) under nitrogen atmosphere at 0 °C for 15 min, *tert*-But-OK (0.41 mL) was added and the reaction mixture stirred at 0°C for a further 30 min. The mixture was poured into 100 mL of water and extracted with EtOAc (3 × 50 mL). The organic layer was dried over anhydrous sodium sulfate, filtered and evaporated under vacuum and further purified by silica gel column chromatography using hexane–CH_2Cl_2 (9:1) to yield an oil (400 mg). The oil (100 mg) was dissolved in EtOH, 5% Pd/C (10 mg) added and the mixture hydrogenated at 30 psi for 30 min to yield **10** (75 mg, 75% yield). The structure of compound **10** was confirmed as 1-(3,4-methylenedioxyphenyl)-decane by spectroscopic analysis.

1-(3,4-Methylenedioxyphenyl)-decane (**10**): light brown oil, UV λ_{max} (methanol) 286 nm; ^{1}H NMR ($CDCl_3$) δ: 6.71 (1 H, d, $J_{15,16}$ = 7.8 Hz, H-15), 6.64 (1 H, dd, $J_{16,12}$ = 1.5 Hz, $J_{16,15}$ = 7.8 Hz, H-16), 6.60 (1 H, d, $J_{12,16}$ = 1.5 Hz, H-12), 5.90 (2H, s, OCH_2O), 2.54 (2H, t, $J_{10,9}$ = 7.3 Hz, H-10), 1.55 (2 H, t, $J_{9,10}$ = 7.3 Hz, H-9) 1.14–1.54 (14 H, m, H-2,3,4,5,6,7,8) 0.83 (3 H, t, H-1); ^{13}C NMR ($CDCl_3$) δ 147.2 (C, C-14), 145.3 (C, C-13), 136.8 (C, C-11), 121.0 (CH, C-16), 108.8 (CH, C-12), 108.6 (CH, C-15), 100.6 (CH_2, OCH_2O), 35.7 (CH_2, C-10), 31.9, 31.8, 29.7, 29.6, 29.5, 29.3, 29.2, 22.7 (CH_2, C-2 to C-9), 14.14 (CH_3, C-1); MS m/z (%): 262 (M^+, 70), 261 (45), 250 (32), 135 (100), 123 (65); anal. C 78.65%, H 9.82%, calc. for $C_{17}H_{26}O_2$, C 78.77%, H 9.55%.

Melanocyte proliferation assay

RPMI 1640 medium was purchased from ICN (Costa Mesa, CA), trichloroacetic acid was from Aldrich (Gillingham, Dorset, UK) and 96-well microtitre plates and tissue culture flasks from Costar (Cambridge, MA). Melan-a, a copiously pigmented non-tumorigenic mouse melanocyte cell line, was a gift from Professor D. C. Bennett, St George's Hospital Medical School, University of London. Culture procedures were carried out as described earlier (Bennett et al 1987) with minor modifications. Subconfluent melan-a cultures (passage number 23–30), maintained in a flask with RPMI 1640 growth medium supplemented with 5% fetal bovine serum (FBS), 2 mM L-glutamine, penicillin 100 U mL^{-1}, streptomycin 100 μg mL^{-1} and 200 nM 12-*O*-tetradecanoyl phorbol-13-acetate (TPA), were trypsinized (250 μg mL^{-1} trypsin at 37°C for 5–10 min) and inoculated into 96-well microtitre plates at 6×10^3 cells/well in medium containing 0 nM TPA and 5% FBS. Test compounds (dissolved first in methanol and diluted in medium) were added to the melan-a cells at final concentrations of 0 (negative control), 0.1, 1, and 10 μM compound and 0.01% v/v methanol. Six replicate wells of each concentration were set up, together with negative control (vehicle only, 12 wells) and positive control (20 and 200 nM TPA, 6 wells). Sulforhodamine B (SRB) assay, which is a colorimetric end-point microplate assay that quantifies only viable cells by staining their cellular protein content, was performed after 4 days of incubation at 37°C according to methods given elsewhere (Skehan et al 1990; Lin et al 1999a, b).

Statistical analysis of cell culture data

Cell growth was expressed as % of the mean value obtained for the control (vehicle only) incubations. Statistical comparisons between compounds and control were made using one-way anaylsis of variance followed by a post-hoc Dunnett's *t*-test.

Results and Discussion

Since the intended target for this drug development program is ultimately vitiligo in man, it would have been desirable to conduct the biological experiments on a human melanocyte culture. However, it is well known that culturing human melanocytes is inherently difficult. For instance, they cannot be passaged for more than three cycles and will not survive during routine frozen storage (Bennett et al 1987). Also, the genetic heterogeneity inherited from different donors could lead to non-reproducible results and complicate comparisons between test groups. We therefore chose to use melan-a cells, an immortalised, pigmented, genetically homogenous, non-tumorigenic mouse melanocyte cell line (Bennett et al 1987), as an in-vitro model for our screening programme. After initial positive results in this line (Lin et al 1999b), we found that piperine also stimulates the replication of human melanoblasts (Lin et al 1998). Our initial studies (Lin et al 1999b) demonstrated the ability of a hot-water extract of *P. nigrum* fruit and piperine to stimulate melanocyte proliferation in-vitro, and of piperine to stimulate pigmentation in-vivo (Soumyanath et al 2006). Sequential Soxhlet extraction of *P. nigrum* fruit with solvents of increasing polarity (hexane, chloroform, methanol and water) yielded extracts containing 25.3, 36.8, 32.5 and 0.6% w/w piperine, respectively, as determined by HPLC. When tested at concentrations equivalent to 1 μM piperine, the chloroform extract, but not the others, showed a greater activity than pure piperine (data not shown). This was confirmed in a dose–response curve (Figure 3) in which the chloroform extract was more active than piperine at concentrations of up to 5 μM piperine, showing the presence of other active compounds. Five compounds (**1–5**) were isolated from the chloroform extract (Figure 2). Based on their characteristics under EI MS, FABMS and ^{1}H and ^{13}C NMR spectroscopy, these compounds (Figure 1) were identified as piperine (**1**), guineensine (**2**), pipericide (**3**), (2E)-N-feruloyltyramine (**4**) and N-isobutyl-2E-4E-dodecadienamide (**5**). The melanocyte stimulatory activity of compounds **2–5** has not previously been reported.

In addition to the above five piperamides isolated from fruit extract of *P. nigrum*, two compounds, piperlongumine (**6**) and safrole (**7**), were obtained from commercial sources. Three compounds were synthesized in the laboratory, namely piperettine (**8**), piperlonguminine (**9**) and 1-(3,4-methylenedi-

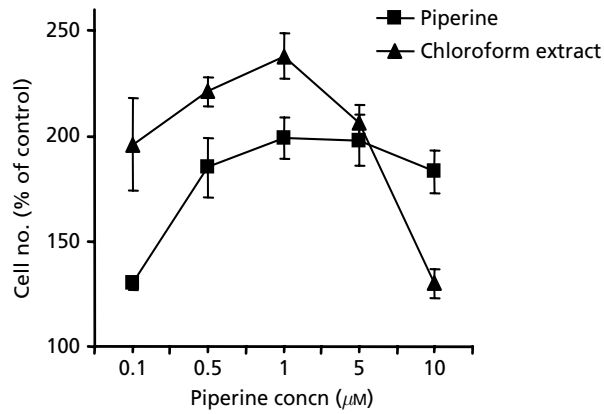


Figure 3 Dose–response curve showing the greater effect on melanocyte proliferation of a chloroform extract of *P. nigrum* fruit containing piperine, as compared with an equivalent concentration of pure piperine. Piperine concentration in the chloroform extract was determined by reversed-phase HPLC. Bars represent s.e.m.

oxyphenyl)-decane (**10**). Compounds **7–9** are all found in *P. nigrum* (Parmar et al 1997). Compound **6** is a major alkaloid in the related species *P. longum* (long pepper) (Chatterjee & Dutta 1963) and was included in this study due to its structural relationship to **1** and its commercial availability. Compound **10** was prepared to determine whether the presence of a methylenedioxyphenyl group and long side chain were sufficient for activity. The effects of these **10** compounds on melanocyte proliferation are shown in Figure 4. Of the compounds occurring naturally in *P. nigrum*, **1–3, 8** and **9** stimulated melanocyte proliferation in-vitro, whereas **4, 5** and **7** did not. The *P. longum* alkaloid, piperlongumine (**6**), was not only inactive but showed toxicity to the cells at 1 μM and above. Compound **10** was also inactive. Novel results reported here on compounds **2–5** and **7**, and those from earlier studies on other compounds (Lin et al 1999b; Venkatasamy et al 2004, Soumyanath et al 2006) allow some structure–activity relationships to be proposed. The main features of the piperine molecule are a methylenedioxyphenyl group, a piperidine amide function and a linking chain consisting of a *trans*-conjugated diene moiety. In this study, only those *P. nigrum* compounds that contained both the methylenedioxyphenyl moiety and an amide function stimulated melanocyte proliferation. The methylenedioxyphenyl group is a common feature shared by **1–3, 8** and **9** and all these amides displayed various degrees of melanocyte stimulatory activity. Compounds **4–6**, which lack this distinctive feature, had no stimulatory effects on melan-a cells. In particular, compound **5**, a close analogue of **2** and **3** lacking this group, did not stimulate melanocyte proliferation. Thus, it is rational to propose that the methylenedioxyphenyl moiety is essential for melanocyte growth promotion. However, it is not clear whether this group is directly responsible for activation of PKC seen with piperine (Lin et al 1999b). For example, both 3,4-methylenedioxymethylamphetamine (MDMA or Ecstasy), which contains this functional group, and its parent compound amphetamine (lacking this function) are activators of PKC in-vitro (Kramer, et al 1998; Park et al 2003). Similarly, in this study, safrole (**7**) has a methylenedioxyphenyl group but was not active. This result and the inactivity of **10** suggest that a methylenedioxyphenyl group with a side chain is insufficient for activity, supporting the need for the terminal amide group. The exact character of the amino portion of the amide function appears less critical. *P. nigrum* alkaloids containing either a piperidine amide (as in **1** and **8**) or an isobutylamide group (as found in **2, 3** and **9**) were both active. In separate studies on synthetic derivatives of piperine (some also naturally occurring), we have found that activity is retained in analogues of piperine with pyrollidinyl or aliphatic amide substituents (Venkatasamy et al 2004). Interestingly, replacement of the amide group of piperine with various ester moieties retained activity, although the corresponding free acid, piperinic acid, was inactive (Venkatasamy et al 2004). Extending the length of the chain connecting the amide and methylenedioxyphenyl functions to a conjugated *trans*-triene (**8**) gave similar activity to **1**. In our earlier studies, we

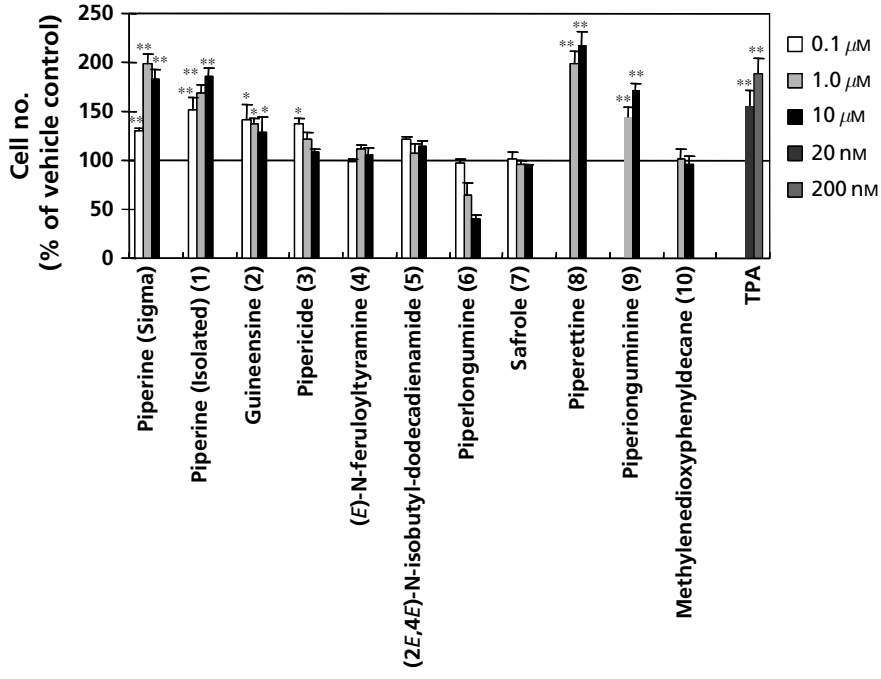


Figure 4 Effect of compounds **1–10** (0.1–10μM) on the in-vitro proliferation of melan-a mouse melanocytes. Cell number is expressed as a % of the value obtained in control incubations containing medium with added vehicle only. TPA at 20 and 200 nM was added as a positive control. $*P < 0.05$ and $**P < 0.01$ when compared to vehicle treatment (one-way analysis of variance, Dunnett's *t*-test).

reported that replacing the dienyl function with a 4- or 6-carbon aliphatic group retained strong activity (Venkatasamy et al 2004). However, in this study, introducing an aliphatic component adjacent to the dienyl group in the linking chain (**2, 3**) led to some loss of potency. The activity (though weaker than **1**) of compounds **2** and **3** led us to investigate the potential activity of **10**. This compound was inactive at all the concentrations tested, supporting the requirement for the amide (or other carbonyl) function at the end of the chain. Interestingly, isomerization of the *trans, trans* double bonds to its geometric isomers (isopiperine, chavicine and isochavicine) resulted in loss of melanocyte stimulatory activity (Soumyanath et al 2006), showing that a particular relative location of the methylenedioxyphenyl and carbonyl functions, or the flexibility to adopt that shape may be essential for the melanocyte stimulatory activity of these compounds.

It is worth noting that cells stimulated with piperine and its melanocyte stimulating analogues show an increase in total melanin (eumelanin) in culture compared with controls (Lin et al 1999b; Venkatasamy et al 2004). However, this appears to be due to the increased number of cells rather than stimulation of melanin synthesis per-se, since melanin content per cell is not increased. The effect of piperine and its analogues on eumelanogenesis has significant clinical implications, given that repigmentation in the skin depends on the amount and type of melanin produced. The positive results obtained from piperine and some of its analogues in a sparsely pigmented animal mouse strain (Soumyanath et al 2006) attest to the potential of this class of molecules to stimulate repigmentation either with or without concurrent ultraviolet irradiation.

Conclusions

This study reports the relative melanocyte stimulatory activity of several amides found in black pepper, *Piper nigrum*, and of related compounds. The presence of a methylenedioxyphenyl and amide group is essential for activity in the naturally occurring compounds, while the length of the chain separating them influences potency. This demonstration that several amide components of *P. nigrum* stimulate melanocyte proliferation supports the traditional use of black pepper in the treatment of vitiligo and highlights the potential for piperine and its analogues to be developed into new pharmaceutical treatments for this distressing skin condition.

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