



Structure–Activity Relationship of Piperine and its Synthetic Analogues for their Inhibitory Potentials of Rat Hepatic Microsomal Constitutive and Inducible Cytochrome P450 Activities

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Abstract—Inhibitors of drug metabolism have important implications in pharmaco-toxicology and agriculture. We have reported earlier that piperine, a major alkaloid of black and long peppers inhibits both constitutive and inducible cytochrome P450 (CYP)-dependent drug metabolising enzymes. In the present study, an attempt has been made to prepare several novel synthetic analogues so as to relate various modifications in the parent molecule to the inhibition of CYP activities. Two types of mono-oxygenase reactions arylhydrocarbon hydroxylase (AHH) and 7-methoxycoumarin-*O*-demethylase (MOCD) have been studied. Inhibition studies were investigated in rat microsomal fraction prepared from untreated, 3MC- and PB- treated rat liver in vitro. Modifications were introduced into the piperine molecule: (i) in the phenyl nucleus, (ii) in the side chain and (iii) in the basic moiety. Thus, 38 compounds have been subjected to such studies, and simultaneously an attempt has also been made to arrive at the structure–activity relationship of synthetic analogues. In general, most of the inhibitory potential of the parent molecule is lost with modification in either of the three components of piperine. Saturation of the side chain resulted in significantly enhanced inhibition of CYP while modifications in the phenyl and basic moieties in few analogues offered maximal selectivity in inhibiting either constitutive or inducible CYP activities. Thus few novel analogues as CYP inactivators have been synthesized which may have important consequences in pharmacokinetics and bioavailability of drugs. © 2000 Elsevier Science Ltd. All rights reserved.

Introduction

Piperine (*trans-trans*-isomer of 1-piperoyl piperidine) is a major ingredient of piper species, *Piper nigrum* Linn and *Piper longum* Linn, which are commonly used as spices and in various traditional systems of medicines.¹ Earlier studies from this laboratory have demonstrated that piperine inhibits several constitutive and inducible cytochrome P450 (CYP) activities in vitro and in vivo.^{2–7} We postulated that the use of piperine in the form of piper species in several traditional herbal formulations might have been responsible for the enhancement of

drug bioavailability consequent to modulation of drug metabolism.² Subsequently, it was shown to enhance the bioavailability of phenytoin in healthy volunteers,⁸ reduce aflatoxin B1 binding of DNA⁹ and protected hepatoma cells⁴ and V79 constructs of rat CYP2B1¹⁰ in cultures from cytotoxicity and genotoxicity of AFB₁ by impairing CYP mediated activation of the mycotoxin. Piperine also produced differential inhibition of glucuronidation in guinea pig enterocytes and rat liver, while conjugated double bonds appeared essential for in vitro inhibition of hepatic UDP-glucose dehydrogenase⁶ irrespective of the oxidation state of piperidine or methylenedioxyphenyl (MDP) rings. Piperine, thus, appears pharmacologically an important molecule despite the fact that it is a natural compound of very low toxicological consequences¹¹ and has been in use the world over for the palatability of food in the form of black pepper. At least four major metabolites of piperine from human urine have been reported, viz. 5-(3,4-dihydroxy phenyl)-2,4-pentadienoic acid piperidide and its 4-hydroxy-piperidine analogues and their respective

Keywords: piperine analogues; rat microsomes; cytochrome P450 inhibition; structure–activity relationship.

Abbreviations: AHH, arylhydrocarbon hydroxylase; BP, benzo(a)-pyrene; CYP, cytochrome P450; MOC, 7-methoxycoumarin; MOCD, 7-methoxycoumarin-*O*-demethylase; 3MC, 3-methylcholanthrene; MDP, methylenedioxyphenyl; PB, phenobarbital.

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tetrahydro-analogues¹² without any studies of their effect on drug metabolism. We became primarily interested to know whether or not any structural modifications in the parent molecule piperine would result in differential inhibition of rat hepatic constitutive and inducible CYP activities. In addition, such studies might also prove useful in developing specific inactivators of certain CYPs from natural lead bioactive molecules such as piperine.

A number of distinct CYPs are present in normal hepatic and extrahepatic tissues whose relative population may be altered due to the *in vivo* exposure to drugs and chemicals. For instance, 3-methylcholanthrene (3MC) induces members of CYP1A family and phenobarbital (PB) induces several members of CYP2 and CYP3 families.¹³ Accordingly, the disposition of drugs and CYP substrates would proceed along pathways different from normal tissues. Therefore, the inhibition or induction of drug metabolising enzymes has important consequences on the pharmacokinetics of drugs and hence their bioavailability. For that matter, a large number of inhibitors of CYP have reportedly been evaluated for the management of breast or uterine cancer, inflammation and other diseases.^{13–15} In this concern use of aromatase inhibitors¹⁶ and development of synergists as inhibitors of CYPs have already shown their due benefits. Moreover, in clinical therapeutics, the treatment regimens ought to be changed because of the inducibility of particular CYPs towards a drug substrate (s). It is, therefore, desirable to search for potential CYP inhibitors with maximal isoenzymic selectivity which indeed appears extremely difficult in view of the diversity and overlapping substrate specificity of various CYP isoforms.

Piperine structure (Fig. 1) consists of three important components, viz. methylenedioxyphenyl (MDP) ring, side chain with conjugated double bonds and a basic piperidine moiety attached through a carbonylamide linkage to side chain. Each of these moieties might influence the constitutive and inducibility characteristics of various CYPs. Furthermore, an MDP ring appears to be a common functional group of several naturally occurring compounds of pharmacological importance¹⁷ contributing significantly to modulate drug metabolism.¹⁸ The natural alkaloid consumed world-wide produces differential inhibition of CYPs.^{2,19} When compared to another MDP-containing insecticide synergist piperonyl butoxide, the latter produced a strong biphasic effect, an initial inhibition followed by induction.¹⁹ Though piperine also induced the CYP1A1 activity by transcription activation, the overall inhibition of benzo(a)pyrene metabolism and AHH activity

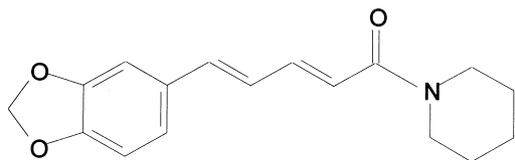


Figure 1. Structure of piperine.

appeared to be the consequence of direct interaction of piperine with CYP1A1 gene product.⁷ Thus apparent shortcomings, if any, in piperine molecule could perhaps be overcome by introducing various structural modifications in the molecule.

In this study we, therefore, have introduced several modifications in piperine so as to correlate its structure to the inhibition of constitutive and inducible rat hepatic CYP activities. For this purpose microsomes from untreated-, 3MC and PB-treated rat liver and two types of diagnostic substrates benzo(a)pyrene and 7-methoxycoumarin have been used. Piperine earlier was reported to inhibit atleast two CYP-dependent marker reactions, viz. arylhydrocarbon (benzo(a)pyrene) hydroxylase (AHH) and 7-methoxycoumarin *O*-demethylase (MOCD) in H4IIEC3 cells.⁴ The former reaction primarily is mediated by members of CYP family which are inducible by polycyclic aromatic hydrocarbons, i.e. CYP1A.²⁰ The latter is catalyzed by constitutive, phenobarbital or dexamethasone inducible CYP forms.²¹ Further, MOCD activity was observed in preparations of SD1 cells containing only CYP2B1, the major PB-inducible CYP of rat liver and not in XEM1 cells containing only CYP1A1.²¹ It may be mentioned that dexamethasone and PB-inducible CYPs involve several members of CYP2 and CYP3 families. Measurement of the activities of AHH and MOCD assayed in the present study offer a simple system of choice for *in vitro* screening of a large number of synthetic analogues of piperine as CYP inhibitors in contrast to the measurement of hexobarbital induced sleeping time in intact animals.²² In this study we, therefore, report the synthesis of several piperine analogues (Table 1) and attempted to correlate their structures with the inhibition of constitutive and inducible CYP activities. The study may be found very useful in developing new analogues as selective inactivators of CYPs.

Results

Structure–activity relationship of substituted phenylpentadienoic acid derivatives with the inhibition of hepatic microsomal monooxygenase activities *in vitro*

The specific activities of AHH and MOCD in microsomes from untreated, 3MC- and PB-treated rat liver, and the effect of piperine thereon are given in Table 2. These values are taken as controls for comparing the CYP activities of corresponding microsomes under the influence of various piperine analogues. Piperine as such produced concentration-dependent and equipotent inhibition of both constitutive and inducible AHH and MOCD activities. Similarly, the influence of various piperine analogues on the CYP activities of liver microsomes from untreated, 3MC- and PB-treated rats was investigated *in vitro* at three different concentrations of 10, 30 and 100 μ M (Tables 3 and 4). The monooxygenases inhibitory potential of each compound was compared with the parent molecule piperine by evaluating the IC₅₀ values, the concentration which brings about 50% of enzymatic inhibition.

Table 1.

Compound	Structure	MF	Mp (°C)	Compound	Structure	MF	Mp (°C)
8		C ₁₇ H ₂₁ NO ₂	135	23		C ₁₇ H ₁₉ NO ₃	113
9		C ₁₆ H ₁₉ NO ₂	137	24		C ₂₄ H ₂₇ NO ₃	116
10		C ₁₇ H ₂₂ N ₂ O ₂	201	25		C ₂₃ H ₂₅ NO ₃	155
11		C ₁₇ H ₂₆ N ₂ O ₂	183	26		C ₂₃ H ₂₇ NO ₃	183
12		C ₁₆ H ₁₇ NO ₃	147	27		C ₁₉ H ₂₅ NO ₄	214
13		C ₁₆ H ₁₉ NO ₃	144	28		C ₁₈ H ₂₃ NO ₄	190
14		C ₁₇ H ₂₃ NO ₃	a	29		C ₂₁ H ₂₇ NO ₂	164
15		C ₁₆ H ₂₁ NO ₃	a	30		C ₂₀ H ₂₅ NO ₂	117
16		C ₁₆ H ₂₃ NO ₃	a	31		C ₂₁ H ₂₉ NO ₂	137
17		C ₁₆ H ₂₃ NO ₃	a	32		C ₁₅ H ₁₇ NO ₃	89
18		C ₁₈ H ₂₃ NO ₃	118	33		C ₁₄ H ₁₅ NO ₃	146
19		C ₁₇ H ₂₁ NO ₃	148	34		C ₁₉ H ₂₅ NO ₂	122
20		C ₁₇ H ₂₃ NO ₃	114	35		C ₁₇ H ₂₃ NO ₂	89
21		C ₁₉ H ₂₇ NO ₃	148	36		C ₁₈ H ₂₅ NO ₂	86
22		C ₁₈ H ₂₁ NO ₃	162	37		C ₂₀ H ₂₉ NO ₂	71

^aSemi-solid.

Table 2. Influence of piperine on hepatic microsomal AHH and MOCD activities of untreated and inducers — pretreated rats^a

Compound	Monooxygenase activities (pmol/min/mg protein)			
	AHH		MOCD	
	Untreated	3MC-inducible	Untreated	PB-inducible
Control	57 ± 7	530 ± 47	388 ± 25	1048 ± 81
Piperine (μM):				
10	46.7 ± 5.1	439 ± 50	310 ± 23	880 ± 65
30	29.1 ± 3.2	312 ± 40	241 ± 27	618 ± 46
100	17 ± 2.5	138 ± 14	128 ± 11	419 ± 44

^aThe data are mean values ± SD (*n* = 8) from rat liver microsomal preparations. These enzyme activities were used as controls for comparison of inhibitory efficacy of the above monooxygenases by various synthetic analogues shown in Tables 3 and 4. Conditions for preparation of microsomes and enzyme assays are given in Materials and Methods. *P* > 0.01 (Student's '*t*' test).

(i) Influence of substituted derivatives of phenylpentadienoic acid on microsomal constitutive and 3MC-inducible AHH activity of rat liver in vitro. Piperine elicited a strong concentration related inhibition of both constitutive and inducible AHH activities (Tables 3). All the three structural components in the piperine molecule appeared obligatory for inhibition of both constitutive and inducible AHH activities (Table 3).

(a) Influence of modifications of the basic moiety on AHH activity. Presence of piperidine moiety in piperine offered an appropriate combination for inhibiting both the constitutive and the 3MC-inducible AHH activities. Replacement of piperidine moiety by pyrrolidine or *n*-butyl amines (**12** and **13**) rendered the inducible AHH activity insensitive to these analogues while the constitutive one was inhibited poorly.

(b) Influence of modifications in aromatic ring on AHH activity. Replacement of the MDP ring by either ethylenedioxyphenyl, 3-methoxy-4-benzyloxyphenyl, 4-methoxyphenyl, 3,4-dimethoxyphenyl, 3,4,5-trimethoxyphenyl and 2,2-dimethyl-3,4-dihydrobenzopyran produced compounds (**8**, **18**, **22**, **24**, **27** and **29**) which in general did not influence the inducible AHH while the constitutive one exhibited poor sensitivity compared to strong inhibition elicited by piperine. The inhibitory effect in general was remarkably reduced from null effect in the inducible form to a moderate inhibition in the constitutive enzyme. The studies suggested that various compounds synthesised by modifying the phenyl ring without disturbing the side chain and the basic moiety again had no influence on the inducible AHH activity. Furthermore, this effect again was found to be comparable with compounds having modifications in both the phenyl and the basic moieties (**9**, **19**, **20**, **23**, **26**, **28**, **30** and **31**). An exception was found with analogue

Table 3. Comparative influence of piperine and its synthetic analogues on the constitutive and 3MC-inducible rat hepatic microsomal AHH activity in vitro^a

Compounds	AHH activity (pmol 3-OH-BP formed per min per mg protein)							
	Untreated				3MC-inducible			
	10 μM	30 μM	100 μM	IC ₅₀	10 μM	30 μM	100 μM	IC ₅₀
Piperine	46.7 ± 5.1	29.1 ± 3.2	17.1 ± 1.5	35	439 ± 50	312 ± 40	138 ± 14	45
Analogues								
12	53.5 ± 4.8	37.6 ± 3.5	34.5 ± 3.5	80	525 ± 50	549 ± 34	548 ± 25	NI ^b
13	59.8 ± 6.0	40.5 ± 3.9	33.1 ± 4.1	105	581 ± 32	653 ± 47	617 ± 51	NI
22	51.9 ± 6.8	50.2 ± 4.9	43.9 ± 5.1	>100	464 ± 37	418 ± 55	444 ± 48	NI
23	54.1 ± 5.6	53.0 ± 6.0	47.3 ± 4.1	NI	510 ± 37	490 ± 40	474 ± 44	NI
24	52.5 ± 4.5	30.3 ± 3.5	28.6 ± 7.5	100	500 ± 30	513 ± 35	520 ± 36	NI
25	49.0 ± 3.7	28.8 ± 4.5	21.7 ± 1.9	35	505 ± 67	551 ± 59	525 ± 61	NI
26	55.9 ± 4.7	48.5 ± 5.9	34.8 ± 4.2	>100	495 ± 61	576 ± 53	525 ± 48	NI
8	53.6 ± 3.9	46.3 ± 3.5	32.4 ± 2.5	>100	521 ± 56	510 ± 42	490 ± 30	NI
9	55 ± 5.0	49.0 ± 3.9	43.8 ± 4.8	NI	503 ± 47	508 ± 42	511 ± 50	NI
10	57 ± 4.8	51.9 ± 4.4	38.2 ± 4.1	>100	525 ± 71	540 ± 47	500 ± 37	NI
18	57.6 ± 3.0	48.5 ± 5.1	39.9 ± 2.1	NI	490 ± 39	474 ± 27	413 ± 43	NI
19	53.0 ± 4.5	57.0 ± 4.1	53.0 ± 2.5	NI	459 ± 51	444 ± 38	464 ± 25	NI
20	54.2 ± 6.9	49.1 ± 6.7	41.1 ± 3.1	NI	495 ± 46	510 ± 35	511 ± 21	NI
27	53 ± 3.7	54.2 ± 5.1	41.1 ± 5.9	>100	454 ± 61	510 ± 47	505 ± 48	NI
28	48.5 ± 6.8	43.9 ± 5.6	37.1 ± 4.2	>100	484 ± 51	459 ± 60	459 ± 49	NI
29	54.7 ± 4.6	45.0 ± 3.4	30.8 ± 4.6	105	444 ± 29	479 ± 50	490 ± 45	NI
30	51.3 ± 3.1	41.6 ± 2.9	35.9 ± 4.7	>100	485 ± 34	479 ± 37	515 ± 42	NI
31	47.3 ± 4.0	34.2 ± 2.9	28.5 ± 3.0	100	459 ± 45	408 ± 39	357 ± 31	>100
32	55.9 ± 4.0	42.8 ± 4.1	30.2 ± 4.2	>100	525 ± 45	291 ± 21	133 ± 11	40
33	53.6 ± 6.8	50.7 ± 5.9	31.9 ± 4.1	>100	459 ± 36	270 ± 19	127 ± 16	35
34	53.6 ± 4.9	48.5 ± 4.2	35.9 ± 4.0	>100	490 ± 51	464 ± 49	479 ± 45	NI
14	49.6 ± 3.4	18.2 ± 2.4	7.41 ± 0.4	23	492 ± 54	540 ± 45	490 ± 39	NI
15	46.2 ± 3.9	34.7 ± 3.1	25.9 ± 3.4	70	469 ± 45	464 ± 51	479 ± 48	NI
16	46.7 ± 3.9	34.2 ± 2.7	18.8 ± 1.1	50	474 ± 40	449 ± 50	469 ± 47	NI
17	48.4 ± 4.9	29.1 ± 3.0	10.3 ± 0.9	32	505 ± 39	439 ± 39	428 ± 27	NI

^aTest compounds were dissolved in 50% methanol and introduced in 10 μL of the vehicle in 1 mL of the assay system before initiation of the reaction with the substrate. Controls received the vehicle only. Assays were performed in duplicate and data are mean ± SD of three experiments. Specific activities of untreated controls are given in Table 2.

^bNI = no inhibition.

Table 4. Comparative influence of piperine and its synthetic analogues on the constitutive and PB-inducible rat hepatic microsomal 7-methoxycoumarin *O*-demethylase activity in vitro^a

Compounds	MOCD activity (pmol 7-hydroxycoumarin formed /min/mg protein)							
	Untreated				PB-inducible			
	10 μ M	30 μ M	100 μ M	IC ₅₀	10 μ M	30 μ M	100 μ M	IC ₅₀
Piperine	310 \pm 23	241 \pm 27	128 \pm 11	45	880 \pm 65	618 \pm 46	419 \pm 44	47
Analogues								
12	335 \pm 30	339 \pm 26	312 \pm 22	100	1032 \pm 72	922 \pm 80	728 \pm 46	90
13	314 \pm 24	353 \pm 31	427 \pm 37	NI	1100 \pm 98	1048 \pm 87	964 \pm 100	NI
22	415 \pm 49	376 \pm 30	144 \pm 15	80	954 \pm 79	828 \pm 80	545 \pm 45	100
23	314 \pm 23	303 \pm 32	229 \pm 23	> 100	713 \pm 62	482 \pm 45	367 \pm 41	25
24	367 \pm 42	326 \pm 50	240 \pm 35	> 100	938 \pm 80	838 \pm 76	645 \pm 56	> 100
25	400 \pm 34	349 \pm 30	244 \pm 19	> 100	891 \pm 70	796 \pm 68	660 \pm 56	> 100
26	334 \pm 34	353 \pm 25	244 \pm 18	> 100	985 \pm 89	681 \pm 68	639 \pm 56	> 100
8	380 \pm 37	320 \pm 31	278 \pm 25	> 100	991 \pm 68	680 \pm 62	550 \pm 44	90
9	388 \pm 31	331 \pm 52	321 \pm 37	NI ^b	ND ^c	ND	ND	
18	322 \pm 27	291 \pm 23	213 \pm 20	> 100	1017 \pm 97	744 \pm 62	440 \pm 26	70
19	361 \pm 23	272 \pm 22	233 \pm 19	> 100	1048 \pm 79	880 \pm 71	629 \pm 80	> 100
20	376 \pm 40	323 \pm 29	241 \pm 24	> 100	765 \pm 67	514 \pm 45	356 \pm 32	28
27	353 \pm 37	314 \pm 29	225 \pm 20	> 100	775 \pm 63	681 \pm 59	513 \pm 48	90
28	361 \pm 26	337 \pm 27	229 \pm 21	> 100	911 \pm 99	796 \pm 59	629 \pm 42	> 100
29	361 \pm 48	295 \pm 25	140 \pm 17	65	796 \pm 67	597 \pm 53	338 \pm 21	40
30	376 \pm 26	334 \pm 30	198 \pm 14	> 100	859 \pm 81	451 \pm 36	335 \pm 27	27
31	369 \pm 41	349 \pm 33	353 \pm 29	NI	943 \pm 78	954 \pm 69	838 \pm 81	NI
32	369 \pm 18	334 \pm 34	256 \pm 26	> 100	1037 \pm 51	890 \pm 46	713 \pm 37	> 100
33	330 \pm 31	302 \pm 19	244 \pm 12	> 100	1079 \pm 90	943 \pm 72	744 \pm 45	> 100
34	450 \pm 40	318 \pm 27	202 \pm 18	100	870 \pm 43	702 \pm 50	325 \pm 39	50
14	303 \pm 21	132 \pm 12	109 \pm 11	25	796 \pm 68	503 \pm 41	367 \pm 33	27
15	357 \pm 49	326 \pm 29	198 \pm 20	100	964 \pm 90	796 \pm 77	492 \pm 50	85
16	341 \pm 31	124 \pm 16	97 \pm 11	23	1048 \pm 89	681 \pm 43	352 \pm 37	50
17	392 \pm 40	204 \pm 21	115 \pm 16	39	954 \pm 81	618 \pm 56	272 \pm 29	45

^aSpecific activities of untreated controls are given in Table 2. Other conditions were the same as described in Table 3.

^bNI, no inhibition.

^cND, not determined.

25 where replacement of MDP by 3-methoxy-4-benzyl-oxyphenyl and piperidine by pyrrolidine resulted in inhibition of only constitutive activity which was comparable to piperine.

(c) Influence of modifications of the side chain on AHH activity. Having modified the two terminals of the piperine molecule, we focused our attention on the central olefinic part of the molecule. Two types of major modifications were introduced in this case, e.g. removal of one double bond, (**32**) and saturation of the conjugated double bonds (**14**). These modifications, without any change in other parts of the parent molecule, offered interesting features. Removal of a double bond (**32**) resulted into marked loss of inhibitory effect on constitutive AHH while it retained its inhibitory effect on the inducible AHH almost equipotent to piperine. A similar effect was observed when the basic moiety of this molecule was replaced by pyrrolidine moiety (**33**). On the contrary, modification of MDP to benzopyranyl in **32**, i.e. **34** resulted in complete abolition of both the constitutive and inducible AHH.

Therefore, presence of conjugated double bonds in piperine appeared essential for overall inhibition of CYP activities and the level of saturation may tilt the balance of inhibition singularly either to constitutive or inducible forms of CYPs. For instance, saturation of double bonds of piperine (**14**) rendered it ineffective towards 3MC-inducible form of CYP while it elicited

pronounced inhibition of constitutive AHH activity. The potentiation of this inhibition of constitutive activity was almost higher by 2-fold compared to piperine. Further, it is important to note that modification of basic moiety in **14** resulted into compounds (**15**, **16**, **17**) having similar or less inhibitory effect as that of piperine on the constitutive AHH while such modifications in the parent molecule have rendered them almost ineffective (**12**, **13**).

Besides the above mentioned compounds, we also synthesised some other analogues of piperine, viz. **11**, **21** and **37**, where modifications were introduced in all the three parts of the molecules. Such compounds did not show any differential or specific inhibitory effects against enzyme activities assayed (not shown).

(ii) Influence of substituted derivatives of phenylpentadienoic acid on microsomal constitutive and PB-inducible MOCD activity of rat liver in vitro. Like AHH activity, modifications introduced in the piperine molecule also affected both the constitutive and PB-inducible MOCD activities (Table 4).

(a) Influence of modifications of the basic moiety on MOCD activity. Replacement of piperidine in piperine by pyrrolidine and *n*-butylamine (**12** and **13**) had no influence on the constitutive with inducible MOCD activities, unlike the strong inhibition produced by piperine. We also observed a similar effect on AHH activity discussed above.

(b) Influence of the modifications in the aromatic ring on MOCD activity. Modification of the MDP ring almost abolished the inhibitory influence on both the constitutive and inducible MOCD activities. For instance, replacement of MDP by either ethylenedioxyphenyl, 3-methoxy-4-benzyloxyphenyl, 4-methoxyphenyl, 3,4-dimethoxyphenyl, 3,4,5-trimethoxyphenyl, and 2,2-dimethyl-3,4-dihydrobenzopyran produced compounds (**8**, **18**, **22**, **24**, **27** and **29**) which in general did not influence the PB-inducible MOCD activity while the constitutive one exhibited poor sensitivity with an exception of **29** which was sensitive to induced activity as much as piperine. Further, when both the MDP and piperidine moieties were modified (**9**, **19**, **20**, **23**, **25**, **26**, **28**, **30** and **31**), most of the compounds lost their inhibitory potentials towards both constitutive and inducible MOCD with the exception of **20**, **23** and **30** which exhibited pronounced inhibitory effect only on inducible MOCD where the IC_{50} values were half of piperine.

(c) Influence of modifications of the side chain on MOCD activity. Decrease in length of the side chain of piperine (removal of one double bond) without altering the MDP or the basic moiety formed compound (**32**) which had no influence on both the constitutive and inducible MOCD activities. This compound otherwise inhibited specifically the inducible AHH activity (see Table 3). A modification of the basic moiety in **32** led to **33** having similar effects as **32**. However, a modification in the MDP of **32** resulted in **34** which is capable of inhibiting PB-inducible MOCD as much as piperine but has no effect on the constitutive one. Such modifications are likely to yield more compounds which might impart differential specificity to the PB-inducible CYPs.

It is again interesting to note that saturation of double bonds of piperine, i.e. **14** leads to potentiation of the inhibition of both the constitutive and inducible MOCD activities as observed with AHH activity. However, replacement of basic moiety in **14** by pyrrolidine (**15**) resulted into a complete loss of inhibitory potential while its replacement by *n*-butylamide (**16**) elicited relatively stronger inhibition of only constitutive activity while replacement by *N,N*-diethylamide (**17**) exhibited sensitivity almost comparable to piperine. Other analogues of piperine, where modifications were introduced in all the three parts of the molecules (**11**, **21** and **37**) had no influence on MOCD activity as has been observed earlier at least against AHH activity (not shown).

Influence of piperine analogues **14**, **16** and **17** on the constitutive and inducible AHH and MOCD activities of the H4IIEC3/G⁻ hepatoma cells in culture

To investigate the initial interaction of these analogues with the mono-oxygenase activities, the cell cultures were exposed to the above compounds for 4 h. Piperine mediated inhibition intensity of the monoxygenase activities in the hepatoma cells in culture was compared with three tetrahydropiperine derivatives (Table 5). Like in vitro inhibition of constitutive AHH, these compounds elicited inhibition stronger than piperine in cell cultures while the BA-inducible activity in comparison

Table 5. Influence of piperine analogues on monoxygenase activities of hepatoma H4IIEC3/G⁻ cells in culture^{a,c}

Compound	Enzyme activity (pmol/min/mg protien)			
	Arylhydrocarbon hydroxylase		7-Methoxycoumarindemethylase	
	Control	BA-treated	Control	PB-treated
DMSO	68.4 ± 2.3	75 ± 4	16.5 ± 1.5	13.5 ± 3.0
Piperine	6.4 ± 2.3	60 ± 5	13.0 ± 1.7	8.5 ± 1.0
14	2.8 ± 1.0	63 ± 6	7.0 ± 0.1	7.5 ± 1.5
16	5.3 ± 0.8	70 ± 10	10.0 ± 1.5	12 ± 1.0
17	3.6 ± 0.1	ND ^b	8.0 ± 0.5	9.5 ± 2.0

^aCells were grown in 90 mm culture dishes near confluency. Cultures were incubated with medium containing 50 μM of piperine analogues for 4 h. In case of pretreatment of cultures to BA (20 μM, 18 h) or PB (2 mM, 3 days), the experimental protocols were staggered so that the treatment with the above compounds started at the same time. Other conditions were the same as described in Materials and Methods.

^bND, not determined.

^cData are mean ± SD from three culture plates.

was not affected significantly. These compounds, nevertheless appeared equipotent in the inhibition of MOCD activity from untreated and PB-treated cultures. These results correlated well with the in vitro microsomal inhibition of mono-oxygenases by piperine. Further, long-term influence of these analogues on the inhibition or induction of AHH and MOCD has been investigated in cell cultures. Cell cultures were exposed to the medium containing 60 μM analogues for 27 h (Fig. 2). Both piperine and **14** induced AHH activity by about 70% while the inducibility was of low magnitude with **16** and **17**. However, MOCD continued exhibiting marginal impairment even after 27 h of exposure with these analogues while the magnitude of inhibition remained relatively higher with **16** and **17** compared to piperine.

Influence of piperine analogues on hexobarbital induced sleeping time in mice

Intraperitoneal administration of the above selected compounds viz. **14**, **16** and **17** potentiated hexobarbital-induced sleeping time over piperine (Fig. 3).

Comparative effect of piperine analogues on the kinetics of MOC demethylation by rat liver microsomes in vitro

Microsomes from untreated rat liver were used. Kinetics of enzyme inhibition in the presence and absence of piperine and two selected analogues using Lineweaver–Burk double reciprocal plot and Dixon plot of analysis has been determined. The values of the kinetic data are given in Table 6. All the three analogues caused non-competitive inhibition. The V_{max} decreased with increasing inhibitor concentrations while the apparent K_m of 151 μM was almost similar when dealkylation of MOC was studied in the presence and absence of piperine and its analogues. The values of half-maximal K_i of enzyme inactivation obtained for **14** and **17** were much lower than piperine, and correlated with the intensity of inhibition of MOC dealkylation in hepatoma cell cultures (Table 5).

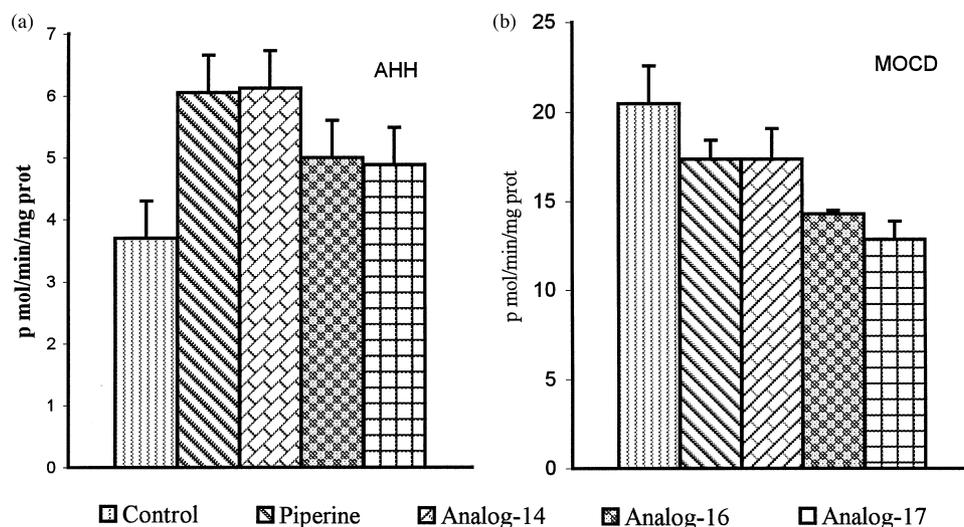


Figure 2. Influence of piperine and its synthetic analogues on monoxygenase activities in rat hepatoma cells. Rat Reuber hepatoma H4IIEC3/G– cells (1×10^6) were seeded in 90 mm tissue culture plates and allowed to grow for 48 h. The medium was changed with fresh medium and cultures were treated with 60 μ M piperine or analogues in 15 μ L DMSO/plate as vehicle. After 27 h of exposure, medium was removed, cultures washed with PBS \times 2. Cells were scrapped and collected in PBS and centrifuged. The cell pellet was stored in liquid nitrogen. Before assay of monoxygenases the pellet was suspended in 0.5 mL of 50 mM Tris–HCl, pH 7.4. Other conditions were the same as described in Materials and Methods. The data are mean \pm SD from four culture plates. $P < 0.05$ (Student's t test).

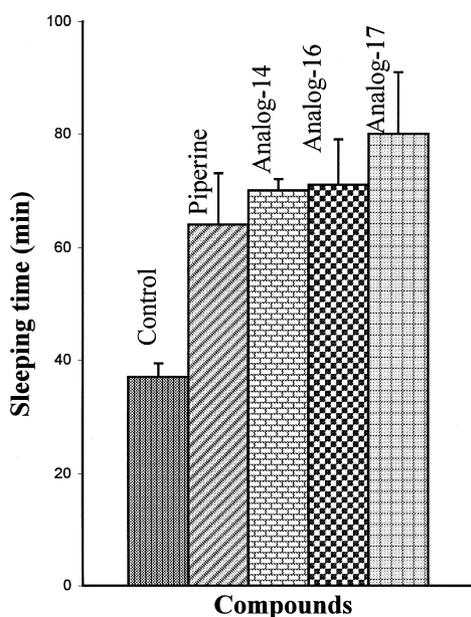


Figure 3. Influence of piperine analogues on the hexobarbital-induced sleeping time in mice. Compounds were administered (5 mg/kg body wt, ip) 30 min before hexobarbital (60 mg/kg body wt, ip). Data are mean \pm SD ($n = 4$). P values < 0.01 (Student's t test).

Discussion

The present studies have been undertaken to relate the role of various functional groups in piperine molecule to the inhibition of constitutive and inducible CYP activities of rat liver by employing the oxidation of marker CYP substrates of CYP1A and CYP2 gene families. The results suggested that (i) all the three components of piperine, viz. MDP ring, side chain and the piperidine moiety together, are essential for maximal inhibition of both the constitutive and inducible AHH and MOCD

Table 6. Comparative effect of piperine analogues on the kinetics of MOC demethylation by rat liver microsomes in vitro^a

Compound	V_{max} (pmol/min/mg protein)				K_m (μ M)	K_i (μ M)
	0 μ M	15 μ M	30 μ M	60 μ M		
Piperine	278	242	194	125	151	50
14	283	193	133	96	150	35
17	284	ND ^b	143	100	152	42

^aValues derived from Lineweaver–Burk double reciprocal and Dixon plots of MOC dealkylation by microsomal MOCD from untreated rat liver in the presence and absence of indicated inhibitors.

^bND, not determined.

activities and that (ii) the modification of any one moiety in the piperine molecule may not only alter the status of inhibition but also could elicit differential inhibition of the two types of monoxygenase activities examined.

Piperine was shown earlier to inhibit the oxidation of benzo(a)pyrene, 7-ethoxy-*O*-coumarin and ethylmorphine in untreated, 3MC- and PB-treated rat liver.² In this study we examined the oxidation of two types of CYP substrates B(a)P and MOC by using microsomes from untreated, 3MC- and PB- pre-treated rat liver. Because we have earlier reported that piperine inhibits at least two CYP-dependent marker reactions, viz. arylhydrocarbon B(a)P hydroxylase (AHH) and 7-methoxycoumarin *O*-demethylase (MOCD) in H4IIEC3 cells.³⁹ The former reaction primarily is mediated by members of CYP family which are inducible by polycyclic aromatic hydrocarbons, i.e. CYP1A.²⁰ The later is catalyzed by constitutive, phenobarbital or dexamethasone inducible CYP forms comprising members of CYP2 and CYP3 families.^{13,21} Further, MOCD activity was observed in preparations of genetically constructed V79 cells (SD1) containing only CYP2B1, the major PB-inducible CYP

of rat liver and not in XEM1 cells containing only CYP1A1.²¹

From the two CYP marker reactions studied against several analogues of piperine, some interesting features emerged about the structure–activity relationship on the inhibition of mono-oxygenase activities examined. For instance all the three structural components in piperine molecule occurring in nature are essential for overall inhibition. In the case of AHH various compounds with modified MDP ring in general were insensitive to both constitutive and inducible AHH with only the exception of **25**. However, the inhibition becomes selective in favour of the constitutive AHH only when the conjugated side chain is saturated with or without any alteration in the basic moiety (**14**, **15**, **16**, **17**). This suggested that differences in the protein domain in active site architecture exist between the constitutive and inducible CYP1A1 enzymes. Saturation of the side chain of piperine induces flexibility in the molecule which may facilitate interaction of the inactivator with protein domain and may thereby enhance constraints for orientation of the CYP substrates to the active site. On the other hand, compounds **32** and **33** are short by one double bond which not only confers rigidity in the molecule but also renders them selective inhibitors of only inducible AHH activity. Saturation of the conjugated double bonds to tetrahydro-derivatives of MDP ring appear to result in higher flexibility of the side chain which perhaps acts as a handle to orient MDP group to the active site of the CYP450 anchored in a strong hydrophobic environment. Thus, altering the functional groups in piperine would determine its interaction with the hydrophobic environment of the active site and hence its potential to determine the specificity and extent of inhibition. In addition, the lipophilic nature of the synthetic analogues also influences strongly the inhibition of AHH and MOCD. This is evidenced from the facts that compounds **14** and **16** have displayed higher inhibition than their unsaturated counterpart **11**. The latter although, it is a tetrahydro derivative, has displayed null inhibition due to quaternary ammonium salt which imparts higher water solubility to the compound. This also suggests the significance of 5-carbon ring or piperidine molecule in supplementing the hydrophobicity of piperine. Synthesis of such compounds thus appear useful in understanding the environment of active site of different CYP enzymes.

Certain substituted methylenedioxy benzenes are also known as synergists for a number of classes of pesticides of different structure types⁴⁰ which act by inhibition of drug biotransformation. MDP ring is generally considered to require hepatic metabolism for inhibition of microsomal oxidation through an active metabolite carbene.^{41,42} Part of the inhibitory action of MDP-related compounds is due to the metabolite intermediate (MI) complexation of CYP. Food flavouring agent isosafrol forms MI complex selectively with PB-inducible CYP2B1 and MC-inducible 1A2.¹³ This inhibition in general was not observed with various compounds we synthesised with change in substitutions in phenyl nucleus. The importance of the methylenedioxy carbon

in the induction of CYP450 has also been demonstrated earlier⁴³ despite the fact that these compounds are found potent inhibitors of mixed function oxidases both in vitro and in vivo.^{44,45} The noncompetitive inhibition of mixed function oxidases by MDP compounds essentially appears due to the binding of MDP metabolite to reduced CYP.⁴⁶ We observed earlier that piperine is not a suicidal inhibitor of monooxygenases. It was found that despite its ability to activate moderately CYP1A1 gene transcription, the alkaloid regulate CYP1A1 gene expression posttranslationally where it inhibits its catalytic activity by binding with the enzyme without destroying the AHH.⁷ However, it is not known that which CYP form (s) is involved in the metabolism of piperine.

In contrast to the B(a)P oxidation by CYP1A family, MOC is a preferred substrate for liver constitutive and PB-inducible CYPs.²¹ PB induces several CYP members of CYP2 (A1, B1, B2, B4, C5, C6) and CYP3 (A2, A4) families.¹³ We do not know the relative preference of each isoform to MOC dealkylation. Under such circumstances it is not easy to design inhibitors of sufficient selectivity to target individual CYP isoforms because of several forms of CYP and wide range of overlapping substrates specificity. However, after introducing structural alterations in piperine a number of compounds synthesised exhibited preferential selectivity either towards constitutive or inducible CYPs (**25**, **29**, **30**). These compounds relatively exhibited maximal selectivity and higher CYP inactivating potency than the parent molecule as evidenced by their effect on microsomal MOCD activities. However, unlike insensitivity to the MC-inducible AHH activity, the tetrahydroderivatives (**14**, **15**, **16**, **17**) were equipotent to both constitutive and inducible forms while analogues **14** and **17** exhibited higher sensitivity than piperine. It appears that the presence of the side chain with saturated double bonds linked through amide linkage appeared to impart specificity for inhibiting different forms of CYP450s. This in turn would again depend upon the amino acids located in the putative substrate recognition sites of CYP which regulate the accessibility of the substrates to generate MI which would sequester CYP to modulate drug biotransformation. In fact, it would require further studies to test these selected analogues for their specificity and selectivity with individual CYP members.

Besides studying the relationship of functional groups of piperine with the inhibition of CYP activities, we attempted to compare the potency of some analogues such as **14**, **16** and **17** with piperine in different systems in vitro and in vivo. These compounds were insensitive to 3MC-inducible but expressed higher sensitivity to constitutive AHH and as well as to constitutive and inducible MOCD. This type of response was also evident from experiments on sleeping time in mice, and monooxygenase inhibition in the hepatoma cells pretreated with inducers. Poor inducibility of AHH by **16** and **17** compared to piperine in this regard was interesting when cells were exposed to these analogues for longer period while they inhibited the enzymes strongly

during initial period. Like piperine,² the noncompetitive nature of inhibition was not altered by tetrahydroderivatives, the K_i values however, were further lowered compared to piperine although K_m value was similar in each case. Similarly, other selective compounds such as **20**, **23**, **25** and **35** could be tested for selectivity and specificity against certain pesticides as synergists or for enhancing the bioavailability of some therapeutically important drugs.

Conclusion

In conclusion, the structure of piperine is ideally suited to affect the microsomal oxidation of large number of compounds. Piperine has a number of inherent advantages in that it is a simple molecule, can be used with intact cells in culture and in vivo, easily available commercially, and a highly indispensable ingredient of spices in platability of food used for ages. Presence of piperidine function at the terminal end of conjugated double bond in the side chain and MDP ring offered differential sensitivity in inhibiting the CYP450 activities examined in the present study. By introducing modifications at different positions desired inhibitors of drug metabolism with strong implications in agriculture and pharmaco-toxicology could be developed. We have earlier reported that presence of unsaturated double bonds in the side chain of piperine molecule are responsible for inhibition of UDP-glucose dehydrogenases⁶ and certain dehydrogenase complex associated with electron transport chain⁴⁷ in vitro. This drawback is obviously removed by saturating the double bond and

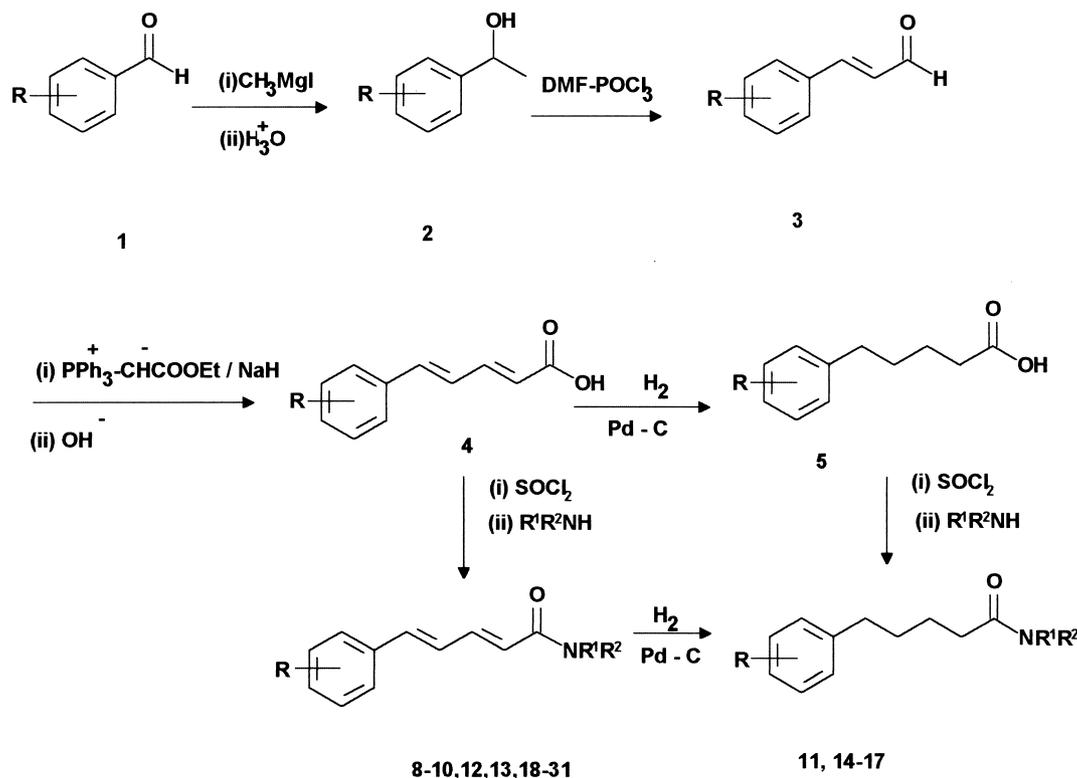
simultaneously increasing the potential drug inhibitory property of the molecule. Modifications of piperine molecule may thus prove useful in the development of selective CYP inhibitors.

Experimental

Chemistry

General methods. Reagents for chemical synthesis were of AR grade and obtained commercially. All reactions were monitored by TLC carried out on 0.25 mm E. Merck silica gel plates using UV light. Silica gel of mesh size 60–120 was used for column chromatography. ¹H NMR spectra were determined at either 60 MHz or 90 MHz using Varian F-60 or Jeol Fx-90 spectrometers, respectively. Mass spectra were determined on Jeol MSD-300 mass spectrometer while IR spectra were recorded on Perkin–Elmer FT-IR spectrometer.

Preparation of substituted aryl pentadienoic acid amides and other derivatives. The structure of piperine (Fig. 1) may be divided into three main components, i.e. methylenedioxyphenyl part, a conjugated side chain and piperidine moiety. For the preparation of its synthetic analogues modifications were envisaged in all the three components. The key intermediate, the substituted phenyl-2E,4E-pentadienoic acid was constructed starting from corresponding benzaldehyde in a five-step reaction sequence via cinnamaldehyde intermediate as depicted in Scheme 1. The amides were readily obtained from the carboxylic acids through acyl chloride formation



Scheme 1.

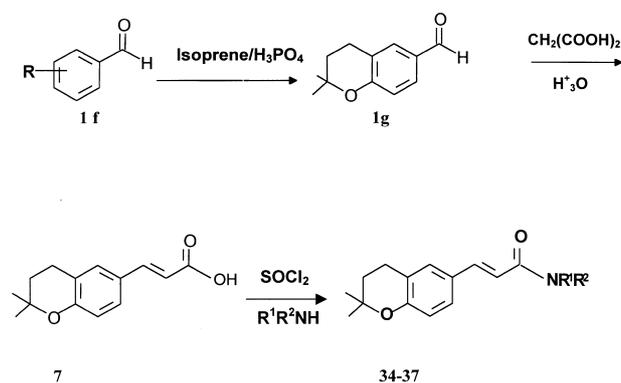
and condensation with appropriate amines. For the synthesis of pyrano analogues, the required benzopyran moiety was prepared from the corresponding phenols by condensing with isoprene in presence of an acid (Scheme 3) which is followed by the same reaction sequence as described in Scheme 1. The synthesis of substituted phenyl-2E-propenoic acid is shown in Schemes 2 and 3. The tetrahydro-analogues were obtained by catalytic reduction of the phenylpentadienoic acid or the phenylpentadienoamides in presence of palladium-charcoal (10%). The structure analysis was carried out mainly through elemental analysis, IR, PMR and MS studies. Various compounds synthesized through Schemes 1–3 are given in Table 1.

Preparation of α -methyl-4-methoxy benzyl alcohol (2a).

To an ethereal solution of Mg metal (2.6 g, 110 mmol) and methyl iodide (9.8 mL, 130 mmol) added an ethereal solution of *p*-anisaldehyde (1a) (15 mL, 110 mmol) and the contents stirred for 2 h at 0–5 °C. The reaction mixture worked up by adding saturated aqueous solution of ammonium chloride (10 mL), followed by dilution with water (100 mL), separation of organic layer followed by extraction of aqueous layer with solvent ether (2×100 mL). The combined organic layer washed with water (2×20 mL), dried over anhydrous sodium sulfate and concentrated in vacuo to give a gummy mass (2a) (16.0 g, 95%) analysed for C₉H₁₂O₂, MS (%) M⁺ at *m/z* 152 (36), 137 (57), 134 (100), 109 (62), 106 (14), 103 (10), 94 (50), 91 (62) and 78 (19). ν cm⁻¹ (KBr) 3352, 2968, 1612, 1588, 1510, 1450, 1422, 1300, 1242, 1174, 1068, 1030, 1016, 888 and 810. ¹H NMR (CDCl₃) δ 1.45 (3H, d, *J*=6.5 Hz, CH₃-C-OH), 3.80 (3H, s, OCH₃), 4.80 (1H, q, *J*=6.5 Hz CH₃-CHOH), 6.90 (2H, d, *J*=8.5 Hz 2×Ar-H) and 7.28 (2H, d, *J*=8.5 Hz, 2×Ar-H).

Preparation of 3-(4-methoxy phenyl)-2E-propenal (3a).

To a chilled solution of (2a) (12.2 g, 80 mmol) in DMF (15 mL) added phosphoryl chloride (7 mL) slowly at 0–5 °C for 1 h.²³ The contents were stirred further for 2 h and then allowed to attain room temperature followed by heating on a water bath for 3 h. The reaction mixture cooled and a saturated solution of sodium acetate (15 mL) added, followed by dilution with water (150 mL). The contents of the reaction mixture were extracted with ethylacetate (5×100 mL), the organic layer washed with water (3×30 mL) and dried over anhydrous sodium sulfate to give crude product which on CC over SiO₂ and elution with pet.ether:ethyl acetate (9:1) gave yellow crystalline compound (3a) (8.4 g, 65%), mp 58 °C (lit. mp. 56–57 °C),²⁴ analysed for

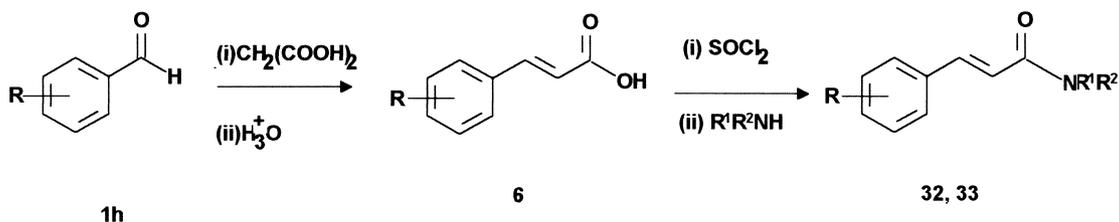


Scheme 3. R¹R²NH: piperidine, pyrrolidine, diethylamine, *n*-butylamine, *n*-pentylamine, isopropylamine, isobutylamin, *n*-methylpiperazine and *n*-hexylamine. S. No. R (a) 4-methoxy; (b) 3,4-dimethoxy; (c) 3,4-ethylenedioxy; (d) 4-benzyloxy-3-methoxy; (e) 3,4,5-trimethoxy; (f) 4-hydroxy; (g) [2H]-2,2-dimethyl-3,4-dihydro pyranyl; (h) 3,4-methylenedioxy.

C₁₀H₁₀O₂, MS (%) M⁺ at *m/z* 162. ν cm⁻¹ (KBr) 1694, 1650, 1600, 1582, 1500, 1462, 1334, 1246, 1124, 996 and 818. ¹H NMR (CDCl₃) δ : 3.90 (3H, s, OCH₃), 6.57 (1H, d, *J*=16.0 Hz and 7.0 Hz, -CH=CH-CO), 6.78 (2H, d, *J*=8.5 Hz, 2×Ar-H), 7.43 (1H, d, *J*=16.0 Hz, -CH=CH-CO), 7.46 (2H, d, *J*=8.5 Hz, 2×Ar-H) and 9.73 (1H, d, *J*=7.0 Hz, =CH-CHO).

Preparation of 5-(4-methoxy phenyl)-2E,4E-pentadienoic acid (4a).

To a stirring mixture of (3a) (6.5 g, 40 mmol) and the ylide, prepared from ethyl bromoacetate (4.8 mL, 44 mmol) and triphenyl phosphine (11.7 g, 44 mmol), in dry dimethoxy ethane (100 mL) was added sodium hydride (2.0 g) in small proportions. The progress of the reaction monitored by TLC; after the completion of the reaction, the contents poured carefully in ethyl acetate to quench the excess of sodium hydride, followed by addition of water, the organic layer separated and the aqueous layer extracted with ethyl acetate (3×125 mL). The combined organic layer washed with water (3×40 mL), dried over anhydrous sodium sulfate and concentrated in vacuo. The crude solid product taken up in 10% methanolic KOH solution (140 mL) and the contents refluxed on water bath for 6 h. On cooling, the contents were diluted with water (300 mL) and extracted with ethyl acetate (3×25 mL). The aqueous phase washed with petroleum ether (30 mL) and then acidified with 2N HCl solution. The resulting precipitate filtered, washed with ice cold water and dried to give acid (4a) (6.7 g, 82%) crystallised from ethyl acetate:petroleum ether (9:1) as colourless compound, mp 183 °C (lit. mp 182–183 °C),^{23,25,26} analysed



Scheme 2.

for $C_{12}H_{12}O_3$, MS (%) M^+ at m/z 204. vcm^{-1} (KBr) 2960, 1688, 1668, 1612, 1550, 1440, 1320, 1040, 960 and 810. ^1H NMR (acetone- d_6) δ 3.90 (3H, s, OCH_3), 6.10 (1H, d, $J=15.0$ Hz, $-\text{CH}=\text{CH}-\text{CO}$), 6.60–7.13 (3H, m, olefinic and Ar–H) and 7.40–8.20 (4H, m, olefinic and Ar–H).

Preparation of 5-(4-methoxy phenyl)-2E,4E-pentadienoic acid piperidine amide (8). To the acid (**4a**) (2.0 g, 10 mmol) in dry methylene chloride (50 mL) added freshly distilled thionyl chloride (0.8 mL) and refluxed for 1 h, excess of thionyl chloride removed in vacuo and thereafter condensed with methylenechloride solution of piperidine (1 mL) and stirred for 30 min. The organic layer washed with water (2×25 mL), dried over anhydrous sodium sulfate and concentrated to give crude product which on CC over SiO_2 and elution with pet. ether:ethyl acetate (4:1), yielded colourless crystalline compound **8** (2.56 g, 94%), mp 135°C (lit. mp 97°C),²⁵ (analysed for $C_{17}H_{21}NO_2$; found C 75.2481, H 7.8004, N 5.1637; calcd C 75.2463, H 7.7999, N 5.1618) MS (%) M^+ at m/z 271 (48), 187 (100), 159 (39), 129 (40), 115 (60) and 84 (10). vcm^{-1} (KBr) 2930, 1640, 1600, 1560, 1450, 1320, 1250, 1110, 1050 and 970. ^1H NMR (CDCl_3) δ : 1.50 (6H, bs, $-\text{N}-\text{CH}_2-(\text{CH}_2)_3$), 3.36 (4H, m, $\text{N}-(\text{CH}_2)_2$), 3.82 (3H, s, OCH_3), 6.36 (1H, d, $J=15.0$ Hz, $\text{CH}=\text{CH}-\text{CO}$) 6.80–7.00 (6H, m, olefinic and Ar–H) and 7.25 (1H, dd, $J=15.0$ Hz and 7.0 Hz, $-\text{CH}-\text{CH}=\text{CH}-\text{CO}$).

Preparation of 5-(4-methoxyphenyl)-2E,4E-pentadienoic acid pyrrolidine amide (9). Compound (**9**) was prepared from acid (**4a**) (1.0 g, 5 mmol) using thionyl chloride (0.4 mL) and pyrrolidine (0.5 mL, 6 mmol) by the same method as described for **8** to give a crude product which on CC over SiO_2 and elution with pet. ether: ethyl acetate (4:1) furnished white crystalline compound (1.18 g, 91.8%), mp. 137°C (analysed for $C_{16}H_{19}NO_2$; found C 74.6824, H 7.4421, N 5.4451; calcd C 74.6804, H 7.4418, N 5.4431). MS (%) M^+ at m/z 257 (35) 187 (100) 159 (12) 129 (16) 116 (32) and 70 (38). vcm^{-1} (KBr) 2955, 1630, 1600, 1520, 1485, 1436, 1310, 1250, 1184, 1136, 1022, 838 and 732. ^1H NMR (CDCl_3) δ : 1.93 (4H, m, $(\text{CH}_2)_2$), 3.56 (4H, m, $-\text{N}(\text{CH}_2)_2$) 3.85 (3H, s, OCH_3), 6.23 (1H, d, $J=15$ Hz, $\text{CO}-\text{CH}=\text{CH}$), 6.66–7.00 and 7.20–7.75 (7H, m, olefinic and Ar-H).

Preparation of 5-(4-methoxy phenyl)-2E,4E-pentadienoic acid N-methyl piperazine amide (10). Compound (**10**) was prepared from acid (**4a**) (2.0 g, 10 mmol) using thionyl chloride (0.8 mL) and N-methyl piperazine (1.0 mL, 11 mmol) by the method as described for **8** to give a crude product which on CC over SiO_2 and elution with pet. ether:ethyl acetate furnished white crystalline compound, (2.45 g, 86%), mp $201-202^\circ\text{C}$ (analysed for $C_{17}H_{22}N_2O_2$; found C 71.3139, H 7.7420, N 9.7852; calcd C 71.3011, H 7.7429, N 9.7823). MS (%) M^+ at m/z 286 (46), 187 (100), 159 (31), 144 (28), 116 (14) and 99 (15). vcm^{-1} (KBr) 3250, 2930, 1640, 600, 1560, 1420, 1310, 1250, 1020 and 970. ^1H NMR (CDCl_3) δ 2.95 (3H, s, $-\text{N}-\text{CH}_3$), 3.20 (4H, m, $-\text{N}-(\text{CH}_2)_2$), 3.50 (4H, m, $-\text{N}-(\text{CH}_2)_2$), 3.92 (3H, s, OCH_3), 6.30 (1H, d, $J=15.0$ Hz $\text{CH}=\text{CH}-\text{CO}$), 6.80–7.00 (6H, m, olefinic

and Ar–H) and 7.30 (1H, dd, $J=15.0$ Hz and 7.0 Hz, $-\text{CH}-\text{CH}=\text{CH}-\text{CO}$).

Preparation of 5-(4-methoxy phenyl) pentanoic acid N-methyl piperazine amide (11). To the compound (**10**) (0.57 g, 2 mmol) in ethylacetate (30 mL) added Pd/C (5%, 30 mg) and hydrogenated the contents at 30 psi. Work up of the reaction mixture afforded **11** (0.54 g, 93%), mp 183°C (analysed for $C_{17}H_{26}N_2O_2$; found C 70.3172, H 9.0280, N 9.6507; calcd C 70.3112, H 9.0237, N 9.6465). MS (%) M^+ at m/z 290 (32), 191 (100) 163 (46) 148 (10) 120 (48) and 99(15). vcm^{-1} (KBr) 2972, 1640, 1560, 1450, 1350, 1310, 1250, 1128, 1010, 930 and 827. ^1H NMR (CDCl_3) δ 1.60 (4H, bs, $(\text{CH}_2)_2$), 2.50 (4H, m, $\text{Ar}-\text{CH}_2-\text{CH}_2-\text{CO}$), 3.10 (3H, bs, $-\text{N}-\text{CH}_3$), 3.40–3.70 (8H, m, $2 \times -\text{N}(\text{CH}_2)_2$) 3.86 (3H, s, OCH_3) and 6.60–7.00 (4H, m, Ar–H).

Preparation of 5-(3,4-methylenedioxy phenyl)-2E,4E-pentadienoic acid (piperic acid) 4h. Piperine (28.0 g, 98 mmol), mp 132°C , dissolved in ethylene glycol (200 mL) and refluxed at 180°C after adding potassium hydroxide (25 g) and after the completion of the reaction the contents diluted with sufficient amount of water and acidified with 2N HCl. The resulting precipitate filtered and dried to give crude product which on crystallisation from ethanol gave **4h** as pale yellow solid (13.8 g, 65%) mp 217°C (lit. mp 217°C).²⁷

Preparation of 5-(3,4-methylenedioxyphenyl)-2E,4E-pentadienoic acid pyrrolidine amide (12). Compound (**12**) was prepared from **4h** (2.2 g, 10 mmol) using thionylchloride (0.9 mL) and pyrrolidine (0.95 mL, 11 mmol) by the same method as described for **8** to give a crude product which on crystallisation with pet. ether:ethylacetate (4:1) furnished a pale yellow crystalline compound (2.46 g, 90%) mp 147°C (lit. mp. $144-146^\circ\text{C}$)²⁸ (analysed for $C_{16}H_{17}NO_3$; found C 70.8444, H 6.3169, N 5.1666; calcd C 70.8315, H 6.3152, N 5.1626) ;MS (%) M^+ at m/z 271 (11) 201 (66) 173 (100) 143 (10) and 70 (9). vcm^{-1} (KBr) 1642, 1616, 1598, 1505, 1490, 1450, 1418, 1364, 1252, 1194, 1148, 1142, 1038, 994, 932 and 844. ^1H NMR (CDCl_3) δ 1.90 (4H, m, $(\text{CH}_2)_2$), 3.52 (4H, m, $-\text{N}(\text{CH}_2)_2$), 5.93 (2H, s, $\text{O}-\text{CH}_2-\text{O}$), 6.20 (1H, d, $J=15$ Hz, $\text{CO}-\text{CH}=\text{CH}$), 6.62–7.50 (6H, m, olefinic and Ar–H).

Preparation of 5-(3,4-methylenedioxy phenyl)-2E,4E-pentadienoic acid n-butyl amide (13). It was prepared from (**4h**) (4.4 g, 20 mmol) using thionyl chloride and n-butyl amine (2 mL, 20 mmol) employing the process as described for the preparation of **8**, to give the amide (**13**) (4.6 g, 84%), mp 144°C (lit. mp $151-152^\circ\text{C}$)²⁸ (analysed for $C_{16}H_{19}NO_3$; found C 70.3120, H 7.0071, N 5.1296; calcd C 70.3019, H 7.0062, N 5.1245); MS (%) M^+ at m/z 273 (100), 216 (14), 201 (17), 173 (87), 152 (11), 143 (30), 135 (12), 115 (71) and 96 (7). vcm^{-1} (KBr) 3328, 2936, 1640, 1550, 1506, 1490, 1466, 1442, 1398, 1364, 1316, 1248, 1208, 1192, 1100, 1042, 942 and 812. ^1H NMR (CDCl_3) δ 0.97 (3H, d, $J=6.5$ Hz CH_2-CH_3), 1.48 (4H, m, $-\text{C}-(\text{CH}_2)_2$), 3.36 (2H, m, $-\text{NH}-\text{CH}_2-\text{CH}_2$), 5.92 (1H, d, $J=15.0$ Hz $-\text{CH}=\text{CH}-\text{CO}$), 5.96 (2H, s, $-\text{O}-\text{CH}_2-\text{O}-$), 6.64–7.00 (5H, m, olefinic and Ar-H) and 7.24–7.52 (1H, m, $\text{CH}=\text{CH}-\text{CO}$).

Preparation of 5-(3,4-methylenedioxy phenyl) pentanoic acid (tetrahydro piperic acid) 5h. Piperic acid (**4h**) (6.70 g, 31 mmol) was dissolved in methanol (100 mL) and to it was added Pd/C (5%, 200 mg) and subjected to hydrogenation at 40 psi to yield tetrahydro piperic acid (**5h**) (6.85 g), mp 95 °C (lit. mp 100–101 °C)²⁹ analysed for C₁₂H₁₄O₄.

Preparation of 5-(3,4-methylenedioxy phenyl) pentanoic acid piperidine amide (14). Compound **5h** (2.0 g, 9 mmol) was condensed with piperidine (0.9 mL) as described for **8** to yield a gummy mass (2.4 g, 92%) (analysed for C₁₇H₂₃NO₃; found C 70.5700, H 8.0118, N 4.8434; calcd C 70.5619, H 8.0110, N 4.8404). MS (%) M⁺ at *m/z* 289 (50), 205 (100), 177 (47), 147 (57), 119 (32) and 84 (16). vcm⁻¹(KBr) 2935, 1640, 1565, 1440, 1250, 1020 and 870. ¹H NMR(CDCl₃) δ: 1.56 (10H, m, -NCH₂ (CH₂)₃ and -C-(CH₂)₂), 2.30 (2H, t, *J* = 7.0 Hz, COCH₂), 2.54 (2H, t, *J* = 7.0 Hz, Ar-CH₂), 3.46 (4H, m, -N-(CH₂)₂), 5.82 (2H, s, -O-CH₂-O-) and 6.62 (3H, m, Ar-H).

Preparation of 5-(3,4-methylenedioxy phenyl) pentanoic acid pyrrolidine amide (15). To **5h** (2.0 g, 9 mmol) dissolved in dry methylene chloride (40 mL) was added thionyl chloride (0.8 mL) and the resulting acid chloride condensed with pyrrolidine (0.9 mL) and worked up as described for compound **8** to furnish a gummy mass (**15**) (2.40 g, 94%) (analysed for C₁₆H₂₁NO₃; found C 69.7801, H 7.6874, N 5.0893; calcd C 69.7943, H 7.6870, N 5.0870). MS (%) M⁺ at *m/z* 275 (66), 148 (27), 126 (98), 113 (100), 105 (16), 98 (57) and 70 (75). vcm⁻¹ (KBr) 2960, 1640, 1550, 1455, 1350, 1305, 1268, 1120, 930 and 840. ¹H NMR (CDCl₃) δ 1.38–1.94 (8H, m, -(CH₂)₄) 2.23 (2H, t, *J* = 7.0 Hz, -COCH₂), 2.58 (2H, t, *J* = 7.0 Hz, Ar-CH₂), 3.33 (4H, m, -N(CH₂)₂), 5.90 (2H, s, -O-CH₂-O-) and 6.65 (3H, bs, 3×Ar-H).

Preparation of 5-(3,4-methylenedioxy phenyl)-pentanoic acid *n*-butyl amide (16). (**5h**) (2.0 g, 9 mmol) was condensed with *n*-butyl amine (1 mL) by the method as described for **8** to yield **16** as a gummy mass (2.0 g, 98%) (analysed for C₁₆H₂₃NO₃; found C 69.2940, H 8.3588 N 5.0522; calc. C 69.2871, H 8.3579, N 5.0500). MS (%) M⁺ at *m/z* 277 (49), 205 (100), 177 (33), 144 (36) and 119 (28). vcm⁻¹ (K Br) 3260, 2932, 1638, 1557, 1448, 1351, 1300, 1252, 1119, 1056, 930 and 835. ¹H NMR (CDCl₃) δ 0.96 (3H, d, *J* = 6.5 Hz, CH₃), 1.16–1.84 (8H, bm, 4×CH₂), 2.16 (2H, m, -CH₂-CO), 2.53 (2H, t, *J* = 6.5 Hz, Ar-CH₂), 3.30 (2H, q, *J* = 6.5 Hz, -NH-CH₂), 5.88 (2H, s, -O-CH₂-O-) and 6.66 (3H, m, Ar-H).

Preparation of 5-(3,4-methylenedioxy phenyl)pentanoic acid diethyl amide (17). The compound was prepared from tetrahydro piperic acid **5h** (2.0 g, 9 mmol) and diethylamine (1 mL) by the method as described for **8**, as a gummy mass (2.30 g, 92%) (analysed for C₁₆H₂₃NO₃; found C 69.2871, H 8.3579, N 5.0500). MS (%) M⁺ at *m/z* 277 (61), 205 (18), 175 (8), 134 (78), 127 (39), 114 (100), 99 (78) and 71(47). vcm⁻¹ (KBr) 2957, 1642, 1553, 1457, 1361, 1241, 1128, 1053, 927 and 843. ¹H NMR (CCl₄) δ: 1.13 (3H, t, *J* = 6.5 Hz, -CH₂-CH₃), 1.18 (3H, t, *J* = 6.5 Hz, -CH₂-CH₃), 1.79 (4H, m, -(CH₂)₂-), 2.39 (2H, t, *J* =

6.5 Hz, CH₂-CH₂-CO), 2.69 (2H, t, *J* = 6.5 Hz, Ar-CH₂-CH₂), 3.29 (4H, q, *J* = 6.5 Hz, -N (CH₂)₂), 5.86 (2H, s, -O-CH₂-O-) and 6.59 (3H, bs, Ar-H).

Preparation of α-methyl-3,4-dimethoxy benzyl alcohol (2b). It was prepared from 3,4-dimethoxy benzaldehyde (**1b**) (20 g, 100 mmol) and Grignard reagent [Mg metal, 3.0 g, 120 mmol) and methyl iodide (9.0 mL) as per the procedure described for compound **2a** to give a gummy mass (**2b**) (18.8 g, 90%), analysed for C₁₀H₁₄O₃, MS (%) M⁺ at *m/z* 182 (60), 166 (86), 164 (5), 138 (100), 123 (29), 107 (20) and 77 (23). vcm⁻¹(KBr) 3476, 2932, 1596, 1508, 1454, 1418, 1366, 1312, 1262, 1234, 136, 1018, 838, 756, and 720. ¹H NMR (CDCl₃) δ 1.42 (3H, d, *J* = 7.0 Hz, -CH₃-CHOH), 3.84 (6H, s, 2×OCH₃), 4.74 (1H, q, *J* = 7.0 Hz, CHOH-CH₃) and 6.88 (3H, s, Ar-H).

Preparation of 3-(3,4-dimethoxyphenyl)-2E-propenal (3b). It was prepared from **2b** (15.0 g, 80 mmol) and POCl₃ (14 mL) and DMF (40 mL) as described for **3a** to give crude product which on crystallisation from ethyl acetate:pet.ether (1:9) gave **3b** (9.8 g, 63%), mp 85 °C (lit. mp 81 °C)³⁰ analysed for C₁₁H₁₂O₃, MS (%) M⁺ at *m/z* 192. vcm⁻¹ (KBr) 1705, 1638, 1595, 1350, 1135, 1010 and 830. ¹H NMR (CDCl₃) δ 3.86 (6H, s, 2×OCH₃) 6.56 (1H, dd, *J* = 16.0 Hz and 7.0 Hz, -CH=CH-CHO), 6.88–7.14 (3H, m, 3×Ar-H), 7.36 (1H, d, *J* = 16.0 Hz, -CH=CH-CHO) and 9.65 (1H, d, *J* = 7.0 Hz, -CH=CH-CHO).

Preparation of 5-(3,4-dimethoxy phenyl)-2E,4E-pentadienoic acid (4b). This compound was prepared from **3b** (9.0 g, 46 mmol) by Wittig reaction as described for **4a** to give **4b** (9.0 g, 83%), crystallised from ethylacetate, mp 166 °C (lit. mp 203–205 °C)³⁰ analysed for C₁₃H₁₄O₄, MS (%) M⁺ at *m/z* 234 (87), 220 (19), 189 (100), 174 (56), 158 (38), 145 (19), 131 (32), 115 (66), 103 (52), 91 (74) and 77 (41). vcm⁻¹ (KBr) 2936, 1682, 1628, 1518, 1444, 1320, 1212, 1168, 1140, 1024, 886 and 827. ¹H NMR (CDCl₃) δ 3.86 (6H, s, 2×OCH₃), 5.92 (1H, d, *J* = 15.0 Hz, -CH=CH-CO), 6.84–7.16 (5H, m, olefinic and Ar-H) and 7.24–7.56 (1H, m, -CH-CH=CH-CO).

Preparation of 5-(3,4-dimethoxy phenyl)-2E,4E-pentadienoic acid piperidine amide (18). Compound **4b** (2.0 g, 8.5 mmol) was condensed with piperidine (0.9 mL) by the method described for compound **8** to yield **18** (2.40 g, 91%), a crystalline solid, mp 118 °C (lit. mp 110 °C)²⁵ (analysed for C₁₈C₂₃NO₃; found C 71.7441, H 7.6922, N 4.6503; calcd C 71.7351, H 7.6917, N 4.6475). MS (%) M⁺ at *m/z* 301 (49), 217 (100), 159 (57), 114 (17) and 84 (16). vcm⁻¹ (KBr) 2930, 1635, 1605, 1565, 1513, 1452, 1440, 1312, 1250, 1131 1025, 870 and 808. ¹H NMR (CDCl₃) δ 1.66 (6H, bs, N-(CH₂)₂-(CH₂)₃), 3.59 (4H, bs, -N (CH₂)₂), 3.92 and 3.94 (6H, 2×s, 2×OCH₃), 6.43 (1H, d, *J* = 15.0 Hz, -CH=CH-CO), 6.70–7.06 (5H, m, olefinic and Ar-H) and 7.24–7.75 (1H, m, CH=CH-CH=CH-CO).

Preparation of 5-(3,4-dimethoxy phenyl)-2E,4E-pentadienoic acid pyrrolidine amide (19). Compound **4b** (2.0 g, 8.5 mmol) was condensed with pyrrolidine (0.9 mL) as

per the procedure described for **8** to furnish a crystalline solid (2.25 g, 92%), mp 148 °C (analysed for C₁₇C₂₁NO₃; found C 71.0803, H 7.3666, N 4.8767; calcd C 71.0569, H 7.3657, N 4.8744) MS (%) M⁺ at *m/z* 287 (41), 217 (100), 189 (66), 174 (22), 98 (37) and 70 (35). $\nu_{\text{cm}^{-1}}$ (KBr) 2965, 1640, 1605, 1560, 1514, 1450, 1322, 1250, 1020, and 870. ¹H NMR (CDCl₃) δ 1.85 (4H, m, N-CH₂-(CH₂)₂), 3.46 and 3.49 (4H, 2×t, *J*=6.0 Hz, -N(CH₂)₂), 3.82 and 3.86 (6H, 2×s, 2×OCH₃), 6.18 (1H, d, *J*=15.0 Hz, -CH=CH-CO), 6.62–7.10 (5H, m, olefinic and Ar-H) and 7.20–7.79 (1H, m, CH=CH-CH=CH-CO).

Preparation of 5-(3,4-dimethoxy phenyl)-2E,4E-pentadienoic acid *n*-butyl amide (20). Compound **4b** (1.5 g, 6.4 mmol) was made to react with *n*-butylamine (0.7 mL) as per the procedure described for **8** to give **20** (1.6 g, 86%), a crystalline solid, mp 114 °C (analysed for C₁₇H₂₃NO₃; found C 70.5704, H 8.0119, N 4.8434; calcd C 70.5619, H 8.0110, N 4.8404). MS (%) M⁺ at *m/z* 289 (44), 217 (9), 189 (23), 188 (100), 157 (16), 151 (13), 99 (10) and 71 (38). $\nu_{\text{cm}^{-1}}$ (KBr) 3240, 2950, 1650, 1510, 1450, 1380, 1250, 1170, 1025, 1005 and 870. ¹H NMR (CDCl₃) δ 0.92 (3H, t, *J*=6.5 Hz, -CH₂-CH₃), 1.46 (4H, m, -NCH₂-(CH₂)₂), 3.31 (2H, m, -NCH₂-CH₂), 3.85 (6H, s, 2×OCH₃), 5.94 (1H, d, *J*=14.0 Hz, -CH=CH-CO), 6.62–7.00 (5H, m, olefinic and Ar-H) and 7.17–7.62 (1H, m, =CH-CH=CH-CO).

Preparation of 5-(3,4-dimethoxy phenyl)-2E,4E-pentadienoic acid-*n*-hexylamine amide (21). Compound **4b** (1.5 g, 6.4 mmol) was condensed with *n*-hexylamine (0.8 mL) by the procedure described for **8** to give **21** (1.8 g, 94%), a crystalline solid, mp 148 °C (analysed for C₁₉H₂₇NO₃; found C 71.9014, H 8.5740, N 4.4182; calcd C 71.8935, H 8.5731, N 4.4126). MS (%) M⁺ at *m/z* 317 (40.0), 217 (92.0), 189 (100), 159 (14.0), 114 (10.0), 89 (15.0) and 63 (13.0). $\nu_{\text{cm}^{-1}}$ (KBr) 3250, 2950, 2450, 1650, 1610, 1510, 1440, 1260, 1140, 1020, 990 and 850. ¹H NMR (CDCl₃) δ 0.90 (3H, t, *J*=6.5 Hz, -CH₂-CH₃), 1.34–1.71 (8H, m, (CH₂)₄), 3.33 (2H, m, -NH-CH₂), 3.94 (6H, s, 2×OCH₃), 5.97 (1H, d, *J*=14.0 Hz, -CH=CH-CO), 6.73–7.10 (5H, m, olefinic and Ar-H) and 7.26 (1H, m, CH-CH=CH-CO).

Preparation of 3,4-ethylenedioxy benzaldehyde (1c). To a solution of 3,4-dihydroxy benzaldehyde (6.9 g, 50 mmol), in dry acetone (110 mL) was added 1,2-dibromoethane (6 mL) and anhydrous potassium carbonate (**5g**) and the contents refluxed for 48 h to give after usual work up, 3,4-ethylenedioxy benzaldehyde (**1c**) (7.5 g, 90%), crystallised from hexane:acetone, mp 54–55 °C, analysed for C₉H₈O₃, MS (%) M⁺ at *m/z* 164 (100) 135 (10) 119 (4) 91 (3) and 79 (22). $\nu_{\text{cm}^{-1}}$ (KBr) 1680, 1608, 1576, 1500, 1458, 1394, 1288, 1206, 1108, 1040, 944, 910 and 862. ¹H NMR (CDCl₃) δ : 4.24 (4H, s, -O-CH₂-CH₂-O-), 6.89 (1H, d, *J*=8.5 Hz, Ar-H), 7.35 (2H, m, Ar-H) and 9.71 (1H, s, CHO).

Preparation of α -methyl-3,4-ethylenedioxy benzyl alcohol (2c). This compound was prepared from 3,4-ethylenedioxy benzaldehyde (5.0 g, 30 mmol) and Grignard reagent (mg metal, 0.86 g, 36 mmol) and methyl iodide

(4 mL) in solvent ether (80 mL) by the procedure described for **2a** to give a semisolid (5.1 g, 94%), analysed for C₁₀H₁₂O₃, MS (%) M⁺ at *m/z* 180 (10), 164 (100), 136 (90), 106 (66) and 92 (90). $\nu_{\text{cm}^{-1}}$ (KBr) 3400, 2968, 1584, 1500, 1432, 1284, 1260, 1198, 1154, 1054, and 868. ¹H NMR (CDCl₃) δ 1.44 (3H, d, *J*=7.0 Hz, CH₃), 4.21 (4H, s, -OCH₂-CH₂O-), 4.76 (1H, q, *J*=7.0 Hz, CHOH) and 6.96 (3H, m, 3× Ar-H).

Preparation of 3-(3,4-ethylenedioxy phenyl)-2E-propenal (3c). It was prepared from **2c** (4.0 g, 22 mmol), POCl₃ (4 mL) and DMF (8 mL) by the procedure described for **3a** to give a yellow solid (2.8 g, 67%), mp 63–64 °C, analysed for C₁₁H₁₀O₃, MS (%) M⁺ at *m/z* 190 (100), 162 (16), 134 (22), 106 (42) and 78 (53). $\nu_{\text{cm}^{-1}}$ (KBr) 2928, 1668, 1612, 1576, 1502, 1452, 1436, 1394, 1288, 1202, 1112, 1036, 968, 912 and 876. ¹H NMR (CDCl₃) δ 4.28 (4H, s, -OCH₂CH₂O-), 6.56 (1H, dd, *J*=15.0 and 7.0 Hz, CH=CH-CHO), 6.88–7.12 (3H, m, 3× Ar-H), 7.36 (1H, d, *J*=15.0 Hz, -CH=CH-CHO) and 9.60 (1H, d, *J*=7.0 Hz, =CH-CHO).

Preparation of 5-(3,4-ethylenedioxy phenyl)-2E,4E-pentadienoic acid (4c). This compound was prepared from **3c** (4.0 g, 21 mmol) by the procedure described for **4a** to furnish **4c** (4.0 g, 82%), mp 178–80 °C, analysed for C₁₃H₁₂O₄, MS (%) M⁺ at *m/z* 232. $\nu_{\text{cm}^{-1}}$ (KBr) 2968, 1704, 1608, 1582, 1502, 1462, 1432, 1374, 1288, 1256, 1158, 1054, 860 and 810. ¹H NMR (CDCl₃ and DMSO-*d*₆) δ 4.28 (4H, s, -OCH₂CH₂O-), 6.10 (1H, d, *J*=15.0 Hz, -CH=CH-CHO) and 6.72–7.60 (6H, m, olefinic and Ar-H).

Preparation of 5-(3,4-ethylenedioxy phenyl)-2E,4E-pentadienoic acid piperidine amide (22). Compound **4c** (1.4 g, 6 mmol) was condensed with piperidine (0.6 mL) by the procedure described for **8** to yield **22** (1.4 g, 78%), mp 162 °C (analysed for C₁₈H₂₁NO₃; found C 72.2207, H 7.0710, N 4.6813; calcd C 72.2181, H 7.0701, N 4.6788). MS (%) M⁺ at *m/z* 299 (62), 215 (90), 189 (100), 162 (80), 114 (31) and 84 (80). $\nu_{\text{cm}^{-1}}$ (KBr) 2990, 1630, 1590, 1500, 1420, 1300, 1256, 1150, 990, 926 and 880. ¹H NMR (CDCl₃) δ : 1.66 (6H, m, -N-C-(CH₂)₃), 3.60 (4H, m, -N-(CH₂)₂), 4.26 (4H, s, -OCH₂CH₂O-), 6.17 (1H, d, *J*=14.0 Hz, -CH=CH-CO), 6.62–7.10 (5H, m, olefinic and Ar-H) and 7.29–7.79 (1H, m, -CH=CH-CO).

Preparation of 5-(3,4-ethylenedioxy phenyl)- 2E,4E-pentadienoic acid pyrrolidine amide (23). Compound **4c** (1.6 g, 7 mmol) was reacted with pyrrolidine (0.8 mL) by the procedure described for **8** to yield **23** (1.90 g, 95%), mp 113 °C (analysed for C₁₇H₁₉NO₃ found C 71.5612, H 6.7121, N 4.9101 calc. C 71.5589, H 6.7113, N 4.9088) MS (%) M⁺ at *m/z* 285 (40), 215 (18), 188 (100), 98 (14) and 70 (23). $\nu_{\text{cm}^{-1}}$ (KBr) 2960, 1645, 1600, 1577, 1510, 1400, 1285, 1248, 1190, 1114, 1052 and 775. ¹H NMR (CDCl₃) δ : 1.94 (4H, m, -N-C-(CH₂)₂), 3.57 (4H, t, *J*=7.0 Hz, N-(CH₂)₂), 4.30 (4H, s, -OCH₂CH₂O-), 6.25 (1H, d, *J*=14.0 Hz, -CH=CH-CO), 6.73–7.16 (5H, m, Olefinic and Ar-H) and 7.30–7.70 (1H, m, -CH=CH-CO).

Preparation of 4-benzyloxy-3-methoxy benzaldehyde (1d). To a solution of 4-hydroxy-3-methoxy benzaldehyde

(13.0 g, 85 mmol) in acetone (250 mL) was added benzylbromide (12.0 mL, 88 mmol) and anhydrous potassium carbonate (10 g) and refluxed for 24 h. The usual work up afforded **1d** (19.0 g, 92%), mp 67 °C, analysed for C₁₅H₁₄O₃ MS (%) M⁺ at *m/z* 242 (100), 227 (18), 213 (21) 181 (29), and 91 (50). $\nu_{\text{cm}^{-1}}$ (KBr) 2928, 1674, 1598, 1586, 1500, 1460, 1428, 1400, 1386, 1236, 1184, 1128, 916 and 818. ¹H NMR (CDCl₃) δ : 4.12 (3H, s, OCH₃), 5.44 (2H, s, -OCH₂-Ar), 7.20 (1H, d, *J*=8.5 Hz, Ar-H), 7.48–7.84 (7H, m, Ar-H) and 9.92 (1H, s, Ar-CHO).

Preparation of α -methyl-4-benzyloxy-3-methoxy benzyl alcohol (2d). It was prepared from **1d** (14.5 g, 60 mmol) and magnesium iodide (62 mmol) as described for **2a** to furnish a semisolid (14.0 g, 90%), analysed for C₁₆H₁₈O₃, MS (%) M⁺ at *m/z* 259 (10), 241 (4), 151 (16), 121 (14) and 91 (100). $\nu_{\text{cm}^{-1}}$ (KBr) 3410, 2972, 1594, 1500, 1454, 1414, 1382, 1258, 1222, 1150, 1115 and 1072. ¹H NMR (CDCl₃) δ 1.38 (3H, d, *J*=7.0 Hz, CHOH-CH₃), 3.83 (3H, s, OCH₃), 4.72 (1H, q, *J*=7.0 Hz, CHOH-CH₃), 5.04 (2H, s, Ar-CH₂O), 6.76 (1H, s, Ar-H), 6.82 (2H, d, *J*=8.5 Hz, 2 \times Ar-H) and 7.16–7.52 (5H, m, 5 \times Ar-H).

Preparation of 3-(4-benzyloxy-3-methoxy phenyl)-2E-propenal (3d). This compound was prepared from **2d** (11 g, 43 mmol) and Vilsmeier reagent as described for **3a** to yield a crystalline solid **3d** (7.0 g, 64%), mp 91 °C (lit. mp. 90 °C)³¹ analysed for C₁₇H₁₆O₃, MS (%) M⁺ at *m/z* 268 (51), 242 (23), 178 (41), 162 (9), 147 (20), 124 (10) and 91 (100). (KBr) 2940, 1666, 1620, 1598, 1502, 1480, 1460, 1426, 1384, 1260, 1220, 1168, 1120, 1028 and 972. ¹H NMR (CDCl₃) δ 3.88 (3H, s, OCH₃), 5.20 (2H, s, Ar-CH₂O), 6.56 (1H, dd, *J*=15.0 and 7.0 Hz, CH=CH-CHO), 6.84–7.14 (3H, m, Ar-H), 7.22–7.54 (6H, m, olefinic and Ar-H) and 9.60 (1H, d, *J*=7.0 Hz, -CH-CHO).

Preparation of 5-(4-benzyloxy-3-methoxyphenyl)-2E,4E-pentadienoic acid (4d). This compound was prepared from **3d** (5.0 g, 19 mmol) by Wittig reaction as described for **4a** to yield **4d** (4.8 g, 81%), a crystalline solid, mp 196 °C, analysed for C₁₉H₁₈O₄, MS (%) M⁺ at *m/z* 310 (20), 297 (18), 266 (24), 219 (16), 175 (30) 115 (11) and 91 (100). $\nu_{\text{cm}^{-1}}$ (KBr) 2932, 1666, 1618, 1592, 1504, 1454, 1422, 1352, 1308 and 1266. ¹H NMR (CDCl₃) δ 3.90 (3H, s, OCH₃), 5.14 (2H, s, Ar-CH₂O), 5.92 (1H, d, *J*=14.0 Hz, CH=CH-CO), 6.73–7.10 (5H, m, Ar-H), 7.21–7.70 (6H, m, olefinic and Ar-H).

Preparation of 5-(4-benzyloxy-3-methoxyphenyl)-2E,4E-pentadienoic acid piperidine amide (24). Compound **4d** (1.55 g, 5 mmol) was condensed with piperidine (0.6 mL, 6 mmol) by the method described for **8** to furnish a solid (1.8 g, 95%) crystallised from ethylacetate:pet.ether (9:1) to give a crystalline solid mp 116–17 °C (lit. mp. 118 °C)³¹ (analysed for C₂₄H₂₇NO₃; found C 76.3667, H 7.2103, N 3.7142; calcd C 76.3651, H 7.2091, N 3.7106); MS (%) M⁺ at *m/z* 377 (42) 286 (100) 258 (12) 201 (13) and 91 (11). $\nu_{\text{cm}^{-1}}$ (KBr) 2928, 2856, 1620, 1510, 1450, 1390, 1368, 1266 and 1196. ¹H NMR (CDCl₃) δ : 1.50 (6H, bs, 3 \times CH₂), 3.44 (4H, bs, -N(CH₂)₂), 3.98 (3H, s, OCH₃) 5.04 (2H, s, Ar-CH₂), 6.34 (1H, d, *J*=15 Hz,

CO-CH=CH), 6.84–7.00 (6H, m, olefinic and Ar-H) and 7.28–7.78 (5H, bs, olefinic and Ar-H).

Preparation of 5-(4-benzyloxy-3-methoxy phenyl)-2E,4E-pentadienoic acid pyrrolidine amide (25). Compound **4d** (1.55 g, 5 mmol) was condensed with pyrrolidine (0.6 mL) by the method described for **8** to yield **25** (1.7 g, 94%), mp 155 °C (analysed for C₂₃H₂₅NO₃; found C 76.0120, H 6.9336, N 3.8551; calcd C 76.0075, H 6.9327, N 3.8538). MS (%) M⁺ at *m/z* 363 (19), 273 (44), 244 (5), 201 (17), 155 (3), 145 (4), 131 (6), 115 (14) and 91 (100). $\nu_{\text{cm}^{-1}}$ (KBr) 2914, 1636, 1610, 1589, 1504, 1420, 1384, 1354, 1260, 1230 1138 and 980. ¹H NMR (CDCl₃) δ : 1.90 (4H, bs, -N-C-(CH₂)₂), 3.52 (4H, bs, -N-(CH₂)₂-), 3.88 (3H, s, OCH₃), 5.14 (2H, s, Ar-CH₂O), 6.24 (1H, d, *J*=15.0 Hz, -CH=CH-CO), 6.68–7.08 (5H, m, olefinic and Ar-H) and 7.16–7.80 (6H, m, olefinic and Ar-H).

Preparation of 5-(4-benzyloxy-3-methoxy phenyl)-2E,4E-pentadienoic acid isobutyl amide (26). Compound **4d** (1.55 g, 5 mmol) was condensed with isobutyl amine (0.6 mL) by the method described for **8** to furnish a solid (1.65 g, 90%), mp 183 °C (analysed for C₂₃H₂₇NO₃; found C 75.5910, H 7.4472, N 3.8347; calcd C 75.5883, H 7.4461, N 3.8325). MS (%) M⁺ at *m/z* 365 (14), 293 (3), 274 (34), 201 (18), 175 (17), 143 (12), 131 (6), 115 (17) and 91 (100). $\nu_{\text{cm}^{-1}}$ (KBr) 2956, 1642, 1610, 1594, 1512, 1458, 1414, 1332, 1248, 1234 and 1122. ¹H NMR (CDCl₃): 0.92 (6H, d, *J*=6.5 Hz, -CH-(CH₃)₂), 1.80 (1H, m, CH-(CH₃)₂), 3.20 (2H, t, *J*=6.5 Hz, -NH-CH₂), 3.90 (3H, s, OCH₃), 5.20 (2H, s, Ar-CH₂O), 5.94 (1H, d, *J*=14.0 Hz, -CH=CH-CO), 6.70–7.08 (5H, m, olefinic and Ar-H). and 7.24–7.80 (6H, m, olefinic and Ar-H).

Preparation of α -methyl-3,4,5-trimethoxy benzyl alcohol (2e). This compound was prepared from 3,4,5-trimethoxy benzaldehyde **1e** (10.0 g, 51 mmol) by reacting with methyl magnesium iodide (53 mmol) as described for **2a** to yield a semisolid **2e** (9.80 g, 90%) analysed for C₁₁H₁₆O₄, MS (%) M⁺ at *m/z* 212 (100), 196 (52), 168 (80), 153 (31), 137 (32), 122 (11), 108 (13), 94 (13) and 77 (13). $\nu_{\text{cm}^{-1}}$ (KBr) 3480, 2972, 1594, 1502, 1456, 1420, 1328, 1234, 1118, 1000 and 832. ¹H NMR (CDCl₃) δ 1.44 (3H, d, *J*=7.0 Hz, CH₃-CHOH), 3.76 (3H, s, OCH₃), 3.82 (6H, s, 2 \times OCH₃), 4.76 (1H, q, *J*=7.0 Hz, CHOH-CH₃) and 6.56 (2H, s, 2 \times Ar-H).

Preparation of 3-(3,4,5-trimethoxy phenyl)-2E-propenal (3e). This compound was prepared from **2e** (8.5 g, 40 mmol) by reacting with Vilsmeier reagent as described for **3a** to give a yellow crystalline solid (5.9 g, 66%), mp 110–111 °C³² analysed for C₁₂H₁₄O₄. MS (%) M⁺ at *m/z* 222, 207, 179 and 151. $\nu_{\text{cm}^{-1}}$ (KBr) 1695, 1638, 1595, 1570, 1350, 1135, 1010 and 830 ¹H NMR (CDCl₃) δ 3.88 (9H, s, 3 \times OCH₃), 6.46 (1H, dd, *J*=16.0 Hz and 7.0 Hz, -CH=CH-CHO), 6.80 (2H, bs, 2 \times Ar-H), 7.40 (1H, d, *J*=16.0 Hz, CH=CH-CHO) and 9.70 (1H, d, *J*=7.0 Hz, =CH-CHO).

Preparation of 5-(3,4,5-trimethoxy phenyl)-2E,4E-pentadienoic acid (4e). This compound was prepared from **3e** (5.1 g, 23 mmol) through Wittig reaction as described for **4a** to give (4.6 g, 76%), mp 190 °C³³ analysed

for $C_{14}H_{16}O_5$ MS (%) M^+ at m/z 264 vcm^{-1} (KBr) 2932, 1700, 1626, 1605, 1506, 1424, 1338, 1232, 1160, 1120, 1010, 974 and 846. ^1H NMR (CDCl_3) δ : 3.81 (9H, s, $3 \times \text{OCH}_3$), 6.20 (1H, d, $J=15.0$ Hz, $-\text{CH}=\text{CH}-\text{CO}$), 6.70–7.10 (4H, m, olefinic and Ar–H), and 7.30–7.84 (1H, m, $\text{CH}=\text{CH}-\text{CO}$).

Preparation of 5-(3,4,5-trimethoxy phenyl)-2E,4E-pentadienoic acid piperidine amide (27). Compound **4e** (1.6 g, 6 mmol) was condensed with piperidine (0.5 mL) as described for **6** to furnish **27** (1.86 g, 94%), mp 214°C (analysed for $C_{19}H_{25}NO_4$; found C 68.8644, H 7.6040, N 4.2296; calcd C 68.8601, H 7.6031, N 4.2264). MS (%) M^+ at m/z 331 (37), 277 (70), 247 (14), 219 (23), 189 (12), 112 (11) and 84 (100). vcm^{-1} (KBr) 2930, 1640, 1605, 1560, 1440, 1350, 1250, 1140, 1010, 960 and 870. ^1H NMR (CDCl_3) δ : 1.60 (6H, bs, $-\text{N}-\text{C}-(\text{CH}_2)_3$), 3.62 (4H, m, N $(\text{CH}_2)_2$), 3.89 (3H, s, OCH_3), 3.94 (6H, s, $2 \times \text{OCH}_3$), 6.47 (1H, d, $J=15.0$ Hz, $\text{CH}=\text{CH}-\text{CO}$), 6.66–7.00 (4H, m, olefinic and Ar–H) and 7.23–7.72 (1H, m, $-\text{CH}=\text{CH}-\text{CO}$).

Preparation of 5-(3,4,5-trimethoxy phenyl)-2E,4E-pentadienoic acid pyrrolidine amide (28). Compound **4e** (1.6 g, 6 mmol) was condensed with pyrrolidine (0.6 mL) as described for **8** to yield **28** (1.80 g, 95%), a crystalline solid, mp 190°C (analysed for $C_{18}H_{23}NO_4$; found C 68.1212, H 7.3047, N 4.4150; calcd C 68.1190, H 7.3040, N 4.4132). MS (%) M^+ at m/z 317 (57), 219 (100), 189 (63), 159 (12), 98 (35), 70 (16). vcm^{-1} (KBr) 2960, 1640, 1605, 1560, 1450, 1330, 1250, 1135, 1015, 970 and 860. ^1H NMR (CDCl_3) δ : 1.95 (4H, m, $-\text{N}-\text{C}-(\text{CH}_2)_2$), 3.64 (4H, t, $J=6.5$ Hz, $-\text{N}(\text{CH}_2)_2$), 3.85 (3H, s, OCH_3), 3.92 (6H, s, $2 \times \text{OCH}_3$), 6.26 (1H, d, $J=15.0$ Hz, $\text{CH}=\text{CH}-\text{CO}$), 6.65–6.93 (4H, m, olefinic and Ar–H) and 7.23–7.60 (1H, m, $-\text{CH}=\text{CH}-\text{CO}$).

Preparation of (2H)-2,2-dimethyl-3,4-dihydro-6-formyl benzopyran (1g). To a stirring solution of 4-hydroxy benzaldehyde **1f** (25 g, 205 mmol) and orthophosphoric acid (20 mL) in hexane (200 mL), a slow addition of freshly distilled isoprene (25 mL) in *n*-hexane (40 mL) was made in 9 h at room temperature and the reaction mixture stirred further for 24 h. The reaction mixture was worked up by dilution with water, followed by extraction of the aqueous layer with solvent ether (2×100 mL). The combined organic layer washed with water, dried over anhydrous sodium sulfate and concentrated to give crude resinous product which on CC over SiO_2 and elution with hexane:benzene (4:1) afforded **1g** (11.5 g, 30%), as a gummy mass³⁴ analysed for $C_{12}H_{14}O_2$, MS (%) M^+ at m/z 190 (36), 189 (73), 160 (33), 146 (38), 134 (100) and 106 (13). vcm^{-1} (KBr) 2940, 1682, 1606, 1576, 1488, 1432, 1384, 1362, 1352, 1324, 1270, 1236, 1114, 1108, 874 and 818. ^1H NMR (CDCl_3) δ : 1.35 (6H, bs, $-\text{C}(\text{CH}_3)_2$), 1.85 (2H, t, $J=6.0$ Hz, $-\text{CH}_2-\text{CH}_2-\text{Ar}$), 2.85 (2H, t, $J=6.0$ Hz, $\text{Ar}-\text{CH}_2-\text{CH}_2$), 6.87 (1H, d, $J=8.5$ Hz, Ar–H), 7.64 (2H, bs, Ar–H) and 9.80 (1H, s, Ar–CHO).

Preparation of 1-[(2H)-2,2-dimethyl-3,4-dihydro-benzopyran-6yl]-ethanol (2g). This compound was prepared from **1g** (7.0 g, 37 mmol) and methyl magnesium iodide (39 mmol) reagent by the method as described for **2a** to give **2g** (7.3 g, 94%) a semisolid, analysed for $C_{13}H_{18}O_2$,

MS (%) M^+ at m/z 207 (48), 188 (100), 121 (80) and 96 (61). vcm^{-1} (KBr) 3352, 2932, 1618, 1588, 1494, 1450, 1370, 1348, 1252, 1208, 1150, 1120, 1070, 926 and 878. ^1H NMR (CDCl_3) δ : 1.28 (6H, s, $(\text{CH}_3)_2$), 1.44 (3H, d, $J=6.5$ Hz, CH_3-CHOH), 1.76 (2H, t, $J=7.0$ Hz, $\text{Ar}-\text{CH}_2-\text{CH}_2$), 2.76 (2H, t, $J=7.0$ Hz, $\text{Ar}-\text{CH}_2-\text{CH}_2$), 4.76 (1H, q, $J=6.5$ Hz, $\text{CHOH}-\text{CH}_3$) 6.72 (1H, d, $J=8.5$ Hz, Ar–H) and 7.04 (2H, bs, Ar–H).

Preparation of 3-[(2H)-2,2-dimethyl-3,4-dihydro-benzopyran-6yl]-2-propenal (3g). This compound was prepared from **2g** (6.20 g, 30 mmol) and Vilsmeier reagent by the method as described for **3a** to give **3g** (4.2 g, 65%), a semi solid, analysed for $C_{14}H_{16}O_2$, MS (%) M^+ at m/z 216 (33), 188 (30), 174 (28), 160 (48), 146 (31), 103 (40) and 69 (100). vcm^{-1} (KBr) 2968, 2916, 1664, 1606, 1574, 1492, 1426, 1386, 1365, 1336, 1244, 1106, 944 and 800. ^1H NMR (CDCl_3) δ : 1.24 (6H, s, $\text{C}(\text{CH}_3)_2$), 1.76 (2H, t, $J=7.0$ Hz, $\text{Ar}-\text{CH}_2-\text{CH}_2$), 2.72 (2H, t, $J=7.0$ Hz, $\text{Ar}-\text{CH}_2-\text{CH}_2$), 6.48 (1H, dd, $J=14.0$ and 7.0 Hz, $\text{CH}=\text{CH}-\text{CHO}$), 6.74 (1H, d, $J=8.5$ Hz, Ar–H), 7.20 (2H, bs, $2 \times \text{Ar}-\text{H}$), 7.32 (1H, d, $J=15.0$ Hz, $\text{Ar}-\text{CH}=\text{CH}-\text{CHO}$) and 9.56 (1H, d, $J=7.0$ Hz, $\text{Ar}-\text{CHO}$).

Preparation of 5-[(2H)-2,2-dimethyl-3,4-dihydro-benzopyran-6yl]-2E,4E-pentadienoic acid (4g). This compound was prepared from **3g** (3.90 g, 18 mmol) by Wittig reaction as described for **4a** to give **4g** (4.1 g, 89%), a crystalline solid, mp 217°C , analysed for $C_{16}H_{18}O_3$, MS (%) M^+ at m/z 258 (57), 213 (19), 203 (26), 171 (10), 157 (100), 145 (26), 129 (82), 115 (54), 102 (13) and 91 (33). vcm^{-1} (KBr) 2944, 1644, 1598, 1494, 1408, 1388, 1362, 1214, 1120, 970 and 866. ^1H NMR (CD_3OD) δ : 1.46 (6H, s, $\text{C}(\text{CH}_3)_2$), 1.90 (2H, t, $J=7.0$ Hz, $\text{Ar}-\text{CH}_2-\text{CH}_2$), 2.87 (2H, t, $J=7.0$ Hz, $\text{Ar}-\text{CH}_2-\text{CH}_2$), 5.95 (1H, d, $J=15.0$ Hz, $\text{CH}=\text{CH}-\text{CO}$), 6.53–7.00 (5H, m, olefinic and Ar–H) and 7.43–7.80 (1H, m, $\text{CH}=\text{CH}-\text{CHO}$).

Preparation of 5-(2H)-2,2-dimethyl-3,4-dihydro-benzopyran-6yl-2E,4E-pentadienoic acid piperidine amide (29). Compound **4g** (1.0 g, 4 mmol) was condensed with piperidine (0.6 mL) as described for **8** to yield **29** (1.16 g, 89%), mp 164°C (analysed for $C_{21}H_{27}NO_2$; found C 77.5093, H 8.3624, N 4.3055; calcd C 77.5023, H 8.3617, N 4.3038) MS (%) M^+ at m/z 325 (85), 297 (37), 241 (100), 213 (48), 189 (39), 111 (92) and 84 (92). vcm^{-1} (KBr) 2950, 1650, 1610, 1548, 1447, 1383, 1314, 1010, 972 and 865. ^1H NMR (CDCl_3) δ : 1.34 (6H, bs, $-\text{C}-(\text{CH}_3)_2$), 1.59–2.16 (6H, m, $-\text{N}-(\text{CH}_2)_3$), 1.80 (2H, t, $J=6$ Hz, $\text{Ar}-\text{C}-\text{CH}_2$), 2.72 (2H, t, $J=6.0$ Hz, $\text{Ar}-\text{CH}_2$), 3.52 (4H, m, $-\text{N}-(\text{CH}_2)_2$), 6.16 (1H, d, $J=14.0$ Hz, $-\text{CH}=\text{CH}-\text{CO}$), 6.59–6.82 and 6.92–7.69 (6H, m, olefinic and Ar–H).

Preparation of 5-[(2H)-2,2-dimethyl-3,4-dihydrobenzopyran-6yl]-2E,4E-pentadienoic acid pyrrolidine amide (30). Compound **4g** (1.0 g, 4 mmol) was condensed with pyrrolidine (0.5 mL) as described for the **8** to give **30** (0.98 g, 85%), mp 117°C (analysed for $C_{20}H_{25}NO_2$, found C 77.1400, H 8.0917, N 4.5004, calcd C 77.1363, H 8.0910, N 4.4977) MS (%) M^+ at m/z 311 (73), 241 (100), 184 (50), 158 (40), 127 (31), 97 (31) and 70 (46). vcm^{-1} (KBr) 2972, 1653, 1610, 1540, 1450, 1385, 1364, 1320, 1250, 1040, 972 and 865. ^1H NMR (CDCl_3) δ : 1.32

(6H, s, $-\text{C}-(\text{CH}_3)_2$), 1.62 (4H, m, $2 \times \text{CH}_2$), 1.80 (2H, t, $J=6.5$ Hz, $\text{Ar}-\text{CH}_2-\text{CH}_2$), 2.75 (2H, t, $J=6.5$ Hz, $\text{Ar}-\text{CH}_2-\text{CH}_2$), 3.58 (2H, m, $-\text{N}-(\text{CH}_2)_2$), 6.40 (1H, d, $J=14.0$ Hz, $-\text{CH}=\text{CH}-\text{CO}$), 6.60–6.92 (3H, m, olefinic and $\text{Ar}-\text{H}$) and 7.04–7.68 (3H, m, olefinic and $\text{Ar}-\text{H}$)

Preparation of 5-[(2H)-2,2-dimethyl-3,4-dihydrobenzopyran-6yl]-2E,4E-pentadienoic acid *n*-pentyl amide (31). Compound **4g** (1.0 g, 4 mmol) was condensed with *n*-pentyl amine (0.6 mL) as described for **8** to yield **31**, a solid (1.20 g, 92%), mp 137 °C (analysed for $\text{C}_{21}\text{H}_{29}\text{NO}_2$; found C 77.0317, H 8.9267, N 4.2793; calcd C 77.0253, H 8.9258, N 4.2774). MS (%) M^+ at m/z 327 (22), 241 (23), 185 (22), 175 (13), 157 (77), 128 (49), 96 (28) and 69 (100). vcm^{-1} (KBr) 3270, 2910, 1640, 1600, 1490, 1380, 1260, 1125, 1000, 920 and 805. $^1\text{H NMR}$ (CDCl_3) δ 0.90 (3H, t, $J=6.0$ Hz, $-\text{CH}_2-\text{CH}_3$), 1.16–1.56 [12H, bs, $-\text{NH}-\text{CH}_2-(\text{CH}_2)_3$ and $\text{C}-(\text{CH}_3)_2$], 1.80 (2H, t, $J=6.0$ Hz, $\text{Ar}-\text{CH}_2-\text{CH}_2$), 2.72 (2H, t, $J=6.0$ Hz, $\text{Ar}-\text{CH}_2-\text{CH}_2$), 3.34 (2H, m, $-\text{N}-(\text{CH}_2)_2$), 5.94 (1H, d, $J=15.0$ Hz, $-\text{CH}=\text{CH}-\text{CO}$), 6.52–6.82 and 6.90–7.66 (6H, m, olefinic and $\text{Ar}-\text{H}$).

Preparation of 3-(3,4-methylenedioxy phenyl)-2E-propenoic acid (6). To **1h** (4.5 g, 30 mmol) in pyridine (25 mL) and piperidine (1 mL) was added malonic acid (3.7 g, 36 mmol) and contents stirred for 24 h, followed by heating on water bath for 6 h. The contents were cooled, poured in ice-cold water, acidified with 2 N HCl. The resulting precipitate filtered, washed with water and air dried to give **6** (6.2 g, 95%), crystallised from ethyl acetate:*n*-hexane (9:1), mp. 244–646 °C (lit. mp 247 °C).³⁵

Preparation of 3-(3,4-methylenedioxy phenyl)-2E-propenoic acid piperidine amide (32). Compound **6** (2.1 g, 11 mmol) was condensed with piperidine (1.2 mL) as described for **8** to yield **32** (2.80 g, 91%), mp 89 °C (analysed for $\text{C}_{15}\text{H}_{17}\text{NO}_3$; found C 69.4901, H 6.6089, N 5.4071; calcd C 69.4804, H 6.6078, N 5.4017) MS (%) M^+ at m/z 259 (4), 147 (21), 111 (100) and 84 (29). vcm^{-1} (KBr) 2940, 1642, 1588, 1494, 1436, 1350, 1298, 1250, 1216, 1100, 1018, 972 and 808. $^1\text{H NMR}$ (CDCl_3) δ 1.64 (6H, bs, $\text{N}-\text{C}-(\text{CH}_2)_3$), 3.60 (4H, bs, $-\text{N}-(\text{CH}_2)_2$), 5.94 (2H, s, $-\text{OCH}_2\text{O}-$), 6.70 (1H, d, $J=15.0$ Hz, $\text{CH}=\text{CH}-\text{CO}$), 6.76 (1H, d, $J=8.5$ Hz, $\text{Ar}-\text{H}$), 6.98 (1H, d, $J=8.5$ Hz, $\text{Ar}-\text{H}$), 7.02 (1H, s, $\text{Ar}-\text{H}$) and 7.52 (1H, d, $J=15.0$ Hz, $\text{CH}=\text{CH}-\text{CO}$).

Preparation of 3-(3,4-methylenedioxy phenyl)-2E-propenoic acid pyrrolidine amide (33). Compound **6** (2.0 g, 10 mmol) was condensed with pyrrolidine (1.0 mL) as described for **8** to yield **33** (2.4 g, 90%), mp 146 °C (analysed for $\text{C}_{14}\text{H}_{15}\text{NO}_3$; found C 68.5603, H 6.1644, N 5.7133; calcd C 68.5569, H 6.1638, N 5.7106). MS (%) M^+ at m/z 245 (15) 174 (32) 147 (9) 98 (30) and 70 (100). vcm^{-1} (KBr) 2960, 1646, 1594, 1496, 1454, 1414, 1354, 1320, 1278, 1242, 1198, 1018, 992, 928 and 826. $^1\text{H NMR}$ (CDCl_3) δ 1.92 (4H, bs, $\text{N}-\text{C}-(\text{CH}_2)_2$), 3.56 (4H, m, $-\text{N}-(\text{CH}_2)_2$), 5.96 (2H, s, $-\text{OCH}_2\text{O}-$), 6.50 (1H, d, $J=15.0$ Hz, $\text{CH}=\text{CH}-\text{CO}$), 6.78 (1H, d, $J=8.5$ Hz, $\text{Ar}-\text{H}$), 6.97 (1H, d, $J=8.5$ Hz, $\text{Ar}-\text{H}$), 7.00 (1H, s, $\text{Ar}-\text{H}$) and 7.56 (1H, d, $J=15.0$ Hz, $\text{CH}=\text{CH}-\text{CO}$).

Preparation of 3-[(2H)-2,2-dimethyl-3,4-dihydrobenzopyran-6yl]-2E-propenoic acid (7). Compound **7** was prepared from **1g** (6.0 g, 32 mmol) and malonic acid (4.2 g, 40 mmol) as described for **6** to give crystalline compound **7**³⁶ (6.7 g, 90%), mp 188 °C analysed for $\text{C}_{14}\text{H}_{16}\text{O}_3$, MS (%) M^+ at m/z 232 (93), 215 (13), 188 (6), 176 (100) and 131 (20). vcm^{-1} (KBr) 2964, 1674, 1609, 1574, 1494, 1430, 1386, 1370, 1304, 1286, 1260, 1234, 1150, 932 and 820. $^1\text{H NMR}$ (CDCl_3) δ 1.32 (6Hs, $-\text{C}(\text{CH}_3)_2$), 1.82 (2H, t, $J=7.0$ Hz, $\text{Ar}-\text{CH}_2-\text{CH}_2$), 2.74 (2H, t, $J=7.0$ Hz, $\text{Ar}-\text{CH}_2-\text{CH}_2$), 6.72 (1H, d, $J=15.0$ Hz, $\text{CH}=\text{CH}-\text{CO}$), 6.80 (1H, d, $J=8.5$ Hz, $\text{Ar}-\text{H}$), 7.24 (1H, s, $\text{Ar}-\text{H}$), 7.28 (1H, d, $J=8.5$ Hz, $\text{Ar}-\text{H}$) and 7.68 (1H, d, $J=15.0$ Hz, $-\text{CH}=\text{CH}-\text{CO}$).

Preparation of 3-[(2H)-2,2-dimethyl-3,4-dihydrobenzopyran-6yl]-2E-propenoic acid piperidine amide (34). Compound **7** (0.93 g, 4 mmol) was condensed with piperidine (0.6 mL) as described for **8** to give **34** (1.10 g, 92%), mp 122 °C (analysed for $\text{C}_{19}\text{H}_{25}\text{NO}_2$; found C 76.2221, H 8.4160, N 4.6802; calcd C 76.2191, H 8.4156, N 4.6781) MS (%) M^+ at m/z 299 (100), 214 (93), 187 (31), 158 (60) 84 (61) and 69 (30). vcm^{-1} (KBr) 2932, 1640, 1598, 1578, 1494, 1434, 1382, 1368, 1270, 1244, 1232, 1214, 1110, 1010, 982 and 830. $^1\text{H NMR}$ (CDCl_3) δ : 1.23 (6H, s, $-\text{C}(\text{CH}_3)_2$), 1.56 (6H, bs, $\text{N}-\text{C}-(\text{CH}_2)_3$), 1.72 (2H, t, $J=6.5$ Hz, $\text{Ar}-\text{CH}_2-\text{CH}_2$), 2.72 (2H, t, $J=6.5$ Hz, $\text{Ar}-\text{CH}_2-\text{CH}_2$), 3.50 (4H, m, $\text{NH}-(\text{CH}_2)_2$), 6.66 (1H, d, $J=15.0$ Hz, $\text{CH}=\text{CH}-\text{CO}$), 6.62 (1H, d, $J=8.5$ Hz, $\text{Ar}-\text{H}$), 7.06–7.24 (2H, m, $2 \times \text{Ar}-\text{H}$) and 7.43 (1H, d, $J=15.0$ Hz, $-\text{CH}=\text{CH}-\text{CO}$).

Preparation of 3-[(2H)-2,2-dimethyl-3,4-dihydrobenzopyran-6yl]-2E-propenoic acid isopropyl amide (35). Compound **7** (1.2 g, 5 mmol) was condensed with isopropyl amine (0.7 mL) by the method as described for **8** to give **35** (1.24 g, 91%), mp 89 °C (analysed for $\text{C}_{17}\text{H}_{23}\text{NO}_2$; found C 74.6898, H 8.4823, N 5.1255; calcd C 74.6900, H 8.4816, N 5.1236); MS (%) M^+ at m/z 273 (100), 215 (61), 188 (58), 159 (51) and 58 (48). vcm^{-1} (KBr) 3260, 2968, 1654, 1612, 1580, 1496, 1420, 1384, 1370, 1304, 1236, 1200, 1152, 1112, 1000, 920 and 874. $^1\text{H NMR}$ (CDCl_3) δ 1.20 (6H, d, $J=6.5$ Hz, $-\text{C}(\text{CH}_3)_2$), 1.33 (6H, s, $(\text{CH}_3)_2$), 1.76 (2H, t, $J=6.50$ Hz, $\text{Ar}-\text{CH}_2-\text{CH}_2$), 2.70 (2H, t, $J=6.5$ Hz, $\text{Ar}-\text{CH}_2$), 4.13 (1H, m, $\text{CH}-\text{N}$), 6.13 (1H, d, $J=15.0$ Hz, $\text{CH}=\text{CH}-\text{CO}$), 6.63 (1H, d, $J=8.5$ Hz, $\text{Ar}-\text{H}$), 6.95–7.23 (2H, m, $\text{Ar}-\text{H}$) and 7.43 (1H, d, $J=15.0$ Hz, $-\text{CH}=\text{CH}-\text{CO}$).

Preparation of 3-[(2H)-2,2-dimethyl-3,4-dihydrobenzopyran-6yl]-2E-propenoic acid *n*-butylamide (36). Compound **7** (1.2 g, 5 mmol) was condensed with *n*-butylamine (0.6 mL) by the method as described for **8** to give **36** (1.29 g, 90%), mp 86 °C (analysed for $\text{C}_{18}\text{H}_{25}\text{NO}_2$; found C 75.2301, H 8.7677, N 4.8771; calcd C 75.2253, H 8.7673, N 4.8736). MS (%) M^+ at m/z 287 (100), 242 (79), 214 (79), 187 (21), 170 (12), 158 (60) 130 (30) and 72 (14). vcm^{-1} (KBr) 3200, 2932, 1648, 1616, 1580, 1544, 1450, 1424, 1384, 1370, 1308, 1264, 1236, 1152, 968 and 852. $^1\text{H NMR}$ (CDCl_3) δ 0.92 (3H, t, $J=6.5$ Hz, $-\text{CH}_2-\text{CH}_3$), 1.33 (6H, s, $-\text{C}(\text{CH}_3)_2$), 1.49 (4H, m, $-\text{C}-(\text{CH}_2)_2$), 1.79 (2H, t, $J=6.5$ Hz, $\text{Ar}-\text{CH}_2-\text{CH}_2$), 2.74 (2H, t, $J=6.5$ Hz, $\text{Ar}-\text{CH}_2-\text{CH}_2$), 3.34 (2H, m, $\text{NH}-\text{CH}_2$), 6.38 (1H, d,

$J = 15.0$ Hz, $\text{CH}=\text{CH}-\text{CO}$), 6.66 (1H, d, $J = 8.5$ Hz, Ar-H), 7.09–7.42 (2H, m, Ar-H) and 7.67 (1H, d, $J = 15.0$ Hz, $-\text{CH}=\text{CH}-\text{CO}$).

Preparation of 3-[(2H)-2,2-dimethyl-3,4-dihydrobenzopyran-6yl]-2E-propenoic acid *n*-hexylamide (37). Compound 7 (0.93 g, 4 mmol) was condensed with hexylamine (0.5 mL) as described for 8 to give 37 (1.15 g, 91%), mp 71 °C (analysed for $\text{C}_{20}\text{H}_{29}\text{NO}_2$; found C 76.1600, H 9.2666, N 4.4443; calcd C 76.1505, H 9.2657, N 4.4402). MS (%) M^+ at m/z 315 (82), 258 (16), 215 (92), 187 (22), 173 (100) and 99 (19). $\nu_{\text{cm}^{-1}}$ (KBr) 3190, 2900, 1654, 1608, 1573, 1540, 1494, 1283, 1381, 1367, 1306, 1238, 1214, 1154, 1120, 928 and 818. ^1H NMR (CDCl_3) δ : 0.89 (3H, d, $J = 6.5$ Hz, $-\text{CH}_3$), 1.30 (6H, s, $-\text{C}(\text{CH}_3)_2$), 1.33 (8H, m, $-\text{C}(\text{CH}_2)_4-\text{CH}_3$), 1.76 (2H, t, $J = 6.0$ Hz, Ar- CH_2-CH_2), 2.73 (2H, t, $J = 6.0$ Hz, Ar- CH_2), 3.33 (2H, m, NH- CH_2) 6.23 (1H, d, $J = 15.0$ Hz, $\text{CH}=\text{CH}-\text{CO}$), 6.70 (1H, d, $J = 8.5$ Hz, Ar-H), 7.10–7.33 (2H, m, Ar-H), and 7.54 (1H, d, $J = 15.0$ Hz, $-\text{CH}=\text{CH}-\text{CO}$).

Biology

Chemicals. Chemicals and cell culture medium were purchased from the following sources: NADPH from Sigma Chemie, München, FRg; 7-methoxycoumarin (MOC) from Aldrich Chemie, Steinheim, FRg and purified as described earlier.²¹ The sources of piperine and other chemicals are described elsewhere.^{2,6} All other chemicals used were of analytical grade and available locally.

Animals and treatment. Adult male albino Charles Foster rats (200 ± 20 g, body wt) and male Swiss albino mice (25 ± 2 g, body wt) used were bred in the animal house of this institute. The animals were maintained on standard commercial pellet food (Hindustan Lever Ltd., Bombay) and water ad libitum. Rats were treated with 3MC and PB for inducibility of CYP activities as described earlier.²

Preparation of microsomes. Liver whole homogenate (25%, w/v) in 0.25 M sucrose was prepared from overnight fasted rats and centrifuged at 10,000 g for 15 min. The post-mitochondrial supernatant was recentrifuged and pellet discarded each time. Microsomal fraction was prepared from the supernatant by Ca^{++} -precipitation.³⁷ The pellet was resuspended in 0.25 M sucrose/1 mM phosphate buffer, pH 7.6 so as to obtain protein 30–40 mg/mL. The preparation was stored in small portions at -70 °C.

Assay of hepatic microsomal 7-methoxycoumarin *O*-demethylase (MOCD) and arylhydrocarbon hydroxylase (AHH) activities. MOCD activity was determined according to Reen et al.²¹ Briefly, the assay system in a total volume of 1 mL contained 40 mM Tris-HCl buffer pH 7.6, 5 mM MgCl_2 , 0.25 mg NADPH, 0.1–0.2 mg microsomal protein and piperine or test compound in 10 μL of 50% methanol. The reaction was started with 0.4 mM 7-methoxycoumarin in 10 μL of 50% methanol. The assay system was incubated for 10 min at 37 °C in a constant shaking water bath. The reaction was

terminated with 75 μL of 15% cold TCA (w/v). The product 7-OH-coumarin was extracted and measured fluorometrically at 396 nm excitation and 520 nm emission.

The activity of arylhydrocarbonhydroxylase (AHH) was measured according to Wiebel et al.³⁸ Briefly, the reaction mixture in a total volume of 1 mL contained 50 mM Tris-HCl buffer, pH 7.6, 3 mM MgCl_2 , 0.6 mM NADPH and 0.5 to 2.5 mg microsomal protein. Piperine or test compound was added in 10 μL of 50% methanol. The reaction was started under subdued light with 0.1 mM benzo(a) pyrene in 20 μL of 50% methanol and incubated at 37 °C for 30 min in a shaking water bath. The reaction was terminated and the relative fluorescence of the aqueous phase was measured at 396 nm excitation and 520 nm emission using 3-OH-benzo(a)pyrene as reference standard.

Sleeping time. The hexobarbital induced sleeping time was determined as the time required for the mice to regain their rightening reflex.² The control animals received only the vehicle.

Cell culture and treatment. H4IIEC3/G– cells used in the present study are the descendants of rat Reuber hepatoma. Their source, growth characteristics and incubation conditions are described earlier.^{4,39} Cells were seeded at a density of 1×10^6 cells per 90 mm plastic dishes in DMEM containing fetal calf serum and antibiotics and allowed to grow for 48 h. Cultures were exposed for 24 h to fresh medium containing test compound delivered in 15 μL DMSO. Control plates received only the vehicle. Cells were washed with phosphate buffer saline (PBS) and harvested in 1 mL PBS and centrifuged. The pellet was stored in liquid nitrogen and used for assay of monooxygenase activities after suspension in 50 mM Tris-HCl, pH 7.4.

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