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Hypolipidaemic and antiplatelet activity of phenoxyacetic acid derivatives related to α-asarone

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Abstract

The phenoxyacetic acid derivatives **1–6** [2-methoxy-4-(2-propenyl)phenoxyacetic acid (**1**); 2-methoxy-5-nitro-4-(2-propenyl)phenoxyacetic acid (**2**); methyl 2-methoxy-4-(2-propenyl)phenoxyacetate (**3**); ethyl 2-methoxy-4-(2-propenyl)phenoxyacetate (**4**); methyl 2-methoxy-5-nitro-4-(2-propenyl)phenoxyacetate (**5**); ethyl 2-methoxy-5-nitro-4-(2-propenyl)phenoxyacetate (**6**)] related to α-asarone have been reported previously as hypolipidaemic agents in diet-induced hyperlipidaemic mice. We have aimed to expand the pharmacological profile of these derivatives by investigating their hypolipidaemic activity in rats and mice under different experimental conditions. The antiplatelet activity was tested also in-vitro from blood derived from consenting healthy volunteers. In normolipidaemic rats, compounds **2**, **3** and **5** at oral doses of 40 and 80 mg kg^{-1} significantly decreased total cholesterol and LDL-cholesterol levels. Moreover, analogues **3** and **5** administered to hypercholesterolaemic rats at the same doses for seven days also produced a reduction in the content of these same lipoproteins. In neither case were the high-density lipoprotein cholesterol and triglyceride concentrations affected. However, practically all tested compounds were found to be hypocholesterolaemic agents, and were shown to effectively lower low-density lipoprotein cholesterol and triglyceride levels in Triton-induced hyperlipidaemic mice at oral doses of 50 and 100 mg kg^{-1}. In all tests, all animals appeared to be healthy throughout the experimental period in their therapeutic ranges. Triton-induced hypercholesterolaemic mice appeared to be a desirable model for this class of hypolipidaemic drugs. On the other hand, compounds **1**, **2**, **4** and **5** significantly inhibited ADP-induced aggregation in-vitro. These findings indicated that all of these compounds appeared to be promising for the treatment of human hyperlipidaemia and thrombotic diseases.

Introduction

Pharmacological intervention to decrease plasma levels of cholesterol has been proven to reduce disease frequency and mortality related to cardiac events. However, drug therapy for dyslipidaemia is a life-long treatment and 75% of patients receiving hypolipidaemic drugs for over two years discontinued the medication due to clinically significant adverse reactions (Safeer & Lacivita 2000). Thus for innovative hypolipidaemic agents to be successful they should possess lower adverse effects.

α-Asarone, (*E*)-1,2,4-trimethoxy-5-(1-propenyl) benzene, is the active compound found in *Guatteria gaumeri* (Annonaceae) bark extract (Enríquez et al 1980). It significantly decreased cholesterol and triglyceride serum levels when given orally to mice, rats and hamsters (Gómez et al 1987; Chamorro et al 1993; Garduño et al 1997). Other pharmacological effects attributed to this molecule, such as anticoagulant (Rubio-Poo et al 1991), anti-thrombotic and antiplatelet (Poplawski et al 2000) effects have been presented. Unfortunately, several toxicity findings such as in-vitro hepatotoxicity (López et al 1993), genotoxicity (Chamorro et al 1998) and teratogenicity (Salazar et al 1992) have been described for α-asarone.

To establish a structure–activity relationship, a new series of α-asarone and clofibrate related phenoxyacetic acid derivatives (compounds **1–6** in Figure 1) were prepared and evaluated in mice (Labarrios et al 1999). The results showed that by modifying the methoxy group into an acetic moiety, including its methyl and ethyl esters and corresponding C-5 nitrated derivatives, some of these compounds were more active than clofibrate in hyperlipidaemic
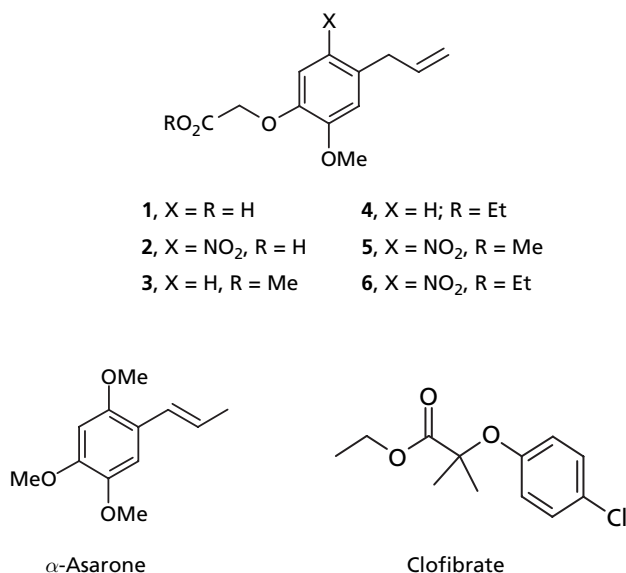
Figure 1   Chemical structure of test compounds **1–6**, α-asarone and clofibrate.

mice. Other alcohol derivatives prepared for that same series showed virtually no hypolipidaemic activity.

As with other kinds of drugs, cholesterol lowering drugs have been shown to be effective in some models or animal species while ineffective for others. An example would be statins inhibiting cholesterol synthesis in mice while no hypocholesterolaemic response was seen in rats (Huff & Burnett 1997). On the other hand, fibrates decreased cholesterol levels in rats but the hypercholesterolaemic response did not occur in rabbits (Krause & Princen 1998).

Accordingly and aiming to expand the pharmacological profile of these innovative derivatives (**1–6**), we have described their hypolipidaemic activity in different models, and their antiplatelet activity.

Materials and Methods

Source of compounds

The test compounds were prepared according to procedures described by Labarrios et al (1999). They were completely characterized by TLC, MP, IR, ^{1}H and ^{13}C NMR, MS and elemental analyses. Triton WR-1339 (Tyloxapol) and clofibrate were obtained from Sigma (St Louis, MO). All other chemicals used were standard commercial high purity materials.

Solutions and doses

For hypolipidaemic studies, compounds **1–6** were suspended in a 1:9 Tween 80:water solution, and given by gavage at doses of 0, 40 or 80 mg kg^{-1} for rats and 0, 50 or 100 mg kg^{-1} for mice, once a day, at the same hour each day throughout the experiments. Doses were selected based on previous studies where their effectiveness and that of other

α-asarone analogues were proven in rats (Cruz et al 2001) and in mice (Labarrios et al 1999). The same solution used to suspend compounds **1–6** was used for clofibrate. The dose given was 100 mg kg^{-1} and was taken from studies confirming its effectiveness (Seri et al 1980). The concentration of solutions was adjusted so that rats and mice could be given 5 mL kg^{-1}. Animals in the control group received a similar volume of vehicle. Solutions were freshly prepared before administration.

For the antiplatelet studies, the solution preparation is described below.

Trial subjects

Hypolipidaemic activity was studied in Wistar Albino male rats (200–250 g; Centro de Investigación y de Estudios Avanzados), and CD1 male mice (25–30 g; Birmex, S.A. de C.V, Mexico City). All animals were housed in hanging metal cages and maintained at 24 ± 2°C and a 50 ± 10% r.h. with 12-h light/dark cycle (lights on 08:00 h, lights off 20:00 h). They were fed on standard pellet diets (Rodent Diet 5001, PMI Nutrition International, Inc., Brentwood, MO) and drinking water was freely available, unless experimental conditions required diet changes.

All animals were treated in accordance with ethical principles and regulations specified by the Animal Care and Use Committee of our Institution and the Standards of the National Institutes of Health of Mexico.

The animals were randomly divided into groups of six animals. Animal distribution into groups and treatments was randomized.

Healthy male donors (25–50 years), who had taken no medication for at least two weeks before the experiment, were tested for antiplatelet activity.

Normolipidaemic rats

Compounds were administered by gavage every day for 28 days to normal animals fed on standard pellet diets.

Hypercholesterolaemic rats

To induce hypercholesterolaemia, a 50% (w/w) corn oil emulsion was prepared with 1% cholesterol and 1% cholic acid added. Rats were orally administered this preparation for seven days, 1 h before the drugs were given.

Triton WR 1339-induced hypercholesterolaemic mice

Hyperlipidaemia was induced in mice by a single 400 mg kg^{-1} intraperitoneal injection of Triton WR 1339 (Tyloxapol). Mice were treated with the drug 1 h before, and 22 and 48 h after the Tyloxapol injection.

Lipid measurement

At the end of each treatment, rats and mice were fasted for 12 h before blood samples were collected. For Tyloxapol-treated mice blood samples were taken 24 h after the Tyloxapol

injection. For all animals, blood samples were collected by periorbital plexus bleeding under ether anaesthesia and centrifuged at 3000 g for 10 min. Total cholesterol, high-density lipoprotein cholesterol (HDL-C) and triglyceride levels were determined using a Wiener lab, Selectra II automatic analyser. Low-density lipoprotein cholesterol (LDL-C) was calculated using the Friedewald equation (Friedewald et al 1972).

In-vitro platelet aggregation

For each assay blood was collected by venipuncture from four healthy volunteers using sodium citrate (final concn 0.38%) as anticoagulant. The study protocol complied with the Bioethical Committee of the Centro Médico Nacional Siglo XXI – IMSS, Mexico City. Platelet-rich plasma (PRP) was obtained by centrifuging blood samples for 20 min at 150 g, and a portion of PRP sera was used for further centrifugation at 2000 g for 10 min. The supernatant was collected and labelled as platelet-poor plasma (PPP). Platelet concentration was measured and adjusted for a final concentration of $2.0 \times 10^5 \, \mu L^{-1}$ with PPP.

Aggregation was performed on a platelet aggregometer (Chronolog model 500, Chrono-Log, Averton, PA) following Born's method (Born 1962). The test compounds were dissolved in 95% ethanol, and 1 μL of this solution (upon final dilution PRP was reported as not influencing platelet response) was fixed to final concentrations with 0.45 mL PRP and placed into the aggregometer at 37°C under a consistent stirring rate (1000 rev min^{-1}) for 2 min for pre-incubation, followed by the addition of 5 μL agonist. The agonist was 10 mM ADP (Chronolog, Havertown, PA). Aggregation percentages were recorded for 6 min, and the results were expressed as a percentage of the mean agonist response with control vehicle only. α-Asarone and acetylsalicylic acid (aspirin) were used as reference drugs.

Statistics

Hypolipidaemic and platelet aggregation inhibition data were evaluated with analysis of variance. When significant differences were seen, Duncans´s test was used to determine group differences. In all cases the significance level was $P < 0.05$.

Results

Hypolipidaemic activity

All the rats and mice appeared to be healthy throughout the experiment. No deleterious effects were seen in any treated animal.

Table 1 summarizes the hypolipidaemic activity data of compounds **1**–**6** on normolipidaemic rats. Compounds **2**, **3** and **5** significantly reduced total cholesterol and LDL-C, while only agent **2** exhibited these effects at the 40 mg kg^{-1} dose. No dose–effect relationship was found for compound **2**. Compounds **3** and **5** showed the most significant hypolipidaemic effect. In addition to reducing total cholesterol, and decreasing LDL-C by 98.3 and 89.3%, compound **3** significantly increased HDL-C by 50.3%. On the other hand, compound **5** caused the highest LDL-C reduction values (132.4 and 129.8%) at 40 and 80 mg kg^{-1} doses, respectively, and caused the highest HDL-C increase of all compounds tested (88.7%). Phenoxyacetic derivative **6**, at the 40 and 80 mg kg^{-1} doses, significantly reduced serum LDL-C levels by 98.3 and 98.7%, respectively, without modifying total cholesterol or HDL-C levels. No compound was seen to reduce triglyceride except compound **3** at the 80 mg kg^{-1} dose. Clofibrate, the positive reference standard, significantly decreased total cholesterol and LDL-C levels.

The effects of the phenoxyacetic derivatives given to rats treated with the cholesterol rich emulsion for seven days are shown in Table 2. Analogues **3**, **5** and **6** significantly reduced

Table 1 Hypolipidaemic effects of α-asarone derivatives **1**–**6** in normolipidaemic rats[a]

Compound	Dose (mg kg^{-1})	Total cholesterol	Low-density lipoprotein cholesterol	High-density lipoprotein cholesterol	Triglycerides
No treatment	–	100.00 ± 0.63[b]	100.00 ± 0.68[c]	100.00 ± 0.77[d]	100.00 ± 0.04[e]
1	40	1.91 ± 0.58	3.57 ± 0.50	$+5.06 \pm 0.36$	$+18.18 \pm 0.07$
	80	$+1.36 \pm 0.67$	$+1.50 \pm 0.69$	$+1.03 \pm 0.22$	$+34.55 \pm 0.05$*
2	40	24.27 ± 1.82*	60.91 ± 2.00*	$+5.05 \pm 0.31$	$+1.82 \pm 0.12$
	80	5.45 ± 1.12	16.12 ± 1.03	$+0.40 \pm 0.32$	21.82 ± 0.03
3	40	$+2.52 \pm 1.29$	98.29 ± 0.01*	$+2.85 \pm 0.37$	$+10.91 \pm 0.04$
	80	26.11 ± 0.79*	89.30 ± 0.01*	$+50.32 \pm 0.80$*	32.73 ± 0.07*
4	40	$+1.36 \pm 0.51$	0.14 ± 0.51	2.59 ± 0.37	$+25.45 \pm 0.07$
	80	5.52 ± 1.06	5.14 ± 0.95	10.48 ± 0.34	$+5.45 \pm 0.06$
5	40	32.04 ± 0.36*	132.38 ± 0.28*	$+88.75 \pm 0.35$*	1.82 ± 0.03
	80	36.06 ± 0.54*	129.81 ± 0.48*	$+45.15 \pm 0.53$*	20.00 ± 0.05
6	40	0.20 ± 0.66	98.39 ± 0.02*	$+3.10 \pm 0.31$	$+10.91 \pm 0.10$
	80	$+3.07 \pm 0.70$	98.72 ± 0.01*	$+7.50 \pm 0.44$	$+16.36 \pm 0.04$
Clofibrate	100	31.90 ± 0.77*	80.02 ± 0.25*	$+11.94 \pm 0.65$	14.54 ± 0.04

[a]Expressed as percent change from the non-treated group (mean ± s.e.). A positive sign (+) means increase. n = 6. Drugs were administered orally for 28 days. Blood was obtained by periorbital plexus bleeding. Serum proteins were analysed as described in **Materials and Methods**. Absolute values: [b]14.67 ± 0.63 mmol L^{-1}; [c]7.01 ± 0.68 mmol L^{-1}; [d]7.73 ± 0.28 mmol L^{-1}; [e]0.55 ± 0.09 mmol L^{-1}. Data were analysed by analysis of variance and then by Duncans's test. *$P < 0.05$, significantly different vs no-treatment group.

Table 2 Hypolipidaemic effects of α-asarone derivatives **1–6** in hypercholesterolaemic rats induced with high cholesterol emulsion[a]

Compound	Dose (mg kg^{-1})	Total cholesterol	Low-density lipoprotein cholesterol	High-density lipoprotein cholesterol	Triglycerides
Normal diet	–	30.36 ± 0.35*	1.21 ± 1.26	7.43 ± 0.37	11.24 ± 0.07
Cholesterol emulsion	–	100.00 ± 0.81[b]	100.00 ± 0.19[c]	100.00 ± 0.19[d]	100.00 ± 0.09[e]
1 + Cholesterol emulsion	40	+4.61 ± 1.01*	+1.21 ± 1.27	7.43 ± 0.37	+9.84 ± 0.07
	80	+31.97 ± 2.35	30.96 ± 2.40*	+10.38 ± 0.58	+26.76 ± 0.14
2 + Cholesterol emulsion	40	3.56 ± 1.20	14.30 ± 1.37	1.68 ± 0.27	5.63 ± 0.08
	80	6.75 ± 0.90	13.90 ± 0.93	9.68 ± 0.28	+4.23 ± 0.08
3 + Cholesterol emulsion e	40	28.08 ± 0.67*	45.40 ± 0.63*	6.03 ± 0.42	1.41 ± 0.07
	80	19.38 ± 1.39*	35.39 ± 1.41*	0.70 ± 0.39	15.49 ± 0.05
4 + Cholesterol emulsion	40	+6.94 ± 1.81	+1.07 ± 1.59	0.42 ± 0.54	+15.49 ± 0.11
	80	+17.86 ± 2.43*	+3.89 ± 3.15	0.28 ± 0.33	+19.72 ± 0.11
5 + Cholesterol emulsion	40	32.9 ± 0.35*	49.63 ± 1.93*	12.20 ± 0.34	+11.67 ± 0.13
	80	4.86 ± 1.92	16.86 ± 2.88*	+1.82 ± 0.49	+22.53 ± 0.09
6 + Cholesterol emulsion	40	+28.12 ± 0.88	47.28 ± 0.77*	1.96 ± 0.23	9.86 ± 0.09
	80	24.32 ± 1.02	41.84 ± 1.17*	0.14 ± 0.35	53.52 ± 0.06
Clofibrate	100	33.77 ± 0.77*	46.65 ± 0.25*	22.24 ± 0.91	37.48 ± 0.03*

[a]Expressed as percent change from the cholesterol emulsion group (mean ± s.e.). A positive sign (+) means increase. n = 6. Drugs were administered orally for seven days. Blood was obtained by periorbital plexus bleeding. Serum proteins were analysed as described in **Materials and Methods**. Absolute values: [b]21.05 mmol L^{-1}; [c]14.89 mmol L^{-1}; [d]7.13 mmol L^{-1}; [e]14.89 mmol L^{-1}. Data were analysed by analysis of variance and then by Duncans's test. *$P < 0.05$, significantly different vs no-treatment group.

Table 3 Hypolipidaemic effects of α-asarone derivatives **1–6** in mice on Tyloxapol[a]

Compound	Dose (mg kg^{-1})	Total cholesterol	Low-density lipoprotein cholesterol	High-density lipoprotein cholesterol	Triglycerides
Normal diet	–	75.32 ± 2.34**	73.20 ± 0.54**	74.76 ± 2.44**	87.91 ± 0.30**
Tyloxapol	400	100.00 ± 10.22[b]	100.00 ± 4.66[c]	100.00 ± 10.33[d]	100.00 ± 3.21[e]
1 + Tyloxapol	50	50.33 ± 31.24**	39.18 ± 4.13**	59.14 ± 6.17*	41.09 ± 12.86*
	100	43.81 ± 16.93*	36.56 ± 6.47	47.08 ± 9.71*	58.87 ± 2.93**
2 + Tyloxapol	50	32.77 ± 15.66*	57.24 ± 18.56	19.00 ± 14.22	29.10 ± 14.22*
	100	39.93 ± 12.81*	50.47 ± 17.20	31.51 ± 8.76	43.24 ± 3.30*
3 + Tyloxapol	50	30.78 ± 29.21*	46.61 ± 4.02**	22.94 ± 18.94	28.80 ± 1.43*
	100	54.10 ± 8.86**	43.73 ± 5.58**	42.57 ± 3.94**	59.53 ± 2.44**
4 + Tyloxapol	50	20.66 ± 11.78	34.26 ± 10.90	31.01 ± 9.88	17.70 ± 26.27
	100	35.92 ± 19.22*	48.07 ± 6.33**	29.10 ± 16.86	38.51 ± 3.61*
5 + Tyloxapol	50	49.64 ± 12.68**	50.74 ± 3.38**	48.56 ± 8.65*	58.13 ± 2.84**
	100	10.38 ± 11.59	100.96 ± 9.42**	+43.43 ± 19.22	1.70 ± 1.38
6 + Tyloxapol	50	35.20 ± 15.61*	80.12 ± 3.34**	7.44 ± 15.70	44.52 ± 2.41**
	100	25.17 ± 9.54*	111.61 ± 7.77**	+18.50 ± 15.84	25.60 ± 0.32**
Clofibrate	100	38.45 ± 4.51*	28.21 ± 7.43	35.2 ± 10.92	45.2 ± 2.57*

[a]Expressed as percent change from the Tyloxapol group (mean ± s.e.). A positive sign (+) means increase. n = 6. Hyperlipidaemia was induced in mice by a single 400 mg kg^{-1} intraperitoneal injection of Triton WR 1339 (Tyloxapol). Mice were treated with the drug 1 h before, and 22 and 48 h after the Tyloxapol injection. Blood was obtained by periorbital plexus bleeding. Serum proteins were analysed as described in **Materials and Methods**. Absolute values: 131.93 mmol L^{-1}; [c]46.65 mmol L^{-1}; [d]75.92 mmol L^{-1}; [e]23.67 mmol L^{-1}. Data were analysed by using the analysis of variance and then by Duncans's test. *$P < 0.05$, significantly different vs no-treatment group; **$P < 0.01$, significantly different vs no-treatment group.

serum total cholesterol and LDL-C, respectively. The highest total cholesterol reduction was achieved with analogue **5** (32.9%). In this model only compound **6** showed a triglyceride lowering effect; HDL-C was not affected. Similar results were obtained for commercial clofibrate.

Table 3 shows the results for the effects of the phenoxyacetic derivatives in mice with Tyloxapol-induced hyperlipidaemia. All compounds were seen to significantly reduce total cholesterol with rates as low as 54.1%. For LDL-C most compounds, with exception of compound **2**, resulted in a significant decrease with any given dose with values as low as 111.6% with compound **6**, for example.

HDL-C rates also decreased, albeit the decreases were significant only with compounds **1**, **3**, and **5**.

For triglycerides, compounds **1**, **3** and **5** were the most effective. They showed decreases of 58.9, 59.5 and 58.1%,

Table 4 Percent of inhibition of human platelet aggregation induced by ADP after incubation with compounds 1–6 and other agents in-vitro

Group	Concn (μM)	% Inhibition[a]
Control	–	0
α-Asarone	200	13.64 ± 1.07*
	400	16.70 ± 1.07*
	600	17.03 ± 2.16*
Acetylsalicylic acid	200	4.31 ± 1.25
	400	21.92 ± 1.65*
	600	25.90 ± 0.51*
1	200	7.50 ± 3.73
	400	5.87 ± 1.94
	600	11.77 ± 2.04*
2	200	15.71 ± 2.16*
	400	14.70 ± 2.13*
	600	25.08 ± 2.32*
3	200	6.94 ± 1.97
	400	4.91 ± 2.34
	600	0.6 ± 2.15
4	200	33.80 ± 2.27
	400	39.40 ± 2.96*
	600	37.20 ± 2.78*
5	200	38.20 ± 1.07*
	400	38.80 ± 2.87*
	600	45.00 ± 0.84*
6	200	32.60 ± 0.81
	400	31.40 ± 3.74
	600	35.80 ± 4.25

[a]Expressed as percent change from the α-asarone control group (mean $\pm$ s.e.). Platelet aggregation was performed as described in **Material and Methods**. The test compounds were dissolved in 95% ethanol and 1 μL of each solution was fixed and placed into the aggregometer followed by the addition of 5 μL agonist. Aggregation percentages were recorded for 6 min. Data were analysed by analysis of variance and then by Duncans's test. *$P < 0.05$, significantly different from the results for control group. Absolute value: 69.94 ± 1.16.

respectively. Other compounds showed significant differences when Tyloxapol was injected. Clofibrate reduced total cholesterol and triglyceride levels. However, LDL-C was not affected.

Platelet aggregation activity

α-Asarone, at 400 and 600 μM, inhibited ADP-induced aggregation of platelets by 13.6 and 16.7%, respectively. Compounds 1, 2, 4 and 5 also caused a significant inhibition (Table 4). Compounds 2 and 5 caused this effect with all doses, while compound 6 was ineffective.

Discussion

Phenoxyacetic acid derivatives of α-asarone (1–6) have been shown to be efficient hypolipidaemic agents in normolipidaemic rats. This investigation suggested that compounds 2, 3 and 5 maintained their activity and most of them decreased LDL-C. In contrast, compounds 1 and 6 lost their effect in this model.

Hypolipidaemic activity for some of these compounds was shown in exogenously-induced hyperlipidaemic animals. Compound 5 was the most active in decreasing total cholesterol concentration. This was consistent with studies conducted by Labarrios et al (1999) in mice, showing a good correlation between rats and mice. However, it did not apply to all hypocholesterolaemic agents.

Tyloxapol-treated mice clearly showed the hypolipidaemic activity of these analogues; these findings were the closest to those seen in mice with a diet given for six days (Labarrios et al 1999). All tested compounds were found to be hypocholesterolaemic agents in this experimental model at oral doses of 50 and 100 mg kg^{-1}, and were shown to effectively lower triglyceride levels. For example, compound 3 showed a strong reduction of triglycerides by up to 59.5%.

In all three tests conducted in this study, the hypolipidaemic activity achieved with clofibrate was nearly comparable with the results derived with compounds 1–6.

Mice fed hypercholesterolaemic agents have been used frequently as a suitable model for exogenously-induced hypercholesterolaemia (Hall et al 1984; Poplawski et al 2000; Silva et al 2001). Drug responses observed in man are now being seen in such models (Krause & Princen 1998). Moreover, Triton WR-1339-injected rodents are known to have increased hepatic cholesterol synthesis. Hence, drugs with an inhibitory action on 3-hydroxy-3-methyl-glutaryl CoA (HMG-Co-A) reductase were indeed effective (Endo et al 1979).

In spite of the significant activity in lowering cholesterol levels by most of the analogues in the series 1–6, a strong effect of decreasing HDL-cholesterol level was observed. An exception, however, were compounds 3 and 5, which afforded a 50.3 and 88.7% increase, respectively, in normolipidaemic rats after 28 days of administration. Evidence has accumulated that the HDL fraction transports cholesterol out of the tissues to the liver for biliary excretion, hence a reduction of cholesterol levels would be associated with an enhancement of HDL-cholesterol (Hall et al 1984). However, clinical studies have shown that commercially available agents such as probucol do not significantly elevate HDL cholesterol concentration in plasma (Kannel & Sytkowsky 1987).

Recent studies with α-asarone have shown that the hypolipidaemic effect of this compound might be due to the inhibition of hepatic HMG-CoA reductase, the rate-limiting enzyme in cholesterol biosynthesis (Rodríguez-Páez et al 2003). The corresponding analogues of α-asarone may possibly have a similar mechanism of action, but their precise mechanism requires research. On the other hand, phenoxyacetic analogues have been shown to be effective in lowering lipids under all conditions tested, and under exogenously- or endogenously-induced normolipidaemic and hyperlipidaemic conditions. However, the effect of α-asarone analogues 1–6 was probably not only attributable to their hypocholesterolaemic effect, but also to non-lipid mechanisms of action. This is becoming increasingly recognized for 3-hydroxy-3-methylglutaryl coenzyme A (HMG-Co-A) reductase inhibitors. The non-lipid factors may include stabilization of arterial plaques, endothelial normalization, anti-inflammatory effects and inhibition of platelet thrombus formation (Dajani et al 2002).
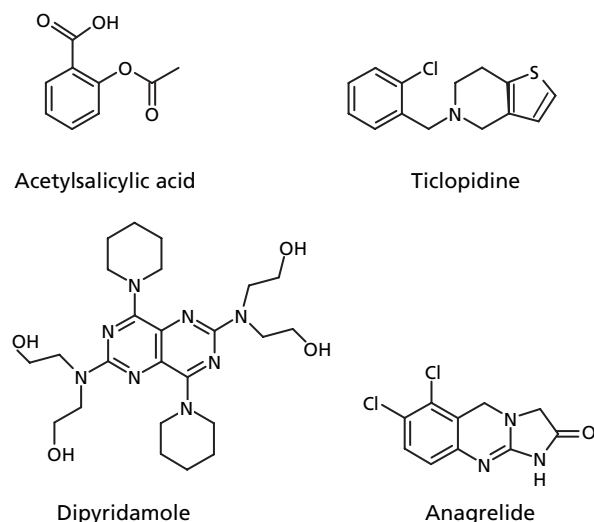
Figure 2 Chemical structure of the antiplatelet drugs: acetylsalicylic acid (aspirin), ticlopidine, dipyridamole and anagrelide.

In association with our research on α-asarone, we decided to analyse the influence of alterations of this molecule on the antiplatelet activity, because changes in structure might influence their activity and specificity. In ADP-induced aggregation, analogues of α-asarone showed inhibitory effects similar to those of acetylsalicylic acid, but the chemical structure of these analogues was different from that of currently known antiplatelet drugs, such as acetylsalicylic acid, ticlopidine, dipyridamole and anagrelide (Figure 2). These analogues may have a potential antiplatelet aggregation role by acting as ADP-receptor antagonists (Yang et al 2004).

Conclusions

The α-asarone analogues, methyl and ethyl ethers **1–6**, administered orally to normolipidaemic and hypercholesterolaemic rats and Tyloxapol-induced hypercholesterolaemic mice, proved to be effective lipid lowering agents. Although the series **1–6** did not increase the HDL-cholesterol content, an effect that would promote reverse cholesterol transport (Meyers & Kashyap 2004), their hypolipidaemic potential was not compromised necessarily (Zuñiga et al 2005). A similar analysis could be done with respect to the fact that these compounds did not reduce the triglyceride levels in the rat models (Zuñiga et al 2005).

These compounds represent a unique structural series which appear to possess hypolipidaemic activity. The role of different functional groups in eliciting the observed activity may not be understood until the mechanism of action on lipid metabolism is known.

It is noteworthy that these new hypocholesterolaemic agents were readily obtained by a short and efficient synthesis and from commercially available starting materials (Diaz et al 1993).

A number of changes in the structure of these phenoxyacetic compounds may be suggested and expected to display

hypocholesterolaemic activity. Indeed, we have prepared a series of compounds whilst looking for the minimal pharmacophore features associated with the potent hypocholesterolaemic activity of the **1–6** series (Cruz et al 2003; Zúñiga et al 2005). This series was structurally characterized by the phenoxyacetic scaffold (as acid or ester derivatives), the propenyl side-chain had been changed for a methyl or an ethyl group, and the methoxy group in position C-2 of the aryl ring was kept or removed, or even replaced for a nitro group. Despite the structural simplicity of this series, a powerful hypocholesterolaemic activity was shown, lowering the mice serum cholesterol and LDL levels by as much as 40%. These results supported the SAR hypothesis of the high pharmacophore value of the phenoxyacetic frame, and the important effect of the aromatic ring side-chain in enhancing such activity. An evaluation of this series under the same hypolipidaemic protocols as shown herein for the series **1–6** is necessary. The compounds **1–6** appeared to be promising antiplatelet drugs.

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