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Original article

Analogues of phospholipids: synthesis and biological evaluation of a series of 3-phosphocholine glyceric acid derivatives

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Summary — A series of phospholipid analogues derived from 3-phosphocholine glyceric acid (**1a–e**) have been prepared and tested as lysophosphatidylcholine or PAF analogues according to their structure. Of these compounds, hexadecyl 3-phosphocholine glycerate (**1a**), the most similar to the model lysophosphatidylcholine, showed some interesting immunomodulating activities, while hexadecyl 2-acetyl-3-phosphocholine glycerate (**1b**), most similar to PAF, had no immunomodulating activity but was a weak agonist of platelet aggregation. Other compounds of the series with modification of the side chain showed little activity in either category.

Résumé — Analogues des phospholipides: synthèse et évaluation biologique d'une série de composés dérivés de l'acide 3-phosphorylcholine glycérique. *Une série de phospholipides analogues dérivés de l'acide 3-phosphocholine glycérique (**1a–e**) ont été préparés et testés comme lysolécithine ou PAF analogues selon leurs structures. De tous ces composés, le **1a**, le plus proche du modèle lysolécithine, montre une activité intéressante comme immunomodulateur; alors que le **1b** plus proche du PAF n'a pas montré d'activité comme immunomodulateur mais une faible activité agoniste de l'agrégation des plaquettes sanguines. Les autres composés de la série, résultant de modifications de la chaîne, ont montré une faible activité dans les deux domaines.*

phosphoryl glyceric acid derivatives / synthesis / platelet aggregation activity / immunoenhancing activity / experimental infections

Introduction

The important role of lysophosphatidylcholines as immunoenhancing compounds [1] and the identification of the structure of the platelet activating factor (PAF) as 1-*O*-alkyl 2-*O*-acetyl sn-glyceryl-3-phosphocholine, in which the alkyl component is comprised largely of C-18 and C-16 homologues, has stimulated a large amount of research in the area of phospholipid analogues.

PAF is a potent activator of various inflammatory cells such as platelets, neutrophils, basophils, and is an important mediator of anaphylaxis and inflammation.

In order to prove the structural requirements for activity (commonly measured as stimulation of platelet aggregation) and to enhance the desirable biological action or to discover antagonists, PAF analogues in a number of classes have been prepared. The most numerous of these analogues are those in which the 1-*O*-alkyl and 2-*O*-acetyl groups have been varied. Homologues in position 1 with an alkoxy-chain length from C1 to C20 were described and their agonist activity correlated with hydrophobicity parameters [2]. The removal of the ether oxygen in the same position [3] or its replacement with a sulphur [4] or nitrogen [5] atom as well as the replacement of the alkoxy chain with a chain containing multiple ether linkages [6], resulted in a decrease biological response; on the contrary, increasing the degree of unsaturation has negligible effects [7]. A highly selective synthetic agonist was obtained by diastereoselectively introducing a methyl group at C-1 of the glycerol moiety [8].

Some analogues carrying strongly modified groups in position 2 including ethoxy, methylaminocarbonyl and nitrate [9] surprisingly maintained a good degree of activity, whereas some others, such as trifluoro- and trichloro-acetyl [10], fluoro and chloro [11] did not. Systematic replacement of a single oxygen at each of the four possible positions to give the carba analogues has been accomplished [12, 13, 14, 15]. Also, the phosphocholine portion of the molecule has been altered: the phosphate moiety has been replaced

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with a neutral sulphonylbismethylene group [16] or a carboxylate [17] and the polar trimethyl ammonium with a thiazolium [18], *N*-methylmorpholinium, pyridinium, *N*-methyl pyrrolidinium group [19]. A programme aimed at investigating carbamate analogues of lysophospholipids has been successful in the search for a specific PAF receptor antagonist [18].

To our knowledge, phosphatidylcholine or PAF analogues with a higher oxidation level of the glycerol moiety have never been prepared. In the present communication, we wish to report the synthesis and the activity of a series of 3-phosphocholine glyceric acid derivatives **1a–e** on the platelet aggregation. Some of them were also tested for their ability to modify the immunological response. We reasoned that compounds **1a, c–e** could mimic the natural lysophosphatidylcholine behaviour as immunoenhancing compounds.

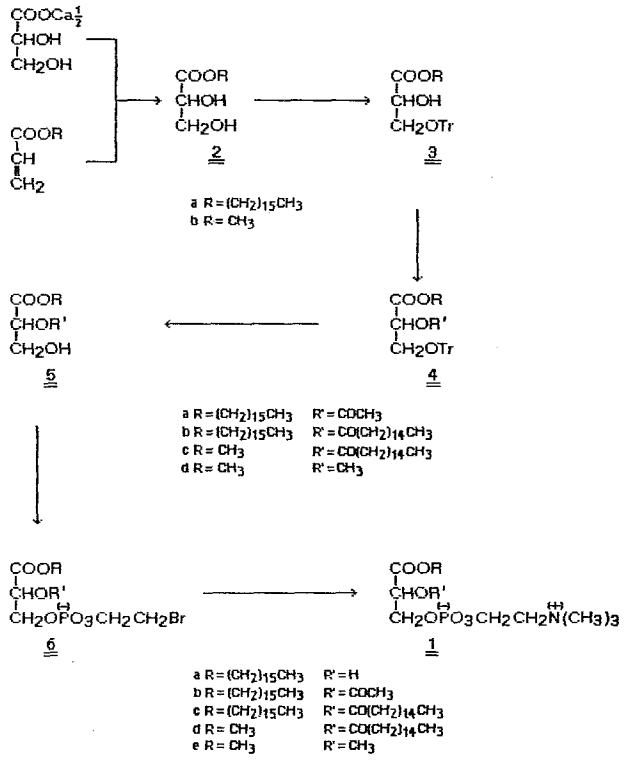
Chemistry

Compounds **1a–e** were prepared as racemic mixtures by a common synthetic strategy that involved the initial preparation of hexadecyl and methyl glycerate as reported in scheme 1. The only successful synthesis of hexadecyl glycerate (**2a**) started from the commercially available calcium glycerate and hexadecyl iodide in hexamethylphosphoric triamide. Methyl glycerate (**2b**) was easily prepared from methyl acrylate by hydroxylation to the *vic*-diol using the potassium chlorate-osmium tetroxide oxidizing system in THF/H_2O, followed by fractional distillation of the crude product.

Selective protection of the primary hydroxyl was achieved with trityl chloride in $CHCl_3$/pyridine to yield **3a, b**. Acylation with palmitoyl or acetyl chloride in the usual manner gave **4a, b, c**, respectively, after purification by column chromatography and recrystallization.

Methyl 2-methoxy-3-trityloxypropionate (**4d**) was prepared from **3b**, by treatment with methyl iodide and silver oxide in DMSO [20].

The deprotection of the compounds **4a–d** was effected under different experimental conditions depending on the substituent. When R and R' were long chain residues (**4b**) better yields were obtained by catalytic hydrogenation (MeOH, Pd/C), even if deprotection is achieved with 6 N HCl in $CHCl_3$/-acetone [21] the percentage of acyl migration from C-2 to C-3 [22] being very limited (< 5%). This compound was also stable enough to be chromatographed and the isomeric purity was easily monitored by TLC (hexane/EtOAc, 9/1) and ^1H NMR. When subjected to catalytic hydrogenation, **4c** proved to be unreactive, deprotection was achieved with 6 N HCl in $CHCl_3$/acetone; the percentage of isomerization was in this case about 20% (monitored by TLC after separation of both isomers and NMR identification). Pure **5c** was obtained by column chromatography. On the contrary, in the case of **5a**, prepared from **4a**, the main difficulty was the separation of the isomers owing to the high percentage of isomerization occurring during purification of large amounts of the product. Thus, we obtained pure **5a** and its isomer *via* fast silica gel column chromatography on limited amounts of crude **5a**. Finally, good yields of **5d** were only obtained from **4d** upon refluxing it with boric acid in trimethyl borate [23].



Scheme 1.

The introduction of the phosphocholine moiety was performed by phosphorylation of the primary hydroxyl group of the isomeric pure **5a–d** with 2-bromoethyl dichlorophosphate in Et_2O [24] or $CHCl_3$ [25] to give compounds **6b–d**, followed by treatment with anhydrous trimethylamine in ethyl methyl ketone.

Compound **1a**, prepared *via* the same phosphorylation and quaternization reactions reported above starting from hexadecyl 2-benzylglycerate (from **3a** and benzyl bromide followed by acid detritylation) could also be obtained directly from **2a** avoiding protection of the secondary hydroxyl and subsequent catalytic hydrogenolysis (purity checked by HPLC).

In all cases, the crude products were suspended in MeOH/H_2O and treated with Ag_2CO_3. Careful purification (column chromatography) gave pure **1a–e**.

Results and Discussion

Platelet aggregation

Compounds **1a–e** were tested for their ability to induce platelet aggregation on rabbit platelet rich plasma (PRP). Only **1b**, whose structure was more strictly related to PAF, showed agonistic activity, being 10^{-3} times less active than PAF (see Experimental).

On the assumption that the other compounds could act as PAF antagonists, we tested their ability to inhibit PAF-induced platelet aggregation on rabbit PRP. The results obtained at a 10^{-4} M concentration of **1a, c–e** indicated that the compounds were very poor PAF antagonists, never exceeding 30% of inhibition of the aggregation caused by 10^{-8} M PAF.

Immunomodulating activity

Higher animals have many defense mechanisms against bacterial infections and the role of macrophages is important in these host-defense mechanisms. The hypothesis that many immunological adjuvants act by the formation of lysolecithin through activation of phospholipase A in macrophages is supported by the fact that phosphatidylcholine itself enhances antibody production in response to soluble proteins and particulate antigens [26].

Thus, we tested compounds **1a, c, d, e** *in vivo* for their capacity to affect the course of experimental infections (bacterial or fungal) in mice compared to the natural α-palmitoyl-γ-lysolecithin [27, 28].

In the case of the bacterial infection, compounds were administered intraperitoneally (ip) to mice the day before the ip administration of sublethal doses of *E coli* ISM. In previous experiments, it was observed that the mice died within 18–24 h after infection and survivals were definitive both in control and treated animals. Results listed in table I represent the percentage of mortality with 3 doses of compounds. The protection achieved after administration of **1a, c, d** was not statistically significative in comparison to the controls ($P < 0.1$, χ^2) but when the treatment was repeated -3 and -1 d prior to the infection, the results for **1a, d** were more clearcut, as may be seen in table II (P values by Fisher test).

Compound **1a**, the most similar to the model phosphatidylcholine, appeared most active. It was therefore also tested against fungal infection by *Candida albicans* administered iv. The results obtained are reported in table III as an average of 2 experiments with 10 mice/doses. The mean survival time increased from 6.5 to 10.7 d ('U' Mann–Witney P = 0.01) at the dose of 5 mg/kg.

The immunotherapeutic action of these compounds, intrinsically lacking antibacterial activity, was con-

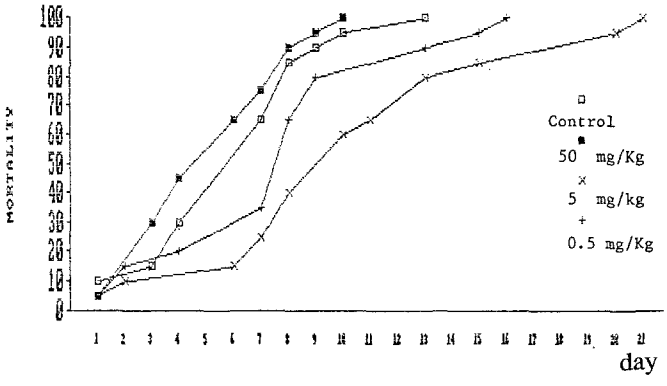
Table I. Protective effect of **1a, c, d, e** compared to lysophosphatidylcholine (lysoPC) against bacterial challenge with *E coli* ISM. Average of 3 experiments with 15 animals/dose/compound. Compounds were administered ip 1 day before injection. Inoculum size 0.5 ml of a suspension of 1.4 x 10^5 CFU/ml.

	% of mortality				
Compounds	***1a***	***1c***	***1d***	***1e***	*LysoPC*
0.5 mg/kg	62.2	46.6	51.1	46.6	66.6
5 mg/kg	55.5	60.0	37.8	60.0	35.5
50 mg/kg	48.9	66.6	46.6	73.3	82.2
Controls	68.9	73.3	57.8	60.0	66.6

Table II. Protective effect of **1a, d** against bacterial infection with *E coli* ISM administered ip at days 3 and 1 prior to the challenge. Average of 3 experiments with 10 mice/dose/compound.

	% of mortality	
Compounds	***1a***	***1d***
0.5 mg/kg	70.0	36.6
5 mg/kg	60.0	30.0
50 mg/kg	0.0 ($p < 0.01$)	16.6 ($p < 0.01$)
Controls	80.0	53.3

Table III. Protective effect of **1a** administered ip on day 0 and +2 against fungal infection of *C albicans* (administered iv; 0.5 ml of a suspension of 2 x 10^6 CFU/ml.



	Test of Mann–Whitney	
	MST	*P*
Control	6.5±0.65	
50 mg/kg	5.6±0.56	>0.25
5 mg/kg	10.7±1.2	<0.01
0.5 mg/kg	8.1±0.9	>0.25

Average of 2 experiments with 10 mice/dose/compound.

firmed by preliminary results obtained in the proliferation of lymphocytes of $B_6D_2F_1$ mice ip treated with **1a, d** induced by the mitogens PHA and ConA [29]. A statistically significant activity was found ($P < 0.05$, 'Dunnett's' test) with both the mitogens utilized when the administrations were repeated on d –5, –3, –1 before removal of spleens*.

Preliminary toxicological studies on BDF_1 mouse showed that **1a** was well tolerated at 250 mg/kg in a single administration or at 50 mg/kg for 14 daily repeated administrations.

In conclusion, the pharmacological tests, even if very limited for the time being, showed that among the compounds included in the series studied, the most active one for platelet aggregation was **1b**, whose structure is the most similar to PAF. The same is true for the immunomodulating activity which reaches a maximum for compound **1a**, again the most similar to the model lysolecithin. In other compounds, the modification of the side chain caused a decrease of activity. It seems that the structural change on C-1 in both PAF and lysolecithin that converted them from glycerol to glyceric acid derivatives does not influence the biological activity in a dramatic way. Further studies on the pharmacological activity of other, well planned glyceric acid derivatives therefore seem to be worthwhile.

Experimental protocols

Chemistry

Melting points were determined with a Buchi capillary apparatus and are uncorrected. ^{1}H NMR spectra were recorded on a Perkin-Elmer R24B spectrometer. IR spectra were recorded on a Perkin–Elmer 681 spectrophotometer. TLC analyses were carried out on Merck glass plates precoated with silica gel. Preparative MPLC was carried out using a Buchi 680 system. HPLC analysis were performed with a Perkin–Elmer Series 3 liquid chromatograph (LiChrosorb-NH_2 Merck column eluted with CH_3CN/H_2O 86/14 at 30°C, flow rate 1 ml/min; λ 220 nm).

*Hexadecyl glycerate (**2a**)*
To a solution of hexadecyl iodide (44.3 g, 0.126 mol) in HMPT (200 ml) calcium glycerate (36 g, 0.126 mol) was added and the solution was stirred at room temperature for 3 d. The solution was then poured in H_2O (1.5 l) and the crude product filtered, washed with water and dried *in vacuo*. Recrystallization from light petroleum gave pure **2a** (40 g, 96% yield); Mp 67–68°C. IR (Nujol) 3300, 1730, 1470 cm^{-1}. ^{1}H NMR ($CDCl_3$): δ 0.85 (t, 3H), 1.25 (br, m, 28H), 3.9 (d, 2H), 4.2 (m, 3H).

*Foresta P (Biological Laboratories, Sigma Tau SpA); private communication

*Methyl glycerate (**2b**)*
To a solution of methyl acrylate (100 g, 1.16 mol) in 1:1 THF/H_2O (2 l), $KClO_3$ (244 g, 1.99 mol) and a catalytic amount of OsO_4 were added. The suspension was stirred for 18 h at room temperature. The organic layer was separated, the aqueous one concentrated *in vacuo* to 400 ml and extracted with AcOEt (3 x 300 ml). The organic extracts were combined, dried and evaporated *in vacuo*. The resulting oil was distilled (112–114°C, 8 Torr) obtaining 92.8 g of pure **2b** (yield 66.4%).

*Hexadecyl 3-(triphenylmethyl)glycerate (**3a**)*
To a solution of **2a** (30 g, 0.09 mol) in $CHCl_3$ (60 ml) and pyridine (35 ml) chlorotriphenylmethane (27.3 g, 0.1 mol) was added in 30 min. After stirring at 60°C for 16 h the solution was cooled to 5°C, diluted with $CHCl_3$ (200 ml) and H_2O (100 ml) and acidified to pH = 2 (2 N HCl). The organic layer was separated, washed with water, dried and evaporated *in vacuo*. The crude product was recrystallized from EtOH giving 49 g of **3a** (yield 93%). Mp 62–63°C. IR (Nujol) 3500, 1740, 1490 cm^{-1}. ^{1}H NMR ($CDCl_3$): δ 0.85 (t, 3H), 1.25 (m, 28H), 2.75 (s, 1H), 3.6 (d, 2H), 4.1 (m, 3H), 7.2 (br, m, 15H).

*Methyl 3–(triphenylmethyl)glycerate (**3b**)*
The compound was prepared as described above for **3a** starting from **2b**. Yield 91%. Mp 120–122°C. IR (Nujol) 3500, 1740 cm^{-1}. ^{1}H NMR ($CDCl_3$): δ 3.4 (d, 2H), 3.75 (s, 3H), 4.2 (t, 1H), 7.3 (m, 15H).

*Hexadecyl 2-acetyl-3-(triphenylmethyl)glycerate (**4a**)*
To a stirred solution of **3a** (100 g, 0.175 mol) in $CHCl_3$ (380 ml) and pyridine (80 ml) cooled at 0°C acetyl chloride (13.0 ml, 0.184 mol) was added dropwise. After stirring overnight, the solution was acidified with 2 N HCl to pH = 2 and diluted with 300 ml of $CHCl_3$. The organic layer was separated, washed with 5% $NaHCO_3$ and H_2O, dried and evaporated *in vacuo*. The crude product was recrystallized from EtOH (100 g, 94% yield): mp 58–61°C. IR (film) 2920, 1750, 1450 1230 cm^{-1}. ^{1}H NMR ($CDCl_3$): δ 0.85 (t, 3H), 1.25 (br, m, 28H), 2.12 (s, 3H), 3.45 (d, 2H), 4.05 (t, 2H), 5.1 (t, 1H), 7.25 (m, 15H).

*Hexadecyl 2-hexadecanoyl-3-(triphenylmethyl)glycerate (**4b**)*
To a stirred solution of **3a** (65 g, 0.11 mol) in $CHCl_3$ (200 ml) and pyridine (20 ml) hexadecanoyl chloride (30.8 g, 0.11 mol) was added dropwise at 0°C. Work-up as described for **4a** give pure **4b** (54 g, 59% yield): mp 43–45°C. IR (Nujol) 1745 cm^{-1}. ^{1}H NMR ($CDCl_3$): δ 0.85 (t, 6H), 1.25 (m, br, 54H), 2.3 (t, 2H), 3.5 (d, 2H), 4.1 (t, 2H), 5.2 (t, 1H), 7.2 (m, 15H).

*Methyl 2-methyl-3-(triphenylmethyl)glycerate (**4d**)*
To a solution of **3b** (120 g, 0.33 mol) in DMSO (480 ml) containing Ag_2O (84 g, 0.36 mol), methyl iodide (110 ml, 1.77 mol) was added dropwise with external heating at 60°C. The suspension was stirred for 2 h at the same temperature, then cooled, poured in H_2O, filtered and extracted with Et_2O (3 x 150 ml). The organic phase was dried and evaporated *in vacuo* and the crude product recrystallized from EtOH (57 g, 44.6% yield). mp 125–127°C. IR (film) 2920, 1745 cm^{-1}. ^{1}H NMR ($CDCl_3$): δ 3.3 (s + m, 5H), 3.55 (s, 3H), 3.8 (t, 1H), 7.3 (m, 15H).

*Hexadecyl 2-acetylglycerate (**5a**)*
A solution of **4a** (100 g, 0.162 mol) in 1:1 $CHCl_3$/acetone (400 ml) was acidified with 6 N HCl (100 ml) and stirred overnight. Then $CHCl_3$ (300 ml) was added, the solution washed with 10% $NaHCO_3$ and H_2O until neutral, dried and

evaporated *in vacuo*. The residue was dissolved in hexane, some insoluble matter removed by filtration and the filtrate evaporated again. The crude product (65 g) containing about 20% of the 3-acetyl isomer was quickly chromatographed portionwise (MPLC; silica gel, eluted with 8:2 hexane/AcOEt) giving **5a** (41 g overall, 67% yield). TLC hexane/AcOEt 8/2 R_f = 0.19 (iodine) [3-acetyl isomer R_f = 0.27]. IR (film) 3500, 1750, 1450 1225 cm^{-1}. ^1H NMR ($CDCl_3$): δ 0.85 (t, 3H), 1.25 (m, 28H), 2.1 (s, 3H), 3.9 (d, 2H), 4.15 (t, 2H), 5.1 (t, 1H).

Hexadecyl 2-hexadecanoylglycerate ***(5b)***
A suspension of **4b** (54 g, 0.067 mol) and 10% Pd/C (4 g) in EtOH (500 ml) was hydrogenated at 3 atm and 60°C for 3 h. After removal of the catalyst, the solvent was distilled *in vacuo* and the crude product purified by MPLC (silica gel, eluted with 9:1 Bz/AcOEt). Yield of **5b** 47%: mp 67–69°C (hexane/AcOEt, 9/1) R_f = 0.26 IR (Nujol) 1735, 1460 1370 cm^{-1}. ^1H NMR ($CDCl_3$): δ 0.85 (t, 6H), 1.25 (br, m, 54H), 2.3 (t, 2H), 3.6 (d, 2H), 4.1 (t, 2H), 5.2 (t, 1H).

Methyl 2-hexadecanoylglycerate ***(5c)***
To a solution of **3b** (100 g, 0.275 mol) in $CHCl_3$ (600 ml) and pyridine (125 ml), hexadecanoyl chloride (80 g, 0.29 mol) was added and the solution stirred for 18 h at room temperature. The obtained mixture was washed with 6 N HCl and H_2O, evaporated *in vacuo* and the residue dissolved in 1:1 $CHCl_3$/acetone (200 ml) and treated with 6 N HCl (50 ml). After 3 h the mixture was worked-up as described and the crude product purified by MPLC (silica gel, with 8:2 benzene/AcOEt as eluent). Yield of **5c** 26 g (27%). TLC (toluene/AcOEt 8/2). R_f = 0.40 [3-hexadecanoyl isomer R_f = 0.48]. IR (Nujol) 3500, 1740 cm^{-1}. ^1H NMR ($CDCl_3$): δ 0.90 (t, 3H), 1.25 (m, 28H), 2.4 (t, 2H), 3.8 (s, 3H), 3.95 (d, 2H), 5.15 (t, 1H).

Methyl 2-methylglycerate ***(5d)***
A solution of **4d** (56 g, 0.14 mol) in trimethyl borate (560 ml) and boric acid (133 g) was refluxed until complete disappearance of the starting compound (TLC, 1:1 $CHCl_3$/acetone). The solution was concentrated *in vacuo*, diluted with chloroform (500 ml) and filtered. The filtrate was evaporated *in vacuo* and the crude product purified by column chromatography (silica gel, eluted with 1:1 $CHCl_3$/acetone). 15.2 of **5d** was obtained (yield 80%). TLC ($CHCl_3$/MeOH 1/1). R_f = 0.6 IR (film) 3480, 1740 cm^{-1}. ^1H NMR ($CDCl_3$): δ 3.5 (s, 3H), 3.8 (m, 6H).

Hexadecyl 3-(2-bromoethyl)phosphoglycerate ***(6a)***
To a solution of 2-bromoethyl dichlorophosphate [24] (87.7 g, 0.364 mol) in anhydrous diethyl ether (800 ml) cooled at 0°C, pyridine (180 ml) and then **2a** (40 g, 0.12 mol) in Et_2O (2.4 l) were added. The solution was gently refluxed for 4 h, then water (300 ml) was added and the resulting mixture stirred for an additional h. The solvent was evaporated, the residue dissolved in 2:1 $CHCl_3$/MeOH (2.4 l) and H_2O (500 ml) and the pH adjusted to 5. The organic layer was separated and the aqueous one washed with 2:1 $CHCl_3$/MeOH (2 x 200 ml). The organic extracts were combined, dried and evaporated *in vacuo*; the crude gummy product was purified by silica gel chromatography using 9:3 $CHCl_3$/MeOH as eluent: 10 g of pure **6a** was obtained (yield 16.5%). Mp 103–105°C. TLC ($CHCl_3$/MeOH 9/3). R_f = 0.34 (iodine, molybdenum spray). IR (Nujol) 3400, 1740 1470, 1225, 1075 cm^{-1}. ^1H NMR ($CDCl_3$): δ 0.85 (t, 3H), 1.25 (br, m, 28H), 3.6 (m, 2H), 4.17 (m, 7H).

Hexadecyl 3-(2-bromoethylphospho)-2-hexadecanoyl glycerate ***(6c)***
To a solution of 2-bromoethyl dichlorophosphate (17.1 g, 0.07 mol) in anhydrous Et_2O (300 ml) cooled at 0°C, pyridine (28.5 ml) and **5b** (13.5 g, 0.024 mol) in Et_2O (800 ml) were added. Work-up as described above for the preparation of **6a**, gave 4.5 g of **6c** (yield 36.7%): mp 172–175°C. TLC ($CHCl_3$/MeOH 9/1). R_f = 0.39 (iodine, molybdenum spray). IR (Nujol) 3400, 1740 1435, 1210, 1115 1070 cm^{-1}. ^1H NMR ($CDCl_3$): δ 0.85 (t, 6H), 1.25 (br, m, 54H), 2.3 (t, 2H), 3.7 (t, 2H), 4.2 (m, 6H), 5.2 (t, 1H).

Methyl 3-(2-bromoethylphospho)-2-hexadecanoyl glycerate ***(6d)***
A stirred solution of **5c** (25 g, 0.07 mol) in pyridine (75 ml) was added to a solution of 2-bromoethyl dichlorophosphate (25 ml) in pyridine at 0°C. After 24 h at room temperature, the solution was poured in 0.1 N KCl (1.2 l) and stirred for 2 h. The pH was then adjusted to 1.5 with 6 N HCl and the solution extracted with $CHCl_3$. The dried organic layer was evaporated *in vacuo* and the crude product chromatographed (silica gel, eluted with 9:2 $CHCl_3$/MeOH) to give 7.1 g (18.3% yield) of 6d. TLC ($CHCl_3$/MeOH 9/2). R_f = 0.32 (iodine, molybdenum spray) IR (film) 1740 1350, 1180, 1100 cm^{-1}. ^1H NMR ($CDCl_3$): δ 0.9 (t, 3H), 1.25 (br, m, 26H), 2.3 (t, 2H), 3.75 (s + m, 5H), 4.2 (m, 4H), 5.15 (t, 1H).

Hexadecyl 3-phosphocholineglycerate ***(1a)***
A solution of **6a** (10 g, 0.07 mol) and anhydrous trimethylamine (60 ml, 0.64 mol) in ethyl methyl ketone (250 ml) was stirred for 2 h at room temperature and then heated at 60°C overnight. The solvent was then evaporated and the residue dissolved in 9:1 MeOH/H_2O (1 l) and stirred for 3 h with Ag_2CO_3 (6.3 g, 0.023 mol). The suspension was filtered on Dicalite and the filtrate evaporated *in vacuo*. The crude product was chromatographed (silica gel, eluted with 60:25:4 $CHCl_3$/MeOH/H_2O) and a colourless solid was obtained (2.6 g, 26.3% yield): mp 180°C. TLC (same eluent). R_f = 0.27 (molybdenum spray) HPLC R_t = 6.6 min. IR (Nujol) 3300, 1745, 1255, 1210, 1060 cm^{-1}. ^1H NMR ($CDCl_3$): δ 0.85 (t, 3H), 1.25 (m, 28H), 3.3 (s, 9H), 4.1 (m, 9H). Anal $C_{24}H_{50}NO_7P$ (C, H, P).

Hexadecyl 2-acetyl-3-phosphocholineglycerate ***(1b)***
The phosphorylation reaction was performed as described above for compound **6a**. **5a** (30 g) was treated with 2-bromoethyl dichlorophosphate in Et_2O-pyridine to obtain **6b** (12 g). TLC ($CHCl_3$/MeOH 9/2). R_f = 0.4 IR (film) 1750, 1230, 1080, 1020 cm^{-1}. ^1HNMR ($CDCl_3$): δ 0.85 (t, 3H), 1.25 (s, 28H), 2.1 (s, 3H), 3.5 (t, 2H), 4.1 (m, 6H), 5.2 (t, 1H). Compound **1b** was prepared from **6b** by replacing the bromo substituent with trimethylamine as described for the preparation of **1a**. Pure **1b** (2.1 g) was obtained as described for **1a**. TLC ($CHCl_3$/MeOH/H_2O, 60/25/4) R_f = 0.34 (molybdenum spray, iodine). IR (film) 1750, 1080, 1020 cm^{-1}. ^1H NMR ($CDCl_3$): δ 0.85 (t, 3H), 1.25 (m, 28H), 2.1 (s, 3H), 3.15 (s, 9H), 3.6 (t, 2H), 4.2 (m, 6H), 5.15 (t, 1H). Anal $C_{26}H_{52}NO_8P$ (C, H, P).

Hexadecyl 2-hexadecanoyl-3-phosphocholineglycerate ***(1c)***
Starting from **6c**, the reaction was carried out following the procedure described for **1a** and led to the title compound **1c** (53% yield). Mp 67–71°C. TLC ($CHCl_3$/MeOH, 9/3) R_f = 0.53 (molybdenum spray, iodine). IR (Nujol) 3300, 1740, 1240, 1080 cm^{-1}. ^1H NMR ($CDCl_3$): δ 0.85 (t, 6H), 1.25 (br, m, 54H), 2.3 (t, 2H), 3.3 (s, 9H), 3.7 (m, 2H), 4.2 (m, 6H), 5.2 (t, 1H). Anal $C_{40}H_{80}NO_8P$ (C, H, N, P).

Methyl 2-hexadecanoyl-3-phosphocholineglycerate ***(1d)***
Compound **1d** was prepared starting from **6d** as described above for **1a**. Purification by column chromatography under the same conditions, gave 1.3 g (20.8% yield) of waxy **1d**,

TLC ($CHCl_3$/MeOH/H_2O, 65/25/4) R_f = 0.28. IR (Nujol) 1745, 1225, 1080 cm^{-1}. ^1H NMR ($CDCl_3$): 0.9 (t, 3H), 1.25 (m, 26H), 2.3 (t, 2H), 3.2 (s, 9H), 3.8 (m, 5H), 4.2 (m, 4H), 5.1 (t, 1H). Anal $C_{25}H_{50}NO_8P$ (C, H, N, P).

*Methyl 2-methyl-3-phosphocholineglycerate (**1e**)*
To a cold solution (0–5°C) of triethylamine (37 ml) in $CHCl_3$ (100 ml) 25 g (0.104 mol) of 2-bromoethyl dichlorophosphate and then 10 g (0.074 mol) of **5d** in $CHCl_3$ (100 ml) were added dropwise. The solution was stirred for 4 h at room temperature and then maintained at 60°C overnight.

To the cooled solution, 0.1 N KCl (300 ml) was added and stirring was continued for 1 h. Then MeOH (500 ml) was added and the pH adjusted to 3 with 6 N HCl. The organic layer was separated and the aqueous one washed with $CHCl_3$. The organic phases were combined, dried and brought to dryness. The residue was dissolved in butanone (300 ml) filtered, and the filtrate was concentrated to half its original volume, cooled to –40°C and treated with anhydrous trimethylamine (70 ml). The resulting solution was stirred at room temperature (1 h) and then at 55°C for 18 h. Work-up as described for compound **1a** gave 1.9 g of pure title compound after column chromatography (silica gel eluted with $CHCl_3$/MeOH/H_2O 30/60/4). TLC (same eluent). R_f = 0.16 (molybdenum spray, iodine). IR (Nujol) 3260, 1740, 1260, 1070 cm^{-1}. ^1H NMR ($CDCl_3$): δ 3.25 (s, 9H), 3.5 (s, 3H), 3.65 (m, 2H), 3.8 (s, 3H), 4.25 (m, 5H). Anal $C_{10}H_{22}NO_7P$ (C, H, N, P).

Biological and pharmacological methods

Platelet aggregation
Agonist effect. Platelet aggregation studies were performed by the method of Born [30]. Blood was collected into 3.2% sodium citrate by cardiac puncture from conscious male New Zealand rabbits. PRP was obtained by centrifugation of blood at 300 *g* for 10 min. Platelet aggregation was monitored by continuous recording of light transmission in a dual channel aggregometer (Chronolog). Aggregations induced by analogues were compared to that obtained with PAF which produced 100% aggregation (5×10^{-8} M).
Antagonist effect on aggregation. To pre-incubated and stirred PRP the compounds **1a, c–e** were added until a final 10^{-4} M concentration was reached; after 2 min PAF acether was added (10^{-8} M). Inhibition of aggregation was measured and compared to a control aggregation induced by PAF-acether (10^{-8} M).

Experimental infection
Male COBS-CD1 mice weighing about 20 g, obtained from a commercial breeder were used. *E coli ISM* was stored in liquid nitrogen and diluted in isotonic saline before infection. Normally 0.5 ml of 1.4×10^5 CFU/ml of suspension was used.

Candida albicans were maintained on slants of Sabouraud medium and transferred weekly to fresh slants. Normally, 0.5 ml of 2×10^6 CFU/ml of suspension was used.

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