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(54) Title: USE OF POLYUNSATURATED KETONES FOR THE TREATMENT OF PSORIASIS

(57) **Abrégé/Abstract:**

Psoriasis is a common, chronic, inflammatory skin disorder. This invention provides the use of a compound of formula (I) R-CO-X (Wherein R is a C₁₆₋₂₄ unsaturated hydrocarbon group optionally interrupted α , β , γ , or δ to the carbonyl group by a heteroatom or group of heteroatoms selected from S, O, N, SO, SO₂ said hydrocarbon group comprising at least 5 non-conjugated double bonds; and X is an electron withdrawing group) in the manufacture of a medicament for the treatment of psoriasis.



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(54) Title: USE OF POLYUNSATURATED KETONES FOR THE TREATMENT OF PSORIASIS

(57) Abstract: Psoriasis is a common, chronic, inflammatory skin disorder. This invention provides the use of a compound of formula (I) R-CO-X (Wherein R is a C₁₆₋₂₄ unsaturated hydrocarbon group optionally interrupted α , β , γ , or δ to the carbonyl group by a heteroatom or group of heteroatoms selected from S, O, N, SO, SO₂ said hydrocarbon group comprising at least 5 non-conjugated double bonds; and X is an electron withdrawing group) in the manufacture of a medicament for the treatment of psoriasis.



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USE OF POLYUNSATURATED KETONES FOR THE TREATMENT OF PSORIASIS

5 This invention relates to the use of certain
polyunsaturated long-chain ketones for the treatment of
psoriasis and in particular to ketones carrying electron
withdrawing substituents alpha to the carbonyl
functionality in such treatment.

10 Psoriasis is a common, chronic, inflammatory skin
disorder. Psoriatic tissue is characterised by chronic
inflammation in both epidermis and dermis, the disease
being further characterised by hyperplasia of epidermal
keratinocytes, fibroblast activation, alteration of
eicosanoid metabolism, and leukocyte infiltration.

15 Effective treatments for psoriasis such as
cyclosporin A, steroids, methotrexate and
photochemotherapy all have immunosuppressive activity
and are thus not ideal treatments due to their side
effects. Scientists have therefore pursued other
20 potential treatments for this disease.

It has been observed that psoriatic tissue exhibits
elevated levels of arachidonic acid and eicosanoids.
This suggests that phospholipase A₂ (PLA₂) may be
involved in the pathogenesis of psoriasis.

25 The phospholipases are a group of enzymes that
release unsaturated fatty acids from the sn2 position of
membrane phospholipids. Once released, the fatty acids
are converted by various enzymes into biologically very
important signalling molecules. Release of arachidonate
30 initiates the arachidonate cascade leading to the
synthesis of eicosanoids such as prostaglandins.
Eicosanoids are important in a variety of physiological
processes and play a central role in inflammation. In
Inflammation, Vol. 18, No.1 1994, Andersen et al
35 identify the presence of certain phospholipases in
psoriatic human skin.

It is therefore believed that inhibition of

phospholipase enzymes should have potential in curing some of the inflammatory symptoms, including epidermal hyperproliferation due to increased leukotriene production, related to eicosanoid production and cell activation in both epidermis and dermis in psoriasis.

In J. Chem. Soc. Perkin Trans. 1, 2000, 2271-2276 several structurally different compounds are reported as inhibitors of cPLA₂ in vitro. The compounds tested were based around (all-Z)-eicosa-5,8,11,14,17-pentaenoic acid (EPA) and (all-Z)-docosa-4,7,10,13,16,19-hexaenoic acid (DHA). The paper suggests that preliminary studies show that in vitro the compounds are active as enzyme inhibitors.

The compounds in J. Chem. Soc. Perkin Trans. 1, 2000, 2271-2276 have however not been tested in vivo and there is thus no way of predicting their in vivo effects. In addition, when devising a treatment for a disease it is necessary to ensure selectivity. There are a very large number of phospholipase enzymes known and more enzymes of this type are being discovered as medical science develops. Since phospholipases control a wide variety of different intracellular functions it is necessary to develop inhibitors of these enzymes that are selective for the particular phospholipase whose activity is to be altered. Compounds which inhibit a large number of phospholipase enzymes are of little commercial interest since the benefits of a desired enzymic inhibition will be opposed by the presence of many unwanted and potentially dangerous side effects caused by unwanted enzyme inhibitions. There remains a need therefore, to provide highly selective inhibitors of phospholipase enzymes.

The present inventors have surprisingly found that compounds of somewhat similar structure or the same structure as those identified in the Perkin Transactions paper are selective for IVa PLA enzymes and are therefore ideal candidates for the treatment of

psoriasis in the absence of side effects. Given that there are a total of 23 enzymes in the phospholipase group and each enzyme fulfils a different physiological and pathological function this is surprising. Moreover, the compounds of the invention have surprisingly been found to be particularly potent in reducing eicosanoid production, by for example, the inhibition of group IVa PLA₂.

Thus, viewed from one aspect the invention provides the use of a compound of formula (I)

10 R-CO-X (I)

(wherein R is a C₁₆₋₂₄ unsaturated hydrocarbon group optionally interrupted α , β , γ , or δ to the carbonyl group by a heteroatom or group of heteroatoms selected from S, O, N, SO, SO₂, said hydrocarbon group comprising at least 5 non-conjugated double bonds; and

15 X is an electron withdrawing group)
for treatment of psoriasis and manufacture of a medicament for such treating.

Viewed from another aspect the invention relates to a method of treating psoriasis comprising administering to an animal, preferably a mammal, e.g. human, an effective amount of a compound of formula (I) as hereinbefore described.

Viewed from another aspect the invention provides use of a compound of formula (I) as hereinbefore described for use for inhibiting the enzyme IVa PLA₂ and in manufacture of a medicament for such inhibiting.

Viewed from yet another aspect, the invention provides a pharmaceutical composition comprising a compound of formula (I) and a pharmaceutically acceptable excipient.

30 The group R preferably comprises 5 to 7 double bonds, preferably 5 or 6 double bonds, e.g. 5 double bonds which should be non-conjugated. It is also

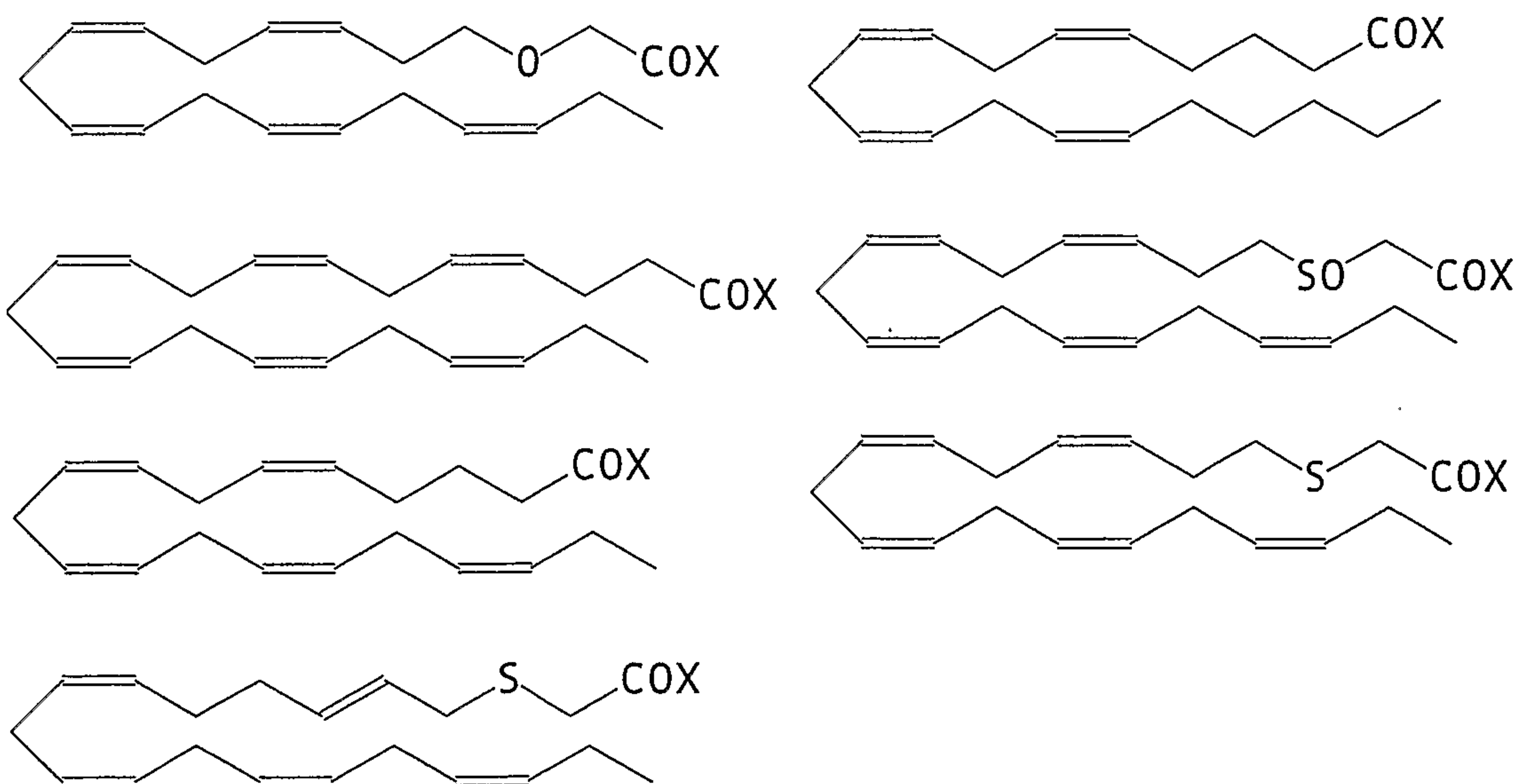
preferred if the double bonds do not conjugate with the carbonyl functionality.

The double bonds present in the group R may be in the cis or trans configuration however, it is preferred if the majority of the double bonds present (i.e. at least 50%) are in the cis configuration. In further advantageous embodiments all the double bonds in the group R are in the cis configuration or all double bonds are in the cis configuration except the double bond nearest the carbonyl group which may be in the trans configuration.

The group R may have between 16 and 24 carbon atoms, preferably 19 to 21 carbon atoms.

The group R may carry a heteroatom or group of heteroatoms positioned α , β , γ , or δ to the carbonyl, preferably β or γ to the carbonyl. Preferably the heteroatom is O or S or a sulphur derivative such as SO.

Specifically preferred RCOX groups are those of formula



The R group may carry up to three substituents

selected from halo or C₁₋₆-alkyl. If present the substituents are preferably non-polar, and small, e.g. a methyl group. It is preferred however, if the R group remains unsubstituted.

5 The group X is an electron withdrawing group. Suitable groups in this regard include O-C₁₋₆ alkyl, CN, CO₂-C₁₋₆ alkyl, phenyl, CHal₃, CHal₂H, CHalH₂ wherein Hal represents a halogen, e.g. fluorine, chlorine, bromine or iodine, preferably fluorine.

10 In a preferred embodiment the electron withdrawing group is CHal₃, especially CF₃.

Highly preferred compounds for use in the invention are EPACOCF₃, EPASCOCF₃ and AKH 217 as depicted below.

15 Compounds of formula (I) may be manufactured using known chemical synthetic routes. It is convenient to begin synthesis from the commercially available compounds arachidonic acid, EPA or DHA. Conversion of the acid functionality of these compounds into, for example a -COCF₃ group can be achieved readily, e.g. by
20 converting the carboxylic acid into its corresponding acid chloride and reacting the same with trifluoroacetic anhydride in the presence of pyridine.

Introduction of a heteroatom into the carbon chain is also achieved readily. Conveniently, for example,
25 the starting acid is reduced to an alcohol and, if required, converted to the corresponding thiol. The nucleophilic thiol may then be reacted with a group such as BrCH₂COCF₃ thereby introducing the carbonyl and electron withdrawing species. Complete synthetic
30 protocols may be found in J. Chem. Soc., Perkin Trans 1, 2000, 2271-2276 or J. Immunol., 1998, 161, 3421.

35 The compounds of formula (I) may be formulated into medicaments using conventional techniques well known to the skilled pharmaceutical chemist. Thus, the compounds may be formulated with well known excipients or pharmaceutical carriers.

The medicaments of the invention may also comprise

other conventional additives such as antioxidants, preservatives, colouring, flavouring etc.

5 The medicaments of the invention may be formulated as tablets, pills, powder, capsules, emulsions but are preferably in the form of creams or ointments. The mode of administration may be any known mode, such as oral, nasal, transmucosal, parenteral, topical, intradermal etc. However, it is advantageous if the medicament is applied topically, i.e. directly to the infected part of
10 the human skin.

The amount of the medicament required to effect a successful treatment will, of course, depend on the patient and on the severity of the psoriasis. The dose will be readily determined by the skilled chemist.

15 The compounds of the invention may be used to treat psoriasis in combination with other known pharmaceuticals for said purpose and this forms a further aspect of the invention.

The invention is described further below with
20 reference to the following non-limiting examples and figures.

Figure 1 shows the relative inhibition of IVa PLA₂ enzyme activity for a number of compounds of the invention in comparison to commercial compounds.
25 Recombinant IVa PLA₂ enzyme was preincubated with inhibitor (5 μ M) for 10 minutes and then assayed in the mixed-micelle enzyme activity assay. The control was not pretreated with inhibitor. Results are given as % of control and are mean of duplicate determinations from 1
30 out of 4 representative experiments.

Figure 2 shows concentration dependent inhibition of IVa PLA₂ in the mixed-micelle enzyme activity assay. Increasing inhibition of IVa PLA₂ by EPACOCF₃, EPASCOCF₃ and AACOCF₃ are shown in Figure B, and increasing
35 inhibition of IVa PLA₂ by EPACH(OH)COCF₃, DHACOCF₃ and MAFP are shown in Figure A. Results are given as % of control and are mean of duplicate determinations from

one out of 2 to 4 representative experiments.

Figure 3A shows that calcium ionophore A_{23187} stimulates extracellular release of [3H]-labelled lipid in a concentration-dependent manner. HaCaT cells were treated with A_{23187} for 1 hour, arachidonic acid and eicosanoids were extracted from cell media using Bond Elut C18 columns and contents of [3H]-labelled lipids in media were determined by scintillation counting. Each column in the figure represents the average of triplicate determinations from one out of 3 representative experiments.

Figure 3B shows that calcium ionophore A_{23187} dosedependently also stimulates production of prostaglandin E_2 (PGE_2), a product of cyclooxygenase modification of arachidonic acid (AA). PGE_2 accumulation in medium was assayed by PGE_2 enzyme immunoassay (EIA).

Figure 4 shows that the fatty acid derivative EPACOCF₃ dosedependently is more powerful than the commercially available cyclooxygenase 2-selective inhibitor NS-398 in inhibiting A_{23187} -induced PGE_2 release in LPS-stimulated HaCat cells (IC_{50} values 180 nM and 240 nM, respectively).

Figure 5 shows that $TNF\alpha$ or $IL-1\beta$ stimulated NF-kB activation is dose-dependently inhibited by AKH217 (91% and 81% respectively).

MATERIALS AND METHODS

Materials

Calcium ionophore A_{23187} , Sigmacoat, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) and bovine serum albumin were obtained from Sigma (St. Louis, MO, USA). Phosphatidylcholine, 1-stearoyl-2-arachidonyl and [3H] arachidonic are from Amersham (Buckinghamshire, UK). Aluminium sheets silica gel 60, ethyl acetate, iso-octane and acetic acid were purchased from Merck (Darmstadt, Germany). $TNF\alpha$ was a generous gift from Professor Terje Espevik, Norwegian University

of Science and Technology, NTNU and IL-1 β was purchased from Roche Molecular Biochemicals.

5 AACOCF₃ is from BIOMOL (Plymouth Meeting, PA, USA), and MAFP is from Cayman (Ann Arbor, MI, USA). All fatty acid compounds were stored under N₂ at -80°C.

Cell culture

10 The spontaneously immortalized human skin keratinocyte cell line HaCaT was kindly provided by Prof. N. E. Fusenig (Heidelberg, Germany). Cells were grown in Dulbecco's Modified Eagle's Medium (DMEM) with 1 g glucose/l (Gibco BRL, Life Technologies Ltd, Paisley, Scotland), supplemented with 5% fetal calf serum (FCS) 15 (HyClone Laboratories, Inc., Utah, USA), 0.3 mg/ml L-glutamine (Sigma Chemical Company, St. Louis, Mo, USA), 0.1 mg/ml gentamicine (Sigma) and 1 μ g/ml fungizone (Gibco). Confluent cells were stimulated with A₂₃₁₈₇, IL-1 β (10mg/ml) or TNF α (10mg/ml) in 0.5% (v/v) FCS for 1 20 hour before harvesting. Passages 40-80 of the cells were used. Generation of HaCat transfectants expressing luciferase under strict control of transcription factor NF-kB is described elsewhere (Anthonsen et al, J. Biol. Chem. 2001, 276, 30527). The reporter plasmid pBIIX 25 contains two copies of the HIV NF-kB sequence cloned upstream of the mouse *fos* promoter driving expression of the *Photinus pyralis* luciferase gene.

EIA detection was performed according to manufacturer description, Cayman Chemical, MI, USA. A 30 dilution of 1:10 was used for PGE₂ measurement. Microplate Manager Software (Bio-Rad Laboratory) calculated sample data.

Luciferase Assay

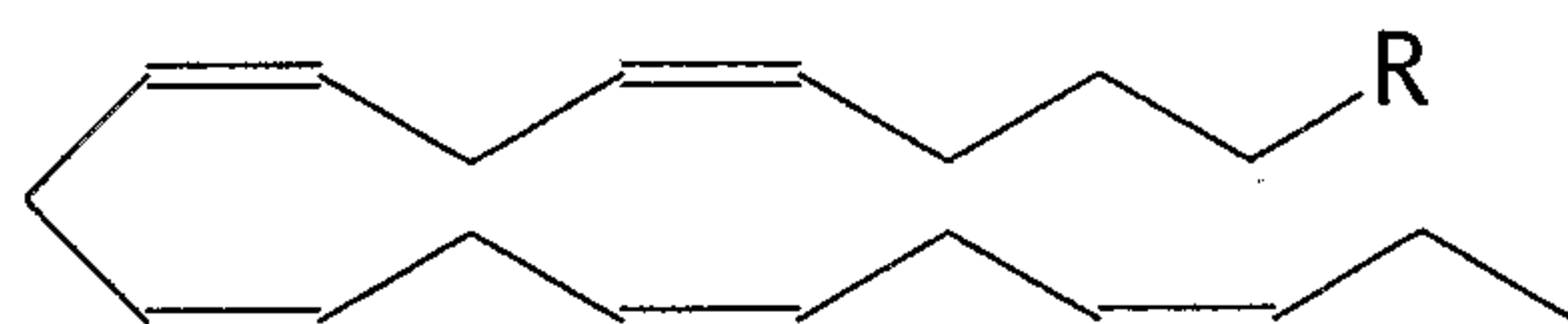
35 Cells were seeded in 24-round multiwell plates (2.8 x 10⁵ cells/well). Treated cells were washed two times with phosphate-buffered saline and lysed, and luciferase

activities were determined using the Luciferase Reporter Assay system (Promega) and Turner Luminometer model TD-20/20 (Turner Designs) as described by the manufacturer.

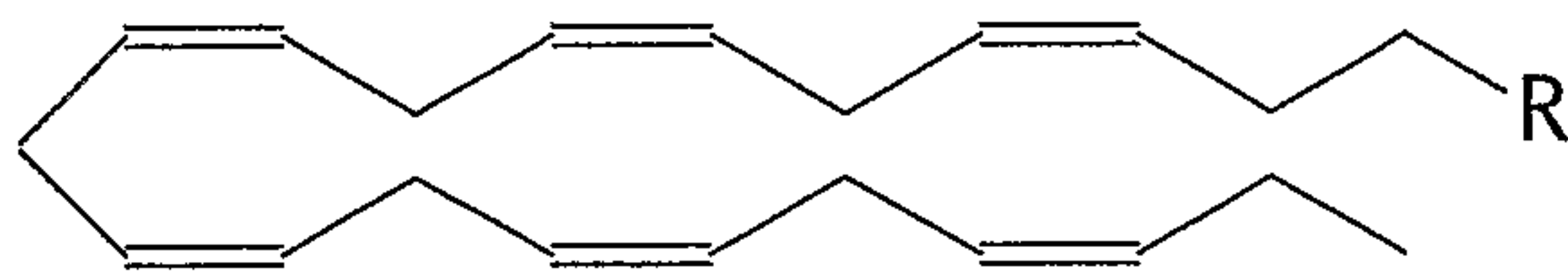
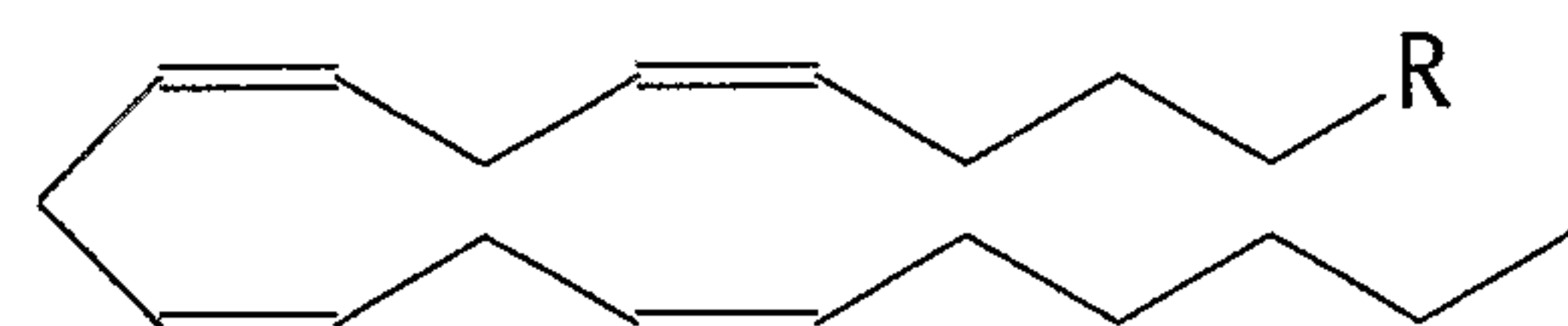
5 Synthesis of (all-Z)-eicosa-5,8,11,14,17-pentaenoic acid (EPA) and (all-Z)-docosa-4,7,10,13,16,19-hexaenoic acid (DHA) derivatives

The derivatives used in the enzyme assays are shown below. EPACOCF₃ was prepared as described in J. Immunol., 1998, 161, 3421. AACOCF₃ and MAFP were bought from suppliers as mentioned above. The remaining derivatives were prepared as described in J. Chem. Soc. Perkin Trans. 1, 2000, 2271-2276.

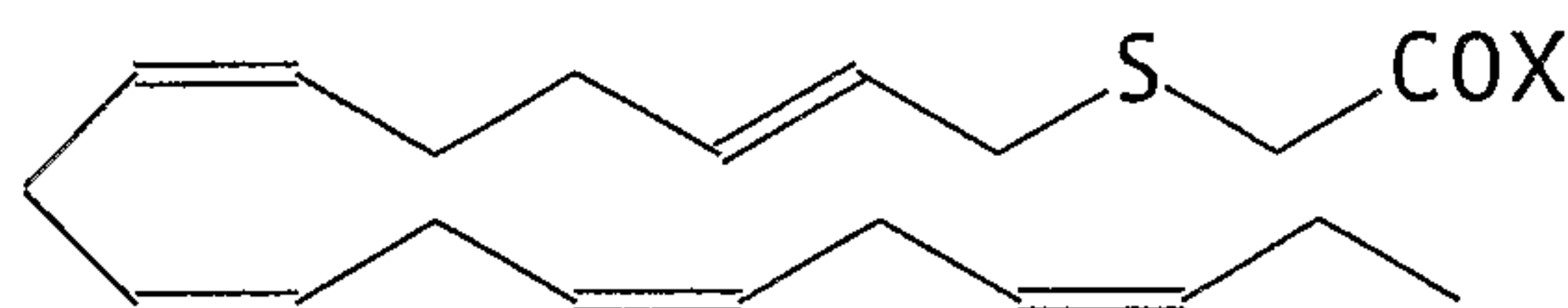
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R = COCF₃ ⇒ EPACOCF₃R = COCH₃ ⇒ EPACOCH₃

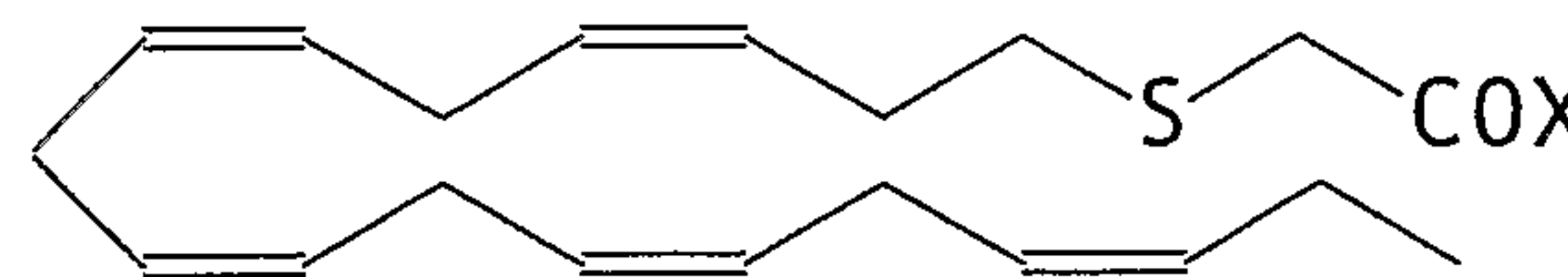
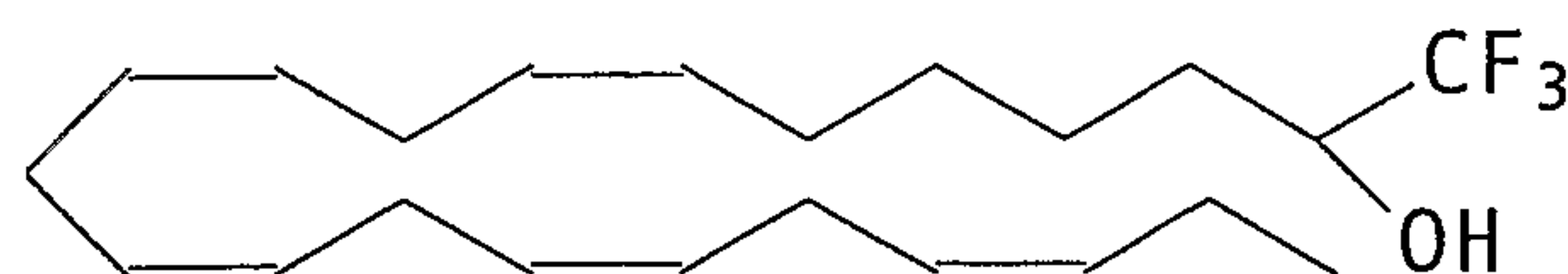
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R = COCF₃ ⇒ DHACOCF₃R = COCF₃ ⇒ AACOCF₃R = POF-CH₃ ⇒ MAFP

25

X = CF₃ ⇒ AKH217

30

X = CF₃ ⇒ EPASCOCF₃EPACH(OH)CF₃

35

Mixed-micelle assay of cPLA₂ activity

Sources of IV PLA₂ enzyme activity were insect cells over

expressing recombinant human IV PLA₂ (10 µg IV PLA₂ protein/10⁶ cells; Bac PAK Baculovirus expression system; CLONTECH Laboratories, Palo Alto, CA, USA). Cytosolic fractions of insect cells were prepared as described in Schalkwijk et al (1992) Eur. J. Biochem. 210, 169-176. The protein contents of the cytosolic fractions were measured with Bio-Rad protein assay (Bio-Rad Laboratories GmbH, Munich, Germany) using bovine serum albumin as standard. The inhibitory derivatives were added 10 minutes prior to substrate addition. The preincubation of inhibitors was performed at room temperature. IV PLA₂ enzyme activity was analyzed using [14C]-L-3 Phosphatidylcholine, 1-stearoyl-2-arachidonyl as substrate according to Wijkander et. al. (Eur. J. Biochem. 202, 873-880, 1991). After 30 min the reaction was stopped and centrifuged, the CHCl₃ phase was evaporated with N₂ gas to dryness and then resuspended in CHCl₃:MeOH (9:1, v/v). Thin layer chromatography (TLC) separated free arachidonic acid from phospholipids on aluminum sheets silica gel 60 developed with ethyl acetate: iso-octane:acetic acid: water (55: 75: 8:100, v/v/v/v) (Gronnich et al, J. Clin. Invest., 93, 1224-1233, 1994). Phosphor-Imager quantified free arachidonic acid and phospholipids, and IV PLA₂ activity was expressed as decreased arachidonic acid release by enzyme incubated with inhibitor compared to no inhibitor.

Arachidonic acid and eicosanoid detection

Confluent cells were labelled with 1 µCi/ml [³H] arachidonic acid in media supplemented with 0.5 % (v/v) FCS 24 hours before cell induction and inhibition. About 90 % of the radioactive arachidonic acid were incorporated in the cell membranes. Extracellular [³H] arachidonic acid was removed by washing the cells 3 times in media. The HaCaT cells were then preincubated with inhibitor for 1 hour and stimulated with calcium

ionophore for 1 hour. The cell media were collected and cleared by centrifugation. Arachidonic acid and eicosanoids were extracted from media using Bond Elut C18 octadecyl columns (500 mg) (Varian SPP, Harbor City, CA) as described by Powell, Anal. Biochem. 164, 117-131, 1987; with modifications previously described Brekke, Cytokine, 4, 269-280, 1992. The samples were collected in glass tubes precoated with Sigmacoat. The ethyl acetate solution of samples were completely dried with N₂, redissolved in 0,5 ml fresh ethyl acetate and aliquots of 50 µl (triplicates) samples were subjected to liquid β-scintillation counting (Beckman LS 1701) in 5 ml Ready Protein liquid (Beckman).

The amount of PGE₂ in cell culture media from calcium ionophore stimulated HaCaT cells was measured using an enzyme immunoassay (EIA; Cayman). The assay is based on the competition between free PGE₂ and a PGE₂-acetylcholinesterase for a limited amount of PGE₂ monoclonal antibody. The media were diluted 1:10 before analyzing of the PGE₂ contents. Microplate Manager Software (Bio-Rad Laboratory) calculated the sample data.

MTT assay

Confluent cells were pretreated with inhibitors in serum free medium for 1 hour, and then treated with stimulating agent for 1 hour. Conversion of substrate [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide] was measured as optical density at 580 nm after 4 hours according to Mosmann [Mosmann T, J.Immunol. Methods 65, 55-63, 1983]. For each concentration of inhibitor nine parallels were measured.

RESULTS

IV PLA₂ enzyme activity

In order to investigate the action of the fatty acid

derivatives as inhibitors of IV PLA₂, we measured the IV PLA₂ activity in the mixed micelle assay with recombinant IV PLA₂ as enzyme source, as described in materials and methods. The synthetic fatty acid derivatives we made are listed above, together with the commercial available inhibitors, which we used for comparison. EPACOCF₃, EPASCOCF₃ and AKH-217 seem to have the same potency as IV PLA₂ inhibitors as AACOCF₃ (i.e. 75-80 % inhibition) (Figure 1). MAFP and DHACOCF₃ were poorer IV PLA₂ inhibitors (50% and 30% inhibition respectively). The compound EPACH(OH)CF₃ was also tested which results in severe attenuation of inhibitory effect (10% inhibition). EPACOHCH₃ was made as a control compound with methyl instead of the trifluoromethyl (CF₃) group. EPACOHCH₃ showed no inhibition (figure 1). The IC₅₀ values of EPACOCF₃, EPASCOCF₃ and AACOCF₃ were measured to be 2.9 ± 1.9 , 3.5 ± 0 μ M and 5.8 ± 1.9 μ M respectively (Figure 2B). While the IC₅₀ values of DHACOCF₃, MAFP and EPACH(OH)CF₃ were determined to be 21.3 ± 1.5 μ M, 24 ± 1.4 μ M and 43 ± 7.1 μ M respectively (Figure 2A).

Kinetic studies with the inhibitors in the mixed micelle assay were performed in order to see if the time course was linear. A peak was achieved in two minutes (results not shown), indicating that the inhibitors are very fast acting.

In summary EPACOCF₃, EPASCOCF₃ and AKH217 seem to have similar or perhaps slightly higher potency as the commercially available compound AACOCF₃ in inhibiting IVa PLA₂.

Arachidonic acid and eicosanoid detection.

In order to evaluate the effect of EPA and DHA derivatives in a more biological system, we utilized the HaCaT cells as a model system [Sjursen et al, Cytokine, 12, 8, 1189-1194, 2000]. The calcium ionophore A₂₃₁₈₇ has

been shown to induce arachidonic acid release in many cell types, probably by increasing the intracellular Ca^{2+} -concentration and thereby inducing the association of cPLA₂ with cellular membranes [Kramer and Sharp, FEBS Lett, 410, 49-53, 1997]. In HaCaT cell, the ionophore induced a dose response release of [3H]-labelled arachidonic acid (Figure 3A).

Concentrations higher than 10 μM of A₂₃₁₈₇ were toxic as determined by MTT assay.

The next step in evaluating our synthetic fatty acid inhibitors was to examine their ability to reduce the extracellular release of PGE₂ in response to A₂₃₁₈₇ in HaCaT cells. Before the cell experiments were performed, we evaluated the toxicity of the inhibitors. MTT assay showed that concentrations of 25 μM and higher of the fatty acid compounds are toxic to HaCaT cells (results not shown).

HaCat cells upregulate expression of cyclooxygenase 2 message when treated with LPS (200 ng/ml, 5% human serum) for 30 min. (unpublished results). Upon ionophore stimulation for one hour, PGE₂ accumulates in medium (Figure 3B). The fatty acid derivative EPACOCF₃ was compared to NS-398, a cox-2 selective inhibitor, in its potency to reduce PGE₂ production by preventing release of its precursor AA. HaCat cells were pretreated with LPS (200 ng/ml, 5% human serum) for 30 min., treated with either inhibitors at different concentrations (0.2-25 μM) for another 30 min and stimulated with A₂₃₁₈₇, 30 min. Finally, PGE₂ EIA was performed on cell media. The dose-response curve was constructed with respect to percent PGE₂ production without inhibitor. As can be observed in Figure 4, EPACOCF₃ is much more potent than NS-398 in inhibiting PGE₂ production (IC₅₀ values of 180 nM and 240 nM, respectively), and indicates that substrate deprivation may be more powerful than activity inhibition for the cyclooxygenase.

In order to determine if inhibition of IVa PLA₂ has

any biologic consequence, HaCat cells were stimulated with the proinflammatory cytokines IL-1 β or TNF α . As a measure of inflammation, activation of the transcription factor NF-kB was analysed. We have shown earlier that

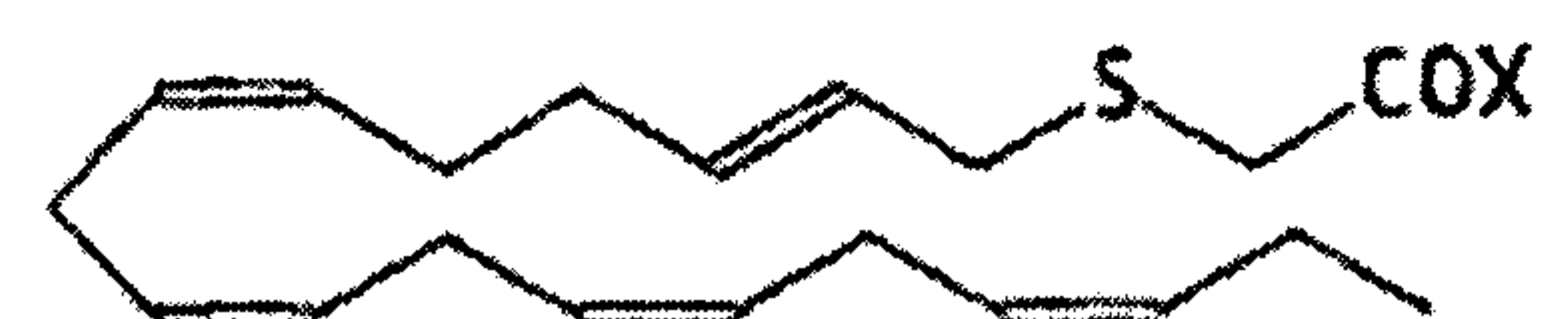
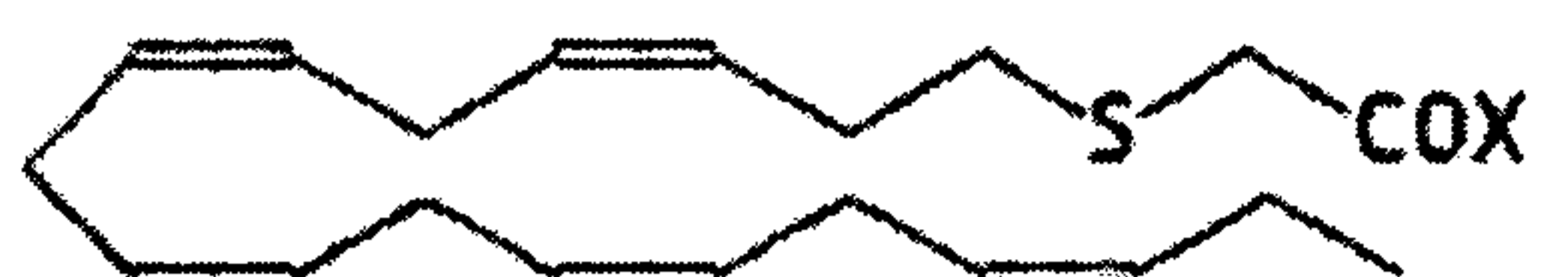
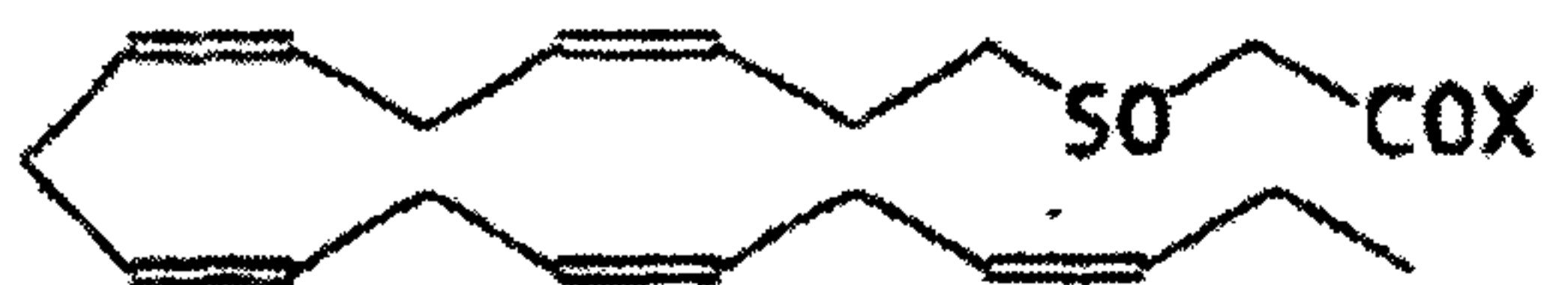
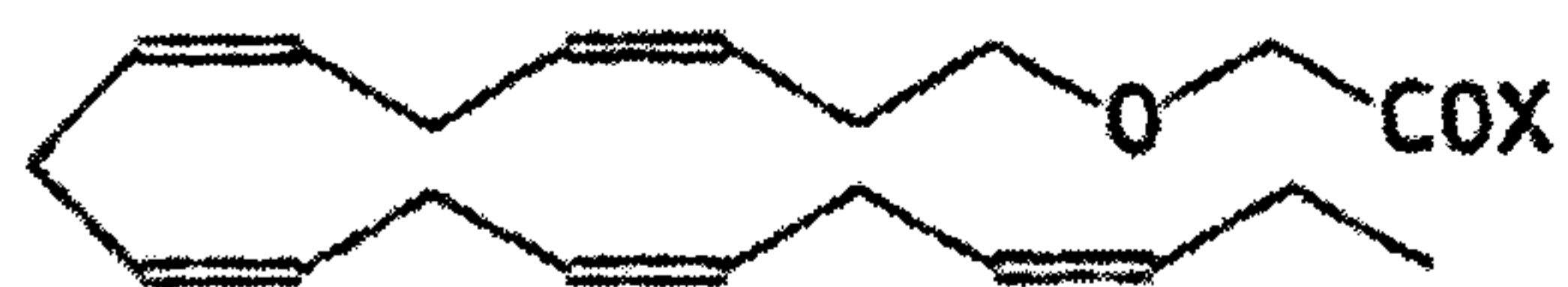
5 TNF α or IL-1 β activates NF-kB in HaCat cells (Thommesen et al, J. Immunol., 1998, 161, 3421). NF-kB activation was analysed as luciferase expression. Treatment of the stably transfected HaCat-pBIIIX cells with TNF α or IL-1 β for 1 h enhanced NF-kB-dependent expression (not shown).

10 In the presence of inhibitors AKH217, IL-1 β stimulated luciferase expression was dose-dependently inhibited by 81%. TNF α stimulated NF-kB activation was inhibited dose-dependently AKH217 by 91% (Figure 5) thus confirming that our synthetic fatty acid inhibitors may

15 be useful in inhibiting inflammatory responses.

Claims

1. Use of a compound of the formula:

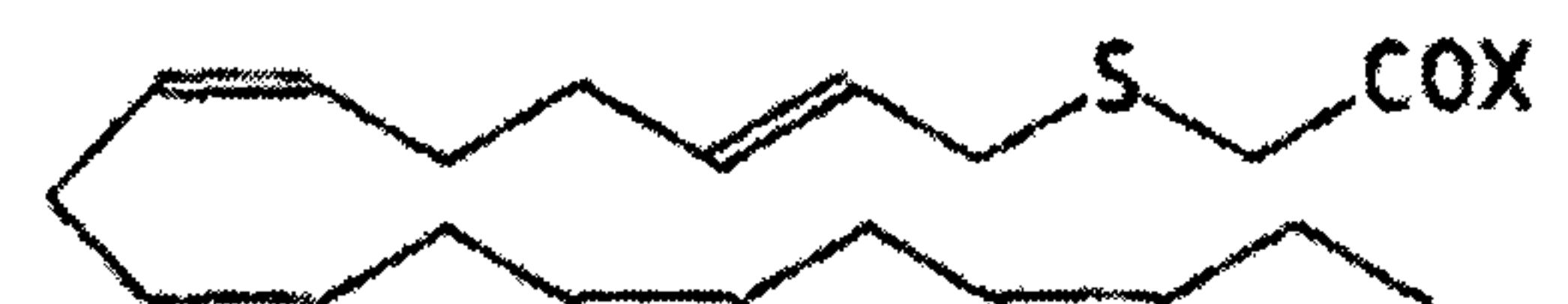
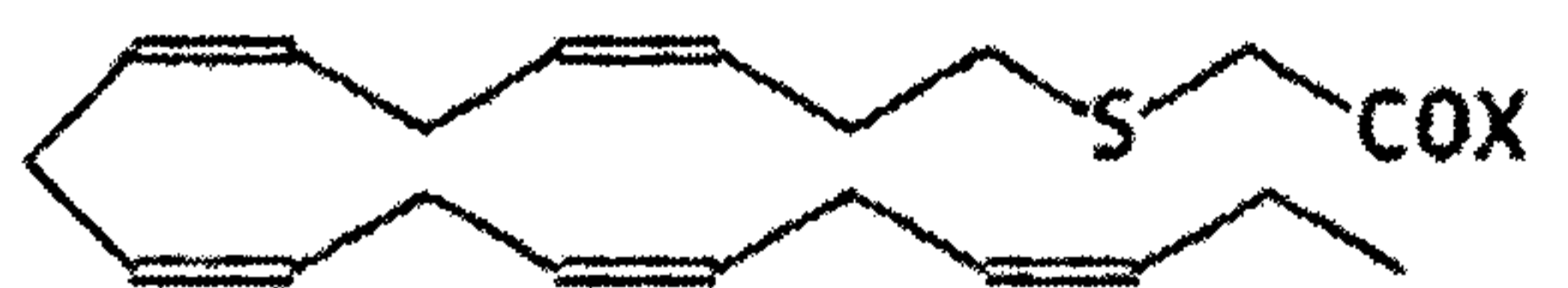
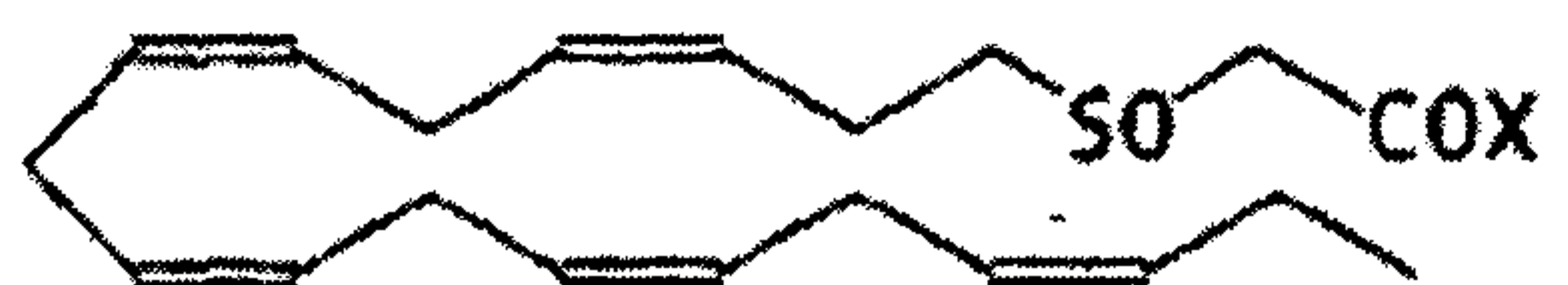
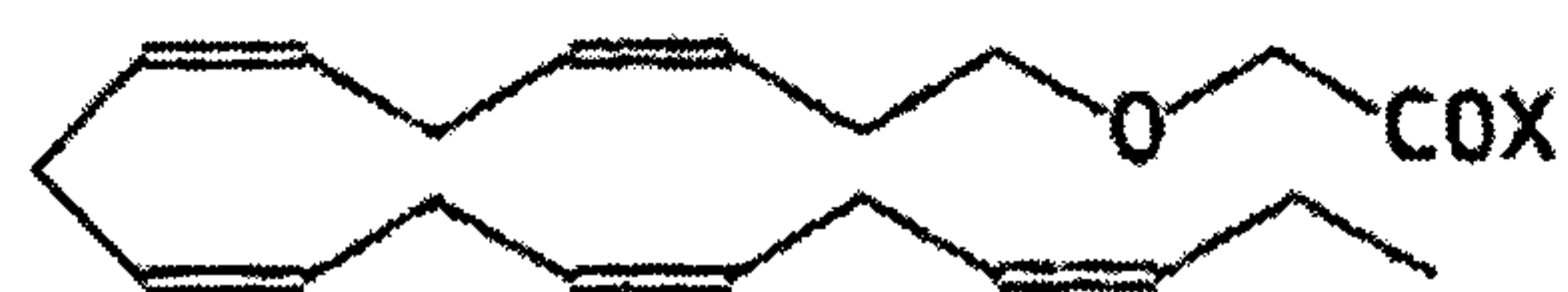


or

wherein X is CF₃,

in manufacture of a medicament for treatment of psoriasis.

2. Use of a compound of the formula:



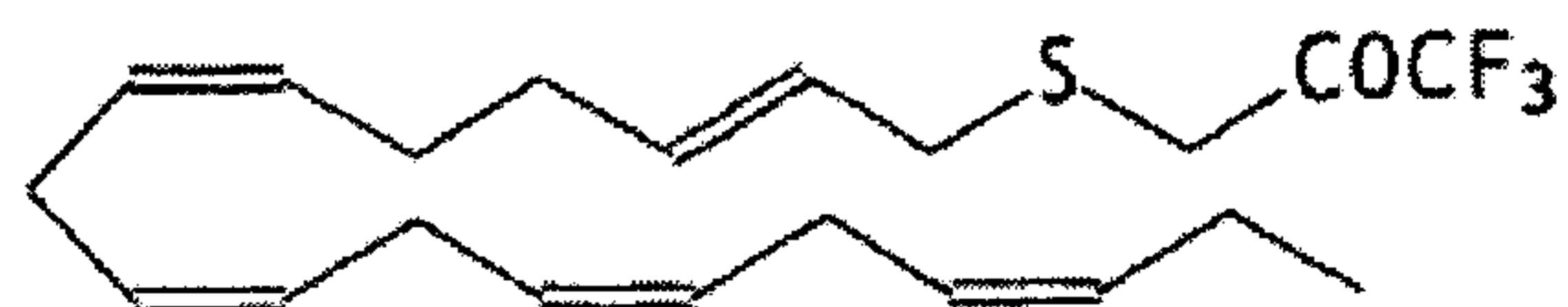
or

wherein X is CF₃,

for treatment of psoriasis.

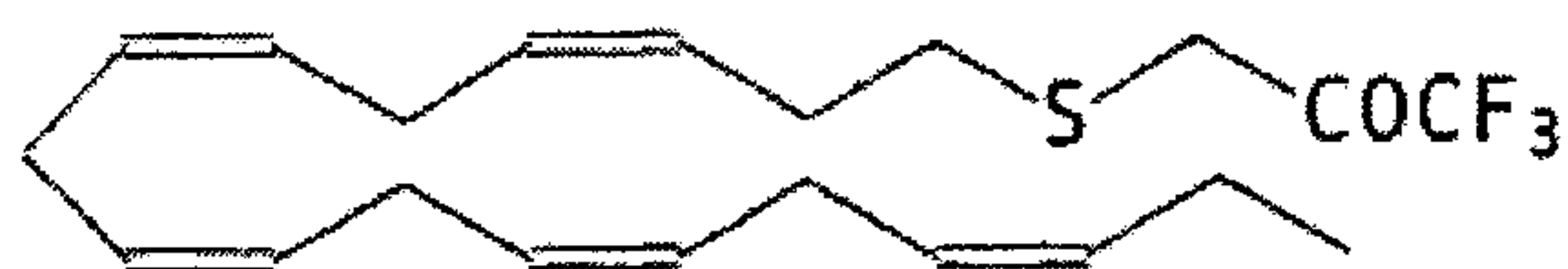
16

3. Use of claim 1 or 2, wherein the compound is:



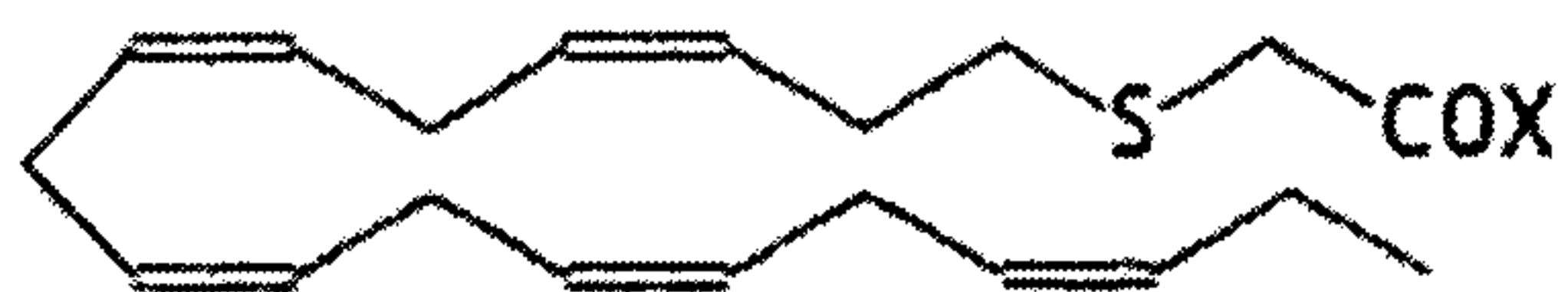
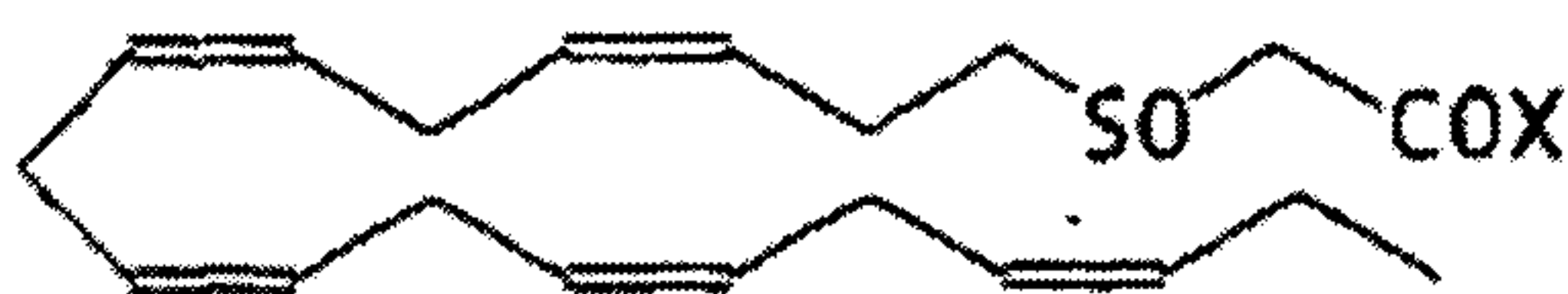
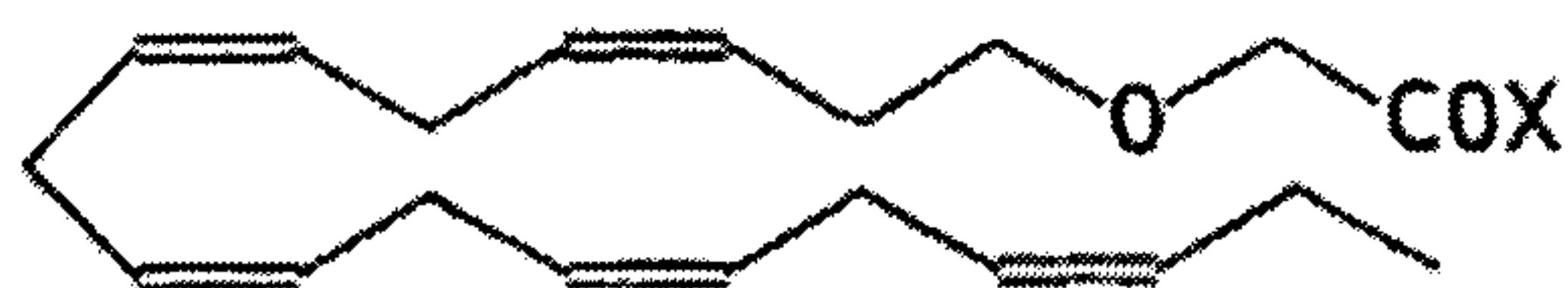
or

5



4. A pharmaceutical composition for use in treatment of psoriasis, the composition comprising a pharmaceutically acceptable carrier or excipient and a compound of the formula:

10



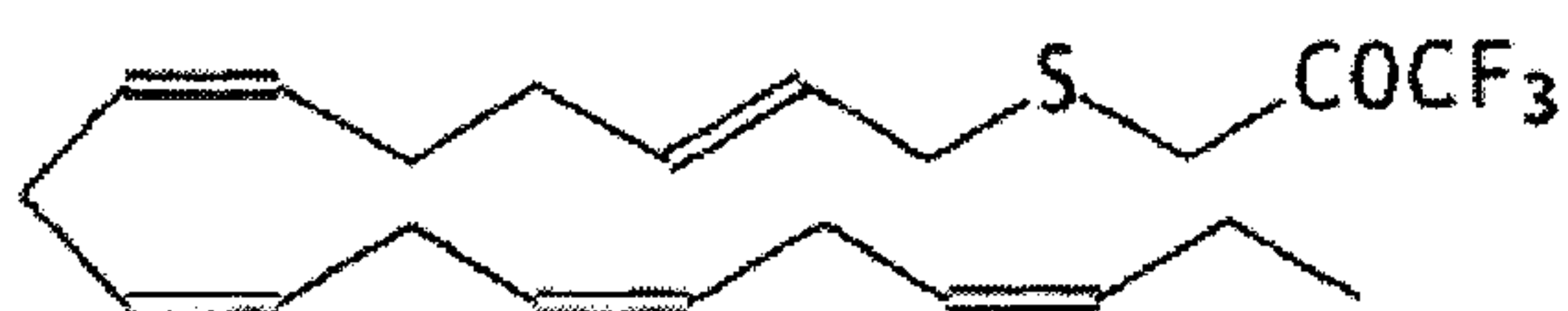
or



wherein X is CF₃.

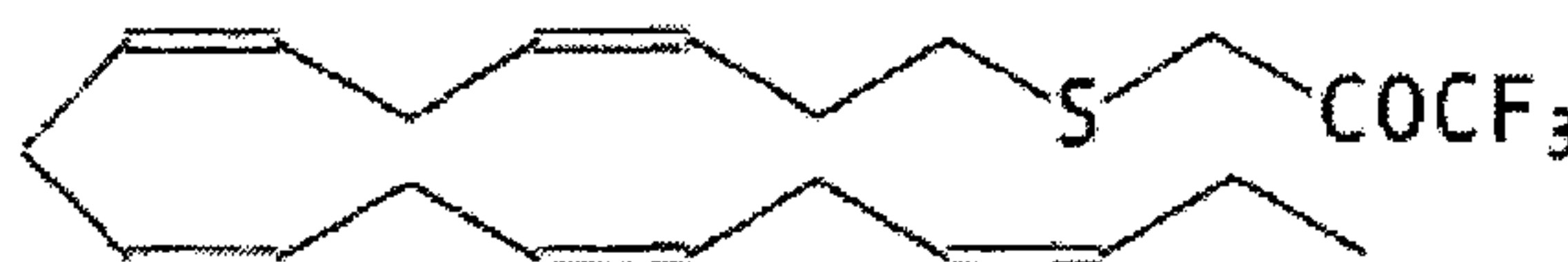
15

5. The composition of claim 4, wherein the compound is:



or

20



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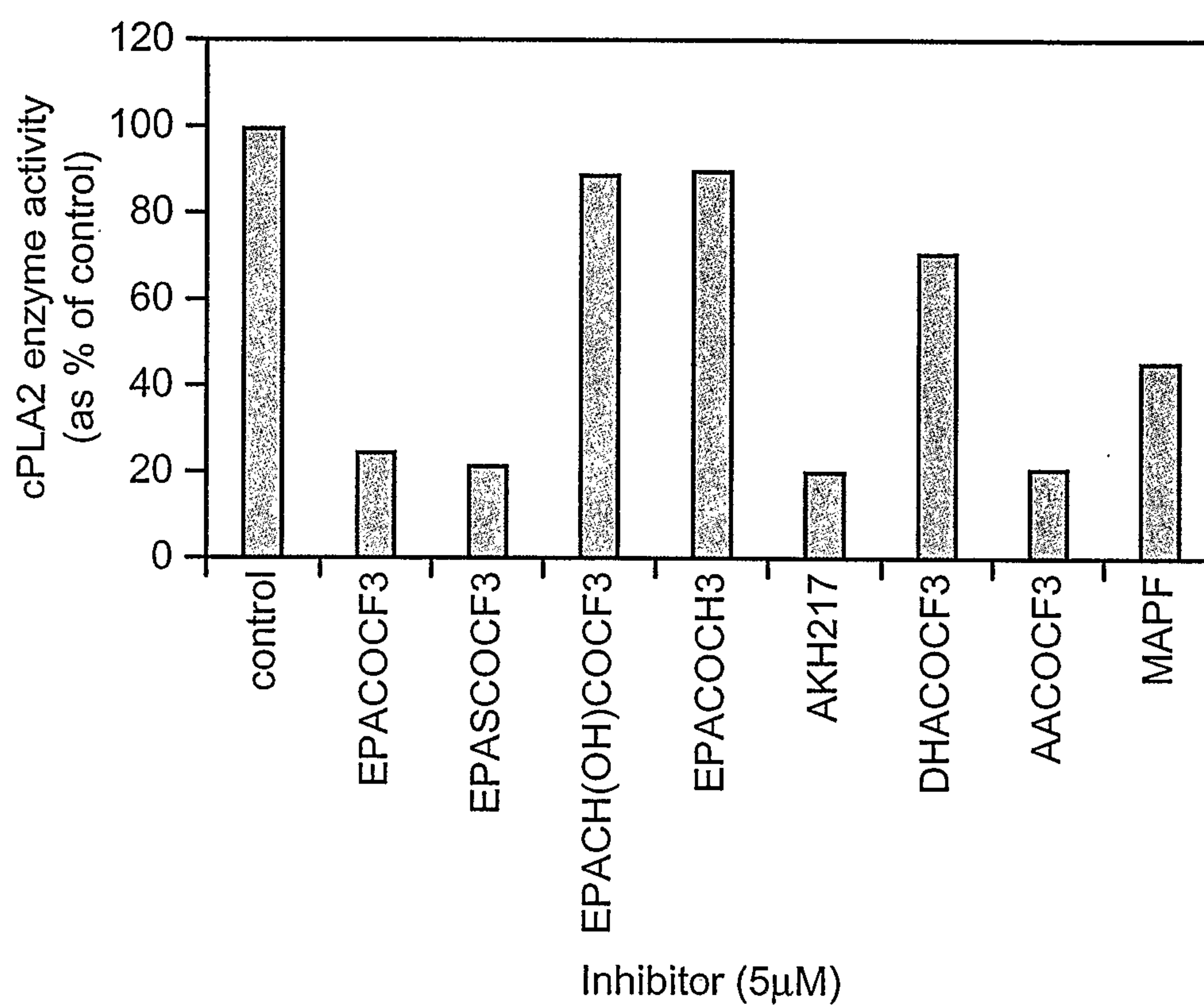


FIG. 1

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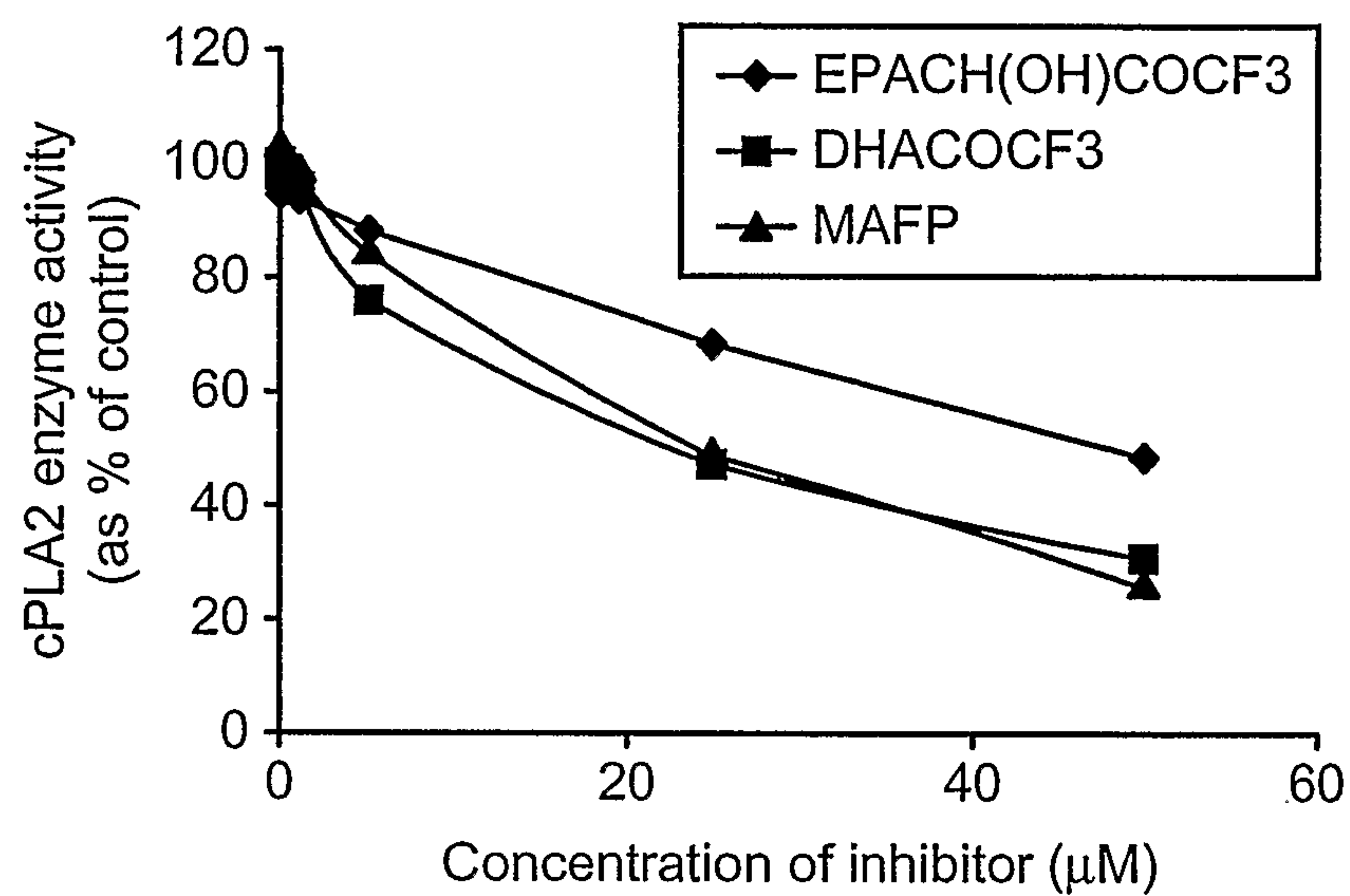


FIG. 2A

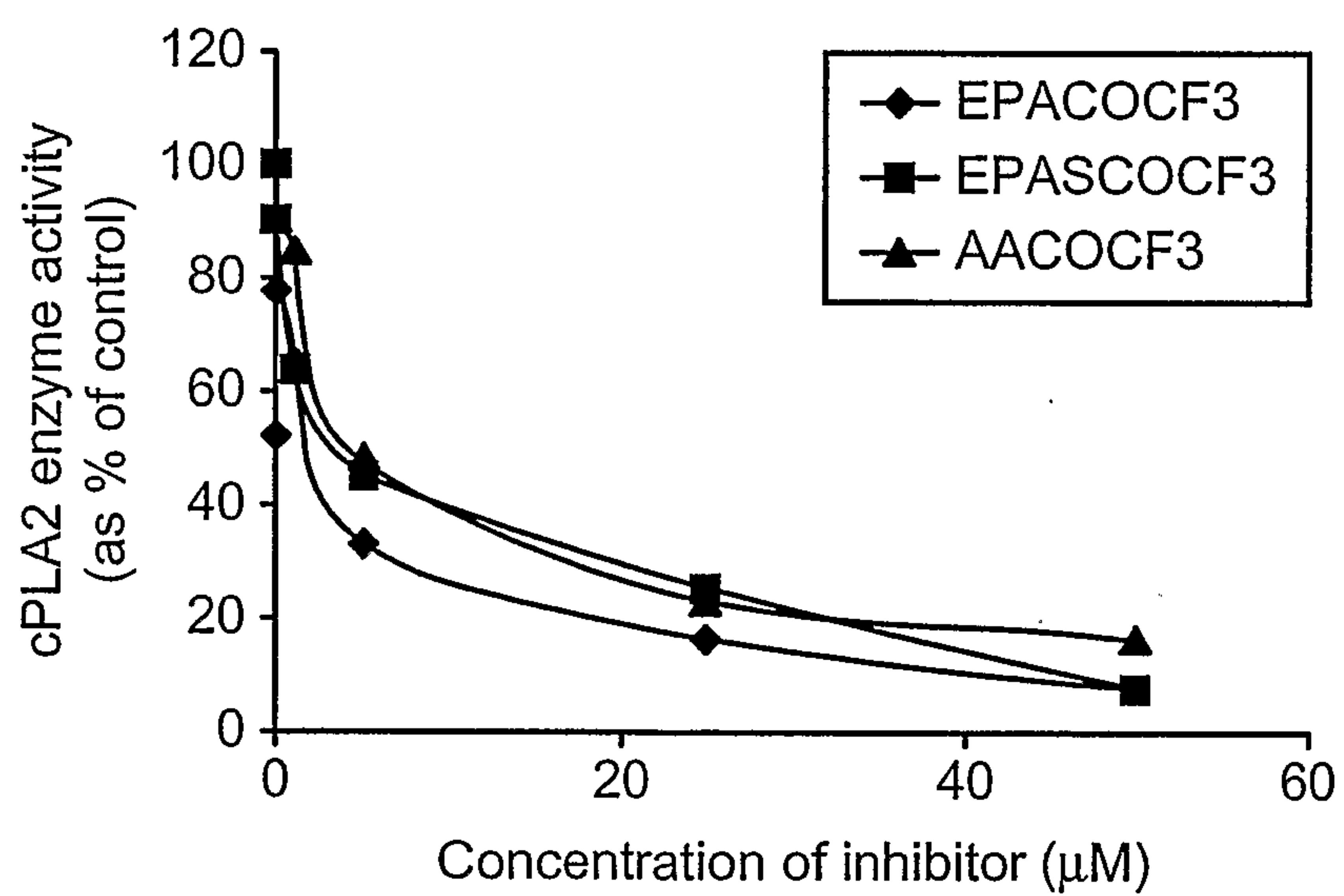


FIG. 2B

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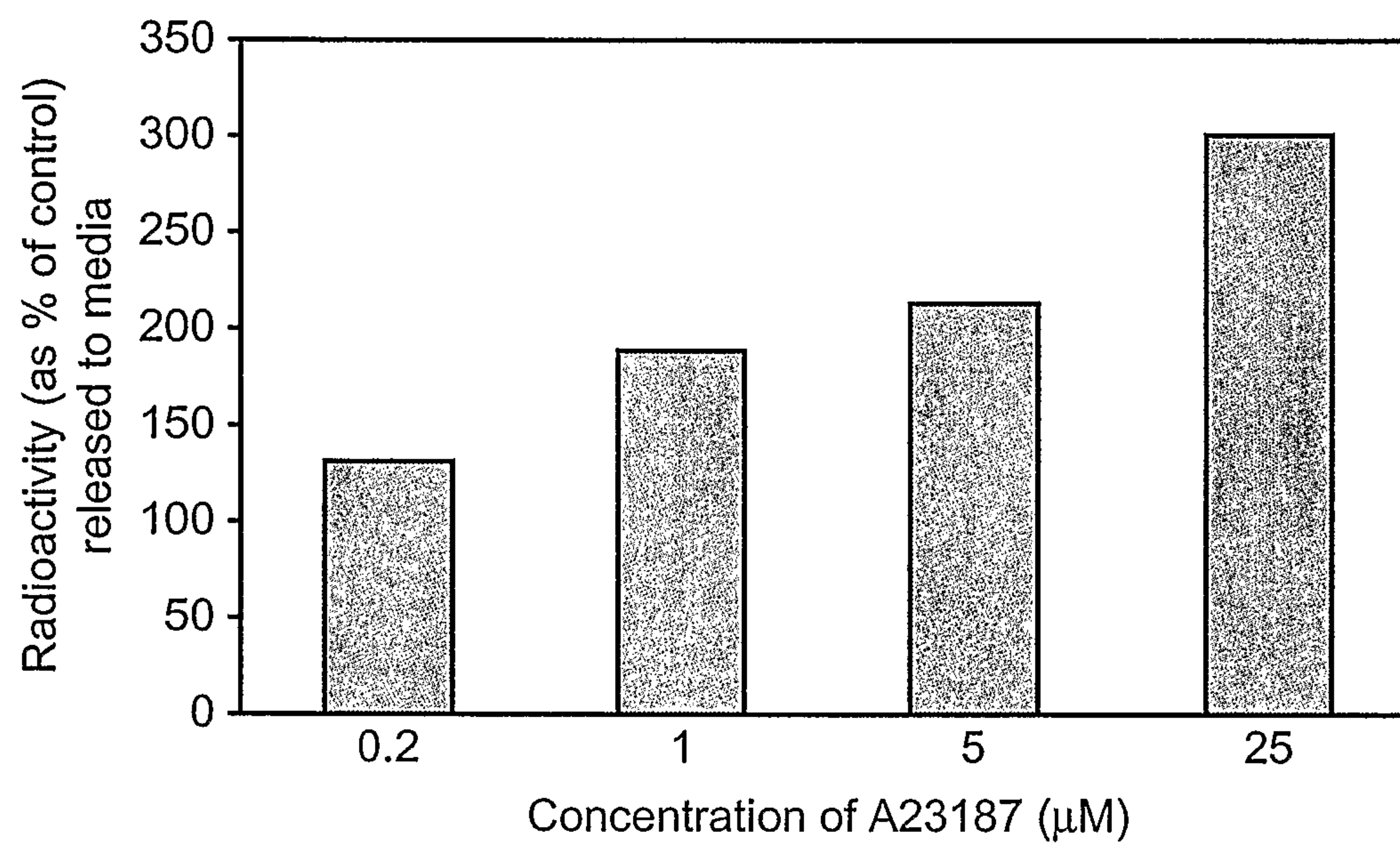


FIG. 3A

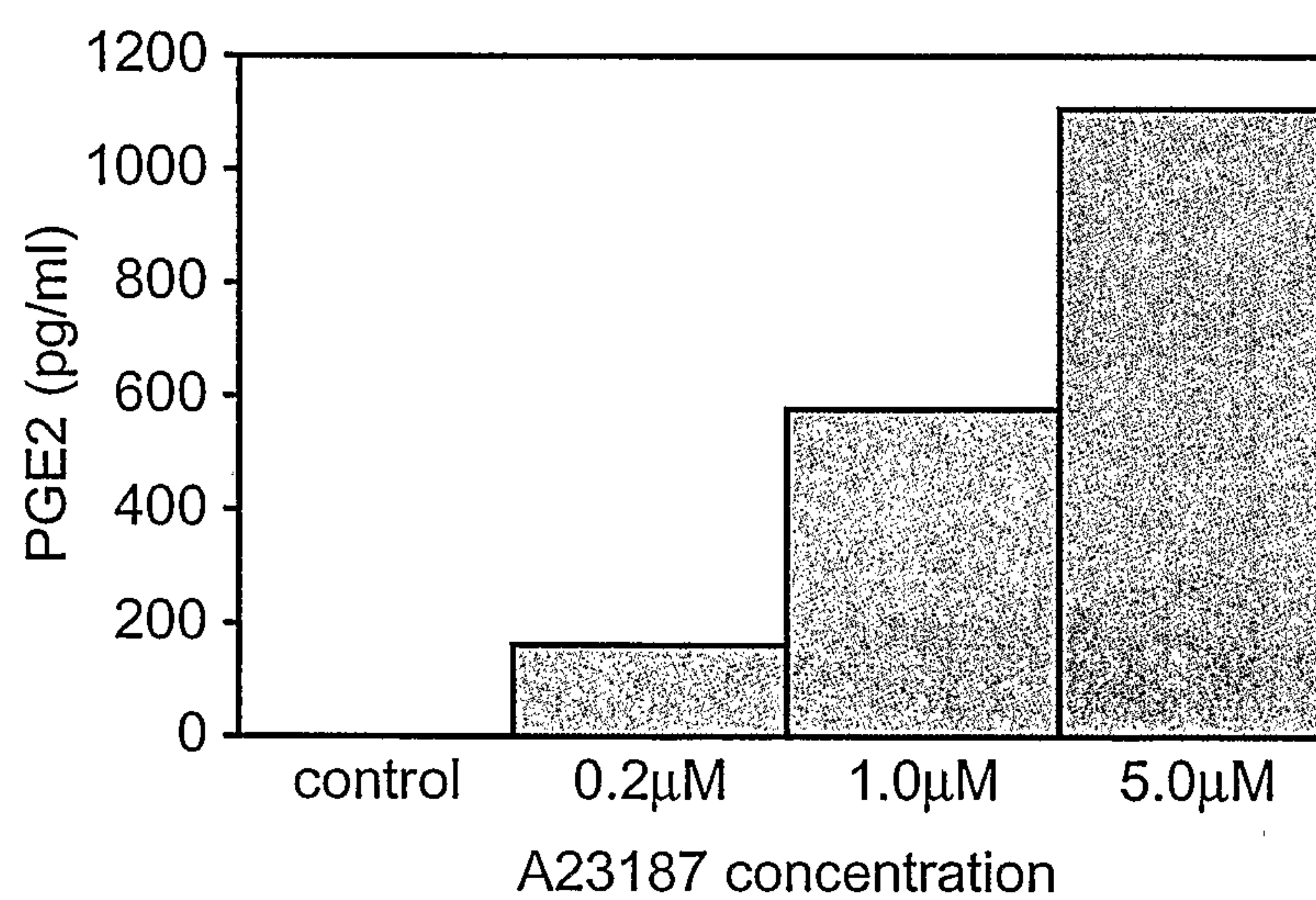


FIG. 3B

4 / 4

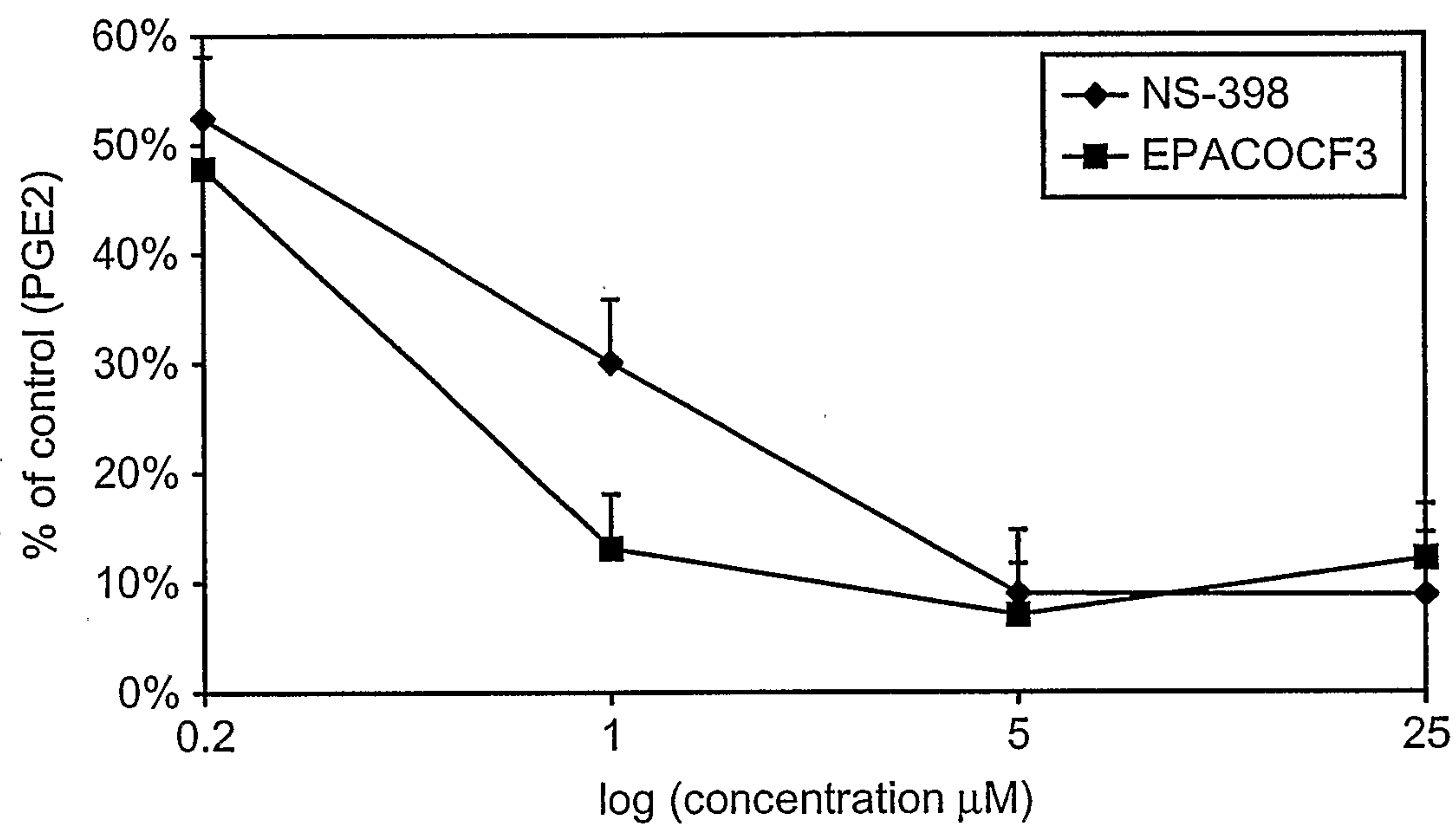


FIG. 4

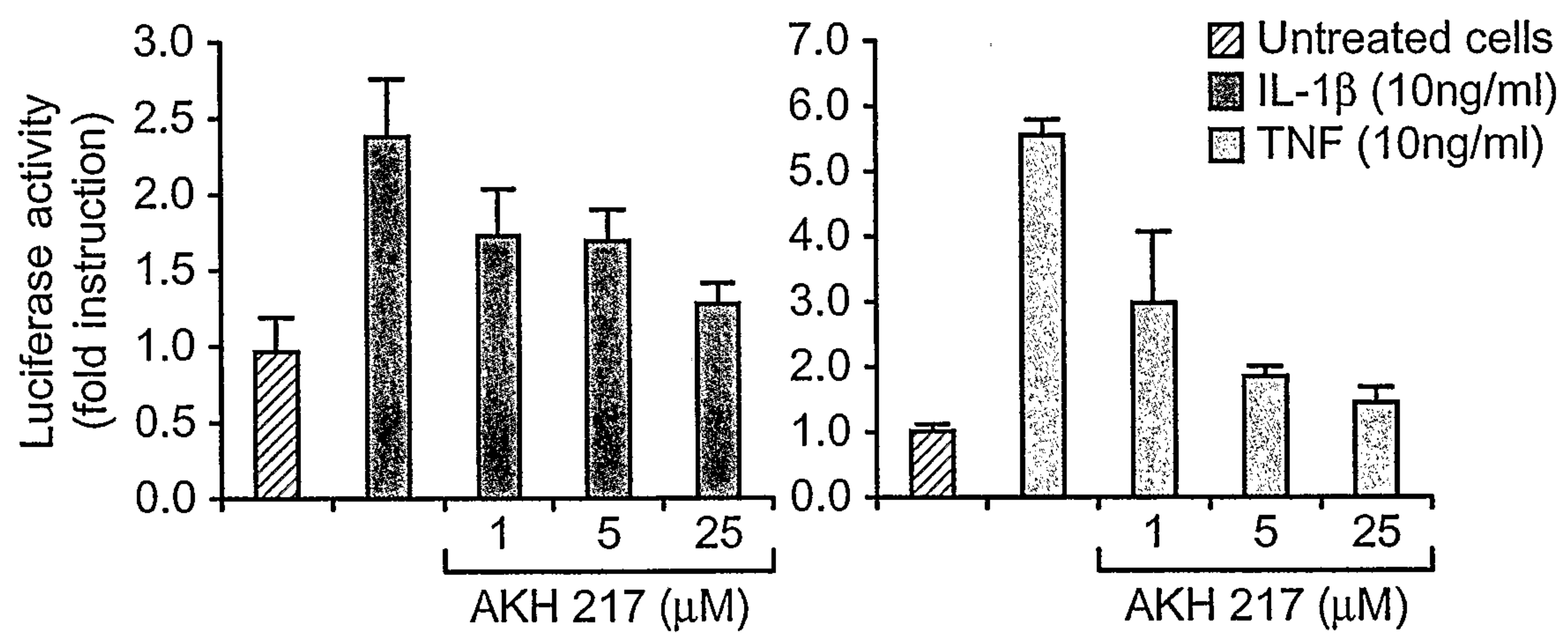


FIG. 5