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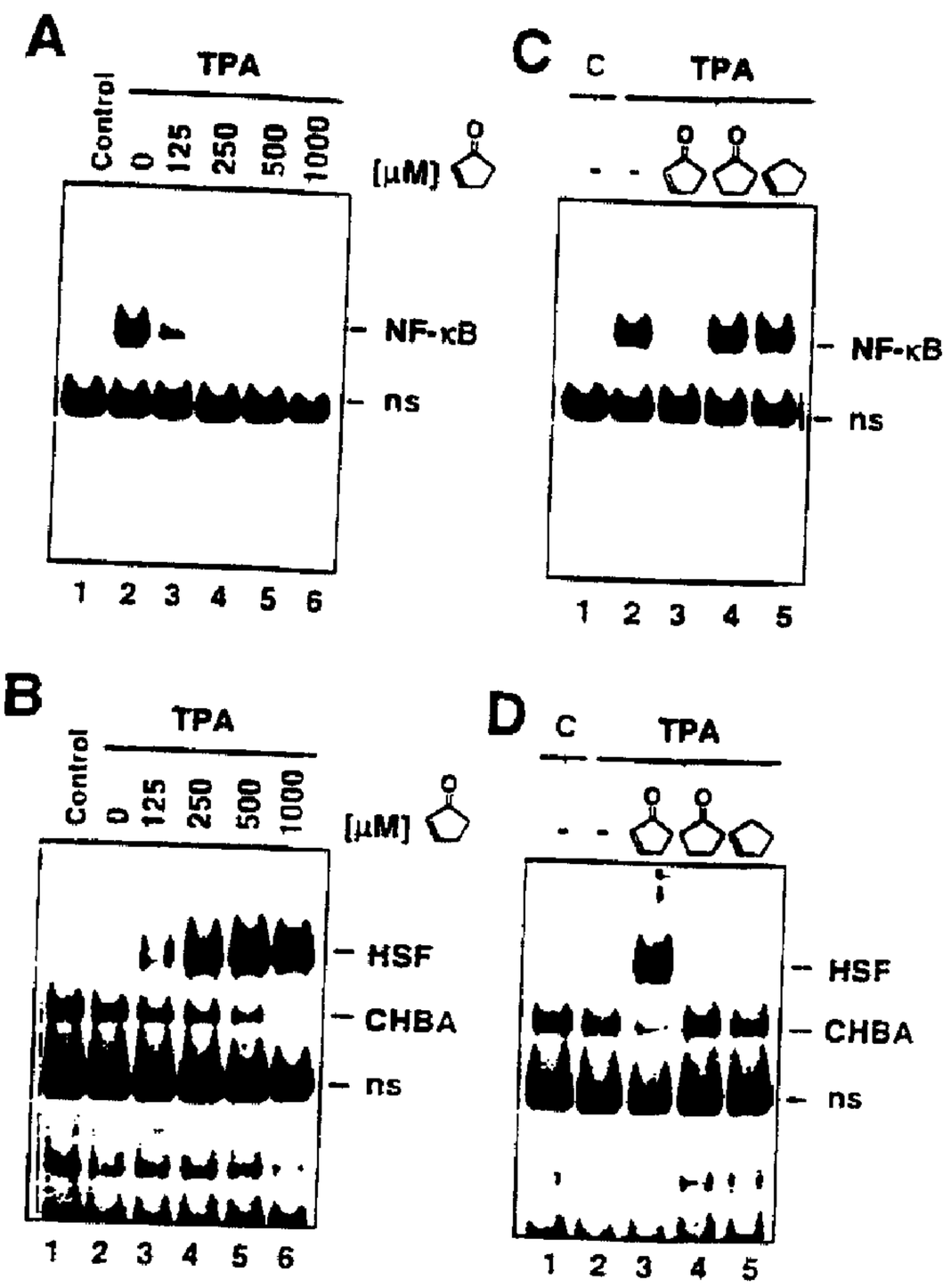
(54) Title: 2-CYCLOPENTEN-1-ONE AND ITS DERIVATIVES AS INHIBITORS OF THE NF-κB FACTOR



(57) Abrégé/Abstract:
2-Cyclopenten-1-one and its derivatives comprising the cyclopentenone nucleus as inhibitors of the NF-κB factor, with anti-inflammatory, anti-proliferative, immuno-suppressive, cytoprotective and antiviral activity, the substituents being selected among the ones which do not affect the NF-κB inhibitory activity.

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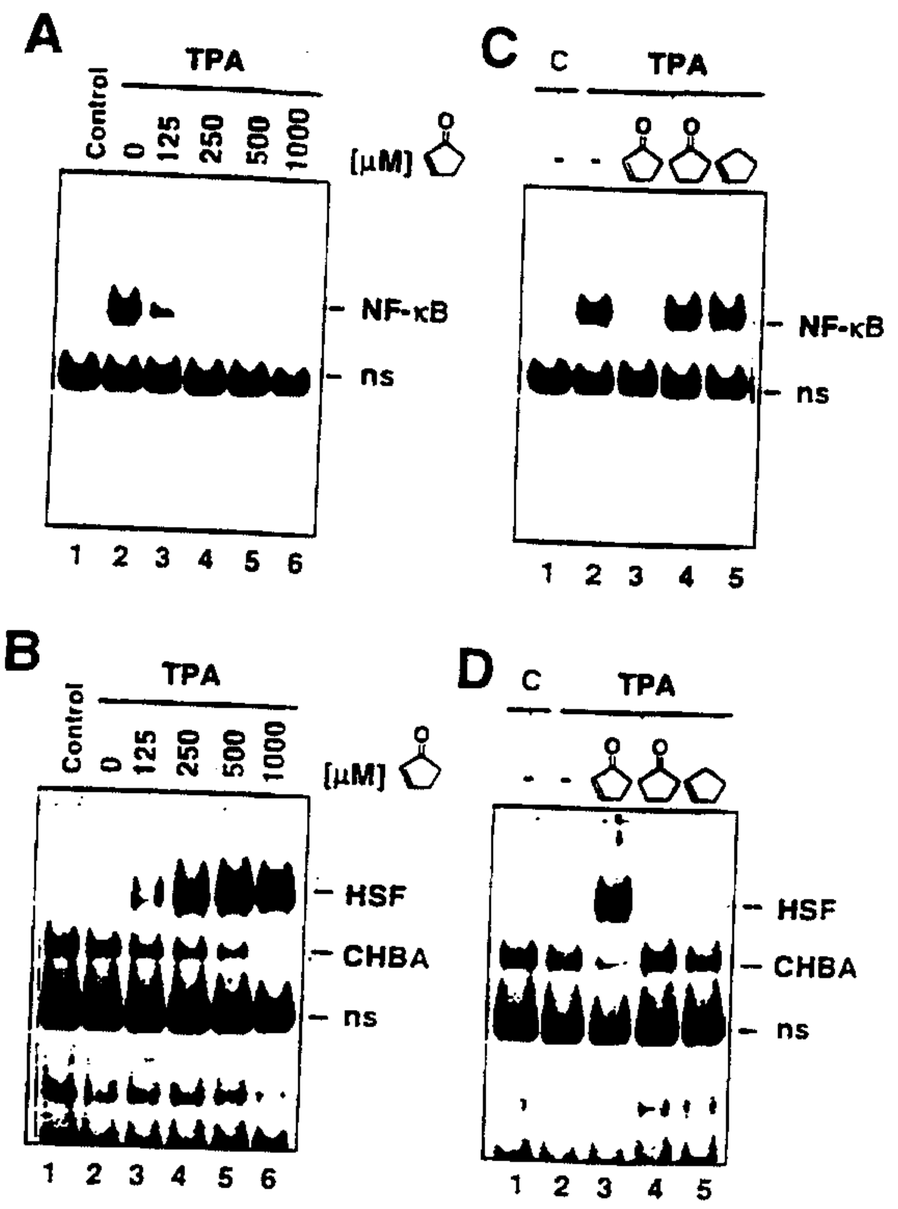
(57) Abstract

2-Cyclopenten-1-one and its derivatives comprising the cyclopentenone nucleus as inhibitors of the NF-*k*B factor, with anti-inflammatory, anti-proliferative, immuno-suppressive, cytoprotective and antiviral activity, the substituents being selected among the ones which do not affect the NF-*k*B inhibitory activity.



2-CYCLOPENTEN-1-ONE AND ITS DERIVATIVES AS INHIBITORS OF THE NF-*k*B FACTOR

FIELD OF INVENTION

The present invention relates to 2-cyclopenten-1-one and its derivatives as inhibitors of the transcription factor Nuclear Factor-kappaB (NF-*k*B). In particular the invention relates to 2-cyclopenten-1-one and its derivatives comprising the cyclopentenone nucleus as inhibitors of the NF-*k*B factor with anti-inflammatory, anti-proliferative, immuno-suppressive, cytoprotective and antiviral activity.

BACKGROUND OF THE INVENTION

NF-*k*B (Nuclear Factor - *k*B) is an eucariotic transcription factor of the *rel* family, which is normally located in the cytoplasm in an inactive complex, whose predominant form is a heterodimer composed of p50 and p65 subunits, bound to inhibitory proteins of the I*k*B family, usually I*k*B-alpha (D. Thanos and T. Maniatis, and Cell 80:529-532, 1995).

NF-*k*B is activated in response to different stimuli, among which phorbol esters, inflammatory cytokines, UV radiation, bacterial and viral infections. Stimulation triggers the release of NF-*k*B from I*k*B in consequence of the phosphorylation and the following degradation of the I*k*B-alpha protein (P.A. Baeuerle and T. Henkel, Annu. Rev. Immunol. 12: 141-179, 1994). Once it is activated, NF-*k*B translocates to the nucleus where it binds to DNA at specific *k*B-sites and induces the transcription of a variety of genes encoding proteins involved in controlling the immune and inflammatory responses, among which a variety of interleukins, the tumor necrosis factor alpha, the NO

synthase and the cyclo-oxigenase 2 (S. Grimm and P.A. Baeuerle, Biochem. J. 290: 297-308, 1993). Accordingly, NF-*k*B is considered an early mediator of the immune and inflammatory responses and it is involved in the control of cell proliferation and in the pathogenesis of various human diseases, among which rheumatoid arthritis (H. Beker et al., Clin. Exp. Immunol. 99: 325, 1995), ischemia (A. Salminen et al. Biochem. Biophys. Res. Comm. 212: 939, 1995), arteriosclerosis (A.S. Baldwin. Annals Rev. Immunol. 14: 649, 1996), as well as in the pathogenesis of the acquired immunodeficiency syndrome (AIDS), due to the enhanced human immunodeficiency virus (HIV-1) transcription in the presence of activated NF-*k*B. The increase of HIV-1 virus RNAs transcription by NF-*k*B is caused by the presence of *k*B-sites in the (LTR) (Long Terminal Repeats) sequences of the virus genome (M.J. Lenardo and D. Baltimore, Cell 58: 227-229, 1989).

It is also known that prostaglandins (PGs) are a class of naturally occurring cyclic 20-carbon fatty acids that are synthetized by various types of eukaryotic cells in response to external stimuli and play an important role in a variety of physiological responses. Since their discovery, PGs were shown to act as microenvironmental hormones and intracellular signal mediators and to control a large number of physiological and pathological processes, including cell proliferation and differentiation, the immune response, inflammation, cytoprotection and the febrile response. In particular, type A and J PGs, which possess a cyclopentenonic structure, are strong inhibitors of virus replication ("Stress Proteins: Induction and Function" Schlesinger MJ, Garaci E., Santoro M.G. ed.s, Springer-Verlag, Heidelberg-Berlin, 27-44, 1990). Particularly, it has been recently demonstrated that

cyclopentenonic prostaglandins inhibit HIV-1 virus replication, by blocking the viral RNAs transcription (C. Rozera et al. J. Clin. Invest. 97: 1795, 1996).

It is also known that the Heat Shock Proteins (HSPs), also called stress proteins (Proc. Natl. Acad. Sci. USA 86, 8407-8411, 1989), are a family of polypeptides synthetized by eukaryotic and prokaryotic cells in response to heat shock or other kinds of environmental stresses. The HSPs are encoded by a cellular subgroup of genes, identified as stress genes.

The authors have shown that the cyclopentenone prostaglandin PGA inhibits the activation of NF-*k*B in human cells by inhibiting the phosphorylation and degradation of the inhibitory I*k*B-alpha protein (A. Rossi, G. Elia and M.G. Santoro, Cold Spring Harbour, New York 1-5 May, 1996, Abstract p. 255).

The authors have also recently shown that inhibition of NF-*k*B activation is one of the molecular mechanisms used by cyclopentenonic prostaglandins to cause a selective and reversible block of HIV-1 virus RNAs transcription.

SUMMARY OF THE INVENTION

It has now been found that 2-cyclopenten-1-one, the structure constituting the center nucleus of PGA, possesses an activity which is analogous to PGA, that is, it is able to inhibit NF-*k*B activation, even though it does not contain the corresponding acid function and aliphatic lateral chains. Therefore it is found that the lateral chains, which are present in the PGA with their substituents and double bonds, in particular the acid function, which implies the fatty acid nature of prostaglandins, can be eliminated without substantially

modifying the herein above described specific activity. It is also found that the alpha,ß-unsatured carbonyl group in the cyclopentenone ring is the key structure necessary for NF-*k*B inhibition.

Furthermore it has been found that the inhibition of NF-*k*B by the cyclopentenone group is related to the ability to activate the HSF transcription factor (Heat Shock Transcription Factor), which is responsible for the synthesis of HSPs (Heat Shock Proteins).

In view of the fact that NF-*k*B inhibition is associated with HSF activation, it is evident that molecules containing the cyclopentenone nucleus, which is active in inhibiting NF-*k*B, will be inducers of the HSF factor and therefore they will be inducers of heat shock proteins.

It is therefore an object of the present invention the 2-cyclopenten-1-one, and its substituted derivatives comprising the cyclopentenone nucleus, as inhibitors of NF-*k*B, the substituents being selected among the ones which do not affect the NF-*k*B inhibitory activity.

Another object of the present invention is the 2-cyclopenten-1-one and its pharmacologically acceptable derivatives as inhibitors of NF-*k*B.

Another object of the invention is the 2-cyclopenten-1-one and its derivatives as inhibitors of NF-*k*B with anti-inflammatory, anti-proliferative, immuno-suppressive, cytoprotective and antiviral activity.

A further object of the invention are pharmaceutical compositions comprising 2-cyclopenten-1-one and/or its pharmaceutically acceptable derivatives to make medicaments with anti-inflammatory, anti-proliferative, immuno-suppressive, cytoprotective and antiviral activity. In particular with antiviral activity against the HIV-1 virus and viruses whose transcription is controlled by NF-*k*B,

including herpesviruses.

BRIEF DESCRIPTION OF FIGURES

Fig. 1A shows the dose-dependent inhibition of NF-*k*B activation by 2-cyclopenten-1-one.

Fig. 1B shows the activation of the HSF factor (Heat Shock Transcription Factor) by 2-cyclopenten-1-one in association with NF-*k*B inhibition.

Fig. 1C shows the specificity of the chemical structure which is responsible for NF-*k*B inhibition.

Fig. 1D shows the specificity of the chemical structure which is responsible for HSF activation.

DETAILED DESCRIPTION OF THE INVENTION

The 2-cyclopenten-1-one is a known product, which can be synthetized according to the process described in Beilstein (*Daene, Eder*, A. **539** [1939] 207, 211).

According to the present invention 2-cyclopente-1-one, preferably in concentrations ranging between 100 and 500 uM, is able to inhibit NF-*k*B activation in human cells (Fig. 1A).

Inhibition tests have been carried out in type T lymphoid human cells (Jurkat cell line), as well as in other human cell lines. NF-*k*B activation was stimulated with 12-o-tetradecanoyl-phorbol-13-acetate (TPA). 2-Cyclopenten-1-one was also effective in inhibiting NF-*k*B activation after other types of stimulation, including stimulation by tumor necrosis factor alpha or viral infection, and in different types of human cells (data not shown). It is demonstrated that NF-*k*B inhibition is associated with the activation of HSF factor (Fig. 1B).

It is also proved that the ability to inhibit the NF-*k*B factor is

specific for 2-cyclopenten-1-one, whereas similar molecules, such as cyclopentanone and cyclopentene, do not inhibit NF-*k*B (Fig. 1C) and do not activate HSF (Fig. 1D).

Based on these results it is possible to use 2-cyclopenten-1-one, as well as its pharmaceutically acceptable derivatives, as active substances to produce medicaments, in particular medicaments having activity in inhibiting the NF-*k*B factor, and in particular:

- anti-inflammatory and immunosuppressive medicaments, in view of the role of NF-*k*B in stimulating the inflammatory and immune responses;
- cytoprotective medicaments, in view of the role of NF-*k*B in ischemia and oxidative damages;
- antiproliferative medicaments, in view of the role of NF-*k*B in cell proliferation;
- antiviral medicaments, in view of the role of NF-*k*B in activating the viral RNAs transcription.

The following examples are reported to illustrate the invention. They should be considered in any case non limiting the scope of the invention itself.

The reagents used in the examples, including 2-cyclopenten-1-one, cyclopentene, cyclopentanone and 12-o-tetradecanoyl-phorbol-13-acetate (TPA), were products of Sigma Aldrich. ^{32}P e ^{35}S were produced by AMERSHAM. Fetal calf serum and cellular culture media were produced by GIBCO.

EXAMPLE 1

The effect of the treatment with 2-cyclopenten-1-one on NF-*k*B activation by TPA has been tested in Jurkat cells by using the procedures described hereinbelow and shown in Fig. 1.

Dose-response effect

The cells were prepared according to the method described in C. Amici et al. (Cancer Research 55, 4452-4457, 1995).

The cells were treated with 2-cyclopenten-1-one at different concentrations (125-100uM) for 1 hour and then were stimulated with TPA (25 ng/ml).

After 3 hours the whole-cell extracts were prepared and subjected to EMSA (Electrophoretic Mobility Shift Assay) as described for NF-*k*B in U. Zabel et al. (J. Biol. Chem. 266: 242, 1991) and HSF in C. Amici et al. (Cancer Res. 55: 4452, 1995), to determine NF-*k*B and HSF activation respectively. The positions of the complex NF-*k*B-DNA (NF-*k*B) and the non-specific binding (ns) are indicated in Fig. 1A.

The positions of the complex HSF-DNA (HSF), the HSF-DNA constitutive activity (CHBA) and the proteins-DNA non-specific interactions (ns) are indicated in Fig. 1B. The line "control" indicates the non-TPA-stimulated cells as a control of non-activated NF-*k*B.

As evident, 2-cyclopenten-1-one is able to inhibit NF-*k*B activation by TPA even at the lower concentration of 125 uM. At the concentration of 500 um the NF-*k*B band is absent (Fig. A). In correlation with NF-*k*B inhibition, in the same samples it is evident the activation of HSF starting from the concentration of 125 uM (Fig. 1B).

Specificity of the inhibitory effect (Fig. 1C)

The cells were treated for 1 hour with the same concentration (500 uM) of: 2-cyclopenten-1-one (line 3), cyclopentanone (line 4) or cyclopentene (line 5), and then were stimulated with TPA (25 ng/ml). C represents the non-TPA-stimulated control. After 3 hours the whole-cell extracts were prepared and subjected to EMSA to verify the

activation of NF-*k*B (Fig. 1C) and of HSF (Fig. 1D) respectively. As evident, (i) TPA activates NF-*k*B (line 2); (ii) 2-cyclopenten-1-one inhibits TPA-induced NF-*k*B activation (line 3); cyclopentanone (line 4) and cyclopentene (line 5) do not inhibit NF-*k*B activation.

In addition, as shown in Fig. 1D, in the same samples inhibition of NF-*k*B, shown in Fig. 1C, is associated with activation of HSF. These results clearly show that the alpha,ß-unsaturated carbonyl group is the key structure trigging HSF activation and its presence is necessary to inhibit NF-*k*B activation.

CLAIMS:

1. Use of 2-cyclopenten-1-one or a substituted derivative thereof comprising a cyclopentenonic nucleus in the preparation of a medicament for treating a non-viral disorder that can be treated using an inhibitor of NF-κB, with the proviso that the derivative is not a prostaglandin or a derivative presenting two adjacent aliphatic side chains in the cyclopentenonic nucleus.

2. Use of 2-cyclopenten-1-one or a substituted derivative thereof comprising a cyclopentenonic nucleus in the preparation of a medicament for treating a non-viral disorder by inhibiting NF-κB activation with the proviso that the derivative is not a prostaglandin or a derivative presenting two adjacent aliphatic side chains in the cyclopentenonic nucleus.

3. Use according to claim 1 or 2, wherein the disorder is an inflammatory disorder.

4. Use according to claim 1 or 2, wherein the disorder is an immune disorder.

5. Use according to claim 1 or 2, wherein the disorder is a disorder involving cell proliferation.

6. Use according to claim 1 or 2, wherein the disorder is a disorder treatable with a cytoprotective agent.

7. Use according to claim 6, wherein the disorder is not a gastric ulcer.

8. Use according to claim 1 or 2, wherein the disorder is ischemia.

9. Use according to claim 1 or 2, wherein the disorder is oxidative cell damage.

10. Use according to claim 1 or 2, wherein the disorder is arteriosclerosis.

11. Use according to claim 1 or 2, wherein the disorder is rheumatoid arthritis.

12. Use of 2-cyclopenten-1-one or a substituted derivative thereof comprising a cyclopentenonic nucleus in the preparation of a medicament for treating a non-viral disorder by inhibiting NF-κB activation with the proviso that the derivative is not a prostaglandin or a derivative presenting two adjacent aliphatic side chains in the cyclopentenonic nucleus, wherein the disorder is selected from:

a) an inflammatory disorder,

b) an immune disorder,

c) a disorder involving cell proliferation,

d) ischemia,

e) arteriosclerosis, and

f) rheumatoid arthritis.

13. Use according to any one of claims 1 to 12, wherein the 2-cyclopenten-1-one or a substituted derivative is used at a concentration ranging between 100 and 500 µm.

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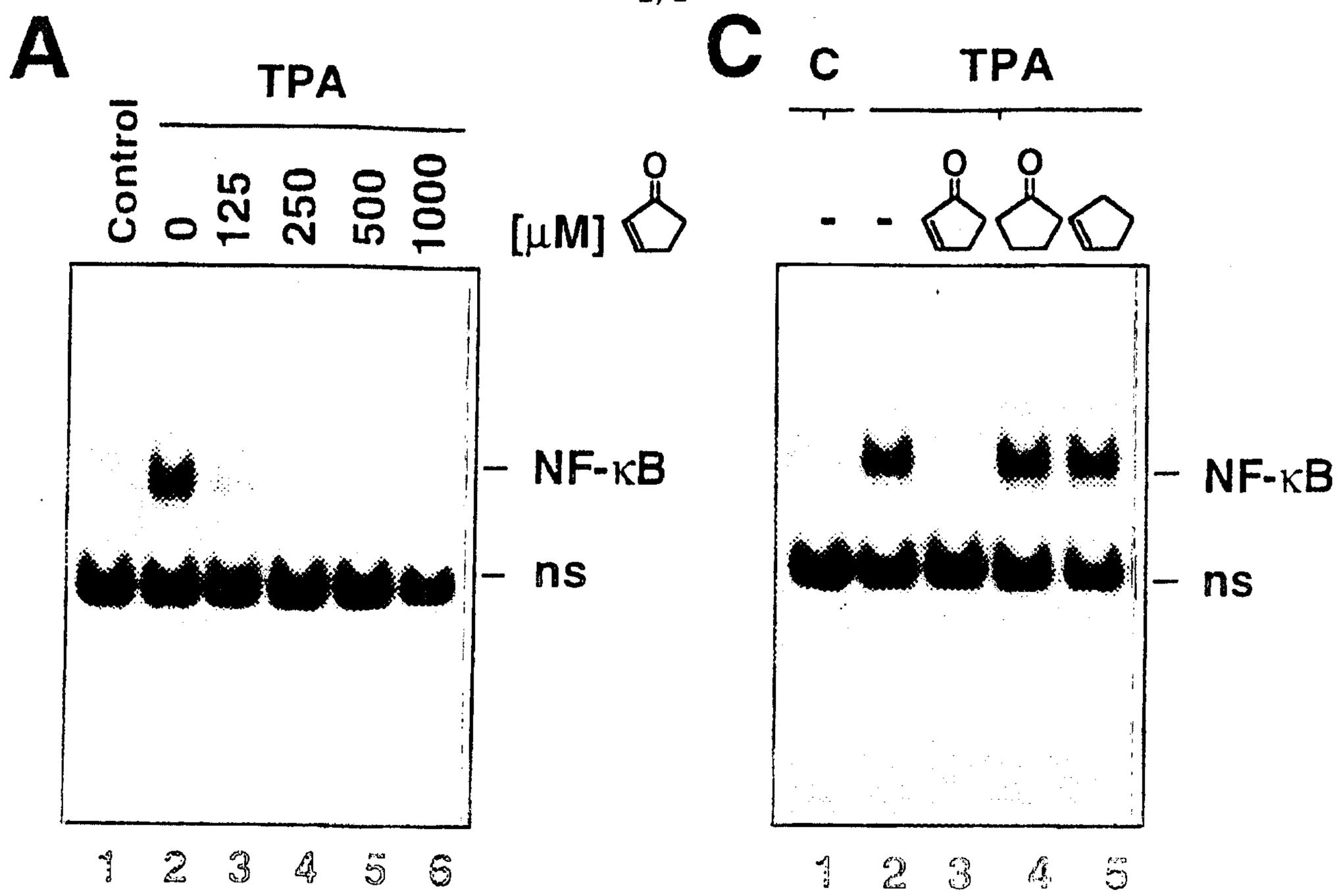
A
TPA
Control
0
125
250
500
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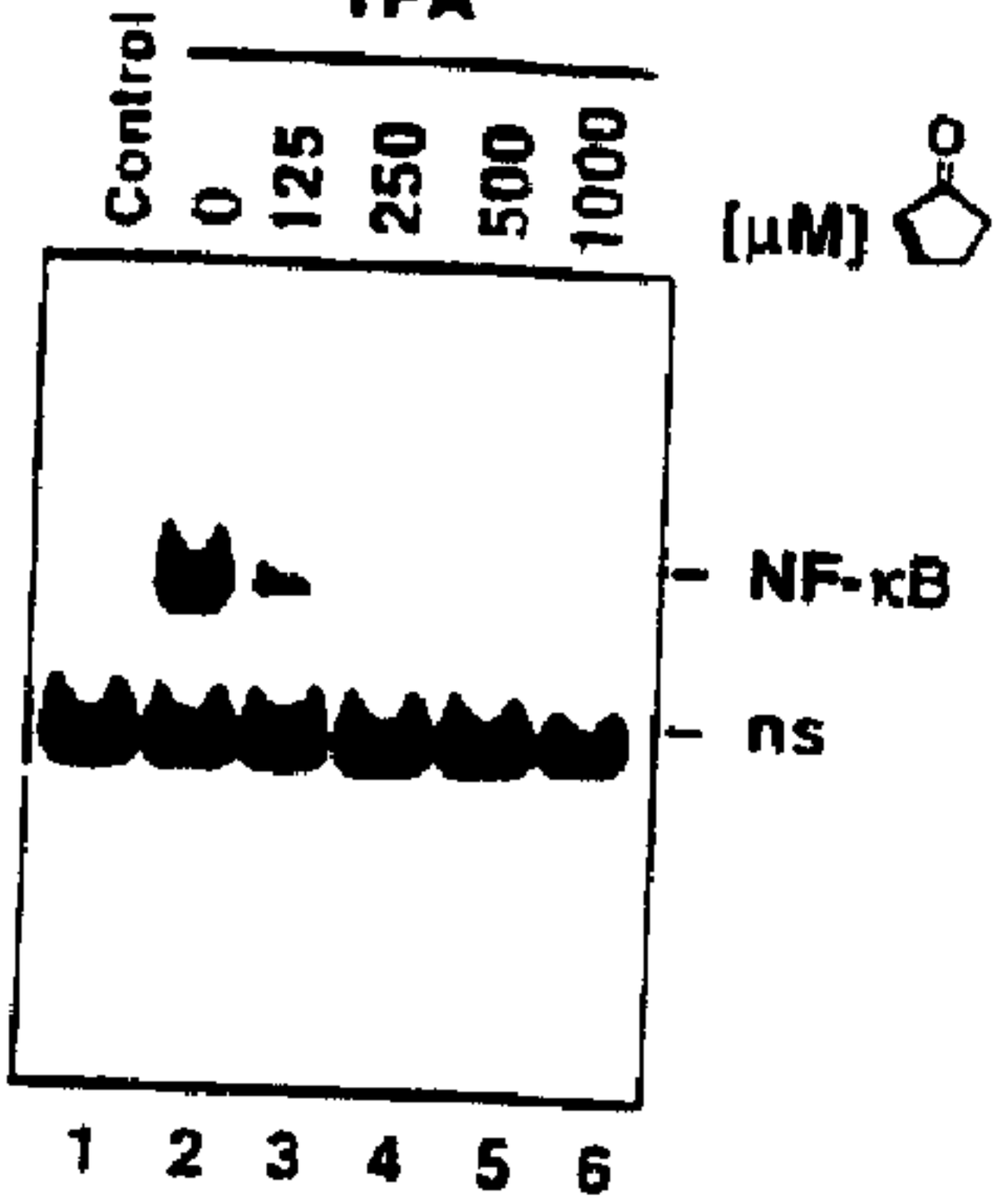
[μM]
NF-κB
ns
1 2 3 4 5 6
C
C
TPA
NF-κB
ns
1 2 3 4 5
B
TPA
Control
0
125
250
500
1000
[μM]
HSF
CHBA
ns
1 2 3 4 5 6
D
C
TPA
HSF
CHBA
ns
1 2 3 4 5

FIGURE 1

A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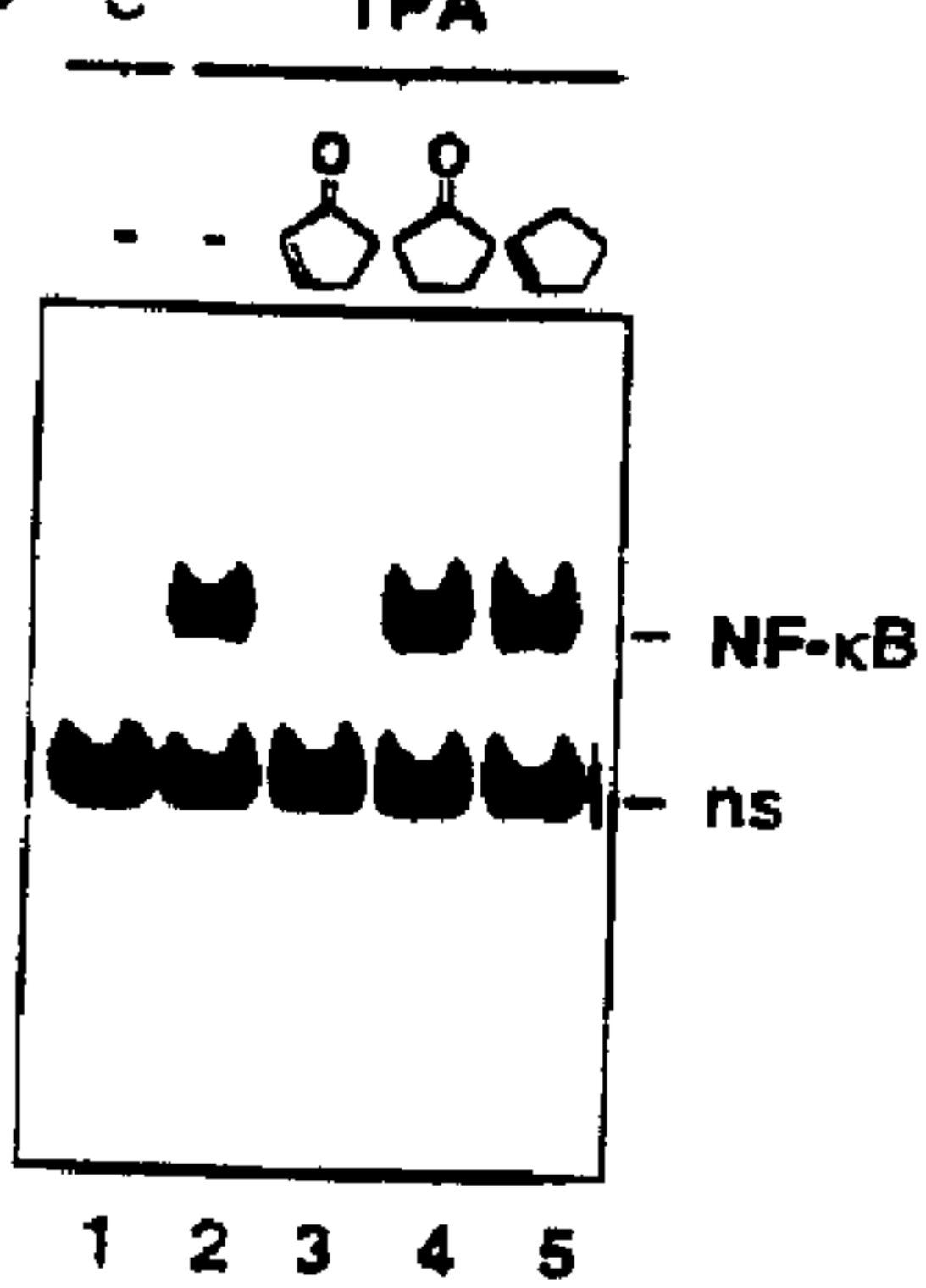
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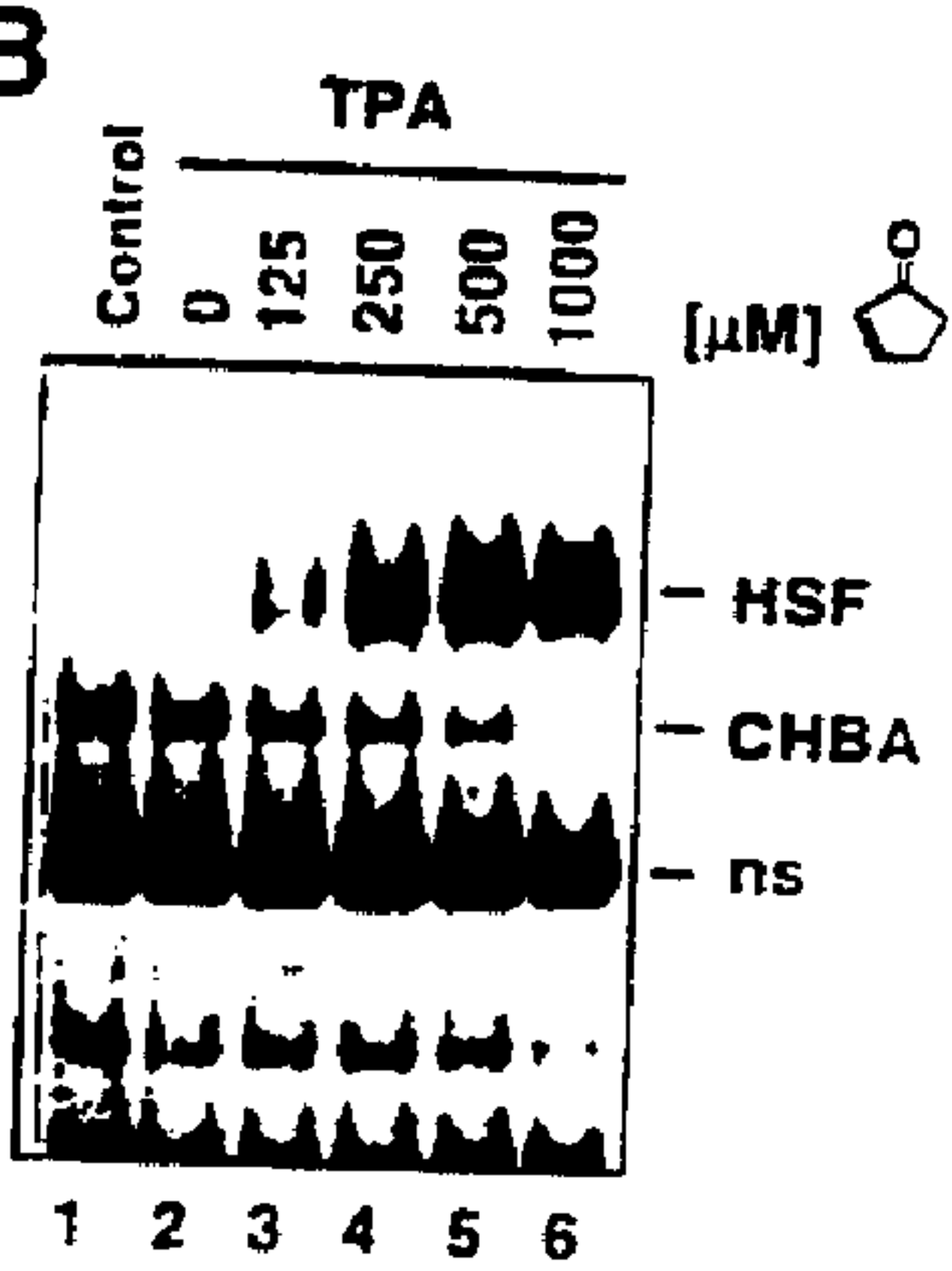
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HSF
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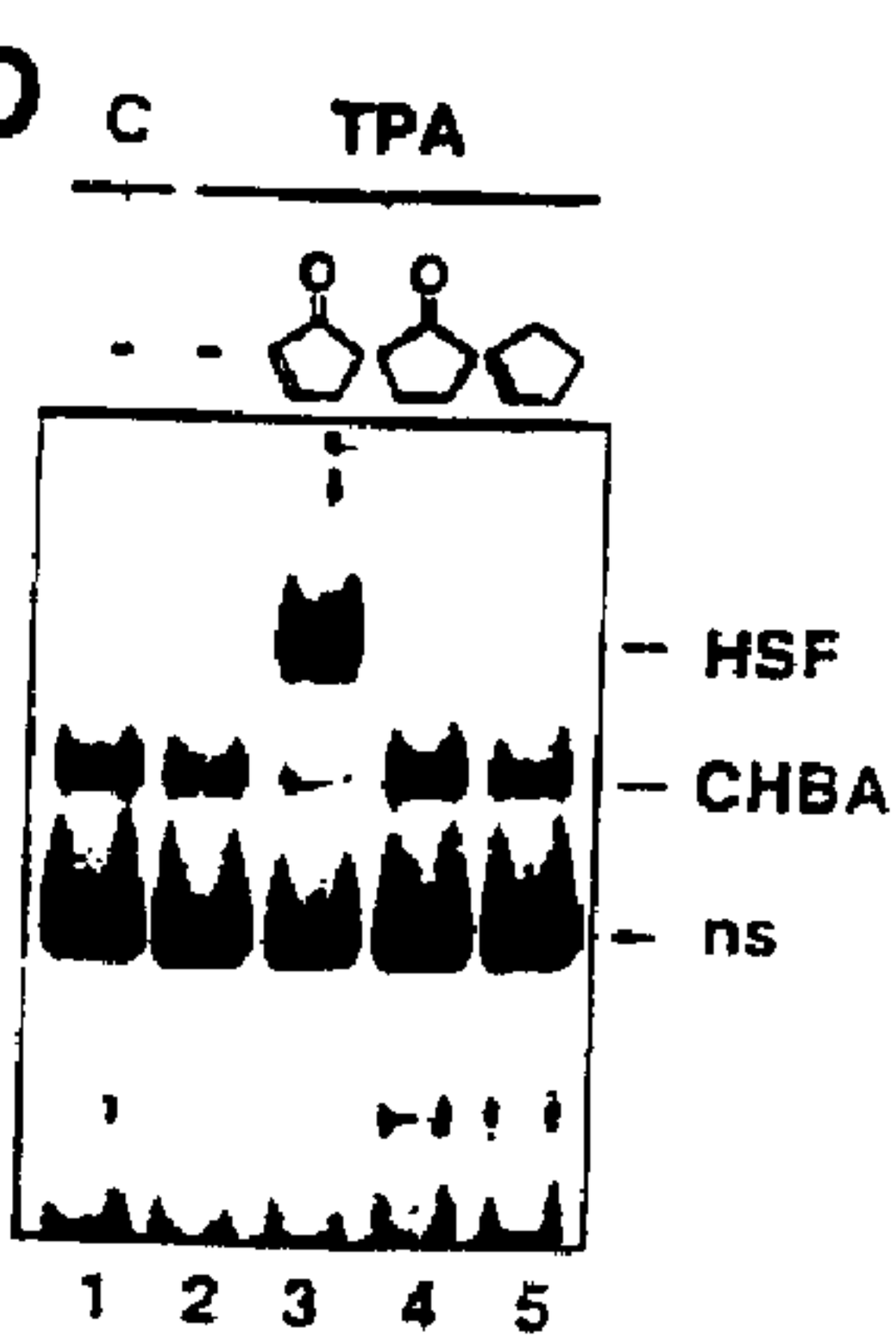
D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