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(54) **INHIBITEURS DU FACTEUR NF-KB EN TANT QU'ACTIVATEURS DE HSF ET INDUCTEURS DE PROTEINES DE STRESS**

(54) **INHIBITORS OF NF-KAPPAB AS ACTIVATORS OF HSF AND INDUCERS OF HEAT SHOCK PROTEINS**

(57) Inhibiteurs du facteur NF-kB et composés dérivés correspondants acceptables sur le plan pharmaceutique conçus pour être utilisés en tant qu'activateurs du facteur HSF servant à la transcription et à la traduction des gènes du stress, avec production de hsp70, et présentant, en particulier, une activité anti-inflammatoire, antiproliférative, immunosuppressive, cytoprotectrice et antivirale.

(57) Inhibitors of the NF-kB factor and corresponding pharmaceutically acceptable derivative compounds to be used as activators of the HSF factor for the transcription and translation of heat shock genes, with production of hsp70, particularly with anti-inflammatory, anti-proliferative, immuno-suppressive, cytoprotective and antiviral activity.

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(54) **Title:** INHIBITORS OF THE NF–*k*B FACTOR AS ACTIVATORS OF HSF AND INDUCERS OF HEAT SHOCK PROTEINS

(57) **Abstract**

Inhibitors of the NF–*k*B factor and corresponding pharmaceutically acceptable derivative compounds to be used as activators of the HSF factor for the transcription and translation of heat shock genes, with production of hsp70, particularly with anti–inflammatory, anti–proliferative, immuno–suppressive, cytoprotective and antiviral activity.

INHIBITORS OF THE NF-*k*B FACTOR AS ACTIVATORS OF HSF AND INDUCERS OF HEAT SHOCK PROTEINS

Field of invention

The present invention relates to inhibitors of the NF-*k*B factor as activators of HSF and iducers of heat shock proteins. Particularly, the invention refers to said inhibitors as activators of HSF with anti-inflammatory, anti-proliferative, immuno-suppressive, cytoprotective and antiviral activity.

Background art

NF-*k*B (Nuclear Factor - kappaB or Nuclear Factor - κB) is an eukaryotic 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 (D. Thanos and T. Maniatis, Cell 80:529-532, 1995).

NF-*k*B is activated in response to different stimuli, among which 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 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 stress genes transcription is regulated by the trancriptional factor HSF (heat shock transcription factor) which is activated in consequence of a temperature raising, an environmental stress or after exposition to some biological molecules (R.I. Morimoto et al., J. Biol. Chem. vol. 267, 21987-21990, 1992; C. Amici et al., Proc. Natl. Acad Sci USA, vol. 89, 6227-6231, 1992). The cytoprotective role of stress proteins has been described in various kinds of pathologies, among which ischemia, (M.S. Marber et al., J. Cli. Invest. 93, march 1994, 1087-1094), trauma, inflammation and viral replication (U. Feige et al., "Stress-Inducible Cellular response" Birkhauser. Verlag, Basel, 1996) can be mentioned.

It is also known that, in the pathogenesis of the viral infection, the stress proteins HSP interfere at various levels with the virus replication, and particularly a cytoprotective role of the HSP70 protein has been characterized in some experimental models of acute infection (M.G. Santoro, Experientia, Vol. 50, 1039-1047, 1994). The possibility to selectively activate some "heat shock" (hs) genes and to manipulate the cellular stress response to the host advantage is suggested by recent studies which demonstrate that prostaglandins are able to induce HSP70 synthesis in a non-stress situation and to protect the host cell during virus infection (M.G. Santoro, Experientia, Vol. 50, 1039-1047, 1994).

The authors have recently shown that the induction of HSP70 synthesis is one of the molecular mechanisms used by cyclopentenonic prostaglandins to cause a selective and reversible block of the protein synthesis in infection models with single strand negatively polarized RNA viruses (C. Amici et al., J. Virol. 68, 6890-6899, 1994). Moreover they 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, Proc. Natl. Acad. Sci. USA, vol. 94, 746-750, 1997). The authors have also recently shown that inhibition of NF-*k*B after treatment with cyclopentenonic prostaglandins, sodium arsenite or thermal shock is strictly related to the activation of the transcriptional factor HSF (Rossi et al., Proc. Natl. Acad. Sci. USA, vol. 94, 746-750, 1997). The authors have also found that the inhibition of the activation of NF-*k*B is one of the molecular mechanisms used by cyclopentenonic prostaglandins to cause a selective and reversible block of HIV-1 virus RNAs transcription. Finally, it is known that the serin protease inhibitors are also inhibitors of the activation of NF-*k*B (T.S. Finco et al., Proc. Natl. Acad. Sci. USA, vol. 91, 11884-11888, 1994).

Summary of the invention

It has now been found, and it is an object of the present invention, that the inhibitors of the activation of NF-*k*B induce the activation of the HSF factor and the trancription and translation of heat shock genes, with production of hsp70.

Another object of the invention resides in the inhibitors of serin protease, which are strong inhibitors of the activation of NF-*k*B and induce the activation of the HSF factor and the trancription and translation of heat shock genes, with production of hsp70, the HSF activation being strictly related to the inhinition of NF-*k*B, both time and dose dependent.

A further object of the invention resides in the inhibition of the activation of NF-*k*B with related induction of the activation of the HSF factor by 3,4-dichloro-iso-cumarine (DCIC), Tosyl-L-Phenylalanine-chloromethylketone (TPCK), N_{α}-Tosyl-Lysine-chloromethylketone (TLCK), N-acethyl-DL-Phenylalanine-β-napthylester (APNE) and N-benzoyl-L-Thyroxine-ethylester (BTEE), 3,4-dichloro-iso-cumarine (DCIC) being preferred.

Another object of the invention is the use of inhibitors of NF-*k*B and corresponding pharmaceutically acceptable derivative compounds as inducers of the activation of the HSF factor.

A further object of the invention is the use of inhibitors of NF-*k*B and corresponding pharmaceutically acceptable derivative compounds as inducers of HSF as medicaments with antiviral activity. In particular antiviral activity against single strand negatively polarized RNA viruses and DNA viruses (e.g.

herpesvirus).

A further object of the invention is the use of inhibitors of NF-*k*B and corresponding pharmaceutically acceptable derivative compounds as inducers of HSF as medicaments with anti-inflammatory, anti-proliferative, immuno-suppressive, cytoprotective and antiviral activity.

Further objects of the invention are pharmaceutical compositions comprising inhibitors of NF-*k*B and corresponding pharmaceutically acceptable derivative compounds as inducers of HSF as medicaments with the above mentioned activity, in particular antiviral activity against HIV-1 virus and viruses whose replication is controlled by HSF and HSP.

Further objects of the invention will be evident from the following detailed description of the invention.

Brief description of figures

Fig. 1A shows the activation of the HSF factor (Heat Shock Factor) by autoradiography.

Fig. 1B shows the activation of the HSF factor by quantitative determination.

Fig. 1C shows the induction and transcription of the heat shock genes by DCIC in human leukemia by autoradiography.

Fig. 1D shows the induction and transcription of the heat shock genes by DCIC in human leukemia by quantitative determination.

Fig. 2A shows the antiviral activity of DCIC.

Fig. 2B shows the induction of the HSP70 and the inhibition of the synthesis of the viral proteins by DCIC.

Fig. 3A shows that the HSF activation by DCIC (A) is strictly related to the inhibition of NF-*k*B.

Fig. 3B shows that the HSF activation by TLCK (B) is strictly related to the inhibition of NF-*k*B.

Fig. 3C shows that the HSF activation by TPCK (C) is strictly related to the inhibition of NF-*k*B.

detailed description of the invention

According to the present invention, the inhibitors of NF-*k*B induce the activation of the HSF factor and the transcription and translation of heat shock genes, with

production of hsp70. Among these inhibitors the serin protease inhibitors can be mentioned, which are strong inhibitors of the activation of NF-*k*B and which turn out to be inducers of the activation of the HSF factor and of the transcription and translation of heat shock genes, with production of hsp70, the HSF activation being strictly related to the inhibition of NF-*k*B, both time and dose dependent.

Among these serin protease inhibitors there are comprised: 3,4-dichloro-iso-cumarine (DCIC), Tosyl-L-Phenylalanine-chloromethylketone (TPCK), N_α-Tosyl-Lysine-chloromethylketone (TLCK), N-acethyl-DL-Phenylalanine-β-napthylester (APNE) and N-benzoyl-L-Thyroxine-ethylester (BTEE). All these products are known, e.i. marketed by Sigma, Aldrich and Fluka.

Moreover, the induction of hsp70 synthesis by the inhibitors of NF-*k*B is found to be associated with high antiviral activity, as previously known for other inducers of this protein. In MA104 cells infected with the Vesicular Stomatitis Virus (VSV) (1-10 P.F.U./cell) the treatment with DCIC, started 1 hour after infection, causes a dose-dependent reduction in the production of infectious viral particles. As in the case of other hsp70 inducers, the block in the replication of the virus is caused by the selective inibition of the synthesis of viral proteins, associated with the synthesis of hsp70 protein. These results indicate that it is possible to use the inhibitors of NF-*k*B, and particularly the inhibitors of serin protease, as activators of HSF to induce the synthesis of hsp70 and to inhibit the viral replication. Therefore it is possible to use the inhibitors of NF-*k*B in the pharmaceutical field, and particularly the inhibitors of serin protease, or corresponding pharmaceutically acceptable derivative products having anti-inflammatory, anti-proliferative, immuno-suppressive, cytoprotective and antiviral activity. Particularly, antiviral activity against negative strand RNA viruses and DNA viruses (e.g. herpesvirus).

According to the present invention DCIC, preferably in concentration ranging between 5 and 45 μM, is able to activate the transcription factor HSF and to selectively induce the transcription and translation of the HSP70 gene. In particular induction tests have been carried out in human leukemia cells (JURKAT cell line), as shown in Fig. 1.

The HSP70 synthesis is induced also in other types of human cells (HEp-2, HeLa) and in monkey epithelial cells (MA104 cells) (Fig.2). Moreover, the induction of

HSP70 synthesis is found to be associated with high antiviral activity. Infact, in MA104 cells infected with the Vesicular Stomatitis Virus (VSV) (1-10 P.F.U./cell) the treatment with DCIC, started 1 hour after infection, causes a dose-dependent reduction in the production of infectious viral particles (Fig.2A). As in the case of other HSP70 inducers, the block in the replication of the virus is caused by the selective inibition of the synthesis of viral proteins, associated with the synthesis of HSP70 protein (Fig.2B). These results confirm the antiviral activity of DCIC as inducer of HSP70 and show the possibility of using DCIC to induce the synthesis of HSP70 and to inhibit the viral replication. Based on these results, it is possible to use DCIC and the other inhibitors of NF-*k*B, as well as the other inhibitors of serin protease, as active substances to produce medicaments, in particular medicaments having antiviral activity against negative strand RNA viruses (e.g. influenza, parainfluenza viruses and rhabdoviruses) and other viruses, sensitive to the antiviral activity of HSP proteins.

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 DCIC, 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 DCIC on the HSF activation, on the heat shock gene transcription and on the synthesis of the proteins have been evaluated in JURKAT cells with the methods described hereinbelow and shown in Fig. 1.

Kinetics of activation

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

Whole-cell extracts, prepared at different times after treatment with 5μM of DCIC in ethanol were subjected to EMSA (Electrophoretic Mobility Shift Assay), as described in C.Amici et al. Cancer Research 55, 4452-4457, 1995. The positions of HSF, CHBA (HFS-DNA constitutive activity) (Fig. 1A) and NS (proteins-DNA non-specific interaction) are indicated. The levels of HSF DNA-binding activity in cells treated with DCIC were quantitated with a Molecular Dynamics

PhosphorImager (MDP). The HSF values were normalized to the level of HSF DNA-binding activity at 3 h after treatment, which was given a value of 100% (Fig. 1B).

As evident, DCIC is able to activate HSF. The activation is prolonged for the following 12 hours, with a maximum at 3 hours from the beginning of the treatment.

Transcription rate of HSP70 gene.

The transcription rates were measured by Nuclear Run-On assay (C.Amici et al., Cancer Research 55, 4452-4457, 1995). The ^{32}P-labelled RNA was hybridized on nitrocellulose filters containing plasmids for the following human genes: hsp90 (pUCHS801; Stress-Gen Biotechnologies Co., Victoria British Columbia Canada); hsp70 (pH,2,3; B.Wu et al., Mol. Cell. Biol. 5, 330 (1985)); grp78/BiP (glucose-regulated 78 protein) (pHG 23,1; C.Amici et al., Proc. Natl. Acad. Sci. USA 89, 6227, 1992); hsc70 (heat shock cognate 70) (pHA 7,6; C.Amici et al., Proc. Natl. Acad. Sci. USA 89, 6227, 1992); GAPDH (rat glyceraldehyde phosphate dehydrogenase) (GAPDH, 1400 bp, PstI; A.Rossi and M.G.Santoro, Biochem. J., 308, 455, 1995). The vector plasmid (Bluescript) was used as a non-specific hybridization control. Following hybridization, the filters were visualized by autoradiography (Fig. 1C) and the radioactivity was quantitated by MDP analysis (Fig. 1D). The values are expressed as arbitrary units obtained by comparing transcription rates to control levels. As evident, DCIC is able to selectively activate the hsp70 and hsp90 heat shock gene transcription. The transcription is prolonged at high levels for at least 9 hours from the beginning of the treatment.

EXAMPLE 2

The effect of DCIC on the replication of Vesicular Stomatitis Virus (VSV) and on the HSP70 protein synthesis was evaluated as described in the following and illustrated in Fig. 2.

Confluent nonolayers of monkey kidney MA104 cells, grown in RPMI-1640 medium supplemented with 5% FCS (fetal calf serum) and antibiotics, were infected with VSV (Indiana serotype, Orsay; 1 P.F.U./cell). After 1 h at 37°C, the viral inoculum was removed and cells were kept at 37°C in RPMI-1640 medium containing 2% FCS and different concentrations of DCIC in ethanol or control

diluent. VSV titers were determined 12 h post infection (p.i.) by cytopathic effect 50% (CPE 50%) assay, as described in F.Pica et al., Antiviral Res., vol. 20, 193, 1993 and illustrated in Fig. 2A. Uninfected (U) or VSV-infected (VSV) MA104 cells were treated with 5 µM (lanes 2 and 7), 15 µM (lanes 3 and 8), 30 µM (lanes 4 and 9) and 45 µM (lanes 5 and 10) DCIC, or with control diluent (lanes 1 and 6), soon after VSV infection and labeled with [^{35}S]-methionine (8 µCi/2x10^5 cells, 1 h pulse starting 5 h p.i.). Equal amounts of protein were analyzed on 10% SDS/PAGE gel and processed by autoradiography. The position of hsp70, identified by western blot analysis using anti-human hsp70 antibodies, is indicated by the arrow. VSV proteins L, G, N, NS and M are indicated.

DCIC, at concentrations ranging between 5 and 45µM, inhibits the production of VSV infectious virions from 50% to more than 98% with respect to the control, under the indicated conditions. The inhibition is mediated by a selective block of the viral protein synthesis, combined with the induction of HSP70.

Table 1 shows that further 4 inhibitors of serin protease, besides DCIC, activate HSF at the minimal inhibitory concentration of NF-*k*B.

Table 1

Inhibitor of protease	NF-*k*B inhibition IC_{90} (µM)	Activation of HSF
(DCIC)	5,5	+
(TPCK)	12	+
(TLCK)	135	+
(APNE)	300	+
(BTEE)	400	+

Effect of the inhibitors of serin protease on NF-*k*B and HSF.

The JURKAT cells were incubated with the above listed compounds or the reference diluent at different concentrations for 1 hour and then were stimulated with TPA (25 ng/ml). After 1 hour at 37°C the whole-cell extracts were prepared and subjected to EMSA to determine NF-*k*B and HSF activation. The levels of binding-DNA activity of NF-*k*B were quantified with Molecular Dynamics PhosphorImager analysis. All the compounds activated HSF at the same concentrations at which they inhibited NF-*k*B. 90% inhibitory concentration IC_{90}.

EXAMPLE 3

The effect of the treatment with DCIC, TLCK and TPCK on the inhibition of the NF-*k*B factor by TPA and on the activation of HSF was evaluated as described in the following and illustrated in Fig. 3.

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 DCIC, TLCK and TPCK at different concentrations for 1 h and then treated with TPA (25 ng/ml). After 3 hours the whole-cells 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. 266:252, 1991) and for HSF in C.Amici et al. Cancer Research 55, 4452-4457, 1995.

In Fig. 3 (lower panels) the positions of the complex NF-*k*B-DNA (NF-*k*B) and the non-specific bonding (ns) are indicated.

In Fig. 3 (upper panels) the positions of the complex HSF-DNA (HSF), of the constitutive activity HSF-DNA (CHBA) and the non-specific interactions of proteins-DNA (ns) are indicated. The line "control" refers to cells non stimulated with TPA as reference of non activated NF-*k*B.

As evident, DCIC, TLCK and TPCK activate HSF at the concentration that inhibits the NF-*k*B activation by TPA.

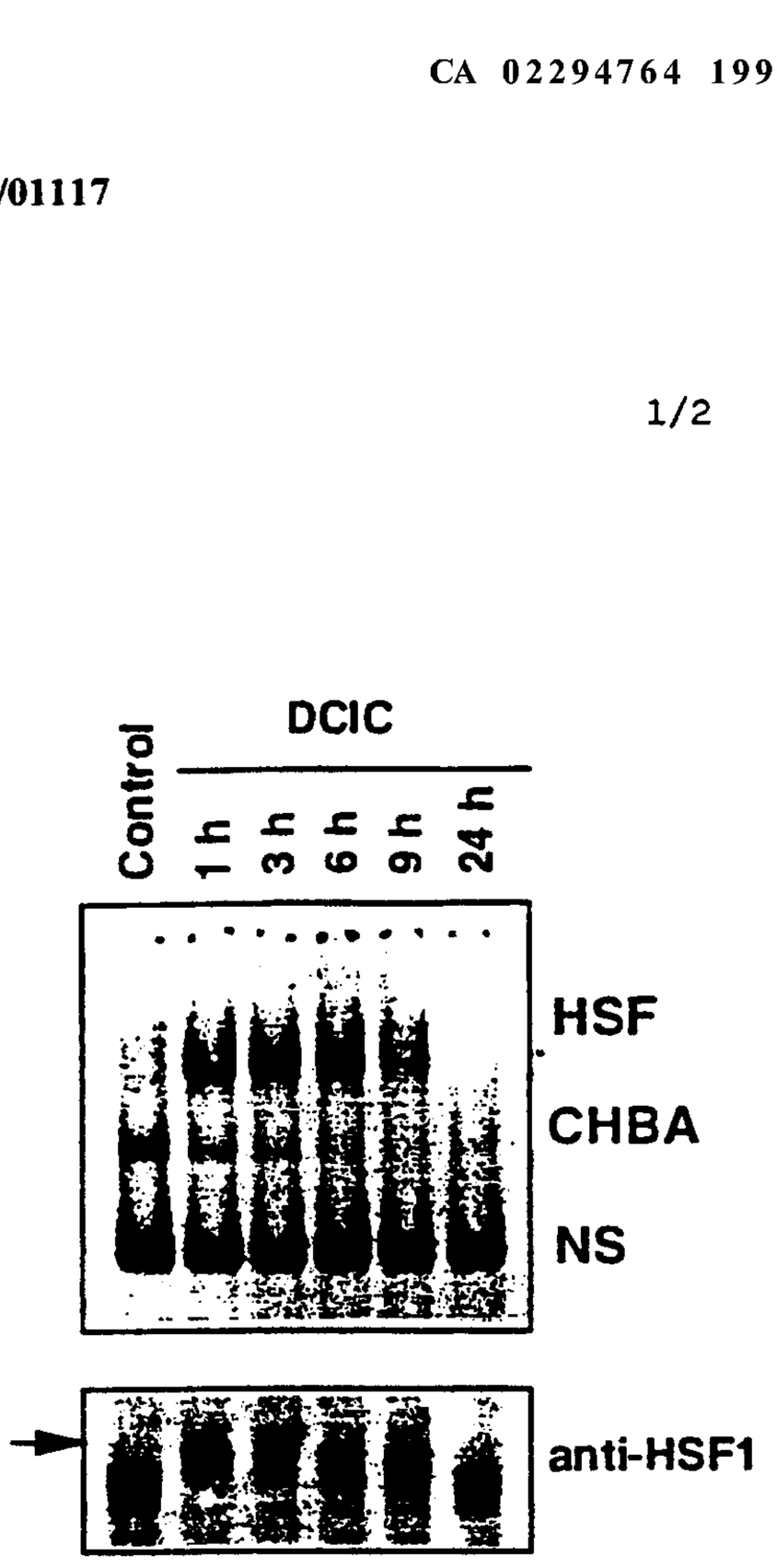
CLAIMS

1. Compound selected among N-acethyl-DL-Phenylalanine-β-napthylester and N-benzoyl-L-Thyroxine-ethylester and corresponding pharmaceutically acceptable derivatives and mixtures thereof to be used as activators of the HSF factor for the transcription and translation of heat shock genes, with production of hsp70.
2. Compound selected among N-acethyl-DL-Phenylalanine-β-napthylester and N-benzoyl-L-Thyroxine-ethylester and corresponding pharmaceutically acceptable derivatives and mixtures thereof to be used as anti-inflammatory, anti-proliferative, immuno-suppressive, cytoprotective and antiviral agents.
3. Compound selected among N-acethyl-DL-Phenylalanine-β-napthylester and N-benzoyl-L-Thyroxine-ethylester and corresponding pharmaceutically acceptable derivatives and mixtures thereof to be used as antiviral agents against HIV-1 and viruses whose replication is controlled HSF and HSP.
4. Pharmaceutical compositions comprising at least one of the following compounds: N-acethyl-DL-Phenylalanine-β-napthylester, N-benzoyl-L-Thyroxine-ethylester and corresponding pharmaceutically acceptable derivatives and mixtures thereof for use as inducers of the HSF activation.
5. Pharmaceutical compositions comprising at least one of the following compounds: N-acethyl-DL-Phenylalanine-β-napthylester, N-benzoyl-L-Thyroxine-ethylester and corresponding pharmaceutically acceptable derivatives and mixtures thereof for use as anti-inflammatory, anti-proliferative, immuno-suppressive, cytoprotective and antiviral agents.
6. Pharmaceutical compositions comprising at least one of the following compounds: N-acethyl-DL-Phenylalanine-β-napthylester, N-benzoyl-L-Thyroxine-ethylester and corresponding pharmaceutically acceptable derivatives and mixtures thereof for use as antiviral agents against HIV-1 and viruses whose replication is controlled HSF and HSP.
7. Use of inhibitors of serin proteases, which inhibit NF-*k*B, and corresponding pharmaceutically acceptable derivatives and mixtures thereof for the preparation of medicaments having activity as inducers of the HSF activation.

AMENDED SHEET

8. Use of inhibitors of NF-*k*B and corresponding pharmaceutically acceptable derivatives and mixtures thereof for the preparation of medicaments having cytoprotective activity.
9. Use of 3,4-dichloro-iso-coumarine and corresponding pharmaceutically acceptable derivatives and mixtures thereof for the preparation of medicaments having activity as inducers of the HSF activation.
10. Use of 3,4-dichloro-iso-coumarine for the preparation of medicaments having cytoprotective activity.
11. Use of 3,4-dichloro-iso-coumarine for the preparation of medicaments having antiviral activity against viruses whose replication is controlled by HSF and HSP.
12. Use of at least one of the following compounds: Tosyl-L-Phenylalanine-chloromethylketone, N_{α}-Tosyl-Lysine-chloromethylketone and corresponding pharmaceutically acceptable derivatives and mixtures thereof for the preparation of medicaments having cytoprotective activity.
13. Use of at least one of the following compounds: N-acethyl-DL-Phenylalanine-β-napthylester, N-benzoyl-L-Thyroxine-ethylester and corresponding pharmaceutically acceptable derivatives and mixtures thereof for the preparation of medicaments having activity as inducers of the HSF activation.
14. Use of at least one of the following compounds: N-acethyl-DL-Phenylalanine-β-napthylester, N-benzoyl-L-Thyroxine-ethylester and corresponding pharmaceutically acceptable derivatives and mixtures thereof for the preparation of medicaments having anti-inflammatory, anti-proliferative, immuno-suppressive, cytoprotective and antiviral activity.
15. Use of at least one of the following compounds: N-acethyl-DL-Phenylalanine-β-napthylester, N-benzoyl-L-Thyroxine-ethylester and corresponding pharmaceutically acceptable derivatives and mixtures thereof for the preparation of medicaments having antiviral activity against HIV-1 and viruses whose replication is controlled HSF and HSP.

A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CHBA
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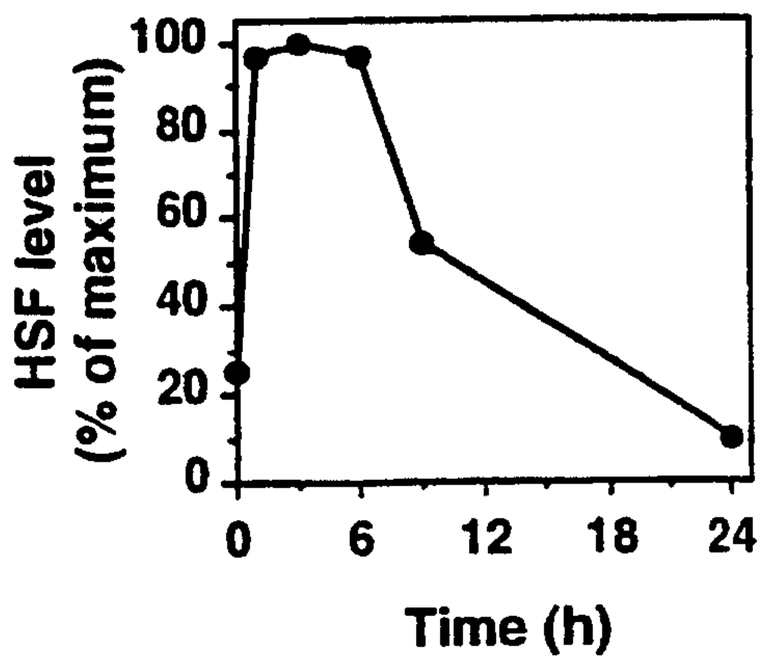
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0
6
12
18
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Time (h)

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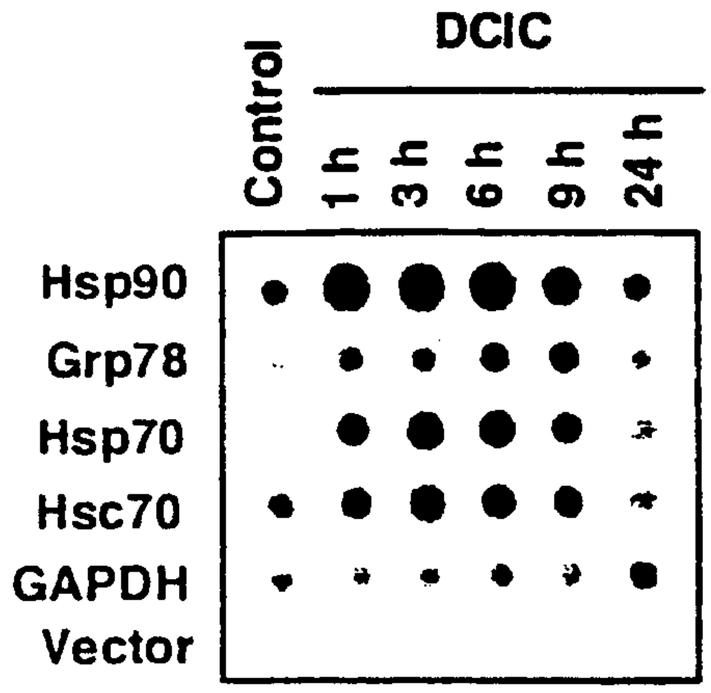
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Grp78
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Hsc70
GAPDH
Vector

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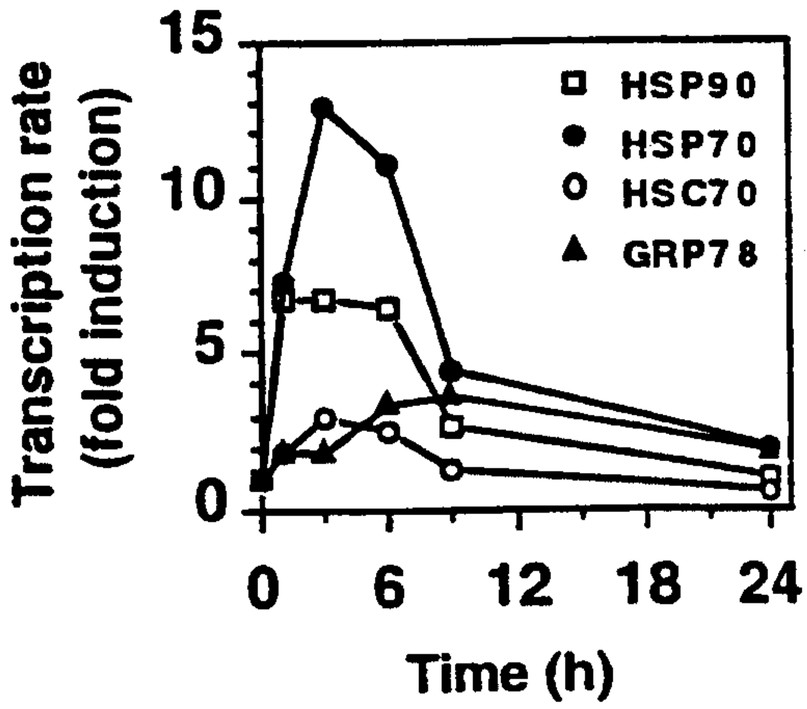
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(fold induction)
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10
5
0
HSP90
HSP70
HSC70
GRP78
0
6
12
18
24
Time (h)

Fig. 1

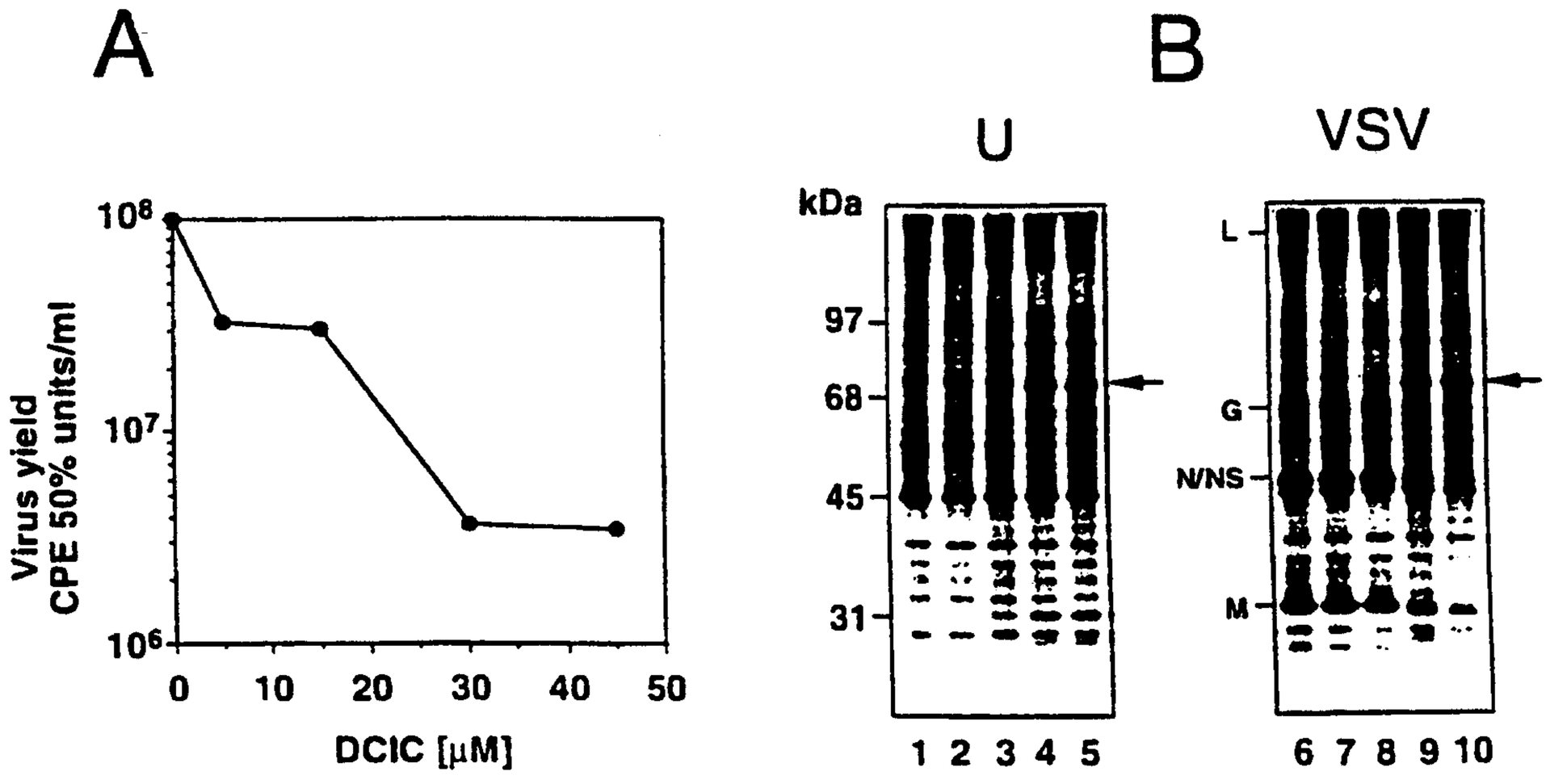
A
Virus yield
CPE 50% units/ml
10^8
10^7
10^6
0
10
20
30
40
50
DCIC [μM]
B
U
VSV
kDa
97
68
45
31
L
G
N/NS
M
1 2 3 4 5
6 7 8 9 10

Fig. 2

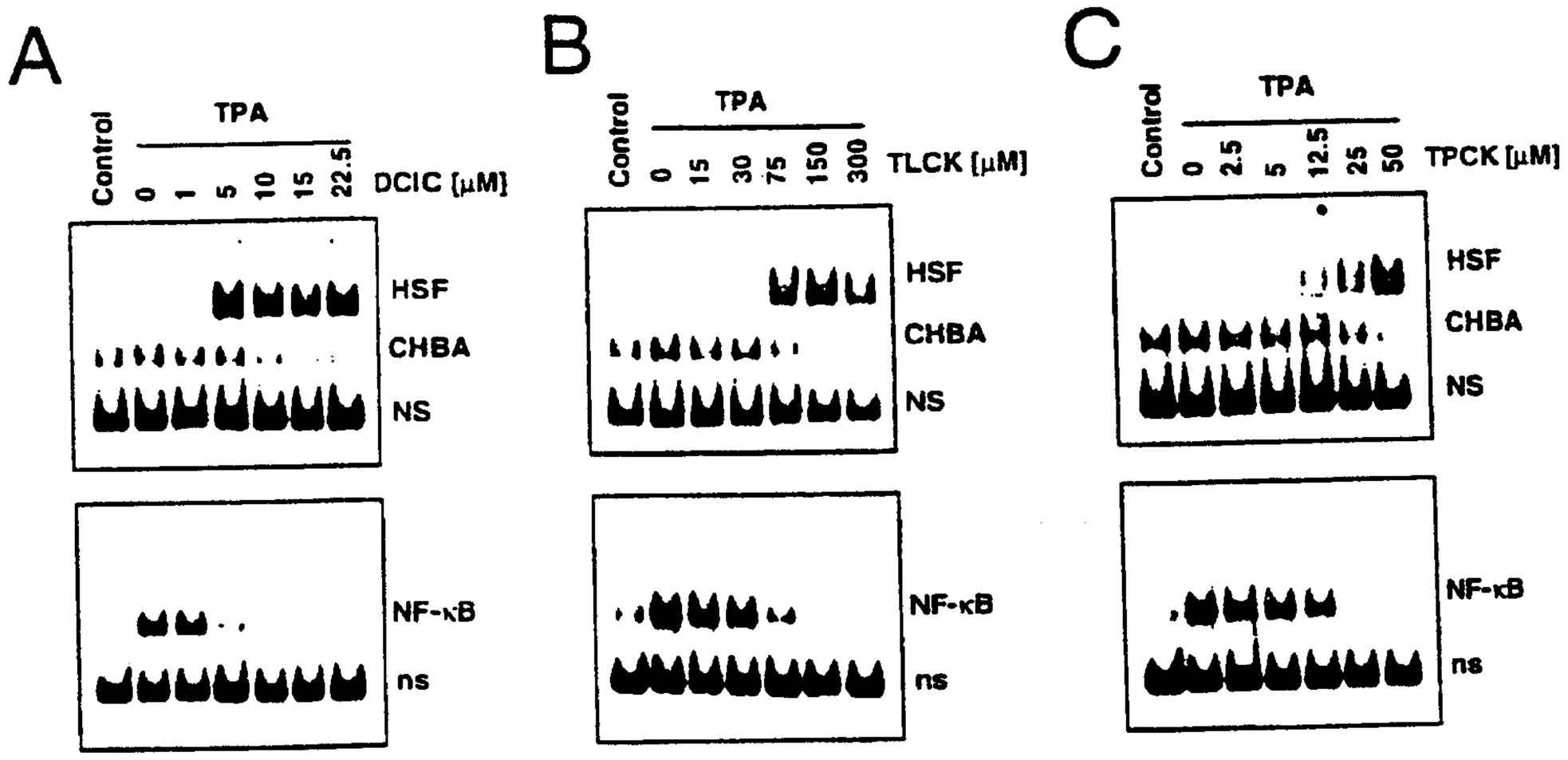
A
Control
TPA
0 1 5 10 15 22.5
DCIC [μM]
HSF
CHBA
NS
NF-κB
ns
B
Control
TPA
0 15 30 75 150 300
TLCK [μM]
HSF
CHBA
NS
NF-κB
ns
C
Control
TPA
0 2.5 5 12.5 25 50
TPCK [μM]
HSF
CHBA
NS
NF-κB
ns

Fig. 3