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DESCRIPTION

[0001] The present invention relates to tetrahydroisoquinolinone derivatives, a pharmaceutical composition comprising the same and the use of these derivatives in the inhibition of the Hsp70 protein. The compounds are useful in the treatment or inhibition of cancer, autoimmune disease, rheumatoid arthritis, inflammatory bowel disease and psoriasis.

[0002] Multiple myeloma is a hematological B cell malignancy. The vast majority of the affected cells are localized within the bone marrow, where they promote bone destruction and impair normal hematopoiesis. Improved methods in stem cell therapies and novel drugs such as proteasome inhibitors and derivatives of thalidomide have lead to significant improvement in overall survival of myeloma patients, which currently stands at about five years. Nevertheless, multiple myeloma remains incurable and its clinical cause is usually characterized by good initial response to treatment followed after some time by relapse and an eventual development of general resistance to current therapies.

[0003] It has been demonstrated that the heat shock protein 70 (Hsp70) plays an essential pathogenic role in multiple myeloma. In recent years, increasing evidence has suggested Hsp70 as a potential anti-cancer target. It has been previously observed that dual targeting of the Hsp70 isoforms Hsp72 and Hsp73 induces tumor-specific apoptosis. However, to date only a limited number of Hsp70 inhibitors is available, while efficient and selective pharmacological agents are almost completely missing.

[0004] US 2009/0068144 A1 discloses tetrahydroisoquinolin-1-one derivatives for the treatment of cancer. The exemplified compounds bear a 4-carboxylic acid substituent being present as free acid or for example as -CONH lower alkyl moiety.

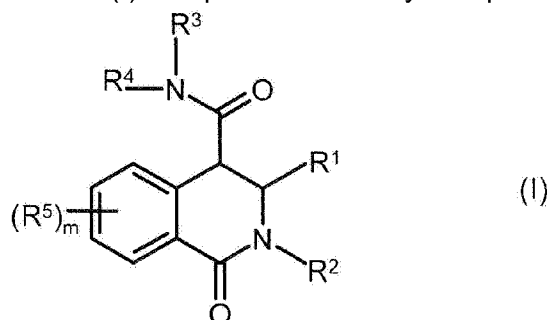
[0005] US 2005/0124614 A1 discloses 3,4-dihydroisoquinolin-1-ones that are said to be activators of caspases and inducers of apoptosis. Most of the exemplified compounds bear no or only small substituents, like methyl or propyl at position 2 of the tetrahydroisoquinolinone ring. Furthermore, the carboxamide substituent at position 4 may bear relatively small substituents like hydrogen and alkyl.

[0006] US 2010/0227866 A1 discloses a broad variety of tetrathydroisoquinolin-1-one derivatives which are said to be useful as therapeutic agents for irritable bowel syndrome.

[0007] There is therefore still a need for further compounds exhibiting improved Hsp70 protein inhibiting activity and being useful for the treatment of cancer, in particular of multiple myeloma.

[0008] The present inventors have found that certain tetrahydroisoquinolin-1-one derivatives bearing specific substituents exhibit high Hsp70 protein inhibition activity and therefore are useful in the treatment of cancer, in particular in the treatment of multiple myeloma.

[0009] The present invention therefore relates to a compound represented by the following formula (I) or a pharmaceutically acceptable salt thereof



wherein

R¹ is phenyl, pyridinyl or pyrimidinyl, each of which may be substituted by one or more substituents Y;

R² is phenyl, pyridinyl or pyrimidinyl, each of which may be substituted by one or more substituents Y';

R³ is phenyl, pyridinyl or pyrimidinyl, each of which may be substituted by one or more substituents Y'';

Y, Y' and Y'' are independently selected from halogen, C₁₋₆ alkyl, C₁₋₆ haloalkyl, hydroxy, C₁₋₆ alkoxy, C₁₋₆ haloalkoxy, nitro, -NR⁶R⁷, -CO-R⁸, -CO-NR⁶R⁷, -COOR⁶, and -CO-NR⁶-CO-R⁸;

R⁴ is hydrogen or C₁₋₄ alkyl;

R⁵ is halogen, hydroxy, C₁₋₆ alkyl, C₁₋₆ alkoxy, C₁₋₆ haloalkyl, C₁₋₆ haloalkoxy, nitro or -NR⁶R⁷;

R⁶ and R⁷ are independently selected from hydrogen and C₁₋₆ alkyl;

R⁸ is C₁₋₆ alkyl;

m is an integer of 0 to 3, wherein if m is 2 or 3 the substituents R⁵ may be selected independently of each other;

and which is a *trans* enantiomer.

These compounds exhibit high cell death-inducing potential in the multiple myeloma cell line INA-6 and at the same time show low toxicity.

[0010] Among these compounds 2,3-bis(4-methoxyphenyl)-N-(6-methylpyridin-2-yl)-1-oxo-1,2,3,4-tetrahydroisoquinoline-4-carboxamide and 2,3-bis(4-methoxyphenyl)-N-pyridin-2-yl-1-oxo-1,2,3,4-tetrahydroisoquinoline-4-carboxamide are reported catalog compounds having CAS registry numbers 442858-86-8 and 442858-72-2, respectively. These compounds are

therefore excluded from the scope of the compound claim but since their ability to inhibit the Hsp70 protein and their utility in the treatment of cancer and other diseases has not yet been reported they are included in the scope of the medical use claims.

[0011] In the context of the present invention the term "halogen" includes fluoro, chloro, bromo and iodo, preferably fluoro and chloro.

[0012] The term "C₁₋₆ alkyl" includes methyl, ethyl, *n*-propyl, iso-propyl, *n*-butyl, iso-butyl, *tert*-butyl, pentyl and hexyl, preferably methyl, ethyl, *n*-propyl and iso-propyl, more preferably methyl and ethyl.

[0013] The term "C₁₋₆ haloalkyl" includes the above described C₁₋₆ alkyl groups being substituted with one or more halogen atoms which are defined as above. A suitable haloalkyl is, for example, trifluoromethyl.

[0014] The term "C₁₋₆ alkoxy" includes alkoxy groups containing the above described C₁₋₆ alkyl residues, such as methoxy, ethoxy, *n*-propoxy and iso-propoxy, in particular methoxy and ethoxy.

[0015] The term "C₁₋₆ haloalkoxy" includes a haloalkoxy group comprising a C₁₋₆ haloalkyl moiety as described above.

[0016] The term "C₁₋₄ alkyl" defines methyl, ethyl, *n*-propyl, iso-propyl, *n*-butyl, iso-butyl and *tert*-butyl, preferably methyl and ethyl.

[0017] The pyridinyl and pyrimidinyl residues represented by R¹, R² and R³ may be attached to the remaining molecule by any of their carbon atoms. Preferably these residues are pyridin-2-yl, pyrimidin-2-yl or pyrimidin-4-yl.

[0018] R¹ preferably is phenyl. R² preferably is phenyl. Most preferably, both, R¹ and R² are phenyl.

[0019] R³ preferably is phenyl or pyridinyl, in particular pyridin-2-yl or pyridin-3-yl.

[0020] R¹, R² and R³ may be substituted by one or more substituents which are independently selected from halogen, C₁₋₆ alkyl, C₁₋₆ haloalkyl, hydroxyl, C₁₋₆ alkoxy and C₁₋₆ haloalkoxy, preferably from halogen, C₁₋₆ alkyl and C₁₋₆ alkoxy. For the substituents Y and Y' in R¹ and R² halogen, methyl, ethyl, methoxy and ethoxy are preferred. For Y" in R³ methyl and ethyl are preferred.

[0021] The number of substituents Y, Y' and Y" is not particularly limited, however, for R¹ and

R^2 each one substituent Y and Y', respectively, is preferred and for R^3 one or two substituents Y'' are preferred.

[0022] Y, Y' and Y'' may be present at any carbon atom of the phenyl, pyridinyl or pyrimidinyl represented by R^1 , R^2 and R^3 , respectively. Preferably, R^1 and R^2 , if substituted, bear at least one substituent at ortho or para position to the position at which R^1 and R^2 are attached to the tetrahydroisoquinolin-1-one ring. Preferably, R^1 and R^2 do not bear substituents only at one or both of their meta positions. Most preferably, R^1 and/or R^2 bear one substituent at para position.

[0023] In a further preferred embodiment of the present invention, R^1 and/or R^2 bear one substituent selected from halogen, methoxy and ethoxy at para position to the position at which R^1 and R^2 are attached to the tetrahydroisoquinolin-1-one ring. In this embodiment both, R^1 and R^2 preferably are phenyl. More preferably R^1 and/or R^2 are phenyl or 4-methoxyphenyl. Most preferred R^1 is 4-methoxyphenyl and R^2 is phenyl.

[0024] One or more substituents Y'' may be present at any carbon atom of the phenyl, pyridinyl and pyrimidinyl represented by R^3 . Preferably R^3 bears one substituent Y'' in meta position to the position at which R^3 is attached to the carboxamide moiety or two substituents Y'' both at meta position to the position at which R^3 is attached to the carboxamide moiety. Thus, preferred substituents R^3 are pyridin-2-yl, 6-methylpyridin-2-yl, *m*-tolyl and 3,5-dimethylphenyl. These preferred substituents R^3 are preferably further combined with the above described preferred substituents R^1 and R^2 , in particular with phenyl and 4-methoxyphenyl for R^1 and/or R^2 .

[0025] The tetrahydroisoquinolin-1-one moiety in the compound of the present invention may bear up to three further substituents R^5 . If there are two or three substituents R^5 , these may be selected independently of each other. Those compounds which do not contain any substituent R^5 are preferred.

[0026] As the compound of the present invention has two chiral centers, four stereoisomers are possible. Among these the *trans*-enantiomers show higher binding activity, they are preferred over the *cis*-enantiomers. A cell-viability assay revealed that the *trans*-R,R enantiomer was more active than the *trans*-S,S enantiomer. Therefore, the *trans*-R,R enantiomer of the compound of the present invention is most preferred.

[0027] The following compounds are particularly preferred:

trans-N-(6-Methylpyridin-2-yl)-1-oxo-2,3-diphenyl-1,2,3,4-tetrahydroisoquinoline-4-carboxamide,

trans-3-(4-Methoxyphenyl)-N-(6-methylpyridin-2-yl)-1-oxo-2-phenyl-1,2,3,4-tetrahydroisoquinoline-4-carboxamide,

trans-2-(4-Methoxyphenyl)-N-(6-methylpyridin-2-yl)-1-oxo-3-phenyl-1,2,3,4-tetrahydroisoquinoline-4-carboxamide,

trans-2,3-Bis(4-methoxyphenyl)-1-oxo-N-(m-tolyl)-1,2,3,4-tetrahydroisoquinoline-4-carboxamide,

trans-N-(3,5-Dimethylphenyl)-2,3-bis(4-methoxyphenyl)-1-oxo-1,2,3,4-tetrahydroisoquinoline-4-carboxamide,

trans-2,3-Bis(4-fluorophenyl)-N-(6-methylpyridin-2-yl)-1-oxo-1,2,3,4-tetrahydroisoquinoline-4-carboxamide,

trans-2,3-Bis(4-chlorophenyl)-N-(6-methylpyridin-2-yl)-1-oxo-1,2,3,4-tetrahydroisoquinoline-4-carboxamide,

trans-3-(4-Methoxyphenyl)-1-oxo-2-phenyl-N-(pyrimidin-2-yl)-1,2,3,4-tetrahydroisoquinoline-4-carboxamide,

trans-3-(4-Methoxyphenyl)-1-oxo-2-phenyl-N-(pyridin-3-yl)-1,2,3,4-tetrahydroisoquinoline-4-carboxamide, and

trans-N-(4,6-Dimethylpyridin-2-yl)-3-(4-methoxyphenyl)-1-oxo-2-phenyl-1,2,3,4-tetrahydroisoquinoline-4-carboxamide.

[0028] The compounds of the present invention exhibit high inhibition of the Hsp70 protein. Therefore, they are suitable for the preparation of pharmaceutical compositions comprising these compounds. The choice of a pharmaceutical composition depends on various factors such as the mode of drug administration, such as oral, systemic or parenteral administration. The preferred manner of administration is oral or parenteral using a convenient daily dosage regimen, which can be adjusted according to the degree of affliction. Oral compositions can take the form of tablets, pills, capsules, semi-solids, powders, sustained release formulations, solutions, suspensions, elixirs, aerosols, or any other appropriate compositions.

[0029] The compositions are comprised of in general, a compound of formula (I) in combination with at least one pharmaceutically acceptable excipient. Such excipient may be any solid, liquid, semi-solid or, in the case of an aerosol composition, gaseous excipient that is generally available to one of skill in the art.

[0030] In general, in the case of oral or parenteral administration to adult humans weighing approximately 80 kg, a daily dose of preferably from about 10 mg to about 10,000 mg, more preferably from about 20 mg to about 1,000 mg, should be appropriate, although the upper limit may be exceeded when indicated. The daily dosage can be administered as singly dose

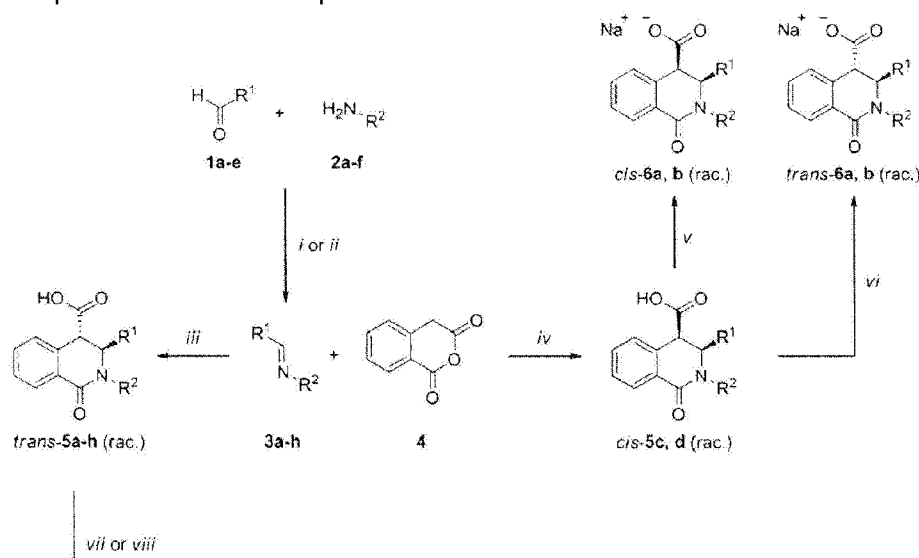
or in separate doses, or for parenteral administration, it may be given as continuous infusion.

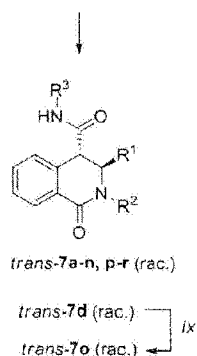
[0031] The compounds of this invention can be administered in combination with known anticancer agents or with other pharmaceutically active ingredients. Combinations of two or more pharmaceutically active ingredients may be administered in the form of a combination preparation containing a fixed dose of each of the pharmaceutically active ingredients or in the form of a combination of several pharmaceutical compositions each containing one of the pharmaceutically active ingredients.

[0032] Furthermore, the present invention relates to the compound of formula (I) as defined above for use in the treatment of inhibition of a disease being susceptible to the inhibition of the Hsp70 protein. Specific diseases which can be treated or inhibited by administration of the compound of formula (I) are cancer, in particular multiple myeloma, autoimmune disease, rheumatoid arthritis, inflammatory bowel disease and psoriasis.

[0033] The tetrahydroisoquinolin-1-one derivatives of the present invention can for example be synthesized via a reaction between homophthalic anhydride and imines as shown in Scheme 1 below. For this purpose a variety of imines can be produced in the first step. Besides the well-known reaction of amines and aldehydes in conventional organic solvents such as ethanol which can be utilized for the syntheses of **3a-c**, a further procedure, using ethyl lactate as a solvent component, can be applied. By adding water to ethyl L-lactate the polarity of the resulting solvent can be adjusted for synthesizing aryl imines like **3d-h**, which purely crystallize straight out of the solution in good yields.

[0034] Scheme 1. Reagents and conditions: (i) EtOH_{abs}, rt, 3 h; (ii) ethyl L-lactate/water (8:2 and 9:1, respectively), rt; (iii) TiCl₄, DIPEA, CH₂Cl₂_{abs}, 0°C/rt, 3 h; (iv) CHCl₃_{abs}, rt, 2 h; (v) NaHCO₃ (1 M), EtOH, rt, 30 min; (vi) NaOH (8M), EtOH, rt, 30 min; (vii) H₂NR³, *i*-butyl chloroformate, NaHCO₃, CH₃CN_{abs}, 0°C/rt; (viii) H₂NR³, TCBoc-chloride, NaHCO₃ and NEt₃, respectively, CH₃CN_{abs}, 0°C/rt; (ix) BBr₃, CH₂Cl₂_{abs}, 0°C/rt. - The substitution pattern of compounds *trans*-**7a-r** is provided in Table 1.





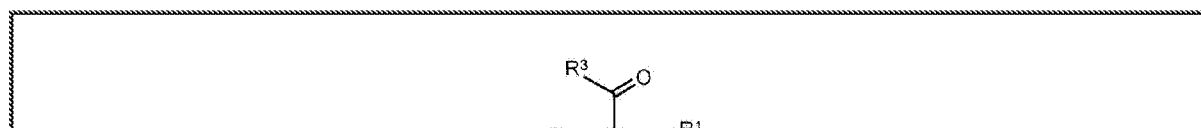
1a $R^1 = C_6H_5$	2a $R^2 = C_6H_5$	3a, 5a $R^1 = R^2 = C_6H_5$
1b $R^1 = p-(H_3CO)-C_6H_4$	2b $R^2 = p-(H_3CO)-C_6H_4$	3b, 5b, 6b $R^1 = p-(H_3CO)-C_6H_4, R^2 = C_6H_5$
		3c, 5c, 6c $R^1 = C_6H_5, R^2 = p-(H_3CO)-C_6H_4$
		3d, 5d, 6d $R^1 = R^2 = p-(H_3CO)-C_6H_4$
1c $R^1 = p-F-C_6H_4$	2c $R^2 = p-F-C_6H_4$	3e, 5e $R^1 = R^2 = p-F-C_6H_4$
1d $R^1 = p-Cl-C_6H_4$	2d $R^2 = p-Cl-C_6H_4$	3f, 5f $R^1 = R^2 = p-Cl-C_6H_4$
1e $R^1 = 3,5-(H_3C)_2-C_6H_3$	2e $R^2 = 3,5-(H_3C)_2-C_6H_3$	3g, 5g $R^1 = R^2 = 3,5-(H_3C)_2-C_6H_3$
	2f $R^2 = p-(C_6H_5O)-C_6H_4$	3h, 5h $R^1 = p-(H_3CO)-C_6H_4$
		$R^2 = p-(C_6H_5O)-C_6H_4$

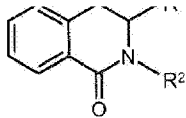
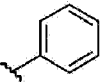
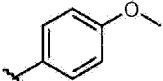
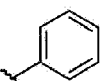
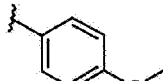
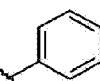
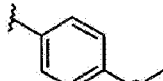
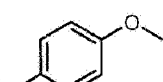
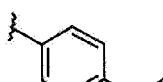
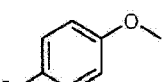
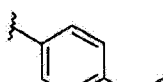
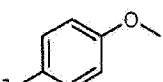
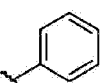
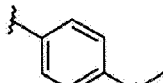
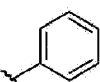
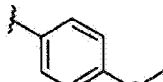
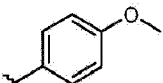
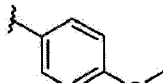
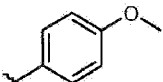
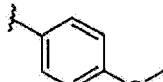
[0035] Several methods for the synthesis of isoquinolone carboxylic acids out of homophthalic anhydride are known using e.g. Lewis acids, protic acids, bases, ionic liquids and heterogeneous catalysts in order to enhance stereoselectivity and yield. The direct conversion with imines bearing discriminating substituents is known to give the *cis*-diastereomer specifically. Hence, the synthesis of *cis-5c* and *cis-5d* can be carried out without further auxiliaries and results in good yield and selectivity (d.e. 96 %). The *trans*-selective conversion (d.e. 96 %) can be achieved by adding $TiCl_4$ and diisopropyl ethyl amine (DIPEA). Diastereomeric assignment can be accomplished by the coupling constant J_{AB} of isoquinolone hydrogen H-3 and H-4, which is 5-6 Hz for the *cis*-isomer and 0-2 Hz for the *trans*-isomer.

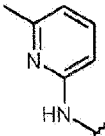
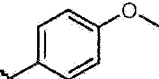
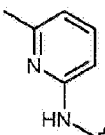
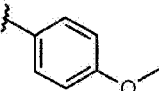
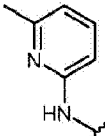
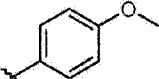
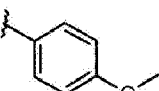
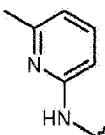
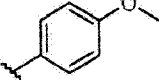
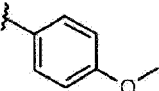
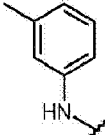
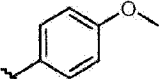
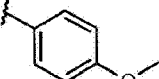
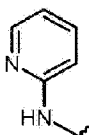
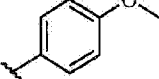
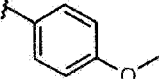
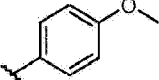
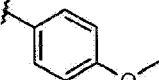
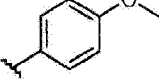
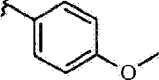
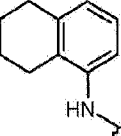
[0036] The sodium salts *trans-6b*, *cis-6c* and *cis-6d* can be obtained *via* deprotonation with $NaHCO_3$. The known epimerization after treatment with $NaOH$ can be utilized for the syntheses of the sodium salts *trans-6c* and *trans-6d*. The corresponding carboxamides can be synthesized via mixed anhydrides using *i*-butyl chloroformate and 2,2,2-trichloro-1,1-dimethylethyl chloroformate (TCBoc-chloride), respectively, followed by the *in situ* reaction with corresponding amines. Using TCBoc-chloride avoids the formation of isoquinolone carboxylester side-product, as no alcohol or other nucleophilic species is eliminated. The phenol *trans-7o* can be synthesized by cleaving both methoxy groups of *trans-7d* by means of BBr_3 .

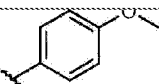
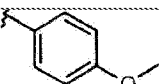
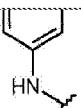
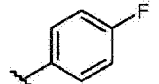
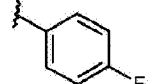
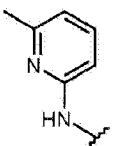
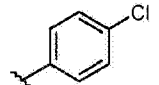
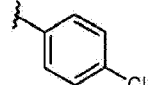
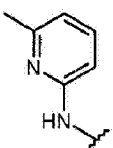
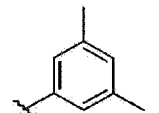
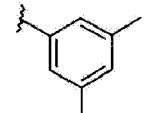
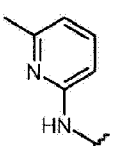
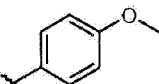
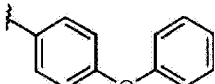
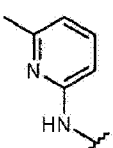
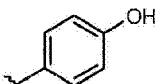
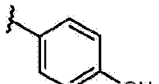
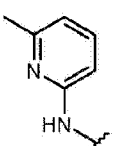
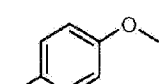
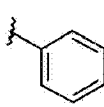
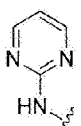
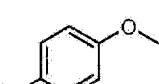
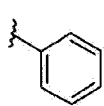
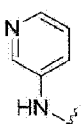
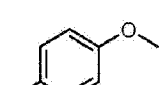
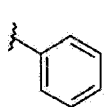
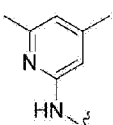
[0037] The compounds of the below examples and comparative examples including their effectivity in the inhibition of the Hsp70 protein as measured by the cell viability assay described below are summarized in the following Table 1.

Table 1



				
Compd.	R ¹	R ²	R ³	INA-6 EC ₅₀ (±sdv) [μM] ^a
<i>trans</i> -5a			-OH	6.9 (±0.3)
<i>trans</i> -5b			-OH	1.3 (±0.2)
<i>cis</i> -5c			-OH	>100
<i>trans</i> -5c			-OH	57 (±15)
<i>cis</i> -5d			-OH	>100
<i>trans</i> -5d			-OH	10.1 (±0.1)
<i>trans</i> -6b			Na ⁺ ⁻O-	2.0 (±0.1)
<i>cis</i> -6c			Na ⁺ ⁻O-	>100
<i>trans</i> -6c			Na ⁺ ⁻O-	64 (±19)
<i>cis</i> -6d			Na ⁺ ⁻O-	>100
<i>trans</i> -6d			Na ⁺ ⁻O-	7.9 (±0.8)

Compd.	R ¹	R ²	R ³	INA-6 EC ₅₀ (±sdv) [μM] ^a
<i>trans</i> -7a				2.3 (±0.6)
<i>trans</i> -7b				0.41 (±0.03)
<i>trans</i> -7c				_ b
<i>trans</i> -7d				0.68 (±0.04)
<i>trans</i> -7e				0.88 (±0.10)
<i>trans</i> -7f				0.53 (±0.03)
<i>trans</i> -7g				7.1 (±0.3)
<i>trans</i> -7h				>100
<i>trans</i> -7i				>100
				

Compd.	R ¹	R ²	R ³	INA-6 EC ₅₀ (±sdv) [μM] ^a
<i>trans</i> -7j				0.95 (±0.09)
<i>trans</i> -7k				6.4 (±0.7)
<i>trans</i> -7l				2.9 (±0.3)
<i>trans</i> -7m				43 (±12) ^b
<i>trans</i> -7n				>100 ^b
<i>trans</i> -7o				30 (±2)
<i>trans</i> -7p				0.20 (±0.01)
<i>trans</i> -7q				0.57 (±0.02)
<i>trans</i> -7r				0.34 (±0.04)

^a All EC₅₀ values are the mean of at least three determinations

^b Precipitated under assay-conditions

[0038] The data in the above table demonstrate that replacing the prior art substituents in position 4 of the tetrahydroisoquinolin-1-one derivatives by the substituents according to the invention significantly lowers the EC₅₀ values of the compounds thereby indicating an increased effectivity in the inhibition of the Hsp70 protein. If, for example, the R³ substituent -OH in compound *trans-5a* is replaced by a methylpyridinylamino substituent in the compound *trans-7a*, the EC₅₀ value decreases from 6.9 to 2.3 µM. Similarly, if in compound *trans-5b* the -OH substituent for R³ is replaced by methylpyridinylamino in compound *trans-7b*, the EC₅₀ value decreases from 1.3 to 0.41 µM. A further example is compound *trans-5d* which bears a -OH substituent as R³ (and which therefore is not according to the invention) and which exhibits a EC₅₀ value of 10.1 µM. Replacing the -OH substituent by methylpyridinylamino (*trans-7d*), methylphenylamino (*trans-7e*) and pyridinylamino (*trans-7f*) results in a decrease in the EC₅₀ value to 0.68, 0.88 and 0.53 µM, respectively. On the other hand, amino substituents for R³ which are not according to the invention result in compounds having significantly increased EC₅₀ values of 7.1 µM (*trans-7g*) and above 100 µM (*trans-7h* and *trans-7i*).

Cell viability assay

[0039] The effect of pharmacological Hsp70 inhibition on cell viability was analyzed after 72 hours of treatment in both non-malignant (mononuclear cells from the peripheral blood) and malignant lymphocytes (multiple myeloma cells). The mononuclear cells were separated from the peripheral blood of healthy donors by Ficoll-Hypaque density gradient centrifugation. The human cell line INA-6 represents a well-established model for multiple myeloma. The percentage of viable and apoptotic cell fractions was assessed using an annexin V - FITC/propidium iodide (PI) staining kit (Bender MedSystems, Vienna, Austria) according to manufacturer's instructions. In brief, cells were washed in PBS, incubated for 10 min in 100 ml binding buffer (10 mM HEPES/NaOH, pH 7.4, 140 mM NaCl, 2.5 mM CaCl₂) containing 2.5 ml annexin V-FITC mix and 1 mg/ml propidium iodide (PI), subsequently diluted with 100 ml binding buffer and analyzed by flow cytometry (FACSCalibur/CELLQuest; Becton Dickinson, Heidelberg, Germany). Whereas viable cells are negative for both, annexin V-FITC and PI, early apoptotic cells show a positive annexin V-FITC staining, and cells in a late apoptotic stage which lose their membrane integrity additionally incorporate DNA-binding PI. To establish kill curves, concentrations of the tested compounds were titrated, and the measured viable cell fractions were further analyzed using the Prism Calculation Software (GraphPad Software Inc., La Jolla, USA).

[0040] Apoptosis assays. Hsp70-dependent prevention of apoptosis was investigated by analyses of the apoptosis-inducing factors (AIF) and of the caspases 9 and 3 in INA-6 cells. INA-6 cells were treated with 0.6 µM of compound *trans-7b* for 8 hours prior to analyze nuclear translocation of AIF. Staining of the nuclear protein lamin A was used as a loading control.

Upon treatment of INA-6 cells with 0.6 μ M of compound *trans-7b* for 24 hours cleavage and activation of the caspases 9 and 3 were analyzed by Western. Analysis of cleavage of the bona fide caspase substrate PARP served as a control for caspase activation. Co-treatment with the pan-caspase inhibitor Z-VAD-FMK (50 μ M) was employed as another specificity control.

[0041] Western blot analysis. Protein expression levels were determined with Western blotting procedures (M. Chatterjee, et al., Blood 2002, 100, 3311-3318). Following separation by SDS-PAGE, proteins were transferred onto nitrocellulose membranes (Schleicher & Schuell, Dassel, Germany) and stained with antibodies against Hsp72, Hsp73, Hsp90 (all from Enzo Life Science, Lörrach, Germany), AIF, lamin A, caspases 9 and 3, PARP (all from Santa Cruz, Heidelberg, Germany). An anti- α tubulin antibody (Sigma, Deisenhofen, Germany) was employed to assess equal loading. Secondary antibodies used were anti-rat (Enzo Life Science), anti-rabbit and anti-mouse (both from GE Healthcare, Little Chalfont, UK).

[0042] As demonstrated by the data in Figure 1, *trans-7b* treatment decreases HSP72 and HSP73 but not HSP90 expression, and inhibits HSP70/HSP90 multi-chaperone activity leading to downregulation of constitutive expression levels of well-defined HSP70-dependent client proteins in myeloma cells. The heat shock proteins HSP72 and HSP73 critically interact with HSP90 in order to mediate an essential chaperone function for a multitude of proteins including various signaling intermediates. According to an established HSP72/73-dependent client protein signature and its role for survival in MM, the proposed anti-HSP72/73 effect of *trans-7b* in the INA-6 multiple myeloma (MM) cell line model was evaluated. INA-6 cells were incubated either with DMSO as a solvent control or with the compound *trans-7b* (0.6 μ M), and additionally incubated with the pan-caspase inhibitor Z-VAD-FMK (50 μ M) prior to Western blot analyses of HSP72, HSP73 or HSP90 expression (Figure 1A), of the expression of several well-defined HSP70/HSP90 chaperon-dependent signaling intermediates or their substrates (Figure 1B), or of the HSP70-dependent nuclear translocation of the apoptosis-inducing factor (AIF) (Figure 1C). For Western analyses either whole cell pellets (Figure 1A and 1B) or nuclear pellets (Figure 1C) were dissolved in lysis buffer (20 mM HEPES (pH 7.9), 350 mM NaCl, 1 mM MgCl₂, 0.5 mM EDTA, 0.1 mM EGTA, 1% NP40, 0.5 mM dithiothreitol (DTT), 1 mM Na₃VO₄, 0.1 mM PMSF and 1 μ g/ml aprotinin). Lysates were cleared by centrifugation, measured by quantitative protein assay (Bio-Rad, München, Germany) and subjected to SDS gel electrophoresis. Proteins were transferred onto nitrocellulose membranes (Schleicher & Schuell, Dassel, Germany), incubated with primary antibodies against HSP72, HSP73, HSP90 (from Stressgen Bioreagents, Ann Arbor, USA), AKT, C-Raf, RIP, IKK α , p-MEK, MEK, p-ERK, ERK, p-STAT3, STAT3, AIF (from Cell Signaling Technology, Frankfurt a.Main, Germany), Lamin A, β -actin (from Sigma-Aldrich, Deisenhofen, Germany), or α -tubulin (from Biozol Eching, Germany) according to standard procedures and visualized with secondary anti-rabbit horseradish peroxidase (HRP)-conjugated antibodies using an enhanced chemoluminescence detection system (ECL, Amersham, Freiburg, Germany). The WB analyses show that in contrast to HSP90 (and α -tubulin which was stained as a loading control), the constitutive expression level of the major HSP70 isoforms HSP72 and HSP73 are reduced indicating their binding to *trans-7b* and their subsequent degradation (Figure 1A). In addition, reduced expression levels of a multitude of signaling proteins, which have been shown to be dependent

on the HSP70/HSP90 multi-chaperone function, have been observed. Thus, AKT, C-Raf, RIP, IKK α and MEK levels were strongly downregulated after *trans-7b* treatment. Likewise, p-MEK as a Raf substrate, p-ERK as a MEK substrate as well as pSTAT3 as a janus kinase substrate are downregulated (Figure 1B). Because HSP90 expression remains unchanged (Figure 1A), these results clearly show that *trans-7b* disrupts HSP70-mediated chaperone function. In addition to signaling intermediates, the apoptosis-inducing factor (AIF), which is a pro-apoptotic factor that is physiologically inactive through HSP70 binding, was analyzed upon treatment with *trans-7b*. Western analyses revealed that activated AIF was shifting from the cytosol to the nucleus 8 hours after *trans-7b* treatment. This finding indicates that binding of HSP70 to AIF is prevented by *trans-7b*.

[0043] As demonstrated by the data in Figure 2, *trans-7b* treatment leads to activation of the caspases 9 and 3, and induction of apoptosis in malignant INA-6 MM cells but not in non-malignant peripheral blood mononuclear cells (PBMCs). In Figure 2A Western blot analyses of caspases 9 and 3, and the caspase substrate PARP (upper panel), and viability analyses (lower panel) of INA-6 MM cells upon treatment with *trans-7b* for 24 hours are shown. INA-6 cells were either incubated with DMSO (as a solvent control) or with 0.6 μ M of *trans-7b*, and additionally incubated either with DMSO or the pan-caspase inhibitor Z-VAD-FMK prior to analyses by Western or viability assessment (without Z-VAD-FMK co-treatment) by Annexin V/propidium iodide double staining. For Western analyses INA-6 cell pellets were dissolved in lysis buffer (20 mM HEPES (pH 7.9), 350 mM NaCl, 1 mM MgCl₂, 0.5 mM EDTA, 0.1 mM EGTA, 1% NP40, 0.5 mM dithiothreitol (DTT), 1 mM Na₃VO₄, 0.1 mM PMSF and 1 μ g/ml aprotinin). Lysates were cleared by centrifugation, measured by quantitative protein assay (Bio-Rad, München, Germany) and subjected to SDS gel electrophoresis. Proteins were transferred onto nitrocellulose membranes (Schleicher & Schuell, Dassel, Germany), incubated with antibodies specific for caspase 9, caspase 3 or PARP (Cell Signaling Technology, Frankfurt a.Main, Germany) according to standard procedures and visualized with secondary anti-rabbit horseradish peroxidase (HRP)-conjugated antibodies using an enhanced chemoluminescence detection system (ECL, Amersham, Freiburg, Germany). Western analyses reveal sequential caspase 9 and 3 cleavage leading to their activation as indicated by specific PARP cleavage. Annexin V / propidium iodide (PI) staining was performed using a fluorescein isothiocyanate (FITC) conjugated recombinant human annexin /propidium iodide kit (BenderMedSystems, Vienna, Austria) according to the manufacturer's instructions. INA-6 cells were washed in PBS, incubated for 15 minutes in 100 ml binding buffer (10 mM HEPES/NaOH, pH 7.4, 140 mM NaCl, 2.5 mM CaCl₂) containing 2.5 mL annexin V-FITC mix and 1 mg/ml PI and analyzed by flow cytometry (FACSCalibur/CELLQuest; Becton Dickinson, Heidelberg, Germany). Whereas early apoptosis is characterized by positive annexin V-FITC staining reaction, cells in a late apoptotic stage lose their membrane integrity and additionally incorporate PI. Thus, the fraction of viable cells remains negative for both annexin V-FITC and PI. In comparison with the DMSO control (100%) viability of *trans-7b*-treated INA-6 cells were drastically decreased. In contrast, if INA-6 MM cells were concomitantly incubated with the pan-caspase inhibitor Z-VAD-FMK (50 μ M), which sufficiently inhibited caspase activation and PARP cleavage, induction of apoptosis was largely prevented indicating that *trans-7b*-induced apoptosis is specifically dependent on caspase activation. Based on the above described

experiments also viability analyses of INA-6 in comparison with non-malignant PBMCs were performed choosing a longer incubation time (72 hours) as shown in Figure 2B or C. Raw data of three independent experiments have been calculated to establish means, standard deviation and dose-effect curves of *trans-7b*. The resulting experimental data show that INA-6 tumor cells are highly sensitive (in a low micromolar range) towards *trans-7b* treatment, whereas primary PBMCs lack any cytotoxic effect even in higher micromolar concentrations.

[0044] The data in Figure 3 shows that concomitant inhibition of HSP72/73 by *trans-7b* strongly enhanced the apoptotic effect of the HSP90 inhibitor NVP-AUY922. The heat shock protein HSP90, which critically cooperates with HSP72 and HSP73 to mediate a chaperone function for a multitude of proteins, is regarded as a reasonable therapeutic target in MM. Synergistic effects of a combinatorial HSP90/HSP70 inhibition in MM were recently demonstrated. Therefore, potential effects of a concomitant treatment of *trans-7b* with the HSP90 inhibitor NVP-AUY922 on viability of INA-6 cells were analyzed. INA-6 cells were incubated either with DMSO (as a solvent control), sublethal concentrations of AUY922 (7.5 nM) or *trans-7b* (3.5 μ M) or with a combination of both. Viability was measured by Annexin V-FITC/ propidium iodide double staining (BenderMedSystems, Vienna, Austria) according to the manufacturer's instructions. Cells were washed in PBS, incubated for 15 minutes in 100 ml binding buffer (10 mM HEPES/NaOH, pH 7.4, 140 mM NaCl, 2.5 mM CaCl₂) containing 2.5 mL annexin V-FITC mix and 1 mg/ml PI and analyzed by flow cytometry (FACSCalibur/CELLQuest; Becton Dickinson, Heidelberg, Germany). Whereas early apoptosis is characterized by positive annexin V-FITC staining reaction, cells in a late apoptotic stage lose their membrane integrity and additionally incorporate PI. Thus, the fraction of viable cells remains negative for both annexin V-FITC and PI. Raw data of three independent measurements were set in relation to their respective DMSO controls, and means and standard deviations were calculated using the Graphpad Prism software (Graphpad, La Jolla, USA). The experimental data clearly show that treatment with the HSP72/73 inhibitor *trans-7b* strongly enhanced the apoptotic effect of the HSP90 inhibitor NVP-AUY922.

General procedures for the syntheses of the imines

[0045] Method A: To a solution of the aniline derivative (1 eq) in abs. ethanol the corresponding aldehyde (1 eq) was added and stirred for 3 h at room temperature. The solvent was evaporated and the crude product was purified by crystallization (**3a-c**). Method B: The aniline derivative (1 eq) was dissolved in a mixture of ethyl L-lactate and water, and the corresponding aldehyde (1 eq) was added. The solution was stirred till all compounds were dissolved. The immediately formed crystals were filtered, washed with cold water and dried *in vacuo* to give the product. (**3d-h**)

General procedure for the syntheses of the *cis*-isoquinolone carboxylic acids (*cis*-5c-d)

[0046] To a solution of an imine (1 eq) in abs. chloroform homophthalic anhydride (1 eq) was added under Ar atmosphere and stirred for 2 h at room temperature. The formed precipitate was filtered and the crude purified by crystallization.

General procedure for the syntheses of the *trans*-isoquinolone carboxylic acids (*trans*-5a-h)

[0047] Homophthalic anhydride (1 eq) was dissolved in abs. dichloromethane under Ar atmosphere. The solution was cooled to 0°C and TiCl₄ (1 eq) was added under stirring. After 5 min N,N-diisopropylethylamine (1 eq) was added and the solution was stirred for 30 min. The corresponding imine dissolved in dichloromethane (1.5 eq) was added dropwise at 0°C. The reaction mixture was allowed to warm to room temperature and stirred for 3 h. The solvent was evaporated and the crude product was purified by column chromatography (silica gel 60, first eluent chloroform/ethanol/formic acid 10:0.3:0.1, second eluent chloroform/ethyl acetate/n-hexane/formic acid 7:5:0.25:0.05, and third eluent petroleum ether/ethyl acetate/formic acid 3:2:0.02 if necessary).

General procedure for the syntheses of the *trans*-1,2,3,4-tetrahydroisoquinoline-4-carboxamides (*trans*-7a-n, p-r)

[0048] The respective 1-oxo-1,2,3,4-tetrahydroisoquinoline-4-carboxylic acid (1 eq) was dissolved in abs. acetonitrile (5 mL) under Ar atmosphere and cooled to 0°C. Either solid NaHCO₃ or NEt₃ was added. Afterwards 1.5 eq of *i*-butyl chloroformiate and 2,2,2-trichloro-1,1-dimethylethyl chloroformiate, respectively, was added. The mixture was stirred for 30 min at 0°C, the corresponding amine (1.5 eq) was added, and the solution was stirred for additional 2 h while the mixture was allowed to warm-up to room temperature.

[0049] Using the above general procedures the following compounds were prepared as comparative examples and examples according to the invention. These examples are intended as being illustrative only and should not be construed as limiting.

Comparative Example 1

[0050] *trans*-2,3-Diphenyl-1-oxo-1,2,3,4-tetrahydroisoquinoline-4-carboxylic acid (*trans*-5a): Homophthalic anhydride (15 mmol, 2.4 g), N,N-diisopropylethylamine (15 mmol, 2.6 mL), titanium tetrachloride (15 mmol, 1.7 mL), **3a** (22.5 mmol, 4.1 g); yield: 41 % (47 %), colorless solid; mp: 226-229°C.

Comparative Example 2

[0051] ***trans*-3-(4-Methoxyphenyl)-1-oxo-2-phenyl-1,2,3,4-tetrahydroisoquinoline-4-carboxylic acid (*trans*-5b):** Homophthalic anhydride (15 mmol, 2.4 g), N,N-diisopropylethylamine (15 mmol, 2.6 mL), titanium tetrachloride (15 mmol, 1.7 mL), **3b** (22.5 mmol, 4.8 g); yield: 44 %; colorless solid; mp: 171-173°C.

Comparative Example 3

[0052] ***cis*-2-(4-Methoxyphenyl)-1-oxo-3-phenyl-1,2,3,4-tetrahydroisoquinoline-4-carboxylic acid (*cis*-5c):** Homophthalic anhydride (40 mmol, 6.5 g), **3c** (60 mmol, 12.7 g); the crude product was purified by crystallization from ethyl acetate. Yield: 48 % (91 %); colorless solid; mp: 208-209°C.

Comparative Example 4

[0053] ***trans*-2-(4-Methoxyphenyl)-1-oxo-3-phenyl-1,2,3,4-tetrahydroisoquinoline-4-carboxylic acid (*trans*-5c):** Homophthalic anhydride (31 mmol, 5.0 g), N,N-diisopropylethylamine (31 mmol, 5.3 mL), titanium tetrachloride (31 mmol, 3.4 mL), **3c** (46 mmol, 9.8 g); yield: 44 % (63 %); colorless amorphous solid; mp: 106-118°C.

Comparative Example 5

[0054] ***cis*-2,3-Bis(4-methoxyphenyl)-1-oxo-1,2,3,4-tetrahydroisoquinoline-4-carboxylic acid (*cis*-5d):** Homophthalic anhydride (30 mmol, 4.9 g), **3d** (30 mmol, 7.2 g); the crude product was purified by crystallization from ethanol. Yield: 44%; colorless solid; mp: 232-233°C.

Comparative Example 6

[0055] ***trans*-2,3-Bis(4-methoxyphenyl)-1-oxo-1,2,3,4-tetrahydroisoquinoline-4-carboxylic acid (*trans*-5d):** Homophthalic anhydride (19 mmol, 3.1 g), N,N-diisopropylethylamine (19 mmol, 3.2 mL), titanium tetrachloride (19 mmol, 2.1 mL), **3d** (28 mmol, 6.8 g); yield: 47 %; colorless solid; mp: 201-203°C.

Comparative Example 7

[0056] ***trans*-2,3-Bis(4-fluorophenyl)-1-oxo-1,2,3,4-tetrahydroisoquinoline-4-carboxylic acid (*trans*-5e):** Homophthalic anhydride (13 mmol, 2.2 g), N,N-diisopropylethylamine (13

mmol, 2.3 mL), titanium tetrachloride (13 mmol, 1.5 mL), **3e** (16 mmol, 3.5 g); yield: 49%; colorless amorphous solid; mp: 116-118°C.

Comparative Example 8

[0057] ***trans*-2,3-Bis(4-chlorophenyl)-1-oxo-1,2,3,4-tetrahydroisoquinoline-4-carboxylic acid (*trans*-5f)**: Homophthalic anhydride (9 mmol, 1.5 g), N,N-diisopropylethylamine (9 mmol, 1.6 mL), titanium tetrachloride (9 mmol, 1.0 mL), **3f** (11 mmol, 2.8 g); yield: 39 %; colorless amorphous solid; mp: 116-127°C.

Comparative Example 9

[0058] ***trans*-2,3-Bis(3,5-dimethylphenyl)-1-oxo-1,2,3,4-tetrahydroisoquinoline-4-carboxylic acid (*trans*-5g)**: Homophthalic anhydride (11 mmol, 1.8 g), **3g** (13.5 mmol, 3.2 g); the crude product was dissolved in acetonitrile and crystallization initiated by adding petroleum ether to give pure *trans*-5g. Yield: 58%; colorless solid; mp: 269-271 °C.

Comparative Example 10

[0059] ***trans*-3-(4-Methoxyphenyl)-1-oxo-2-(4-phenoxyphenyl)-1,2,3,4-tetrahydroisoquinoline-4-carboxylic acid (*trans*-5h)**: Homophthalic anhydride (8 mmol, 1.3 g), N,N-diisopropylethylamine (8 mmol, 1.4 mL), titanium tetrachloride (8 mmol, 0.9 mL), **3h** (10 mmol, 3.0 g); yield: 46 %; colorless amorphous solid; mp: 109-115°C.

Comparative Example 11

[0060] **Sodium *trans*-3-(4-methoxyphenyl)-1-oxo-2-phenyl-1,2,3,4-tetrahydroisoquinoline-4-carboxylate (*trans*-6b)**: Compound *trans*-5b (0.4 mmol, 150 mg) was suspended in ethanol and a solution of NaHCO₃ (0.4 mmol, 400 µL, 1 M) was added. After stirring for 30 min at room temperature the solvent was evaporated to give pure *trans*-6b. Yield: 96 %; colorless solid; mp: 97-99°C.

Comparative Example 12

[0061] **Sodium *cis*-2-(4-methoxyphenyl)-1-oxo-3-phenyl-1,2,3,4-tetrahydroisoquinoline-4-carboxylate (*cis*-6c)**: Compound *cis*-5c (0.5 mmol, 200 mg) was suspended in ethanol and a solution of NaHCO₃ (0.5 mmol, 540 µL, 1M) was added. After stirring for 30 min at room

temperature the solvent was evaporated to give pure *cis*-**6c**. Yield: 98 %; colorless solid; mp: 234-236°C.

Comparative Example 13

[0062] **Sodium *trans*-2-(4-methoxyphenyl)-1-oxo-3-phenyl-1,2,3,4-tetrahydroisoquinoline-4-carboxylate (*trans*-**6c**)**: Compound *cis*-**5c** (0.5 mmol, 200 mg) was suspended in ethanol and a solution of NaOH (0.5 mmol, 70 µL, 8M) was added. After stirring for 30 min at room temperature the solvent was evaporated to give pure *trans*-**6c**. Yield: 97 %; colorless solid; mp: 207-209 °C.

Comparative Example 14

[0063] **Sodium *trans*-2,3-bis(4-methoxyphenyl)-1-oxo-1,2,3,4-tetrahydroisoquinoline-4-carboxylate (*cis*-**6d**)**: Compound *cis*-**5d** (0.5 mmol, 200 mg) was suspended in ethanol and a solution of NaHCO₃ (0.5 mmol, 500 µL, 1M) was added. After stirring for 30 min at room temperature the solvent was evaporated to give pure *cis*-**6d**. Yield: 98 %; colorless solid; mp: 208-209°C.

Comparative Example 15

[0064] **Sodium *cis*-2,3-bis(4-methoxyphenyl)-1-oxo-1,2,3,4-tetrahydroisoquinoline-4-carboxylate (*trans*-**6d**)**: Compound *cis*-**5d** (1 mmol, 400 mg) was suspended in ethanol and a solution of NaOH (1 mmol, 125 µL, 8M) was added. After stirring for 30 min at room temperature the solvent was evaporated. The residue was dissolved in acetone and crystallization initiated by adding diethyl ether to give pure *trans*-**6d**. Yield: 89 %; colorless solid; mp: 216-218°C.

Example 1

[0065] ***trans*-N-(6-Methylpyridin-2-yl)-1-oxo-2,3-diphenyl-1,2,3,4-tetrahydroisoquinoline-4-carboxamide (*trans*-**7a**)**: Quantities: *trans*-**5a** (0.9 mmol, 300 mg), NaHCO₃ (0.9 mmol, 75 mg), 2,2,2-trichloro-1,1-dimethylethyl chloroformate (1.3 mmol, 315 mg) and 2-amino-6-picoline (1.3 mmol, 135 µL). The precipitate was filtered, suspended in water and dried *in vacuo*. Yield: 82 %; colorless solid; mp: 265-266°C.

Example 2

[0066] ***trans*-3-(4-Methoxyphenyl)-N-(6-methylpyridin-2-yl)-1-oxo-2-phenyl-1,2,3,4-tetrahydroisoquinoline-4-carboxamide (*trans*-7b)**: Quantities: *trans*-5b (1 mmol, 375 mg), NaHCO₃ (2 mmol, 170 mg), *i*-butyl chloroformate (1.5 mmol, 195 µL) and 2-amino-6-picoline (1.5 mmol, 150 µL). The precipitate was filtered and extracted with CHCl₃ using a soxhlet extractor. The resulting organic phase was evaporated. Crystallization from ethyl acetate and toluene gave *trans*-7b. Yield: 55 %; colorless solid; mp: 265-268°C.

Example 3

[0067] ***trans*-2-(4-Methoxyphenyl)-N-(6-methylpyridin-2-yl)-1-oxo-3-phenyl-1,2,3,4-tetrahydroisoquinoline-4-carboxamide (*trans*-7c)**: Quantities: *trans*-5c (2 mmol, 750 mg), NaHCO₃ (4 mmol, 335 mg), *i*-butyl chloroformate (3 mmol, 390 µL) and 2-amino-6-picoline (3 mmol, 305 µL). The precipitate was filtered and extracted with CHCl₃ using a soxhlet extractor. The obtained organic phase was evaporated. Crystallization from ethyl acetate gave *trans*-7c. Yield: 54 %; colorless solid; mp: 284-286°C.

Example 4

[0068] ***trans*-2,3-Bis(4-methoxyphenyl)-N-(6-methylpyridin-2-yl)-1-oxo-1,2,3,4-tetrahydroisoquinoline-4-carboxamide (*trans*-7d)**: Quantities: *trans*-5d (3.5 mmol, 1.4 g), NaHCO₃ (7 mmol, 585 mg), 2,2,2-trichloro-1,1-dimethylethyl chloroformate (5.2 mmol, 1.25 g) and 2-amino-6-picoline (5.2 mmol, 525 µL). The precipitate was filtered and extracted with CHCl₃ using a soxhlet extractor. The obtained organic phase was evaporated. Crystallization from MeOH gave *trans*-7d. Yield: 71 %; colorless solid; mp: 252-254°C.

Example 5

[0069] ***trans*-2,3-Bis(4-methoxyphenyl)-1-oxo-N-(*m*-tolyl)-1,2,3,4-tetrahydroisoquinoline-4-carboxamide (*trans*-7e)**: Quantities: *trans*-5d (2 mmol, 810 mg), NaHCO₃ (4 mmol, 335 mg), *i*-butyl chloroformate (3 mmol, 390 µL) and *m*-toluidine (3 mmol, 320 µL). The reaction mixture was evaporated, dissolved in CHCl₃ and extracted with aqueous formic acid (5 %). The organic layer was dried over Na₂SO₄, concentrated *in vacuo* to a few mL and *trans*-7e was precipitated by adding Et₂O. Yield: 64 %; colorless solid; mp: 254-256°C.

Example 6

[0070] ***trans*-2,3-Bis(4-methoxyphenyl)-1-oxo-N-(pyridin-2-yl)-1,2,3,4-**

tetrahydroisoquinoline-4-carboxamide (trans-7f): Quantities: *trans*-5d (2 mmol, 810 mg), NaHCO₃ (4 mmol, 335 mg), *i*-butyl chloroformate (3 mmol, 390 µL) and 2-aminopyridine (3 mmol, 280 mg). The precipitate was filtered, dissolved in CHCl₃ and extracted with water. The organic layer was dried over Na₂SO₄ and concentrated *in vacuo*. Crystallization from ethyl acetate gave *trans*-7f. Yield: 62 %; colorless solid; mp: 219-229°C.

Comparative Example 16

[0071] *trans*-2,3-Bis(4-methoxyphenyl)-N-methyl-1-oxo-1,2,3,4-tetrahydroisoquinoline-4-carboxamide (trans-7g): Quantities: *trans*-5d (1.2 mmol, 500 mg), NaHCO₃ (1.2 mmol, 105 mg), 2,2,2-trichloro-1,1-dimethylethyl chloroformate (1.9 mmol, 445 mg) and methylamine (1.9 mmol, 0.9 µL, 2M in THF). The solvent was reduced *in vacuo* and the residue was purified by column chromatography (silica gel 60, n-hexane/ethyl acetate/formic acid 1:2:0.1) and crystallized from ethyl acetate to give *trans*-7g. Yield: 74 %; colorless solid; mp: 202-203°C.

Comparative Example 17

[0072] *trans*-2,3-Bis(4-methoxyphenyl)-N,N-dimethyl-1-oxo-1,2,3,4-tetrahydroisoquinoline-4-carboxamide (trans-7h): Quantities: *trans*-5d (1.0 mmol, 400 mg), NEt₃ (1.0 mmol, 140 µL), 2,2,2-trichloro-1,1-dimethylethyl chloroformate (1.5 mmol, 360 mg) and dimethylamine (2.0 mmol, 1.0 mL, 2M in THF). The solvent was reduced *in vacuo* and the residue was purified by column chromatography (silica gel 60, 1st n-hexane/ethyl acetate/formic acid 1:10:0.1 and 2nd chloroform/ethanol 1 0:0.5) to give *trans*-7h. Yield: 90 %; colorless amorphous solid; mp: 105-107°C.

Comparative Example 18

[0073] *trans*-2,3-Bis(4-methoxyphenyl)-1-oxo-N-(5,6,7,8-tetrahydronaphthalen-1-yl)-1,2,3,4-tetrahydroisoquinoline-4-carboxamide (trans-7i): Quantities: *trans*-5d (1.2 mmol, 500 mg), NEt₃ (1.2 mmol, 175 µL), 2,2,2-trichloro-1,1-dimethylethyl chloroformate (1.9 mmol, 445 mg) and 5,6,7,8-tetrahydro-1-naphthylamine (1.9 mmol, 260 ML). The reaction mixture was concentrated *in vacuo*, dissolved in CHCl₃ and extracted with aqueous formic acid (5 %). The organic layer was dried over Na₂SO₄ and evaporated. Crystallization from ethyl acetate gave *trans*-7i. Yield: 63%; colorless solid; mp: 131-133°C.

Example 7

[0074] ***trans*-N-(3,5-Dimethylphenyl)-2,3-bis(4-methoxyphenyl)-1-oxo-1,2,3,4-tetrahydroisoquinoline-4-carboxamide (*trans*-7j)**: Quantities: *trans*-5d (1.2 mmol, 500 mg), NEt₃ (1.2 mmol, 175 μ L), 2,2,2-trichloro-1,1-dimethylethyl chloroformate (1.9 mmol, 445 mg) and 3,5-dimethylaniline (1.9 mmol, 230 μ L). The precipitate was filtered, dissolved in CHCl₃ and extracted with aqueous formic acid (5 %). The organic layer was dried over Na₂SO₄ and evaporated to give *trans*-7j. Yield: 91 %; colorless solid; mp: 286-288°C.

Example 8

[0075] ***trans*-2,3-Bis(4-fluorophenyl)-N-(6-methylpyridin-2-yl)-1-oxo-1,2,3,4-tetrahydroisoquinoline-4-carboxamide (*trans*-7k)**: Quantities: *trans*-5e (1.3 mmol, 500 mg), NEt₃ (1.3 mmol, 185 μ L), 2,2,2-trichloro-1,1-dimethylethyl chloroformate (2.0 mmol, 475 mg) and 2-amino-6-picoline (2.00 mmol, 200 μ L). The precipitate was filtered and crystallized from CHCl₃ to give *trans*-7k. Yield: 90 %; colorless crystals; mp: 275-277°C.

Example 9

[0076] ***trans*-2,3-Bis(4-chlorophenyl)-N-(6-methylpyridin-2-yl)-1-oxo-1,2,3,4-tetrahydroisoquinoline-4-carboxamide (*trans*-7l)**: Quantities: *trans*-5f (1 mmol, 410 mg), NEt₃ (1 mmol, 140 μ L), 2,2,2-trichloro-1,1-dimethylethyl chloroformate (1.5 mmol, 360 mg) and 2-amino-6-picoline (1.5 mmol, 150 μ L). The precipitate was filtered, dissolved in CHCl₃ and extracted with aqueous formic acid (5 %). The organic layer was dried over Na₂SO₄ and evaporated. Crystallization from ethyl acetate gave *trans*-7l. Yield: 77 %; colorless solid; mp: 241-242°C.

Example 10

[0077] ***trans*-2,3-Bis(3,5-dimethylphenyl)-N-(6-methylpyridin-2-yl)-1-oxo-1,2,3,4-tetrahydroisoquinoline-4-carboxamide (*trans*-7m)**: Quantities: *trans*-5g (1 mmol, 400 mg), NEt₃ (1 mmol, 140 μ L), 2,2,2-trichloro-1,1-dimethylethyl chloroformate (1.5 mmol, 360 mg) and 2-amino-6-picoline (1.5 mmol, 150 μ L). The precipitate was filtered, dissolved in CHCl₃ and extracted with aqueous formic acid (5 %). The organic layer was dried over Na₂SO₄ and evaporated. Crystallization from ethyl acetate gave *trans*-7m. Yield: 94 %; colorless solid; mp: 281-283°C.

Comparative Example 19

[0078] ***trans*-3-(4-Methoxyphenyl)-N-(6-methylpyridin-2-yl)-1-oxo-2-(4-phenoxyphenyl)-1,2,3,4-tetrahydroisoquinoline-4-carboxamide (*trans*-7n):** Quantities: *trans*-5h (1 mmol, 465 mg), NEt₃ (1 mmol, 140 µL), 2,2,2-trichloro-1,1-dimethylethyl chloroformate (1.5 mmol, 360 mg) and 2-amino-6-picoline (1.5 mmol, 150 µL). The precipitate was filtered, dissolved in CHCl₃ and extracted with aqueous formic acid (5 %). The organic layer was dried over Na₂SO₄ and evaporated. Crystallization from CHCl₃ gave *trans*-7n. Yield: 83 %; colorless solid; mp: 240-242°C.

Example 11

[0079] ***trans*-2,3-Bis(4-hydroxyphenyl)-N-(6-methylpyridin-2-yl)-1-oxo-1,2,3,4-tetrahydroisoquinoline-4-carboxamide (*trans*-7o):** Compound *trans*-7d (2 mmol, 1.0 g) was dissolved in abs. dichloromethane under Ar atmosphere and cooled to 0°C. Borane tribromide (24 mmol, 2.3 mL) was added dropwise to the stirred solution. After 2h the reaction was quenched with an excess of MeOH. The solvent evaporated and the residue was purified by column chromatography (silica gel 60, chloroform/ethanol 10:1) to give *trans*-7o. Yield: 61 %; colorless solid; mp: 298-301°C (decompn.).

Example 12

[0080] ***trans*-3-(4-Methoxyphenyl)-1-oxo-2-phenyl-N-(pyrimidin-2-yl)-1,2,3,4-tetrahydroisoquinoline-4-carboxamide (*trans*-7p):** Quantities: *trans*-5b (0.7 mmol, 250 mg), *N,N*-diisopropylethylamine (0.7 mmol, 115 µL), 2,2,2-trichloro-1,1-dimethylethyl chloroformate (1.0 mmol, 240 mg) and 2-aminopyrimidine (1.3 mmol, 130 mg). The solvent was reduced *in vacuo* and the residue was purified by column chromatography (silica gel 60, chloroform/ethanol 10:1) and crystallized from toluene to give *trans*-7p. Yield: 48 %; colorless solid; mp: 273-275°C.

Example 13

[0081] ***trans*-3-(4-Methoxyphenyl)-1-oxo-2-phenyl-N-(pyridin-3-yl)-1,2,3,4-tetrahydroisoquinoline-4-carboxamide (*trans*-7q):** Quantities: *trans*-5b (1.3 mmol, 500 mg), NEt₃ (1.3 mmol, 180 µL), 2,2,2-trichloro-1,1-dimethylethyl chloroformate (2.0 mmol, 480 mg) and 3-aminopyridine (2.7 mmol, 250 mg). The solvent was reduced *in vacuo* and the residue was purified by column chromatography (silica gel 60, chloroform/ethanol 10:1) and crystallized from ethyl acetate to give *trans*-7q. Yield: 86 %; colorless solid; mp: 219-221 °C.

Example 14

[0082] ***trans*-N-(4,6-Dimethylpyridin-2-yl)-3-(4-methoxyphenyl)-1-oxo-2-phenyl-1,2,3,4-tetrahydroisoquinoline-4-carboxamide (*trans*-7r)**: Quantities: *trans*-5b (0.8 mmol, 300 mg), NEt₃ (0.8 mmol, 110 µL), 2,2,2-trichloro-1,1-dimethylethyl chloroformate (1.2 mmol, 290 mg) and 2-amino-4,6-dimethylpyridine (1.6 mmol, 195 mg). The precipitate was filtered and crystallized from ethyl acetate to give *trans*-7r. Yield: 45 %; colorless solid; mp: 265-267°C.

REFERENCES CITED IN THE DESCRIPTION

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Patent documents cited in the description

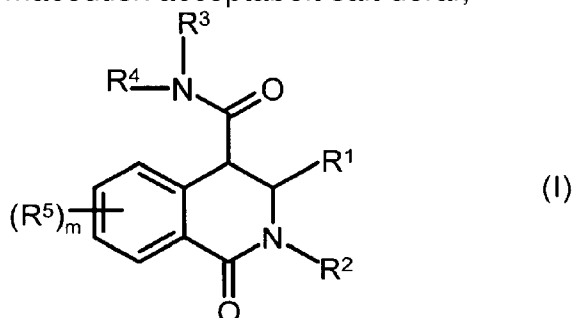
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P a t e n t k r a v

1. Forbindelse, der er repræsenteret ved den følgende formel (I), eller et farmaceutisk acceptabelt salt deraf,



5 hvor

R¹ er phenyl, pyridinyl eller pyrimidinyl, hvor hver af dem kan være substitueret med en eller flere substituenten Y;

R² er phenyl, pyridinyl eller pyrimidinyl, hvor hver af dem kan være substitueret med en eller flere substituenten Y';

10 R³ er phenyl, pyridinyl eller pyrimidinyl, hvor hver af dem kan være substitueret med en eller flere substituenten Y'';

Y, Y' og Y'' uafhængigt er valgt blandt halogen, C₁₋₆-alkyl, C₁₋₆-halogenalkyl, hydroxy, C₁₋₆-alkoxy, C₁₋₆-haloalkoxy, nitro, -NR⁶R⁷, -CO-R⁸, -CO-NR⁶R⁷, -COOR⁶ og -CO-NR⁶-CO-R⁸;

15 R⁴ er hydrogen eller C₁₋₄-alkyl;

R⁵ er halogen, hydroxy, C₁₋₆-alkyl, C₁₋₆-alkoxy, C₁₋₆-halogenalkyl, C₁₋₆-halogenalkoxy, nitro eller -NR⁶R⁷;

R⁶ og R⁷ uafhængigt er valgt blandt hydrogen og C₁₋₆-alkyl;

R⁸ er C₁₋₆-alkyl;

20 og

m er et heltal på 0 til 3, hvor, hvis m er 2 eller 3, kan substituenterne R⁵ vælges uafhængigt af hinanden;

og der er en *trans*-enantiomer,

25 forudsat at forbindelsen med formelen (I) ikke er 2,3-bis(4-methoxyphenyl)-N-(6-methylpyridin-2-yl)-1-oxo-1,2,3,4-tetrahydroisoquinolin-4-carboxamid eller 2,3-bis(4-methoxyphenyl)-1-oxo-N-(pyridin-2-yl)-1,2,3,4-tetrahydroisoquinolin-4-carboxamid.

2. Forbindelse ifølge krav 1, hvor R¹ og R² er phenyl.
3. Forbindelse ifølge krav 1 eller 2, hvor R³ er phenyl eller pyridinyl.
- 5 4. Forbindelse ifølge et af de foregående krav, hvor Y og Y' uafhængigt er valgt blandt halogen, methyl, ethyl, methoxy og ethoxy.
5. Forbindelse ifølge et af de foregående krav, hvor Y'' er methyl eller ethyl.
- 10 6. Forbindelse ifølge et af de foregående krav, hvor R¹ og R², hvis de er substitueret, bærer mindst en substituent i ortho- eller paraposition til den position, hvor R¹ og R² er bundet til tetrahydroisoquinolin-1-on-ringen.
- 15 7. Forbindelse ifølge et af de foregående krav, hvor R¹ og R² bærer en substituent, der er valgt blandt halogen, methoxy og ethoxy, i paraposition til den position, hvor R¹ og R² er bundet til tetrahydroisoquinolin-1-on-ringen.
- 20 8. Forbindelse ifølge et af de foregående krav, hvor R³ er pyridin-2-yl, 6-methylpyridin-2-yl, m-tolyl eller 3,5-dimethylphenyl.
9. Forbindelse ifølge et af de foregående krav, hvor m er 0.
10. Forbindelse ifølge et af de foregående krav, der er
trans-N-(6-methylpyridin-2-yl)-1-oxo-2,3-diphenyl-1,2,3,4-tetrahydroiso-
 25 quinolin-4-carboxamid,
trans-3-(4-methoxyphenyl)-N-(6-methylpyridin-2-yl)-1-oxo-2-phenyl-1,2,3,4-
 tetrahydroisoquinolin-4-carboxamid,
trans-2-(4-methoxyphenyl)-N-(6-methylpyridin-2-yl)-1-oxo-3-phenyl-1,2,3,4-
 tetrahydroisoquinolin-4-carboxamid,
 30 *trans*-2,3-Bis(4-methoxyphenyl)-1-oxo-N-(m-tolyl)-1,2,3,4-tetrahydroiso-
 quinolin-4-carboxamid,
trans-N-(3,5-dimethylphenyl)-2,3-bis(4-methoxyphenyl)-1-oxo-1,2,3,4-tetrahy-
 droisoquinolin-4-carboxamid,
trans-2,3-Bis(4-fluorphenyl)-N-(6-methylpyridin-2-yl)-1-oxo-1,2,3,4-tetrahyd-
 35 roisoquinolin-4-carboxamid,

trans-2,3-Bis(4-chlorophenyl)-N-(6-methylpyridin-2-yl)-1-oxo-1,2,3,4-tetrahydroisoquinolin-4-carboxamid,

trans-3-(4-methoxyphenyl)-1-oxo-2-phenyl-N-(pyrimidin-2-yl)-1,2,3,4-tetrahydroisoquinolin-4-carboxamid,

5 *trans*-3-(4-methoxyphenyl)-1-oxo-2-phenyl-N-(pyridin-3-yl)-1,2,3,4-tetrahydroisoquinolin-4-carboxamid eller

trans-N-(4,6-dimethylpyridin-2-yl)-3-(4-methoxyphenyl)-1-oxo-2-phenyl-1,2,3,4-tetrahydroisoquinolin-4-carboxamid.

10 **11.** Farmaceutisk sammensætning omfattende en forbindelse med formlen (I) eller et farmaceutisk acceptabelt salt deraf ifølge et af de foregående krav, forudsat at forbindelsen med formlen (I) ikke er 2,3-bis(4-methoxyphenyl)-N-(6-methylpyridin-2-yl)-1-oxo-1,2,3,4-tetrahydroisoquinolin-4-carboxamid eller 2,3-bis(4-methoxyphenyl)-N-pyridin-2-yl-1-oxo-1,2,3,4-tetrahydroisoquinolin-4-carboxamid.

15 **12.** Forbindelse med formlen (I) eller et farmaceutisk acceptabelt salt deraf ifølge et af kravene 1 til 10 indbefattende 2,3-bis(4-methoxyphenyl)-N-(6-methylpyridin-2-yl)-1-oxo-1,2,3,4-tetrahydroisoquinolin-4-carboxamid og 2,3-bis(4-methoxyphenyl)-N-pyridin-2-yl-1-oxo-1,2,3,4-tetrahydroisoquinolin-4-carboxamid til anvendelse ved behandling eller inhibering af cancer, autoimmune sygdom, reumatoid arthritis, inflammatorisk tarmsygdom eller psoriasis.

20 **13.** Forbindelse til anvendelse ifølge krav 12 til anvendelse ved behandling eller inhibering af myelomatose.

DRAWINGS

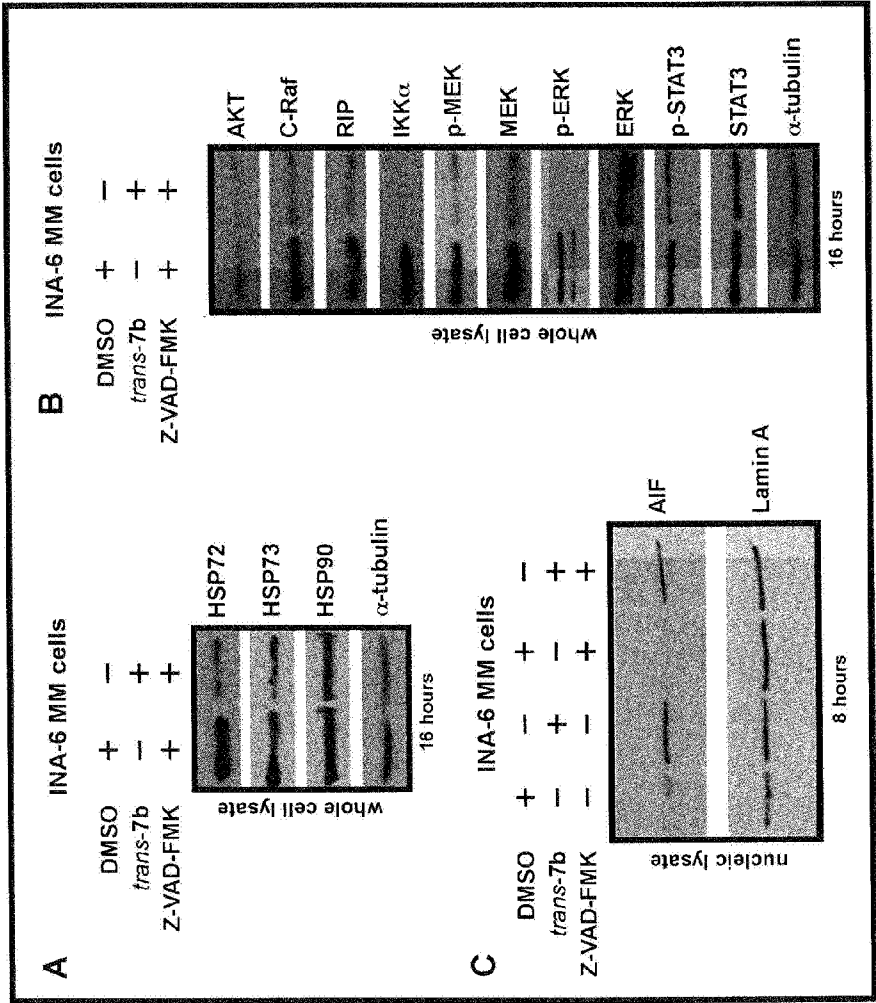
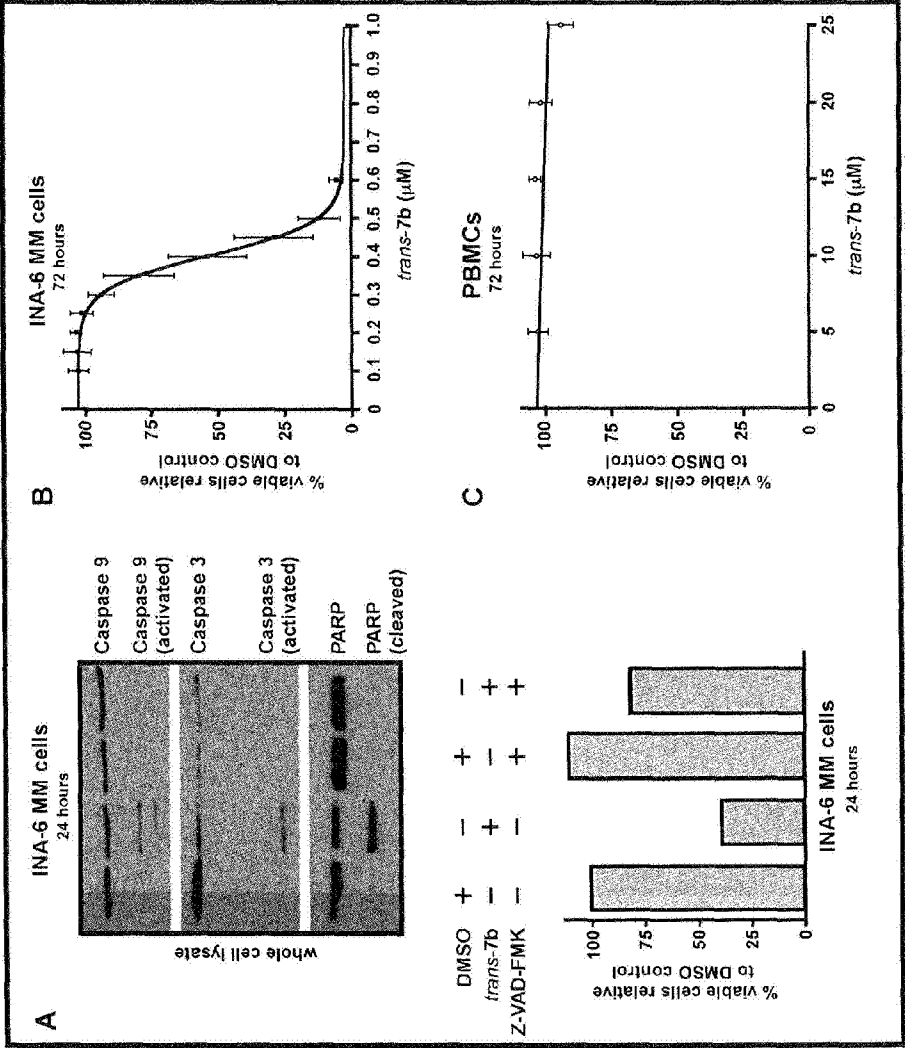


Figure 1

Figure 2



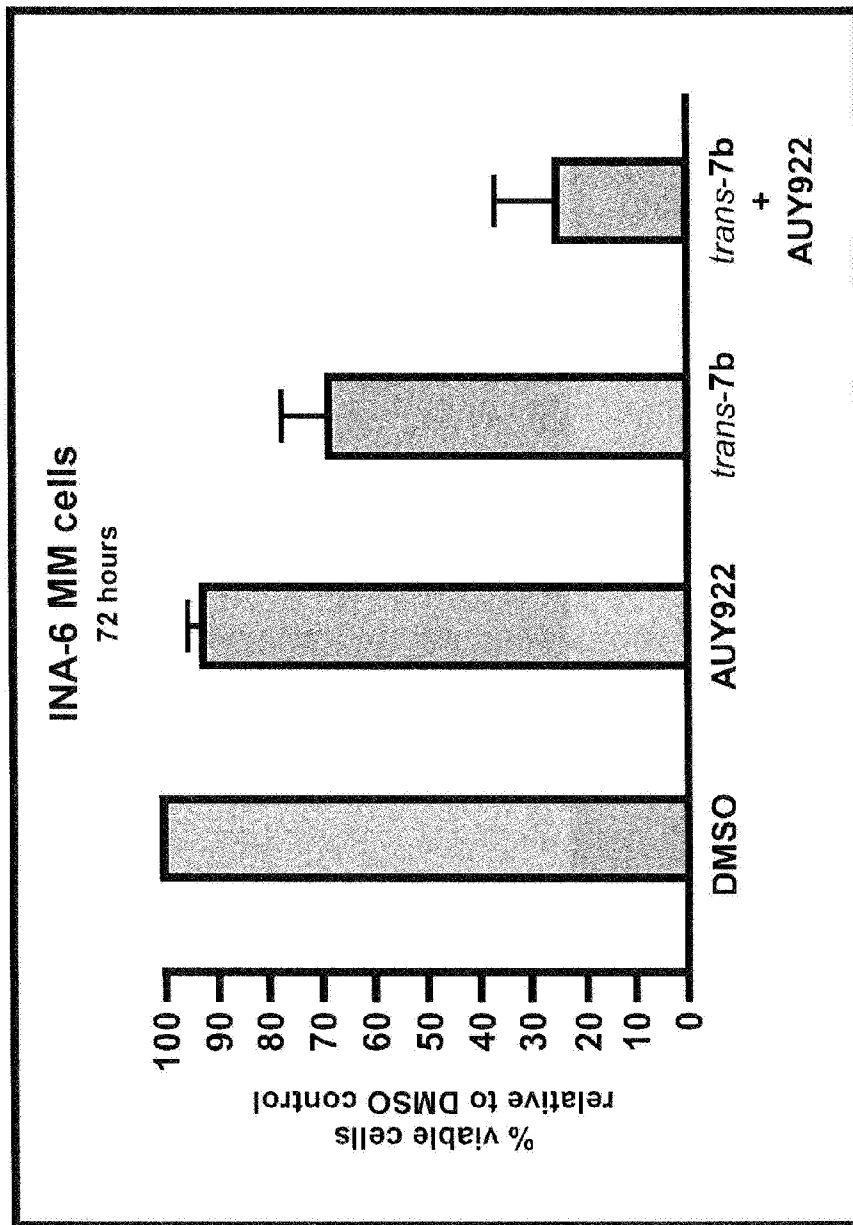


Figure 3