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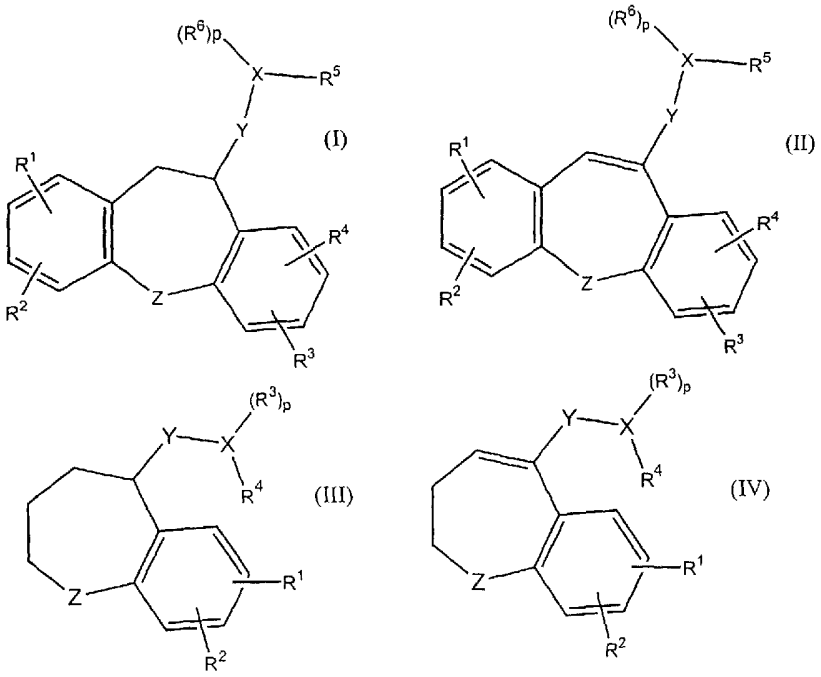
(54) Title: COUMPOUNDS MODULATING THE ACTIVITY OF GAPDH AND/OR IAMT



(57) Abstract: Novel compounds and their use for modulationof L-Isoaspartyl (D-Aspartyl) 0-Metyltransferase and/or glyceraldehyde-3-phosphate dehydrogenase activity enable the prevention treatment or alleviation of diabetes, autoimmune diseases and neuro-degenerative diseases.. Compounds are of any of the formulae I - IV below:

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Compounds modulating the activity of GAPDH and/or IAMT

Technical field of the invention

The present invention relates to novel compounds and their use as modulators of glyceraldehyde-3-phosphate dehydrogenase (GAPDH) and L-Isoaspartyl (D-Aspartyl) O-Methyltransferase (IAMT) activity, thereby preventing treating or alleviating diabetes (type I and type II diabetes), autoimmune diseases, or neurodegenerative diseases in a mammal.

Background of the invention

Effect of the compounds on Diabetes

Insulin is stored in secretory vesicles closely juxtaposed to the pancreatic β-cell plasma membrane. Fuel secretagogues including D-glucose must be metabolized by pancreatic β-cells to facilitate the release of insulin, which is mediated by the fusion of secretory granules with pancreatic β-cell plasma membranes. However, the chemical mechanism coupling secretagogue metabolism with β-cell secretory granule-plasma membrane fusion are presently unknown.

Modulation of proteins which participate in fusion between pancreatic β-cell plasma membranes and secretory granules may have implications for the regulation of insulin secretion and for the treatment of insulin secretory defects in type II diabetes mellitus.

Glyceraldehyde-3-phospate dehydrogenase (GAPDH, EC 1.2.1.12) catalyzes the conversion of glyceraldehyde-3-phosphate to 1,3-bisphosphoglycerate in the glycolytic pathway and converts NAD+ to the high-energy carrier NADH. For over three decades, GAPDH was studied for its pivotal role in glycolysis.

However, recent evidence has demonstrated that mammalian GAPDH displays a number of diverse activities unrelated to its glycolytic function. These include roles for GAPDH in apoptosis, microtubule assembly, nuclear RNA export, protein phosphotransferase/kinase reactions, the translational control of gene expression, DNA

replication, DNA repair, membrane fusion and exocytosis as recently reviewed by Sirover (Sirover 1999).

The multiple activities of GAPDH indicate that diverse forms of GAPDH may exist having different activities. The GAPDH gene family appears to contain one active gene and a diversity of pseudogenes. mRNA studies indicate that the human chromosome 12 locus encodes a single mRNA specifying a solitary protein. However, the expression of a single GAPDH mRNA and protein presents an apparent paradox with respect to experimental data demonstrating both the functional diversity of GAPDH, the subcellular localization of these diverse activities and immunoflourescence studies demonstrating proliferative dependent alterations in GAPDH localizations in vivo.

In this context recent evidence indicates that the functional diversity of the protein may at least partially be related to the oligomeric structure of GAPDH in vivo. GAPDH largely exists as a tetramer with minor populations of dimers and monomers. Interestingly GAPDH appears to exert different functions depending on its oligomeric organization. Thus GAPDH exhibits glycolytic activity as a dimer and tetramer but not as a monomer. Further the tetrameric form of the molecule appears to be related to apoptosis but not the dimeric form.

The compounds described in the present invention modulate the oligomeric structure of GAPDH potentially affecting the insulin secreting capacity of β-cells.
Of interest in this context, it has recently been shown that that fusion between naturally occurring subcellular fractions involved in insulin excretion (exocytosis) can be accelerated dramatically by a brain cytosolic protein constituent that is chemically, chromatographically and immunologically similar to an isoform of GAPDH (Han et al 1998).

Increased apoptosis of β-cells is a characteristic feature of both type I and type II diabetes. Treatments decreasing apoptosis of these cells are expected to have a beneficial effect on diabetics.

We show here, that the compounds described in the present invention, exert anti-apoptotic effect on β-cells, which will be beneficial for patients affected by diabetes. The

anti-apoptotic effect of the compounds is thought to be caused by their effect on GAPDH (oligomerisation) and or their effect on IAMT Activity (IAMT trascription).

In addition to this, the putative effect of the compounds on insulin secretion may further increase their positive effect on diabetics.

Effect of compounds on autoimmune diseases (e.g. type I diabetes)

Autoimmune diseases are characterized by immune recognition of specific antigens in the patient's own tissue or organs. These antigens are commonly referred to as autoantigens. Depending on the localisation of the target autoantigen and distribution of autoimmune reactions in the organism, autoimmune diseases may be classified as either organ specific or systemic. It is not known why some proteins are prone to become autoantigens. Various possibilities have been suggested: molecular mimicry by bacteria and viruses or release of proteins or peptides from an immune-privileged tissue upon its damage or posttranslational modifications of otherwise tolerated antigens.

The potential role of posttranslational modifications in autoimmunity has been reviewed by Doyle (Doyle and Mamula 2001). One such posttranslational modification is spontaneous isomerisation or optical inversion of an amino acid within a protein. Aspartic acid and aspargine will in some proteins undergo this spontaneous reaction in an age dependent fashion, resulting in the formation of L-iso-Asp, D-iso-Asp or D-Asp containing proteins. This reaction has been suggested to play an important role in autoimmunity (Mamula et al 1999; Young et al 2001; patent application WO 01/13110).

Foreign as well as self protein antigens must be broken down within endosomes or lysosomes of the antigen presenting cell (APC), to generate suitable peptides that will form complexes with class II major histocompatibility complex molecules (MHCII) for presentation to T cells. It has recently been shown that a specific protease called Asparginyl Endo Peptidase (AEP) initially cleaves antigen at asparginyl residues and that antigen is further processed by other proteases such as Cathepsin D and E (Antoniou et al 2000; Hewitt et al 1997; Manoury et al 1998; Manoury 2001). AEP processing appears to be a central event in immune reactions, as AEP-cleavage determines whether certain antigens are presented.

These findings are very important, because they can offer a mechanistic explanation for why isomerisation or optical inversion can induce autoimmunity. In the normal situation, AEP cleavage within an aspargine containing antigen fragment will prevent the formation of self-peptides that can form complexes with MHCII for presentation to T cells preventing an immune response to self-protein. If self-proteins are isomerised/ optically inverted, AEP cleavage is hindered and iso-Asp/ D-Asp containing epitopes within a self-antigen is presented on MHCII leading to T-cell responses and autoimmunity.

The enzyme L-Isoaspartyl (D-Aspartyl) O-Methyltransferase (IAMT, PIMT or PCMT), EC 2.1.1.77, is an ubiquitous, mainly cytosolic enzyme which catalyzes transfer of the reactive methyl group of S-adenosyl L-methionine onto the α-carboxyl group of L-isoaspartyl or D-aspartyl sites in peptides and proteins. Almost every known organism has IAMT or a homologue thereof. IAMT fulfils an important role as repair mechanism for isomerised proteins in the body. IAMT deletion mutants have been shown to possess distinct phenotypes. Mice lacking a functional IAMT gene, exhibit growth retardation and die of fatal seizures at an average age of 42 days (Kim et al 1997). Furthermore these mice have an increased amount of iso-aspartyl containing histone H2B, a possible explanation for the anti-histone antibodies found in systemic lupus erythematosus patients (Young et al 2001).

No studies yet exist on whether the IAMT "repair-system" is altered in any form in autoimmunity. However, amino acid polymorphisms have been identified in human IAMT, which may affect the enzymes ability to recognise its substrates (David et al 1997; Tsai and Clarke 1994).

IAMT expression levels have been shown to affect apoptosis. An increased IAMT expression level rescues cells from apoptosis, whereas decreased or missing IAMT expression induces elevated levels of apoptosis (Hüebscher et al 1999; patent application WO 98/15647). Connections between apoptosis and autoimmunity has been made; in multiple sclerosis a decrease in T-cell apoptosis is observed in the patient group versus healthy individuals (Macchi et al 1999; Zang et al 1999). In other reports an increase in apoptosis has been linked to autoimmunity, where cell death within a tissue provides a supply of putative autoantigens (Rodenburg et al 2000).

The present invention provides a way to regenerate an aspartyl residue to regain cleavage sites for proteases by increasing IAMT activity in tissue cells (prone for attacks by the immune system) or antigen presenting cells (APC), thereby preventing autoantigen presentation.

This is a very different approach than the apoptotic decrease achieved through increased IAMT activity described in patent application WO 98/15647. According to WO 98/15647 neurodegenerative diseases are associated with increased apoptosis, which imply that neurodegenerative diseases can be relived through an increase in IAMT activity as this results in decreased apoptosis. A decrease in apoptosis can also be a disease-causing factor; autoimmunity is mentioned briefly as an example of this in WO 98/15647. This would imply that a decrease in IAMT activity (increase in apoptosis) should have a positive effect on autoimmune diseases. Thus increasing IAMT activity in antigen presenting cells (APC) to alleviate autoimmunity as disclosed in the present patent application is the opposite approach as would be expected from what was taught in patent application WO 98/15647. Furthermore the self-antigen presenting cells have no direct connection to apoptosis, as the process of presenting an autoantigen will not necessarily lead to cell death.

Not only regulation of IAMT activity in APC can have a positive effect in alleviation or treatment of autoimmune diseases. It has been shown that T-cells, which lack IAMT hyper-proliferate upon antigen stimulation (Doyle et al 2001). This proliferation is not due to a decrease in apoptosis. Thus, as for the APC, an increase of IAMT activity in T-cells of an autoimmune patient can have a positive effect, by decreasing the immune response to potential autoimmune stimuli.

Summary of the invention

The present invention comprises compounds of the general formula I,III and IV and their pharmaceutical use to prevent, treat or alleviate diseases in mammals.

Furthermore, the present invention comprises pharmaceutical use of compounds of the general formula I, II, III and IV to prevent, treat or alleviate diseases in subjects suffering from these involving insulin secretory defects and in a preferred embodiment type II diabetes mellitus , autoimmune diseases and in a preferred embodiment type I

diabetes mellitus and neurodegenerative diseases. In a preferred embodiment the present invention comprises Alzheimer's disease.

The present invention is based on the discovery that molecules or compounds that interfere with the ability of dehydrogenases such as GAPDH to oligomerize are able to modulate insulin secretion from β-cells, and decrease the apoptosis of such cells. These abilities render such compounds appropriate for the treatment of diabetes.

In another aspect, the present invention relates to L-Isoaspartyl (D-Aspartyl) O-Methyltransferase (IAMT) in connection with autoimmune diseases. A method is provided for preventing or alleviating an autoimmune response or disease in a mammalian, through up-regulation of IAMT activity in one or more cell types e.g. antigen presenting cells, T-cells or cells prone for an autoimmune attack.

Associated with the preventing method, a method for diagnosis or risk assessment in relation to autoimmunity is provided in the present invention. The method either utilizes screening for genetic polymorphisms in the IAMT gene or quantification of IAMT gene transcription level, protein level or activity, in a sample.

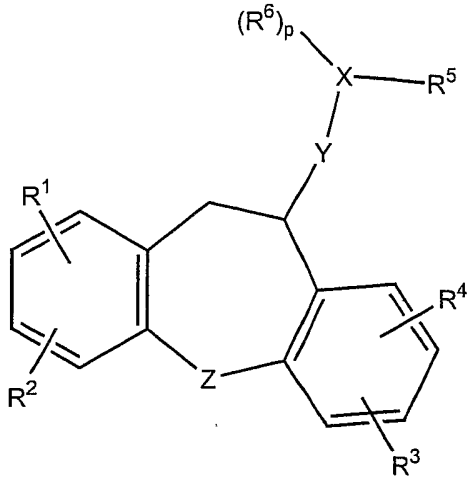
The IAMT protein or derivatives thereof, preferably in a suitable pharmaceutical composition, can according to the present invention be used to prevent, treat or alleviate an autoimmune response or disease.

Compounds able to regulate IAMT activity, can according to the invention be used to treat or prevent an autoimmune condition. These compounds are preferably provided in a suitable pharmaceutical composition.

Another way to modulate IAMT activity and thereby influence an autoimmune response according to the invention, is to provide a IAMT encoding nucleic acid sequence or a functional derivative thereof to a patient. Especially a pharmaceutical composition including an expression vector with the IAMT gene regulated by a specific promoter is presented in the present invention.

Detailed description of the invention

In a first aspect the present invention comprises a compound of the general formula I



(I)

wherein:

R^1, R^2, R^3 and R^4 independently are hydrogen, halogen such as fluoro, chloro, bromo or iodo, hydroxy, cyano, nitro, trifluoromethyl, optionally substituted nitrogen (NR'R'' where R', R'' = optionally substituted C_{1-20}-alkyl or C_{2-20}-alkenyl or C_{4-20}-alkadienyl or C_{6-20}-alkatrienyl or aryl or heteroaryl), optionally substituted sulfur (SR' where R' = optionally substituted C_{1-20}-alkyl or C_{2-20}-alkenyl or C_{4-20}-alkadienyl or C_{6-20}-alkatrienyl or aryl or heteroaryl), optionally substituted C_{1-20}-alkyl or C_{2-20}-alkenyl or C_{4-20}-alkadienyl or C_{6-20}-alkatrienyl or C_{1-20}-alkoxy or aryl or heteroaryl or ester (-COOR'' where R''= optionally substituted C_{1-20}-alkyl or C_{2-20}-alkenyl or C_{4-20}-alkadienyl or C_{6-20}-alkatrienyl or aryl or heteroaryl) ;

R^5, R^6 are, each independently of the other and of R^1, R^2, R^3 and R^4, hydrogen, optionally substituted lower alkyl, optionally substituted lower alkenyl, optionally substituted lower alkynyl, optionally substituted alkoxy, optionally substituted aryl, optionally substituted heteroaryl, provided that not both of R^5 and R^6 are hydrogen;

X is $(CH_m)_n$, –O-, -N- or –S-;

m = 0, 1, 2;

n = 1,2,3;

p is 0 or 1;

Y is C=O, $(CH_m)_n$, –O-, -N (W)- or –S-; where W is H,OH or C_{1-6} alkyl;

m = 0, 1, 2;

n = 1,2,3; and

Z is C, N(W), O or S, where W is independently as defined above,

or a pharmaceutically acceptable salt thereof, optionally together with a pharmaceutically acceptable carrier, diluent or excipient.

In one embodiment of the present invention R^1, R^2, R^3 and R^4 of compound I independently are chosen from hydrogen, halogen, trifluoromethyl, hydroxy, cyano, nitro, optionally substituted nitrogen (NR'R'' where R', R'' = optionally substituted C_{1-6}-alkyl or C_{2-8}-alkenyl or C_{4-10}-alkadienyl or aryl or heteroaryl), optionally substituted sulfur (SR' where R' = optionally substituted C_{1-6}-alkyl or C_{2-8}-alkenyl or C_{4-10}-alkadienyl or aryl or heteroaryl), optionally substituted C_{1-6}-alkyl or C_{2-8}-alkenyl or C_{4-10}-alkadienyl or C_{1-6}-alkoxy or aryl or heteroaryl or ester (-COOR'' where R''= optionally substituted C_{1-6}-alkyl or C_{2-8}-alkenyl or C_{4-10}-alkadienyl or aryl or heteroaryl)and R^5, R^6 are, each independently of the other and of R^1, R^2, R^3 and R^4, hydrogen, optionally substituted lower alkyl, optionally substituted lower alkenyl, optionally substituted lower alkynyl, optionally substituted aryl.

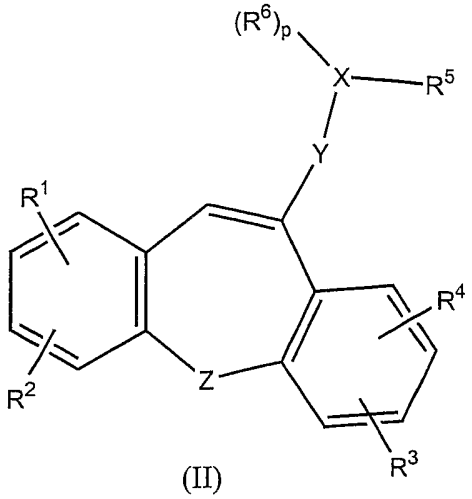
In another embodiment of the present invention R^1, R^2, R^3 and R^4 of compound I independently are chosen from hydrogen, halogen, trifluoromethyl, nitro, optionally substituted nitrogen (NR'R'' where R', R'' = optionally substituted C_{1-6}-alkyl or aryl), optionally substituted C_{1-6}-alkyl or optionally substituted C_{3-6}-alkynyl or optionally substituted C_{1-6}-alkoxy or aryl and R^5, R^6 are each independently of the other and of R^1, R^2, R^3 and R^4, hydrogen, optionally substituted lower alkyl, optionally substituted C_{3-6}-alkynyl, optionally substituted aryl.

In a preferred embodiment of the present invention X of compound I is N or O.

In another preferred embodiment of the present invention Y of compound I is C=O or CH_2.

In a still another preferred embodiment of the present invention Z of compound I is O or N(W).

In another aspect the present invention relates to the compound of the general formula II



(II)

wherein:

R^1, R^2, R^3 and R^4 independently are hydrogen, halogen such as fluoro, chloro, bromo or iodo, hydroxy, cyano, nitro, trifluoromethyl, optionally substituted nitrogen (NR'R'' where R', R'' = optionally substituted C_{1-20}-alkyl or C_{2-20}-alkenyl or C_{4-20}-alkadienyl or C_{6-20}-alkatrienyl or aryl or heteroaryl), optionally substituted sulfur (SR' where R' = optionally substituted C_{1-20}-alkyl or C_{2-20}-alkenyl or C_{4-20}-alkadienyl or C_{6-20}-alkatrienyl or aryl or heteroaryl), optionally substituted C_{1-20}-alkyl or C_{2-20}-alkenyl or C_{4-20}-alkadienyl or C_{6-20}-alkatrienyl or C_{1-20}-alkoxy or aryl or heteroaryl or ester (-COOR'' where R''= optionally substituted C_{1-20}-alkyl or C_{2-20}-alkenyl or C_{4-20}-alkadienyl or C_{6-20}-alkatrienyl or aryl or heteroaryl) ;

R^5, R^6 are, each independently of the other and of R^1, R^2, R^3 and R^4, hydrogen, optionally substituted lower alkyl, optionally substituted lower alkenyl, optionally substituted lower alkynyl, optionally substituted alkoxy, optionally substituted aryl, optionally substituted heteroaryl, provided that not both of R^5 and R^6 are hydrogen;

X is $(CH_m)_n$, –O-, -N- or –S-;

m = 0, 1, 2;

n = 1,2,3;

p is 0 or 1;

Y is C=O, $(CH_m)_n$, –O-, -N(W)- or –S-; provided that when X is N,

Y is C=0, wher W is H,OH or C_{1-6} alkyl;

m = 0, 1, 2;

n = 1,2,3; and

Z is C, N(W), O or S, wherein W is independently as defined above;

or a pharmaceutically acceptable salt thereof, optionally together with a pharmaceutically acceptable carrier, diluent or excipient.

In one embodiment of the present invention R^1, R^2, R^3 and R^4 of compound II independently are chosen from hydrogen, halogen, trifluoromethyl, hydroxy, cyano, nitro, optionally substituted nitrogen (NR'R'' where R', R'' = optionally substituted C_{1-6}-alkyl or C_{2-8}-alkenyl or C_{4-10}-alkadienyl or aryl or heteroaryl), optionally substituted sulfur (SR' where R' = optionally substituted C_{1-6}-alkyl or C_{2-8}-alkenyl or C_{4-10}-alkadienyl or aryl or heteroaryl), optionally substituted C_{1-6}-alkyl or C_{2-8}-alkenyl or C_{4-10}-alkadienyl or C_{1-6}-alkoxy or aryl or heteroaryl or ester (-COOR'' where R''= optionally substituted C_{1-6}-alkyl or C_{2-8}-alkenyl or C_{4-10}-alkadienyl or aryl or heteroaryl)and R^5, R^6 are, each independently of the other and of R^1, R^2, R^3 and R^4, hydrogen, optionally substituted lower alkyl, optionally substituted lower alkenyl, optionally substituted lower alkynyl, optionally substituted aryl.

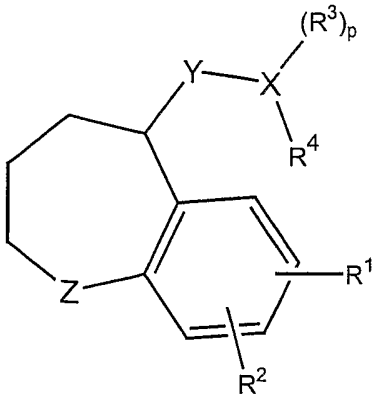
In another embodiment of the present invention R^1, R^2, R^3 and R^4 of compound II R^1, R^2, R^3 and R^4 independently are chosen from hydrogen, halogen, trifluoromethyl, nitro, optionally substituted nitrogen (NR'R'' where R', R'' = optionally substituted C_{1-6}-alkyl or aryl), optionally substituted C_{1-6}-alkyl or optionally substituted C_{3-6}-alkynyl or optionally substituted C_{1-6}-alkoxy or aryl and R^5, R^6 are, each independently of the other and of R^1, R^2, R^3 and R^4, hydrogen, optionally substituted lower alkyl, optionally substituted C_{3-6}-alkynyl, optionally substituted aryl.

In a preferred embodiment of the present invention X of compound II is N or O.

In another preferred embodiment of the present invention Y of compound II is C=O or CH_2.

In yet another preferred embodiment of the present invention Z of compound II is O or N(W).

In a further aspect the present invention relates to a compound of the general formula III



(III)

wherein:

R^1, R^2 independently are hydrogen, halogen such as fluoro, chloro, bromo or iodo, hydroxy, cyano, nitro, trifluoromethyl, optionally substituted nitrogen (NR'R'' where R', R'' = optionally substituted C_{1-20}-alkyl or C_{2-20}-alkenyl or C_{4-20}-alkadienyl or C_{6-20}-alkatrienyl or aryl or heteroaryl), optionally substituted sulfur (SR' where R' = optionally substituted C_{1-20}-alkyl or C_{2-20}-alkenyl or C_{4-20}-alkadienyl or C_{6-20}-alkatrienyl or aryl or heteroaryl), optionally substituted C_{1-20}-alkyl or C_{2-20}-alkenyl or C_{4-20}-alkadienyl or C_{6-20}-alkatrienyl or C_{1-20}-alkoxy or aryl or heteroaryl or ester (-COOR'' where R''= optionally substituted C_{1-20}-alkyl or C_{2-20}-alkenyl or C_{4-20}-alkadienyl or C_{6-20}-alkatrienyl or aryl or heteroaryl) ;

R^3, R^4 are, each independently of the other and of R^1, R^2, hydrogen, optionally substituted lower alkyl, optionally substituted lower alkenyl, optionally substituted lower alkynyl, optionally substituted alkoxy, optionally substituted aryl, optionally substituted heteroaryl, provided that not both of R^3 and R^4 are hydrogen;

X is $(CH_m)_n$, –O-, -N- or –S-;

m = 0, 1, 2;

n = 1,2,3;

p is 0 or 1;

Y is C=O, $(CH_m)_n$, –O-, -N(W)- or –S-, where W is H, OH or C_{1-6} alkyl;

m = 0, 1, 2;

n = 1,2,3; and

Z is C, N(W), O or S, where W is independently as defined above;

or a pharmaceutically acceptable salt thereof, optionally together with a pharmaceutically acceptable carrier, diluent or excipient.

In one embodiment of the present invention R^1 and R^2 of compound III independently are chosen from hydrogen, halogen, trifluoromethyl, hydroxy, cyano, nitro, optionally substituted nitrogen (NR'R'' where R', R'' = optionally substituted C_{1-6}-alkyl or C_{2-8}-alkenyl or C_{4-10}-alkadienyl or aryl or heteroaryl), optionally substituted sulfur (SR' where R' = optionally substituted C_{1-6}-alkyl or C_{2-8}-alkenyl or C_{4-10}-alkadienyl or aryl or heteroaryl), optionally substituted C_{1-6}-alkyl or C_{2-8}-alkenyl or C_{4-10}-alkadienyl or C_{1-6}-alkoxy or aryl or heteroaryl or ester (-COOR'' where R''= optionally substituted C_{1-6}-alkyl or C_{2-8}-alkenyl or C_{4-10}-alkadienyl or aryl or heteroaryl)and R^3, R^4 are, each independently of the other and of R^1, R^2, hydrogen optionally substituted lower alkyl, optionally substituted lower alkenyl, optionally substituted lower alkynyl, optionally substituted aryl.

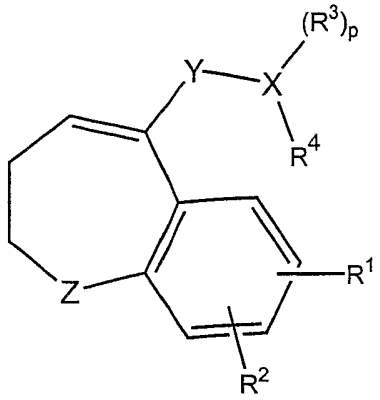
In another embodiment of the present invention R^1, R^2 of compound III independently are chosen from hydrogen, halogen, trifluoromethyl, nitro, optionally substituted nitrogen (NR'R'' where R', R'' = optionally substituted C_{1-6}-alkyl or aryl), optionally substituted C_{1-6}-alkyl or optionally substituted C_{3-6}-alkynyl or optionally substituted C_{1-6}-alkoxy or aryl and R^3, R^4 are, each independently of the other and of R^1, R^2, hydrogen, optionally substituted lower alkyl, optionally substituted C_{3-6}-alkynyl, optionally substituted aryl.

In a preferred embodiment of the present invention X of compound III is N or O.

In another preferred embodiment of the present invention Y of compound III is C=O or CH_2.

In still another preferred embodiment of the present invention Z of compound III is O or N(W).

In a still further aspect the present invention relates to the compound of the general formula IV



(IV)

wherein:

R^1, R^2 independently are hydrogen, halogen such as fluoro, chloro, bromo or iodo, hydroxy, cyano, nitro, trifluoromethyl, optionally substituted nitrogen (NR'R'' where R', R'' = optionally substituted C_{1-20}-alkyl or C_{2-20}-alkenyl or C_{4-20}-alkadienyl or C_{6-20}-alkatrienyl or aryl or heteroaryl), optionally substituted sulfur (SR' where R' = optionally substituted C_{1-20}-alkyl or C_{2-20}-alkenyl or C_{4-20}-alkadienyl or C_{6-20}-alkatrienyl or aryl or heteroaryl), optionally substituted C_{1-20}-alkyl or C_{2-20}-alkenyl or C_{4-20}-alkadienyl or C_{6-20}-alkatrienyl or C_{1-20}-alkoxy or aryl or heteroaryl or ester (-COOR'' where R''= optionally substituted C_{1-20}-alkyl or C_{2-20}-alkenyl or C_{4-20}-alkadienyl or C_{6-20}-alkatrienyl or aryl or heteroaryl) ;

R^3, R^4 are, each independently of the other and of R^1, R^2, hydrogen, optionally substituted lower alkyl, optionally substituted lower alkenyl, optionally substituted lower alkynyl, optionally substituted alkoxy, optionally substituted aryl, optionally substituted heteroaryl, provided that not both of R^3 and R^4 are hydrogen;

X is $(CH_m)_n$, –O-, -N- or –S-;

m = 0, 1, 2;

n = 1,2,3;

p is 0 or 1;

Y is C=O, $(CH_m)_n$, –O-, -N(W)- or –S-, where W is H, OH, or C_{1-6} alkyl;

m = 0, 1, 2;

n = 1,2,3; and

Z is C, N, O or S, where W is independently as defined above;

or a pharmaceutically acceptable salt thereof together with a pharmaceutically acceptable carrier, diluent or excipient.

In one embodiment of the present invention R^1, R^2 of compound IV independently are chosen from hydrogen, halogen, trifluoromethyl, hydroxy, cyano, nitro, optionally substituted nitrogen (NR'R'' where R', R'' = optionally substituted C_{1-6}-alkyl or C_{2-8}-alkenyl or C_{4-10}-alkadienyl or aryl or heteroaryl), optionally substituted sulfur (SR' where R' = optionally substituted C_{1-6}-alkyl or C_{2-8}-alkenyl or C_{4-10}-alkadienyl or aryl or heteroaryl), optionally substituted C_{1-6}-alkyl or C_{2-8}-alkenyl or C_{4-10}-alkadienyl or C_{1-6}-alkoxy or aryl or heteroaryl or ester (-COOR'' where R''= optionally substituted C_{1-6}-alkyl or C_{2-8}-alkenyl or C_{4-10}-alkadienyl or aryl or heteroaryl)and R^3, R^4 are, each independently of the other and of R^1, R^2, hydrogen, optionally substituted lower alkyl, optionally substituted lower alkenyl, optionally substituted lower alkynyl, optionally substituted aryl.

In another embodiment of the present invention R^1, R^2 of compound IV independently are chosen from hydrogen, halogen, trifluoromethyl, nitro, optionally substituted nitrogen (NR'R'' where R', R'' = optionally substituted C_{1-6}-alkyl or aryl), optionally substituted C_{1-6}-alkyl or optionally substituted C_{3-6}-alkynyl or optionally substituted C_{1-6}-alkoxy or aryl and R^3, R^4 are, each independently of the other and of R^1, R^2, hydrogen, optionally substituted lower alkyl, optionally substituted C_{3-6}-alkynyl, optionally substituted aryl.

In a preferred embodiment of the present invention X of compound IV is N or O.

In another preferred embodiment of the present invention Y of compound IV is C=O or CH_2.

In still another preferred embodiment of the present invention Z of compound IV is O or N(W).

In a more preferred embodiment of the present invention X of compound I is S.

In a another preferred embodiment of the present invention X of compound I is O.

In a most preferred embodiment of the present invention X of compound I is N.

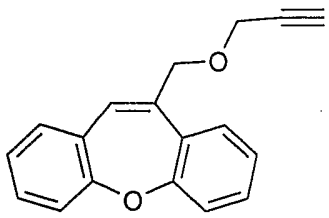
In a more preferred embodiment of the present invention Y of compound I is S.

In another preferred embodiment of the present invention Y of compound I is N.

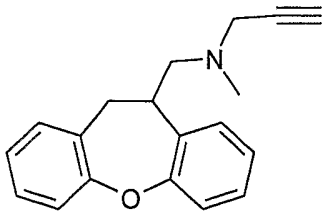
In yet another preferred embodiment of the present invention Y of compound I is O.

In a most preferred embodiment of the present invention Y of compound I is CH_2.

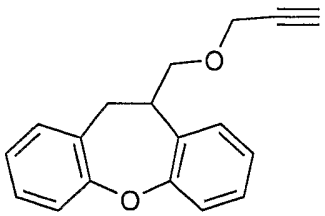
In a more preferred embodiment the present invention relates to the compounds NBC-1, NBC-2, NBC-3, NBC-4, NBC-5, NBC-6, NBC-7, NBC-8, NBC-9, NBC-10 and NBC-11.



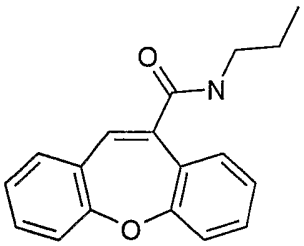
NBC-1



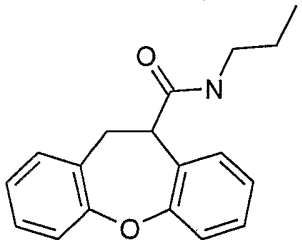
NBC-2



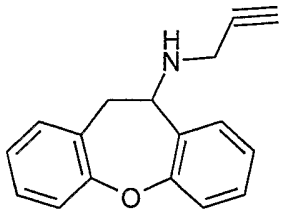
NBC-3



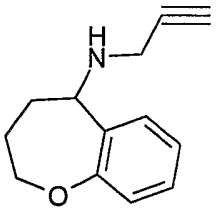
NBC-4



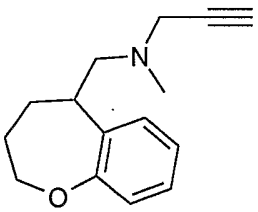
NBC-5



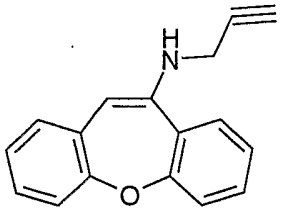
NBC-6



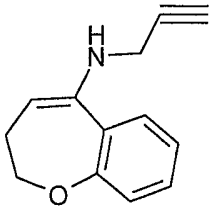
NBC-7



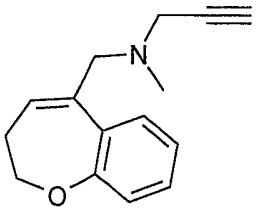
NBC-8



NBC-9

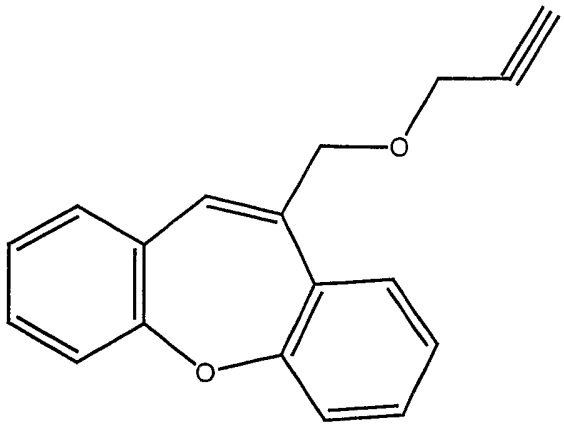


NBC-10

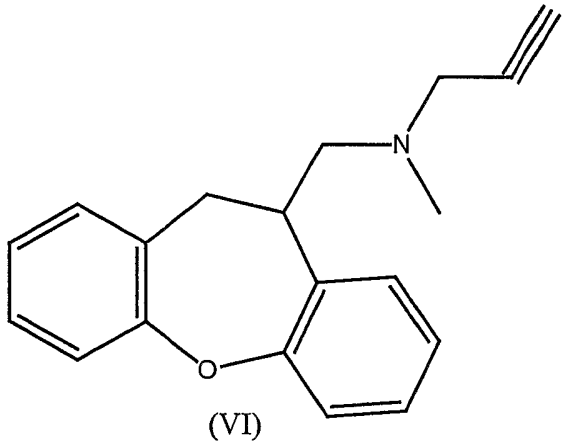


NBC-11

In a most preferred embodiment the present invention relates to the compounds



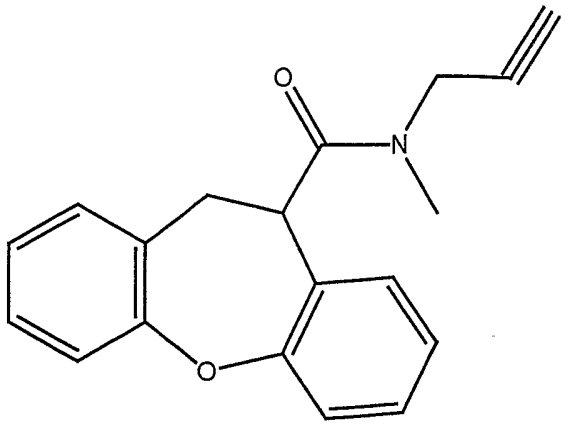
(V) 10-Prop-2-ynyloxymethyl-dibenzo[*b*,*f*]oxepine ;



(VI)

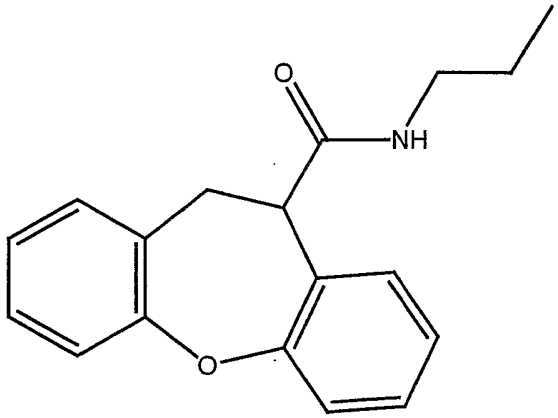
(10,11-Dihydro-dibenzo[*b*,*f*]oxepin-10-ylmethyl)-methyl-prop-2-ynyl-amine;

5



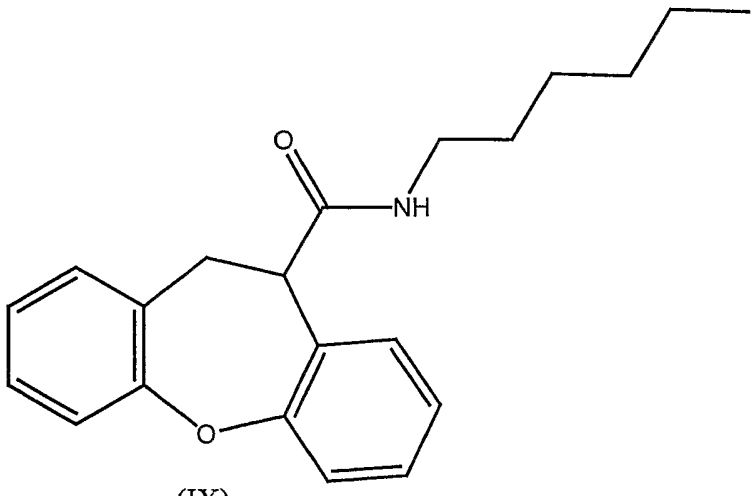
(VII)

10,11-Dihydro-dibenzo[*b*,*f*]oxepine-10-carboxylic acid methyl-prop-2-ynyl-amide;



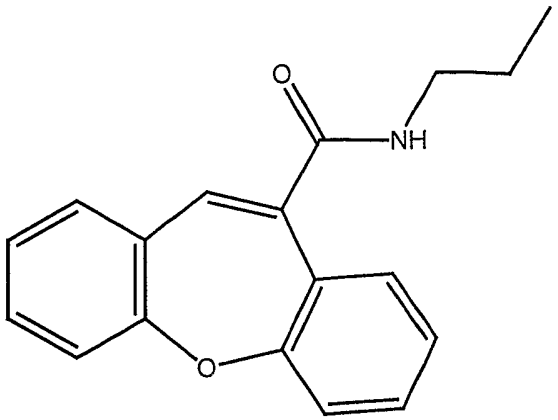
(VIII)

10,11-Dihydro-dibenzo[*b*,*f*]oxepine-10-carboxylic acid propylamide;



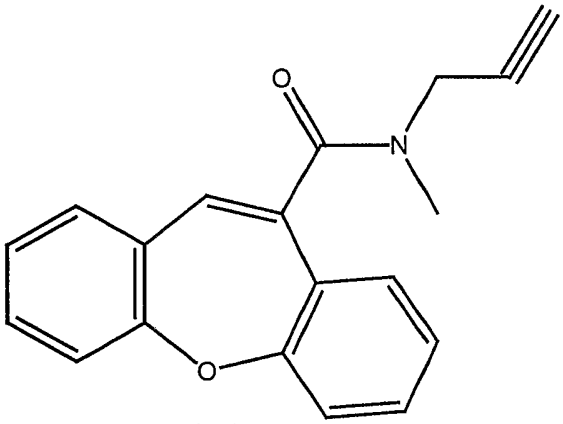
(IX)

10,11-Dihydro-dibenzo[*b*,*f*]oxepine-10-carboxylic acid hexylamide;



(X)

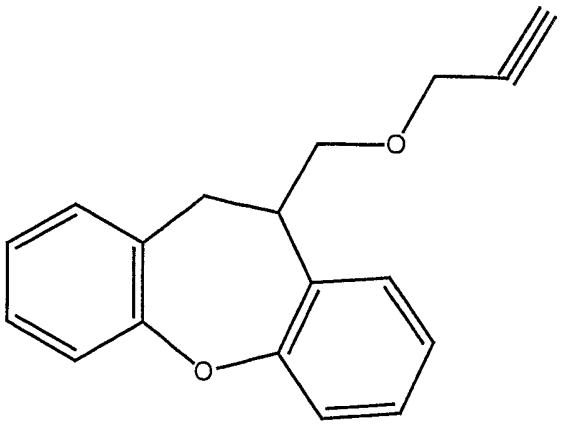
Dibenzo[*b,f*]oxepine-10-carboxylic acid propylamide;



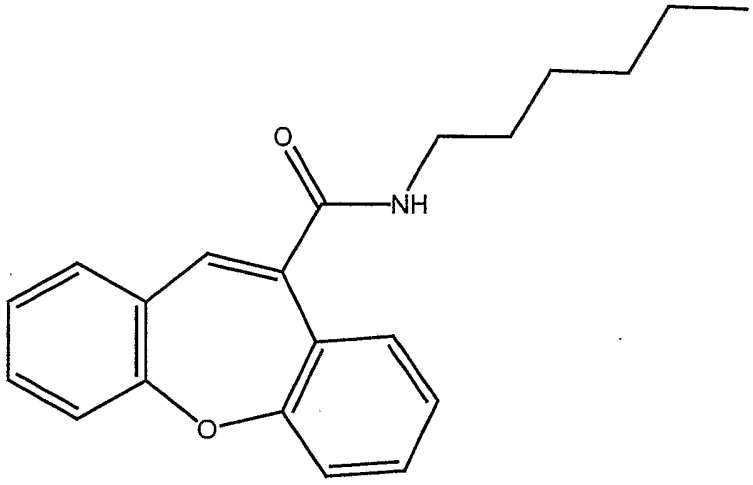
(XI)

Dibenzo[*b,f*]oxepine-10-carboxylic acid methyl-prop-2-ynyl-amide;

5



(XII)
10-Prop-2-ynyloxymethyl-10,11-dihydro-dibenzo[*b*,*f*]oxepine;



(XIII)
Dibenzo[*b*,*f*]oxepine-10-carboxylic acid hexylamide

As used herein "divalent aliphatic radicals" are, for example, lower alkylene radicals and, as a component of an amino group disubstituted by a divalent aliphatic radical, also aza-, oxa- or thia-lower alkylene radicals, such as 3- or 4-aza-lower alkylene that is unsubstituted or N-substituted by lower alkyl, hydroxy-lower alkyl, lower alkoxy-lower alkyl or by lower alkanoyl, 3- or 4-oxa-lower alkylene or optionally S-oxidised 3- or 4-thia-lower alkylene.

As used herein "A functional derivative of IAMT protein", means a derivative derivable from the respective natural form of IAMT by modification, e.g. by mutagenesis like amino acid substitution, deletion, insertion or addition, or by chemical

modification, said derivative substantially showing biological activity by preventing or alleviating an autoimmune response either by decreasing or enhancing IAMT activity.

As used herein the term "C_{1-20}-alkyl" is intended to mean a linear, cyclic or branched hydrocarbon group having 1 to 20 carbon atoms, such as methyl, ethyl, propyl, iso-propyl, cyclopropyl, butyl, *tert*-butyl, iso-butyl, cyclobutyl, pentyl, cyclopentyl, hexyl, cyclohexyl, hexadecyl, heptadecyl, octadecyl, nonadecyl. Analogously, the term "C_{1-6}-alkyl" is intended to mean a linear, cyclic or branched hydrocarbon group having 1 to 6 carbon atoms, such as methyl, ethyl, propyl, iso-propyl, pentyl, cyclopentyl, hexyl, cyclohexyl, and the term "C_{1-4}-alkyl" is intended to cover linear, cyclic or branched hydrocarbon groups having 1 to 4 carbon atoms, e.g. methyl, ethyl, propyl, iso-propyl, cyclopropyl, butyl, *iso*-butyl, *tert*-butyl, cyclobutyl.

Preferred embodiments of C_{1-20}-alkyl are methyl, ethyl, propyl, iso-propyl, butyl, *tert*-butyl, iso-butyl, pentyl, cyclopentyl, hexyl and cyclohexyl.

Especially preferred embodiments are methyl, ethyl, propyl, iso-propyl, *tert*-butyl, iso-butyl and cyclohexyl.

Similarly, the terms "C_{2-20}-alkenyl", "C_{4-20}-alkadienyl", and "C_{6-20}-alkatrienyl" as used in the present invention are intended to mean a linear, cyclic or branched hydrocarbon group having 2 to 20, 4 to 20, and 6 to 20, carbon atoms, respectively, and comprising one, two, and three unsaturated bonds, respectively. Examples of alkenyl groups are vinyl, allyl, butenyl, pentenyl, hexenyl, heptenyl, octenyl, heptadecaenyl. Examples of alkadienyl groups are butadienyl, pentadienyl, hexadienyl, heptadienyl, heptadecadienyl. Examples of alkatrienyl groups are hexatrienyl, heptatrienyl, octatrienyl, heptadecatrienyl.

Preferred embodiments of C_{2-20}-alkenyl, C_{4-20}-alkadienyl, and C_{6-20}-alkatrienyl are vinyl, allyl and butenyl.

Similarly, the terms "C_{3-20}-alkynyl", as used in the present invention are intended to mean a linear, cyclic or branched hydrocarbon group having 3 to 20 carbon atoms, and comprising one triple bond. Examples of alkynyl groups are propargyl, butyn-1-yl, butyn-2-yl, pentyn-1-yl, pentyn-2-yl, hexyn-1-yl, hexyn-2-yl, hexyn-3-yl.

In the context of the present invention, i.e. in connection with the terms "alkyl", "alkenyl", "alkadienyl", and "alkatrienyl", the term "optionally substituted" is intended to mean that the group in question may be substituted one or several times, preferably 1-

3 times, with group(s) selected from hydroxy, C_{1-6}-alkoxy, carboxy, C_{1-6}-alkoxycarbonyl, C_{1-6}-alkylcarbonyl, formyl, aryl, aryloxycarbonyl, arylcarbonyl, heteroaryl, amino, mono- and di(C_{1-6}-alkyl)amino, carbamoyl, mono- and di(C_{1-6}-alkyl)-aminocarbonyl, amino-C_{1-6}-alkyl-aminocarbonyl, mono- and di(C_{1-6}-alkyl)amino-C_{1-6}-alkyl-aminocarbonyl, C_{1-6}-alkylcarbonylamino, guanidino, carba-mido, C_{1-6}-alkanoyloxy, sulphono, C_{1-6}-alkylsulphonyloxy, nitro, sulfanoyl, trihalogenalkyl, halogen such as fluoro, chloro, bromo or iodo, where aryl and heteroaryl may be substituted with C_{1-6}-alkyl, hydroxy, C_{1-6}-alkoxy, amino, mono- and di(C_{1-6}-alkyl)amino, nitro, cyano or halogen.

Preferably, the substituents of R_1 and R_2 are selected from hydroxy, C_{1-6}-alkoxy, carboxy, C_{1-6}-alkoxycarbonyl, C_{1-6}-alkylcarbonyl, formyl, aryl, aryloxy-carbonyl, arylcarbonyl, heteroaryl, amino, mono- and di(C_{1-6}-alkyl)amino, carbamoyl, mono- and di(C_{1-6}-alkyl)aminocarbonyl, amino-C_{1-6}-alkyl-aminocarbonyl, mono- and di(C_{1-6}-alkyl)amino-C_{1-6}-alkyl-aminocarbonyl, C_{1-6}-alkylcarbonylamino, guanidino, carbamido, trihalogenalkyl, halogen such as fluoro, chloro, bromo or iodo, where aryl and heteroaryl may be substituted with C_{1-6}-alkyl, hydroxy, C_{1-6}-alkoxy, amino, mono- and di(C_{1-6}-alkyl)amino, nitro, cyano or halogen.

Especially preferred embodiments are hydroxy, C_{1-6}-alkoxy, carboxy, aryl, heteroaryl, amino, mono- and di(C_{1-6}-alkyl)amino, mono- and di(C_{1-6}-alkyl)amino, nitro, cyano and halogen such as fluoro, chloro, bromo or iodo.

In the present invention the term "aryl" is intended to mean an aromatic carbocyclic ring or ring system, exemplified by, but not limited to phenyl, benzyl, biphenyl, phenoxy-phenyl, naphthyl, anthracyl, phenanthracyl, pyrenyl, benzopyrenyl, fluorenyl and xanthenyl.

Preferred embodiments of aryl are phenyl, biphenyl, phenoxy-phenyl and benzyl.

The term "heteroaryl" is intended to mean an aryl group where one or more of the carbon atoms have been replaced with heteroatoms, e.g. nitrogen, sulphur, and/or oxygen atoms. Examples of such heteroaryl groups include but are not limited to oxazolyl, isoxazolyl, thiazolyl, isothiazolyl, pyrrolyl, imidazolyl, pyrazolyl, pyridinyl, pyrazinyl, pyridazinyl, piperidinyl, coumaryl, furyl, quinolyl, indolyl, phenoxazonyl, thiophenyl.

Preferred heteroaryl groups are pyrazinyl, pyridinyl, thiophenyl, piperidinyl.

In the present invention, i.e. in connection with the terms "aryl" and "heteroaryl", the term "optionally substituted" is intended to mean that the group in question may be substituted one or several times, preferably 1-5 times, with group(s) selected from C_{1-6}-alkyl, C_{2-8}-alkenyl, C_{4-10}-alkadienyl, C_{6-16}-alkatrienyl, C_{3-8}-alkynyl, hydroxy, C_{1-6}-alkoxy, carboxy, C_{1-6}-alkoxycarbonyl, C_{1-6}-alkylcarbonyl, formyl, aryl, aryloxycarbonyl, arylcarbonyl, heteroaryl, amino, mono- and di(C_{1-6}-alkyl)amino; carbamoyl, mono- and di(C_{1-6}-alkyl)aminocarbonyl, amino-C_{1-6}-alkyl-aminocarbonyl, mono- and di(C_{1-6}-alkyl)amino-C_{1-6}-alkyl-aminocarbonyl, C_{1-6}-alkyl-carbonylamino, guanidino, carbamido, C_{1-6}-alkanoyloxy, sulphono, C_{1-6}-alkyl-sulphonyloxy, sulfonamidyl, mono- and di(C_{1-6}-alkyl)sulfonamidyl,nitro, cyano, sulfanoyl, trihalogenalkyl, halogen such as fluoro, chloro, bromo or iodo.

Preferred embodiments are hydroxy, nitro, cyano, C_{1-6}-alkyl, C_{1-6}-alkoxy, carboxy, C_{1-6}-alkoxycarbonyl, C_{1-6}-alkylcarbonyl, aryl, amino, mono- and di(C_{1-6}-alkyl)amino, heteroaryl and halogen such as fluoro, chloro, bromo or iodo.

The compound of the formula (I) can exist in several tautameric forms all of which are included in the present invention and the above definitions.

As used herein "desmethyl and/ or despropargyl derivative" means the compound of regard without the methyl and/ or propargyl group.

As used herein "lower" in connection with radicals and compounds, means having up to and including 7, preferably up to and including 4, carbon atoms.

Lower alkoxy is, for example, C_1 -C_7 alkoxy, preferably C_1 –C_4 alkoxy, such as methoxy, ethoxy, propyloxy, isopropyloxy or butyloxy, but may also be isobutyloxy, sec-butyloxy, tert-butyloxy or a C_5 -C_7 alkoxy group, such as a pentyloxy, hexyloxy or heptyloxy group.

Lower alkyl is, for example, C_1 -C_7 alkyl, preferably C_1 –C_4 alkyl, such as methyl, ethyl, propyl, isopropyl or butyl, but may also be isobutyl, sec-butyl, tert-butyl or a C_5 -C_7 alkyl group, such as a pentyl, hexyl or heptyl group.

Use of compounds modulating dehydrogenases, insulin secretion and β-cell apoptosis to treat diabetes

The fundamental aspect of the present invention is the ability to influence insulin secretion and apoptosis of β–cells by oligomeric modulation of dehydrogenases as GAPDH by the use of oligomeric modulators thus preventing, treating or alleviating diabetes.

As used herein "Dehydrogenases" is intended to include enzymes which catalyse oxidation by the removal of hydrogen, e.g., lactate dehydrogenase (LADH), glyceraldehyde-3-phosphate dehydrogenase, etc.

As used herein the term "GAPDH oligomeric modulators" is intended to include those compounds or compositions which prevent, inhibit or interfere with GAPDH oligomer formation e.g. tetramer of GAPDH, wherein "oligomer" preferably refers to more than two, and even more and more preferably more than three, monomeric sub-units. Also included are those compounds which enhance or promote the formation of a lower form of the dehydrogenase, e.g. a dimer or monomer.

As used herein "Oligomeric modulation" is intended to include the prevention or inhibition of, or interference with dehydrogenase oligomer, e.g. tetramer formation. Also included is the enhancement or promotion of the formation of a lower form of the dehydrogenase e.g. a dimer or monomer.

Use of compounds modulating L-Isoaspartyl (D-Aspartyl) O-Methyltransferase to treat autoimmune diseases

Another fundamental aspect of the present invention is the ability to influence an autoimmune response, preferably preventing, treating or alleviating it, through the regulation of IAMT activity. Preference is given to mammalian IAMT, in particular human, canis, canine,feline and rodent IAMT (Swiss Prot accession nr. Human P22061, Mouse P23506, Rat P22062, Dog and Cat are still un-dissolved).

As used herein, "antibody" means polyclonal, monoclonal or humanized antibodies, including Fc fragments, Fab fragments, chimeric antibodies or other antigen-specific antibody fragments.

As used herein "autoantigen / self-antigen", means a molecule produced and used by an individual self, which is recognized by an autoantibody, eliciting an immune response possibly leading to an autoimmune disease.

As used herein "A functional derivative of IAMT protein", means a derivative derivable from the respective natural form of IAMT by modification, e.g. by mutagenesis like amino acid substitution, deletion, insertion or addition, or by chemical modification, said derivative substantially showing biological activity by preventing or alleviating an autoimmune response either by decreasing or enhancing IAMT activity.

As used herein "patient" means an individual consulted by a medical practitioner.

As used herein "molecule", means any chemical compound either synthetic or natural occurring, including DNA, RNA, peptides, proteins or fragments thereof as well as small inorganic and organic compounds.

As used herein "regulator of IAMT activity", means a molecule affecting the basal activity of IAMT at any level. For example IAMT agonists, catalyst, antagonists, gene expression enhancers or inhibitors, RNA stabilisers, inhibitors or activators of molecules interacting with IAMT.

As used herein, "sequence independent context", means that the sequence, surrounding the L-iso-aspartyl and/ or D-aspartyl residue(s), can be composed of virtually any of the 20 natural occurring amino acids or derivatives thereof, in a random order, producing a peptide or protein or a peptide like structure.

As used herein, "suitable promoter", means an inducible or constitutively active promoter operably linked to a coding region. The promoter is only transcribed under certain conditions, for example in certain tissues, cells or as a reaction to a certain disease possibly by induction through molecules generated as a result of the disease.

In one aspect the present invention relates to the pharmaceutical use of one of the compounds I, III, IV, V or VI for the treatment of a disease in a mammal.

In a preferred embodiment of the present invention said mammal is human.

In another aspect of the present invention said pharmaceutical use relates to a method the for preventing, treating or alleviating diabetes (Type I and Type II diabetes).

In yet another aspect of the present invention said pharmaceutical use relates to a method for preventing, treating or alleviating an autoimmune response and/ or disease in a mammal comprising regulating L-Isoaspartyl (D-Aspartyl) O-Methyltransferase

(IAMT) or glycerylaldehyde-3-phosphate dehydrogenase (GAPDH) activity by administering a molecule with such a regulatory effect.

In a preferred embodiment said method comprises the regulation occuring within one or more cell types such as antigen presenting cells, T-cells or cells that become targets for an autoimmune attack by the immune system.

In a preferred embodiment of the present invention said method comprises the up-regulation of IAMT or its activity.

In a preferred embodiment the present invention comprises use of a regulator of IAMT or GAPDH activity for the preparation of a composition for the prevention, treatment or alleviation of an autoimmune response and/ or disease in a mammal.

In another aspect the present invention comprises a method to identify compounds which are potential regulators of IAMT or activity comprising the steps of

a) providing a test system suitable for determining IAMT activity comprising IAMT, one or more peptides prone for isomerisation/ optical inversion and an antibody, which recognizes either iso-aspartyl or D-aspartyl in a sequence independent context;
b) exposing a candidate compound to the test system;
c) exposing a control compound to the test system;
d) determining the amount of iso-aspartyl or D-aspartyl containing peptides in the test system versus the control system; and
e) identifying compounds which result in a decreased amount of iso-aspartyl or D-aspartyl containing peptides compared to the control.

In one embodiment said method comprises the use of a compound, for the preparation of a composition for the prevention, treatment or alleviation of an autoimmune response and/ or disease in a mammal.

In another embodiment the present invention comprises said use, wherein the compound is selected from the compounds I, II, II or IV.

In a preferred embodiment the present invention comprises said use, wherein the compound is selected from the compounds V (NBC-1) or VI (NBC-2).

In another preferred embodiment the present invention comprises the use of a pharmaceutical composition comprising a compound as defined above, optionally together with at least one pharmaceutically acceptable carrier and/ or excipient.

In a further embodiment the present invention comprises the use of IAMT or a functional derivate thereof for the preparation of a composition for the prevention, treatment or alleviation of an autoimmune response and/ or disease in a mammal.

In still another embodiment the present invention comprises the use of IAMT or a functional derivate thereof for the preparation of a composition for the prevention, treatment or alleviation of diabetes in a mammal.

In yet another embodiment the present invention comprises the use of a pharmaceutical composition comprising IAMT or a functional derivative thereof in an amount effective for preventing or alleviating an autoimmune response and/ or disease in a mammal, optionally together with at least one pharmaceutically acceptable carrier and/ or excipient.

In another preferred embodiment the present invention comprises the use of a pharmaceutical composition comprising IAMT or a functional derivative thereof in an amount effective for preventing or alleviating diabetes (type I and type II diabetes) in a mammal, optionally together with at least one pharmaceutically acceptable carrier and/ or excipient.

In still another preferred embodiment the present invention comprises the use of a composition comprising an IAMT or GAPDH encoding nucleic acid sequence or a functional derivative thereof for the use in diagnosing and/ or treatment of an autoimmune disease in a mammal, optionally together with at least one pharmaceutically acceptable carrier and/ or excipient.

In a further preferred embodiment the present invention comprises the use a composition comprising an IAMT or GAPDH encoding nucleic acid sequence or a functional derivative thereof for the use in diagnosing and/ or treatment of diabetes (type I and type II diabetes) in a mammal, optionally together with at least one pharmaceutically acceptable carrier and/ or excipient

In yet another preferred embodiment the present invention comprises the use of a said composition, wherein the IAMT encoding nucleic acid sequence is situated in an expression vector comprising a suitable promoter for expression of IAMT.

In another aspect the present invention comprises a method for diagnosing an autoimmune disease or assessing an individuals risk of developing an autoimmune disease, comprising screening for genetic polymorphisms in the IAMT or GAPDH gene.

In one embodiment the present invention comprises a method of diagnosing diabetes (type I and type II diabetes) or assessing an individuals risk of developing an autoimmune disease, comprising screening for genetic polymorphisms in the IAMT or GAPDH gene.

In another embodiment the present invention comprises the a method of diagnosing diabetes or assessing an individuals risk of developing diabetes, comprising screening for genetic polymorphisms in the IAMT or GAPDH gene.

In still another embodiment the present invention comprises the a method of diagnosing an autoimmune disease or assessing an individuals risk of developing an autoimmune disease, comprising quantification of IAMT or GAPDH on gene transcription level, protein level or activity, in a biological sample from a patient versus a control.

A method of diagnosing an autoimmune disease or assessing an individuals risk of developing diabetes (type I and type II diabetes), comprising quantification of IAMT or GAPDH on gene transcription level, protein level or activity, in a biological sample from a patient versus a control.

In one embodiment of the present invention the administration of a molecule with a regulatory effect on IAMT activity, within one or more cell types will enable prevention, treatment or alleviation of an autoimmune response or an autoimmune disease. Especially cell-types associated with the immune system, such as B-cells, dendritic cells, macrophages, mast cells, monocytes, neutrophils, NK cells or T-cells are considered, most preferred are T-cells and antigen presenting cells, such as dendritic cells, macrophages and B-cells. Other cell-types of importance, are cells that become targets for an autoimmune attack by the immune system, such as, but not limited to, pancreatic β-cells, Schwann cells, mucus secretory cells such as goblet cells, salivary gland cells or other endocrine gland cells.

In a preferred embodiment of the present invention, the activity of IAMT is up-regulated.

In another embodiment a regulator of IAMT activity is used for the preparation of a composition for the prevention, treatment or alleviation of an autoimmune response and/ or disease in a mammal.

A humoral or cell mediated immune response directed toward a self-antigen/ autoantigen, is considered to be an autoimmune response. An autoimmune response often leads to an autoimmune disease. The present invention provide means for therapeutic interventions or disease prevention of autoimmune diseases such as, but not limited to, celiac disease, Crohns disease, insulin dependent diabetes mellitus, Grave's disease, multiple sclerosis, myasthenia gravis, psoriasis, rheumatoid arthritis, Sjogren's syndrome, systemic lupus erythematosus or ulcerative colitis.

The present invention provides a method for identifying regulators of IAMT activity from candidate or test compounds or agents (*e.g.*, peptides, peptidomimetics, small molecules or other drugs), which have a modulatory (*i.e.*, stimulatory or inhibitory) effect on, for example, expression or activity of IAMT.

Cell-based screening assays for IAMT has been described in WO 98/15647, either measuring the level of gene expression using a reporter protein, mRNA or protein levels with techniques generally know in the art. Furthermore a direct assessment of IAMT activity was described utilizing S-adenosyl-L-[methyl-^{3}H]-methonine, followed by measuring the incorporation of methyl-^{3}H into the substrate (L-iso-aspartyl) by fluorography. A similar technique measuring IAMT activity is described in the ISOQUANT kit from Promega utilizing a scintillation counter or HPLC.

In one embodiment of the present invention, a test system for IAMT activity is provided, not utilizing radioactivity or time-consuming HPLC techniques. The preferred test system is cell-based, containing L-iso-aspartyl and/ or D-aspartyl peptides and expressing IAMT. The cell, for example, can be a yeast cell, a cell of mammalian origin or a tissue section. A cell-free system can also be applied when testing compounds acting directly on IAMT, L-iso-aspartyl or D-aspartyl. The test system is contacted with the test compound and the ability of the test compound to regulate IAMT activity is determined by measuring substrate conversion utilizing an immunoassay. Antibodies, which recognize either L-iso-aspartyl or D-aspartyl in a sequence independent context,

constitutes a part of the test system and will enable a fast determination of a compounds effect on IAMT activity. A reduced level of antibody binding, as compared to suitable controls, means a decrease in L-iso-aspartyl and/ or D-aspartyl containing peptides, which correlate with an increase in IAMT activity. Antibody binding can be assessed by techniques generally know in the art, for example Western blot, ELISA, RIA, immuno-precipitation or histology.

The method for measuring IAMT activity as described above can be provided as a kit. This will include a suitable test system, for example a cell free system containing IAMT protein and L-iso-aspartyl and/ or D-aspartyl containing peptides or a cellular system (e.g. e-coli, yeast, mammalian cell-lines, primary cell cultures or tissue sections) containing and expressing endogenous (homologous) and/ or exogenous (heterologous) IAMT encoding nucleic acid. The expression can be coupled to an easy detectable reporter protein, such as, but not limited to, β-galactosidase, chloramphenicol acetyltransferase (CAT), Green Fluorescent Protein, or luciferase. Furthermore the kit includes a context independent antibody recognizing a L-iso-aspartyl or D-aspartyl, and possibly a second antibody with specificity towards the first antibody. For competition measurements a synthetic or natural occurring peptide containing one or more L-iso-aspartyl or D-aspartyl residues might be supplied either in a labelled or unlabelled form. The antibodies may be used with or without modifications. The antibodies may be labelled by joining them, either covalently or non-covalently, with a reporter molecule. Suitable reporter molecules or labels, which may be used for ease of detection, include radionuclides, enzymes, fluorescent, chemiluminescent, or chromogenic agents as well as substrates, cofactors, inhibitors, magnetic particles, and the like. Antibodies or synthetic peptides of the kit might be immobilised, preferably on a solid surface like a micro-titter plate, possibly by conjugation to a suitable protein carrier like BSA, thyroglobulin, ovalbumin or keyhole limpet hemocyanine.

In another aspect the present invention comprises a method for diagnosing an autoimmune disease or assessing an individuals risk of developing diabetes (type I and type II diabetes), comprising detecting the IAMT polymorphy

22132

AGATCCGCCGCTCGAAACAGCTGACCCAGCGACGACTGCGG

AGATCCGCCGCTCGAAACAG*G*TGACCCAGCGACGACTGCGG

at position 22132 of the PCMT1 (IAMT) gene in a biological sample from a patient versus a control.

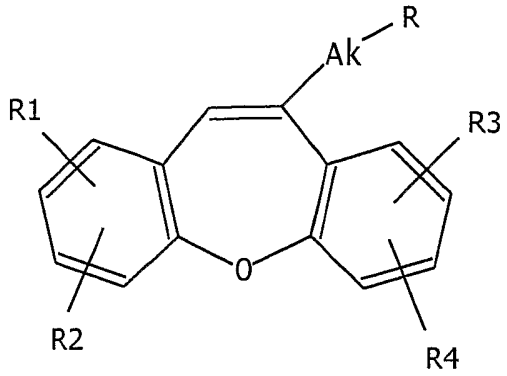
The invention also encompasses molecules that serve to regulate IAMT activity for use in prevention, alleviation or treatment of autoimmune disorders. IAMT agonists, IAMT catalysts, compounds that stimulate the synthesis or expression of endogenous IAMT and compounds that inhibit inhibitors of IAMT activity (*i.e.*, an inhibitor of a IAMT antagonist), IAMT antagonists, as well as IAMT protein or a functional derivative thereof, are contemplated.

Neuro-rescuing compounds, such as (-)-deprenyl, (-)-desmethyldeprenyl, (-)-2-heptyl-N-methylpropargylamine or structural analogs for example 10-aminoaliphatyl-dibenz[b,f]oxepines, can have IAMT regulating activity and are covered by the present invention.

In one embodiment of the present invention the use of compounds identified by the method for determining regulators of IAMT activity, provided in the present invention (see the above description), are used for preparation of a composition with a preventing, treating or alleviating effect on an autoimmune response and/ or disease.

Another embodiment of the present invention, is the use of 10-amino-aliphatyl-dibenz[b,f]oxepines (formula XIV) for the preparation of various compositions for preventing, alleviating or treating an diabetes in a mammal.

Another embodiment of the present invention, is the use of 10-amino-aliphatyl-dibenz[b,f]oxepines (formula XIV) for the preparation of various compositions for preventing, alleviating or treating an autoimmune response and/ or disease in a mammal.



Formula XIV

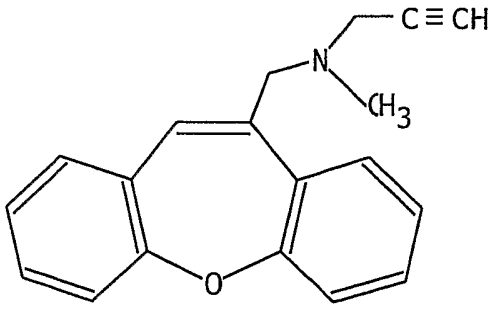
wherein Ak is a divalent aliphatic radical,

R is an amino group that is unsubstituted or mono- or di-substituted by monovalent aliphatic and/or araliphatic radicals or disubstituted by divalent aliphatic radicals, and

R1, R2, R3 and R4 are each, independently of the others, hydrogen, lower alkyl, lower alkoxy, halogen or trifluoromethyl.

Compounds of formula XV are disclosed in patent EP 0726265, hereafter incorporated by reference. Further classes of compounds may be envisaged, e.g. compounds involving substitutions, side chain alterations and ring modifications of the above-mentioned oxepines. Such further compounds may be tested applying the method for identifying regulators of IAMT activity, provided in the present invention.

A preferred embodiment of the present invention, is the use of N-(dibenz [b,f]oxepin-10-ylmethyl)-N-methyl-N-prop-2-ynylamine (Formula XV) for the preparation of a composition for preventing, alleviating or treating an autoimmune response and/ or disease in a mammal.



Formula XV

Metabolites of N-(dibenz [b,f]oxepin-10-ylmethyl)-N-methyl-N-prop-2-ynylamine are also covered in the present invention, specifically the N-desmethyl, N-despropargyl and N-desmethyl-despropargyl derivatives.

Moreover the invention is directed to the use of IAMT protein or a functional derivative thereof for the preparation of a composition for preventing, alleviating or treating an autoimmune response and/ or disease.

One way to gain control of an autoimmune disease could be through the use of compounds for the preparation of a pharmaceutical composition, which decrease T-cell proliferation and/ or autoantigen presentation on MHC II molecules, thereby preventing, alleviating or treating an autoimmune response. Preferably the compounds are chosen among those identified by the the method for identifying regulators of IAMT activity, provided in the present invention, or among the oxepines described in the above or the IAMT protein or a functional derivative thereof.

The invention furthermore relates to pharmaceutical compositions for the treatment, prevention or alleviation of an autoimmune response and/ or disease. Such a pharmaceutical composition contains an IAMT protein, functional derivative thereof or a regulator of IAMT activity suitable for administration to a patient, preferably a mammal, more preferably a human.

Also contemplated are pharmaceutical compositions for prevention, alleviation or treatment of an autoimmune response and/ or disease, involving combination therapies comprising, administering an effective amount of IAMT protein, functional derivative thereof or a IAMT modulator in combination with other therapeutic agents. Other therapeutic agents can be, for example, anti-inflammatory drugs (e.g. NSAIDs, Phosphosugars or COX-2 inhibitors), anti-diabetes agents, immunotherapeutic agents, insulin-releasing agents (e.g. GLP-1, nateglinide, repaglinide, sulfonylurea, vasopressin), cytokines (e.g. interferons, interleukins, tumor necrosis factor, Fas ligand, cytokine antagonist (i.e. antibodies or receptors to TNF-α, IL-1, IL-6 or IL-12) or protease inhibitors (e.g. cysteine protease inhibitor, DPP IV antagonist, serine-protease inhibitor).

When administered to a patient, an IAMT protein, functional derivative thereof or a regulator of IAMT activity is preferably administered as a component of a composition that optionally comprises a pharmaceutically acceptable carrier, excipient or vehicle. In a preferred embodiment, these compositions are administered orally. Other

administration routes may be, but is not limited to, depot injection, implantation, intracavitary, intramuscular, intravenous, nasal, subcutaneous, time-release mode or transdermal. The pharmaceutical composition is formulated to be compatible with its intended route of administration.

Compositions for oral administration might require an enteric coating to protect the composition(s) from degradation within the gastrointestinal tract. In another example, the composition(s) can be administered in a liposomal formulation to shield the IAMT protein, functional derivative thereof or an IAMT modulator disclosed herein, from degradative enzymes, facilitate the molecule's transport in the circulatory system, and effect delivery of the molecule across cell membranes to intracellular sites.

Pharmaceutical compositions applicable in gene therapy approaches can also be used in accordance with the present invention to modulate the expression of an IAMT protein or an IAMT regulator (including IAMT antisense) and accordingly treat, alleviate or prevent an autoimmune response and/ or disease. Any of the methods for gene therapy available in the art can be used in accordance with the present invention. IAMT encoding nucleic acid sequences can be assessed through, but not limited to, Genbank accession nr. D13892, D25545, D25546, M60320, M26686, D11475, hereafter incorporated by reference.

A recipient's cells or heterologous cells can be engineered to express IAMT protein, IAMT regulator or a combination. The cells can be grown as an implant in an experimental animal or in tissue culture using techniques known in the art. Once altered genetically, the engineered cells can then be administered to a subject using procedures known in the art. Alternatively, one can use gene therapy to transfect the recipient's cells *in vivo*.

The present invention encompasses expression vectors comprising a nucleic acid sequence encoding an IAMT protein or an IAMT regulator of the invention. Any type of plasmid, cosmid, YAC or viral vector can be used to prepare the recombinant construct. Alternatively, vectors can be used, which selectively target a tissue or cell type, *e.g.* viruses that infect antigen presenting cells or T-cells. Further specificity can be realized by using a tissue-specific or cell-specific promoter in the expression vector.

In one embodiment, an expression vector containing a nucleic acid sequence encoding an IAMT protein or an IAMT regulator to be introduced for purposes of gene therapy, comprises an inducible promoter operably linked to the coding region, such

that expression of the nucleic acid sequence can be controlled using an appropriate inducer or inhibitor of transcription.

In another embodiment, the vector contains a promoter, which expresses the cloned construct constitutively. The promoter can be down-regulated using a suppressor molecule. Alternatively, the vector contains a promoter, such that an inducing molecule initiates or increases expression of the cloned nucleic acid sequence.

In a preferred embodiment, the vector contains a specific promoter. Such a promoter can for example restrict expression to occur in a specific tissue or organ, such as, but not limited to, skin, muscle, intestine, lung, cartilage, bone, brain or certain areas of the brain, pancreas, liver, kidney or thymus. Specific cell types can also be a target for such a promoter, for example cells associated with the immune system, such as B-cells, dendritic cells, macrophages, mast cells, monocytes, neutrophils, NK cells or T-cells, antigen presenting cells, such as dendritic cells, macrophages and B-cells. Other cell-types, such as, but not limited to, pancreatic β-cells, Schwann cells, epithelia cells, mucus secretory cells such as goblet cells, salivary gland cells or other endocrine gland cells. A vector containing a disease-specific promoter, such that expression is largely limited to diseased tissues or tissues surrounding diseased tissues is also a possibility. A disease specific promoter could be controlled through certain cytokines, antibodies or other molecules released as reaction to a certain disease.

Formulations of nucleic acid sequences for gene therapeutic methods can be, but are not limited to, naked DNA, nucleic acid sequence encapsulated into liposomes or liposomes combined with viral envelope receptor proteins, DNA coupled to a polylysine-glycoprotein carrier complex, and nucleic acid precipitants.

The present invention additionally encompasses methods of diagnosing or assessing an individual's risk developing an autoimmune disease, associated with irregularities connected to IAMT.

The gene encoding IAMT protein is know to contain polymorphisms, where at least one has been shown to result in different enzyme activities (David, Szumlanski, DeVry, Park-Hah, Clarke, Weinshilboum, and Aswad 1997; Tsai and Clarke 1994).

A study connecting such genetic polymorphisms to autoimmune diabetes (IDDM) has been conducted. A polymorphism in the human IAMT1 (PCMT1) gene promoter was identified.

In addition to this whilst screening, are amino acid 22 Ile/Leu, amino acid 119 Val/Ile and amino acid 205 Lys/Arg, their connection to autoimmunity will be determined, as well as new exon or intron polymorphisms, which might emerge through the study.

In one embodiment the results from such a study will produce a method for diagnosing or assessing an individual risk developing an autoimmune disease. Methods for determination of genetic polymorphism in genomic DNA includes, but is not limited to, direct comparison of sequences of different genomes, pulsed field gel electrophoresis, alterations in restriction enzyme cleavage patterns or polymerase chain reaction with designed primers. The screening of genetic polymorphism in the IAMT gene can be performed on any biological material containing genomic DNA, for example blood, erythrocytes, hair, salvia or tissue samples.

In another embodiment of the present invention irregularities connected with IAMT gene transcription level, protein level or activity will be utilized for diagnosing or assessing an individual's risk developing an autoimmune disease.

For quantitative determination of IAMT gene transcription level in an individual, the amount of IAMT mRNA in a sample can be measured utilizing techniques generally know in the art and include for example rtPCR, micro arrays or Northern blot techniques. A decreased IAMT gene transcription level compared to a control, for example a group of healthy individuals, indicate a risk of autoimmunity or possible diagnosis of autoimmunity.

The IAMT protein level indirectly reflects gene transcription level as well as mRNA stability. Techniques for measuring proteins levels are generally known in the art and include for example Western blot analysis, ELISA, RIA, immuno-precipitation, histology, micro arrays and the like.

A method for quantification of IAMT activity, utilizing antibodies, which recognize L-iso-aspartyl or D-aspartyl in a sequence independent context, has already been disclosed in this invention, and can also be applied for diagnostic means. The decrease in L-iso-aspartyl or D-aspartyl containing peptides supplied to a sample, provided from an individual to be diagnosed, assess the activity of endogenous IAMT in the individual. Other methods to assess IAMT activity can also be utilized in relation to diagnosis of an autoimmune disease, for example the method described in the ISOQUANT kit from Promega. A decreased IAMT level compared to a control, for

example a group of healthy individuals, indicate a risk of autoimmunity or possible diagnosis of autoimmunity.

Preferably any of the, in the above, described measurements performed for diagnosis are determined against suitable controls, e.g. healthy individuals or cell lines where IAMT baseline expressions are known.

The diagnostic measurements can be performed on biological samples such as, but not limited to, human body fluids (e.g. blood, serum or urine samples) or extracts from cells or tissue samples. Another possibility is to isolate specific cell types from blood or tissue samples, where IAMT play a role in connection with autoimmunity, such as T-cells or antigen presenting cells. Cells circulating in the blood can be isolated using FACS. Cells can also be cultured from an area affected by an autoimmune response, followed by selection for one or more specific cell types, e.g. macrophages, dendritic cells or the like.

Chemical Synthesis :

1 : Synthesis of Dibenzo[b,f]oxepine-10-carboxylic acid (I)

Sodium (s, 2.3 g, 1.5 eq.) is added to ethanol (abs., 60 ml). When evolution of hydrogen had ceased, 2-phenoxy-phenylacetonitrile (13.7 g, 65.5 mmol, 1 eq.) in ethanol (abs., 20 ml), and subsequently diethyloxalate (11.5 g, 1.2 eq.) was added. Left at room temperature for 18 h. The reaction mixture was acidified using 1 M HCl and extracted with ethylacetate (3 * 200 ml). The combined organic phases were dried (Na_2SO_4), filtered and concentrated *in vacuo* to give a slightly yellow oil (20 g). Glacial acetic acid (7 ml/g, 140 ml) was added and the mixture stirred at room temperature to 30 minutes, whereupon water (3.5 ml/g, 70 ml) and sulphuric acid (concentrated, 3.5 g/ml, 70 ml) was added. The resulting mixture was heated to reflux and left for 5 hours. The reaction mixture was cooled at room temperature, and left under vigorous stirring over night. The reaction mixture (orange) was filtered and the yellow precipitate was washed with water and dried on the filter, yielding 12.05 g (77 %) of dibenzo[b,f]oxepine-10-carboxylic acid, (I).

2 : Synthesis of Dibenzo[b,f]oxepin-10-yl-methanol (2)

Dibenzo[b,f]oxepine-10-carboxylic acid (I, 7.02 g, 29.5 mmol) was dissolved in dimethoxyethane (75 ml) and cooled to –15 ° C using a ethyleneglycol/dry ice bath. N-methylmorpholine (3.25 ml) was added and subsequently under stirring and drop wise isobutylchloroformate (3.82 ml) was added at a rate to keep the temperature at –15 ° C. After 20 minutes, the reaction mixture was filtered and the filtrate cooled to –15 ° C, and under vigorous stirring sodiumborohydride (2.2 g in 22 ml water) was added drop wise to avoid excessive gas evolution. Upon completion of addition, the reaction mixture was left at –15 ° C for 30 minutes, when it was quenched by adding 1 M hydrochloric acid. pH was adjusted to 13 using 1 M sodiumhydroxide, and the resulting mixture extracted with ethylacetate (3 * 200 ml). The combined organic phases were dried (Na_2SO_4), filtered and concentrated *in vacuo* to give (2) as a colourless oil (6.57 g, 99%).

3 : Synthesis of dibenzo[b,f]oxepin-10-ylmethyl-methyl-prop-2-ynyl-amine, monomaleic acid salt (3)

Dibenzo[b,f]oxepin-10-yl-methanol (2, 3.8 g, 16.8 mmol) is dissolved in dimethylformamide (dry, 80 ml). N-methyl-morpholine (1.3 eq., 2.4 ml) and subsequently methanesulfunylchloride (1.2 eq, 1.57 ml) was added, and the reaction mixture was stirred for 3 hours at room temperature, when N-methylpropargylamine (2.5 eq., 3.6 ml) was added at the mixture stirred at 40 ° C for 1 hour. N-methylpropargylamine (0.5 ml) was added and the temperature increased to 60 ° C for another 30 minutes. The reaction mixture was concentrated *in vacuo* to give a brown oil, which was redissolved in diethylether (200 ml) and extracted using water (2 * 200 ml), brine (3 * 200 ml). The organic phase was dried (Na_2SO_4), filtered and concentrated *in vacuo* to give dibenzo[b,f]oxepin-10-ylmethyl-methyl-prop-2-ynyl-amine as a brown oil (3.5 g), which was then redissolved in boiling ethanol (abs., 100 ml). To the boiling solution was added maleic acid (3 g) in ethanol (abs., 75 ml) and the resulting solution was left at –20 ° C for 18 h, when the formed precipitate was collected and washed using ice-cold ethanol to give (3) as off-white needles (2.4 g, 68 % from (2)). The composition of the formed salt was determined by HPLC to be 92 % maleic acid mono-salt.

4 : Synthesis of 10,11-Dihydro-dibenzo[b,f]oxepine-10-carboxylic acid (4)

Dibenzo[b,f]oxepine-10-carboxylic acid (I, 4 g, 17 mmol) was dissolved in methanol (100 ml) in 2 L round bottomed flask. Ar (g) was introduced and Pd/C (10%, 2 g) was added. Hydrogen was introduced and the mixture heated to 45 ° C for 18 hours. The mixture was filtered through celite, the filtercake was washed using methanol (200 ml) and the combined organic solution was evaporated to colourless oil, which settles as semi crystalline white solid upon standing to give (4) (4.3 g, 107 %)

5 : Synthesis of Dibenzo[b,f]oxepine-10-carboxylic acid methyl-prop-2-ynyl-amide (5)

Dibenzo[b,f]oxepine-10-carboxylic acid (I, 550 mg, 2.3 mmol) was dissolved in dichloromethane (30 ml). HOBt (1.3 eq., 400 mg), EDCI (1.1 eq., 460 mg) and DIPEA

(3 eq., 1 ml) was added and the mixture was left to preactivate for 30 minutes. N-methyl-propargyl amine (3 eq., 0.55 ml) was added and the reaction left for 18 hours at room temperature. The reaction mixture was concentrated, the residue redissolved in ethylacetate (100 ml) and extracted using 1 M HCl (4 * 100 ml) and 0.2 M NaOH (4 * 100 ml). The organic phase was dried (Na_2SO_4), filtered and concentrated *in vacuo* to give (5) as a yellow oil (g, %).

6 : Synthesis of Dibenzo[b,f]oxepine-10-carboxylic acid propylamide (6)

Dibenzo[b,f]oxepine-10-carboxylic acid (I, 550 mg, 2.3 mmol) was dissolved in dichloromethane (30 ml). HOBt (1.3 eq., 400 mg), EDCI (1.1 eq., 460 mg) and DIPEA (3 eq., 1 ml) was added and the mixture was left to preactivate for 30 minutes. Proylamine (3 eq., 0.50 ml) was added and the reaction left for 18 hours at room temperature. The reaction mixture was concentrated, the residue redissolved in ethylacetate (100 ml) and extracted using 1 M HCl (4 * 100 ml) and 0.2 M NaOH (4 * 100 ml). The organic phase was dried (Na_2SO_4), filtered and concentrated *in vacuo* to give (6) as a yellow oil which crystallises upon standing (g, %).

7 : Synthesis of Dibenzo[b,f]oxepine-10-carboxylic acid hexylamide (7)

Dibenzo[b,f]oxepine-10-carboxylic acid (I, 550 mg, 2.3 mmol) was dissolved in dichloromethane (30 ml). HOBt (1.3 eq., 400 mg), EDCI (1.1 eq., 460 mg) and DIPEA (3 eq., 1 ml) was added and the mixture was left to preactivate for 30 minutes. Hexylamine (3 eq., 0.80 ml) was added and the reaction left for 18 hours at room temperature. The reaction mixture was concentrated, the residue redissolved in ethylacetate (100 ml) and extracted using 1 M HCl (4 * 100 ml) and 0.2 M NaOH (4 * 100 ml). The organic phase was dried (Na_2SO_4), filtered and concentrated *in vacuo* to give (7) as a yellow oil (g, %).

8 : Synthesis of 10,11-Dihydro-dibenzo[b,f]oxepine-10-carboxylic acid methyl-prop-2-ynyl-amide (8)

10,11-Dihydro-dibenzo[b,f]oxepine-10-carboxylic acid (4, 550 mg, 2.3 mmol) was dissolved in dichloromethane (30 ml). HOBt (1.3 eq., 400 mg), EDCI (1.1 eq., 460 mg) and DIPEA (3 eq., 1 ml) was added and the mixture was left to preactivate for 30 minutes. N-methyl-propargylamine (3 eq., 0.55 ml) was added and the reaction left for 18 hours at room temperature. The reaction mixture was concentrated, the residue redissolved in ethylacetate (100 ml) and extracted using 1 M HCl (4 * 100 ml) and 0.2 M NaOH (4 * 100 ml). The organic phase was dried (Na_2SO_4), filtered and concentrated *in vacuo* to give (8) as a colourless oil (g, %)

9 : Synthesis of 10,11-Dihydro-dibenzo[b,f]oxepine-10-carboxylic acid hexylamide (9)

10,11-Dihydro-dibenzo[b,f]oxepine-10-carboxylic acid (4, 550 mg, 2.3 mmol) was dissolved in dichloromethane (30 ml). HOBt (1.3 eq., 400 mg), EDCI (1.1 eq., 460 mg) and DIPEA (3 eq., 1 ml) was added and the mixture was left to preactivate for 30 minutes. Propylamine (3 eq., 0.50 ml) was added and the reaction left for 18 hours at room temperature. The reaction mixture was concentrated, the residue redissolved in ethylacetate (100 ml) and extracted using 1 M HCl (4 * 100 ml) and 0.2 M NaOH (4 * 100 ml). The organic phase was dried (Na_2SO_4), filtered and concentrated *in vacuo* to give (9) as a colourless oil which crystallises upon standing. Recrystallisation from ethanol/water (6:4) gave (9) as a white crystals (280 mg, 49%)

10 : Synthesis of 10,11-Dihydro-dibenzo[b,f]oxepine-10-carboxylic acid hexylamide (10)

10,11-Dihydro-dibenzo[b,f]oxepine-10-carboxylic acid (4, 550 mg, 2.3 mmol) was dissolved in dichloromethane (30 ml). HOBt (1.3 eq., 400 mg), EDCI (1.1 eq., 460 mg) and DIPEA (3 eq., 1 ml) was added and the mixture was left to preactivate for 30 minutes. Hexylamine (3 eq., 0.80 ml) was added and the reaction left for 18 hours at room temperature. The reaction mixture was concentrated, the residue redissolved in ethylacetate (100 ml) and extracted using 1 M HCl (4 * 100 ml) and 0.2 M NaOH (4 * 100 ml). The organic phase was dried (Na_2SO_4), filtered and concentrated *in vacuo* to give (10) as a colourless oil which crystallises upon standing. Recrystallisation from ethanol/water (6:4) gave (10) as white crystals (340 mg, 54%)

In the following further description of the invention reference is made to the accompanying drawings in which:-

Figure 1: Shows the ability of compounds NBC1 and NBC2 to increase cell number/viability of MIN6 cells in a dose-dependent manner. MIN6 cells were treated for three days with 0, 5, 50, 500, 5000nM of compounds NBC1 and NBC2. Cell number/viability was then measured with AlamarBlue assay. Histograms indicate the mean+SD of 5 replicates.

Figure 2: Shows that the combination of IL-1beta + IFNgamma induces cell death in MIN-6 cells.

Cells were treated for three days with IL-1beta or INFgamma alone, or with the combination of them at different concentrations. Histograms indicate the mean+SD of 5 replicates. Cell survival was determined by AlamarBlue assay.

Figure 3: Shows the effect of the compound NBC2 on survival of MIN6 cells exposed to IL-1beta+ IFNgamma. Cells were pretreated for three days with 50 or 500nM of the compound C and then exposed to 100ng/ml IL-1beta+25ng/ml IFNgamma for 24 additional hours. Cell survival was determined by AlamarBlue assay.

A) Rate of cell survival after 24hr-cytokine exposure in cultures without compound.

B) Rate of cell survival in cultures pre and co treated with compound compared to control. Histograms indicate the mean+SD of 5 replicates.

Figure 4: Shows the effect of CGP3466 and NBC-2 on apoptosis of serum-deprived MIN6 cells.

A) CGP3466 treated MIN6 cells.

B) NBC-2 treated MIN6 cells.

Cells were cultured with serum (control) or without serum (Deprivation) to induce apoptosis. Cells deprived from serum were cultured in the presence of vehicle (Deprivation) or titrated concentrations of CGP3466B or NBC-2 as indicated. Apoptosis was assessed using a combination of DAP1 and FLICA staining to count the number of total cells and apoptotic cells respectively. % apoptotic cells significantly lower than vehicle-treated cells (**), $P<0.01$; (*), $p<0.05$.

Example 1: Effect of compounds on β-cell viability

To investigate benefits of the compounds NBC1 and NBC2 in diabetes, we set up in vitro experiments with MIN6 cells, a pancreatic beta-cell line which has conserved physiological functions like glucose-inducible insulin secretion. As a first experiment, cells seeded at 30,000 cells in 96-well-plate in high glucose DMEM complemented with 10% FCS, were treated for three days with 0, 5, 50, 500 or 5000nM with compounds NBC1 and NBC2. Then, cell growth in different conditions was examined with Alamarblue™ assay that measures cell metabolic activity and gives quantitative information on cell number/viability in well.

As illustrated by figure 1, normal cultures of MIN6 cells treated with lead and analogs showed an increase of cell number/viability compared to control, which was dose-dependent. From 50 to 5000nM, the gain was statistically significant. Nevertheless, a slight curve inflexion was noticed at 5000nM for all of them, suggesting that compounds may become toxic for cells at high concentration.

In some diabetes forms, especially in type 1, insulin-secreting pancreatic beta cells are progressively destroyed by the action of inflammatory cytokines produced by infiltrating T-cells. We studied whether lead and analogs could rescue beta cells from cytokines-induced cell death. As indicated by figure 2, treatment of MIN6 for three days with the combination of IL-1beta plus IFNgamma induced a massive beta-cells

destruction whereas IL-1beta or IFNgamma alone had no effect or mild-effect, respectively. When MIN6 cells were pretreated for two days with 50 or 500nM of lead or analog C and then incubated 24hours with 100ng/ml IL-1beta + 25ng/ml IFNgamma, cell survival was increased about 10-15% compared to the condition where cells exposed to cytokines, didn't receive compounds (figure 9). The gain provided by the treatment with compound NBC2 was statistically significant.

These experiments strongly suggest that compounds NBC1 and NBC2 increase beta-cell survival in both physiological and pathological situations and could bring substantial gains in treatment for diabetes.

Example 2: In vitro studies of the effect of CGP3466B (Formula XV maleate salt) and the structurally related compound NBC-2 on the apoptosis of ß- cells

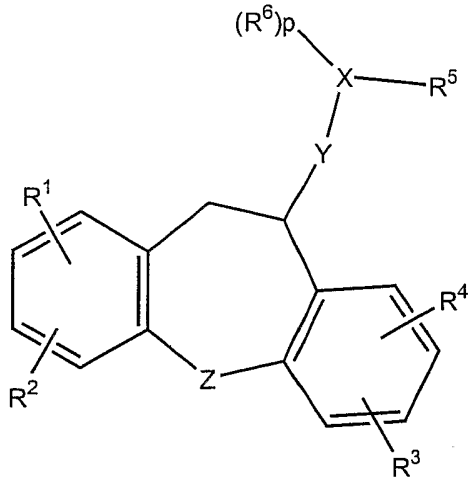
To examine the protection effect of CGP3466B and NBC-2 on pancreatic islets, *in vitro* studies were carried out with the mouse pancreatic ß-cell line MIN6. The MIN6 cell is a mouse ß-cell line that has conserved physiological functions like glucose-inducible insulin secretion. It has been reported that serum deprivation is an external stress that induces apoptosis in MIN6 cells. We therefore decided to examine the effect of CGP3466B and NBC-1 on MIN6 apoptosis induced by serum-withdrawal.

In the first assay, MIN6 cells were seeded in 96-well-plate in Dulbecco's modified Eagle medium (DMEM) with high glucose supplemented with 10% FCS (full medium) at 37°C, 5% CO_2. After 24 hours, cells were washed 3 times with DMEM only and subsequently maintined in serum-free medium in presence of 5, 50, 500, 5000 nM compounds or vehicle for 3 days at 37°C, 5% CO2. Medium and compounds were replaced every day. At the end of the experiment, apoptotic cells were detected with the cell-permeable and non-cytotoxic fluorochrome inhibitor of caspases (FLICA, Chemicon International, CA, USA) that binds covalently to the active caspases and produces a green fluorescence. All the cells were counterstained with 4', 6-Diamidino-2-phenylindole dihydrochloride hydrate (DAP1, Sigma, USA). To determine the rate of apoptosis, the number of FLICA-labelled cells was counted and expressed as a percentage of the total number of counted cells (more than 500 cells/well).

After 3 days of culture without serum, the staining of MIN6 cells with the fluorescent inhibitor of caspases FLICA showed that 30-40% of cells were apoptotic (Fig. 1A). The rate of apoptosis was significantly decreased when cells were treated with CGP3466B or NBC-2. NBC-2 was significantly more potent than CGP3466B and could significantly reduce apoptosis at 50nM (in contrast CGP3466B only displayed significant anti-apoptotic effects at 5000 nM).

Claims

1. A compound of the general formula I



(I)

wherein

R^1, R^2, R^3 and R^4 independently are hydrogen, halogen such as fluoro, chloro, bromo or iodo, hydroxy, cyano, nitro, trifluoromethyl, optionally substituted nitrogen (NR'R'' where R', R'' = optionally substituted C_{1-20}-alkyl or C_{2-20}-alkenyl or C_{4-20}-alkadienyl or C_{6-20}-alkatrienyl or aryl or heteroaryl), optionally substituted sulfur (SR' where R' = optionally substituted C_{1-20}-alkyl or C_{2-20}-alkenyl or C_{4-20}-alkadienyl or C_{6-20}-alkatrienyl or aryl or heteroaryl), optionally substituted C_{1-20}-alkyl or C_{2-20}-alkenyl or C_{4-20}-alkadienyl or C_{6-20}-alkatrienyl or C_{1-20}-alkoxy or aryl or heteroaryl or ester (-COOR'' where R''= optionally substituted C_{1-20}-alkyl or C_{2-20}-alkenyl or C_{4-20}-alkadienyl or C_{6-20}-alkatrienyl or aryl or heteroaryl) ;

R^5, R^6 are, each independently of the other and of R^1, R^2, R^3 and R^4, hydrogen, optionally substituted lower alkyl, optionally substituted lower alkenyl, optionally substituted lower alkynyl, optionally substituted alkoxy, optionally substituted aryl, optionally substituted heteroaryl, provided that not both of R^5 and R^6 are hydrogen;

X is $(CH_m)_n$, –O-, -N- or –S-;

m = 0, 1, 2;

n + 1,2,3;

p is 0 or 1;

Y is C=O, $(CH_m)_n$, –O-, -N(W)- or –S-; where W is hydrogen, OH, or C_{1-6} alkyl;

m = 0, 1, 2;

n = 1,2,3; and

Z is C, N(W), O, S, where W is independently defined above,

or a pharmaceutically acceptable salt thereof, optionally together with a pharmaceutically acceptable carrier, diluent or excipient.

2. A compound according to claim 1 wherein R^1, R^2, R^3 and R^4 independently are chosen from hydrogen, halogen, trifluoromethyl, hydroxy, cyano, nitro, optionally substituted nitrogen (NR'R'' where R', R'' = optionally substituted C_{1-6}-alkyl or C_{2-8}-alkenyl or C_{4-10}-alkadienyl or aryl or heteroaryl), optionally substituted sulfur (SR' where R' = optionally substituted C_{1-6}-alkyl or C_{2-8}-alkenyl or C_{4-10}-alkadienyl or aryl or heteroaryl), optionally substituted C_{1-6}-alkyl or C_{2-8}-alkenyl or C_{4-10}-alkadienyl or C_{1-6}-alkoxy or aryl or heteroaryl or ester (-COOR'' where R''= optionally substituted C_{1-6}-alkyl or C_{2-8}-alkenyl or C_{4-10}-alkadienyl or aryl or heteroaryl)and R^5, R^6 are each independently of the other and of R^1, R^2, R^3 and R^4, hydrogen, optionally substituted lower alkyl, optionally substituted lower alkenyl, optionally substituted lower alkynyl, optionally substituted aryl.

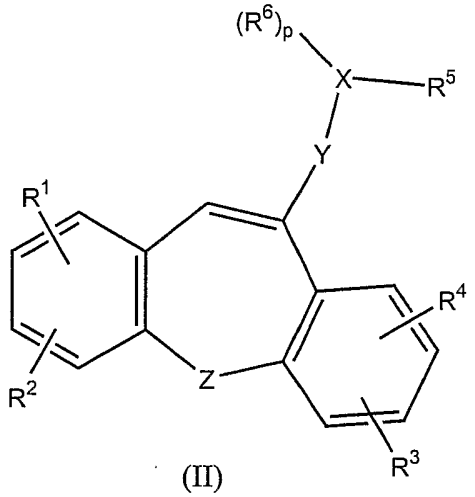
3. A compound according to claim 1 wherein R^1, R^2, R^3 and R^4 independently are chosen from hydrogen, halogen, trifluoromethyl, nitro, optionally substituted nitrogen (NR'R'' where R', R'' = optionally substituted C_{1-6}-alkyl or aryl), optionally substituted C_{1-6}-alkyl or optionally substituted C_{3-6}-alkynyl or optionally substituted C_{1-6}-alkoxy or aryl and R^5, R^6 are, each independently of the other and of R^1, R^2, R^3 and R^4, hydrogen, optionally substituted lower alkyl, optionally substituted C_{3-6}-alkynyl, optionally substituted aryl.

4. A compound according to claim 1 wherein X is N or O.

5. A compound according to claim 1 wherein Y is C=O or CH_2.

6. A compound according to claim 1 wherein Z is O or N(W).

7. A compound of the general formula II



(II)

wherein

R^1, R^2, R^3 and R^4 independently are hydrogen, halogen such as fluoro, chloro, bromo or iodo, hydroxy, cyano, nitro, trifluoromethyl, optionally substituted nitrogen (NR'R'' where R', R'' = optionally substituted C_{1-20}-alkyl or C_{2-20}-alkenyl or C_{4-20}-alkadienyl or C_{6-20}-alkatrienyl or aryl or heteroaryl), optionally substituted sulfur (SR' where R' = optionally substituted C_{1-20}-alkyl or C_{2-20}-alkenyl or C_{4-20}-alkadienyl or C_{6-20}-alkatrienyl or aryl or heteroaryl), optionally substituted C_{1-20}-alkyl or C_{2-20}-alkenyl or C_{4-20}-alkadienyl or C_{6-20}-alkatrienyl or C_{1-20}-alkoxy or aryl or heteroaryl or ester (-COOR'' where R''= optionally substituted C_{1-20}-alkyl or C_{2-20}-alkenyl or C_{4-20}-alkadienyl or C_{6-20}-alkatrienyl or aryl or heteroaryl) ;

R^5, R^6 are, each independently of the other and of R^1, R^2, R^3 and R^4, hydrogen optionally substituted lower alkyl, optionally substituted lower alkenyl, optionally substituted lower alkynyl, optionally substituted alkoxy, optionally substituted aryl, optionally substituted heteroaryl, provided that not both of R^5 and R^6 are hydrogen;

X is $(CH_m)_n$, –O-, -N- or –S-;

m = 0, 1, 2;

n = 1,2,3;

p = 0 or 1;

Y is C=O, $(CH_m)_n$, –O-, -N(W)- or –S-, provided that when X is N, Y is C=0, where W is hydrogen, OH, or C_{1-6} alkyl;

m = 0, 1, 2;

n = 1,2,3; and

Z is C, N(W), O or S, where W is independently as defined above,

or a pharmaceutically acceptable salt thereof, optionally together with a pharmaceutically acceptable carrier, diluent or excipient.

8. A compound according to claim 7 wherein R^1, R^2, R^3 and R^4 independently are chosen from hydrogen, halogen, trifluoromethyl, hydroxy, cyano, nitro, optionally substituted nitrogen (NR'R'' where R', R'' = optionally substituted C_{1-6}-alkyl or C_{2-8}-alkenyl or C_{4-10}-alkadienyl or aryl or heteroaryl), optionally substituted sulfur (SR' where R' = optionally substituted C_{1-6}-alkyl or C_{2-8}-alkenyl or C_{4-10}-alkadienyl or aryl or heteroaryl), optionally substituted C_{1-6}-alkyl or C_{2-8}-alkenyl or C_{4-10}-alkadienyl or C_{1-6}-alkoxy or aryl or heteroaryl or ester (-COOR'' where R''= optionally substituted C_{1-6}-alkyl or C_{2-8}-alkenyl or C_{4-10}-alkadienyl or aryl or heteroaryl)and R^5, R^6 are each independently of the other and of R^1, R^2, R^3 and R^4, hydrogen optionally substituted lower alkyl, optionally substituted lower alkenyl, optionally substituted lower alkynyl, optionally substituted aryl.

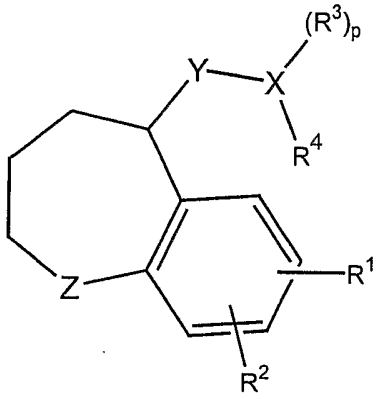
9. A compound according to claim 7 wherein R^1, R^2, R^3 and R^4 independently are chosen from hydrogen, halogen, trifluoromethyl, nitro, optionally substituted nitrogen (NR'R'' where R', R'' = optionally substituted C_{1-6}-alkyl or aryl), optionally substituted C_{1-6}-alkyl or optionally substituted C_{3-6}-alkynyl or optionally substituted C_{1-6}-alkoxy or aryl and R^5, R^6 are each independently of the other and of R^1, R^2, R^3 and R^4, hydrogen, optionally substituted lower alkyl, optionally substituted C_{3-6}-alkynyl, optionally substituted aryl.

10. A compound according to claim 7 wherein X is N or O.

11. A compound according to claim 7 wherein Y is C=O or CH_2.

12. A compound according to claim 7 wherein Z is O or N(W).

13. A compound of the general formula III



(III)

wherein

R^1, R^2 independently are hydrogen, halogen such as fluoro, chloro, bromo or iodo, hydroxy, cyano, nitro, trifluoromethyl, optionally substituted nitrogen (NR'R'' where R', R'' = optionally substituted C_{1-20}-alkyl or C_{2-20}-alkenyl or C_{4-20}-alkadienyl or C_{6-20}-alkatrienyl or aryl or heteroaryl), optionally substituted sulfur (SR' where R' = optionally substituted C_{1-20}-alkyl or C_{2-20}-alkenyl or C_{4-20}-alkadienyl or C_{6-20}-alkatrienyl or aryl or heteroaryl), optionally substituted C_{1-20}-alkyl or C_{2-20}-alkenyl or C_{4-20}-alkadienyl or C_{6-20}-alkatrienyl or C_{1-20}-alkoxy or aryl or heteroaryl or ester (-COOR'' where R''= optionally substituted C_{1-20}-alkyl or C_{2-20}-alkenyl or C_{4-20}-alkadienyl or C_{6-20}-alkatrienyl or aryl or heteroaryl) ;

R^3, R^4 are each independently of the other and of R^1, R^2, hydrogen, optionally substituted lower alkyl, optionally substituted lower alkenyl, optionally substituted lower alkynyl, optionally substituted alkoxy, optionally substituted aryl, optionally substituted heteroaryl, provided that not both of R^3 and R^4 are hydrogen;

X is $(CH_m)_n$, –O-, -N- or –S-;
m = 0, 1, 2;
n = 1,2,3;
p is 0 or 1;
Y is C=O, $(CH_m)_n$, –O-, -N(W)- or –S-, where W is hydrogen, OH, or C_{1-6} alkyl;
m = 0, 1, 2;
n = 1,2,3; and
Z is C, N(W), O or S, where W is as defined above,
or a pharmaceutically acceptable salt thereof together with a pharmaceutically acceptable carrier, diluent or excipient.

14. A compound according to claim 13 wherein R^1, R^2 independently are chosen from hydrogen, halogen, trifluoromethyl, hydroxy, cyano, nitro, optionally substituted nitrogen (NR'R'' where R', R'' = optionally substituted C_{1-6}-alkyl or C_{2-8}-alkenyl or C_{4-10}-alkadienyl or aryl or heteroaryl), optionally substituted sulfur (SR' where R' = optionally substituted C_{1-6}-alkyl or C_{2-8}-alkenyl or C_{4-10}-alkadienyl or aryl or heteroaryl), optionally substituted C_{1-6}-alkyl or C_{2-8}-alkenyl or C_{4-10}-alkadienyl or C_{1-6}-alkoxy or aryl or heteroaryl or ester (-COOR'' where R''= optionally substituted C_{1-6}-alkyl or C_{2-8}-alkenyl or C_{4-10}-alkadienyl or aryl or heteroaryl)and R^3, R^4 are each independently of the other and of R^1, R^2, hydrogen, optionally substituted lower alkyl, optionally substituted lower alkenyl, optionally substituted lower alkynyl, optionally substituted aryl.

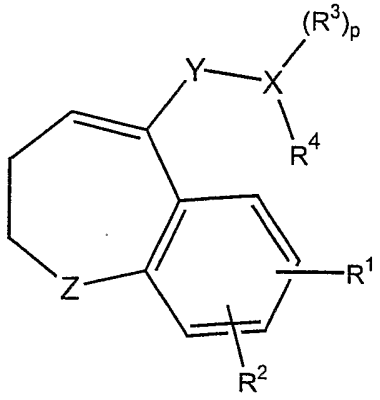
15. A compound according to claim 13 wherein R^1, R^2 independently are chosen from hydrogen, halogen, trifluoromethyl, nitro, optionally substituted nitrogen (NR'R'' where R', R'' = optionally substituted C_{1-6}-alkyl or aryl), optionally substituted C_{1-6}-alkyl or optionally substituted C_{3-6}-alkynyl or optionally substituted C_{1-6}-alkoxy or aryl and R^3, R^4 are each independently of the other and of R^1, R^2, hydrogen, optionally substituted lower alkyl, optionally substituted C_{3-6}-alkynyl, optionally substituted aryl.

16. A compound according to claim 13 wherein X is N or O.

17. A compound according to claim 13 wherein Y is C=O or CH_2.

18. A compound according to claim 13 wherein Z is O, N(W).

19. A compound of the general formula IV.



(IV)

wherein

R^1, R^2 independently are hydrogen, halogen such as fluoro, chloro, bromo or iodo, hydroxy, cyano, nitro, trifluoromethyl, optionally substituted nitrogen (NR'R'' where R', R'' = optionally substituted C_{1-20}-alkyl or C_{2-20}-alkenyl or C_{4-20}-alkadienyl or C_{6-20}-alkatrienyl or aryl or heteroaryl), optionally substituted sulfur (SR' where R' = optionally substituted C_{1-20}-alkyl or C_{2-20}-alkenyl or C_{4-20}-alkadienyl or C_{6-20}-alkatrienyl or aryl or heteroaryl), optionally substituted C_{1-20}-alkyl or C_{2-20}-alkenyl or C_{4-20}-alkadienyl or C_{6-20}-alkatrienyl or C_{1-20}-alkoxy or aryl or heteroaryl or ester (-COOR'' where R''= optionally substituted C_{1-20}-alkyl or C_{2-20}-alkenyl or C_{4-20}-alkadienyl or C_{6-20}-alkatrienyl or aryl or heteroaryl) ;

R^3, R^4 are, each independently of the other and of R^1, R^2, hydrogen, optionally substituted lower alkyl, optionally substituted lower alkenyl, optionally substituted lower alkynyl, optionally substituted alkoxy, optionally substituted aryl, optionally substituted heteroaryl, provided that not both of R^3 and R^4 are hydrogen;

X is $(CH_m)_n$, –O-, -N- or –S-;

m = 0, 1, 2;

n = 1,2,3;

p is 0 or 1;

Y is C=O, $(CH_m)_n$, –O-, -N(W)- or –S-, where W is hydrogen, OH, or C_{1-6} alkyl;

m = 0, 1, 2;

n = 1,2,3; and

Z is C, N(W), O, S, where W is independently as defined above;

or a pharmaceutically acceptable salt thereof, optionally together with a pharmaceutically acceptable carrier, diluent or excipient.

20. A compound according to claim 19 wherein R^1, R^2 independently are chosen from hydrogen, halogen, trifluoromethyl, hydroxy, cyano, nitro, optionally substituted nitrogen (NR'R'' where R', R'' = optionally substituted C_{1-6}-alkyl or C_{2-8}-alkenyl or C_{4-10}-alkadienyl or aryl or heteroaryl), optionally substituted sulfur (SR' where R' = optionally substituted C_{1-6}-alkyl or C_{2-8}-alkenyl or C_{4-10}-alkadienyl or aryl or heteroaryl), optionally substituted C_{1-6}-alkyl or C_{2-8}-alkenyl or C_{4-10}-alkadienyl or C_{1-6}-alkoxy or aryl or heteroaryl or ester (-COOR'' where R''= optionally substituted C_{1-6}-alkyl or C_{2-8}-alkenyl or C_{4-10}-alkadienyl or aryl or heteroaryl)and R^3, R^4 are each independently of the other and of R^1, R^2, hydrogen, optionally substituted lower alkyl, optionally substituted lower alkenyl, optionally substituted lower alkynyl, optionally substituted aryl.

21. A compound according to claim 19 wherein R^1, R^2 independently are chosen from hydrogen, halogen, trifluoromethyl, nitro, optionally substituted nitrogen (NR'R'' where R', R'' = optionally substituted C_{1-6}-alkyl or aryl), optionally substituted C_{1-6}-alkyl or optionally substituted C_{3-6}-alkynyl or optionally substituted C_{1-6}-alkoxy or aryl and R^3, R^4 are, each independently of the other and of R^1, R^2, hydrogen, optionally substituted lower alkyl, optionally substituted C_{3-6}-alkynyl, optionally substituted aryl.

22. A compound according to claim 19 wherein X is N or O.

23. A compound according to claim 19 wherein Y is C=O or CH_2.

24. A compound according to claim 19 wherein Z is O or N(W).

25. A compound according to claim 1 wherein X is S.

26. A compound according to claim 1 wherein X is N(W).

27. A compound according to claim 1 wherein X is O.

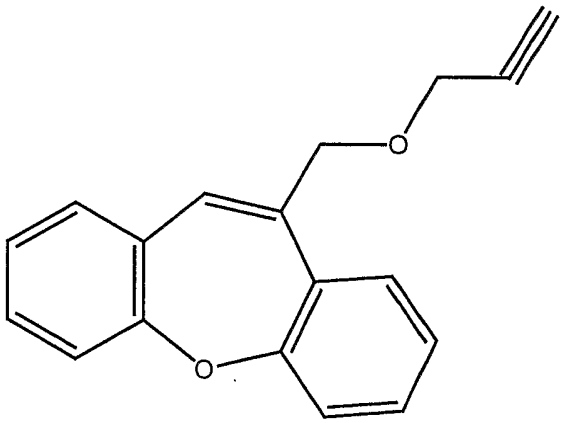
28. A compound according to claim 1 wherein Y is S.

29. A compound according to claim 1 wherein Y is N(W).

30. A compound according to claim 1 wherein Y is O.

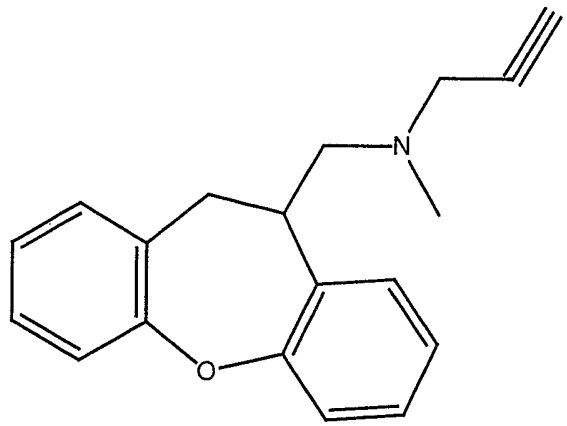
31. A compound according to claim 1 wherein Y is CH_2.

32. A compound of the formula V



(V)

33. A compound of the formula VII



(VI)

34. A compound according to any preceding claim for the treatment of a disease.

35. A compound according to any one of preceding claims for the treatment diabetes (Type I and/or Type II diabetes).

36. A use of a compound according to any preceding claim as a regulator of IAMT or GAPDH activity for the preparation of a composition for the prevention, treatment or alleviation of an autoimmune response and/ or disease in a mammal.

37. A pharmaceutical composition comprising a compound as defined in any one of claims 1 to 33, formulated together with at least one pharmaceutically acceptable carrier and/or excipient for pharmaceutical administration.

wherein:

R^1, R^2, (all formulae) R^3 and R^4 (Formulae I and II) independently are hydrogen, halogen such as fluoro, chloro, bromo or iodo, hydroxy, cyano, nitro, trifluoromethyl, optionally substituted nitrogen (NR'R'' where R', R'' = optionally substituted C_{1-20}-alkyl or C_{2-20}-alkenyl or C_{4-20}-alkadienyl or C_{6-20}-alkatrienyl or aryl or heteroaryl), optionally substituted sulfur (SR' where R' = optionally substituted C_{1-20}-alkyl or C_{2-20}-alkenyl or C_{4-20}-alkadienyl or C_{6-20}-alkatrienyl or aryl or heteroaryl), optionally substituted C_{1-20}-alkyl or C_{2-20}-alkenyl or C_{4-20}-alkadienyl or C_{6-20}-alkatrienyl or C_{1-20}-alkoxy or aryl or heteroaryl or ester (-COOR'' where R''= optionally substituted C_{1-20}-alkyl or C_{2-20}-alkenyl or C_{4-20}-alkadienyl or C_{6-20}-alkatrienyl or aryl or heteroaryl) ;

R^3 and R^4 (Formulae III and IV) R^5, R^6 (Formulae I and II) are, each independently of the other and of R^1, R^2, R^3 and R^4 , hydrogen, optionally substituted lower alkyl, optionally substituted lower alkenyl, optionally substituted lower alkynyl, optionally substituted alkoxy, optionally substituted aryl, optionally substituted heteroaryl, provided that not both of R^3 and R^4 (Formulae III and IV) or R^5 and R^6 (Formulae I and II) are hydrogen;

X is $(CH_m)_n$, –O-, -N- or –S-;

m = 0, 1, 2;

n = 1,2,3;

p is 0 or 1;

Y is C=O, $(CH_m)_n$, –O-, -N(W)- or –S-; where W is hydrogen, OH, or C_{1-6} alkyl;

m = 0, 1, 2;

n = 1,2,3; and

Z is C, N(W), O, S, where W is independently as defined above,

or a pharmaceutically acceptable salt thereof, optionally together with a pharmaceutically acceptable carrier, diluent or excipient.

Figure 1

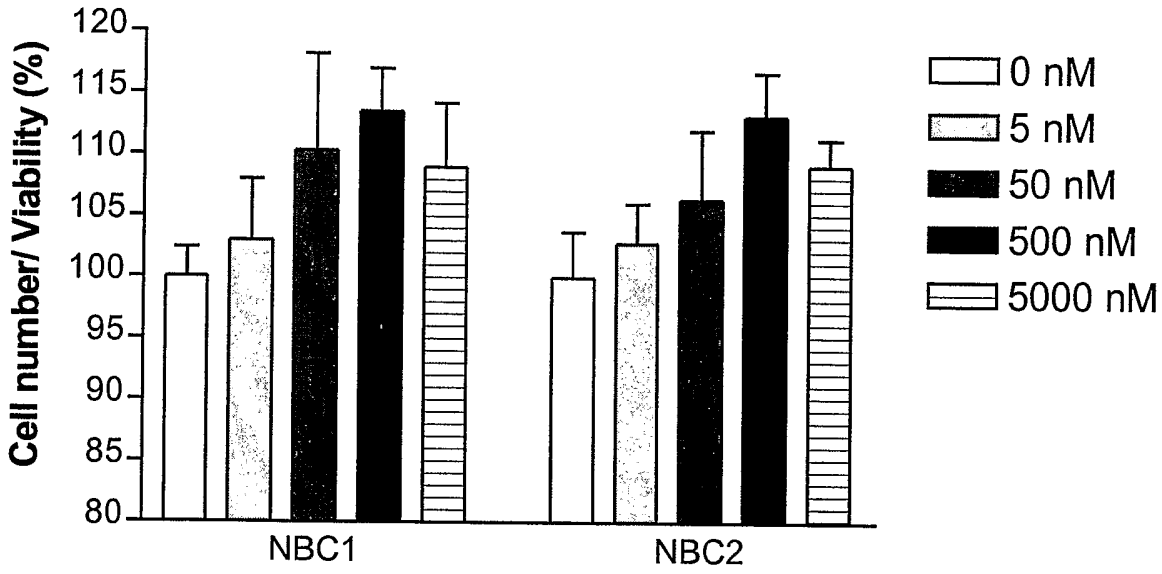
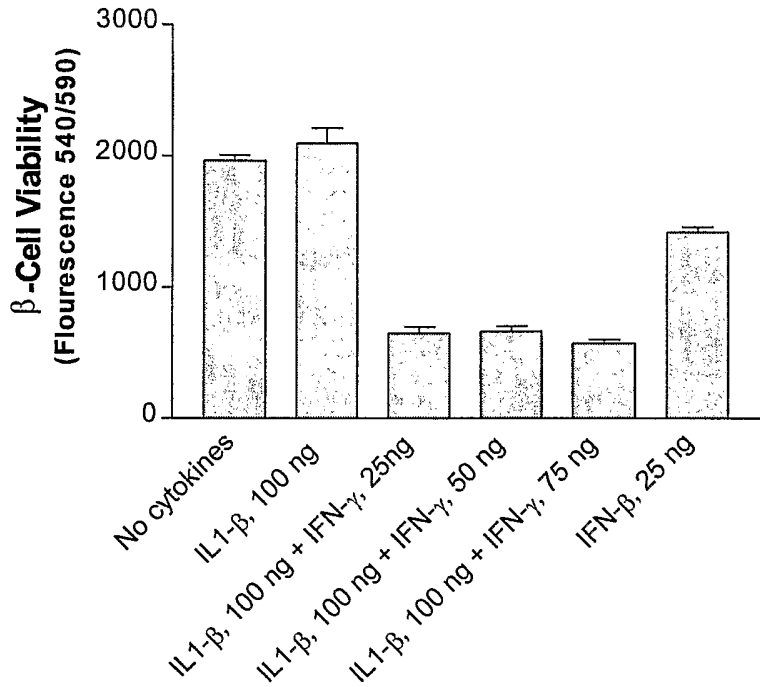

Cell number/ Viability (%)
120
115
110
105
100
95
90
85
80
NBC1
NBC2
0 nM
5 nM
50 nM
500 nM
5000 nM

Figure 2


β-Cell Viability
(Flourescence 540/590)
3000
2000
1000
0
No cytokines
IL1-β, 100 ng
IL1-β, 100 ng + IFN-γ, 25ng
IL1-β, 100 ng + IFN-γ, 50 ng
IL1-β, 100 ng + IFN-γ, 75 ng
IFN-β, 25 ng

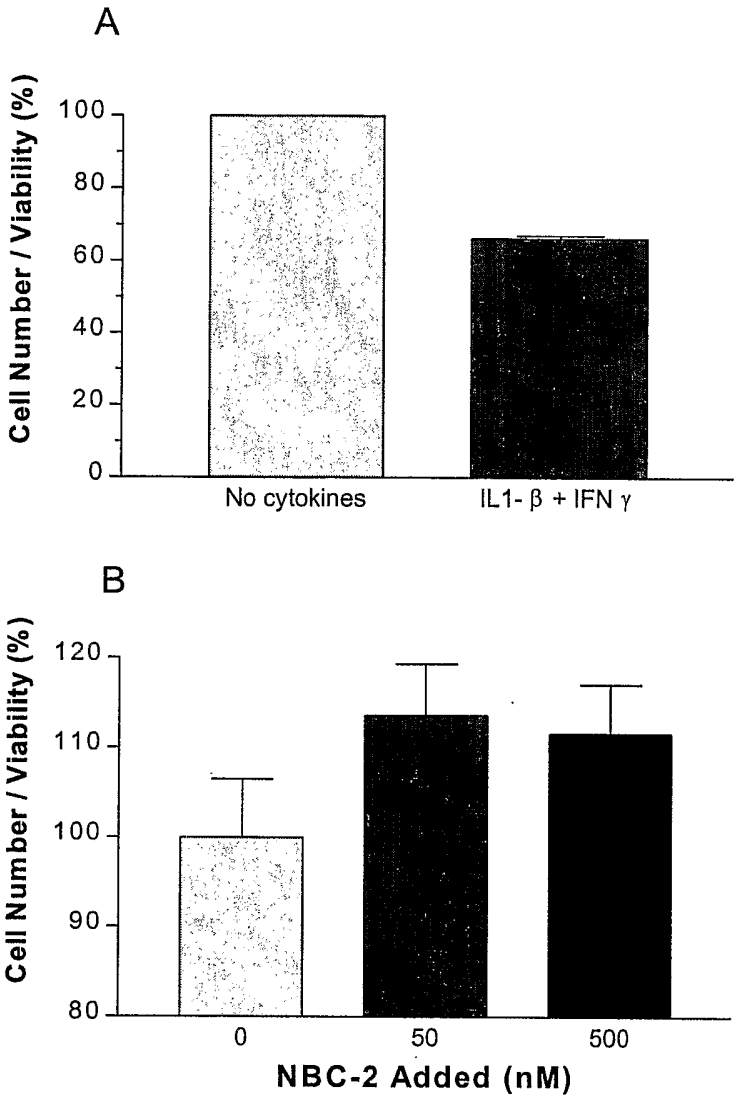
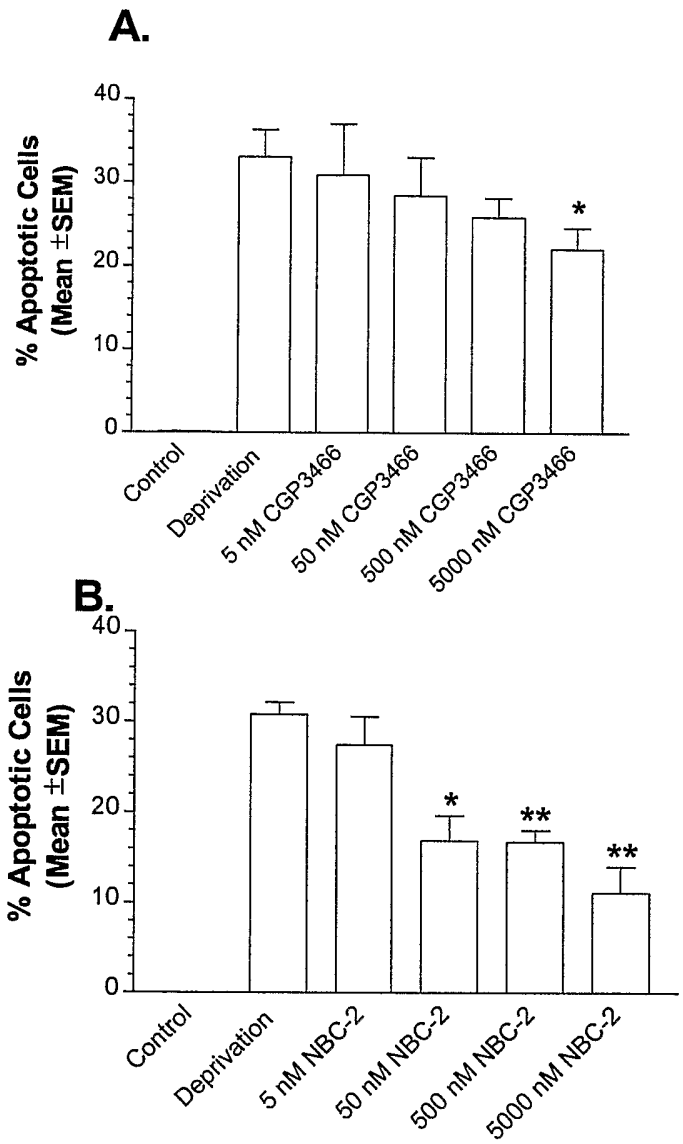

A
Cell Number / Viability (%)
100
80
60
40
20
0
No cytokines
IL1- β + IFN γ
B
Cell Number / Viability (%)
120
110
100
90
80
0
50
500
NBC-2 Added (nM)

Figure 3

Figure 4


A.
% Apoptotic Cells (Mean ±SEM)
40
30
20
10
0
*
Control
Deprivation
5 nM CGP3466
50 nM CGP3466
500 nM CGP3466
5000 nM CGP3466
B.
% Apoptotic Cells (Mean ±SEM)
40
30
20
10
0
*
**
**
Control
Deprivation
5 nM NBC-2
50 nM NBC-2
500 nM NBC-2
5000 nM NBC-2