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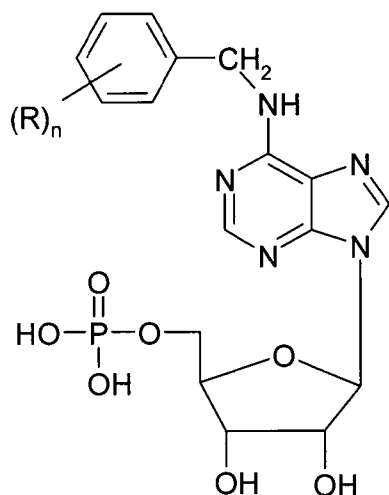
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(54) Title: SUBSTITUTION DERIVATIVES OF N⁶-BENZYLADENOSINE-5'-MONOPHOSPHATE, METHODS OF PREPARATION THEREOF, USE THEREOF AS MEDICAMENTS, AND THERAPEUTIC PREPARATIONS CONTAINING THESE COMPOUNDS



I

(57) Abstract: The invention relates to substitution derivatives of N⁶-benzyladenosine-5'-monophosphate of the general formula (I), wherein (R)_n represents 1 to 4 substituents (n is in the range 1 - 4), which can be the same or different, and R is selected from the group comprising C₁ to C₈ alkyl, C₁ to C₈ alkoxy, amino, halogen, hydroxy, mercapto and nitro groups, and the pharmaceutically acceptable salts thereof. This invention also relates to methods of their preparation, their use as medicaments and in other applications, and a therapeutic composition containing these derivatives.

Fig. 7



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Substitution derivatives of N⁶-benzyladenosine-5'-monophosphate, methods of preparation thereof, use thereof as medicaments, and therapeutic preparations containing these compounds

5 Field of Art

The invention relates to novel substitution derivatives of N⁶-benzyladenosine-5'-monophosphate, having anticancer, antimitotic, immunosuppressive and proapoptotic properties for animal cells, including human cells. This invention also relates to
10 methods of their preparation and their use as drugs; therapeutic compositions, which contain these derivatives as active compounds, and the use of these derivatives for the preparation of drugs and in pharmaceutical biotechnologies.

Background Art

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Cytokinins are important plant hormones that are defined by their ability to promote cell division in plant tissue culture in the presence of auxins (Skoog *et al.*, Science 148, 532-533, 1965). Cytokinins so far found in plants are adenine derivatives substituted at the N⁶-position with either an isoprenoid or an aromatic sidechain.
20 Isoprenoid *trans*-zeatin (tZ) is the most abundant naturally occurring cytokinin. The abundance of other isoprenoid cytokinins (N⁶-isopentenyladenine, iP, *cis*-zeatin, cZ) and derivatives with a saturated sidechain, such as dihydrozeatin (DHZ), varies between plant species. While isoprenoid cytokinins are ubiquitous in plants, aromatic cytokinins (represented by N⁶-benzyladenine, BA, and its hydroxylated derivatives,
25 the topolins) have only been identified, as yet, in a limited group of plant taxa (Horgan *et al.*, Phytochemistry 14, 1005-1008, 1975; Strnad, Physiol. Plant. 101, 674-688, 1997; Strnad *et al.*, Plant Physiol. 99, 74-80, 1992). The most abundant appears to be *ortho*-topolin riboside, which is present at micro-molar concentrations in poplar leaves after daybreak (Hewett *et al.*, Planta 114, 119-129, 1973). Both
30 families of cytokinins occur in several forms: free bases, ribosides, riboside-5'-phosphates, 3-, 7-, 9- and O-glucosides, and amino acid conjugates.

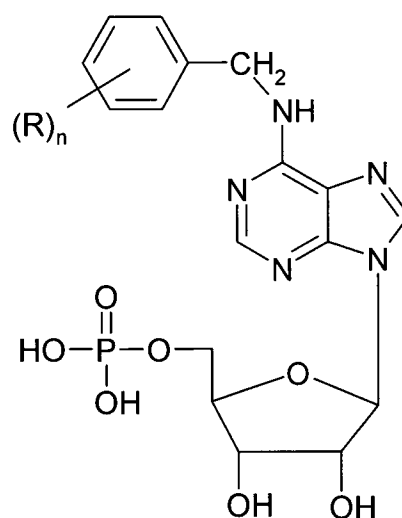
Knowledge that cytokinins play key roles in the regulation of plant growth and differentiation led to the postulation that they could also affect growth and differentiation in animals, and have a potential utility for treating human diseases that involve dysfunctional cell proliferation and/or differentiation. The ability of cytokinin bases to induce or promote the differentiation of human cells has been demonstrated in both keratinocytes (Berge et al., Ann. N. Y. Acad. Sci. 1067, 332-336, 2006) and several leukaemia cell lines, including HL-60 and K-562 (Ishii et al., Biochim. Biophys. Acta. 1643, 11-24, 2003). However, while cytokinin bases induce differentiation at relatively high concentrations (25-100 μ M), their ribosides cause rapid apoptosis of leukemia cell lines at low micromolar concentrations (Mlejnek, J. Cell Biochem. 83, 678-689, 2001). Cell death in HL-60 is preceded by depletion of adenosine triphosphate, activation of caspases and mitochondrial depolarization (Mlejnek, J. Cell Biochem. 83, 678-689, 2001; Ishii et al., Biochim. Biophys. Acta. 1643, 11-24, 2002). The caspase inhibition shifts the activity of N⁶-isopentenyladenosine (iPR) in HL-60 to growth inhibitory and differentiating activity (Ishii et al., Biochim. Biophys. Acta. 1643, 11-24, 2002). It was recently shown that kinetin riboside (KR) could be a suitable drug candidate against multiple myeloma (Tiedemann et al., J. Clin. Invest. 118, 1750-1764, 2008). In several multiple myeloma models, KR induced rapid suppression of cyclin D1 and D2 transcription followed by cell-cycle arrest and tumour cell-selective apoptosis (Tiedemann et al., J. Clin. Invest. 118, 1750-1764, 2008). Cytotoxic effects of iPR, KR and N⁶-benzyladenosine (BAR) against human cell lines derived from solid tumours were reported by Cabelo et al. (Int. J. Cancer 120, 2744-2748, 2008), Choi et al. (Cancer Lett. 261, 37-45, 2008), Laezza et al. (Int. J. Cancer. 124, 1322-1329, 2009), Meisel et al. (FEBS Lett. 433, 265-268, 1998) and Spinola et al. (Int. J. Cancer 120, 2744-2748, 2007). Depending on the cell line and cytokinin used, the treatment resulted in cell cycle block (either G1 or G2/M phase) and/or apoptosis. The *in vivo* anticancer activity of iPR, KR and BAR was also demonstrated in several animal and xenograft models of cancer (Choi et al., Cancer Lett. 261, 37-45, 2008; Laezza et al., FASEB J. 20, 412-418, 2006; Tiedemann et al., J. Clin. Invest. 118, 1750-1764, 2008). iPR and BAR also showed a promising activity against diverse malignancies in a small clinical trial (Mittelman et al., Ann. N. Y. Acad. Sci. 255, 225-234, 1975).

Micromolar concentrations of both cytokinins ribosides and cytokinin bases are also able to induce cell death with features of apoptosis (activation of caspase-like proteases and fragmentation of DNA) in plant cell cultures (Mlejnek and Procházka, *Planta* 215, 158-166, 2002). The cell death is also preceded by depletion of adenosine triphosphate and reactive oxygen species production. The cytotoxic concentrations are higher than endogenous cytokinin levels in plant tissue but yet in the concentration range used in plant biotests (Carimi et al., *Planta* 216, 413-421, 2003; Mlejnek et al., *Plant. Cell. Environ.* 26, 1723-1735, 2003; *Plant. Sci.* 168, 389-395, 2005). Cytotoxic activity of natural cytokinins and their analogues (Doležal et al., *Bioorg. Med. Chem.* 14, 875-884, 2006; *Bioorg. Med. Chem.* 15, 3737-3747, 2007 ; CZ 294538) in mammalian and human experimental systems was demonstrated repeatedly.

It is an object of this invention to provide new anticancer and proapoptotic heterocyclic compounds derived from phenyl-substituted N⁶-benzyladenosine-5'-monophosphate having improved selectivity and bioavailability than their adenosine analogues known heretofore. Hence they can be used for development of new generation of anticancer, antimitotic and proapoptotic drugs, particularly as anticancer drugs, as well as for other medical applications.

Disclosure of the Invention

Object of this invention are substitution derivatives of N⁶-benzyladenosine-5'-monophosphate of the general formula I



I

wherein

5

(R)_n represents 1 to 4 substituents R (n is within the range of 1-4), which can be the same or different,

and R is selected from the group comprising C₁ to C₈ alkyl, C₁ to C₈ alkoxy, amino, halogen, hydroxy, mercapto and nitro group,

10

and pharmaceutically acceptable salts thereof.

The pharmaceutically acceptable salts of compounds of the general formula I comprise salts with alkali metals, ammonium or amines, or addition salts with acids.

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The pharmaceutically acceptable salts of compounds of the general formula I are selected preferably from the group comprising sodium and ammonium salts.

As used herein, the generic substituent groups have meanings defined in this legend, wherein:

20

C₁ to C₈ alkyl denotes branched or unbranched alkyl chain containing 1 to 8 carbon atoms, preferably selected from the group comprising methyl, ethyl, propyl, isopropyl, butyl, isobutyl, pentyl, isopentyl, hexyl, and isohexyl,

C₁ to C₈ alkoxy denotes the group -O-R_a, wherein R_a is C₁ to C₈ alkyl or C₃ to C₈ cycloalkyl,

amino denotes the group -NH₂,

halogen is selected from the group comprising fluorine, bromine, chlorine and iodine atom,

hydroxy denotes the group -OH,

mercapto denotes the group -SH,

nitro denotes the group -NO₂.

- 10 In the preferred embodiment, the derivatives of the general formula I comprise compounds wherein one of R is hydroxyl in the position 2 of the phenyl ring.

It is a further object of this invention to provide substitution derivatives of N⁶-benzyladenosine-5'-monophosphate of the general formula I for use as medicaments.

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It is an object of this invention to provide substitution derivatives of N⁶-benzyladenosine-5'-monophosphate of the general formula I for use as inhibitors of protein kinases, preferably human kinase EPHB2.

- 20 It is another object of this invention to provide substitution derivatives of N⁶-benzyladenosine-5'-monophosphate of the general formula I for use for inhibiting cell proliferation and/or inducing apoptosis.

- 25 It is a further object of this invention to provide substitution derivatives of N⁶-benzyladenosine-5'-monophosphate of the general formula I for use as medicaments for the treatment of disorders which involve aberrant cell proliferation and/or aberrant cell death, such as cancer, leukemia, restenosis, rheumatoid arthritis, lupus (psoriasis), type I diabetes, multiple sclerosis, Alzheimer's disease, parasitoses caused by animals, fungi and protists, polycystic kidney disease, graft rejection (host versus graft disease), graft versus host disease, and gout.
- 30

Another object of the invention is also the use of the substitution derivatives of N⁶-benzyladenosine-5'-monophosphate of the general formula I in the manufacture of a medicament for the treatment of disorders which involve aberrant cell proliferation and/or aberrant cell death, such as cancer, leukemia, restenosis, rheumatoid arthritis, lupus, type I diabetes, multiple sclerosis, Alzheimer's disease, parasitoses caused by animals, fungi and protists, polycystic kidney disease, graft rejection (host versus graft disease), graft versus host disease, and gout.

It is likely that inhibition by the compounds of the invention of the catalytic activity of protein kinases is mediated by interaction of the compounds at the ATP-binding site of the enzyme. Such compounds are particularly desirable for reducing excessive cell growth, since they allow inhibition of the kinase activity regardless of the cause underlying the excessive kinase activity leading to excessive cell proliferation. Thus, the compounds of the invention are active in situations in which the excessive kinase activity results from the kinase being a mutated hyperactive form of the kinase and situations in which the kinase is present at excessive levels. Such compounds can also block excessive kinase activity in situations in which the cyclin regulating the kinase is present at excessive levels or its binding to the kinase is enhanced. Furthermore, compounds which block kinase activity by interacting with the ATP binding site of the enzyme are also useful for inhibiting kinase activity in the situations in which a natural protein inhibitor of the kinase complexes is mutated.

Studies carried out on the derivatives of the invention have demonstrated, in addition, a strong effect on apoptosis of many cancer cell lines. It has been seen that apoptosis can be induced at stage G₁ or G₂ and following the damage of the DNA, some cells stop at stage G₁ and the p53-dependent apoptotic pathway is then induced. In other situations, it seems that cells stop at G₂/M stage in response to the damage caused to the DNA, and the activation of a p53-independent apoptotic path is observed. This path has proved particularly significant in the therapy of tumours in which lack of active p53 is observed. By the application of the derivatives of the invention, the p53-independent apoptosis will be stimulated in cells, which have stopped at stage G₂ through the damage to the DNA using agents such as mitoxantrone or cis-platinum.

The inhibitors of this invention can thus increase the therapeutic potential of the anti-tumor agents currently used.

This invention also concerns substitution derivatives of N⁶-benzyladenosine-5'-monophosphate of the general formula I, for use as medicaments for suppressing immunostimulation, e.g. arthritis, or in suppression of transplant rejection in mammals.

This invention also concerns substitution derivatives of N⁶-benzyladenosine-5'-monophosphate of the general formula I, in the manufacture of a medicament for suppressing immunostimulation, e.g. in the treatment of arthritis or in suppression of transplant organ rejection in mammals.

A further object of this invention is the use of substitution derivatives of N⁶-benzyladenosine-5'-monophosphate of the general formula I in tissue cultures for regulation of tissue proliferation and morphogenesis.

A further object of this invention is the use of substitution derivatives of N⁶-benzyladenosine-5'-monophosphate of the general formula I in preparation of affinity absorption matrices, immobilised enzymes for process control, immunoassay reagents, diagnostic samples, as well as compounds and oligonucleotides labeled by ¹⁴C, ³H, avidin or biotin.

This invention also concerns the use of substitution derivatives of N⁶-benzyladenosine-5'-monophosphate of the general formula I for *in vitro* cloning of plant and animal, preferably mammalian (except for the human), embryonic cells and embryos (esp. oocytes).

This invention further concerns the use of substitution derivatives of N⁶-benzyladenosine-5'-monophosphate of the general formula I as growth regulators of plants, microorganisms, yeast, fungi and animals, preferably mammals except for the human.

The invention also includes a therapeutic composition, which comprises at least one substitution derivative of N⁶-benzyladenosine-5'-monophosphate of the general formula I, or its pharmaceutically acceptable salt, and at least one pharmaceutically acceptable carrier. The therapeutic compositions can be, e.g., pharmaceutical compositions, growth regulating compositions, etc.

The following derivatives of formula I are particularly preferred, namely:

	6-(2-fluorobenzylamino)purine	riboside-5'-monophosphate,	6-(3-
10	fluorobenzylamino)purine	riboside-5'-monophosphate,	6-(4-
	fluorobenzylamino)purine	riboside-5'-monophosphate,	6-(2-
	bromobenzylamino)purine	riboside-5'-monophosphate,	6-(3-
	bromoobenzylamino)purine	riboside-5'-monophosphate,	6-(4-
	bromobenzylamino)purine	riboside-5'-monophosphate,	6-(2-
15	riboside-5'-monophosphate,	6-(3-iodobenzylamino)purine	riboside-5'-
	monophosphate,	6-(4-iodobenzylamino)purine	riboside-5'-monophosphate,
	chlorobenzylamino)purine	riboside-5'-monophosphate,	6-(3-
	chlorobenzylamino)purine	riboside-5'-monophosphate,	6-(4-
	chlorobenzylamino)purine	riboside-5'-monophosphate,	6-(2-
20	nitrobenzylamino)purine	riboside-5'-monophosphate,	6-(3-nitrobenzylamino)purine
	riboside-5'-monophosphate,	6-(4-nitrobenzylamino)purine	riboside-5'-
	monophosphate,	6-(5-nitro-2-methylbenzylamino)purine	riboside-5'-monophosphate,
	6-(2-methylbenzylamino)purine	riboside-5'-monophosphate,	6-(3-
	methylbenzylamino)purine	riboside-5'-monophosphate,	6-(4-
25	methylbenzylamino)purine	riboside-5'-monophosphate,	6-(2-
	methoxybenzylamino)purine	riboside-5'-monophosphate,	6-(3-
	methoxybenzylamino)purine	riboside-5'-monophosphate,	6-(4-
	methoxybenzylamino)purine	riboside-5'-monophosphate,	6-(2-
	hydroxybenzylamino)purine	riboside-5'-monophosphate,	6-(3-
30	hydroxybenzylamino)purine	riboside-5'-monophosphate,	6-(4-
	hydroxybenzylamino)purine	riboside-5'-monophosphate,	6-(2-
	ethoxybenzylamino)purine	riboside-5'-monophosphate,	6-(3-

	ethoxybenzylamino)purine	riboside-5'-monophosphate,	6-(4-
	ethoxybenzylamino)purine	riboside-5'-monophosphate,	6-(4-
	ethylbenzylamino)purine	riboside-5'-monophosphate,	6-(4-pentylbenzylamino)purine
	riboside-5'-monophosphate,	6-(4-pentyloxybenzylamino)purine	riboside-5'-
5	monophosphate, 6-(4-octylbenzylamino)purine	riboside-5'-monophosphate,	6-(2,5-
	diaminobenzylamino)purine	riboside-5'-monophosphate,	6-(3,5-
	dibromobenzylamino)purine	riboside-5'-monophosphate,	6-(3,5-dibromo-4-
	methoxybenzylamino)purine	riboside-5'-monophosphate,	6-(2,3-
	dichlorobenzylamino)purine	riboside-5'-monophosphate,	6-(2,4-
10	dichlorobenzylamino)purine	riboside-5'-monophosphate,	6-(2,5-
	dichlorobenzylamino)purine	riboside-5'-monophosphate,	6-(2,6-
	dichlorobenzylamino)purine	riboside-5'-monophosphate,	6-(3,4-
	dichlorobenzylamino)purine	riboside-5'-monophosphate,	6-(3,5-
	dichlorobenzylamino)purine	riboside-5'-monophosphate,	6-(2,3,4,5-
15	tetrafluorobenzylamino)purine	riboside-5'-monophosphate,	6-(2-chloro-3,6-
	difluorobenzylamino)purine	riboside-5'-monophosphate,	6-(5-chloro-2-
	fluorobenzylamino)purine	riboside-5'-monophosphate,	6-(2,3,4-
	trifluorobenzylamino)purine	riboside-5'-monophosphate,	6-(2,3,5-
	trifluorobenzylamino)purine	riboside-5'-monophosphate,	6-(2,4,5-
20	trifluorobenzylamino)purine	riboside-5'-monophosphate,	6-(3,4,5-
	trifluorobenzylamino)purine	riboside-5'-monophosphate,	6-(2,3,6-
	trifluorobenzylamino)purine	riboside-5'-monophosphate,	6-(3-chloro-2,6-
	difluorobenzylamino)purine	riboside-5'-monophosphate,	6-(2-chloro-6-
	fluorobenzylamino)purine	riboside-5'-monophosphate,	6-(2,6-
25	difluorobenzylamino)purine	riboside-5'-monophosphate,	6-(2,4-
	difluorobenzylamino)purine	riboside-5'-monophosphate,	6-(3,4-
	difluorobenzylamino)purine	riboside-5'-monophosphate,	6-(2,5-
	difluorobenzylamino)purine	riboside-5'-monophosphate,	6-(3,5-
	difluorobenzylamino)purine	riboside-5'-monophosphate,	6-(2-chloro-6-fluoro-3-
30	methylbenzylamino)purine	purine riboside-5'-monophosphate,	6-(6-chloro-2-fluoro-
	3-methylbenzylamino)purine	riboside-5'-monophosphate,	6-(2,3-difluoro-4-
	methylbenzylamino)purine	riboside-5'-monophosphate,	6-(2,6-difluoro-3-

	methylbenzylamino)purine	riboside-5'-monophosphate,	6-(3-chloro-2,6-
	difluorobenzylamino)purine	riboside-5'-monophosphate,	6-(3-fluoro-4-methyl
	benzylamino)purine	riboside-5'-monophosphate,	6-(4-fluoro-3-
	methylbenzylamino)purine	riboside-5'-monophosphate,	6-(5-fluoro-2-
5	methylbenzylamino)purine	riboside-5'-monophosphate,	6-(2-chloro-3,6-
	difluorobenzylamino)purine	riboside-5'-monophosphate,	6-(2-chloro-4-
	fluorobenzylamino)purine	riboside-5'-monophosphate,	6-(3,4-
	dihydroxybenzylamino)purine	riboside-5'-monophosphate,	6-(3,5-
	dihydroxybenzylamino)purine	riboside-5'-monophosphate,	6-(3,4-
10	dihydroxybenzylamino)purine	riboside-5'-monophosphate,	6-(2,4-
	dihydroxybenzylamino)purine	riboside-5'-monophosphate,	6-(2,5-
	dihydroxybenzylamino)purine	riboside-5'-monophosphate,	6-(2,6-
	dihydroxybenzylamino)purine	riboside-5'-monophosphate,	6-(3,4-
	dimethoxybenzylamino)purine	riboside-5'-monophosphate,	6-(3,5-
15	dimethoxybenzylamino)purine	riboside-5'-monophosphate,	6-(2,3-
	dimethoxybenzylamino)purine	riboside-5'-monophosphate,	6-(2,4-
	dimethoxybenzylamino)purine	riboside-5'-monophosphate,	6-(2,5-
	dimethoxybenzylamino)purine	riboside-5'-monophosphate,	6-(2,6-
	dimethoxybenzylamino)purine	riboside-5'-monophosphate,	6-(2-hydroxy-3-
20	methoxybenzylamino)purine	riboside-5'-monophosphate,	6-(2-hydroxy-4-
	methoxybenzylamino)purine	riboside-5'-monophosphate,	6-(2-hydroxy-5-
	methoxybenzylamino)purine	riboside-5'-monophosphate,	6-(2-hydroxy-6-
	methoxybenzylamino)purine	riboside-5'-monophosphate,	6-(3-hydroxy-2-
	methoxybenzylamino)purine	riboside-5'-monophosphate,	6-(3-hydroxy-4-
25	methoxybenzylamino)purine	riboside-5'-monophosphate,	6-(3-hydroxy-5-
	methoxybenzylamino)purine	riboside-5'-monophosphate,	6-(3-hydroxy-6-
	methoxybenzylamino)purine	riboside-5'-monophosphate,	6-(4-hydroxy-2-
	methoxybenzylamino)purine	riboside-5'-monophosphate,	6-(4-hydroxy-3-
	methoxybenzylamino)purine	riboside-5'-monophosphate,	6-(4-hydroxy-5-
30	methoxybenzylamino)purine	riboside-5'-monophosphate,	6-(4-hydroxy-6-
	methoxybenzylamino)purine	riboside-5'-monophosphate,	6-(2-hydroxy-3,4-
	dimethoxybenzylamino)purine	riboside-5'-monophosphate,	6-(2-hydroxy-3,5-

	dimethoxybenzylamino)purine	riboside-5'-monophosphate,	6-(2-hydroxy-3,6-
	dimethoxybenzylamino)purine	riboside-5'-monophosphate,	6-(2-hydroxy-4,5-
	dimethoxybenzylamino)purine	riboside-5'-monophosphate,	6-(2-hydroxy-4,6-
	dimethoxybenzylamino)purine	riboside-5'-monophosphate,	6-(2-hydroxy-5,6-
5	dimethoxybenzylamino)purine	riboside-5'-monophosphate,	6-(3-hydroxy-4,5-
	dimethoxybenzylamino)purine	riboside-5'-monophosphate,	6-(3-hydroxy-4,6-
	dimethoxybenzylamino)purine	riboside-5'-monophosphate,	6-(3-hydroxy-2,4-
	dimethoxybenzylamino)purine	riboside-5'-monophosphate,	6-(3-hydroxy-2,5-
	dimethoxybenzylamino)purine	riboside-5'-monophosphate,	6-(3-hydroxy-2,6-
10	dimethoxybenzylamino)purine	riboside-5'-monophosphate,	6-(4-hydroxy-2,3-
	dimethoxybenzylamino)purine	riboside-5'-monophosphate,	6-(4-hydroxy-2,5-
	dimethoxybenzylamino)purine	riboside-5'-monophosphate,	6-(4-hydroxy-2,6-
	dimethoxybenzylamino)purine	riboside-5'-monophosphate,	6-(4-hydroxy-3,5-
	dimethoxybenzylamino)purine	riboside-5'-monophosphate,	6-(4-hydroxy-3,6-
15	dimethoxybenzylamino)purine	riboside-5'-monophosphate,	6-(2,3-dihydroxy-4-
	methoxybenzylamino)purine	riboside-5'-monophosphate,	6-(2,3-dihydroxy-5-
	methoxybenzylamino)purine	riboside-5'-monophosphate,	6-(2,3-dihydroxy-6-
	methoxybenzylamino)purine	riboside-5'-monophosphate,	6-(2,4-dihydroxy-3-
	methoxybenzylamino)purine	riboside-5'-monophosphate,	6-(2,4-dihydroxy-5-
20	methoxybenzylamino)purine	riboside-5'-monophosphate,	6-(2,4-dihydroxy-6-
	methoxybenzylamino)purine	riboside-5'-monophosphate,	6-(2,5-dihydroxy-3-
	methoxybenzylamino)purine	riboside-5'-monophosphate,	6-(2,5-dihydroxy-4-
	methoxybenzylamino)purine	riboside-5'-monophosphate,	6-(2,5-dihydroxy-6-
	methoxybenzylamino)purine	riboside-5'-monophosphate,	6-(2,6-dihydroxy-3-
25	methoxybenzylamino)purine	riboside-5'-monophosphate,	6-(2,6-dihydroxy-4-
	methoxybenzylamino)purine	riboside-5'-monophosphate,	6-(2,6-dihydroxy-5-
	methoxybenzylamino)purine	riboside-5'-monophosphate,	6-(3,4-dihydroxy-2-
	methoxybenzylamino)purine	riboside-5'-monophosphate,	6-(3,4-dihydroxy-5-
	methoxybenzylamino)purine	riboside-5'-monophosphate,	6-(3,4-dihydroxy-6-
30	methoxybenzylamino)purine	riboside-5'-monophosphate,	6-(3,5-dihydroxy-2-
	methoxybenzylamino)purine	riboside-5'-monophosphate,	6-(3,5-dihydroxy-4-
	methoxybenzylamino)purine	riboside-5'-monophosphate,	6-(3,5-dihydroxy-6-

	methoxybenzylamino)purine	riboside-5'-monophosphate,	6-(2,3,4-
	trimethoxybenzylamino)purine	riboside-5'-monophosphate,	6-(2,4,5-
	trimethoxybenzylamino)purine	riboside-5'-monophosphate,	6-(2,4,6-
	trimethoxybenzylamino)purine	riboside-5'-monophosphate,	6-(3,4,5-
5	trimethoxybenzylamino)purine	riboside-5'-monophosphate,	6-(2-hydroxy-3,4,5-
	trimethoxybenzylamino)purine	riboside-5'-monophosphate,	6-(2-hydroxy-3,4,6-
	trimethoxybenzylamino)purine	riboside-5'-monophosphate,	6-(2-hydroxy-4,5,6-
	trimethoxybenzylamino)purine	riboside-5'-monophosphate,	6-(2,4,6-
	trimethoxybenzylamino)purine	riboside-5'-monophosphate,	6-(2,3,4-
10	trihydroxybenzylamino)purine	riboside-5'-monophosphate,	6-(2,4,6-
	trihydroxybenzylamino)purine	riboside-5'-monophosphate,	6-(2,3,4-
	trihydroxybenzylamino)purine	riboside-5'-monophosphate,	6-(3,4,5-
	trihydroxybenzylamino)purine	riboside-5'-monophosphate,	6-(2,4,6-
	trihydroxybenzylamino)purine	riboside-5'-monophosphate,	6-(2-hydroxy-3-
15	chlorobenzylamino)purine	riboside-5'-monophosphate,	6-(2-hydroxy-4-
	chlorobenzylamino)purine	riboside-5'-monophosphate,	6-(2-hydroxy-5-
	chlorobenzylamino)purine	riboside-5'-monophosphate,	6-(2-hydroxy-6-
	chlorobenzylamino)purine	riboside-5'-monophosphate,	6-(2-hydroxy-3-
	iodobenzylamino)purine	riboside-5'-monophosphate,	6-(2-hydroxy-4-
20	iodobenzylamino)purine	riboside-5'-monophosphate,	6-(2-hydroxy-5-
	iodobenzylamino)purine	riboside-5'-monophosphate,	6-(2-hydroxy-6-
	iodobenzylamino)purine	riboside-5'-monophosphate,	6-(2-hydroxy-3-
	bromobenzylamino)purine	riboside-5'-monophosphate,	6-(2-hydroxy-4-
	bromobenzylamino)purine	riboside-5'-monophosphate,	6-(2-hydroxy-5-
25	bromobenzylamino)purine	riboside-5'-monophosphate,	6-(2-hydroxy-6-
	bromobenzylamino)purine	riboside-5'-monophosphate,	6-(2-hydroxy-3-
	fluorobenzylamino)purine	riboside-5'-monophosphate,	6-(2-hydroxy-4-
	fluorobenzylamino)purine	riboside-5'-monophosphate,	6-(2-hydroxy-5-
	fluorobenzylamino)purine	riboside-5'-monophosphate,	6-(2-hydroxy-6-
30	fluorobenzylamino)purine	riboside-5'-monophosphate,	6-(2-hydroxy-3-
	methylbenzylamino)purine	riboside-5'-monophosphate,	6-(2-hydroxy-4-
	methylbenzylamino)purine	riboside-5'-monophosphate,	6-(2-hydroxy-5-

	methylbenzylamino)purine	riboside-5'-monophosphate,	6-(2-hydroxy-6-
	methylbenzylamino)purine	riboside-5'-monophosphate,	6-(2,3-dihydroxy-4-
	chlorobenzylamino)purine	riboside-5'-monophosphate,	6-(2,3-dihydroxy-5-
	chlorobenzylamino)purine	riboside-5'-monophosphate,	6-(2,5-dihydroxy-4-
5	chlorobenzylamino)purine	riboside-5'-monophosphate,	6-(2,6-dihydroxy-4-
	chlorobenzylamino)purine	riboside-5'-monophosphate,	6-(2,6-dihydroxy-4-
	iodobenzylamino)purine	riboside-5'-monophosphate,	6-(2,6-dihydroxy-3-
	chlorobenzylamino)purine	riboside-5'-monophosphate,	6-(2,6-dihydroxy-3-
	bromobenzylamino)purine	riboside-5'-monophosphate,	6-(2,6-dihydroxy-3-
10	iodobenzylamino)purine	riboside-5'-monophosphate,	6-(2,6-dihydroxy-3-
	fluorobenzylamino)purine	riboside-5'-monophosphate,	6-(2,6-dihydroxy-3,5-
	dichlorobenzylamino)purine	riboside-5'-monophosphate,	6-(2,6-dihydroxy-3,5-
	dibromobenzylamino)purine	riboside-5'-monophosphate,	6-(2,6-dihydroxy-3,5-
	diiodobenzylamino)purine	riboside-5'-monophosphate,	6-(2,6-dihydroxy-3,5-
15	difluorobenzylamino)purine	riboside-5'-monophosphate,	6-(4,5-dimethoxy-2-
	nitrobenzylamino)purine	riboside-5'-monophosphate,	6-(3,4-
	dimethylbenzylamino)purine	riboside-5'-monophosphate,	6-(2,3-
	dimethylbenzylamino)purine	riboside-5'-monophosphate,	6-(2,4-
	dimethylbenzylamino)purine	riboside-5'-monophosphate,	6-(2,6-
20	dimethylbenzylamino)purine	riboside-5'-monophosphate,	6-(2,6-dimethyl-4-
	hydroxybenzylamino)purine	riboside-5'-monophosphate,	6-(3,5-dimethyl-4-
	hydroxybenzylamino)purine	riboside-5'-monophosphate,	6-(2-fluoro-4-
	hydroxybenzylamino)purine	riboside-5'-monophosphate,	6-(3-fluoro-4-
	methylbenzylamino)purine	riboside-5'-monophosphate,	6-(3,4-
25	dinitrobenzylamino)purine	riboside-5'-monophosphate,	6-(3,5-
	dinitrobenzylamino)purine	riboside-5'-monophosphate,	6-(2-methyl-5-
	nitrobenzylamino)purine	riboside-5'-monophosphate,	6-(3-methyl-4-
	nitrobenzylamino)purine	riboside-5'-monophosphate,	6-(3,4-diiodo-4-
	hydroxybenzylamino)purine	riboside-5'-monophosphate,	6-(2-chloro-3,4-
30	dimethoxybenzylamino)purine	riboside-5'-monophosphate,	6-(4-chloro-3,5-
	dinitrobenzylamino)purine	riboside-5'-monophosphate,	6-(2-chloro-4-
	fluorobenzylamino)purine	riboside-5'-monophosphate,	6-(3-chloro-4-

	fluorobenzylamino)purine	riboside-5'-monophosphate,	6-(2-chloro-6-
	methylbenzylamino)purine	riboside-5'-monophosphate,	6-(3-chloro-2-
	methylbenzylamino)purine	riboside-5'-monophosphate,	6-(3-chloro-4-
	methylbenzylamino)purine	riboside-5'-monophosphate,	6-(5-chloro-2-
5	methoxybenzylamino)purine	riboside-5'-monophosphate,	6-(2-chloro-4-
	fluorobenzylamino)purine	riboside-5'-monophosphate,	6-(2-chloro-5-
	nitrobenzylamino)purine	riboside-5'-monophosphate,	6-(2-chloro-6-
	nitrobenzylamino)purine	riboside-5'-monophosphate,	6-(4-chloro-3-
	nitrobenzylamino)purine	riboside-5'-monophosphate,	6-(5-chloro-2-
10	nitrobenzylamino)purine	riboside-5'-monophosphate,	6-(3-bromo-4-
	hydroxybenzylamino)purine	riboside-5'-monophosphate,	6-(3,5-dibromo-4-
	hydroxybenzylamino)purine,	6-(3-bromo-4-methoxybenzylamino)purine,	6-(4-
	butoxybenzylamino)purine	riboside-5'-monophosphate,	6-(4-/t-
	butyl/benzylamino)purine	riboside-5'-monophosphate,	6-(4-t-butyl-2,6-
15	dimethylbenzylamino)purine	riboside-5'-monophosphate,	6-(2-
	aminobenzylamino)purine	riboside-5'-monophosphate,	6-(3-
	aminobenzylamino)purine	riboside-5'-monophosphate,	6-(4-
	aminobenzylamino)purine	riboside-5'-monophosphate,	6-(2-amino-3-
	chlorobenzylamino)purine	riboside-5'-monophosphate,	6-(2-amino-4-
20	chlorobenzylamino)purine	riboside-5'-monophosphate,	6-(2-amino-5-
	chlorobenzylamino)purine	riboside-5'-monophosphate,	6-(2-amino-6-
	chlorobenzylamino)purine	riboside-5'-monophosphate,	6-(3-amino-2-
	chlorobenzylamino)purine	riboside-5'-monophosphate,	6-(3-amino-4-
	chlorobenzylamino)purine	riboside-5'-monophosphate,	6-(3-amino-5-
25	chlorobenzylamino)purine	riboside-5'-monophosphate,	6-(3-amino-6-
	chlorobenzylamino)purine	riboside-5'-monophosphate,	6-(2,6-diamino-3-
	chlorobenzylamino)purine	riboside-5'-monophosphate,	6-(2,6-diamino-4-
	chlorobenzylamino)purine	riboside-5'-monophosphate,	6-(4-amino-3-
	chlorobenzylamino)purine	riboside-5'-monophosphate,	6-(4-amino-5-
30	dichlorobenzylamino)purine	riboside-5'-monophosphate,	6-(5-amino-2-
	methylbenzylamino)purinee	riboside-5'-monophosphate,	6-(2-amino-3-
	nitrobenzylamino)purinee	riboside-5'-monophosphate,	6-(4-amino-3-

	nitrobenzylamino)purine	riboside-5'-monophosphate,	6-(2,4,5-
	trichlorobenzylamino)purine	riboside-5'-monophosphate,	6-(2,4,5-
	trichlorobenzylamino)purine	riboside-5'-monophosphate,	6-(2,4,6-
	trichlorobenzylamino)purine	riboside-5'-monophosphate,	6-(2,3,4-
5	trichlorobenzylamino)purine	riboside-5'-monophosphate,	6-(2,3,5-
	trichlorobenzylamino)purine	riboside-5'-monophosphate,	6-(2,3,6-
	trichlorobenzylamino)purine	riboside-5'-monophosphate,	6-(2,5,6-
	trichlorobenzylamino)purine riboside-5'-monophosphate.		

10 The starting material for the preparation of the compounds of the general formula I is 6-chloropurine riboside-5'-monophosphate. Another starting material for the preparation of the compounds of the general formula I is 6-bromopurine riboside-5'-monophosphate; both starting materials are known from literature or commercially available.

15 Another starting material for the preparation of the compounds of the general formula I is 6-fluoropurine riboside-5'-monophosphate, which can be prepared from 6-chloropurine riboside-5'-monophosphate by reaction with triethylamine under the formation of quaternary ammonium salt, which can be converted by reaction with tetrabutylammonium triphenyldifluorosilicate in dimethylformamid to 6-fluoropurine
 20 riboside-5'-monophosphate (Gurvich et al., Nucleos.Nucleot. 18: 2327 (1999)).

Yet another starting material for the preparation of the compounds of the general formula I are substituted benzylamines. Those having one or more hydroxyl groups, are not commercially available and may be prepared by demethylation of appropriate methoxyderivatives using 48% HBr in N₂ atmosphere.

25 This invention also concerns preparation of substitution derivatives of N⁶-benzyladenosine-5'-monophosphate of the general formula I, wherein R and n have the above mentioned meanings, and which may be prepared from 6-halogenopurine riboside-5'-monophosphate, wherein halogen is selected from the group containing bromine, chlorine or fluorine, by a nucleophilic substitution with benzylamine
 30 substituted by one to four R substitutents, which can be the same or different, wherein R has the above mentioned meaning.

THERAPEUTIC COMPOSITIONS

The therapeutic composition comprises about 1% to about 95% of the active ingredient, single-dose forms of administration preferably comprising about 20% to about 90% of the active ingredient and administration forms, which are not single-dose preferably comprising about 5% to about 20% of the active ingredient. Unit dose forms may be, for example, coated tablets, tablets, ampoules, vials, suppositories or capsules. Other forms of administration are, for example, ointments, creams, pastes, foams, tinctures, lipsticks, drops, sprays, dispersions and the like. Examples are capsules containing from about 0.05 g to about 1.0 g of the active ingredient.

The pharmaceutical compositions of the present invention are prepared in a manner known per se, for example by means of conventional mixing, granulating, coating, dissolving or lyophilizing processes.

Preferably, solutions of the active ingredient, and in addition also suspensions or dispersions, especially isotonic aqueous solutions, dispersions or suspensions, are used, if possible for these to be prepared before use, for example in the case of lyophilised compositions which comprise the active substance by itself or together with a carrier, for example mannitol. The pharmaceutical compositions can be sterilised and/or comprise excipients, for example preservatives, stabilisers, wetting agents and/or emulsifiers, solubilizing agents, salts for regulating the osmotic pressure and/or buffers, and they are prepared in a manner known per se, for example by means of conventional dissolving or lyophilising processes. The solutions or suspensions mentioned can comprise viscosity-increasing substances, such as sodium carboxymethylcellulose, dextran, polyvinylpyrrolidone or gelatine. Suspensions in oil comprise, as the oily component, vegetable, synthetic or semi-synthetic oils customary for injection purposes. Oils which may be mentioned are, in particular, liquid fatty acid esters which contain, as the acid component, a long-chain fatty acid having 8 - 22, in particular 12-22, carbon atoms, for example lauric acid, tridecylic acid, myristic acid, pentadecylic acid, palmitic acid, margaric acid, stearic acid, arachidonic acid, behenic acid or corresponding unsaturated acids, for example oleic acid, elaidic acid, euric acid, brasidic acid or linoleic acid, if appropriate with the addition of antioxidants, for example vitamin E, β -carotene or 3,5-di-*tert*-butyl-4-hydroxytoluene. The alcohol component of these fatty acid esters has not more than 6

carbon atoms and is mono- or polyhydric, for example mono-, di- or trihydric alcohol, for example methanol, ethanol, propanol, butanol, or pentanol, or isomers thereof, but in particular glycol and glycerol. Fatty acid esters are, for example: ethyl oleate, isopropyl myristate, isopropyl palmitate, "Labrafil M 2375" (polyoxyethylene glycerol trioleate from Gattefoscé, Paris), "Labrafil M 1944 CS" (unsaturated polyglycolated glycerides prepared by an alcoholysis of apricot kernel oil and made up of glycerides and polyethylene glycol esters; from Gattefoscé, Paris), "Labrasol" (saturated polyglycolated glycerides prepared by an alcoholysis of TCM and made up of glycerides and polyethylene glycol esters; from Gattefoscé, Paris) and/or "Miglyol 812" (triglyceride of saturated fatty acids of chain length C_8 to C_{12} from Hüls AG, Germany), and in particular vegetable oils, such as cottonseed oil, almond oil, olive oil, castor oil, sesame oil, soybean oil and, in particular, groundnut oil.

The preparation of the injection compositions is carried out in the customary manner under sterile conditions, as are bottling, for example into ampoules or vials, and closing of the containers.

For example, pharmaceutical compositions for oral use can be obtained by combining the active ingredient with one or more solid carriers, if appropriate granulating the resulting mixture, and, if desired, processing the mixture or granules to tablets or coated tablet cores, if appropriate by addition of additional excipients.

Suitable carriers are, in particular, fillers, such as sugars, for example lactose, sucrose, mannitol or sorbitol, cellulose preparations and/or calcium phosphates, for example tricalcium diphosphate, or calcium hydrogen phosphate, and furthermore binders, such as starches, for example maize, wheat, rice or potato starch, methylcellulose, hydroxypropylmethylcellulose, sodium carboxymethylcellulose and/or polyvinylpyrrolidone, and/or, if desired, disintegrators, such as the above mentioned starches, and furthermore carboxymethyl-starch, cross-linked polyvinylpyrrolidone, alginic acid or a salt thereof, such as sodium alginate. Additional excipients are, in particular, flow regulators and lubricants, for example salicylic acid, talc, stearic acid or salts thereof, such as magnesium stearate or calcium stearate, and/or polyethylene glycol, or derivatives thereof.

Coated tablet cores can be provided with suitable coatings which, if appropriate, are resistant to gastric juice, the coatings used being, inter alia, concentrated sugar

solutions, which, if appropriate, comprise gum arabic, talc, polyvinylpyrrolidone, polyethylene glycol and/or titanium dioxide, coating solutions in suitable organic solvents or solvent mixtures or, for the preparation of coatings which are resistant to gastric juice, solutions of suitable cellulose preparations, such as acetylcellulose phthalate or hydroxypropylmethylcellulose phthalate. Dyes or pigments can be
5 admixed to the tablets or coated tablet coatings, for example for identification or characterisation of different doses of active ingredient.

Pharmaceutical compositions, which can be used orally, are also hard capsules of gelatine and soft, closed capsules of gelatine and a plasticiser, such as glycerol or
10 sorbitol. The hard capsules can contain the active ingredient in the form of granules, mixed for example with fillers, such as maize starch, binders and/or lubricants, such as talc or magnesium stearate, and stabilisers if appropriate. In soft capsules, the active ingredient is preferably dissolved or suspended in suitable liquid excipients, such as greasy oils, paraffin oil or liquid polyethylene glycol or fatty acid esters of
15 ethylene glycol or propylene glycol, it being likewise possible to add stabilisers and detergents, for example of the polyethylene sorbitan fatty acid ester type.

Other oral forms of administration are, for example, syrups prepared in the customary manner, which comprise the active ingredient, for example, in suspended form and in a concentration of about 5% to 20%, preferably about 10% or in a similar
20 concentration which results in a suitable individual dose, for example, when 5 or 10 ml are measured out. Other forms are, for example, also pulverulent or liquid concentrates for preparing of shakes, for example in milk. Such concentrates can also be packed in unit dose quantities.

Pharmaceutical compositions, which can be used rectally, are, for example,
25 suppositories that comprise a combination of the active ingredient with a suppository base. Suitable suppository bases are, for example, naturally occurring or synthetic triglycerides, paraffin hydrocarbons, polyethylene glycols or higher alkanols.

Compositions which are suitable for parental administration are aqueous solutions of an active ingredient in water-soluble form, for example of water-soluble salt, or
30 aqueous injection suspensions, which comprise viscosity-increasing substances, for example sodium carboxymethylcellulose, sorbitol and/or dextran, and, if appropriate, stabilizers. The active ingredient can also be present here in the form of a lyophilisate,

if appropriate, together with excipients, and be dissolved before parenteral administration by addition of suitable solvents. Solutions such as are used, for example, for parental administration can also be used as infusion solutions. Preferred preservatives are, for example, antioxidants, such as ascorbic acid, or microbicides, such as sorbic or benzoic acid.

Ointments are oil-in-water emulsions which comprise not more than 70%, preferably 20 - 50% of water or aqueous phase. The fatty phase consists, in particular, hydrocarbons, for example vaseline, paraffin oil or hard paraffins, which preferably comprise suitable hydroxy compounds, such as fatty alcohols or esters thereof, for example cetyl alcohol, or wool wax alcohols, such as wool wax, to improve the water-binding capacity. Emulsifiers are corresponding lipophilic substances, such as sorbitan fatty acid esters (Spans), for example sorbitan oleate and/or sorbitan isostearate. Additives to the aqueous phase are, for example, humectants, such as polyalcohols, for example glycerol, propylene glycol, sorbitol and/or polyethylene glycol, or preservatives and odoriferous substances.

Tinctures and solutions usually comprise an aqueous-ethanolic base to which, humectants for reducing evaporation, such as polyalcohols, for example glycerol, glycols and/or polyethylene glycol, and re-oiling substances, such as fatty acid esters with lower polyethylene glycols, i.e. lipophilic substances soluble in the aqueous mixture to substitute the fatty substances removed from the skin with ethanol, and, if necessary, other excipients and additives, are admixed.

The invention also relates to a process or method for treatment of the disease states mentioned above. The compounds can be administered prophylactically or therapeutically as such or in the form of pharmaceutical compositions, preferably in an amount, which is effective against the diseases mentioned. With a warm-blooded animal, for example a human, requiring such treatment, the compounds are used, in particular, in the form of pharmaceutical composition. A daily dose of about 0.1 to about 5 g, preferably 0.5 g to about 2 g, of a compound of the present invention is administered here for a body weight of about 70 kg.

Brief Description of Drawings

Fig. 1. shows the inhibition of growth of cancer cell lines CEM and HL60 by the compounds of the general formula I. The cytotoxicity was measured using Calcein AM assay. The activity is presented as percentage of maximum activity (in the absence of inhibitor). The dose-dependent antiproliferative activity. ZR: zeatin riboside-5'-monophosphate; compound **21**: *ortho*-topolin riboside-5'-monophosphate (= 6-(2-hydroxybenzylamino)purine riboside-5'-monophosphate); **2**: 6-(3-fluorobenzylamino)purine riboside-5'-monophosphate; **5**: 6-(3-chlorobenzylamino)purine riboside-5'-monophosphate; **28**: 6-(2-hydroxy-3-methoxybenzylamino)purine riboside-5'-monophosphate.

Fig. 2 shows the effect of compound **21** on DNA fragmentation. y-axis is the percentage of apoptotic sub-G1 fraction. Cancer cell line CEM was incubated with compound **21** in concentrations corresponding to IC50 (1.3 μ M, ●), triple IC50 (▲) and fivefold IC50 (■). Control cells (○) were cultivated with DMSO vehiculum.

Fig. 3 displays induction of activity of caspase-3 by compound **21** (10 μ M).

Fig. 4 shows the effect of compound **21** on HL-60 cell nuclear morphology. **a**) Nuclei of cells cultivated at standard conditions in medium without compound **21**; **b**) nuclei of cells cultivated in medium containing 5 μ M of compound **21** for 24 hours.

Fig. 5 shows induction of protein p21^{WAF-1} in MCF-7 cells after treatment with different concentrations of compound **28**.

Fig. 6 shows p21^{WAF-1} induction in MCF-7 cells 0.5-24 hours after the application of compound **28** in 1 μ M concentration.

Examples of Carrying Out the Invention

The following examples serve to illustrate the invention and should not be construed as limiting the scope thereof.

The starting material for the compounds of the formula I is available from commercial sources (Sigma-Aldrich, Fluka, Olchemim, etc.). Melting points were determined on a Koffler block and are uncorrected. Evaporations were carried out on a rotary evaporator under vacuum at temperatures below 80 °C. The ¹H NMR spectra

(σ , ppm; J, Hz) were measured on Varian VXR-400 (400 MHz) or on Varian Unity 300 (300 MHz) instruments. All spectra were obtained at 25 °C using tetramethylsilane as an internal standard. Electron impact mass spectra m/z (rel.%, composition, deviation) were measured on a VG 7070E spectrometer (70 eV, 200 °C, direct inlet). Quadrupole mass spectra were measured on a Micromass ZMD detector with electrospray ionization. Merck silica gel Kieselgel 60 (230-400 mesh) was used for column chromatography. All compounds gave satisfactory elemental analyses (± 0.4 %).

10 EXAMPLE 1

General method for the preparation of compounds of the general formula I

Compounds were prepared from 6-chloropurine-9 β -D-ribose-5'-*O*-monophosphate, disodium salt dihydrate, by nucleophilic substitution with the corresponding substituted benzylamine in the presence of N,N-diisopropyl-N-ethylamine in methanol. The reaction was carried out at 90 °C for a period of 12 hours under nitrogen atmosphere. The solvent was removed by vacuum evaporation and the raw product was further purified by *RP* C18 flash chromatography (mobile phase 15% methanol in water) followed by crystallization from 2-propanol. Purity of the final product was 95 % (HPLC) and the yield 60-70 %.

Table 1 Compounds Prepared by the Method of Example 1 (listed compounds are anhydrous disodium salts)

No.	Substituent at the position 6 of purine	Elemental analyses calculated/found			ES-MS [M-H] ⁺
		%C	%H	%N	
1	2-fluorobenzylamino	40.9/40.8	3.4/3.4	14.0/13.9	454
2	3-fluorobenzylamino	40.9/50.0	3.4/3.4	14.0/14.1	454
3	4-fluorobenzylamino	40.9/40.9	3.4/3.3	14.0/14.0	454
4	2-chlorobenzylamino	39.6/39.8	3.3/3.2	13.6/13.5	470

5	3-chlorobenzylamino	39.6/39.8	3.3/3.3	13.6/13.4	470
6	4-chlorobenzylamino	39.6/39.6	3.3/3.2	13.6/13.7	470
7	2-bromobenzylamino	36.4/36.5	3.1/3.0	12.5/12.3	515
8	3-bromobenzylamino	36.4/36.2	3.1/3.2	12.5/12.3	515
9	4-bromobenzylamino	36.4/36.5	3.1/3.1	12.5/12.6	515
10	2-iodobenzylamino	33.6/33.4	2.8/2.7	11.5/11.4	562
11	3-iodobenzylamino	33.6/33.5	2.8/2.8	11.5/11.3	562
12	2-methylbenzylamino	43.6/43.7	4.1/4.1	14.1/14.0	450
13	3-methylbenzylamino	43.6/43.4	4.1/4.0	14.1/13.8	450
14	4-methylbenzylamino	43.6/43.8	4.1/4.2	14.1/14.0	450
15	2-methoxybenzylamino	42.3/42.3	3.9/3.8	13.7/13.7	466
16	3-methoxybenzylamino	42.3/42.2	3.9/3.9	13.7/13.5	466
17	4-methoxybenzylamino	42.3/42.2	3.9/3.8	13.7/13.8	466
18	2-aminobenzylamino	41.1/41.0	3.9/3.9	16.9/16.7	451
19	3-aminobenzylamino	41.1/41.2	3.9/4.0	16.9/16.7	451
20	4-aminobenzylamino	41.1/41.1	3.9/3.9	16.9/17.1	451
21	2-hydroxybenzylamino	41.1/41.0	3.9/3.9	16.9/16.7	451
22	3-hydroxybenzylamino	41.1/41.2	3.9/4.0	16.9/16.7	451
23	4-hydroxybenzylamino	41.1/41.1	3.9/3.9	16.9/17.1	451
24	2,4-dichlorobenzylamino	37.1/37.0	2.9/2.8	12.7/12.7	504
25	3,4-dichlorobenzylamino	37.1/37.2	2.9/2.9	12.7/12.5	504
26	2,3-dihydroxybenzylamino	39.8/39.9	3.5/3.5	13.6/13.5	468
27	3,5-dihydroxybenzylamino	39.8/39.8	3.5/3.4	13.6/13.3	468
28	2-hydroxy-3-methoxybenzylamino	41.0/41.2	3.8/3.8	13.3/13.3	482
29	2-hydroxy-5-methoxybenzylamino	41.0/41.1	3.8/3.7	13.3/13.2	482
30	3-hydroxy-4-methoxybenzylamino	41.0/41.0	3.8/3.9	13.3/13.1	482
31	2,3-dimethoxybenzylamino	42.1/42.0	4.1/4.0	12.9/12.8	496
32	2,4-dimethoxybenzylamino	42.1/42.1	4.1/4.0	12.9/12.9	496

33	3,4-dimethoxybenzylamino	42.1/42.0	4.1/4.1	12.9/12.7	496
34	3,5-dimethoxybenzylamino	42.1/42.1	4.1/4.0	12.9/12.8	496
35	4-hydroxy-3,5-dimethoxybenzylamino	40.9/40.8	4.0/4.1	12.6/12.4	512
36	2,4-difluorobenzylamino	39.5/39.8	3.1/3.2	13.5/13.2	472
37	3,5-difluorobenzylamino	39.5/39.4	3.1/3.1	13.5/13.3	472
38	2,3,4-trifluorobenzylamino	38.1/38.3	2.8/2.8	13.1/12.9	490
39	2,4,5-trifluorobenzylamino	38.1/38.2	2.8/2.7	13.1/13.0	490
40	2,3,6-trifluorobenzylamino	38.1/38.1	2.8/2.8	13.1/12.8	490
41	3-chloro-4-fluorobenzylamino	38.3/38.4	3.0/3.0	13.1/12.9	488
42	2-hydroxy-3-methylbenzylamino	42.3/42.3	3.9/3.8	13.7/13.5	466
43	2-hydroxy-3-fluorobenzylamino	39.6/39.4	3.3/3.3	13.6/13.5	470
44	2-hydroxy-3-iodobenzylamino	32.8/32.7	2.7/2.7	11.2/11.1	578
45	2-hydroxy-5-methoxybenzylamino	41.0/41.0	3.8/3.8	13.3/13.0	482
46	2-hydroxy-5-methylbenzylamino	42.3/42.4	3.9/3.9	13.7/13.9	466
47	2-hydroxy-5-chlorobenzylamino	38.4/38.5	3.2/3.2	13.2/13.0	486
48	2-hydroxy-5-fluorobenzylamino	39.6/39.4	3.3/3.3	13.6/13.5	470
49	2-amino-5-fluorobenzylamino	39.7/39.7	3.5/3.4	16.3/16.0	469

EXAMPLE 2

6-fluoropurine riboside-5'-monophosphate (3 mmol) was dissolved in n-propanol (15 ml). Subsequently, 3.15 mmol of 2,3-dihydroxybenzylamine hydrobromide and 10 mmol of triethylamine was added. The reaction was carried out at 90 °C for a period of 12 hours. After cooling, the precipitated product was filtered off, washed with cold
 5 (5 °C) n-propanol (2 x 5 ml) and was further purified by *RP* C18 flash chromatography (mobile phase 15-20% methanol in water) followed by crystallization from ethanol. TLC: chloroform-methanol-ammonia (90:9:0.1). Yield 65-70 %.

10 **Table 2** Compounds Prepared by the Method of Example 2 (listed compounds are anhydrous disodium salts)

No	Substituent at the position 6 of purine	Elemental analyses calculated/found			ES-MS [M-H] ⁺
		%C	%H	%N	
26	2,3-dihydroxybenzylamino	39.8/39.6	3.5/3.5	13.6/13.5	468
27	3,5-dihydroxybenzylamino	39.8/39.8	3.5/3.5	13.6/13.4	468
28	2-hydroxy-3-methoxybenzylamino	41.0/41.2	3.8/3.8	13.3/13.3	482
30	3-hydroxy-4-methoxybenzylamino	41.0/41.0	3.8/3.9	13.3/13.1	482
31	2,3-dimethoxybenzylamino	42.1/42.0	4.1/4.0	12.9/12.8	496
32	2,4-dimethoxybenzylamino	42.1/42.1	4.1/4.0	12.9/12.9	496
33	3,4-dimethoxybenzylamino	42.1/42.0	4.1/4.1	12.9/12.7	496
34	3,5-dimethoxybenzylamino	42.1/42.1	4.1/4.0	12.9/12.8	496
35	4-hydroxy-3,5-dimethoxybenzylamino	40.9/40.8	4.0/4.1	12.6/12.4	512

EXAMPLE 3 *In vitro* Cytotoxic Activity of Novel Derivatives

15 Because toxic compounds negatively influence metabolic processes in cells, many standard cytotoxicity assays are based on measurement of metabolisation rate of

various artificial substrates. Resulting product is then quantified e.g. by means of spectrometry. The assays can be easily modified for use in 96-well plates. For evaluation of cytotoxic effect of the novel substituted 9-substituted cytokinin derivatives of this invention, a microtiter assay based on quantification of
5 metabolisation of Calcein AM was used. The assay is widely used in drug screening programs and in chemosensitivity testing. In the living cells, Calcein AM is enzymatically hydrolysed and accumulation of resulting calcein is manifested by green fluorescence.

In the routine screening the following cell lines have been used: CEM (T-
10 lymphoblastoid leukemia), HL-60 (promyelocytic leukemia), K-562 (human erythroleukemia), MCF-7 (breast carcinoma), HOS (human osteogenic sarcoma), and G-361 (human malignant melanoma). Normal human fibroblasts BJ were used as a control for routine screening of the compounds. The cells were kept in Nunc/Corning 80 cm² plastic bottles and grown in cell culture medium (DMEM
15 containing 5g/l glucose, 2mM glutamine, 100 U/ml penicilline, 100 µg/ml streptomycine, 10% fetal bovine serum and sodium hydrogencarbonate), the compounds were prepared according to the procedure of the present invention.

The cell suspensions were prepared and diluted according to the particular cell type and the expected target cell density (2.500-30.000 cells per well based on cell growth
20 characteristics) and pipetted (80µl) into 96-well plates. Inoculates were allowed a pre-incubation period of 24 hours at 37°C and 5% CO₂ for stabilisation. Tested compound was added in total volume of 20 µl of water at time zero. Usually, test compound was evaluated at six 3-fold dilutions. In routine testing, the highest concentration tested was 166.6 µM. All drug concentrations were tested in triplicates.
25 Incubations of cells with the test compounds lasted for 72 hours at 37 °C, in 5% CO₂ atmosphere and 100% humidity. At the end of incubation period Calcein AM in PBS was added into final concentration of 1 µg / ml. After another 1 hour of incubation fluorescence (FD) was measured with the Labsystem FIA Reader Fluoroscan Ascent (UK). Growth inhibition (GI) was estimated using the following equitation: $GI =$
30 $(\text{mean } FD_{\text{drug exposed wells}} - \text{mean } FD_{\text{blank}}) / (\text{mean } FD_{\text{control wells}} - \text{mean } FD_{\text{blank}}) \times 100\%$.
The GI₅₀ value, the drug concentration causing 50% reduction of esterase activity, was calculated from the obtained dose response curves (Fig. 1).

Cytotoxicity of novel compounds was tested on panel of cell lines of different histogenetic and species origin (Table 3). We show here that equal activities were found in all tumour cell lines tested, however, the non-malignant cells, e.g. BJ fibroblasts and normal human lymphocytes, were resistant to new compounds induced cytotoxicity. The effective novel derivatives killed tumour cells in concentrations close to 0.1-50 μ M.

Tab. 3. Cytotoxicity of New Substitution Derivatives of N⁶-benzyladenosine-5'-monophosphate for Different Cancer Cell Lines

	Cell line tested / GI50 (μ mol/L)						
	HOS	K-562	MCF7	BJ	G-361	CEM	HL60
adenosine-5'-monophosphate (control)	>166.7	>166.7	>166.7	>166.7	>166.7	>166.7	>166.7
zeatin riboside-5'-monophosphate (control)	>166.7	>166.7	>166.7	>166.7	>166.7	>166.7	>166.7
Substituent at N⁶-position of the purine ring of the compound of formula I							
2-hydroxybenzylamino	2.5	4	10.5	43.2	13.5	1.3	0.48
3-hydroxybenzylamino	>166.7	92.4	107.9	>166.7	>166.7	49.8	19.0
4-hydroxybenzylamino	>166.7	>166.7	>166.7	>166.7	>166.7	19.4	9.7
2-methoxybenzylamino	20.1	8.4	112.9	>166.7	>166.7	2.4	2.1
3-methoxybenzylamino	>166.7	121.5	48.1	>166.7	>166.7	4.6	5.1
2-chlorobenzylamino	>166.7	56	>166.7	>166.7		1.9	1.4
3-chlorobenzylamino	>166.7	23.4	>166.7	>166.7		1.5	0.5
4-chlorobenzylamino	>166.7	12.6	>166.7	>166.7		4.9	5.3
2-fluorobenzylamino	>166.7	31.7	>166.7			3.9	3.3
3-fluorobenzylamino	>166.7	7.2	14.7	>166.7	14.6	3.2	0.83
4-fluorobenzylamino	12.1	6.1	12.3	>166.7		1.2	0.69
2-methylbenzylamino	>166.7	>166.7	144.4	>166.7		11.4	3.1
3-methylbenzylamino	>166.7	160.2	71.7	>166.7		11.6	6.6
2-bromobenzylamino	>166.7	9.7	45.6	>166.7		2.3	5.9
3-bromobenzylamino	>166.7	19.1	>166.7	>166.7		5.1	4.2
4-bromobenzylamino	>166.7	49.9	>166.7	>166.7		17.6	41.3
2,4-dimethoxybenzylamino	>166.7	>166.7	>166.7	>166.7		>166.7	41
2-chloro-4-fluorobenzylamino	>166.7	9.6	>166.7	>166.7		12.7	7.9
3-chloro-4-fluorobenzylamino	>166.7	4.3	99.1	>166.7		1.4	3.1
2,3-dimethoxybenzylamino	>166.7	>166.7	>166.7	>166.7		>166.7	91

2,4-dichlorobenzylamino	>166.7	81.5	121.5	>166.7		81.2	72.4
2,4-difluorobenzylamino	>166.7	5.9	>166.7	>166.7		4.9	2.8
2,3,4-trifluorobenzylamino	>166.7	>166.7	>166.7	>166.7		58.6	11.3
3,4-dichlorobenzylamino	111.5	6.1	82.8	131.8		3.1	1.0
3,5-difluorobenzylamino	>166.7	>166.7	>166.7	>166.7		21.1	6.9
3,5-dimethoxybenzylamino	>166.7	>166.7	>166.7	>166.7			19.8
2,3,6-trifluorobenzylamino	>166.7	>166.7	>166.7	>166.7			11.3
2-hydroxy-3-methoxybenzylamino	24.1	2.1	30.4	69.7	27.9	0.3	0.5
3-hydroxy-4-methoxybenzylamino	>166.7	>166.7	>166.7	>166.7		79.3	68.9
2-hydroxy-5-methoxybenzylamino	6.4	15.4	21	87.9	12.3	0.9	0.54
2-hydroxy-5-chlorobenzylamino	10.6	15.7	18	63.4	12.5	0.6	0.47
2-hydroxy-5-fluorobenzylamino	9.1	13.4	16	58.7	10.7	0.5	0.53

EXAMPLE 4 Induction of cancer cell apoptosis

To analyse the mechanisms of induced cytotoxicity by the novel compounds, it is important to distinguish apoptosis from the other major form of cell death, necrosis. First, at tissue level, apoptosis produces little or no inflammation, since the resulting cell fragments are engulfed by neighbouring cells, especially by macrophages, rather than being released into the extracellular fluid. In contrast, in necrosis, cellular content is released into the extracellular fluid, and thus has an irritant effect on the nearby cells, causing inflammation. Second, at the cellular level, apoptotic cells exhibit shrinkage and blebbing of the cytoplasm, preservation of structure of cellular organelles including the mitochondria, condensation and margination of chromatin, fragmentation of nuclei, and formation of apoptotic bodies, though not all of these are seen in all cell types. Third, at the molecular level, a number of biochemical processes take an important role in induction of apoptosis. However, majority of them is not well understood, and they result in activation of proteases and nucleases, which finally destruct key biological macromolecules - proteins and DNA.

Effect of novel compounds on activity of caspases-3

In the course of apoptosis, caspase cascade is activated. Caspase-3 is an effector caspase that plays a role in the induction of some changes typical for apoptosis (chromatin condensation, DNA fragmentation). Leukemia cell line CEM was

cultured in cell culture medium (DMEM containing 5g/l glucose, 2mM glutamine, 100 U/ml peniciline, 100 µg/ml streptomycine, 10% fetal calf serum and sodium bicarbonate). 24 hours before threatment, cell suspensions were centrifuged, diluted to concentration of 500000 cells per ml with fresh medium and pipetted in 50 µL aliquotes into 96-well plate. After 12 and 24 hours of incubation with the test compound, the activity of caspase-3 was measured using commecial kit (Apo-ONE, Promega) that is based on quantification of hydrolysis of fluorogenic substrate Z-DEVD-R110 (excitation 480 nm / emission 520 nm). Reaction specifity was confirmed in a control experiment, when both test compound and caspase-3 inhibitor Ac-DEVD-CHO were added. All experiments were done in triplicate.

Figure 3 shows that ortho-topolin riboside-5'-monophosphate (compound 21) activates caspase-3 and that level of the activation is time dependent.

EXAMPLE 5 Analysis of apoptosis using flow cytometry (DNA fragmentation induction)

One of the hallmarks of apoptosis is fragmentation of DNA. It can be measured by flowcytometry of the cells stained with propidium iodide. Because the fragmentation results in a loss of a fraction of the nuclear DNA, apoptotic cells have a lower DNA content than cells in G1 phase of cell cycle and can be detected as a subG1 peak in the histogram.

Leukemia cell line CEM was cultured in cell culture medium (DMEM containing 5g/l glucose, 2mM glutamine, 100 U/ml peniciline, 100 µg/ml streptomycine, 10% fetal calf serum and sodium bicarbonate). 24 hours before threatment, cell suspensions were centrifuged, diluted with fresh medium to concentration of 100000 cells per ml with fresh medium and pipetted in 10 ml aliquotes Petri dishes. After an incubation for a given time period, the cells were collected by centrifugation (4°C , 600 g), washed with PBS (4°C) and fixed with 70% ethanol at -20°C overnight. Low molecular weight DNA fragmentse were extracted with citrate buffer (11,36 g trisodium citrate dihydrate /l). The nuclei were stained with the solution of propidium iodide in PBS (10 µg/ml) containing RNase A (60 IU per sample). After

1 hour incubation the samples were analyzed by flowcytometry (excitation 488 nm, emission above 620 nm).

Figure 2 shows that in leukemia cell line CEM induction of DNA fragmentation is dependent on the concentration of compound **21** and the time of the treatment. The effect was visible already after 12 hours.

EXAMPLE 6 Detection of apoptosis on the basis of cell morphology

Cell culture. A suspension of human promyelocytic HL-60 cells was cultured in RPMI-1640 medium supplemented with a 10% calf foetal serum and antibiotics in 5% CO₂ atmosphere at 37 °C. Cell number was determined using hemacytometer. HL-60 cells were obtained from ECACC.

Cell viability assay. Double staining with fluorescein diacetate (FDA, 2 µg/ml) and propidium iodide (PI, 10 µg/ml) directly in growth medium. The percentage of dead cells (i.e., red stained cells) was evaluated using fluorescence microscopy (Mlejnek and Kolman 1999, Chem. Biol. Interact. 1999, 117: 219-239).

Morphological analysis of cell nuclei. Cells were harvested, washed in PBS, and fixed in an ice-cold methanol + acetic acid (3:1) fixation mixture at -20°C for 12 hours. Aliquots of cells were spread on glass slides and stained with Hoechst 33342 (2 µg/ml) in PBS/glycerol (v/v, 70:30). The morphology of cell nuclei was examined by a fluorescence microscope Olympus BX60 (Mlejnek and Kuglík, J. Cell. Biochem. 2000, 77:6-17). The demonstration of apoptotic cells by this method is shown in Fig. 4.

EXAMPLE 7 Induction of expression p21^{WAF-1} natural cyclin-dependent kinases inhibitor by action of compound **28** in breast cancer cell line MCF-7 – molecular mechanism of action

*p21^{WAF-1} protein level changes in relation to compound **28** concentration*

MCF-7 cells cultured at 37°C in 5% CO₂ atmosphere in DMEM medium supplemented with 10% foetal calf serum were treated with compound **28** concentration ranges of 0-100 µM. Compound **28** was added into the medium from

the 100 mM stock solution in DMSO. Cells were harvested 12 hours after incubation with compound **28**, centrifuged (1000 rpm, 4°C, 5 min), washed twice by ice-cold PBS and re-centrifuged. These washed cells were then lysed by 1x CSB (loading buffer for SDS-PAGE, i.e. electrophoresis of proteins in polyacrylamide gel containing SDS). Cell lysate proteins were separated by SDS-PAGE and transferred onto a nitrocellulose (NC) membrane. NC membrane was blocked by 5% skimmed milk, 0.1% Tween 20 in PBS. Protein level of p21^{WAF-1} and actin (as a loaded protein volume control) were detected immunochemically using commercially available specific monoclonal antibodies anti-p21^{WAF-1} (Ab-1, Calbiochem) and anti-Actin (Clone AC-40, Sigma-Aldrich). The primary antibodies bind to target proteins were detected using rabbit secondary peroxidase-labeled antibody (RAM-Px, DAKO), followed by chemiluminescence (ECL, Amersham-Pharmacia). The efficient p21^{WAF-1} protein induction is achieved in MCF-7 cells by treating these cells with compound **28** in the concentration range and in different treatment times (Fig. 5 and 6).

EXAMPLE 8 Immunosuppressive activity

One of the most important parameters of specific cellular immunity is the proliferative response of lymphocytes to antigens or polyclonal mitogens. The majority of normal mammalian peripheral lymphocytes comprise resting cells. Antigens or nonspecific polyclonal mitogens have the capacity to activate lymphoid cells and this is accompanied by dramatic changes of intracellular metabolism (mitochondrial activity, protein synthesis, nucleic acids synthesis, formation of blastic cells and cellular proliferation). Compounds with ability to selectively inhibit lymphocyte proliferation are potent immunosuppressants. Variety of *in vitro* assays was developed to measure proliferative response of lymphocytes. The most commonly used is ³H-thymidine incorporation method.

During cell proliferation, DNA has to be replicated before the cell is divided into two daughter cells. This close association between cell doublings and DNA synthesis is very attractive for assessing cell proliferation. If labelled DNA precursors are added to the cell culture, cells that are about to divide incorporate the labelled nucleotide into their DNA. Traditionally, those assays usually involve the use of radiolabelled

nucleosides, particularly tritiated thymidine ($[^3\text{H}]\text{-TdR}$). The amount of $[^3\text{H}]\text{-TdR}$ incorporated into the cellular DNA is quantified by liquid scintillation counting.

Human heparinized peripheral blood was obtained from healthy volunteers by cubital vein puncture. The blood was diluted in PBS (1:3) and mononuclear cells were separated by centrifugation in Ficoll-Hypaque density gradient (Pharmacia, 1.077 g/ml) at 2200 rpm for 30 minutes. Following centrifugation, lymphocytes were washed in PBS and resuspended in cell culture medium (RMPI 1640, 2mM glutamine, 100 U/ml penicillin, 100 $\mu\text{g/ml}$ streptomycin, 10% fetal calf serum and sodium bicarbonate).

The cells were diluted at target density of 1.100.000 cells/ml and were added by pipette (180 μl) into 96-well microtiter plates. Four-fold dilutions of the intended test concentration were added at time zero in 20 μl aliquots to the microtiter plate wells. Usually, test compound was evaluated at six 4-fold dilutions. In routine testing, the highest well concentration was 266.7 μM . All drug concentrations were examined in duplicates. All wells with exception of unstimulated controls were activated with 50 μl of concanavalin A (25 $\mu\text{g/ml}$). Incubations of cells with test compounds lasted for 72 hours at 37 $^{\circ}\text{C}$, in 5% CO_2 atmosphere and 100% humidity. At the end of incubation period, the cells were assayed by using the $[^3\text{H}]\text{-TdR}$:

Cell cultures were incubated with 0.5 μCi (20 μl of stock solution 500 $\mu\text{Ci/ml}$) per well for 6 hours at 37 $^{\circ}\text{C}$ and 5% CO_2 . The automated cell harvester was used to lyse cells in water and adsorb the DNA onto glass-fiber filters in the format of microtiter plate. The DNA incorporated $[^3\text{H}]\text{-TdR}$ was retained on the filter while unincorporated material passes through. The filters were dried at room temperature overnight, sealed into a sample bag with 10-12 ml of scintillant. The amount of $[^3\text{H}]\text{-TdR}$ present in each filter (in cpm) was determined by scintillation counting in a Betaplate liquid scintillation counter. The effective dose of immunosuppressant (ED) was calculated using the following equation: $\text{ED} = (\text{CCPM}_{\text{drug exposed well}} / \text{mean CCPM}_{\text{control wells}}) \times 100\%$. The ED_{50} value, the drug concentration inhibiting proliferation of 50% of lymphocytes, was calculated from the obtained dose response curves.

To evaluate immunosuppressive activity of substituted adenines, their ability to inhibit polyclonal mitogen induced proliferation of normal human lymphocytes was

analyzed (Table 4). Our data demonstrate that these compounds have only marginal activity on ^3H -thymidine incorporation in non-activated lymphocytes, nonetheless, they efficiently inhibit proliferation of activated lymphocytes. Effective immunosuppressive dose of new derivatives under in vitro conditions was close to

5 10-40 μM .

Tab. 4. Immunosuppressive activity of novel derivatives

SUBSTITUENT AT 6-POSITION OF PURINE	Human lymphocytes ED ₅₀ (μM)
2-hydroxybenzylamino	6.7
3-hydroxybenzylamino	12.8
2-fluorobenzylamino	6.3
3-fluorobenzylamino	11.5
4-fluorobenzylamino	13.4
2-methylbenzylamino	7.3
3-methylbenzylamino	7.5
3,4-dihydroxybenzylamino	9.4
2-hydroxy-3-chlorobenzylamino	3.2
2-hydroxy-3-methoxybenzylamino	1.2
2-hydroxy-4-methoxybenzylamino	2.1
2-chlorobenzylamino	8.7
3-chlorobenzylamino	3.2
4-chlorobenzylamino	3.3

10

EXAMPLE 9 The effect of novel compounds on plant cell division

Stimulatory effect of newly prepared derivatives was tested using cytokinin-dependent tobacco callus. The cytokinin-dependent tobacco callus *Nicotiana*

15 *tabacum* L. cv. Wisconsin 38 was maintained at 25 °C in darkness on modified MS medium, containing per 1 liter: 4 μmol of nicotinic acid, 2.4 μmol of pyridoxine

hydrochloride, 1.2 μmol of thiamine, 26.6 μmol of glycine, 1.37 μmol of glutamine, 1.8 μmol of myo-inositol, 30 g of sucrose, 8 g of agar, 5.37 μmol of NAA and 0.5 μmol of the compound tested. Subcultivation was carried out every three weeks. Fourteen days before the bioassay, the callus tissue was transferred to the media without 6-benzylaminopurine. The biological activity was determined from the increase of the fresh callus weight after four weeks of cultivation. Five replicates were prepared for each concentration of the compound tested and the entire test was repeated twice. Kinetin (K), which is known to be highly active cytokinin, was used in each experiment as a control. The compounds to be tested were dissolved in dimethylsulfoxide (DMSO) and the solution brought up to 10^{-3} M with distilled water. This stock solution was further diluted with the respective media used for the biotest to a concentration ranging from 10^{-8} M to 10^{-4} M. The final concentration of DMSO did not exceed 0.2 % and therefore did not affect the biological activity in the assay system used. The stimulatory activity was calculated from the dose-dependent fresh weight callus growth curves.

From the obtained data, the concentration with the highest activity was selected for each compound tested. Relative activity of the compound at this concentration was calculated (Table 3). The activity obtained for 10^{-6} M of the control substance kinetin was postulated as 100 % of biological activity.

Novel Substitution Derivatives of N^6 -benzyladenosine-5'-monophosphate functioning as cytokinins should exhibit stimulatory effect on cell division of plant cells. The results in Table 5 show that some of the newly prepared cytokinin derivatives of the general formula I showed strong increase of the cytokinin activity in the callus bioassay in comparison to the classical cytokinin - 10^{-6} M concentration of the control substance kinetin was postulated as 100 %.

Table 5. The effect of Novel Substitution Derivatives of N^6 -benzyladenosine-5'-monophosphate on the Growth of Cytokinin-Dependent Tobacco Callus *Nicotiana tabacum* L. cv. Wisconsin 38

N^6 -Substituent	Concentration with highest activity (mol.l^{-1})	Activity (%) [10^{-5}mol.l^{-1} KR = 100%]

2-fluorobenzylamino	10^{-6}	100±9
3-fluorobenzylamino	10^{-5}	103±6
2-chlorobenzylamino	10^{-6}	121±4
3-chlorobenzylamino	10^{-5}	109±5
2-bromobenzylamino	10^{-5}	100±4
3-bromobenzylamino	10^{-6}	102±11
2-methylbenzylamino	10^{-6}	108±4
2-methoxylbenzylamino	10^{-5}	108±1
3-methoxylbenzylamino	10^{-6}	102±1
3-chloro-4-fluorobenzylamino	10^{-6}	107±4
2-chloro-4-fluorobenzylamino	10^{-5}	108±4
3,5-difluorobenzylamino	10^{-6}	105±3
2,4,5-trifluorobenzylamino	10^{-5}	105±3

Example 10 *In vivo* anticancer activity

Screening of biological activity of compound **21** was performed on survival model of P388D1 leukemia transplanted intraperitoneally with $2 \cdot 10^5$ cells. DBA-2 mice were used as a host. One day following the leukemia transplantation, treatment with **21** was initiated. The drug was given orally by gastric gavages in total volume of 200 μ l/dose twice daily at dose 250 mg/kg in two chemotherapy cycles (Days 1-3 and 7-9). The compound **21** was solubilized in 5% N-methyl-2-pyrrolidine, 30% polyethylene glycol 400, 65% tartar buffer (pH 3.0). Survival analysis of treated animals (10 mice/group) was preformed in comparison with vehicle treated mice using Kaplan-Meier method and the significance was evaluated by the log-rank test. Body weight of experimental animals was evaluated in parallel in order to reflect toxicity and efficacy of therapy. Comparative analysis of body weight was performed using non-parametric t-test.

Results of our analysis demonstrated that compound **21** is reducing body weight of treated animals, which is in the model of intraperitoneally transplanted P388D1 leukemia indicative for both toxicity and efficacy of the treatment (reduction of

ascites and intraperitoneal tumor formation). Survival analysis demonstrated significantly better survival of mice treated with **21** ($P=0.0409$) that has been translated to longer mean survival time (15.4 days for drug treated group versus 13.9 days for vehicle treated group). Results of our analysis demonstrated that compound **21** has good tolerability in animals. There was only slight reduction the body weight of treated animals, which was however significant in one time point (Day 52) only.

Tumor volume analysis showed significantly smaller tumors in mice treated with **21** ($P=0.0409$) that has been translated to longer mean survival time (15.4 days for drug treated group versus 13.9 days for vehicle treated group).

EXAMPLE 11 Dry Capsules

5000 capsules, each of which contains 0.25 g of a compound of the formula **I** as an active ingredient, are prepared as follows:

Composition

Active ingredient	1250 g
Talc	180 g
Wheat starch	120 g
Magnesium stearate	80 g
Lactose	20 g

Preparation process: The powdered substances mentioned are pressed through a sieve of mesh width 0.6 mm. Portions of 0.33 g of the mixture are transferred to gelatine capsules with the aid of a capsule-filling machine.

EXAMPLE 12 Soft Capsules

5000 soft gelatine capsules, each of which contains 0.05 g of a compound of the formula **I** as an active ingredient, are prepared as follows:

Composition

Active ingredient 250 g

Lauroglycol 2 litres

Preparation process: The powdered active ingredient is suspended in Lauroglykol[®] (propylene glycol laurate, Gattefossé S.A., Saint Priest, France) and ground in a wet-pulveriser to a particle size of about 1 to 3 μ m. Portions of in each case 0.419 g of the mixture are then transferred to soft gelatine capsules by means of a capsule-filling machine.

EXAMPLE 13 Soft Capsules

10

5000 soft gelatine capsules, each of which contains 0.05 g of a compound of the formula I as an active ingredient, are prepared as follows:

Composition

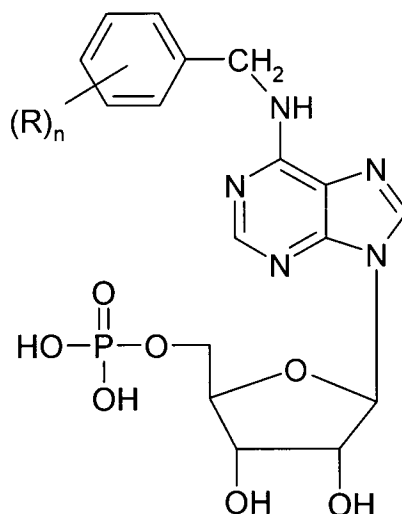
15 Active ingredient 250 g
PEG 400 1 litre
Tween 80 1 litre

Preparation process: The powdered active ingredient is suspended in PEG 400 (polyethylene glycol of Mr between 380 and about 420, Sigma, Fluka, Aldrich, USA) and Tween[®] 80 (polyoxyethylene sorbitan monolaurate, Atlas Chem. Inc., Inc., USA, supplied by Sigma, Fluka, Aldrich, USA) and ground in a wet-pulveriser to a particle size of about 1 to 3 mm. Portions of in each case 0.43 g of the mixture are then transferred to soft gelatine capsules by means of a capsule-filling machine.

25

CLAIMS

1. Substitution derivatives of N⁶-benzyladenosine-5'-monophosphate of the general formula I,



I

wherein

(R)_n represents 1 to 4 substituents R, which can be the same or different,

10 and R is selected from the group comprising C₁ to C₈ alkyl, C₁ to C₈ alkoxy, amino, halogen, hydroxy, mercapto and nitro groups, and their pharmaceutically acceptable salts.

2. Substitution derivatives of N⁶-benzyladenosine-5'-monophosphate of the formula I

15 according to claim 1, wherein the compounds are selected from the group containing

6-(2-fluorobenzylamino)purine	riboside-5'-monophosphate,	6-(3-
fluorobenzylamino)purine	riboside-5'-monophosphate,	6-(4-
fluorobenzylamino)purine	riboside-5'-monophosphate	, 6-(2-
bromobenzylamino)purine	riboside-5'-monophosphate,	6-(3-
20 bromobenzylamino)purine	riboside-5'-monophosphate,	6-(4-
bromobenzylamino)purine	riboside-5'-monophosphate,	6-(2-iodobenzylamino)purine
riboside-5'-monophosphate,	6-(3-iodobenzylamino)purine	riboside-5'-

	monophosphate, 6-(4-iodobenzylamino)purine	riboside-5'-monophosphate, 6-(2-chlorobenzylamino)purine	riboside-5'-monophosphate, 6-(3-chlorobenzylamino)purine	riboside-5'-monophosphate, 6-(4-chlorobenzylamino)purine	riboside-5'-monophosphate, 6-(2-nitrobenzylamino)purine
5	nitrobenzylamino)purine	riboside-5'-monophosphate, 6-(3-nitrobenzylamino)purine	riboside-5'-monophosphate, 6-(4-nitrobenzylamino)purine	riboside-5'-monophosphate, 6-(5-nitro-2-methylbenzylamino)purine	riboside-5'-monophosphate, 6-(2-methylbenzylamino)purine
	methylbenzylamino)purine	riboside-5'-monophosphate, 6-(3-methylbenzylamino)purine	riboside-5'-monophosphate, 6-(4-methylbenzylamino)purine	riboside-5'-monophosphate, 6-(2-methoxybenzylamino)purine	riboside-5'-monophosphate, 6-(3-methoxybenzylamino)purine
10	methoxybenzylamino)purine	riboside-5'-monophosphate, 6-(3-methoxybenzylamino)purine	riboside-5'-monophosphate, 6-(4-methoxybenzylamino)purine	riboside-5'-monophosphate, 6-(2-hydroxybenzylamino)purine	riboside-5'-monophosphate, 6-(3-hydroxybenzylamino)purine
15	hydroxybenzylamino)purine	riboside-5'-monophosphate, 6-(4-hydroxybenzylamino)purine	riboside-5'-monophosphate, 6-(2-ethoxybenzylamino)purine	riboside-5'-monophosphate, 6-(3-ethoxybenzylamino)purine	riboside-5'-monophosphate, 6-(4-ethoxybenzylamino)purine
20	ethylbenzylamino)purine	riboside-5'-monophosphate, 6-(4-pentylbenzylamino)purine	riboside-5'-monophosphate, 6-(4-pentyloxybenzylamino)purine	riboside-5'-monophosphate, 6-(2,5-diaminobenzylamino)purine	riboside-5'-monophosphate, 6-(3,5-dibromobenzylamino)purine
25	methoxybenzylamino)purine	riboside-5'-monophosphate, 6-(2,3-dichlorobenzylamino)purine	riboside-5'-monophosphate, 6-(2,4-dichlorobenzylamino)purine	riboside-5'-monophosphate, 6-(2,5-dichlorobenzylamino)purine	riboside-5'-monophosphate, 6-(2,6-dichlorobenzylamino)purine
30	dichlorobenzylamino)purine	riboside-5'-monophosphate, 6-(3,4-dichlorobenzylamino)purine	riboside-5'-monophosphate, 6-(3,5-dichlorobenzylamino)purine	riboside-5'-monophosphate, 6-(2,3,4,5-tetrafluorobenzylamino)purine	riboside-5'-monophosphate, 6-(2-chloro-3,6-

	difluorobenzylamino)purine	riboside-5'-monophosphate,	6-(5-chloro-2-
	fluorobenzylamino)purine	riboside-5'-monophosphate,	6-(2,3,4-
	trifluorobenzylamino)purine	riboside-5'-monophosphate,	6-(2,3,5-
	trifluorobenzylamino)purine	riboside-5'-monophosphate,	6-(2,4,5-
5	trifluorobenzylamino)purine	riboside-5'-monophosphate,	6-(3,4,5-
	trifluorobenzylamino)purine	riboside-5'-monophosphate,	6-(2,3,6-
	trifluorobenzylamino)purine	riboside-5'-monophosphate,	6-(3-chloro-2,6-
	difluorobenzylamino)purine	riboside-5'-monophosphate,	6-(2-chloro-6-
	fluorobenzylamino)purine	riboside-5'-monophosphate,	6-(2,6-
10	difluorobenzylamino)purine	riboside-5'-monophosphate,	6-(2,4-
	difluorobenzylamino)purine	riboside-5'-monophosphate,	6-(3,4-
	difluorobenzylamino)purine	riboside-5'-monophosphate,	6-(2,5-
	difluorobenzylamino)purine	riboside-5'-monophosphate,	6-(3,5-
	difluorobenzylamino)purine	riboside-5'-monophosphate,	6-(2-chloro-6-fluoro-3-
15	methylbenzylamino)purine	purine riboside-5'-monophosphate,	6-(6-chloro-2-fluoro-
	3-methylbenzylamino)purine	riboside-5'-monophosphate,	6-(2,3-difluoro-4-
	methylbenzylamino)purine	riboside-5'-monophosphate,	6-(2,6-difluoro-3-
	methylbenzylamino)purine	riboside-5'-monophosphate,	6-(3-chloro-2,6-
	difluorobenzylamino)purine	riboside-5'-monophosphate,	6-(3-fluoro-4-methyl
20	benzylamino)purine	riboside-5'-monophosphate,	6-(4-fluoro-3-
	methylbenzylamino)purine	riboside-5'-monophosphate,	6-(5-fluoro-2-
	methylbenzylamino)purine	riboside-5'-monophosphate,	6-(2-chloro-3,6-
	difluorobenzylamino)purine	riboside-5'-monophosphate,	6-(2-chloro-4-
	fluorobenzylamino)purine	riboside-5'-monophosphate,	6-(3,4-
25	dihydroxybenzylamino)purine	riboside-5'-monophosphate,	6-(3,5-
	dihydroxybenzylamino)purine	riboside-5'-monophosphate,	6-(3,4-
	dihydroxybenzylamino)purine	riboside-5'-monophosphate,	6-(2,4-
	dihydroxybenzylamino)purine	riboside-5'-monophosphate,	6-(2,5-
	dihydroxybenzylamino)purine	riboside-5'-monophosphate,	6-(2,6-
30	dihydroxybenzylamino)purine	riboside-5'-monophosphate,	6-(3,4-
	dimethoxybenzylamino)purine	riboside-5'-monophosphate,	6-(3,5-
	dimethoxybenzylamino)purine	riboside-5'-monophosphate,	6-(2,3-

	dimethoxybenzylamino)purine	riboside-5'-monophosphate,	6-(2,4-
	dimethoxybenzylamino)purine	riboside-5'-monophosphate,	6-(2,5-
	dimethoxybenzylamino)purine	riboside-5'-monophosphate,	6-(2,6-
	dimethoxybenzylamino)purine	riboside-5'-monophosphate,	6-(2-hydroxy-3-
5	methoxybenzylamino)purine	riboside-5'-monophosphate,	6-(2-hydroxy-4-
	methoxybenzylamino)purine	riboside-5'-monophosphate,	6-(2-hydroxy-5-
	methoxybenzylamino)purine	riboside-5'-monophosphate,	6-(2-hydroxy-6-
	methoxybenzylamino)purine	riboside-5'-monophosphate,	6-(3-hydroxy-2-
	methoxybenzylamino)purine	riboside-5'-monophosphate,	6-(3-hydroxy-4-
10	methoxybenzylamino)purine	riboside-5'-monophosphate,	6-(3-hydroxy-5-
	methoxybenzylamino)purine	riboside-5'-monophosphate,	6-(3-hydroxy-6-
	methoxybenzylamino)purine	riboside-5'-monophosphate,	6-(4-hydroxy-2-
	methoxybenzylamino)purine	riboside-5'-monophosphate,	6-(4-hydroxy-3-
	methoxybenzylamino)purine	riboside-5'-monophosphate,	6-(4-hydroxy-5-
15	methoxybenzylamino)purine	riboside-5'-monophosphate,	6-(4-hydroxy-6-
	methoxybenzylamino)purine	riboside-5'-monophosphate,	6-(2-hydroxy-3,4-
	dimethoxybenzylamino)purine	riboside-5'-monophosphate,	6-(2-hydroxy-3,5-
	dimethoxybenzylamino)purine	riboside-5'-monophosphate,	6-(2-hydroxy-3,6-
	dimethoxybenzylamino)purine	riboside-5'-monophosphate,	6-(2-hydroxy-4,5-
20	dimethoxybenzylamino)purine	riboside-5'-monophosphate,	6-(2-hydroxy-4,6-
	dimethoxybenzylamino)purine	riboside-5'-monophosphate,	6-(2-hydroxy-5,6-
	dimethoxybenzylamino)purine	riboside-5'-monophosphate,	6-(3-hydroxy-4,5-
	dimethoxybenzylamino)purine	riboside-5'-monophosphate,	6-(3-hydroxy-4,6-
	dimethoxybenzylamino)purine	riboside-5'-monophosphate,	6-(3-hydroxy-2,4-
25	dimethoxybenzylamino)purine	riboside-5'-monophosphate,	6-(3-hydroxy-2,5-
	dimethoxybenzylamino)purine	riboside-5'-monophosphate,	6-(3-hydroxy-2,6-
	dimethoxybenzylamino)purine	riboside-5'-monophosphate,	6-(4-hydroxy-2,3-
	dimethoxybenzylamino)purine	riboside-5'-monophosphate,	6-(4-hydroxy-2,5-
	dimethoxybenzylamino)purine	riboside-5'-monophosphate,	6-(4-hydroxy-2,6-
30	dimethoxybenzylamino)purine	riboside-5'-monophosphate,	6-(4-hydroxy-3,5-
	dimethoxybenzylamino)purine	riboside-5'-monophosphate,	6-(4-hydroxy-3,6-
	dimethoxybenzylamino)purine	riboside-5'-monophosphate,	6-(2,3-dihydroxy-4-

	methoxybenzylamino)purine	riboside-5'-monophosphate,	6-(2,3-dihydroxy-5-
	methoxybenzylamino)purine	riboside-5'-monophosphate,	6-(2,3-dihydroxy-6-
	methoxybenzylamino)purine	riboside-5'-monophosphate,	6-(2,4-dihydroxy-3-
	methoxybenzylamino)purine	riboside-5'-monophosphate,	6-(2,4-dihydroxy-5-
5	methoxybenzylamino)purine	riboside-5'-monophosphate,	6-(2,4-dihydroxy-6-
	methoxybenzylamino)purine	riboside-5'-monophosphate,	6-(2,5-dihydroxy-3-
	methoxybenzylamino)purine	riboside-5'-monophosphate,	6-(2,5-dihydroxy-4-
	methoxybenzylamino)purine	riboside-5'-monophosphate,	6-(2,5-dihydroxy-6-
	methoxybenzylamino)purine	riboside-5'-monophosphate,	6-(2,6-dihydroxy-3-
10	methoxybenzylamino)purine	riboside-5'-monophosphate,	6-(2,6-dihydroxy-4-
	methoxybenzylamino)purine	riboside-5'-monophosphate,	6-(2,6-dihydroxy-5-
	methoxybenzylamino)purine	riboside-5'-monophosphate,	6-(3,4-dihydroxy-2-
	methoxybenzylamino)purine	riboside-5'-monophosphate,	6-(3,4-dihydroxy-5-
	methoxybenzylamino)purine	riboside-5'-monophosphate,	6-(3,4-dihydroxy-6-
15	methoxybenzylamino)purine	riboside-5'-monophosphate,	6-(3,5-dihydroxy-2-
	methoxybenzylamino)purine	riboside-5'-monophosphate,	6-(3,5-dihydroxy-4-
	methoxybenzylamino)purine	riboside-5'-monophosphate,	6-(3,5-dihydroxy-6-
	methoxybenzylamino)purine	riboside-5'-monophosphate,	6-(2,3,4-
	trimethoxybenzylamino)purine	riboside-5'-monophosphate,	6-(2,4,5-
20	trimethoxybenzylamino)purine	riboside-5'-monophosphate,	6-(2,4,6-
	trimethoxybenzylamino)purine	riboside-5'-monophosphate,	6-(3,4,5-
	trimethoxybenzylamino)purine	riboside-5'-monophosphate,	6-(2-hydroxy-3,4,5-
	trimethoxybenzylamino)purine	riboside-5'-monophosphate,	6-(2-hydroxy-3,4,6-
	trimethoxybenzylamino)purine	riboside-5'-monophosphate,	6-(2-hydroxy-4,5,6-
25	trimethoxybenzylamino)purine	riboside-5'-monophosphate,	6-(2,4,6-
	trimethoxybenzylamino)purine	riboside-5'-monophosphate,	6-(2,3,4-
	trihydroxybenzylamino)purine	riboside-5'-monophosphate,	6-(2,4,6-
	trihydroxybenzylamino)purine	riboside-5'-monophosphate,	6-(2,3,4-
	trihydroxybenzylamino)purine	riboside-5'-monophosphate,	6-(3,4,5-
30	trihydroxybenzylamino)purine	riboside-5'-monophosphate,	6-(2,4,6-
	trihydroxybenzylamino)purine	riboside-5'-monophosphate,	6-(2-hydroxy-3-
	chlorobenzylamino)purine	riboside-5'-monophosphate,	6-(2-hydroxy-4-

	chlorobenzylamino)purine	riboside-5'-monophosphate,	6-(2-hydroxy-5-
	chlorobenzylamino)purine	riboside-5'-monophosphate,	6-(2-hydroxy-6-
	chlorobenzylamino)purine	riboside-5'-monophosphate,	6-(2-hydroxy-3-
	iodobenzylamino)purine	riboside-5'-monophosphate,	6-(2-hydroxy-4-
5	iodobenzylamino)purine	riboside-5'-monophosphate,	6-(2-hydroxy-5-
	iodobenzylamino)purine	riboside-5'-monophosphate,	6-(2-hydroxy-6-
	iodobenzylamino)purine	riboside-5'-monophosphate,	6-(2-hydroxy-3-
	bromobenzylamino)purine	riboside-5'-monophosphate,	6-(2-hydroxy-4-
	bromobenzylamino)purine	riboside-5'-monophosphate,	6-(2-hydroxy-5-
10	bromobenzylamino)purine	riboside-5'-monophosphate,	6-(2-hydroxy-6-
	bromobenzylamino)purine	riboside-5'-monophosphate,	6-(2-hydroxy-3-
	fluorobenzylamino)purine	riboside-5'-monophosphate,	6-(2-hydroxy-4-
	fluorobenzylamino)purine	riboside-5'-monophosphate,	6-(2-hydroxy-5-
	fluorobenzylamino)purine	riboside-5'-monophosphate,	6-(2-hydroxy-6-
15	fluorobenzylamino)purine	riboside-5'-monophosphate,	6-(2-hydroxy-3-
	methylbenzylamino)purine	riboside-5'-monophosphate,	6-(2-hydroxy-4-
	methylbenzylamino)purine	riboside-5'-monophosphate,	6-(2-hydroxy-5-
	methylbenzylamino)purine	riboside-5'-monophosphate,	6-(2-hydroxy-6-
	methylbenzylamino)purine	riboside-5'-monophosphate,	6-(2,3-dihydroxy-4-
20	chloroobenzylamino)purine	riboside-5'-monophosphate,	6-(2,3-dihydroxy-5-
	chlorobenzylamino)purine	riboside-5'-monophosphate,	6-(2,5-dihydroxy-4-
	chlorobenzylamino)purine	riboside-5'-monophosphate,	6-(2,6-dihydroxy-4-
	chlorobenzylamino)purine	riboside-5'-monophosphate,	6-(2,6-dihydroxy-4-
	iodobenzylamino)purine	riboside-5'-monophosphate,	6-(2,6-dihydroxy-3-
25	chlorobenzylamino)purine	riboside-5'-monophosphate,	6-(2,6-dihydroxy-3-
	bromobenzylamino)purine	riboside-5'-monophosphate,	6-(2,6-dihydroxy-3-
	iodobenzylamino)purine	riboside-5'-monophosphate,	6-(2,6-dihydroxy-3-
	fluoroobenzylamino)purine	riboside-5'-monophosphate,	6-(2,6-dihydroxy-3,5-
	dichlorobenzylamino)purine	riboside-5'-monophosphate,	6-(2,6-dihydroxy-3,5-
30	dibromobenzylamino)purine	riboside-5'-monophosphate,	6-(2,6-dihydroxy-3,5-
	diiodobenzylamino)purine	riboside-5'-monophosphate,	6-(2,6-dihydroxy-3,5-
	difluorobenzylamino)purine	riboside-5'-monophosphate,	6-(4,5-dimethoxy-2-

	nitrobenzylamino)purine	riboside-5'-monophosphate,	6-(3,4-
	dimethylbenzylamino)purine	riboside-5'-monophosphate,	6-(2,3-
	dimethylbenzylamino)purine	riboside-5'-monophosphate,	6-(2,4-
	dimethylbenzylamino)purine	riboside-5'-monophosphate,	6-(2,6-
5	dimethylbenzylamino)purine	riboside-5'-monophosphate,	6-(2,6-dimethyl-4-
	hydroxybenzylamino)purine	riboside-5'-monophosphate,	6-(3,5-dimethyl-4-
	hydroxybenzylamino)purine	riboside-5'-monophosphate,	6-(2-fluoro-4-
	hydroxybenzylamino)purine	riboside-5'-monophosphate,	6-(3-fluoro-4-
	methylbenzylamino)purine	riboside-5'-monophosphate,	6-(3,4-
10	dinitrobenzylamino)purine	riboside-5'-monophosphate,	6-(3,5-
	dinitrobenzylamino)purine	riboside-5'-monophosphate,	6-(2-methyl-5-
	nitrobenzylamino)purine	riboside-5'-monophosphate,	6-(3-methyl-4-
	nitrobenzylamino)purine	riboside-5'-monophosphate,	6-(3,4-diiodo-4-
	hydroxybenzylamino)purine	riboside-5'-monophosphate,	6-(2-chloro-3,4-
15	dimethoxybenzylamino)purine	riboside-5'-monophosphate,	6-(4-chloro-3,5-
	dinitrobenzylamino)purine	riboside-5'-monophosphate,	6-(2-chloro-4-
	fluorobenzylamino)purine	riboside-5'-monophosphate,	6-(3-chloro-4-
	fluorobenzylamino)purine	riboside-5'-monophosphate,	6-(2-chloro-6-
	methylbenzylamino)purine	riboside-5'-monophosphate,	6-(3-chloro-2-
20	methylbenzylamino)purine	riboside-5'-monophosphate,	6-(3-chloro-4-
	methylbenzylamino)purine	riboside-5'-monophosphate,	6-(5-chloro-2-
	methoxybenzylamino)purine	riboside-5'-monophosphate,	6-(2-chloro-4-
	fluorobenzylamino)purine	riboside-5'-monophosphate,	6-(2-chloro-5-
	nitrobenzylamino)purine	riboside-5'-monophosphate,	6-(2-chloro-6-
25	nitrobenzylamino)purine	riboside-5'-monophosphate,	6-(4-chloro-3-
	nitrobenzylamino)purine	riboside-5'-monophosphate,	6-(5-chloro-2-
	nitrobenzylamino)purine	riboside-5'-monophosphate,	6-(3-bromo-4-
	hydroxybenzylamino)purine	riboside-5'-monophosphate,	6-(3,5-dibromo-4-
	hydroxybenzylamino)purine,	6-(3-bromo-4-methoxybenzylamino)purine,	6-(4-
30	butoxybenzylamino)purine	riboside-5'-monophosphate,	6-(4-
	butoxybenzylamino)purine	riboside-5'-monophosphate,	6-(4-t-
	butyl/benzylamino)purine	riboside-5'-monophosphate,	6-(4-t-butyl-2,6-

	dimethylbenzylamino)purine	riboside-5'-monophosphate,	6-(2-
	aminobenzylamino)purine	riboside-5'-monophosphate,	6-(3-
	aminobenzylamino)purine	riboside-5'-monophosphate,	6-(4-
	aminobenzylamino)purine	riboside-5'-monophosphate,	6-(2-amino-3-
5	chlorobenzylamino)purine	riboside-5'-monophosphate,	6-(2-amino-4-
	chlorobenzylamino)purine	riboside-5'-monophosphate,	6-(2-amino-5-
	chlorobenzylamino)purine	riboside-5'-monophosphate,	6-(2-amino-6-
	chlorobenzylamino)purine	riboside-5'-monophosphate,	6-(3-amino-2-
	chlorobenzylamino)purine	riboside-5'-monophosphate,	6-(3-amino-4-
10	chlorobenzylamino)purine	riboside-5'-monophosphate,	6-(3-amino-5-
	chlorobenzylamino)purine	riboside-5'-monophosphate,	6-(3-amino-6-
	chlorobenzylamino)purine	riboside-5'-monophosphate,	6-(2,6-diamino-3-
	chlorobenzylamino)purine	riboside-5'-monophosphate,	6-(2,6-diamino-4-
	chlorobenzylamino)purine	riboside-5'-monophosphate,	6-(4-amino-3-
15	chlorobenzylamino)purine	riboside-5'-monophosphate,	6-(4-amino-5-
	dichlorobenzylamino)purine	riboside-5'-monophosphate,	6-(5-amino-2-
	methylbenzylamino)purine	riboside-5'-monophosphate,	6-(2-amino-3-
	nitrobenzylamino)purine	riboside-5'-monophosphate,	6-(4-amino-3-
	nitrobenzylamino)purine	riboside-5'-monophosphate,	6-(2,4,5-
20	trichlorobenzylamino)purine	riboside-5'-monophosphate,	6-(2,4,5-
	trichlorobenzylamino)purine	riboside-5'-monophosphate,	6-(2,4,6-
	trichlorobenzylamino)purine	riboside-5'-monophosphate,	6-(2,3,4-
	trichlorobenzylamino)purine	riboside-5'-monophosphate,	6-(2,3,5-
	trichlorobenzylamino)purine	riboside-5'-monophosphate,	6-(2,3,6-
25	trichlorobenzylamino)purine	riboside-5'-monophosphate,	6-(2,5,6-
	trichlorobenzylamino)purine	riboside-5'-monophosphate.	

3. A method of preparation of a substitution derivative of N⁶-benzyladenosine-5'-monophosphate of the general formula I according to claim 1 or 2, which comprises a

30 nucleophilic substitution of 6-halogenopurine riboside-5'-monophosphate, wherein halogen is selected from the group containing bromine, chlorine or fluorine, with

benzylamine substituted by one to four substituents R, which can be the same or different.

4. Substitution derivatives of N⁶-benzyladenosine-5'-monophosphate of the general formula I according to claim 1 or 2 for use as medicaments.

5. Substitution derivatives of N⁶-benzyladenosine-5'-monophosphate of the general formula I according to claim 1 or 2 for use as protein kinase inhibitors.

6. Substitution derivatives of N⁶-benzyladenosine-5'-monophosphate of the general formula I according to claim 1 or 2 for use for treating disorders involving aberrant cell proliferation and/or aberrant cell death.

7. Substitution derivatives of N⁶-benzyladenosine-5'-monophosphate of the general formula I according to claim 1 or 2 for use in the treatment of a disorder selected from the group comprising cancer, leukemia, restenosis, rheumatoid arthritis, lupus, type I diabetes, multiple sclerosis, Alzheimer's disease, parasitoses caused by animals, fungi and protists, polycystic kidney disease, graft rejection, graft versus host disease, and gout.

8. Use of substitution derivatives of N⁶-benzyladenosine-5'-monophosphate of the general formula I according to claim 1 or 2 in the manufacture of a medicament for the treatment of disorders which involve aberrant cell proliferation and/or aberrant cell death, such as cancer, leukemia, restenosis, rheumatoid arthritis, lupus, type I diabetes, multiple sclerosis, Alzheimer's disease, parasitoses caused by animals, fungi and protists, polycystic kidney disease, graft rejection, graft versus host disease, or gout.

9. Substitution derivatives of N⁶-benzyladenosine-5'-monophosphate of the general formula I according to claim 1 or 2 for use as medicaments for suppressing immunostimulation or in suppression of transplant rejection.

10. Use of substitution derivatives of N⁶-benzyladenosine-5'-monophosphate of the general formula I according to claim 1 or 2 in the manufacture of a medicament for the treatment of disorders which involve suppressing immunostimulation or in suppression of transplant rejection.

5

11. Use of substitution derivatives of N⁶-benzyladenosine-5'-monophosphate of the general formula I according to claim 1 or 2 in tissue cultures for regulation of tissue proliferation and morphogenesis.

10 12. Use of substitution derivatives of N⁶-benzyladenosine-5'-monophosphate of the general formula I according to claim 1 or 2 in affinity absorption matrices, immobilised enzymes for process control, immunoassay reagents, diagnostic samples, as well as compounds and oligonucleotides labeled by ¹⁴C, ³H, avidin or biotin.

15

13. Use of substitution derivatives of N⁶-benzyladenosine-5'-monophosphate of the general formula I according to claim 1 or 2 for *in vitro* cloning of plant and mammalian, preferably mammalian, except for the human, embryonic cells and embryos.

20

14. Use of substitution derivatives of N⁶-benzyladenosine-5'-monophosphate of the general formula I according to claim 1 or 2 as growth regulators of plants, microorganisms, yeast, fungi and animals, preferably mammals except for the human.

25

15. A therapeutic composition, characterized in that it contains at least one substitution derivative of N⁶-benzyladenosine-5'-monophosphate of the general formula I according to claim 1 or 2 or its pharmaceutically acceptable salt, and at least one pharmaceutically acceptable carrier.

30

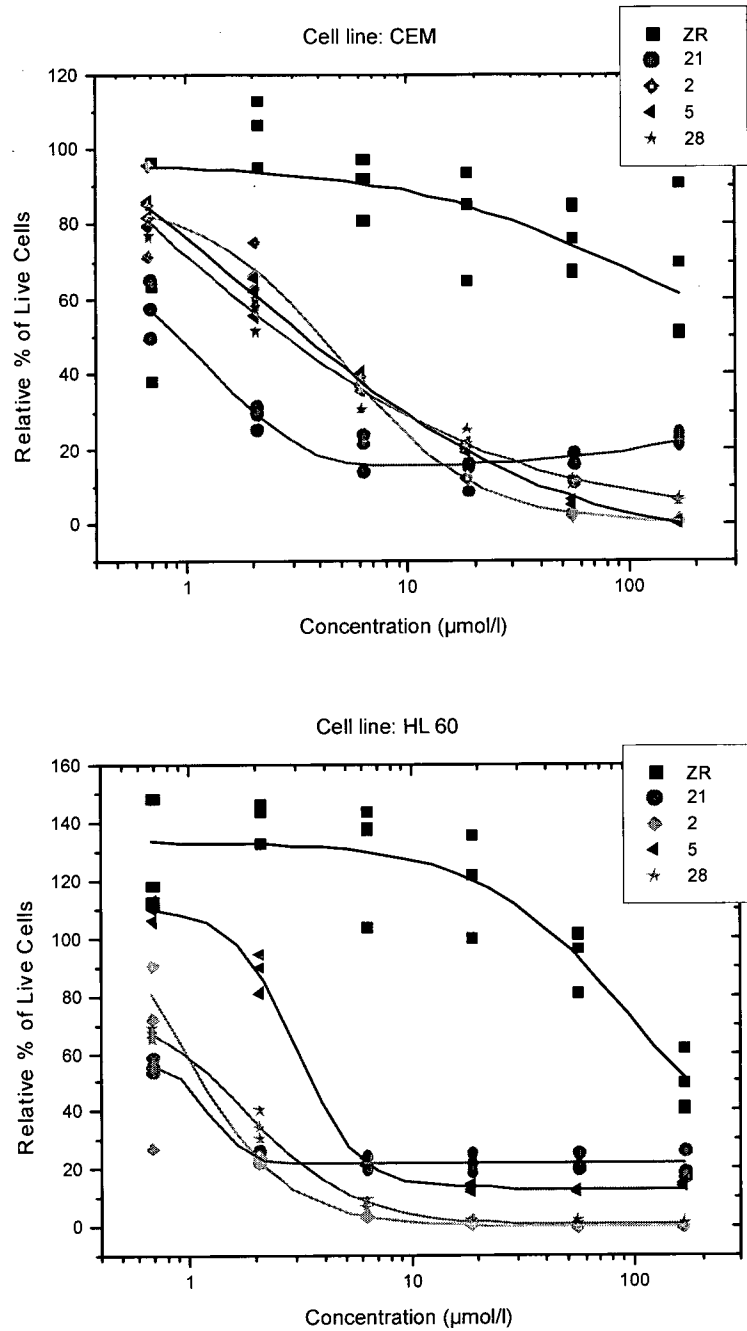


Fig. 1

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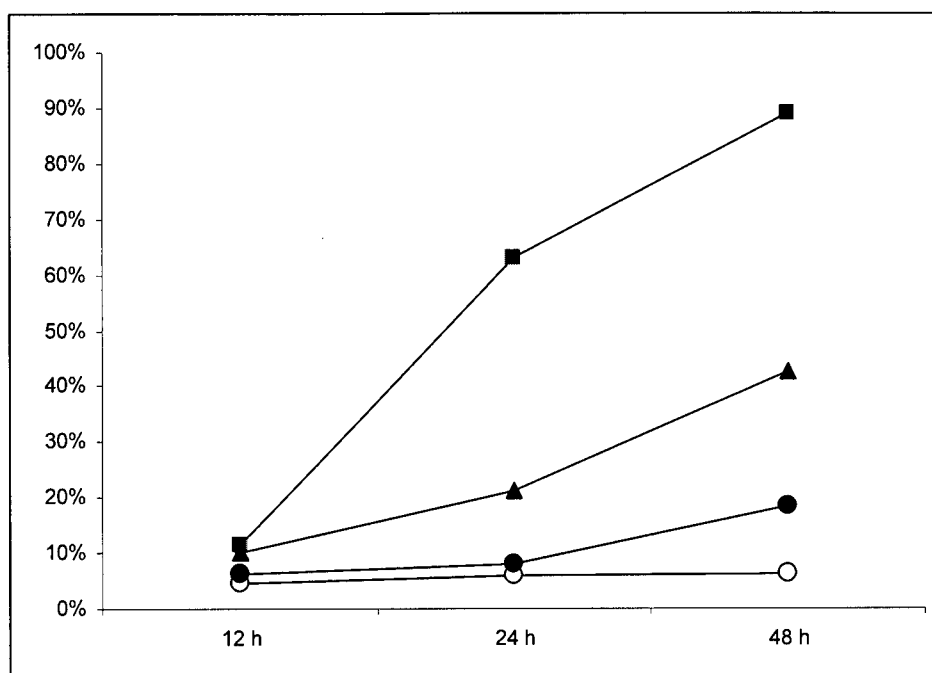


Fig. 2

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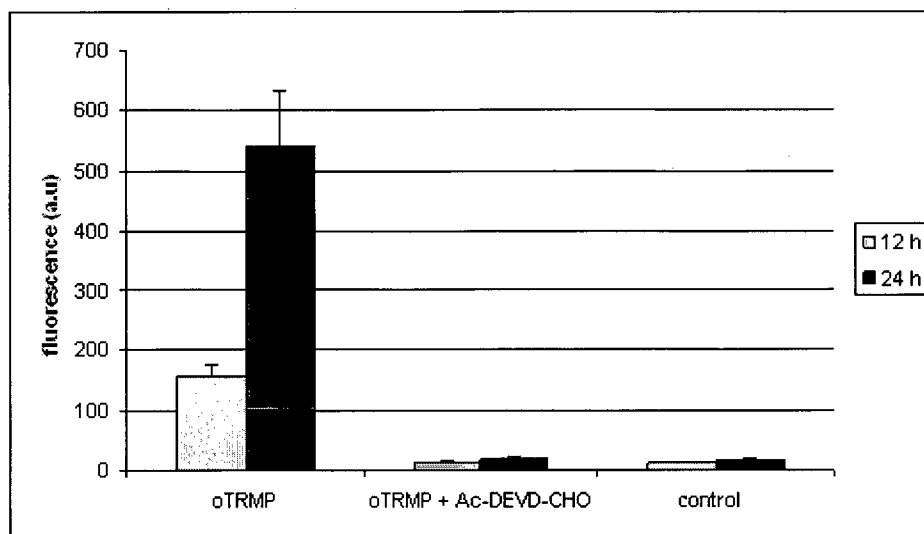


Fig. 3

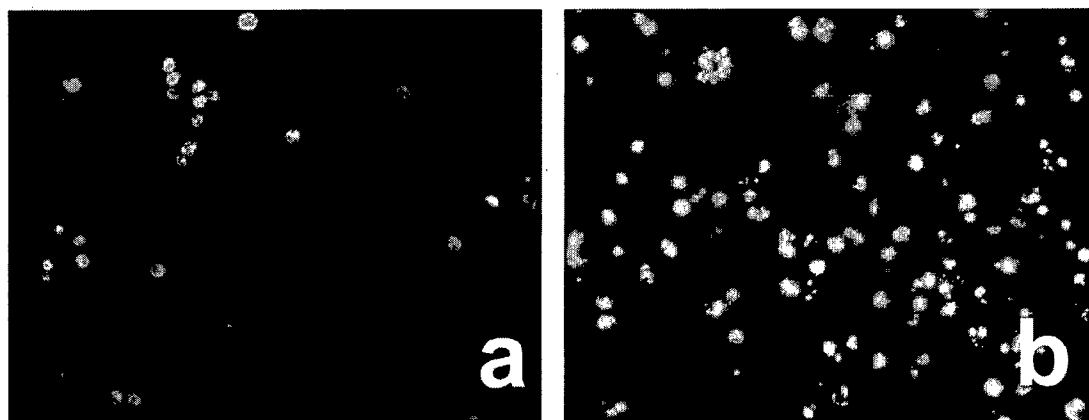


Fig. 4

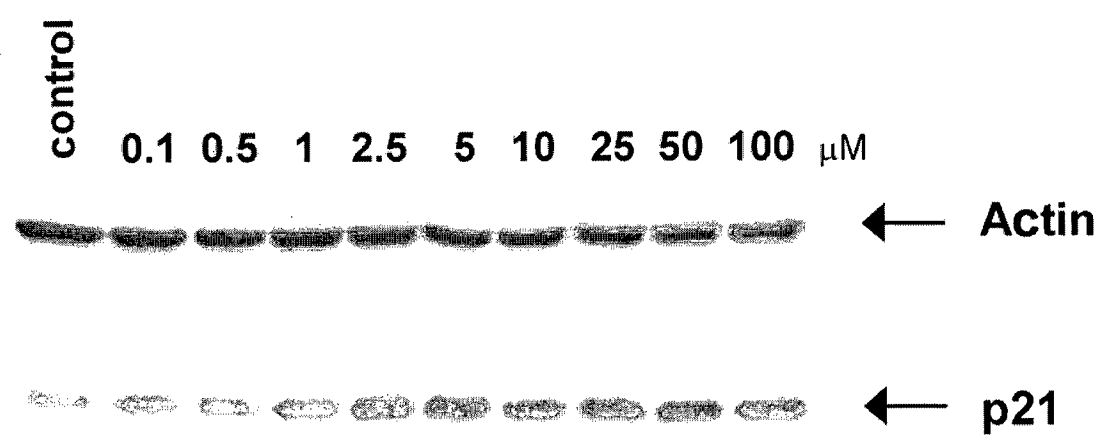


Fig. 5

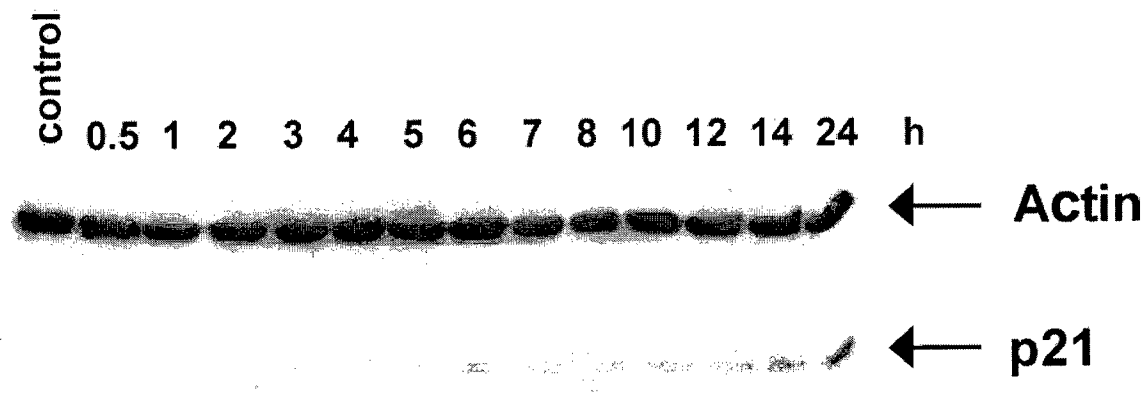
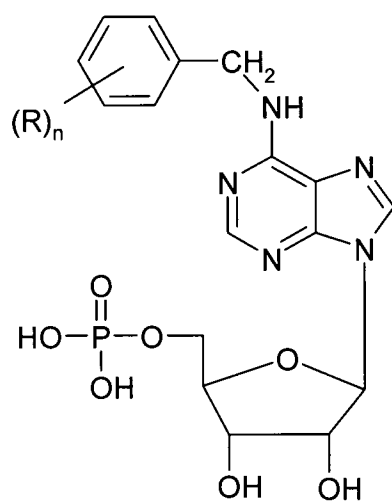


Fig. 6

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Fig. 7