Senolytic compounds

Description

The present invention relates to the novel use of compounds as senolytic according to the preambles of claims 1 and 26, to novel compounds according to the preamble of claim 28, as well as to a medicament according to claim 32.

Compounds containing the 3-substituted pyrimido-[5,6-e]-1,2,4-triazine-5,7-dione as core structure (general formula (I) have been reported biologically active in multiple bioassays thus complying with the definition of frequent hitters or pan-assay interference compounds (PAINS) (Baell et al., 2010; Baell et al., 2014; Dahlin et al., 2016; Vidler et al., 2018; Baell et al., 2018) The reported bioactivities include antibacterial (Levenberg and Linton, 1966; Latuasan and Berends, 1961; Nagamatsu et al., 1993), antifungal (Li et al., 2019), and anticancer assays (Wei et al., 2010; Raoof et al., 2013; Franci et al., 2017). Further bioactivities as protein target kinases (Hayward et al., 2010), chaperones (Zhou et al., 2009), transcription factor TCF4/ß-catenin (Wei et al., 2010; Mao et al., 2014), and tyrosyl-DNA phosphodiesterase 2 (Raoof et al., 2013) have been identified. The core structure was first discovered in the natural product toxoflavin, a toxic metabolite of *Pseudomonas cocovenenans* (Latuasan and Berends, 1961). Therefore, the substances are also referred to as toxoflavins. There have been several accounts on the redox activity of 3-substituted pyrimido-triazines (Light and Walsh, 1980; Stewart et al., 1981; Walsh et al., 1978; Yoneda et al., 1975; Todorovic et al., 2010), however, a detailed and quantitative characterization of the mechanism responsible for activity and a general technical functionality conferred by this mechanism is missing so far.

Yoneda et al. (1971), Yoneda et al. (1973), and Yoneda et al. (1975) describe some of the toxoflavins, their synthesis method, and their reactivity.

JP H09255681 A describes an antitumor agent comprising a 7-azapteridine derivative of a pyrimido-[5,6-e]-1,2,4-triazine-5,7-dione.

Likewise, WO 2010/014798 A2 describes pyrimido-[5,6-e]-1,2,4-triazine-5,7-diones and their use as anti-cancer drug.

DE 103 01 788 A1 describes various pyrimido-[5,6-e]-1,2,4-triazine-5,7-diones, their manufacturing method and their use as drug for treating diabetes.

WO 2012/006104 A2 describes pyrimido-[5,6-e]-1,2,4-triazine-5,7-diones and their use as drug for treating tuberculosis.

WO 2018/140762 A1 describes pyrimido-[5,6-e]-1,2,4-triazine-5,7-diones and their use as drug for treating human immunodeficiency virus (HIV) infections, in particular for inhibiting HIV-1 integrase multimerization.

WO 2010/072807 A2 describes pyrimido-[5,6-e]-1,2,4-triazine-5,7-diones and their use as inhibitors of cystathionine beta synthase which, among other biochemical effects, allow reduction of the neurotoxic overproduction of endogenous hydrogen sulfide.

Senescent cells are cells no longer performing their native biological functions. Similar to apoptosis as another cell-cycle exit program, senescence is a natural cellular stress-responsive mechanism executed when DNA has been damaged or other severe cellular insults were encountered. Senescent cells remain viable and metabolically active, but are unable to proliferate. This condition is entered upon high energy radiation, oxidative stress, chemotherapeutic agents, mutagenesis, and viral infection, among others. Natural aging is also linked to senescence, since after a certain number of divisions, cells stop replicating and enter senescence (replicative senescence). Senescent cells may promote inflammation and chronic disease due to their senescence-associated secretory phenotype (SASP). In tumor therapy, tumor cell (and bystander cell) senescence may occur as an alternative to apoptotic cell death. On the one hand, this is a desired therapeutic effect to stop tumor cell proliferation; on the other hand, senescent cells undergo profound epigenomic reprogramming, which might equip them with latent stemness capacity. Upon occasional cell-cycle re-entry out of senescence, these changes render tumor cells cancer stem cells, accounting for particularly aggressive relapses. The *tumor stem cell program* of post-senescent cells is a pivotal and detrimental capability to re-initiate tumor growth, translating into poor patient outcome. Accordingly, these previously senescent cells behave more aggressively regarding tumor growth than pre-senescent tumor cells, namely tumor cells that have never been in senescence.

For these reasons, chemical compounds that selectively eliminate senescent cells are urgently needed. Some prototypic compounds with hints for senescence-targeting capacity are known, but mostly poorly characterized regarding their senescence-selective activity, and only a few

are currently approved (or about to be approved) on the market. Most known senolytics (*i.e.,* compounds inducing cell death preferentially in senescent cells) exert their senolytic efficacy by inhibiting anti-apoptotic proteins of the Bcl-2 family, which are often found upregulated in stress-surviving senescent cells, but underlying mechanisms of other potential senolytics are still not fully understood. Senolytics appear to have enormous potential across numerous medical fields in which the lasting persistence of senescent cells contributes to disease, especially as cancer therapeutics and in the arena of age-related diseases.

WO 2016/118014 A2 describes an anti-senescent peptide and methods for the use of this peptide in the treatment of age-related disorders.

WO 2020/084105 A2 (CA 3 117 553 A1) describes a composition comprising one or more inhibitors capable of inhibiting at least two of cyclooxygenase-1 (COX-1), cyclooxygenase-2 (COX-2) and lipoxygenase or a composition comprising one or more inhibitors capable of inhibiting an enzyme with arachidonate-Co A ligase activity, specifically long-chain-fatty-acid-Co A ligase (ACSL) 1, ACSL3, ACSL4, ACSL5, ACSL6, SLC27A2 or ACSBG2, or a combination thereof for use in selectively eliminating senescent cells. This patent application further relates to an in vitro method of identifying senescent cells in a subject and to a method of identifying candidate compounds for the selective elimination of senescent cells.

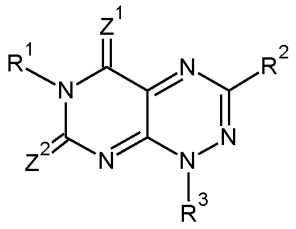
Letfus et al. (2020) describes toxoflavin derivatives that were used to design and synthesize new KDM4C inhibitors. These inhibitors showed anticancer efficacy.

KR 20170042011 A describes an anti-aging composition comprising kaempferol as an active ingredient. The composition is effective in preventing, improving or treating aging. It increases expression of SIRT1, adjusts p53/p21, p16/Rb, and m-TOR mechanisms.

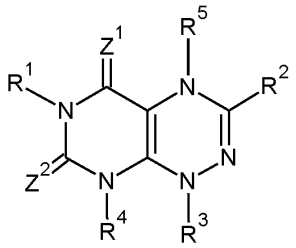
Wu et al. (2024) describes the toxoflavin analogue D43 that displayed a significant dose-dependent inhibitory effect on the proliferation of triple-negative breast cancer (TNBC) cells (MDA-MB-231 and HCC1806). Additionally, D43 inhibited DNA synthesis in TNBC cells, leading to cell cycle arrest at the G2/M phase. Furthermore, D43 consistently promoted intracellular ROS generation, induced DNA damage, and resulted in apoptosis in TNBC cells. The authors concluded that D43 is a potent anticancer agent that elicits significant antiproliferation, oxidative stress, apoptosis, and DNA damage effects in TNBC cells, and D43 holds promise as a potential candidate for the treatment of TNBC.

It is an object of the present invention to provide a novel use for already known substances as well as to provide novel substances that are suited to be used as senolytic.

This object is achieved with the novel medical use (i.e., in-vivo use) of a compound according to claim 1 as senolytic. Such a compound has a chemical structure according to general formula (I), according to any of general formulae (II), (III), or (IV), or is a pharmaceutically acceptable salt or hydrate thereof:



(I)



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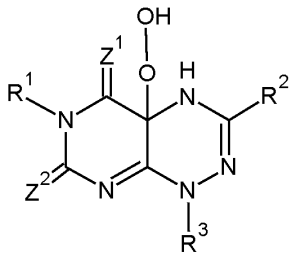

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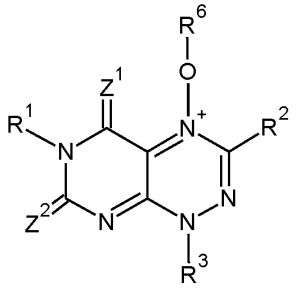


(IV)

In this context, the residues have the following meanings:

R^1 = H, C_1-C_{10} alkyl (in particular C_2-C_9 alkyl, in particular C_3-C_8 alkyl, in particular C_4-C_7 alkyl, in particular C_5-C_6 alkyl), C_3-C_{20} cycloalkyl (in particular C_4-C_{19} cycloalkyl, in particular C_5-C_{18} cycloalkyl, in particular C_6-C_{17} cycloalkyl, in particular C_7-C_{16} cycloalkyl, in particular C_8-C_{15} cycloalkyl, in particular C_9-C_{14} cycloalkyl, in particular C_{10}-C_{13} cycloalkyl, in particular C_{11}-C_{12} cycloalkyl), heteroatom-substituted C_3-C_{20} cycloalkyl (in particular heteroatom-substituted C_4-C_{19} cycloalkyl, in particular heteroatom-substituted C_5-C_{18} cycloalkyl, in particular heteroatom-substituted C_6-C_{17} cycloalkyl, in particular heteroatom-substituted C_7-C_{16} cycloalkyl, in particular heteroatom-substituted C_8-C_{15} cycloalkyl, in particular heteroatom-substituted C_9-C_{14} cycloalkyl, in particular heteroatom-substituted C_{10}-C_{13} cycloalkyl, in particular heteroatom-substituted C_{11}-C_{12} cycloalkyl), CF_3, CF_2H, CFH_2, optionally substituted piperidine, C_1-C_{10} alkyl (in particular C_2-C_9 alkyl, in particular C_3-C_8 alkyl, in particular C_4-C_7 alkyl, in particular C_5-C_6 alkyl) carrying a primary, secondary or tertiary amino substituent, OH, CH_2-O-galactosyl, optionally substituted C_6-C_{20} aryl (in particular optionally substituted C_7-C_{19} aryl, in particular optionally substituted C_8-C_{18} aryl, in particular optionally substituted C_9-C_{17} aryl, in particular optionally substituted C_{10}-C_{16} aryl, in particular optionally substituted C_{11}-C_{15} aryl, in particular optionally substituted C_{12}-C_{14} aryl), or optionally substituted C_5-C_{20} heteroaryl (in particular optionally substituted C_6-C_{19} heteroaryl, in particular optionally substituted C_7-C_{18} heteroaryl, in particular optionally substituted C_8-C_{17} heteroaryl, in particular optionally substituted C_9-C_{16} heteroaryl, in particular optionally substituted C_{10}-C_{15} heteroaryl, in particular optionally substituted C_{11}-C_{14} heteroaryl, in particular optionally substituted C_{12}-C_{13} heteroaryl),

R^2 = optionally substituted C_1-C_{10} alkyl (in particular optionally substituted C_2-C_9 alkyl, in particular optionally substituted C_3-C_8 alkyl, in particular optionally substituted C_4-C_7 alkyl, in particular optionally substituted C_5-C_6 alkyl), optionally substituted C_7-C_{30} alkyl aryl (in particular optionally substituted C_8-C_{29} alkyl aryl, in particular optionally substituted C_9-C_{28} alkyl aryl, in particular optionally substituted C_{10}-C_{27} alkyl aryl, in particular optionally substituted C_{11}-C_{26} alkyl aryl, in particular optionally substituted C_{12}-C_{25} alkyl aryl, in particular optionally substituted C_{13}-C_{24} alkyl aryl, in particular optionally substituted C_{14}-C_{23} alkyl aryl, in particular optionally substituted C_{15}-C_{22} alkyl aryl, in particular optionally substituted C_{16}-C_{21} alkyl aryl, in particular optionally substituted C_{17}-C_{20} alkyl aryl, in particular optionally substituted C_{18}-C_{19} alkyl aryl), optionally substituted C_6-C_{30} alkyl heteroaryl (in particular optionally substituted C_7-C_{29} alkyl heteroaryl, in particular optionally substituted C_8-C_{28} alkyl heteroaryl, in particular optionally substituted C_9-C_{27} alkyl heteroaryl, in particular optionally

substituted C_{10}-C_{26} alkyl heteroaryl, in particular optionally substituted C_{11}-C_{25} alkyl heteroaryl, in particular optionally substituted C_{12}-C_{24} alkyl heteroaryl, in particular optionally substituted C_{13}-C_{23} alkyl heteroaryl, in particular optionally substituted C_{14}-C_{22} alkyl heteroaryl, in particular optionally substituted C_{15}-C_{21} alkyl heteroaryl, in particular optionally substituted C_{16}-C_{20} alkyl heteroaryl, in particular optionally substituted C_{17}-C_{19} alkyl heteroaryl), optionally substituted C_6-C_{20} aryl (in particular optionally substituted C_7-C_{19} aryl, in particular optionally substituted C_8-C_{18} aryl, in particular optionally substituted C_9-C_{17} aryl, in particular optionally substituted C_{10}-C_{16} aryl, in particular optionally substituted C_{11}-C_{15} aryl, in particular optionally substituted C_{12}-C_{14} aryl), optionally substituted C_6-C_{20} aryl nitro (in particular optionally substituted C_7-C_{19} aryl nitro, in particular optionally substituted C_8-C_{18} aryl nitro, in particular optionally substituted C_9-C_{17} aryl nitro, in particular optionally substituted C_{10}-C_{16} aryl nitro, in particular optionally substituted C_{11}-C_{15} aryl nitro, in particular optionally substituted C_{12}-C_{14} aryl nitro), optionally substituted C_2-C_{10} alkenyl (in particular optionally substituted C_2-C_9 alkenyl, in particular optionally substituted C_3-C_8 alkenyl, in particular optionally substituted C_4-C_7 alkenyl, in particular optionally substituted C_5-C_6 alkenyl), optionally substituted C_8-C_{30} alkenyl aryl (in particular optionally substituted C_8-C_{29} alkenyl aryl, in particular optionally substituted C_9-C_{28} alkenyl aryl, in particular optionally substituted C_{10}-C_{27} alkenyl aryl, in particular optionally substituted C_{11}-C_{26} alkenyl aryl, in particular optionally substituted C_{12}-C_{25} alkenyl aryl, in particular optionally substituted C_{13}-C_{24} alkenyl aryl, in particular optionally substituted C_{14}-C_{23} alkenyl aryl, in particular optionally substituted C_{15}-C_{22} alkenyl aryl, in particular optionally substituted C_{16}-C_{21} alkenyl aryl, in particular optionally substituted C_{17}-C_{20} alkenyl aryl, in particular optionally substituted C_{18}-C_{19} alkenyl aryl), optionally substituted C_8-C_{30} alkenyl aryl nitro (in particular optionally substituted C_8-C_{29} alkenyl aryl nitro, in particular optionally substituted C_9-C_{28} alkenyl aryl nitro, in particular optionally substituted C_{10}-C_{27} alkenyl aryl nitro, in particular optionally substituted C_{11}-C_{26} alkenyl aryl nitro, in particular optionally substituted C_{12}-C_{25} alkenyl aryl nitro, in particular optionally substituted C_{13}-C_{24} alkenyl aryl nitro, in particular optionally substituted C_{14}-C_{23} alkenyl aryl nitro, in particular optionally substituted C_{15}-C_{22} alkenyl aryl nitro, in particular optionally substituted C_{16}-C_{21} alkenyl aryl nitro, in particular optionally substituted C_{17}-C_{20} alkenyl aryl nitro, in particular optionally substituted C_{18}-C_{19} alkenyl aryl nitro), optionally substituted C_7-C_{30} alkenyl heteroaryl (in particular optionally substituted C_8-C_{29} alkenyl heteroaryl, in particular optionally substituted C_9-C_{28} alkenyl heteroaryl, in particular optionally substituted C_{10}-C_{27} alkenyl heteroaryl, in particular optionally substituted C_{11}-C_{26} alkenyl heteroaryl, in particular

optionally substituted C_{12}-C_{25} alkenyl heteroaryl, in particular optionally substituted C_{13}-C_{24} alkenyl heteroaryl, in particular optionally substituted C_{14}-C_{23} alkenyl heteroaryl, in particular optionally substituted C_{15}-C_{22} alkenyl heteroaryl, in particular optionally substituted C_{16}-C_{21} alkenyl heteroaryl, in particular optionally substituted C_{17}-C_{20} alkenyl heteroaryl, in particular optionally substituted C_{18}-C_{19} alkenyl heteroaryl), optionally substituted C_2-C_{10} alkinyl (in particular optionally substituted C_2-C_9 alkinyl, in particular optionally substituted C_3-C_8 alkinyl, in particular optionally substituted C_4-C_7 alkinyl, in particular optionally substituted C_5-C_6 alkinyl), optionally substituted C_8-C_{30} alkinyl aryl (in particular optionally substituted C_8-C_{29} alkinyl aryl, in particular optionally substituted C_9-C_{28} alkinyl aryl, in particular optionally substituted C_{10}-C_{27} alkinyl aryl, in particular optionally substituted C_{11}-C_{26} alkinyl aryl, in particular optionally substituted C_{12}-C_{25} alkinyl aryl, in particular optionally substituted C_{13}-C_{24} alkinyl aryl, in particular optionally substituted C_{14}-C_{23} alkinyl aryl, in particular optionally substituted C_{15}-C_{22} alkinyl aryl, in particular optionally substituted C_{16}-C_{21} alkinyl aryl, in particular optionally substituted C_{17}-C_{20} alkinyl aryl, in particular optionally substituted C_{18}-C_{19} alkinyl aryl), optionally substituted C_7-C_{30} alkinyl heteroaryl (in particular optionally substituted C_8-C_{29} alkinyl heteroaryl, in particular optionally substituted C_9-C_{28} alkinyl heteroaryl, in particular optionally substituted C_{10}-C_{27} alkinyl heteroaryl, in particular optionally substituted C_{11}-C_{26} alkinyl heteroaryl, in particular optionally substituted C_{12}-C_{25} alkinyl heteroaryl, in particular optionally substituted C_{13}-C_{24} alkinyl heteroaryl, in particular optionally substituted C_{14}-C_{23} alkinyl heteroaryl, in particular optionally substituted C_{15}-C_{22} alkinyl heteroaryl, in particular optionally substituted C_{16}-C_{21} alkinyl heteroaryl, in particular optionally substituted C_{17}-C_{20} alkinyl heteroaryl, in particular optionally substituted C_{18}-C_{19} alkinyl heteroaryl), optionally substituted C_{11}-C_{30} heteroaryl aryl (in particular optionally substituted C_{12}-C_{29} heteroaryl aryl, in particular optionally substituted C_{13}-C_{28} heteroaryl aryl, in particular optionally substituted C_{14}-C_{27} heteroaryl aryl, in particular optionally substituted C_{15}-C_{26} heteroaryl aryl, in particular optionally substituted C_{16}-C_{25} heteroaryl aryl, in particular optionally substituted C_{17}-C_{24} heteroaryl aryl, in particular optionally substituted C_{18}-C_{23} heteroaryl aryl, in particular optionally substituted C_{19}-C_{22} heteroaryl aryl, in particular optionally substituted C_{20}-C_{21} heteroaryl aryl), optionally substituted C_9-C_{30} cyclyl aryl (in particular optionally substituted C_{10}-C_{29} cyclyl aryl, in particular optionally substituted C_{11}-C_{28} cyclyl aryl, in particular optionally substituted C_{12}-C_{27} cyclyl aryl, in particular optionally substituted C_{13}-C_{26} cyclyl aryl, in particular optionally substituted C_{14}-C_{25} cyclyl aryl, in particular optionally substituted C_{15}-C_{24} cyclyl aryl, in particular optionally substituted C_{16}-C_{23} cyclyl aryl, in particular

optionally substituted C_{17}-C_{22} cyclyl aryl, in particular optionally substituted C_{18}-C_{21} cyclyl aryl, in particular optionally substituted C_{19}-C_{20} cyclyl aryl), optionally substituted C_8-C_{30} heterocyclyl aryl (in particular optionally substituted C_9-C_{29} heterocyclyl aryl, in particular optionally substituted C_{10}-C_{29} heterocyclyl aryl, in particular optionally substituted C_{11}-C_{28} heterocyclyl aryl, in particular optionally substituted C_{12}-C_{27} heterocyclyl aryl, in particular optionally substituted C_{13}-C_{26} heterocyclyl aryl, in particular optionally substituted C_{14}-C_{25} heterocyclyl aryl, in particular optionally substituted C_{15}-C_{24} heterocyclyl aryl, in particular optionally substituted C_{16}-C_{23} heterocyclyl aryl, in particular optionally substituted C_{17}-C_{22} heterocyclyl aryl, in particular optionally substituted C_{18}-C_{21} heterocyclyl aryl, in particular optionally substituted C_{19}-C_{20} heterocyclyl aryl), optionally substituted C_3-C_{20} cycloalkyl (in particular optionally substituted C_4-C_{19} cycloalkyl, in particular optionally substituted C_5-C_{18} cycloalkyl, in particular optionally substituted C_6-C_{17} cycloalkyl, in particular optionally substituted C_7-C_{16} cycloalkyl, in particular optionally substituted C_8-C_{15} cycloalkyl, in particular optionally substituted C_9-C_{14} cycloalkyl, in particular optionally substituted C_{10}-C_{13} cycloalkyl, in particular optionally substituted C_{11}-C_{12} cycloalkyl), halogen, OH, C_1-C_{10} alkoxy (in particular C_2-C_9 alkoxy, in particular C_3-C_8 alkoxy, in particular C_4-C_7 alkoxy, in particular C_5-C_6 alkoxy), C_6-C_{20} aryloxy (in particular C_7-C_{19} aryloxy, in particular C_8-C_{18} aryloxy, in particular C_9-C_{17} aryloxy, in particular C_{10}-C_{16} aryloxy, in particular C_{11}-C_{15} aryloxy, in particular C_{12}-C_{14} aryloxy), amino, substituted amino, CN, carboxy, optionally substituted C_1-C_{10} alkyl carboxy (in particular optionally substituted C_2-C_9 alkyl carboxy, in particular optionally substituted C_3-C_8 alkyl carboxy, in particular optionally substituted C_4-C_7 alkyl carboxy, in particular optionally substituted C_5-C_6 alkyl carboxy), optionally substituted C_6-C_{20} aryl carboxy (in particular optionally substituted C_7-C_{19} aryl carboxy, in particular optionally substituted C_8-C_{18} aryl carboxy, in particular optionally substituted C_9-C_{17} aryl carboxy, in particular optionally substituted C_{10}-C_{16} aryl carboxy, in particular optionally substituted C_{11}-C_{15} aryl carboxy, in particular optionally substituted C_{12}-C_{14} aryl carboxy), carbamido, optionally substituted C_5-C_{20} heteroaryl (in particular optionally substituted C_6-C_{19} heteroaryl, in particular optionally substituted C_7-C_{18} heteroaryl, in particular optionally substituted C_8-C_{17} heteroaryl, in particular optionally substituted C_9-C_{16} heteroaryl, in particular optionally substituted C_{10}-C_{15} heteroaryl, in particular optionally substituted C_{11}-C_{14} heteroaryl, in particular optionally substituted C_{12}-C_{13} heteroaryl), optionally substituted C_2-C_{20} heterocyclyl (in particular optionally substituted C_3-C_{19} heterocyclyl, in particular optionally substituted C_4-C_{19} heterocyclyl, in particular optionally substituted C_5-C_{18}

heterocyclyl, in particular optionally substituted C_6-C_{17} heterocyclyl, in particular optionally substituted C_7-C_{16} heterocyclyl, in particular optionally substituted C_8-C_{15} heterocyclyl, in particular optionally substituted C_9-C_{14} heterocyclyl, in particular optionally substituted C_{10}-C_{13} heterocyclyl, in particular optionally substituted C_{11}-C_{12} heterocyclyl), heteroatom-substituted C_6-C_{20} cycloalkyl (in particular heteroatom-substituted C_7-C_{19} cycloalkyl, in particular heteroatom-substituted C_8-C_{18} cycloalkyl, in particular heteroatom-substituted C_9-C_{17} cycloalkyl, in particular heteroatom-substituted C_{10}-C_{16} cycloalkyl, in particular heteroatom-substituted C_{11}-C_{15} cycloalkyl, in particular heteroatom-substituted C_{12}-C_{14} cycloalkyl, in particular heteroatom-substituted C_{13}-C_{14} cycloalkyl), optionally substituted SH, sulfonamido, or sulfone,

R^3 = C_1-C_{10} alkyl (in particular C_2-C_9 alkyl, in particular C_3-C_8 alkyl, in particular C_4-C_7 alkyl, in particular C_5-C_6 alkyl), C_3-C_{20} cycloalkyl (in particular C_4-C_{19} cycloalkyl, in particular C_5-C_{18} cycloalkyl, in particular C_6-C_{17} cycloalkyl, in particular C_7-C_{16} cycloalkyl, in particular C_8-C_{15} cycloalkyl, in particular C_9-C_{14} cycloalkyl, in particular C_{10}-C_{13} cycloalkyl, in particular C_{11}-C_{12} cycloalkyl), heteroatom-substituted C_3-C_{20} cycloalkyl (in particular heteroatom-substituted C_4-C_{19} cycloalkyl, in particular heteroatom-substituted C_5-C_{18} cycloalkyl, in particular heteroatom-substituted C_6-C_{17} cycloalkyl, in particular heteroatom-substituted C_7-C_{16} cycloalkyl, in particular heteroatom-substituted C_8-C_{15} cycloalkyl, in particular heteroatom-substituted C_9-C_{14} cycloalkyl, in particular heteroatom-substituted C_{10}-C_{13} cycloalkyl, in particular heteroatom-substituted C_{11}-C_{12} cycloalkyl), CF_3, CF_2H, CFH_2, optionally substituted piperidine, C_1-C_{10} alkyl (in particular C_2-C_9 alkyl, in particular C_3-C_8 alkyl, in particular C_4-C_7 alkyl, in particular C_5-C_6 alkyl) carrying a primary, secondary or tertiary amino substituent, OH, CH_2-O-galactosyl, optionally substituted C_6-C_{20} aryl (in particular optionally substituted C_7-C_{19} aryl, in particular optionally substituted C_8-C_{18} aryl, in particular optionally substituted C_9-C_{17} aryl, in particular optionally substituted C_{10}-C_{16} aryl, in particular optionally substituted C_{11}-C_{15} aryl, in particular optionally substituted C_{12}-C_{14} aryl), or optionally substituted C_5-C_{20} heteroaryl (in particular optionally substituted C_6-C_{19} heteroaryl, in particular optionally substituted C_7-C_{18} heteroaryl, in particular optionally substituted C_8-C_{17} heteroaryl, in particular optionally substituted C_9-C_{16} heteroaryl, in particular optionally substituted C_{10}-C_{15} heteroaryl, in particular optionally substituted C_{11}-C_{14} heteroaryl, in particular optionally substituted C_{12}-C_{13} heteroaryl),

R^4, R^5 = independently from each other H, C_1-C_{10} alkyl (in particular C_2-C_9 alkyl, in particular C_3-C_8 alkyl, in particular C_4-C_7 alkyl, in particular C_5-C_6 alkyl), C_3-C_{20} cycloalkyl (in particular C_4-C_{19} cycloalkyl, in particular C_5-C_{18} cycloalkyl, in particular C_6-C_{17} cycloalkyl, in particular C_7-C_{16} cycloalkyl, in particular C_8-C_{15} cycloalkyl, in particular C_9-C_{14} cycloalkyl, in particular C_{10}-C_{13} cycloalkyl, in particular C_{11}-C_{12} cycloalkyl), heteroatom-substituted C_3-C_{20} cycloalkyl (in particular heteroatom-substituted C_4-C_{19} cycloalkyl, in particular heteroatom-substituted C_5-C_{18} cycloalkyl, in particular heteroatom-substituted C_6-C_{17} cycloalkyl, in particular heteroatom-substituted C_7-C_{16} cycloalkyl, in particular heteroatom-substituted C_8-C_{15} cycloalkyl, in particular heteroatom-substituted C_9-C_{14} cycloalkyl, in particular heteroatom-substituted C_{10}-C_{13} cycloalkyl, in particular heteroatom-substituted C_{11}-C_{12} cycloalkyl), CF_3, CF_2H, CFH_2, optionally substituted piperidine, C_1-C_{10} alkyl (in particular C_2-C_9 alkyl, in particular C_3-C_8 alkyl, in particular C_4-C_7 alkyl, in particular C_5-C_6 alkyl)carrying a primary, secondary or tertiary amino substituent, OH, CH_2-O-galactosyl, optionally substituted C_6-C_{20} aryl (in particular optionally substituted C_7-C_{19} aryl, in particular optionally substituted C_8-C_{18} aryl, in particular optionally substituted C_9-C_{17} aryl, in particular optionally substituted C_{10}-C_{16} aryl, in particular optionally substituted C_{11}-C_{15} aryl, in particular optionally substituted C_{12}-C_{14} aryl), or optionally substituted C_5-C_{20} heteroaryl (in particular optionally substituted C_6-C_{19} heteroaryl, in particular optionally substituted C_7-C_{18} heteroaryl, in particular optionally substituted C_8-C_{17} heteroaryl, in particular optionally substituted C_9-C_{16} heteroaryl, in particular optionally substituted C_{10}-C_{15} heteroaryl, in particular optionally substituted C_{11}-C_{14} heteroaryl, in particular optionally substituted C_{12}-C_{13} heteroaryl), or a protecting group chosen from i) *tert*-butyloxycarbonyl, ii) trityl, iii) acetyl, iv) C_1-C_{10} acyl (in particular C_2-C_9 acyl, in particular C_3-C_8 acyl, in particular C_4-C_7 acyl, in particular C_5-C_6 acyl) residues, and v) monosubstituted or disubstituted compounds according to general formula (II), wherein R^4 and R^5 denote H,

R^6 = absent (i.e., the oxygen atom carries a negative charge) or a protecting group chosen from C_1-C_{10} acyl (in particular C_2-C_9 acyl, in particular C_3-C_8 acyl, in particular C_4-C_7 acyl, in particular C_5-C_6 acyl), acetyl, ester residues, benzoyl, benzyl, trityl, and ether residues, and

Z^1, Z^2 = independently from each other O or S.

The invention relates, in an aspect, to a compound according to general formula (I), a derivative of such compound according to any of general formulae (II), (III), or (IV), or a pharmaceutically acceptable salt or hydrate of such compound or derivative, for in-vivo use as senolytic.

In an embodiment, the compound is used in the delay of a medical condition comprising senescent cells. In an embodiment, the compound is used in the delay of a condition comprising senescent cells. In an embodiment, such a condition is ageing. Such condition comprising senescent cells preferably occurs in subjects, more preferably in humans or animals.

In an embodiment, senescent cells are killed. In an embodiment, senescent cells are removed.

In an embodiment, the treatment, prevention and/or delay comprises killing of senescent cells. In such embodiment, killing of senescent cells is particularly beneficial for such medical condition.

In another embodiment, the treatment, prevention and/or delay comprises removal of senescent cells. In such embodiment, removal of senescent cells is particularly beneficial for such medical condition.

In an embodiment, the compound is used in the treatment and/or prevention of a disease comprising senescent cells. In an embodiment, the compound is used for the delay of a disease comprising senescent cells. In another embodiment, the disease comprising senescent cells comprises removal of senescent cells. In such embodiment, killing and/or removal of senescent cells is beneficial.

In an embodiment, the disease is caused or aggravated by senescence.

In an embodiment, the compound, the derivative, or the pharmaceutically acceptable salt or hydrate of such compound or derivative is administered to a patient in an effective dosage as a senolytic.

In an embodiment, the compound, the derivative, or the pharmaceutically acceptable salt or hydrate of such compound or derivative is administered to a patient intravenously in a dosage in a range from 0.05 mg to 10 mg, preferably between 0.1 mg to 4 mg, per kg bodyweight of the patient to be treated.

In an embodiment, the compound, the derivative, or the pharmaceutically acceptable salt or hydrate of such compound or derivative is administered to a patient in such a dosage that a number of viable senescence-associated beta-galactosidase positive cells in a tissue sample obtained from the patient is equal to or less than 40 %, (in particular 0 % to 40 %, in particular 5 % to 35 %, in particular 10 % to 30 %, in particular 15 % to 25 %, in particular 17 % to 20 %) of the number of viable senescence-associated beta-galactosidase positive cells in a comparable control sample obtained from at least one control subject suffering from the same disease as the patient but not having been treated with the compound, the derivative, or the pharmaceutically acceptable salt or hydrate of such compound or derivative, wherein a viability of senescence-associated beta-galactosidase positive cells is determined by a dye exclusion test, wherein the dye exclusion test on the tissue sample of the patient is carried out at least 22 hours (in particular 22 hours to 8 days, in particular 24 hours to 7 days, in particular 36 hours to 6 days, in particular 48 hours to 5 days, in particular 72 hours to 4 days) after administration of the compound, the derivative, or the pharmaceutically acceptable salt or hydrate of such compound or derivative described herein.

In an embodiment, the compound, the derivative, or the pharmaceutically acceptable salt or hydrate of such compound or derivative is administered to a patient in an effective senolytic dosage that a number of viable senescence-associated beta-galactosidase positive cells (="senescent cells") in a tissue sample obtained from the patient is equal to or less than 20 % (in particular 0 % to 20 %, in particular 5 % to 15 %, in particular 7 % to 12 %, in particular 8 % to 10 %) of the number of viable senescence-associated beta-galactosidase positive cells in a comparable control sample obtained from at least one control subject suffering from the same disease as the patient but not having been treated with the compound, the derivative, or the pharmaceutically acceptable salt or hydrate of such compound or derivative, wherein a viability of senescence-associated beta-galactosidase positive cells is determined by a dye exclusion test, wherein the dye exclusion test on the tissue sample of the patient is carried out at least 22 hours (in particular 22 hours to 8 days, in particular 24 hours to 7 days, in particular 36 hours to 6 days, in particular 48 hours to 5 days, in particular 72 hours to 4 days) after administration of the compound, the derivative, or the pharmaceutically acceptable salt or hydrate of such compound or derivative, preferably wherein the comparison between the treated and the untreated patient is carried out in accordance with the beta-galactosidase assay as described in prior art, and the dye exclusion test is carried out according to prior art

The compound or the pharmaceutically acceptable salt or hydrate of such compound is in particular for use in treating a disease in which the removal of senescent cells is beneficial, wherein the compound or the pharmaceutically acceptable salt or hydrate of such compound

is in particular administered to a patient in such a dosage that a number of viable senescence-associated beta-galactosidase positive cells in a tissue sample obtained from the patient is equal to or less than 40 % (in particular 0 % to 40 %, in particular 5 % to 35 %, in particular 10 % to 30 %, in particular 15 % to 25 %, in particular 17 % to 20 %) of the number of viable senescence-associated beta-galactosidase positive cells in a comparable control sample obtained from at least one control subject suffering from the same disease as the patient but not having been treated with the compound or the pharmaceutically acceptable salt or hydrate of such compound. In this context, a viability of senescence-associated beta-galactosidase positive cells is determined by a dye exclusion test, wherein the dye exclusion test on the tissue sample of the patient is carried out at least 22 hours (in particular 22 hours to 8 days, in particular 24 hours to 7 days, in particular 36 hours to 6 days, in particular 48 hours to 5 days, in particular 72 hours to 4 days) after administration of the compound or the pharmaceutically acceptable salt or hydrate of such compound.

In an embodiment, the tissue sample is a sample of tumor tissue. In an embodiment, the tissue sample is a blood sample.

Particularly appropriate dyes for carrying out the dye exclusion tests are Ghost Dye Red 780, Trypan Blue, and propidium iodide. Other commercially available dyes for selectively staining necrotic or dead cells are likewise appropriate.

Ghost Dye Red 780 is an amine reactive viability dye that can be used to discriminate viable from non-viable mammalian cells in flow cytometry applications. Ghost Dye Red 780 irreversibly binds free amines available on the cell surface as well as intracellular free amines exposed in cells with compromised cell membranes. Necrotic cells with compromised membranes will react with significantly more Ghost Dye Red 780 dye than viable cells in the same sample and therefore will exhibit much greater fluorescence intensity allowing exclusion of these cells from analysis. An excitation laser emitting red light having a wavelength from 633 nm to 640 nm is Appropriate for exciting Ghost Dye Red 780. The dye will emit fluorescence light having a wavelength of 780 nm. Any dye having comparable or similar cell binding properties as well as fluorescence properties like Ghost Dye Red 780 is likewise suited for carrying out the dye exclusion test. Therefore, the term "Ghost Dye red 780" in this paragraph can be simply replaced by "a dye" or "the dye" without deviating from the present disclosure.

As will be shown in connection with cell culture experiments further below in the present disclosure, the cell viability could well be detected 22 hours after subjecting a cell culture to

the presently claimed compounds or the compounds, the use of which is presently claimed, respectively. Therefore, a cell viability test carried out on a sample taken from a patient to whom any of the compounds has been administered, can be reliably carried out after expiry of at least 22 hours after administration of the compound.

In an embodiment, residues R^4 and R^5 denote hydrogen. Then, the compounds according to general formula (II) represent the reduced form of the compounds according to general formula (I). They can also be denoted as reduced derivative of the compounds according to general formula (I).

The compounds according to general formula (III) represent the hydroperoxy derivative of the compounds according to general formula (I).

The compounds according to general formula (IV) represent the N-oxide derivatives of the compounds according to general formula (I).

Thus, the compounds according to general formula (I), (II), (III), and (IV) are closely interrelated. As will be explained below in more detail, they represent different forms of one and the same molecule during a redox cycle that is passed through by the molecule within a senescent cell to exhibit its senolytic activity.

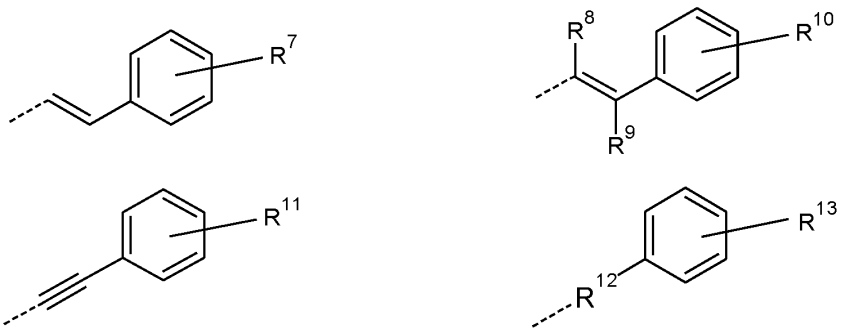
The compounds according to general formula (I) are able to produce hydrogen peroxide (H_2O_2, HP) via a redox mechanism. Therefore, it will be referred to them in the following also as “peroxygenins” (POG). In the presence of a suitable biochemical reducing agent such as dithiothreitol (DTT), reduced nicotinamide adenine dinucleotide (NADH), reduced nicotinamide adenine dinucleotide phosphate (NADPH), or dihydrolipoic acid (DHLA), the oxidized form ox-POG can be reduced to the reduced form red-POG, which then forms a hydroperoxy species with molecular oxygen (confer Figure 1A). The original form of POG is recovered by release of HP, which means that the formation of HP occurs in a catalytic cycle driven by a reducing agent. POG activation is promoted by senescent cells, because senescent cells are metabolically altered compared to healthy cells and specifically exhibit higher glucose consumption and higher oxidative stress (reactive oxygen species, ROS, like HP). Indeed, the inventors found that cells undergoing senescence generate significantly higher cellular levels of the reducing equivalent NADH. Importantly, glutathione (GSH) levels were found to be depleted in senescent cells; accordingly, GSSG/GSH ratios were strongly increased due to oxidative stress.

In consequence, POG are efficiently reduced by NADH in senescent cells, generating high concentrations of HP, which are only inefficiently detoxified by the already reduced GSH levels, thereby selectively killing senescent cells (confer Figure 1A). Such mode of activation applies to all types of senescence, thus, conceptually constituting POG as a novel pan-senolytic drug. The terms “drug” or “compound” or “agent” can be used interchangeably.

In an embodiment, Z^1 is O and Z^2 is S. In an embodiment, Z^1 is S and Z^2 is O. In an embodiment, Z^1 is O and Z^2 is O. In an embodiment, Z^1 is S and Z^2 is S.

Generally, the nitro group is a particularly appropriate substituent of all hydrocarbon systems (such as alkyl and alkenyl chains, cycloalkyl, heterocyclyl, aryl, and heteroaryl groups) of the various residues, in particular of residue R^2. In an embodiment, the nitro group serves as substituent of any of the hydrocarbon systems of the various residues, in particular of residue R^2. In case of an optionally substituted aryl nitro (such as an optionally substituted nitrostyryl) or alkenyl aryl nitro (such as an optionally substituted alkenyl nitrostyryl), a further substituent (besides the already present nitro group) can be another nitro group or a different substituent.

In an embodiment, residue R^2 is a residue according to any of the following formulae, wherein the dashed line indicates a bond by which the residue R^2 is covalently bound to the triazine ring of the compound according to general formulae (I), (II), (III), or (IV):



In this context, the residues have the following meanings:

R^7, R^{10}, R^{11}, R^{13} = independently from each other H, NO_2, optionally substituted C_1-C_{10} alkyl (in particular optionally substituted C_2-C_9 alkyl, in particular optionally substituted C_3-C_8 alkyl, in particular optionally substituted C_4-C_7 alkyl, in particular optionally substituted C_5-C_6 alkyl), optionally substituted C_2-C_{10} alkenyl (in particular optionally substituted C_2-C_9 alkenyl, in particular optionally substituted C_3-C_8 alkenyl, in particular optionally substituted C_4-

C_7 alkenyl, in particular optionally substituted C_5-C_6 alkenyl), optionally substituted C_3-C_{20} cycloalkyl (in particular optionally substituted C_4-C_{19} cycloalkyl, in particular optionally substituted C_5-C_{18} cycloalkyl, in particular optionally substituted C_6-C_{17} cycloalkyl, in particular optionally substituted C_7-C_{16} cycloalkyl, in particular optionally substituted C_8-C_{15} cycloalkyl, in particular optionally substituted C_9-C_{14} cycloalkyl, in particular optionally substituted C_{10}-C_{13} cycloalkyl, in particular optionally substituted C_{11}-C_{12} cycloalkyl), heteroatom-substituted C_3-C_{20} cycloalkyl (in particular heteroatom-substituted C_4-C_{19} cycloalkyl, in particular heteroatom-substituted C_5-C_{18} cycloalkyl, in particular heteroatom-substituted C_6-C_{17} cycloalkyl, in particular heteroatom-substituted C_7-C_{16} cycloalkyl, in particular heteroatom-substituted C_8-C_{15} cycloalkyl, in particular heteroatom-substituted C_9-C_{14} cycloalkyl, in particular heteroatom-substituted C_{10}-C_{13} cycloalkyl, in particular heteroatom-substituted C_{11}-C_{12} cycloalkyl), halogen, OH, C_1-C_{10} alkoxy (in particular C_2-C_9 alkoxy, in particular C_3-C_8 alkoxy, in particular C_4-C_7 alkoxy, in particular C_5-C_6 alkoxy), C_6-C_{20} aryloxy (in particular C_7-C_{19} aryloxy, in particular C_8-C_{18} aryloxy, in particular C_9-C_{17} aryloxy, in particular C_{10}-C_{16} aryloxy, in particular C_{11}-C_{15} aryloxy, in particular C_{12}-C_{14} aryloxy), amino, substituted amino, CN, carboxy, optionally substituted C_1-C_{10} alkyl carboxy (in particular optionally substituted C_2-C_9 alkyl carboxy, in particular optionally substituted C_3-C_8 alkyl carboxy, in particular optionally substituted C_4-C_7 alkyl carboxy, in particular optionally substituted C_5-C_6 alkyl carboxy), optionally substituted C_6-C_{20} aryl-carboxy (in particular optionally substituted C_7-C_{19} aryl carboxy, in particular optionally substituted C_8-C_{18} aryl carboxy, in particular optionally substituted C_9-C_{17} aryl carboxy, in particular optionally substituted C_{10}-C_{16} aryl carboxy, in particular optionally substituted C_{11}-C_{15} aryl carboxy, in particular optionally substituted C_{12}-C_{14} aryl carboxy), carbamido, optionally substituted C_5-C_{20} heteroaryl (in particular optionally substituted C_6-C_{19} heteroaryl, in particular optionally substituted C_7-C_{18} heteroaryl, in particular optionally substituted C_8-C_{17} heteroaryl, in particular optionally substituted C_9-C_{16} heteroaryl, in particular optionally substituted C_{10}-C_{15} heteroaryl, in particular optionally substituted C_{11}-C_{14} heteroaryl, in particular optionally substituted C_{12}-C_{13} heteroaryl), optionally substituted C_2-C_{20} heterocyclyl (in particular optionally substituted C_3-C_{19} heterocyclyl, in particular optionally substituted C_4-C_{19}

heterocyclyl, in particular optionally substituted C_5-C_{18} heterocyclyl, in particular optionally substituted C_6-C_{17} heterocyclyl, in particular optionally substituted C_7-C_{16} heterocyclyl, in particular optionally substituted C_8-C_{15} heterocyclyl, in particular optionally substituted C_9-C_{14} heterocyclyl, in particular optionally substituted C_{10}-C_{13} heterocyclyl, in particular optionally substituted C_{11}-C_{12} heterocyclyl), optionally substituted C_6-C_{20} aryl (in particular optionally substituted C_7-C_{19} aryl, in particular optionally substituted C_8-C_{18} aryl, in particular optionally substituted C_9-C_{17} aryl, in particular optionally substituted C_{10}-C_{16} aryl, in particular optionally substituted C_{11}-C_{15} aryl, in particular optionally substituted C_{12}-C_{14} aryl), optionally substituted SH, sulfonamido, or sulfone,

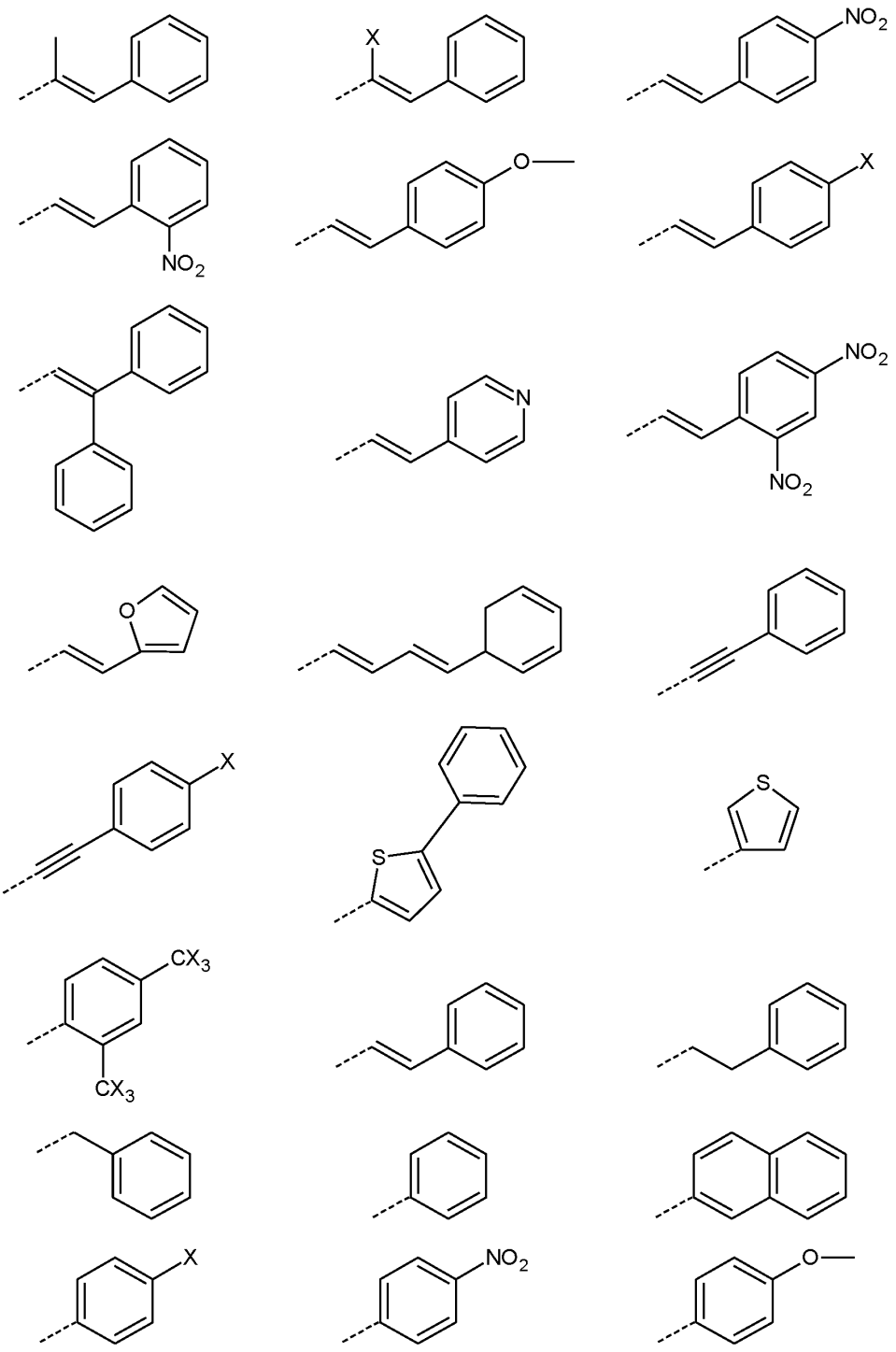
R^8, R^9 = independently from each other H, optionally substituted C_1-C_{10} alkyl (in particular optionally substituted C_2-C_9 alkyl, in particular optionally substituted C_3-C_8 alkyl, in particular optionally substituted C_4-C_7 alkyl, in particular optionally substituted C_5-C_6 alkyl), optionally substituted C_2-C_{10} alkenyl (in particular optionally substituted C_2-C_9 alkenyl, in particular optionally substituted C_3-C_8 alkenyl, in particular optionally substituted C_4-C_7 alkenyl, in particular optionally substituted C_5-C_6 alkenyl), optionally substituted C_3-C_{20} cycloalkyl (in particular optionally substituted C_4-C_{19} cycloalkyl, in particular optionally substituted C_5-C_{18} cycloalkyl, in particular optionally substituted C_6-C_{17} cycloalkyl, in particular optionally substituted C_7-C_{16} cycloalkyl, in particular optionally substituted C_8-C_{15} cycloalkyl, in particular optionally substituted C_9-C_{14} cycloalkyl, in particular optionally substituted C_{10}-C_{13} cycloalkyl, in particular optionally substituted C_{11}-C_{12} cycloalkyl), heteroatom-substituted C_3-C_{20} cycloalkyl (in particular heteroatom-substituted C_4-C_{19} cycloalkyl, in particular heteroatom-substituted C_5-C_{18} cycloalkyl, in particular heteroatom-substituted C_6-C_{17} cycloalkyl, in particular heteroatom-substituted C_7-C_{16} cycloalkyl, in particular heteroatom-substituted C_8-C_{15} cycloalkyl, in particular heteroatom-substituted C_9-C_{14} cycloalkyl, in particular heteroatom-substituted C_{10}-C_{13} cycloalkyl, in particular heteroatom-substituted C_{11}-C_{12} cycloalkyl), halogen, OH, C_1-C_{10} alkoxy (in particular C_2-C_9 alkoxy, in particular C_3-C_8 alkoxy, in particular C_4-C_7 alkoxy, in particular C_5-C_6 alkoxy), C_6-C_{20} aryloxy (in particular C_7-C_{19} aryloxy, in particular C_8-C_{18} aryloxy, in particular C_9-C_{17} aryloxy, in particular C_{10}-C_{16}

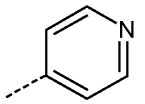
aryloxy, in particular C_{11}-C_{15} aryloxy, in particular C_{12}-C_{14} aryloxy), amino, substituted amino, CN, carboxy, optionally substituted C_1-C_{10} alkyl carboxy (in particular optionally substituted C_2-C_9 alkyl carboxy, in particular optionally substituted C_3-C_8 alkyl carboxy, in particular optionally substituted C_4-C_7 alkyl carboxy, in particular optionally substituted C_5-C_6 alkyl carboxy), optionally substituted C_6-C_{20} aryl-carboxy (in particular optionally substituted C_7-C_{19} aryl carboxy, in particular optionally substituted C_8-C_{18} aryl carboxy, in particular optionally substituted C_9-C_{17} aryl carboxy, in particular optionally substituted C_{10}-C_{16} aryl carboxy, in particular optionally substituted C_{11}-C_{15} aryl carboxy, in particular optionally substituted C_{12}-C_{14} aryl carboxy), carbamido, optionally substituted C_5-C_{20} heteroaryl (in particular optionally substituted C_6-C_{19} heteroaryl, in particular optionally substituted C_7-C_{18} heteroaryl, in particular optionally substituted C_8-C_{17} heteroaryl, in particular optionally substituted C_9-C_{16} heteroaryl, in particular optionally substituted C_{10}-C_{15} heteroaryl, in particular optionally substituted C_{11}-C_{14} heteroaryl, in particular optionally substituted C_{12}-C_{13} heteroaryl), optionally substituted C_2-C_{20} heterocyclyl (in particular optionally substituted C_3-C_{19} heterocyclyl, in particular optionally substituted C_4-C_{19} heterocyclyl, in particular optionally substituted C_5-C_{18} heterocyclyl, in particular optionally substituted C_6-C_{17} heterocyclyl, in particular optionally substituted C_7-C_{16} heterocyclyl, in particular optionally substituted C_8-C_{15} heterocyclyl, in particular optionally substituted C_9-C_{14} heterocyclyl, in particular optionally substituted C_{10}-C_{13} heterocyclyl, in particular optionally substituted C_{11}-C_{12} heterocyclyl), optionally substituted C_6-C_{20} aryl (in particular optionally substituted C_7-C_{19} aryl, in particular optionally substituted C_8-C_{18} aryl, in particular optionally substituted C_9-C_{17} aryl, in particular optionally substituted C_{10}-C_{16} aryl, in particular optionally substituted C_{11}-C_{15} aryl, in particular optionally substituted C_{12}-C_{14} aryl), optionally substituted SH, sulfonamido, or sulfone, CF_3, CF_2H, or CFH_2, and

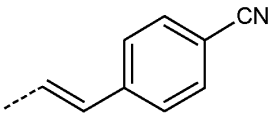
R^{12} = independently from each other optionally substituted C_1-C_{10} alkyl (in particular optionally substituted C_2-C_9 alkyl, in particular optionally substituted C_3-C_8 alkyl, in particular optionally substituted C_4-C_7 alkyl, in particular optionally substituted C_5-C_6 alkyl), optionally substituted C_2-C_{10} alkenyl (in particular optionally substituted C_2-C_9 alkenyl, in particular

optionally substituted C_3-C_8 alkenyl, in particular optionally substituted C_4-C_7 alkenyl, in particular optionally substituted C_5-C_6 alkenyl), optionally substituted C_2-C_{10} alkinyl (in particular optionally substituted C_2-C_9 alkinyl, in particular optionally substituted C_3-C_8 alkinyl, in particular optionally substituted C_4-C_7 alkinyl, in particular optionally substituted C_5-C_6 alkinyl), optionally substituted C_6-C_{20} aryl (in particular optionally substituted C_7-C_{19} aryl, in particular optionally substituted C_8-C_{18} aryl, in particular optionally substituted C_9-C_{17} aryl, in particular optionally substituted C_{10}-C_{16} aryl, in particular optionally substituted C_{11}-C_{15} aryl, in particular optionally substituted C_{12}-C_{14} aryl), optionally substituted C_5-C_{20} heteroaryl (in particular optionally substituted C_6-C_{19} heteroaryl, in particular optionally substituted C_7-C_{18} heteroaryl, in particular optionally substituted C_8-C_{17} heteroaryl, in particular optionally substituted C_9-C_{16} heteroaryl, in particular optionally substituted C_{10}-C_{15} heteroaryl, in particular optionally substituted C_{11}-C_{14} heteroaryl, in particular optionally substituted C_{12}-C_{13} heteroaryl), optionally substituted C_3-C_{20} cycloalkyl (in particular optionally substituted C_4-C_{19} cycloalkyl, in particular optionally substituted C_5-C_{18} cycloalkyl, in particular optionally substituted C_6-C_{17} cycloalkyl, in particular optionally substituted C_7-C_{16} cycloalkyl, in particular optionally substituted C_8-C_{15} cycloalkyl, in particular optionally substituted C_9-C_{14} cycloalkyl, in particular optionally substituted C_{10}-C_{13} cycloalkyl, in particular optionally substituted C_{11}-C_{12} cycloalkyl), optionally substituted C_3-C_{20} cyclyl (in particular optionally substituted C_4-C_{19} cyclyl, in particular optionally substituted C_5-C_{18} cyclyl, in particular optionally substituted C_6-C_{17} cyclyl, in particular optionally substituted C_7-C_{16} cyclyl, in particular optionally substituted C_8-C_{15} cyclyl, in particular optionally substituted C_9-C_{14} cyclyl, in particular optionally substituted C_{10}-C_{13} cyclyl, in particular optionally substituted C_{11}-C_{12} cyclyl), or optionally substituted C_2-C_{20} heterocyclyl (in particular optionally substituted C_3-C_{19} heterocyclyl, in particular optionally substituted C_4-C_{19} heterocyclyl, in particular optionally substituted C_5-C_{18} heterocyclyl, in particular optionally substituted C_6-C_{17} heterocyclyl, in particular optionally substituted C_7-C_{16} heterocyclyl, in particular optionally substituted C_8-C_{15} heterocyclyl, in particular optionally substituted C_9-C_{14} heterocyclyl, in particular optionally substituted C_{10}-C_{13} heterocyclyl, in particular optionally substituted C_{11}-C_{12} heterocyclyl).

In an embodiment, residue R^2 is a residue according to any of the following formulae, wherein the dashed line indicates a bond by which the residue R^2 is covalently bound to the triazine ring of the compound according to general formulae (I), (II), (III), or (IV):







In this context, residue X has one of the following meanings: F, Cl, Br, or I, in particular F or Br, in particular F.

In an embodiment, residue R^1 is a methyl residue. In an embodiment, residue R^1 is a hydrogen atom.

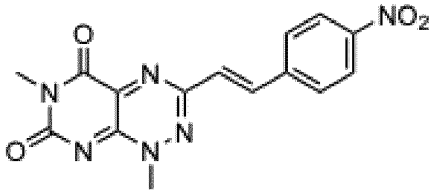
In an embodiment, residue R^3 is a methyl residue.

In an embodiment, residue R^1 is a methyl residue or a hydrogen atom and residue R^3 is chosen from methyl, ethyl, isopropyl, phenyl, and substituted phenyl.

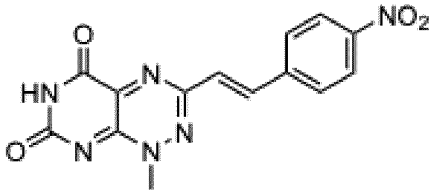
In an embodiment, residue R^1 is a methyl residue or a hydrogen atom and R^3 is a methyl residue.

In an embodiment, the optionally substituted phenyl, in particular the optionally substituted phenyl of residue R^2 or residue R^3, is a phenyl residue substituted with a nitro group or a cyano group.

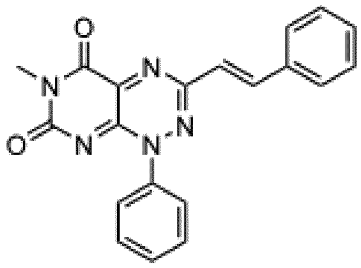
In an embodiment, the compound has the following structure:



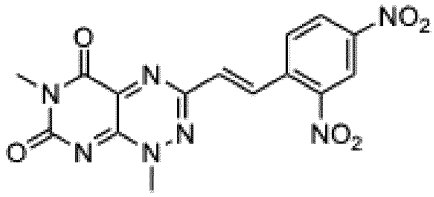
In an embodiment, the compound has the following structure:



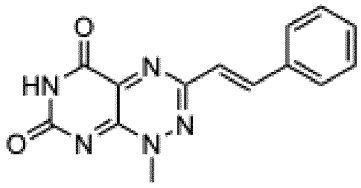
In an embodiment, the compound has the following structure:



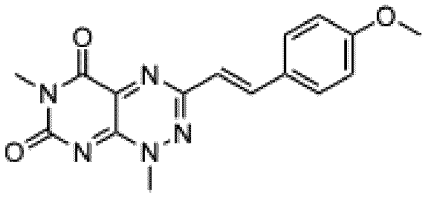
In an embodiment, the compound has the following structure:



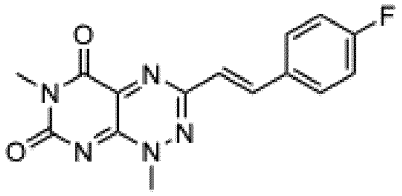
In an embodiment, the compound has the following structure:



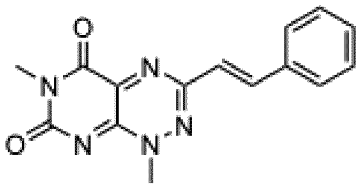
In an embodiment, the compound has the following structure:



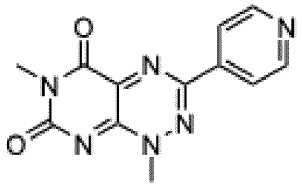
In an embodiment, the compound has the following structure:



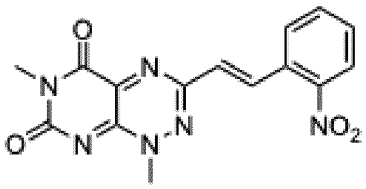
In an embodiment, the compound has the following structure:



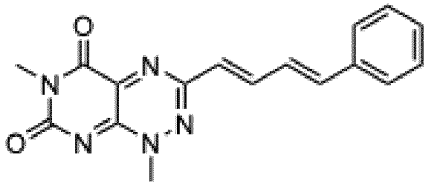
In an embodiment, the compound has the following structure:



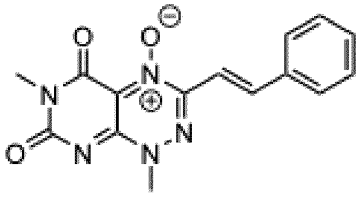
In an embodiment, the compound has the following structure:



In an embodiment, the compound has the following structure:

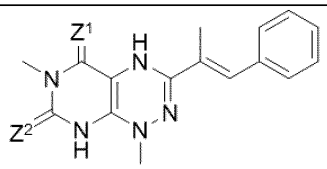
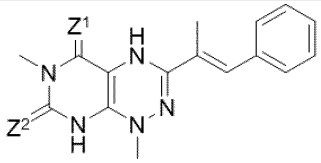
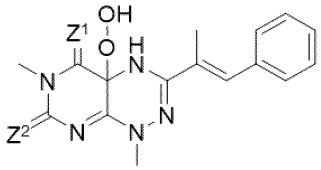
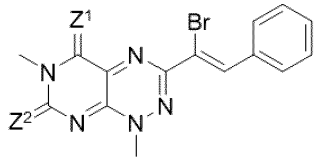
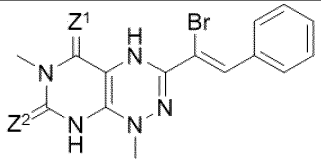
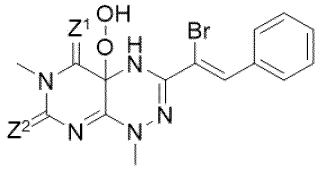
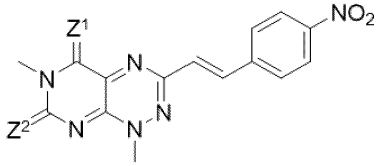
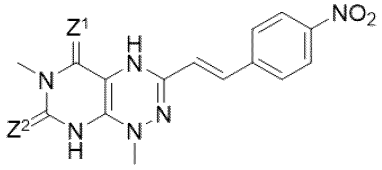


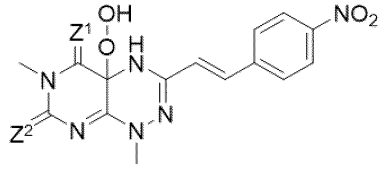
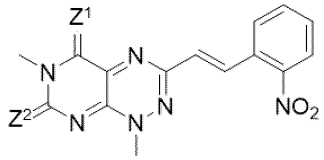
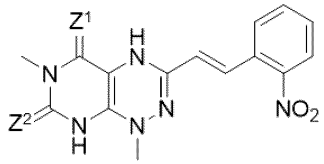
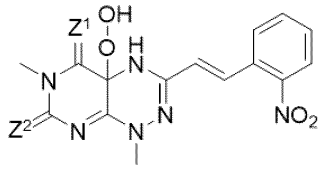
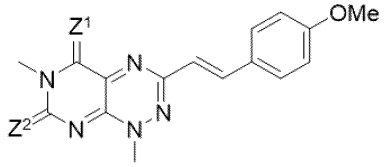
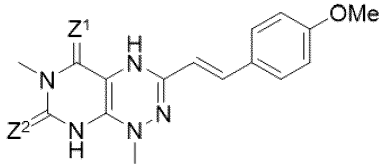
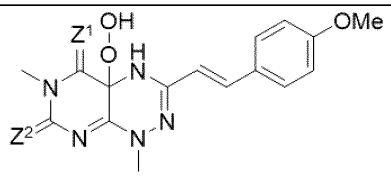
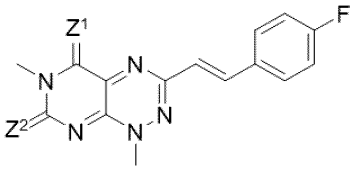
In an embodiment, the compound has the following structure:

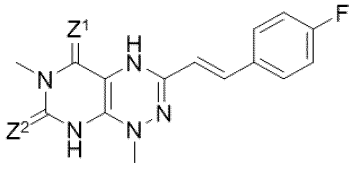
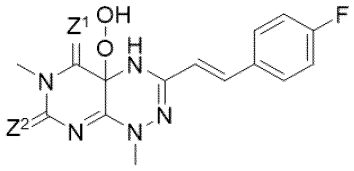
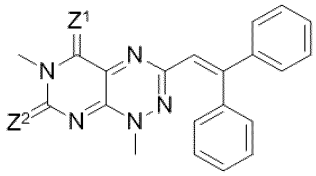
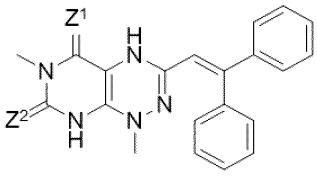
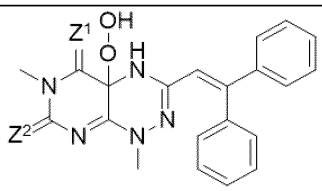
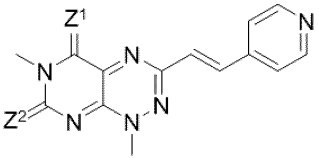
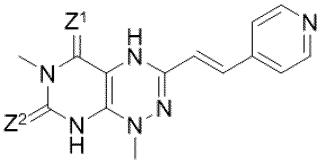
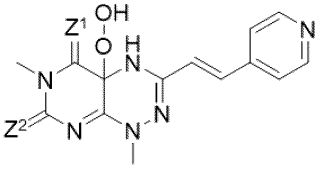


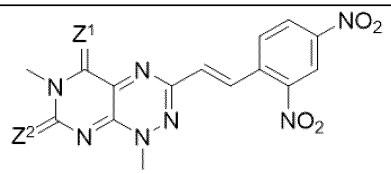
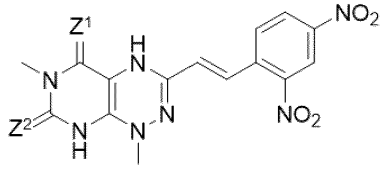
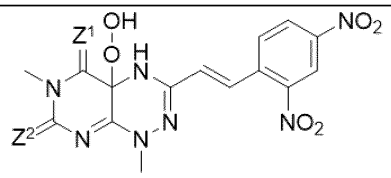
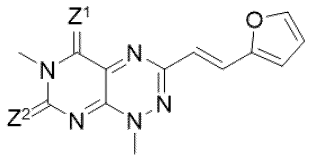
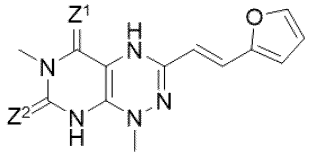
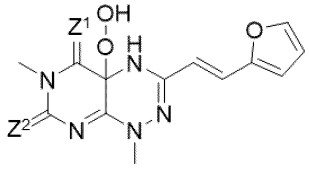
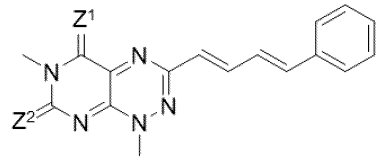
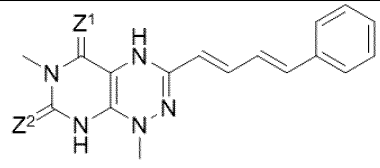
Particular appropriate examples of compounds for the claimed use are listed in the following Table 1.

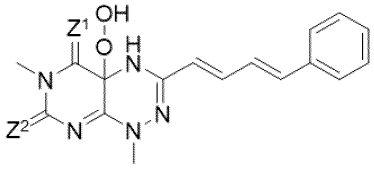
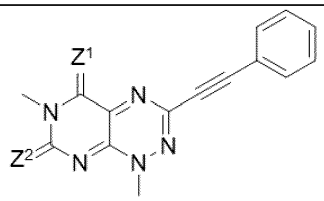
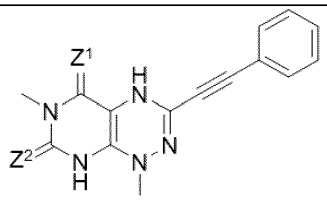
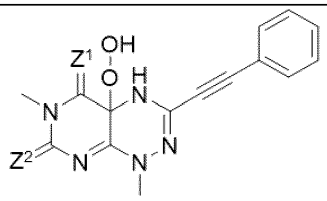
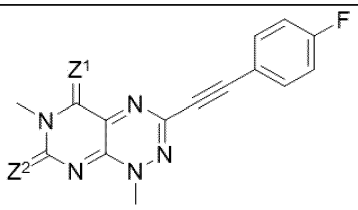
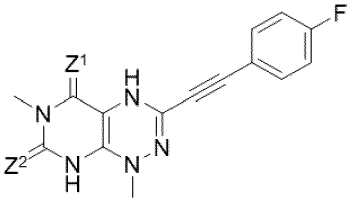
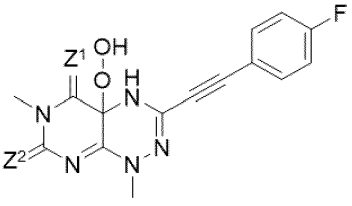
Table 1: Particular appropriate examples of compounds for the claimed use.

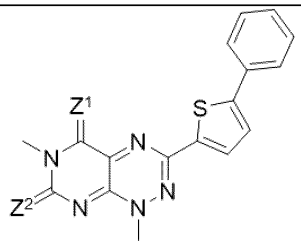
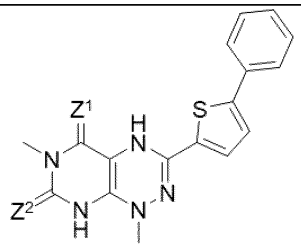
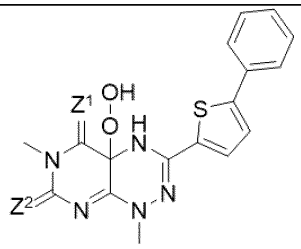
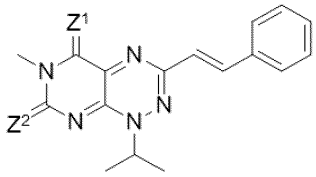
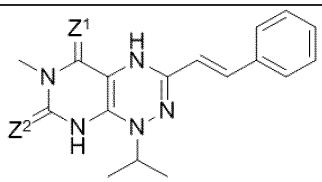
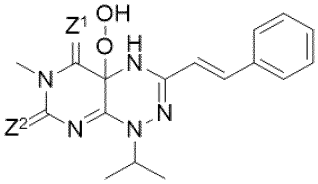
No.	Name	Structure
1	alpha-methylstyryl POG	
1.1	red-alpha-methylstyryl POG	
1.2	hydroperoxy-alpha-methylstyryl POG	
2	alpha-bromostyryl POG	
2.1	red-alpha-bromostyryl POG	
2.2	hydroperoxy-alpha-bromostyryl POG	
3	p-nitrostyryl POG	
3.1	red-p-nitrostyryl POG	

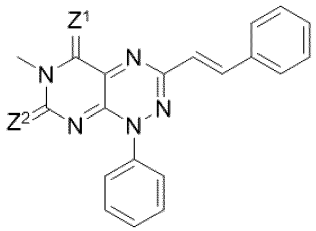
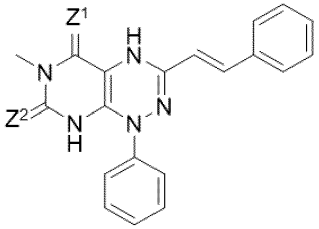
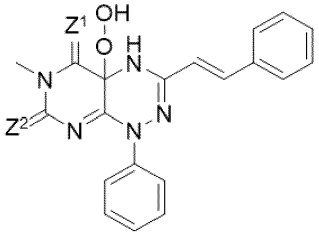
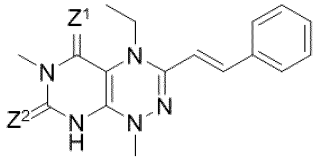
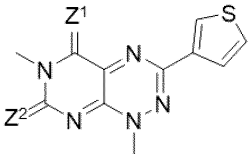
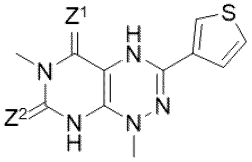
No.	Name	Structure
3.2	hydroperoxy-p-nitrostyryl POG	
4	o-nitrostyryl POG	
4.1	red-o-nitrostyryl POG	
4.2	hydroperoxy-o-nitrostyryl POG	
5	p-methoxystyryl POG	
5.1	red-p-methoxystyryl POG	
5.2	hydroperoxy-p-methoxystyryl POG	
6	p-fluorostyryl POG	

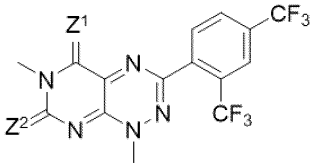
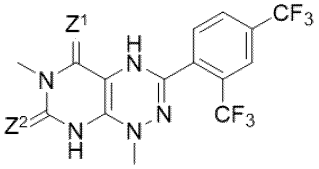
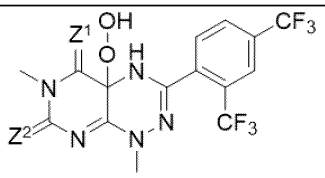
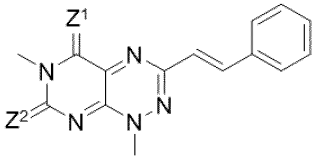
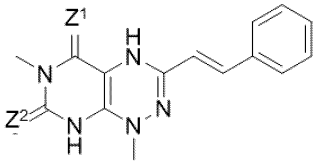
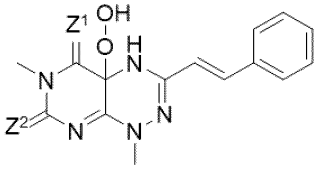
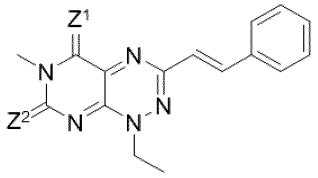
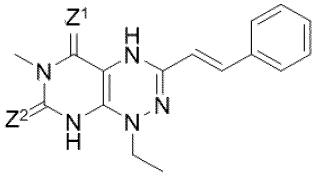
No.	Name	Structure
6.1	red-p-fluorostyryl POG	
6.2	hydroperoxy-p-fluorostyryl POG	
7	diphenylstyryl POG	
7.1	red-diphenylstyryl POG	
7.2	hydroperoxy-diphenylstyryl POG	
8	4-pyridinestyryl POG	
8.1	red-4-pyridinestyryl POG	
8.2	hydroperoxy-4-pyridinestyryl POG	

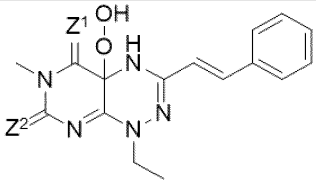
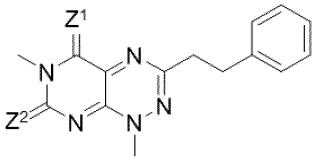
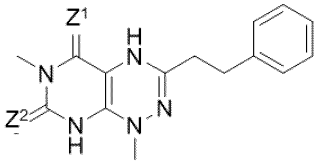
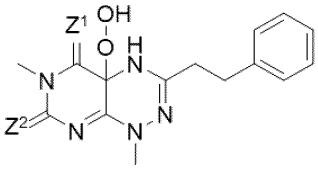
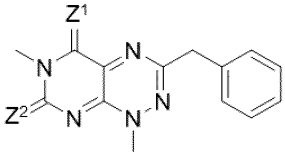
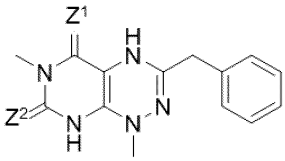
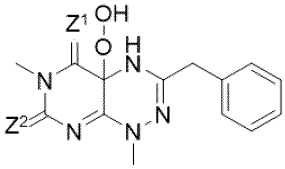
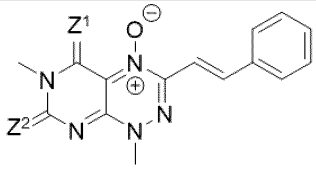
No.	Name	Structure
9	dinitrostyryl POG	
9.1	red-dinitrostyryl POG	
9.2	hydroperxy-dinitrostyryl POG	
10	furanacroleinstyryl POG	
10.1	red-furanacroleinstyryl POG	
10.2	hydroperoxy-furanacroleinstyryl POG	
11	2m-styryl POG	
11.1	red-2m-styryl POG	

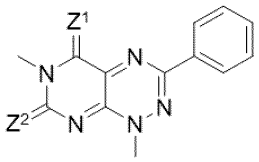
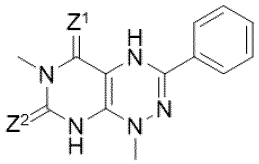
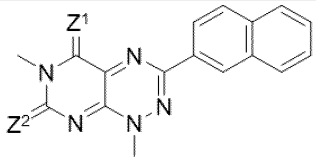
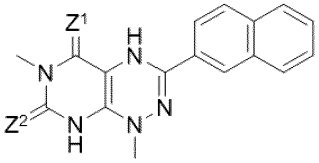
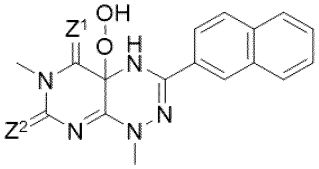
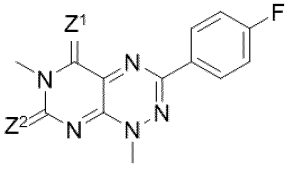
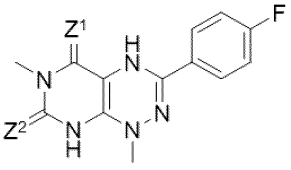
No.	Name	Structure
11.2	hydroperoxy-2m-styryl POG	
12	ethine POG	
12.1	red-ethine POG	
12.2	hydroperoxy-ethine POG	
13	p-fluoroethine POG	
13.1	red-p-fluoroethine POG	
13.2	hydroperoxy-p-fluoroethine POG	

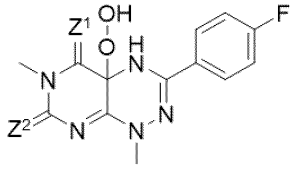
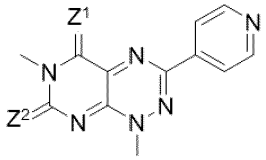
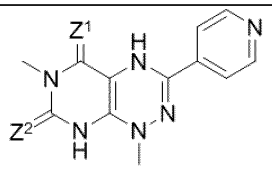
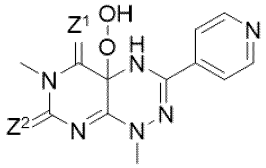
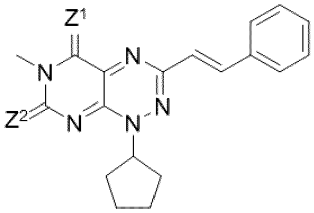
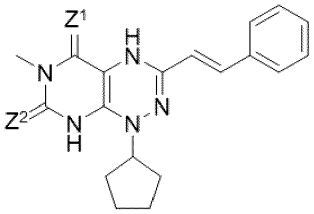
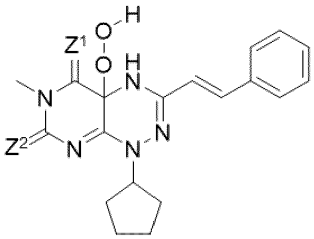
No.	Name	Structure
14	phenyl-thiophenyl POG	
14.1	red- phenyl-thiophenyl POG	
14.2	hydroperoxy-phenyl-thiophenyl POG	
15	N1-iPr-styryl POG	
15.1	red-N1-iPr-styryl POG	
15.2	hydroperoxy-N1-iPr-styryl POG	

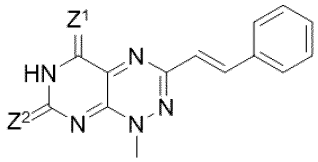
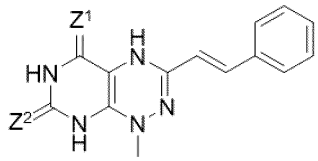
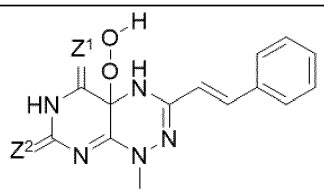
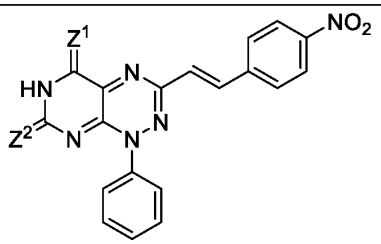
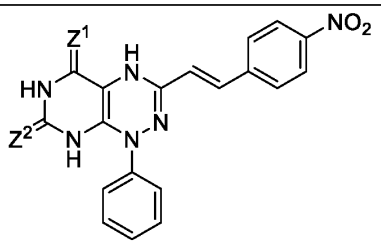
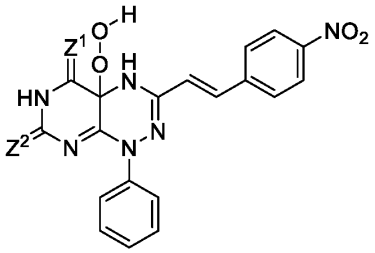
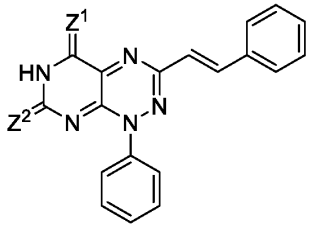
No.	Name	Structure
16	N1-phenyl-styryl POG	
16.1	red-N1-phenyl POG	
16.2	hydroperoxy-N1-phenyl POG	
17	N3-ethyl-styryl POG (POG-10)	
18	thiophene POG	
18.1	red-thiophene POG	
18.2	hydroperoxy-thiophene POG	

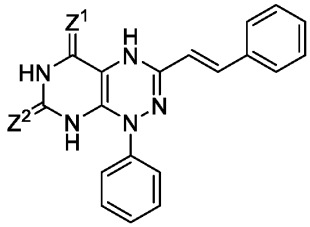
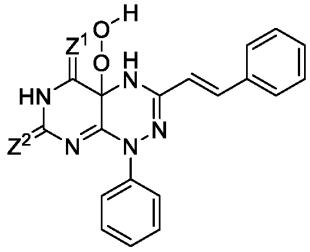
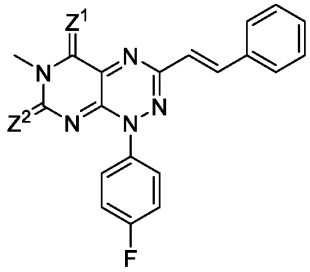
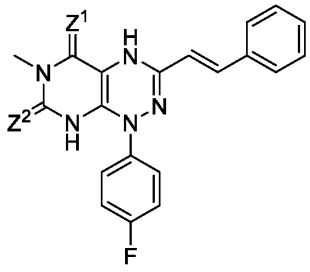
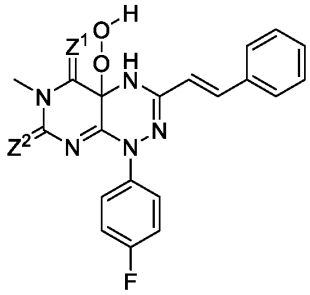
No.	Name	Structure
19	di-trifluoromethylphenyl POG	
19.1	red-di-trifluoromethylphenyl POG	
19.2	hydroperoxy-di-trifluoromethylphenyl POG	
20	styryl POG POG-4	
20.1	red-styryl POG	
20.2	hydroperoxy-styryl POG	
21	N1-ethylstyryl POG	
21.1	red-N1-ethylstyryl POG	

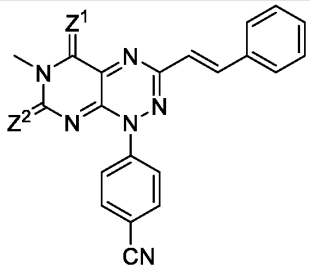
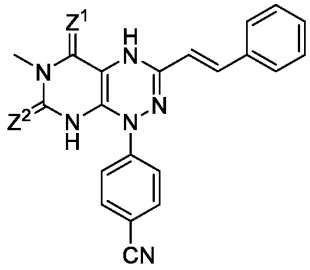
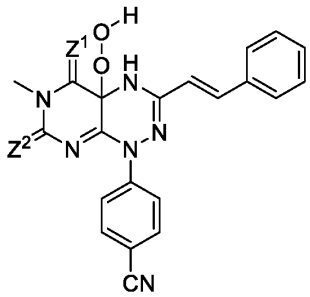
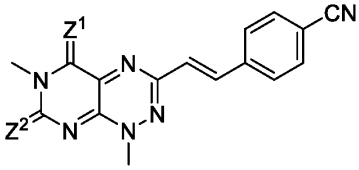
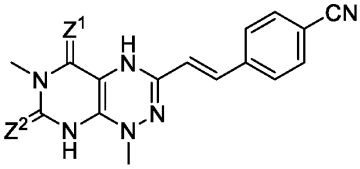
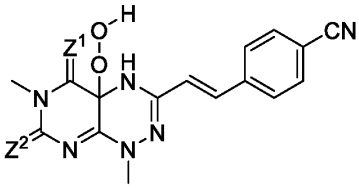
No.	Name	Structure
21.2	hydroperoxy-N1-ethylstyryl POG	
22	hydrostyryl POG	
22.1	red-hydrostyryl POG	
22.2	hydroperoxy-hydrostyryl POG	
23	benzyl POG	
23.1	red-benzyl POG	
23.2	hydroperoxy-benzyl POG	
24	N-oxide styryl POG	

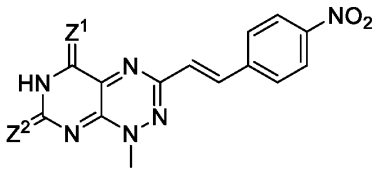
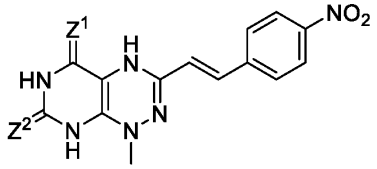
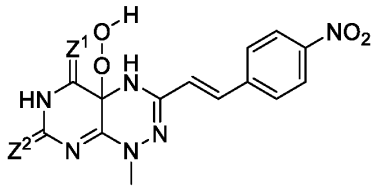
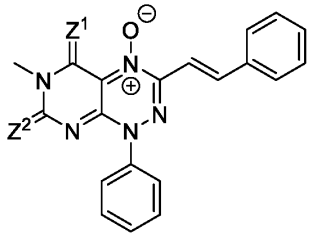
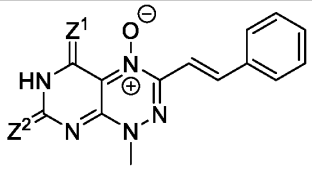
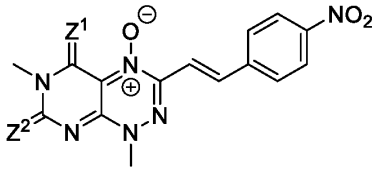
No.	Name	Structure
25	phenyl POG	
25.1	red-phenyl POG	
25.2	hydroperoxy-phenyl POG	
26	naphthalene POG	
26.1	red-naphthalene POG	
26.2	hydroperoxy-naphthalene POG	
27	p-fluorophenyl POG	
27.1	red-p-fluorophenyl POG	

No.	Name	Structure
27.2	hydroperoxy-p-fluorophenyl POG	
28	4-pyridinyl POG	
28.1	red-4-pyridinyl POG	
28.2	hydroperoxy-4-pyridinyl POG	
29	1-N-cyclopentylstyryl POG	
29.1	red-1-N-CyclopentylstyrylPOG	
29.2	hydroperoxy-1-N-CyclopentylstyrylPOG	

No.	Name	Structure
30	6-N-demethylstyryl POG	
30.1	red-6-N-demethylstyryl POG	
30.2	hydroperoxy-6-N-demethylstyryl POG	
31	6-N-demethyl-N1-phenyl-p-nitrostyryl POG	
31.1	red-6-N-demethyl-N1-phenyl-p-nitrostyryl POG	
31.2	hydroperoxy-6-N-demethyl-N1-phenyl-p-nitrostyryl POG	
32	6-N-demethyl-N1-phenyl-styryl POG	

No.	Name	Structure
32.1	red-6-N-demethyl-N1-phenyl-styryl POG	
32.2	hydroperoxy-6-N-demethyl-N1-phenyl-styryl POG	
33	N1-fluorophenyl-styryl POG	
33.1	red-N1-fluorophenyl-styryl POG	
33.2	hydroperoxy-N1-fluorophenyl-styryl POG	

No.	Name	Structure
34	N1-cyanophenyl-styryl POG	
34.1	red-N1-cyanophenyl-styryl POG	
34.2	hydroperoxy-N1-cyanophenyl-styryl POG	
35	p-cyanostyryl POG	
35.1	red-p-cyanostyryl POG	
35.2	hydroperoxy-p-cyanostyryl POG	

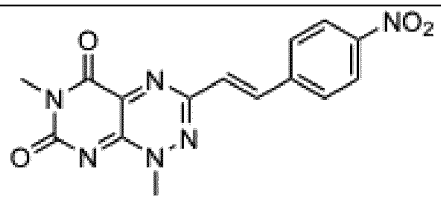
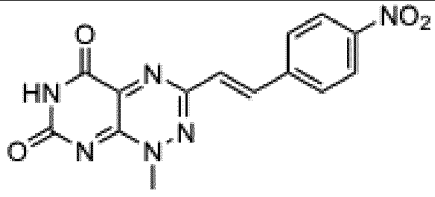
No.	Name	Structure
36	6-N-demethyl-p-nitrostyryl POG	
36.1	red-6-N-demethyl-p-nitrostyryl POG	
36.2	hydroperoxy-6-N-demethyl-p-nitrostyryl POG	
37	N1-phenyl-N-oxide-styryl POG	
38	6-N-demethyl-N-oxide-styryl POG	
39	p-nitro-N-oxide-styryl POG	

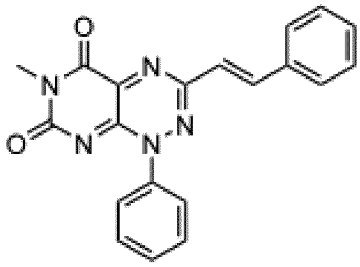
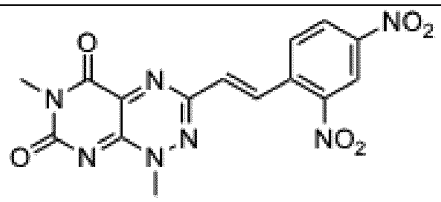
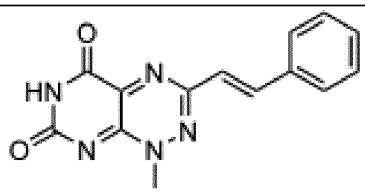
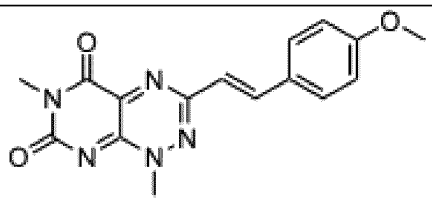
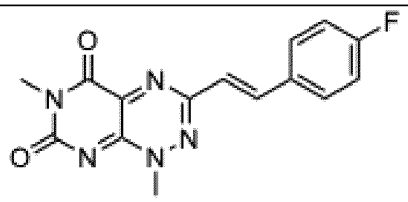
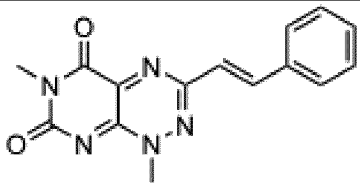
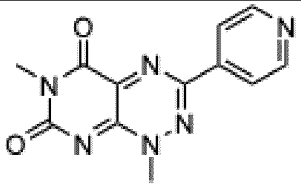
In the formulae of Table 1, Z^1 and Z^2 denote independently from each other O or S. Thus, the formulae listed in Table 1 represent four different variants (V1, V2, V3, and V4) of each example (V1: Z^1 = O and Z^2 = S; V2: Z^1 = S and Z^2 = O; V3: Z^1 = O and Z^2 = O; and V4: Z^1 = S and Z^2 = S). All variants shall be deemed to be individually disclosed in Table 1.

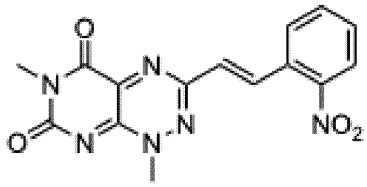
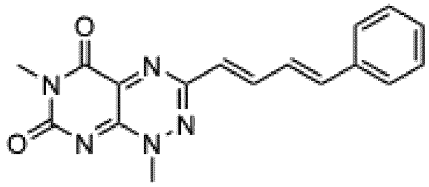
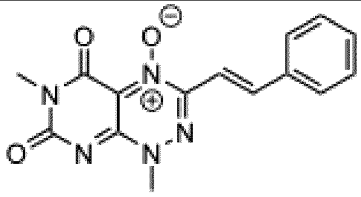
Individual substances falling under general formulae (I), (II), (III), or (IV) were tested with respect to their capability of generating hydrogen peroxide as well as with respect to their senolytic potency. The capability of generating hydrogen peroxide was measured in an

aqueous fluorescence assay at pH 6.9 containing final concentrations of 131 µM europium(III) chloride, 41.6 µM tetracyclin, 26 mM 3-morpholinpropylsulfonic acid, 1 mM NADH (nicotinamide adenine dinucleotide, reduced form) or 1 mM DTT (dithiothreitol), 2 µM of the substance and 0.5% DMSO (dimethyl sulfoxide). Substances were dissolved in DMSO and added last to the assay. Hydrogen peroxide generation was measured via fluorescence intensity (λ_{ex} = 405 nm, λ_{em} = 616 nm). The senolytic potency was tested as will also be described further below in more detail. Briefly, the amount of viable senescent-associated beta-galactosidase positive cells after treatment was compared with the amount of viable senescent-associated beta-galactosidase positive cells prior to the treatment. The lower the relative number of viable cells after treatment, the higher was the senolytic potency. These substances are listed in the following Table 2. They bear different groups as residues R^2 and R^3, thus illustrating beneficial properties of these substances over a broad range of substituents as residues R^2 and R^3. In an embodiment, each individual compound listed in Table 2 is the compound for the medical use as claimed in here. Thus, these compounds are particularly appropriate example compounds for the presently claimed use.

Table 2: Substances falling under general formulae (I), (II), (III), or (IV) and having a high hydrogen peroxide generation capability and/or a high senolytic potency. POG-4 (substance no. 20 of Table 1 as well as substance no. 8 of Table 2) was used as lead structure. Its hydrogen peroxide generation capability was set to 100 %. The marker "++" indicates a senolytic potency toward senescent cells that is at least as good as that of POG-4. The marker "+++" indicates a senolytic potency that is better than that of POG-4. The marker "--" indicates a senolytic potency less good than that of POG-4.

No.	Structure	H_2O_2 generation	Senolytic potency
1		161%	++
2		161%	++

No.	Structure	H_2O_2 generation	Senolytic potency
3		161%	+++
4		142%	--
5		122%	++
6		103%	++
7		103%	++
8		100%	++
9		98%	--

No.	Structure	H_2O_2 generation	Senolytic potency
10		90%	--
11		81%	++
12		73% (Prodrug)	++

The compounds described herein show high specificity for senescent cells and efficacy in killing senescent cells without significantly harming non-senescent cells. In an embodiment, POG has significant and specific senolytic activity associated with essentially non-existent or non-existent toxicity to non-senescent cells.

In other words, the compounds described herein can specifically and effectively kill senescent cells while leaving non-senescent cells essentially unharmed. This is a significant advantage over known senolytics, such as Navitoclax, which kills both senescent and non-senescent cells at the senolytically active dosage or concentration. For example, the senolytically active dosage can be 0.25 mg/kg bodyweight up to 7.5 mg/kg bodyweight (in particular 0.5 mg/kg bodyweight up to 7 mg/kg bodyweight, in particular 1 mg/kg bodyweight up to 6 mg/kg bodyweight, in particular 2 mg/kg bodyweight up to 5 mg/kg bodyweight, in particular 3 mg/kg bodyweight up to 4 mg/kg bodyweight) in a mouse model.

In a cell culture a concentration of, e.g., 1 µM, 2 µM, 3 µM, 4 µM, 5 µM, 6 µM, 7 µM, 8 µM, 9 µM, 10 µM, 15 µM, 20 µM, 25 µM, 30 µM, 35 µM, 40 µM, 45 µM, 50 µM, 55 µM, 60 µM, 65 µM, 70 µM, 75 µM, 80 µM, 85 µM, 90 µM, 95 µM,100 µM or more is an appropriate senolytically active concentration.

In an embodiment, DMSO is used as solvent or co-solvent for the compounds. In an embodiment, 2-hydroxypropyl-beta-cyclodextrin is used as solvent or co-solvent instead of DMSO. Any POG dissolved in a solution containing 2-hydroxypropyl-beta-cyclodextrin, in particular in a solution of 20 % to 50 %, in particular 30 % to 40 %, 2-hydroxypropyl-beta-cyclodextrin will be denoted as POG-CD.

In an embodiment, compounds, such as POG-4, POG #18 or POG-CD (cf. Table 3 for their structures) are highly effective in killing senescent cells.

In an embodiment, these compounds are administered such to obtain a concentration of 1 µM, 2 µM, 3 µM, 4 µM, 5 µM, 6 µM, 7 µM, 8 µM, 9 µM, 10 µM, 15 µM, 20 µM, 25 µM, 30 µM, 35 µM, 40 µM, 45 µM, 50 µM, 55 µM, 60 µM, 65 µM, 70 µM, 75 µM, 80 µM, 85 µM, 90 µM, 95 µM,100 µM or more, preferably 4 µM to 8 µM. In an embodiment, these compounds are administered in a senolytically active dosage as explained above. In these examples, only about 10% of the senescent cells remain viable. It is particularly remarkable that even at a concentration of 4 µM, POG reduces viability of senescent cells to less than 10%. An approximating similar effect can only be achieved with a much higher concentration of Navitoclax (40 µM), which kills senescent but also non-senescent cells at this concentration.

In an embodiment, the compound has a significantly higher and more specific senolytic activity than prior art senolytics.

Generally, a senolytic can be used in the treatment of diseases or conditions in which the removal of senescent cells is beneficial. Such diseases or conditions are diseases or (pathological) conditions that are caused or exacerbated by senescent cells. The treatment, i.e., the therapeutic treatment, encompasses also a prophylactic treatment, i.e., a prevention of a disease or condition, or a delay of a disease or condition. Exemplary diseases or conditions in which the removal of senescent cells is beneficial are cancer and premalignant precursor lesions, viral and/or bacterial infection diseases, neurodegenerative diseases (e.g., Alzheimer's disease), age-related diseases/conditions, autoimmune diseases, and inflammatory processes (such as inflammations). Further exemplary diseases or conditions in which the removal of senescent cells has been show in the prior art to be beneficial are diffuse large B-cell lymphoma (DLBCL), melanoma, lung adenocarcinoma, Ras-mutated tumors, glioblastoma, small cell lung cancer (SCLC), triple negative breast cancer (TNBC), pancreatic ductal adenocarcinoma (PDAC), glaucoma (Liton et al., 2005), idiopathic pulmonary fibrosis (Yanai et al., 2015; Schafer et al., 2017), atherosclerosis (Uryga et al., 2016; Childs et al., 2016), liver cirrhosis and non-alcoholic fatty liver disease (NAFLD) (Krizhanovsky et al., 2008;

Kim et al., 2013; Ogrodnik et al., 2017; Wiemann et al., 2002), glomerulosclerosis (Melk et al., 2003; Melk et al., 2004; Baker et al., 2016), type-2 diabetes (Chen et al., 2009; Helman et al., 2016), cachexia (Berry et al., 2017; Xu et al., 2015; Baker et al., 2016), sarcopenia (Sousa-Victor et al., 2014; Cosgrove et al., 2014; Chang et al., 2016), and osteoarthritis (Price et al., 2002; Kuyinu et al., 2016; Jeon et al., 2017). Yet further exemplary diseases will be listed further below.

In an embodiment, the compound is used as senolytic medicament against any or all of these diseases/conditions.

Senescent cells can release pro-inflammatory substances. These substances can promote inflammatory reactions and contribute to the development of any or all of these diseases or conditions.

The invention also relates to a medicament comprising a compound described herein for use in the treatment of cancer and any types of cancer.

The invention also relates to a pharmaceutical composition comprising a compound for in-vivo use as senolytic.

In an embodiment, the pharmaceutical composition is used in the treatment, prevention or delay of a condition comprising senescent cells.

Combinations of in vitro and in vivo studies (refer to the Figures and examples) show selective killing of senescent cells in vitro and the dependence of cell killing or the triggering of a clinically relevant long-term effect in vivo. In particular, different genetic test scenarios in vivo were chosen that either allow senescence development or not and accordingly see surprising effects of POG (effective on senescence ability) in vivo. The person skilled in the art would not have expected the compounds to be such specific to senescent cells. In particular, the high selectivity and effectiveness with negligible or non-significant toxicity is surprising and beneficial.

For example, the compounds exhibit senolytic activity in a variety of senescence scenarios, such as including replicative senescence, virus-induced senescence, therapy-induced senescence, oncogene-induced senescence, oncogene-inhibition induced senescence and against various cancer cell lines and cancer models in vivo, without any significant damage to

non-senescent cells or critical organismic toxicity being observed in the animal model. (refer to the exemplary embodiments).

In an embodiment, the compound is for use as senolytic against senescent cells in replicative senescence (RS) and/or in virus-induced senescence (VIS) and/or in oncogene-induced senescence (OIS), and/or in oncogene inhibition-induced senescence (OIIS) and/or in therapy-induced senescence (TIS). In this context, it is important to make a distinction between an anti-tumor activity of POG and an anti-senescence activity of POG such as a senolytic activity against cells having oncogene-induced senescence. Notably, the so far reported anti-tumor activity of POG is only directed against proliferating tumor cells and requires high concentrations (in cell culture: > 10 µM). POG applied in such concentration would expectedly harm healthy tissue in vivo (in mice, adverse effects (in particular oxidative stress) have been observed in vivo in concentrations of > 0.5 mg POG/kg of bodyweight), i.e., such dose range is likely to be very toxic.

The senolytic activity of POG can already be exploited at much lower concentrations of less than 1 µM, hence, in vitro less than 10 % of the concentration necessary for reportedly exhibiting an anti-tumor activity in proliferating cancer cells. At low concentration (250 ug POG/kg bodyweight in mice), no relevant organ toxicity was observed in mouse models in vivo. An inhibition of never-senescent, proliferating tumor cells could not be observed at such low concentrations, while senolytic activity was evident with respect to cells being in either of the four above-mentioned states of senescence, i.e. RS, VIS, OIS, and TIS. Consequently, the compounds, the use of which is presently claimed, are particularly appropriate to be used as senolytic in case of age-related diseases, virus infections, for the prevention of tumors (in case of OIS) as well as therapeutic senolytic in the context of tumor drug therapy (in case of TIS).

In an embodiment, the compound is used in a concentration in which it does not show any activity against proliferating tumor cells. This concentration is dependent on the concrete experimental setup or the physiologic conditions of the patient to be treated, respectively. For instance, concentrations as low as 0.8 µM produced 50 % death of senescent cells, while > 95 % of the proliferating cells remained viable under this condition. In in-vivo experiments conducted in mice, a dose of two times 250 µg POG per kg bodyweight showed a senolytic effect. Thus, in an embodiment, the concentration is 100 µg to 1000 µg POG per kg bodyweight, in particular 200 µg to 900 µg per kg bodyweight, in particular 300 µg to 800 µg per kg bodyweight, in particular 400 µg to 700 µg per kg bodyweight, in particular 500 µg to 600 µg per kg bodyweight. Typically, the concentration of the compound is at most 10 % of the reported concentration for the POG used as anti-tumor agent against proliferating tumor cells,

and can be closely linked to the senescent status of the cells as indicated by the senescence-associated beta-galactosidase activity marker (SA-β-gal), i.e. it kills in a SA-β-gal-proportional manner, thereby leaving never-senescent SA-β-gal-negative cells largely unaffected. Notably, it is intrinsic to the mode of POG activation (see below) that POG is preferentially activated in the redox context of senescent cells as compared to proliferating non-senescent cells, thereby further explaining the cytotoxic efficacy of POG at a much lower concentration against senescent cells.

Different concentrations and dosages of the compounds are suitable and achieve the described senolytic effect determined by the methods described herein.

The development of senescence can also be traced, for example, on the basis of numerous cellular senescence markers that indicate senescent cells and are overexpressed in the lung cells of old mice (Figure 14). These senescence markers also include the overexpression of the CDK inhibitor p16INK4a, histone methylation (H3K9me3). Treatment with POG not only eradicated the senescent cells, but also their disease-underlying profibrogenic action, the formation of collagen cuffs around the airway.

The precedingly mentioned concentrations or dosages for mice can be converted into appropriate equivalent dosages for other species, such as humans, e.g., by applying conversion factors known from the scientific literature such as a publication by Nair and Jacob (2016).

In an embodiment, the compound is for sequential use in treating cancer following a chemotherapeutic agent. Thus, the compound is used as senolytic for treating tumor cells in TIS. The number of tumor cells that undergo TIS during clinically applicable anti-cancer therapy (such as chemotherapy, radiation or targeted therapeutics) can be significantly higher than the number of senescent cells naturally seen to occur under other triggers. Consequently, the use of the compounds as senolytic against therapy-induced senescent tumor cells has a significantly higher likelihood to quantitatively eliminate residual cancer cells, i.e. tumor cells that survived chemotherapy or other anti-cancer therapies before (and, hence, bear the risk to account for a subsequent tumor relapse). The risk of recurrence or relapse and (a) the ability to enter senescence under primary chemotherapy and (b) to favor a disease relapse due to this (cell-autonomous, non-cell-autonomous) can also be related.

For example, recurrences or relapse after chemotherapy are known in the prior art for several cancers, including glioblastomas, ovarian carcinomas, diffuse large B-cell lymphomas, breast

carcinomas, and non-small cell lung carcinomas. A skilled person in the art would know in which tumor diseases and chemotherapeutic agents the described effect occurs. The inventors were able to show effectiveness of the compounds in different tumor cell lines and chemotherapeutic agents.

In an embodiment, the compound is for use in treating cancer that has previously been treated with a chemotherapeutic agent. Thus, the compound is used as senolytic for treating therapy-induced senescence of tumor cells. It could be shown that the number of tumor cells that undergo therapy-induced senescence is significantly higher than the number of senescent tumor cells that reached the status of senescence due to other triggers. Consequently, the use of the compounds as senolytic against therapy-induced senescent tumor cells has a significantly higher effect with respect to cancer therapy than the use of the compounds as senolytic against tumor cells that have reached the status of senescence due to non-therapeutic triggers.

Senescence in primary, non-malignant ("healthy") cells can be enforced in response to the expression of certain activated oncogenes. This type of senescence is referred to as oncogene-induced senescence. In a general embodiment, the compounds, the use of which is presently claimed, are used as senolytic against any senescent cells (irrespective of the trigger of senescence). In a more specific embodiment, the compounds, the use of which is presently claimed, are specifically not used as senolytic against oncogene-induced senescent cells (i.e., senescent cells that have reached their senescent status due to oncogene-induced senescence).

In an embodiment, the compounds, the use of which is presently claimed, are used as senolytic against (mutant) Ras-driven or (mutant) Braf-driven cancers, e.g., in the treatment and/or prevention of Ras-driven or Braf-driven cancers. Mutation-activated Ras genes and Braf genes are oncogenes, because they cooperate with other genetic lesions to transform normal cells into tumor cells, and to eventually develop full-blown cancer. The inventors were able to show that POG, the use of which is presently claimed, are particularly potent in killing cells that reached a status of OIS in response to Ras or Braf activation.

POG is also a redox-sensitive, NADH-activatable cytotoxic compound activated in vitro by NADH. POG generates H_2O_2 in vitro in the presence of NADH, indicating strong activation by NADH. The inventors were able to show that POG selectively targets Ras and Braf mutated senescent cells. The inventors were further able to show, that POG targets OIS cells and are particularly selectively effective on Ras and Braf mutant senescent cells.

In an embodiment, administration of POG is associated with a significant improvement in blood parameters in aging subjects.

In an embodiment, POG selectively targets and/or exhibits potent activity against Ras and Braf mutant senescent cells.

POG cannot only eliminate macroscopic senescent lesions, but also act as a cancer delaying and preventing drug by degrading incipient senescent cells that, if not eliminated early, could otherwise become the origin of full-blown and even metastatic malignant tumors (confer the examples).

In an embodiment, POG delays the progression of disease.

The inventors were able to show that POG administration can also extend healthy lifespan in aging subjects and “overall-survival” in cancer-induced subjects and anti-cancer treated subjects. In an embodiment, POG administration can improve age-related blood parameters and promotes physical recovery. In an embodiment, POG administration can improve rotarod performance in aging mice, indicating improved physical endurance.

In an embodiment, POG targets senescent cells in the lung and significantly reduces the number of senescent cells in the lung of aging mice.

In an embodiment, POG administration reduces peribronchial pulmonary fibrosis and exhibits antifibrotic activity in aging mice.

Administration of POG can also improve markers typically associated with age-related loss of function, such as red blood cells, hemoglobin, urea and serum protein levels.

In an embodiment, one or more weeks, preferably 4 weeks, after administration of POG senescent cells and peribronchial fibrosis are significantly reduced in aged subjects.

In an embodiment, administration of POG improves markers associated with age-related loss of function, such as red blood cells, hemoglobin, urea and serum protein levels.

In an embodiment, administration of POG causes no or negligible toxicity.

In an embodiment, the compound delays medical conditions of aging.

Administration of POG has also no apparent adverse effects in vivo, such as on body weight or white blood cells, liver function and glucose metabolism (confer examples).

In an embodiment, administration of POG treatment is associated with no or substantially no adverse effects on body weight and blood parameters.

In an aspect, the invention relates to a cosmetic use of a compound as senolytic for treating conditions related to aging.

For the subject matter of a cosmetic use, all parts of the invention disclosed herein apply in an equivalent manner.

In an embodiment, the compounds are used for treating replicative senescence, i.e., as senolytic against cells that reached their senescent state due to replicative senescence (RS). RS cells are known as a major driver of organismic aging and age-related pathologies. Despite their relative sparsity in tissues, they account via their SASP for chronic “inflammaging”, leading to age-related organ damage such as chronic obstructive pulmonary disease or liver fibrosis. Hence, eliminating pro-inflammatory RS cells by senolytics can help to slow the aging process in a system-wide fashion.

In an embodiment, the compounds are used for treating virus-induced senescence, i.e., as senolytic against cells that reached their senescent state due to virus-induced senescence. In this setting, it is – similar to RS – the therapeutic goal, to selectively eliminate senescent cells because of their contribution to inflammation-mediated organ damage, as known from the cytokine storm attributed to the SARS-CoV-2 virus in severe COVID-19, in which senolytics were shown to mitigate the course of disease.

A pharmaceutically acceptable salt of the compounds explained above is, e.g., an acid salt of a substance containing an amine or other basic group. A salt can be obtained by reacting the substance with a suitable organic or inorganic acid, such as hydrogen chloride, hydrogen bromide, acetic acid, perchloric acid and the like. Non-limiting examples of such salts are hydrochlorides, hydrobromides, sulfates, methanesulfonates, nitrates, maleates, acetates, citrates, fumarates, tartrates, succinates, benzoates and salts with amino acids such as glutamic acid. Salts of compounds containing a carboxylic acid or other acidic functional group can be prepared by reacting with a suitable base. Non-limiting examples of such salts are alkali

metal salts (e.g., sodium or potassium), alkaline earth metal salts (e.g., calcium or magnesium), aluminum salts, ammonium salts, and salts of organic bases such as trimethylamine, triethylamine, morpholine, pyridine, piperidine, picoline, dicyclohexylamine, N,N'-dibenzylethylenediamine, 2-hydroxyethylamine, bis-(2-hydroxyethyl)amine, tri-(2-hydroxyethyl)amine, procaine, dibenzylpiperidine, dehydroabietylamine, glucamine, N-methylglucamine, collidine, quinine, quinoline, and basic amino acids such as lysine and arginine. Descriptions of suitable pharmaceutically acceptable salts can be found in Handbook of Pharmaceutical Salts, Properties, Selection and Use, Wiley VCH (2002). For therapeutic use, salts of the compounds are those wherein the counter-ion is pharmaceutically acceptable. However, salts of acids and bases which are non-pharmaceutically acceptable may also find use, for example, in the preparation or purification of a pharmaceutically acceptable compound.

In an embodiment, the compound is for use in a specific patient group. This specific patient group comprises or consists of patients that have been classified by gene expression analysis to have a high susceptibility against the compounds as explained herein. In an embodiment, the specific patient group excludes patients in whom the efficacy of the compounds as described in here can considered to be below a predeterminable threshold or is expected to be not existent. Such a patient group comprises or consists of patients that have a tumor carrying a deactivated p53 gene. Due to this deactivated p53 gene, the tumor will not go into senescence so that the compounds described herein will not be able to apply their senolytic activity, i.e., they will not be effective against such tumor.

In an aspect, the present invention relates to a method of treatment, namely, a method of inducing senolysis in a patient in need thereof, wherein the method comprises administering an effective amount of a compound according to any of general formulae (I), (II), (III), or (IV) as indicated above, or a pharmaceutically acceptable salt or hydrate of such compound or derivative to the patient. In this context, the variable residues of general formulae (I), (II), (III), or (IV) have the meanings as indicated above.

In an embodiment, the method of inducing senolysis is a method of treating cancer in the patient, in particular by senolysis of senescent tumor cells that have reached their senescent state due to therapy-induced senescence, e.g., by subjecting the patient to a chemotherapy.

In another aspect, the invention relates to compounds for in-vivo use as senolytic intended for treating cancer or cancer-like disorders, such as a cellular proliferative disorder or cancer metastasis.

"Cancer", as used herein, is a disease characterized by the uncontrolled growth of abnormal cells. Cancer refers to any type of cancerous growth or carcinogenic process, metastatic tissue or malignant transformed cells, tissues or organs, regardless of histopathological type or invasive stage. Cancer cells can spread locally or to other parts of the body via the bloodstream and lymphatic system. Cancer cells spreading to other parts to the body are termed “metastatic cells” or “metastatic tumor cells”.

The terms "cancer," "proliferative disorder" or “tumor” or "cellular proliferative disorder" or "tumor disease" or "malignant tumor" may be understood interchangeably to refer to any disorder in which the proliferative capabilities of affected cells differ from the normal proliferative capabilities of unaffected cells, e.g. the terms include solid and liquid, e.g. general or circulating tumors, premalignant and malignant cancers and tumors. An example of a cell proliferative disorder is neoplasia. Malignant cells develop as a result of a multistep process. A cell proliferative disorder as described herein may be a neoplasm, commonly referred to as a tumor. The term "carcinoma" refers to a malignant new growth made up of epithelial cells tending to infiltrate surrounding tissues, and to give rise to metastases.

The invention provides, in an aspect, a method of preventing, treating, and/or managing a solid tumor in a patient, the method comprising administering to a patient in need thereof a prophylactically effective regimen or a therapeutically effective regimen, the regimen comprising administering to the patient a compound of the invention, or a pharmaceutically acceptable salt thereof, wherein the patient has been diagnosed with a solid tumor. Examples of solid tumors are colorectal cancer, pancreatic cancer, breast cancer, rectal cancer, liver, lung, breast, lymphatic system, digestive organs (e.g. colon), urogenital organs (e.g. kidney, urothelial cells), prostate and throat, malignant organ systems including tumors such as sarcomas, adenocarcinomas and cancer. Examples for breast cancer that can be treated with the presently described compounds are ductal carcinoma in situ (DCIS), lobular carcinoma in situ (LCIS), invasive ductal carcinoma (IDC), invasive ductal carcinoma including tubular, medullary, mucinous, papillary, and cribriform carcinomas, invasive lobular carcinoma (ILC), inflammatory breast cancer, male breast cancer, Paget’s Disease of the nipple, phyllodes tumors of the breast, recurrent and/or metastatic breast cancer. Adenocarcinoma includes most malignant tumors such as colon cancer, rectal cancer, renal cell cancer, liver cancer, non-small cell lung cancer, small intestine cancer and esophageal cancer. In certain forms the cancer is a melanoma, e.g. an advanced stage melanoma. Metastatic lesions of the cancer can also be treated or prevented with the methods and compositions of the invention. Examples of other types of cancer that can be treated are, leukemias, bone cancer, skin

cancer, head and neck cancer, skin or intraocular melanoma, uterine cancer, ovarian cancer, anal cancer, stomach cancer, testicular cancer, faropius duct cancer, endometrial cancer, cervical cancer, vaginal cancer, vulvar cancer, esophageal cancer, small intestine cancer, endocrine cancer, thyroid cancer, parathyroid cancer, adrenal cancer, soft tissue sarcoma, urethral cancer, penile cancer, solid tumor in childhood, lymphoma, bladder cancer, kidney or ureter cancer, renal pelvis cancer, neoplasm of the central nervous system (CNS), primary CNS lymphoma, tumor angiogenesis, spinal axis tumor, brainstem glioma, pituitary adenoma, Kaposi's sarcoma, epidermoid carcinoma, squamous cell carcinoma, including a combination of environmental cancer and cancer including treatment of metastatic cancer, e.g. metastatic cancer that expresses PD-L1 (Iwai et al. Int. Immunol., 2005) as well as “Cancers of unknown primary (CUP)” can be performed with the inhibitory compound described in this invention.

In an aspect, the present invention relates to an in-vitro method for senolysis. This method comprising subjecting senescent cells to a compound according to general formula (I) as indicated above, to a derivative of such compound according to any of general formulae (II), (III), or (IV) as indicated above, or to a pharmaceutically acceptable salt or hydrate of such compound or derivative. In this context, the variable residues of general formulae (I), (II), (III), or (IV) have the meanings as indicated above.

In an embodiment, the compound, the derivative or the pharmaceutically acceptable salt or hydrate of such compound or derivative is used in in such a dose that a number of viable senescence-associated beta-galactosidase positive cells is equal to or less than 20 % (in particular 0 % to 20 %, in particular 5 % to 15 %, in particular 7 % to 12 %, in particular 8 % to 10 %) of the number of viable senescence-associated beta-galactosidase positive cells prior to subjecting the senescent cells to the compound, the derivative, or the pharmaceutically acceptable salt or hydrate of such compound or derivative. In this context, a viability of senescence-associated beta-galactosidase positive cells is determined by a dye exclusion test carried out at least 22 hours (in particular 22 hours to 8 days, in particular 24 hours to 7 days, in particular 36 hours to 6 days, in particular 48 hours to 5 days, in particular 72 hours to 4 days) after subjecting the senescent cells to the compound, the derivative, or the pharmaceutically acceptable salt or hydrate of such compound or derivative.

For the subject of this aspect of the invention, all parts of the invention disclosed herein apply.

Reference is made to the explanations given above with respect to particularly appropriate dyes for the dye exclusion test.

The term “in vitro” as used throughout herein also encompasses the term “ex vivo”. Thus, the above explained in-vitro method for senolysis is also appropriate in ex-vivo methods. An example of such an ex-vivo method is the field of organ transplantation. Here, the senolytic compounds can be used, e.g., for preparing and optimizing organs to be transplanted (by removing senescent cells from such organs). Cell therapies such as immune therapies and cell transplantations are further examples for an ex vivo application in which senolysis can be beneficial and in which the presently described compounds can well be used as senolytic.

The term “senolytic” as used herein, refers to a class of molecules or compounds selectively (preferentially or to a greater degree) killing, destroying, removing, significantly reducing viability, facilitating selective destruction of senescent cells or inducing death of senescent cells. Senolytic is also known to skilled person in the art.

A senolytic drug or compound for use as senolytic refers to a substance selectively targeting senescent cells. This targeting of senescent cells is beneficial for the delay, treatment, and/or prevention of a condition, medical condition or disease described herein. For example, the compound or molecule comprising senolytic activity kills or destroys or removes a senescent cell in a medical, non-medical, biologically, clinically, and/or statistically significant manner compared to its capability to kill or to destroy or to remove a non-senescent cell. Targeting of senescent cells by compounds described herein can lead to killing such cells. The compounds described herein are sufficient to kill an appropriate number of senescent cells necessary for the delay, treatment, and/or prevention of a condition, medical condition or disease described herein. A senolytic is also a drug that selectively kills senescent cells without harming non-senescent cells.

Typically, senescent cells can release inflammatory molecules into the surrounding tissue. This can, for example, cause other cells to also go into senescence. The removal of senescent cells with senolytics is beneficial for the treatment, prevention and delay of a variety of conditions, including but not limited to medical conditions as described herein. As shown in the examples, the senolytic compound or drug has an effect on a senescent cell, but has no effect on non-senescent tissue or non-senescent cells, such as proliferating cells.

A senolytic compound or drug used in an amount and for a time sufficient that selectively kills, destroys, removes, facilitates selective destruction or induces death of established senescent cells is not harming a non-senescent cell, for example does not kill, destroy, or cause death of non-senescent cell.

Senescent cells are cells known to skilled person as cells or aged cells that have stopped dividing and do not die. This condition is known as cell senescence, preferentially triggered by various stress factors such as DNA damage, oxidative stress or critical shortening of the telomeres (the protective caps at the ends of the chromosomes). They are metabolically active but have lost their original function and SASP.

For example, senescent cells can be distinguished from non-senescent cells by testing the cells with respect to an expression of senescence-associated beta-galactosidase. Senescence-associated beta-galactosidase positive cells are also senescent cells, wherein senescence-associated beta-galactosidase negative cells are also non-senescent cells. Cytochemical assays for distinguishing senescence-associated beta-galactosidase positive cells from senescence-associated beta-galactosidase negative cells are well known to a person skilled in the art and are, e.g., described by Dimri et al. (1995), Bassaneze et al. (2008), Gary et al. (2005), and Itahana et al. (2007).

Feature of senescent cells can be: (i) senescent cells stop dividing but remain metabolically active (cell cycle arrest), (ii) senescent cells show altered gene expression, including upregulation of genes associated with inflammation and cell cycle arrest (iii) senescent cells secrete a variety of pro-inflammatory cytokines, growth factors and proteases (SASP) (secretory phenotype) and/or (iv) senescent cells are often resistant to programmed cell death mechanisms (apoptosis), which means that they can remain in the tissue and accumulate (resistance to apoptosis). SASP can affect the surrounding tissue and contribute to chronic inflammation. Typically, a senescent cell releases inflammatory molecules into the surrounding tissue. This can, for example, cause other cells to also go into senescence. The removal of a senescent cell with a senolytic compound or molecule is beneficial for the treatment, prevention and delay of a variety of medical conditions. Non-senescent cells may be proliferating cells, quiescent cells, functional terminally differentiated non-dividing cells or functional terminally differentiated partially dividing cells.

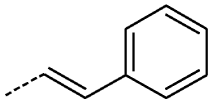
The senolytic compound or drug may alter, for example, either or both of a cell survival signaling pathway (e.g., Akt pathway) or an inflammatory pathway, for example, by antagonizing a protein within the cell survival and/or inflammatory pathway in a senescent cell. In certain embodiments, the senolytic compound or drug described herein alter at least one signaling pathway in a manner that induces (initiates, stimulates, triggers, activates, promotes) and results in (i.e., causes, leads to) death of the senescent cell. In some embodiments, senolytic compounds or drugs may be used in treatments described as useful for treating a cancer.

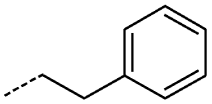
As used herein "treatment" or "treating" or “therapy” includes any beneficial or desirable effect on the symptoms or pathology of a disease or pathological condition, and may include even minimal reductions in one or more measurable markers of the disease or condition being treated. Treatment can involve optionally either the reduction or amelioration of symptoms of the disease or condition, or the delaying of the progression of the disease or condition. The effect may be prophylactic in view of completely or partially preventing a disease and/or a symptom, for example by reducing the risk of a subject having a particular disease, disease progression, disease development in an undesired course, or symptom, "Treatment" does not necessarily indicate complete eradication or cure of the disease or condition, or associated symptoms thereof.

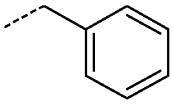
As used herein, "prevent," and similar words such as "prevented," "preventing" or "prophylactic" etc., indicate an approach for preventing, inhibiting, or reducing the likelihood of the occurrence or recurrence or relapse of, a disease or condition. It also refers to delaying the onset or recurrence of a disease or condition or delaying the occurrence or recurrence or relapse of the symptoms of a disease or condition. As used herein, "prevention" and similar words also includes reducing the intensity, effect, symptoms and/or burden of a disease or condition prior to onset or recurrence or relapse of the disease or condition.

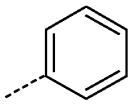
In the present invention, "therapy" includes arbitrary treatments of diseases or conditions in mammals, in particular, humans, for example, the following treatments (a) to (c): (a) prevention of onset of a disease, condition or symptom in a patient; (b) Inhibition of a symptom of a condition, that is, prevention of progression of the symptom; (c) Amelioration of a symptom of a condition, that is, induction of regression of the disease or symptom.

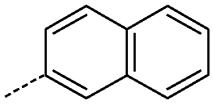
In an aspect, the present invention relates to a compound according to general formula (I) as indicated above, a derivative of such compound according to any of general formulae (II), (III), or (IV) as indicated above, or a pharmaceutically acceptable salt or hydrate of such compound or derivative. In this context, the variable residues of general formulae (I), (II), (III), or (IV) generally have the meanings as indicated above. However, certain limitations apply to the possible meanings of residue R^2 that will be explained in the following. In this context, the dashed line indicates a bond by which the residue R^2 is covalently bound to the triazine ring of the compound according to general formulae (I), (II), (III), or (IV).

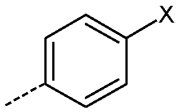
i) R^2 is not  if residue R^1 is a methyl residue and residue R^3 is a methyl or ethyl residue,

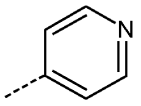
ii) R^2 is not  if residue R^1 is a methyl residue and residue R^3 is a methyl residue,

iii) R^2 is not  if residue R^1 is a methyl residue and residue R^3 is a methyl residue,

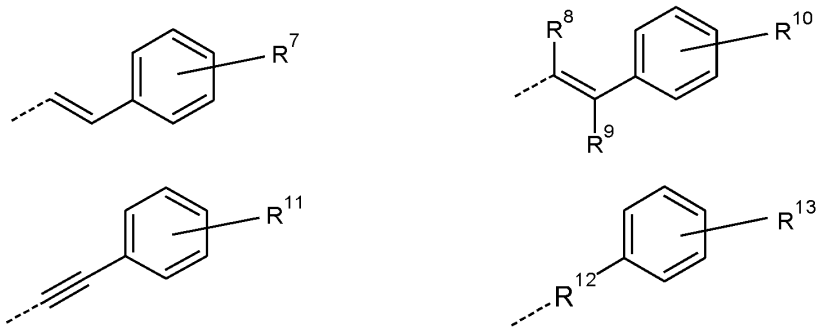
iv) R^2 is not  if residue R^1 is a methyl residue and residue R^3 is a methyl residue,

v) R^2 is not  if residue R^1 is a methyl residue and residue R^3 is a methyl residue,

vi) R^2 is not  if residue R^1 is a methyl residue and residue R^3 is a methyl residue, wherein X denotes F, Cl, Br, or I, and

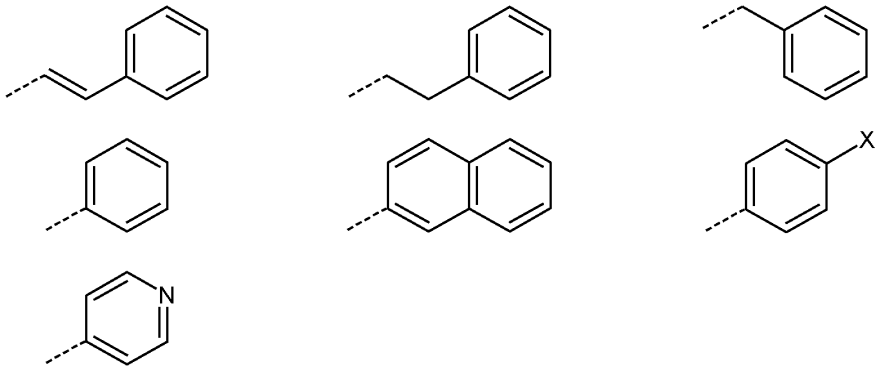
vii) R^2 is not  if residue R^1 is a methyl residue and residue R^3 is a methyl residue.

In an embodiment, residue R^2 is a residue according to any of the following formulae, wherein the dashed line indicates a bond by which the residue R^2 is covalently bound to the triazine ring of the compound according to general formulae (I), (II), (III), or (IV):



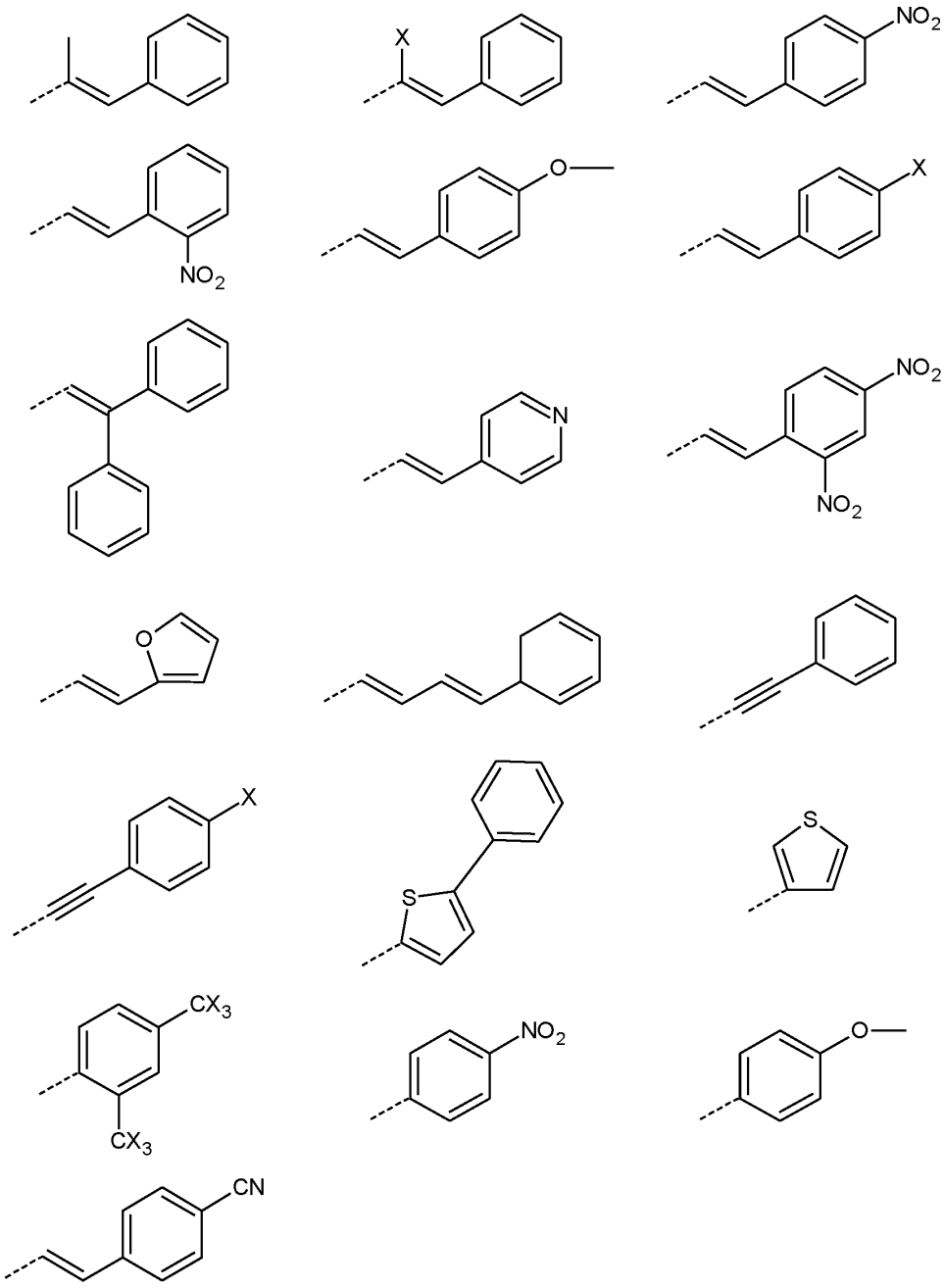
In this context, the residues R^7, R^8, R^9, R^{10}, R^{11}, R^{12}, and R^{13} have the meanings as indicated above. The limitations explained above with respect to possible meanings of residue R^2 also apply for this embodiment and the embodiments explained in the following.

In an embodiment, residue R^2 does not comply with any of the following formulae, wherein the dashed line indicates a bond by which the residue R^2 is covalently bound to the triazine ring of the compound according to general formulae (I), (II), (III), or (IV):



In this context, residue X has one of the following meanings: F, Cl, Br, or I, in particular F or Br, in particular F.

In an embodiment, residue R^2 is a residue according to any of the following formulae, wherein the dashed line indicates a bond by which the residue R^2 is covalently bound to the triazine ring of the compound according to general formulae (I), (II), (III), or (IV):



In this context, residue X has one of the following meanings: F, Cl, Br, or I, in particular F or Br, in particular F.

In an embodiment, the compound is any of compounds no. 1, 1.1, 1.2, 2, 2.1, 2.2, 3, 3.1, 3.2, 4, 4.1, 4.2, 5, 5.1, 5.2, 6, 6.1, 6.2, 7, 7.1, 7.2, 8, 8.1, 8.2, 9, 9.1, 9.2, 10, 10.1, 10.2, 11, 11.1, 11.2, 12, 12.1, 12.2, 13, 13.1, 13.2, 14, 14.1, 14.2, 15, 15.1, 15.2, 16, 16.1, 16.2, 17, 18, 18.1,

18.2, 19, 19.1, 19.2, 29, 29.1, 29.2, 30, 30.1, 30.2, 31, 31.1, 31.2, 32, 32.1, 32.2, 33, 33.1, 33.2, 34, 34.1, 34.2, 35, 35.1, 35.2, 36, 36.1, 36.2, 37, 38, and 39 (any compound being present as variant V1, V2, V3, or V4) of Table 1 listed above.

In an embodiment, the compound is any of compounds no. 1 to 15 of Table 2 listed above. Compound 1 of Table 2 is a particularly appropriate compound. Compound 2 of Table 2 is another particularly appropriate compound. Compound 3 of Table 2 is another particularly appropriate compound. Compound 4 of Table 2 is another particularly appropriate compound. Compound 5 of Table 2 is another particularly appropriate compound. Compound 6 of Table 2 is another particularly appropriate compound. Compound 7 of Table 2 is another particularly appropriate compound. Compound 8 of Table 2 is another particularly appropriate compound. Compound 9 of Table 2 is another particularly appropriate compound. Compound 10 of Table 2 is another particularly appropriate compound. Compound 11 of Table 2 is another particularly appropriate compound. Compound 12 of Table 2 is another particularly appropriate compound.

In an aspect, the present invention relates to a medicament comprising at least one compound according to the preceding explanations. The compound is typically present in the medicament in a pharmaceutically active amount. The medicament optionally comprises at least one other pharmaceutically active compound. The medicament typically also comprises pharmaceutically inactive compounds such as binders and/or excipients.

In an aspect, the present invention relates to a first method for manufacturing a compound as defined above. This method comprises the following steps:

a) adding a nitrite such as sodium nitrite to an acidic solution of 6-(1-methyl-2-((styryl)methylene)hydrazinyl)-3-methyluracil, filtering-off a precipitate, evaporating the filtrate to dryness and optionally washing it with water;

b) combining the precipitate and the dried and optionally washed filtrate and suspending the combination in an organic solvent such as ethanol;

c) adding thiophenol and stirring the mixture; filtering-off a precipitate, drying the precipitate to obtain an oxidized form of the compound, in particular ox-POG-4.

In an embodiment, the first manufacturing method further comprises the following steps:

d) adding thiophenol under inert atmosphere (such as a nitrogen atmosphere) to a suspension to the oxidized form of the compound in an organic solvent such as ethanol and stirring the mixture;

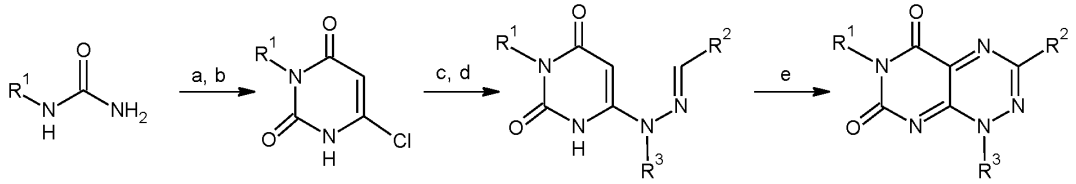
e) filtering-off the formed precipitate, optionally washing it with an organic solvent such as ethanol (in particular under an inert atmosphere such as a nitrogen atmosphere) and drying the resulting product to obtain a reduced form of the compound, in particular red-POG-4.

In an embodiment, the first manufacturing method further comprises the following steps:

f) adding 1,8-Diazabicyclo(5.4.0)undec-7-ene (DBU) to a suspension of the reduced form of the compound in an organic solvent, such as chloroform, in particular degassed chloroform;

g) adding ethyl iodide; washing the reaction mixture; drying the organic phase and obtaining another exemplary embodiment of the compound, in particular (E)-4-ethyl-1,6-dimethyl-3-styryl-4,8-dihydropyrimido[5,4-e][1,2,4]triazine-5,7(1H,6H)-dione (POG-10).

In an aspect, the present invention relates to a second method for manufacturing a compound as defined above. This method is illustrated by the following general synthesis scheme (in which the residues have the meanings as defined above) and comprises the subsequently explained steps:



a) adding diethyl malonate and N-substituted urea **1** to a solution of sodium metal in an organic solvent such as ethanol; at least partially dissolving the formed precipitate in an organic solvent such as ethanol; dissolving the resulting solid in water or an aqueous solution and acidifying the solution; removing the solvent and filtering-off the precipitate; optionally washing the precipitate to obtain N-substituted barbituric acid;

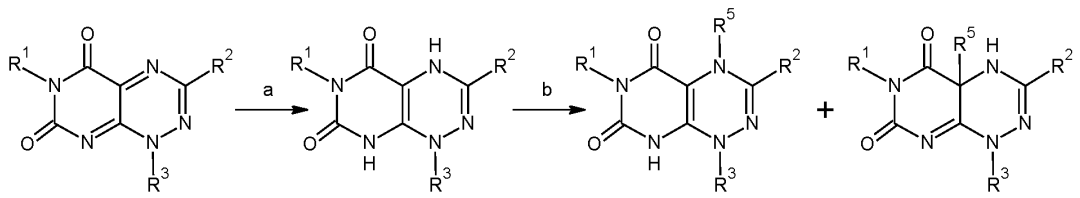
b) adding $POCl_3$ to the N-substituted barbituric acid and removing excess $POCl_3$; pouring the product over ice water and stirring it; filtering the formed precipitate to obtain compound **2** (3-N-substituted 6-chloro-uracil 2);

c) adding substituted hydrazine to the 3-N-substituted 6-chloro-uracil **2** in an organic solvent such as ethanol; filtering-off the formed precipitate and optionally washing it to obtain 3-N-substituted 6-hydrazino-uracil;

d) adding an aldehyde to a solution of the 3-N-substituted 6-hydrazino-uracil in an organic solvent such as ethanol, optionally containing a catalytic amount of an acid such as HCl; stirring the resulting mixture; filtering-off the formed precipitate to obtain an aldehyde hydrazone **3**;

e) adding a saturated aqueous solution of $NaNO_2$ to a solution of the aldehyde hydrazone **3** in aqueous acidic solution such as acetic acid; stirring the reaction mixture to obtain a mixture of the compound (POG) and its 4-N oxide; isolating the 4-N oxide or adding thiophenol to the mixture, stirring the resulting mixture, and removing the solvent to obtain the corresponding compound (POG).

In an aspect, the present invention relates to a third method for manufacturing a compound as defined above, namely 4-N and 4a-substituted POG. This method is illustrated by the following general synthesis scheme (in which the residues have the meanings as defined above) and comprises the subsequently explained steps:

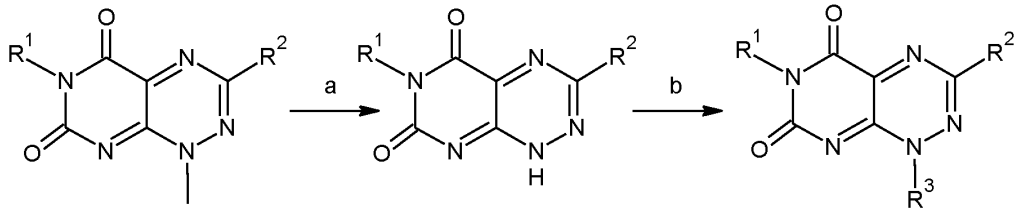


ox-POG **red-POG** **4-N substituted POG** **4a-substituted POG**

a) adding thiophenol to a suspension of an oxidized form of the compound (ox-POG) in an organic solvent, e.g., ethanol, under an inert atmosphere such as a nitrogen atmosphere; stirring the mixture; filtering-off the obtained precipitate and optionally washing it to obtain the reduced form of the compound (red-POG);

b) adding DBU to a suspension of the reduced form of the compound (red-POG) in an organic solvent such as chloroform, in particular degassed chloroform; adding an alkyl iodide and stirring the mixture; drying the product to obtain a mixture of an 4-N substituted compound and 4a-substituted compound (4-N substituted POG and 4a-substituted POG).

In an aspect, the present invention relates to a fourth method for manufacturing a compound as defined above, namely 1-N substituted POG. This method is illustrated by the following general synthesis scheme (in which the residues have the meanings as defined above) and comprises the subsequently explained steps:

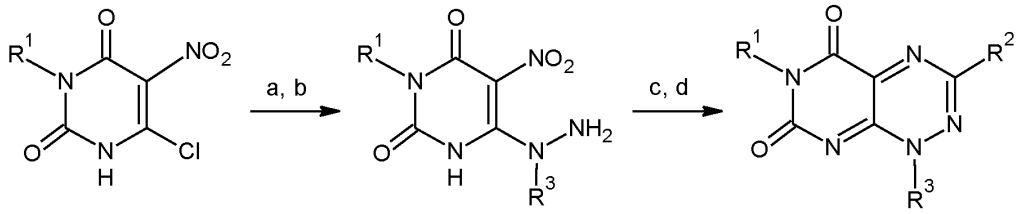


1-N methyl POG **1-N demethyl POG** **1-N substituted POG**

a) heating a solution of POG in an organic solvent such as dimethylformamide (DMF) to obtain 1-N demethyl POG.

b) adding anhydrous potassium carbonate or DBU to the 1-N demethyl POG in an organic solvent such as 1,4-dioxane; adding an appropriate alkyl bromide; stirring the mixture; filtering-off the precipitated potassium carbonate; extracting the mixture with an organic solvent such as ethyl acetate and drying the combined extracts to obtain the corresponding 1-N substituted POG.

In an aspect, the present invention relates to a fifth method for manufacturing a compound as defined above, namely 1-N substituted POG. This method is illustrated by the following general synthesis scheme (in which the residues have the meanings as defined above) and comprises the subsequently explained steps:



4 **5** **1-N substituted POG**

a) adding a hydrazine with a protecting group such as a tert-butyloxycarbonyl protecting group (boc) to compound **4** in an organic solvent such as DCM; stirring the mixture and filtering-

off the formed precipitate; optionally washing the precipitate; drying the precipitate to obtain 3-N substituted 5-nitro 6-(2-N-Boc-hydrazino)-uracil;

b) suspending the 3-N substituted 5-nitro 6-Boc-hydrazino-uracil in an organic solvent such as DCM; adding an acid such as trifluoroacetic acid (TFA); stirring the reaction mixture and removing the solvent to obtain compound **5**;

c) adding the corresponding aldehyde to a solution of 3-N substituted 5-nitro 6-hydrazino-uracil **5** in an organic solvent such as ethanol, optionally containing a catalytic amount of an acid such as HCl; filtering-off the formed precipitate to obtain the corresponding aldehyde nitro hydrazone;

d) suspending the aldehyde nitro hydrazone in an aqueous mixture of an alcohol such as ethanol in water; adding zinc dust and a chloride such as NH_4Cl; adding an acid such as HCl; stirring the reaction mixture; filtering-off, optionally washing, and drying the formed precipitate to obtain the corresponding 1-N substituted POG derivative.

All embodiments of the uses can be combined in any desired way and can be transferred either individually or in any arbitrary manner to the compound, to the various methods, and to the medicament. Likewise, all embodiments of the compound can be combined in any desired way and can be transferred either individually or in any arbitrary manner to the use, to the various methods, and to the medicament. Furthermore, all embodiments of the methods can be combined in any desired way and can be transferred either individually or in any arbitrary manner to the compound, to the use, and to the medicament. Finally, all embodiments of the medicament can be combined in any desired way and can be transferred either individually or in any arbitrary manner to the use, the compound, and to the various methods.

Further details of aspects of the present invention will be explained in the following making reference to exemplary embodiments and accompanying Figures. These are not intended to limit the scope of the invention but represent preferred embodiments of aspects of the invention provided for greater illustration. In the Figures:

Figure 1A shows a scheme on the mechanism of action of POG in senescent cells;

Figure 1B is an overview illustrating the pan-senolytic activity of POG in all types of cellular senescence;

Figure 1C shows a schematic view of the proposed mechanism of action for POG in senescent cells.

Figure 2A shows a plot illustrating the generation of hydrogen peroxide by POG in a fluorescence-based europium tetracycline assay at different POG concentrations;

Figure 2B shows a plot illustrating the generation of hydrogen peroxide by POG in a fluorescence-based europium tetracycline assay at different NADH concentrations;

Figure 3 shows a plot illustrating the redox potential of cells driven into senescence;

Figure 4 shows two plots illustrating the viability of non-senescent and senescent cells after treatment with POG;

Figure 5 shows a plot illustrating the effect of POG in non-senescent cells with differing redox potential;

Figure 6 shows a plot illustrating the senolytic activity of the known senolytic Navitoclax;

Figure 7A shows a plot illustrating the effect of POG on physical performance in aged mice;

Figure 7B shows a plot illustrating the outcome of a POG-assisted anticancer treatment *in vivo;*

Figure 8A shows a first plot illustrating the effect of POG on the number of senescent liver cells of mice of different ages;

Figure 8B shows a second plot illustrating the effect of POG on overall viability of liver cells isolated from mice, wherein POG selectively kills only the senescent subpopulation isolated from aged mice;

Figure 9A shows a first plot illustrating the effect of POG on N-nitroso-N-methylurea-(NMU)-induced senescent lesions in mouse embryo fibroblasts;

Figure 9B shows a second plot illustrating the effect of POG on NMU-induced cell transformation of mouse embryo fibroblasts;

Figure 10 shows a plot illustrating the effect of POG on DMBA-induced OIS cells (mouse embryo fibroblasts);

Figure 11 illustrates the effect of POG on NMU-induced tumorigenesis in vivo;

Figure 12 illustrates the senolytic activity of POG against virus-induced senescence (VIS);

Figure 13 illustrates the generation of hydrogen peroxide by POG and phenylethine POG in the presence of DTT;

Figure 14 illustrates the efficacy of POG against senescent cells;

Figure 15A illustrates the effect of irradiation and of palbocilib on the NADH and GSH concentration in cells;

Figure 15B illustrates the effect of ADR and on a mutation in the Ras gene on the NADH and GSH concentration in cells;

Figure 16 illustrates the viability of p53 +/+ cells after gamma irradiation (IRR) or after treatment with palbocilib, and subsequent treatment with POG;

Figures 17A to 17H and 17J to 17K illustrate the specificity of compounds against senescent cells compared to non-senescent cells;

Figure 18 illustrates the low specificity of senolytics known from prior art against senescent cells;

Figure 19 illustrates the therapy-induced senescence in cells of melanoma cell line A375 by vemurafenib;

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Figure 20 illustrates POG acting as a redox-sensitive compound in vitro;

Figure 21 shows POG treatment in old mice improves markers associated with an age-dependent functional decline;

Figure 22A illustrates POG targets OIS cells;

Figure 22B shows POG preferentially targets Braf-V600E mutant senescent fibroblasts;

Figure 23A shows POG targets senescent Braf-V600E induced skin lesions *in vivo*;

Figure 23B illustrates early senolytic POG intervention delays Braf-V600E-driven melanomagenesis in vivo;

Figure 24 shows side-by-side comparison of the senolytic activities of POG and Navitoclax in OIS induced OHT-inducible ER:Ras-G12V-transgenic Tig3 cells;

Figure 25A illustrates no impact of POG on body weight in aging mice;

Figure 25B shows no overt adverse effects of POG in vivo; and

Figure 26 illustrates POG-mediated senolysis of Braf mutant cells.

Aspects of the invention are further described by the following examples. These are not intended to limit the scope of the invention but represent preferred embodiments of aspects of the invention provided for greater illustration.

Figure 1 shows a scheme on the mechanism of action of POG in senescent cells. As already explained above, the oxidized form of POG (ox-POG) can be reduced to the reduced form of POG (red-POG) in the presence of a suitable biochemical reducing agent such as DTT or NADH. Red-POG then forms a hydroperoxy species with molecular oxygen. The original form of POG is recovered by release of hydrogen peroxide. The released hydrogen peroxide leads to apoptosis of the senescent cells.

The redox potential of senescent cells (i.e., the concentration of NADH or the NADH/NAD^+ ratio) is significantly higher than the redox potential of non-senescent cells, thereby preferentially activating POG in senescent as compared to proliferating never-senescent cells and explaining, at least in part, the preferential cytotoxic action of POG in senescent cells.

Non-tumor cells enter a state of senescence by natural ageing (so-called replicative senescence (RS)) or due to a (high-titer) virus infection (so-called virus-induced senescence (VIS)). Primary, non-malignant cells can develop senescence when an activated oncogene such as mutant Ras or BRaf is expressed in these cells (so-called oncogene-induced senescence (OIS)).

Tumor cells can be transferred to a senescent state by chemotherapy or radiation therapy (so-called therapy-induced senescence (TIS)).

In a senescent state, the cells show increases in glycolysis, the tricarboxylic acid cycle (TCA cycle) metabolism, and lipid oxidation. These factors lead to increased cellular NADH levels and thus to an altered redox potential of the cells. The oxidative stress elicited in senescent cells results in the depletion of the cellular GSH pool. Both the elevated NADH- and massively decreased GSH levels present in senescent cells result in net activation of POG (that is production of cytotoxic H_2O_2) selectively in senescent cells.

The pan-senolytic effect of POG in all types of cellular senescence identified by the inventors is illustrated in Figure 1B that will be explained in the following paragraphs.

RS: Low-passage human lung fibroblasts (IMR-90) were stably transduced with ana RS-ablating hTERT-overexpressing or an empty vector as a control, and subsequently passaged until the vector-transduced cells had quantitatively entered RS at passage number 53. Increased passage numbers reflect the need of replating (repassaging) the cells due to their proliferative capacity, which was exhausted here at passage 53.

VIS: The human alveolar epithelial adenocarcinom cell line A549 as well as VIS-deficient VeroB4 cells were infected with a high-titer GFP-expressing lentivirus (pCDH-CMV-MCS-EF1-GFP; MOI = 50) to induce VIS.

TIS: Eμ-myc;Bcl2 lymphoma cells (with or without catalase overexpression) and senescence-/p53-deficient Eμ-myc; Bcl2 lymphoma cells were treated with ADR (0.02 μg/ml) or solvent.

OIIS: A375^{BRafV600E}, A375$^{BRafV600E;PLX4032\text{-}resistant}$ and MEWO$^{BRaf\text{-}WT}$ melanoma cell lines were exposed to PLX4032 (1.5 µM) or solvent (WT = wild-type).

OIS: ER:Ras^{G12V} Tig3 cells were treated with OHT or solvent as in C. Primary mouse embryo fibroblasts (MEF) harboring ana OHT-inducible wild-type-p53 allele were treated with or without OHT and subsequently transduced with a Ras^{G12V} overexpressing pBABE vector to induce OIS only in OHT-treated wild-type-p53 cells.

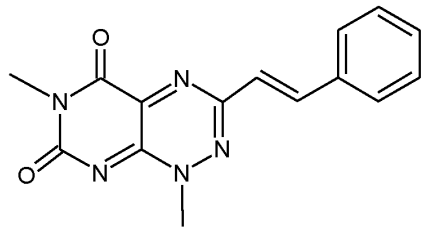
All cells were analyzed at day 10 after start of the treatment and oncogene induction, respectively. Shown in grey in the first (left) column are mean percentages of senescence-associated beta-galactosidase (SA-β-gal)-)positive cells for each treatment. Proliferating cells and their senescent counterpart were exposed to the same increasing concentrations of POG (from left to right: murine Eμ-myc lymphoma cells and primary mouse fibroblasts = 0, 0.2, 0.4, 0.8, and 1.2 µM POG, for VIS and RS = 0, 2, 3, 4, and 5 µM POG, and for all others = 0, 1, 2, 3, and 4 µM POG) for 22 h before measuring their viabilities using a dye exclusion test.

Viability of solvent-treated cells were set to 100% for each treatment and viabilities of POG-treated cells were normalized relative to solvent-treated controls. The asterix indicates lymphoma cells engineered to overexpress catalase that are capable to enter TIS but lack sensitivity to POG.

Quantification of hydrogen peroxide generated by peroxygenins

For a confirmation of the POG redox cycling, hydrogen peroxide formed by peroxygenin from elemental oxygen was quantified. For this purpose, a fluorescence-based europium tetracycline assay in 3-(N-morpholino)propanesulfonic acid (MOPS) buffer at pH 6.9, close to the physiological pH, was adapted for monitoring of hydrogen peroxide released from POG-4 (Wolfbeis et al., 2002; Dürkop et al., 2005; Wu et al., 2005; Schrenkhammer et al., 2007) Reportedly, this assay is based on the formation of an quaternary pentagonal bipyramidal Eu(III)-tetracycline (Tc) complex of the stoichiometry Eu_2Tc_2 (Karthikeyan et al., 2004) in which one equivalent of hydrogen peroxide forms a bridged peroxide complex (μ-H_2O_2 ligand) (Dehaen et al. 2009) and upon excitation at 405 nm fluoresces at 616 nm. First, the sensitivity of the assay toward hydrogen peroxide was investigated. For that, different hydrogen peroxide concentrations, ranging from 10 µM to 1000 µM, were used to analyze the fluorescence signal (Figures 2A and 2B, curves 6 to 14 and 26 to 34; curves 6 and 26:1000 µM, curves 7 and 27: 800 µM, curves 8 and 28: 400 µM, curves 9 and 29: 200 µM, curves 10 and 30: 100 µM, curves 11 and 31: 50 µM, curves 12 and 32: 25 µM, curves 13 and 33: 10 µM, curves 14 and 33: 0 µM).

Next, the same assay conditions were applied to quantify hydrogen peroxide formation from POG-4 (V3) (referred to in the following simply as POG-4) having the following formula:



POG-4

POG-4 was added to the assay buffer at various concentrations (Figure 2A; curve 1: 5 µM, curve 2: 2 µM, curve 3: 1 µM, curve 4: 0.5 µM, curve 5: 0.1 µM) with 1 mM NADH. In contrast to pure hydrogen peroxide, incubation of POG-4 with NADH produced a steadily increased fluorescence, suggesting the continuous generation of H_2O_2 during the observed time frame of 300 min. Higher concentrations of POG-4 led to more rapidly growing and higher fluorescence intensities, corresponding to higher amounts of generated hydrogen peroxide.

At a concentration of 5 µM POG-4, the maximum concentration was reached at approximately 120 minutes and then slightly decreased.

Comparing the time-dependent generation of fluorescence signals from H_2O_2 (curves 6 to 14) and from POG-4 (curves 1 to 5) it can be concluded that the formation of the Eu_2Tc_2-H_2O_2 complex is significantly faster than the generation of hydrogen peroxide by POG-4.

Figure 2B shows the result of an equivalent experiment in which the POG concentration was kept constant at 1 µM, wherein the NADH concentration was varied from 0.1 mM to 5 mM (Figure 2B; curve 21: 5 mM, curve 22: 2 mM, curve 23: 1 mM, curve 4: 0.5 mM, curve 5: 0.1 mM). A higher NADH concentration generally resulted in a higher hydrogen peroxide concentration, wherein the hydrogen peroxide formation curves of 2 mM NADH and 5 mM NADH were quite similar (within the limits of measurement accuracy).

The senolytic effects of POG-4 were investigated in several established senescence models. In these models, cells were driven into therapy-induced senescence (TIS), virus-induced senescence (VIS), or replicative senescence (RS).

In a TIS-model, it could be shown that senescent cells indeed contained increased NADH levels compared to the control (Figure 3 illustrating an Eμ-myc cell line after TIS with doxorubicin (Adriamycin, ADR) and control (UT).

The senescent state was documented by specific measurement of increased SA-β-galactosidase activity. In the same TIS model, senescent cells were found to be significantly more sensitive to POG-4 than non-senescent tumor cells, highlighting the selectivity of the presently claimed and described senolytics for senescent cells. Figure 4 shows the results of a 22-hours cytotoxicity assay visualizing the difference in viability rate between senescent cells treated with POG-4 (ADR) and non-senescent cells treated with POG-4 (UT) (upper panel). Eμ-myc;Bcl2 lymphoma cells (with or without ectopic catalase overexpression, introduced via stable retroviral gene transfer) and senescence-/p53-deficient Eμ-myc;Bcl2 lymphoma cells were treated with ADR (0.02 μg/ml) or solvent for 10 d. Proliferating cells and their senescent counterpart were exposed to the same increasing concentrations of POG for 22 h before measuring their viabilities using a dye exclusion test (Ghost Dye Red 780, 13-0865-T500, Tonbo). Viability of solvent-treated cells were set to 100% for each treatment and viabilities of POG-treated cells were normalized relative to solvent-treated controls.

The effect was shown to be based on the action of hydrogen peroxide, as the POG-4 did not exhibit senolytic activity when the senescent cells were protected against hydrogen peroxide by the expression of catalase, an enzyme cleaving hydrogen peroxide (lower panel of Figure 4).

Furthermore, it was demonstrated that the activity of POG-4 relied on increased levels of the reducing equivalent (NADH) in cells. Increasing cellular NADH levels sensitizes cells towards POG-mediated killing in a H_2O_2-dependent fashion. Primary mouse embryo fibroblasts (MEF) were stably transduced with a catalase-overexpressing retrovirus or empty virus and exposed to 500 nM rotenone (an NADH dehydrogenase inhibitor used to increase cellular NADH levels via inhibition of the mitochondrial complex I) 2 h prior to POG exposure (1 μM) before measuring cell viabilities 22 h later using a dye exclusion assay (Ghost Dye Red 780, 13-0865-T500, Tonbo). Figure 5 illustrates the results in form of means ± standard deviation (SD).

For a direct side-by-side comparison of the senolytic activities of POG-4 and Navitoclax in TIS and OIIS (oncogene-inhibition induced senescence), respectively, murine Bcl2-protected Eμ-myc lymphoma cells (n = 4, upper panel of Figure 6) as well as the human BRafV600E mutant melanoma cell line A375 (lower panel of Figure 6) were treated for 10 d with either ADR (0.02 μg/ml) or PLX4032 (1.5 μM) (an inhibitor of the mutant B-Raf enzyme) or its solvents. Induction

of senescence was verified using the SA-β-gal assay before exposing cells with POG. Cell viabilities were measured after 22 h using the dye exclusion assay (Ghost Dye Red 780, 13-0865-T500, Tonbo). Figure 6 illustrates the results in form of means ± standard deviation (SD). In the upper panel of Figure 6, unfilled circles denote proliferating cells treated with POG-4, filled circles denote senescent cells treated with POG-4, triangles standing on the base denote proliferating cells treated with Navitoclax, and standing on the apex denote senescent cells treated with Navitoclax.

To quantify the global impact of POG on age-related physical decline, a rotarod performance test was conducted, which captures the time mice can run on a rotating rod before falling off due to exhaustion. As expected, older DMSO solvent-only-exposed control mice (10-months old) dropped after much shorter periods off the rod compared to untreated young mice (10-weeks old), while POG administration significantly extended the running time achieved in the older age group, albeit not to the extent seen in young mice (Figure 7A; solvent: solvent-treated mice; POG: POG-4-treated mice).

For the experiments, the results of which are shown in Figure 7A, C57/BL6 mice (10-months old, “old mice”) were treated twice with POG (0.25 mg/kg body weight, *i.v.* injected every fortnight; $n = 11$) or solvent ($n = 11$), before their physical performance was assessed using a rotarod device one month later (from 4 to 40 rpm in 180 s; acceleration rate: 0.2 rpm/s). For comparison a cohort of untreated young mice (8-weeks old; $n = 10$) was measured in parallel. Each datapoint represents the maximum time the (previously trained) animal was able to remain on the rotating rod from 5 consecutive runs per mouse.

The inventors also analyzed the impact of a sequential POG exposure on overall survival in chemotherapy-exposed mice bearing senescence-capable *vs.* senescence-incapable lymphomas. Strikingly, most mice harboring Eμ-*myc* transgenic lymphomas generated from a $p53^{+/+}$ background as a senescence-capable control lived significantly longer, when POG, (2 x 0.25 mg/kg bodyweight), as compared to DMSO only, was administered following exposure to the senescence-inducing chemotherapeutic agent cyclophosphamide (CTX). Mice bearing senescence-incapable Eμ-*myc* lymphomas failed to realize such benefit due to lack of both *p53* alleles (Figure 7B). In essence, POG treatment led to reduction of senescent cells *in vivo*, and extended “healthy lifespan” in aging mice and “overall survival” of anticancer-treated mice.

Figure 7B shows the overall survival of 7-day CTX-treated Bcl2-protected Eμ-myc ($n = 7$) or Eμ-myc;p53-null ($n = 4$) lymphoma-bearing mice randomly assigned to sequential exposure to POG or solvent, presented as matched pair analysis ($P = 0.0282$ for Eμ-myc comparison; $P >$

0.05 for Eμ-myc.p53-/- comparison). CTX treatment: 1 x 300 mg/kg body weight via *i.p.*, POG treatment: 2 x 0.25 mg/kg body weight via the *i.v.* application route. Figure 7B illustrates means ± SD, wherein an unpaired two-tailed Student's t test was done for statistical analysis (*P < 0.05).

Additionally, the inventors were able to show that the percentage of senescent liver cells from old mice decreased significantly upon treatment with POG-4 (Figures 8A and 8B; Solvent: non-treated mice; POG: POG-4-treated mice) due to the senolytic effect of POG-4. Liver cell preparations from young (10-weeks old) and old (20-months old) C57/BL6 mice were treated *ex vivo* for 22 h with POG (1.5 μM) or solvent. Cells were fixed and stained for SA-β-gal, TUNEL (Terminal deoxynucleotidyl transferase dUTP nick end labeling) and DAPI (4′,6-diamidino-2-phenylindole). Percentage of senescent (SA-β-gal-positive) cells present in the viable (TUNEL-negative) liver cell population (Figure 8A) and overall viability of the liver cell population (Figure 8B) was quantified via fluorescence microscopy.

The inventors found that POG-4 is not only able to kill senescent tumor cells but that it is capable to prevent Ras-driven tumor formation in-vivo (Figures 9A, 9B, 10, and 11).

Primary mouse fibroblasts (MEF) were treated for 5 weeks with 2 mM N-nitroso-N-methylurea (NMU) or solvent to mutagenize their genomes. Selective killing of senescent subpopulation was assessed by measuring the percentage of SA-β-gal positive cells after POG-4 exposure (1.0 μM for 22 h). Figure 9A shows means ± standard deviation (SD). Figure 9B shows that POG-4 ablates NMU-induced transformation of primary MEF in vitro. 2 x 10^4 of viable NMU-pre-treated MEF with or without subsequent POG-4 exposure (1.0 μM) from the experiment of Figure 9A were seeded in 0.3 % soft-agar and cultivated for 10 weeks, before formation of colonies (>50 cells/colony) was quantified. Again, means ± SD are illustrated

POG-mediated senolysis of DMBA-induced OIS cells was tested; the results are depicted in Figure 10. Like NMU, 7,12-dimethyl-benzoanthracen (DMBA) is known to induce oncogenic mutations across all Ras isoforms. MEF were mutagenized with DMBA (0.25 μg/ml) for 7 days before cells were exposed to increasing concentrations of POG 1 week later. Cell viabilities were measured 22 h later using a dye exclusion assay (right; (Ghost Dye Red 780, 13-0865-T500, Tonbo). SA-β-gal stainings (left) were performed 2 days after POG exposure. Filled circles denote experiments conducted with solvent; filled squares denote experiments conducted with DMBA.

POG prevents NMU-induced Ras-driven tumor formation in vivo.

The interference of POG-4 with NMU-induced tumorigenesis was established *in vivo* (Figure 11). For this purpose, 30 days old C57/BL6 mice were mutagenized for 5 consecutive weeks with 30 mg NMU/kg bodyweight (b.w.) intraperitoneally (i.p.) prior to treatment with POG-4 (2 x 0.25 mg kg^{-1} body weight intravenously (i.v.) injected within one week) or solvent, respectively. The mice were subsequently monitored for 300 d and T-cell lymphoma development was plotted as the time between first NMU treatment and tumor manifestation in a Kaplan Meier format. Log-rank [Mantel-Cox] test was used to compare solvent- and POG-treated cohorts. Figure 11 illustrates that POG-4 interferes with NMU-induced tumorigenesis in vivo ($p = 0.0258$).

POG-4 was also found to exhibit senolytic activity against virus-induced (VIS) senescence (Figure 12). The adenocarcinomic alveolar epithelial cell line A549 as well as VIS-deficient VeroB4 cells were infected with a high-titer GFP-expressing lentivirus (pCDH-CMV-MCS-EF1-GFP; multiplicity of infection [MOI] = 50). At day 6, the cells were stained for SA-β-gal before treating them with increasing concentrations of POG-4. After 22 h, the cell viability was measured using a dye exclusion test. Figure 12 illustrates means ± SD.

The experiments explained in the preceding paragraphs prove that peroxygenins act effectively as pan-senolytics. While most of the experiments were conducted with POG-4 (also denoted as styryl POG or styrenyl POG, i.e., a POG containing a styrenyl residue in position R^2), many novel, hitherto unreported POG derivatives like phenylethinyl POG (also referred to as ethine POG; confer structure no. 12 in Table 1) as well as all other POG listed in Table 1 were synthesized and many of them were successfully tested (cf. Table 2). Phenylethinyl-POG was found to be more potent in the generation of hydrogen peroxide than styrenyl POG in the EuTc assay (Figure 13). In this assay, hydrogen peroxide generation by 500 nM POG-4 (filled circles) or by 500 nM phenylethinyl POG (filled triangles) in the presence of 1 mM DTT was compared. A blank sample is illustrated by filled squares.

POG extends healthy lifespan in aging mice. The efficacy of POG can also be seen from Figure 14 that illustrates the results of experiments in which lung cells of old mice were subjected to POG-4. The Liver Dissociation Kit (130-105-807, Miltenyi Biotec) and gentleMACS™ Dissociators (Miltenyi Biotec) were used for mechanical dissociation of freshly isolated mouse liver tissue. Viable cells were exposed to POG (1.5 µM) for 22 h, formalin-fixed and stained for SA-β-gal activity together with the In Situ Cell Death Detection Kit-TMR red (TUNEL, 12156792910, Sigma-Aldrich) according to the manufacturer's instructions, with 4′,6-diamidino-2-phenylindole (DAPI) as a counterstain. Percentage of senescent (SA-β-gal-positive) cells present in the viable (TUNEL-negative) cell population was quantified *via*

light/fluorescence microscopy. POG treatment targets senescent cells residing in the lungs of aged mice. Lung tissues isolated from young (12-week old, n = 4) and old C57BL/6 mice (20-month old) treated with POG (n = 5) or solvent (n = 6) was fixed and stained for markers of senescence (p16INK4A, H3K9me3) and fibrotic tissue (COL3A1) and quantified. The formation of senescence can be monitored by numerous cellular senescence markers which indicate senescent cells. These senescent markers are over-expressed also in the lung cells of old mice as could be shown by immunohistology. These senescence markers include over-expression of the CDK-inhibitor p16INK4a and of collagen type III alpha 1 chain gene (Col3A1), as well as epigenetic histone-methylation (H3K9me3).

Reflecting the established age-related link of cellular senescence as a driver of fibrotic pulmonary disease, aged mice presented with signs of peribronchial lung fibrosis as indicated by staining for collagen type III alpha-1 (col3A1), coinciding with strongly positive staining for senescence markers p16INK4a and H3K9me3. While these markers could not be observed in the lung cells of young mice of 12 weeks age (Y, topmost bars in the plots of Figure 14), they were prominently present in lung cells of old mice of 20 months age (O, middle bars in the plots of Figure 14). After subjecting the lung cells of the old mice to POG-4, these senescent cells were eradicated almost completely or their number at least significantly reduced (O+P, lowest bars in the plots of Figure 14).

Strikingly, four weeks after POG treatment, the aged group exhibited a profound reduction of senescent cells and peribronchial fibrosis, underscoring the power of POG to not only stabilize otherwise progressive fibrosis but to exert anti-fibrotic activity as a net effect. To quantify the global impact of POG on age-related physical decline, the inventors conducted a rotarod performance test, which captures the time mice are able to run on a rotating roll before falling off due to physical exhaustion. As expected, older solvent-only-exposed control mice dropped off the rod after much shorter periods compared to equally control-treated young mice, while POG administration significantly extended the maximal running time achieved in the older age group, albeit not fully to the extent seen in young mice (Figure 7A). Notably, there was only modest toxicity with the doses used throughout the experiments, and no critical weight loss or blood-based signals of overt organ toxicity were observed (Figure 25A and B). Interestingly, POG exposure improved a number of markers typically associated with an age-dependent functional decline, such as red blood cell count, hemoglobin, urea, and serum protein levels as well as iron deposition in the live (Figure 21). Hence, POG administration is feasible without overt toxicities, improves age-related blood parameters, and even promotes physical re-strengthening.

In all studied scenarios of senescence, senescence was strictly associated with an increase of NADH concentrations and decrease of GSH concentrations within cells. Figure 15A illustrates these findings exemplarily. In untreated mouse embryo fibroblasts (UT), the amount of senescent cells was 4.8 ± 1.3 %, as revealed by an SA-β-gal test carried out as explained before (data not shown). 10 days after gamma irradiation with 10 Gy using a Cs137 source (GSR-D1, Eckert & Ziegler, Leipzig), the amount of SA-β-gal positive (i.e., senescent) cells dramatically increased to 70.5 ± 6.9 % (data not shown). After treatment with palbocilib (20 µM for 10 days) (a highly selective and reversible inhibitor of cyclin-dependent kinases (CDKs) 4 and 6, which are essential for cell cycle progression and cell proliferation), the amount of SA-β-gal positive (i.e., senescent) cells increased to even 84.8 ± 7.0 % (data not shown). At the same time, the NADH concentration in the cells that were subjected to gamma irradiation treatment (IRR) or treatment with palbocilib was significantly higher than that in untreated (UT) MEF cells (Figure 15A, left panel). In contrast, the GSH concentration (measured after 10 days) in the cells that were subjected to irradiation treatment (IRR) or treatment with palbocilib was significantly lower than that in untreated (UT) cells (Figure 15A, right panel).

Figure 15B shows additional results on the NADH and GSH concentration in senescent cells with respect to non-senescent cells. The experiments were performed on the human BRafV600E mutant melanoma cell line A375, on primary mouse embryo fibroblasts (MEF), and on Tig3 cells. In A375 cells, senescence was induced by treatment with Adriamycin (ADR) (the control was the solvent without ADR). In MEF, senescence was induced by transforming MEF with the RasG12V mutant located on a vector (the control was the plein vector used for transformation (i.e., without the RasG12V gene)). In Tig3 cells carrying the RasG12V gene, senescence was induced via treatment with the chemotherapeutic 4-hydroxytamoxifen (4-OHT) (the control was the solvent without 4-OHT). In all cases of induced senescence, the NADH concentration was significantly higher than that in the control, whereas the GSH concentration was significantly lower than that in the control.

p53-proficient (p53 +/+) MEF were pre-treated and subsequently exposed to POG before measuring their viabilities. Figure 16 illustrates the viability rates of p53 +/+ mouse embryo fibroblasts after irradiation with gamma rays (i.e., irradiation-induced senescence; with 10 Gy using a Cs137 source (GSR-D1, Eckert & Ziegler, Leipzig)) or treatment with palbocilib (20µM) and subsequent treatment with POG-4 (POG). While the senolytic effect of POG was pronounced in the cell cultures after gamma irradiation or treatment with palbocilib, no effect on the viability was observed in case of untreated cells. POG enforced death of these senescent cells in a dose-dependent manner, while equally treated but genetically senescence-incapable p53-deficient MEF experienced little cytotoxicity. This underlines the

high specificity of POG against senescent cells as it specifically targets senescent cells, while non-senescent cells remain unaffected by POG treatment. This further shows that POG operates as a pan-senolytic agent in any type of cellular senescence.

Figures 17A to 17 K illustrates the high specificity of the claimed compounds against senescent cells, while not being harmful towards non-senescent cells at the senolytic dosage. In Figures 17 and 18, “Prol.” denotes proliferating (i.e., non-senescent) cells and “Sen.” denotes senescent cells. The compounds listed in the following Table 3 were tested in the experiments the results of which are depicted in Figures 17A to 17K:

Table 3: Compounds tested in the experiments the results of which are depicted in Figures 17A to 17K.

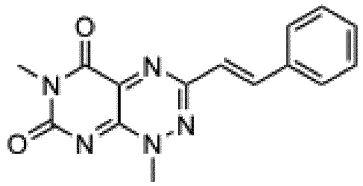
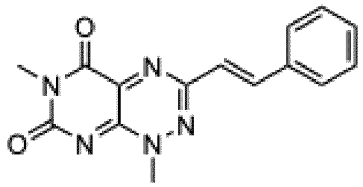
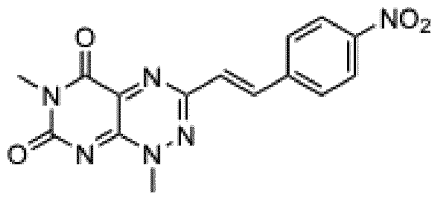
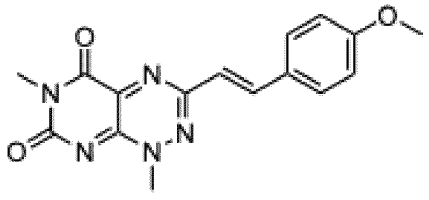
Figure	POG ID	Compound	Structure
17A	CD	POG-4 in 30 % 2-hydroxypropyl-beta-cyclodextrin solution (CD-POG)	
17A-17K	#1	Substance no. 8 of Table 2 (POG-4; in Figures 17A to 17K simply denoted as POG))	
17B	#3	Substance no. 1 of Table 2	
17C	#4	Substance no. 6 of Table 2	

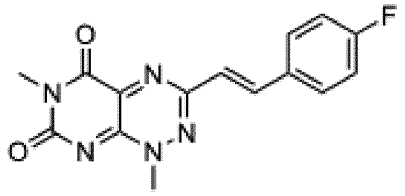
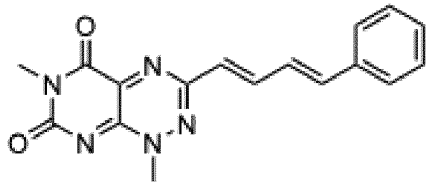
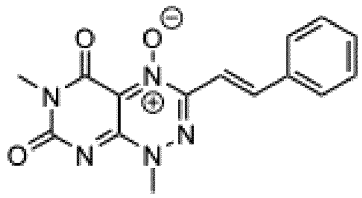
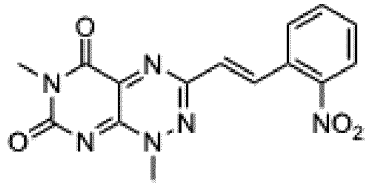
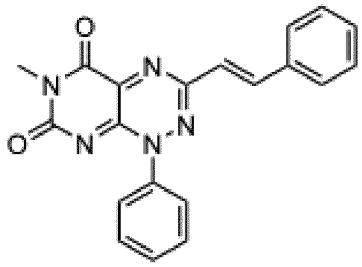
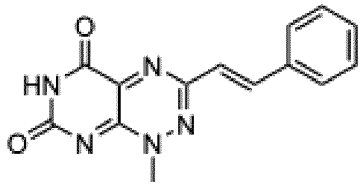
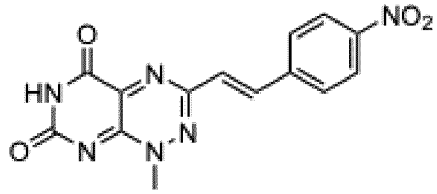
Figure	POG ID	Compound	Structure
17D	#5	Substance no. 7 of Table 2	
17E	#6	Substance no. 11 of Table 2	
17F	#13	Substance no. 12 of Table 2	
17G	#15	Substance no. 10 of Table 2	
17H	#18	Substance no. 3 of Table 2	
17J	#26	Substance no. 5 of Table 2	

Figure	POG ID	Compound	Structure
17K	27	Substance no. 2 of Table 2	

POG-CD was prepared by first dissolving 2-hydroxypropyl-beta-cyclodextrin (30 % w/v) in either water or 0.9 % sterile saline solution at room temperature. Secondly, POG-4 was dissolved in this 2-hydroxypropyl-beta-cyclodextrin (HP-β-CD) solution, under the exclusion of light, at room temperature. Compared to a solution of POG in DMSO, solving of POG (like POG-4) in HP-β-CD has various advantages. First, HP-β-CD is a physiologic buffer. Thus, no harmful substances like DMSO are needed for preparing a POG solution. Second, POG has a significantly higher solubility in HP-β-CD than in DMSO. This enables significantly higher dosages of POG.

Human melanoma A375 cells were treated with the BRafV600 inhibitor Vemurafenib (1.5 µM) for 10 d before exposing senescent cells with POG-4 and POG derivatives from a senolytic screening. Viabilities were measured using GhostDye Red after 22 h. Upon treatment with 8 µM POG-4 (POG) and, for example, POG-CD only 10 % of the senescent cells remain viable. Beside POG-CD, further compounds were tested, including POG #18 (N1-phenyl-styryl POG), where upon treatment with 8 µM of these compounds only 10% and less of the senescent cells remained viable. Remarkably, at a concentration of 4 µM POG #18 (N1-phenyl-styryl POG), less than 10% of the senescent cells remained viable. Similar efficiency of selective killing of senescent cells (while largely sparing non-senescent proliferating cells) of POG-4 at 4 µM (or even lower at 2 µM of POG #18, N1-phenyl-styryl POG) requires much higher concentrations of other senolytic compounds (Navitoclax at 20 µM, Fisetin and Dasatinib/Quercetin at 80 µM).

Figure 18 shows senolytic activity of POG compared to other senolytic agents. Human melanoma A375 cells were treated with the BRafV600 inhibitor Vemurafenib (1.5 µM) for 10 d before exposing cells with POG, Navitoclax, Fisetin or Dasatinib/Quercetin (1:9). Viabilities were measured using GhostDye Red after 22 h. A viability of 90 – 95% in compound-treated proliferating cells was set as a threshold for assessing the amount of killing of the senescent cell population at the respective dose. Specificity and effectivity of the other known senolytics dasatinib/quercetin (D/Q) and fisetin are substantially less than compounds disclosed herein

as seen from Figure 18, while these substances are not as efficient as navitoclax at the tested concentrations.

Figure 19 shows SA-β-gal staining of Vemurafenib treated A375 cells at the time of senolytic treatments and illustrates the effect of the kinase inhibitor vemurafenib on A375 cells. After treatment for 10 d with vemurafenib (1.5 µM, OIIS) or its solvents only, 3.3 ± 1.2 % of the cells are SA-β-gal positive (i.e., they are senescent). However, after treatment with vemurafenib, almost all cells (98.0 ± 1.7 %) are SA-β-gal positive. This illustrates the high efficacy of vemurafenib in therapy-induced senescence. The SA-β-gal test was carried out as explained above in more detail. Cell viabilities were measured after 22 h using a dye exclusion assay (cf. Figure 6 for more details).

The senolytic effects of POG were demonstrated in-vitro and in-vivo toward senescent cells of various cell lines and cancer cell lines including B-cell lymphoma (Eμ-myc, Figure 1B), human diffuse large B-cell lymphoma (DLBCL, patient-derived cell line, data not shown), melanoma cells (A375, Figure 1B), lung adenocarcinoma (A549, Figure 1B), lung fibroblast cells (IMR90, Tig-3, MEF, Figure 1B, Figure 16) indicating the generality of the claimed use. Further, the selective senolytic effects of POG were demonstrated in-vivo in several cancer models, organs and tissues including mouse B cell lymphoma (Eμ-myc) (Figure 7B) and N-nitroso-methyl urea (NMU) induced T-cell lymphoma (Figure 11). The specific senolytic effects in therapy-induced senescence has been demonstrated for Doxorubicin (Adriamycin, ADR, Figure 1B), Cyclophosphamide (CDX, Figure 7B), Vemurafenib (PLX4032, Figure 1B, Figure 4), Palbociclib (Figure 16),. With this representative selection of chemotherapeutics, the main groups of chemotherapeutics in clinical use today are covered, including alkylating agents (Cyclophosphamide), DNA-intercalating drugs (Doxorubicin), anti-metabolites (5-Fluorouracil) as classical anti-cancer drugs as well as kinase inhibitors (Vemurafenib: Raf inhibitor; Palbociclib: CDK-inhibitor) as examples for modern targeted chemotherapeutics. The senolytic effects of POG were also demonstrated for senescent cells generated by gamma-irradiation (IRR, Figure 16).POG is a redox-sensitive, NADH-activatable cytotoxic compound. As shown in Figure 20, POG is activated by NADH in vitro. H_2O_2 generation by ox-POG (straight lines) or toxoflavin (dashed lines) in the presence of NADH (curve 1), DTT (curve 2), or solvent (curve 3) using the europium-tetracycline assay. NADH, Nicotinamide-adenine-dinucleotide. Importantly, the physiologically relevant reductant NADH was able to directly activate ox-POG considerably stronger than toxoflavin in a cell-free system, where it supported sustained high-level H2O2 production. In living cells, H2O2 is counter-regulated by GSH in a dose-dependent manner, suggesting that POG activity in cells as measured by H2O2 production is determined by high NADH and low GSH concentrations (Figure 1C, 2A, 2B, 20) ,.

In Figure 21 it is shown that POG treatment in old mice improves markers associated with an age-dependent functional decline. Analysis of blood collected from young (Y; 12-week old; n = 7) and old C57BL/6 mice (20-month old) treated with POG (n = 10) or solvent (n = 4). POG exposure improved a number of markers typically associated with an age-dependent functional decline, such as red blood cell count, hemoglobin, urea, and serum protein levels as well as iron deposition in the liver.

POG selectively targets Ras- and Braf mutant-induced senescent cells in vitro (Figures 22A and 22B). POG targets OIS cells (Figure 22A). Wildtype and senescence-ablated, p53-deficient MEF were transduced with a Ras-G12V encoding or empty retrovirus to induce OIS. 10 days later, cells were exposed to POG and viabilities measured. MEF entering OIS in response to oncogenic H-Ras-G12V(4) expression were effectively targeted by POG, as expected from their profoundly elevated NADH and lowered GSH levels.

As shown in Figure 22B, POG preferentially targets Braf-V600E mutant senescent fibroblasts. The inventors also tested LSL-Braf-V600E MEF (carrying a loxP-flanked transcriptional termination stop cassette [loxP-STOP-loxP; LSL] at the endogenous braf locus with an activating V600E knock-in mutation), whose pro-senescent oncogenic Braf-V600E expression was unlocked to natural levels by retroviral Cre provision. Conditional activation of this endogenous onco-allele evoked SA-ß-gal-positive senescence in a large proportion of the cells, further underscored by high p16INK4a expression. MEF with a Cre-inducible endogenous LSL-Braf-V600E mutation were transduced with an empty vector- or Cre-recombinase encoding retrovirus. Three weeks later cells were treated with POG and measured for viability measured. As seen before regarding Ras activation by chemical cancerogens, senescent cells were preferentially targeted by the addition of POG (Figure 22B). POG also exerted dose-dependent senolytic activity in NHEM that entered senescence upon lentiviral transfer of Braf-V600E alleles (Figure 26). Figure 26 illustrates POG-mediated senolysis of Braf mutant cells. NHEM (normal human epidermal melanocytes) were stably transduced with a lentivirus overexpressing the Braf-V600E mutant to induce OIS. After three weeks, cells were exposed to POG before measuring their viabilities using a dye exclusion assay.

In essence, POG selectively eliminated human and murine cells that entered OIS in response to oncogenic Ras and Braf moieties expressed from stably virus-transferred or endogenous alleles, thereby insinuating its potential to cancel imminent full-blown tumor formation that may emerge from OIS-escaping cells in vivo.

POG reduces Ras- or Braf-driven tumor formation in vivo (Figures 23A and 23B). Figures 23A and 23B show that early senolytic POG treatment interferes with Ras- and Braf-mutant-driven tumorigenesis in vivo. To probe POG as a candidate cancer-preventive agent that eliminates incipient but potentially cancer-founding OIS lesions throughout the body, we interrogated a variety of suitable tumor-prone models. We first focused on LSL-Braf-V600E mice intercrossed with another mouse strain engineered to express tyrosinase-driven Cre recombinase fused to a modified, 4-OHT-responsive estrogen receptor (Tyr-Cre:ERT2), thus unlocking melanocyte-preferential Braf-V600E expression upon administration of 4-OHT, especially when provided topically onto the skin. Senescent cell nevi, as known from human Braf-V600E-positive moles, form in this model and reflect premalignant precursor lesions, which are promoted to full-blown, even metastasizing melanomas by additional cooperating, senescence-disabling genetic defects, e.g. PTEN depletion or overexpression of H3K9me3-inactivating demethylases such as JMJD2c or LSD1. POG vs. DMSO-only was locally applied to topically 4-OHT-pretreated Tyr-Cre:ERT2;LSL-Braf-V600E mice. The quantification of black nevus-reminiscent spots per area residing in the skin of the depilated and drug-exposed back of the mice unveiled less melanocytic lesions in POG-exposed as compared to solvent-only-treated mice (Figure 23A). POG targets senescent Braf-V600E induced skin lesions in vivo. LSL-Braf-V600E;Tyr-Cre transgenic mice were topically exposed to OHT to induce mutant Braf expression in the skin. Two months later skin was exposed to topical POG or solvent (n = 3 each) and analyzed 4 weeks later. Images of skin shown are representative of n = 7 FFPE sections per mouse and nevi density was quantified (Figure 23A). Senescence markers were found drastically reduced in 4-OHT-Braf-V600E-induced mice that subsequently received POG treatment.

It was determined whether POG-mediated senolytic reduction of Braf-V600E-driven nevus formation would actually translate into lower incidence of full-blown melanoma in vivo. Early senolytic POG intervention delays Braf-V600E-driven melanomagenesis in vivo is shown in Figure 23B. LSL-Braf-V600E;Tyr-Cre transgenic mice homozygous for the tamoxifen-inducible Wildtype-p53-knock-in allele were treated with topical OHT for 3 weeks prior to treatment with POG or solvent before OHT exposure was discontinued. Melanoma development plotted as the time between start of the OHT treatment and manifestation of a visible tumor located at the treated skin area in the Kaplan Meier format. Time window of POG treatment is marked as grey bar. (Median survival: Solvent cohort = 89 days, POG cohort = 122 days after start of OHT treatment). Macroscopic nevus-reminiscent skin hyperpigmentation was not yet observed at this rather early time-point, compared to the reported nevus onset at around two month of 4-OHT exposure. To test whether these incipient OIS lesions would be sensitive to senolytic

removal, POG or DMSO as a solvent-only control were administered topically and systemically together with 4-OHT during the fourth week. After this, drug applications were stopped, leading to p53 inactivation in the skin, thereby potentially promoting melanoma formation from pre-existing Braf-V600E melanocytic lesions. Strikingly, by day 103 after drug cessation, all (6/6) animals of the control group had developed full-blown melanoma, all of them with lymph-node and lung metastases, while about 85% of the POG-exposed mice (6/7) were still melanoma-free (Figure 23B).

These data unmask the power of POG to not only eliminate macroscopic senescent lesions but to serve as a cancer-delaying and -preventive agent applied to deplete incipient senescent cells, which, if not eliminated early, may otherwise become the origin of full-blown and even metastatic malignancies.

Figure 24 represents a side-by-side comparison of the senolytic activities of POG and Navitoclax (as also shown in Figure 6). OIS was elicited in OHT-inducible ER:Ras-G12V-transgenic Tig3 cells. Induction of senescence was verified using the SA-ß-gal assay before exposing cells to POG. Cell viabilities were measured after 22 h using a dye exclusion assay. Compared to the established senolytic agent, the Bcl2 family inhibitor Navitoclax, POG was significantly less toxic to non-senescent cells with equivalent senolytic activity within the senolytic dose range tested.

No overt adverse effects of POG in vivo are found (Figures 25A and 25B). Figure 25A shows that POG treatment does not impact on body weight in aging mice. Body weight was measured in C57BL/6 mice (10-month-old) from Figure 7A treated with solvent or POG (n = 11). Further, as seen in Figure 25B, POG treatment does not show any adverse effects on white blood cells, liver functions and glucose metabolism in aged mice. C57BL/6 mice (20-month-old) were treated with POG (2 x 0.25 mg/kg body weight; n = 10) or solvent (n = 4) before full blood and serum was collected 4 weeks later and a complete blood count and a metabolic panel analysis was performed. With the doses used throughout the experiments, no critical weight loss or blood-based signals of overt organ toxicity were observed. Hence, POG administration is feasible without overt toxicities, improves age-related blood parameters, and even promotes physical re-strengthening.

Materials and methods

Synthesis protocol 1

(E)-1,6-Dimethyl-3-styrylpyrimido[5,4-e][1,2,4]triazine-5,7(1H,6H)-dione (ox -POG-4)

Sodium nitrite (2.3 g, 34 mmol) was added at 0 °C to a solution of 6-(1-methyl-2-((styryl)methylene)hydrazinyl)-3-methyluracil (8.0 g, 28 mmol) in glacial acetic acid and water. After 2 h the precipitate was filtered off, the filtrate was evaporated to dryness and washed with little water. Both residues were combined, suspended in EtOH and thiophenol (approx. 2 eq. thiophenol per N-oxide present in the mixture) was added. The mixture was stirred for 45 min at 90 °C. The mixture was allowed to cool to room temperature and the red precipitate was filtered off and dried in vacuo to afford ox-POG-4 (5.7 g, 19 mmol, 69%).

^{1}H-NMR (300 MHz, DMSO-d_6): δ 7.80-7.75 (m, 3H), 7.47-7.39 (m, 3H), 7.29 (d, 1H, ^{3}J = 16 Hz), 3.98 (s, 3H), 3.28 (s, 3H).

^{13}C-NMR (75 MHz, DMSO-d_6): δ 158.9, 154.0, 151.9, 149.3, 146.0, 137.4, 135.0, 129.6, 128.9, 127.8, 122.0, 42.4, 28.3

HRMS (ESI) [*m/z*]: calculated: 296.1142 $(M+H)^+$; found: 296.1141 $(M+H)^+$

CHN analysis: calculated N 23.7, C 61.0, H 4.4; found: N 23.7, C 61.7, H 4.5.

Absorption maximum: 329 nm, extinction coefficient: 93109.1 L/mol*cm (329 nm).

(E)-1,6-Dimethyl-3-styryl-4,8-dihydropyrimido[5,4-e][1,2,4]triazine-5,7(1H,6H)-dione (red-POG-4)

Thiophenol (405 µL, 3.9 mmol) was added under nitrogen atmosphere to a suspension of ox-POG-4 (499 mg, 1.7 mmol) in degassed EtOH. The mixture was stirred 3 h at room temperature. The dark brown precipitate was filtered off, washed three times with degassed EtOH under nitrogen and dried in vacuo to afford red-POG-4 (440 mg, 1.5 mmol, 88%).

^{1}H-NMR (300 MHz, DMSO-d_6): δ 10.95 (s, 1H), 7.63-7.24 (m, 6H), 6.93 (s, 1H), 6.46 (d, 1H, ^{3}J = 16 Hz), 3.09 (s, 3H), 2.98 (s, 3H).

Due to oxidation sensitivity, no LCMS analysis could be made.

Absorption maximum: 298 nm, extinction coefficient: 93327.3 L/mol*cm (298 nm).

(E)-4-Ethyl-1,6-dimethyl-3-styryl-4,8-dihydropyrimido[5,4-e][1,2,4]triazine-5,7(1H,6H)-dione (POG-10)

1,8-Diazabicyclo(5.4.0)undec-7-ene (DBU) (670 µL, 4.5 mmol) was added to a suspension of red-POG-4 (670 mg, 2.3 mmol) in degassed $CHCl_3$. After 10 min ethyl iodide was added and stirred for 16 h at 60 °C. The reaction mixture was concentrated, and the remaining chloroform was washed three times each with 1M hydrochloric acid, brine and water. The organic phase was dried over sodium sulfate and evaporated to dryness. The residual was purified by preparative HPLC (water/MeCN) to afford POG-10 (54 mg, 0.166 mmol, 7%) as yellow powder.

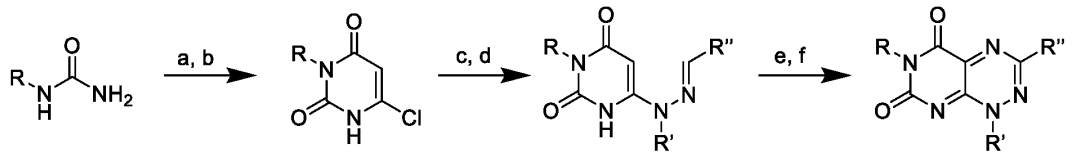
^{1}H-NMR (300 MHz, DMSO-d_6): δ 10.45 (s, 1H), 7.59-7.52 (m, 3H), 7.47-7.40 (m, 3H), 6.85 (d, 1H, ^3J = 16 Hz), 4.09 (dq, 1H, ^2J =12 Hz, ^3J =7.0 Hz), 3.61-3.50 (m, 4H), 3.18 (s, 3H), 1.24 (t, 3H, ^3J = 7.0 Hz).

^{13}C-NMR (75 MHz, DMSO-d_6): δ 157.9, 154.2, 151.2, 137.8, 136.8, 134.6, 129.8, 129.1, 127.5, 117.2, 101.4, 63.7, 57.0, 27.6, 8.7

HRMS (ESI) [*m/z*]: calculated: 326.1612 $(M+H)^+$; found: 326.1625 $(M+H)^+$

Synthesis protocol 2

General synthesis scheme



Reaction conditions: (a) Na, EtOH, diethyl malonate, N-substituted urea 1, µW, 170 °C, 6 min; (b) $POCl_3$, µW, 140 °C, 5 min; (c) R'$NHNH_2$, EtOH, 100 °C, 10 min; (d) R"CHO, EtOH, HCl, room temperature (rt), 1 h (e) $NaNO_2$, AcOH, H_2O, 0 °C to rt, over night (o.n.); (f) thiophenol, EtOH, reflux, 45 min.

N-Substituted barbituric acid (step a)

Clean sodium metal (1 eq) was dissolved completely in EtOH. To this solution, diethyl malonate (1 eq) was added followed by N-substituted urea 1 (1 equivalent (eq)), after which a white precipitate formed immediately. A second portion of EtOH was then added and stirred to partially bring the precipitate into solution. The reaction mixture was then heated by microwave irradiation (µW) to 150 °C for 12 min. The resulting solid was returned to solution by addition of H2O and then acidified with 37 % HCl and refrigerated for 3 h. The solvent was removed in vacuo to yield a slurry of H2O and precipitate which was then filtered off and washed with cold

H2O to remove impurities. This process was repeated with the mother liquor (the filtrate from the washing step) to yield white crystals.

3-N-substituted 6-chloro-uracil 2 (step b)

To 1-N-substituted barbituric acid (1 eq) and H_2O, $POCl_3$ (12.5 eq) was added dropwise at 0 °C. The resulting mixture was heated by microwave irradiation at 140 °C for 5 min. Excess $POCl_3$ was removed via rotary evaporation and the resulting yellow oil was poured over ice water and stirred for one hour. The obtained precipitate, compound **2**, was filtered and collected as a yellow powder.

3-N-substituted 6-hydrazino-uracil (step c)

To the 3-N-substituted 6-chlorouracil **2** (1 eq) substituted hydrazine (2.2 eq) was added in EtOH. The reaction was heated by microwave irradiation to 100 °C for 10 min. The precipitate was filtered and washed with cold EtOH to obtain pure product as white solid.

Aldehyde hydrazones 3 (step d)

The corresponding aldehyde (1.5 eq) was added to a solution of 3-N-substituted 6-hydrazino-uracil (1 eq) in EtOH and a catalytic amount of HCl. The resulting mixture was allowed to stir for several hours. The separated precipitate was then filtered to yield the aldehyde hydrazone **3**.

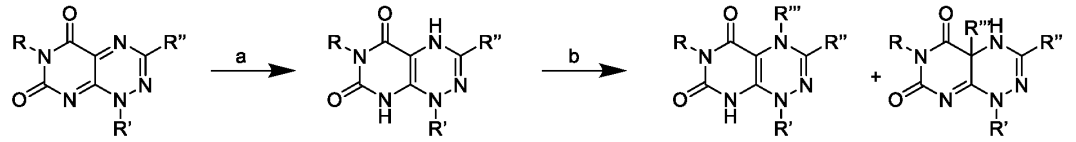
Ring closure and 4-N-oxide reduction (POG, step e and f)

Saturated aqueous solution of $NaNO_2$ (2 eq) was added to a solution of the aldehyde hydrazones **3** (1eq) in AcOH at 0 °C. After 10 min the reaction mixture was brought to room temperature and stirred for several hours during which time precipitate was formed. The precipitate as well as the filtrate was determined to be a mixture of both products and its 4-N-oxide. To the mixture of POG and its N-oxide in MeOH or EtOH, thiophenol (2 eq) was given and stirred at room temperature for 8-9 hours depending on substituent. After the reaction was complete, the solvent was removed via rotary evaporation and the resulting solid was recrystallized from EtOH to yield the corresponding POG derivative.

Isolation of 4-N-oxides

For the isolation of the N-oxide proceed as described above up to step e and omit the reduction with thiophenol (step f). The mixture of POG and its corresponding 4-N-oxide was separated by normal phase chromatography in methanol/dichloromethane (MeOH/DCM) and the pure fractions were combined.

General synthesis scheme for 4-N and 4a-substituted POG



Reaction conditions: (a) thiophenol, EtOH, rt, 3 h, inert atmosphere; (b) DBU, DCM, R'''-I, 60 °C, 16 h.

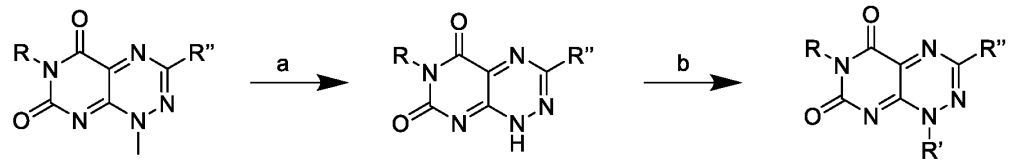
Dihydro POG (step a)

Thiophenol (2 eq) was added to a suspension of ox-POG (1 eq) in degassed EtOH under nitrogen atmosphere. The mixture was stirred for 3 h at room temperature. The dark brown precipitate was filtered off, washed three times with degassed EtOH under nitrogen and dried in vacuo to afford red-POG.

4-N and 4a-substituted POG (step b)

DBU (2 eq) was added to a suspension of red-POG (1 eq) in degassed $CHCl_3$. After 10 min alkyl iodide (1.5 eq) was added and stirred for 16 h at 60 °C. The reaction mixture was concentrated, and the remaining chloroform was washed three times each with 1M hydrochloric acid, brine, and water. The organic phase was dried over sodium sulfate and was evaporated to dryness. The residue was purified by preparative HPLC (water/MeCN) to afford 4-N substituted POG and 4a-substituted POG as yellow powders.

General procedures for 1-N substituted POG



Reaction conditions: (a) DMF, 140 °C, 3 h; (b) K_2CO_3 or DBU, 1,4 dioxane, R-Br, 120 °C, 3-8 h.

1-N-demethylPOG (step a)

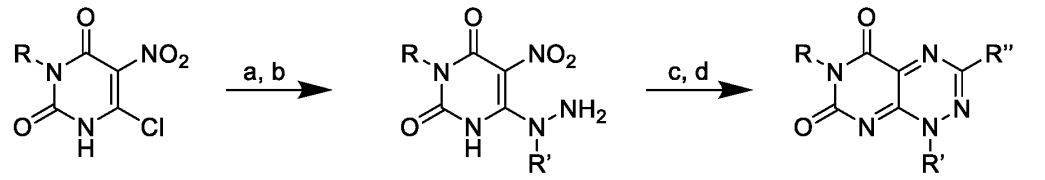
A solution of POG (18.6 mmol) in DMF was heated at 140 °C for 3 hours. Concentration of the solution *in vacuo* and recrystallisation of the residue from EtOH gave 1-N dimethyl POG.

1-N substituted POG (step b)

To 1-N demethyl POG (1 eq) in 1,4-dioxane and anhydrous potassium carbonate or DBU (2 eq), an appropriate alkyl bromide (3 eq) was added and the stirring mixture was heated under reflux at 120 °C for 3-8 hours, depending on substituent. After cooling, the precipitated potassium carbonate was filtered off and the filtrate was concentrated *in vacuo*. A solution of the residue in water was extracted with ethyl acetate and the combined extracts were dried over anhydrous $MgSO_4$. Then the extract was evaporated *in vacuo* to leave a solid, which was recrystallized to afford the corresponding pure 1-N substituted POG derivatives.

Synthesis protocol 3

General synthesis scheme



4 **5** **1-N substituted POG**

Reaction conditions: (a) DCM, R'NHNHBoc, rt, o.n.; (b) DCM, TFA, rt, o.n.; (c) R"CHO, EtOH, HCl, rt, 1 h; (d) $EtOH/H_2O$, Zn, NH_4Cl, 100 °C, 1 h.

3-N substituted 5-nitro 6-(2-N-Boc-hydrazino)-uracil (step a)

To compound **4** (1 eq) in DCM the corresponding boc-protected hydrazine (1.5 eq) was added and stirred at room temperature overnight. The formed precipitate was filtered off, washed several times with cold DCM, and dried under reduced pressure.

3-N substituted 5-nitro 6-hydrazino-uracil (step b)

3-N substituted 5-nitro 6-Boc-hydrazino-uracil (1 eq) was suspended in DCM and TFA (20% (v/v)) was added. The reaction mixture was stirred at room temperature until the reaction was completed. The solvent was removed under reduced pressure to obtain compound **5**.

Aldehyde nitro hydrazones (step c)

The corresponding aldehyde (1.5 eq) was added to a solution of 3-N substituted 5-nitro 6-hydrazino-uracil **5** (1 eq) in EtOH and a catalytic amount of HCl. The resulting mixture was stirred for several hours. The separated precipitate was then filtered off to yield the corresponding aldehyde nitro hydrazone.

1-N substituted POG (step d)

The aldehyde nitro hydrazone (1 eq) was suspended in EtOH/H_2O and both Zn dust (4 eq) and NH_4Cl (2 eq) were added. The reaction mixture was heated for 1 h at 100 °C and then cooled to room temperature. 1N HCl-solution was added and the reaction mixture was stirred for additional 1 h at room temperature. The resulting precipitate was filtered, washed with cold EtOH and dried under reduced pressure to obtain the corresponding pure 1-N substituted POG derivatives.

Europium-tetracycline assay for hydrogen peroxide determination

MOPS buffer (pH 6,9): 3-morpholinpropylsulfonic acid (MOPS) (1.09 g, 5.20 mmol) was dissolved in 180 mL water (Milli-Q-pore). pH was adjusted to 6.9 with 10 M sodium hydroxide solution and filled up with water to 200 mL in order to obtain a 26 mM MOPS buffer.

Eu_2Tc_2 solution: $EuCl_3$ hexahydrate (9.1 mg, 24.8 µmol) and tetracycline hydrochloride (3.8 mg, 7.9 µmol) were dissolved in MOPS buffer (95 mL) each. By mixing both solutions 1:1 a solution with 131 µM $EuCl_3$ and 41.6 µM tetracycline in MOPS buffer was obtained.

Hydrogen peroxide calibration solution: 30% (w/v) hydrogen peroxide was diluted in water to 160 mM, 80 mM, 40 mM, 20 mM, 10 mM, 5 mM and 2 mM hydrogen peroxide.

Protocol: The assay was performed in cuvettes with a base area of 1 cm x 1 cm. Measurement of fluorescence intensity was carried out with a fluorescence spectrometer FP-6500 by Jasco at λ_{em} = 616 nm (λ_{ex} = 405 nm). During measurements the solution inside the cuvette was stirred.

H_2O_2 calibration: DTT (10 µL, 200 mM; 1mM final concentration) were added to Eu_2Tc_2 (2 mL) solution in a cuvette and equilibrated by stirring for 5 min at 37 °C until the fluorescence signal was constant. The measurement was started and after 50 s H_2O_2 calibration solution (10 µL) was added. The calibration was made using 1000 µM, 800 µM, 400 µM, 200 µM, 100 µM, 50 µM 25 µM and 10 µM hydrogen peroxide. All measurements were repeated three times and a mean was calculated.

Measurements of H_2O_2 generation: protocol was the same as for calibration, but POG-4 solution (10 µL) was used instead of hydrogen peroxide. The final concentrations were 500 nM, 300 nM, 150 nM and 71 nM of POG-4. All measurements were repeated three times and a mean was calculated.

Beta-Galactosidase assay

SA-beta-galactosidase stainings were performed as previously described (Schmitt et al., 2002), and images were obtained using an Evos Core microscope (AMG).

Cell viability assay

Adherent cells were stained with the amine-reactive viability dye Ghost Dye Red 780 (13-0865-T500, Tonbo) by adding 1:5000 diluted GhostDye Red in PBS for 20 minutes at 37°C. Cells were then trypsinized by adding 2x trypsin/EDTA for 10 minutes, washed with PBS and fixed in 2% formalin before analysis using a Guava easyCyte HT12 (Luminex) benchtop flow cytometer.

List of references cited in the preceding sections or deemed otherwise to be relevant

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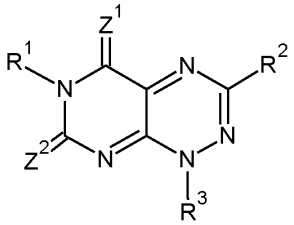
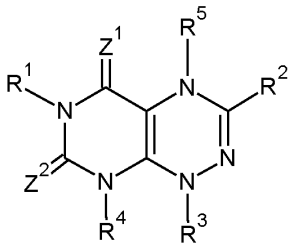
Claims

1. Compound according to general formula (I), a derivative of such compound according to any of general formulae (II), (III), or (IV), or a pharmaceutically acceptable salt or hydrate of such compound or derivative, for in-vivo use as senolytic in treating a disease in which the removal of senescent cells is beneficial:



(I)



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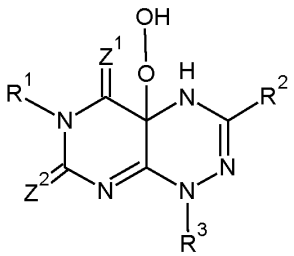

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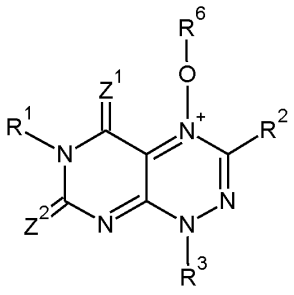


(IV)

wherein

R^1 = H, C_1-C_{10} alkyl, C_3-C_{20} cycloalkyl, heteroatom-substituted C_3-C_{20} cycloalkyl, CF_3, CF_2H, CFH_2, optionally substituted piperidine, C_1-C_{10} alkyl carrying a primary, secondary or tertiary amino substituent, OH, CH_2-O-galactosyl, optionally substituted C_6-C_{20} aryl, or optionally substituted C_5-C_{20} heteroaryl,

R^2 = optionally substituted C_1-C_{10} alkyl, optionally substituted C_7-C_{30} alkyl aryl, optionally substituted C_6-C_{30} alkyl heteroaryl, optionally substituted C_6-C_{20} aryl, optionally substituted C_6-C_{20} aryl nitro, optionally substituted C_2-C_{10} alkenyl, optionally substituted C_8-C_{30} alkenyl aryl, optionally substituted C_8-C_{30} alkenyl aryl nitro, optionally substituted C_7-C_{30} alkenyl heteroaryl, optionally substituted C_2-C_{10} alkinyl, optionally substituted C_8-C_{30} alkinyl aryl, optionally substituted C_7-C_{30} alkinyl heteroaryl, optionally substituted C_{11}-C_{30} heteroaryl aryl, optionally substituted C_9-C_{30} cyclyl aryl, optionally substituted C_8-C_{30} heterocyclyl aryl, optionally substituted C_3-C_{20} cycloalkyl, halogen, OH, C_1-C_{10} alkoxy, C_6-C_{20} aryloxy, amino, substituted amino, CN, carboxy, optionally substituted C_1-C_{10} alkyl carboxy, optionally substituted C_6-C_{20} aryl-carboxy, carbamido, optionally substituted C_5-C_{20} heteroaryl, optionally substituted C_2-C_{20} heterocyclyl, heteroatom-substituted C_6-C_{20} cycloalkyl, optionally substituted SH, sulfonamido, or sulfone,

R^3 = C_1-C_{10} alkyl, C_3-C_{20} cycloalkyl, heteroatom-substituted C_3-C_{20} cycloalkyl, CF_3, CF_2H, CFH_2, optionally substituted piperidine, C_1-C_{10} alkyl carrying a primary, secondary or tertiary amino substituent, OH, CH_2-O-galactosyl, optionally substituted C_6-C_{20} aryl, or optionally substituted C_5-C_{20} heteroaryl,

R^4, R^5 = independently from each other H, C_1-C_{10} alkyl, C_3-C_{20} cycloalkyl, heteroatom-substituted C_3-C_{20} cycloalkyl, CF_3, CF_2H, CFH_2, optionally substituted piperidine, C_1-C_{10} alkyl carrying a primary, secondary or tertiary amino substituent, OH, CH_2-O-galactosyl, optionally substituted C_6-C_{20} aryl, or optionally substituted C_5-C_{20} heteroaryl, or a protecting group chosen from i) *tert*-butyloxycarbonyl, ii) trityl, iii) acetyl, iv) C_1-C_{10} acyl residues, and v) monosubstituted or disubstituted compounds according to general formula (II), wherein R^4 and R^5 denote H,

R^6 = absent or a protecting group chosen from C_1-C_{10} acyl, acetyl, ester residues, benzoyl, benzyl, trityl, and ether residues, and

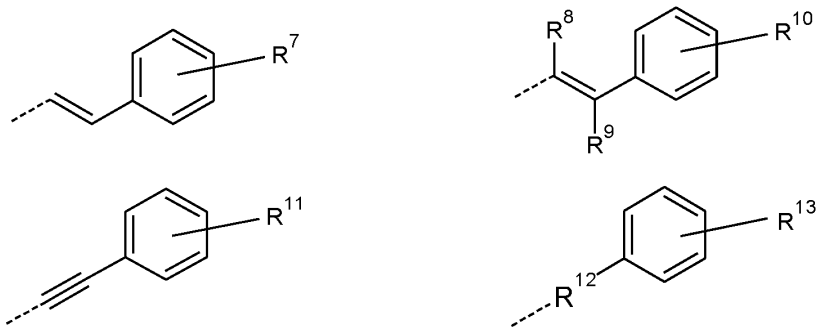
Z^1, Z^2 = independently from each other O or S.

2. Compound, derivative, or pharmaceutically acceptable salt or hydrate of such compound or derivative for use according to claim 1, **characterized** in that the compound, the derivative, or the pharmaceutically acceptable salt or hydrate of such compound or derivative is administered to a patient in an effective dosage as a senolytic.

3. Compound, derivative, or pharmaceutically acceptable salt or hydrate of such compound or derivative for use according to claim 1 or 2, **characterized** in that the compound, the derivative, or the pharmaceutically acceptable salt or hydrate of such compound or derivative is administered to a patient intravenously in a dosage in a range from 0.05 mg to 10 mg, preferably between 0.1 mg to 4 mg, per kg bodyweight of a patient to be treated

4. Compound, derivative, or pharmaceutically acceptable salt or hydrate of such compound or derivative for use according to any of the preceding claims, **characterized** in that the compound, the derivative, or the pharmaceutically acceptable salt or hydrate of such compound or derivative is administered to a patient in such a dosage that a number of viable senescence-associated beta-galactosidase positive cells in a tissue sample obtained from the patient is equal to or less than 20 % of the number of viable senescence-associated beta-galactosidase positive cells in a comparable control sample obtained from at least one control subject suffering from the same disease as the patient but not having been treated with the compound, the derivative, or the pharmaceutically acceptable salt or hydrate of such compound or derivative, wherein a viability of senescence-associated beta-galactosidase positive cells is determined by a dye exclusion test, wherein the dye exclusion test on the tissue sample of the patient is carried out at least 22 hours after administration of the compound, the derivative, or the pharmaceutically acceptable salt or hydrate of such compound or derivative.

5. Compound, derivative, or pharmaceutically acceptable salt or hydrate of such compound or derivative for use according any of the preceding claims, **characterized** in that residue R^2 is a residue according to any of the following formulae, wherein the dashed line indicates a bond by which the residue R^2 is covalently bound to the triazine ring of the compound according to general formulae (I), (II), (III), or (IV):



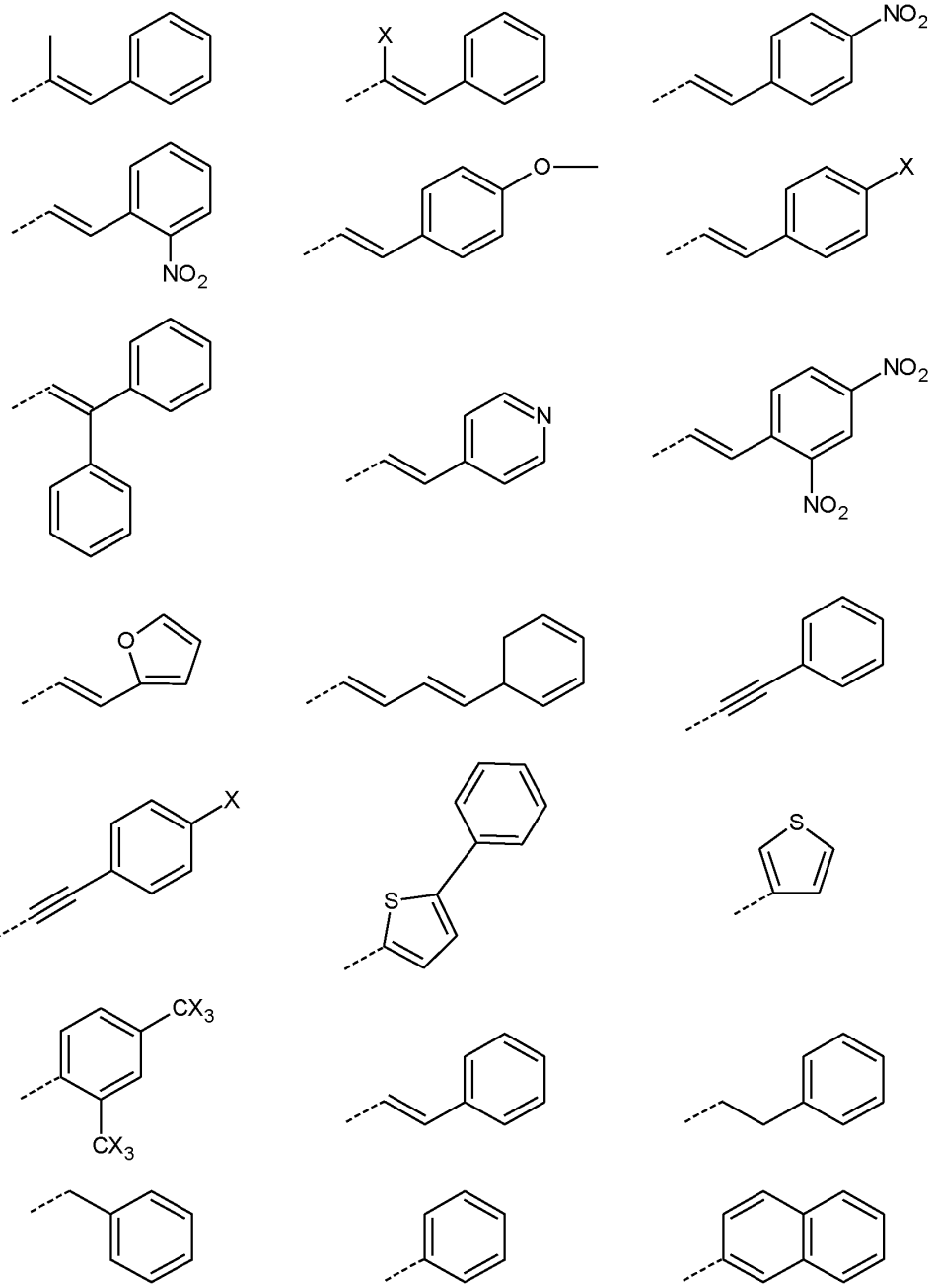
wherein

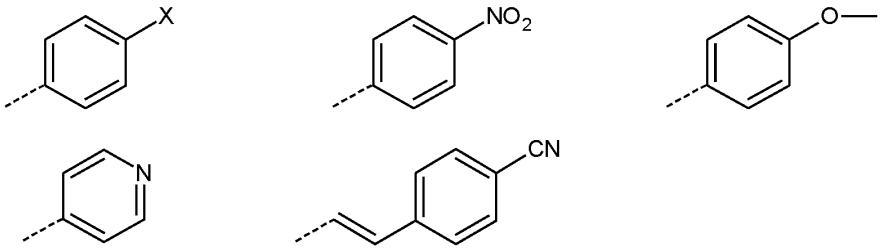
R^7, R^{10}, R^{11}, R^{13} = independently from each other H, NO_2, optionally substituted C_1-C_{10} alkyl, optionally substituted C_2-C_{10} alkenyl, optionally substituted C_3-C_{20} cycloalkyl, heteroatom-substituted C_6-C_{20} cycloalkyl, halogen, OH, C_1-C_{10} alkoxy, C_6-C_{20} aryloxy, amino, substituted amino, CN, carboxy, optionally substituted C_1-C_{10} alkyl carboxy, optionally substituted C_6-C_{20} aryl-carboxy, carbamido, optionally substituted C_5-C_{20} heteroaryl, optionally substituted C_2-C_{20} heterocyclyl, optionally substituted C_6-C_{20} aryl, optionally substituted SH, sulfonamido, or sulfone,

R^8, R^9 = independently from each other H, optionally substituted C_1-C_{10} alkyl, optionally substituted C_2-C_{10} alkenyl, optionally substituted C_3-C_{20} cycloalkyl, heteroatom-substituted C_6-C_{20} cycloalkyl, halogen, OH, C_1-C_{10} alkoxy, C_6-C_{20} aryloxy, amino, substituted amino, CN, carboxy, optionally substituted C_1-C_{10} alkyl carboxy, optionally substituted C_6-C_{20} aryl-carboxy, carbamido, optionally substituted C_5-C_{20} heteroaryl, optionally substituted C_2-C_{20} heterocyclyl, optionally substituted C_6-C_{20} aryl, optionally substituted SH, sulfonamido, or sulfone, CF_3, CF_2H, or CFH_2, and

R^{12} = independently from each other optionally substituted C_1-C_{10} alkyl, optionally substituted C_2-C_{10} alkenyl, optionally substituted C_2-C_{10} alkinyl, optionally substituted C_6-C_{20} aryl, optionally substituted C_5-C_{20} heteroaryl, optionally substituted C_3-C_{20} cycloalkyl, optionally substituted C_3-C_{20} cyclyl, or optionally substituted C_2-C_{20} heterocyclyl.

6. Compound, derivative, or pharmaceutically acceptable salt or hydrate of such compound or derivative for use according to any of the preceding claims, **characterized** in that residue R^2 is a residue according to any of the following formulae, wherein the dashed line indicates a bond by which the residue R^2 is covalently bound to the triazine ring of the compound according to general formulae (I), (II), (III), or (IV):

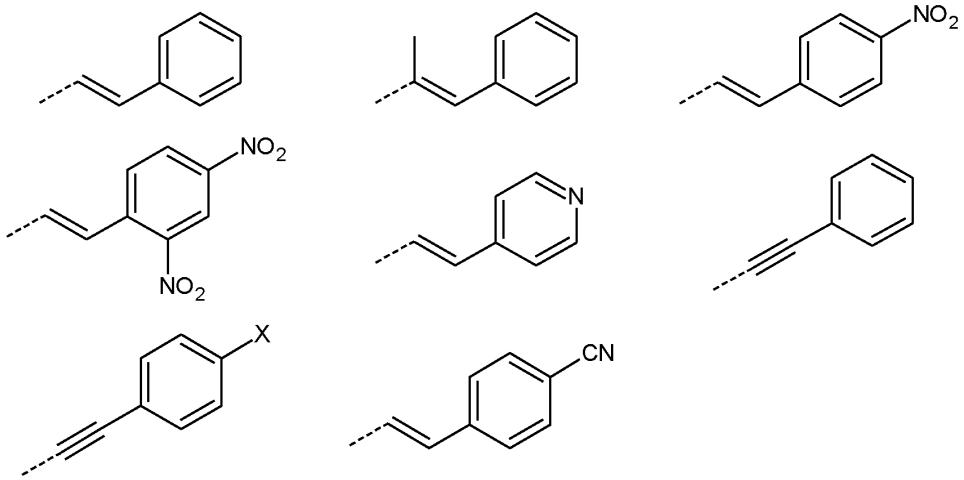




wherein

X = F, Cl, Br, or I.

7. Compound, derivative, or pharmaceutically acceptable salt or hydrate of such compound or derivative for use according to any of the preceding claims, **characterized** in that residue R^2 is a residue according to any of the following formulae, wherein the dashed line indicates a bond by which the residue R^2 is covalently bound to the triazine ring of the compound according to general formulae (I), (II), (III), or (IV):



wherein

X = F, Cl, Br, or I.

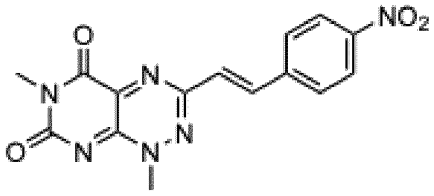
8. Compound, derivative, or pharmaceutically acceptable salt or hydrate of such compound or derivative for use according to any of the preceding claims, **characterized** in that residue R^1 is a methyl residue or a hydrogen.

9. Compound, derivative, or pharmaceutically acceptable salt or hydrate of such compound or derivative for use according to any of the preceding claims, **characterized** in that residue R^3 is a methyl residue.

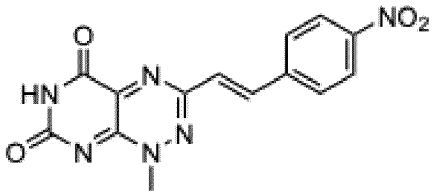
10. Compound, derivative, or pharmaceutically acceptable salt or hydrate of such compound or derivative for use according to any of the preceding claims, **characterized** in that residue R^1 is a methyl residue or a hydrogen and residue R^3 is chosen from methyl, ethyl, isopropyl, and phenyl.

11. Compound, derivative, or pharmaceutically acceptable salt or hydrate of such compound or derivative for use according to any of the preceding claims, **characterized** in that residue R^1 is a methyl residue or a hydrogen and R^3 is a methyl residue.

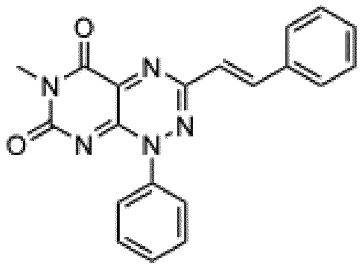
12. Compound, derivative, or pharmaceutically acceptable salt or hydrate of such compound or derivative for use according to any of the preceding claims, **characterized** in that the compound has the following structure:



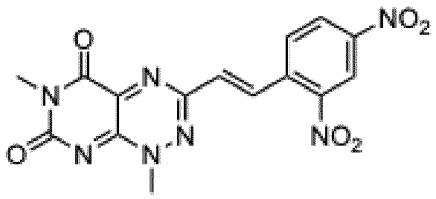
13. Compound, derivative, or pharmaceutically acceptable salt or hydrate of such compound or derivative for use according to any of claims 1 to 11, **characterized** in that the compound has the following structure:



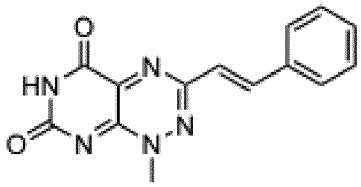
14. Compound, derivative, or pharmaceutically acceptable salt or hydrate of such compound or derivative for use according to any of claims 1 to 11, **characterized** in that the compound has the following structure:



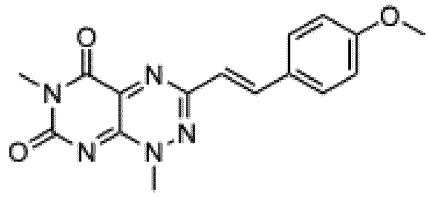
15. Compound, derivative, or pharmaceutically acceptable salt or hydrate of such compound or derivative for use according to any of claims 1 to 11, **characterized** in that the compound has the following structure:



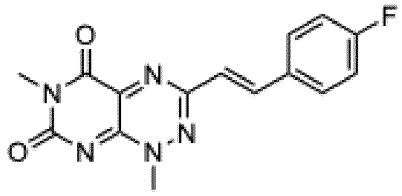
16. Compound, derivative, or pharmaceutically acceptable salt or hydrate of such compound or derivative for use according to any of claims 1 to 11, **characterized** in that the compound has the following structure:



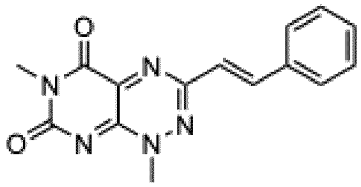
17. Compound, derivative, or pharmaceutically acceptable salt or hydrate of such compound or derivative for use according to any of claims 1 to 11, **characterized** in that the compound has the following structure:



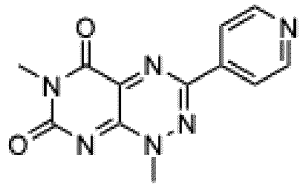
18. Compound, derivative, or pharmaceutically acceptable salt or hydrate of such compound or derivative for use according to any of claims 1 to 11, **characterized** in that the compound has the following structure:



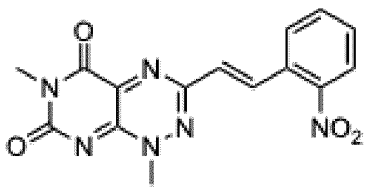
19. Compound, derivative, or pharmaceutically acceptable salt or hydrate of such compound or derivative for use according to any of claims 1 to 11, **characterized** in that the compound has the following structure:



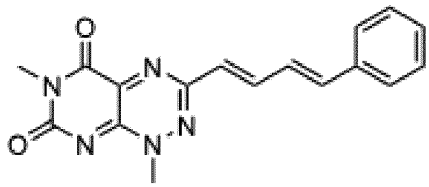
20. Compound, derivative, or pharmaceutically acceptable salt or hydrate of such compound or derivative for use according to any of claims 1 to 11, **characterized** in that the compound has the following structure:



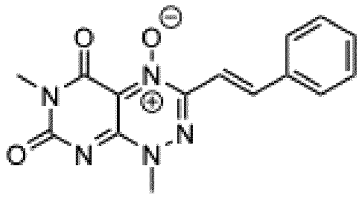
21. Compound, derivative, or pharmaceutically acceptable salt or hydrate of such compound or derivative for use according to any of claims 1 to 11, **characterized** in that the compound has the following structure:



22. Compound, derivative, or pharmaceutically acceptable salt or hydrate of such compound or derivative for use according to any of claims 1 to 11, **characterized** in that the compound has the following structure:



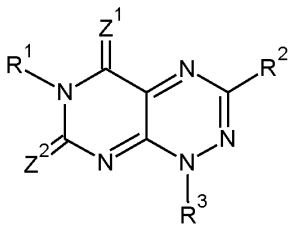
23. Compound, derivative, or pharmaceutically acceptable salt or hydrate of such compound or derivative for use according to any of claims 1 to 11, **characterized** in that the compound has the following structure:



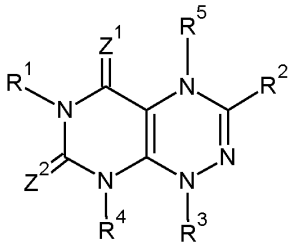
24. Compound, derivative, or pharmaceutically acceptable salt or hydrate of such compound or derivative for use according to any of the preceding claims, **characterized** in that the compound is for use in treating cancer that has previously been treated with a chemotherapeutic agent.

25. Compound, derivative, or pharmaceutically acceptable salt or hydrate of such compound or derivative for use according to claim 24, **characterized** in that the previous treatment with a chemotherapeutic agent has resulted in tumor cells that are in a state of therapy-induced senescence.

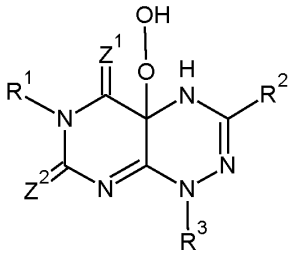
26. In-vitro method for senolysis, the method comprising subjecting senescent cells to a compound according to general formula (I), to a derivative of such compound according to any of general formulae (II), (III), or (IV), or to a pharmaceutically acceptable salt or hydrate of such compound or derivative:



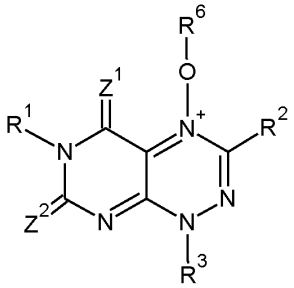
(I)



(II)



(III)



(IV)

wherein

R^1 = H, C_1-C_{10} alkyl, C_3-C_{20} cycloalkyl, heteroatom-substituted C_3-C_{20} cycloalkyl, CF_3, CF_2H, CFH_2, optionally substituted piperidine, C_1-C_{10} alkyl carrying a primary, secondary or tertiary amino substituent, OH, CH_2-O-galactosyl, optionally substituted C_6-C_{20} aryl, or optionally substituted C_5-C_{20} heteroaryl,

R^2 = optionally substituted C_1-C_{10} alkyl, optionally substituted C_7-C_{30} alkyl aryl, optionally substituted C_6-C_{30} alkyl heteroaryl, optionally substituted C_6-C_{20} aryl, optionally substituted C_6-C_{20} aryl nitro, optionally substituted C_2-C_{10} alkenyl, optionally substituted C_8-C_{30} alkenyl aryl, optionally substituted C_8-C_{30} alkenyl aryl nitro, optionally substituted C_7-C_{30} alkenyl heteroaryl, optionally substituted C_2-C_{10} alkinyl, optionally substituted C_8-C_{30} alkinyl aryl, optionally substituted C_7-C_{30} alkinyl heteroaryl, optionally substituted C_{11}-C_{30} heteroaryl aryl, optionally substituted C_9-C_{30} cyclyl aryl, optionally substituted C_8-C_{30} heterocyclyl aryl, optionally substituted C_3-C_{20} cycloalkyl, halogen, OH, C_1-C_{10}

alkoxy, C_6-C_{20} aryloxy, amino, substituted amino, CN, carboxy, optionally substituted C_1-C_{10} alkyl carboxy, optionally substituted C_6-C_{20} aryl-carboxy, carbamido, optionally substituted C_5-C_{20} heteroaryl, optionally substituted C_2-C_{20} heterocyclyl, heteroatom-substituted C_6-C_{20} cycloalkyl, optionally substituted SH, sulfonamido, or sulfone,

R^3 = C_1-C_{10} alkyl, C_3-C_{20} cycloalkyl, heteroatom-substituted C_3-C_{20} cycloalkyl, CF_3, CF_2H, CFH_2, optionally substituted piperidine, C_1-C_{10} alkyl carrying a primary, secondary or tertiary amino substituent, OH, CH_2-O-galactosyl, optionally substituted C_6-C_{20} aryl, or optionally substituted C_5-C_{20} heteroaryl,

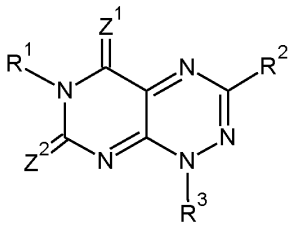
R^4, R^5 = independently from each other H, C_1-C_{10} alkyl, C_3-C_{20} cycloalkyl, heteroatom-substituted C_3-C_{20} cycloalkyl, CF_3, CF_2H, CFH_2, optionally substituted piperidine, C_1-C_{10} alkyl carrying a primary, secondary or tertiary amino substituent, OH, CH_2-O-galactosyl, optionally substituted C_6-C_{20} aryl, or optionally substituted C_5-C_{20} heteroaryl, or a protecting group chosen from i) *tert*-butyloxycarbonyl, ii) trityl, iii) acetyl, iv) C_1-C_{10} acyl residues, and v) monosubstituted or disubstituted compounds according to general formula (II), wherein R^4 and R^5 denote H,

R^6 = absent or a protecting group chosen from C_1-C_{10} acyl, acetyl, ester residues, benzoyl, benzyl, trityl, and ether residues and

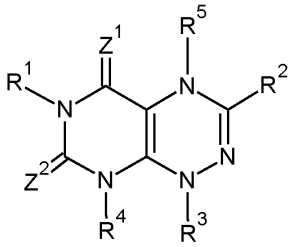
Z^1, Z^2 = independently from each other O or S.

27. In-vitro method for senolysis according to claim 26, **characterized** in that the compound, the derivative or the pharmaceutically acceptable salt or hydrate of such compound or derivative is used in such a dose that a number of viable senescence-associated beta-galactosidase positive cells is equal to or less than 20 % of the number of viable senescence-associated beta-galactosidase positive cells prior to subjecting the senescent cells to the compound, the derivative, or the pharmaceutically acceptable salt or hydrate of such compound or derivative, wherein a viability of senescence-associated beta-galactosidase positive cells is determined by a dye exclusion test carried out at least 22 hours after subjecting the senescent cells to the compound, the derivative, or the pharmaceutically acceptable salt or hydrate of such compound or derivative.

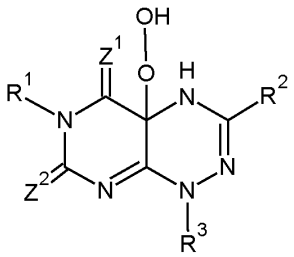
28. Compound according to general formula (I), a derivative of such compound according to any of general formulae (II), (III), or (IV), or a pharmaceutically acceptable salt or hydrate of such compound or derivative:



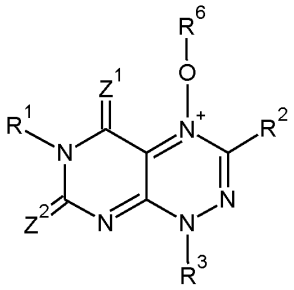
(I)



(II)



(III)



(IV)

wherein

R^1 = H, C_1-C_{10} alkyl, C_3-C_{20} cycloalkyl, heteroatom-substituted C_3-C_{20} cycloalkyl, CF_3, CF_2H, CFH_2, optionally substituted piperidine, C_1-C_{10} alkyl carrying a primary, secondary or tertiary amino substituent, OH, CH_2-O-galactosyl, optionally substituted C_6-C_{20} aryl, or optionally substituted C_5-C_{20} heteroaryl,

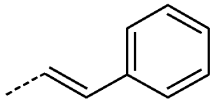
R^2 = optionally substituted C_6-C_{30} alkyl heteroaryl, optionally substituted C_6-C_{20} aryl, optionally substituted C_6-C_{20} aryl nitro, optionally substituted C_8-C_{30} alkenyl aryl, optionally substituted C_8-C_{30} alkenyl aryl nitro, optionally substituted C_7-C_{30} alkenyl heteroaryl, optionally substituted C_8-C_{30} alkinyl aryl, optionally substituted C_7-C_{30} alkinyl heteroaryl, optionally substituted C_{11}-C_{30} heteroaryl aryl, optionally substituted C_9-C_{30} cyclyl aryl, optionally substituted C_8-C_{30} heterocyclyl aryl, halogen, OH, C_1-C_{10} alkoxy, C_6-C_{20} aryloxy, amino, substituted amino, CN, carboxy, optionally substituted C_1-C_{10} alkyl carboxy, optionally substituted C_6-C_{20} aryl-carboxy, carbamido, optionally substituted SH, sulfonamido, or sulfone,

R^3 = C_1-C_{10} alkyl, C_3-C_{20} cycloalkyl, heteroatom-substituted C_3-C_{20} cycloalkyl, CF_3, CF_2H, CFH_2, optionally substituted piperidine, C_1-C_{10} alkyl carrying a primary, secondary or tertiary amino substituent, OH, CH_2-O-galactosyl, optionally substituted C_6-C_{20} aryl, or optionally substituted C_5-C_{20} heteroaryl,

R^4, R^5 = independently from each other H, C_1-C_{10} alkyl, C_3-C_{20} cycloalkyl, heteroatom-substituted C_3-C_{20} cycloalkyl, CF_3, CF_2H, CFH_2, optionally substituted piperidine, C_1-C_{10} alkyl carrying a primary, secondary or tertiary amino substituent, OH, CH_2-O-galactosyl, optionally substituted C_6-C_{20} aryl, or optionally substituted C_5-C_{20} heteroaryl, or a protecting group chosen from i) *tert*-butyloxycarbonyl, ii) trityl, iii) acetyl, iv) C_1-C_{10} acyl residues, and v) monosubstituted or disubstituted compounds according to general formula (II), wherein R^4 and R^5 denote H,

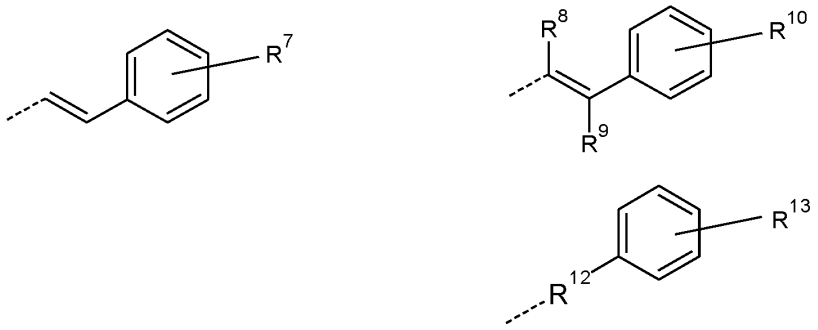
R^6 = absent or a protecting group chosen from C_1-C_{10} acyl, acetyl, ester residues, benzoyl, benzyl, trityl, and ether residues, and

Z^1, Z^2 = independently from each other O or S,

with the proviso that residue R^2 does not comply with formula  if residue R^1 is a methyl residue and residue R^3 is a methyl or ethyl residue,

wherein the dashed line indicates a bond by which the residue R^2 is covalently bound to the triazine ring of the compound according to general formulae (I), (II), (III), or (IV).

29. Compound, derivative, or pharmaceutically acceptable salt or hydrate of such compound or derivative according to claim 28, **characterized** in that residue R^2 is a residue according to any of the following formulae, wherein the dashed line indicates a bond by which the residue R^2 is covalently bound to the triazine ring of the compound according to general formulae (I), (II), (III), or (IV):



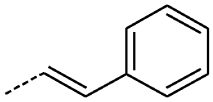
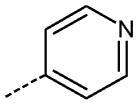
wherein

R^7, R^{10}, R^{13} = independently from each other H, NO_2, optionally substituted C_1-C_{10} alkyl, optionally substituted C_2-C_{10} alkenyl, optionally substituted C_3-C_{20} cycloalkyl, halogen, OH, C_1-C_{10} alkoxy, C_6-C_{20} aryloxy, amino, substituted amino, CN, carboxy, optionally substituted C_1-C_{10} alkyl carboxy, optionally substituted C_6-C_{20} aryl-carboxy, carbamido, optionally substituted C_5-C_{20} heteroaryl, optionally substituted C_2-C_{20} heterocyclyl, heteroatom-substituted C_6-C_{20} cycloalkyl, optionally substituted C_6-C_{20} aryl, optionally substituted SH, sulfonamido, or sulfone,

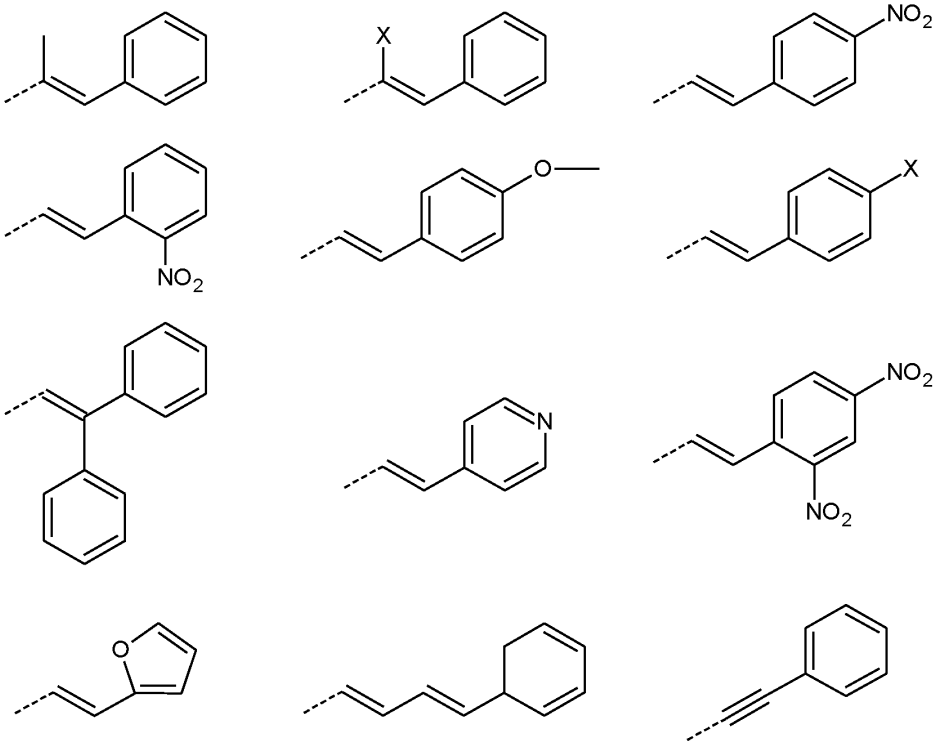
R^8, R^9 = independently from each other H, optionally substituted C_1-C_{10} alkyl, optionally substituted C_2-C_{10} alkenyl, optionally substituted C_3-C_{20} cycloalkyl, halogen, OH, C_1-C_{10} alkoxy, C_6-C_{20} aryloxy, amino, substituted amino, CN, carboxy, optionally substituted C_1-C_{10} alkyl carboxy, optionally substituted C_6-C_{20} aryl-carboxy, carbamido, optionally substituted C_5-C_{20} heteroaryl, optionally substituted C_2-C_{20} heterocyclyl, heteroatom-substituted C_6-C_{20} cycloalkyl, optionally

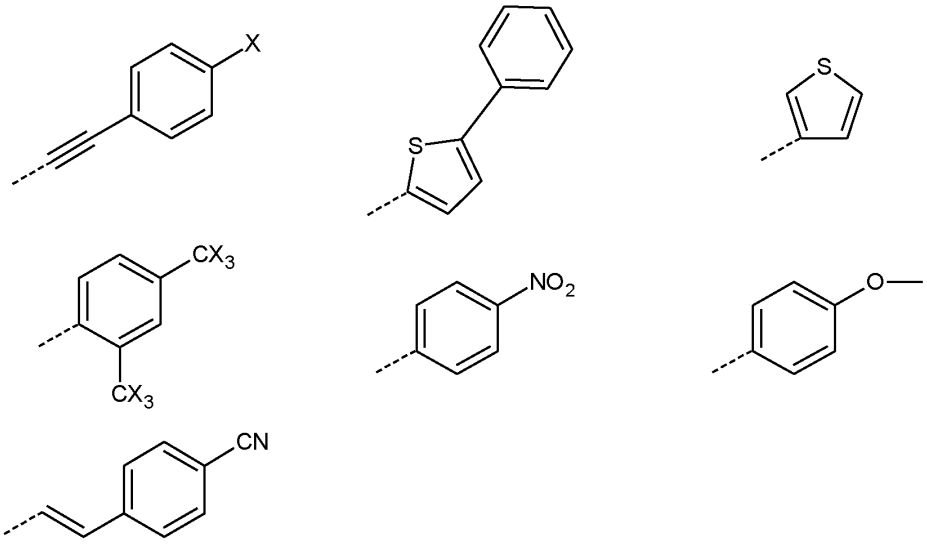
substituted C_6-C_{20} aryl, optionally substituted SH, sulfonamido, or sulfone, CF_3, CF_2H, or CFH_2, and

R^{12} = independently from each other optionally substituted C_2-C_{10} alkenyl, optionally substituted C_6-C_{20} aryl, optionally substituted C_5-C_{20} heteroaryl, optionally substituted C_3-C_{20} cycloalkyl, optionally substituted C_3-C_{20} cyclyl, or optionally substituted C_2-C_{20} heterocyclyl.

30. Compound, derivative, or pharmaceutically acceptable salt or hydrate of such compound or derivative according to claim 28 or 29, **characterized** in that in that that residue R^2 does not comply with formula  or fomula .

31. Compound, derivative, or pharmaceutically acceptable salt or hydrate of such compound or derivative according to claim 28 or 30, **characterized** in that that residue R^2 is a residue according to any of the following formulae, wherein the dashed line indicates a bond by which the residue R^2 is covalently bound to the triazine ring of the compound according to general formulae (I), (II), (III), or (IV):





wherein

X = F, Cl, Br, or I.

32. Medicament, comprising at least one compound, derivative, or pharmaceutically acceptable salt or hydrate of such compound or derivative according to any of claims 28 to 31.