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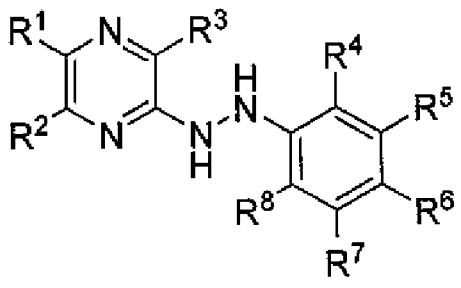
(54) **Title**: SUBSTITUTED 2-(2-PHENYLHYDRAZINYL)PYRAZINE, PROCESS FOR ITS PREPARATION, ITS USE AND A PHARMACEUTICAL COMPOSITION CONTAINING THE SAME



I

(57) **Abstract**: Substituted 2-(2-phenylhydrazinyl)pyrazine of general formula I, wherein each R^1, R^2 is independently H or CN; R^3 is CH_3 or $CONH_2$; each R^4, R^5, R^6, R^7, R^8 is independently H, CI, or NO_2. The invention further provides a method for preparing such substances, in which the appropriate substituted chloropyrazine is treated with a substituted phenylhydrazine under conditions of microwave synthesis. The compounds of general formula I are characterized by their low toxicity and high activity against mycobacteria. They can also be used in pharmaceutical preparations as antitubercular drugs.

Substituted 2-(2-phenylhydrazinyl)pyrazine, process for its preparation, its use and a pharmaceutical composition containing the same

Field of the Invention

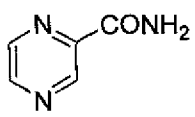
The present invention relates to novel compounds based on substituted phenylhydrazinyl-pyrazines, processes for their preparation, and their use as antituberculous drugs and pharmaceutical preparations containing them.

Background of the Invention

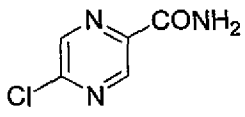
Substituted pyrazine derivatives belong to compounds having significant biological activity, they are used as essential drugs (Miniyar P.B., Murumkar P.R., Patil P.S., et al.: Mini Rev. Med. Chem. **13**, 1607-25, 2013; Ferreira S.B., Kaiser C.R. Exp. Opin. Ther. Patents **22**, 1033-51, 2012).

Tuberculosis (TB), caused by *Mycobacterium tuberculosis complex* (MTB), has for many years belonged to the most widespread infectious diseases in the world. Its incidence and mortality has so far failed to reduce despite existing antituberculotic therapy (World Health Organization, Stop TB Partnership. Tuberculosis Global Facts 2014). The reason is the emergence and development of increasingly resistant forms of the disease agent and co-infection with HIV, the incidence in the Czech Republic is increasing (Ústav zdravotnických informací a statistiky ČR; Tuberkulóza a respirační nemoci 2012, Prha ÚZIS, 2013, 111pp.). Therefore, it is necessary to develop new more efficient low-molecular antituberculotic drugs active against increasingly occurring resistant and/or multiresistant mycobacterial strains.
The most important first line anti-tuberculosis drugs (along with isoniazide, rifampicin and ethambutol) include pyrazinamide (PZA). Only PZA, or rifampicin, are active against latent forms of TB (*i.e.* semidormant mycobacteria occurring in acidic environment tissues). Thanks to PZA is the treatment shortened from 9-12 to 6 months, which is due to the ability to kill mycobacteria persisting in an acidic environment (Zhang H., et al. The FEBS Journal **275**, 753-762, 2008). PZA does not act by a single mechanism, but we recognize still new ways of its action: a) PZA is a prodrug, which is converted with the enzyme nikotinamidase/pyrazinamidase (PncA) to the pyrazinoic acid (POA), as its active form. There is an accumulation of POA and intracellular acidification (Doležal M., Kešetovič D., Zitko J. Curr. Pharm. Design, 2011, vol. 17, no. 32, p. 3506–3514). This disturbance leads to

influencing the activity of enzymatic systems and disrupting the processes that are dependent on the pH gradient at the interface organelle / cytoplasmic compartment (Zhang H., et al. The FEBS Journal **275**, 753-762, 2008). Furthermore, POA acts by inhibiting protein and RNA synthesis, serine uptake, disruption of membrane potential in the acidic pH (Wright H.T., Reynolds K.A. Curr. Opin. Microbiol. **10**, 447-453, 2007); b) PZA influences RpsA - an enzyme system responsible for regulating biological processes, including cell adhesion, migration and differentiation (Zimic M., et al. Microb. Drug Resist. **18**, 372-375, 2012; Sayahi H., et al. Chemistry & Biodiversity **9**, 2582-2596, 2012). Through this process PZA inhibits *trans*-translation, which is a process to prevent the creation of non-functional proteins, and is associated with stress survival, virulence and recovery of nutrient starvation (Shi W., Zhang X., Jiang X., et al. Science **333**, 1630-1632, 2011); c) estimated competition of PZA (and 5-chloro-pyrazine-2-carboxamide) with NADPH for binding to the complex FAS I (Fatty Acid Synthase). The synthesis of mycolic acids, which are part of the cell wall of mycobacteria, is inhibited. Also POA is binding to FAS I, but in different location of the enzyme (Zimic M., et al. Microb. Drug Resist. **18**, 372-375, 2012; Sayahi H., et al. Chemistry & Biodiversity **9**, 2582-2596, 2012). Recently it has been found that FAS I is the primary goal of the pyrazinamide derivatives, including 5-chloropyrazinamide, which also inhibits FAS complex II. FAS I is involved in the synthesis of short chain-mycolic acids, FAS II mediates the synthesis of long chain-mycolic acids. One of the key enzymes of FAD II is the enoyl-ACP reductase (Ngo S.C., et al. Antimicrob. Agents Chemother. **51**, 2430-2435, 2007).



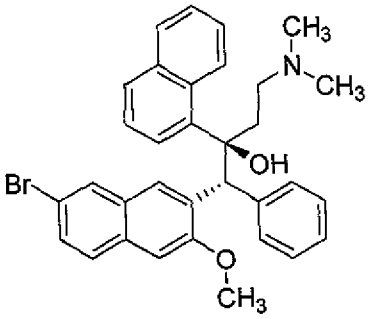
pyrazinamide



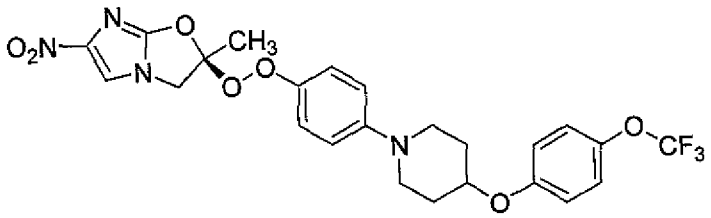
5-chloropyrazine-2-carboxamide

Pharmaceutical research and development has neglected the field of antituberculotics (AT); for almost 40 years there have been no launch of a new drug in this indication group; currently (2015), only 3 new antituberculotics (entirely new structures), bedaquilline, delamanid and pretomanid, are in the third stage of clinical trials worldwide. However, these new ATs must be combined with, *e.g.*, PZA or rifampicin. Due to problems of TB therapy, any new substance is highly expected since resistance develops gradually to actually used

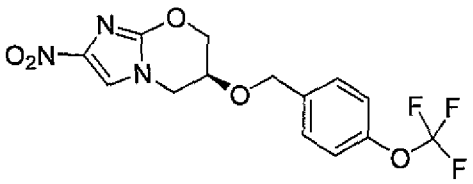
ATs and it is only a matter of time when no effective drugs will be available against TB (Matthias Stehr M., Elamin A.A., Singh M. Curr. Top. Med. Chem. **14**, 110-129, 2014).



bedaquilline

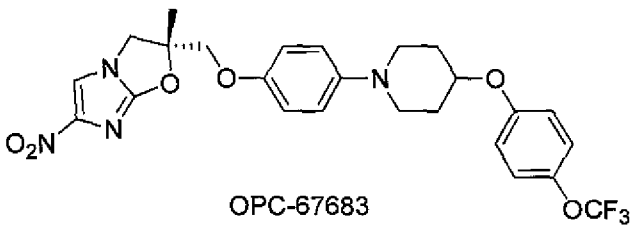


delamanid



pretomanid

For the reasons mentioned above there persist attempts to find an AT which would act against MDR-TB strains, or against latent forms of TB. They must be structurally novel compounds which act by novel mechanisms other than the currently used AT, or with an improved pharmacokinetic profile. New potential ATs, which are now in preclinical and clinical stage of development, are often substances which contain a nitro moiety in their molecule. Relevant examples include the nitroimidazoles pretomanid, OPC-67683 and delamanid. Another promising group of AT are oxazolidinones, for example linezolid and sutezolid, containing, *inter alia*, the carboxamide functional moiety (Matthias Stehr M., Elamin A.A., Singh M. Curr. Top. Med. Chem. **14**, 110-129, 2014).



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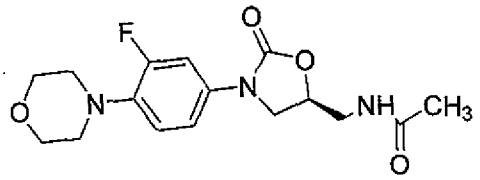
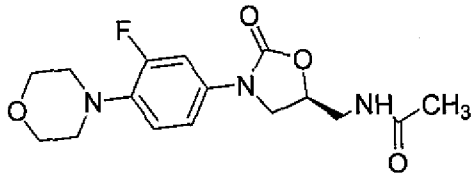
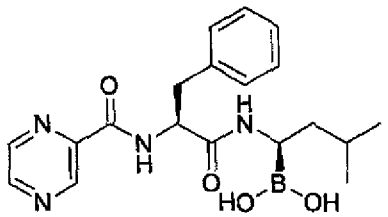


linezolid

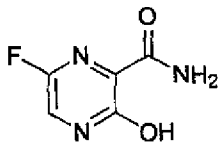


sutezolid

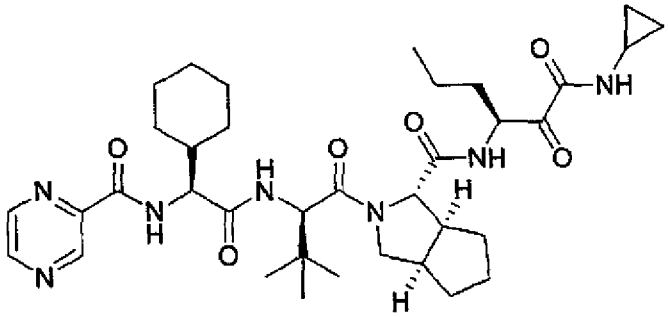
For all these substances, the nitro or carboxamide moieties have proved essential for the antimycobacterial activity, but the mechanisms of action were different for each other (Matthias Stehr M., Elamin A.A., Singh M. Curr. Top. Med. Chem. **14**, 110-129, 2014). The pyrazinecarboxamide motif can be found in a wide range of modern drugs such as the antineoplastic agent bortezomib (Palle Raghavendracharyulu Venkata, Kadaboina Rajasekhar, Murki Veerendeer et al., Bortezomib and proces for producing same. US2010226597 (2010)), the antivirotic favipiravir (Furuta Y, Takahashi K, Shirakib K, et al. T-705 (favipiravir) and related compounds: Novel broad-spectrum inhibitors of RNA viral infections. Antiviral Res. **82**, 95-102, 2009), or telaprevir (Zeuzem S., Andreone P., Pol S., et al. Telaprevir for Retreatment of HCV Infection. New Engl. J. Med. **25**, 2417-28, 2011).



bortezomib



favipiravir



telaprevir

A PZA fragment is also contained in a large number of drugs which are in the clinical trial phase, for example pyrazine derivatives patented as kinase inhibitors (Charrier J.D., Durrant S.J., Kay D., et al. Compounds useful as inhibitors of ATR kinase; US2014113005 (2014);

Song Y., Xu Q., Jia Z., et al. Preparation of pyrazine derivatives for use as Syk kinase activity inhibitors. US20130131040 (2013); Boyle R.G., Boyce R.J. Preparation of 1-(substituted phenyl)-3-(5-cyano-pyrazin-2-yl) urea compounds as Chk-1 kinase inhibitors. WO2013072502 (2013); Collins I., Lainchbury M., Matthews T.P., Reader J.C. Preparation of 5-(pyridin-2-ylamino)pyrazine-2-carbonitrile compounds as CHK1 inhibitors. WO2013068755 (2013); Collins I., Lainchbury M., Matthews T.P., Reader J.C. 5-(pyridine-2-yl-aminopyrazine-2-carbonitrile compounds and their therapeutic use. AU2012335409 (2014)).

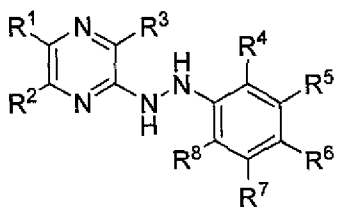
Over the past decades, methods of microwave synthesis methods and their applications suitable for the development of new drugs have got the forefront of interest of pharmaceutical chemists. These reactions usually provide a higher yield, require significantly shorter reaction time, and reduce the consumption of solvents compared to conventional methods of organic synthesis. In some cases, the desired products can be obtained only by using a microwave synthesis. Advantages of these reactions can be explained by the interaction of microwaves and molecules; the possibility of reaching a temperature greater than the boiling point of the solvent at atmospheric pressure (in a sealed system) is also very advantageous (Hayes B.L. Microwave Synthesis: Chemistry at the Speed of Light; CEM Publishing: Matthews, NC, USA, 2002; De la Hoz A., Diaz-Ortiz A., Moreno A. Microwaves in organic synthesis. Thermal and non-thermal microwave effects. Chem. Soc. Rev. **34**, 164–178, 2005). During microwave heating, microwaves act directly on the molecules present in the reaction mixture and heating is hence not dependent on the thermal conductivity of the container, unlike in classical heating. The transmission of energy from the microwave heating to the substance proceeds by two basic mechanisms, either by dipole rotation, or by ion conductivity. Dipole rotation is an interaction of the spin at which polar molecules attempt to align with rapidly changing electric field of the microwaves. This rotational motion of molecules results in energy transfer. The second way is to transmit energy through the ion conduction. If free ions or ionic groups are present in the reaction mixture, these will move in the solution under the influence of an electric field, which leads to energy expenditure due to the increased extent of precipitation and a conversion of kinetic energy into heat occurs. The temperature of the substance also affects the ionic conductivity - as the temperature rises, energy transfer becomes more efficient (Hayes B.L. Microwave Synthesis: Chemistry at the Speed of Light; CEM Publishing: Matthews, NC, USA, 2002; Lidström P., Tierney J., Wathey B., Westman J.

Microwave assisted organic synthesis - a review. Tetrahedron. **57**, 9225-9283, 2001). Also the choice of solvents for microwave-assisted synthesis can be critical to the outcome of the reaction. The important characteristics of used solvents include their polarity, the rule for which is that the more polar solvent, the higher the ability to interact with microwaves will be. Even solvents having a low boiling point can be used in this synthesis because the microwave energy reaches and exceeds the boiling point of most of solvents in a few seconds. Using of pressurized reaction vessels further ensures recovery of solvents with a low boiling point. Another important factor in selecting the solvent is how efficiently the solvent molecules interact with microwaves and convert the microwave energy into thermal energy. The microwave synthesis can be applied even in systems without using any solvent. There are three main types of reactions without solvent: reaction mixtures adsorbed on mineral oxides, reactions of a phase transfer catalyst and a clean reactions (Hayes B.L. Microwave Synthesis: Chemistry at the Speed of Light; CEM Publishing: Matthews, NC, USA, 2002).

The compound 5-chloro-6-methyl-pyrazine-2,3-dicarbonitrile (II) was used in the past for the preparation of a series of substituted 5-benzylamino-6-methylpyrazine-2,3-dicarbonitriles; the obtained products did not show significant biological properties in the tests (Jandourek, O.; Dolezal, M.; Paterova, P.; Kubicek, V.; Pesko, M.; Kunes, J.; Coffey, A.; Guo, J.; Kralova, K. Molecules **19**, 651-671,2014). Also, the compound 3-chloropyrazine-2-carboxamide (III) was used in the past for the preparation of a series of substituted 3-(alkylamino)pyrazine-2-carboxamides; the products obtained did not show significant biological properties in the assays (Jandourek, O.; Dolezal, M.; Kunes, J.; Kubicek, V.; Paterova, P.; Pesko, M.; Buchta, V.; Kralova, K.; Zitko, J. Molecules **19**, 9318-9338, 2014).

Disclosure of the invention

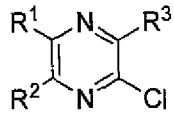
The invention relates to novel substituted 2-(2-phenylhydrazinyl)pyrazine of the general formula I



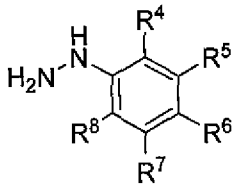
I

wherein each R^1, R^2 is independently H or CN; R^3 is CH_3 or $CONH_2$; each R^4, R^5, R^6, R^7, R^8 is independently H, Cl, or NO_2.

Another object of the invention is a method for preparing of a substituted 2-(2-phenylhydrazinyl)pyrazine of general formula I, consisting in that the substituted chloropyrazine of general formula



wherein each R^1, R^2 is independently H or CN; R^3 is CH_3 or $CONH_2$, is reacted with a substituted phenylhydrazine of general formula



wherein each R^4, R^5, R^6, R^7, R^8 is independently H, Cl, or NO_2, in a polar solvent, under the conditions of microwave synthesis to form a substituted phenylhydrazinylpyrazine of general formula I.

From the above physical conditions of microwave synthesis, we have chosen, as the best, the following reaction conditions: polar solvent: methanol, time 30 min., temperature 140 °C, pressure 15 kPa and an output of 120 W.

Another object of the invention relates to the use of the above mentioned substituted 2-(2-fenylhydrazinyl)pyrazine of the formula I according to the invention for use as an antituberculotic against *Mycobacterium tuberculosis* and against its atypical strains (mycobacteriosis pathogens), including pathogenic strains isolated from the sick patients.

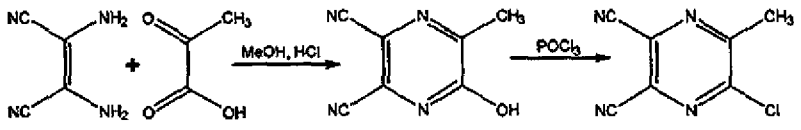
All compounds of the general formula I contain, in their molecule, the hydrazino group, but their toxicity in the tests carried out is very low. These substances are exceptional with by their low toxicity since the hydrazine group in the molecule of a drug usually bears higher toxicity or irritation. Based on current knowledge of pharmaceutical chemistry such

substitution in the field of synthesis of new promising drugs previously has always been considered undesirable.

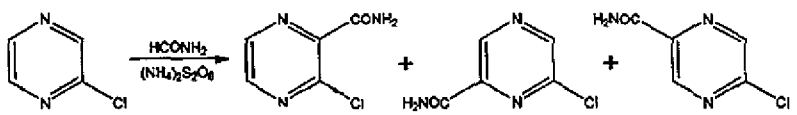
An indispensable role for the effect is also played by theselected a six-membered heterocyclic pyrazine ring, or the pyrazinecarboxamide or pyrazinecarbonitrile fragment, because such moiety represents the aza analog of nicotinamide, which plays a crucial role in the metabolism of mycobacteria.

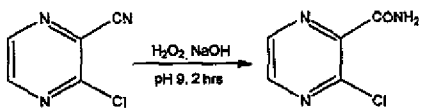
The essence of the invention consists is a combination of the six-membered heterocycle pyrazine and an aromatic part, linked each other with a linking hydrazine bridge. The heteroaromatic ring is substituted with a carboxamide or carbonitrile moieties, while the aromatic ring is unsubstituted, or substituted with nitro groups or chlorine atoms in various positions.

The starting compounds II and III are accessible by conventional methods of organic synthesis (Takematsu T., Segawa H., Miura, T. et al. A. 2,3-Dicyanopyrazines. USP4259489, 1981; Dlabal K., Palat K., Lycka A., Odlerova Z. Synthesis and ^{1}H- and ^{13}C-NMR spectra of sulfur derivatives of pyrazine derived from amidation product of 2-chloropyrazine and 6-chloro-2-pyrazinecarbonitrile. Tuberculostatic activity. Collect. Czechoslov. Chem. Commun. **55**, 2493–2500, 1990).



II

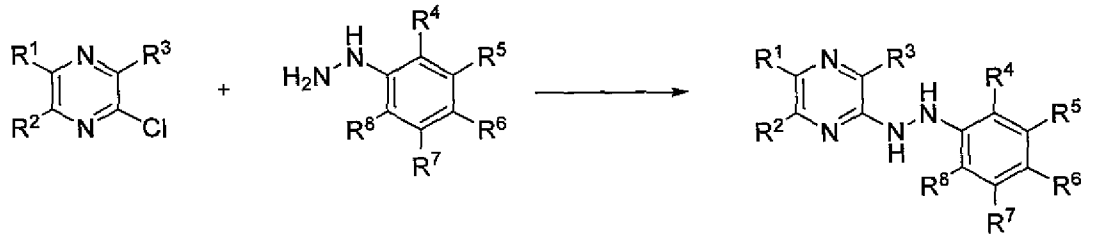




III

The final products of general formula I were in turn obtained by amino(hydrazino)dehalogenation of the corresponding substituted chloropyrazine by the reaction with an appropriately substituted phenylhydrazine (Scheme 1), wherein R^1 a R^2 is H

(hydrogen atom) or -CN (carbonitrile group), R^3 is $-CH_3$ (methyl) or $-CONH_2$ (carboxamide) group and R^4, R^5, R^6, R^7, R^8 are H (hydrogen atom), Cl (chlorine) or $-NO_2$ (nitro moiety), under the conditions of microwave synthesis. Their preparation is not synthetically difficult, and the raw material from which they are prepared is readily accessible and inexpensive.



Scheme 1

The synthesis of the products of general formula I was carried out in the focused microwave field apparatus CEM Discover (CEM Corporation, Matthews, NC, USA). Using of the technology of microwaves, which is a part of the electromagnetic waves with wavelengths of 1 cm to 1 m (mainly used frequency of 2450 MHz), greatly facilitates and accelerates all reactions. In fact, microwaves easily and quickly change the polarity of the electromagnetic field used. The resulting oscillation act on the polar compound and, by oscillating the reaction material, give it energy. This might lead to the erosion of bonds and increasing of the kinetic energy of the molecules. This in fact increases the temperature and it is just the thermic effect which is of significant benefit of the methodology used (Hayes B.L. Microwave Synthesis: Chemistry at the Speed of Light; CEM Publishing: Matthews, NC, USA, 2002; De la Hoz A., Diaz-Ortiz A., Moreno A. Microwaves in organic synthesis. Thermal and non-thermal microwave effects. Chem. Soc. Rev. **34**, 164–178, 2005). The apparatus used is equipped with a so-called focused field, which is advantageous since the microwaves are aimed directly to the reaction mixture placed in a waveguide. Another positive aspect of the method used is also time, energy and solvents saving.

The prepared compounds of general formula I have been evaluated *in vitro* against the strains of *M. kansasii* 235/80, *M. avium* 80/72, *M. avium* 152/73 and *M. tuberculosis* H37Rv using a fluid Sula's semisynthetic medium (Trios, Prague, Czech Republic) by the microdilution method in comparison with pyrazinamide (PZA) at pH 5.6. The tested compounds were dissolved in dimethylsulfoxide (DMSO) and diluted with the medium to final concentrations

of 100, 50, 25, 12,5, 6,25, 3,125 and 1,563 μg/mL. The results were read after two or three weeks. Pyrazinamide (PZA) was used as the standard. The results are shown in Table 1. Results indicate high antimycobacterial activity, for all tested compounds of formula I it was 2-16x higher than that of the used standard PZA. High activity was discovered against *M. kansasii* for compounds **1-5** of general formula I and against *M. avium* in the case of compounds **2-4** of general formula I.

Table 1 Antimycobacterial activity of compounds of general formula I

Strain	**Compound Evaluated (code)** MIC ($\mu g.L^{-1}$)										
	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	PZA
M. tuberculosis H37Rv	**6.25**	**12.5**	**12.5**	**1.56**	**12.5**	**12.5**	**6.25**	**12.5**	**12.5**	**12.5**	25
M. kansasii My 235/80	**1.56**	**12.5**	**12.5**	**12.5**	**12.5**	>100	>100	>100	>100	>100	>100
M. avium 80/72	>100	**50**	**50**	**50**	**100**	>100	>100	>100	>100	>100	>100
M. avium 152/73	>100	**25**	**12.5**	**12.5**	**100**	>100	>100	>100	>100	>100	>100

All compounds corresponding to general formula I were also tested for their antifungal activity *in vitro* using the microdilution broth assay.

Medium used: RPMI 1640 with glutamine

Incubation duration: 24 – 48 hrs. (for TM: 48 – 72 hrs.)

Incubation method: static, in the dark, humid atmosphere

Reading: visual / photometric (OD 540 nm); MIC = IC_{80}, for filamentous fungi: IC_{50} (inhibition of the control)

pH / buffer: 7.0 / MOPS (0,165 M)

Temperature: 35 °C

Strains evaluated (code, number):

CA – *Candida albicans* ATCC 44859

CT – *Candida tropicalis* 156

CK – *Candida krusei* E28

CG – *Candida glabrata* 20/I

TA – *Trichosporon asahii* 1188

AF – *Aspergillus fumigatus* 231

AC – *Absidia corymbifera* 272

TM - *Trichophyton mentagrophytes* 445

Standards: FLZ – Fluconazole, AMB – Amphotericin B

The results are shown in Table 2. The results show antifungal activity in virtually most of the tested compounds of general formula I. High antifungal activity was discovered of for the compounds **1**, **2** and **4** of general formula I against yeasts and filamentous fungi.

Tab. 2 Antimycotic activity of compounds with general formula I

strain (code)		Compound Evaluated (code) MIC/IC_{80} /IC_{50} (μmol.L^{-1})											
		1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**FLZ**	**AMB**
CA	24h	**15.62**	**125**	>500	**125**	>500	>500	**500**	**500**	>250	>125	0.25	0.016
	48h	**31.25**	**500**	>500	**250**	>500	>500	>500	>500	>250	>125	0.5	0.063
CT	24h	**62.5**	**250**	>500	**250**	>500	>500	>500	>500	>250	>125	0.5	0.063
	48h	**250**	>500	>500	**500**	>500	>500	>500	>500	>250	>125	>128	0.063
CK	24h	**3.9**	**0.49**	**7.81**	**31.25**	>500	>500	**500**	**500**	>250	>125	16	0.125
	48h	**7.81**	**3.9**	**15.62**	**250**	>500	>500	>500	>500	>250	>125	32	0.125
CG	24h	**62.5**	**250**	>500	**15.62**	>500	>500	**500**	>500	>250	>125	4	0.031
	48h	**62.5**	**250**	>500	**31.25**	>500	>500	>500	>500	>250	>125	16	0.125
TA	24h	**62.5**	**250**	>500	**15.62**	>500	**500**	**500**	>500	>250	>125	0.25	1
	48h	**125**	**250**	>500	**62.5**	>500	**500**	>500	>500	>250	>125	0.5	2
AF	24h	**31.25**	**15.62**	**62.5**	**3.9**	>500	>500	**500**	**250**	>250	>125	>128	0.25
	48h	**62.5**	**250**	>500	**15.62**	>500	>500	>500	>500	>250	>125	>128	0.125
AC	24h	**31,25**	**15,62**	**125**	**7,81**	>500	**500**	**500**	**125**	>250	>125	>128	1
	48h	**31.25**	**15.62**	>500	**15.62**	>500	>500	**500**	**250**	>250	>125	>128	2
TM	72h	**500**	**0.49**	**1.65**	**0.49**	**250**	**500**	**500**	**500**	>250	>125	8	1
	120h	**500**	**7.81**	**7.81**	**3.9**	**250**	**500**	**500**	**500**	>250	>125	16	1

The prepared derivatives corresponding to general formula I were tested for their antibacterial activity *in vitro*, using the microdilution broth test.

Medium used: Müeller Hinton broth

Incubation duration: 24 – 48 hrs.

Incubation method: static, in the dark, humid atmosphere

Reading: visual / photometric (OD 540 nm); MIC = IC_{80} (80 % of inhibition of the control)

Temperature: 35 °C

Strains evaluated (code, number):

SA – *Staphylococcus aureus* CCM 4516/08

MRSA - *Staphylococcus aureus* (methicilin resistant) H 5996/08

SE – *Staphylococcus epidermis* H 6966/08

EF – *Enterococcus sp.* J 14365/08

EC – *Escherichia coli* CCM4517

KP – *Klebsiella pneumoniae* D 11750/08

KP-E - *Klebsiella pneumoniae* (ESBL positive) J 14368/08

PA – *Pseudomonas aeruginosa* CCM 1961

Standards: NEOM – Neomycin sulphate, BAC – bacitracin, PEN – penicillin G, CIPR – ciprofloxacin, PHEN – phenoxymethylpenicillin.

The results are shown in Table 3. The results show that selective antibacterial activity was discovered for virtually most of the compounds of general formula I tested against staphylococci and enterococci.

Tab. 3 Antibacterial activity of compounds with general formula I

Strain (code)		Compound Evaluated (code) MIC/IC_{80} (μmol.L^{-1})														
		1	2	3	4	5	6	7	8	9	10	NEOM	BA	PEN	CIPR	PHEN
SA	24h	62.5	15.62	62.5	7.81	62.5	>500	500	500	500	>250	3.9	15.3	0.24	0.98	0.24
	48h	62.5	62.5	125	15.62	250	>500	500	500	500	>250	3.9	31.2	0.24	0.98	0.24
MRSA	24h	62.5	7.81	125	15.62	62.5	>500	500	500	500	>250	0.98	15.3	125	500	250
	48h	62.5	62.5	125	15.62	250	>500	>500	>500	500	>250	0.98	31.2	125	500	500
SE	24h	15.62	31.25	62.5	7.81	31.25	500	31.25	62.5	62.5	31.25	3.9	15.6	31.25	250	62.5
	48h	15.62	62.5	62.5	15.62	31.25	500	31.25	62.5	62.5	31.25	7.81	31.2	125	250	250
EF	24h	250	250	125	500	500	500	250	62.5	250	62.5	250	31.2	7.81	0.98	7.81
	48h	250	>500	125	500	500	>500	250	62.5	500	62.5	250	31.2	15.62	0.98	7.81
EC	24h	>500	>500	>500	>500	>500	>500	>500	>500	>500	>250	0.98	>500	125	0.06	>500
	48h	>500	>500	>500	>500	>500	>500	>500	>500	>500	>250	0.98	>500	125	0.06	>500
KP	24h	>500	>500	>500	>500	>500	>500	>500	>500	>500	>250	0.98	>500	250	0.12	>500
	48h	>500	>500	>500	>500	>500	>500	>500	>500	>500	>250	0.98	>500	500	0.12	>500
KP-E	24h	>500	>500	>500	>500	>500	>500	>500	>500	>500	>250	0.98	>500	>500	>500	>500
	48h	>500	>500	>500	>500	>500	>500	>500	>500	>500	>250	0.98	>500	>500	>500	>500
PA	72h	>500	>500	>500	500	>500	>500	>500	>500	>500	>250	7.81	>500	>500	3.9	>500
	120h	>500	>500	>500	500	>500	>500	>500	>500	>500	>250	15.62	>500	>500	7.81	>500

The prepared derivatives corresponding to the general formula I were also tested for antiviral activity at Rega Institute for Medical Research, Katholieke Universiteit, Leuven, Belgium (Prof. Dr. Lieve Naesens and co-workers). The viral infections against which efficacy was evaluated included: Influenza virus A (H1N1; H3N2) and B, Herpes simplex virus-1 and -2, Vesicular stomatitis virus, Vaccinia virus, Parainfluenza-3 virus, Reovirus-1, Sindbisvirus, Coxsackie virus B4, Punta Toro virus, Respiratory syncytial virus, Feline corona virus, Feline herpes virus. Zanamivir, ribavirin, amantadin, rimantadin, ribavirin, and ganciclovir, inter alia, were used as standards. The results are shown in Table 4, wherein the given EC_{50} values [μM] is the effective concentration at which a 50% inhibition of the studied virus induced cytopathic effect occurs. Only for compounds **5** and **6** corresponding to the general formula I antiviral effects have been discovered.

Tab. 4 Antiviral activity of compounds with general formula I

Compd. Code / Standard	EC_{50} [μM]			
	Influenza A/H1N1		Vesicular stomatitis virus	Feline herpes virus
	visual CPE score	MTS		
5	**4.0**	**2.4**	**8.9 / 8.9**	**5.0 / 9,2**
6	-	-	-	**4.0 / 8.2**
Zanamivir	4.0	1.5	-	-
Ribavirin	12	10	-	-
Amantadin	100	201	-	-
Rimantadin	20	7.3	-	-
Ribavirin	-	-	**25.0 / 112.0**	-
Ganciclovir	-	-		**0.9 / 3.8**

The most active compounds of general formula I were subjected to basic toxicity MTT tests on human hepatocytes, or on a CRFK cell culture (Crandel-Ress feline cells). The results are shown in Tables 5 and 6. The results show that even at the highest concentration used (20 μM) of the evaluated potential antitubercular compounds corresponding to the general formula I hepatocyte viability is not affected. Standard methodology was used (Owen T.C. USP5185450, 1993; Crandell R.A., Fabricant C.G., Nelson-Rees W.A. In Vitro **9**, 176-185, 1973).

Table 5 Cytotoxicity of selected compounds of general formula I evaluated on human hepatocytes Hep G2 (data are sorted from highest to lowest toxicity)

Compound	Cytotoxicity IC_{50} (μM)
3	21.9
5	27.8
7	481.4
6	863.7

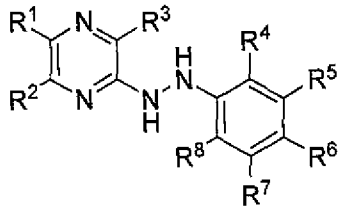
Table 6 Cytotoxicity of selected compounds of general formula I evaluated on CRFK cell culture (cell line derived from kidney cells of domestic cats)

Compound	Cytotoxicity CC_{50} (μM)
1	37
3	54
4	33
5	48

6	24
10	>100

Examples

The following examples illustrate the process of the present invention relating to the preparation and use of substituted 2-(2-fenylhydrazinyl)pyrazines of general formula I



I

wherein the symbols R^1, R^2, R^3, R^4, R^5, R^6, R^7, and R^8 are as defined above.

Thin Layer Chromatography (TLC) was used for monitoring and controlling thesynthetic reactions and for verifying the purity of the products. Hexane and ethyl acetate (1:1) were used as mobile phases. The reaction progress was checked with TLC plates (Merck, Darmstadt, Germany), silica gel 60 F_{254}, using 254 nm wavelenght UV detection.

For chemicals used for the following reactions the basic physical constants have been validated. The solvents used for the preparation of starting compounds II and III (acetone, toluene, and chloroform), were thoroughly purified and dried. A conventional, commercially available, anhydrous methanol was used for the preparation of the final synthesized products.

Newly synthesized products were separated and purified by using of preparative chromatograph CombiFlash® Rf (Teledyne Isco, Inc. Lincoln, Nebraska, USA). Melting point confirmation of pure product was determined with open capillary apparatus Stuart Scientific, SMP30 (Bibby Scientific Ltd., Staffordshire, UK) and was uncorrected.

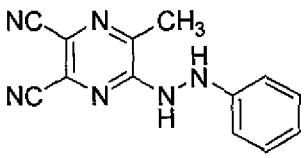
Identity of compounds prepared was recorded and checked by means of ^{1}H-NMR and ^{13}C-NMR spectra on Varian Mercury VX-BB 300 and Varian VNMR S500 spectrometers (Varian Corp., Palo Alto, CA, USA). The spectra were recorded in deuterated $CDCl_3$ or DMSO at the correct room temperature and the frequency of 300 MHz for ^{1}H and 75 MHz for ^{13}C; and 500 MHz for ^{1}H and 125 MHz for ^{13}C, respectively. Chemical shifts were reported in ppm (δ) in ppm units and were applied indirectly to tetramethylsilane as a signal of solvent (2.49 for ^{1}H and 39.7 for ^{13}C in DMSO-*d*6).

Infrared spectra were recorded in deuterated $CDCl_3$ or DMSO with spectrometer FT-IR Nicolet 6700 (Thermo Scientific, Waltham, MA, USA) using attenuated total reflectance (ATR) methodology.

Elemental analyses were measured with an EA 1110 CHNS Analyzer (Fisons Instruments S. p. A., Carlo Erba, Milano, Italy). All measured values are given in percent.

Experimental lipophilicity parameter log *k* was ascertained using an Agilent Technologies 1200 SL HPLC system with a SL G1315C Diode-Array Detector, ZORBAX XDB-C18 5 μm, 4 × 4 mm, Part No. 7995118-504 chromatographic pre-column and ZORBAX Eclipse XDB-C18 5 μm, 4.6 × 250 mm, Part No. 7995118-585 column (Agilent Technologies Inc., Colorado Springs, CO, USA). The separation process was controlled by Agilent ChemStation, version B.04.02 extended by spectral module (Agilent Technologies Inc.). A solution of MeOH (HPLC grade, 70%) and H_2O (HPLC-Milli-Q Grade, 30%) was used as mobile phase. The total flow of the column was 1.0 mL/min, injection 20 μL, column temperature 30 °C. Detection wavelength λ= 210 nm and monitor wavelength λ= 270 nm were chosen for this measurement. The KI methanol solution was used for the dead time (TD) determination. Retention times (TR) of synthesized compounds were measured in minutes. The capacity factors *k* were calculated using Microsoft Excel according to the formula $k = (TR - TD)/TD$, where TR is the retention time of the solute and TD denotes the dead time obtained *via* an unretained analyte. Log *k*, calculated from the capacity factor *k*, is used as the lipophilicity index converted to log *P* scale. Values of log *P* and Clog *P* were calculated with the PC programme CS ChemBioDraw Ultra 13.0 (CambridgeSoft, Cambridge, MA, USA).

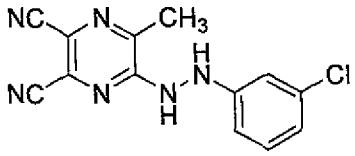
Example 1: 5-Methyl-6-(2-phenylhydrazinyl)pyrazine-2,3-dicarbonitrile (**1**)



Compound **1** is prepared by reaction of phenylhydrazine (3 mmol) with 5-chloro-6-methylpyrazine-2,3-dicarbonitrile (II, 1 mmol) in 3 *mL* of methanol and pyridine (1 mmol). The reaction is performed in a microwave reactor at the temperature 140°C, pressure 15 kPa and an output of 120 W during 30 min. After completing the reaction, product **1** was isolated and purified by column chromatography on silica gel (mobile phase: hexane / ethyl acetate 1:1), yield 29%. Analytical data for compound **1**: Brown-red crystalline solid; Mp. = 158.1-159.7°C; Elemental analysis calculated for $C_{13}H_{10}N_6$ (m.w. 250.26): 62.39 % C, 4.03 % H, 33.58 % N; found 62.49 % C, 4.30 % H, 33.31 % N; IR (ATR-Ge, cm^{-1}): 3398 (-NH-), 3297 (-NH-), 2238 (-CN), 1603, 1554, 1485, 1445, 1393 (pyr); ^{1}H-NMR (300 MHz, $CDCl_3$) δ 10.03

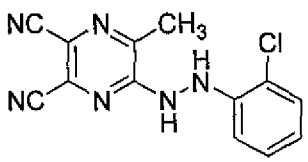
(1H, bs, NH), 8.16 (1H, bs, NH), 7.21-7.06 (2H, m, H2′, H6′), 6.84-6.65 (3H, m, H3′, H4′, H5′), 2.50 (3H, s, CH_3); ^{13}C NMR (75 MHz, DMSO) δ 153.8, 148.2, 146.7, 130.1, 129.1, 119.3, 119.0, 115.5, 114.8, 112.6, 21.2; Lipophilicity: calc. value log *P* = 2.07; experimental determined value log *k* = 0.2218.

Example 2: 5-(2-(3-chlorophenyl)hydrazinyl)-6-methylpyrazine-2,3-dicarbonitrile (**2**)



Compound **2** is prepared by reaction of 3-chlorophenylhydrazine (3 mmol) with 5-chloro-6-methylpyrazine-2,3-dicarbonitrile (II, 1 mmol) in 3 mL of methanol and pyridine (1 mmol). The reaction is performed in a microwave reactor at the temperature 140°C, pressure 15 kPa and an output of 120 W during 30 min. After completing the reaction, product **2** was isolated and purified by column chromatography on silica gel (mobile phase: hexane / ethyl acetate 1:1), yield 65%. Analytical data for compound **2**: Green-brown crystalline solid; Mp. = 177.0-177.8°C; Elemental analysis calculated for $C_{13}H_9ClN_6$ (m.w. 284.70): 54.84% C, 3.19% H, 29.52% N; found 54.95% C, 2.95% H, 29.54% N; IR (ATR-Ge, cm^{-1}): 3314 (-NH-), 3285 (-NH-), 2231 (-CN), 1599, 1549, 1485, 1430, 1398 (pyr); ^1H-NMR (300 MHz, $CDCl_3$) δ 10.05 (1H, bs, NH), 8.46 (1H, bs, NH), 7.16 (1H, t, *J* = 8.0 Hz, H5′), 6.89-6.71 (3H, m, H2′, H4′, H6′), 2.52 (3H, s, CH_3); ^{13}C NMR (75 MHz, DMSO) δ 153.6, 149.9, 147.0, 133.9, 130.7, 130.0, 119.3, 118.8, 115.5, 114.8, 111.8, 111.2, 21.3; Lipophilicity: calc. value log *P* = 2.63; experimental determined value log *k* = 0.2499.

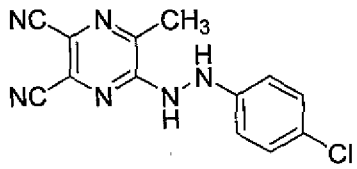
Example 3: 5-(2-(2-chlorophenyl)hydrazinyl)-6-methylpyrazine-2,3-dicarbonitrile (**3**)



Compound **3** is prepared by reaction of 2-chlorophenylhydrazine (3 mmol) with 5-chloro-6-methylpyrazine-2,3-dicarbonitrile (II, 1 mmol) in 3 mL of methanol and pyridine (1 mmol). The reaction is performed in a microwave reactor at the temperature 140°C, pressure 15 kPa and an output of 120 W during 30 min. After completing the reaction, product **3** was isolated and purified by column chromatography on silica gel (mobile phase: hexane / ethyl acetate

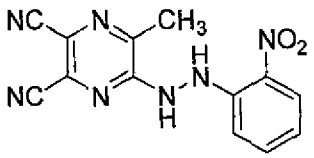
1:1), yield 67%. Analytical data for compound **3**: Green-brown crystalline solid; Mp. > 250.0°C (decomp.); Elemental analysis calculated for $C_{13}H_9ClN_6$ (m.w. 284.70): 54.84% C, 3.19% H, 29.52% N; found 54.78% C, 3.22% H, 29.34% N; IR (ATR-Ge, cm^{-1}): 3289 (-NH-), 2231 (-CN), 1594, 1564, 1488, 1442, 1398 (pyr); ^{1}H-NMR (300 MHz, $CDCl_3$) δ 10.17 (1H, bs, NH), 7.90 (1H, bs, NH), 7.34 (1H, dd, J = 7.8 Hz, J = 1.1 Hz, H3′), 7.12 (1H, t, J = 7.8 Hz, H5′), 6.88 (1H, dd, J = 7.8 Hz, J = 1.1 Hz, H6′), 6.83-6.75 (1H, m, H4′), 2.54 (3H, s, CH_3); ^{13}C NMR (75 MHz, DMSO) δ 153.6, 146.8, 143.7, 130.0, 129.5, 128.0, 120.3, 119.4, 117.7, 115.4, 114.8, 113.4, 21.2; Lipophilicity: calc. value log P = 2.63; experimental determined value log k = 0.1793.

Example 4: 5-(2-(4-chlorophenyl)hydrazinyl)-6-methylpyrazine-2,3-dicarbonitrile (**4**)



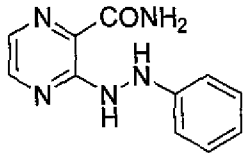
Compound **4** is prepared by reaction of 4-chlorophenylhydrazine (3 mmol) with 5-chloro-6-methylpyrazine-2,3-dicarbonitrile (II, 1 mmol) in 3 mL of methanol and pyridine (1 mmol). The reaction is performed in a microwave reactor at the temperature 140°C, pressure 15 kPa and an output of 120 W during 30 min. After completing the reaction, product **4** was isolated and purified by column chromatography on silica gel (mobile phase: hexane / ethyl acetate 1:1), yield 21%. Analytical data for compound **4**: Light-brown crystalline solid; Mp. = 152.0-152.8°C; Elemental analysis calculated for $C_{13}H_9ClN_6$ (m.w. 284.70): 54.84% C, 3.19% H, 29.52% N; found 55.11% C, 2.89% H, 29.32% N; IR (ATR-Ge, cm^{-1}): 3355 (-NH-), 3306 (-NH-), 2225 (-CN), 1598, 1557, 1491, 1437, 1397 (pyr); ^{1}H-NMR (300 MHz, $CDCl_3$) δ 10.06 (1H, bs, NH), 8.36 (1H, bs, NH), 7.22-7.13 (2H, m, AA′, BB′, H2′, H6′), 6.86-6.77 (2H, m, AA′, BB′, H3′, H5′), 2.51 (3H, s, CH_3); ^{13}C NMR (75 MHz, DMSO) δ 153.7, 147.3, 146.9, 130.0, 128.9, 122.6, 119.2, 115.5, 114.8, 114.2, 21.2; Lipophilicity: calc. value log P = 2,63; experimental determined value log k = 0.2333.

Example 5: 5-(2-(2-nitrophenyl)hydrazinyl)-6-methylpyrazine-2,3-dicarbonitrile (**5**)



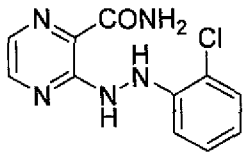
Compound **5** is prepared by reaction of 2-nitrophenylhydrazine (3 mmol) with 5-chloro-6-methylpyrazine-2,3-dicarbonitrile (II, 1 mmol) in 3 mL of methanol and pyridine (1 mmol). The reaction is performed in a microwave reactor at the temperature 140°C, pressure 15 kPa and an output of 120 W during 30 min. After completing the reaction, product **5** was isolated and purified by column chromatography on silica gel (mobile phase: hexane / ethyl acetate 1:1), yield 38%. Analytical data for compound **5**: Dark-brown crystalline solid; Mp. = 189.9-190.6°C; Elemental analysis calculated for $C_{13}H_7N_7O_2$ (m.w. 295.26): 52.88% C, 3.07% H, 33.21% N; found 52.99% C, 2.90% H, 33.21% N; IR (ATR-Ge, cm^{-1}): 3410 (-NH-), 3327 (-NH-), 2232 (-CN), 1614, 1558, 1489, 1445, 1401 (pyr), 1516, 1341 ($-NO_2$); ^{1}H-NMR (300 MHz, $CDCl_3$) δ 10.40 (1H, bs, NH), 9.58 (1H, bs, NH), 8.22-8.06 (1H, m, Ar), 7.62-7.47 (1H, m, Ar), 7.32-7.19 (1H, m, Ar), 6.98-6.84 (1H, m, Ar), 2.36 (3H, s, CH_3); ^{13}C NMR (75 MHz, DMSO) δ 154.8; 146.4; 144.6; 136.7; 132.5; 130.5; 126.1; 118.6; 117.4; 115.7; 115.3; 114.7; 21.2; Lipophilicity: calc. value log *P* = 1.15; experimental determined value log *k* = -0.0512.

Example 6: 3-(2-phenylhydrazinyl)pyrazine-2-carboxamide (**6**)



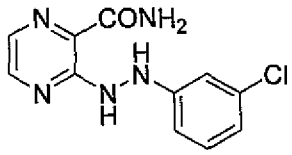
Compound **6** is prepared by reaction of phenylhydrazine (3 mmol) with 3-chloropyrazine-2-carboxamide (III, 1 mmol) in 3 mL methanol and pyridine (1 mmol). The reaction is performed in a microwave reactor at the temperature 140°C, pressure 15 kPa and an output of 120 W during 30 min. After completing the reaction, product **6** was isolated and purified by column chromatography on silica gel (mobile phase: hexane / ethyl acetate 1:1), yield 42%. Analytical data for compound **6**: Orange crystalline solid; Mp. = 161.3-162.0°C; Elemental analysis for $C_{11}H_{11}N_5O$ (m.w. 229,24): 57.63% C, 4.84% H, 30.55% N; found 57.88% C, 4.94% H, 30.52% N; IR (ATR-Ge, cm^{-1}): 3444 (-NH-), 3292 ($-CONH_2$), 1669 (-C=O), 1604, 1533, 1482, 1413 (pyr); ^{1}H-NMR (300 MHz, $CDCl_3$) δ 10.01 (1H, bs, NH), 8.30 (1H, bs, NH), 8.24 (1H, d, *J* = 2.3 Hz, H5), 7.95-7.90 (2H, H6, NH_2), 7.87 (1H, bs, NH_2), 7.16-7.04 (2H, m, H2′, H6′), 6.76-6.62 (3H, m, H3′, H4′, H5′); ^{13}C NMR (75 MHz, DMSO) δ 168.7, 155.7, 149.6, 146.7, 132.2, 129.0, 127.2, 118.7, 112.2; Lipophilicity: calc. value log *P* = -0.22; experimental determined value log *k* = -0.2382.

Example 7: 3-(2-(2-chlorophenyl)hydrazinyl)pyrazine-2-carboxamide (7)



Compound **7** is prepared by reaction of 2-chlorophenylhydrazine (3 mmol) with 3-chloropyrazine-2-carboxamide (III, 1 mmol) in 3 mL methanol and pyridine (1 mmol). The reaction is performed in a microwave reactor at the temperature 140°C, pressure 15 kPa and an output of 120 W during 30 min. After completing the reaction, product **7** was isolated and purified by column chromatography on silica gel (mobile phase: hexane / ethyl acetate 1:1), yield 17%. Analytical data for compound **7**: Brown crystalline solid; Mp. = 193.6-194.7°C; Elemental analysis calculated for $C_{11}H_{10}ClN_5O$ (m.w. 263.68): 50.10% C, 3.82% H, 26.56% N; found 50.36% C, 3.94% H, 26.68% N; IR (ATR-Ge, cm^{-1}): 3448 (-NH-), 3315 (-$CONH_2$), 1679 (-C=O), 1595, 1536, 1490, 1412 (pyr); ^{1}H-NMR (300 MHz, $CDCl_3$) δ 10.06 (1H, bs, NH), 8.31 (1H, bs, NH), 8.26 (1H, *J* = 2.4 Hz, H5), 7.96 (1H, d, *J* = 2.4 Hz, H6), 7.88 (1H, bs, NH_2), 7.67 (1H, bs, NH_2), 7.28 (1H, dd, *J* = 7.8 Hz, *J* = 1.5 Hz, H3′), 7.10-7.05 (1H, m, H5′), 6.78 (1H, dd, *J* = 7.8 Hz, *J* = 1,5 Hz, H6′), 6.72 (1H, dt, *J* = 7.8 Hz, *J* = 1.5 Hz, H4′); ^{13}C NMR (75 MHz, DMSO) δ 168.5, 155.3, 146.4, 145.1, 132.6, 129.4, 128.0, 127.5, 119.5, 117.4, 113.0; Lipophilicity: calc. value log *P* = 0,34; experimental determined value log *k* = 0.0872.

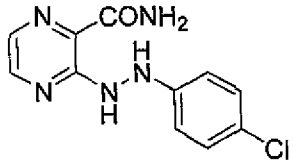
Example 8: 3-(2-(3-chlorophenyl)hydrazinyl)pyrazine-2-carboxamide (**8**)



Compound **8** is prepared by reaction of 3-chlorophenylhydrazine (3 mmol) with 3-chloropyrazine-2-carboxamide (III, 1 mmol) in 3 mL methanol and pyridine (1 mmol). The reaction is performed in a microwave reactor at the temperature 140°C, pressure 15 kPa and an output of 120 W during 30 min. After completing the reaction, product **8** was isolated and purified by column chromatography on silica gel (mobile phase: hexane / ethyl acetate 1:1), yield 24%. Analytical data for compound **8**: Dark brown crystalline solid; Mp. = 119.5-120.9°C; Elemental analysis calculated for $C_{11}H_{10}ClN_5O$ (m.w. 263.68): 50.10% C, 3.82% H,

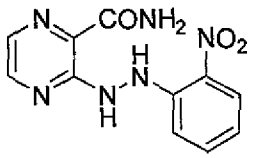
26.56% N; found 50.31% C, 3.71% H, 26,55% N; IR (ATR-Ge, cm^{-1}): 3445 (-NH-), 3253 (-$CONH_2$), 1671 (-C=O), 1598, 1522, 1476, 1413 (pyr); ^{1}H-NMR (300 MHz, $CDCl_3$) δ ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.34 (2H, bs, NH2), 7.92 (2H, bs, H5, H6), 7.63 – 6.82 (4H, m, H2′, H4′, H5′, H6′), 5.71 (2H, bs, NH); ^{13}C NMR (75 MHz, DMSO) δ 168.89, 152.20, 146.20, 140.24, 134.40, 132.36, 129.72, 126.57, 122.94, 120.26, 118.52; Lipophilicity: calc. values log P = 0.34; experimental determined values log k = 0.5898.

Example 9: 3-(2-(4-chlorophenyl)hydrazinyl)pyrazin-2-carboxamide (**9**)



Compound **9** is prepared by reaction of 4-chlorophenylhydrazine (3 mmol) with 3-chloropyrazine-2-carboxamide (III, 1 mmol) in 3 mL methanol and pyridine (1 mmol). The reaction is performed in a microwave reactor at the temperature 140 °C, pressure 15 kPa and an output of 120 W during 30 min. After completing the reaction, product **9** was isolated and purified by column chromatography on silica gel (mobile phase: hexane / ethyl acetate 1:1), yield 23%. Analytical data for compound **9**: Brown crystalline solid; Mp. = 155.3-156.2°C; Elemental analysis calculated for $C_{11}H_{10}ClN_5O$ (m.w. 263.68): 50.10% C, 3.82% H, 26.56% N; found 50.27% C, 3.72% H, 26.35% N; IR (ATR-Ge, cm^{-1}): 3453 (-NH-), 3202 (-$CONH_2$), 1686 (-C=O), 1596, 1527, 1491, 1405 (pyr); ^{1}H-NMR (300 MHz, $CDCl_3$) δ 11.40 (1H, bs, NH), 8.45 (1H, bs, NH), 8.40 (1H, d, J = 2.3 Hz, H5), 8.05 (1H, d, J = 2.3 Hz, H6), 8.01 (1H, bs, NH), 7.75-7.67 (2H, m, AA′, BB′, H2′, H6′), 7.40-7.32 (2H, m, AA′, BB′, H3′, H5′); ^{13}C NMR (75 MHz, DMSO) δ 168.9, 151.7, 145.8, 138.3, 132.9, 128.9, 127.7, 126.1, 121.4; Lipophilicity: calc. values log P = 0.34; experimental determined values log k = 0.5996.

Example 10: 3-(2-(2-nitrophenyl)hydrazinyl)pyrazine-2-carboxamide (**10**)



Compound **10** is prepared by reaction of 2-nitrophenylhydrazine (3 mmol) with 3-chloropyrazine-2-carboxamide (III, 1 mmol) in 3 mL methanol and pyridine (1 mmol). The reaction is performed in a microwave reactor at the temperature 140 °C, pressure 15 kPa and

an output of 120 W during 30 min. After completing the reaction, product **10** was isolated and purified by column chromatography on silica gel (mobile phase: hexane / ethyl acetate 1:1), yield 17%. Analytical data for compound **10**: Red-braun crystalline solid; Mp. = 237.2-238.2°C; Elemental analysis calculated for $C_{11}H_{10}N_6O_3$ (m.w. 274.24): 48.18% C, 3.68% H, 30.65% N; found 48.27% C, 3.73% H, 3.73% N; IR (ATR-Ge, cm^{-1}): 3455 (-NH-), 3279 (-$CONH_2$), 1683 (-C=O), 1615, 1575, 1498, 1439 (pyr); ^{1}H-NMR (300 MHz, $CDCl_3$) δ 10.24 (1H, bs, NH), 9.53 (1H, bs, NH), 8.35 (1H, bs, NH2), 8.29 (1H, *J* = 1.8 Hz, H5), 8.10 (1H, dd, *J* = 8.1 Hz, H3′), 8.03 (1H, d, *J* = 1.8 Hz, H6), 7.92 (1H, bs, NH2), 7.50 (1H, t, *J* = 8.1 Hz, H5′), 7.17 (1H, d, *J* = 8.1 Hz, H6′), 6.83 (1H, t, *J* = 8.1 Hz, H4′); ^{13}C NMR (75 MHz, DMSO) δ 168.3, 155.0, 146.5, 146.4, 136.7, 133.3, 131.7, 128.0, 126.0, 117.7, 115.2; Lipophilicity: calc. value log *P* = 0.35; experimental determined value log *k* = -0.3118.

Examples of pharmaceutical formulations – tablets

In the manufacture of solid dosage forms, the procedure technologies conventional in this art, a dry or wet granulation, is followed, which is well-known to a person skilled in the art. Commonly employed and recognized excipients providing the dosage form with the desired physical properties are used.

Examples for dry granulation:

Example 11 (content of the active ingredient 100 mg):

Active ingredient of the general formula I (**1** or **6**)	100.0 mg
Microcrystalline cellulose	75.0 mg
Carboxymethyl starch sodium	3.5 mg
Magnesium stearate	0.5 mg
Colloidal silica gel	0.5 mg

Example 12 (content of the active ingredient 200 mg):

Active ingredient of the general formula I (**1** nebo **6**)	200.0 mg
Microcrystalline cellulose	95.0 mg
Carboxymethyl starch sodium	7.0 mg
Magnesium stearate	1.0 mg

Colloidal silica gel	1.0 mg

Example 13 (content of the active ingredient 300 mg):

Active ingredient of the general formula I (**1** or **6**)	300.0 mg
Microcrystalline cellulose	115.0 mg
Carboxymethyl starch sodium	10.5 mg
Magnesium stearate	1.5 mg
Colloidal silica gel	1.5 mg

Example 14 (content of the active ingredient 400 mg):

Active ingredient of the general formula I (**1** or **6**)	400.0 mg
Microcrystalline cellulose	130.0 mg
Carboxymethyl starch sodium	14.5 mg
Magnesium stearate	2.0 mg
Colloidal silica gel	2.0 mg

Example 15 (content of the active ingredient 500 mg):

Active ingredient of the general formula I (**1** or **6**)	500.0 mg
Microcrystalline cellulose	140.0 mg
Carboxymethyl starch sodium	17.5 mg
Magnesium stearate	2.5 mg
Colloidal silica gel	2.5 mg

The active ingredient is mixed with the individual ingredients and the tableting blend is compressed in a tablet machine in the usual manner.

Examples of wet granulation:

Example 16 (content of the active ingredient 100 mg):

Active ingredient of the general formula I (**1** or **6**)	100.0 mg
Potato starch	48.0 mg
Lactose	27.0 mg
Povidone	3.0 mg

Carboxymethyl starch sodium	4.0 mg
Magnesium stearate	0.2 mg
Talc	1.8 mg

Example 17 (content of the active ingredient 200 mg):

Active ingredient of the general formula I (**1** or **6**)	200.0 mg
Potato starch	60.8 mg
Lactose	34.2 mg
Povidone	6.0 mg
Carboxymethyl starch sodium	8.0 mg
Magnesium stearate	0.4 mg
Talc	3.6 mg

Example 18 (content of the active ingredient 300 mg):

Active ingredient of the general formula I (**1** or **6**)	300.0 mg
Potato starch	73.6 mg
Lactose	41.4 mg
Povidone	9.0 mg
Carboxymethyl starch sodium	12.0 mg
Magnesium stearate	0.6 mg
Talc	5.4 mg

Example 19 (content of the active ingredient 400 mg):

Active ingredient of the general formula I (**1** or **6**)	400.0 mg
Potato starch	82.3 mg
Lactose	46.8 mg
Povidone	12.0 mg
Carboxymethyl starch sodium	16.0 mg
Magnesium stearate	0.8 mg
Talc	7.2 mg

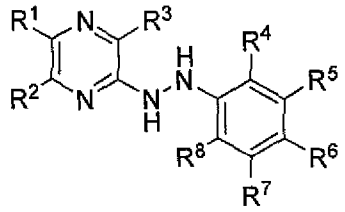
Example 16 (content of the active ingredient 500 mg):

Active ingredient of the general formula I (**1** or **6**)	500.0 mg
Potato starch	96.0 mg
Lactose	54.0 mg
Povidone	15.0 mg
Carboxymethyl starch sodium	20.0 mg
Magnesium stearate	1.0 mg
Talc	9.0 mg

The active ingredient is gradually mixed with lactose, potato starch, the mixture is granulated in an aqueous solution of povidone, the dried granulate is mixed with sodium carboxymethyl starch, magnesium stearate and talc and the resulting blend is compressed in a tablet machine in the usual way.

CLAIMS

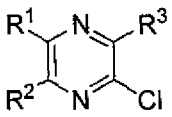
1. Substituted 2-(2-phenylhydrazinyl)pyrazines of the general formula I



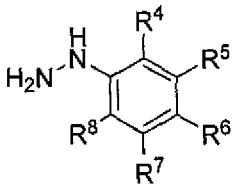
I

wherein each R^1, R^2 is independently H or CN; R^3 is CH_3 or $CONH_2$; each R^4, R^5, R^6, R^7, R^8 is independently H, Cl, or NO_2.

2. A process for preparing 2-(2-phenylhydrazinyl)pyrazines of general formula I according to claim 1, characterized in that a substituted chloropyrazine of general formula



wherein each R^1, R^2 is independently H or CN; R^3 is CH_3 or $CONH_2$, is reacted with a substituted phenylhydrazine of general structure



wherein each R^4, R^5, R^6, R^7, R^8 is independently H, Cl, or NO_2, in a polar solvent, under the conditions of microwave synthesis to form a substituted 2-(2-phenylhydrazinyl)pyrazine of general formula I.

3. The process according to claim 2, characterized in that the microwave synthesis is carried out in a polar solvent preferably in methanol, at 140°C and 15 kPa pressure and power of 120 W for 30 minutes.

4. Substituted 2-(2-phenylhydrazinyl)pyrazines of general formula I according to claim 1 for use as a medicament.

5. Substituted 2-(2-phenylhydrazinyl)pyrazines of general formula I according to claim 1 for use as an antituberculotic drug.

6. Substituted 2-(2-phenylhydrazinyl)pyrazines of general formula I according to claim 1 for use as an antibacterial drug for the treatment of mycobacteriosis caused by *M. kansasii* and/or *M. avium*.

7. Substituted 2-(2-phenylhydrazinyl)pyrazines of general formula I according to claim 1 for use as an antifungal drug for the treatment of infections caused by yeasts and filamentous fungi.

8. Substituted 2-(2-phenylhydrazinyl)pyrazines of general formula I according to claim 1 for use as an antibacterial drug for the treatment of infections caused by staphylococci and enterococci.

9. Substituted 2-(2-phenylhydrazinyl)pyrazines of general formula I according to claim 1 for use as an antiviral drug for the treatment of viral infections.

10. Use of substituted 2-(2-phenylhydrazinyl)pyrazines of general formula I according to claim 1 for the formulation of a drug for the treatment of tuberculosis.

11. Use of substituted 2-(2-phenylhydrazinyl)pyrazines of general formula I according to claim 1 for the formulation of a drug for the treatment of mycobacteriosis caused by *M. kansasií* and/or *M. avium*.

12. Use of substituted 2-(2-phenylhydrazinyl)pyrazines of general formula I according to claim 1 for the formulation of a drug for the treatment of fungal infections caused by yeasts and filamentous fungi.

13. Use of substituted 2-(2-phenylhydrazinyl)pyrazines of general formula I according to claim 1 for the formulation of a drug for the treatment of bacterial infections caused by staphylococci and enterococci.

14. Use of substituted 2-(2-phenylhydrazinyl)pyrazines of general formula I according to claim 1 for the formulation of drug for the treatment of viral infections.

INTERNATIONAL SEARCH REPORT

International application No
PCT/CZ2015/000127

A. CLASSIFICATION OF SUBJECT MATTER
INV. C07D241/04 A61K31/4965 A61P31/00
ADD.

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
C07D A61K A61P

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
EPO-Internal, CHEM ABS Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	SILVANA C NGO ET AL: "Inhibition of isolated mycobacterium tuberculosis fatty acid synthase I by pyrazinamide analogs", ANTIMICROBIAL AGENTS AND CHEMOTHERAPY, AMERICAN SOCIETY FOR MICROBIOLOGY, US , vol. 51, no. 7 1 July 2007 (2007-07-01), pages 2430-2435, XP002698058, ISSN: 0066-4804, DOI: 10.1128/AAC.01458-06 Retrieved from the Internet: URL:http://aac.asm.org/content/51/7/2430 [retrieved on 2007-05-07] p. 2433, Table 2; ----- -/--	1-14

[X] Further documents are listed in the continuation of Box C. [ ] See patent family annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

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"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
18 December 2015	24/03/2016

Name and mailing address of the ISA/	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Fax: (+31-70) 340-3016	Wolf, Claudia

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INTERNATIONAL SEARCH REPORT

International application No
PCT/CZ2015/000127

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	MAKAM PARAMESHWAR ET AL: "2-(2-Hydrazinyl)thiazole derivatives: Design, synthesis andin vitroantimycobacterial studies", EUROPEAN JOURNAL OF MEDICINAL CHEMISTRY, vol. 69, 1 July 2007 (2007-07-01), pages 564-576, XP028762830, ISSN: 0223-5234, DOI: 10.1016/J.EJMECH.2013.08.054 p. 566, Table 1; p. 568, table 2; -----	1-14

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