(12) STANDARD PATENT
(19) AUSTRALIAN PATENT OFFICE

(11) Application No. AU 2006343832 B2

(54) Title
New benzothiazinone derivatives and their use as antibacterial agents

(51) International Patent Classification(s)
C07D 417/04 (2006.01) ***A61P 31/04*** (2006.01)
A61K 31/5415 (2006.01) ***C07D 491/10*** (2006.01)

(21) Application No: **2006343832** (22) Date of Filing: **2006.05.24**

(87) WIPO No: **WO07/134625**

(43) Publication Date: **2007.11.29**
(44) Accepted Journal Date: **2011.07.21**

(71) Applicant(s)
LEIBNIZ INSTITUTE FOR NATURAL PRODUCT RESEARCH AND INFECTION BIOLOGY E.V. HANS-KNOLL-INSTITUT (HKI)

(72) Inventor(s)
Mollmann, Ute;Makarov, Vadim A.;Cole, Stewart T.

(74) Agent / Attorney
Houlihan2, Level 1 70 Doncaster Road, Balwyn North, VIC, 3104

(56) Related Art
WO 2005/092872 A1
US 3522247

(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) **World Intellectual Property Organization**
International Bureau

(43) **International Publication Date**
29 November 2007 (29.11.2007)

PCT

(10) **International Publication Number**
WO 2007/134625 A1

(51) **International Patent Classification:**
C07D 417/04 (2006.01) ***A61K 31/5415*** (2006.01)
C07D 491/10 (2006.01) ***A61P 31/04*** (2006.01)

(21) **International Application Number:** PCT/EP2006/004942

(22) **International Filing Date:** 24 May 2006 (24.05.2006)

(25) **Filing Language:** English

(26) **Publication Language:** English

(71) **Applicant** *(for all designated States except US)*: **LEIBNIZ INSTITUTE FOR NATURAL PRODUCT RESEARCH AND INFECTION BIOLOGY E.V. HANS-KNÖLL-INSTITUT (HKI)** [DE/DE]; Beutenbergstr. 11a, 07747 Jena (DE).

(72) **Inventors; and**

(75) **Inventors/Applicants** *(for US only)*: **MAKAROV, Vadim, A.** [RU/RU]; Olimpiski Prospekt 10/1/17, Moskow, 129090 (RU). **COLE, Stewart T.** [GB/FR]; 25-28 Rue du Docteur Roux, F-75724 Paris Cedex (FR). **MÖLLMANN, Ute** [DE/DE]; Schlendorfer Oberweg 17, 07749 Jena (DE).

(74) **Agent: BOCK, Gerhard**; Patent- und Rechtsanwälte Bock-Bieber-Donath Partnerschaftsgesellschaft, Hans-Knöll-Strasse 1, 07745 Jena (DE).

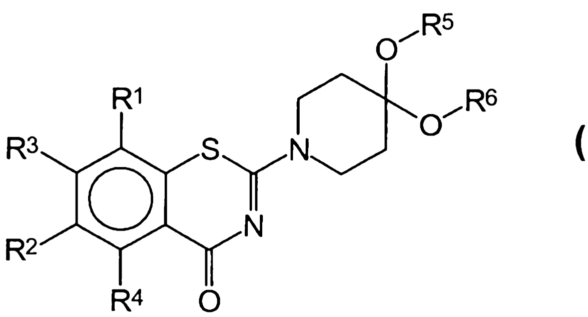
(81) **Designated States** *(unless otherwise indicated, for every kind of national protection available)*: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BW, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KM, KN, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, LY, MA, MD, MG, MK, MN, MW, MX, MZ, NA, NG, NI, NO, NZ, OM, PG, PH, PL, PT, RO, RU, SC, SD, SE, SG, SK, SL, SM, SY, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, YU, ZA, ZM, ZW.

(84) **Designated States** *(unless otherwise indicated, for every kind of regional protection available)*: ARIPO (BW, GH, GM, KE, LS, MW, MZ, NA, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European (AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HU, IE, IS, IT, LT, LU, LV, MC, NL, PL, PT, RO, SE, SI, SK, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

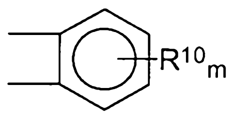
Published:
— *with international search report*

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

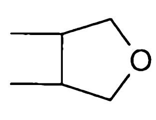
(54) **Title:** NEW BENZOTHIAZINONE DERIVATIVES AND THEIR USE AS ANTIBACTERIAL AGENTS



(I)



(II)



(III)

(57) **Abstract:** Novel benzothiazin derivatives of formula (I) and their use as antibacterial agents in infectious diseases caused by bacteria, especially tuberculosis (TB) and leprosy caused by mycobacteria, wherein R^1 and R^2 are, independently each from other, NO_2, CN, $CONR^7R^8$, $COOR^9$, CHO, halogen, NR^7R^8, $SO_2NR^7R^8$, SR^9, OCF_3, mono-, di or trifluoromethyl; R^3 and R^4 are, independently each from other, H, a saturated or unsaturated, linear or branched aliphatic radical having 1-7 chain members, cycloalkyl having 3-6 carbon atoms, benzyl, SR^9, OR^9; R^5 and R^6 are, independently each from other, a saturated or unsaturated, halogenated or unhalogenated, linear or branched aliphatic radical having 1-8 chain members, cycloalkyl having 3-6 carbon atoms, phenyl, or R^5 and R^6 together represent a bivalent radical $-(CR^9{}_2)m-$, or R^5 and R^6 together represent bivalent radicals: formula (II) or (III), wherein m is 1-4, or represent bivalent radicals a saturated or unsaturated mono or polyheterocycles with heteroatoms N, S, O and substituted by $(R^{10})x$, wherein x is 1-4.

WO 2007/134625 A1

2006343832 09 Dec 2010

NEW BENZOTHIAZINONE DERIVATIVES AND THEIR USE AS ANTIBACTERIAL AGENTS

Description

The present invention relates to novel benzothiazin derivatives and their use as antibacterial agents in infectious diseases of mammals (humans and animals) caused by bacteria, especially diseases like tuberculosis (TB) and leprosy caused by *mycobacteria*.

Thiazinone, their derivatives and their use as antibacterial agents, especially against *mycobacteria*, laid open for public in AR 24 25 67 Al, AU 37 04 400 Al, CA 13 22 551 Cl or EP 0 245 901 Bl for instance.

As known, there is a threatful worldwide increase in tuberculosis infections with *mycobacteria* which developed resistance against the available therapeutics (B.R.Bloom, J.L.Murray, tuberculosis: commentary on a reemergent killer. Science 257, 1992, 1055-1064). Extremely dangerous is the development of multidrug resistant (MDR) *mycobacteria*. These are *mycobacteria*, resistant at least against two of the most active tuberculosis drugs, isoniazid and rifampicin, but also against streptomycin, pyranzinamid and ethambutol. The proportion of MDR-TB in some countries is already more than 20%. Together with the increased number of TB diseases generally, worldwide it causes about 3.000.000 deaths annually.

For the treatment of such diseases, like TB or leprosy there is an urgent need for new drugs with new mechanisms of actions, especially to overcome drug resistance and to overcome the known dramatic side effects of the available drugs.

Any prior art reference or statement provided in the specification is not to be taken as an admission that such art constitutes, or is to be understood as constituting, part of the common general knowledge in New Zealand.

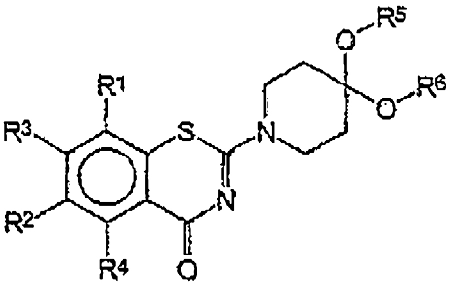
Where the terms "comprise", "comprises", "comprised" or "comprising" are used in this specification, they are to be interpreted as specifying the presence of the stated features, integers, steps or components referred to, but not to preclude the presence or addition of one or more other feature, integer, step, component or group thereof.

2006343832 09 Dec 2010

Aspects of the invention

The present invention seeks to provide new compounds with activity against mycobacteria as potential new tuberculosis drugs.

The present invention provides a compound of the formula I

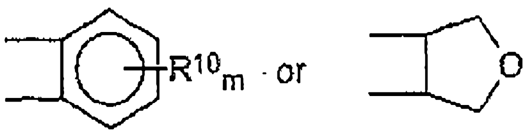


(I)

wherein R^1 and R^2 are, independently from each other, NO_2, CN, $CONR^7R^8$, $COOR^9$, CHO, halogen, NR^7R^8, $SO_2NR^7R^8$, SR^9, OCF_3, mono-, di or trifluoromethyl;

R^3 and R^4 are, independently from each other, H, a saturated or unsaturated, linear or branched aliphatic radical having 1-7 chain members, cycloalkyl having 3-6 carbon atoms, benzyl, SR^9, OR^9;

R^5 and R^6 are, independently from each other, a saturated or unsaturated, halogenated or unhalogenated, linear or branched aliphatic radical having 1-8 chain members, cycloalkyl having 3-6 carbon atoms, phenyl, or R^5 and R^6 together represent a bivalent radical $-(CR^9{}_2)_m-$, or R^5 and R^6 together represent bivalent radicals:



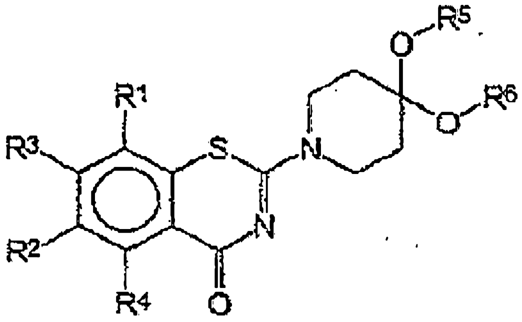
wherein m is 1-4, or represents bivalent radicals, a saturated or unsaturated, mono or polyheterocycles with heteroatoms N, S, O and substituted by $(R^{10})x$, wherein x is 1-4;

R^7, R^8 and R^9 are, independently from each other, H, a saturated or unsaturated, halogenated or unhalogenated, linear or branched aliphatic radical having 1-7 chain members, mono-, di or trifluoromethyl, halogen, phenyl, or R^3 and R^4 together represent a bivalent radical $-(CH_2)_n-$ wherein n is 2-7;

R^{10} is H or a saturated or unsaturated, halogenated or unhalogenated, linear or branched aliphatic radical having 1-7 chain members, NO_2, NR^7R^8, CN, $CONR^7R^8$, $COOR^9$, CHO, halogen, $SO_2NR^7R^8$, SR^9, OR^9, OCF_3, mono-, di or trifluoromethyl, benzyl or phenyl.

The present invention also provides a compound of formula (I)

2006343832 09 Dec 2010



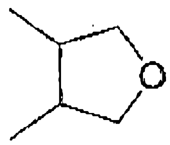
(I)

or a salt thereof,

wherein R^1 and R^2 are independently from each other NO_2, CN, trifluoromethyl, halogen, $CONR^7R^8$, $COOR^9$, CHO, $SO_2NR^7R^8$ or OCF_3;

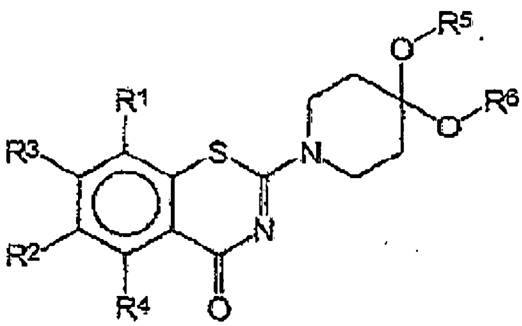
R^3 and R^4 are independently from each other H or a methyl group;

R^5 and R^6 are independently from each other a linear or branched aliphatic radical having 1-8 chain members, or R^5 and R^6 together represent a bivalent radical $-(CR^9{}_2)_m-$ wherein m is 1-4, or R^5 and R^6 together represent the bivalent radical:



R^7, R^8 and R^9 are independently H or a linear or branched aliphatic radical having 1-7 chain members, or phenyl.

The present invention also provides a process for preparation of a compound according to formula (I)

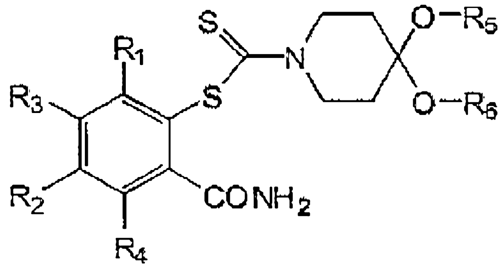


(I)

comprising the following step:

treating a compound of the following formula:

COMS ID No: ARCS-302678 Received by IP Australia: Time (H:m) 17:37 Date (Y-M-d) 2010-12-09



wherein the substituents R^1, R^2, R^5 and R^6 have the same meanings given in claim 1 and wherein R^3 and R^4 are hydrogen, with H_2O/EtOH to obtain a compound according to formula (I).

In a preferred embodiment the invention concerns compounds of the formula (I) selected from the group consisting of:

2-(4-R^5-4-R^6-piperidin-l-yl)-8-nitro-6-trifluoromethyl-l,3-benzothiazin-4-one,

6-cyano-2-(4-R^5-4-R^6-piperidin-l-yl)-8-nitro-l,3-benzothiazin-4-one,

6-amido-2-(4-R^5-4-R^6-piperidin-l-yl)-8-nitro-l,3-benzothiazin-4-one,

2-(l,4-dioxa-8-azaspiro[4.5]dec-8-yl)-8-R^1-6-R^2-l,3-benzothiazin-4-one,

2-(2-methyl-1,4-dioxa-8-azaspiro[4.5]dec-8-y1)-8-R^1-6-R^2-1,3- benzothiazin-4-one,

2-[(2*R*)-2-methyl-l,4-dioxa-8-azaspiro[4.5]dec-8-yl]-8-R^1-6-R^2-l,3-benzothiazin-4-one,

2-[(2*S*)-2-methyl-l,4-dioxa-8-azaspiro[4.5]dec-8-yl]-8-R^1-6-R^2-l,3-benzothiazin-4-one,

2-(2,3-dimethyl-l,4-dioxa-8-azaspiro[4.5]dec-8-yl)-8-R^1-6-R^2-l,3-benzothiazin-4-one,

2-(l,5-dioxa-9-azaspiro[5.5]undec-9-yl)-8-$R^1$6-R^2-1,3-benzothiazin-4-one,

wherein R^1, R^2, R^5 and R^6 have the above meanings,

The present invention is even more particularly concerned with at least one compound selected from the group consisting of 2-(l,4-dioxa-8-azaspiro[4.5]dec-8-yl)-6,8-dinitro-l,3-benzothiazin-4-one, 2-(2-methyl-1,4-dioxa-8-azaspiro[4.5]dec-8-yl)-6,8-dinitro-1,3-benzothiazin-4-one,

2-(4,4-diethoxypiperidin-1-yl)-6,8-dinitro-1,3-benzothiazin-4-one,

7-methyl-2-(2-methyl-1,4-dioxa-8-azaspiro[4.5]dec-8-yl)-6,8-dinitro-1,3-benzothiazin-4-one,

2-(2-methyl-1,4-dioxa-8-azaspiro[4.5]dec-8-yl)-8-nitro-6-(trifluoromethyl)-1,3-benzothiazin-4-one,

2-(2,3-dimethyl-1,4-dioxa-8-azaspiro[4.5]dec-8-yl)-8-nitro-6-(trifluoromethyl)-1,3-benzothiazin-4-one,

2-(1,5-dioxa-9-azaspiro[5.5]undec-9-yl)-8-nitro-6-(trifluoromethyl)-1,3-benzothiazin-4-one,

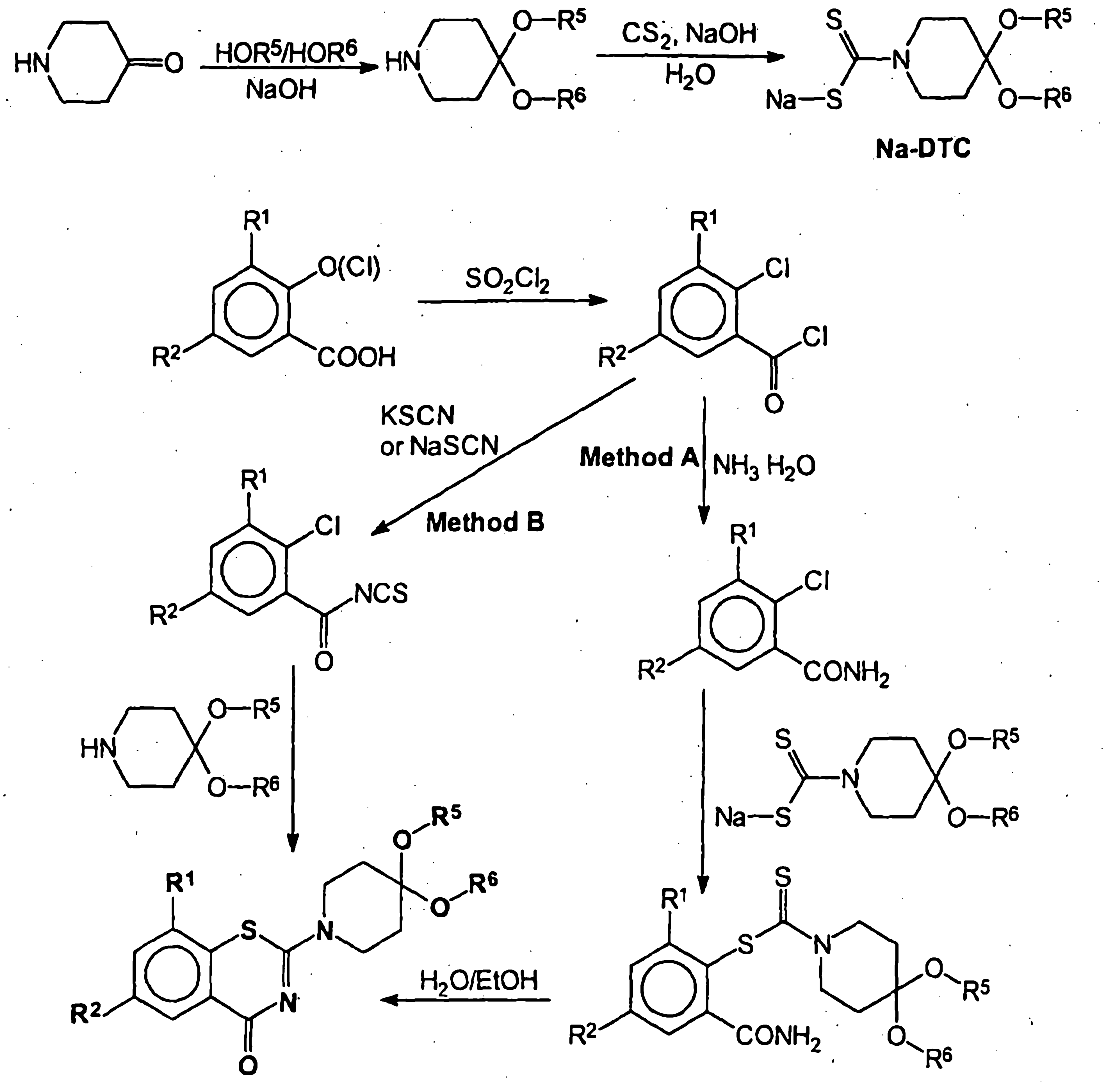
2-(1,5-dioxa-9-azaspiro[5.5]undec-9-yl)-8-nitro-4-oxo-1,3-benzothiazine-6-carbonitrile,

2-(2-methyl-1,4-dioxa-8-azaspiro[4.5]dec-8-yl)-8-nitro-4-oxo-1,3-benzothiazine-6-carbonitrile,

8-amino-2-(2-methyl-1,4-dioxa-8-azaspiro[4.5]dec-8-yl)-4-oxo-1,3-benzothiazine-6-carbonitrile and

8-amino-2-(2-methyl-1,4-dioxa-8-azaspiro[4.5]dec-8-yl)-6-(trifluoromethyl)-1,3-benzo-thiazin-4-one.

For the synthesis of the aimed compounds we developed our original method of 1,3-benzothiazin-4-one synthesis with usage of dithiocarbamate derivatives as intermediate (method A). The classical method of 1,3-benzothiazin-4-one synthesis with usage of thiocyanate salts (method B) is usable too. Both are presented in the scheme below.



Surprisingly the compounds of the invention exhibit strong antibacterial activity, especially against mycobacteria with minimal inhibitory concentrations (MIC) in the range of 0,23pg/ml->10μg/ml for fast growing mycobacteria, of 0,195-1,56 μg/ml for *M. tuberculosis*, including multiresistant strains determined by the classical method and of 0,030μg/ml for *M. tuberculosis* H37Rv determined by the Alamar Blue method. Surprisingly the compounds of the invention demonstrate a high level of selectivity for mycobacteria only which reduces the potential for adverse side effects dramatically.

The compounds of the invention are non-mutagenic at 5mg/ml in the SOS chromotest.

The compounds of the invention are *in vivo* therapeutically active in the murine model of tuberculosis infection superior compared to the main antituberculosis drug isoniazid used as a positive control. 100% of mice survived. All control animals died until day 33.

The compound of the invention (especially compound no 2 = example 1 in the embodiments), is non toxic after per os administration of doses ranging up to 2000 mg/kg was the compound was well endured by animals in the first and 24 next coming hours after introducing. During 7 days of investigations the compound 2 did not cause changes in general state and behavior of the mice, it did not affect motor and reflex activity, active and calm cycles, grooming, food consumption, there were no cases of animal death. LD_{50} for compound 2 is > 2000 mg/kg.

Thus, the compounds of the invention are useful for the treatment of tubercular infection and other mycobacterial infections, in humans and in animals.

Accordingly, the invention concerns pharmaceutical compositions comprising a compound of the formula I.
The invention relates furthermore to a compound of the formula I for use in a method for the treatment of bacterial infections in mammals. Preferred compounds of the formula I for use in such method are those specifically listed above.

The compounds of the invention are formulated for use by preparing a dilute solution or suspension in pharmaceutically acceptable aqueous, organic or aqueous-organic medium for topical or parenteral administration by intravenous, subcutaneous or intramuscular injection, or for intranasal application; or are prepared in tablet, capsule or aqueous suspension form with conventional excipients for oral administration or as suppositorium.

The compounds can be used in dosages from 0,001 – 1000 mg/kg body weight.

The examples which follow in the subsequent experimental part serve to illustrate the invention but should not be construed as a limitation thereof.

The structures of the compounds of the invention were established by modes of synthesis and elementary analysis, and by nuclear magnetic resonance and/or mass spectra, as well as by X-ray analysis.

Embodiments

Starting materials

Chemicals and solvents were purchased from Lancaster Synthesis (Lancashire, England) or from Aldrich (Sigma-Aldrich Company, St-Louis, US) and were used in the synthesis without additional purification. Melting points were determined according to the BP procedure and are uncorrected (Electrothermal 9001, GB). If analyses are indicated only by the symbols of the elements, analytical results are within ±0.3% of the theoretical values (Carlo-Erba 5500, Italy). NMR spectra were determined with a Varian Unity Plus 400 (USA). Shifts for ^{1}H NMR are reported in ppm downfield from TMS (δ). Mass spectra were obtained using a Finnigan SSQ-700 (USA) instrument with direct inject. Reactions and purity of compounds were controlled by TLC with usage Silicagel 60 F_{254} aluminium sheets (Merck Co, Germany).

Example 1

2-(1,4-dioxa-8-azaspiro[4.5]dec-8-yl)-8-nitro-6-(trifluoromethyl)-1,3-benzothiazin-4-one, (compound 1)
Method A.

To a stirred 50 mL solution of 25% aqueous ammonia was added drop-wise a solution of 5 g of 2-chloro-3-nitro-5-trifluoromethylbenzoyl chloride (D.E Welch, R.R.Baron, B.A.Burton, J. Med. Chem. 12; 2; 1969; 299-303) in acetonitrile (10 mL) at –20°C. 10 min later, 50 ml of ethyl acetate was added. The organic phase was separated, washed twice in water, dried over Na_2SO_4, treated by activated carbon, filtered and concentrated in vacum. The crude product was purified by crystallization from ethanol. The yield of 2-chloro-3-nitro-5-(trifluoromethyl)benzamide was 92%. mp 195-197°C (methanol).

Anal. Calcd. for $C_8H_4ClF_3N_2O_3$:	C, 35.78; H, 1.50; N, 10.43
Found:	C, 36.01; H, 1.53; N, 10.39

0.5 g of 2,2-chloro-3-nitro-5-(trifluoromethyl)benzamide was dissolved in a 25 ml of ethanol. The reaction mixture was treated with of 0.5 g of 1,4-dioxa-8-azaspiro[4.5]decane-8-carbodithioic acid sodium salt dihydrate (Z. Ge, R. Li, T. Cheng, Synth. Commun., 29, 18, 1999, 3191 - 3196) and stored for 18 h at room temperature. It was then poured into 50 ml of cooled water and the resulting yellow precipitate was filtered off. Pure final product was obtained after recrystallization twice from ethanol. 2-(Aminocarbonyl)-6-nitro-4-(trifluoromethyl)phenyl-1,4-dioxa-8-azaspiro[4.5]decane-8-carbodithioate is light yellow crystalline solid. Yield 0.47g %. mp 138-140°C.

Anal. Calcd. for $C_{11}H_{12}N_4O_2S_2$:	C, 42.57; H, 3.57; N, 9.31; S, 14.21
Found:	C, 42.61; H, 3.67; N, 9.22; S, 14.30

0.4 g of 2-(aminocarbonyl)-6-nitro-4-(trifluoromethyl)phenyl-1,4-dioxa-8-azaspiro[4.5]decane-8-carbodithioate was dissolved in a 25 ml of ethanol. The reaction mixture was treated with of 0.32 g of Na_2HPO_4 x $12H_2O$ and refluxed for 6 h. It was then cooled and ligth yellow precipitate was filtered off and washed by 30 ml methanol. Pure final product was obtained after recrystallization twice from ethanol. 2-(1,4-Dioxa-8-aza-spiro[4.5]dec-8-yl)-8-nitro-6-trifluoromethyl)-1,3-benzothiazin-4-one is light yellow crystalline solid. Yield 0.47g %. mp 211-212°C.
R_f ((hexane-acetone; 2/1) - 0.35
MS m/z 417 (M^+).
^1H NMR (DMSO-d_6) δ 8.83 and 8.77 (two 1H, two s, 2CH), 3.80 (8H, broad s, $N(CH_2CH_2)_2C$), 2.02 (4H, broad s, OCH_2CH_2O) ppm.

Anal. Calcd. for $C_{16}H_{14}F_3N_3O_5S$:	C, 46.04; H, 3.38; N, 10.07; S, 7.68
Found:	C, 45.94; H, 3.37; N, 10.09; S, 7.76

Method B. The procedure in detail was the same as described in J. Imrich, P. Kristian, Coll. Czech. Chem. Commun., 47, 1982, 3268-3282; D. Koscik, P. Kristian, J. Gonda, E. Dandarova, Coll. Czech. Chem. Commun., 48, 1983, 3315-3328; D. Koscik, P. Kristian, O. Forgac, Coll. Czech. Chem. Commun., 48, 1983, 3427-3432; T. H. Cronin, H. – J .E. Hess, Pat. US 3522247. Yield of 2-(1,4-dioxa-8-aza-spiro[4.5]dec-8-yl)-8-nitro-6-trifluoromethyl)-1,3-benzo-thiazin-4-one is 0.21%. The compound is identical by spectroscopical data to the compound synthesized by method A.

Example 2

2-(2-methyl-1,4-dioxa-8-azaspiro[4.5]dec-8-yl)-8-nitro-6-(trifluoro-methyl)-1,3-benzothiazin-4-one, (compound 2)

Following the procedure of Example 1. Light yellow crystalline solid. Yield 54%. mp 192-3°C.
R_f (hexane-acetone; 2/1) - 0.30.
MS m/z 431 (M^+).
^1H NMR (DMSO-d_6) δ 8.81 and 8.77 (two 1H, two s, 2CH), 4.24 (1H, m, CH), 4.11 (1H, m, CH), 4.06 (4H, broad s, $N(CH_2)_2$), 3,47 (1H, t, CH), 3.27 (1H, s, CH), 1.80 (4H, broad d, $C(CH_2)_2$), 1.23 (3H, d, CH_3) ppm.

Anal. Calcd. for $C_{17}H_{16}N_3O_5S$:	C, 47.33; H, 3.74; N, 9.74; S, 7.43
Found:	C, 47.36; H, 3.80; N, 9.87; S, 7.51

Example 3

2-(1,4-dioxa-8-azaspiro[4.5]dec-8-yl)-6,8-dinitro-1,3-benzothiazin-4-one, (compound 4)

Following the procedure of Example 1 with usage of 2-hydroxy-3,5-dinitrobenzoic acid as starting material. Light yellow crystaline solid. Yield 43%. mp 271-3°C (EtOH/DMF)..
R_f (hexane-acetone; 2/1) - 0.25.
MS m/z 394 (M^+).
^1H NMR (DMSO-d_6) δ 9.15 and 9.12 (two 1H, two s, 2CH), 3.86 (8H, broad s, $N(CH_2CH_2)_2C$), 2.97 (4H, broad s, OCH_2CH_2O) ppm.

Anal. Calcd. for $C_{15}H_{14}N_4O_7S$: C, 45.68; H, 3.58; N, 14.21; S, 8.13
Found: C, 45.34; H, 3.56; N, 14.30; S, 7.98

Example 4

2-(2-methyl-1,4-dioxa-8-azaspiro[4.5]decyl)-6,8-dinitro-1,3-benzothiazin-4-one, (compound 4)

Following the procedure of Example 1 with usage of 2-hydroxy-3,5-dinitrobenzoic acid as starting material. Yellow crystalline solid. Yield 57%. mp 139-142°C (EtOH/DMF).
R_f (hexane-acetone; 2/1) - 0.50.
MS m/z 408 (M^+).
^1H NMR (DMSO-d_6) δ 9.08 and 9.11 (two 1H, two s, 2CH), 4.23 (1H, m, CH), 4.10 (1H, m, CH), 4.06 (4H, broad s, $N(CH_2)_2$), 3,43 (1H, t, CH), 3.27 (1H, s, CH), 1.80 (4H, broad d, $C(CH_2)_2$), 1.20 (3H, d, CH_3) ppm.

Anal. Calcd. for $C_{16}H_{16}N_4O_7S$: C, 47.06; H, 3.95; N, 13.72; S, 7.85
Found: C, 46.87; H, 3.91; N, 13.57; S, 7.83

Example 5

2-(2,3-dimethyl-1,4-dioxa-8-azaspiro[4.5]dec-8-yl)-8-nitro-6-(trifluoromethyl)-1,3-benzothiazin-4-one, (compound 5)

Following the procedure of Example 1 with usage of 2-hydroxy-3-nitro-5-trifluoromethylbenzoic acid as starting material. Light yellow crystalline solid. Yield 58%. mp 205-207°C (EtOH/DMF).
R_f (hexane-acetone; 2/1) - 0.55.
MS m/z 44522 (M^+).

^{1}H NMR (DMSO-d_6) δ 8.82 and 8.77 (two 1H, two s, 2CH), 3.86 (4H, broad c, $N(CH_2)_2$), 3,45-3.53 (2H, m, 2CH), 2.41 (4H, broad d, $C(CH_2)_2$), 1.13-1.17 (6H, m, $2CH_3$) ppm.

Anal. Calcd. for $C_{18}H_{18}F_3N_3O_5S$: C, 48.54; H, 4.07; N, 9.43; S, 7.20
Found: C, 48.66; H, 4.12; N, 9.32; S, 7.46

Example 6

2-(4,4-diethoxypiperidin-1-yl)-6,8-dinitro-1,3-benzothiazin-4-one, (compound 6)

Following the procedure of Example 1 with usage as starting material 2-hydroxy-3,5-dinitrobenzoic acid. Yellow crystalline solid. Yield 32%. mp 179-181°C (*i*-PrOH).
R_f (hexane-acetone; 2/1) - 0.30.
MS m/z 424 (M^+).
^{1}H NMR (DMSO-d_6) δ 9.08 and 9.11 (two 1H, two s, 2CH), 3.60-3.67 (4H, m, $N(CH_2)_2$) 2.11-2.08 (4H, m, $C(CH_2)_2$), 3.47 and 3.57 (two 2H, q, $2OCH_2$), 1.16 (6H, t, $2CH_3$), ppm.

Anal. Calcd. for $C_{17}H_{20}N_4O_7S$: C, 48.11; H, 4.75; N, 13.20; S, 7.56
Found: C, 48.12; H, 4.73; N, 13.41; S, 7.67

Example 7

2-(7,12-dioxa-3-azaspiro[5.6]dodec-3-yl)-6,8-dinitro-1,3-benzothiazin-4-one, (compound 7)

Following the procedure of Example 1 with usage as starting material 2-hydroxy-3,5-dinitrobenzoic acid. Yellow crystalline solid. Yield 51%. mp 193-195°C (*i*-PrOH/DMF).
R_f (hexane-acetone; 2/1) - 0.45.
MS m/z 422 (M^+).
^{1}H NMR (DMSO-d_6) δ 8.97 and 9.16 (two 1H, two s, 2CH), 3.57-3.74 (8H, m, $4CH_2$), 1.93-2.35 (8H, m, $4CH_2$)ppm.

Anal. Calcd. for $C_{17}H_{18}N_4O_7S$: C, 48.34; H, 4.30; N, 13.26; S, 7.56
Found: C, 48.21; H, 4.43; N, 13.30; S, 7.66

Example 8

2-(1,4-dioxa-8-azaspiro[4.5]dec-8-yl)-7-methyl-6,8-dinitro-1,3-benzothiazin-4-one, (compound 8)

Following the procedure of Example 1 with usage as starting material 2-hydroxy-4-methyl-3,5-dinitrobenzoic acid. Yellow crystalline solid. Yield 51%. mp 207-210°C (*i*-PrOH/DMF).
R_f (hexane-acetone; 2/1) - 0.30.
MS m/z 408 (M^+).
^{1}H NMR (DMSO-d_6) δ 8.77 (1H, s, CH), 3.86 (8H, broad s, $N(CH_2CH_2)_2C$), 2.97 (4H, broad c, OCH_2CH_2O), 2.79 (3H, s, CH_3) ppm. ppm.

Anal. Calcd. for $C_{16}H_{16}N_4O_7S$: C, 47.06; H, 3.95; N, 13.72; S, 7.85
Found: C, 47.12; H, 4.01; N, 13.69; S, 7.94

Example 9

2-(1,4-dioxa-8-azaspiro[4.5]dec-8-yl)-8-nitro-4-oxo-1,3-benzothiazine-6-carbonitrile, (compound 9)

To a stirred solution of 5 g (19 mmol) 2-hydroxy-5-iodobenzoic acid in 50ml DMF was added by small portions dry 2.5 g (22 mmol) of CuCN (I). The reaction mixture was refluxed during 5 h, 100 ml of water and 50 ml ethylacetate were added. After it conc. Hydrochloric acid was added up to pH ~ 3 very carefully under good ventilation. The organic phase was separated, washed twice in water, dried over Na_2SO_4, treated by activated carbon, filtered and concentrated in vacum. The crude product was purified by crystallization from water. The yield of 5-cyano-2-hydroxybenzoic acid was 71%. Following the procedure of Example 1. Yield 44%. mp 217-220°C (EtOH/DMF).

R_f (hexane-acetone; 2/1) - 0.50.
MS m/z 374 (M^+).
^{1}H NMR (DMSO-d_6) δ 8.74 and 8.67 (two 1H, two s, 2CH), 3.41 (8H, broad s, $N(CH_2CH_2)_2C$), 2.93 (4H, broad s, OCH_2CH_2O) ppm.

Anal. Calcd. for $C_{16}H_{14}N_4O_5S$: C, 51.33; H, 3.77; N, 14.97; S, 8.57
Found: C, 51.30; H, 3.84; N, 14.89;S, 8.62

Example 10

2-(2-methyl-1,4-dioxa-8-azaspiro[4.5]dec-8-yl)-8-nitro-4-oxo-1,3-benzothiazine-6-carbonitrile, (compound 10)
Following the procedure of Example 9. Yellow crystalline solid. Yield 34%. mp 251-253°C (EtOH/DMF).
R_f (hexane-acetone; 2/1) - 0.40.
MS m/z 388 (M^+).
^{1}H NMR (DMSO-d_6) δ 8.73 and 8.61 (two 1H, two s, 2CH), 4.23 (1H, m, CH), 4.11 (1H, m, CH), 4.07 (4H, broad s, $N(CH_2)_2$), 3,51 (1H, t, CH), 3.27 (1H, s, CH), 1.81 (4H, broad d, $C(CH_2)_2$), 1.22 (3H, d, CH_3) ppm ppm.

Anal. Calcd. for $C_{17}H_{16}N_4O_5S$: C, 52.57; H, 4.15; N, 14.43; S, 8.26
Found: C, 52.42; H, 4.08; N, 14.50; S, 8.27

Example 11

2-(1,5-dioxa-9-azaspiro[5.5]undec-9-yl)-8-nitro-4-oxo-1,3-benzothiazine-6-carbonitrile, (compound 11)

Following the procedure of Example 9. Yellow crystalline solid. Yield 40%. mp 230-232°C (EtOH/DMF).
R_f (hexane-acetone; 2/1) - 0.15.

MS m/z 388 (M^+).
^1H NMR (DMSO-d_6) δ 8.74 and 8.61 (two 1H, two s, 2CH), 3.29-3.65 (6H, m, $3CH_2$), 2.38 (4H, broad s, $2CH_2$), 1.82-1.93 (4H, m, $2CH_2$) ppm.

Anal. Calcd. for $C_{17}H_{16}N_4O_5S$:	C, 52.57; H, 4.15; N, 14.43; S, 8.26
Found:	C, 52.52; H, 4.11; N, 14.59; S, 8.13

Example 12

Determination of the *in vitro* inhibitory activity of the compounds of the invention against mycobacteria.

The antibacterial activities of the compounds against *Mycobacterium smegmatis* SG 987, *M. aureum* SB66, *M. vaccae* IMET 1010670 and *M. fortuitum* B were tested by determination of minimal inhibitory concentrations (MIC) by the micro broth dilution method in Mueller-Hinton broth (Difco) according to the NCCLS guidelines [National Committee for Clinical Laboratory Standards: Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically; 5th Ed.; Villanova, Ed.; Approved standard Document M7-A5. NCCLS, (2000)]

Activity against *M. tuberculosis* H37Rv was tested by the following method for determination of minimal inhibitory concentrations (MIC) and minimal bactericidal concentrations (MBC):

Strains were inoculated onto solid Lowenstein-Jensen medium. After 21 days, the cultures grown were used to prepare an inoculum suspension corresponding to 5 x 10^8 microbial cells/ml). With 0,2 ml of that suspension tubes with 2 ml liquid Shkolnikova medium, containing corresponding concentrations of compounds under study – from 100,0 to 0,195 мg/ml, were inoculated. After 14 days of incubation at 37 °C the tubes with liquid medium were centrifuged for 15min. at 3000 RPM. After discarding the supernatant, the sediment was resuspended in 0,8 ml of sterile 0,9% NaCl. 0,1 ml of the suspension was used to prepare smears subsequently stained by the Ziehl-Neelsen method. The remaining sediment was inoculated in 0,2 ml volumes into three tubes with solid drug free Lowenstein-Jensen medium to determine minimal bactericidal concentrations (MBC). The results were read after 21-28 days of cultivation at 37 °C. Controls were tubes cultured with test-strains not treated with the studied agents.

Minimal bactericidal concentration of drugs (MBC) was considered as the drug concentration completely inhibiting the growth of mycobacteria on the solid medium. The bacteriostatic effect (MIC) was characterized by the presence of only individual mycobacteria in the smear and a strong decrease in the number of colonies grown on solid media compared to the controls.

The results are presented in Tables 1 and 2.

Table 1: Antimicrobial activity of compounds as of the formula I determined by minimal inhibitory concentrations MIC [µg/ml]

Compound.	**M. smegmatis**	**M.vaccae**	**M.fortuitum**
1	12,5 ng/ml	3,12ng/ml	12,5 ng/ml
2	1,56 ng/ml	0,76pg/ml	0,023 pg/ml
3	0,2 µg/ml	0,0015 µg/ml	0,006 µg/ml
4	0,2 µg/ml	0,003 µg/ml	0,003 µg/ml
5	6,25ng/ml	0,078ng/ml	0,078ng/ml
6	>10 µg/ml	0,04 µg/ml	0,08 µg/ml
7	0,78 µg/ml	0,003 µg/ml	0,003 µg/ml
8	0,4 µg/ml	0,025 µg/ml	0,025 µg/ml
9	0,05µg/ml	3,12 ng/ml	25 ng/ml
10	25 ng/ml	3,12 ng/ml	12,5 ng/ml
11	0,05 µg/ml	6,25 ng/ml	25 ng/ml

Table 2: Antimicrobial activity of compounds of the formula I against *Mycobacterium tuberculosis* H37Rv and clinical isolates 6341 and 6374 as determined by minimal inhibitory concentrations (MIC) and minimal bactericidal concentrations (MBC)

Strain	**Compound**	**MBC** (µg/mL)	**MIC** (µg/mL)	**MBC** (µg/mL) mean	**MIC** (µg/mL) mean
H37Rv	10	0,58	0,39	0,71	0,45
6341		0,78	0,58		
6374		0,78	0,39		
H37Rv	9	0,29	0,195	0,75	0,52
6341		1,17	0,78		
6374		0,78	0,58		
H37Rv	2	0,58	0,39	0,45	0,29
6341		0,39	0,29		
6374		0,39	0,195		
H37Rv	5	0,58	0,39	0,45	0,39
6341		0,39	<0,39		
6374		0,39	<0,39		
H37Rv	1	0,58	0,39	1,75	1,17
6341		2,34	1,56		
6374		2,34	1,56		
H37Rv	Isoniazid (INH)	1,15	0,97	1,15	0,97
6341		>100	>100	Not active, >100	
6374		>100	>100	Not active, > 100	

Example 13

Determination of the *in vivo* inhibitory activity of the compounds of the invention against *Mycobacterium tuberculosis* in the murine TB model

To determine the chemotherapeutic efficacy we used BALB/c line mice with experimental hematogenously disseminated tuberculosis. The mice were obtained from the Central Animal Nursery of the Russian Academy of Medical Sciences. In this study we included mice after quarantine, standardized by weight (20-25 g) and male only. The mice were infected with a 2-week virulent culture of *Mycobacterium tuberculosis* H37Rv by intravenous injection (into tail vein) of the mycobacterial suspension at a dose of 5×10^6 CFU (Colony Forming Unit) in 0,5 ml saline. All the experimental animals were divided into groups depending on the treatment regimen used (Table 3). Tested drug doses were selected based on the data from literature and on results of previous investigations.

Table 3:

№ group	Compound	Dose (mg/kg)	Number of animals per group
3	2	12	10
4	2	25	10
5	Isoniazid (INH)	25	10
6	without treatment		10

Treatment was started the next day after infection. The drugs were introduced orally as suspension in carboxymethylcellulose/water with a small quantity PEG-400.

Chemotherapy was administered daily 6 times per week (except Sunday).

The animals were killed with ether narcosis. To determine the efficacy of each treatment regimen we registered macroscopical changes in parenchymal organs of the mice, growth of mycobacteria from pathologic material on solid media, as well as a bacterioscopical index of organ injury. We carried out a qualitative and quantitative analysis of macroscopical changes in the liver, spleen and lungs and calculated an injury index (using a four-score scale).

Macroscopical evaluation of the efficacy of each treatment regimen was expressed in the efficacy index, calculated using a formula.

$$\text{Efficacy index} = 100\% - \frac{\text{Injury index of the studied group}}{\text{Injury index of the control group}} \times 100$$

Microbiological examination included culture for determination of CFU in parenchymal organs. For this purpose, we homogenisated the right lung and separately the spleen with 6% sulfuric acid, centrifuged, washed by water and saline. The yield (about 0,5 mL) was diluted by 1,0 mL of saline and homogenisated. This suspension (0,5 mL) of test organs was diluted 100 and 1000 times by saline and was distributed on solid Finn-2 medium. The cultures were incubated at 37°C for 1 months and read weekly starting from day 10. After 28 days CFU's were counted.

Data of macroscopical and microbiological examinations of parenchymal organs of mice which died during the experiment were also considered in the overall assessment of the experimental results which are represented in tables 4-6.

Table 4: Indexes of organ injury in mice and treatment efficacy

Group	Drug	Dose (mg/kg)	Injury index	Efficacy index (%)
3	Compound 2	12	2,1	44,7
4	Compound 2	25	1,0	78
5	INH, Isoniazid	25	1,2	70,5
6	Control	--	3,8	--

Table 5: Results of microbiological examination of right lung and spleen of experimental mice (42 days after inoculation of the culture medium)

Group	Compound	Dose (mg/kg)	right lung Culture, without dilution CFU	spleen Culture, without dilution CFU
3	2	12	~ 60	~ 60
4	2	25	~ 35	~ 35
5	INH, Isoniazid	25	~ 40	~ 40
6	Control	--	> 120 (total growth)	> 120 (total growth)

Table 6: Survival of animals

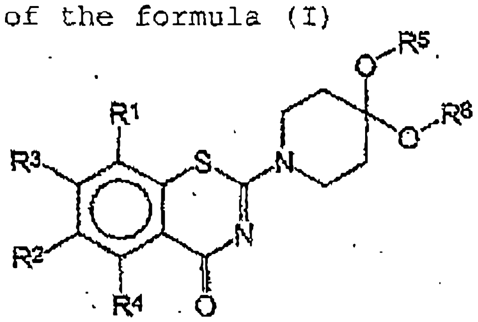
Day of Treatment	Group 3 Compound 2	Group 4 Compound 2	Group 5 INH	Group 6 Control
1	10	10	10	10
2	10	10	10	10
3	10	10	10	10
4	10	10	10	10
5	10	10	10	10
6	10	10	10	10
7	10	10	10	10
8	10	10	10	10
9	10	10	10	10
10	10	10	10	10
11	10	10	10	10
12	10	10	10	10
13	10	10	10	9
14	10	10	10	9
15	10	10	10	9
16	10	10	10	9
17	10	10	10	9
18	10	10	10	9
19	10	10	10	9
20	10	10	10	8
21	10	10	10	8
22	10	10	10	8
23	10	10	10	8
24	10	10	10	8
25	10	10	10	5
26	10	10	10	4
27	100%	100%	100%	40%

All control animals died until day 33

2006343832 09 Dec 2010

The claims defining the invention are as follows:

1. A compound of the formula (I)



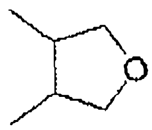
(I)

or a salt thereof,

wherein R^1 and R^2 are independently from each other NO_2, CN, trifluoromethyl, halogen, $CONR^7R^8$, $COOR^9$, CHO, $SO_2NR^7R^8$ or OCF_3;

R^3 and R^4 are independently from each other H or a methyl group;

R^5 and R^6 are independently from each other a linear or branched aliphatic radical having 1-8 chain members, or R^5 and R^6 together represent a bivalent radical $-(CR^9{}_2)_m-$ wherein m is 1-4, or R^5 and R^6 together represent the bivalent radical:



R^7, R^8 and R^9 are independently H or a linear or branched aliphatic radical having 1-7 chain members, or phenyl.

2. A compound according to formula (I) of claim 1, wherein R^1 represents NO_2, R^2 is CF_3, R^3 and R^4 are H, and R^5 and R^6 have the meanings given in claim 1.

2006343832 09 Dec 2010

3. A compound according to formula (I) of claim 1, wherein R^1 represents NO_2, R^2 is CN, R^3 and R^4 are H, and R^5 and R^6 have the meanings given in claim 1.

4. A compound according to formula (I) of claim 1, wherein R^1 and R^2 represents NO_2, R^3 and R^4 are H, and R^5 and R^6 have the meanings given in claim 1.

5. A compound according to formula (I) of claim 1, wherein R^5 and R^6 are independently C_{1-8} alkyl groups.

6. A compound selected from the group consisting of
2-(1,4-dioxa-8-azaspiro[4.5]dec-8-yl)-8-nitro-6-(trifluoromethyl)-1,3-benzothiazin-4-one,
2-(7,12-dioxa-3-azaspiro[5.6]dodec-3-yl)-6,8-dinitro-1,3-benzothiazin-4-one,
2-(1,4-dioxa-8-azaspiro[4.5]dec-8-yl)-7-methyl-6,8-dinitro-1,3-benzothiazin-4-one,
2-(1,4-dioxa-8-azaspiro[4.5]dec-8-yl)-8-nitro-4-oxo-1,3-benzothiazine-6-carbonitrile,
2-(1,4-dioxa-8-azaspiro[4.5]dec-8-yl)-6,8-dinitro-1,3-benzothiazin-4-one,
2-(2-methyl-1,4-dioxa-8-azaspiro[4.5]dec-8-yl)-6,8-dinitro-1,3-benzothiazin-4-one,
2-(4,4-diethoxypiperidin-1-yl)-6,8-dinitro-1,3-benzothiazin-4-one,
7-methyl-2-(2-methyl-1,4-dioxa-8-azaspiro[4.5]dec-8-yl)-6,8-dinitro-1,3-benzothiazin-4-one,
2-(2-methyl-1,4-dioxa-8-azaspiro[4.5]dec-8-yl)-8-nitro-6-(trifluoromethyl)-1,3-benzothiazin-4-one,
2-(2,3-dimethyl-1,4-dioxa-8-azaspiro[4.5]dec-8-yl)-8-nitro-6-(trifluoromethyl)-1,3-benzothiazin-4-one,
2-(1,5-dioxa-9-azaspiro[5.5]undec-9-yl)-8-nitro-6-(trifluoromethyl)-1,3-benzothiazin-4-one,
2-(1,5-dioxa-9-azaspiro[5.5]undec-9-yl)-8-nitro-4-oxo-1,3-benzothiazine-6-carbonitrile,
2-(2-methyl-1,4-dioxa-8-azaspiro[4.5]dec-8-yl)-8-nitro-4-oxo-1,3-benzothiazine-6-carbonitrile.

7. 2-(2-methyl-1,4-dioxa-8-azaspiro[4.5]dec-8-yl)-8-nitro-6-(trifluoromethyl)-1,3-benzothiazin-4-one.

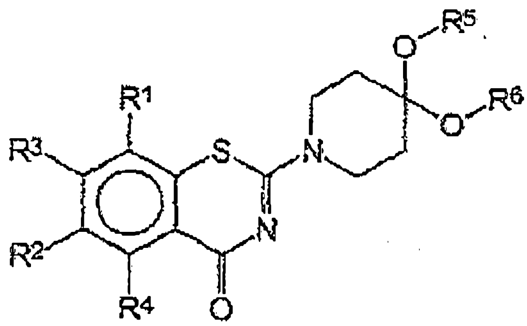
8. Use of a compound of formula (I) or a salt thereof according to any one of the preceding claims for the preparation of a pharmaceutical composition.

9. Use of a compound according to any one of claims 1-7 for the preparation of a medicament for the therapeutic or prophylactic treatment of tuberculosis infection or leprosy infection in mammals.

10. Pharmaceutical composition comprising a compound according to any one of claims 1-7.

11. A compound according to any one of claims 1-7 for use in a method for the therapeutic or prophylactic treatment of tuberculosis infection or leprosy infection in mammals.

12. Process for the preparation of a compound according to formula (I)

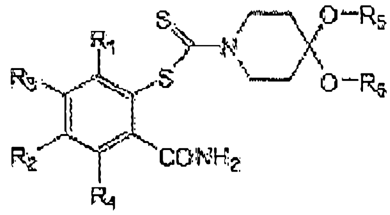


(I)

comprising the following step:

treating a compound of the following formula:

09 Dec 2010
2006343832



wherein the substituents R^1, R^2, R^5 and R^6 have the same meanings given in claim 1 and wherein R^3 and R^4 are hydrogen, with H_2O/EtOH to obtain a compound according to formula (I).

13. A compound prepared by the process of claim 12.

14. A compound of any one of claims 1 to 7 substantially as hereinbefore described with reference to any of the Examples.

15. A process of claim 12 substantially as hereinbefore described with reference to any of the Examples.