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ORIGINAL PAPER

Design, synthesis, antitubercular and antiviral properties of new spirocyclic indole derivatives

Gökçe Cihan-Üstündağ[1] · Lieve Naesens[2] · Dilek Şatana[3] · Gonca Erköse-Genç[3] · Emel Mataracı-Kara[4] · Gültaze Çapan[1]

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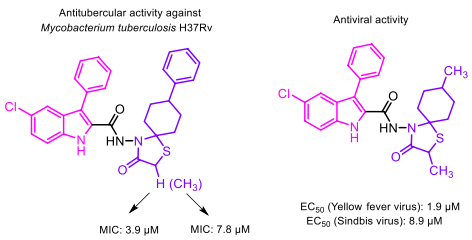
Abstract

A series of indole-based spirothiazolidinones have been designed, synthesized and evaluated, in vitro, for their antitubercular, antiviral, antibacterial, and antifungal activities. The structures of the new compounds were established by IR, ^{1}H NMR, ^{13}C NMR (proton decoupled, APT, and DEPT), electrospray ionization mass spectrometry, and microanalysis. Compounds bearing a phenyl substituent at position 8 of the spiro ring, exhibited significant antitubercular activity against *Mycobacterium tuberculosis* H37Rv ATCC 27294 at concentrations of 3.9 and 7.8 µM. Still, some of the tested compounds displayed activity on mycobacteria with MIC values of 16 and 31 µM. Four of the indole-spirothiazolidinone derivatives were found to be moderately active against Punta Toro virus, yellow fever virus or Sindbis virus in Vero cells. The antiviral EC_{50} values were in the range of 1.9–12 µM and the selectivity index (ratio of cytotoxic to antivirally effective concentration) was above 10 in some cases. The most potent effect was seen with the compound that is methylated at positions 2 and 8 of the spirothiazolidinone system.

Graphic abstract



Keywords Heterocycles · Spirothiazolidinone · Antitubercular activity · Antiviral activity · Cytotoxicity · Drug research

Introduction

Tuberculosis (TB) is a highly infectious disease caused by the bacillus *Mycobacterium tuberculosis.* For the past 5 years, it has been the leading cause of mortality from a

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✉ Gökçe Cihan-Üstündağ
gokcechn@istanbul.edu.tr

[1] Department of Pharmaceutical Chemistry, Faculty of Pharmacy, Istanbul University, Istanbul 34116, Turkey

[2] Department of Microbiology, Immunology and Transplantation, Rega Institute for Medical Research, KU Leuven, 3000 Louvain, Belgium

[3] Department of Microbiology and Clinical Microbiology, Istanbul Faculty of Medicine, Istanbul University, Istanbul 34116, Turkey

[4] Department of Pharmaceutical Microbiology, Faculty of Pharmacy, Istanbul University, 34116 Istanbul, Turkey

single infectious disease, ranking above HIV/AIDS [1]. Major problems associated with the currently available TB treatment include long treatment duration, inadequate compliance, concurrent HIV infection, and increasing incidence of multidrug-resistant (MDR) and extensively drug-resistant (XDR) tuberculosis [2–5]. This emergence of difficult to treat strains necessitates the discovery and development of novel antitubercular drugs. After this domain has been inactive for several decades, two new drugs became available, i.e., the mitochondrial ATP synthase inhibitor bedaquiline and mycolic acid biosynthesis inhibitor delamanid, which received accelerated approval for the treatment of MDR tuberculosis in 2012 and 2014, respectively [6]. Besides, diverse novel drug candidates are in preclinical or clinical development [6–8].

Indole-2-carboxamides incorporating an alicyclic system (Fig. 1a) have been extensively studied by different research groups [9–14]. This type of compounds was found to be highly active against both drug-susceptible and drug-resistant strains of *Mycobacterium tuberculosis* by acting on the MmpL3 transporter protein. In previous investigations, we have identified the indole-spirothiazolidinone system (Fig. 1b) as a promising scaffold against the *Mycobacterium tuberculosis* H37Rv strain [15, 16]. Some of these analogues exhibited in vitro antitubercular activity with GI (growth inhibition) values of 91–95% at a MIC (minimum inhibitory concentration) value of 6.25 μg/cm^3. Recently, we reported on the synthesis of novel 5-fluoro-3-phenyl-1*H*-indole derivatives containing a 4-thiazolidinone nucleus (instead of spirothiazolidinone system) [17]. Two molecules (Fig. 1c) displayed notable antitubercular activity at concentrations tenfold lower than those that produced cytotoxicity in mammalian cell lines.

Furthermore, several spirothiazolidinone compounds synthesized in our laboratory were found to be efficient inhibitors of membrane fusion mediated by influenza virus hemagglutinin (HA) [18–20]. As demonstrated in Fig. 1d, these compounds have a similar backbone structure, consisting of an aromatic/polycyclic ring linked to a non-aromatic spiro system via an amide bridge. Some analogues displayed low micromolar activity against the influenza A/H3N2 subtype with a favorable selectivity index.

Based on these insights and our objective to optimize the antimicrobial activity of indolyl thiazolidinones and spirothiazolidinones, we here report the chemical synthesis, structural characterization and in vitro antitubercular, antiviral, antibacterial, and antifungal evaluation of new 5-chloro-3-phenyl-*N*-(2,7,8,9-substituted/nonsubstituted-3-oxo-1-thia-4-azaspiro[4.4]nonan/[4.5]decan-4-yl)-1*H*-indole-2-carboxamides **4a**–**4i, 5a**–**5h** (Fig. 1e).

Results and discussion

Chemistry and structural characterization

The synthetic pathways for the preparation of the spirothiazolidinones **4a**–**4i** and **5a**–**5h** are illustrated in Scheme 1. Thus, the diazonium salt, formed by the reaction of 4-chloroaniline with $NaNO_2$ and HCl, was reacted with ethyl 2-benzyl-3-oxobutanoate to obtain compound **1** [21] according

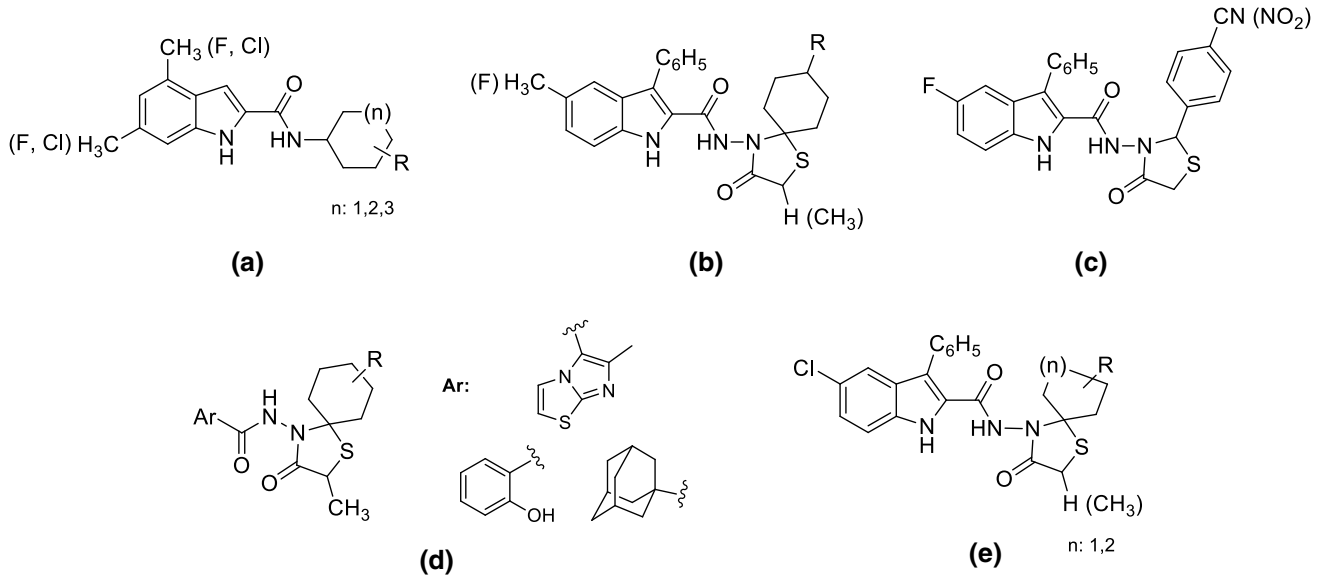
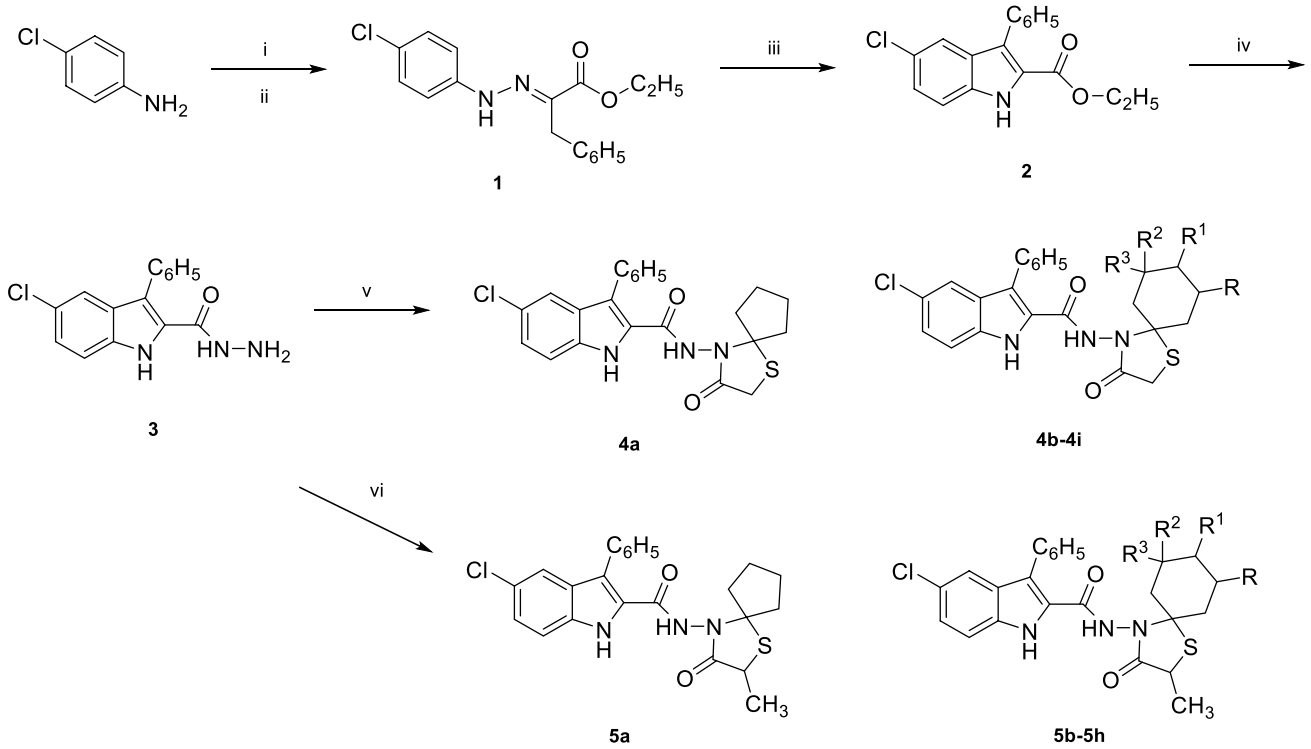


Fig. 1 Structures of **a**, **b**, **c** indole-2-carboxamide-based antitubercular agents and **d** the spirothiazolidinone compounds acting as influenza A virus fusion inhibitors

Scheme 1



(i) 7% $NaNO_2$, EtOH, conc. HCl, 0 °C; (ii) ethyl 2-benzyl-3-oxo-butanoate, KOH, EtOH, 0 °C; (iii) conc. HCl, reflux, 4 h; (iv) $H_2NNH_2{\cdot}H_2O$, EtOH, reflux, 6 h; (v) cyclopentanone/3,4,5-(non)substituted cyclohexanone, mercaptoacetic acid, dry toluene, reflux, 5–6 h; (vi) cyclopentanone/3,4,5-(non)substituted cyclohexanone, 2-mercaptopropionic acid, dry toluene, reflux, 5–6 h. **4b**, **5b**: R,R^1,R^2,R^3=H; **4c**, **5c**: vR=CH_3;R^1,R^2,R^3,=H; **4d**, **5d**: R^1=CH_3;R,R^2,R^3=H; **4e**, **5e**: R^1=C_2H_5;R,R^2,R^3=H; **4f**, **5f**: R^1=C_3H_7;R,R^2,R^3=H; **4g**, **5g**: R,R^2,R^3=CH_3;R^1=H; **4h**, **5h**: R^1=C_6H_5;R,R^2,R^3=H; **4i**:R^1=$C(CH_3)_3$;R,R^2,R^3=H

to the Japp–Klingemann reaction. The Fischer indole synthesis was carried out in acidic medium to cyclize **1** into ethyl 5-chloro-3-phenyl-1*H*-indole-2-carboxylate **2** [22]. Subsequent exposure of **2** to an excess of hydrazine hydrate afforded compound **3** [23]. The target spirocyclic compounds **4a**–**4i**, **5a**–**5h** were synthesized by treatment of the key intermediate **3** with appropriate cyclic ketones and mercapto acids in a one-pot reaction [15].

The structures of the new compounds were characterized by IR, ^{1}H NMR, ^{13}C NMR (proton decoupled, APT, and DEPT), electrospray ionization mass spectrometry (ESI-MS), and combustion analysis. The IR spectra of **4a**–**4i** and **5a**–**5h** exhibited the ring and the exocyclic C=O bands in the 1692–1713 cm^{-1} and 1651–1670 cm^{-1}, respectively. The shifts observed in the amide bands when compared to that of **3** (1636 cm^{-1}) and the presence of additional lactam bands provided definite proof for the aimed cyclization. Observation of NH signals assigned to the indole NH (δ = 12.11–12.18 ppm) and amide NH (9.97–0.21 ppm) together with the absence of the NH_2 resonance of the intermediate hydrazide **3** in the ^{1}H NMR spectra of **4** and **5**, provided further evidence for the formation of new adducts. The S–CH_2 (**4a**–**4i**) and S–CH (**5a**–**5h**) protons of the newly formed spiroalkane residue resonated at about 3.50-3.65 and 3.87-3.93 ppm, respectively. The S–CH_2 protons of **4a**–**4i** appeared as singlets except for the methylene hydrogens of compound **4g** which were observed as separate doublets with large coupling constants (J = 16.1 Hz) due to the geminal coupling resulting from the chiral centers of the spirothiazolidinone ring. The ^{1}H NMR spectra of **5a**–**5h** displayed the thiazolidinone S–CH protons as quartets or broad/distorted singlets and doublets. Assignment of the indole protons was achieved on the basis of the values and coupling constants reported for the 2,3,5-trisubstituted indole ring [15, 16, 24, 25]. The carbon resonances were assigned by chemical shifts and comparison with previously reported ^{13}C NMR data for compounds having a similar backbone structure [15, 19, 26]. CH_3, CH_2, CH, and C signals were assigned by

Table 1 Antitubercular activity against *M. tuberculosis* H37Rv ATCC 27294

Compound	R	R^1	R^2	R^3	MIC[a]/μM
4a	–	–	–	–	1000
4b	H	H	H	H	16
4c	CH_3	H	H	H	16
4d	H	CH_3	H	H	31
4e	H	C_2H_5	H	H	250
4f	H	C_3H_7	H	H	31
4g	CH_3	H	CH_3	CH_3	500
4h	H	C_6H_5	H	H	3.9
4i	H	$C(CH_3)_3$	H	H	16
5a	–	–	–	–	250
5b	H	H	H	H	250
5c	CH_3	H	H	H	500
5d	H	CH_3	H	H	1000
5e	H	C_2H_5	H	H	1000
5f	H	C_3H_7	H	H	1000
5g	CH_3	H	CH_3	CH_3	1000
5h	H	C_6H_5	H	H	7.8
Rifampicin	–	–	–	–	<0.1 μg/cm^3

The experiment was performed twice and the same results were obtained

[a]MIC, the actual minimum inhibitory concentration required to inhibit the growth of 100% of organisms

APT and DEPT experiments. Observation of the typical C5 ($\delta = 70.59$–76.40 ppm) and C=O (167.64–170.57 ppm) resonances of the spirothiazolidinone structure in the ^{13}C NMR spectra of **4** and **5**, verified the formation of the expected spiro ring system.

Deprotonated $[M-H]^-$ or protonated $[M+H]^+$ ions observed in the ESI-MS confirmed the molecular weight of the compounds. Additional $[(M-H)+2]^-$ or $[(M+H)+2]^+$ isotope peaks approximately one-third the intensity of the molecular ion peak resulting from the ^{37}Cl isotope further confirmed the new structures.

Antitubercular activity

The antitubercular activity of compounds **4a**–**4i** and **5a**–**5h** was tested in vitro against *M. tuberculosis* H37Rv ATCC 27294 using the microdilution method. The lowest concentration of compound that inhibited 100% of mycobacterial growth in the culture was defined as the MIC. Rifampicin was used as the reference drug. Compounds were assayed using twofold dilutions starting at 1000 μM. As shown in Table 1, compounds **4 h** and **5h**, bearing a phenyl substituent at position 8 of the spiro ring, exhibited the highest anti-TB activity at concentrations of 3.9 and 7.8 μM, respectively. Most of the compounds in series **4** (**4b**, **4c**, **4d**, **4f**, and **4i**) displayed some activity on mycobacteria with MIC values of 16 and 31 μM.

Looking at the chemical structures of the active compounds, it can be observed that the presence of a methyl group at position 2 of the spirocyclic system (series **5**) led to a significant reduction in antitubercular activity. Introduction of a bulky aromatic substituent (C_6H_5) at position 8 of the ring, as in **4h** and **5h**, enhanced the antitubercular activity.

Antiviral activity

Compounds **4a**–**4i** and **5a**–**5h** were evaluated against a variety of DNA and RNA viruses in cell culture, namely: herpes simplex virus type-1 (HSV-1) and type-2 (HSV-2), an acyclovir-resistant thymidine kinase-deficient (TK^-) mutant of HSV-1, vaccinia virus, human adenovirus-2, human coronavirus, vesicular stomatitis virus, Coxsackie B4 virus, respiratory syncytial virus, parainfluenza-3 virus, reovirus, Sindbis virus, Punta Toro virus, yellow fever virus, and influenza A and influenza B virus. The cytopathic effect reduction assays revealed that compounds **4b**, **4c**, **5b**, and **5d** were moderately active against Punta Toro virus, yellow fever virus or Sindbis virus in Vero cells (Table 2). The antiviral EC_{50} values were in the range of 1.9–12 μM and the selectivity index (SI: ratio of cytotoxic to antivirally effective concentration) was above 10 in some cases (see values between brackets in Table 2). The most potent effect was seen with compound **5d** that is methylated at positions 2 and 8 of the spirothiazolidinone system. Of note, no antiviral activity was obtained for the analogues carrying a spiro-fused cyclopentane ring instead of cyclohexane (i.e., **4a** and **5a**) or a bulkier group than methyl on the cyclohexane residue. Introduction of a methyl group at position 2 of the ring system (e.g., compare compounds **4b** and **5b**) seemed to have a slightly positive effect on antiviral activity.

The test compounds did not exhibit activity against any of the other DNA- or RNA-viruses tested. Nevertheless, this broad antiviral testing allowed to determine the compounds' cytotoxic activity in different mammalian cell lines (Table 3). In general, the compounds endowed with antiviral activity (**4b**, **4c**, **5b**, and **5d**) tended to be less cytotoxic than the inactive derivatives.

Antibacterial and antifungal activity

The broad antibacterial and antifungal activity of compounds 4a–4i and 5a–5 h was further assessed using *Pseudomonas aeruginosa* ATCC 27853, *Escherichia coli* ATCC 25922, *Klebsiella pneumoniae* ATCC 4352, *Proteus mirabilis* ATCC 14153, *Staphylococcus aureus* ATCC 29213, *Staphylococcus epidermidis* ATCC 12228, *Enterococcus faecalis* ATCC 29212, *Candida albicans* ATCC 10231, *Candida parapsilosis* ATCC 22019, and *Candida tropicalis* ATCC

Table 2 Antiviral activity in Vero[a] cell cultures infected with diverse RNA viruses

Compound	R	R^1	R^2	R^3	Antiviral assays in Vero cells						
					Antiviral EC_{50}^b value/μM						MCC[c]/μM
					Parain-fluenza-3 virus	Reovirus-1	Sindbis virus	Cox-sackie B4 virus	Punta Toro virus	Yellow Fever virus	
4a	–	–	–	–	> 100	> 100	> 100	> 100	> 100	> 100	> 100
4b	H	H	H	H	> 100	> 100	> 100	> 100	11 [9]	> 100	100
4c	CH_3	H	H	H	> 100	> 100	> 100	> 100	12 [8]	> 100	100
4d	H	CH_3	H	H	> 100	> 100	> 100	> 100	> 100	> 100	20
4e	H	C_2H_5	H	H	> 100	> 100	> 100	> 100	> 100	> 100	20
4f	H	C_3H_7	H	H	> 100	> 100	> 100	> 100	> 100	> 100	20
4g	CH_3	H	CH_3	CH_3	> 100	> 100	> 100	> 100	> 100	> 100	20
4h	H	C_6H_5	H	H	> 100	> 100	> 100	> 100	> 100	> 100	20
4i	H	$C(CH_3)_3$	H	H	> 100	> 100	> 100	> 100	> 100	> 100	20
5a	–	–	–	–	> 100	> 100	> 100	> 100	> 100	> 100	> 100
5b	H	H	H	H	> 100	> 100	> 100	> 100	> 100	5.6 [> 18]	> 100
5c	CH_3	H	H	H	> 100	> 100	> 100	> 100	> 100	> 100	> 100
5d	H	CH_3	H	H	> 100	> 100	8.9 [≥ 2]	> 100	> 100	1.9 [≥ 11]	≥20
5e	H	C_2H_5	H	H	> 100	> 100	> 100	> 100	> 100	> 100	20
5f	H	C_3H_7	H	H	> 100	> 100	> 100	> 100	> 100	> 100	20
5g	CH_3	H	CH_3	CH_3	> 100	> 100	> 100	> 100	> 100	> 100	> 100
5h	H	C_6H_5	H	H	> 100	> 100	> 100	> 100	> 100	> 100	20
DS-10,000[d]	–	–	–	–	> 100	> 100	0.8	34	4.0	15	> 100
Ribavirin	–	–	–	–	111	111	8.9	>250	111	>250	>250
Mycophenolic acid	–	–	–	–	0.4	1.5	0.4	> 100	1.7	0.8	> 100

Values shown are the mean of two independent tests. In square brackets, the selectivity index (i.e., ratio of MCC to antiviral EC_{50}) is given

[a]Vero: African green monkey kidney cells

[b]EC_{50}: 50% effective concentration, producing 50% inhibition of virus-induced cytopathic effect, as determined by microscopy

[c]MCC: minimal cytotoxic concentration, or compound concentration causing minimal changes in cell morphology, as assessed by microscopy

[d]DS-10,000: dextran sulfate with MW 10,000; for this compound, data are expressed in μg per cm^3

750. The compounds were assayed using twofold dilutions from 2500 to 156 μM. Neither of the test compounds produced antimicrobial activity below this concentration range.

Conclusion

In the search for effective antimicrobial agents, we achieved the synthesis of novel spirothiazolidinone derivatives **4a**–**4i** **5a**–**5h** with the 5-chloro-3-phenyl-1*H*-indole scaffold. Structures of the new compounds were characterized and confirmed by spectrometric methods (IR, ^{1}H NMR, ^{13}C NMR, and ESI-MS) and elemental analysis. Compounds **4a**–**4i** and **5a**–**5h** were evaluated for in vitro antitubercular, antiviral, antibacterial, and antifungal activity against various viral, bacterial, and fungal strains. Compounds **4h** and **5h**, bearing a bulky phenyl group at position 8 of the spiro ring, displayed appreciable anti-TB activity against *M. tuberculosis* H37Rv ATCC 27294 with MIC values of 3.9 and 7.8 μM, respectively. Compounds **4b**, **4c**, **5b**, and **5d** exhibited inhibitory effect on the replication of Punta Toro virus, yellow fever virus or Sindbis virus in Vero cells. The antiviral EC_{50} values were in the range of 1.9–12 μM and the selectivity index (SI: ratio of cytotoxic to antivirally effective concentration) was above 10 in some cases. The most potent effect was seen with compound **5d** that is methylated at positions 2 and 8 of the spirothiazolidinone system. Neither of the indole-spirothiazolidinone compounds showed activity against any of the bacterial or fungal strains tested, at concentrations below 2500–156 μM.

Experimental

All purchased solvents and chemicals were of analytical grade and used as received. Melting points were determined in open capillary tubes with a Buchi B-540 melting point apparatus. Microanalyses were performed on a Thermo

Table 3 Cytotoxic activity in diverse mammalian cell lines[a]

Compound	MCC[b]/μM				CC_{50}[c]/μM
	HEL	HeLa	Vero	MDCK	MDCK
4a	> 100	> 100	> 100	> 100	> 100
4b	100	100	100	≥20	63
4c	100	100	100	≥ 20	64
4d	20	20	20	4	2.3
4e	20	20	20	4	9.9
4f	20	20	20	4	2.4
4g	20	20	20	4	20
4h	20	20	20	4	2.3
4i	20	20	20	4	2.3
5a	> 100	> 100	> 100	≥ 100	> 100
5b	100	≥100	> 100	100	> 100
5c	> 100	> 100	> 100	> 100	> 100
5d	20	20	≥ 20	≥ 20	> 100
5e	20	20	20	4	18
5f	20	20	20	4	2.3
5g	≥ 100	100	> 100	100	> 100
5h	20	20	20	4	2.3
DS-10,000[d]	ND	> 100	> 100	ND	ND
Ribavirin	ND	≥ 250	>250	> 100	> 100
Ganciclovir	> 100	ND	ND	ND	ND
Brivudin	> 250	ND	ND	ND	ND

ND not done

[a]*HEL* human embryonic lung fibroblast cells, *HeLa* human cervix carcinoma cells, *Vero* African green monkey kidney cells, *MDCK* Madin–Darby canine kidney cells

[b]*MCC* minimal cytotoxic concentration, or compound concentration causing minimal changes in cell morphology, as assessed by microscopy

[c]CC_{50} 50% cytotoxic concentration, assessed by the spectroscopic MTS cell viability assay

[d]*DS-10,000* dextran sulfate with MW 10,000; for this compound, data are expressed in μg per cm^3

Finnigan Flash EA 1112 elemental analyzer and their results were found to be in good agreement (±0.3%) with the calculated values. IR spectra were recorded in KBr discs ($\bar{\nu}$/cm^{-1}) on a Shimadzu IRAffinity-1 FTIR spectrophotometer. ^{1}H NMR (DMSO-d_6), ^{13}C NMR (APT, DMSO-d_6) were run on Varian^{UNITY}INOVA (500 MHz) instrument. ^{13}C NMR (DEPT, DMSO-d_6) were run Bruker ARX (300 MHz). Chemical shifts are reported as δ (ppm) relative to TMS as internal standard and coupling constants (J) are given in hertz (Hz). MS (ESI±) were determined on a Finnigan LCQ Advantage Max mass spectrometer (*: broad/distorted, ind.:indole, ar.:aromatic, sp.: spirodecane).

Ethyl 2-[2-(4-chlorophenyl)hydrazinylidene]-3-phenylpropanoate (1) [21] To a solution of 4-chloroaniline (0.02 mol) in 10 cm^3 ethanol, 10 cm^3 water, and 6 cm^3 conc. HCl, 30 cm^3 aqueous $NaNO_2$ solution (7%) was added dropwise at 0 °C with stirring. The resulting solution of diazonium salt was poured into a cooled (0 °C) mixture of ethyl 2-benzyl-3-oxobutanoate (0.02 mol), 10 cm^3 ethanol, 10 cm^3 water, and 5.4 g KOH while stirring. The resulting mixture was refrigerated overnight. The red oily solid residue thus obtained was separated, washed with water, and used without further purification. Yield: 72.4%; m.p.: 90–93 °C (Ref. [21] 87–93 °C).

Ethyl 5-chloro-3-phenyl-1*H*-indole-2-carboxylate (2) [22] A solution of **1** (0.02 mol) in 20 cm^3 conc. HCl was heated under reflux on a water bath (70–80 °C) for 4 h. The crude product was filtered off, washed with water until tested neutral to litmus and used without further purification. Yield: 67.8%; m.p.: 160–162 °C (Ref. [22] 158–160 °C).

5-Chloro-3-phenyl -1*H*-indole-2-carbohydrazide (3) [23] A mixture of **2** (0.02 mol), 20 cm^3 ethanol, and 8 cm^3 $H_2NNH_2{\cdot}H_2O$ (98%) was heated under reflux on a water bath (70–80 °C) for 6 h. The resulting brown solid was filtered off and recrystallized from ethanol. Yield: 53.7%; m.p.: 227–230 °C (Ref. [23] 234–236 °C).

General procedure for the synthesis of 5-chloro-3-phenyl-*N*-(2,7,8,9-substituted/nonsubstituted-3-oxo-1-thia-4-azaspiro[4.4]nonan/[4.5]decan-4-yl)-1*H*-indole-2-carboxamides 4a–4i, 5a–5h

A mixture of **3** (0.0025 mol), an appropriate cyclohexanone/cyclopentanone (0.003 mol), and mercaptoacetic acid or 2-mercaptopropionic acid (0.01 mol) in 20 cm^3 dry toluene was heated to reflux with a heating mantle for 5–6 h using a Dean–Stark water separator. Excess toluene was evaporated in vacuo. The resulting residue was treated with saturated $NaHCO_3$ solution until CO_2 evolution ceased and was allowed to stand overnight or in some cases refrigerated until solidification. The solid thus obtained was washed with water, dried, and recrystallized from ethanol or ethyl acetate.

5-Chloro-*N*-(3-oxo-1-thia-4-azaspiro[4.4]nonan-4-yl)-3-phenyl-1*H*-indole-2-carboxamide (4a, $C_{22}H_{20}ClN_3O_2S$) White crystals; yield: 87.7%; m.p.: 297–300 °C; R_f=0.46 (EtOAc/$CHCl_3$ 1:1); IR (KBr): $\bar{\nu}$=3277 (N–H), 1709, 1651 (C=O) cm^{-1}; ^{1}H NMR (DMSO-d_6, 500 MHz): δ=1.58–1.69 (4H, m, CH_2-sp.), 1.72–1.74 (2H, m, CH_2-sp.), 1.94 (2H, s*, CH_2-sp.), 3.65 (2H, s, S–CH_2-sp.), 7.29 (1H, dd, J=8.5, 2.0 Hz, H6-ind.), 7.37 (1H, tt, J=7.3, 1.5 Hz, 3-C_6H_5(H4)-ind.), 7.45 (2H, t, J=7.3 Hz, 3-C_6H_5(H3,H5)-ind.), 7.51–7.54 (4H, m, H4, H7, 3-C_6H_5(H2,H6)-ind.), 10.10 (1H, s, CONH), 12.13 (1H, s, NH) ppm; ^{13}C NMR (APT, DMSO-d_6, 125 MHz): δ=22.40, 29.05, 37.91 (CH_2-sp.), 76.40

(C5-sp.), 114.62, 114.67 (CH-ar.), 118.64 (C-ar.), 119.32, 119.41 (CH-ar.), 124.69 (CH-ar.), 125.50 (C-ar.), 127.75 (CH-ar.), 127.81 (C-ar.), 128.03 (C-ar.), 128.79 (CH-ar.), 130.44 (CH-ar.), 133.27 (C-ar.), 134.65 (C-ar.), 162.08 (CO–NH), 167.87 (CO-sp.) ppm; MS (ESI-): *m/z* (%) = 424.5 ($[M-H]^-$, 100), 426.3 ($[(M-H)+2]^-$, 36.5).

5-Chloro-*N*-(3-oxo-1-thia-4-azaspiro[4.5]decan-4-yl)-3-phenyl-1*H*-indole-2-carboxamide (4b, $C_{23}H_{22}ClN_3O_2S$) White crystals; yield: 76.9%; m.p.: 229–232 °C; R_f = 0.48 (EtOAc/$CHCl_3$ 1:1); IR (KBr): $\bar{\nu}$ = 3279 (N–H), 1709, 1651 (C=O) cm^{-1}; ^{1}H NMR (DMSO-d_6, 500 MHz): δ = 1.01–1.11 (2H, m, CH_2-sp.), 1.35–1.42 (2H, m, CH_2-sp.), 1.56 (1H, d*, *J* = 13.2 Hz, CH_2-sp.), 1.64–1.72 (5H, m, CH_2-sp.), 3.58 (2H, s, S–CH_2-sp.), 7.30 (1H, dd, *J* = 8.8, 2.4 Hz, H6-ind.), 7.37 (1H, tt, *J* = 7.3, 1.5 Hz, 3-C_6H_5(H4)-ind.), 7.46 (2H, t, *J* = 7.6 Hz, 3-C_6H_5(H3,H5)-ind.), 7.51–7.56 (4H, m, H4, H7, 3-C_6H_5(H2,H6)-ind.), 10.09 (1H, s, CONH), 12.14 (1H, s, NH) ppm; ^{13}C NMR (APT, DMSO-d_6, 125 MHz): δ = 23.33, 24.31, 28.29, 37.10 (CH_2-sp.), 72.63 (C5-sp.), 114.56, 114.64 (CH-ar.), 118.50 (C-ar.), 119.31, 119.39 (CH-ar.), 124.54, 124.66 (CH-ar.), 125.47 (C-ar.), 127.41 (CH-ar.), 127.82 (C-ar.), 128.23 (C-ar.), 128.90 (CH-ar.), 130.51 (CH-ar.), 133.38 (C-ar.), 134.62 (C-ar.), 162.14 (CO–NH), 167.73 (CO-sp.) ppm.

5-Chloro-*N*-(7-methyl-3-oxo-1-thia-4-azaspiro[4.5]decan-4-yl)-3-phenyl-1*H*-indole-2-carboxamide (4c, $C_{24}H_{24}ClN_3O_2S$) White powder; yield: 83.4%; m.p.: 268–270 °C; R_f = 0.52 (EtOAc/$CHCl_3$ 1:1); IR (KBr): $\bar{\nu}$ = 3231 (N–H), 1711, 1651 (C=O) cm^{-1}; ^{1}H NMR (DMSO-d_6, 500 MHz): δ = 0.76–0.85 (1H, m, CH/CH_2-sp.), 0.87 (3H, d, *J* = 6.4 Hz, 7-CH_3-sp.), 1.24–1.70 (8H, m, CH/CH_2-sp.), 3.58 (2H, s, S–CH_2-sp.), 7.29 (1H, dd, *J* = 8.8, 2.0 Hz, H6-ind.), 7.37 (1H, tt, *J* = 7.3, 1.5 Hz, 3-C_6H_5(H4)-ind.), 7.45 (2H, t, *J* = 7.3 Hz, 3-C_6H_5(H3,H5)-ind.), 7.49 (1H, d, *J* = 2.0 Hz, H4-ind.), 7.52-7.54 (3H, m, H7, 3-C_6H_5(H2,H6)-ind.), 10.06 (1H, s, CONH), 12.14 (1H, s, NH) ppm; ^{13}C NMR (APT, DMSO-d_6, 125 MHz): δ = 22.42 (7-CH_3-sp.), 22.88, 28.36 (CH_2-sp.), 29.97 (C7-sp.), 32.98, 36.54, 45.20 (CH_2-sp.), 72.59 (C5-sp.), 114.56, 114.63 (CH-ar.), 118.50 (C-ar.), 119.28, 119.37 (CH-ar.), 124.54 (CH-ar.), 125.45 (C-ar.), 127.42 (CH-ar.), 127.85 (C-ar.), 128.25 (C-ar.), 128.91 (CH-ar.), 130.51 (CH-ar.), 133.43 (C-ar.), 134.62 (C-ar.), 162.18 (CO–NH), 167.66 (CO-sp.) ppm.

5-Chloro-*N*-(8-methyl-3-oxo-1-thia-4-azaspiro[4.5]decan-4-yl)-3-phenyl-1*H*-indole-2-carboxamide (4d, $C_{24}H_{24}ClN_3O_2S$) White powder; yield: 91.2%; m.p.: 292–293 °C; R_f = 0.51 (EtOAc/$CHCl_3$ 1:1); IR (KBr): $\bar{\nu}$ = 3285 (N–H), 1709, 1651 (C=O) cm^{-1}; ^{1}H NMR (DMSO-d_6, 500 MHz): δ = 0.89, 0.95 (3H, 2d, *J* = 6.4 Hz, 8-CH_3-sp.), 1.08–1.32 (3H, m, CH/CH_2-sp.), 1.64–1.75 (6H, m, CH/CH_2-sp.), 3.57, 3.58 (2H, 2 s, S–CH_2-sp.), 7.29, 7.30 (1H, 2dd, *J* = 8.5, 2.4 Hz, H6-ind.), 7.36 (1H, tt, *J* = 8.0, 1.5 Hz, 3-C_6H_5(H4)-ind.), 7.46 (2H, t, *J* = 7.8 Hz, 3-C_6H_5(H3,H5)-ind.), 7.51-7.55 (4H, m, H4, H7, 3-C_6H_5(H2,H6)-ind.), 10.05, 10.06 (1H, 2 s, CONH), 12.11, 12.12 (1H, 2 s, NH) ppm; ^{13}C NMR (proton decoupled and DEPT, DMSO-d_6, 75 MHz): δ = 22.23 (8-CH_3-sp.), 28.19 (CH_2-sp.), 30.70 (C8-sp.), 31.73, 36.76 (CH_2-sp.), 72.39 (C5-sp.), 114.50 (CH-ar.), 118.43 (C-ar.), 119.25 (CH-ar.), 124.52 (CH-ar.), 125.38 (C-ar.), 127.26 (CH-ar.), 127.73 (C-ar.), 128.06 (C-ar.), 128.76 (CH-ar.), 130.37 (CH-ar.), 133.26 (C-ar.), 134.53 (C-ar.), 162.00 (CO–NH), 167.69 (CO-sp.) ppm; MS (ESI-): *m/z* (%) = 452.6 ($[M-H]^-$, 100), 454.4 ($[(M-H)+2]^-$, 34.3).

5-Chloro-*N*-(8-ethyl-3-oxo-1-thia-4-azaspiro[4.5]decan-4-yl)-3-phenyl-1*H*-indole-2-carboxamide (4e, $C_{25}H_{26}ClN_3O_2S$) White flakes; yield: 88.2%; m.p.: 236–237 °C; R_f = 0.53 (EtOAc/$CHCl_3$ 1:1); IR (KBr): $\bar{\nu}$ = 3283 (N–H), 1713, 1651 (C=OC=O) cm^{-1}; ^{1}H NMR (DMSO-d_6, 500 MHz): δ = 0.89 (3H, t, *J* = 7.3 Hz, 8-$CH_2C\underline{H}_3$-sp.), 1.04–1.35 (5H, m, CH/CH_2-sp., 8-$C\underline{H}_2CH_3$-sp.), 1.62–1.96 (6H, m, CH/CH_2-sp.), 3.58, 3.59 (2H, 2 s, S–CH_2-sp.), 7.30 (1H, dd, *J* = 8.8, 2.0 Hz, H6-ind.), 7.36 (1H, tt, *J* = 7.3, 1.5 Hz, 3-C_6H_5(H4)-ind.), 7.45 (2H, t, *J* = 7.3 Hz, 3-C_6H_5(H3,H5)-ind.), 7.51-7.56 (4H, m, H4, H7, 3-C_6H_5(H2,H6)-ind.), 10.09, 10.10 (1H, 2 s, CONH), 12.13 (1H, s, NH) ppm; ^{13}C NMR (APT, DMSO-d_6, 125 MHz): δ = 11.94 (8-$CH_2\underline{C}H_3$-sp.), 28.29, 28.35, 29.22, 29.41, 36.84 (CH_2-sp., 8-$\underline{C}H_2CH_3$-sp.), 37.45 (C8-sp.), 72.75 (C5-sp.), 114.55, 114.63 (CH-ar.), 118.48 (C-ar.), 119.29, 119.38 (CH-ar.), 124.64 (CH-ar.), 125.47 (C-ar.), 127.40 (CH-ar.), 127.81 (C-ar.), 128.24 (C-ar.), 128.87 (CH-ar.), 130.48 (CH-ar.), 133.38 (C-ar.), 134.60 (C-ar.), 162.11 (CO–NH), 167.78 (CO-sp.) ppm; MS (ESI−): *m/z* (%) = 466.8 ([M-H]$^-$, 100), 468.5 ($[(M-H)+2]^-$, 30.9).

5-Chloro-3-phenyl-*N*-(8-propyl-3-oxo-1-thia-4-azaspiro[4.5]decan-4-yl)-1*H*-indole-2-carboxamide (4f, $C_{26}H_{28}ClN_3O_2S$) Beige powder; yield: 75.7%; m.p.: 233-236 °C; R_f = 0.55 (EtOAc/$CHCl_3$ 1:1); IR (KBr): $\bar{\nu}$ = 3291, 3217 (N–H), 1707, 1661 (C=O) cm^{-1}; ^{1}H NMR (DMSO-d_6, 500 MHz): δ = 0.88 (3H, t, *J* = 7.3 Hz, 8-$CH_2CH_2C\underline{H}_3$-sp.), 1.05–1.34 (7H, m, CH/CH_2-sp., 8-$C\underline{H}_2C\underline{H}_2CH_3$-sp.), 1.60–1.82 (6H, m, CH/CH_2-sp.), 3.58 (2H, s, S–CH_2-sp.), 7.29 (1H, dd, *J* = 8.8, 2.0 Hz, H6-ind.), 7.35 (1H, tt, *J* = 7.3, 1.5 Hz, 3-C_6H_5(H4)-ind.), 7.45 (2H, t, *J* = 7.8 Hz, 3-C_6H_5(H3,H5)-ind.), 7.51 (1H, d, *J* = 2.0 Hz, H4-ind.), 7.53–7.56 (3H, m, H7, 3-C_6H_5(H2,H6)-ind.), 10.10 (1H, s, CONH), 12.13 (1H, s, NH) ppm; ^{13}C NMR (APT, DMSO-d_6, 125 MHz): δ = 14.58 (8-$CH_2CH_2\underline{C}H_3$-sp.), 19.99, 28.30, 28.36, 29.77 (CH_2-sp., 8-$\underline{C}H_2\underline{C}H_2CH_3$-sp.), 35.25 (C8-sp.), 36.85, 38.76 (CH_2-sp., 8-$\underline{C}H_2\underline{C}H_2CH_3$-sp.), 72.74 (C5-sp.),

114.55, 114.61 (CH-ar.), 118.47 (C-ar.), 119.30, 119.38 (CH-ar.), 124.54 (CH-ar.), 125.47 (C-ar.), 127.30 (CH-ar.), 127.67 (C-ar.), 128.28 (C-ar.), 128.84 (CH-ar.), 130.51 (CH-ar.), 133.42 (C-ar.), 134.61 (C-ar.), 162.12 (CO–NH), 167.77 (CO-sp.) ppm.

5-Chloro-*N*-(7,7,9-trimethyl-3-oxo-1-thia-4-azaspiro[4.5]-decan-4-yl)-3-phenyl-1*H*-indole-2-carboxamide (4g, $C_{26}H_{28}ClN_3O_2S$) White crystals; yield: 85.1%; m.p.: 264-266.5 °C; $R_f = 0.56$ (EtOAc/$CHCl_3$ 1:1); IR (KBr): $\bar{\nu} = 3277$, 3219 (N–H), 1705, 1695, 1665 (C=O) cm^{-1}; ^1H NMR (DMSO-d_6, 500 MHz): $\delta = 0.76$ (1H, t, $J = 12.7$ Hz, CH/CH_2-sp.), 0.90 (6H, s*, 7-$(CH_3)_2$-sp.), 1.04 (3H, s*, 9-CH_3-sp.), 1.20-1.34 (2H, m, CH/CH_2-sp.), 1.58-1.84 (4H, m, CH/CH_2-sp.), 3.50 (1H, d, $J = 16.1$ Hz, S–CH_2-sp.), 3.61 (1H, d, $J = 16.1$ Hz, S–CH_2-sp.), 7.29 (1H, dd, $J = 8.6$, 2.0 Hz, H6-ind.), 7.37 (1H, t*, $J = 7.3$ Hz, 3-C_6H_5(H4)-ind.), 7.44–7.48 (3H, m, H4, 3-C_6H_5(H3,H5)-ind.), 7.51–7.54 (3H, m, H7, 3-C_6H_5(H2,H6)-ind.), 9.97 (1H, s, CONH), 12.13 (1H, s, NH) ppm; ^{13}C NMR (APT, DMSO-d_6, 125 MHz): $\delta = 22.24$, 25.97, 27.64 (7-$(CH_3)_2$, 9-CH_3-sp.), 28.50, 32.24 (CH_2-sp.), 34.09 (C9-sp.), 45.23, 46.80, 47.31 (CH_2-sp., C7-sp.), 71.87 (C5-sp.), 114.56, 114.64 (CH-ar.), 118.50 (C-ar.), 119.28, 119.38 (CH-ar.), 124.58 (CH-ar.), 125.45 (C-ar.), 127.44 (CH-ar.), 127.96 (C-ar.), 128.18 (C-ar.), 128.99 (CH-ar.), 130.54 (CH-ar.), 133.47 (C-ar.), 134.62 (C-ar.), 162.40 (CO–NH), 167.64 (CO-sp.) ppm.

5-Chloro-3-phenyl-*N*-(8-phenyl-3-oxo-1-thia-4-azaspiro[4.5]-decan-4-yl)-1*H*-indole-2-carboxamide (4h, $C_{29}H_{26}ClN_3O_2S$) Beige powder; yield: 72.8%; m.p.: 285–287 °C; $R_f = 0.58$ (EtOAc/$CHCl_3$ 1:1); IR (KBr): $\bar{\nu} = 3277$, 3237 (N–H), 1707, 1663 (C=O) cm^{-1}; ^1H NMR (DMSO-d_6, 500 MHz): $\delta = 1.60$–1.91 (8H, m, CH_2-sp.), 2.47–2.49 (1H, m, CH-sp.), 3.64 (2H, s, S–CH_2-sp.), 7.21 (1H, tt*, $J = 7.3$, 1.5 Hz, 8-C_6H_5(H4)-sp.), 7.25 (2H, d*, $J = 7.3$ Hz, 8-C_6H_5-sp.), 7.29–7.34 (3H, m, H6-ind., 8-C_6H_5-sp.), 7.41 (1H, tt*, $J = 7.8$, 1.5 Hz, 3-C_6H_5(H4)-ind.), 7.50–7.61 (6H, m, H4, H7, 3-C_6H_5(H2,H3,H5,H6)-ind.), 10.18 (1H, s, CONH), 12.17 (1H, s, NH) ppm; ^{13}C NMR (APT, DMSO-d_6, 125 MHz): $\delta = 28.38$, 30.92, 37.12 (CH_2-sp.), 41.95 (C8-sp.), 72.10 (C5-sp.), 114.57, 114.64 (CH-ar.), 118.60 (C-ar.), 119.34, 119.42 (CH-ar.), 124.62 (CH-ar.), 125.51 (C-ar.), 126.98 (8-ar.(CH)-sp.), 127.09 (CH-ar.), 127.73 (8-ar.(CH)-sp.), 127.88 (C-ar.), 128.28 (C-ar.), 128.95 (CH-ar.), 130.57 (CH-ar.), 133.45 (C-ar.), 134.63 (C-ar.), 146.26 (8-ar.(C)-sp.), 162.18 (CO–NH), 167.84 (CO-sp.) ppm.

***N*-(8-*tert*-Butyl-3-oxo-1-thia-4-azaspiro[4.5]-decan-4-yl)-5-chloro-3-phenyl-1*H*-indole-2-carboxamide (4i, $C_{27}H_{30}ClN_3O_2S$)** White crystals; yield: 79.3%; m.p.: 230-232 °C; $R_f = 0.57$ (EtOAc/$CHCl_3$ 1:1); IR (KBr): $\bar{\nu} = 3285$ (N–H), 1702, 1662 (C=O) cm^{-1}; ^1H NMR (DMSO-d_6, 500 MHz): $\delta = 0.84$ (9H, s, 8-$C(CH_3)_3$-sp.), 0.90-0.96 (1H, m, CH/CH_2-sp.), 1.12–1.22 (2H, m, CH/CH_2-sp.), 1.64-1.72 (6H, m, CH/CH_2-sp.), 3.58 (2H, s, S–CH_2-sp.), 7.30 (1H, dd, $J = 8.8$, 2.0 Hz, H6-ind.), 7.36 (1H, tt, $J = 7.3$, 1.5 Hz, 3-C_6H_5(H4)-ind.), 7.45 (2H, t, $J = 7.3$ Hz, 3-C_6H_5(H3,H5)-ind.), 7.50 (1H, d, $J = 2.0$ Hz, H4-ind.), 7.52-7.56 (3H, m, H7, 3-C_6H_5(H2,H6)-ind.), 10.13 (1H, s, CONH), 12.14 (1H, s, NH) ppm; ^{13}C NMR (APT, DMSO-d_6, 125 MHz): $\delta = 24.14$ (CH_2-sp.), 27.80 (8-C($\underline{C}H_3$)$_3$-sp.), 28.28 (CH_2-sp.), 32.39 (8-$\underline{C}(CH_3)_3$-sp.), 37.11 (CH_2-sp.), 45.96 (C8-sp.), 72.57 (C5-sp.), 114.60 (CH-ar.), 118.42 (C-ar.), 119.27, 119.35 (CH-ar.), 124.64 (CH-ar.), 125.45 (C-ar.), 127.40 (CH-ar.), 127.84 (C-ar.), 128.38 (C-ar.), 128.90 (CH-ar.), 130.51 (CH-ar.), 133.44 (C-ar.), 134.58 (C-ar.), 162.13 (CO–NH), 167.79 (CO-sp.) ppm; MS (ESI-): *m/z* (%) = 494.5 ([M-H]$^-$, 100), 496.3 ([(M-H) + 2]$^-$, 37.6).

5-Chloro-*N*-(2-methyl-3-oxo-1-thia-4-azaspiro[4.4]-nonan-4-yl)-3-phenyl-1*H*-indole-2-carboxamide (5a, $C_{23}H_{22}ClN_3O_2S$) Beige powder; yield: 85.4%; m.p.: 270–272 °C; $R_f = 0.60$ (EtOAc/$CHCl_3$ 1:1); IR (KBr): $\bar{\nu} = 3283$ (N–H), 1709, 1651 (C=O) cm^{-1}; ^1H NMR (DMSO-d_6, 500 MHz): $\delta = 1.42$ (3H, d, $J = 6.8$ Hz, 2-CH_3-sp.), 1.59-1.77 (6H, m, CH_2-sp.), 1.91-2.08 (2H, m, CH_2-sp.), 3.93 (1H, q, $J = 6.8$ Hz, S–CH-sp.), 7.29 (1H, dd, $J = 8.8$, 2.0 Hz, H6-ind.), 7.36 (1H, tt, $J = 7.8$, 1.5 Hz, 3-C_6H_5(H4)-ind.), 7.44 (2H, t, $J = 7.8$ Hz, 3-C_6H_5(H3,H5)-ind.), 7.50-7.55 (4H, m, H4, H7, 3-C_6H_5(H2,H6)-ind.), 10.14 (1H, s, CONH), 12.13 (1H, s, NH) ppm; ^{13}C NMR (APT, DMSO-d_6, 125 MHz): $\delta = 19.81$ (2-CH_3-sp.), 22.47 (CH_2-sp.), 37.93, 38.06 (C2-sp.), 38.25, 38.37 (CH_2-sp.), 74.96 (C5-sp.), 114.60, 114.66 (CH-ar.), 118.64 (C-ar.), 119.33, 119.41 (CH-ar.), 124.63, 124.74 (CH-ar.), 125.49 (C-ar.), 127.48 (CH-ar.), 127.84 (C-ar.), 128.02 (C-ar.), 128.81 (CH-ar.), 130.48 (CH-ar.), 133.27 (C-ar.), 134.65 (C-ar.), 162.05 (CO–NH), 170.57 (CO-sp.) ppm.

5-Chloro-*N*-(2-methyl-3-oxo-1-thia-4-azaspiro[4.5]-decan-4-yl)-3-phenyl-1*H*-indole-2-carboxamide (5b, $C_{24}H_{24}ClN_3O_2S$) White crystals; yield: 90.7%; m.p.: 245–248 °C; $R_f = 0.62$ (EtOAc/$CHCl_3$ 1:1); IR (KBr): $\bar{\nu} = 3285$ (N–H), 1711, 1651 (C=O) cm^{-1}; ^1H NMR (DMSO-d_6, 500 MHz): $\delta = 1.02$–1.11 (2H, m, CH_2-sp.), 1.31–1.38 (2H, m, CH_2-sp.), 1.41 (3H, d, $J = 6.8$ Hz, 2-CH_3-sp.), 1.56 (1H, d, $J = 12.7$ Hz, CH_2-sp.), 1.62–1.75 (5H, m, CH_2-sp.), 3.93 (1H, q, $J = 6.4$ Hz, S–CH-sp.), 7.30 (1H, dd, $J = 8.8$, 2.0 Hz, H6-ind.), 7.37 (1H, t, $J = 7.3$ Hz, 3-C_6H_5(H4)-ind.), 7.47 (2H, t, $J = 7.3$ Hz, 3-C_6H_5(H3,H5)-ind.), 7.50-7.55 (4H, m, H4, H7, 3-C_6H_5(H2,H6)-ind.), 10.12 (1H, s, CONH), 12.14 (1H, s, NH) ppm; ^{13}C NMR (APT, DMSO-d_6, 125 MHz): $\delta = 20.01$ (2-CH_3-sp.), 23.59, 24.31 (CH_2-sp.), 37.12 (C2-sp.), 38.02 (CH_2-sp.), 71.29 (C5-sp.), 114.56, 114.63 (CH-ar.), 118.50 (C-ar.), 119.31, 119.39 (CH-ar.), 124.63

(CH-ar.), 125.46 (C-ar.), 127.42 (CH-ar.), 127.84 (C-ar.), 128.23 (C-ar.), 128.90 (CH-ar.), 130.54 (CH-ar.), 133.37 (C-ar.), 134.62 (C-ar.), 162.11 (CO–NH), 170.36 (CO-sp.) ppm.

5-Chloro-*N*-(2,7-dimethyl-3-oxo-1-thia-4-azaspiro[4.5]decan-4-yl)-3-phenyl-1*H*-indole-2-carboxamide (5c, $C_{25}H_{26}ClN_3O_2S$) White crystals; yield: 86.0%; m.p.: 262–264 °C; R_f = 0.65 (EtOAc/$CHCl_3$ 1:1); IR (KBr): $\bar{\nu}$ = 3235 (N–H), 1694, 1661 (C=O) cm^{-1}; ^{1}H NMR (DMSO-d_6, 500 MHz): δ = 0.75–0.80 (1H, m, CH/CH_2-sp.), 0.87 (3H, d, J = 6.0 Hz, 7-CH_3-sp.), 1.23–1.68 (8H, m, CH/CH_2-sp.), 1.41 (3H, d, J = 6.3 Hz, 2-CH_3-sp.), 3.88 (1H, s*, S–CH-sp.), 7.29 (1H, dd, J = 8.6, 2.0 Hz, H6-ind.), 7.37 (1H, tt, J = 7.3, 1.5 Hz, 3-C_6H_5(H4)-ind.), 7.45–7.49 (3H, m, H4, 3-C_6H_5(H3,H5)-ind.), 7.52-7.54 (3H, m, H7, 3-C_6H_5(H2,H6)-ind.), 10.07 (1H, s, CONH), 12.12 (1H, s, NH) ppm; ^{13}C NMR (APT, DMSO-d_6, 125 MHz): δ = 20.01, 20.03 (2-CH_3-sp.), 22.38, 22.48 (7-CH_3-sp.), 22.72, 23.12 (CH_2-sp.), 29.86, 30.25 (C7-sp.), 32.98, 36.66 (CH_2-sp.), 37.27, 37.38 (C2-sp.), 37.71, 45.41, 46.10 (CH_2-sp.), 71.22, 71.26 (C5-sp.), 114.55, 114.63 (CH-ar.), 118.52 (C-ar.), 119.29, 119.39 (CH-ar.), 124.56, 124.65 (CH-ar.), 125.45 (C-ar.), 127.42 (CH-ar.), 127.88 (C-ar.), 128.25 (C-ar.), 128.90 (CH-ar.), 130.55 (CH-ar.), 133.43 (C-ar.), 134.62 (C-ar.), 162.14 (CO–NH), 170.30 (CO-sp.) ppm; MS (ESI-): *m/z* (%) = 466.7 ([M-H]$^-$, 100), 468.5 ([(M-H) + 2]$^-$, 33.4).

5-Chloro-*N*-(2,8-dimethyl-3-oxo-1-thia-4-azaspiro[4.5]decan-4-yl)-3-phenyl-1*H*-indole-2-carboxamide (5d, $C_{25}H_{26}ClN_3O_2S$) White crystals; yield: 95.1%; m.p.: 223-225.5 °C; R_f = 0.65 (EtOAc/$CHCl_3$ 1:1); IR (KBr): $\bar{\nu}$ = 3337, 3262 (N–H), 1694, 1670 (C=O) cm^{-1}; ^{1}H NMR (DMSO-d_6, 500 MHz): δ = 0.89, 0.96 (3H, 2d, J = 6.4 Hz, 8-CH_3-sp.), 1.03-1.36 (3H, m, CH/CH_2-sp.), 1.41 (3H, d, J = 6.8 Hz, 2-CH_3-sp.), 1.50-1.74 (6H, m, CH/CH_2-sp.), 3.87 (1H, q*, J = 6.8 Hz, S–CH-sp.), 7.30, 7.31 (1H, 2dd, J = 8.8, 2.0 Hz, H6-ind.), 7.37 (1H, tt, J = 7.3, 1.5 Hz, 3-C_6H_5(H4)-ind.), 7.46 (2H, t, J = 7.3 Hz, 3-C_6H_5(H3,H5)-ind.), 7.50 (1H, d, J = 2.0 Hz, H4-ind.), 7.52–7.58 (3H, m, H7, 3-C_6H_5(H2,H6)-ind.), 10.10 (1H, s, CONH), 12.13 (1H, s, NH) ppm; ^{13}C NMR (proton decoupled and DEPT, DMSO-d_6, 75 MHz): δ = 20.24 (2-CH_3-sp.), 22.25 (8-CH_3-sp.), 30.71 (C8-sp.), 31.60, 32.02, 36.91 (CH_2-sp.), 37.10 (C2-sp.), 37.78 (CH_2-sp.), 71.05 (C5-sp.), 114.50 (CH-ar.), 118.43 (C-ar.), 119.26 (CH-ar.), 124.52 (CH-ar.), 125.38 (C-ar.), 127.27 (CH-ar.), 127.75 (C-ar.), 128.08 (C-ar.), 128.76 (CH-ar.), 130.41 (CH-ar.), 133.27 (C-ar.), 134.53 (C-ar.), 161.98 (CO–NH), 170.33 (CO-sp.) ppm.

5-Chloro-*N*-(8-ethyl-2-methyl-3-oxo-1-thia-4-azaspiro[4.5]decan-4-yl)-3-phenyl-1*H*-indole-2-carboxamide (5e, $C_{26}H_{28}ClN_3O_2S$) White needles; yield: 87.2%; m.p.: 198–200 °C; R_f = 0.64 (EtOAc/$CHCl_3$ 1:1); IR (KBr): $\bar{\nu}$ = 3383 (N–H), 1711, 1653 (C=O) cm^{-1}; ^{1}H NMR (DMSO-d_6, 500 MHz): δ = 0.89 (3H, t, J = 7.3 Hz, 8-$CH_2C\underline{H}_3$-sp.), 1.02–1.24 (5H, m, CH/CH_2-sp., 8-$C\underline{H}_2CH_3$-sp.), 1.41 (3H, d, J = 6.8 Hz, 2-CH_3-sp.), 1.62-1.88 (6H, m, CH/CH_2-sp.), 3.87 (1H, q*, J = 6.3 Hz, S–CH-sp.), 7.30 (1H, dd, J = 8.8, 2.0 Hz, H6-ind.), 7.37 (1H, tt, J = 7.8, 1.5 Hz, 3-C_6H_5(H4)-ind.), 7.46 (2H, t, J = 7.8 Hz, 3-C_6H_5(H3,H5)-ind.), 7.51 (1H, d, J = 2.0 Hz, H4-ind.), 7.52–7.56 (3H, m, H7, 3-C_6H_5(H2,H6)-ind.), 10.13 (1H, s, CONH), 12.13 (1H, s, NH) ppm; ^{13}C NMR (APT, DMSO-d_6, 125 MHz): δ = 11.92 (8-$CH_2\underline{C}H_3$-sp.), 20.35 (2-CH_3-sp.), 29.23, 29.67, 36.98 (CH_2-sp., 8-$\underline{C}H_2CH_3$-sp.), 37.14, 37.27 (C2-sp.), 37.42 (C8-sp.), 37.85 (CH_2-sp., 8-$\underline{C}H_2CH_3$-sp.), 71.42, 71.71 (C5-sp.), 114.55, 114.61 (CH-ar.), 118.50 (C-ar.), 119.32, 119.39 (CH-ar.), 124.56, 124.64 (CH-ar.), 125.46 (C-ar.), 127.39 (CH-ar.), 127.85 (C-ar.), 128.24 (C-ar.), 128.88 (CH-ar.), 130.51 (CH-ar.), 133.39 (C-ar.), 134.60 (C-ar.), 162.07 (CO–NH), 170.41 (CO-sp.) ppm.

5-Chloro-*N*-(2-methyl-8-propyl-3-oxo-1-thia-4-azaspiro[4.5]-decan-4-yl)-3-phenyl-1*H*-indole-2-carboxamide (5f, $C_{27}H_{30}ClN_3O_2S$) Beige crystals; yield: 89.1%; m.p.: 194–197 °C; R_f = 0.67 (EtOAc/$CHCl_3$ 1:1); IR (KBr): $\bar{\nu}$ = 3287, 3184 (N–H), 1713, 1692, 1653 (C=O) cm^{-1}; ^{1}H NMR (DMSO-d_6, 500 MHz): δ = 0.88 (3H, t, J = 7.3 Hz, 8-$CH_2CH_2C\underline{H}_3$-sp.), 1.00–1.34 (7H, m, CH/CH_2-sp., 8-$C\underline{H}_2C\underline{H}_2CH_3$-sp.), 1.42 (3H, d, J = 6.8 Hz, 2-CH_3-sp.), 1.60–1.84 (6H, m, CH/CH_2-sp.), 3.89 (1H, d*, J = 5.8 Hz, S–CH-sp.), 7.30 (1H, dd, J = 8.8, 2.0 Hz, H6-ind.), 7.36 (1H, tt, J = 7.3, 1.5 Hz, 3-C_6H_5(H4)-ind.), 7.45 (2H, t, J = 7.3 Hz, 3-C_6H_5(H3,H5)-ind.), 7.50 (1H, d, J = 2.0 Hz, H4-ind.), 7.52-7.56 (3H, m, H7, 3-C_6H_5(H2,H6)-ind.), 10.13 (1H, s, CONH), 12.13 (1H, s, NH) ppm; ^{13}C NMR (APT, DMSO-d_6, 125 MHz): δ = 14.57 (8-$CH_2CH_2\underline{C}H_3$-sp.), 20.00 (CH_2-sp., 8-$\underline{C}H_2\underline{C}H_2CH_3$-sp.), 20.31 (2-$CH_3$-sp.), 29.62, 30.05 ($CH_2$-sp., 8-$\underline{C}H_2\underline{C}H_2CH_3$-sp.), 35.26 (C8-sp.), 37.00 (CH_2-sp., 8-$\underline{C}H_2\underline{C}H_2CH_3$-sp.), 37.16, 37.29 (C2-sp.), 37.86, 38.79 (CH_2-sp., 8-$\underline{C}H_2\underline{C}H_2CH_3$-sp.), 71.39, 71.75 (C5-sp.), 114.53, 114.60 (CH-ar.), 118.50 (C-ar.), 119.32, 119.39 (CH-ar.), 124.55, 124.64 (CH-ar.), 125.47, 125.52 (C-ar.), 127.36 (CH-ar.), 127.66 (C-ar.), 128.27 (C-ar.), 128.84 (CH-ar.), 130.55 (CH-ar.), 133.32, 133.43 (C-ar.), 134.61, 134.66 (C-ar.), 162.08 (CO–NH), 170.37, 170.41 (CO-sp.) ppm; MS (ESI-): *m/z* (%) = 494.7 ([M − H]$^-$, 100), 496.4 ([(M − H) + 2]$^-$, 34.2).

5-Chloro-*N*-(2,7,7,9-tetramethyl-3-oxo-1-thia-4-azaspiro[4.5]-decan-4-yl)-3-phenyl-1*H*-indole-2-carboxamide (5g, $C_{27}H_{30}ClN_3O_2S$) White crystals; yield: 75.5%; m.p.: 266-268 °C; R_f = 0.68 (EtOAc/$CHCl_3$ 1:1); IR (KBr): $\bar{\nu}$ = 3287 (N–H), 1705, 1655 (C=O) cm^{-1}; ^{1}H NMR (DMSO-d_6, 500 MHz): δ = 0.76 (1H, t, J = 12.7 Hz, CH/CH_2-sp.), 0.90

(6H, s*, 7-$(CH_3)_2$-sp.), 1.03 (3H, d, $J=6.4$ Hz, 9–CH_3-sp.), 1.24–1.42 (2H, m, CH/CH_2-sp.), 1.38 (3H, d, $J=6.8$ Hz, 2-CH_3-sp.), 1.62–1.80 (4H, m, CH/CH_2-sp.), 3.87 (1H, s*, S–CH-sp.), 7.29, 7.30 (1H, 2dd, $J=8.8$, 2.0 Hz, H6-ind.), 7.38 (1H, t*, $J=7.3$ Hz, 3-C_6H_5(H4)-ind.), 7.45–7.48 (3H, m, H4, 3-C_6H_5(H3,H5)-ind.), 7.50–7.54 (3H, m, H7, 3-C_6H_5(H2,H6)-ind.), 10.02 (1H, s, CONH), 12.13, 12.17 (1H, 2 s, NH) ppm; ^{13}C NMR (APT, DMSO-d_6, 125 MHz): $\delta=20.05$ (2-CH_3-sp.), 22.23, 25.68, 26.21, 27.69 (7-$(CH_3)_2$, 9-CH_3-sp.), 32.27 (CH_2-sp.), 36.84, 36.96 (C9-sp.), 37.46, 37.57 (C2-sp.), 45.22, 46.80, 47.29 (CH_2-sp., C7-sp.), 70.59 (C5-sp.), 114.56, 114.63 (CH-ar.), 118.52 (C-ar.), 119.29, 119.38 (CH-ar.), 124.58, 124.67 (CH-ar.), 125.44 (C-ar.), 127.44 (CH-ar.), 127.98 (C-ar.), 128.18, 128.24 (C-ar.), 128.97 (CH-ar.), 130.54 (CH-ar.), 133.46, 133.48 (C-ar.), 134.61 (C-ar.), 162.44 (CO–NH), 170.24 (CO-sp.) ppm; MS (ESI+): *m/z* (%) = 497.1 ($[M+H]^+$, 31.2), 426.3 ($[(M+H)+2]^+$, 10.7).

5-Chloro-*N*-(2-methyl-8-phenyl-3-oxo-1-thia-4-azaspiro[4.5]-decan-4-yl)-3-phenyl-1*H*-indole-2-carboxamide (5h, $C_{30}H_{28}ClN_3O_2S$) Beige powder; yield: 82.3%; m.p.: 213–215 °C; $R_f=0.69$ (EtOAc/$CHCl_3$ 1:1); IR (KBr): $\bar{\nu}=3289$ (N–H), 1705, 1663 (C=O) cm^{-1}; ^{1}H NMR (DMSO-d_6, 500 MHz): $\delta=1.45$ (3H, d, $J=6.8$ Hz, 2-CH_3-sp.), 1.58-2.00 (8H, m, CH_2-sp.), 2.45-2.51 (2H, m, CH-sp., DMSO-d6), 3.92 (1H, d*, $J=5.6$ Hz, S–CH-sp.), 7.20 (1H, tt*, $J=7.3$, 1.5 Hz, 8-C_6H_5(H4)-sp.), 7.25 (2H, d*, $J=6.8$ Hz, 8-C_6H_5-sp.), 7.30-7.34 (3H, m, H6-ind., 8-C_6H_5-sp.), 7.42 (1H, tt*, $J=7.3$, 1.5 Hz, 3-C_6H_5(H4)-ind.), 7.51-7.61 (6H, m, H4, H7, 3-C_6H_5(H2,H3,H5,H6)-ind.), 10.21 (1H, s, CONH), 12.18 (1H, s, NH) ppm; ^{13}C NMR (APT, DMSO-d_6, 125 MHz): $\delta=20.03$ (2-CH_3-sp.), 30.76, 31.24 (CH_2-sp.), 37.38 (C2-sp.), 38.10 (CH_2-sp.), 41.95 (C8-sp.), 70.78 (C5-sp.), 114.57, 114.63 (CH-ar.), 118.61 (C-ar.), 119.34, 119.43 (CH-ar.), 124.59, 124.69 (CH-ar.), 125.49 (C-ar.), 126.97 (8-ar.(CH)-sp.), 127.08 (CH-ar.), 127.43 (8-ar.(CH)-sp.), 127.91 (C-ar.), 128.27 (C-ar.), 128.95 (CH-ar.), 130.63 (CH-ar.), 133.45 (C-ar.), 134.63 (C-ar.), 146.28 (8-ar.(C)-sp.), 162.14 (CO–NH), 170.47 (CO-sp.) ppm; MS (ESI-): *m/z* (%) = 528.8 ($[M-H]^-$, 100), 530.8 ($[(M-H)+2]^-$, 33.8).

Antitubercular activity assays

The microdilution method was performed according to a standard protocol from the Clinical and Laboratory Standard Institute (CLSI) [27, 28]. The resazurin microtitre assay (REMA) has been developed as a colorimetric and standard method for drug susceptibility testing. The minimum inhibitory concentrations (MICs) were determined according to color changes at the end of incubation [29–31]. The strain used, i.e., *Mycobacterium tuberculosis* H37Rv ATCC 27294 is susceptible to all common antimycobacterial drugs.

Middlebrook 7H9 broth medium (Becton and Dickinson, USA) was used and the medium was adjusted to pH 7.0 at 25 °C. Each bottle was controlled for sterility before it was used. Resazurin purchased from Sigma-Aldrich (St Louis, USA) was dissolved in sterile distilled water to a final concentration of 0.02% and sterilized by filtration, then stored at 4 °C until use. Rifampicin purchased from Becton–Dickinson (BD, USA) was dissolved in sterile distilled water to a final concentration of 1 μg/cm^3 (critical concentration). The synthesized compounds were dissolved in 100% dimethyl sulfoxide according to CLSI methods [27, 28]. Stock solutions were obtained by 40-fold dilution in DMSO followed by sterile filtration. From here, working stocks at 4000 μM were obtained by diluting 1/10 in MB7H9 medium. The final concentrations were 1000 μM to 0.49 μM for the synthesized compounds. For rifampicin, the critical concentration (1 μg/cm^3) was used [27, 28].

Inoculum suspensions of mycobacteria were prepared according to the CLSI guidelines as described previously. The isolates were subcultured on Löwenstein Jensen medium and incubated at 37 °C for 20–25 days. A few colonies from freshly grown *M. tuberculosis* were suspended in Middlebrook 7H9 broth medium to obtain 1.0 McFarland turbidity, then diluted ten times with the same medium.

The broth microdilution test was performed in sterile 96-well U-shaped microdilution plates (LP Italiano SPA, Milano, Italy). Rows A–F contained 100 mm^3 of the compound dilutions, whereas rows G (positive control) and H (negative control) contained 100 mm^3 medium. One hundred mm^3 of the corresponding inoculum was added to all wells except for row H. The microplates were incubated at 35 °C for about 7–10 days, when mycobacterial growth was clearly visible as a white sediment in the positive control. Microbial growth was confirmed by Ehrlich–Ziehl–Neelsen acid-fast stain. Resazurin solution (30 mm^3) was added to each well and the plates were incubated for one additional day. At that time, the first purple colored well in which no growth was visible, was defined as the compounds' MIC value (Table 1).

Antiviral activity assays

Stock solutions of the test and reference compounds were prepared in 100% DMSO at 5–25 mM. During incubation with the cells, the highest test concentration was 100 μM (or 250 μM for ribavirin). The antiviral reference compounds were: ganciclovir, brivudin, zanamivir, amantadine, ribavirin, dextran sulfate-10,000, and mycophenolic acid. Antiviral evaluation was carried out with a broad panel of viruses using cytopathic effect (CPE) reduction assays. Human influenza A (H1N1 and H3N2) and B viruses were examined on Madin–Darby canine kidney (MDCK) cells. Respiratory syncytial virus, vesicular stomatitis virus and Coxsackie B4 virus were evaluated on human cervix carcinoma

HeLa cells. African Green Monkey Vero cells were used for parainfluenza-3 virus, reovirus-1, Sindbis virus, Coxsackie B4 virus, Punta Toro virus and yellow fever virus. Human embryonic lung (HEL) fibroblast cells were infected with herpes simplex virus types 1 and 2, vaccinia virus, human adenovirus-2, and human coronavirus 229E.

Semiconfluent cell cultures in 96-well plates were infected with virus at a multiplicity of infection of 100 $CCID_{50}$ (50% cell culture infective dose) per well. Together with the virus, fourfold dilutions of the test or reference compounds were added. The plates were incubated at 37 °C (or 35 °C for influenza- and coronavirus) until far advanced CPE was visible, i.e., during 3–6 days or 10 days in the case of adenovirus-2. Then, microscopy was performed to score the CPE and calculate the 50% antivirally effective concentration (EC_{50}). Microscopy was also done to assess cytotoxicity, expressed as the compound concentration causing minimal changes in cell morphology (minimal cytotoxic concentration; MCC).

Antibacterial and antifungal activity assays

Antimicrobial activity against *Pseudomonas aeruginosa* ATCC 27853, *Escherichia coli* ATCC 25922, *Klebsiella pneumoniae* ATCC 4352, *Proteus mirabilis* ATCC 14153, *Staphylococcus aureus* ATCC 29213, *Staphylococcus epidermidis* ATCC 12228, *Enterococcus faecalis* ATCC 29212, *Candida albicans* ATCC 10231, *Candida parapsilosis* ATCC 22019, and *Candida tropicalis* ATCC 750 was determined by the microbroth dilutions technique using CLSI recommendations [32, 33]. Serial twofold dilutions ranging from 2500 µM to 1.22 µM were prepared in the test medium, i.e., Mueller–Hinton broth for bacteria and RPMI-1640 medium for yeast strains. The inoculum was prepared using a 4–6 h broth culture of each bacteria type and 24 h culture of yeast strains adjusted to a turbidity equivalent to 0.5 McFarland standard, diluted in broth media to give a final concentration in the test tray of 5×10^5 cfu/cm^3 for bacteria and 5×10^3 cfu/cm^3 for yeast. The trays were covered and placed into plastic bags to prevent evaporation. The bacteria trays were incubated at 35 °C for 18–20 h while the yeast-containing trays were incubated at 35 °C for 46–50 h. The MIC was defined as the lowest concentration of compound giving complete inhibition of visible growth. Amikacin and fluconazole were used as reference antibiotics for bacteria and yeast, respectively; their MIC values were within the accuracy range of the CLSI guidelines [34].

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