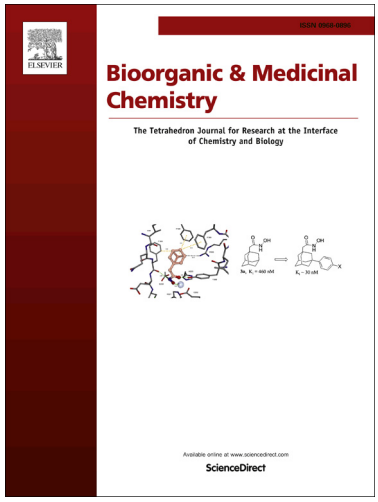
Accepted Manuscript



Ring-substituted 8-hydroxyquinoline-2-carboxanilides as potential antimycobacterial agents

Jiri Kos, Iveta Zadrazilova, Eoghan Nevin, Michal Soral, Tomas Gonec, Peter Kollar, Michal Oravec, Aidan Coffey, Jim O'Mahony, Tibor Liptaj, Katarina Kralova, Josef Jampilek

PII: S0968-0896(15)00541-6
DOI: http://dx.doi.org/10.1016/j.bmc.2015.06.047
Reference: BMC 12403

To appear in: *Bioorganic & Medicinal Chemistry*

Received Date: 16 April 2015
Revised Date: 13 June 2015
Accepted Date: 18 June 2015

Please cite this article as: Kos, J., Zadrazilova, I., Nevin, E., Soral, M., Gonec, T., Kollar, P., Oravec, M., Coffey, A., O'Mahony, J., Liptaj, T., Kralova, K., Jampilek, J., Ring-substituted 8-hydroxyquinoline-2-carboxanilides as potential antimycobacterial agents, *Bioorganic & Medicinal Chemistry* (2015), doi: http://dx.doi.org/10.1016/j.bmc.2015.06.047

Ring-substituted 8-hydroxyquinoline-2-carboxanilides as potential antimycobacterial agents

Jiri Kos[a], Iveta Zadrazilova[a], Eoghan Nevin[b], Michal Soral[c], Tomas Gonec[a], Peter Kollar[d], Michal Oravec[e], Aidan Coffey[b], Jim O'Mahony[b], Tibor Liptaj[c], Katarina Kralova[f], Josef Jampilek[a,*]

[a]*Department of Chemical Drugs, Faculty of Pharmacy, University of Veterinary and Pharmaceutical Sciences, Palackeho 1/3, 612 42 Brno, Czech Republic*

[b]*Department of Biological Sciences, Cork Institute of Technology, Bishopstown, Cork, Ireland*

[c]*Department of NMR Spectroscopy and Mass Spectrometry, Faculty of Chemical and Food Technology, Slovak University of Technology in Bratislava, Radlinskeho 9, 812 37 Bratislava, Slovakia*

[d]*Department of Human Pharmacology and Toxicology, Faculty of Pharmacy, University of Veterinary and Pharmaceutical Sciences, Palackeho 1/3, 612 42 Brno, Czech Republic*

[e]*Global Change Research Centre AS CR, Belidla 986/4a, 603 00 Brno, Czech Republic*

[f]*Institute of Chemistry, Faculty of Natural Sciences, Comenius University, Mlynska dolina Ch-2, 842 15 Bratislava, Slovakia*

* Corresponding author: E-mail: josef.jampilek@gmail.com

Abstract:

In this study, a series of twenty-two ring-substituted 8-hydroxyquinoline-2-carboxanilides was prepared and characterized. Primary *in vitro* screening of the synthesized compounds was performed against *Mycobacterium tuberculosis* H37Ra, *M. avium* complex and *M. avium* subsp. *paratuberculosis.* Some of the tested compounds showed the antimycobacterial activity against *M. avium* subsp. *paratuberculosis* comparable with or higher than that of rifampicin. 8-Hydroxy-*N*-[3-(trifluoromethyl)phenyl]- and 8-hydroxy-*N*-[4-(trifluoromethyl)phenyl]quinoline-2-carboxamide showed MIC = 24 μM against all tested mycobacterial strains. 3-Methoxyphenyl- and 3-methylphenyl derivatives expressed MIC = 27 or 29 μM also against all the tested strains. Their activity against *M. avium* subsp. *paratuberculosis* was 4-fold higher than that of rifampicin. 2-Bromophenyl- and 2-(trifluoromethyl)phenyl derivatives had MIC = 23 or 24 μM against *M. tuberculosis*. A significant decrease of mycobacterial cell metabolism (viability of *M. tuberculosis* H37Ra) was observed using MTT assay. Screening of cytotoxicity of the compounds was performed using the THP-1 cells, and no significant lethal effect was observed up to tested concentration 30 μM. The structure-activity relationships are discussed.

Keywords: 8-hydroxyquinolines; *in vitro* antimycobacterial activity; MTT assay; *in vitro* cytotoxicity; structure-activity relationships.

1. Introduction

Tuberculosis (TB) remains one of the world's deadliest communicable diseases. *Mycobacterium tuberculosis*, the pathogen responsible for TB, uses diverse strategies to survive in a variety of host lesions and to evade immune surveillance. In 2013, an estimated 9.0 million people developed TB and 1.5 million died from the disease, 360 000 of whom were HIV-positive. Multidrug-resistant TB strains (MDR-TB) are a great worldwide problem. Drug resistance surveillance data indicate that in 2013 approximately 480 000 people developed MDR-TB worldwide. Among TB patients reported by national TB programmes in 2013, there were an estimated 300 000 cases of MDR-TB. Extensively drug-resistant TB (XDR-TB, defined as MDR-TB plus resistance to any fluoroquinolone and any second-line injectable drugs) has been identified in 100 countries globally in 2013, and the average proportion of MDR-TB cases with XDR-TB was 9.0%. Also non-tuberculous mycobacteria (NTM), for example, *M. avium*, *M. kansasii*, *M. abscessus*, *M. fortuitum*, *M. chelonae*, *M. smegmatis*, which become the main human pathogens and cause difficult-to-treat or incurable diseases ending in death of mainly HIV-positive patients, are a threat. NTM can cause a broad spectrum of diseases, such as pulmonary disease, lymphadenitis, skin and soft tissue disease, gastrointestinal and skeletal infections. The number of infections that can be associated with specific species as well as the number of new species as etiological agents has exploded in the last few years due to the increase in the number of immunocompromised patients. The emergence of MDR-TB and NTM makes the discovery of new molecular scaffolds a priority to achieve effective control of both TB and NTM.[1–4]

The quinoline nucleus can be considered as a privileged structure;[5] quinoline-based compounds exhibit various activities such as anti-inflammatory, cardiovascular, anticonvulsant, antiproliferative, antiprotozoan, antifungal and antibacterial.[6–12] Quinoline analogues[6–8,13,14] and especially 8-hydroxyquinolines have been also recognized as promising antimycobacterial agents.[15–18] The best known quinoline anti-TB derivative, bedaquiline (Sirturo™) belonging to diarylquinolines, a new class of antitubercular drugs, was approved by FDA for treatment of MDR-TB.[19] Structural modifications of quinolines by various groups have led to the identification of potent anti-TB compounds exhibiting significant activity especially against strains of *M. tuberculosis*.[13,14,20-29] It was found that these anti-TB quinolines exhibit various mechanisms of action; for example, they inhibit mycobacterial ATP synthase,[23,30] mycobacterial FtsZ protein,[31] glutathione *S*-transferase,[32] enoyl-ACP reductase[20,21,33] or decaprenylphosphoryl-β-D-ribose-2′-epimerase;[24] also other sites of actions

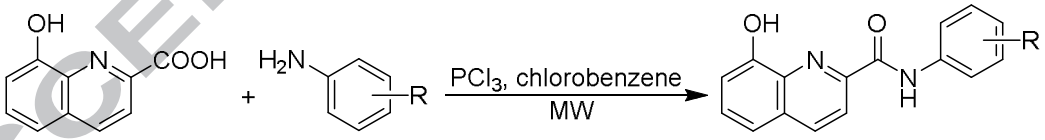
of 8-hydroxyquinolines can be found due to the bidentate metal-chelating property of 8-hydroxyquinolines.[15–18]

The aim of this contribution is synthesis and antimycobacterial screening of 8-hydroxyquinoline-2-carboxanilides with following analysis of the structure-activity relationships as a continuation of the investigation of recently designed ring-substituted quinoline analogues.[9-11,34-37] Based on the above mentioned facts all the presented compounds were designed to bear an 8-hydroxyquinoline fragment and an amide moiety. Both structural features are important functional groups that are able to interact with a number of enzymes/receptors via hydrogen bonds and in this manner to affect the biological response.[38-40]

2. Results and Discussion

2.1 Chemistry and physicochemical properties

All studied compounds **1**–**8c** were prepared according to Scheme 1 by modified microwave-assisted (MW) synthesis;[41,42] thus the synthesis of the target compounds was carried out in only one step with yields 66–76%. At first the carboxyl group was activated with phosphorus trichloride. The final amide was immediately formed by aminolysis of the acyl chloride by ring-substituted aniline in dry chlorobenzene. All the crude target compounds were recrystallized from ethanol.



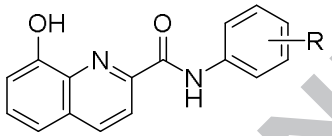
R = H (**1**), OCH_3 (**2a-c**), CH_3 (**3a-c**), F (**4a-c**), Cl (**5a-c**), Br (**6a-c**), CF_3 (**7a-c**), NO_2 (**8a-c**)

Scheme 1. Synthesis of ring-substituted 8-hydroxyquinoline-2-carboxanilides **1**–**8c**

Within structure-activity relationship studies various parameters describing physicochemical properties are investigated. Lipophilicity is a property that has a major effect on solubility, absorption, distribution and biotransformation as well as pharmacological activity, because drugs cross biological membranes through passive transport, which strongly depends on their lipophilicity. Lipophilicity of the studied compounds was determined

by RP-HPLC as capacity factor logarithm (log *k*) and calculated as log *P* using ACD/Percepta ver. 2012 (Advanced Chemistry Development, Inc., Toronto, ON, Canada, 2012). The corresponding values of ring-substituted 8-hydroxyquinoline-2-carboxanilides **1**–**8c** are shown in Table 1.

Table 1. Structure of discussed 8-hydroxyquinoline-2-carboxanilides **1**–**8c**, experimentally determined values of lipophilicity log *k*, predicted values of compound lipophilicity log *P* and electronic Hammett's σ parameters of R substituents, *in vitro* antimycobacterial activity (MIC) of compounds in comparison with rifampicin (RIF) standard and *in vitro* cytotoxicity assay (IC_{50}) of selected compounds.



Comp.	R	log *k*	log *P*[a]	σ[a]	MIC [μM]			IC_{50} [μM]
					MT	MAC	MAP	
1	H	0.7600	2.55	0	30	30	30	>30
2a	2-OCH_3	0.7935	2.67	-0.28	27	51	51	>30
2b	3-OCH_3	0.8164	2.61	0.12	27	27	27	>30
2c	4-OCH_3	0.7129	2.51	-0.27	849	425	849	>30
3a	2-CH_3	0.6944	2.90	-0.17	29	54	29	>30
3b	3-CH_3	0.9686	2.90	-0.07	29	29	29	>30
3c	4-CH_3	0.9521	2.90	-0.17	29	54	29	>30
4a	2-F	0.6806	2.59	0.06	28	53	28	>30
4b	3-F	0.9420	2.76	0.34	53	53	53	>30
4c	4-F	0.8598	2.59	0.06	53	53	53	>30
5a	2-Cl	0.9566	3.07	0.22	27	837	27	>30
5b	3-Cl	1.1718	3.28	0.37	50	50	50	>30
5c	4-Cl	1.1543	3.05	0.23	837	50	837	>30
6a	2-Br	1.0536	3.16	0.22	23	729	23	>30
6b	3-Br	1.2357	3.31	0.39	44	44	44	>30
6c	4-Br	1.2347	3.19	0.23	729	44	729	>30
7a	2-CF_3	0.9147	3.36	0.51	24	752	24	>30
7b	3-CF_3	1.3206	3.44	0.43	24	24	24	>30
7c	4-CF_3	1.3653	3.27	0.51	24	24	24	>30
8a	2-NO_2	1.1277	2.73	0.77	97	808	97	>30
8b	3-NO_2	0.9845	2.56	0.71	26	808	808	>30
8c	4-NO_2	1.0495	2.45	0.78	26	808	404	>30
RIF	–	–	–	–	10	10	109	–

[a]calculated using ACD/Percepta ver. 2012 (Advanced Chemistry Development, Inc., Toronto, ON, Canada, 2012); MT = *M. tuberculosis* H37Ra ATCC 25177, MAC = *M. avium* complex CIT19/06 (clinical isolate) and MAP = *M. avium* subsp. *paratuberculosis* CIT03 (clinical isolate).

The highest experimental lipophilicity was found for 8-hydroxy-*N*-(4-trifluoromethylphenyl)quinoline-2-carboxamide (**7c**), while *N*-(2-fluorophenyl)-8-hydroxyquinoline-2-carboxamide (**4a**) showed the lowest log *k* value. The results obtained with the discussed compounds show that the experimentally-determined lipophilicities (log *k*) of the *ortho*-substituted derivatives showed poor match, while the *meta*- and *para*-substituted compounds showed higher match with the predicted log *P* values. In this case the most significant deviations can be observed for nitro substituted derivatives; when 2-NO_2 (**8a**) is eliminated, correlation coefficient of the dependence of log *k* on log *P* values improves to r = 0.7502 (n = 6) and after elimination of 3-NO_2 (**8b**) and 4-NO_2 (**8c**) correlation coefficient of the dependence of log *k* on log *P* values improves to r = 0.9759 (n = 13), which reflects intra/intermolecular interactions of the nitro moiety. The influence of R substituents on lipophilicity of *ortho*-substituted derivatives is as follows: F < CH_3 < H < OCH_3 < CF_3 < Cl < Br < NO_2; for *meta*- and *para*-substituted derivatives it is as follows: OCH_3 / H < F < CH_3 < NO_2 < Cl < Br < CF_3. Within the individual series the lipophilicity determined by log *k* values increases for halogens, methyl and trifluoromethyl substituents as follows: *ortho* < *para* < *meta*; for methoxy substituent as follows: *para* < *ortho* < *meta*, and for NO_2 as follows: *meta* < *para* < *ortho*.

Similarly poor correlation between experimental and calculated lipophilicity values was found also for different derivatives of 8-hydroquinolines.[9-11,35,36,43] This is probably caused by the fact that between the hydroxyl moiety in $C_{(8)}$ of the quinoline nucleus and nitrogen of quinoline intramolecular H-bond affecting lipophilicity, solubility and basicity/acidity is formed.[44] Based on the presented results, it can be stated that log *k* values specify lipophilicity within individual series of the studied compounds better than log *P* values.

The presence of different substituents in the anilide part of the discussed compounds results in a wide range of electronic properties. Electronic parameters (expressed as Hammett's σ parameters) of compounds **1**–**8c** were predicted using ACD/Percepta software, see Table 1; they ranged from -0.28/-0.27 (compound **2a**,**c** 2-OCH_3, 4-OCH_3) to 0.77/0.78 (compound **8a**,**c** 2-NO_2, 4-NO_2).

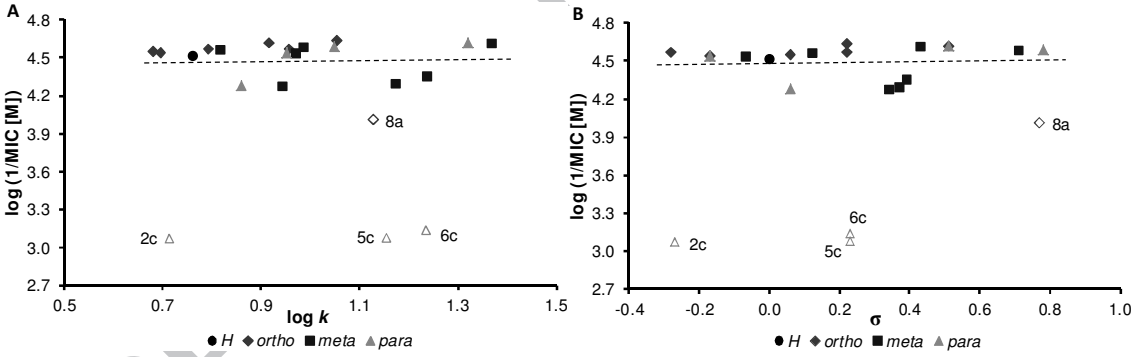
2.2 In vitro antimycobacterial evaluation

The evaluation of the *in vitro* antimycobacterial activity of the compounds was performed against *Mycobacterium tuberculosis* H37Ra ATCC 25177 (MT) and clinical isolates of *M. avium* complex CIT19/06 (MAC) and *M. avium* subsp. *paratuberculosis* CIT03 (MAP), see Table 1. To reduce risk and make manipulation in the laboratory easier, surrogate model pathogens for *M. tuberculosis* can be used in laboratory studies. Avirulent *M. tuberculosis* strain H37Ra is very closely related to human-infecting *M. tuberculosis* strains (e.g., H37Rv), making it a good model for study especially because of the lower risk for laboratory workers.[45] Because of *M. tuberculosis,* the pathogenic role of NTM in humans was overshadowed for a long time. *Mycobacterium avium* complex includes ubiquitous atypical mycobacteria found in the environment that can easily infect immunosuppressed patients and cause disseminated disease, producing fever, sweats, weight loss and anaemia.[46] *M. avium* subsp. *paratuberculosis* is suspected to be a causative agent in gastrointestinal diseases; it is resistant to standard antimycobacterial therapy, but may be susceptible to some standard antibiotics, however, resistance to these antibiotics develops quickly.[47] The activity of compounds was expressed as the minimum inhibitory concentration (MIC) that is defined for mycobacteria as a 90% or greater (IC_{90}) reduction of growth in comparison with the control. The MIC/IC_{90} value is routinely and widely used in bacterial assays and is a standard detection limit according to the Clinical and Laboratory Standards Institute (CLSI).

Most of the target compounds showed activity against all three tested mycobacterial strains. With respect to *M. tuberculosis* a greater number of more efficient compounds showing higher effect than *para*-substituted compounds can be found among *ortho*- and *meta*-substituted derivatives, except for $C'_{(4)}$ substituted derivatives **3c** (R = 4-CH_3), **7c** (R = 4-CF_3) and **8c** (R = 4-NO_2), which activity was comparable with that of *ortho*- and *meta*-substituted compounds. Compounds **6a** (R = 2-Br, MIC = 23 μM), **7a** (R = 2-CF_3, MIC = 24 μM), **7b** (3-CF_3, MIC = 24 μM) and **7c** (4-CF_3, MIC = 24 μM) exhibited the highest activity. The antimycobacterial activity of the compounds against *M. tuberculosis* was expressed as log(1/MIC [M]), and the dependences of the activity on lipophilicity expressed as log *k* and electronic properties expressed as Hammett's σ constants are illustrated in Figures 1A and 1B. When really inactive *para*-substituted compounds **2c**, **5c** and **6c** (R = 4-OCH_3, 4-Cl, 4-Br) are eliminated (marked by empty symbols), it is evident that neither lipophilicity nor electronic properties possess any significant effect on the antitubercular activity. Compounds **4b**,**c** (R = 3-, 4-F), **5b** (R = 3-Cl) and **6b** (R = 3-Br) had slightly lower activity than other 14 illustrated compounds. Similar trends can be found for the dependences of the activity

against *M. avium* subsp. *paratuberculosis* on physicochemical parameters, therefore these dependences are not illustrated. It is important to note that nitro substituted derivatives **8a**–**c** expressed different activity than it could be expected. As mentioned in section 2.1, all nitro derivatives **8a**–**c** showed poor correlation between experimental and calculated lipophilicity values. Especially $C'_{(2)}$ substituted derivative **8a** has the highest experimentally determined lipophilicity (log k = 1.1277) along with the strongest electron-withdrawing properties but the lowest antitubercular activity within the series. Probably the above mentioned interactions of the nitro moiety with the amide bond limited binding of the nitro substituted derivatives to target sites in mycobacterial cells. These interactions are most evident for compound **8a**, therefore this compound is also eliminated (marked by empty symbols in Figures 1A and 1B) from SAR discussion. Similar observations for compound **8a** were made in respect to *M. avium* subsp. *paratuberculosis*.

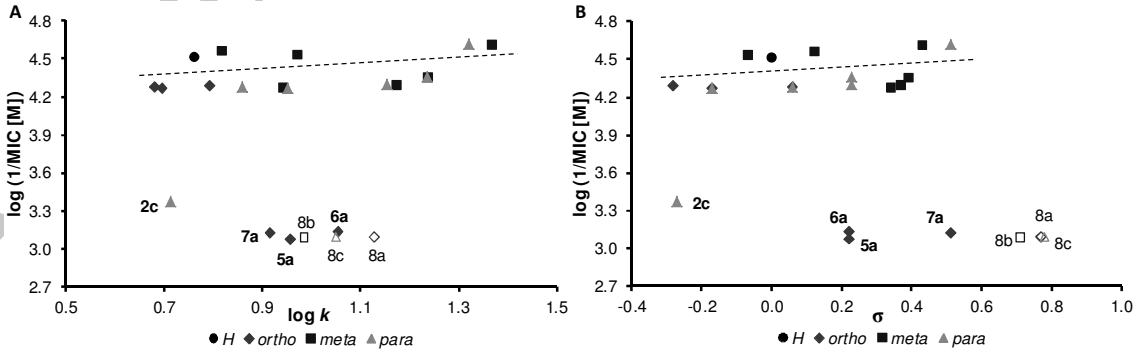
Figure 1. Dependence of antitubercular activity against *M. tuberculosis* expressed as log 1/MIC [M] of tested compounds on lipophilicity expressed as log k (**A**) and on Hammett's σ constants of R substituent (**B**). (Inactive compounds are marked by empty symbols.)



Compounds **6a** (R = 2-Br, MIC = 23 μM), **7b** (R = 3-CF_3, MIC = 24 μM) and **7c** (R = 4-CF_3, MIC = 24 μM) showed the highest activity against *M. avium* subsp. *paratuberculosis*. Based on the results it can be stated that *ortho*- and especially *meta*-substituted derivatives expressed higher activity than *para*-substituted ones. Only **7c** showed high effect within the $C'_{(4)}$ substituted compounds. Similarly as for the previous strain, electronic properties show that the character of individual substituents R does not influence the antimycobacterial activity significantly.

Compounds **7b** (3-CF_3, MIC = 24 μM) and **7c** (4-CF_3, MIC = 24 μM) showed the highest activity against *M. avium* complex. The nitro moiety in all three positions (compounds **8a**-**c**) is the least favoured substituent, therefore these compounds are not considered for SAR discussion. Most effective compounds can be found among *meta*-substituted derivatives. The antimycobacterial activity of the compounds against *M. avium* complex was expressed as log(1/MIC [M]), and the dependences of the activity on lipophilicity expressed as log *k* and electronic properties expressed as Hammett's σ constants are illustrated in Figures 2A and 2B. Generally, it seems that within the whole discussed series of compounds dependences of antimycobacterial activity against *M. avium* complex on lipophilicity and electronic properties increase slightly with increasing of lipophilicity values and electron-withdrawing effect of substituents; although for the *meta*-substituted compounds this dependence is very slight, see Figures 2A and 2B. The significant dependence of antimycobacterial activity on lipophilicity and electronic properties of the *ortho*- and *para*-substituents can be observed. The *ortho*-substituted derivatives are completely ineffective when lipophilicity (log *k*) is >0.8 along with Hammett's σ constants of R substituents >0.1 (see compounds **5a**, **6a**, **7a**), contrary to the properties of the *para*-substituted compounds that are ineffective when log *k* <0.8 and σ < -0.2 (compound **2c**), see Figures 2A and 2B.

Figure 2. Dependence of antimycobacterial activity against *M. avium* complex expressed as log 1/MIC [M] of tested compounds on lipophilicity expressed as log *k* (**A**) and on Hammett's σ constants of R substituent (**B**). (Compounds **8a**–**c** not included in SAR discussion are marked by empty symbols.)



Generally all the prepared compounds showed relatively high lipophilicity (log *P* = 2.5–3.4 or log *k* = 0.68–1.36) that enables the compounds to permeate through

hydrophobic mycobacterial cell wall. Compound **2c** (R = 4-OCH_3) was completely inactive against all three mycobacterial strains; compounds **5c**, **6c**, **8b** and **8c** (R = 4-Cl, 4-Br, 3-NO_2, 4-NO_2) were ineffective against two of the tested mycobacterial strains. It can be stated that there is no any significant difference between antimycobacterial activities against *M. tuberculosis* and *M. avium* subsp. *paratuberculosis*, nevertheless *ortho*-substituted derivatives seem to be favourable, see **4a>4b=4c**, **5a>5b>>>5c**, **6a>6b>>>6c**, except **7a** (2-CF_3) and **8a** (2-NO_2) substituted derivatives. On the other hand, the *meta*-substituted derivatives showed the highest potency against *M. avium* complex. When the compounds showed potency against *M. avium* complex, they were effective also against *M. tuberculosis* and *M. avium* subsp. *paratuberculosis*. Thus, it can be postulated that compounds with potency against all three strains were received by the substitution of $C'_{(3)}$ position of aniline. On the other hand also the hydroxyl moiety in $C_{(8)}$ of quinoline seems to play a significant role for activity, because its absence in quinoline-2-carboxanilides led to a decrease of antimycobacterial effect.[37] Similar essential contribution of the hydroxyl moiety amplifying antimycobacterial potency was also observed for 1-hydroxynaphthalene-2-carboxanilides[41] and 6-hydroxynaphthalene-2-carboxanilides[42] *versus* naphthalene-2-carboxanilides.[37]

2.3 MTT assay

Additionally, a standard MTT assay was performed for the selected most effective compounds, the MICs of which were previously determined through alamarBlue assays, see Table 1. The MTT assay is a well-characterized method of assessing cell growth through measurement of respiration. For the purpose of this assay, a measured viability of *M. tuberculosis* H37Ra of less than 70%, as determined by MTT, after exposure to the MIC of each test compound (as determined by the alamarBlue assay) was considered a positive result. As such, a low level of cell viability may suggest inhibition of cell growth through respiratory inhibition.[48] Model compounds selected from 14 derivatives with the highest antitubercular activity, namely 3-methoxy (**2b**, 30.9 %), 3-methyl (**3b**, 66.4 %), 2-fluoro (**4a**, 26.9 %), 2-chloro (**5a**, 21.4 %), 2-bromo (**6a**, 26.9 %), 2-trifluoromethyl (**7a**, 53.4 %), 3-trifluoromethyl (**7b**, 18.8 %), 4-trifluoromethyl (**7c**, 33.1 %), 3-nitro (**8b**, 7.0 %) and 4-nitro (**8c**, 17.3 %) derivatives, showed less than 70% viability of *M. tuberculosis* H37Ra at the lowest tested concentration (8 μg/mL, i.e. *ca.* 26 μM). Similar effect was observed previously with ring-substituted naphthalene-1-carboxanilides[49] where 3-methoxy and 4-methyl derivatives showed more than 80% reduction in activity at the lowest tested concentration

(8 μg/mL, *i.e.*, *ca.* 30 μM) after four hours of incubation, similar to the reduction observed in the rifampicin and ciprofloxacin standards and 2-methoxy and 3-fluoro derivatives achieved similar levels of inhibition at 16 μg/mL (*ca.* 60 μM) concentration.[20,33]

Based on the fact that the change in colour of alamarBlue is caused by a decrease of mycobacterial cell metabolism, it may be hypothesized that the mechanism of action of these ring-substituted 8-hydroxyquinoline-2-carboxanilides could be connected with affection of mycobacterial energy metabolism;[38] nevertheless, another possible site of action of the studied compounds in the mycobacteria cannot be excluded.[15-18]

2.4 In vitro cytotoxicity assay

The preliminary *in vitro* screening of the cytotoxicity of the compounds was performed using the human monocytic leukemia THP-1 cell line. The cytotoxicity was evaluated as the IC_{50} value (compound concentration causing 50% inhibition of cell population proliferation), see Table 1. A compound is considered as cytotoxic when it demonstrates a toxic effect on cells at the concentration up to 10 μM,[50] and the highest tested concentration that was used for the toxicity assay was three times this value. Treatment with 30 μM of the discussed compounds did not lead to significant lethal effect on THP-1 cells. Based on these observations it can be concluded that the discussed amides can be considered as non-toxic agents for subsequent design of novel antimycobacterial agents.

3. Conclusions

A series of twenty-two ring-substituted 8-hydroxyquinoline-2-carboxanilides were prepared by means of microwave-assisted synthesis and subsequently characterized. All the compounds were tested for their *in vitro* antimycobacterial activity against *M. tuberculosis* and clinical isolates of *M. avium* complex and *M. avium* subsp. *paratuberculosis.* Some of the tested compounds showed the antimycobacterial activity against *M. avium* subsp. *paratuberculosis* comparable with or higher than that of rifampicin. 8-Hydroxy-*N*-[3-(trifluoromethyl)phenyl]- (**7b**) and 8-hydroxy-*N*-[4-(trifluoromethyl)phenyl]quinoline-2-carboxamide (**7c**) showed MIC = 24 μM against all three tested mycobacterial strains. 3-Methoxyphenyl- (**2b**) and 3-methylphenyl (**3b**) derivatives expressed MIC = 27 or 29 μM

also against all the tested strains. 2-Bromophenyl- (**6a**) and 2-(trifluoromethyl)phenyl (**7a**) derivatives showed MIC = 23 or 24 μM against *M. tuberculosis*. All the compounds possess high lipophilicity; they can permeate through hydrophobic mycobacterial cell wall. Based on the results it can be postulated that compounds with potency against all three strains were received by the substitution of $C'_{(3)}$ position of aniline. The performed MTT assay of the selected compounds shows that they cause a decrease of mycobacterial cell metabolism; probably they influence the mycobacterial respiratory chain. All the compounds were tested for their *in vitro* cytotoxicity against the THP-1 cells and within this preliminary screening they demonstrated insignificant toxicity up to tested concentration 30 μM, therefore it can be concluded that the discussed anilides can be considered as promising agents for subsequent design of novel antimycobacterial agents.

4. Experimental

4.1. Chemistry

All reagents were purchased from Aldrich (Sigma-Aldrich, St. Louis, MO, USA). TLC experiments were performed on alumina-backed silica gel 40 F254 plates (Merck, Darmstadt, Germany). The plates were illuminated under UV (254 nm) and evaluated in iodine vapour. The melting points were determined on Kofler hot-plate apparatus HMK (Franz Kustner Nacht KG, Dresden, Germany) and are uncorrected. Infrared (IR) spectra were recorded on a Smart MIRacle™ ATR ZnSe for Nicolet™ Impact 410 FT-IR spectrometer (Thermo Electron Corporation, West Palm Beach, FL, USA). The spectra were obtained by accumulation of 256 scans with 2 cm^{-1} resolution in the region of 4000–600 cm^{-1}. All ^{1}H- and ^{13}C-NMR spectra were recorded on an Agilent VNMRS 600 MHz system (Agilent Technologies, Santa Clara, CA, USA) equipped with a triple resonance HCN probe at 25 °C in DMSO-d_6. ^{1}H and ^{13}C chemical shifts and ^{13}C-^{19}F coupling constants were determined from the standard ^{1}H and ^{13}C spectra with digital resolution 0.3 Hz or better. Chemical shifts (δ) are reported in ppm. When necessary, additional experiments were done: ^{13}C-APT (Attached Proton Test) for discrimination between CH and quaternary carbons; DQF COSY, HSQC and HMBC for through-bond ^{1}H-^{1}H and one- and multiple-bond ^{1}H-^{13}C correlations. Mass spectra were measured using a LTQ Orbitrap Hybrid Mass Spectrometer (Thermo Electron Corporation) with direct injection into an APCI source (400 °C) in the negative mode.

General procedure for synthesis of 8-hydroxy-*N*-(substituted-phenyl)quinoline-2-carboxamides 1–8c. 8-Hydroxyquinoline-2-carboxylic acid (5.3 mM) was suspended in dry chlorobenzene (30 mL) at ambient temperature and phosphorus trichloride (2.7 mM, 0.5 eq.), and the corresponding substituted aniline (5.3 mM, 1 eq.) was added dropwise. The reaction mixture was transferred to the microwave reactor, where the synthesis was performed (1st phase: 10 min, 100 °C, 100 W; 2nd phase: 15 min, 120 °C, 500 W; 3rd phase: 20 min, 130 °C, 500 W). Then the mixture was cooled to 50 °C, and then the solvent was removed to dryness under reduced pressure. The residue was washed with hydrochloric acid and water. The crude product was recrystallized from isopropanol. Studied compounds **1**–**8c** are presented in Table 1.

8-Hydroxy-*N*-phenylquinoline-2-carboxamide (**1**). Yield 78%; mp 197 °C; IR (cm^{-1}): 3336, 3046, 1679, 1600, 1575, 1527, 1505, 1461, 1444, 1429, 1389, 1366, 1310, 1287, 1235, 1197, 1129, 1117, 1082, 931, 906, 879, 847, 814, 802, 761, 752, 722, 692; ^{1}H-NMR (DMSO-d_6), δ: 7.183 (tt, 1H, *J*=7.4, 1.1 Hz), 7.228 (dd, 1H, *J*=7.6, 1.2 Hz), 7.447 (m, 2H), 7.524 (dd, 1H, *J*=8.1, 1.2 Hz), 7.608 (t, 1H, *J*=7.9 Hz), 7.901 (m, 2H), 8.272 (d, 1H, *J*=8.5 Hz), 8.571 (d, 1H, *J*=8.5 Hz), 10.404 (s, 1H), 11.171 (s, 1H); ^{13}C-NMR (DMSO-d_6), δ: 112.03, 117.64, 118.95, 120.70 (2C), 124.13, 128.77 (2C), 129.69, 129.72, 136.36, 138.10, 138.25, 147.28, 153.70, 162.09; HR-MS: $C_{16}H_{11}N_2O_2$ $[M-H]^-$ calculated 263.0826 m/z, found 263.0822 m/z.

8-Hydroxy-*N*-(2-methoxyphenyl)quinoline-2-carboxamide (**2a**). Yield 71%; mp 187–189 °C; IR (cm^{-1}): 3446, 3372, 1679, 1601, 1538, 1505, 1485, 1456, 1432, 1326, 1289, 1236, 1198, 1135, 1105, 1083, 1046, 1030, 929, 888, 848, 813, 744, 722, 680; ^{1}H-NMR (DMSO-d_6), δ: 3.349 (s, 3H), 7.027 (td, 1H, *J*=7.7, 1.3 Hz), 7.151 (dd, 1H, *J*=8.3, 1.3 Hz), 7.211 (dd, 1H, *J*=7.6, 1.2 Hz), 7.243(ddd, 1H, *J*=9.1, 7.5, 1.6 Hz), 7.510 (dd, 1H, *J*=8.2, 1.2 Hz), 7.587 (t, 1H, *J*=7.9 Hz), 7.800 (dd, 1H, *J*=7.8, 1.6 Hz), 8.221 (d, 1H, *J*=8.5 Hz), 8.544 (d, 1H, *J*=8.5 Hz), 10.374 (s, 1H), 10.885 (s, 1H); ^{13}C-NMR (DMSO-d_6), δ: 55.66, 111.50, 112.13, 117.65, 118.84, 120.26, 124.95, 126.14, 126.22, 129.56, 129.77, 136.64, 137.95, 147.44, 152.05, 153.78, 162.28; HR-MS: $C_{17}H_{13}N_2O_3$ $[M-H]^-$ calculated 293.0932 m/z, found 293.0928 m/z.

8-Hydroxy-*N*-(3-methoxyphenyl)quinoline-2-carboxamide (**2b**). Yield 69%; mp 199–201 °C; IR (cm^{-1}): 3331, 3307, 1684, 1657, 1605, 1592, 1525, 1504, 1456, 1416, 1391, 1358, 1328, 1286, 1265, 1247, 1224, 1201, 1159, 1081, 1053, 1033, 994, 874, 843, 822, 774, 765, 758, 736, 723, 702, 688; ^{1}H-NMR (DMSO-d_6), δ: 3.813(s, 3H), 6.768 (dd, 1H, *J*=8.3, 2.5 Hz), 7.230 (dd, 1H, *J*=7.6, 1.3 Hz), 7.349 (t, 1H, *J*=8.2 Hz), 7.499 (dm, 1H, *J*=8.1 Hz), 7.524 (d, 1H, *J*=8.2 Hz), 7.577 (t, 1H, *J*=2.2 Hz), 7.609 (t, 1H, *J*=7.9 Hz), 8.269 (d, 1H, *J*=8.5 Hz), 8.572 (d, 1H, *J*=8.5 Hz), 10.413 (s, 1H), 11.133 (s, 1H); ^{13}C-NMR (DMSO-d_6), δ: 55.12, 106.38, 109.60, 112.08, 112.91, 117.66, 118.96, 129.59, 129.71, 129.76, 136.36, 138.13, 139.44, 147.24, 153.71, 159.58, 162.13; HR-MS: $C_{17}H_{13}N_2O_3$ $[M-H]^-$ calculated 293.0932 m/z, found 293.0927 m/z.

8-Hydroxy-*N*-(4-methoxyphenyl)quinoline-2-carboxamide (**2c**). Yield 66%, mp 209 °C; IR (cm^{-1}): 3334w, 3208w, 1675m, 1627m, 1593m, 1528s, 1510s, 1464s, 1410w, 1302w, 1287w, 1230s, 1174m, 1163m, 1110m, 1086m, 1036s, 937w, 855m, 828s, 815s, 762s, 748s, 723s, 677w; ^{1}H-NMR (DMSO-d_6), δ: 3.779(s, 3H), 7.017 (m, 2H), 7.217 (dd, 1H, *J*=7.6, 1.2 Hz), 7.511 (dd, 1H, *J*=8.2, 1.2 Hz), 7.594 (t, 1H, *J*=7.9 Hz), 7.795 (m, 2H), 8.253 (d, 1H, *J*=8.5 Hz), 8.553 (d, 1H, *J*=8.5 Hz), 10.357 (s, 1H), 11.605 (s, 1H); ^{13}C-NMR (DMSO-d_6), δ: 55.22, 111.94, 113.93(2C), 117.63, 118.92, 122.26(2C), 129.61(2C), 131.30, 136.36, 138.04, 147.46, 153.66, 155.89, 161.74; HR-MS: $C_{17}H_{13}N_2O_3$ $[M-H]^-$ calculated 293.0932 m/z; found 293.0925 m/z.

8-Hydroxy-*N*-(2-methylphenyl)quinoline-2-carboxamide (**3a**). Yield 73%; mp 139–140 °C; IR (cm^{-1}): 3177, 3043, 2960, 1661, 1615, 1587, 1538, 1523, 1456, 1428, 1367, 1325, 1247, 1208, 1144, 1106, 1083, 1067, 1050, 933, 882, 846, 814, 758, 710; ^{1}H-NMR (DMSO-d_6), δ: 2.303 (s, 3H), 7.211 (dd, 1H, *J*=7.6, 1.0 Hz), 7.232 (td, 1H, *J*=7.3, 1.2 Hz), 7.291 (t, 1H, *J*=7.5 Hz), 7.347(d, 1H, *J*=7.5 Hz), 7.451 (d, 1H, *J*=7.7 Hz), 7.525(d, 1H, *J*=8.3 Hz), 7.602 (t, 1H, *J*=7.9 Hz), 8.230 (d, 1H, *J*=8.5 Hz), 8.562 (d, 1H, *J*=8.5 Hz), 10.240 (s, 1H), 11.055 (s, 1H); ^{13}C-NMR (DMSO-d_6), δ: 17.96, 111.84, 117.61, 118.91, 126.16, 126.25, 126.49, 129.63, 129.65, 130.43, 133.72, 135.81, 136.44, 137.99, 147.28, 153.64, 162.27; HR-MS: $C_{17}H_{13}N_2O_2$ $[M-H]^-$ calculated 277.0983 m/z, found 277.0977 m/z.

8-Hydroxy-*N*-(3-methylphenyl)quinoline-2-carboxamide (**3b**). Yield 75%; mp 203–204 °C; IR (cm^{-1}): 3342, 3312, 3049, 1686, 1656, 1612, 1538, 1505, 1489, 1462, 1392, 1358, 1327, 1308, 1289, 1224, 1181, 1163, 1117, 1084, 1046, 940, 897, 881, 853, 779, 758, 741, 724, 692; ^{1}H-NMR (DMSO-d_6), δ: 2.368 (s, 3H), 7.000 (dm, 1H, *J*=7.5 Hz), 7.226 (dd, 1H,

J=7.6, 1.2 Hz), 7.321 (t, 1H, *J*=7.7 Hz), 7.520 (dd, 1H, *J*=8.1, 1.2 Hz), 7.606 (t, 1H, *J*=7.9 Hz), 7.720 (s, 1H), 7.729 (d, 1H, *J*=7.7 Hz), 8.265 (d, 1H, *J*=8.5 Hz), 8.568 (d, 1H, *J*=8.5 Hz), 10.407 (s, 1H), 11.107 (s, 1H); ^{13}C-NMR (DMSO-d_6), δ: 21.17, 112.04, 117.64, 117.81, 118.94, 121.17, 124.81, 128.62, 129.68, 129.71, 136.37, 137.99, 138.10, 138.18, 147.33, 153.72, 162.02; HR-MS: $C_{17}H_{13}N_2O_2$ $[M-H]^-$ calculated 277.0983 m/z, found 277.0980 m/z.

8-Hydroxy-*N*-(4-methylphenyl)quinoline-2-carboxamide (**3c**). Yield 68%; mp 208 °C; IR (cm^{-1}): 3457, 3204, 2917, 1683, 1630, 1590, 1527, 1501, 1464, 1404, 1319, 1297, 1260, 1230, 1185, 1163, 1086, 1047, 1019, 980, 931, 889, 851, 809, 761, 749, 724; ^{1}H-NMR (DMSO-d_6), δ: 2.321(s, 3H), 7.221 (dd, 1H, *J*=7.6, 1.3 Hz), 7.248 (m, 2H), 7.521 (dd, 1H, *J*=8.2, 1.2 Hz), 7.604 (t, 1H, *J*=7.9 Hz), 7.779 (m, 2H), 8.259 (d, 1H, *J*=8.5 Hz), 8.556 (d, 1H, *J*=8.5 Hz), 10.382 (s, 1H), 11.110 (s, 1H); ^{13}C-NMR (DMSO-d_6), δ: 20.530, 112.00, 117.64, 118.94, 120.67(2C), 129.18(2C), 129.66, 129.67, 133.16, 135.73, 136.36, 138.08, 147.38, 153.69, 162.92; HR-MS: $C_{17}H_{13}N_2O_2$ $[M-H]^-$ calculated 277.0983m/z; found 277.0988 m/z.

***N*-(2-fluorophenyl)-8-hydroxyquinoline-2-carboxamide** (**4a**). Yield 68%; mp 145 °C; IR (cm^{-1}): 3489, 3376, 1689, 1618, 1594, 1537, 1505, 1455, 1359, 1327, 1290, 1252, 1232, 1194, 1169, 1121, 1083, 1031, 887, 848, 757, 722; ^{1}H-NMR (DMSO-d_6), δ: 11.168 (s, 1H), 10.352 (s, 1H), 8.576 (d, 1H, *J*=8.5 Hz), 8.230 (d, 1H, *J*=8.5 Hz), 7.745 (td, 1H, *J*=7.8, 1.8 Hz), 7.608 (dd, 1H, *J*=7.7, 8.1 Hz), 7.523 (dd, 1H, *J*=8.2, 1.2 Hz), 7.40-7.28 (m, 3H), 7.219 (dd, 1H, *J*=7.7, 1.2 Hz); ^{13}C-NMR (DMSO-d_6), δ: 162.54, 155.81 (*J*=247.4 Hz), 153.74, 146.76, 138.06, 136.46, 129.82, 129.73, 127.22 (*J*=9.2 Hz), 127.20, 125.05 (*J*=12.2 Hz), 124.45 (*J*=3.6 Hz), 118.92, 115.95(*J*=19.8 Hz), 117.60, 111.93; HR-MS: $C_{16}H_{10}FN_2O_2$ $[M-H]^-$ calculated 281.0732 m/z, found 281.0737 m/z.

***N*-(3-fluorophenyl)-8-hydroxyquinoline-2-carboxamide** (**4b**). Yield 72%; mp 197 °C; IR (cm^{-1}): 3333, 1686, 1612, 1599, 1528, 1504, 1463, 1443, 1391, 1365, 1325, 1295, 1276, 1237, 1200, 1174, 1145, 1111, 1083, 965, 872, 846, 775, 760, 748, 722, 684; ^{1}H-NMR (DMSO-d_6), δ: 11.265 (s, 1H), 10.376 (s, 1H), 8.572 (d, 1H, J=8.5 Hz), 8.263 (d, 1H, *J*=8.5 Hz), 7.866 (dt, 1H, *J*=11.6, 2.2 Hz), 7.698 (ddd, 1H, *J*=8.1, 1.9, 0.9 Hz), 7.611 (t, 1H, *J*=7.9 Hz), 7.522 (dd, 1H, *J*=8.2, 1.2 Hz), 7.484 (dt, 1H, *J*=6.8, 8.2 Hz), 7.233 (dd, 1H, *J*=7.6, 1.2 Hz), 7.012 (td, 1H, *J*=8.4, 2.5 Hz); ^{13}C-NMR (DMSO-d_6), δ: 162.35, 162.33 (*J*=241.6 Hz), 153.67, 146.87, 140.01(*J*=11.0 Hz), 138.20, 136.32, 130.43 (*J*=9.5 Hz), 129.87,

129.77, 118.95, 117.68, 116.33 (*J*=2.6 Hz), 112.10, 110.61 (*J*=20.9 Hz), 107.30 (*J*=26.1 Hz); HR-MS: $C_{16}H_{10}FN_2O_2$ $[M-H]^-$ calculated 281.0732 m/z, found 281.0736 m/z.

***N*-(4-fluorophenyl)-8-hydroxyquinoline-2-carboxamide** (**4c**). Yield 71%; mp 200 °C; IR (cm^{-1}): 3318, 1677, 1655, 1609, 1528, 1528, 1504, 1464, 1405, 1359, 1309, 1286, 1225, 1189, 1156, 1133, 1089, 1083, 932, 886, 854, 827, 760, 723, 678; ^{1}H-NMR (DMSO-d_6), δ: 11.206 (s, 1H), 10.349 (s, 1H), 8.565 (d, 1H, *J*=8.5 Hz), 8.260 (d, 1H, *J*=8.5 Hz), 7.909 (m, 2H), 7.604 (dd, 1H, *J*=8.1, 7.7 Hz), 7.519 (dd, 1H, *J*=8.2, 1.2 Hz), 7.290 (m, 2H), 7.226 (dd, 1H, *J*=7.6, 1.2 Hz); ^{13}C-NMR (DMSO-d_6), δ: 162.05, 158.57 (*J*=240.9 Hz), 153.64, 147.12, 138.12, 136.33, 134.60 (*J*=2.7 Hz), 129.74, 129.68, 122.56 (2C, *J*=8.0 Hz), 118.93, 117.65, 115.41(2C, *J*=22.4 Hz), 111.99; HR-MS: $C_{16}H_{10}FN_2O_2$ $[M-H]^-$ calculated 281.0732 m/z, found 281.0728 m/z.

***N*-(2-chlorophenyl)-8-hydroxyquinoline-2-carboxamide** (**5a**). Yield 71%; mp 151–152 °C; IR (cm^{-1}): 3355, 1695, 1595, 1581, 1535, 1505, 1461, 1433, 1362, 1328, 1310, 1290, 1237, 1198, 1136, 1115, 1088, 1052, 1032, 885, 848, 743, 724, 668; ^{1}H-NMR (DMSO-d_6), δ: 11.224 (s, 1H), 10.322 (s, 1H), 8.571 (d, 1H, *J*=8.5 Hz), 8.229 (d, 1H, *J*=8.5 Hz), 7.746 (dd, 1H, *J*=7.9, 1.6 Hz), 7.630 (dd, 1H, *J*=8.0, 1.4 Hz), 7.610 (t, 1H, *J*=7.9 Hz), 7.528 (dd, 1H, *J*=8.2, 1.1 Hz), 7.458 (td, 1H, *J*=7.6, 1.5 Hz), 7.352 (td, 1H, *J*=7.8, 1.6 Hz), 7.233 (dd, 1H, *J*=7.6, 1.2 Hz); ^{13}C-NMR (DMSO-d_6), δ: 162.57, 153.72, 146.77, 138.07, 136.50, 134.58, 129.80, 129.79, 129.67, 129.14, 128.09, 127.63, 127.59, 118.86, 117.62, 111.97; HR-MS: $C_{16}H_{10}ClN_2O_2$ $[M-H]^-$ calculated 297.0436 m/z, found 297.0437 m/z.

***N*-(3-chlorophenyl)-8-hydroxyquinoline-2-carboxamide** (**5b**). Yield 75%; mp 227 °C; IR (cm^{-1}): 3342, 1689, 1589, 1524, 1502, 1481, 1464, 1405, 1324, 1296, 1222, 1191, 1169, 1118, 1097, 1078, 977, 881, 855, 817, 786, 765, 722, 685; ^{1}H-NMR (DMSO-d_6), δ: 11.243 (s, 1H), 10.354 (s, 1H), 8.573 (d, 1H, *J*=8.5 Hz), 8.261 (d, 1H, *J*=8.5 Hz), 8.060 (t, 1H, *J*=2.1 Hz), 7.874 (ddd, 1H, *J*=8.2, 2.0, 0.9 Hz), 7.614 (dd, 1H, *J*=7.7, 8.1 Hz), 7.524 (dd, 1H, *J*=8.2, 1.1 Hz), 7.478 (t, 1H, *J*=8.1 Hz), 7.240 (ddd, 1H, *J*=8.0, 2.1, 0.9 Hz), 7.236 (dd, 1H, *J*=7.6, 1.2 Hz); ^{13}C-NMR (DMSO-d_6), δ: 162.33, 153.65, 146.82, 139.74, 138.20, 136.30, 133.11, 130.50, 129.88, 129.76, 123.80, 119.98, 118.93, 118.90, 117.67, 112.07; HR-MS: $C_{16}H_{10}ClN_2O_2$ $[M-H]^-$ calculated 297.0436 m/z, found 297.0432 m/z.

***N*-(4-chlorophenyl)-8-hydroxyquinoline-2-carboxamide** (**5c**). Yield 75%; mp 272–273 °C; IR (cm^{-1}): 3390, 3316, 1686, 1651, 1589, 1528, 1502, 1466, 1399, 1362, 1307, 1283, 1226, 1187, 1090, 1008, 889, 839, 804, 748, 722, 671; ^{1}H-NMR (DMSO-d_6), δ: 11.229 (s, 1H), 10.356 (s, 1H), 8.563 (d, 1H, *J*=8.5 Hz), 8.255 (d, 1H, *J*=8.5 Hz), 7.938 (m, 2H), 7.605 (dd, 1H, *J*=8.1, 7.7 Hz), 7.515 (dd, 1H, *J*=8.1, 1.2 Hz), 7.502 (m, 2H, *J*=7.9 Hz), 7.226 (dd, 1H, *J*=7.6, 1.2 Hz); ^{13}C-NMR (DMSO-d_6), δ: 162.19, 153.66, 146.98, 138.16, 137.22, 136.32, 129.81, 129.72, 128.71 (2C), 127.79, 122.17 (2C), 118.93, 117.66, 112.04; HR-MS: $C_{16}H_{10}ClN_2O_2$ $[M-H]^-$ calculated 297.0436 m/z, found 297.0433 m/z.

***N*-(2-bromophenyl)-8-hydroxyquinoline-2-carboxamide** (**6a**). Yield 76%; mp 176–177 °C; IR (cm^{-1}): 3452, 3336, 1696, 1592, 1575, 1532, 1504, 1460, 1431, 1362, 1328, 1305, 1235, 1196, 1134, 1109, 1087, 1045, 1021, 848, 814, 758, 723, 668; ^{1}H-NMR (DMSO-d_6), δ: 11.208 (s, 1H), 10.306 (s, 1H), 8.571 (d, 1H, *J*=8.5 Hz), 8.223 (d, 1H, *J*=8.5 Hz), 7.786 (dd, 1H, *J*=8.0, 1.4 Hz), 7.700 (dd, 1H, *J*=7.9, 1.6 Hz), 7.610 (t, 1H, *J*=7.9 Hz), 7.528 (dd, 1H, *J*=8.2, 1.1 Hz), 7.499 (td, 1H, *J*=7.7, 1.4 Hz), 7.283 (td, 1H, *J*=7.7, 1.7 Hz), 7.219 (dd, 1H, *J*=7.7, 1.2 Hz); ^{13}C-NMR (DMSO-d_6), δ: 162.56, 153.72, 146.80, 138.07, 136.50, 136.10, 132.80, 129.80, 129.78, 128.50, 128.24, 128.05, 120.10, 118.86, 117.62, 111.95; HR-MS: $C_{16}H_{10}BrN_2O_2$ $[M-H]^-$ calculated 340.9931 m/z, found 340.9935 m/z.

***N*-(3-bromophenyl)-8-hydroxyquinoline-2-carboxamide** (**6b**). Yield 75%; mp 235 °C; IR (cm^{-1}): 3445, 3279, 3057, 1626, 1584, 1536, 1504, 1504, 1478, 1409, 1329, 1306, 1230, 1190, 1163, 1123, 1072, 994, 881, 850, 722, 747, 725, 677; ^{1}H-NMR (DMSO-d_6), δ: 11.228 (s, 1H), 10.347 (s, 1H), 8.572 (d, 1H, *J*=8.5 Hz), 8.257(d, 1H, *J*=8.5 Hz), 8.183 (t, 1H, *J*=2.0 Hz), 7.924 (ddd, 1H, *J*=8.1, 1.9, 1.0 Hz), 7.612 (dd, 1H, *J*=7.8, 8.1 Hz), 7.522 (dd, 1H, *J*=8.3, 1.2 Hz), 7.415 (t, 1H, *J*=8.0 Hz), 7.368 (ddd, 1H, *J*=7.9, 1.9, 1.1 Hz), 7.233 (dd, 1H, *J*=7.7, 1.2 Hz); ^{13}C-NMR (DMSO-d_6), δ: 162.31, 153.65, 146.82, 139.88, 138.21, 136.30, 130.81, 129.88, 129.76, 126.70, 122.81, 121.54, 119.28, 118.94, 117.68, 112.08; HR-MS: for $C_{16}H_{10}BrN_2O_2$ $[M-H]^-$ calculated 340.9931 m/z, found 340.9937 m/z.

***N*-(4-bromophenyl)-8-hydroxyquinoline-2-carboxamide** (**6c**). Yield 75%; mp 283–284 °C; IR (cm^{-1}): 3386, 3316, 1686, 1650, 1585, 1524, 1500, 1393, 1305, 1280, 1224, 1179, 1161, 1111, 1072, 1005, 933, 887, 837, 822, 811, 801, 720; ^{1}H-NMR (DMSO-d_6), δ: 11.221 (s, 1H), 10.356 (s, 1H), 8.565 (d, 1H, *J*=8.5 Hz), 8.255 (d, 1H, *J*=8.5 Hz), 7.888 (m, 2H), 7.631 (m, 2H), 7.607 (t, 1H, *J*=7.9 Hz), 7.519 (dd, 1H, *J*=8.2, 1.2 Hz), 7.227 (dd, 1H, *J*=7.6, 1.2 Hz);

^{13}C-NMR (DMSO-d_6), δ: 162.19, 153.65, 146.96, 138.16, 137.63, 136.32, 131.62 (2C), 129.82, 129.72, 122.51 (2C), 118.93, 117.66, 115.87, 112.04; HR-MS: $C_{16}H_{10}BrN_2O_2$ $[M-H]^-$ calculated 340.9931 m/z, found 343.9932 m/z.

8-Hydroxy-*N*-(2-trifluoromethylphenyl)quinoline-2-carboxamide (**7a**). Yield 71%; mp 142–143 °C; IR (cm^{-1}): 3312, 1666, 1590, 1524, 1505, 1483, 1459, 1365, 1315, 1280, 1240, 1202, 1170, 1117, 1056, 1032, 935, 894, 857, 817, 754, 733, 668; ^{1}H-NMR (DMSO-d_6), δ: 11.221 (s, 1H), 10.269 (s, 1H), 8.569 (d, 1H, *J*=8.5 Hz), 8.208 (d, 1H, *J*=8.5 Hz), 7.867 (dm, 1H, *J*=7.9 Hz), 7.807 (t, 1H, *J*=7.8 Hz), 7.706 (dm, 1H, *J*=7.8 Hz), 7.612 (dd, 1H, *J*=7.7, 8.2 Hz), 7.592 (tm, 1H, *J*=7.7 Hz), 7.529 (dd, 1H, *J*=8.2, 1.2 Hz), 7.218 (dd, 1H, *J*=7.6, 1.2 Hz); ^{13}C-NMR (DMSO-d_6), δ: 163.49, 153.69, 146.62, 138.07, 136.49, 135.36 (q, *J*=2.9 Hz), 133.32, 130.53, 129.83, 129.78, 127.49, 126.61 (q, *J*=4.9 Hz), 126.03 (q, *J*=29.4 Hz), 123.60 (q, *J*=273.5 Hz), 118.80, 117.60, 111.91; HR-MS: $C_{17}H_{10}F_3N_2O_2$ $[M-H]^-$ calculated 331.0699 m/z, found 331.0703 m/z.

8-Hydroxy-*N*-(3-trifluoromethylphenyl)quinoline-2-carboxamide (**7b**). Yield 68%; mp 213–214 °C; IR (cm^{-1}): 3293, 1691, 1657, 1614, 1541, 1493, 1393, 1329, 1224, 1161, 1111, 1096, 880, 854, 794, 754, 724, 694; ^{1}H-NMR (DMSO-d_6), δ: 11.373 (s, 1H), 10.343 (s, 1H), 8.575 (d, 1H, *J*=8.5 Hz), 8.267 (d, 1H, *J*=8.5 Hz) 8.321 (t, 1H, *J*=2.2 Hz), 8.199 (dm, 1H, *J*=8.1 Hz), 7.689 (t, 1H, *J*=8.0 Hz), 7.613 (t, 1H, *J*=7.9 Hz), 7.527 (dm, 1H, *J*=7.6 Hz), 7.522 (dd, 1H, *J*=8.3, 1.2 Hz), 7.237 (dd, 1H, *J*=7.6, 1.1 Hz); ^{13}C-NMR (DMSO-d_6), δ: 162.54, 153.66, 146.75, 139.08, 138.22, 136.31, 130.05, 129.91, 129.79, 129.54 (q, *J*=31.5 Hz), 124.11 (q, *J*=272.2 Hz), 124.08, 120.40 (q, *J*=3.9 Hz), 118.94, 117.69, 116.57 (q, *J*=4.0 Hz), 112.08; HR-MS: $C_{17}H_{10}F_3N_2O_2$ $[M-H]^-$ calculated 331.0699 m/z, found 331.0698 m/z.

8-Hydroxy-*N*-(4-trifluoromethylphenyl)quinoline-2-carboxamide (**7c**). Yield 72%; mp 253 °C; IR (cm^{-1}): 3315, 1692, 1662, 1614, 1595, 1530, 1501, 1464, 1409, 1360, 1318, 1228, 1183, 1108, 1063, 1016, 933, 852, 837, 755, 722; ^{1}H-NMR (DMSO-d_6), δ: 11.378 (s, 1H), 10.398 (s, 1H), 8.577 (d, 1H, *J*=8.5 Hz), 8.273 (d, 1H, *J*=8.5 Hz), 8.146 (m, 2H), 7.818 (m, 2H), 7.617 (t, 1H, *J*=7.9 Hz), 7.526 (dd, 1H, *J*=8.2, 1.3 Hz), 7.238 (dd, 1H, *J*=7.6, 1.3 Hz); ^{13}C-NMR (DMSO-d_6), δ: 162.57, 153.69, 146.75, 141.89, 138.23, 136.32, 129.95, 129.81, 126.07 (q, 2C, *J*=3.8 Hz), 124.34 (q, *J*=271.3 Hz), 124.05 (q, *J*=32.2 Hz),

120.45 (2C), 118.98, 117.69, 112.13; HR-MS: $C_{17}H_{10}F_3N_2O_2$ $[M-H]^-$ calculated 331.0699 m/z, found 331.0704 m/z.

8-Hydroxy-*N*-(2-nitrophenyl)quinoline-2-carboxamide (**8a**). Yield 79%; mp 239–241 °C; IR (cm^{-1}): 3462, 3287, 1697, 1606, 1581, 1548, 1499, 1464, 1447, 1427, 1363, 1338, 1292, 1271, 1234, 1198, 1143, 1132, 1115, 1088, 935, 862, 847, 809, 783, 753, 736, 724, 668; ^{1}H-NMR (DMSO-d_6), δ: 7.247 (d, 1H, *J*=7.7 Hz), 7.502 (t, 1H, *J*=7.7 Hz), 7.545 (d, 1H, *J*=8.2 Hz), 7.632(t, 1H, *J*=7.8 Hz), 7.845 (t, 1H, *J*=7.7 Hz), 7.934 (d, 1H, *J*=8.1 Hz), 8.101 (d, 1H, *J*=8.1 Hz), 8.206 (d, 1H, *J*=8.5 Hz), 8.590 (d, 1H, *J*=8.5 Hz), 10.219 (s, 1H), 11.826 (s, 1H); ^{13}C-NMR (DMSO-d_6), δ: 112.08, 117.73, 118.92, 125.23, 126.01, 126.21, 129.92, 130.07, 130.84, 134.21, 136.45, 138.30, 142.99, 146.20, 153.69, 162.46; HR-MS: $C_{16}H_{10}N_3O_4$ $[M-H]^-$ calculated 308.0677 m/z, found 308.0673 m/z.

8-Hydroxy-*N*-(3-nitrophenyl)quinoline-2-carboxamide (**8b**). Yield 73%; mp 273–276 °C; IR (cm^{-1}): 3463, 3276, 1680, 1594, 1525, 1502, 1456, 1429, 1347, 1295, 1275, 1230, 1186, 1164, 1124, 1076, 946, 894, 882, 833, 803, 755, 735, 724, 668; ^{1}H-NMR (DMSO-d_6), δ: 7.244 (dd, 1H, *J*=7.6, 1.2 Hz), 7.538 (dd, 1H, *J*=8.1, 1.0 Hz), 7.627 (t, 1H, *J*=8.0 Hz), 7.746 (t, 1H, *J*=8.1 Hz), 8.030 (dd, 1H, *J*=8.2, 2.1 Hz), 8.278 (d, 1H, *J*=8.5 Hz), 8.350 (dd, 1H, *J*=8.2, 1.8 Hz), 8.594 (d, 1H, *J*=8.5 Hz), 8.854 (t, 1H, *J*=2.1 Hz), 10.360 (s, 1H), 11.469 (s, 1H); ^{13}C-NMR (DMSO-d_6), δ: 112.10, 114.55, 117.70, 118.53, 118.95, 126.37, 129.82, 130.00, 130.25, 136.29, 138.26, 139.47, 146.53, 147.99, 153.66, 162.66; HR-MS: $C_{16}H_{10}N_3O_4$ $[M-H]^-$ calculated 308.0677 m/z, found 308.0672 m/z.

8-Hydroxy-*N*-(4-nitrophenyl)quinoline-2-carboxamide (**8c**). Yield 75%; mp 297–300 °C; IR (cm^{-1}): 3253, 3116, 1690, 1595, 1567, 1535, 1501, 1464, 1409, 1328, 1303, 1244, 1177, 1113, 1083, 977, 942, 914, 847, 802, 748, 690; ^{1}H-NMR (DMSO-d_6), δ: 7.242 (dd, 1H, *J*=7.7, 1.2 Hz), 7.537 (dd, 1H, *J*=8.2, 1.2 Hz), 7.631 (t, 1H, *J*=7.9 Hz), 8.189 (m, 2H), 8.273 (d, 1H, *J*=8.5 Hz), 8.347 (m, 2H), 8.593 (d, 1H, *J*=8.5 Hz), 10.442 (s, 1H), 11.531 (s, 1H); ^{13}C-NMR (DMSO-d_6), δ: 112.51, 117.99, 119.23, 120.48(2C), 125.08(2C), 130.11, 130.37, 136.55, 138.55, 143.03, 144.62, 146.65, 153.85, 163.05; HR-MS: $C_{16}H_{10}N_3O_4$ $[M-H]^-$ calculated 308.0677 m/z, found 308.0675 m/z.

4.2. Lipophilicity determination by HPLC (Capacity factor k/calculated log k)

A HPLC system Agilent 1200 equipped with DAD detector (Agilent, Santa Clara, CA, USA) was used. A chromatographic column Symmetry® C_{18} 5 μm, 4.6×250 mm, Part No. WAT054275, (Waters Corp., Milford, MA, USA) was used. The HPLC separation process was monitored and evaluated by EZChrom Elite software ver. 3.3.2 (Agilent). Isocratic elution by a mixture of MeOH p.a. (60%) and H_2O-HPLC Mili-Q grade (40%) as a mobile phase was used. The total flow of the column was 1.0 mL/min, injection 20 μL, column temperature 40 °C and sample temperature 10 °C. The detection wavelength 210 nm was chosen. The KI methanolic solution was used for the dead time (t_D) determination. Retention times (t_R) were measured in minutes. The capacity factors *k* were calculated according to formula $k = (t_R - t_D)/t_D$, where t_R is the retention time of the solute, whereas t_D denotes the dead time obtained using an unretained analyte. Log *k*, calculated from the capacity factor *k*, is used as the lipophilicity index converted to log *P* scale. The log *k* values of the individual compounds are shown in Table 1.

4.3. In vitro antimycobacterial evaluation

Mycobacterium tuberculosis H37Ra ATCC 25177 and well characterised clinical isolates of *M. avium* complex CIT19/06, and *M. avium* subsp. *paratuberculosis* CIT03 were grown in Middlebrook broth (MB), supplemented with Oleic-Albumin-Dextrose-Catalase supplement (OADC, Becton Dickinson, UK) and mycobactin J (2 μg/mL). Identification of these isolates was performed using biochemical and molecular protocols. At log phase growth, a culture sample (10 mL) was centrifuged at 15,000 rpm/20 min using a bench top centrifuge (Model CR 4-12, Jouan Inc., UK). Following removal of the supernatant, the pellet was washed in fresh Middlebrook 7H9GC broth and re-suspended in fresh supplemented MB (10 mL). The turbidity was adjusted to match McFarland standard No. 1 (3×10^8 cfu) with MB broth. A further 1:20 dilution of the culture was then performed in MB broth. The antimicrobial susceptibility of all three mycobacterial species was investigated in a 96-well plate format. In these experiments, sterile deionised water (300 μL) was added to all outer-perimeter wells of the plates to minimize evaporation of the medium in the test wells during incubation. Each evaluated compound (100 μL) was incubated with each of the mycobacterial species (100 μL). Dilutions of each compound were prepared in duplicate. For all synthesized compounds, final concentrations ranged from 1,000 μg/mL to 8 μg/mL. All compounds were prepared in DMSO and subsequent dilutions were made in supplemented MB. The plates were sealed with parafilm and incubated at 37 °C, for 5 days in the case of *M. avium complex*,

7 days in the case of *M. tuberculosis* and 11 days in the case of *M. avium paratuberculosis*. Following incubation, a 10% addition of alamarBlue (AbD Serotec, Kidlington, UK) was mixed into each well and readings at 570 nm and 600 nm were taken, initially for background subtraction and subsequently after 24 h re-incubation. The background subtraction is necessary for strongly coloured compounds, where the colour may interfere with the interpretation of any colour change. For non-interfering compounds, a blue colour in the well was interpreted as an absence of growth and a pink colour was scored as growth. The minimum inhibitory concentration (MIC) was defined as the lowest concentration of the compound at which no visible bacterial growth was observed, i.e. the MIC is the lowest concentration that prevented a visual colour change from blue to pink. The MIC value is routinely and widely used in bacterial assays and is a standard detection limit according to the Clinical and Laboratory Standards Institute (CLSI, www.clsi.org). Rifampicin (Sigma-Aldrich) was used as the standard, as it is a clinically used antimycobacterial drug. The results are summarized in Table 1.

4.4. MTT assay

For the MTT assay, the outer wells of a 96-well plate were filled with 200 µL of sterile water, and the inner wells were filled with 100 µL of the tested compound at the concentration to be examined. Compounds were prepared as previously stated and diluted in Middlebrook media to achieve the desired concentration. *Mycobacterium tuberculosis* H37Ra ATCC 25177 was suspended in ODAC supplemented Middlebrook broth at a MacFarland standard of 1.0 and then diluted through a 1:20 dilution using Middlebrook broth as a diluent. The diluted mycobacteria (100 µL) were added to each well containing the compound to be tested. A negative growth control was composed of 100 µL of DMSO and 100 µL of media, and the diluted mycobacteria in broth absent of inhibiting compounds were used as a positive growth control. All compounds and controls were prepared in triplicate. Plates were incubated at 37°C for 7 days. After the incubation period, 10% well volume of MTT reagent was mixed into each well and incubated at 37 °C for 24 hours. The reagent and media were then aspirated from the wells, to which 50 µL 99% propan-2-ol was then added, and plates were read at 570 nm. The absorbance readings from the cells, grown in the presence of the tested compounds, were compared with uninhibited cell growth (using DMSO as the blank) to determine the relative percent viability. The percent viability was determined through the MTT assay. The percent viability is calculated through comparison of a measured value

against that of the uninhibited control: %viability = $OD_{570}E/OD_{570}P \times 100$, where $OD_{570}E$ is the reading from the compound-exposed cells, while $OD_{570}P$ is the reading from the uninhibited cells (positive control). Cytotoxic potential is determined by a percent viability of <70%.

4.5. In vitro cytotoxicity assay

Human monocytic leukemia THP-1 cells were used for *in vitro* toxicity assay. Cells were obtained from the European Collection of Cell Cultures (ECACC, Salisbury, UK) and routinely cultured in RPMI 1640 medium supplemented with 10% fetal bovine serum, 2% L-glutamine, 1% penicillin and streptomycin at 37 °C with 5% CO_2. Cells were passaged at approximately 1 week intervals. Cytotoxicity of the compounds was determined using a WST-1 assay kit (Roche Diagnostics, Mannheim, Germany) according to the manufacturer's instructions. The tested compounds were dissolved in DMSO and added in five increasing concentrations (0.37, 1.1, 3.3, 10, and 30 μM) to the cell suspension in the culture RPMI 1640 medium. The maximum concentration of DMSO in the assays never exceeded 0.1%. Subsequently, the cells were incubated for 24 h at 37 °C with 5% CO_2. For WST-1 assays, cells were seeded into 96-well plates (5×10^4 cells/well in 100 μL culture medium) in triplicate in serum-free RPMI 1640 medium and measurements were taken 24 h after the treatment with the compounds. The median inhibition concentration values, IC_{50}, were deduced through the production of a dose-response curve. All data were evaluated using GraphPad Prism 5.00 software (GraphPad Software, San Diego, CA, USA). The results are summarized in Table 1.

Acknowledgments

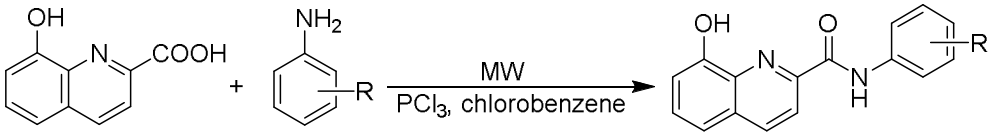
This study was supported by IGA VFU Brno 37/2014/FaF, 304/2015/FaF, 320/2015/FaF, 322/2015/FaF, Slovak Grant Agency VEGA 1/0770/15, by Project APVV-0061-11 and by the Irish Department of Agriculture Fisheries and Food (FIRM): Refs. 08RDCIT601 & 08RDCIT617. The system of high-performance liquid chromatography coupled to an LTQ Orbitrap XL high-resolution mass spectrometer forms a part of the National Infrastructure CzeCos/ICOS (LM2010007).

References

1. World Health Organization. *Global tuberculosis report 2014*. WHO Press, Geneva, Switzerland, 2014.
2. Wagner, D.; Young, L. S. *Infection* **2004**, *32*, 257.
3. Koul, A.; Arnoult, E.; Lounis, N.; Guillemont, J.; Andries, K. *Nature* **2011**, *469*, 483.
4. Baquero, F.; Coque, T.M.; de la Cruz, F. *Antimicrob. Agents Chemother.* **2011**, *55*, 3649.
5. Polanski, J.; Kurczyk, A.; Bak, A.; Musiol, R. *Curr. Med. Chem.* **2012**, *19*, 1921.
6. Kumar, S.; Bawa, S.; Gupta, H. *Mini-Rev. Med. Chem.* **2009**, *9*, 1648.
7. Pati, B.; Banerjee, S. *J. PharmaSciTech* **2014**, *3*, 59.
8. Marella, A.; Tanwar, O.P.; Saha, R.; Ali, M.R.; Srivastava, S.; Akhter, M.; Shaquiquzzaman, M.; Alam, M.M. *Saudi. Pharm. J.* **2013**, *21*, 1.
9. Musiol, R.; Jampilek, J.; Buchta, V.; Niedbala, H.; Podeszwa, B.; Palka, A.; Majerz-Maniecka, K.; Oleksyn, B.; Polanski, J. *Bioorg. Med. Chem.* **2006**, *14*, 3592.
10. Musiol, R.; Serda, M.; Hensel-Bielowka, S.; Polanski, J. *Curr. Med. Chem.* **2010**, *17*, 1960.
11. Cieslik, W.; Musiol, R.; Nycz, J.; Jampilek, J.; Vejsova, M.; Wolff, M.; Machura, B.; Polanski, J. *Bioorg. Med. Chem.* **2012**, *20*, 6960.
12. Sahu, K.B.; Ghosh, S.; Banerjee, M.; Maity, A.; Mondal, S.; Paira, R.; Saha, P.; Naskar, S.; Hazra, A.; Banerjee, S.; Samanta, A.; Mondal N.B. *Med. Chem. Res.* **2013**, *22*, 94.
13. Beena; Rawat, D.S. *Med. Res. Rev.* **2013**, *33*, 693.
14. Keri, R.S.; Patil, S.A. *Biomed. Pharmacother.* **2014**, *68*, 1161.
15. Fraser, S.S.R.; Creanor, J. *Biochem. J.* **1975**, *147*, 401.
16. Hongmanee, P.; Rukseree, K.; Buabut, B.; Somsri, B.; Palittapongarnpim, P. *Antimicrob. Agents Chemother.* **2007**, *51*, 1105.
17. Darby, C.M.; Nathan, C.F. *J. Antimicrob. Chemother.* **2010**, *65*, 1424.
18. Prachayasittikul, V.; Prachayasittikul, S.; Ruchirawat, S.; Prachayasittikul, V. *Drug Des. Devel. Ther.* **2013**, *7*, 1157.
19. Sirturo. http://www.sirturo.com/. (April 9, 2015)
20. Gratraud, P.; Surolia, N.; Besra, G.S.; Surolia, A.; Kremer, L. *Antimicrob. Agents Chemother.* **2008**, *52*, 1162.
21. Thomas, K.D.; Adhikari, A.V.; Chowdhury, I.H.; Sumesh, E.; Pal, N.K. *Eur. J. Med. Chem.* **2011**, *46*, 2503.
22. Thomas, K.D.; Adhikari, A.V.; Telkar, S.; Chowdhury, I.H.; Mahmood, R.; Pal, N.K.; Row, G.; Sumesh E. *Eur. J. Med. Chem.* **2011**, *46*, 5283.

23. Khan, S.R.; Singh, S.; Roy, K.K.; Akhtar, M.S.; Saxena, A.K.; Krishnan, M.Y. *Int. J. Antimicrob. Agents* **2013**, *41*, 41.
24. Naik, M.; Humnabadkar, V.; Tantry, S.J.; Panda, M.; Narayan, A.; Guptha, S.; Panduga, V.; Manjrekar, P.; Jena, L.K.; Koushik, K.; Shanbhag, G.; Jatheendranath, S.; Manjunatha, M.R.; Gorai, G.; Bathula, C.; Rudrapatna, S.; Achar, V.; Sharma, S.; Ambady, A.; Hegde, N.; Mahadevaswamy, J.; Kaur, P.; Sambandamurthy, V.K.; Awasthy, D.; Narayan, C.; Ravishankar, S.; Madhavapeddi, P.; Reddy, J.; Prabhakar, K.; Saralaya, R.; Chatterji, M.; Whiteaker, J.; McLaughlin, B.; Chiarelli, L.R.; Riccardi, G.; Pasca, M.R.; Binda, C.; Neres, J.; Dhar, N.; Signorino-Gelo, F.; McKinney, J.D.; Ramachandran, V.; Shandil, R.; Tommasi, R.; Iyer, P.S.; Narayanan, S.; Hosagrahara, V.; Kavanagh, S.; Dinesh, N.; Ghorpade, S.R. *J. Med. Chem.* **2014**, *57*, 5419.
25. Marriner, G.A.; Nayyar, A.; Uh, E.; Wong, S.Y.; Mukherjee, T.; Via, L.E.; Carroll, M.; Edwards, R.L.; Grubber, T.D.; Choi, I.; Lee, J.; Arora, K.; England, K.D.; Boshoff, H.I.M.; Barry, C.E. *Top. Med. Chem.* **2011**, *7*, 47.
26. Ballell, L.; Bates, R.H.; Young, R.J.; Alvarez-Gomez, D.; Alvarez-Ruiz, E.; Barroso, V.; Blanco, D.; Crespo, B.; Escribano, J.; Gonzalez, R.; Lozano, S.; Huss, S.; Santos-Villarejo, A.; Martin-Plaza, J.J.; Mendoza, A.; Rebollo-Lopez, M.J.; Remuinan-Blanco, M.; Lavandera, J.L.; Perez-Herran, E.; Gamo-Benito, F.J.; Garcia-Bustos, J.F.; Barros, D.; Castro, J.P.; Cammack, N. *ChemMedChem* **2013**, *8*, 313.
27. Upadhayaya, R.S.; Vandavasi, J.K.; Kardile, R.A.; Lahore, S.V.; Dixit, S.S.; Deokar, H.S.; Shinde, P.D.; Sarmah, M.P.; Chattopadhyaya, J. *Eur. J. Med. Chem.* **2010**, *45*, 1854.
28. Nayyar, A.; Monga, V.; Malde, A.; Coutinho, E.; Jain, R. *Bioorg. Med. Chem.* **2007**, *15*, 626.
29. Nayyar, A.; Jain R. *Ind. J. Chem.* **2008**, *47B*, 117.
30. Bueno, R. V.; Braga, R. C.; Segretti, N. D.; Ferreir, E. I.; Trossini, G. H.; Andrade, C. H. *Curr. Pharm. Des.* **2014**, 20, 4474.
31. Mathew, B.; Ross, L.; Reynolds, R.C. *Tuberculosis* **2013**, *93*, 398.
32. Paritala, H.; Carroll, K.S. *Infect. Disord. Drug Targets* **2013**, *13*, 85.
33. Joshi, S.D.; More, U.A.; Dixit, S.R.; Dubey, D.; Tripathi, A.; Kulkarni, V.H. *Ind. Am. J. Pharm. Res.* **2014**, *4*, 864–877.
34. Jampilek, J.; Dolezal, M.; Kunes, J.; Buchta, V.; Silva, L.; Kralova, K. *Med. Chem.* **2005**, *1*, 591.

35. Musiol, R.; Jampilek, J.; Kralova, K.; Richardson, D.R.; Kalinowski, D.; Podeszwa, B.; Finster, J.; Niedbala, H.; Palka, A.; Polanski, J. *Bioorg. Med. Chem.* **2007**, *15*, 1280.
36. Musiol, R.; Tabak, D.; Niedbala, H.; Podeszwa, B.; Jampilek, J.; Kralova, K.; Dohnal, J.; Finster, J.; Mencel, A.; Polanski, J. *Bioorg. Med. Chem.* **2008**, *16*, 4490.
37. Gonec, T.; Bobal, P.; Pesko, M.; Guo, J.H.; Kralova, K.; Kos, J.; Coffey, A.; Kollar, P.; Imramovsky, A.; Jampilek, J. *Molecules* **2012**, *17*, 613.
38. Zumla, A.; Nahid, P.; Cole, S. T. *Nat. Rev. Drug Discov.* **2013**, *12*, 388.
39. Pattabiraman, V. R.; Bode, J. W. *Nature* **2011**, *480*, 471.
40. Lavecchia, A.; Di Giovanni, C. *Curr. Med. Chem.* **2013**, *20*, 2839.
41. Gonec, T.; Kos, J.; Zadrazilova, I.; Pesko, M.; Keltosova, S.; Tengler, J.; Bobal, P.; Kollar, P.; Cizek, A.; Kralova, K.; Jampilek, J. *Bioorg. Med. Chem.* **2013**, *21*, 6531.
42. Kos, J.; Nevin, E.; Soral, M.; Kushkevych, I.; Gonec, T.; Bobal, P.; Kollar, P.; Coffey, A.; O´Mahony, J.; Liptaj, T.; Kralova, K.; Jampilek, J. *Bioorg. Med. Chem.* **2015**, *23*, 2035.
43. Musiol, R.; Jampilek, J.; Podeszwa, B.; Finster, J.;Tabak, D.; Dohnal, J.; Polanski, J. *Cent. Eur. J. Chem.* **2009**, *7*, 586.
44. Palmer, M.H. *The Structure and Reactions of Heterocyclic Compounds*. Edward Arnold Publisher, London, UK, 1967; pp. 105–144.
45. Zheng, H.; Lu, L.; Wang, B.; Pu, S.; Zhang, X.; Zhu, G.; Shi, W.; Zhang, L.; Wang, H.; Wang, S.; Zhao, G.; Zhang, Y. *PLoS One* **2008**, *3*, e2375.
46. Havlik, J. A.; Horsburgh, C. R.; Metchock, B.; Williams, P.; Fann, S. A.; Thompson, S. E. *J. Infect. Dis.* **1992**, *165*, 577.
47. Rath, T.; Roderfeld, M.; Blocher, S.; Rhode, A.; Basler, T.; Akineden, O.; Abdulmawjood, A.; Halwe, J. M.; Goethe, R.; Bulte, M.; Roeb, E. *BMC Gastroenterology* **2011**, *11*, 34.
48. Bueno, J. Antitubercular in vitro drug discovery: Tools for begin the search. In: *Understanding Tuberculosis-New Approaches to Fighting against Drug Resistance*, Cardona, P.J. (Ed.). InTech, Rijeka, Croatia, 2012; pp. 147–168.
49. Gonec, T.; Kos, J.; Nevin, E.; Govender, R.; Pesko, M.; Kushkevych, I.; Oravec, M.; Kollar, P.; O´Mahony, J.; Kralova, K.; Coffey, A.; Jampilek, J. *Molecules* **2014**, *19*, 10386.
50. Suffness, M.; Douros, J. *J. Nat. Prod.* **1982**, *45*, 1.



Optimal:

R = 2-,3-,4-CF_3, 3-OCH_3, 3-CH_3, 2-Br, 2-Cl

Small library (22 compounds):

R = H, OCH_3, CH_3, F, Cl, Br, CF_3, NO_2

MIC = **23-29** μM (*M.tuberculosis*, *M. avium* complex, *M. avium* subsp. *paratuberculosis*)