Journal Pre-proof

Design, synthesis, and evaluation of new 2-(quinoline-4-yloxy)acetamide-based antituberculosis agents

Ana Flávia Borsoi, Josiane Delgado Paz, Bruno Lopes Abbadi, Fernanda Souza Macchi, Nathalia Sperotto, Kenia Pissinate, Raoní S. Rambo, Alessandro Silva Ramos, Diana Machado, Miguel Viveiros, Cristiano Valim Bizarro, Luiz Augusto Basso, Pablo Machado

PII: S0223-5234(20)30146-X

DOI: https://doi.org/10.1016/j.ejmech.2020.112179

Reference: EJMECH 112179

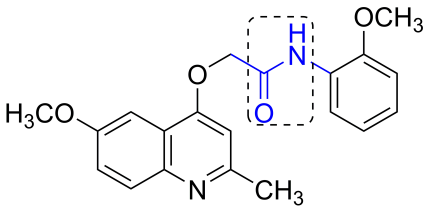
To appear in: *European Journal of Medicinal Chemistry*

Received Date: 20 December 2019

Revised Date: 12 February 2020

Accepted Date: 20 February 2020

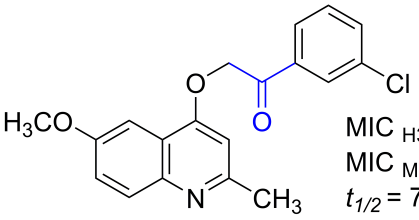
Please cite this article as: Ana.Flá. Borsoi, J.D. Paz, B.L. Abbadi, F.S. Macchi, N. Sperotto, K. Pissinate, Raoní.S. Rambo, A.S. Ramos, D. Machado, M. Viveiros, C.V. Bizarro, L.A. Basso, P. Machado, Design, synthesis, and evaluation of new 2-(quinoline-4-yloxy)acetamide-based antituberculosis agents, *European Journal of Medicinal Chemistry* (2020), doi: https://doi.org/10.1016/j.ejmech.2020.112179.



GSK358607A

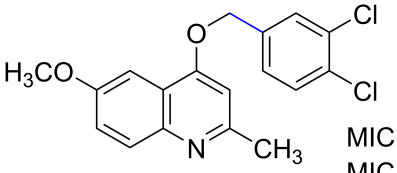
MIC $_{H37Rv}$ = 0.44 μM
$t_{1/2}$ = 16.7 min

Molecular Simplification



3h

MIC $_{H37Rv}$ = 1.8 μM
MIC $_{MDR-TB}$ = 0.5 μM
$t_{1/2}$ = 78.2 min



4k

MIC $_{H37Rv}$ = 0.3 μM
MIC $_{MDR-TB}$ = 0.2 μM
$t_{1/2}$ = 30.6 min

Design, synthesis, and evaluation of new 2-(quinoline-4-yloxy)acetamide-based antituberculosis agents

Ana Flávia Borsoi[a,b], Josiane Delgado Paz[a,c], Bruno Lopes Abbadi[a], Fernanda Souza Macchi[a,c], Nathalia Sperotto[a,b], Kenia Pissinate[a], Raoní S. Rambo[a], Alessandro Silva Ramos[a], Diana Machado[d], Miguel Viveiros[d], Cristiano Valim Bizarro[a,c], Luiz Augusto Basso[a,b,c,*], Pablo Machado[a,c,*]

[a]Instituto Nacional de Ciência e Tecnologia em Tuberculose, Centro de Pesquisas em Biologia Molecular e Funcional, Pontifícia Universidade Católica do Rio Grande do Sul, 90616-900, Porto Alegre, Rio Grande do Sul, Brazil

[b]Programa de Pós-Graduação em Medicina e Ciências da Saúde, Pontifícia Universidade Católica do Rio Grande do Sul, 90616-900, Porto Alegre, Rio Grande do Sul, Brazil

[c]Programa de Pós-Graduação em Biologia Celular e Molecular, Pontifícia Universidade Católica do Rio Grande do Sul, 90616-900, Porto Alegre, Rio Grande do Sul, Brazil

[d]Global Health and Tropical Medicine, GHTM, Instituto de Higiene e Medicina Tropical, IHMT, Universidade NOVA de Lisboa, UNL, Lisbon, Portugal

*Corresponding author

E-mail addresses:

luiz.basso@pucrs.br (Luiz Augusto Basso); *pablo.machado@pucrs.br* (Pablo Machado)

Abstract

Using a classical molecular simplification approach, a series of 36 quinolines were synthesized and evaluated as *in vitro* inhibitors of *Mycobacterium tuberculosis* (*M. tuberculosis*) growth. Structure–activity relationship (SAR) studies leaded to potent antitubercular agents, with minimum inhibitory concentration (MIC) values as low as 0.3 μM against *M. tuberculosis* H37Rv reference strain. Furthermore, the lead compounds were active against multidrug-resistant strains, without cross-resistance with some first- and second-line drugs. Testing the molecules against a spontaneous mutant strain containing a single mutation in the *qcrB* gene (T313A) indicated that the synthesized quinolines targeted the cytochrome bc_1 complex. In addition, leading compounds were devoid of apparent toxicity to HepG2 and Vero cells and showed moderate elimination rates in human liver S9 fractions. Finally, the selected structures inhibited *M. tuberculosis* growth in a macrophage model of tuberculosis infection. Taken together, these data indicate that this class of compounds may furnish candidates for the future development of antituberculosis drugs.

Keywords: *Mycobacterium tuberculosis*; molecular simplification; multidrug-resistant strains; SAR; intracellular activity; cytochrome bc_1 complex.

1. Introduction

Tuberculosis (TB) is an airborne infectious disease, described among the top 10 causes of death worldwide. The disease has *Mycobacterium tuberculosis* (*M. tuberculosis*) as its main etiological agent, and it was responsible for claiming 1.5 million lives in 2018 [1]. In the same year, about 10.0 million people developed TB, according to the World Health Organization [1]. This public health problem has been aggravated by the emergence of rifampicin-resistant TB (RR-TB), multidrug-resistant TB (MDR-TB), HIV coinfection, and the large number of individuals infected with latent or dormant bacilli [1,2]. The recommended treatment includes a set of four drugs administered in two different combinations for six months. Although it has a high cure rate, the current therapeutic regimen suffers from low adherence of the patients, with increased drug resistance, adverse effects, and the impossibility of co-administration with some antiretroviral drugs [3,4]. In the early 2010s, the approval of bedaquiline [5] and delamanid [6] for the treatment of adult patients with pulmonary MDR-TB ended a period of more than four decades without approval of a new anti-TB drug. This addition to the therapeutic arsenal has been met with caution since these drugs have resulted in certain toxicity events, including drug-induced QT interval prolongation. In addition, the adaptive capacity of *M. tuberculosis* has already led to the emergence of bedaquiline- and delamanid-resistant strains [7], indicating the need for continuous effort to obtain new therapeutic alternatives to TB treatment.

Within this context and as part of our ongoing research, we evaluated the antimycobacterial activity of 2-(quinolin-4-yloxy)acetamides **1** (**Figure 1**) and their derivatives [8,9]. The compounds demonstrated submicromolar activity against resistant and non-resistant *M. tuberculosis* strains by targeting the QcrB subunit of menaquinol cytochrome c oxidoreductase

(bc_1 complex) [10]. Despite their high capacity to inhibit *M. tuberculosis* growth *in vitro*, their good membrane permeability, and their synergistic *in vitro* effect with rifampin, this chemical class has shown moderate metabolic stability [9]. The microsomal instability of 2-(quinolin-4-yloxy)acetamides has been attributed to the amide group lability, which is a probable point of esterase-mediated hydrolysis [11]. Such instability could reduce the pharmacokinetic exposure of these molecules when evaluated in *in vivo* models of tuberculosis. Thus, a change in this chemical group with a reduction in electrophilicity could lead to compounds containing better metabolic profiles. Our hypothesis was that molecular simplification of the attached acetamide group could provide novel compounds with optimized properties (**Figure 1**). The major challenge would be to maintain antimycobacterial activity while altering the amide since this group has been described as an important pharmacophoric point of compounds with potent activity against *M. tuberculosis* growth [8,9,12]. Furthermore, it has been described that quinolines containing ether groups at the 4-position are devoid of antitubercular activity [13].

Therefore, in an attempt to obtain new compounds with activity against drug-susceptible and, mostly, drug-resistant *M. tuberculosis* strains, a new series of simplified 2-methyl-6-methoxy-2-quinolines was synthesized. First, the structural requirements for potency of molecules (SAR) were evaluated using minimal inhibitory concentration (MIC) values. Subsequently, the most active structures against *M. tuberculosis* H37Rv were tested against a panel of well characterized multidrug-resistant strains, while the viability of HepG2 and Vero cells was used as an indicator of the toxicity and selectivity of the compounds. Finally, the *in vitro* metabolic stability and intracellular activity in a macrophage model of *M. tuberculosis* infection were also evaluated.

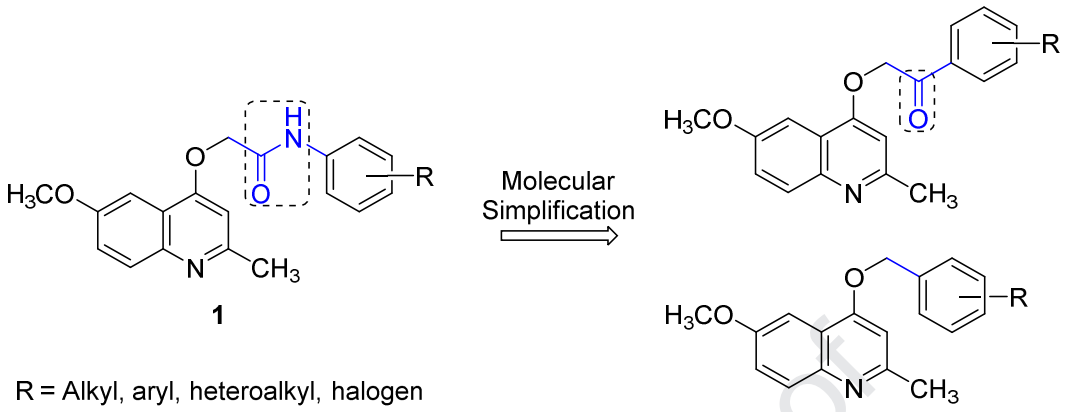
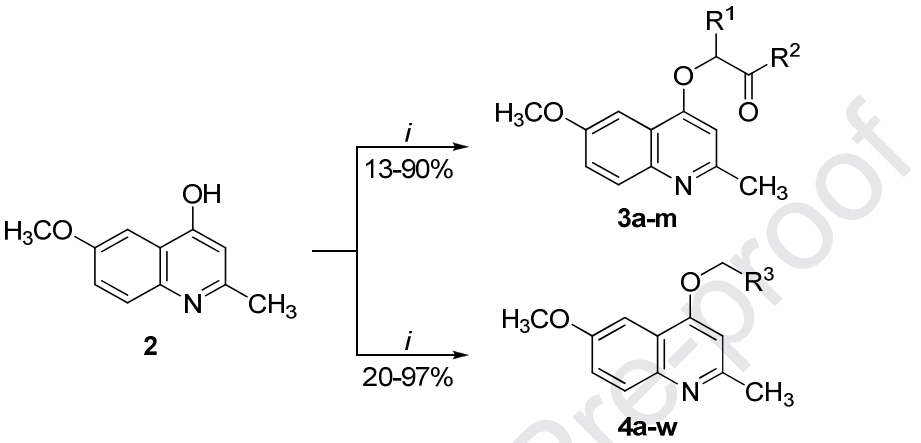


Figure 1. Molecular simplification of the amide group from 2-(quinolin-4-yloxy)acetamides as a strategy for the discovery and optimization of new antimycobacterial compounds with better metabolic properties.

2. Results and discussion

The designed compounds were obtained in two synthetic steps. First, 4-hydroxyquinoline (**2**) was synthesized in a classical Conrad-Limpach cyclocondensation reaction between ethyl 3-oxobutanoate and 4-methoxyaniline, according to an already-reported protocol [9]. The second step was accomplished through *O*-alkylation in a second-order nucleophilic substitution reaction (S_N2). Importantly, the substituents of the alkylating agents were chosen from different electron-donating and electron-withdrawing groups, including bulky alkyl and aryl groups. The quinolines **3a**–**m** were obtained from the reaction of 4-hydroxyquinoline (**2**) and 2-bromo-1-arylethanones using potassium carbonate (K_2CO_3) as a base and dimethylformamide (DMF) as the solvent. The reactants were stirred for 18 h at 25°C, leading to products **3a**–**m** with 13–90% yields (**Scheme 1**). Using the same procedure, the quinolines **4a**–**w** were synthesized by the reaction of 4-hydroxyquinoline (**2**) and benzyl bromides, with 20−97% yields (**Scheme 1**). In

general, the presence of a carbonyl group in the alkylating agent provided products in lower yields when compared to alkylation reactions using benzyl bromides. Spectroscopic and spectrometric data were found to be in agreement with the proposed structures (Supporting Information).



Scheme 1. Reagents and conditions. (*i*) = Alkyl halide, K_2CO_3, DMF, 25°C, 18 h.

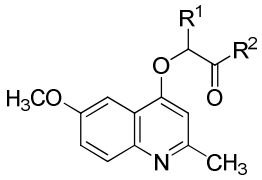
The synthesized compounds **3** and **4** were evaluated in a whole-cell assay against the *M. tuberculosis* H37Rv strain using isoniazid as positive control [14,15]. The quinolines **3a**–**m** presented good activity against the bacillus, with MIC values ranging from 1.3 to 31.1 μM under the tested conditions (**Table 1**). The antimycobacterial activity results showed that carbonyl-containing compounds **3a** and **3h** yielded lower MICs than the first line drug, isoniazid. Positioning the methoxy group at the 4-position of the benzene ring led to a compound with reduced activity against *M. tuberculosis*, whereas the unsubstituted compound **3a** exhibited a MIC of 1.3 μM; the presence of the methoxy group at the 4-position of **3b** (MIC = 7.4 μM) reduced the activity more than 5-fold. Additionally, the presence of the methoxy group at the 3- (**3c**) and 2-positions (**3d**) reduced the activity of the molecules to a greater extent, with MIC

values of 14.8 and 29.6 μM, respectively. By changing the electron-donating group to 4-methyl, the potency was also reduced since quinoline **3e** showed a MIC of 15.6 μM. The same pattern was observed with the use of electron-withdrawing groups as substituents. The 4-fluor- (**3f**) and 4-chloro-substituted (**3g**) compounds showed MICs of 30.7 and 12.9 μM, respectively. Notably, changing the chloro atom from the 4- to 3-position significantly altered the antimycobacterial activity because molecule **3h** exhibited a MIC value of 1.8 μM. This value differed only slightly from **3a** (1.3 μM) and showed increased potency of nearly 7-fold compared to the 4-substituted derivative **3g**. Substitution with chlorine atoms at position 3 and 4 of the benzyl ring reduced the inhibitory activity against *M. tuberculosis*. 3,4-Dichlorophenyl-substituted **3i** showed a MIC of 6.6 μM, which was more than 3.5-fold lower than its monosubstituted analog, **3h**. Additionally, the 4-bromophenyl-substituted **3j** exhibited a MIC of 14.6 μM, denoting that the classic bioisosteric replacement between the chlorine and bromine was able to maintain similar and reduced potencies. The significant increase in the lipophilicity when using 4-*iso*-butyl and 4-phenyl groups did not increase the activity of the molecules against the *M. tuberculosis* H37Rv strain since structures **3k** and **3l** presented MICs of 6.9 and 13.0 μM, respectively. Finally, the presence of an α-methyl group in quinoline **3m** reduced the activity nearly 24-fold, suggesting that changes in this position do not sustain the antimycobacterial activity of this chemical class.

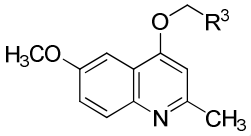
150

151 **Table 1.** ClogP values and *in vitro* activity of quinolines **3** and **4** against the *M. tuberculosis*

152 H37Rv strain.



3a-m



4a-w

153

Entry	R^1	R^2	R^3	ClogP[a]	MIC (μM)
3a	H	Ph	–	4.18	1.3
3b	H	4-MeO-Ph	–	4.40	7.4
3c	H	3-MeO-Ph	–	4.40	14.8
3d	H	2-MeO-Ph	–	4.40	29.6
3e	H	4-Me-Ph	–	4.67	15.6
3f	H	4-F-Ph	–	4.39	30.7
3g	H	4-Cl-Ph	–	4.96	12.9
3h	H	3-Cl-Ph	–	4.96	1.8
3i	H	3,4-$(Cl)_2$-Ph	–	5.58	6.6
3j	H	4-Br-Ph	–	5.11	14.6
3k	H	4-*i*-Bu-Ph	–	6.13	6.9
3l	H	4-Ph-Ph	–	6.06	13.0
3m	Me	Ph	–	4.48	31.1
4a	–	–	Ph	4.99	5.6
4b	–	–	3-MeO-Ph	4.90	32.3

4c	–	–	3,5-$(MeO)_2$-Ph	4.99	29.5
4d	–	–	4-F-Ph	5.13	16.8
4e	–	–	3-F-Ph	5.13	16.8
4f	–	–	2-F-Ph	5.13	19.2
4g	–	–	3,4-$(F)_2$-Ph	5.20	3.9
4h	–	–	4-Cl-Ph	5.67	15.9
4i	–	–	3-Cl-Ph	5.67	7.9
4j	–	–	2-Cl-Ph	5.70	18.8
4k	–	–	3,4-$(Cl)_2$-Ph	6.29	0.3
4l	–	–	2,3-$(Cl)_2$-Ph	6.29	28.7
4m	–	–	3-Cl-4-Br-Ph	6.42	1.6
4n	–	–	4-Br-Ph	5.85	13.9
4o	–	–	3-Br-Ph	5.85	13.9
4p	–	–	4-F_3C-Ph	5.87	7.2
4q	–	–	3-F_3C-Ph	5.87	7.2
4r	–	–	4-O_2N-Ph	4.73	30.8
4s	–	–	4-*i*-Pr-Ph	6.41	1.9
4t	–	–	4-*t*-Bu-Ph	6.81	3.7
4u	–	–	Bn	5.31	34.1
4v	–	–	2-Naphthyl	6.16	7.6
4w	–	–		4.95	30.9
INH	–	–	–	–	2.3

[a]ClogP calculated with ChemBioDraw Ultra, version 13.0.0.3015. INH, Isoniazid.

In the second round of obtaining new anti-TB drug candidates, the antimycobacterial activity of 2-quinolin-4-yloxy derivatives **4a**-**w** against the *M. tuberculosis* H37Rv strain was determined (**Table 1**). In general, dihalogenated- or alkyl-substituted compounds showed the best activities under the tested conditions. The unsubstituted quinoline **4a** showed a MIC of 5.6 µM. The presence of the methoxy group attached at the 3-position of the benzene ring yielded compound **4b**, which exhibited a MIC of 32.3 µM. This result demonstrates that this electron-donating group reduced the activity by more than 5-fold compared to the unsubstituted analogue, **4a**. Similarly, the use of another methoxy group attached at the 5-position of the benzene ring led to molecule **4c**, which presented a MIC of 25.5 µM. Fluorine attached at the 4- (**4d**) and 3-positions (**4e**) generated equipotent compounds, with a MIC of 16.8 µM. Quinoline **4f**, containing a fluorine atom at the 2-position, showed a MIC of 19.2 µM. When two fluorine atoms were positioned at the 3- and 4-positions of the benzene ring, the capacity to inhibit the bacillus growth increased. The MIC presented by difluorinated derivative **4g** was 3.9 µM. This MIC value indicated a slightly superior activity compared to that presented by compound **4a** (MIC = 5.6 µM). The chlorine atom attached at the 4- and 3-positions of **4h** and **4i** yielded compounds with MIC values of 15.9 µM and 7.9 µM, respectively. Once more, the presence of a halogen at the 2-position reduced the potency against *M. tuberculosis* since molecule **4j** exhibited a MIC of 18.8 µM. On the other hand, 3,4-dichlorobenzyl derivative **4k** showed the highest antimycobacterial activity of the series of synthesized quinolines. The presence of chlorine atoms at the 3- and 4-positions in structure **4k** led to a MIC value of 0.3 µM. This finding indicated that compound **4k** was approximately 7.6-fold more effective than isoniazid (MIC = 2.3 µM). The importance of this molecular arrangement is evident by the comparison with the activity of

structure **4l**. Changing the chlorine atom from 4- to 2-position greatly reduced antimycobacterial activity as quinoline **4l** showed MIC of 28.7 µM. In addition, steric properties seem to be directly related to the activity presented by the compounds rather than the physicochemical parameters as CLogP of **4k** and **4l** are identical (CLogP = 6.19). Another finding related to the specificity of the activity showed by molecule **4k** was that exchanging the 4-chlorine with the 4-bromine in quinoline **4m** reduced the activity more than 5-fold, leading to a MIC value of 1.6 µM. Notably, independent of the position of the bromine atom attached to the benzene ring in molecules **4n** and **4o**, the MIC values were 13.9 µM. The same pattern was observed with trifluoromethylated compounds **4p** and **4q**, which exhibited MIC values of 7.2 µM. Additionally, by increasing the polarization of quinoline **4r** with the 4-nitro substituent attached to the aryl group, the activity was greatly reduced. This electron-withdrawing group yielded a compound that inhibited *M. tuberculosis* H37Rv growth, with a MIC of 30.8 µM. By contrast, replacement of the hydrogen of the benzene ring with alkyl groups at the 4-position was well tolerated. However, the 4-*iso*-propyl group in molecule **4s** was able to inhibit *M. tuberculosis*, with a MIC of 1.9 µM. The 4-*tert*-butyl group in **4t** presented a MIC value of 3.7 µM. In particular, these results showed that structure **4s** has a slightly higher potency than isoniazid under the experimental conditions used. Moreover, the use of a methylene group as a spacer greatly reduced the activity of quinoline **4u**. The MIC of this structure was 34.1 µM, which was approximately 6-fold less effective than compound **4a**. Finally, the 2-naphthyl group in **4v** presented a MIC value of 7.6 µM, while benzo[*d*][1,3]dioxole derivative **4w** was able to inhibit *M. tuberculosis*, with a MIC of 30.9 µM. This result reveal that increasing the polarity in the substituent reduced the inhibitory capacity of the molecule nearly 4-fold.

Using the MIC value of 2.3 μM, presented by isoniazid, as a threshold, quinolines **3a**, **3h**, **4k**, and **4s** were selected for inhibitory activity against a panel of multidrug-resistant *M. tuberculosis* strains (**Table 2**). Due to the antimycobacterial activity of compounds **4k** and **4m** and their structural similarity, only 3,4-dichlorobenzyl derivative **4k** was selected. *M. tuberculosis* strains PT2, PT12, and PT20 have been described as resistant to drugs such as isoniazid, rifampin, streptomycin, ethionamide, and rifabutine. Additionally, PT12 and PT20 are also resistant to drugs such as pyrazinamide and ethambutol and PT12 present additional resistance to amikacin and capreomycin. The genome of these strains has been sequenced, and the genotypic alterations responsible for the resistant phenotypes have been already reported [16]. Notably, the evaluated quinolines exhibited similar activities, and they were even more potent against PT2, PT12, and PT20 strains than the *M. tuberculosis* H37Rv strain (**Table 2**). Compounds **3a** and **4k** exhibited similar activities between the different drug-susceptible and drug-resistant *M. tuberculosis* strains. Molecules **3h** and **4s** were 1.9- to 9.5-fold more potent against MDR strains than the drug-susceptible *M. tuberculosis* H37Rv strain. From the results obtained, one can conclude that the synthesized quinolines do not share *in vitro* cross-resistance with some important and clinically useful anti-TB drugs. These data suggest promising potential of these structures against drug-susceptible and MDR *M. tuberculosis* strains, probably involving different molecular targets from known drugs.

In an attempt to shed light on the mechanism of action of synthesized quinolines **3a**, **3h**, **4k**, and **4s**, the MICs of the compounds were determined against a 2-(quinolin-4-yloxy)acetamide-resistant *M. tuberculosis* strain (**Table 2**). Whole genome sequencing of this spontaneous resistant strain has revealed a mutation in the *qcrB* gene which substituted an adenine nucleotide at 937 position by a guanine resulting in the T313A amino acid exchange [10]. The four

evaluated compounds showed MIC values that were at least 4-fold higher than those displayed against the *M. tuberculosis* H37Rv strain. These data suggested the involvement of the *qcrB* gene product in the antimycobacterial activity elicited by these molecules. The *qcrB* gene encodes the *b*-subunit of the cytochrome bc_1 complex, which is part of the respiratory electron transport chain and required for ATP biosynthesis. Therefore, the molecular simplification from 2-(quinolin-4-yloxy)acetamides, which culminates in the withdrawal of amide function in compounds **3** and **4**, maintained the cytochrome bc_1 complex as a possible molecular target for this series of molecules.

Table 2. *In vitro* activity of the selected quinolines against *M. tuberculosis* H37Rv, MDR strains, and the 2-(quinolin-4-yloxy)acetamide-resistant strain. Evaluation of the viability of HepG2 and Vero cells. Metabolic stability evaluated in human liver S9 fractions.

Entry	MIC H37Rv (μM)	MIC PT2 (μM)	MIC PT12 (μM)	MIC PT20 (μM)	MIC[a] *qcrB*-T313A (μM)	CC_{50}[b] HepG2 (μM)	CC_{50}[b] Vero (μM)	Cl_{int}[c] (mL/min/kg)	$t_{1/2}$[d] (min)
3a	1.3	2.0	2.0	1.0	32.5	>20	>20	7.1	87.5
3h	1.8	0.5	0.9	0.2	7.3	>20	>20	7.7	78.2
4k	0.3	0.2	0.5	0.2	14.4	14.4[e];14.9[f]	>20	12.6	30.6
4s	1.9	0.5	1.0	0.2	7.8	13.1[e];13.0[f]	>20	17.4	10.0
INH	2.3	291.7	72.9	145.8	2.3	–	–	–	–
RIF	0.05	>48.6	>48.6	12.2	–	–	–	–	–

[a]2-(Quinolin-4-yloxy)acetamide-resistant spontaneous mutant containing a unique alteration in the *qcrB* gene (ACC to GCC at nucleotide 937 position or T313A amino substitution). [b]The toxicity and selectivity of the compounds was studied on HepG2 and Vero cells. The 50% cytotoxic concentration determined by MTT and Neutral Red assays. [c]Human S9 intrinsic clearance. [d]Half-live. [e]Determined by the MTT method. [f]Determined by the Neutral Red method. INH, Isoniazid. RIF, Rifampin.

In addition, quinolines **3a**, **3h**, **4k**, and **4s** were evaluated for their cytotoxicity using HepG2 and Vero cells (**Table 2**). Cellular viability was determined using MTT and Neutral Red uptake assays [9,17] after exposing the cell lineages to the quinolines for 72 h [18]. While MTT determines mitochondrial activity, neutral red assesses the lysosomal viability of the cells. Incubation of carbonyl-containing compounds **3a** and **3h** at a concentration of 20 μM did not significantly affect the viability of either cell lineage. Furthermore, the viability of the Vero cells exposed to 20 μM of compounds **4k** and **4s** was not affected. By contrast, molecules **4k** and **4s** exhibited CC_{50} (50% cytotoxic concentration) values of 14.4/14.9 μM and 13.1/13.0 μM, respectively, when incubated with HepG2 cells. These findings point out that there is a difference of 6.8–6.9-fold between the antimycobacterial activity elicited by structure **4s** against *M. tuberculosis* H37Rv and its cytotoxic concentration. Taking this data alone, these values could be considered indicative of toxicity and, therefore, motivation for exclusion of **4s** from subsequent trials. However, when considering the MIC values of molecule **4s** against MDR strains, this difference increases to at least 13-fold. Therefore, the viability results suggested that structures **3a**, **3h**, **4k**, and **4s** present a reasonable to high degree of selectivity for *M. tuberculosis*, propelling us to continue our research efforts.

Afterward, the metabolic stability of quinolines **3a**, **3h**, **4k**, and **4s** was determined using human liver S9 fractions (**Table 2**). Compounds **3a**, **3h**, and **4k** showed moderate elimination rates based on human S9 intrinsic clearance values (15< Cl_{int} >5 mL/min/kg) [19]. By contrast, molecule **4s** exhibited a high elimination rate under the evaluated conditions (Cl_{int} >15 mL/min/kg) [19]. Interestingly, carbonyl-containing structures **3a** and **3h** demonstrated half-lives that were at least 2.5-fold longer than ether derivatives **4k** and **4s**. Another finding was that the presence of the 4-*iso*-propylbenzyl group reduced the half-life of quinoline **4s** 3-fold when compared to 3,4-dichlorobenzyl derivative **4k**. This may be related to the activation of the benzene ring by the alkyl group, which is prone to oxidation reactions from microsomal enzymes. However, further studies are needed to confirm such hypothesis. For purposes of comparison, the metabolic stability of 2-(quinolin-4-yloxy)acetamides, determined under the same experimental conditions, showed half-lives with mean values of 18.7 min [8,9]. This data suggests, once more [11], that the presence of an amide function generates a hydrolysis soft spot that can be circumvented by the molecular simplification strategy.

In order to evaluate the ability of the compounds to pass cell membranes and to inhibit the intracellular growth of the bacilli, quinolines **3a**, **3h**, and **4k** were evaluated in a macrophage model of *M. tuberculosis* infection. It is important to mention that these molecules were chosen based on cytotoxicity and metabolic stability studies. Macrophages from the untreated group (0.5% DMSO) showed an increase of around 1.21 $\log_{10}$ CFU, within five days, compared to the early control group. This data denoted the bacteria's ability to multiply intracellularly (**Table 3**). Treatments with molecules **3h** and **4k** prevented bacterial growth and kept the bacterial loads stable inside the macrophages. By contrast, phenyl-substituted compound **3a** was ineffective when statically compared to the early control and the untreated group. The data obtained

suggested that structures **3h** and **4k** were able to have a bacteriostatic effect on intracellular *M. tuberculosis* growth ($P<0.05$). It is noteworthy that these simplified quinolines also exhibited the best activities against MDR *M. tuberculosis* strains, making these lead compounds highly attractive.

Table 3. Intracellular activity of compounds **3a**, **3h**, and **4k** in murine macrophages infected with the virulent *M. tuberculosis* H37Rv strain.

Entry	**Log_{10} CFU/well (Mean ± SD)**
Early Control	3.45 ± 0.07
Untreated	4.66 ± 0.23**
3a (5 µM)	4.06 ± 0.11
3h (5 µM)	3.80 ± 0.43*
4k (5 µM)	3.80 ± 0.17*

SD, standard deviation; *P < 0.05 compared to untreated group (0.5% DMSO); **P < 0.01 compared to early control (EC) group.

3. Conclusion

In summary, herein the design and synthesis of a new series of simplified quinolines was shown, and we demonstrated their *in vitro* antimycobacterial activity. The synthetic procedures were performed using readily accessible reagents and reactants under mild reaction conditions. In addition, the compounds showed selective activity against drug-sensitive and MDR *M. tuberculosis* strains, with MIC values in the low micromolar or submicromolar range. Interestingly, the lead compounds carry out their antitubercular activity by targeting the

cytochrome bc_1 complex. This possible mechanism of action/resistance expands the potential use of these structures for non-replicating forms of *M. tuberculosis*. Furthermore, the design strategy provided molecules that were metabolically more stable than their counterparts, which were able to inhibit the intracellular *M. tuberculosis* growth with a bacteriostatic effect. Finally, the submicromolar activity against MDR *M. tuberculosis* strains elicited by leading quinolines coupled to the metabolic stability and intracellular activity suggests that this class of compounds may yield candidates for the development of new anti-TB drugs. New structural modifications of the compounds, as well as bioavailability studies, are currently underway.

4. Experimental section

4.1 Synthesis and structure: apparatus and analysis

The commercially available reactants and solvents were obtained from commercial suppliers and were used without additional purification. The melting points were measured using a Microquímica MQAPF-302 apparatus. ^{1}H and ^{13}C NMR spectra were acquired on an Avance III HD Bruker spectrometer (Pontifical Catholic University of Rio Grande do Sul). Chemical shifts (δ) were expressed in parts per million (ppm) relative to DMSO-d_6, which was used as the solvent, and to TMS, which was used as the internal standard. High-resolution mass spectra (HRMS) were obtained on an LTQ Orbitrap Discovery mass spectrometer (Thermo Fisher Scientific, Bremer, Germany). This system combines an LTQ XL linear ion-trap mass spectrometer and an Orbitrap mass analyzer. The analyses were performed through the direct infusion of the sample in MeOH/CH_3CN (1:1) with 0.1% formic acid (flow rate of 10 µL/min) in positive-ion mode using electrospray ionization (ESI). For the elemental composition, the calculations used the specific tool included in the Qual Browser module of Xcalibur (Thermo

Fisher Scientific, release 2.0.7) software. The compound purity was determined using an Äkta HPLC system (GE Healthcare® Life Sciences) equipped with a binary pump, manual injector, and UV detector. Unicorn 5.31 software (Build 743) was used for data acquisition and processing. The HPLC conditions were as follows: RP column, 5 µm Nucleodur C-18 (250 × 4.6 mm); flow rate, 1.5 mL/min; UV detection, 254 nm; 100% water (0.1% acetic acid) was maintained from 0 to 7 min, followed by a linear gradient from 100% water (0.1% acetic acid) to 90% acetonitrile/methanol (1:1, v/v) from 7 to 15 min (15–30 min) and subsequently returned to 100% water (0.1% acetic acid) in 5 min (30–35 min) and maintained for an additional 10 min (35–45 min). All the evaluated compounds were ≥90% pure.

*4.2 General procedure for the synthesis of quinolines **3** and **4***

The synthesis of 4-hydroxyquinoline (**2**) was performed in accordance to an already reported procedure [9]. The appropriate alkyl halide (1.2 mmol) was added to a mixture of 4-hydroxyquinoline (1 mmol) and potassium carbonate (K_2CO_3, 3.12 mmol) in DMF (6 mL). The reaction mixture was stirred at 25°C for 18 h. Afterwards, the reaction mixture was diluted in water (10 mL) with concomitant precipitation of the product. The solid was separated using a centrifuge (18,000 RPM, 4°C, 10 min), washed with water (3 × 15 mL), and dried under reduced pressure to afford the products in good purity, which was measured by HPLC experiments. In some cases where the purity of the products was not satisfactory, the solids were washed with ethyl ether or purified by flash chromatography using ethyl acetate and hexane in a ratio of 3:7; 1:1, and, finally, 7:3, respectively.

*4.2.1 2-((6-Methoxy-2-methylquinolin-4-yl)oxy)-1-phenylethan-1-one **(3a)***

Yield 38%; m.p.: 155-156 °C; HPLC 90% (t_R = 14.53 min); ^{1}H NMR (400 MHz, DMSO-d_6) δ 2.52 (s, 3H), 3.89 (s, 3H), 5.89 (s, 2H), 6.93 (s, 1H), 7.35 (dd, J = 9.1, 2.9 Hz, 1H), 7.48 (d, J = 2.9 Hz, 1H), 7.61 (t, J = 7.6 Hz, 2H), 7.68 – 7.84 (m, 2H), 8.04 – 8.13 (m, 2H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 24.7, 55.1, 70.2, 99.5, 119.5, 121.2, 127.7 (2C), 128.6 (4C), 129.3, 133.6, 134.0, 156.0, 156.7, 159.1, 193.2; HRMS (FTMS + pESI) *m/z* calcd. for $C_{19}H_{17}NO_3$ $(M)^+$: 308.1281; found: 308.1273.

4.2.2 2-((6-Methoxy-2-methylquinolin-4-yl)oxy)-1-(4-methoxyphenyl)ethan-1-one ***(3b)***

Yield 58%; m.p.: 145-146 °C; HPLC 97% (t_R = 14.72 min); ^{1}H NMR (400 MHz, DMSO-d_6) δ 2.52 (s, 3H), 3.88 (s, 6H), 5.81 (s, 2H), 6.88 (s, 1H), 7.12 (d, J = 8.7 Hz, 2H), 7.35 (dd, J = 9.1, 2.9 Hz, 1H), 7.48 (d, J = 2.8 Hz, 1H), 7.79 (d, J = 9.1 Hz, 1H), 8.06 (d, J = 8.8 Hz, 2H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 25.0, 55.8, 70.5, 99.8, 102.4, 114.0 (2C), 119.8, 121.4, 127.4, 129.4, 130.3 (3C), 144.1, 156.5, 156.9, 159.4, 163.6, 191.6; HRMS (FTMS + pESI) *m/z* calcd. for $C_{20}H_{19}NO_4$ $(M)^+$: 338.1387; found: 338.1401.

4.2.3 2-((6-Methoxy-2-methylquinolin-4-yl)oxy)-1-(3-methoxyphenyl)ethan-1-one ***(3c)***

Yield 78%; m.p.: 93-95 °C; HPLC 90% (t_R = 15.80 min); ^{1}H NMR (400 MHz, DMSO-d_6) δ 2.53 (s, 3H), 3.86 (s, 3H), 3.89 (s, 3H), 5.88 (s, 2H), 6.93 (s, 1H), 7.29 – 7.31 (m, 1H), 7.35 (dd, J = 9.1, 2.9 Hz, 1H), 7.48 (d, J = 2.9 Hz, 1H), 7.53 (t, J = 7.9 Hz, 1H), 7.57 – 7.59 (m, 1H), 7.68 (dd, J = 6.7, 1.2 Hz, 2H), 7.79 (d, J = 9.1 Hz, 1H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 25.5, 55.8, 55.9, 71.0, 100.2, 103.0, 113.2, 120.2, 120.3, 120.8, 122.0, 130.0, 130.3, 136.1, 144.7, 156.7, 157.4, 159.8, 160.0, 193.8; HRMS (FTMS + pESI) *m/z* calcd. for $C_{20}H_{19}NO_4$ $(M)^+$: 338.1387; found: 338.1379.

4.2.4 2-((6-Methoxy-2-methylquinolin-4-yl)oxy)-1-(2-methoxyphenyl)ethan-1-one ***(3d)***

Yield 57%; m.p.: 140-142 °C; HPLC 90% (t_R = 14.93 min); ^{1}H NMR (400 MHz, DMSO-d_6) δ 2.51 (s, 3H), 3.86 (s, 3H), 3.96 (s, 3H), 5.58 (s, 2H), 6.75 (s, 1H), 7.11 (t, J = 7.5 Hz, 1H), 7.26 (d, J = 8.4 Hz, 1H), 7.34 (dd, J = 9.1, 2.9 Hz, 1H), 7.41 (d, J = 2.8 Hz, 1H), 7.65 (t, J = 7.8 Hz, 1H), 7.78 (d, J = 8.9 Hz, 2H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 24.9, 55.3, 56.0, 73.4, 99.8, 102.2, 112.6, 119.8, 120.7, 121.4, 124.6, 129.5, 129.7, 134.8, 144.2, 156.2, 156.8, 159.1, 159.5, 194.5; HRMS (FTMS + pESI) *m/z* calcd. for $C_{20}H_{19}NO_4$ $(M)^+$: 338.1387; found: 338.1385.

4.2.5 2-((6-Methoxy-2-methylquinolin-4-yl)oxy)-1-(p-tolyl)ethan-1-one ***(3e)***

Yield 25%; m.p.: 138-139 °C; HPLC 90% (t_R = 15.01 min); ^{1}H NMR (400 MHz, DMSO-d_6) δ 2.52 (s, 3H), 3.88 (s, 3H), 5.84 (s, 2H), 6.91 (s, 1H), 7.35 (dd, J = 9.1, 2.9 Hz, 1H), 7.41 (d, J = 8.0 Hz, 2H), 7.48 (d, J = 2.8 Hz, 1H), 7.79 (d, J = 9.1 Hz, 1H), 7.98 (d, J = 8.2 Hz, 2H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 21.2, 25.1, 55.3, 70.3, 100.1, 102.5, 119.8, 121.4, 127.8 (2C), 129.3 (2C), 129.4, 131.8, 144.1, 144.4, 156.3, 156.8, 159.4, 192.7; HRMS (FTMS + pESI) *m/z* calcd. for $C_{20}H_{19}NO_3$ $(M)^+$: 322.1438; found: 322.1451.

4.2.6 1-(4-Fluorophenyl)-2-((6-methoxy-2-methylquinolin-4-yl)oxy)ethan-1-one ***(3f)***

Yield 31%; m.p.: 128-130 °C; HPLC 92% (t_R = 14.69 min); ^{1}H NMR (400 MHz, DMSO-d_6) δ 2.53 (s, 3H), 3.89 (s, 3H), 5.88 (s, 2H), 6.95 (s, 1H), 7.36 (dd, J = 9.2, 2.7 Hz, 1H), 7.42 – 7.49 (m, 3H), 7.80 (d, J = 9.1 Hz, 1H), 8.18 (dd, J = 8.3, 5.8 Hz, 2H). 13C NMR (101 MHz, DMSO-d6) δ 25.0, 55.3, 70.3, 99.7, 102.5, 115.9 (d, J = 22.0 Hz, 2C), 119.8, 121.5, 126.9, 129.6, 131.0

(d, *J* = 5.4 Hz, 2C), 144.2, 156.3, 156.9, 159.3, 165.4 (d, *J* = 252.2 Hz), 192.2. HRMS (FTMS + pESI) *m/z* calcd. for $C_{19}H_{16}FNO_3$ $(M)^+$: 326.1187; found: 326.1188.

4.2.7 1-(4-Chlorophenyl)-2-((6-methoxy-2-methylquinolin-4-yl)oxy)ethan-1-one ***(3g)***

Yield 13%; m.p.: 134-136 °C; HPLC 93% (t_R = 15.26 min); ^{1}H NMR (400 MHz, DMSO-d_6) δ 2.53 (s, 3H), 3.88 (s, 3H), 5.87 (s, 2H), 6.95 (s, 1H), 7.35 (dd, *J* = 9.1, 2.9 Hz, 1H), 7.47 (d, *J* = 2.9 Hz, 1H), 7.69 (d, *J* = 8.6 Hz, 2H), 7.79 (d, *J* = 9.2 Hz, 1H), 8.09 (d, *J* = 8.6 Hz, 2H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 25.0, 55.3, 70.4, 99.7, 102.5, 119.7, 121.5, 128.0, 129.5, 129.9 (2C), 131.9 (2C), 138.8, 144.2, 156.3, 156.9, 159.3, 192.8. HRMS (FTMS + pESI) *m/z* calcd. for $C_{19}H_{16}ClNO_3$ $(M)^+$: 342.0891; found: 342.0897.

4.2.8 1-(3-Chlorophenyl)-2-((6-methoxy-2-methylquinolin-4-yl)oxy)ethan-1-one ***(3h)***

Yield 63%; m.p.: 141-142 °C; HPLC 99% (t_R = 14.76 min); ^{1}H NMR (400 MHz, DMSO-d_6) δ 2.53 (s, 3H), 3.89 (s, 3H), 5.90 (s, 2H), 6.99 (s, 1H), 7.36 (dd, *J* = 9.1, 2.9 Hz, 1H), 1H), 7.47 (d, *J* = 2.9 Hz, 1H), 7.65 (t, *J* = 7.9 Hz, 1H), 7.76 – 7.84 (m, 2H), 8.10 – 8.15 (m, 1H), 8.02 (d, *J* = 7.7 Hz, 1H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 25.4, 55.8, 71.0, 100.2, 103.1, 120.2, 122.1, 127.0, 128.3, 129.9, 131.3, 134.03 134.2, 136.6, 144.5, 156.8, 157.4, 159.8, 193.1; HRMS (FTMS + pESI) *m/z* calcd. for $C_{19}H_{16}ClNO_3$ $(M)^+$: 342.0891; found: 342.0893.

4.2.9 1-(3,4-Dichlorophenyl)-2-((6-methoxy-2-methylquinolin-4-yl)oxy)ethan-1-one ***(3i)***

Yield 53%; m.p.: 149-151 °C; HPLC 95% (t_R = 15.80 min); ^{1}H NMR (400 MHz, DMSO-d_6) δ 2.53 (s, 3H), 3.89 (s, 3H), 5.88 (s, 2H), 6.99 (s, 1H), 7.35 (dd, *J* = 9.1, 2.9 Hz, 1H), 7.46 (d, *J* = 2.9 Hz, 1H), 7.79 (d, *J* = 9.1 Hz, 1H), 7.89 (d, *J* = 8.3 Hz, 1H), 8.01 (dd, *J* = 8.4, 2.0 Hz, 1H),

8.31 (d, J = 2.0 Hz, 1H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 24.9, 55.3, 70.5, 99.7, 102.5, 119.7, 121.6, 129.7, 129.4, 130.0, 131.2, 131.9, 136.6, 144.0, 156.3, 156.9, 159.2, 191.8; HRMS (FTMS + pESI) *m/z* calcd. for $C_{19}H_{15}Cl_2NO_3$ $(M)^+$: 376.0502; found: 376.0508.

4.2.10 1-(4-Bromophenyl)-2-((6-methoxy-2-methylquinolin-4-yl)oxy)ethan-1-one ***(3j)***

Yield 55%; m.p.: 134-136 °C; HPLC 91% (t_R = 15.37 min); ^{1}H NMR (400 MHz, DMSO-d_6) δ 2.53 (s, 3H), 3.89 (s, 3H), 5.86 (s, 2H), 6.95 (s, 1H), 7.35 (dd, J = 9.1, 2.9 Hz, 1H), 7.47 (d, J = 2.9 Hz, 1H), 7.77 – 7.85 (m, 3H), 8.01 (d, J = 8.6 Hz, 2H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 25.0, 55.3, 70.4, 99.7, 102.5, 119.7, 121.5, 128.0, 129.5, 129.9 (2C), 131.9 (2C), 133.2, 144.2, 156.3, 156.9, 159.3, 192.8; HRMS (FTMS + pESI) *m/z* calcd. for $C_{19}H_{16}BrNO_3$ $(M)^+$: 386.0386; found: 386.0391.

4.2.11 1-(4-Isobutylphenyl)-2-((6-methoxy-2-methylquinolin-4-yl)oxy)ethan-1-one ***(3k)***

Yield 90%; m.p.: 130-132 °C; HPLC 96% (t_R = 15.99 min); ^{1}H NMR (400 MHz, DMSO-d_6) δ 0.88 (s, 3H), 0.89 (s, 3H), 1.85 – 1.93 (m, 1H), 2.52 (s, 3H), 2.56 (d, J = 7.2 Hz, 2H), 3.88 (s, 3H), 5.86 (s, 2H), 6.92 (s, 2H), 7.35 (dd, J = 9.1, 2.9 Hz, 1H), 7.35 – 7.42 (m, 2H), 7.48 (d, J = 2.9 Hz, 1H), 7.79 (d, J = 9.1 Hz, 1H), 7.97 – 8.04 (m, 2H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 22.6 (2C), 25.5, 30.0, 45.0, 55.8, 70.8, 100.2, 102.9, 120.3, 122.0, 128.4 (2C), 129.8 (2C), 130.0, 132.6, 144.7, 148.4, 156.7, 157.4, 159.9, 193.5; HRMS (FTMS + pESI) *m/z* calcd. for $C_{23}H_{25}NO_3$ $(M)^+$: 364.1907; found: 364.1894.

4.2.12 1-([1,1'-Biphenyl]-4-yl)-2-((6-methoxy-2-methylquinolin-4-yl)oxy)ethan-1-one ***(3l)***

Yield 38%; m.p.: 160-162 °C; HPLC 97% (t_R = 16.25 min); ^{1}H NMR (400 MHz, DMSO-d_6) δ 2.54 (s, 3H), 3.89 (s, 3H), 5.93 (s, 2H), 6.97 (s, 1H), 7.36 (dd, J = 9.2, 2.9 Hz, 1H), 7.44 – 7.50 (m, 4H), 7.48 – 7.58 (m, 3H), 7.75 – 7.84 (m, 3H), 7.87 – 7.94 (m, 2H), 8.14 – 8.21 (m, 2H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 25.0, 55.3, 70.4, 99.7, 102.5, 119.8, 121.5, 126.9 (2C), 127.0 (2C), 128.5, 128.7 (2C), 129.1 (2C), 129.5, 133.0, 138.8, 144.2, 145.3, 156.3, 156.9, 159.4, 193.0. HRMS (FTMS + pESI) *m/z* calcd. for $C_{25}H_{21}NO_3$ $(M)^+$: 384.1594; found: 384.1596.

4.2.13 2-((6-Methoxy-2-methylquinolin-4-yl)oxy)-1-phenylpropan-1-one ***(3m)***

Yield 71%; m.p.: 142-144 °C; HPLC 94% (t_R = 14.58min); ^{1}H NMR (400 MHz, DMSO-d_6) δ 1.71 (d, J = 6.8 Hz, 3H), 2.48 (s, 3H), 3.87 (s, 3H), 6.30 (q, J = 6.7 Hz, 1H), 6.75 (d, J = 1.2 Hz, 1H), 7.35 (ddd, J = 9.2, 2.9, 1.1 Hz, 1H), 7.43 (dd, J = 2.9, 1.1 Hz, 1H), 7.57 – 7.63 (m, 2H), 7.69 – 7.74 (m, 1H), 7.77 (dd, J = 9.1, 1.1 Hz, 1H), 8.12 (dd, J = 8.4, 1.2 Hz, 2H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 18.1, 25.0, 55.3, 75.1, 99.9, 102.5, 119.9, 121.5, 128.5, 129.0 (2C), 129.5 (2C), 133.95, 134.01, 144.2, 156.3, 156.7, 158.6, 197.2; HRMS (FTMS + pESI) *m/z* calcd. for $C_{20}H_{19}NO_3$ $(M)^+$: 322.1438; found: 322.1433.

4.2.14 4-(Benzyloxy)-6-methoxy-2-methylquinoline ***(4a)***

Yield 49%; m.p.: 140-142 °C; HPLC 90% (t_R = 14.59 min); ^{1}H NMR (400 MHz, DMSO-d_6) δ 2.56 (s, 3H), 3.85 (s, 3H), 5.38 (s, 2H), 7.00 (s, 1H), 7.34 (dd, J = 9.1, 2.9 Hz, 1H), 7.41 – 7.49 (m, 2H), 7.57 (dt, J = 6.3, 1.4 Hz, 2H), 7.78 (d, J = 9.1 Hz, 1H); ^{13}C NMR (101 MHz, DMSO-d_6) δ 25.0, 55.3, 69.5, 99.7, 102.4, 119.9, 121.2, 127.5 (2C), 128.0 (2C), 128.5, 129.6, 136.3, 144.1, 156.2, 156.9, 159.5; HRMS (FTMS + pESI) *m/z* calcd. for $C_{18}H_{17}NO_2$ $(M)^+$: 280.1332; found: 280.1324.

455

456 4.*2.15 6-Methoxy-4-((3-methoxybenzyl)oxy)-2-methylquinoline* ***(4b)***

457 Yield 86%; m.p.: 103-104 °C; HPLC 98% (t_R = 14.63 min); ^{1}H NMR (400 MHz, DMSO-d_6) δ
458 2.56 (s, 3H), 3.79 (s, 3H), 3.86 (s, 3H), 5.35 (s, 2H), 6.91 – 6.97 (m, 1H), 6.99 (m, 1H), 7.10 –
459 7.16 (m, 2H), 7.33 – 7.43 (m, 3H), 7.79 (d, J = 9.1 Hz, 1H). ^{13}C NMR (101 MHz, DMSO-d_6) δ
460 25.6, 55.6, 55.8, 69.9, 100.2, 103.0, 113.5, 114.0, 120.1, 120.4, 121.9, 130.1, 130.3, 138.4, 144.6,
461 156.8, 157.6, 159.9, 160.1; HRMS (FTMS + pESI) *m/z* calcd. for $C_{19}H_{19}NO_3$ $(M)^+$: 310.1438;
462 found: 310.1452.

463

464 *4.2.16 4-((3,5-Dimethoxybenzyl)oxy)-6-methoxy-2-methylquinoline* ***(4c)***

465 Yield 88%; m.p.: 106-108 °C; HPLC 93% (t_R = 15.04 min); ^{1}H NMR (400 MHz, DMSO-d_6) δ
466 2.57 (s, 3H), 3.77 (s, 6H), 3.87 (s, 3H), 5.32 (s, 2H), 6.50 (d, J = 2.2 Hz, 1H), 6.72 (d, J = 2.2 Hz,
467 2H), 7.36 (dd, J = 9.1, 2.9 Hz, 1H), 7.00 (s, 1H), 7.41 (d, J = 2.8 Hz, 1H), 7.79 (d, J = 9.1 Hz,
468 1H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 25.3, 55.6 (3C), 70.0, 100.1, 100.2, 103.0, 105.7 (2C),
469 120.4, 122.0, 129.7, 139.0, 144.1, 156.8, 157.4, 160.2, 161.1 (2C); HRMS (FTMS + pESI) *m/z*
470 calcd. for $C_{20}H_{21}NO_4$ $(M)^+$: 340.1543; found: 340.1534.

471

472 *4.2.17 4-((4-Fluorobenzyl)oxy)-6-methoxy-2-methylquinoline* ***(4d)***

473 Yield 67%; m.p.: 113-114 °C; HPLC 96% (t_R = 15.01 min); ^{1}H NMR (400 MHz, DMSO-d_6) δ
474 2.58 (s, 3H), 3.85 (s, 3H), 5.35 (s, 2H), 6.98 (s, 1H), 7.28 (t, J = 8.9 Hz, 2H), 7.34 (dd, J = 9.0,
475 2.9 Hz, 1H), 7.38 (d, J = 2.7 Hz, 1H), 7.63 (dd, J = 8.6, 5.6 Hz, 2H), 7.80 (d, J = 9.0 Hz, 1H);
476 ^{13}C NMR (101 MHz, DMSO-d_6) δ 25.1, 55.3 (d, J = 1.8 Hz), 68.9, 99.8, 102.5, 115.5 (d, J =
477 21.4 Hz, 2C), 119.9, 121.3, 129.6, 129.9 (d, J = 8.3 Hz, 2C), 132.6 (d, J = 3.0 Hz), 144.1,

156.31, 157.1, 159.5, 162.0 (d, *J* = 243.9 Hz); HRMS (FTMS + pESI) *m/z* calcd. for $C_{18}H_{16}FNO_2$ $(M)^+$: 298.1238; found: 298.1251.

4.2.18 4-((3-Fluorobenzyl)oxy)-6-methoxy-2-methylquinoline ***(4e)***

Yield 84%; m.p.: 79-80 °C; HPLC 98% (t_R = 15.80 min); ^{1}H NMR (400 MHz, DMSO-d_6) δ 2.57 (s, 3H), 3.87 (s, 3H), 5.40 (s, 2H), 6.98 (s, 1H), 7.22 (td, *J* = 8.4, 2.0 Hz, 1H), 7.33 – 7.44 (m, 4H), 7.46 – 7.54 (m, 1H), 7.80 (d, *J* = 9.1 Hz, 1H). ^{13}C NMR (101 MHz, DMSO-d6) δ 25.5, 55.8, 69.2 (d, *J* = 2.1 Hz), 100.2, 103.0, 114.7 (d, *J* = 22.1 Hz), 115.3 (d, *J* = 20.9 Hz), 120.3, 121.9, 123.9 (d, *J* = 2.8 Hz), 130.0, 131.2 (d, *J* = 8.4 Hz), 139.7 (d, *J* = 7.5 Hz), 144.6, 156.8, 157.5, 159.8, 162.7 (d, *J* = 243.7 Hz), 163.9; HRMS (FTMS + pESI) m/z calcd. for $C_{18}H_{16}FNO_2$ (M)+: 298.1238; found: 298.1251.

4.2.19 4-((2-Fluorobenzyl)oxy)-6-methoxy-2-methylquinoline ***(4f)***

Yield 52%; m.p.: 139-141 °C; HPLC 90% (t_R = 14.59 min); ^{1}H NMR (400 MHz, DMSO-d_6) δ 2.58 (s, 3H), 3.83 (s, 3H), 5.42 (s, 2H), 7.07 (s, 1H),7.26 – 7.38 (m, 4H), 7.43 – 7.52 (m, 1H), 7.68 (td, *J* = 7.5, 1.7 Hz, 1H), 7.75 – 7.83 (m, 1H); ^{13}C NMR (101 MHz, DMSO-d_6) δ 24.9, 55.2, 64.0 (d, *J* = 3.8 Hz), 99.7, 102.3, 115.4 (d, *J* = 20.9 Hz), 119.7, 121.1, 123.0 (d, *J* = 14.3 Hz), 124.6 (d, *J* = 3.5 Hz), 129.5, 130.3 (d, *J* = 4.0 Hz), 130.5 (d, *J* = 8.3 Hz), 144.1, 156.2, 156.9, 159.3, 160.3 (d, *J* = 246.4 Hz); HRMS (FTMS + pESI) *m/z* calcd. for $C_{18}H_{16}FNO_2$ $(M)^+$: 298.1238; found: 298.1229.

4.2.20 4-((3,4-Difluorobenzyl)oxy)-6-methoxy-2-methylquinoline ***(4g)***

Yield 90%; m.p.: 119-121 °C; HPLC 95% (t_R = 15.33 min); ^{1}H NMR (400 MHz, DMSO-d_6) δ 2.57 (s, 3H), 3.86 (s, 3H), 5.36 (s, 2H), 6.99 (s, 1H), 7.34 (dd, J = 9.1, 2.9 Hz, 1H), 7.39 (d, J = 2.8 Hz, 1H), 7.39 – 7.47 (m, 1H), 7.46 – 7.55 (m, 1H), 7.65 (ddd, J = 11.4, 7.8, 2.0 Hz, 1H), 7.78 (d, J = 9.1 Hz, 1H); ^{13}C NMR (101 MHz, DMSO-d_6) δ 25.0, 55.3, 68.3, 99.8, 102.5, 116.8 (dd, J = 17.5, 3.9 Hz), 117.7 (d, J = 17.2 Hz), 119.8, 121.4, 124.6 (dd, J = 6.8, 3.5 Hz), 129.55, 134.06 (dd, J = 6.1, 3.8 Hz), 144.09, 148.07 (dd, J = 24.7, 12.6 Hz), 150.52 (dd, J = 24.6, 12.5 Hz), 156.96, 157.80 (d, J = 301.4 Hz, 2C); HRMS (FTMS + pESI) *m/z* calcd. for $C_{18}H_{15}F_2NO_2$ $(M)^+$: 316.1144; found: 316.1144.

4.2.21 4-((4-Chlorobenzyl)oxy)-6-methoxy-2-methylquinoline ***(4h)***

Yield 73%; m.p.: 159-160 °C; HPLC 92% (t_R = 15.61 min); ^{1}H NMR (400 MHz, DMSO-d_6) δ 2.57 (s, 3H), 3.86 (s, 3H), 5.35 (s, 2H), 6.95 (s, 1H), 7.34 (dd, J = 9.1, 2.5 Hz, 1H), 7.39 (s, 1H), 7.50 (d, J = 8.3 Hz, 2H), 7.59 (d, J = 8.2 Hz, 2H), 7.80 (d, J = 9.0 Hz, 1H); ^{13}C NMR (101 MHz, DMSO-d_6) δ 25.1, 55.4, 68.8, 99.8, 102.6, 119.9, 121.4, 128.7 (2C), 129.5, 129.6 (2C), 132.7, 135.4, 144.1, 156.3, 157.1, 159.4; HRMS (FTMS + pESI) *m/z* calcd. for $C_{18}H_{16}ClNO_2$ $(M)^+$: 314.0942; found: 314.0956.

4.2.22 4-((3-Chlorobenzyl)oxy)-6-methoxy-2-methylquinoline ***(4i)***

Yield 90%; m.p.: 101-102 °C; HPLC 93% (t_R = 15.65 min); ^{1}H NMR (400 MHz, DMSO-d_6) δ 2.57 (s, 3H), 3.87 (s, 3H), 5.40 (s, 2H), 6.99 (s, 1H), 7.35 (dd, J = 9.1, 2.9 Hz, 1H), 7.40 (d, J = 2.8 Hz, 1H), 7.41 – 7.51 (m, 2H), 7.54 (d, J = 7.2 Hz, 1H), 7.62 (s, 1H), 7.79 (d, J = 9.1 Hz, 1H); ^{13}C NMR (101 MHz, DMSO-d_6) δ 25.0, 55.3, 68.7, 99.7, 102.5, 119.8, 121.4, 126.1, 127.3,

128.0, 129.4, 130.6, 133.2, 138.8, 143.9, 156.3, 157.0, 159.4; HRMS (FTMS + pESI) *m/z* calcd. for $C_{18}H_{16}ClNO_2$ $(M)^+$: 314.0942; found: 319.0945.

4.2.23 4-((2-Chlorobenzyl)oxy)-6-methoxy-2-methylquinoline ***(4j)***

Yield 86%; m.p.: 86-87 °C; HPLC 94% (t_R = 14.92 min); ^{1}H NMR (400 MHz, DMSO-d_6) δ 2.56 (s, 3H), 3.85 (s, 3H), 5.38 (s, 2H, CH_2), 7.00 (s, 1H), 7.34 (dd, J = 9.1, 2.9 Hz, 1H), 7.37 – 7.40 (m, 2H), 7.41 – 7.49 (m, 2H), 7.57 (d, J = 6.8 Hz, 1H), 7.78 (d, J = 9.1 Hz, 1H); ^{13}C NMR (101 MHz, DMSO-d_6) δ 25.0, 55.2, 67.2, 99.7, 102.4, 119.8, 121.2, 127.4, 129.5, 129.6, 130.0, 130.1, 132.7, 133.5, 144.1, 156.2, 157.0, 159.3; HRMS (FTMS + pESI) *m/z* calcd. for $C_{18}H_{16}ClNO_2$ $(M)^+$: 314.0942; found: 314.0935.

4.2.24 4-((3,4-Dichlorobenzyl)oxy)-6-methoxy-2-methylquinoline ***(4k)***

Yield 63%; m.p.: 141-143 °C; HPLC 97% (t_R = 16.21 min); ^{1}H NMR (400 MHz, DMSO-d_6) δ 2.56 (s, 3H), 3.85 (s, 3H), 5.38 (s, 2H), 7.00 (s, 1H), 7.34 (dd, J = 9.1, 2.9 Hz, 1H), 7.39 (d, J = 2.9 Hz, 1H), 7.45 (t, J = 7.3 Hz, 2H), 7.53 – 7.59 (m, 1H), 7.78 (d, J = 9.1 Hz, 1H); ^{13}C NMR (101 MHz, DMSO-d_6) δ 24.8, 55.1, 67.8, 99.5, 102.2, 119.5, 121.1, 127.5, 129.2, 129.4, 130.4, 130.6, 131.0, 137.3, 143.9, 156.1, 156.7, 159.0; HRMS (FTMS + pESI) *m/z* calcd. for $C_{18}H_{15}Cl_2NO_2$ $(M)^+$: 348.0553; found: 348.0546.

4.2.25 4-((2,3-Dichlorobenzyl)oxy)-6-methoxy-2-methylquinoline ***(4l)***

Yield 93%; m.p.: 127-129 °C; HPLC 99% (t_R = 15.73 min); ^{1}H NMR (400 MHz, DMSO-d_6) δ 2.59 (s, 3H), 3.86 (s, 3H), 5.47 (s, 2H), 7.06 (s, 1H), 7.34 – 7.42 (m, 2H), 7.46 (t, J = 7.9 Hz, 1H), 7.66 – 7.71 (m, 2H), 7.77 – 7.84 (m, 1H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 25.3, 55.9,

68.1, 100.3, 103.1, 120.2, 122.1, 128.7, 129.0, 129.7, 130.8, 131.0, 132.6, 136.6, 144.1, 156.9, 157.5, 159.9; HRMS (FTMS + pESI) *m/z* calcd. for $C_{18}H_{15}Cl_2NO_2$ $(M)^+$: 348.0553; found: 348.0541.

4.2.26 4-((4-Bromo-3-chlorobenzyl)oxy)-6-methoxy-2-methylquinoline ***(4m)***

Yield 96%; m.p.: 150-152 °C; HPLC 99% (t_R = 15.99 min); ^{1}H NMR (400 MHz, DMSO-d_6) δ 2.57 (s, 3H), 3.87 (s, 3H), 5.37 (s, 2H), 6.97 (s, 1H), 7.35 (dd, J = 9.0, 2.8 Hz, 1H), 7.39 (d, J = 2.9 Hz, 1H), 7.47 (dd, J = 8.2, 2.1 Hz, 2H), 7.76 – 7.86 (m, 3H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 25.4, 55.8, 68.5, 100.2, 102.9, 120.2, 121.4, 121.9, 128.3, 129.8, 129.9, 133.7, 134.5, 138.5, 144.4, 156.8, 157.4, 159.8; HRMS (FTMS + pESI) *m/z* calcd. for $C_{18}H_{15}BrClNO_2$ $(M)^+$: 392.0047; found: 392.0035.

4.2.27 4-((4-Bromobenzyl)oxy)-6-methoxy-2-methylquinoline ***(4n)***

Yield 78%; m.p.: 173-174 °C; HPLC 98% (t_R = 15.72 min); ^{1}H NMR (400 MHz, DMSO-d_6) δ 2.55 (s, 3H), 3.86 (s, 3H), 5.36 (s, 2H), 6.98 (s, 1H), 7.34 (dd, J = 9.1, 2.9 Hz, 1H), 7.38 (d, J = 2.8 Hz, 1H), 7.52 (d, J = 8.3 Hz, 2H), 7.60 – 7.67 (m, 2H), 7.78 (d, J = 9.1 Hz, 1H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 24.9, 55.2, 68.7, 99.9, 102.4, 119.7, 121.0, 121.1, 129.4, 129.5 (2C), 131.3 (2C), 135.7, 144.0, 156.2, 156.8, 159.3; HRMS (FTMS + pESI) *m/z* calcd. for $C_{18}H_{16}BrNO_2$ $(M)^+$: 358.0437; found: 358.0455.

4.2.28 4-((3-Bromobenzyl)oxy)-6-methoxy-2-methylquinoline ***(4o)***

Yield 95%; m.p.: 111-112 °C; HPLC 97% (t_R = 15.68 min);); ^{1}H NMR (400 MHz, DMSO-d_6) δ 2.56 (s, 3H), 3.87 (s, 3H), 5.39 (s, 2H), 6.98 (s, 1H), 7.35 (dd, J = 9.1, 2.9 Hz, 1H), 7.39 (d, J =

2.7 Hz, 1H), 7.42 (d, J = 7.7 Hz, 1H), 7.58 (dd, J = 7.8, 1.8 Hz, 2H), 7.74 – 7.82 (m, 2H); ^{13}C NMR (101 MHz, DMSO-d_6) δ 25.0, 55.3, 68.6, 99.7, 102.5, 119.8, 121.4, 121.8, 126.5, 129.5 (2C), 130.2, 130.8, 130.9, 139.1, 144.0, 156.3, 157.0, 159.4; HRMS (FTMS + pESI) *m/z* calcd. for $C_{18}H_{16}BrNO_2$ $(M)^+$: 358.0437; found: 358.0440.

4.2.29 6-Methoxy-2-methyl-4-((4-(trifluoromethyl)benzyl)oxy)quinoline ***(4p)***

Yield 81%; m.p.: 171-173 °C; HPLC 94% (t_R = 15.81 min); ^{1}H NMR (400 MHz, DMSO-d_6) δ 2.57 (s, 3H), 3.88 (s, 3H), 5.52 (s, 2H), 7.04 (s, 1H),7.38 (dd, J = 9.2, 2.9 Hz, 1H), 7.44 (d, J = 2.9 Hz, 1H), 7.76 – 7.84 (m, 5H); ^{13}C NMR (101 MHz, DMSO-d_6) δ 25.0, 55.2, 68.6, 99.7, 102.5, 119.8, 121.4, 123.9 (q, J = 3.8 Hz), 124.1 (q, J = 272.4 Hz), 124.7 (q, J = 3.8 Hz), 129.3 (q, J = 31.8 Hz), 129.6, 129.7 (2C), 131.4, 137.9, 144.1, 156.3, 157.0, 159.3; HRMS (FTMS + pESI) *m/z* calcd. for $C_{19}H_{16}F_3NO_2$ $(M)^+$: 348.1206; found: 348.1191.

4.2.30 6-Methoxy-2-methyl-4-((3-(trifluoromethyl)benzyl)oxy)quinoline ***(4q)***

Yield 90%; m.p.: 103-104 °C; HPLC 96% (t_R = 15.80 min); ^{1}H NMR (400 MHz, DMSO-d_6) δ 2.57 (s, 3H), 3.86 (s, 3H), 5.49 (s, 2H), 7.01 (s, 1H), 7.35 (dd, J = 9.1, 2.9 Hz, 1H,), 7.41 (d, J = 2.8 Hz, 1H), 7.67 – 7.77 (m, 2H), 7.80 (d, J = 9.1 Hz, 1H), 7.89 (d, J = 7.6 Hz, 1H), 7.95 (s, 1H); ^{13}C NMR (101 MHz, DMSO-d_6) δ 25.0, 55.4, 68.7, 102.3, 119.8, 121.4, 124.1 (q, J = 251.5 Hz), 125.5 (q, J = 3.7 Hz, 2C), 127.9 (2C), 128.7 (q, J = 31.7 Hz), 129.6 (2C), 141.2, 144.2, 156.4, 157.0, 159.3; HRMS (FTMS + pESI) *m/z* calcd. for $C_{19}H_{16}F_3NO_2$ $(M)^+$: 348.1206; found: 348.1207.

4.2.31 6-Methoxy-2-methyl-4-((4-nitrobenzyl)oxy)quinoline ***(4r)***

Yield 64%; m.p.: 198-199 °C; HPLC 98% (t_R = 14.94 min); ^{1}H NMR (400 MHz, DMSO-d_6) δ 2.56 (s, 3H), 3.89 (s, 3H), 5.56 (s, 2H), 6.99 (s, 1H), 7.36 (dd, J = 9.2, 2.9 Hz, 1H), 7.45 (d, J = 3.1 Hz, 1H), 7.81 (m, 3H), 8.31 (d, J = 8.4 Hz, 2H); ^{13}C NMR (101 MHz, DMSO-d_6) δ 25.1, 55.4, 68.4, 99.8, 102.6, 119.8, 121.5, 123.8 (2C), 128.2 (3C), 129.6, 144.2, 147.2, 156.4, 157.1, 159.2; HRMS (FTMS + pESI) *m/z* calcd. for $C_{18}H_{16}N_2O_4$ $(M)^+$: 325.1183; found: 325.1199.

4.2.32 4-((4-Isopropylbenzyl)oxy)-6-methoxy-2-methylquinoline ***(4s)***

Yield 80%; m.p.: 124-126 °C; HPLC 91% (t_R = 16.32 min); ^{1}H NMR (400 MHz, DMSO-d_6) δ 1.21 (d, J = 7.0 Hz, 6H), 2.56 (s, 3H), 2.91 (m, 1H), 3.85 (s, 3H), 5.32 (s, 2H), 7.01 (s, 1H),7.24 – 7.43 (m, 4H), 7.48 (d, J = 7.8 Hz, 2H),7.78 (d, J = 9.1 Hz, 1H); ^{13}C NMR (101 MHz, DMSO-d_6) δ 23.7 (2C), 25.0, 33.1, 55.3, 69.6, 99.8, 102.5, 119.8, 121.2, 126.4 (2C), 127.8 (2C), 129.6, 133.6, 144.3, 148.5, 156.1, 156.9, 159.7; HRMS (FTMS + pESI) *m/z* calcd. for $C_{21}H_{23}NO_2$ $(M)^+$: 322.1802; found: 322.1780.

4.2.33 4-((4-(Tert-butyl)benzyl)oxy)-6-methoxy-2-methylquinoline ***(4t)***

Yield 97%; m.p.: 133-134 °C; HPLC 97% (t_R = 15.83 min); ^{1}H NMR (400 MHz, DMSO-d_6) δ 1.29 (s, 9H), 2.57 (s, 3H), 3.84 (s, 3H), 5.32 (s, 2H), 6.99 (s, 1H), 7.30 – 7.36 (m, 1H), 7.37 – 7.41 (m, 1H), 7.42 – 7.50 (m, 4H), 7.77 (dd, J = 9.1, 3.3 Hz, 1H); ^{13}C NMR (101 MHz, DMSO-d_6) δ 25.5, 31.6 (3C), 55.8, 69.9, 100.3, 102.9, 120.4, 121.7, 125.8 (2C), 127.8, 127.9 (2C), 130.0, 133.8, 144.6, 151.0, 156.8, 157.5, 160.1; HRMS (FTMS + pESI) *m/z* calcd. for $C_{22}H_{25}NO_2$ $(M)^+$: 336.1958; found: 336.1974.

4.2.34 6-Methoxy-2-methyl-4-phenethoxyquinoline ***(4u)***

Yield 20%; m.p.: 90-92 °C; HPLC 90% (t_R = 15.80 min); ^{1}H NMR (400 MHz, DMSO-d_6) δ 2.55 (s, 3H), 3.19 (t, J = 6.5 Hz, 2H), 3.85 (s, 3H), 4.40 (t, J = 6.6 Hz, 2H), 6.89 (s, 1H), 7.24 (m, 1H), 7.28 – 7.36 (m, 4H), 7.42 (d, J = 7.2 Hz, 2H), 7.75 (dd, J = 8.2, 1.4 Hz, 1H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 25.0, 34.7, 55.2, 68.6, 99.6, 101.9, 119.7, 121.2, 126.3, 128.2 (2C), 129.1 (2C), 129.5, 138.3, 144.0, 156.1, 157.1, 159.6; HRMS (FTMS + pESI) *m/z* calcd. for $C_{19}H_{19}NO_2$ $(M)^+$: 294.1489; found: 294.1482.

4.2.35 6-Methoxy-2-methyl-4-(naphthalen-2-ylmethoxy)quinoline ***(4v)***

Yield 64%; m.p.: 156-158 °C; HPLC 92% (t_R = 16.14 min); ^{1}H NMR (400 MHz, DMSO-d_6) δ 2.58 (s, 3H), 3.85 (s, 3H), 5.54 (s, 2H), 7.06 (s, 1H), 7.36 (dd, J = 9.2, 2.9 Hz, 1H), 7.44 (d, J = 2.9 Hz, 1H), 7.52 – 7.57 (m, 2H), 7.66 – 7.73 (m, 1H), 7.81 (d, J = 9.1 Hz, 1H), 7.92 – 8.02 (m, 3H), 8.10 (s, 1H); ^{13}C NMR (101 MHz, DMSO-d_6) δ 25.1, 55.3, 69.7, 99.7, 102.6, 119.9, 121.4, 125.5, 126.3, 126.4, 126.5, 127.6, 127.9, 128.3, 129.6, 132.6, 132.8, 133.9, 144.2, 156.3, 157.0, 159.6; HRMS (FTMS + pESI) *m/z* calcd. for $C_{22}H_{19}NO_2$ $(M)^+$: 330.1489; found: 330.1476.

4.2.36 4-(Benzo[d][1,3]dioxol-4-ylmethoxy)-6-methoxy-2-methylquinoline ***(4w)***

Yield 90%; m.p.: 128-130 °C; HPLC 91% (t_R = 14.83 min); ^{1}H NMR (400 MHz, DMSO-d_6) δ 2.56 (s, 3H), 3.85 (s, 3H), 5.25 (s, 2H), 6.05 (s, 2H), 6.94 – 7.00 (m, 2H), 7.05 (d, J = 9.2 Hz, 1H), 7.13 (s, 1H), 7.29 – 7.37 (m, 2H), 7.77 (d, J = 8.9 Hz, 1H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 24.9, 55.1, 69.5, 99.8, 101.0, 102.4, 108.2, 108.3, 119.9, 121.2, 121.6, 129.5, 129.9, 144.1, 147.1, 147.4, 156.2, 156.9, 159.5; HRMS (FTMS + pESI) *m/z* calcd. for $C_{19}H_{17}NO_4$ $(M)^+$: 324.1230; found: 324.1237.

637 *4.3 Susceptibility testing against M. tuberculosis*

638 The compounds were tested for their inhibitory potential against *M. tuberculosis* H37Rv
639 reference strain (ATCC 27294) by the resazurin reduction microplate assay (REMA), as already
640 thoroughly described [9,14,15]. Stock solutions (2 mg mL^{-1}) of the test compounds were made in
641 neat DMSO (Sigma-Aldrich), and aliquots were stored at –20°C. The compounds were further
642 diluted in 1 mL of Difco™ Middlebrook 7H9 broth (Becton Dickinson, BD), supplemented with
643 10% (v/v) BBL™ Middlebrook ADC enrichment (albumin, dextrose, and catalase; BD) and 5%
644 (v/v) DMSO. The maximum concentration tested for each compound ranged from 5 to 40 µg
645 mL^{-1} due to differences in solubility. The compounds were prepared as 10-point, 2-fold serial
646 dilutions directly in 96-well plates. Three independent experiments were performed, and the MIC
647 was considered to be the lowest compound concentration that prevented resazurin (Sigma-
648 Aldrich) reduction, which, otherwise, is indicated by a color conversion from blue to pink. The
649 MIC value reported for each compound was the most frequent value among the three assays or
650 the highest value obtained, and it was expressed in molar concentration (µM).

651

652 *4.4 Susceptibility testing against MDR strains of M. tuberculosis*

653 Compounds **3a**, **3h**, **4s**, and **4k** were further tested by REMA, as described in section 4.3, for
654 their inhibitory potential against three MDR clinical isolates of *M. tuberculosis* [16] and one
655 laboratory strain of *M. tuberculosis* that carries a mutation in the *qcrB* (Rv2196) gene [10]. The
656 clinical isolates (named PT2, PT12, and PT20) were obtained from patients in the Lisbon Health
657 Region, Lisbon, Portugal. INH and RIF were used as control drugs to demonstrate the MDR
658 phenotype of these isolates.

659

4.5 Cytotoxicity investigation

Cellular viability determination after incubation with the test compounds was performed using two different methods: the MTT method and neutral red uptake assay [12]. First, HepG2 and Vero cells were grown in Dulbecco's Modified Eagle Medium (DMEM) supplemented with 10% inactivated fetal bovine serum, 1% antibiotic (gentamicin) and 0.01% antifungal (amphotericin B). Cells were seeded at 4×10^3 (HepG2) or 2×10^3 cells/well (Vero) in a 96-well microtiter plate and incubated for 24 h. Test compounds were diluted in three different concentrations (1, 5 and 20 µM) using 2% DMSO and were incubated with the cell lines for 72 h at 37 °C under 5% CO_2. For the MTT assay, the cultures were incubated with MTT reagent (5 mg/mL) for 4 h. The absorbance was measured with excitation and emission wavelengths of 570 and 655 nm, respectively (SpectraMax M2e, Molecular Devices, USA). The precipitated purple formazan crystals were directly proportional to the number of live cells with active mitochondria. For the neutral red assay, after 72 h of incubation with the compounds, the cells were washed with PBS before the addition of 200 µL of neutral red dye solution (25 µg/mL, Sigma) prepared in serum-free medium. The plate was incubated for an additional 3 h at 37 °C under 5% CO_2. After incubation, cells were washed with PBS, followed by incubation with 100 µL of a desorb solution ($CH_3COOH/EtOH/H_2O$, 1:50:49) for 30 min, with gentle shaking to extract neutral red dye from the viable cells. The absorbance was measured at 562 nm using a microtiter plate reader. The percentage of cell viability for the treated groups was reported by considering the control wells (2% DMSO) as 100% of cell viability: cell viability (%) = (absorbance of treated wells/absorbance of control wells) × 100. Statistical analysis was performed using one-way analysis of variance using GraphPad Prism 5.0 software (San Diego, CA, USA).

4.6 Metabolic stability

In brief, the human liver S9 fraction was prepared by homogenizing liver, centrifuging at 9,000 × *g*, and saving the post-mitochondrial supernatant. It is noteworthy that the S9 fraction contains both cytosolic and membrane-bound drug-metabolizing enzymes. Test compounds at a concentration of 2 µM were incubated with 1 mg/mL of the enzyme preparation containing NADPH at 37°C. Consumption of the compounds from the incubation mixture was measured at 0, 5, 15, and 30 min using the HPLC-MS/MS technique to determine the *in vitro* disappearance half-life. Verapamil was used as the positive control. The intrinsic clearance has been described as low (<5 mL/min/kg), moderate (5 to 15 mL/min/kg), and high (>15 mL/min/kg) [19].

4.7 Intracellular activity in a macrophage model of M. tuberculosis infection

Compounds **3a**, **3h**, and **4k** were evaluated in a macrophage model of *M. tuberculosis* infection, as previously described [9,20], with slight modifications. Murine macrophage RAW 264.7 cells were cultured in RPMI 1640 medium (Gibco), supplemented with 10% heat-inactivated fetal bovine serum (FBS), without penicillin–streptomycin, and about 5×10^4 cells were seeded in each well of a sterile flat-bottom 24-well plate. After an incubation period of 24 h in a bacteriological chamber (at 37°C with 5% CO_2 and a humid atmosphere), the adhered cells were washed once with pre-heated sterile PBS (pH 7.4) to remove non-adherent cells, and the infection occurred as follows. One isolated colony of the *M. tuberculosis* H37Rv strain was cultured in 5 mL of 7H9-ADC broth, supplemented with 0.05% (v/v) Tween 80 (Sigma-Aldrich) and 0.2% (v/v) glycerol (MERCK) until the mid-log phase (OD_{600} ≈0.5). The culture was diluted in pre-heated RPMI medium, and approximately 2.5×10^4 CFU was added to each well. The infection was allowed to continue for 3 h at 37°C with 5% CO_2. Afterwards, the infected cells

were washed twice with sterile PBS to remove non-internalized mycobacteria. Cells of the early control (EC) group were lysed on the day of treatment onset with 1 mL of 0.025% SDS (dissolved in sterile 0.9% saline). Lysates were serially diluted in sterile 0.9% saline and plated on Difco™ Middlebrook 7H10 Agar (BD), supplemented with 10% BBL™ Middlebrook OADC enrichment (oleic acid, albumin, dextrose, and catalase; BD) and 0.5% (v/v) glycerol. Thereafter, the infected cells were treated with 5 µM of each test compound in triplicate. Compounds **3a**, **3h**, and **4k** were first solubilized (4 mM) in neat DMSO, and then diluted in 2 mL of RPMI medium to a final concentration of 5 µM. The final DMSO concentration was maintained at 0.5% in each well. After 5 days of treatment, the RPMI medium was removed, and each well was gently washed with PBS. The treated macrophages were lysed with 0.025% SDS, serially diluted in 0.9% saline, and plated on 7H10 agar. After an incubation period of 2–3 weeks at 37°C, the CFU were counted, setting a limit of detection (LOD) between 20 and 200 CFU per plate. The calculated CFU values were converted into logarithms of CFU before statistical analysis, and the result was expressed as the mean of the $\log_{10}$ CFU values per well ± the standard deviation (mean $\log_{10}$ CFU/well ± SD). Groups were compared by one-way analysis of variance (ANOVA), followed by the Tukey post-test, using GraphPad Prism 5.0 (GraphPad, San Diego, CA, USA). Significance between groups was determined using $P < 0.05$.

Supporting information

^{1}H and ^{13}C NMR spectra of the compounds. This material is available free of charge and can be obtained via the Internet.

Acknowledgments

This work was supported by Banco Nacional de Desenvolvimento Econômico e Social (BNDES) [grant number 14.2.0914.1] and the National Institute of Science and Technology on Tuberculosis (CNPq-FAPERGS-CAPES) [grant numbers: 421703-2017-2 and 17-1265-8]. C. V. Bizarro, L. A. Basso, and P. Machado are Research Career Awardees of the National Research Council of Brazil (CNPq). This study was financed in part by the Coordenação de Aperfeiçoamento de Pessoal de Nivel Superior, Brasil (CAPES), Finance Code 001.

Notes

The authors declare no competing financial interests.

References

1. Global tuberculosis report 2019, World Health Organization. Available from: http:// https://www.who.int/tb/global-report-2019, (accessed in December 2019).

2. M. Pai, M.A. Behr, D. Dowdy, K. Dheda, M. Divangahi, C.C. Boehme, A. Ginsberg, S. Swaminathan, M. Spigelman, H. Getahun, D. Menzies, M. Raviglione. Tuberculosis. Nat Rev Dis Primers 2 (2016) 16076. doi: 10.1038/nrdp.2016.76

3. A. Koul, E. Arnoult, N. Lounis, J. Guillemont, K. Andries. The challenge of new drug discovery for tuberculosis. Nature 469 (2011) 483. doi:10.1038/nature09657.

4. S. Tiberi, R. Buchanan, J.A. Caminero, R. Centis, M.A. Arbex, M.A. Salazar-Lezama, J. Potter, G.B. Migliori. The challenge of the new tuberculosis drugs. Press Medicale. 46 (2017) 41. http://dx.doi.org/10.1016/j.lpm.2017.01.016

5. A.H. Diacon, A. Pym, M. Grobusch, R. Patientia, R. Rustomjee, L. Page-Shipp, et al. The diarylquinoline TMC207 for multidrug-resistant tuberculosis. N Engl J Med. 360 (23) (2009) 2397. doi:10.1056/NEJMoa0808427.

6. M.T. Gler, V. Skripconoka, E. Sanchez-Garavito, H. Xiao, J. L. Cabrera-Rivero, D. E. Vargas-Vasquez, M. Gao, M. Awad, S. K. Park, T. S. Shim, G. Y. Suh, M. Danilovits, H. Ogata, A. Kurve, J. Chang, K. Suzuki, T. Tupasi, W.J. Koh, B. Seaworth, L.J. Geiter, C. D. Wells. Delamanid for Multidrug-Resistant Pulmonary Tuberculosis. New England Journal of Medicine. 366 (2012) 2151. doi:10.1056/NEJMoa1112433.

7. G.V. Bloemberg, P.M. Keller, D. Stucki, A. Trauner, S. Borrell, T. Latshang, M. Coscolla, T. Rothe, R. Hömke, C. Ritter, J. Feldmann, B. Schulthess, S. Gagneux, E.C. Böttger. Acquired Resistance to Bedaquiline and Delamanid in Therapy for Tuberculosis. N Engl J Med. 373 (2015)1986. DOI: 10.1056/NEJMc1505196.

8. K. Pissinate, A.D. Villela, V. Rodrigues-Junior, B.C. Giacobbo, E.S. Grams, B.L. Abbadi, R.V. Trindade, L. Roesler Nery, C.D. Bonan, D.F. Back, M.M. Campos, L.A. Basso, D.S. Santos, P. Machado. 2-(Quinolin-4-yloxy)acetamides Are Active against Drug-Susceptible and Drug-Resistant *Mycobacterium tuberculosis* Strains. ACS Med Chem Lett. 7 (2016) 35. doi: 10.1021/acsmedchemlett.5b00324.

9. B.C. Giacobbo, K. Pissinate, V. Rodrigues-Junior, A.D. Villela, E.S. Grams, B.L. Abbadi, F.T. Subtil, N. Sperotto, R.V. Trindade, D.F. Back, M.M. Campos, L.A. Basso, P. Machado, D.S. Santos. New insights into the SAR and drug combination synergy of 2-(quinolin-4-yloxy)acetamides against *Mycobacterium tuberculosis*. Eur J Med Chem. 126 (2017) 491. doi: 10.1016/j.ejmech.2016.11.048.

10. F.T. Subtil, A.D. Villela, B.L. Abbadi, V.S. Rodrigues-Junior, C.V. Bizarro, L.F.S.M.

Timmers, O.N. de Souza, K. Pissinate, P. Machado, A. López-Gavín, G. Tudó, J. González-Martín, L.A. Basso, D.S. Santos. Activity of 2-(quinolin-4-yloxy)acetamides in *Mycobacterium tuberculosis* clinical isolates and identification of their molecular target by whole genome sequencing. Int J Antimicrob Agents. 51(3) (2018) 378. doi: 10.1016/j.ijantimicag.2017.08.023.

11. E. Pitta, M.K. Rogacki, O. Balabon, S. Huss, F. Cunningham, E. M. Lopez-Roman, J. Joossens, K. Augustyns, L. Ballell, R. H. Bates, P. Van der Veken. Searching for New Leads for Tuberculosis: Design, Synthesis, and Biological Evaluation of Novel 2-Quinolin-4-yloxyacetamides. J Med Chem. 59(14) (2016) 6709. doi: 10.1021/acs.jmedchem.6b00245.

12. F.S. Macchi, K. Pissinate, A.D. Villela, B.L. Abbadi, V. Rodrigues-Junior, D. D. Nabinger, S. Altenhofen, N. Sperotto, A. da Silva Dadda, F.T. Subtil, T.F. de Freitas, A.P. Erhart Rauber, A.F. Borsoi, C.D. Bonan, C.V. Bizarro, L.A. Basso, D.S. Santos, P. Machado. 1H-Benzo[*d*]imidazoles and 3,4-dihydroquinazolin-4-ones: Design, synthesis and antitubercular activity. Eur J Med Chem. 155 (2018) 153. doi: 10.1016/j.ejmech.2018.06.005.

13. J. Escribano, C. Rivero-Hernández, H. Rivera, D. Barros, J. Castro-Pichel, E. Pérez-Herrán, A. Mendoza-Losana, I. Angulo-Barturen, S. Ferrer-Bazaga, E. Jiménez-Navarro, L. Ballell. 4-Substituted thioquinolines and thiazoloquinolines: potent, selective, and Tween-80 in vitro dependent families of antitubercular agents with moderate in vivo activity. ChemMedChem. 6(12) (2011) 2252. doi: 10.1002/cmdc.201100309.

794 14. Ballell L, Bates RH, Young RJ, Alvarez-Gomez D, Alvarez-Ruiz E, Barroso V, et al.
795 Fueling open-source drug discovery: 177 small-molecule leads against tuberculosis.
796 ChemMedChem 8 (2013) 313. doi: 10.1002/cmdc.201200428.

797 15. J. Palomino, A. Martin, M. Camacho, H. Guerra, J. Swings, F. Portaels. Resazurin
798 Microtiter Assay Plate: Simple and Inexpensive Method for Detection of Drug Resistance
799 in *Mycobacterium tuberculosis*. Antimicrob Agents Chemother 46 (2002) 2720. doi:
800 10.1128/AAC.46.8.2720-2722.2002.

801 16. J. Perdigão, H. Silva, D. Machado, R. Macedo, F. Maltez, C. Silva, L. Jordao, I. Couto,
802 K. Mallard, F. Coll, G.A. Hill-Cawthorne, R. McNerney, A. Pain, T.G. Clark, M.
803 Viveiros, I. Portugal. Unraveling Mycobacterium tuberculosis genomic diversity and
804 evolution in Lisbon, Portugal, a highly drug resistant setting. BMC Genomics. 15 (2014)
805 991. doi: 10.1186/1471-2164-15-991.

806 17. G. Repetto, A. del Peso, J.L. Zurita. Neutral red uptake assay for the estimation of cell
807 viability/cytotoxicity. Nat Protoc 3 (2008) 1125. doi: 10.1038/nprot.2008.75.

808 18. Tuberculosis Antimicrobial Acquisition & Coordinating Facility (TAACF): Description
809 of TAACF Assays.

810 19. Kerns, E. H.; Di, L. Drug Like Proprieties: Concept, Structure Design and Methods from
811 Toxicity Optimization Press: Elsevier, London, UK, 2008.

812 20. V.S. Rodrigues-Junior, A.A. dos Santos Junior, A.D. Villela, J.M. Belardinelli, H.R.
813 Morbidoni, L.A. Basso, M.M. Campos, D.S. Santos, IQG-607 abrogates the synthesis of
814 mycolic acids and displays intracellular activity against *Mycobacterium tuberculosis* in

815 infected macrophages, Int J Antimicrob Agents. 43 (2014) 82.
816 https://doi.org/10.1016/j.ijantimicag.2013.08.021.

817

Highlights:

Compounds with minimum inhibitory concentration (MIC) values in the low micromolar or submicromolar range

Activity against drug-susceptible and multidrug-resistant *Mycobacterium tuberculosis* (Mtb) strains

Structures carry out their antitubercular activity by targeting the cytochrome bc_1 complex

Compounds devoid of apparent toxicity to HepG2 and Vero cells

Molecules with improved metabolic properties able to inhibit the intracellular Mtb growth

Declaration of interests

☒ The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

☐The authors declare the following financial interests/personal relationships which may be considered as potential competing interests:

Pablo Machado on behalf of authors.