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Article

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Searching for new leads for Tuberculosis: Design, synthesis and biological evaluation of novel 2-quinolin-4-yloxyacetamides

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Keywords: tuberculosis, mycobacteria, anti-mycobacterial, anti-infective, HTS, quinoline, quinoloxyacetamides

ABSTRACT

In this study, a new series of more than sixty quinoline derivatives has been synthesized and evaluated against *Mycobacterium tuberculosis* (H37Rv). Apart from the SAR exploration around the initial hits, the optimization process focused on the improvement of the physicochemical properties, cytotoxicity and metabolic stability of the series. The best compounds obtained

exhibited MIC values in the low micromolar range, excellent intracellular antimycobacterial activity and an improved physicochemical profile without cytotoxic effects. Further investigation revealed that the amide bond was the source for the poor blood stability observed while some of the compounds exhibited hERG affinity. Compound **83** which contains a benzoxazole ring instead of the amide group was found to be a good alternative, with good blood stability and no hERG affinity, providing new opportunities for the series. Overall, the obtained results suggest that further optimization of solubility and microsomal stability of the series could provide a strong lead for a new anti-TB drug development program.

INTRODUCTION

Tuberculosis (TB) is a worldwide pandemic caused by *Mycobacterium tuberculosis (Mtb)*. The threat it represents to global health is escalating because of the increased prevalence of multidrug resistant TB (MDR-TB) and extensively drug resistant TB (XDR-TB) strains. The World Health Organization has estimated that one third of the world's population is infected with *Mtb*, resulting in 1.5 million TB deaths in 2014.[1]

The first-line drugs for the treatment of drug-susceptible TB are isoniazid, pyrazinamide, rifampicin, ethambutol and streptomycin.[2] The current treatment regimen for drug-sensitive TB consists of a combination of 3-4 first-line drugs that must be taken for 6 months or longer.[3] Infection relapse and emergence of drug-resistant strains have been reported in many cases as often patients partly or completely drop the therapy due to its side effects and long duration.[4] In case of drug-resistant strains, treatment consists of second-line drugs which are administered for 2 years or longer. Apart from the high cost, many second-line drugs are toxic and have severe side effects, posing a significant challenge to health systems.[5]

Consequently, the need for novel, more effective drugs is evident. Ideally, any new drug should be able to shorten the duration of treatment, avoid significant drug-drug interactions with current regimens, be efficacious against MDR-TB and XDR-TB and preferably operate via a new mode of action.[6]

One approach to address this drug discovery need is through high throughput phenotypic screening of small molecule libraries directly against mycobacteria in order to identify a variety of new active scaffolds.[7] A high throughput screening (HTS) campaign performed by GlaxoSmithKline (GSK) delivered several compound families that passed multiple drug-like property filters and were progressed for further profiling.[6] The quinoloxyacetamides (QOA) constitute one of these families and the two most active hit compounds (**1** and **2**, shown in Fig. 1) were selected for further structure-activity relationship (SAR) studies and optimization of their properties. It is noteworthy that quinolines represent a common substructure of several known anti-tubercular drugs, e.g., bedaquiline, mefloquine and fluoroquinolones such as moxifloxacin and gatifloxacin.[8,9,10,11,12]

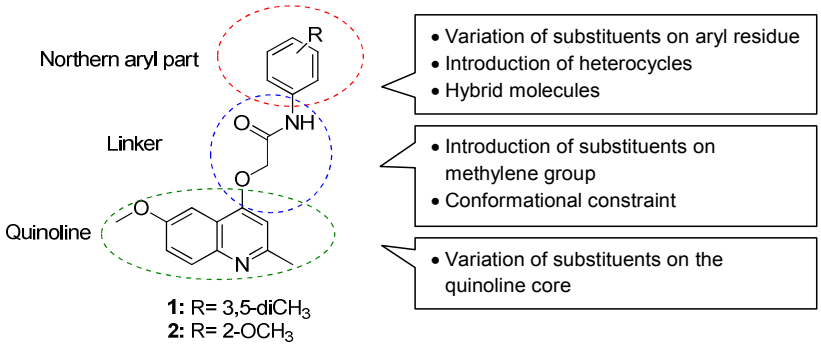
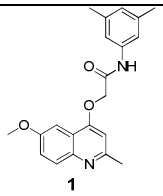
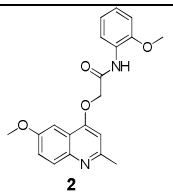


Figure 1. Hit compounds **1**, **2** and SAR design.

As shown in Table 1, the hit compounds **1** and **2** were found to possess significant anti-mycobacterial activity with minimum inhibitory concentration (MIC) values 1.9 μM and 1.4 μM respectively, against *M. tuberculosis* (H37Rv). The cytotoxicity ($HepG_2$) of the initial hits was

also evaluated and hit **1** displayed a level of cytotoxicity (IC_{50} 19.95 μM) while hit **2** did not exhibit cytotoxic effects. Moreover, two key physicochemical parameters (solubility and permeability) were investigated, revealing workable, but suboptimal properties of **1** and **2**. Also, the mouse and human microsomal stabilities were determined, and the results indicated that further optimization was required before progressing to an *in vivo* proof-of-concept.[6]

Table 1. Biological profile for the hit compounds **1** and **2**.[a]

Structure Cmpd	 **1**		 **2**	
MIC (μM)[b]	1.9		1.4	
Cytotoxicity IC_{50} (μM) [c]	19.95		>100.00	
Permeability (nm/sec) [d]	180		120	
Solubility (μM)[e]	26		38	
Microsomal Fraction Stability [f]	Mouse	Human	Mouse	Human
Cl_{int} (mL $min^{-1}g^{-1}$)	18.9	1.3	>30	5.4
$t_{1/2}$ (min)	<5	>30	<3	16

[a]upon re-testing the obtained data were found to differ in some cases from the data published in reference 6; [b]MIC against *Mycobacterium tuberculosis* (H37Rv); [c]$HepG_2$, human caucasian hepatocyte carcinoma; [d]artificial membrane permeability; [e]*in vitro* profiling for kinetic aqueous solubility (CLND, chemiluminescent nitrogen detection); [f]*in vitro* microsomal fraction stability (mouse and human) results: intrinsic clearance (Cl_{int}) and half-life time ($t_{1/2}$) are reported; imidazolam was used as control with Cl_{int} = 27.5 ±0.4 and 6.4 mL $min^{-1}g^{-1}$ in mouse and human, respectively and $t_{1/2}$ = <5 and 9 min in mouse and human, respectively.

Based on the promising initial data, a library of more than sixty novel analogues was designed and synthesized, relying on iterative logic-based SAR exploration of the chemical space around the hits. Along with identifying the structural parameters that govern the anti-mycobacterial properties of these molecules, the primary goals were to optimize physicochemical properties and to improve metabolic stability. For practical reasons, hit **1** was selected as a reference and

divided into three substructures to help organize the SAR exploration: the quinoline, the linker and the northern aryl part (Fig.1). This was done by preparing three compound sub-series in which each of the substructures was modified separately, while keeping the rest of the molecule identical to the reference compound. Practically, the process of compound optimization was organized around iterative cycles of design, synthesis and evaluation. At each stage, experimentally obtained anti-mycobacterial, physico-chemical, and *in vitro* toxicity data were used to refine the decision model used for synthetic planning.

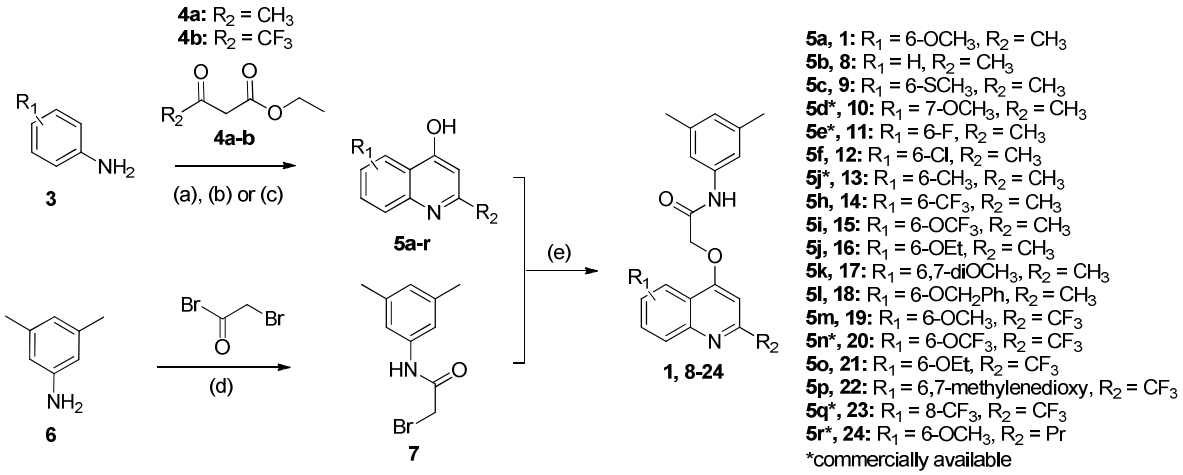
RESULTS AND DISCUSSION

Chemistry. More than 60 novel compounds were synthesized for this study. The target compounds are clustered according to the modification type they contain, relative to reference compound **1**. Lastly, three compounds which contain amide bond replacements are presented.

Modification of the quinoline substitution pattern. An important goal of these compounds was to investigate the contribution to anti-mycobacterial activity of the 6-methoxy and 2-methyl quinoline substituents in **1**. The selection of substituents was carried out in a highly exploratory manner and comprises groups with widely differing impact on the sterics and electronics of the quinoline system. The general synthetic strategy to obtain the target compounds (**1**, **8-24**) consisted of coupling the modified quinoline core **5a-r** to 2-bromo-*N*-(3,5-dimethylphenyl)acetamide **7** in the presence of potassium carbonate (K_2CO_3) (Scheme 1). Construction of the 2-methyl and 2-trifluoromethyl quinolin-4-ols (**5a-c, 5f, 5h-m, 5o-p**) was achieved by condensation of a number of commercially available anilines (**3**) with ethyl acetoacetate (**4a**) or ethyl 4,4,4-trifluoroacetoacetate (**4b**) following a Conrad-Limpach protocol.[13,14,15] The moderate to low yields obtained with the used Conrad-Limpach protocols were considered acceptable for our purposes. However, further optimization of the quinolone

synthesis would be required in case of upscaling. The *N*-aryl haloacetamide building block **7** was obtained in excellent yield by acylation of aniline **6** with bromoacetyl bromide in the presence of triethylamine (TEA).[16]

Scheme 1. Synthesis of compounds with quinoline substitution modifications[a]



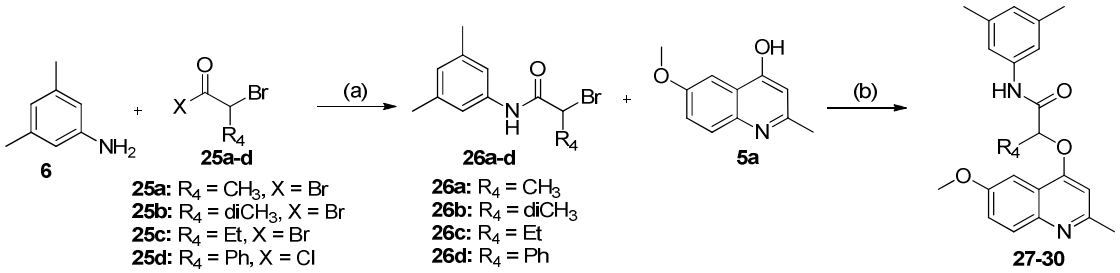
[a]Reagents and conditions: (a) Dowtherm A, H_2SO_4, 240-250 ºC, 35-60 min; (b) 130 °C, 90 min, then Dowtherm A, 250 °C, 1 h; (c) acetic acid, toluene, reflux, 2 h, then Dowtherm A, 240 ºC, 1 h; (d) triethylamine, anhydrous DCM, rt, 2 h; (e) potassium carbonate, anhydrous DMF, rt, 3h - 4d.

Modification of the linker. SAR investigation of the linker region was focused on three main approaches: (1) introduction of substituents on the acetyl's methylene group, (2) conformational constraint of the linker and (3) other modification types (Schemes 2, 3 and 4, respectively).

The synthetic approach to target compounds with the first modification type (**27-30**, summarized in Scheme 2), was analogous to the general strategy described earlier (Scheme 1). The reaction of 3,5-dimethylaniline (**6**) with acyl halides **25a-d** gave intermediates **26a-d**. The bromoalkylacyl bromides **25a-c** were commercially available, while 2-bromo-2-phenylacetyl chloride (**25d**) was prepared from phenylacetyl chloride in the presence of *N*-bromosuccinimide and 2,2′-Azobis(2-methylpropionitrile) (AIBN) according to a literature procedure.[17] The final

products **27-30** were subsequently obtained by alkylation of 2-methyl-6-methoxy-quinolinol (**5a**) with these halides (**26a-d**) in the presence of sodium hydride (NaH).

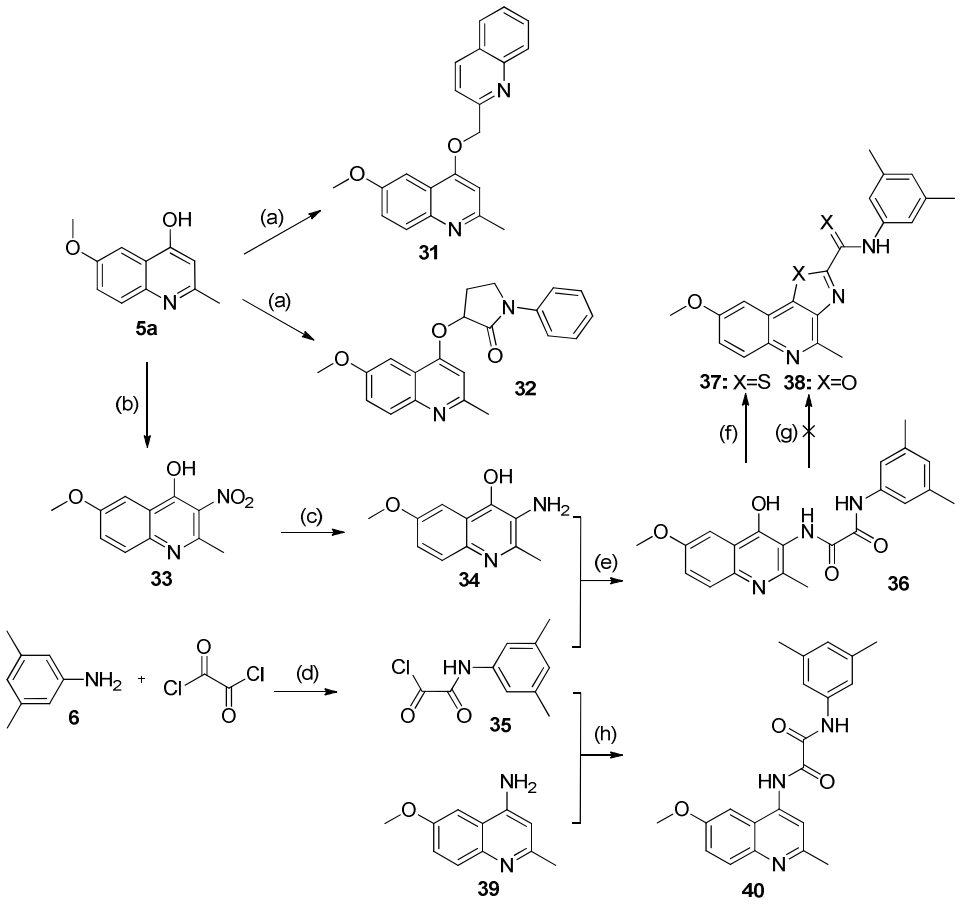
Scheme 2. Synthesis of compounds with introduction of substituents on the linker[a]



[a]Reaction conditions: (a) triethylamine, anhydrous DCM, rt, 2h-overnight; (b) sodium hydride, (potassium iodide), anhydrous DMF, rt, 6-48 h.

Next, four conformationally constrained analogues were prepared (compounds **31**, **32**, **37** and **40**, Scheme 3). While the synthetic preparation of the target compounds **31** and **32** could be achieved using the general alkylation procedure, a more lengthy approach was required for compound **37**. Nitration and subsequent zinc/ammonium chloride (Zn/NH_4Cl) reduction of 2-methyl-6-methoxyquinolin-4-ol (**5a**) yielded 3-aminoquinolin-4-ol **34**.[14] The latter compound was *N*-acylated with intermediate **35**, which was prepared from oxalyl chloride and 3,5-dimethylaniline (**6**). Dehydration of the obtained intermediate **36** with phosphorus pentasulfide (P_4S_{10}) led to the assembly of the annulated thiazole ring of thiazoloquinoline **37**. The associated formation of a thioamide in **37** during P_4S_{10}-mediated dehydration was not considered problematic, and the obtained compound was allowed to enter biological evaluation after purification. Comparable attempts to prepare the oxazole analogue of **37** (compound **38**) by dehydration of **36** with phosphorus pentoxide (P_4O_{10}), were not successful. Compound **40** was obtained from the acylation reaction of intermediate **35** with the commercially available 2-methyl-4-amino-6-methyl quinoline **39**.

Scheme 3. Synthesis of compounds with conformational constraint of the linker[a]

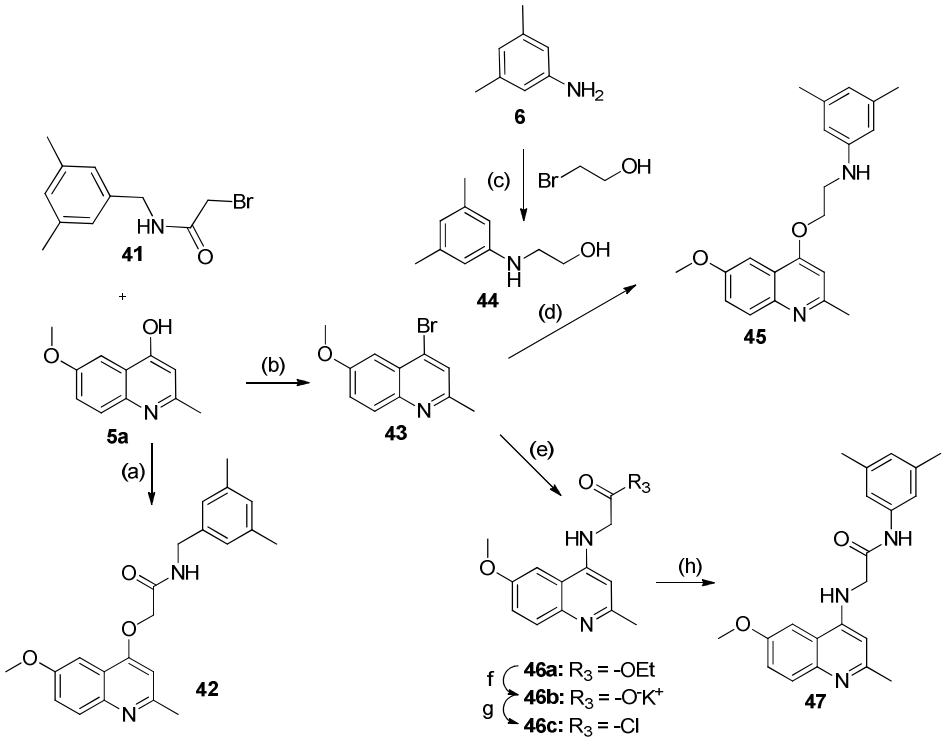


[a]Reaction conditions: (a) 7-(bromomethyl)quinoline or 3-bromo-1-phenylpyrrolidin-2-one, potassium carbonate, anhydrous DMF, rt, 2-72 h; (b) nitric acid, propionic acid, 110 °C, 2 h; (c) Zn, THF/sat. aq. NH_4Cl: 2/1, rt, 1 h; (d) 0 °C, 1 h; (e) anhydrous DCM:DMF (10:1), 0 °C, 1 h; (f) phosphorus pentasulfide, anhydrous pyridine, reflux, overnight; (g) phosphorus pentoxide, anhydrous pyridine, reflux, overnight; (h) sodium hydride, anhydrous DMF, rt, overnight.

Subsequently, three more analogues (**42**, **45**, **47**) were prepared with linker-modifications that were not covered in the aforementioned sets (Scheme 4). The synthetic approach to **42** was completely analogous to the general strategy mentioned earlier. While for the preparation of **45**, the hydroxy group of quinolinol **5a** was converted to bromine by phosphorous tribromide (PBr_3) leading to intermediate **43**. Separately, the reaction of 3,5-dimethylaniline (**6**) and 2-bromoethanol resulted in intermediate **44**, which was subsequently coupled with **43** to yield the target compound **45** using Cu(I)-catalysis (Ullmann reaction).[18] For the synthesis of **47**,

intermediate **43** was coupled with glycine ethyl ester under nucleophilic aromatic substitution conditions to yield the carboxylic ester **46a**.[19] After basic hydrolysis of **46a** in methanol, carboxylate **46b** was obtained in quantitive yield and then it was converted to the acyl chloride **46c** using thionyl chloride ($SOCl_2$). Amide bond formation between intermediate **46c** and 3,5-dimethylaniline (**6**) was achieved using basic acylating conditions to afford final compound **47**.

Scheme 4. Synthesis of compounds which contain other modifications types[a]



[a] Reaction conditions: (a) potassium carbonate, anhydrous DMF, rt, 26 h; (b) PBr_3, DMF, 50 °C, 4 d; (c) 90 °C, 4 h; (d) CuI, TMEDA, cesium carbonate, anhydrous DMF, 95 °C, 2 d; (e) glycine ethyl ester·HCl, phenol, 120 °C, overnight; (f) sodium hydroxide, MeOH, reflux, 90 min; (g) thionyl chloride, anhydrous DCM, reflux, 2 d; (h) 3,5-dimethylaniline (**6**), anhydrous DCM, rt, 16 h.

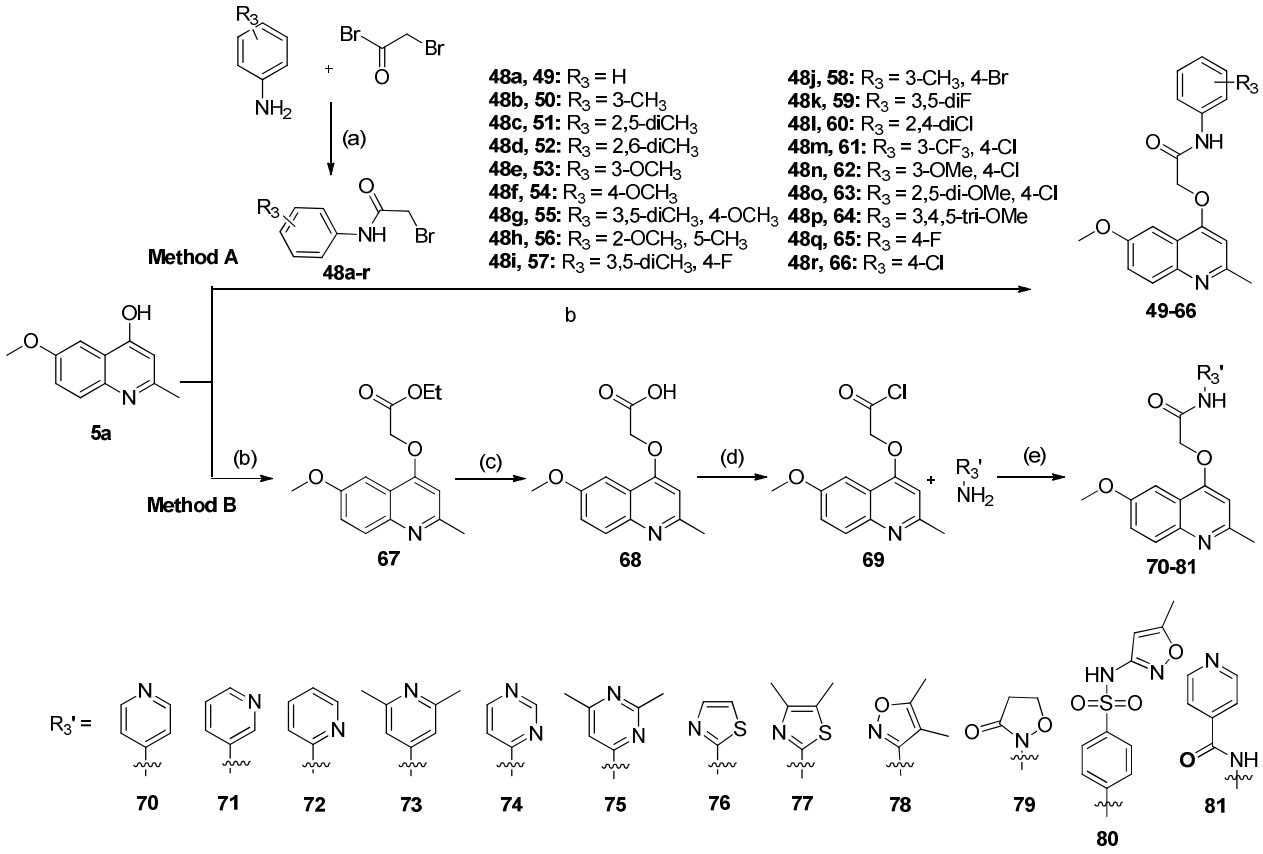
Modification of the northern aryl fragment. Analogues in this section are divided into three categories: (1) derivatives with modified phenyl substitution (**49-66**), (2) compounds with heteroaryl groups (**70-78**) and (3) hybrid molecules in which known anti-tubercular drugs replace the aniline of the hits (**79-81**) (Scheme 5). The synthesis of the first set of compounds (**49-66**)

relied on the general alkylation-based methodology. Thus, haloacetamide intermediates **48a-r** were first prepared by acylation of a number of commercially available anilines coupled with bromoacetyl bromide, as depicted in Scheme 5 (Method A). Subsequently, *O*-alkylation of the quinolinol **5a** with these halides resulted in the final products **49-66**.

The target compounds **70-81** were obtained by an alternative route (Method B) shown in Scheme 5. According to this, quinolinol **5a** was alkylated with ethyl 2-bromoacetate followed by hydrolysis of ester **67** under basic conditions and conversion of the corresponding carboxylic acid **68** to acyl chloride **69** using thionyl chloride ($SOCl_2$). Subsequently, reaction with a number of commercially available heterocyclic amines afforded the final compounds (**70-78**).

Lastly, the same methodology (method B) was used to synthesize three "hybrid" compounds, in which the quinoloxyacetamide core was covalently linked to known anti-TB drugs with available free amines: cycloserine (**79**), sulfamethoxazole (**80**) and isoniazid (**81**).[20]

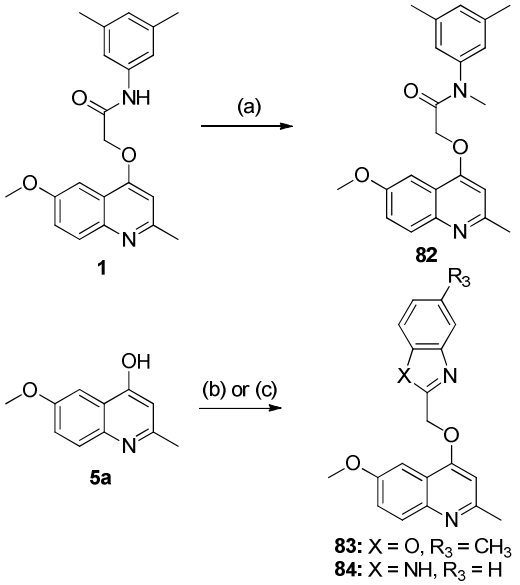
Scheme 5. Synthesis of compounds with modifications of the northern aryl fragment[a]



[a] Reaction conditions: (a) triethylamine, anhydrous DCM, rt, 2-48 h; (b) potassium carbonate, anhydrous DMF, rt, 3-96 h; (c) potassium hydroxide, MeOH or EtOH, reflux, 1.5-3.5 h; (d) thionyl chloride, anhydrous DCM, 20 ºC to 40 ºC, 24-48 h; (e) anhydrous DCM, rt to reflux, 18-48 h.

Amide bond replacements. Lastly, three compounds (**82-84**) which could avoid amide bond hydrolysis were prepared. Deprotonation of compound **1** using sodium hydride (NaH) and subsequent methylation with methyl iodide (MeI) afforded the target compound **82**. Preparation of compounds **83** and **84** was similar to the general alkylation method described previously.

Scheme 6. Synthesis of compounds with amide bond replacements[a]



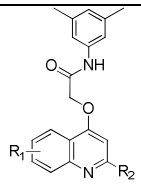
[a] Reaction conditions: (a) methyl iodide, sodium hydride, anhydrous THF, 0 ºC to 20 ºC, overnight; (b) potassium carbonate, anhydrous DMF, rt, overnight; (c) sodium hydride, anhydrous DMF, rt, overnight.

Biological evaluation. The hit compound **1** obtained from high throughput screening (HTS) was resynthesized to confirm activity and evaluated together with 62 novel derivatives. All synthesized compounds were evaluated for their ability to inhibit the growth of *Mtb* H_{37}Rv strain and for cytotoxicity in $HepG_2$ cells. In addition, three physicochemical properties were measured: artificial membrane permeability, kinetic aqueous solubility (CLND, chemiluminescent nitrogen detection) and ChromlogD.[21,22]

Table 2 presents the results for the reference compound **1** and the first set of compounds possessing variations to the substitution pattern of the quinoline system (**8-24**). The main goal was to investigate the contribution to anti-mycobacterial activity of the 6-methoxy and 2-methyl quinoline substituents. Therefore, the 6-methoxy substituent was removed or replaced with halides, methylthio, alkyl or alkoxy substituents (**8, 9, 11-16**, Table 2). In addition, a regio-isomer of compound **1** with the methoxy group shifted from position 6 to 7 was prepared (**10**,

Table 2). Since the 2-methyl substituent of hits **1** and **2** was suspected to be a metabolically labile site, a few analogues possessing a trifluoromethyl group were synthesized (**19-23**, Table 2). Additionally, the 2-methyl group was replaced by a propyl group in an attempt to explore the available space (**24**).

Table 2. Biological profile of the compounds with a modified quinoline part.

Compd			MIC (μM) [a]	Cytotoxicity IC_{50} (μM)[b]	Permeability (nm/sec)[c]	Solubility (μM) [d]	Chrom logD[e]
	R_1	R_2					
1	6-OCH_3	-CH_3	1.9	19.95	180	26	5.64
8	-H	-CH_3	24	>100.00	370	55	5.39
9	6-SCH_3	-CH_3	>125	>100.00	n.d.[f]	3	6.42
10	7-OCH_3	-CH_3	>250	50.12	n.d.[f]	n.d.[f]	5.59
11	6-F	-CH_3	15.6	63.10	310	28	5.79
12	6-Cl	-CH_3	40	>100.00	n.d.[f]	8	6.38
13	6-CH_3	-CH_3	3.9	15.85	520	83	5.95
14	6-CF_3	-CH_3	>250	>100.00	<30	10	6.67
15	6-OCF_3	-CH_3	>250	>100.00	<30	n.d.[f]	6.79
16	6-OEt	-CH_3	>250	>100.00	<30	n.d.[f]	5.98
17	6,7-*di*OCH_3	-CH_3	>250	10.00	605	n.d.[f]	4.84
18	6-OCH_2Ph	-CH_3	>250	>100.00	<10	<1	7.03
19	6-OCH_3	-CF_3	>250	>100.00	<10	15	7.02
20	6-OCF_3	-CF_3	>250	>100.00	<3	34	7.96
21	6-OEt	-CF_3	>250	>100.00	<3	<1	7.55
22	6,7-methylen-dioxy	-CF_3	>250	>100.00	<30	<1	6.73

23	8-CF_3	-CF_3	>250	>100.00	<10	<1	7.73
24	6-OCH_3	-Pr	>250	12.59	n.d.[f]	12	6.44

[a]MIC against *Mycobacterium tuberculosis* (H37Rv); [b]$HepG_2$, human caucasian hepatocyte carcinoma; [c]artificial membrane permeability; [d]*in vitro* profiling for kinetic aqueous solubility (CLND, chemiluminescent nitrogen detection); [e]chromlogD values at pH = 7.4; [f]n.d. = not determined.

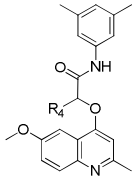
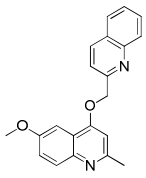
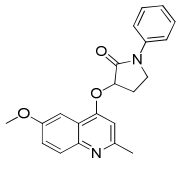
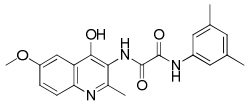
As shown in Table 2, SAR analysis of the quinoline part of the molecule showed that only a methyl can be considered an acceptable alternative for the original 6-methoxy group (**13**, MIC = 3.9 μM). This compound showed slightly better solubility (CLND) and high permeability, although it displayed similar cytotoxicity to **1**. The presence of fluorine or no substituent (-H) at the same position led to decreased activity (**11** and **8**; MIC = 15.6 and 24 μM, respectively). Chlorine resulted in a further drop of the potency (**12**, MIC = 40 μM). All the other substituents in the series were inactive. Steric limitations might be involved here, although other factors most likely contribute, as reflected by the absence of activity for the 6-trifluoromethyl containing analogue **15**.[23] Regarding the methyl group at position 2, its replacement with a trifluoromethyl or a propyl group led to inactive compounds (**19** and **24**). For all the compounds (**19-24**) possessing a trifluoromethyl group in position 2, the extremely poor solubility and higher chromlogD values could play a role in the loss of potency.

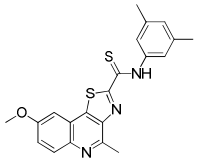
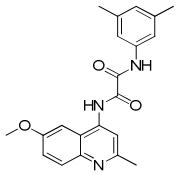
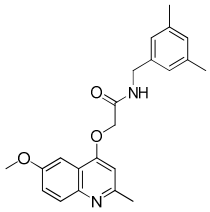
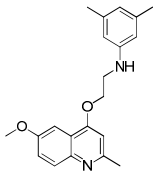
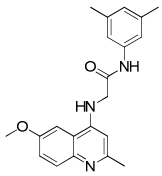
Table 3 presents the results for the second set of compounds possessing modifications on the linker. The first area of exploration was around the acetyl methylene group. Mono-substitution at this prochiral position is the most straightforward way of introducing a chiral center in these compounds without affecting the quinoloxyacetamide basic framework. In addition, this site was also suspected of being a contributor to the fast (oxidative) metabolism observed for the hit compounds. In this initial proof-of-concept compound set, the substituent choice was limited to small aliphatic groups (methyl and ethyl) and a phenyl ring (**27-30**).

Next, four conformationally constrained analogues were prepared (**31**, **32**, **37** and **40**). In compounds **31**, **32** and **37** conformational locking was accomplished by an additional ring closure, while in the case of analogue **40** the oxalyl diamide fragment was expected to rigidify the linker region into a constrained conformation.[24, 25]

Subsequently, three more analogues (**42**, **45**, **47**) were prepared with linker-modifications that were not covered in the aforementioned sets. Compound **42** contains a benzylamide function as a means to avoid potential toxicity and metabolic stability concerns that are associated with anilide groups. Compound **45** possesses a fully reduced linker, while in compound **47** the ether bridge between the linker region and the quinoline residue is replaced by an amine function.

Table 3. Biological profile of the compounds with linker modifications.

Cmpd	Structure	MIC (μM)[a]	Cytotoxicity IC_{50} (μM)[b]	Permeability (nm/sec)[c]	Solubility (μM)[d]	Chrom logD[e]
27	 $R_4 = CH_3$	125	39.81	320	61	5.76
28	$R_4 = diCH_3$	>250	>100.00	n.d.[f]	20	6.37
29	$R_4 = Et$	>250	79.43	390	123	6.28
30	$R_4 = Ph$	>125	>100.00	<30	2	7.13
31		32	31.62	470	21	5.48
32		125	100.00	550	215	4.99
36		>125	>100.00	n.d.[f]	10	3.76

37		>125	>100.00	<30	35	n.d.[f]
40		>125	>100.00	n.d.[e]	10	6.47
42		31	>100.00	<3	92	5.25
45		32	31.62	190	33	6.93
47		47	6.31	310	204	3.59

[a]MIC against *Mycobacterium tuberculosis* (H37Rv); [b]$HepG_2$, human caucasian hepatocyte carcinoma; [c]artificial membrane permeability; [d]*in vitro* profiling for kinetic aqueous solubility (CLND, chemiluminescent nitrogen detection); [e]chromlogD values at pH = 7.4; [f]n.d. = not determined.

Antimycobacterial screening of the compounds with an *alpha*-substituted acetyl group (**27-30**) demonstrated that modifications of this type are detrimental to activity. Similarly, in the second group of compounds the activity was lost or significantly reduced in all cases. In the same way, compounds belonging to the third group (**42**, **45** and **47**) showed some moderate potency giving MIC values of 31, 32 and 47 μM respectively.

To investigate the role of the hydrophobic phenyl ring in the northern part of the molecule, thirty compounds (**49-66**, **70-78** and **79-81**) possessing modifications on the northern aryl part

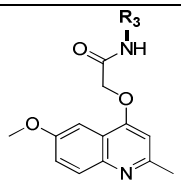
were prepared and data from their biological and physicochemical evalution is presented in Table 4.

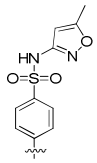
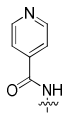
Analogues in this section are divided into three categories: (1) derivatives with modified phenyl substitution (**49-66**), (2) compounds with heteroaryl groups (**70-78**) and (3) hybrid molecules in which 3 known anti-TB drugs replace the aniline of the hits compounds (**79-81**). For the first category, the initial goal was to introduce a diverse set of commercially available anilines. These molecules allow a more thorough investigation of the role of the aryl substituents on the antimycobacterial properties of QOA-based molecules. Furthermore, the metabolic liability of the specific aryl residues present in **1** and **2** had also been proposed as a potential reason for the observed microsomal instability. Therefore, additional value for this series could come from its potential to deliver antimycobacterial products in which positions prone to CYP-mediated oxidation have been blocked or modified.

Similar to the substitutions discussed above, replacement of the phenyl ring with a heteroaryl (**70-78**) offered the possibility of improving the microsomal stability, at least partially, by reducing the overall lipophilicity of the molecule. Commercially available heteroaromatic amines with an identical or similar dimethyl-substitution pattern as present in reference **1** were included in the series to allow direct comparisons.

Regarding the hybrid compounds, we hypothesized that the full hybrid constructs could still possess antimycobacterial properties. In addition, if metabolic cleavage of the amide would take place, either inside bacteria or mediated by host metabolism, a second antimycobacterial compound could be released.

Table 4. Biological profile of the compounds with modifications on the northern aryl.

Cmpd	 R_3	MIC (μM)[a]	Cytotoxicity IC_{50} (μM)[b]	Permeability (nm/sec)[c]	Solubility (μM)[d]	Chrom logD[e]
49	phenyl	2	39.81	370	63	4.28
50	3-CH_3-phenyl	2	39.81	355	193	4.83
51	2,5-*di*CH_3-phenyl	8	>100.00	810	45.5	5.05
52	2,6-*di*CH_3-phenyl	125	>100.00	630	112	4.55
2	2-OCH_3-phenyl	1.4	>100.00	120	38	4.68
53	3-OCH_3-phenyl	6.4	>100.00	470	35	4.49
54	4-OCH_3-phenyl	0.6	>100.00	420	108	4.22
55	3,5-*di*CH_3, 4-OCH_3-phenyl	1	7.94	320	17	4.99
56	2-OCH_3, 5-CH_3-phenyl	2.5	>100.00	n.d.[f]	9.5	5.65
57	3,5-*di*CH_3, 4-F-phenyl	2	>100.00	210	31.5	5.50
58	3-CH_3, 4-Br-phenyl	3.9	15.85	n.d.[f]	<1	5.95
59	3,5-*di*F-phenyl	3	>100.00	n.d.[f]	13	5.13
60	2,4-*di*Cl-phenyl	2	>100.00	n.d.[f]	20.5	6.40
61	3-CF_3, 4-Cl-phenyl	47	79.43	n.d.[f]	17	6.22
62	3-OMe, 4-Cl-phenyl	>125	>100.00	570	13	5.09
63	2,5-*di*OMe, 4-Cl-phenyl	>125	>100.00	n.d.[f]	3	5.80
64	3,4,5-*tri*OMe-phenyl	62	>100.00	620	34	3.95
65	4-F-phenyl	12	63.10	230	104	4.49
66	4-Cl-phenyl	3	>100.00	n.d.[f]	9	5.15
70	pyridin-2-yl	15.65	>100.00	480	29	3.49
71	pyridin-3-yl	62.5	>100.00	330	32	2.46

72	pyridin-4-yl	>250	>100.00	560	91.5	2.50
73	2,6-*di*CH_3-pyridin-4-yl	>250	12.59	n.d.[f]	12	3.01
74	pyrimidin-4-yl	>250	>100.00	525	27.5	2.53
75	2,6-diCH_3-pyrimidin-4-yl	>250	>100.00	12	5	3.24
76	thiazol-2-yl	>250	>100.00	1120	54	3.20
77	4,5-*di*CH_3-thiazol-2-yl	62	>100.00	440	31.5	4.24
78		187	>100.00	625	195.5	3.30
79		>125	>100.00	<10	≥166	0.79
80		125	>100.00	15	≥381	2.20
81		62	>100.00	33	≥375	1.48

[a]MIC against *Mycobacterium tuberculosis* (H37Rv); [b]$HepG_2$, human caucasian hepatocyte carcinoma; [c]artificial membrane permeability; [d]*in vitro* profiling for kinetic aqueous solubility (CLND, chemiluminescent nitrogen detection); [e]chromlogD values at pH = 7.4; [f]n.d. = not determined.

Modification of the substitution pattern on the phenyl ring resulted in a number of active compounds with MIC values in the low micromolar range. Elimination of one or both methyl groups (**50**, **49**) did not affect the activity (MIC = 2 μM), indicating that the methyl groups are not critical for potency. Moreover, solubity and chromlogD values were improved, although cytotoxicity remained at similar levels as **1**. It is worth noting that shifting one methyl group from position 3 to 2 led to decreased activity (**51**, MIC = 8 μM), while shifting both methyls from 3,5 to 2,6 positions led to the practically inactive compound **52** (MIC = 125 μM).

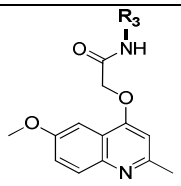
Exploration of the position of the methoxy group of the hit compound **2** indicated that the *para*-position (compound **54**, MIC = 0.6 μM) is more favourable than the *ortho*- (hit compound **2**, 1.2 μM) or *meta*- substitution (compound **53**, MIC = 6.4 μM). Compound **54**, apart from excellent activity, also possessed an improved profile in comparison with the reference compound **1**. More specifically, it did not exhibit cytotoxic effects (IC_{50} >100 μM), had good permeability (420 nm/sec), improved kinetic aqueous solubility (CLND) (108 μM) and chromlogD values (4.22). Compound **55**, where we preserved both methyl groups at positions 3 and 5 (as the reference compound **1**) and a methoxy group at position 4 (as compound **54**), showed very good activity (MIC = 1 μM) but high cytotoxicity (IC_{50} 7.94 μM) and low solubility (17 μM). Several compounds possessing mono-, di- or tri- substituted phenyl rings (**56-60**, **66**) exhibited very good potencies, although they were less active than the reference compounds. Most of them did not show cytotoxic effects (**56**, **57**, **59**, **60**, **66**), but, chromlogD values were generally high (>5) and solubility low (>32).

Introduction of more hydrophilic rings such as pyridine, pyrimidine, thiazole, isoxazolidinone or dimethylisoxazole did not provide the desired solubility improvement although the chromlogD values (2.46-4.24) were lower than the phenyl derivatives, and the antitubercular activity was lost for most of them. Comparison among compounds **70-72** revealed that 2-pyridine (**70**) is the most favourable, however still less active than the hit compounds (**1** and **2**). The last subset of compounds which includes three hybrid molecules (**79-81**), unfortunately, led to loss of activity too.

Additionally, the intracellular activity for the hit compounds **1** and **2** and the most active, non-cytotoxic synthesized compounds (**54**, **56**, **57**, **59**, **60**, **66**) was evaluated. This assay determines the effect of the compounds on mycobacteria growing inside phagocytes/macrophages. Activity

in this assay is considered highly desirable as many of the bacteria during an active *Mtb*-infection are found intracellularly in phagocytotic cell types. The obtained results are shown in Table 5.

Table 5. Intracellular IC_{50} and IC_{90} values for selected compounds.

Cmpd	 R_3	Intracellular IC_{50} (μM)[a]	Intracellular IC_{90} (μM)[a]
1	3,5-*di*CH_3-phenyl	0.05	0.2
2	2-OCH_3-phenyl	0.50	1.58
54	4-OCH_3-phenyl	0.03	0.25
56	2-OCH_3, 5-CH_3-phenyl	0.16	0.63
57	3,5-*di*CH_3, 4-F-phenyl	0.08	0.25
59	3,5-*di*F-phenyl	0.40	2.51
60	2,4-*di*Cl-phenyl	0.79	>50
66	4-Cl-phenyl	0.16	0.50

[a] IC_{50} and IC_{90} against infected Human THP-1 macrophages with *Mycobacterium tuberculosis* (H37Rv)

It is worth noting that 6 out of 8 tested compounds showed excellent intracellular IC_{90} values, ranging from 0.25 to 2.51 μM. More specifically, compound **54**, which had also shown the highest MIC value, and compound **57** exhibited the highest intracellular potencies with IC_{90} values of 0.25 μM. They were followed by compounds **56** and **66** with IC_{90} values 0.50 and 0.63 μM, respectively. Less active but still very potent compounds were the hit compound **2** and compound **59** (IC_{90} 1.58 and 2.51 μM, respectively). Lastly, compound **60,** although possessing a good MIC value, did not reach 90% inhibition at 50 μM (IC_{90} >50 μM) in the intracellular assay.

This could be due to poor permeability of the compounds through the cell membrane or due to bacterial efflux pumps activated by the macrophage or inactivation of the compounds by host cell derived metabolites such as reactive species or acidic pH.[26]

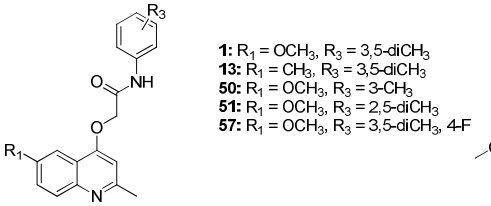
Metabolic stability studies. In view of the fact that microsomal instabillity was identified as a possible liability of the hits, six compounds were selected and the stability in mouse and human microsomal fractions was evaluated before continuing with further synthetic efforts. The selection of compounds was driven by structural criteria in an attempt to identify the metabolic liabilities of the series. Three possible metabolic sites were explored: the methoxy group, the amide bond and the phenyl ring of the reference compound **1**. Therefore, compound **13** which possesses a methyl group instead of methoxy group was selected in order to evaluate its stability. Similarly, compounds **28** and **45**, which have a sterically hindered amide and an amine, respectively, were also chosen. Lastly, compounds **50**, **75** and **57** were selected because they contain substituents in different positions of the phenyl ring.

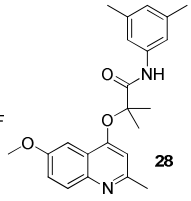
The six selected compounds together with the reference compound **1**, were evaluated for their stability in mouse and human microsomal fractions, and the obtained data are presented in Table 6. All the tested compounds proved to be highly unstable, especially when incubated with mouse microsomal fractions. Comparison of the obtained data with the control without co-factor data indicated that cytochrome P-450 metabolism was not determinant for all the tested compounds apart from compound **45**, signifying that the amide bond is the most susceptible group of the series and that the esterase hydrolysis might be involved.

In order to confirm that esterases are responsible for the rapid metabolism of the compounds, the selected compounds were incubated in fresh whole CD1 mouse blood. A parallel run, after pretreatment of the blood with pan-esterase inhibitor sodium fluoride (NaF), was performed and

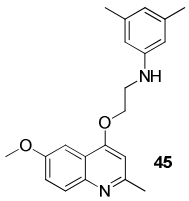
the obtained results are depicted in Table 6. As expected and in accordance with the no co-factor control in the microsomal stability experiment, nearly all the compounds possessing an amide bond were highly unstable. On the other hand, compounds **45** and **28**, which possess an amine or a sterically hindered amide, were stable. In addition, the instability is mitigated after NaF pretreatment suggesting that it is mainly due to the hydrolysis of the amide. Microsomal stability in human fractions was better, which is in agreement with the hypothesis of esterase hydrolysis, since esterases in rodents are more active.[27]

Table 6. Stability of selected compounds in mouse/human microsomal fractions and blood.





28



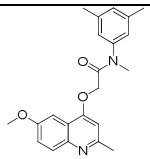
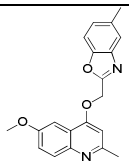
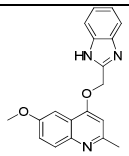
45

Cmpd	Microsomal fraction stability[a] Mouse		Human		Blood stability[b]	
	Clint (mL $min^{-1}g^{-1}$)	$t_{1/2}$ (min)	Clint (mL $min^{-1}g^{-1}$)	$t_{1/2}$ (min)	$t_{1/2}$ (min)	NaF
1	18.9	<5	1.3	>30	<5	Partially stabilized
13	21.9	<5	1.6	>30	<5	Partially stabilized
28	27.3	<5	2.7	19	96	Stable
45	86.5	<5	6.3	>30	>120	Stable
50	18.1	<5	2.5	23	<5	Partially stabilized
51	50.2	<5	2.3	24	<5	Partially stabilized
57	19.3	<5	1.1	>30	<5	Partially stabilized

[a] *in vitro* microsomal fraction stability (mouse and human) results: intrinsic clearance (Cl_{int}) and half-life time ($t_{1/2}$) are reported; imidazolam was used as control with Cl_{int} = 27.5 ±0.4 and 6.4 mL $min^{-1}g^{-1}$ in mouse and human, respectively and $t_{1/2}$ = <5 and 9 min in mouse and human, respectively; [b] blood stability results: half-life time ($t_{1/2}$) and effect in presence of NaF are reported.

In the light of this evidence, further medicinal chemistry effort led to methylation of the amide (**82**) and replacement of the *N*-phenyl amide with benzoxazole (**83**) or benzimidazole (**84**) rings in an effort to overcome the rapid clearance observed in mice microsomal fractions. The ring closure or the methylated amide bond could potentially stabilize the metabolic liability of the series, in theory rendering an improved profile over the previous scaffold. Table 7 presents the obtained data for compounds **82**, **83** and **84**. Lastly, *N*-methylation of the reference compound **1** was explored as a classic approach to reduce amide hydrolysis.

Table 7. Biological profile of compounds **82**, **83** and **84**.

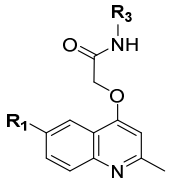
Structure			
Cmpd	**82**	**83**	**84**
MIC (μM) [a]	109.3	5.5	16
Intracellular IC_{90} (μM) [b]	n.d. [c]	5	n.d. [c]
Cytotoxicity IC_{50} (μM) [d]	50.12	>100.00	>100.00
Permeability (nm/sec)[e]	300	86	n.d.[c]
Solubility (μM)[f]	373	1	13
ChromlogD[g]	5.47	6.12	3.59
Microsomal fraction stability[h]			
Cl_{int} [mL $min^{-1}g^{-1}$]	n.d.[g]	46.8 (m), 3.5 (h)	n.d.[c]
$t_{1/2}$ (min)		<5 (m), 15.7 (h)	
Blood stability[i] $t_{1/2}$ (min)	n.d.[g]	>240	n.d.[c]

[a]MIC against *Mycobacterium tuberculosis* (H37Rv); [b]IC_{90} against infected Human THP-1 macrophages with *Mycobacterium tuberculosis* (H37Rv); [c]n.d. = not determined; [d]$HepG_2$, human caucasian hepatocyte carcinoma; [e]artificial membrane permeability; [f]*in vitro* profiling for kinetic aqueous solubility (CLND, chemiluminescent nitrogen detection); [g]chromlogD values at pH = 7.4; [h]*in vitro* microsomal fraction stability results; clearance (Cl_{int}) and half-life time ($t_{1/2}$) is reported; imidazolam was used as control with Cl_{int} = 27.5 ±0.4 and 6.4 mL $min^{-1}g^{-1}$ in mouse and human, respectively and $t_{1/2}$ = <5 and 9 min in mouse and human, respectively (h) = human, (m) = mouse; [i]blood stability results: half-life time ($t_{1/2}$) is reported.

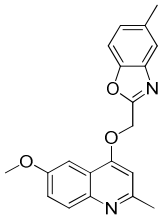
Compound **83,** which possesses a benzoxazole ring instead of anilide, showed significant anti-tubercular activity with an MIC value of 5.5 μM and intracellular IC_{90} of 5 μM. Subsequent testing for blood and microsomal stability revealed the expected improvement in blood stability due to the removal of the labile amide bond. However, solubility of compound **83** was very poor and *in vitro* microsomal clearance was high, suggesting that further medicinal chemistry effort is required in order to overcome these problems.

Lastly, a preliminary evaluation of the cardiosafety of the series was performed by measuring the human ether-a-go-go-related gene (hERG) inhibition of selected compounds. Obtained results are presented in Table 8.

Table 8. Results of hERG binding of selected compounds.



Cmpd	R_1	R_3	hERG (pIC_{50})
1	6-CH_3O-	3,5-*di*CH_3-phenyl	<4.3
2	6-CH_3O-	2-OCH_3-phenyl	5
13	6-CH_3-	3,5-*di*CH_3-phenyl	<4.3
54	6-CH_3O-	4-OCH_3-phenyl	5.2
56	6-CH_3O-	2-OCH_3, 5-CH_3-phenyl	5.3
57	6-CH_3O-	3,5-*di*CH_3, 4-F-phenyl	<4.3
66	6-CH_3O-	4-Cl-phenyl	5.3

83		<4.3

The data in **Table 8** indicate that four out of eight tested compounds displayed a hERG inhibitory potency that was higher than the threshold value used for safety assessment (pIC_{50}= 4.3). Interestingly, three of these (**2**, **54** and **56**) possess a methoxyaniline substituent. The rest of the evaluated compounds did not display potent hERG inhibition (pIC_{50} <4.3). Compound **83**, which was found to be of particular interest due to its blood stability, did not show interaction with hERG.

CONCLUSION

In the presented work, the synthesis of more than sixty novel quinoloxyacetamide derivatives and their biological evaluation against *Mycobacterium tuberculosis* (H37Rv) is reported. Apart from the SAR exploration around the initial hits, the optimization processes focused on the improvement of the physicochemical properties, cytotoxicity and metabolic stability of the hit compounds. Several compounds showed potent anti-tubercular activities with MICs in the low micromolar range with the best compound (**54**) exhibiting a MIC value of 0.6 μM and no measurable cytotoxicity. This compound and other potent, non-cytotoxic analogues also showed excellent intracellular IC_{90} values ranging from 0.25 to 2.51 μM. Furthermore, the metabolic stability of the series was investigated and it was shown that the amide bond is the most labile group. Thus, synthetic efforts were focused on amide replacement and compound **83** which possessed a benzoxazole was identified as a good alternative to improve blood stability (half-life time >240 min). Evaluation of hERG binding indicated that mainly compounds possessing a

methoxyaniline substituent, showed appreciable hERG inhibition, while the optimized compound **83** displayed no activity in the assay. Further medicinal chemistry effort is ongoing to increase solubility and microsomal stability of the series in order to provide a strong lead for a new anti-tubercular drug discovery program.

EXPERIMENTAL SECTION

General Information. Unless otherwise stated, laboratory reagent grade solvents were used. Reagents were purchased from Sigma-Aldrich, Acros Organics, TCI or Enamine and were used without further purification unless otherwise mentioned. Reactions were monitored by TLC on silica gel with detection by UV light (254 nm). TLC analysis was performed using Polygram® precoated silica gel TLC sheets SIL G/UV$_{254}$.

Characterization of all compounds was done using ^{1}H NMR and ^{13}C-NMR spectroscopy and mass spectrometry. ^{1}H NMR (400 MHz) and ^{13}C-NMR (100 MHz) spectra were recorded on a Bruker Avance III Nanobay Ultrashield 400 or a Bruker DPX 400 spectrometer. The chemical shift (δ) values are expressed in parts per million (ppm) and coupling constants are in Hertz (Hz). Minor rotamers of the amide bond, which were less than 10% of the major rotamer, are not reported in the NMR data. $CDCl_3$, CD_3OD or DMSO-*d*$_6$ were used as the standard NMR solvents. Legend: s = singlet, d = doublet, t = triplet, q = quartet, quint = quintet, m = multiplet, dd = doublet of doublets, ddd = doublets of doublets of doublets, br = broad signal. For the measurement of melting points, a Technoterm 7300 (Reichert-Jung Optische Werke) microscope was used.

Purity and mass were verified using a UPLC-MS system and purities of all final products were found to be >95%. UPLC-MS involved the following: Waters Acquity UPLC system coupled to a Waters TQD ESI mass spectrometer and Waters TUV detector. A Waters Acquity UPLC BEH

C18 1.7 μm, 2.1 mm × 50 mm column was used. Solvent A consisted of water with 0.1% formic acid. Solvent B consisted of acetonitrile with 0.1% formic acid. Method A involved the following: flow 0.4 mL/min, 0.15 min isocratic elution (95% A, 5% B), followed by gradient elution during 1.85 min (from 95% A, 5% B to 95% B, 5% A), then 0.25min (0.350 mL/min) isocratic elution (95% B, 5% A). The wavelength for UV detection was 254 nm. Method B: flow 0.4 mL/min, 0.25 min isocratic elution (95% A, 5% B), followed by gradient elution during 4.75 min (95% B, 5% A, then isocratic 0.25 min of isocratic elution (95% B, 5% A) followed by 0.75 min isocratic elution (95% A, 5% B). The wavelength for UV detection was 214nm. For method C, a Waters Acquity UPLC system was coupled to a Waters SQ detector and an Acquity UPLC BEH C18 1.7 μm, 3x50 mm column was used. The concentration of the measured samples was 0.1 mg/ml and flow 0.8 mL/min. The method involved the following: Acetate NH_4 25mM + 10% ACN at pH 6.6 /ACN, 0.0-0.2 min 99.9: 0.1, 0.2-1.0 min 10:90, 1.0-1.8 min 10:90, 1.9-2.0 min 99.9:0.1 at temperature 40ºC. The UV detection was an averaged signal from wavelength of 210 nm to 400 nm. The quasi-molecular ions $[M+H]^+$ or $[M-H]^-$ were detected. Retention time (RT) was indicated for the described method.

For the High Resolution Mass Spectrometry (HRMS) measurements: Positive ion mass spectra were acquired using a QSTAR Elite (AB Sciex Instruments) mass spectrometer, equipped with a turbospray source, over a mass range of 250–700.

When necessary, flash purification was performed on a Biotage ISOLERA One flash system equipped with an internal variable dual-wavelength diode array detector (200-400 nm). For normal phase purifications SNAP cartridges (10-100 g, flow rate of 10-100 mL/min) were used, and reverse-phase purifications were done making use of KP-C18 containing cartridges. Dry sample loading was done by self-packing samplet cartridges using silica or Celite 545,

respectively, for normal and reversed phase purifications. Gradients used varied for each purification. However, typical gradients used for normal phase were gradient of 0−100% ethyl acetate in *n*-heptane or 0-15% methanol in ethyl acetate. For reverse phase a gradient of 5% MeCN in water to 50% MeCN in water was used.

The following section comprises the synthetic procedures and analytical data for all final compounds and some representative intermediates reported in this publication. Complimentary data for the rest of intermediates can be found in the Supporting Information. Synthetic procedures that were used in the preparation of several products are summarized here as "General Procedures".

For the synthesis of the quinolin-4-ols, three related methods were used (General procedures A, B and C).

General procedure A. Formation of 2-methyl-quinolin-4-ols (5a, 5l) and 2-trifluoromethyl-quinolin-4-ols (5m, 5o, 5p). According to Brouet et al., the appropriate substituted aniline (1 eq.) and ethyl acetoacetate or ethyl 4,4,4-trifluoroacetoacetate (1.25-2.5 eq.) were dissolved in Dowtherm A (molarity: 0.5 M) and concentrated sulfuric acid (1-3 drops) was added to the stirred mixture.[1] The reaction vessel was equipped with a short distillation apparatus. The reaction mixture was heated gradually to 240-250 °C for 35-60 min and the produced water/ethanol was removed by distillation as the reaction progressed. Subsequently, the reaction mixture was cooled down to room temperature and poured into n-heptane to give a precipitate. The precipitate was collected by filtration and washed with n-heptane and EtOAc. If necessary, the product was further purified by recrystallization or silica gel flash chromatography.

General procedure B. Formation of 2-methyl-quinolin-4-ols (5a, 5c, 5f, 5h-k). In accordance with the second method described by Tantrizos et al., the appropriate aniline (1 eq.) and ethylacetoacetate (1 eq.) were stirred at 130 °C for 90 min to form the corresponding imine.[15] Then, Dowtherm A (molarity: 0.7-2.3 M) was added to the reaction mixture and it was heated for 1 h at 250°C. Then, the reaction mixture was cooled down to room temperature and poured into n-heptane to give a (sticky) precipitate. The precipitate was collected by filtration and washed with n-heptane and EtOAc. If necessary, the product was further purified by recrystallization or silica gel flash chromatography.

General procedure C. Formation of 2-methyl-quinolin-4-ols (5a, 5b). In line with Escribiano et al., AcOH (1.3 eq.) was added to a solution of the appropriate aniline (1eq.) and ethylacetoacetate (1.2 eq.) in toluene (molarity: 0.5 M).[14] The reaction mixture was held at reflux for 2 h with azeotropic removal of water by means of a Dean-Stark apparatus. The solvent was evaporated under reduced pressure, the liquid residue was dissolved in Dowtherm A (molarity: 0.1M) and heated at 240°C for 1h. A Dean-Stark apparatus was used to remove the ethanol produced during the reaction. The reaction mixture was cooled down to room temperature and poured into n-heptane to give a (sticky) precipitate. The precipitate was collected by filtration and washed with n-heptane and EtOAc. If necessary, the product was further purified by recrystallization or silica gel flash chromatography.

General Procedure D. Preparation of 2-bromo-*N*-substituted-acetamides (7, 26a-d, 41, 48a-r). 2-bromoacetyl bromide (1.2 eq.) was slowly added dropwise to a mixture of R-NH_2 (1 eq.) and triethylamine (1.2 mmol) in anhydrous DCM (Molarity: 1M) at 0°C. The reaction mixture was warmed to room temperature and stirred for an additional 2-48h. After the solvent was removed under reduced pressure, the residue was washed with ice water and separated by

filtration. If necessary, the product was purified by recrystallization or silica gel column chromatography.

General Procedure E. *O*-alkylation of 4-hydroxy quinolines (1, 8-24, 31, 32, 42, 49-67, 83, 84). To a solution of substituted 4-hydroxyquinoline (1 eq.) in anhydrous DMF under nitrogen atmosphere was added K_2CO_3 (3 eq.) and a solution of the suitable halide (1-1.2 eq.) in anhydrous DMF. The reaction mixture was stirred for 3h-4d at room temperature before being poured into water (50 mL) and extracted with ethyl acetate (3 x 50 mL) or filtered in case of precipitation. The combined organic extracts were dried over $MgSO_4$, filtered, concentrated in vacuo and purified by silica gel flash chromatography, if necessary.

General Procedure F. *O*-alkylation of deprotonated 4-hydroxy quinolines (27-30). Sodium hydride (NaH) 60% suspended in mineral oil (1 eq.) was added to a solution of intermediate **7a** in anhydrous DMF under nitrogen atmosphere, and the resulting suspension was stirred for 30 min. Subsequently, a solution of the suitable halide (1 eq.) and in some cases potassium iodide (KI) (1 eq.) in anhydrous DMF was added to the reaction mixture and left stirring for 3h-4d at room temperature. Afterwards, the reaction mixture was poured into water (50 mL) and extracted with ethyl acetate (3 x 50 mL) or filtered in case of precipitation. The combined organic extracts were dried over $MgSO_4$, concentrated under reduced pressure and purified by flash chromatography, if necessary.

General Procedure G. Formation of the acyl chlorides (46c, 69). The appropriate carboxylic acid/carboxylate (1 eq.) was dissolved in anhydrous DCM (Molarity: 0.04-0.3 M) and thionyl chloride was added (1.2-45 eq.) dropwise. The reaction mixture was left stirring for 1-3 days at room temperature or under reflux. The volatiles of the reaction mixture were evaporated under reduced pressure and the obtained acyl chlorides were directly used for next step.

General Procedure H. Formation of the amide bond (47, 70-81). The acyl chlorides (**46c**, **69**) were dissolved in anhydrous DCM (Molarity: 0.04-0.5 M) and the suitable amine (1-2.5 eq.) was added to the solution. In some cases, triethylamine (1-1.2 eq.) was also added to the reaction mixture. Then, the reaction mixture was stirred at room temperature for 7-72 h. If triethylamine was not used, the volatiles were evaporated under reduced pressure and the residue was dissolved in water. The solution was basified to pH 8-9 using an aqueous solution of NaOH (2 N), and the obtained crystals were collected by filtration, washed with water and dried to obtain in most cases the pure product. If necessary, the crude product was recrystallized from EtOAc or purified by column chromatography. If triethylamine was used, DCM was evaporated and the obtained residue was purified by column chromatography.

***N*-(3,5-Dimethylphenyl)-2-((6-methoxy-2-methylquinolin-4-yl)oxy)acetamide (1).** The title compound was prepared using the general procedure E. White solid; yield 85% (237 mg, 0.68 mmol), mp 189-191 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.16 (s, 1H), 7.91 (d, *J* = 9.0 Hz, 1H), 7.42 – 7.32 (m, 2H), 7.18 (s, 2H), 6.80 (s, 1H), 6.60 (s, 1H), 4.80 (s, 2H), 3.96 (s, 3H), 2.64 (s, 3H), 2.29 (s, 6H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 165.3, 159.0, 157.6, 157.3, 145.0, 139.1, 136.6, 130.1, 127.0, 122.2, 119.9, 118.0, 102.3, 99.6, 67.9, 55.7, 25.7, 21.5. UPLC-MS (A) (ESI) RT 1.58 min, *m/z* 351.5 $[M+H]^+$ (>95%). HRMS (ESI) *m/z* calcd for $C_{21}H_{23}N_2O_3$ $[M+H]^+$: 351.1703; found: 351.1694.

6-Methoxy-2-methylquinolin-4-ol (5a). The title compound was prepared using the general procedures A, B and C. Off-white solid; yield 7% (100 mg, 0.53 mmol, procedure A), 20% (153 mg, 0809 mmol, procedure B), 41% (6.237 g, 33.0 mmol, procedure C). ^{1}H NMR (400 MHz, DMSO-*d*$_6$) δ 11.53 (br s, 1H), 7.45 (dd, *J* = 5.9, 2.9 Hz, 2H), 7.24 (dd, *J* = 9.0, 2.9 Hz, 1H), 5.86 (s, 1H), 3.81 (s, 3H), 2.32 (s, 3H). ^{13}C NMR (101 MHz, DMSO-*d*$_6$) δ 176.1, 155.2, 148.5, 134.7,

125.5, 121.7, 119.4, 107.4, 104.2, 55.3, 19.3. UPLC-MS (A) (ESI) RT 1.06 min, *m/z* = 190.1 $[M+H]^+$ (>95%).

2-Bromo-*N*-(3,5-dimethylphenyl)acetamide (7). The title compound was prepared using the general procedure D. Brown solid; yield 97% (3.890 g, 16.07 mmol). ^{1}H NMR (400 MHz, DMSO-*d*$_6$) δ 10.20 (br. s, 1H), 7.20 (s, 2H), 6.73 (s, 1H), 4.00 (s, 2H), 2.24 (d, *J* =0.4 Hz, 6H). ^{13}C NMR (101 MHz, DMSO-*d*$_6$) (major cis/trans amide rotamers) δ 170.6, 138.3, 137.6, 125.0, 117.3, 61.8, 21.1; (minor cis/trans amide rotamers) δ 164.7, 138.5, 137.9, 125.4, 117.0, 30.5, 21.1. UPLC-MS (A) (ESI) RT 1.73 min, *m/z* 242.3:244.3 (1:1) $[M+H]^+$ (>95%).

***N*-(3,5-Dimethylphenyl)-2-((2-methylquinolin-4-yl)oxy)acetamide (8).** The title compound was prepared using the general procedure E. White solid; yield 63% (318 mg, 0.99 mmol). ^{1}H NMR (400 MHz, DMSO-*d*$_6$) δ 10.10 (s, 1H), 8.24 (dd, *J* = 8.3, 1.0 Hz, 1H), 7.87 (dd, *J* = 8.4, 0.5 Hz, 1H), 7.72 (ddd, *J* = 8.4, 6.9, 1.5 Hz, 1H), 7.53 (ddd, *J* = 8.2, 6.9, 1.2 Hz, 1H), 7.27 (s, 2H), 6.89 (s, 1H), 6.75 (s, 1H), 4.99 (s, 2H), 2.60 (s, 3H), 2.25 (s, 6H). ^{13}C NMR (101 MHz, DMSO-*d*$_6$) δ 165.4, 160.2, 159.7, 148.4, 138.2, 137.8, 129.8, 127.9, 125.3, 124.8, 121.8, 119.2, 117.5, 102.0, 67.2, 25.4, 21.1. UPLC-MS (A) (ESI) RT 1.49 min, *m/z* 321.4 $[M+H]^+$ (>95%).

***N*-(3,5-Dimethylphenyl)-2-((2-methyl-6-(methylthio)quinolin-4-yl)oxy)acetamide (9).** The title compound was prepared using the general procedure E. White solid; yield 48% (171 mg, 0.47 mmol). ^{1}H NMR (400 MHz, DMSO-*d*$_6$) δ 10.10 (s, 1H), 7.94 (d, *J* = 2.1 Hz, 1H), 7.79 (d, *J* = 8.8 Hz, 1H), 7.61 (dd, *J* = 8.8, 2.3 Hz, 1H), 7.26 (s, 2H), 6.87 (s, 1H), 6.74 (s, 1H), 4.99 (s, 2H), 2.60 (s, 3H), 2.56 (s, 3H), 2.24 (s, 6H). ^{13}C NMR (101 MHz, DMSO-*d*$_6$) δ 165.4, 159.3, 158.8, 146.4, 138.2, 137.8, 134.9, 128.8, 128.4, 125.3, 119.6, 117.4, 116.7, 102.6, 67.1, 25.3, 21.1, 14.9. UPLC-MS (A) (ESI) RT 1.65 min, *m/z* 367.1 $[M+H]^+$ (>95%).

***N*-(3,5-Dimethylphenyl)-2-((7-methoxy-2-methylquinolin-4-yl)oxy)acetamide (10).** The title compound was prepared using the general procedure E. White solid; yield 34% (93 mg, 0.27 mmol). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.12 (br. s, 1H), 8.03 (d, *J* = 9.1 Hz, 1H), 7.38 (d, *J* = 2.5 Hz, 1H), 7.22 – 7.15 (m, 3H), 6.81 (s, 1H), 6.55 (s, 1H), 4.80 (s, 2H), 3.94 (s, 3H), 2.68 (s, 3H), 2.31 (s, 6H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 165.1, 161.7, 160.6, 160.2, 150.7, 139.1, 136.5, 127.1, 122.2, 118.5, 118.1, 113.8, 106.8, 100.5, 67.7, 55.7, 25.8, 21.5. UPLC-MS (A) (ESI) RT 1.63 min, *m/z* 351.5 $[M+H]^+$ (>95%).

***N*-(3,5-Dimethylphenyl)-2-(6-fluoro-2-methylquinolin-4-yloxy)acetamide (11).** The title compound was prepared using the general procedure E. White solid; yield 84% (643 mg, 1.90 mmol). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.07 – 7.97 (m, 2H), 7.75 (dd, *J* = 9.2, 2.8 Hz, 1H), 7.48 (ddd, *J* = 9.2, 8.2, 2.9 Hz, 1H), 7.20 (s, 2H), 6.82 (s, 1H), 6.69 (s, 1H), 4.82 (s, 2H), 2.70 (s, 3H), 2.31 (s, 6H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 164.8, 160.2 (d, $^{1}J_{C\text{-}F}$ = 246.8 Hz), 159.6 (d, $^{4}J_{C\text{-}F}$ = 2.6 Hz), 146.0, 139.2, 136.4, 131.0 (d, $^{3}J_{C\text{-}F}$ =8.7 Hz), 127.2, 120.4 (d, $^{2}J_{C\text{-}F}$ = 25.3 Hz), 119.9 (d, $^{3}J_{C\text{-}F}$ =9.5 Hz), 118.2, 118.0, 105.2 (d, $^{2}J_{C\text{-}F}$ = 23.6 Hz), 102.6, 67.9, 25.8, 21.5. UPLC-MS (A) (ESI) RT 1.58 min, *m/z* 339.4 $[M+H]^+$ (>95%).

2-((6-Chloro-2-methylquinolin-4-yl)oxy)-*N*-(3,5-dimethylphenyl)acetamide (12). The title compound was prepared using the general procedure E. White solid; yield 31% (113 mg, 0.32 mmol). ^{1}H NMR (400 MHz, DMSO-*d6*) δ 10.10 (s, 1H), 8.22 (d, *J* = 2.3 Hz, 1H), 7.88 (d, *J* = 9.0 Hz, 1H), 7.72 (dd, *J* = 9.0, 2.5 Hz, 1H), 7.25 (s, 2H), 6.97 (s, 1H), 6.74 (s, 1H), 5.00 (s, 2H), 2.59 (s, 3H), 2.24 (s, 6H). ^{13}C NMR (101 MHz, DMSO-*d6*) δ 165.3, 160.6, 159.5, 146.8, 138.1, 137.8, 130.2, 130.1, 129.4, 125.4, 120.8, 120.0, 117.7, 103.0, 67.3, 25.4, 21.1. UPLC-MS (A) (ESI) RT 1.67 min, *m/z* 355.1, 357.1 (3:1) $[M+H]^+$ (>95%).

***N*-(3,5-Dimethylphenyl)-2-((2,6-dimethylquinolin-4-yl)oxy)acetamide (13).** The title compound was prepared using the general procedure E. White solid; yield 95% (365 mg, 1.09 mmol); mp 208-210 ºC. ^{1}H NMR (400 MHz, DMSO-*d*$_6$) δ 10.10 (s, 1H), 7.99 (s, 1H), 7.76 (d, J = 8.5 Hz, 1H), 7.55 (dd, J = 8.6, 2.0 Hz, 1H), 7.27 (s, 2H), 6.83 (s, 1H), 6.75 (s, 1H), 4.97 (s, 2H), 2.57 (s, 3H), 2.50 (s, 3H, overlaps with solvent's peak), 2.25 (s, 6H). ^{13}C NMR (101 MHz, DMSO-*d*$_6$) δ 165.4, 159.9, 158.6, 146.9, 138.2, 137.8, 134.1, 131.7, 127.7, 125.3, 120.5, 119.0, 117.4, 101.9, 67.1, 25.3, 21.2, 21.1. UPLC-MS (A) (ESI) RT 1.64 min, *m/z* 335.5 $[M+H]^+$ (>95%). HRMS (ESI) *m/z* calcd for $C_{21}H_{23}N_2O_2$ $[M+H]^+$: 335.1754; found: 335.1739.

***N*-(3,5-Dimethylphenyl)-2-((2-methyl-6-(trifluoromethyl)quinolin-4-yl)oxy)acetamide (14).** The title compound was prepared using the general procedure E. White solid; yield 66% (226 mg, 0.58 mmol); mp >210 °C. ^{1}H NMR (400 MHz, DMSO-*d*$_6$) δ 10.14 (s, 1H), 8.54 (s, 1H), 8.07 (d, *J* = 8.8 Hz, 1H), 7.98 (dd, *J* = 8.9, 2.1 Hz, 1H), 7.24 (s, 2H), 7.06 (s, 1H), 6.74 (s, 1H), 5.06 (s, 2H), 2.64 (s, 3H), 2.24 (s, 6H). ^{13}C NMR (101 MHz, DMSO-*d*$_6$) δ 165.2, 163.0, 160.8, 149.5, 138.1, 137.8, 125.3, 125.2 (q, $^{3}J_{C\text{-}F}$ = 3.1 Hz), 125.0 (q, $^{2}J_{C\text{-}F}$ = 32.1 Hz), 124.3 (q, $^{1}J_{C\text{-}F}$ = 272.2 Hz), 120.0 (q, $^{3}J_{C\text{-}F}$ = 4.5 Hz), 118.5, 117.5, 103.4, 67.3, 25.6, 21.1. UPLC-MS (A) (ESI) RT 2.08 min, *m/z* 389.2 $[M+H]^+$ (>95%). HRMS (ESI) *m/z* calcd for $C_{21}H_{20}F_3N_2O_2$ $[M+H]^+$: 389.1471; found: 389.1478.

***N*-(3,5-Dimethylphenyl)-2-((2-methyl-6-(trifluoromethoxy)quinolin-4-yl)oxy)acetamide (15).** The title compound was prepared using the general procedure E. White solid; yield 47% (157 mg, 0.39 mmol); mp 241 °C. ^{1}H NMR (400 MHz, DMSO-*d*$_6$) δ 10.12 (s, 1H), 8.09 (d, *J* = 1.4 Hz, 1H), 8.00 (d, *J* = 9.1 Hz, 1H), 7.71 (dd, *J* = 9.1, 2.1 Hz, 1H), 7.24 (s, 2H), 7.00 (s, 1H), 6.74 (s, 1H), 5.03 (s, 2H), 2.61 (s, 3H), 2.24 (s, 6H). ^{13}C NMR (101 MHz, DMSO-*d*$_6$) δ 165.2, 161.0, 160.2, 146.8, 144.9, 138.1, 137.8, 130.6, 125.3, 123.7, 120.2 (q, $^{1}J_{C\text{-}F}$ = 256.9 Hz), 119.4,

117.5, 112.8, 103.1, 67.2, 25.4, 21.1. UPLC-MS (A) (ESI) RT 2.08 min, *m/z* 405.2 $[M+H]^+$ (>95%). HRMS (ESI) *m/z* calcd for $C_{21}H_{20}F_3N_2O_3$ $[M+H]^+$: 405.1421; found: 405.1418.

***N*-(3,5-Dimethylphenyl)-2-((6-ethoxy-2-methylquinolin-4-yl)oxy)acetamide (16).** The title compound was prepared using the general procedure E. White solid; yield 9% (32 mg, 0.09 mmol). ^{1}H NMR (400 MHz, DMSO-*d*$_6$) δ 10.10 (s, 1H), 7.77 (d, *J* = 9.1 Hz, 1H), 7.48 (d, *J* = 2.8 Hz, 1H), 7.34 (dd, *J* = 9.1, 2.9 Hz, 1H), 7.25 (s, 2H), 6.82 (s, 1H), 6.74 (s, 1H), 4.97 (s, 2H), 4.15 (q, *J* = 7.0 Hz, 2H), 2.54 (s, 3H), 2.24 (s, 6H), 1.40 (t, *J* = 7.0 Hz, 3H). ^{13}C NMR (101 MHz, DMSO-*d*$_6$) δ 165.5, 159.4, 156.8, 155.5, 144.1, 138.2, 137.8, 129.5, 125.3, 121.8, 119.8, 117.4, 102.1, 100.7, 67.1, 63.4, 25.1, 21.1, 14.6. UPLC-MS (B) (ESI) RT 4.08 min, *m/z* 365.2 $[M+H]^+$ (>95%).

2-((6,7-Dimethoxy-2-methylquinolin-4-yl)oxy)-*N*-(3,5-dimethylphenyl)acetamide (17). The title compound was prepared using the general procedure E. White solid; yield 70% (244 mg, 0.64 mmol).^{1}H NMR (400 MHz, DMSO-*d*$_6$) δ 10.09 (s, 1H), 7.44 (s, 1H), 7.26 (s, 1H), 7.26 (s, 2H), 6.73 (s, 1H), 6.72 (s, 1H), 4.96 (s, 2H), 3.90 (s, 6H), 2.52 (s, 3H), 2.24 (s, 6H). ^{13}C NMR (101 MHz, DMSO-*d*$_6$) δ 165.6, 159.3, 157.0, 152.1, 148.1, 145.3, 138.2, 137.8, 125.2, 117.3, 113.2, 107.4, 100.6, 99.9, 67.0, 55.6, 55.5, 25.1, 21.1. UPLC-MS (A) (ESI) RT 1.94 min, *m/z* 381.3 $[M+H]^+$ (>95%).

2-(6-(Benzyloxy)-2-methylquinolin-4-yloxy)-*N*-(3,5-dimethylphenyl)acetamide (18). The title compound was prepared using the general procedure E. White solid; yield 48% (100 mg, 0.23 mmol). ^{1}H NMR (400 MHz, DMSO-*d*$_6$) δ 10.09 (s, 1H), 7.80 (d, *J* = 9.1 Hz, 1H), 7.63 (d, *J* = 2.8 Hz, 1H), 7.54 – 7.49 (m, 2H), 7.45 – 7.31 (m, 4H), 7.27 (s, 2H), 6.84 (s, 1H), 6.74 (s, 1H), 5.24 (s, 2H), 4.98 (s, 2H), 2.55 (s, 3H), 2.24 (s, 6H). ^{13}C NMR (101 MHz, DMSO-*d*$_6$) δ 165.5, 159.5, 157.0, 155.3, 144.3, 138.2, 137.8, 136.8, 129.6, 128.4, 127.9, 127.8, 125.3, 121.9, 119.7,

117.4, 102.2, 101.6, 69.6, 67.1, 25.1, 21.1. UPLC-MS (A) (ESI) RT 1.82 min, *m/z* 427.5 $[M+H]^+$ (>95%).

***N*-(3,5-Dimethylphenyl)-2-(6-methoxy-2-(trifluoromethyl)quinolin-4-yloxy)acetamide (19).** The title compound was prepared using the general procedure E. Off-white solid; yield 72% (597 mg, 1.48 mmol); mp 201-202 ºC. ^{1}H NMR (400 MHz, DMSO-*d6*) δ 10.18 (s, 1H), 8.05 (d, *J* = 9.2 Hz, 1H), 7.61 (d, *J* = 2.8 Hz, 1H), 7.57 (dd, *J* = 9.2, 2.9 Hz, 1H), 7.36 (s, 1H), 7.24 (s, 2H), 6.75 (s, 1H), 5.20 (s, 2H), 3.97 (s, 3H), 2.24 (s, 6H). ^{13}C NMR (101 MHz, DMSO-*d6*) δ 165.1, 161.3, 158.6, 145.1 (q, $^2J_{C\text{-}F}$ = 33.5 Hz), 143.4, 138.1, 137.9, 130.8, 125.3, 123.8, 122.3, 121.7 (q, $^1J_{C\text{-}F}$ = 275.4 Hz), 117.3, 99.9, 98.2, 67.6, 55.7, 21.1. UPLC-MS (B) (ESI) RT 4.60 min, *m/z* 405.6 $[M+H]^+$ (>95%). HRMS (ESI) *m/z* calcd for $C_{21}H_{20}F_3N_2O_3$ $[M+H]^+$: 405.1421; found: 405.1426.

***N*-(3,5-Dimethylphenyl)-2-(6-(trifluoromethoxy)-2-(trifluoromethyl)quinolin-4-yloxy)acetamide (20).** The title compound was prepared using the general procedure E. White solid; yield 39% (182 mg, 0.40 mmol). ^{1}H NMR (400 MHz, DMSO-*d6*) δ 10.18 (s, 1H), 8.30 (d, *J* = 9.2 Hz, 1H), 8.24 (bs, 1H), 7.95 (dd, *J* = 8.8, 2.3 Hz, 1H), 7.54 (s, 1H), 7.23 (s, 2H), 6.75 (s, 1H), 5.25 (s, 2H), 2.24 (s, 6H). ^{13}C NMR (101 MHz, DMSO-*d6*) δ 164.8, 162.7, 148.5 (q, $^2J_{C\text{-}F}$ = 33.9 Hz), 147.1, 145.9, 138.1, 137.9, 132.1, 125.5, 125.4, 121.6, 121.3 (q, $^1J_{C\text{-}F}$ = 275.9 Hz), 120.1 (q, $^1J_{C\text{-}F}$ = 258.5 Hz), 117.4, 112.8, 99.1, 67.9, 21.1. UPLC-MS (A) (ESI) RT 2.51 min, *m/z* 459.4 $[M+H]^+$ (>95%).

***N*-(3,5-Dimethylphenyl)-2-(6-ethoxy-2-(trifluoromethyl)quinolin-4-yloxy)acetamide (21).** The title compound was prepared using the general procedure E. White solid; yield 77% (189 mg, 0.45 mmol). ^{1}H NMR (400 MHz, DMSO-*d6*) δ 10.16 (s, 1H), 8.03 (d, *J* = 9.2 Hz, 1H), 7.59 (d, *J* = 2.8 Hz, 1H), 7.54 (dd, *J* = 9.2, 2.9 Hz, 1H), 7.35 (s, 1H), 7.24 (s, 2H), 6.74 (s, 1H), 5.18

(s, 2H), 4.23 (q, *J* = 7.0 Hz, 2H), 2.24 (s, 6H), 1.43 (t, *J* = 7.0 Hz, 3H). ^{13}C NMR (101 MHz, DMSO-*d6*) δ 165.1, 161.2, 157.8, 145.0 (q, $^{2}J_{C\text{-}F}$ = 33.8 Hz), 143.3, 138.1, 137.8, 130.8, 125.3, 124.0, 122.3, 121.7 (q, $^{1}J_{C\text{-}F}$ = 274.7 Hz), 117.3, 100.5, 98.1, 67.6, 63.8, 21.0, 14.5. UPLC-MS (A) (ESI) RT 2.28 min, *m/z* 419.4 $[M+H]^+$ (>95%).

***N*-(3,5-Dimethylphenyl)-2-((6-(trifluoromethyl)-[1,3]dioxolo[4,5-g]quinolin-8-yl)oxy)acetamide (22).** The title compound was prepared using the general procedure E. White solid; yield 43% (217 mg, 0.52 mmol). ^{1}H NMR (400 MHz, DMSO-*d6*) δ 10.11 (s, 1H), 7.63 (s, 1H), 7.47 (s, 1H), 7.29 (s, 1H), 7.23 (s, 2H), 6.74 (s, 1H), 6.29 (s, 2H), 5.12 (s, 2H), 2.24 (s, 6H). ^{13}C NMR (101 MHz, DMSO-*d6*) δ 165.2, 161.3, 152.0, 149.0, 146.2, 145.4 (q, $^{2}J_{C\text{-}F}$ = 33.8 Hz), 138.1, 137.8, 125.4, 121.7 (q, $^{1}J_{C\text{-}F}$ = 274.9 Hz), 117.7, 117.6, 105.2, 102.8, 97.5 (q, $^{3}J_{C\text{-}F}$ = 2.4 Hz), 97.4, 67.6, 21.1. UPLC-MS (A) (ESI) RT 2.17 min, *m/z* 419.4 $[M+H]^+$ (>95%).

2-((2,8-Bis(trifluoromethyl)quinolin-4-yl)oxy)-*N*-(3,5-dimethylphenyl)acetamide (23). The title compound was prepared using the general procedure E. White solid; yield 50% (118 mg, 0.27 mmol). ^{1}H NMR (400 MHz, DMSO-*d6*) δ 10.17 (s, 1H), 8.67 (d, *J* = 7.9 Hz, 1H), 8.35 (d, *J* = 7.2 Hz, 1H), 7.90 (t, *J* = 7.9 Hz, 1H), 7.61 (s, 1H), 7.24 (s, 2H), 6.74 (s, 1H), 5.27 (s, 2H), 2.24 (s, 6H). ^{13}C NMR (101 MHz, DMSO-*d6*) δ 164.8, 163.1, 148.4 (q, $^{2}J_{C\text{-}F}$ = 33.8 Hz), 143.7, 138.1, 137.8, 130.2 (q, $^{3}J_{C\text{-}F}$ = 5.2 Hz), 127.3, 127.1, 126.3 (q, $^{2}J_{C\text{-}F}$ = 29.7 Hz), 125.4, 123.7 (q, $^{1}J_{C\text{-}F}$ = 273.5 Hz), 121.8, 121.1 (q, $^{1}J_{C\text{-}F}$ = 276.6 Hz), 117.4, 99.4, 67.9, 21.0. UPLC-MS (A) (ESI) RT 2.35 min, *m/z* 443.4 $[M+H]^+$ (>95%).

***N*-(3,5-Dimethylphenyl)-2-((6-methoxy-2-propylquinolin-4-yl)oxy)acetamide (24).** The title compound was prepared using the general procedure E. White solid; yield 57% (197 mg, 0.52 mmol). ^{1}H NMR (400 MHz, DMSO-*d6*) δ 10.10 (s, 1H), 7.80 (d, *J* = 9.1 Hz, 1H), 7.50 (d, *J*

= 2.8 Hz, 1H), 7.35 (dd, *J* = 9.1, 2.9 Hz, 1H), 7.24 (s, 2H), 6.84 (s, 1H), 6.73 (s, 1H), 4.99 (s, 2H), 3.89 (s, 3H), 2.81 – 2.72 (m, 2H), 2.23 (s, 6H), 1.80 – 1.66 (m, 2H), 0.91 (t, *J* = 7.4 Hz, 3H). ^{13}C NMR (101 MHz, DMSO-*d*$_6$) δ 165.6, 160.6, 159.5, 156.3, 144.2, 138.2, 137.8, 129.7, 125.3, 121.5, 120.0, 117.4, 101.7, 100.0, 67.2, 55.4, 40.5, 22.3, 21.1, 13.8. UPLC-MS (A) (ESI) RT 2.01 min, *m/z* 379.3 $[M+H]^+$ (>95%).

2-Bromo-2-phenylacetyl chloride (25d). The title compound was prepared according to the literature.[17] Phenylacetylchloride (0,428 ml, 3,23 mmol), *N*-bromosuccinimide (576 mg, 3,23 mmol) and (E)-azobis(isobutyronitrile) (31,9 mg, 0,194 mmol) were taken up in CCl_4 (Volume: 3,7ml) and the resulting mixture was heated at 80°C for 6h. The reaction mixture was then cooled to ambient temperature, followed by addition of n-heptane. The solid that precipitated was removed by filtration and the filtrate was concentrated under reduced pressure to yield the target compound as a yellow oil. The yellow oil was used directly for the next step without further purification.

***N*-(3,5-Dimethylphenyl)-2-((6-methoxy-2-methylquinolin-4-yl)oxy)propanamide (27).** The title compound was prepared using the general procedure F. White solid; yield 49% (140 mg, 0.38 mmol). ^{1}H NMR (400 MHz, DMSO-*d*$_6$) δ 10.09 (s, 1H), 7.77 (d, *J* = 9.1 Hz, 1H), 7.49 (d, *J* = 2.9 Hz, 1H), 7.35 (dd, *J* = 9.1, 2.9 Hz, 1H), 7.24 (s, 2H), 6.74 (s, 1H), 6.73 (s, 1H), 5.16 (q, *J* = 6.5 Hz, 1H), 3.90 (s, 3H), 2.51 (s, 3H), 2.22 (s, 6H), 1.69 (d, *J* = 6.6 Hz, 3H). ^{13}C NMR (101 MHz, DMSO-*d*$_6$) δ 168.8, 158.9, 156.7, 156.2, 144.3, 138.2, 137.8, 129.5, 125.3, 121.5, 119.9, 117.5, 102.3, 100.3, 73.9, 55.4, 25.2, 21.0, 18.4. UPLC-MS (A) (ESI) RT 1.83 min, *m/z* 365.3 $[M+H]^+$ (>95%).

***N*-(3,5-Dimethylphenyl)-2-((6-methoxy-2-methylquinolin-4-yl)oxy)-2-methylpropanamide (28).** The title compound was prepared using the general procedure F. White solid; yield 25%

(99 mg, 0.26 mmol). ^{1}H NMR (400 MHz, DMSO-*d6*) δ 9.82 (s, 1H), 7.76 (d, *J* = 9.1 Hz, 1H), 7.51 (d, *J* = 2.7 Hz, 1H), 7.36 (dd, *J* = 9.1, 2.8 Hz, 1H), 7.21 (s, 2H), 6.72 (s, 1H), 6.54 (s, 1H), 3.91 (s, 3H), 2.47 (s, 3H), 2.21 (s, 6H), 1.74 (s, 6H). ^{13}C NMR (101 MHz, DMSO-*d6*) δ 171.3, 156.8, 156.2, 156.2, 144.5, 138.1, 137.5, 129.5, 125.5, 121.3, 121.2, 118.5, 105.5, 100.9, 81.3, 55.3, 25.1, 24.5, 21.0. UPLC-MS (A) (ESI) RT 1.62 min, *m/z* 379.2 $[M+H]^+$ (>95%).

***N*-(3,5-Dimethylphenyl)-2-((6-methoxy-2-methylquinolin-4-yl)oxy)butanamide (29).** The title compound was prepared using the general procedure F. White solid; yield 15% (60 mg, 0.16 mmol). ^{1}H NMR (400 MHz, DMSO-*d6*) δ 10.09 (s, 1H), 7.77 (d, *J* = 9.1 Hz, 1H), 7.51 (d, *J* = 2.9 Hz, 1H), 7.36 (dd, *J* = 9.1, 2.9 Hz, 1H), 7.23 (s, 2H), 6.73 (s, 1H), 6.72 (s, 1H), 4.96 (t, *J* = 6.3 Hz, 1H), 3.90 (s, 3H), 2.51 (s, 3H, overlaps with solvent's peak), 2.22 (s, 6H), 2.10 (tt, *J* = 8.6, 4.9 Hz, 2H), 1.10 (t, *J* = 7.4 Hz, 3H). ^{13}C NMR (101 MHz, DMSO-*d6*) δ 168.2, 159.2, 156.7, 156.3, 144.3, 138.1, 137.8, 129.5, 125.4, 121.5, 120.0, 117.6, 102.3, 100.3, 78.8, 55.4, 25.9, 25.2, 21.0, 9.6. UPLC-MS (A) (ESI) RT 2.15 min, *m/z* 379.3 $[M+H]^+$ (>95%).

***N*-(3,5-Dimethylphenyl)-2-((6-methoxy-2-methylquinolin-4-yl)oxy)-2-phenylacetamide (30).** The title compound was prepared using the general procedure F. White solid; yield 14% (63 mg, 0.15 mmol). ^{1}H NMR (400 MHz, DMSO-*d6*) δ 10.37 (s, 1H), 7.78 (t, *J* = 8.4 Hz, 3H), 7.60 (d, *J* = 2.9 Hz, 1H), 7.53 – 7.45 (m, 2H), 7.45 – 7.36 (m, 2H), 7.21 (s, 2H), 6.81 (s, 1H), 6.72 (s, 1H), 6.18 (s, 1H), 3.92 (s, 3H), 2.52 (s, 3H), 2.21 (s, 6H). ^{13}C NMR (101 MHz, DMSO-*d6*) δ 166.6, 158.6, 156.7, 156.4, 144.3, 138.0, 137.9, 136.1, 129.6, 128.9, 128.8, 126.9, 125.6, 121.5, 120.0, 117.5, 102.7, 100.5, 78.8, 55.4, 25.2, 21.0. UPLC-MS (A) (ESI) RT 1.79 min, *m/z* 427.2 $[M+H]^+$ (>95%).

6-Methoxy-2-methyl-4-(quinolin-2-ylmethoxy)quinoline (31). The title compound was prepared using the general procedure E. Off-white solid; yield 81% (349 mg, 0.86 mmol). ^{1}H

NMR (400 MHz, DMSO-*d6*) δ 8.46 (d, *J* = 8.5 Hz, 1H), 8.05 (d, *J* = 8.5 Hz, 1H), 8.02 (dd, *J* = 8.2, 1.0 Hz, 1H), 7.86 – 7.75 (m, 3H), 7.64 (ddd, *J* = 8.1, 6.9, 1.2 Hz, 1H), 7.50 (d, *J* = 2.9 Hz, 1H), 7.36 (dd, *J* = 9.1, 2.9 Hz, 1H), 7.03 (s, 1H), 5.65 (s, 2H), 3.89 (s, 3H), 2.53 (s, 3H). ^{13}C NMR (101 MHz, DMSO-*d6*) δ 159.5, 157.0, 156.7, 156.4, 147.0, 144.2, 137.3, 130.0, 129.6, 128.6, 128.0, 127.3, 126.7, 121.4, 119.8, 119.4, 102.7, 99.8, 71.1, 55.4, 25.1. UPLC-MS (A) (ESI) RT 1.44 min, *m/z* 331.4 $[M+H]^+$ (>95%).

3-((6-Methoxy-2-methylquinolin-4-yl)oxy)-1-phenylpyrrolidin-2-one (32). The title compound was prepared using the general procedure E. Light brown solid; yield 20% (75 mg, 0.22 mmol). ^{1}H NMR (400 MHz, DMSO-*d6*) δ 7.83 – 7.73 (m, 3H), 7.46 – 7.41 (m, 2H), 7.39 (d, J = 2.8 Hz, 1H), 7.35 (dd, J = 9.1, 2.9 Hz, 1H), 7.25 – 7.17 (m, 1H), 7.12 (s, 1H), 5.60 (t, J = 8.4 Hz, 1H), 4.06 – 3.88 (m, 2H), 3.87 (s, 3H), 2.94 – 2.81 (m, 1H), 2.58 (s, 3H), 2.36 – 2.22 (m, 1H). ^{13}C NMR (101 MHz, DMSO-*d6*) δ 169.4, 159.0, 156.9, 156.3, 144.3, 139.1, 129.6, 128.8, 124.7, 121.6, 119.8, 119.5, 103.4, 99.7, 75.8, 55.4, 44.1, 25.4, 25.1. UPLC-MS (A) (ESI) RT 1.43 min, *m/z* 349.4 $[M+H]^+$ (>95%).

6-Methoxy-2-methyl-3-nitroquinolin-4-ol (33). A suspension of **5a** (1 g, 5.29 mmol) in propionic acid (12 ml) was heated at 110°C. Nitric acid (0.236 ml, 5.29 mmol) was then added dropwise and the reaction mixture was heated for 2 h at 110 °C with vigorous stirring. The resulting suspension was cooled to room temperature, the solid was collected by filtration, washed with cold ethanol and dried. It was used in the next step without purification. Yield 76% (942 mg, 4.02 mmol). ^{1}H NMR (400 MHz, DMSO-*d6*) δ 12.42 (s, 1H), 7.58 (d, *J* = 9.0 Hz, 1H), 7.53 (d, *J* = 2.9 Hz, 1H), 7.40 (dd, *J* = 9.0, 3.0 Hz, 1H), 3.86 (s, 3H), 2.50 (s, 3H, ovelaps with solvent's peak). ^{13}C NMR (101 MHz, DMSO-*d6*) δ 166.7, 156.6, 145.4, 135.0, 132.9, 126.6, 123.3, 120.4, 105.0, 55.6, 16.8. UPLC-MS (A) (ESI) RT 1.24 min, *m/z* 234.9 $[M+H]^+$ (95%).

3-Amino-6-methoxy-2-methylquinolin-4-ol (34). Zinc (Zn) was purified by stirring 1g of commercial zinc dust with 5 ml of 2% hydrochloric acid (HCl) for 1 min. The acid was removed by filtration and the zinc was washed with one 5 ml portion of 2% hydrochloric acid, three 5 ml portions of distilled water, two 5 ml portions of 95% ethanol, and finally with one 5 ml portion of absolute ether, the wash solutions being removed each time by filtration. Then the material was quickly dried on air. 6-methoxy-2-methyl-3-nitroquinolin-4-ol (1612mg, 6,88 mmol) was dissolved in 2:1 mixture of THF and saturated aqueous NH_4Cl (60ml). The mixture was treated with the zinc at vigorous stirring to keep the Zn dust in a suspension and to prevent it from caking on the bottom of flask. The reaction mixture was stirred at room temperature for 1h. The reaction mixture was filtered through GF/F paper while rinsing with THF. The filtrate was evaporated under reduced pressure and the residue was purified by column chromatography reverse phase using MeOH/H2O 50:50. Yellow solid; yield 16% (227 mg, 1.11 mmol). ^{1}H NMR (400 MHz, DMSO-*d6*) δ 11.34 (s, 1H), 7.45 – 7.37 (m, 2H), 7.12 (dd, *J* = 9.1, 2.9 Hz, 1H), 4.16 (s, 2H), 3.79 (s, 3H), 2.33 (s, 3H). ^{13}C NMR (101 MHz, DMSO-*d6*) δ 167.7, 154.1, 132.0, 129.3, 127.7, 121.6, 120.6, 119.3, 103.0, 55.1, 15.7. UPLC-MS (A) (ESI) RT 0.39 min, *m/z* 205.3 $[M+H]^+$ (>95%).

2-((3,5-Dimethylphenyl)amino)-2-oxoacetyl chloride (35). 3,5-Xylidine (5.14 ml, 41.3 mmol) was added dropwise to oxalyl chloride (34.9 ml, 413 mmol) at 0ºC and the resulting mixture was stirred for 1 h. The excess oxalyl chloride was removed under vacuum. Diethyl ether was added to the residue and the solids were filtered off. The filtrate was evaporated under reduced pressure, and hexane was added to the residue. After vigorous stirring for 30 min, the insoluble material was filtered, washed with pentane and dried under vacuum to give the target

compound. Yield 38% (3.305 g, 15.6 mmol). It was used directly for next step without further purification.

***N1*-(3,5-Dimethylphenyl)-*N2*-(4-hydroxy-6-methoxy-2-methylquinolin-3-yl)oxalamide (36).** The title compound was prepared according to the procedure described by H. Kokatla et al..[28] Compound **34** (200 mg, 0.98 mmol) was dissolved in a mixture of anhydrous DCM/anhydrous DMF: 10/1 (Volume: 22ml) and stirred at room temperature for 5 min. Compound **35** (435 mg, 1.44 mmol) was added to the stirring solution at 0°C and the reaction mixture was allowed to react for 1h at 0°C. Solvents were removed and the residue was purified by silica gel column chromatography. Off-white solid; 54% (201 mg, 0.53 mmol). ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.52 (s, 1H), 9.69 (s, 1H), 7.53 (d, J = 9.0 Hz, 1H), 7.50 – 7.46 (m, 3H), 7.31 (dd, J = 9.0, 3.0 Hz, 1H), 6.81 (s, 1H), 3.84 (s, 3H), 2.32 (s, 3H), 2.27 (s, 6H). UPLC-MS (A) (ESI) RT 1.61 min, *m/z* 380.4 $[M+H]^+$ (>95%).

***N*-(3,5-Dimethylphenyl)-8-methoxy-4-methylthiazolo[4,5-c]quinoline-2-carbothioamide (37).** The title compound was prepared using the procedure described by H. Kokatla et al.[28] according to which compound **36** (100mg, 0.26 mmol) was suspended in anhydrous pyridine (10 ml) and phosphorus pentasulfide (586 mg, 1.31 mmol) was added and the reaction mixture was left stirring under reflux overnight. Pyridine was evaporated and the obtained residue was dissolved in water. The pH was adjusted to 8 using saturated aqueous solution of potasium carbonate and the target compound was extracted with EtOAc. It was purified by silica gel and reverse phase column chromatography. Off-white solid; 19% (20 mg, 0.05 mmol). ^{1}H NMR (400 MHz, $CDCl_3$) δ 10.82 (s, 1H), 8.06 (d, J = 9.1 Hz, 1H), 7.67 (s, 2H), 7.37 (dd, J = 9.2, 2.8 Hz, 1H), 7.25 (d, J = 2.7 Hz, 1H), 6.97 (s, 1H), 3.97 (s, 3H), 3.08 (s, 3H), 2.40 (s, 6H). UPLC-MS (A) (ESI) RT 2.54 min, m/z 394.4 $[M+H]^+$ (>95%).

***N1*-(3,5-Dimethylphenyl)-*N2*-(6-methoxy-2-methylquinolin-4-yl)oxalamide (40).** 6-Methoxy-2methyl-4-quinolinamine (200mg, 1.06 mmol) was allowed to react with sodium hydride (NaH), 60% mineral oil (85mg, 1.28 mmol) in anhydrous DMF (10 ml) for 20 min at room temperature. Subsequently, 2-((3,5-dimethylphenyl)amino)-2-oxoacetyl chloride (**36**) (321mg, 1.06 mmol) was added to the mixture and left stirring overnight at room temperature. The reaction mixture was poured into water (80 ml) and the formed precipitate was filtered and washed with water (10 ml x 3). It was further purified by silica gel column chromatography. White solid; yield 12% (45 mg, 0.12 mmol). ^{1}H NMR (400 MHz, DMSO-*d6*) δ 10.94 (br. s, 1H), 10.72 (s, 1H), 7.87 (d, *J* = 9.1 Hz, 1H), 7.73 (s, 1H), 7.51 (s, 2H), 7.40 (dd, *J* = 9.1, 2.7 Hz, 1H), 7.35 (d, *J* = 2.7 Hz, 1H), 6.83 (s, 1H), 3.91 (s, 3H), 2.63 (s, 3H), 2.28 (s, 6H). ^{13}C NMR (101 MHz, DMSO-*d6*) δ 159.8, 158.2, 156.6, 155.9, 144.3, 139.4, 137.8, 137.4, 130.2, 126.3, 121.7, 121.3, 118.2, 115.4, 101.1, 55.5, 24.7, 21.1. UPLC-MS (A) (ESI) RT 1.66 min, *m/z* 364.4 $[M+H]^+$ (>95%).

***N*-(3,5-Dimethylbenzyl)-2-((6-methoxy-2-methylquinolin-4-yl)oxy)acetamide (42).** The title compound was prepared using the general procedure E. Off-white solid; yield 64% (117 mg, 0.32 mmol). ^{1}H NMR (400 MHz, DMSO-*d6*) δ 8.72 (t, *J* = 6.0 Hz, 1H), 7.79 (d, *J* = 9.1 Hz, 1H), 7.54 (d, *J* = 2.8 Hz, 1H), 7.35 (dd, *J* = 9.1, 2.9 Hz, 1H), 6.85 (s, 3H), 6.80 (s, 1H), 4.86 (s, 2H), 4.32 (d, *J* = 6.0 Hz, 2H), 3.89 (s, 3H), 2.54 (s, 3H), 2.22 (s, 6H). ^{13}C NMR (101 MHz, DMSO-*d6*) δ 166.9, 159.3, 156.8, 156.2, 144.2, 139.1, 137.2, 129.5, 128.2, 124.9, 121.4, 119.7, 102.3, 100.5, 67.2, 55.4, 41.8, 25.1, 20.9. UPLC-MS (A) (ESI) RT 1.60 min, *m/z* 365.5 $[M+H]^+$ (>95%).

4-Bromo-6-methoxy-2-methylquinoline (43). Phosphorous tribromide (1,994 ml, 21,14 mmol) was added slowly to a suspension of 6-methoxy-4-hydroxy-2-methyl-quinoline (**5a**) (1 g,

5,29 mmol) in DMF (20 ml) at ambient temperature. The reaction mixture was stirred under nitrogen at 50°C for 4 days. The mixture was quenched with water and stirred for 30min. The reaction mixture was made basic by addition of $KHCO_3$ and the formed precipitate was filtered and dried. Yellow solid; yield 86% (1.141 g, 4.53 mmol). ^{1}H NMR (400 MHz, DMSO-*d6*) δ 7.89 (d, *J* = 9.1 Hz, 1H), 7.82 (s, 1H), 7.45 (dd, *J* = 9.1, 2.8 Hz, 1H), 7.34 (d, *J* = 2.8 Hz, 1H), 3.93 (s, 3H), 2.60 (s, 3H). ^{13}C NMR (101 MHz, DMSO-*d6*) δ 157.9, 156.3, 143.8, 131.4, 130.6, 126.3, 126.0, 122.7, 104.1, 55.6, 24.0. UPLC-MS (A) (ESI) RT 1.43 min, *m/z* 251.8, 253.8 (1:1) $[M+H]^+$ (>95%).

2-((3,5-Dimethylphenyl)amino)ethanol (44). Glycolbromohydrin (681 mg, 5,45 mmol) was added to 3,5-xylidine (1 g, 8,25 mmol) and the reaction mixture was heated at 90°C for 4h. The resulting solid was dissolved in ethyl acetate, washed with 2M aqueous NaOH, followed by brine and dried over $MgSO_4$. The solvent was removed under reduced pressure and the residue was purified through a silica gel flash column, using n-heptane/EtOAc gradient from 20-40% to yield the desired compound. Brown liquid; yield 43% (587 mg, 3.55 mmol). ^{1}H NMR (400 MHz, DMSO-*d6*) δ 6.18 (s, 2H), 6.17 (s, 1H), 5.23 (t, *J* = 5.7 Hz, 1H), 4.63 (t, *J* = 5.5 Hz, 1H), 3.52 (q, *J* = 6.1 Hz, 2H), 3.04 (q, *J* = 6.1 Hz, 2H), 2.12 (s, 6H). ^{13}C NMR (101 MHz, DMSO-*d6*) δ 148.9, 137.6, 117.6, 110.0, 59.6, 45.6, 21.3. UPLC-MS (A) (ESI) RT 0.97 min, *m/z* 166.0 $[M+H]^+$ (>95%).

***N*-(2-((6-Methoxy-2-methylquinolin-4-yl)oxy)ethyl)-3,5-dimethylaniline (45).** 4-bromo-6-methoxy-2-methylquinoline (**43**) (200 mg, 0,793 mmol) and 2-((3,5-dimethylphenyl) amino) ethanol (**44**) (131 mg, 0,793 mmol), TMEDA (9,22 mg, 0,079 mmol), copper(I)iodide (15,11 mg, 0,079 mmol) and cesium carbonate (517 mg, 1,587 mmol) were placed in anhydrous DMF (4 ml) in a high pressure tube under nitrogen atmosphere. The reaction mixture was stirred at 95

°C for 2 d, cooled down and filtered. The filtrate was evaporated and the residue was dissolved in DCM (50 ml) and washed with brine (30 ml x 3). The organic layer was dried over $MgSO_4$ and evaporated under reduced pressure. The residue was purified by silica gel column chromatography. Light yellow solid; yield 17% (45 mg, 0.13 mmol). ^{1}H NMR (400 MHz, DMSO-*d6*) δ 7.74 (d, *J* = 9.1 Hz, 1H), 7.40 (d, *J* = 2.9 Hz, 1H), 7.31 (dd, *J* = 9.1, 2.9 Hz, 1H), 6.89 (s, 1H), 6.30 (s, 2H), 6.19 (s, 1H), 5.72 (t, *J* = 6.2 Hz, 1H), 4.31 (t, *J* = 5.4 Hz, 2H), 3.83 (s, 3H), 3.57 (q, *J* = 5.6 Hz, 2H), 2.54 (s, 3H), 2.13 (s, 6H). ^{13}C NMR (101 MHz, DMSO-*d6*) δ 159.9, 157.1, 156.5, 148.5, 144.0, 137.7, 129.4, 121.2, 119.9, 117.9, 110.1, 102.0, 100.3, 67.3, 55.4, 41.8, 25.0, 21.3. UPLC-MS (A) (ESI) RT 1.63 min, *m/z* 337.4 $[M+H]^+$ (>95%).

Ethyl 2-((6-methoxy-2-methylquinolin-4-yl)amino)acetate (46a). 4-bromo-6-methoxy-2-methyl quinoline (**43**) (250 mg, 0,992 mmol), glycine ethyl ester hydrochloride (277 mg, 1,983 mmol) and phenol (900 mg, 9,56 mmol) were placed in a round bottom flask and heated at 120°C under magnetic stirring overnight. The reaction mixture was cooled at room temperature and diluted with EtOAc. The formed precipitate was filtered off and washed with EtOAc. The target compound was purified by column chromatography using as eluent EtOAc/n-heptane gradient from 50-100%. Off-white solid; yield 55% (150 mg, 0.55 mmol). ^{1}H NMR (400 MHz, DMSO-*d6*) δ 7.63 (d, *J* = 9.1 Hz, 1H), 7.50 (d, *J* = 2.7 Hz, 1H), 7.33 (t, *J* = 6.3 Hz, 1H), 7.23 (dd, *J* = 9.1, 2.7 Hz, 1H), 6.17 (s, 1H), 4.22 – 4.10 (m, 4H), 3.87 (s, 3H), 2.40 (s, 3H), 1.22 (t, *J* = 7.1 Hz, 3H). ^{13}C NMR (101 MHz, DMSO-*d6*) δ 170.5, 155.9, 155.5, 149.1, 143.5, 129.8, 120.3, 117.8, 100.7, 98.7, 60.5, 55.5, 44.1, 24.9, 14.2. UPLC-MS (A) (ESI) RT 1.27 min, *m/z* 275.4 $[M+H]^+$ (>95%).

Potassium 2-((6-methoxy-2-methylquinolin-4-yl)amino)acetate (46b). In a round bottom flask, ethyl 2-((6-methoxy-2-methylquinolin-4-yl)amino)acetate (**46a**) (150mg, 0,547 mmol) was

dissolved in methanol (Volume: 10 ml). Potassium hydroxide (92 mg, 1,640 mmol) was added to the reaction mixture and left stirring under reflux for 1.5 h. Then, methanol was evaporated under reduced pressure to yield 250mg of the crude which was directly used for the next step. Off-white solid. ^{1}H NMR (400 MHz, MeOD) δ 7.68 (d, *J* = 9.2 Hz, 1H), 7.37 (d, *J* = 2.7 Hz, 1H), 7.24 (dd, *J* = 10.3, 3.8 Hz, 1H), 6.28 (s, 1H), 3.94 (s, 3H), 3.84 (s, 2H), 2.50 (s, 3H). UPLC-MS (A) (ESI) RT 0.89, m/z 247.3 $[M+H]^+$ (>95%).

***N*-(3,5-Dimethylphenyl)-2-((6-methoxy-2-methylquinolin-4-yl)amino)acetamide (47).** The title compound was prepared according to general procedure H. Off-white solid; yield 65% (125 mg, 0.36 mmol). ^{1}H NMR (400 MHz, DMSO-*d*$_6$) δ 9.96 (s, 1H), 7.64 (d, *J* = 9.1 Hz, 1H), 7.54 (d, *J* = 2.7 Hz, 1H), 7.33 (t, *J* = 6.0 Hz, 1H), 7.28 – 7.20 (m, 3H), 6.70 (s, 1H), 6.23 (s, 1H), 4.10 (d, *J* = 6.1 Hz, 2H), 3.89 (s, 3H), 2.40 (s, 3H), 2.22 (s, 6H). ^{13}C NMR (101 MHz, DMSO-*d*$_6$) δ 168.1, 155.9, 155.5, 149.5, 143.5, 138.7, 137.7, 129.7, 124.9, 120.3, 117.9, 117.1, 100.9, 98.7, 55.6, 46.3, 25.0, 21.1. UPLC-MS (A) (ESI) RT 1.53 min, *m/z* 350.4 $[M+H]^+$ (>95%).

2-((6-Methoxy-2-methylquinolin-4-yl)oxy)-*N*-phenylacetamide (49). The title compound was prepared using the general procedure E. Off-white solid; yield 29% (75 mg, 0.23 mmol); mp 221-222 ºC. ^{1}H NMR (400 MHz, DMSO-*d*$_6$) δ 10.26 (s, 1H), 7.78 (d, *J* = 9.1 Hz, 1H), 7.64 (dd, *J* = 8.5, 1.0 Hz, 2H), 7.51 (d, *J* = 2.8 Hz, 1H), 7.39 – 7.29 (m, 3H), 7.14 – 7.05 (m, 1H), 6.86 (s, 1H), 5.01 (s, 2H), 3.90 (s, 3H), 2.55 (s, 3H). ^{13}C NMR (101 MHz, DMSO-*d*$_6$) δ 165.7, 159.5, 156.9, 156.2, 144.2, 138.4, 129.5, 128.8, 123.8, 121.6, 119.7, 119.6, 102.2, 100.1, 67.1, 55.4, 25.1. UPLC-MS (A) (ESI) RT 1.66 min, *m/z* 323.2 $[M+H]^+$ (>95%). HRMS (ESI) *m/z* calcd for $C_{19}H_{19}N_2O_3$ $[M+H]^+$: 323.1390; found: 323.1380.

2-((6-Methoxy-2-methylquinolin-4-yl)oxy)-*N*-(m-tolyl)acetamide (50). The title compound was prepared using the general procedure E. Off-white solid; yield 58% (155 mg, 0.46 mmol);

mp 142-144 ºC. ^{1}H NMR (400 MHz, DMSO-*d*$_6$) δ 10.19 (s, 1H), 7.78 (d, *J* = 9.1 Hz, 1H), 7.57 – 7.46 (m, 2H), 7.41 (d, *J* = 8.0 Hz, 1H), 7.35 (dd, *J* = 9.1, 2.9 Hz, 1H), 7.21 (t, *J* = 7.8 Hz, 1H), 6.91 (d, *J* = 7.5 Hz, 1H), 6.84 (s, 1H), 5.00 (s, 2H), 3.89 (s, 3H), 2.54 (s, 3H), 2.28 (s, 3H). ^{13}C NMR (101 MHz, DMSO-*d*$_6$) δ 165.58, 159.47, 156.85, 156.25, 144.22, 138.30, 138.02, 129.51, 128.65, 124.44, 121.59, 120.15, 119.75, 116.82, 102.19, 100.08, 67.07, 55.40, 25.10, 21.17. UPLC-MS (A) (ESI) RT 1.55 min, *m/z* 337.2 $[M+H]^+$ (>95%). HRMS (ESI) *m/z* calcd for $C_{20}H_{21}N_2O_3$ $[M+H]^+$: 337.1547; found: 337.1533.

***N*-(2,5-Dimethylphenyl)-2-((6-methoxy-2-methylquinolin-4-yl)oxy)acetamide (51).** The title compound was prepared using the general procedure E. White solid; yield 35% (97 mg, 0.28 mmol).^{1}H NMR (400 MHz, DMSO-*d*$_6$) δ 9.57 (s, 1H), 7.79 (d, *J* = 9.1 Hz, 1H), 7.55 (d, *J* = 2.9 Hz, 1H), 7.35 (dd, *J* = 9.1, 2.9 Hz, 1H), 7.30 (s, 1H), 7.11 (d, *J* = 7.7 Hz, 1H), 6.94 (d, *J* = 7.6 Hz, 1H), 6.90 (s, 1H), 5.01 (s, 2H), 3.90 (s, 3H), 2.57 (s, 3H), 2.26 (s, 3H), 2.18 (s, 3H). ^{13}C NMR (101 MHz, DMSO-*d*$_6$) δ 165.9, 159.5, 157.1, 156.5, 144.4, 135.5, 135.3, 130.4, 129.7, 129.0, 126.5, 125.9, 121.9, 119.9, 102.6, 100.4, 67.5, 55.6, 25.3, 20.8, 17.5. UPLC-MS (A) (ESI) RT 1.66 min, *m/z* 351.2 $[M+H]^+$ (>95%).

***N*-(2,6-Dimethylphenyl)-2-((6-methoxy-2-methylquinolin-4-yl)oxy)acetamide (52).** The title compound was prepared using the general procedure E. Off-white solid; yield 73% (204 mg, 0.58 mmol). ^{1}H NMR (400 MHz, DMSO-*d*$_6$) δ 9.67 (s, 1H), 7.79 (d, *J* = 9.1 Hz, 1H), 7.58 (d, *J* = 2.8 Hz, 1H), 7.35 (dd, *J* = 9.1, 2.9 Hz, 1H), 7.12 – 7.06 (m, 3H), 6.90 (s, 1H), 5.03 (s, 2H), 3.89 (s, 3H), 2.57 (s, 3H), 2.17 (s, 6H). ^{13}C NMR (101 MHz, DMSO-*d*$_6$) δ 165.6, 159.3, 156.8, 156.3, 144.2, 135.4, 134.3, 129.5, 127.8, 126.8, 121.7, 119.8, 102.4, 100.4, 67.3, 55.4, 25.0, 18.1. UPLC-MS (A) (ESI) RT 1.45 min, *m/z* 351.5 $[M+H]^+$ (>95%).

2-((6-Methoxy-2-methylquinolin-4-yl)oxy)-*N*-(3-methoxyphenyl)acetamide (53). The title compound was prepared using the general procedure E. White solid; yield 28% (77 mg, 0.22 mmol). ^{1}H NMR (400 MHz, DMSO-*d*$_6$) δ 10.27 (s, 1H), 7.78 (d, *J* = 9.1 Hz, 1H), 7.50 (d, *J* = 2.8 Hz, 1H), 7.38 – 7.32 (m, 2H), 7.24 (t, *J* = 8.1 Hz, 1H), 7.20 – 7.15 (m, 1H), 6.85 (s, 1H), 6.68 (ddd, *J* = 8.1, 2.5, 1.0 Hz, 1H), 5.01 (s, 2H), 3.89 (s, 3H), 3.73 (s, 3H), 2.54 (s, 3H). ^{13}C NMR (101 MHz, DMSO-*d*$_6$) δ 165.7, 159.5, 159.5, 156.9, 156.3, 144.2, 139.6, 129.6, 129.5, 121.6, 119.7, 111.8, 109.2, 105.4, 102.2, 100.1, 67.0, 55.4, 55.0, 25.1. UPLC-MS (A) (ESI) RT 1.48 min, *m/z* 353.5 $[M+H]^+$ (>95%).

2-((6-Methoxy-2-methylquinolin-4-yl)oxy)-*N*-(4-methoxyphenyl)acetamide (54). The title compound was prepared using the general procedure E. White solid; yield 85% (238 mg, 0.68 mmol); mp 205-207 ºC. ^{1}H NMR (400 MHz, MeOD) δ 7.79 (d, *J* = 9.2 Hz, 1H), 7.65 (d, *J* = 2.8 Hz, 1H), 7.55 – 7.46 (m, 2H), 7.36 (dd, *J* = 9.2, 2.9 Hz, 1H), 6.95 – 6.87 (m, 2H), 6.84 (s, 1H), 4.95 (s, 2H), 3.95 (s, 3H), 3.78 (s, 3H), 2.62 (s, 3H). ^{13}C NMR (101 MHz, MeOD) δ 167.8, 161.9, 158.8, 158.7, 158.4, 145.2, 131.9, 129.3, 123.6, 123.5, 121.7, 115.1, 103.3, 101.4, 68.7, 56.1, 55.9, 24.8. UPLC-MS (A) (ESI) RT 1.40 min, *m/z* 353.5 $[M+H]^+$ (>95%). HRMS (ESI) *m/z* calcd for $C_{20}H_{21}N_2O_4$ $[M+H]^+$: 353.1496; found: 353.1494.

2-((6-Methoxy-2-methylquinolin-4-yl)oxy)-*N*-(4-methoxy-3,5-dimethylphenyl)acetamide (55). The title compound was prepared using the general procedure E. White solid; yield 60% (180 mg, 0.47 mmol); mp 202-204 ºC. ^{1}H NMR (400 MHz, DMSO-*d*$_6$) δ 10.05 (s, 1H), 7.78 (d, *J* = 9.1 Hz, 1H), 7.50 (d, *J* = 2.8 Hz, 1H), 7.35 (dd, *J* = 9.1, 2.9 Hz, 1H), 7.28 (s, 2H), 6.83 (s, 1H), 4.97 (s, 2H), 3.89 (s, 3H), 3.61 (s, 3H), 2.54 (s, 3H), 2.19 (s, 6H). ^{13}C NMR (101 MHz, DMSO-*d*$_6$) δ 165.3, 159.5, 156.8, 156.2, 152.7, 144.2, 133.8, 130.4, 129.5, 121.6, 120.1, 119.8,

102.2, 100.1, 67.1, 59.4, 55.4, 25.1, 16.0. UPLC-MS (A) (ESI) RT 1.79 min, *m/z* 381.3 $[M+H]^+$ (>95%). HRMS (ESI) *m/z* calcd for $C_{22}H_{25}N_2O_4$ $[M+H]^+$: 381.1809; found: 381.1798.

2-((6-Methoxy-2-methylquinolin-4-yl)oxy)-*N*-(2-methoxy-5-methylphenyl)acetamide (56). The title compound was prepared using the general procedure E. Off-white solid; yield 32% (94 mg, 026 mmol); mp 193-194 ºC. ^{1}H NMR (400 MHz, DMSO-*d6*) δ 9.32 (s, 1H), 7.93 (s, 1H), 7.81 (d, *J* = 9.1 Hz, 1H), 7.50 (d, *J* = 2.3 Hz, 1H), 7.39 (dd, *J* = 9.1, 2.8 Hz, 1H), 7.00 – 6.87 (m, 3H), 5.04 (s, 2H), 3.91 (s, 3H), 3.80 (s, 3H), 2.56 (s, 3H), 2.23 (s, 3H). ^{13}C NMR (101 MHz, DMSO-*d6*) δ 165.4, 158.9, 157.0, 156.4, 147.1, 144.2, 129.7, 129.3, 126.2, 124.9, 121.5, 121.3, 119.6, 111.1, 102.5, 100.1, 67.0, 55.9, 55.5, 25.1, 20.5. UPLC-MS (A) (ESI) RT 1.61 min, *m/z* 367.3 $[M+H]^+$ (>95%). HRMS (ESI) *m/z* calcd for $C_{21}H_{23}N_2O_4$ $[M+H]^+$: 367.1652; found: 367.1653.

***N*-(4-Fluoro-3,5-dimethylphenyl)-2-((6-methoxy-2-methylquinolin-4-yl)oxy)acetamide (57).** The title compound was prepared using the general procedure E. White solid; yield 68% (198 mg, 0.54 mmol); mp 207-210 ºC. ^{1}H NMR (400 MHz, DMSO-*d6*) δ 10.14 (s, 1H), 7.78 (d, *J* = 9.1 Hz, 1H), 7.50 (d, *J* = 2.8 Hz, 1H), 7.39 – 7.31 (m, 3H), 6.84 (s, 1H), 4.98 (s, 2H), 3.89 (s, 3H), 2.54 (s, 3H), 2.19 (s, 6H). ^{13}C NMR (101 MHz, DMSO-*d6*) δ 165.5, 159.5, 156.9, 156.2, 155.5 (d, $^1J_{C\text{-}F}$ = 238.3 Hz), 144.2, 133.7 (d, $^4J_{C\text{-}F}$ = 3.2 Hz), 129.5, 123.9 (d, $^2J_{C\text{-}F}$ = 18.8 Hz), 121.6, 120.3 (d, $^3J_{C\text{-}F}$ = 4.4 Hz), 119.8, 102.2, 100.1, 67.0, 55.4, 25.1, 14.5 (d, $^3J_{C\text{-}F}$ = 3.6 Hz). UPLC-MS (A) (ESI) RT 1.84 min, *m/z* 369.2 $[M+H]^+$ (>95%). HRMS (ESI) *m/z* calcd for $C_{21}H_{22}N_2O_3$ $[M+H]^+$: 369.1609; found: 369.1610.

***N*-(4-Bromo-3-methylphenyl)-2-((6-methoxy-2-methylquinolin-4-yl)oxy)acetamide (58).** The title compound was prepared using the general procedure E. Yellowish solid; yield 54% (178 mg, 0.43 mmol). ^{1}H NMR (400 MHz, DMSO-*d6*) δ 10.33 (s, 1H), 7.78 (d, *J* = 9.1 Hz, 1H),

7.66 (d, *J* = 2.3 Hz, 1H), 7.53 (d, *J* = 8.7 Hz, 1H), 7.50 (d, *J* = 2.9 Hz, 1H), 7.41 (dd, *J* = 8.7, 2.4 Hz, 1H), 7.35 (dd, *J* = 9.1, 2.9 Hz, 1H), 6.85 (s, 1H), 5.01 (s, 2H), 3.89 (s, 3H), 2.54 (s, 3H), 2.32 (s, 3H). ^{13}C NMR (101 MHz, DMSO-*d*$_6$) δ 165.8, 159.4, 156.8, 156.2, 144.2, 137.9, 137.5, 132.3, 129.5, 121.9, 122.0, 119.7, 119.1, 117.9, 102.2, 100.1, 67.0, 55.4, 25.1, 22.7. UPLC-MS (A) (ESI) RT 1.75 min, *m/z* 415.4, 417.4 (1:1) $[M+H]^+$ (>95%).

***N*-(3,5-Difluorophenyl)-2-((6-methoxy-2-methylquinolin-4-yl)oxy)acetamide (59).** The title compound was prepared using the general procedure E. White solid; yield 53% (151 mg, 0.42 mmol); mp 175-176 °C. ^{1}H NMR (400 MHz, DMSO-*d*$_6$) δ 10.64 (s, 1H), 7.78 (d, *J* = 9.1 Hz, 1H), 7.50 (d, *J* = 2.9 Hz, 1H), 7.44 – 7.31 (m, 3H), 6.97 (tt, *J* = 9.4, 2.4 Hz, 1H), 6.86 (s, 1H), 5.04 (s, 2H), 3.89 (s, 3H), 2.55 (s, 3H). ^{13}C NMR (101 MHz, DMSO-*d*$_6$) δ 166.5, 162.4 (dd, $^1J_{C\text{-}F}$ = 243.5 Hz, $^3J_{C\text{-}F}$ = 15.3 Hz), 159.3, 156.9, 156.3, 144.2, 140.9 (t, $^3J_{C\text{-}F}$ = 13.8 Hz), 129.5, 121.6, 119.7, 102.5 (d, $^2J_{C\text{-}F}$ = 29.7 Hz), 102.3, 100.1, 98.9 (t, $^2J_{C\text{-}F}$ = 26.0 Hz), 66.9, 55.4, 25.1. UPLC-MS (A) (ESI) RT 1.93 min, *m/z* 359.2 $[M+H]^+$ (>95%). HRMS (ESI) *m/z* calcd for $C_{19}H_{17}F_2N_2O_3$ $[M+H]^+$: 359.1202; found: 359.1209.

***N*-(2,4-Dichlorophenyl)-2-((6-methoxy-2-methylquinolin-4-yl)oxy)acetamide (60).** The title compound was prepared using the general procedure E. Off white solid; yield 2% (9 mg, 0.02 mmol). ^{1}H NMR (400 MHz, DMSO-*d*$_6$) δ 9.86 (s, 1H), 7.88 (d, *J* = 8.7 Hz, 1H), 7.79 (d, *J* = 9.1 Hz, 1H), 7.73 (d, *J* = 2.4 Hz, 1H), 7.54 (d, *J* = 2.8 Hz, 1H), 7.47 (dd, *J* = 8.7, 2.4 Hz, 1H), 7.36 (dd, *J* = 9.1, 2.9 Hz, 1H), 6.92 (s, 1H), 5.07 (s, 2H), 3.90 (s, 3H), 2.56 (s, 3H). ^{13}C NMR (101 MHz, DMSO-*d*$_6$) δ 166.2, 159.0, 156.9, 156.3, 144.2, 133.3, 129.9, 129.6, 129.0, 127.8, 127.4, 126.8, 121.7, 119.6, 102.5, 100.0, 67.1, 55.4, 25.1. UPLC-MS (A) (ESI) RT 1.57 min, *m/z* 391.1, 393.1 (1:1) $[M+H]^+$ (>95%).

***N*-(4-Chloro-3-(trifluoromethyl)phenyl)-2-((6-methoxy-2-methylquinolin-4-yl)oxy)acetamide (61).** The title compound was prepared using the general procedure E. White solid; yield 43% (194 mg, 0.46 mmol). ^{1}H NMR (400 MHz, DMSO-*d*$_6$) δ 10.69 (br. s, 1H), 8.24 (d, *J* = 2.5 Hz, 1H), 7.91 (dd, *J* = 8.8, 2.5 Hz, 1H), 7.79 (d, *J* = 9.1 Hz, 1H), 7.70 (d, *J* = 8.8 Hz, 1H), 7.52 (d, *J* = 2.9 Hz, 1H), 7.35 (dd, *J* = 11.4, 5.2 Hz, 1H), 6.88 (s, 1H), 5.05 (s, 2H), 3.89 (s, 3H), 2.55 (s, 3H). ^{13}C NMR (101 MHz, DMSO-*d*$_6$) δ 166.5, 159.3, 156.9, 156.3, 144.2, 137.9, 132.2, 129.5, 126.8 (q, $^{2}J_{C\text{-}F}$ = 30.6 Hz), 124.49, 122.69 (q, $^{1}J_{C\text{-}F}$ = 272.8 Hz), 121.62, 119.70, 118.32 (m, 2C), 102.30, 100.12, 66.95, 55.40, 25.07. UPLC-MS (A) (ESI) RT 1.67 min, *m/z* 425.4, 427.4 (3:1) $[M+H]^+$ (>95%).

***N*-(4-Chloro-3-methoxyphenyl)-2-((6-methoxy-2-methylquinolin-4-yl)oxy)acetamide (62).** The title compound was prepared using the general procedure E. White solid; yield 68% (278 mg, 0.72 mmol). ^{1}H NMR (400 MHz, DMSO-*d*$_6$) δ 10.38 (s, 1H), 7.78 (d, *J* = 9.2 Hz, 1H), 7.56 (d, *J* = 2.3 Hz, 1H), 7.51 (d, *J* = 2.9 Hz, 1H), 7.40 – 7.31 (m, 2H), 7.22 (dd, *J* = 8.6, 2.3 Hz, 1H), 6.86 (s, 1H), 5.02 (s, 2H), 3.90 (s, 3H), 3.82 (s, 3H), 2.55 (s, 3H). ^{13}C NMR (101 MHz, DMSO-*d*$_6$) δ 165.9, 159.4, 156.9, 156.3, 154.5, 144.2, 138.6, 129.8, 129.5, 121.6, 119.7, 115.4, 112.2, 104.2, 102.2, 100.1, 67.0, 55.9, 55.4, 25.1. UPLC-MS (A) (ESI) RT 1.54 min, *m/z* 387.4, 389.4 (3:1) $[M+H]^+$ (>95%).

***N*-(4-Chloro-2,5-dimethoxyphenyl)-2-((6-methoxy-2-methylquinolin-4-yl)oxy)acetamide (63).** The title compound was prepared using the general procedure E. White solid; yield 27% (117 mg, 0.28 mmol). ^{1}H NMR (400 MHz, DMSO-*d*$_6$) δ 9.49 (s, 1H), 8.04 (s, 1H), 7.81 (d, *J* = 9.1 Hz, 1H), 7.48 (d, *J* = 2.7 Hz, 1H), 7.39 (dd, *J* = 9.1, 2.9 Hz, 1H), 7.21 (s, 1H), 6.93 (s, 1H), 5.08 (s, 2H), 3.91 (s, 3H), 3.83 (s, 3H), 3.77 (s, 3H), 2.56 (s, 3H). ^{13}C NMR (101 MHz, DMSO-*d*$_6$) δ 165.8, 158.9, 157.0, 156.4, 148.2, 144.2, 143.2, 129.6, 126.3, 121.3, 119.6, 115.4, 113.3,

106.0, 102.4, 100.1, 66.9, 56.7, 56.4, 55.4, 25.1. UPLC-MS (A) (ESI) RT 1.56 min, *m/z* 417.4, 419.4 (3:1) $[M+H]^+$ (>95%).

2-((6-Methoxy-2-methylquinolin-4-yl)oxy)-*N*-(3,4,5-trimethoxyphenyl)acetamide (64). The title compound was prepared using the general procedure E. Off-white solid; yield 75% (328 mg, 0.80 mmol). ^{1}H NMR (400 MHz, DMSO-*d6*) δ 10.18 (s, 1H), 7.78 (d, *J* = 9.1 Hz, 1H), 7.51 (d, *J* = 2.8 Hz, 1H), 7.35 (dd, *J* = 9.1, 2.9 Hz, 1H), 7.04 (s, 2H), 6.85 (s, 1H), 4.99 (s, 2H), 3.90 (s, 3H), 3.74 (s, 6H), 3.63 (s, 3H), 2.55 (s, 3H). ^{13}C NMR (101 MHz, DMSO-*d6*) δ 165.5, 159.4, 156.9, 156.3, 152.8, 144.2, 134.5, 133.7, 129.5, 121.6, 119.7, 102.2, 100.1, 97.3, 67.0, 60.1, 55.7, 55.4, 25.1. UPLC-MS (A) (ESI) RT 1.36 min, *m/z* 413.5 $[M+H]^+$ (>95%).

***N*-(4-Fluorophenyl)-2-((6-methoxy-2-methylquinolin-4-yl)oxy)acetamide (65).** The title compound was prepared using the general procedure E. White solid; yield 52% (230 mg, 0.68 mmol). ^{1}H NMR (400 MHz, DMSO-*d6*) δ 10.32 (br. s, 1H), 7.78 (d, *J* = 9.1 Hz, 1H), 7.70 – 7.62 (m, 2H), 7.52 (d, *J* = 2.8 Hz, 1H), 7.35 (dd, *J* = 9.1, 2.9 Hz, 1H), 7.23 – 7.14 (m, 2H), 6.86 (s, 1H), 5.00 (s, 2H), 3.89 (s, 3H), 2.55 (s, 3H). ^{13}C NMR (101 MHz, DMSO-*d6*) δ 165.6, 159.4, 15.3 (d, $^1J_{C\text{-}F}$ = 240.4 Hz), 156.9, 156.2, 144.2, 134.7 (d, $^4J_{C\text{-}F}$ = 2.5 Hz), 129.5, 121.6, 121.6 (d, $^3J_{C\text{-}F}$ = 8.1 Hz), 119.7, 115.4 (d, $^2J_{C\text{-}F}$ = 22.4 Hz), 102.2, 100.1, 67.1, 55.4, 25.1. UPLC-MS (A) (ESI) RT 1.42 min, *m/z* 341.4 $[M+H]^+$ (>95%).

***N*-(4-Chlorophenyl)-2-((6-methoxy-2-methylquinolin-4-yl)oxy)acetamide (66).** The title compound was prepared using the general procedure E. White solid; yield 75% (353 mg, 0.99 mmol); mp 224-225 °C. ^{1}H NMR (400 MHz, DMSO-*d6*) δ 10.41 (br. s, 1H), 7.78 (d, *J* = 9.1 Hz, 1H), 7.70 – 7.64 (m, 2H), 7.51 (d, *J* = 2.9 Hz, 1H), 7.43 – 7.37 (m, 2H), 7.35 (dd, *J* = 9.1, 2.9 Hz, 1H), 6.86 (s, 1H), 5.02 (s, 2H), 3.89 (s, 3H), 2.54 (s, 3H). ^{13}C NMR (101 MHz, DMSO-*d6*) δ 165.9, 159.4, 156.9, 156.3, 144.2, 137.3, 129.5, 128.7, 127.3, 121.6, 121.2, 119.7, 102.2, 100.1,

67.1, 55.4, 25.1. UPLC-MS (A) (ESI) RT 1.51 min, *m/z* 357.3, 359.3 (3:1) $[M+H]^+$ (>95%). HRMS (ESI) *m/z* calcd for $C_{19}H_{18}ClN_2O_3$ $[M+H]^+$: 357.1000; found: 357.1001.

2-((6-Methoxy-2-methylquinolin-4-yl)oxy)acetic acid (68). Ethyl 2-((6-methoxy-2-methylquinolin-4-yl)oxy)acetate (**67**) (1 eq.) was dissolved in methanol or ethanol (molarity: 0.1-0.3 M) and potassium hydroxide (2eq.) was added to the solution. The reaction mixture was stirred under reflux for 2.5-3.5 h, and then the solvent was removed under reduced pressure. The obtained residue was dissolved in H_2O and the pH was acidified using CH_3COOH until pH 5-6. The crystallized solid was collected by filtration, washed with H_2O and dried to give the pure target compound. White solid; yield (over 2 steps) 92% (1.252 g, 5.06 mmol). ^{1}H NMR (400 MHz, DMSO-*d6*) δ 7.77 (d, *J* = 9.1 Hz, 1H), 7.40 (d, *J* = 2.9 Hz, 1H), 7.33 (dd, *J* = 9.1, 2.9 Hz, 1H), 6.80 (s, 1H), 4.93 (s, 2H), 3.87 (s, 3H), 2.53 (s, 3H). ^{13}C NMR (101 MHz, DMSO-*d6*) δ 169.4, 159.3, 156.8, 156.3, 144.1, 129.5, 121.6, 119.7, 102.2, 99.6, 64.7, 55.4, 25.0. UPLC-MS (A) ESI RT 0.43 min, *m/z* 248.1 $[M+H]^+$ (>95%).

2-((6-Methoxy-2-methylquinolin-4-yl)oxy)acetyl chloride (69). The title compound was prepared using the general procedure G. Off-white solid; yield 100% (1.275 g, 4.80 mmol). ^{1}H NMR (400 MHz, DMSO-*d6*) δ 16.10 (s, 1H), 8.25 (d, *J* = 9.3 Hz, 1H), 7.74 (dd, *J* = 9.3, 2.8 Hz, 1H), 7.56 (d, *J* = 2.8 Hz, 1H), 7.51 (s, 1H), 5.28 (s, 2H), 3.95 (s, 3H), 2.84 (s, 3H). ^{13}C NMR (101 MHz, DMSO-*d6*) δ 168.2, 164.9, 158.5, 155.8, 134.1, 126.1, 121.6, 120.2, 104.2, 100.9, 66.3, 56.0, 20.1.

2-((6-Methoxy-2-methylquinolin-4-yl)oxy)-*N*-(pyridin-2-yl)acetamide (70). The title compound was prepared using the general procedure H. Off-white solid; yield 51% (61 mg, 0.19 mmol). ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 2.54 (s, 3 H), 3.34 (s, 3 H), 5.12 (s, 2 H), 6.83 (s, 1 H), 7.15 (ddd, *J*=7.33, 4.93, 0.88 Hz, 1 H), 7.35 (dd, *J*=9.09, 2.78 Hz, 1 H), 7.46 (d, *J*=3.03 Hz,

1 H), 7.74 - 7.86 (m, 2 H), 8.06 (d, *J*=8.34 Hz, 1 H), 8.34 - 8.38 (m, 1 H), 10.76 (s, 1 H). ^{13}C NMR (101 MHz, DMSO-d_6) δ ppm 25.1, 55.4, 66.6, 99.8, 102.0, 113.6, 119.7, 119.8, 121.6, 129.5, 138.4, 144.2, 148.1, 151.4, 156.3, 156.8, 159.4, 166.5. UPLC-MS (C) RT 1.06 min, *m/z* 324 $[M+H]^+$ (>95%).

2-((6-Methoxy-2-methylquinolin-4-yl)oxy)-*N*-(pyridin-3-yl)acetamide (71). The title compound was prepared using the general procedure H. Off-white solid; yield 58% (70 mg, 0.22 mmol). ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 2.55 (s, 3 H), 3.90 (s, 3 H), 5.05 (s, 2 H), 6.88 (s, 1 H), 7.30 - 7.43 (m, 2 H), 7.53 (d, *J*=2.8 Hz, 1 H), 7.79 (d, *J*=9.1 Hz, 1 H), 8.03 - 8.14 (m, 1 H), 8.31 (dd, *J*=4.7, 1.4 Hz, 1 H), 8.80 (d, *J*=2.5 Hz, 1 H), 10.48 (s, 1 H). ^{13}C NMR (101 MHz, DMSO-d_6) δ ppm 25.0, 55.4, 66.9, 100.1, 102.2, 119.7, 121.3, 123.7, 126.7, 129.6, 135.0, 141.3, 144.1, 144.7, 156.2, 156.8, 159.3, 166.3. UPLC-MS (C) RT 0.97 min, *m/z* 324 $[M+H]^+$ (>95%).

2-((6-Methoxy-2-methylquinolin-4-yl)oxy)-*N*-(pyridin-4-yl)acetamide (72). The title compound was prepared using the general procedure H. White solid; yield 31% (81 mg, 0.25 mmol). ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.66 (s, 1H), 8.46 (dd, *J* = 4.8, 1.7 Hz, 2H), 7.78 (d, *J* = 9.1 Hz, 1H), 7.62 (dd, *J* = 4.8, 1.7 Hz, 2H), 7.50 (d, *J* = 2.9 Hz, 1H), 7.35 (dd, *J* = 9.2, 2.9 Hz, 1H), 6.86 (s, 1H), 5.07 (s, 2H), 3.89 (s, 3H), 2.54 (s, 3H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 166.9, 159.3, 156.9, 156.3, 150.5, 145.1, 144.2, 129.5, 121.6, 119.7, 113.5, 102.2, 100.0, 66.9, 55.4, 25.1. UPLC-MS (A) (ESI) RT 0.43 min, *m/z* 324.1 $[M+H]^+$ (>95%).

***N*-(2,6-Dimethylpyridin-4-yl)-2-((6-methoxy-2-methylquinolin-4-yl)oxy)acetamide (73).** The title compound was prepared using the general procedure H. White solid; yield 36% (51 mg, 0.15 mmol). ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.77 (s, 1H), 7.81 (d, *J* = 9.1 Hz, 1H), 7.49 (d, *J* = 2.8 Hz, 1H), 7.42 – 7.34 (m, 3H), 6.89 (s, 1H), 5.11 (s, 2H), 3.89 (s, 3H), 2.56 (s, 3H), 2.42 (s, 6H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 167.0, 159.8, 157.1, 156.8, 156.4, 146.9, 143.6, 129.0,

122.0, 119.7, 110.2, 102.4, 100.0, 67.0, 55.4, 24.7, 23.3. UPLC-MS (A) (ESI) RT 1.01 min, *m/z* 352.2 $[M+H]^+$ (>95%).

2-((6-Methoxy-2-methylquinolin-4-yl)oxy)-*N*-(pyrimidin-4-yl)acetamide (74). The title compound was prepared using the general procedure H. White solid; yield 40% (105 mg, 0.32 mmol). ^{1}H NMR (400 MHz, DMSO-*d*$_6$) δ 11.22 (s, 1H), 8.93 (d, *J* = 0.9 Hz, 1H), 8.68 (d, *J* = 5.8 Hz, 1H), 8.03 (dd, *J* = 5.8, 1.2 Hz, 1H), 7.78 (d, *J* = 9.1 Hz, 1H), 7.45 (d, *J* = 2.8 Hz, 1H), 7.35 (dd, *J* = 9.1, 2.9 Hz, 1H), 6.83 (s, 1H), 5.16 (s, 2H), 3.88 (s, 3H), 2.53 (s, 3H). ^{13}C NMR (101 MHz, DMSO-*d*$_6$) δ 167.9, 159.4, 158.5, 158.4, 157.2, 156.9, 156.3, 144.2, 129.5, 121.6, 119.7, 110.0, 102.1, 99.8, 66.6, 55.4, 25.1. UPLC-MS (A) (ESI) RT 1.36 min, *m/z* 325.2 $[M+H]^+$ (>95%).

***N*-(2,6-Dimethylpyrimidin-4-yl)-2-((6-methoxy-2-methylquinolin-4-yl)oxy)acetamide (75).** The title compound was prepared using the general procedure H. White solid; yield 40% (115 mg, 0.33 mmol). ^{1}H NMR (400 MHz, DMSO-*d*$_6$) δ 11.06 (s, 1H), 7.78 (d, *J* = 9.1 Hz, 1H), 7.73 (s, 1H), 7.44 (d, *J* = 2.9 Hz, 1H), 7.35 (dd, *J* = 9.1, 2.9 Hz, 1H), 6.80 (s, 1H), 5.12 (s, 2H), 3.88 (s, 3H), 2.53 (s, 3H), 2.50 (s, 3H, overlaps with solvent's peak) 2.37 (s, 3H). ^{13}C NMR (101 MHz, DMSO-*d*$_6$) δ 168.2, 167.7, 166.6, 159.4, 157.4, 156.8, 156.3, 144.2, 129.5, 121.6, 119.7, 105.6, 102.1, 99.8, 66.5, 55.4, 25.2, 25.1, 23.9. UPLC-MS (A) (ESI) RT 1.13 min, *m/z* 353.2 $[M+H]^+$ (>95%).

2-((6-Methoxy-2-methylquinolin-4-yl)oxy)-*N*-(thiazol-2-yl)acetamide (76). The title compound was prepared using the general procedure H. White solid; yield 59% (266 mg, 0.48 mmol). ^{1}H NMR (400 MHz, DMSO-*d*$_6$) δ 12.51 (s, 1H), 7.79 (d, *J* = 9.1 Hz, 1H), 7.51 (d, *J* = 3.6 Hz, 1H), 7.47 (d, *J* = 2.8 Hz, 1H), 7.35 (dd, *J* = 9.1, 2.9 Hz, 1H), 7.27 (d, *J* = 3.6 Hz, 1H), 6.84 (s, 1H), 5.17 (s, 2H), 3.89 (s, 3H), 2.54 (s, 3H). ^{13}C NMR (101 MHz, DMSO-*d*$_6$) δ 166.0, 159.4,

157.5, 156.9, 156.3, 144.2, 137.7, 129.5, 121.7, 119.7, 113.9, 102.1, 99.9, 66.1, 55.4, 25.1. UPLC-MS (A) (ESI) RT 1.65 min, *m/z* 330.2 $[M+H]^+$ (>95%).

***N*-(4,5-Dimethylthiazol-2-yl)-2-((6-methoxy-2-methylquinolin-4-yl)oxy)acetamide (77).** The title compound was prepared using the general procedure H. White solid; yield 6% (18 mg, 0.05 mmol); ^{1}H NMR (400 MHz, DMSO-*d*$_6$) δ 12.26 (s, 1H), 7.78 (d, *J* = 9.1 Hz, 1H), 7.47 (d, *J* = 2.9 Hz, 1H), 7.35 (dd, *J* = 9.1, 2.9 Hz, 1H), 6.80 (s, 1H), 5.11 (s, 2H), 3.89 (s, 3H), 2.53 (s, 3H), 2.24 (d, *J* = 0.7 Hz, 3H), 2.17 (d, *J* = 0.7 Hz, 3H). UPLC-MS (A) (ESI) RT 1.70 min, *m/z* 358.2 $[M+H]^+$ (>95%).

***N*-(4,5-Dimethylisoxazol-3-yl)-2-((6-methoxy-2-methylquinolin-4-yl)oxy)acetamide (78).** The title compound was prepared using the general procedure H. White solid; yield 61% (169 mg, 0.50 mmol). ^{1}H NMR (400 MHz, DMSO-*d*$_6$) δ 10.56 (br. s, 1H), 7.78 (d, *J* = 9.1 Hz, 1H), 7.51 (d, *J* = 2.8 Hz, 1H), 7.35 (dd, *J* = 9.1, 2.9 Hz, 1H), 6.82 (s, 1H), 5.08 (s, 2H), 3.89 (s, 3H), 2.55 (s, 3H), 2.31 (s, 3H), 1.81 (s, 3H). ^{13}C NMR (101 MHz, DMSO-*d*$_6$) δ 166.5, 166.2, 159.3, 157.6, 156.8, 156.3, 144.2, 129.5, 121.6, 119.7, 106.4, 102.2, 100.2, 66.7, 55.4, 25.1, 10.8, 6.8. UPLC-MS (A) (ESI) RT 1.54 min, *m/z* 342.2 $[M+H]^+$ (>95%).

2-((6-Methoxy-2-methylquinolin-4-yl)oxy)-*N*-(3-oxoisoxazolidin-4-yl)acetamide (79). The title compound was prepared using the general procedure H. Off-white solid; yield 5% (17 mg, 0.05 mmol); mp decomposition >150 ºC. ^{1}H NMR (400 MHz, DMSO-*d*$_6$) δ ppm 8.31 (s, 1 H), 7.95 (d, *J*=4.5 Hz, 1 H), 7.76 (d, *J*=9.0 Hz, 1 H), 7.47 (d, *J*=3.0 Hz, 1 H), 7.33 (dd, *J*=9.2, 2.9 Hz, 1 H), 6.85 (s, 1 H), 4.80 (ABq, $\Delta\delta_{AB}$ =0.004, *J*=14.6 Hz, 2 H), 4.30 (t, *J*=7.8 Hz, 1 H), 4.08 - 4.17 (m, 1 H), 3.92 (s, 3 H), 3.36 - 3.39 (m, 1 H, overlaps with water peak), 2.54 (s, 3 H). ^{13}C NMR (101 MHz, DMSO-*d*$_6$) δ ppm 171.9, 166.9, 159.0, 156.9, 156.4, 144.1, 129.5, 121.7,

119.6, 102.4, 99.7, 73.0, 66.8, 55.5, 55.3, 25.0. UPLC-MS (A) (ESI) RT 0.97 min, *m/z* 332.1 $[M+H]^+$ (>95%). HRMS (ESI) *m/z* calcd for $C_{16}H_{18}N_3O_5$ $[M+H]^+$: 332.1241; found: 332.1241.

2-((6-Methoxy-2-methylquinolin-4-yl)oxy)-*N*-(4-(N-(5-methylisoxazol-3-yl)sulfamoyl) phenyl) acetamide (80). The title compound was prepared using the general procedure H. Off-white solid; yield 44% (165 mg, 0.34 mmol). ^{1}H NMR (400 MHz, DMSO-*d6*) δ 11.38 (br. s, 1H), 10.68 (s, 1H), 7.83 (s, 4H), 7.78 (d, *J* = 9.1 Hz, 1H), 7.49 (d, *J* = 2.8 Hz, 1H), 7.35 (dd, *J* = 9.1, 2.9 Hz, 1H), 6.85 (s, 1H), 6.12 (s, 1H), 5.06 (s, 2H), 3.89 (s, 3H), 2.54 (s, 3H), 2.29 (s, 3H). ^{13}C NMR (101 MHz, DMSO-*d6*) δ 170.2, 166.4, 159.4, 157.6, 156.9, 156.3, 144.1, 142.6, 133.8, 129.4, 128.1, 121.7, 119.7, 119.4, 102.2, 100.0, 95.4, 66.9, 55.4, 25.0, 12.0. UPLC-MS (A) (ESI) RT 1.40 min, *m/z* 483.4 $[M+H]^+$ (>95%).

***N'*-(2-((6-Methoxy-2-methylquinolin-4-yl)oxy)acetyl)isonicotinohydrazide (81).** The title compound was prepared using the general procedure H. Off-white solid; yield 19% (55 mg, 0.15 mmol). ^{1}H NMR (400 MHz, DMSO-*d6*) δ 10.83 (br. s, 1H), 10.54 (s, 1H), 8.78 (dd, *J* = 4.4, 1.6 Hz, 2H), 7.81 – 7.75 (m, 3H), 7.57 (d, *J* = 2.8 Hz, 1H), 7.35 (dd, *J* = 9.1, 2.9 Hz, 1H), 6.94 (s, 1H), 4.99 (s, 2H), 3.90 (s, 3H), 2.58 (s, 3H). ^{13}C NMR (101 MHz, DMSO-*d6*) δ 166.5, 164.1, 159.1, 156.9, 156.3, 150.5, 144.2, 139.3, 129.5, 121.5, 121.3, 119.7, 102.6, 100.4, 66.2, 55.5, 25.1. UPLC-MS (A) (ESI) RT 0.29 min, *m/z* 367.4 $[M+H]^+$ (>95%).

***N*-(3,5-Dimethylphenyl)-2-((6-methoxy-2-methylquinolin-4-yl)oxy)-*N*-methylacetamide (82).** Iodomethane (MeI) (0.021 mL, 0.342 mmol) was added to a solution of N-(3,5-dimethylphenyl)-2-((6-methoxy-2-methylquinolin-4-yl)oxy)acetamide (100mg, 0.285 mmol) and sodium hydride (NaH) 60% suspended in mineral oil (13.70 mg, 0.342 mmol) in anhydrous tetrahydrofuran (THF) (2mL) which was maintained below 5 ºC. There reaction mixture was left stirring overnight at room temperature. THF was evaporated, the obtained residue was dissolved

in water (50 mL) and the target compound was extracted with ethyl acetate (50 mL x 3). The combined organic layers were dried over $MgSO_4$ and evaporated under reduced pressure. The residue was purified by flash chromatography using a Merk pre-packed column (18+2g) and eluent ethyl acetate/cyclohexane 80/20. Yellowish solid; yield 83% (86 mg, 0.237 mmol). ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 7.74 (d, *J*=9.1 Hz, 1 H), 7.31 (dd, *J*=9.1, 3.0 Hz, 1 H), 7.22 (br. s., 1 H), 7.07 (br. s., 2 H), 7.00 (br. s., 1 H), 6.59 (br. s., 1 H), 4.79 (br. s., 2 H), 3.84 (s, 3 H), 3.19 (br. s., 3 H), 2.53 (s, 3 H), 2.25 (s, 6 H). ^{13}C NMR (101 MHz, DMSO-d_6) δ ppm 166.4 (br. s.), 159.8, 157.1, 156.6, 144.5, 142.3 (br. s.), 139.5(br. s.), 129.8 (2 peaks overlap, proven by HSQC), 124.8 (br. s.), 121.9, 120.2, 102.3 (br. s.), 100.2, 66.2, 55.8, 37.5, 25.5, 21.2. UPLC-MS (C) RT 1.23 min, *m/z* 365 $[M+H]^+$ (>95%).

2-(((6-Methoxy-2-methylquinolin-4-yl)oxy)methyl)-5-methylbenzo[d]oxazole (83). The title compound was prepared using the general procedure E. White solid; yield 19% (8.5 mg, 0.025 mmol); mp 231-233 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 7.79 (d, *J*=9.1 Hz, 1 H), 7.66 (d, *J*=8.3 Hz, 1 H), 7.61 (s, 1 H), 7.39 (d, *J*=2.8 Hz, 1 H), 7.33 - 7.38 (m, 1 H), 7.26 (dd, *J*=8.2, 1.1 Hz, 1 H), 7.10 (s, 1 H), 5.74 (s, 2 H), 3.86 (s, 3 H), 2.55 (s, 3 H), 2.43 (s, 3 H). UPLC-MS (C) RT 1.25 min, *m/z* 335 $[M+H]^+$ (>95%). HRMS (ESI) *m/z* calcd for $C_{20}H_{19}N_2O_3$ $[M+H]^+$: 335.1390; found: 335.1376.

4-((1*H*-Benzo[d]imidazol-2-yl)methoxy)-6-methoxy-2-methylquinoline (84).

The title compound was prepared using the general procedure E. Off-white solid; yield 3% (5 mg, 0.016 mmol). ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 12.80 (br. s, 1 H), 7.79 (d, *J*=9.1 Hz, 1 H), 7.66 (d, *J*=7.8 Hz, 1 H), 7.53 (d, *J*=7.1 Hz, 1 H), 7.48 (d, *J*=2.8 Hz, 1 H), 7.35 (dd, *J*=9.1, 3.0 Hz, 1 H), 7.17 - 7.28 (m, 2 H), 7.12 (s, 1 H), 5.59 (s, 2 H), 3.87 (s, 3 H), 2.57 (s, 3 H). UPLC-MS (C) RT 1.05 min, *m/z* 320 $[M+H]^+$ (>95%).

Strain and growth conditions. *M. tuberculosis* H37Rv (ATC25618) wild-type was grown in Middlebrook 7H9-ADC broth (Difco) supplemented with 0.05% Tween 80 and on 7H10-OADC or 7H11-OADC agar (Difco) at 37 °C. Isoniazid and hygromycin were purchased from Sigma-Aldrich. When required, hygromycin (50 μg/ml) was added to the culture medium.

MIC determination. The measurement of the Minimum Inhibitory Concentration (MIC) against *M. tuberculosis* H37Rv for each tested compound was performed in 96-well flat-bottom, polystyrene microtiter plates in a final volume of 200 μl. Ten two-fold drug dilutions in neat DMSO were performed. Middlebrook 7H9 (Difco) was used as medium. Isoniazid (INH) (Sigma Aldrich) was used as a positive control with two-fold dilutions of INH starting at 4 μg/ml placed at row 11 of the plate layout and rifampicin (Sigma Aldrich) was used as no-growth control at concentration of 1 μM, placed at G-12 and H-12 wells. The inoculum (200 μl) was added to the entire plate. All plates were placed in a sealed box to prevent drying out of the peripheral wells and incubated at 37ºC without shaking for six days. A Resazurin solution was prepared by dissolving one tablet of resazurin (Resazurin Tablets for Milk Testing; Ref 330884Y' VWR International Ltd) in 30 ml of sterile PBS (phosphate buffered saline). Of this solution, 25 μl were added to each well. Fluorescence was measured (Spectramax M5 Molecular Devices, Excitation 530nm, Emission 590 nm) after 48 hours to determine the MIC value.

Intracellular IC_{50} and IC_{90} determination. Human THP-1 macrophages differentiated with PMA was used as a model to study the intracellular stages of *Mycobacterium tuberculosis* (Mtb). The assay determines the effect of the compounds on mycobacteria growing inside phagocytes by determining luciferase activity per well, which is related to the number of living bacteria.

Protocol Steps: (a) Bacterial culture and single cell suspension protocol. A single cell suspension of Mtb H37Rv pATB45luc was prepared prior to infection. 25 ml of bacterial culture

grown to log phase was centrifuged at 2800 g for 10 min. After removal of the supernatant, cell clumps were dispersed by vigorously shaking with sterile glass 3mm beads (Sigma) for 2 min. Dispersed cells were then resuspended in 10 ml of RPMI medium and left to decant for 5 min at room temperature. Cells were then centrifuged at 400 g for 5 min. Supernatant was collected and its OD600 was measured. OD ml^{-1} was converted to CFU ml^{-1} considering that 0.125 OD is equal to 10^7 CFU ml^{-1}.

(b) THP1 cell preparation and infection with Mtb. THP1 cells (Human acute monocytic leukemia cell line, ATCC number TIB-202) were maintained in suspension with RPMI-1640 media (Sigma) containing 10% fetal bovine serum (Gibco), 1 mM of Pyruvate (Sigma), 2mM of L-Glutamine (Sigma), and incubated at 37 ºC with 5% CO_2. THP1 cells were routinely subcultured every 3 days at a cell density of 10^5 cells/mL. THP-1 cells were simultaneously differentiated with phorbol myristate acetate (PMA, 40 ng ml-1, Sigma) and infected with a single cell suspension of Mtb H37Rv-pATB45luc in a roller bottle at a MOI of 1:1. Cells were put in a roller bottle apparatus for 4 hours at 37° C at 1.5 rpm. After this step of incubation, infected cells were washed four times by centrifugation at 400 g for 5 min and resuspended in fresh RPMI medium to remove extracellular bacilli. In the last wash, infected cells were resuspended in RPMI medium supplemented with 10% fetal bovine serum (Gibco), 2 mM L-glutamine and pyruvate at a concentration of 2 x 10^5 cells/ml. 50 µl of this cell suspension (typically 10000 cells per well) were dispensed into the wells of 384-well plates (white, flat bottom, Greiner).

(c) Incubation of infected THP-1 cells with tested compounds. Prior to addition of the infected cell suspension, the compounds (250 nL/well) were dispensed into the plates with an Echo liquid handler. The maximum DMSO concentration is 0.5%. Plates were allowed to incubate at 37 °C

at 80% relative humidity for 4 days. Luciferase activity, proportional to bacterial load, was determined by using BrightGlo™ Luciferase Assay System (Promega, Madison, WI) according to the manufacturer's protocol. Resultant luminescence was measured in an Envision Multilabel Plate Reader (PerkinElmer) using the 384-plate Ultra Sensitive luminescence mode, with a measurement time of 200 ms per well. Results were processed by using an Excel spreadsheet and Grafit software. IC_{50} and IC_{90} values were calculated from the dose-response curves by non-linear regression analysis.

$HepG_2$ cytotoxicity assay. $HepG_2$ cells were cultured using Eagle's MEM supplemented with 10% heat-inactivated FBS, 1% NEAA and 1% penicillin/streptomycin. Prior to addition of the cell suspension, 250 nL of test compounds per well were pre-dispensed in TC-treated black clear-bottomed 384 well plates (Greiner, cat.# 781091) with an Echo 555 instrument. After that, 25 μL of $HepG_2$ (ATCC HB-8065) cells (~3000 cells/well) grown to confluency in Eagle's MEM supplemented with 10% heat-inactivated FBS, 1% NEAA and 1% Pencillin/Streptomycin were added to each well with the reagent dispenser. Plates were allowed to incubate at 37 °C with 20% O_2 and 5% CO_2 for 48 h.

After the incubation period (48h), the plates were equilibrated to room temperature before proceeding to develop the luminescent signal. ATP levels measured with CellTiter Glo kit (Promega) were used as cell viability read-out. 25 μL of CellTiter Glo substrate dissolved in the buffer was added to each well. Plates were incubated at room temperature for 10 minutes for stabilization of luminescence signal and read on View Lux with excitation and emission filters of 613 and 655 nm, respectively.

Microsomal fraction stability experimental procedure. Pooled mouse liver microsomes were purchased from Xenotech. Microsomes (final protein concentration 0.5 mg/ml, $MgCl_2$

(final concentration 5 mM) and test compound (final substrate concentration 0.5 µM; final DMSO concentration 0.5 %) in 0.1 M phosphate buffer pH 7.4 were pre-incubated at 37°C prior to the addition of NADPH (final concentration 1 mM) to initiate the reaction. The final incubation volume was 600 µl. All incubations were performed singularly for each test compound. Each compound was incubated for 30 minutes and samples (90 µl) of incubate were taken at 0, 5, 10, 20 and 30 minutes. The reactions were stopped by the addition of sample to 200 µl of acetonitrile:methanol (3:1) containing an internal standard. The terminated samples were centrifuged at 3700 rpm for 15 minutes at 4°C to precipitate the protein. Quantitative analysis: following protein precipitation, the samples were analyzed using specific LC-MS/MS conditions. Data analysis: from a plot of ln peak area ratio (compound peak area/internal standard peak area) against time, the gradient of the line was determined. Subsequently, half-life and intrinsic clearance were calculated using the equations below:

$$\text{Elimination rate constant (k)} = (-\text{gradient})$$

$$\text{Half life } (t_{1/2})(\text{min}) = \frac{0.693}{k}$$

$$\text{Intrinsic Clearance (CLint) (ml/min/g)} = \frac{0.693}{t_{1/2}} \text{ x (ml of incubation/mg microsomal protein) x (mg microsomal protein/g liver)}$$

Blood stability assay. The stability of each compound was assessed in CD1 mouse whole blood collected on the day of the experiment in EDTA tubes. Typically 1 mL of blood was spiked with 2 µL of a 0.5 mM of each test compound solution to produce a 1 µM incubation.

Three separate 300 µl aliquots were then taken from each tube and incubated at 37 ºC. At each time point (0, 5, 10, 20, 30, 60, 120, (240) min), 50 µL of blood were collected from each sample over 50 µL of MilliQ water. Samples were extracted by protein precipitation with 350 µL of 0.1% AcOH acetonitrile methanol 3-1 (v:v) containing 1 µM internal standard and centrifuged

for 10 min. Supernatants were collected prior the injection onto an LC-MS/MS system. Analyte/Internal standard peak area ratios were referenced to the zero time-point samples as 100% in order to determine the percentage of compound remaining for each time-point. Ln plots of the % remaining for each compound were used to determine the half-life for the blood incubations.

The human biological samples were sourced ethically and their research use was in accord with the terms of the informed consents.

Artificial membrane permeability, kinetic aqueous solubility (CLND) and hydrophobicity (chromlog$D_{pH7.4}$) assays. Those assays were performed analogously to previously described.[6]

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The authors gratefully acknowledge the many scientists from GSK DDW, involved in the research.

ABBREVIATIONS USED

ACN, acetonitrile; AcOH, acetic acid; ATP, adenosine triphosphate; CFU, colony-forming unit; Cl_{int}, hepatic intrinsic clearance; CLND, chemiluminescent nitrogen detection; DCM, dichloromethane; DMF, *N,N*-dimethylformamide; DMSO, dimethyl sulfoxide; EDTA, ethylenediaminetetraacetic acid; ESI, electrospray ionization; EtOAc, ethyl acetate; $HepG_2$, hepatocellular carcinoma, human; IC_{50}, half maximal inhibitory concentration; LC-MS, liquid chromatography–mass spectrometry; *Mtb, Mycobacterium Tuberculosis*; MDR-TB, multidrug-resistant tuberculosis; MEM, minimum essential medium; MeOH, methanol; MIC, minimun inhibitory concentration; NADPH, nicotinamide adenine dinucleotide phosphate; NMR, nuclear magnetic resonance; SAR, structure–activity relationship; TB, tuberculosis; THF, tetrahydrofuran; UPLC-MS, ultra performance liquid chromatography-mass spectrometry; XDR-TB, extensively drug-resistant tuberculosis.

ASSOCIATED CONTENT

Supporting Information

Additional data (yield, ^{1}H-NMR, ^{13}C-NMR and UPLC-MS) for the intermediate compounds that were not reported here.

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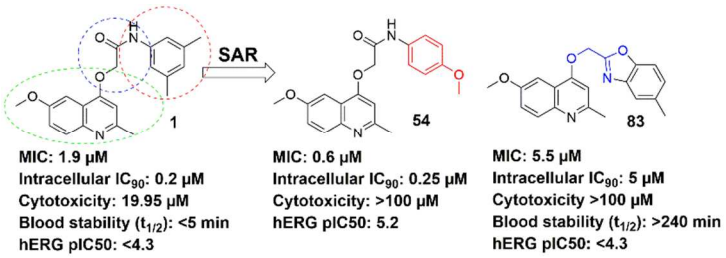
TABLE OF CONTENTS GRAPHIC



1
MIC: 1.9 μM
Intracellular IC_{90}: 0.2 μM
Cytotoxicity: 19.95 μM
Blood stability ($t_{1/2}$): <5 min
hERG pIC50: <4.3

54
MIC: 0.6 μM
Intracellular IC_{90}: 0.25 μM
Cytotoxicity: >100 μM
hERG pIC50: 5.2

83
MIC: 5.5 μM
Intracellular IC_{90}: 5 μM
Cytotoxicity >100 μM
Blood stability ($t_{1/2}$): >240 min
hERG pIC50: <4.3

Graphics for Pitta et al.

1) Figure :

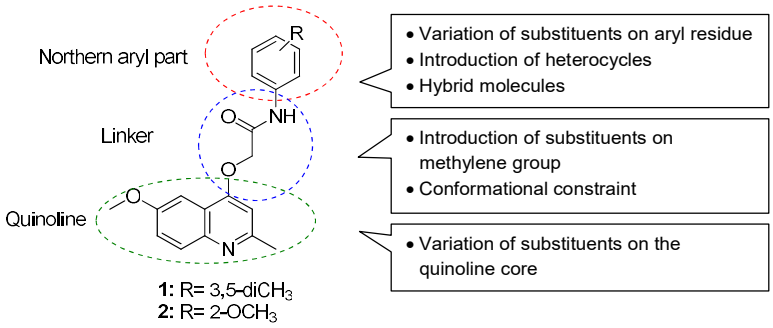
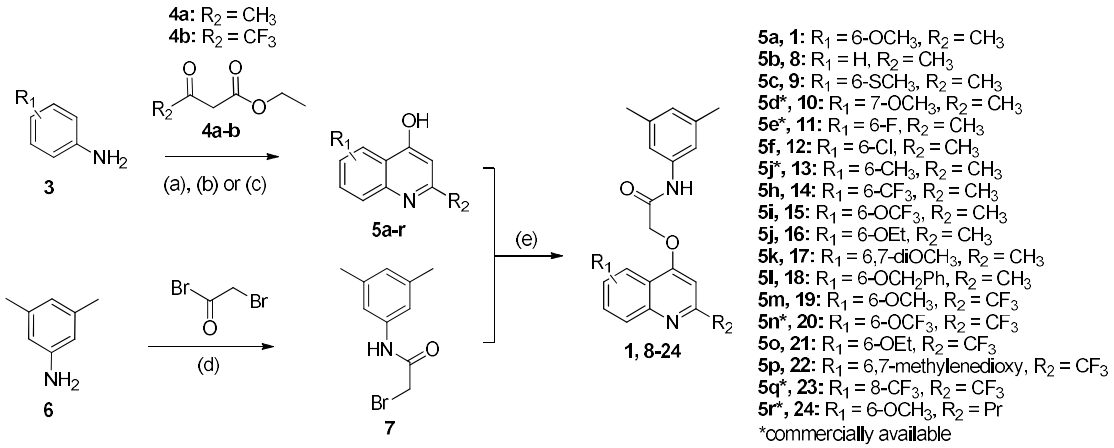


Figure 1. Hit compounds **1**, **2** and SAR design.

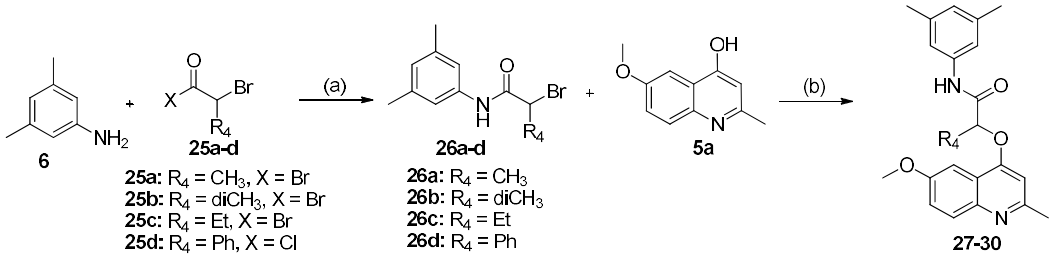
2) Schemes

Scheme 1. Synthesis of compounds with quinoline substitution modifications[a]



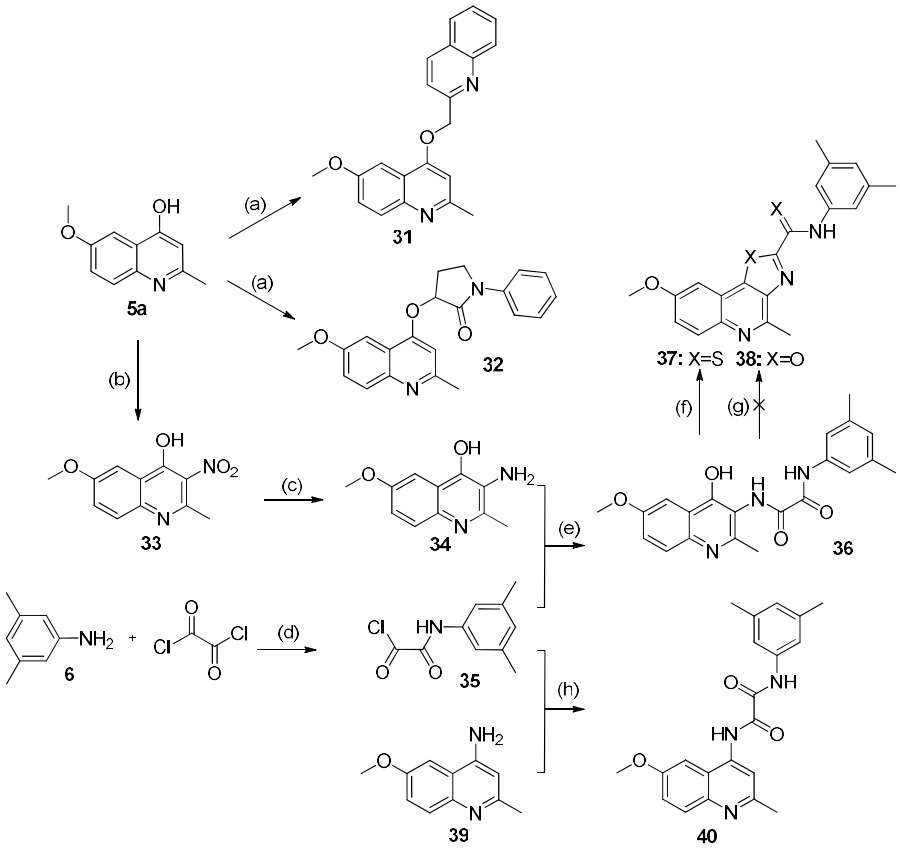
[a]Reagents and conditions: (a) Dowtherm A, H_2SO_4, 240-250 ºC, 35-60 min; (b) 130 °C, 90 min, then Dowtherm A, 250 °C, 1 h; (c) acetic acid, toluene, reflux, 2 h, then Dowtherm A, 240 ºC, 1 h; (d) triethylamine, anhydrous DCM, rt, 2 h; (e) potassium carbonate, anhydrous DMF, rt, 3h - 4d.

Scheme 2. Synthesis of compounds with introduction of substituents on the linker[a]



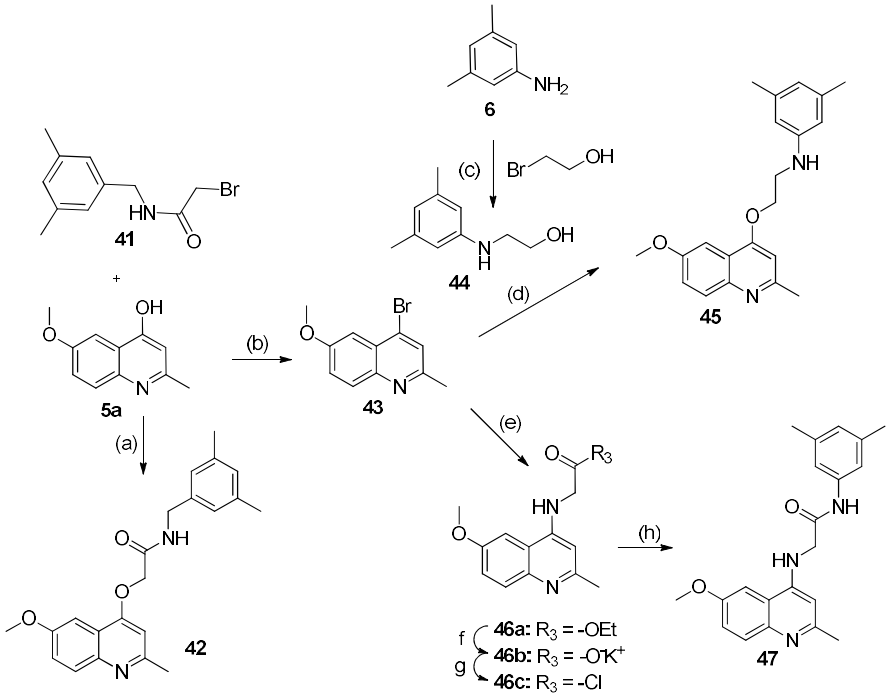
[a]Reaction conditions: (a) triethylamine, anhydrous DCM, rt, 2h-overnight; (b) sodium hydride, (potassium iodide), anhydrous DMF, rt, 6-48 h.

Scheme 3. Synthesis of compounds with conformational constraint of the linker[a]



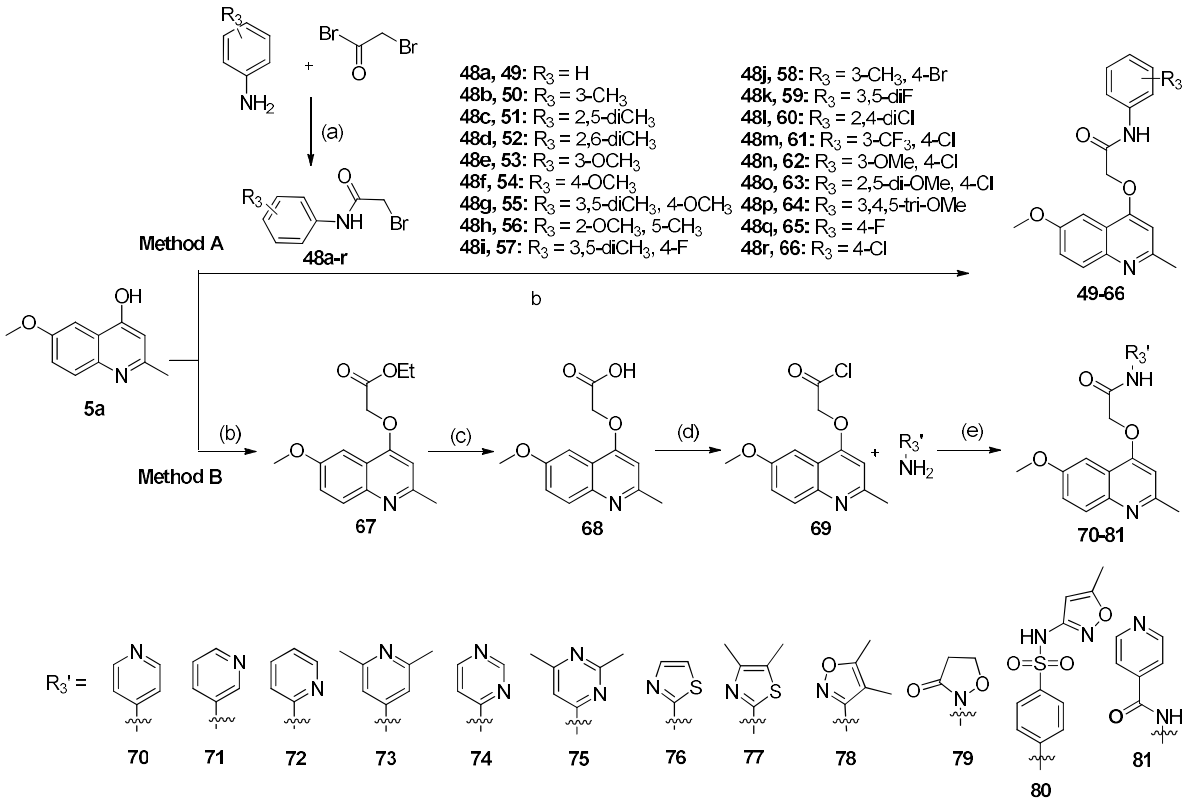
[a]Reaction conditions: (a) 7-(bromomethyl)quinoline or 3-bromo-1-phenylpyrrolidin-2-one,potassium carbonate, anhydrous DMF, rt, 2-72 h; (b) nitric acid, propionic acid, 110 °C, 2 h; (c) Zn, THF/sat. aq. NH_4Cl: 2/1, rt, 1 h; (d) 0 °C, 1 h; (e) anhydrous DCM:DMF (10:1), 0 °C, 1 h; (f) phosphorus pentasulfide, anhydrous pyridine, reflux, overnight; (g) phosphorus pentoxide, anhydrous pyridine, reflux, overnight; (h) sodium hydride, anhydrous DMF, rt, overnight.

Scheme 4. Synthesis of compounds which contain other modifications types[a]



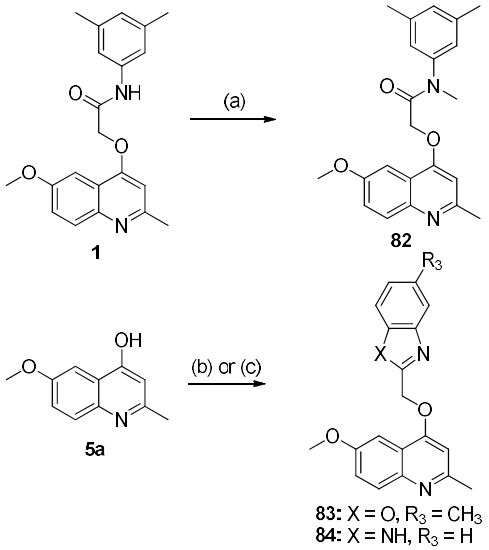
[a] Reaction conditions: (a) potassium carbonate, anhydrous DMF, rt, 26 h; (b) PBr_3, DMF, 50 °C, 4 d; (c) 90 °C, 4 h; (d) CuI, TMEDA, cesium carbonate, anhydrous DMF, 95 °C, 2 d; (e) glycine ethyl ester·HCl, phenol, 120 °C, overnight; (f) sodium hydroxide, MeOH, reflux, 90 min; (g) thionyl chloride, anhydrous DCM, reflux, 2 d; (h) 3,5-dimethylaniline (**6**), anhydrous DCM, rt, 16 h.

Scheme 5. Synthesis of compounds with modifications of the northern aryl fragment[a]



[a] Reaction conditions: (a) triethylamine, anhydrous DCM, rt, 2-48 h; (b) potassium carbonate, anhydrous DMF, rt, 3-96 h; (c) potassium hydroxide, MeOH or EtOH, reflux, 1.5-3.5 h; (d) thionyl chloride, anhydrous DCM, 20 ºC to 40 ºC, 24-48 h; (e) anhydrous DCM, rt to reflux, 18-48 h.

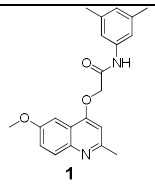
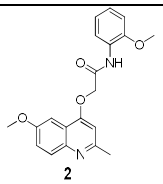
Scheme 6. Synthesis of compounds with amide bond replacements[a]



[a] Reaction conditions: (a) methyl iodide, sodium hydride, anhydrous THF, 0 ºC to 20 ºC, overnight; (b) potassium carbonate, anhydrous DMF, rt, overnight; (c) sodium hydride, anhydrous DMF, rt, overnight.

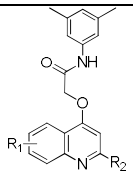
3) Tables

Table 1. Biological profile for the hit compounds **1** and **2**.[a]

Structure Cmpd	 1		 2	
MIC (μM)[b]	1.9		1.4	
Cytotoxicity IC_{50} (μM) [c]	19.95		>100.00	
Permeability (nm/sec) [d]	180		120	
Solubility (μM)[e]	26		38	
Microsomal Fraction Stability [f]	Mouse	Human	Mouse	Human
Cl_{int} (mL $min^{-1}g^{-1}$)	18.9	1.3	>30	5.4
$t_{1/2}$ (min)	<5	>30	<3	16

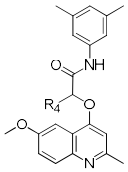
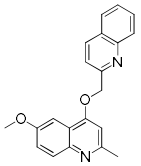
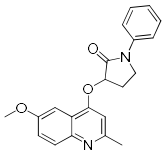
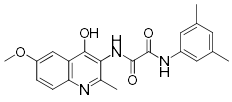
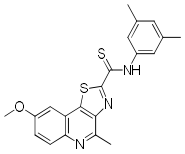
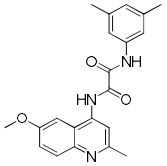
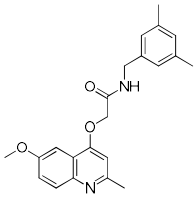
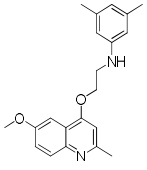
[a]upon re-testing the obtained data were found to differ in some cases from the data published in reference 6; [b]MIC against *Mycobacterium tuberculosis* (H37Rv); [c]$HepG_2$, human caucasian hepatocyte carcinoma; [d]artificial membrane permeability; [e]*in vitro* profiling for kinetic aqueous solubility (CLND, chemiluminescent nitrogen detection); [f]*in vitro* microsomal fraction stability (mouse and human) results: intrinsic clearance (Cl_{int}) and half-life time ($t_{1/2}$) are reported; imidazolam was used as control with Cl_{int} = 27.5 ±0.4 and 6.4 mL $min^{-1}g^{-1}$ in mouse and human, respectively and $t_{1/2}$ = <5 and 9 min in mouse and human, respectively.

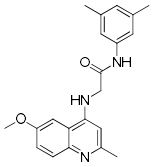
Table 2. Biological profile of the compounds with a modified quinoline part.

Compd			MIC (μM) [a]	Cytotoxicity IC_{50} (μM)[b]	Permeability (nm/sec)[c]	Solubility (μM) [d]	Chrom logD[e]
	R_1	R_2					
1	6-OCH_3	-CH_3	1.9	19.95	180	26	5.64
8	-H	-CH_3	24	>100.00	370	55	5.39
9	6-SCH_3	-CH_3	>125	>100.00	n.d.[f]	3	6.42
10	7-OCH_3	-CH_3	>250	50.12	n.d.[f]	n.d.[f]	5.59
11	6-F	-CH_3	15.6	63.10	310	28	5.79
12	6-Cl	-CH_3	40	>100.00	n.d.[f]	8	6.38
13	6-CH_3	-CH_3	3.9	15.85	520	83	5.95
14	6-CF_3	-CH_3	>250	>100.00	<30	10	6.67
15	6-OCF_3	-CH_3	>250	>100.00	<30	n.d.[f]	6.79
16	6-OEt	-CH_3	>250	>100.00	<30	n.d.[f]	5.98
17	6,7-*di*OCH_3	-CH_3	>250	10.00	605	n.d.[f]	4.84
18	6-OCH_2Ph	-CH_3	>250	>100.00	<10	<1	7.03
19	6-OCH_3	-CF_3	>250	>100.00	<10	15	7.02
20	6-OCF_3	-CF_3	>250	>100.00	<3	34	7.96
21	6-OEt	-CF_3	>250	>100.00	<3	<1	7.55
22	6,7-methylen-dioxy	-CF_3	>250	>100.00	<30	<1	6.73
23	8-CF_3	-CF_3	>250	>100.00	<10	<1	7.73
24	6-OCH_3	-Pr	>250	12.59	n.d.[f]	12	6.44

[a]MIC against *Mycobacterium tuberculosis* (H37Rv); [b]$HepG_2$, human caucasian hepatocyte carcinoma; [c]artificial membrane permeability; [d]*in vitro* profiling for kinetic aqueous solubility (CLND, chemiluminescent nitrogen detection); [e]chromlogD values at pH = 7.4; [f]n.d. = not determined.

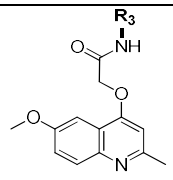
Table 3. Biological profile of the compounds with linker modifications.

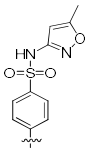
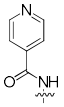
Cmpd	Structure	MIC (μM)[a]	Cytotoxicity IC_{50} (μM)[b]	Permeability (nm/sec)[c]	Solubility (μM)[d]	Chrom logD[e]
27	 $R_4 = CH_3$	125	39.81	320	61	5.76
28	$R_4 = diCH_3$	>250	>100.00	n.d.[f]	20	6.37
29	$R_4 = Et$	>250	79.43	390	123	6.28
30	$R_4 = Ph$	>125	>100.00	<30	2	7.13
31		32	31.62	470	21	5.48
32		125	100.00	550	215	4.99
36		>125	>100.00	n.d.[f]	10	3.76
37		>125	>100.00	<30	35	n.d.[f]
40		>125	>100.00	n.d.[e]	10	6.47
42		31	>100.00	<3	92	5.25
45		32	31.62	190	33	6.93

47		47	6.31	310	204	3.59

[a]MIC against *Mycobacterium tuberculosis* (H37Rv); [b]$HepG_2$, human caucasian hepatocyte carcinoma; [c]artificial membrane permeability; [d]*in vitro* profiling for kinetic aqueous solubility (CLND, chemiluminescent nitrogen detection); [e]chromlogD values at pH = 7.4; [f]n.d. = not determined.

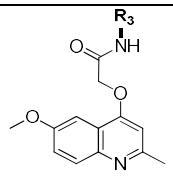
Table 4. Biological profile of the compounds with modifications on the northern aryl.

Cmpd	 R_3	MIC (μM)[a]	Cytotoxicity IC_{50} (μM)[b]	Permeability (nm/sec)[c]	Solubility (μM)[d]	Chrom logD[e]
49	Phenyl	2	39.81	370	63	4.28
50	3-CH_3-phenyl	2	39.81	355	193	4.83
51	2,5-*di*CH_3-phenyl	8	>100.00	810	45.5	5.05
52	2,6-*di*CH_3-phenyl	125	>100.00	630	112	4.55
2	2-OCH_3-phenyl	1.4	>100.00	120	38	4.68
53	3-OCH_3-phenyl	6.4	>100.00	470	35	4.49
54	4-OCH_3-phenyl	0.6	>100.00	420	108	4.22
55	3,5-*di*CH_3, 4-OCH_3-phenyl	1	7.94	320	17	4.99
56	2-OCH_3, 5-CH_3-phenyl	2.5	>100.00	n.d.[f]	9.5	5.65
57	3,5-*di*CH_3, 4-F-phenyl	2	>100.00	210	31.5	5.50
58	3-CH_3, 4-Br-phenyl	3.9	15.85	n.d.[f]	<1	5.95
59	3,5-*di*F-phenyl	3	>100.00	n.d.[f]	13	5.13
60	2,4-*di*Cl-phenyl	2	>100.00	n.d.[f]	20.5	6.40
61	3-CF_3, 4-Cl-phenyl	47	79.43	n.d.[f]	17	6.22
62	3-OMe, 4-Cl-phenyl	>125	>100.00	570	13	5.09
63	2,5-*di*OMe, 4-Cl-phenyl	>125	>100.00	n.d.[f]	3	5.80
64	3,4,5-*tri*OMe-phenyl	62	>100.00	620	34	3.95
65	4-F-phenyl	12	63.10	230	104	4.49
66	4-Cl-phenyl	3	>100.00	n.d.[f]	9	5.15
70	pyridin-2-yl	15.65	>100.00	480	29	3.49
71	pyridin-3-yl	62.5	>100.00	330	32	2.46
72	pyridin-4-yl	>250	>100.00	560	91.5	2.50
73	2,6-*di*CH_3-pyridin-4-yl	>250	12.59	n.d.[f	12	3.01

74	pyrimidin-4-yl	>250	>100.00	525	27.5	2.53
75	2,6-*di*CH_3-pyrimidin-4-yl	>250	>100.00	12	5	3.24
76	thiazol-2-yl	>250	>100.00	1120	54	3.20
77	4,5-*di*CH_3-thiazol-2-yl	62	>100.00	440	31.5	4.24
78		187	>100.00	625	195.5	3.30
79		>125	>100.00	<10	≥166	0.79
80		125	>100.00	15	≥381	2.20
81		62	>100.00	33	≥375	1.48

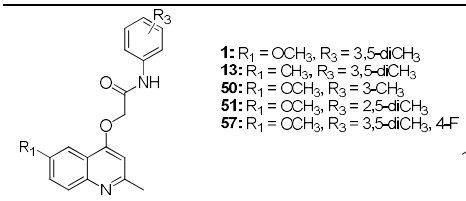
[a]MIC against *Mycobacterium tuberculosis* (H37Rv); [b]$HepG_2$, human caucasian hepatocyte carcinoma; [c]artificial membrane permeability; [d]*in vitro* profiling for kinetic aqueous solubility (CLND, chemiluminescent nitrogen detection); [e]chromlogD values at pH = 7.4; [f]n.d. = not determined.

Table 5. Intracellular IC_{50} and IC_{90} values for selected compounds.

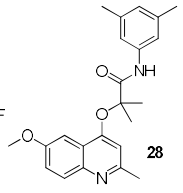
Cmpd	 R_3	Intracellular IC_{50} (μM)[a]	Intracellular IC_{90} (μM)[a]
1	3,5-*di*CH_3-phenyl	0.05	0.2
2	2-OCH_3-phenyl	0.50	1.58
54	4-OCH_3-phenyl	0.03	0.25
56	2-OCH_3, 5-CH_3-phenyl	0.16	0.63
57	3,5-*di*CH_3, 4-F-phenyl	0.08	0.25
59	3,5-*di*F-phenyl	0.40	2.51
60	2,4-*di*Cl-phenyl	0.79	>50
66	4-Cl-phenyl	0.16	0.50

[a]IC_{50} and IC_{90} against infected Human THP-1 macrophages with *Mycobacterium tuberculosis* (H37Rv)

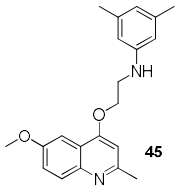
Table 6. Stability of selected compounds in mouse/human microsomal fractions and blood.



1: $R_1 = OCH_3$, $R_3 = 3,5\text{-diCH}_3$
13: $R_1 = CH_3$, $R_3 = 3,5\text{-diCH}_3$
50: $R_1 = OCH_3$, $R_3 = 3\text{-CH}_3$
51: $R_1 = OCH_3$, $R_3 = 2,5\text{-diCH}_3$
57: $R_1 = OCH_3$, $R_3 = 3,5\text{-diCH}_3$, 4-F



28

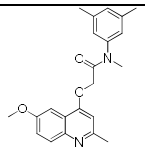
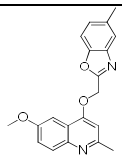
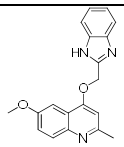


45

Cmpd	Microsomal fraction stability[a] Mouse Clint (mL $min^{-1}g^{-1}$)	$t_{1/2}$ (min)	Human Clint (mL $min^{-1}g^{-1}$)	$t_{1/2}$ (min)	Blood stability[b] $t_{1/2}$ (min)	NaF
1	18.9	<5	1.3	>30	<5	Partially stabilized
13	21.9	<5	1.6	>30	<5	Partially stabilized
28	27.3	<5	2.7	19	96	Stable
45	86.5	<5	6.3	>30	>120	Stable
50	18.1	<5	2.5	23	<5	Partially stabilized
51	50.2	<5	2.3	24	<5	Partially stabilized
57	19.3	<5	1.1	>30	<5	Partially stabilized

[a]*in vitro* microsomal fraction stability (mouse and human) results: intrinsic clearance (Cl_{int}) and half-life time ($t_{1/2}$) are reported; imidazolam was used as control with $Cl_{int} = 27.5 \pm 0.4$ and 6.4 mL $min^{-1}g^{-1}$ in mouse and human, respectively and $t_{1/2} = <5$ and 9 min in mouse and human, respectively; [b]blood stability results: half-life time ($t_{1/2}$) and effect in presence of NaF are reported.

Table 7. Biological profile of compounds **82**, **83** and **84**.

Structure			
Cmpd	**82**	**83**	**84**
MIC (μM) [a]	109.3	5.5	16
Intracellular IC_{90} (μM) [b]	n.d. [c]	5	n.d. [c]
Cytotoxicity IC_{50} (μM) [d]	50.12	>100.00	>100.00
Permeability (nm/sec)[e]	300	86	n.d.[c]
Solubility (μM)[f]	373	1	13
ChromlogD[g]	5.47	6.12	3.59
Microsomal fraction stability[h]			
Cl_{int} [mL $min^{-1}g^{-1}$]	n.d.[g]	46.8 (m), 3.5 (h)	n.d.[c]
$t_{1/2}$ (min)		<5 (m), 15.7 (h)	
Blood stability[i] $t_{1/2}$ (min)	n.d.[g]	>240	n.d.[c]

[a]MIC against Mycobacterium tuberculosis (H37Rv); [b]IC_{90} against infected Human THP-1 macrophages with *Mycobacterium tuberculosis* (H37Rv); [c]n.d. = not determined; [d]$HepG_2$, human caucasian hepatocyte carcinoma; [e]artificial membrane permeability; [f]*in vitro* profiling for kinetic aqueous solubility (CLND, chemiluminescent nitrogen detection); [g]chromlogD values at pH = 7.4; [h]*in vitro* microsomal fraction stability results; clearance (Cl_{int}) and half-life time ($t_{1/2}$) is reported; imidazolam was used as control with Cl_{int} = 27.5 ±0.4 and 6.4 mL $min^{-1}g^{-1}$ in mouse and human, respectively and $t_{1/2}$ = <5 and 9 min in mouse and human, respectively (h) = human, (m) = mouse; [i]blood stability results: half-life time ($t_{1/2}$) is reported.

Table 8. Results of hERG binding of selected compounds.

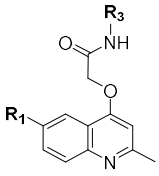
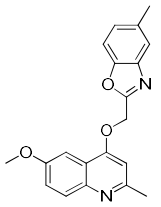
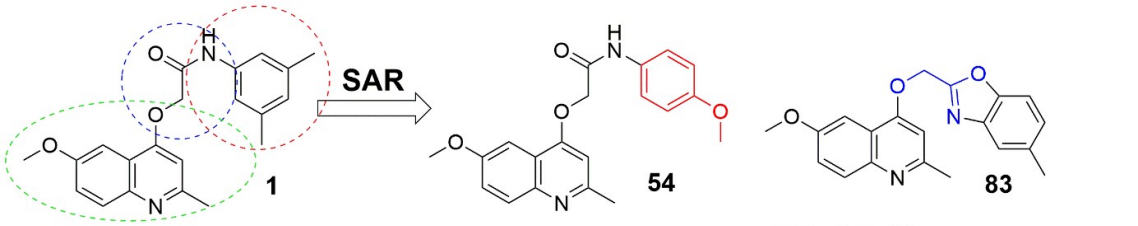
Cmpd	 R_1	R_3	hERG (pIC_{50})
1	6-CH_3O-	3,5-*di*CH_3-phenyl	<4.3
2	6-CH_3O-	2-OCH_3-phenyl	5
13	6-CH_3-	3,5-*di*CH_3-phenyl	<4.3
54	6-CH_3O-	4-OCH_3-phenyl	5.2
56	6-CH_3O-	2-OCH_3, 5-CH_3-phenyl	5.3
57	6-CH_3O-	3,5-*di*CH_3, 4-F-phenyl	<4.3
66	6-CH_3O-	4-Cl-phenyl	5.3
83			<4.3

TABLE OF CONTENTS GRAPHIC



MIC: 1.9 μM
Intracellular IC_{90}: 0.2 μM
Cytotoxicity: 19.95 μM
Blood stability ($t_{1/2}$): <5 min
hERG pIC50: <4.3

MIC: 0.6 μM
Intracellular IC_{90}: 0.25 μM
Cytotoxicity: >100 μM
hERG pIC50: 5.2

MIC: 5.5 μM
Intracellular IC_{90}: 5 μM
Cytotoxicity >100 μM
Blood stability ($t_{1/2}$): >240 min
hERG pIC50: <4.3