Differential characterization using readily accessible NMR experiments of novel N- and O-alkylated quinolin-4-ol, 1,5-naphthyridin-4-ol and quinazolin-4-ol derivatives with antimycobacterial activity

Differential characterization using readily accessible NMR experiments of novel *N*- and *O*-alkylated quinolin-4-ol, 1,5-naphthyridin-4-ol and quinazolin-4-ol derivatives with antimycobacterial activity

Eleni Pitta[a], Olga Balabon[a], Maciej K. Rogacki[a], Jesús Gómez[b], Fraser Cunningham[b], Jurgen Joosens[a], Koen Augustyns[a], Pieter van der Veken[a,*], Robert Bates[b,*]

[a]*Medicinal Chemistry, Department of Pharmaceutical Sciences, University of Antwerp, Universitieitsplein 1, B-2610 Wilrijk, Belgium*

[a]*Diseases of the Developing World (DDW), Tres Cantos Medicines Development Campus (TCMDC), GlaxoSmithKline, Severo Ochoa 2, 28760, Tres Cantos Madrid, Spain*

*Corresponding Authors

For R.B.: phone, +34 650395529; e-mail, robert.h.bates@gsk.com.

For P.V.V: phone, +32 32652708; e-mail, pieter.vanderveken@uantwerpen.be.

Keywords: quinolinol, naphthyridinol, quinazolinol, tuberculosis, alkylation, regioselectivity, NMR

Abstract

During the construction of bioactive molecules, regioselective alkylation of heterocyclic, N/O ambident nucleophiles is a frequently encountered synthetic transformation. In this framework, specific attention is required to unambiguously determine the structures of obtained reaction products. As part of our project on quinoloxyacetamide based antimycobacterial agents, a series of *N*- or *O*- alkylated quinolin-4-ol, 1,5-naphthyridin-4-ol and quinazolin-4-ol derivatives were prepared during the course of which we observed unexpected selectivity issues. After finding that no consistent procedure existed in the literature for assigning regioisomers of this type, we applied three readily accessible NMR experiment types (^{13}C-NMR, HSQC/HMBC and NOE) to resolve any uncertainties regarding the obtained regioisomeric structures. Furthermore, the antimycobacterial activity of all final compounds was evaluated with the best compound (**23**) showing potent antitubercular activity (MIC = 1.25 μM) without cytotoxic effects.

1. Introduction

Despite continuing advances in synthetic organic chemistry, regioselective alkylation of heterocyclic N/O ambident nucleophiles remains a challenging transformation. Many scientists have tried to identify reaction parameters and conditions (e.g., countercation, solvent, temperature, electrophile) that influence the outcome in a predictive manner [1–4]. However, in general the outcome can still vary among different heterocycles and even between nearly identical scaffolds depending on their substitution pattern [5]. Likewise, several theoretical approaches to offer insight in this matter have been proposed. Systematic investigations by Kornblum and Tieckelmann were integrated in Pearson's concept of "Hard and Soft Acids and Bases" (HSAB) which became the most popular approach to rationalize ambident reactivity [6,7]. The HSAB rationale was complemented with the Klopman-Salem theory of charge and orbital control of organic reactions [8,9]. Although these concepts have been widely accepted, they have also been criticized by Gompper and Wagner and by Mayer and co-workers [10,11]. More recently, Marcus analysis has been proposed by Breugst and co-workers as a more successful alternative to rationalize the behavior of ambident nucleophiles [12].

Several of these ambident heteroaromatic scaffold types such as quinolinols, quinazolinols and naphthyridinols have notable value for drug discovery since they often display drug-like properties. As evidence of these properties, they can be found in many drugs such as antibiotics, antimalarials, antidiabetics, anti-inflammatories etc [13–16].

We recently reported the synthesis and antimycobacterial activity of a number of 2-substituted quinolines with a 4-oxyacetyl-derived substituent [17]. Based on the promising results obtained, we decided to explore the Structure-Activity-Relationship (SAR) of this novel class of antimycobacterials in greater detail. Herein, we describe a further expansion of the initial set with compounds lacking the 2-substituent (compounds of type A, Figure 1)

along with replacements of the quinoline system with related bicyclics such as naphthyridine and quinazoline (compounds of types B and C, Figure 1). The rationale for the scaffold replacement approach was to decrease the lipophilicity of the molecules (chromlogD was used as an indicator of lipophilicity) and improve solubility by introducing additional nitrogen atoms [18,19].

During the course of our investigation we were surprised to find that *N*-, *O*- selectivity of our compounds was not always as expected and therefore each scaffold was carefully examined using NMR techniques to fully assign the structure and rationalise the selectivity of the core heterocycles.

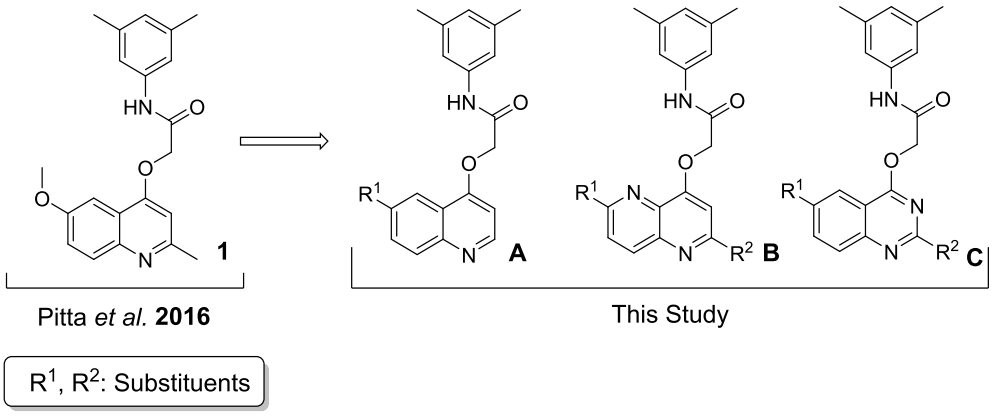


Figure 1. Initial hit compound and design of new compounds.

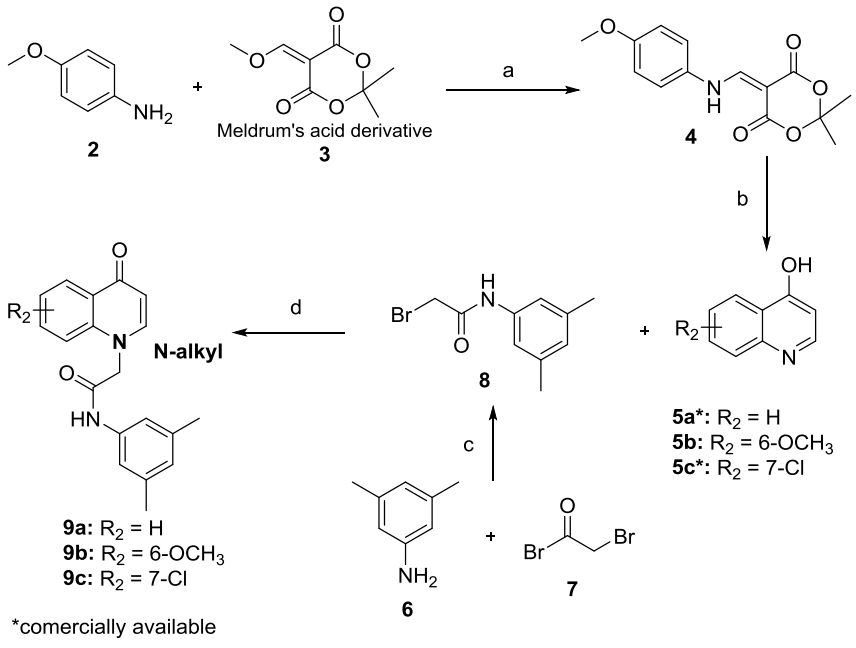
2. Results and discussion

2.1. Chemistry

The general synthetic approach planned for all target compounds of this study consisted of the initial preparation of the required heterocyclic cores equipped with a 4-hydroxyfunction. Subsequently, the *O*-substituent was to be introduced using an alkylation protocol that had proven earlier to regioselectively render the required *O*-alkylated isomers in the 2-substituted quinolone series [17]. The observed *O*-regioselectivity was in agreement with the literature reports of 2-(trifluoro)methyl quinolin-4-ols [20–22]. The alkylation protocol consisted of stirring the heterocyclic scaffold with the required

haloacetyl derivative in *N,N*-dimethylformamide (DMF) at room temperature and using potassium carbonate (K_2CO_3) as a base.

For preparing the heterocyclic core of novel compounds from the quinoline series, the Conrad-Limpach strategy used earlier for the 2-substituted series was considered unsuitable because this methodology performs sluggishly for the production of 2-unsubstituted derivatives [23]. The alternative condensation reaction that was used [24], involved two steps as shown in Scheme 1. First, aniline **2** was added to Meldrum's acid derivative **3** in order to deliver enamine **4**. Afterwards, thermal cyclization of **4** gave the desired 2*H*-quinolin-4-ol (**5b**). The *N*-aryl haloacetamide **8** was obtained in excellent yield by acylation of aniline **6** with bromoacetyl bromide **7** in the presence of triethylamine (Et_3N). Unexpectedly, alkylation of quinolinols **5a-c** with alkyl halide **8** using the previously mentioned alkylation protocol exclusively rendered the *N*-alkylated products (**9a-c**, unambiguous structural identification was carried out as described in the next section).

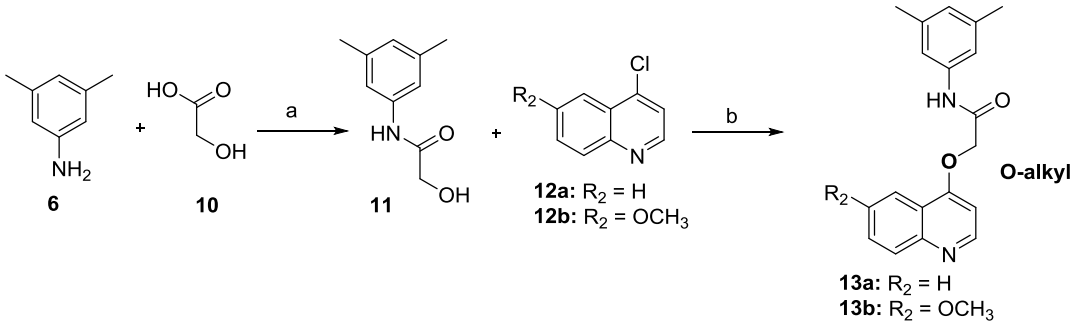


Scheme 1. Synthesis of the *N*-alkylated quinolin-4-ones **9a-c**. Reagents and conditions: (a) $CH(OEt)_3$, EtOH, reflux, 2 h; (b) Dowtherm A, 220 °C, 10 min; (c) Et_3N, anhydrous DCM, rt, 2 h; (d) K_2CO_3, anhydrous DMF, rt, 3 h

A likely explanation for this inverted regioselectivity could be that the 2-methyl substituent exerts sufficient steric hindrance around the quinoline ring nitrogen to render the oxygen

more reactive. In agreement with our findings, Wang and co-workers in 1996 [25] and Oyama *et al.* in 2015 [26] reported exclusively *N*-alkylated products in case of 2*H*-quinolin-4-ols under the same experimental conditions. On the other hand, one can find publications [27,28] where *O*-alkylated products were reported when applying the same protocol, although after careful examination we found that they did not include full, unambiguous characterization data.

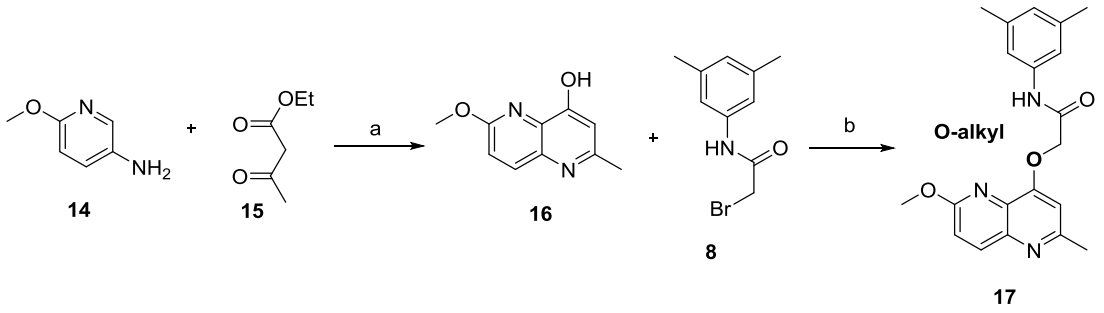
A different synthetic approach was designed to obtain the originally desired *O*-alkylated analogues by employing a S_NAr reaction of the desired alcohols with 4-chloroquinazoline. The synthesis (shown in Scheme 2) began with preparing 2-hydroxyacetamide **11** by heating the corresponding aniline **6** and glycolic acid (**10**) at 130 °C without solvent according to a procedure described by Hung *et al.* [29]. Subsequently, 2-hydroxyacetamide **11** was coupled with 4-chloroquinolines **12a-b**, in an Ullman-type reaction, catalysed by copper iodide/tetramethyl ethylenediamine (CuI/TMEDA), according to Zhou *et al.* [30]. Cesium carbonate (Cs_2CO_3) was used as a base and dimethyl sulfoxide (DMSO) as a solvent. Heating the reaction mixture overnight at 95 °C afforded final products **13a-b**.



Scheme 2. Synthesis of the *O*-alkylated quinolin-4-ols **13a-b**. Reagents and conditions: (a) 130 ºC, 5.5 h; CuI, TMEDA, Cs_2CO_3, anhydrous DMF, 95 ºC, overnight

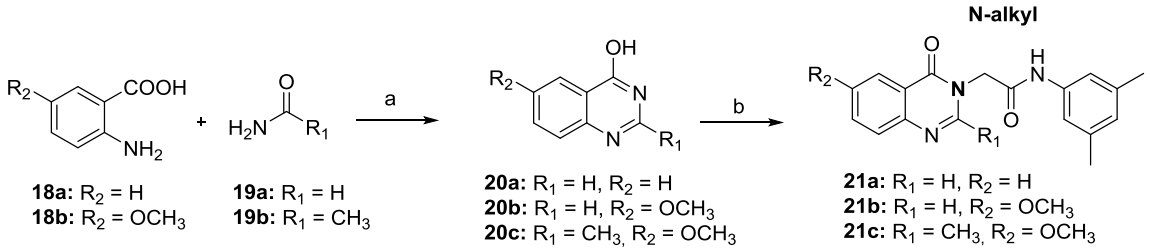
To obtain 6-methoxy-2-methyl-1,5-naphthyridin-4-ol (**16**), a classical Conrad-Limpach protocol [31,32] could be employed as shown is Scheme 3. Thus, 6-methoxypyridin-3-amine (**14**) and ethyl acetoacetate (**15**) were heated at 130 ºC for 3 h, followed by addition of Dowtherm A and heating at 240-250 ºC for 1 h. Subsequently, intermediate **16** was subjected to the selected alkylation protocol with alkyl bromide **8** to obtain the desired *O*-

alkylated analogue **17** (Scheme 3). As previously hypothesized, the presence of a substituent *ortho* to the ring-nitrogen likely accounts for the observed regioselectivity.



Scheme 3. Synthesis of the *O*-alkylated 1,5-naphthyrid-4-ol **17**. Reagents and conditions: (a) 130 ºC, 3h, then Dowtherm A, 250 ºC, 1 h; (b) K_2CO_3, anhydrous DMF, rt, 18 h

For the ring synthesis of 4-hydroxy quinazolines **20a-c**, a Niementowski protocol [33] was employed as shown in Scheme 4. The appropriate anthranilic acids (**18a-b**) and formamide or acetamide (**19a** or **19b**) were heated at 150-165 °C to afford intermediates **20a-c**. Afterwards, their alkylation with alkyl bromide **8** under the standard conditions (K_2CO_3, anhydrous DMF, r.t.) provided the *N(3)*-alkylated products **21a-c** in all cases (2-substituted and 2-unsubstituted). Clearly in this case, steric factors can no longer reasonably explain the observed regioselectivity.



Scheme 4. Synthesis of the *N(3)*-alkylated quinazolin-4-ones **21a-c**. Reagents and conditions: (a) 150-165 ºC, 7-54 h; (b) 8, K_2CO_3, anhydrous DMF, rt, 3h

As shown in Figure 2, 3,4-dihydroquinazoline-4-ones have three potential alkylation sites, i.e., the *N(1)*, *N(3)* and OH groups.

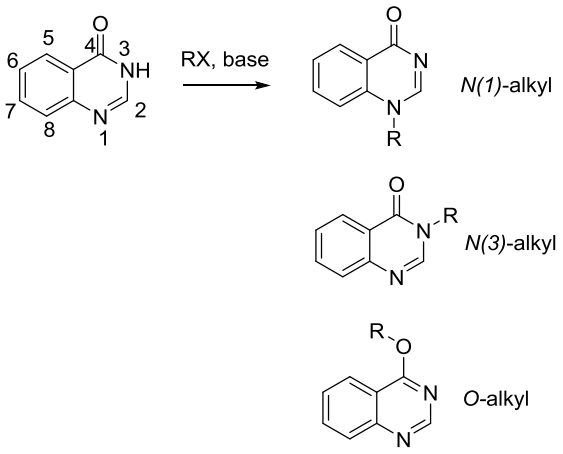
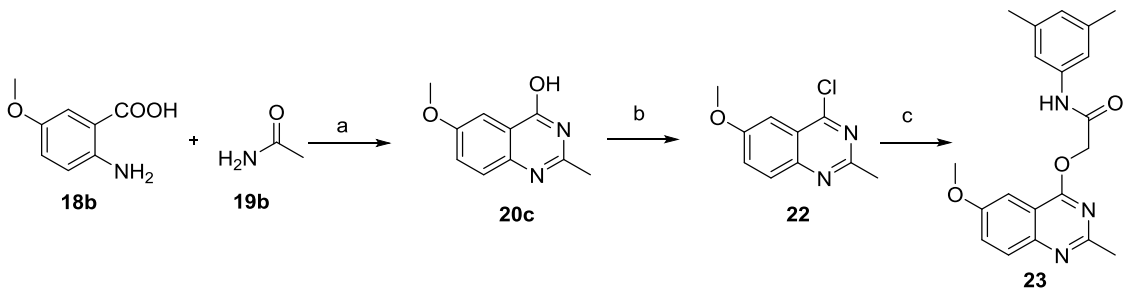


Figure 2. 3,4-dihydroquinazolin-4-one and possible alkylation positions.

It is known that 4-quinazolinones react normally with alkyl halides at *N(3)* of the quinazoline ring and occasionally at the oxygen atom [5]. Therefore, the fact that we obtained *N(3)*-alkylated products (**21a-c**) should not be surprising. As highlighted by Spulak et al. [34], there is likely an artificially high number of *O*-alkylated products in the literature due to mischaracterization or insufficient data which fully, unambiguously characterise the obtained products [35–37].

As shown in Scheme 5, a different synthetic approach was selected to obtain the desired *O*-alkylated quinazoline **23**. First, quinazolin-4-ol **20c** was prepared using a modified Niementowski protocol by means of microwave irradiation [33]. Oxychloro-exchange was achieved by treatment with phosphoryl chloride ($POCl_3$) under reflux for 60 h resulting in 4-chloroquinazoline **22**. Subsequently, alcohol **11** was treated with sodium hydride (NaH) in tetrahydrofuran (THF) for 1 h and 4-chloroquinazoline **22** was added to deliver the desired *O*-alkylated compound **23** [38].



Scheme 5. Synthesis of the *O*-alkylated quinazolin-4-ol **23**. Reagents and conditions: (a) microwave irradiation, 160 ºC, 12 h; (b) $POCl_3$, reflux, 60 h; (c) **11**, NaH, anhydrous THF, rt, 3.5 h

2.2. Structure elucidation

The routine analytical tools that are used for addressing structural identity and purity of compounds in most cases are incapable of unambiguously distinguishing *O*- and *N*-alkylated analogues. Both regioisomers often produce similar ^{1}H NMR spectra and have identical masses. IR spectroscopy has been demonstrated to be helpful to determine the alkylation position in structurally simple molecules [39]. In case of *N*-alkylation, a carbonyl-type group is preserved and its characteristic stretching bands are potentially easy to recognize in a relatively isolated spectral region [40]. However, this is not always true for more complex molecules, especially if other carbonyl groups are present, leading to possible overlap of the key IR signals. Herein, we describe a number of standard 1D and 2D NMR techniques which allowed for the unambiguous characterization of these type of heterocyclic systems. All 1D and 2D NMR experiments described here are pre-programmed on standard, contemporary NMR spectrometers.

^{13}C NMR chemical shifts: The sensitivity of ^{13}C chemical shifts to the carbon's environment has been commonly used for structure determination and differentiation between compounds, as reflected in many examples from the literature. We exploited the fact that the carbon atom of the methylene linker (-CH_2-) directly bonded to an oxygen versus a nitrogen atom will possess a notably different ^{13}C chemical shift as an initial indication of the obtained regioisomer. The desired *O*-analogue will produce a significant and predictable downfield shift of the methylene signal in comparison to the *N*-analogue. The examples provided in Table 1 and 2 demonstrate that typical ^{13}C chemical shifts for *O*-analogues fall into the 50-80 ppm range, while in the case of *N*-analogues, they appear at higher field (typically 40-60 ppm).

Table 1. Comparison of ^{13}C-NMR chemical shifts in DMSO-d_6 of the methylene group (-CH_2-) for the *N*-alkylated quinolin-4-ones **9a-c**, *O*-alkylated quinolin-4-ols **13a-b** and 1,5-naphthyridin-4-ol **17**

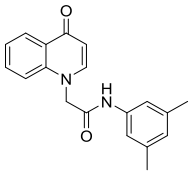
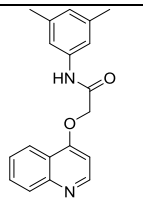
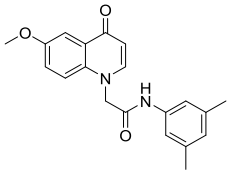
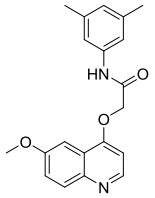
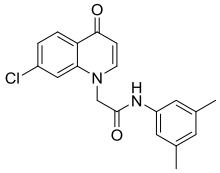
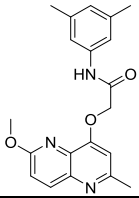
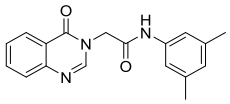
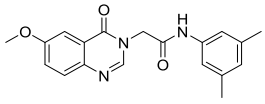
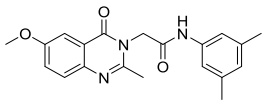
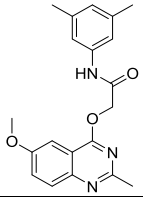
	***N*-alkylated**			***O*-alkylated**		
	Cmpd	**Structure**	**^{13}C shifts (ppm)**	**Cmpd**	**Structure**	**^{13}C shifts (ppm)**
Quinolin-4-ones/ols	**9a**		54.6	**13a**		67.2
	9b		54.7	**13b**		67.1
	9c		54.5			
1,5-Naphthyridin-4-ol				**17**		67.3

Table 2. Comparison of ^{13}C-NMR chemical shifts in DMSO-d_6 of the methylene group (-CH_2-) for the *N*-alkylated quinazolin-4-ones **21a-c** and *O*-alkylated quinazolin-4-ol **23**

	***N*-alkylated**			***O*-alkylated**		
	Cmpd	**Structure**	**^{13}C shifts (ppm)**	**Cmpd**	**Structure**	**^{13}C shifts (ppm)**
Quinazolin-4-ones/ol	**21a**		48.8			
	21b		48.8			
	21c		47.0	**23**		64.5

A faster alternative to ^{13}C NMR acquisition is a highly sensitive 2D NMR Heteronuclear Single Quantum Correlation (HSQC) experiment, which detects correlations between nuclei of two different types separated by one bond [41,42]. HSQC crosspeaks correlate protons

of the methylene linker (-CH_2-) with their attached carbon, facilitating the rapid determination of the ^{13}C chemical shift of interest.

This methodology does not render definitive proof on atom connectivity. However, in a series of structurally related compounds, the complete assignment of some representatives allows a trend in the ^{13}C chemical shifts to be established which can be utilised for preliminary assignment of target compounds.

HSQC & HMBC: ^{1}H-^{13}C HMBC (Heteronuclear Multi-Bond Connectivity) correlates coupled spins across multiple bonds, optimized in a way to detect proton-carbon correlation over two or three bonds [43,44]. Single-bond HSQC and multiple-bond HMBC experiments are complementary and their combination allows full resonance assignment and unambiguous structure determination.

As an example, relevant HMBC correlations of quinolin-4-ols (**9a**, **13a**) and quinazolin-4-ols (**21c**, **23**) are displayed in Figures 3 and 4 respectively, to demonstrate their appropriateness for confident differentiation between *N*- and *O*- analogues. All available NMR data and full resonance assignments are provided in the *Supplementary information*.

For **9a,** consistency with an *N*-linked connectivity was clear due to the presence of H1'/C2 and H1'/C8a crosspeaks, meaning that H1' should be within two or three bond distances from C2 and C8a of the quinolin-4-ol core. This pattern of correlations was impossible for the *O*-alkylated analogue. Instead, the acetyl protons H1' in compound **13a** had a distinctive correlation with C4 of the core, indicating the formation of *O*-alkylated regioisomer (Figure 3).

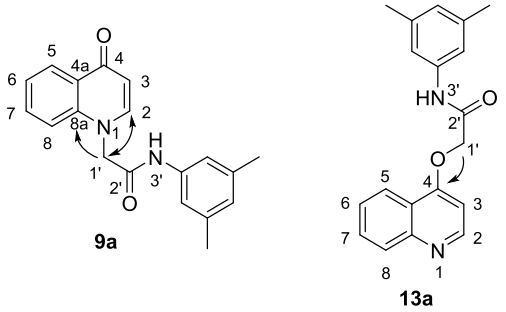


Figure 3. Relevant HMBC connectivities in **9a** and **13a** for *N*- vs *O*- differentiation.

The HMBC spectrum of compound **21c** demonstrated crosspeaks H1'/C2 and H1'/C4, which were consistent with alkylation on the nitrogen atom at position 3. It is worth noting that the correlation of H1' with C4 of the carbonyl group and absence of H11/C8a crosspeak excluded the possibility of alkylation on nitrogen atom at position 1. In contrast, in the regioisomer **23** only one crosspeak was noted, which was proven to represent H1'/C4 correlation, consistent with the *O*-substituted analogue (Figure 4).

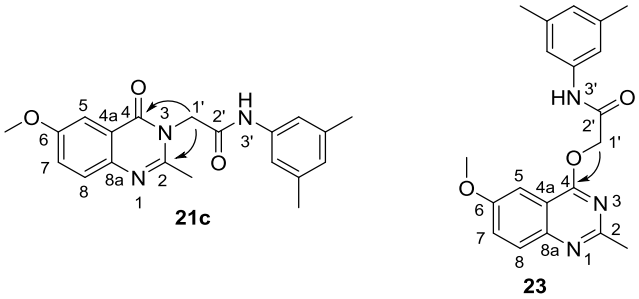


Figure 4. HMBC connectivity in **21c** and **23** for *N*- vs *O*- differentiation.

NOE: Nuclear Overhauser Effect is a transfer of nuclear spin polarization through space, not through chemical bonds. It provides valuable information on the intramolecular distances as the relaxation is strongly dependent on the distance between a pair of nuclei. In our studies, 1D NOE experiments were performed by irradiating the methylene linker to produce an intensive signal, while neighbouring nuclei were identified by weaker signals of the opposite phase (see *Supplementary information*). The peak to be irradiated should possess a relatively isolated signal in ^{1}H NMR in order to produce a precise NOE. 2D NOESY experiments can be used as an alternative to 1D NOE, as it is a pre-programmed NMR experiment which gives connectivity through space for the whole molecule, not only for a selected signal.

In many cases an intrinsic limitation of the NOE approach arises from necessity of a previous full proton and carbon assignments (often by 2D NMR) for correlation of the observed peaks with the compound structure. Nevertheless, when the groups neighbouring to the bond in question are easily assigned by ^{1}H NMR, their chemical shifts

can be used directly as references in 1D NOE. In such case, the NOE approach becomes the fastest unambiguous method to assess the compound structure.

We have used this method to confirm our findings from the previous methods in case of compounds **9a** and **13a**. The presence of H2, H8 and H3' peaks in the NOE spectrum of the quinolin-4-ol derivative **9a** upon irradiation of H1', indicated that H1' is in proximity of those protons. This was consistent with the *N*-alkylated analogue. In contrast, the NOE data of compound **13a** reported H3 and H3' peaks upon irradiation of methylene protons H1', consistent with the *O*-alkylated analogue (Figure 5).

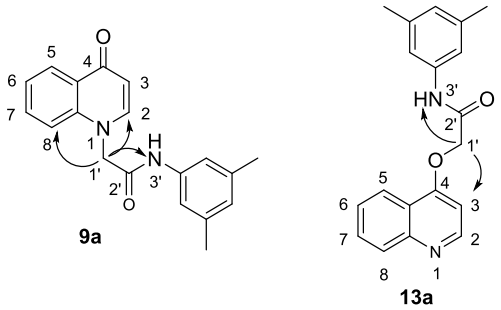


Figure 5. NOE correlations upon irradiation of H1' in **9a** and **13a** for *N*- vs *O*- differentiation.

The quinazolin-4-ols **21c** and **23** are an excellent demonstration of the NOE method potential. The 2-methyl group (2-CH_3) was readily assigned from ^{1}H NMR spectra. In case of quinazolin-4-ol derivative **21c** irradiation of methylene protons H1' resulted in two peaks: H3' (NH of the linker) and 2-CH_3, which gave a direct indication of the linker proximity to the methyl group. This was consistent with alkylation on the nitrogen atom in position 3. It is worth noticing that no H8 peak was observed, which would be expected in case of alkylation on nitrogen atom in position 1. On the other hand, in **23** only a weak H3' signal (corresponding to the acetamide NH) was registered upon irradiation of methylene proton H1', which was in a good agreement with the *O*-alkylated analogue (Figure 6).

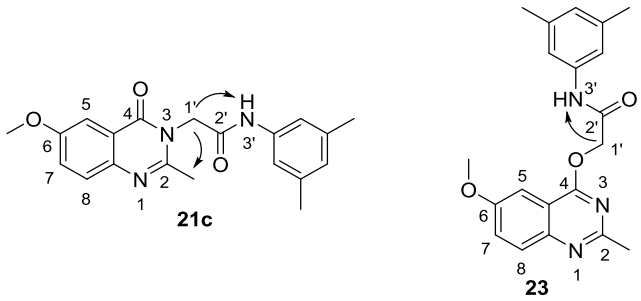


Figure 6. NOE correlations upon irradiation of H1' in **21c** and **23** for *N*- vs *O*- differentiation.

2.3. Biological evaluation

All final compounds were first evaluated for their antimycobacterial activity against *Mycobacterium tuberculosis* H37Rv strain. In parallel, cytotoxicity in $HepG_2$ cells and physicochemical measurements were obtained to further explore the profiles of these molecules. In particular, artificial membrane permeability (AMP), kinetic aqueous solubility (CLND, chemiluminescent nitrogen detection) and hydrophobicity was measured using the chromatography technique to generate ChromlogD (pH 7.4) values. The obtained results are shown in Table 3.

Table 3. Antimycobacterial activity, toxicity and physicochemical properties of final compounds.

	Cmpd	*M.tb.* MIC (μM)[a]	Cytotoxicity pIC_{50}[b]	Permeability (nm/sec)[c]	Solubility CLND (μM) [d]	chromlogD [e]
***O*-alkyl**	**1**	1.9	4.7	180	26	5.64
	13a	>80	<4	460	59	5.03
	13b	10	4.3	280	51	5.30
	17	3.8	<4	510	19	5.57
	23	1.25	<4	370	3	5.59
***N*-alkyl**	**9a**	>250	4.2	450	34	3.54
	9b	>250	<4	190	30	3.72
	9c	>250	4	260	86	4.39
	21a	>250	4.1	600	20	4.29
	21b	>250	<4	n.d. [f]	8	4.50
	21c	250	<4	580	74	4.77

[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

The obtained data demonstrated that removal of both substituents from the quinoline core led to the inactive compound **13a**, while removal of the methyl group at position 2

preserved some antimycobacterial potency (**13b**, MIC = 10 μM). The most interesting compounds, possessing a naphthyridine or quinazoline core (**17, 23**), showed very good antimycobacterial activity with MIC values of 3.8 and 1.25 μM, respectively, comparable with the reference compound **1**. Additionally, these compounds did not exhibit cytotoxicity against $HepG_2$ cells, an improvement over the reference compound **1**. Unfortunately, chromlogD values for compounds **17** and **23** remained at the same level as **1**, while their solubility continued to be very poor (<30 μM). All *N*-alkylated compounds (**9a-c, 21a-c**) completely lost their activity against *Mycobacterium tuberculosis*, suggesting that alkylation at the *O*- is essential for the activity of this series.

3. Conclusion

In continuation of our antimycobacterial research on quinoloxyacetamides, we explored the alkylation of heterocyclic N/O-ambident nucleophilic scaffolds (quinolin-4-ol, naphthyridin-4-ol and quinazolin-4-ol) under the same experimental conditions (K_2CO_3, DMF, rt). Alkylation of 2-unsubstituted quinolinols led solely to *N*-alkylated compounds (**9a-c**), in contrary with the 2-methyl quinolin-4-ols/naphthyridin-4-ol where *O*-alkylated compounds were obtained. All quinazolin-4-ols gave exclusively *N(3)*-alkylated products (**21a-c**) regardless if they were 2-substituted or not. The desired alkoxy- analogues were unambiguously prepared via the conversion of the 4-hydroxy quinazolines/quinolines into the corresponding 4-chloro compounds followed by nucleophilic aromatic substitution by alkoxides. Given the lack of commonly applied methods for the unambiguous assignment of *N/O*-ambident alkylation reaction products in the literature, three NMR methods (^{13}C-NMR chemical shifts, 2D HSQC/HMBC and 1D NOE) were applied for structure determination. Reporting correct structures is of particular importance in medicinal chemistry, and the above mentioned NMR techniques should be utilised for full structural assignment of compounds when tackling similar regioselectivity issues. Furthermore, antimycobacterial activity was evaluated for all final compounds, indicating that *O*-alkylation is essential for

activity, with the most active compound (**23**) exhibiting excellent potency against *M. tuberculosis* H37Rv (MIC = 1.25 μM) without cytotoxic effects. Further research is necessary in order to address lipophilicity and solubility issues of the series.

4. Experimental

4.1. Chemistry

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/UV254.

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 400 DPX 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. For method B, 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.

Where necessary, flash purification was performed on a Biotage ISOLERA One flash system equipped with an internal variable dual-wavelength diode array detector. For normal phase purifications, pre-packed Biotage SNAP and Merck cartridges (10-50 g) 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. HPLC purifications were performed using XBridge columns 19-30 and as eluent acetonitrile in water plus 0.1% ammonium bicarbonate (NH_4HCO_3).

The following section comprises the synthetic procedures and analytical data for all intermediates and final compounds reported in this publication. Complementary data regarding NMR spectra can be found in the *Supporting Information*. Synthetic procedures that were used in the preparation of several products are summarized here as “General Methods”.

General Method A. The appropriate 4-hydroxyquinoline/quinazoline/naphthyridine (1 eq.), 2-bromo-*N*-(3,5-dimethylphenyl)acetamide (1-1.1 eq.) and potassium carbonate (3 eq.) were dissolved in anhydrous DMF (0.09-0.11 M) and stirred for 3-18 h under nitrogen atmosphere at room temperature. Then, the reaction mixture was poured into water and the resulting precipitate was filtered, washed with water and dried. If necessary, it was purified by silica gel column chromatography using *n*-heptane/ethyl acetate as eluent or by recrystallization using ethyl acetate as solvent.

*5-((4-Methoxyphenylamino)methylene)-2,2-dimethyl-1,3-dioxane-4,6-dione (**4**).* A solution of *para*-anisidine (1 g, 8.12 mmol), 2,2-dimethyl-1,3-dioxane-4,6-dione (1.381 g, 9.58 mmol) and triethoxymethane (1.6 ml, 9.58 mmol) in ethanol (10 mL) was heated under reflux for 2 h and then allowed to cool down to room temperature. The resulting precipitate was filtered, washed with cold absolute ethanol and dried under reduced pressure afforded the title compound. Yield 90% (white solid, 2.028 g, 7.31 mmol); TLC, R_f 0.6 (ethyl acetate/*n*-heptane, 50/50); ^{1}H NMR (400 MHz, $CDCl_3$) ppm 11.23 (d, *J*=14.6 Hz, 1 H), 8.55 (d, *J*=14.3 Hz, 1 H), 7.16 - 7.23 (m, 2 H), 6.93 - 6.98 (m, 2 H), 3.84 (s, 3 H), 1.76 (s, 6 H); UPLC-MS (ESI) (A), RT 1.63 min, m/z 278.4 $[M+H]^+$ (>95%).

*6-Methoxyquinolin-4-ol (**5b**).* 5-((4-methoxyphenylamino)methylene)-2,2-dimethyl-1,3-dioxane-4,6-dione (1 g, 3.61 mmol) was added portion wise to Dowtherm A (10 mL) at 220 °C. After bubbling stopped, the mixture was heated for additional 10 min and then allowed to cool down to room temperature. The mixture was poured into *n*-heptane (50 mL), the brown solid was collected by filtration and washed with *n*-heptane (20 mL x 3). It was purified by silica gel column chromatography using *n*-heptane/ethyl acetate and then ethyl acetate/methanol as eluents. Yield 64% (off-white solid, 403 mg, 2.30 mmol); TLC, R_f 0.4 (ethyl acetate/methanol, 80/20); UPLC-MS (ESI) (A), RT 0.56 min, *m/z* 176.4 $[M+H]^+$ (>95%).

*N-(3,5-dimethylphenyl)-2-(4-oxoquinolin-1(4H)-yl)acetamide (**9a**).* The title compound was prepared according to the general method A, using 4-hydroxy-1-azanaphthalene (300 mg, 2.07 mmol), 2-bromo-*N*-(3,5-dimethylphenyl)acetamide (550 mg, 2.27 mmol) and potassium carbonate (857 mg, 6.20 mmol) in anhydrous DMF (24 mL) and the reaction time was 3 h. Yield 84% (white solid, 553 mg, 1.74 mmol); TLC, R_f 0.4 (ethyl acetate/methanol, 90/10); ^{1}H NMR (400 MHz, DMSO-d_6)δ ppm 10.38 (s, 1 H), 8.19 (dd, *J*=8.0, 1.4 Hz, 1 H), 7.98 (d, *J*=7.6 Hz, 1 H), 7.70 (ddd, *J*=8.6, 7.1, 1.5 Hz, 1 H), 7.48 (d, *J*=8.6 Hz, 1 H), 7.37 (t, *J*=7.5 Hz, 1 H), 7.21 (s, 2 H), 6.72 (s, 1 H), 6.10 (d, *J*=7.8 Hz, 1 H), 5.11 (s, 2 H), 2.22 (s, 6 H); ^{13}C NMR (101 MHz, DMSO-d_6) δ ppm 176.5, 165.2, 145.8, 140.5, 138.3, 137.9, 132.1, 126.4, 125.7, 125.2, 123.3, 116.9, 116.0, 108.7, 54.6, 21.0; UPLC-MS (ESI) (A), RT 1.64 min, *m/z* 307.5 $[M+H]^+$ (>95%).

*N-(3,5-dimethylphenyl)-2-(6-methoxy-4-oxoquinolin-1(4H)-yl)acetamide (**9b**).* The title compound was prepared according to the general method A, using 6-methoxyquinolin-4-ol (400 mg, 2,28 mmol), 2-bromo-*N*-(3,5-dimethylphenyl)acetamide (608 mg, 2.51 mmol) and potassium carbonate (947 mg, 6,85 mmol) in anhydrous DMF (25 mL) and the reaction time was 3 h. Yield 51% (white solid, 393 mg, 1.17 mmol; TLC, R_f 0.35 (ethyl acetate/methanol, 90/10); ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 10.35 (s, 1 H), 7.92 (d, *J*=7.6 Hz, 1 H), 7.61 (d, *J*=3.0 Hz, 1 H), 7.45 (d, *J*=9.3 Hz, 1 H), 7.34 (dd, *J*=9.1, 3.0 Hz, 1 H), 7.20 (s, 2 H), 6.72 (s, 1 H), 6.05 (d, *J*=7.6 Hz, 1 H), 5.10 (s, 2 H), 3.84 (s, 3 H), 2.22 (s, 6 H); ^{13}C NMR (101 MHz, DMSO-d_6) δ ppm 175.9, 165.2, 155.5, 144.8, 138.3, 137.9, 135.1, 127.6, 125.2, 121.8, 117.9, 116.9, 107.6, 105.5, 55.4, 54.7, 21.0; UPLC-MS (ESI) (A), RT 1.69 min, *m/z* 337.6 $[M+H]^+$ (>95%).

*2-(7-Chloro-4-oxoquinolin-1(4H)-yl)-N-(3,5-dimethylphenyl)acetamide (**9c**).* The title compound was prepared according to the general method A, using 7-chloro-4-hydroxyquinoline (500 mg, 2.78 mmol), 2-bromo-*N*-(3,5-dimethylphenyl)acetamide (674

mg, 2.78 mmol) and potassium carbonate (1.154 g, 8.35 mmol) in anhydrous DMF (31 mL) and the reaction time was 3 h. Yield 5% (white solid, 45 mg, 0.13 mmol); TLC, R_f 0.63 (ethyl acetate/methanol, 90/10); ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 10.33 (s, 1 H), 8.17 (d, *J*=8.6 Hz, 1 H), 7.98 (d, *J*=7.8 Hz, 1 H), 7.64 (s, 1 H), 7.41 (dd, *J*=8.6, 1.0 Hz, 1 H), 7.21 (s, 2 H), 6.73 (s, 1 H), 6.12 (d, *J*=7.8 Hz, 1 H), 5.12 (s, 2 H), 2.22 (s, 6 H); ^{13}C NMR (101 MHz, DMSO-d_6) δ ppm 175.9, 165.0, 146.2, 141.5, 138.3, 137.9, 137.0, 127.9, 125.2, 125.0, 123.7, 116.9, 115.9, 109.4, 54.5, 21.0; UPLC-MS (ESI) (A), RT 1.81 min, *m/z* 341.5, 343.5 (3:1) $[M+H]^+$ (>95%).

*N-(3,5-dimethylphenyl)-2-hydroxyacetamide (**11**).* A mixture of 3,5-dimethylaniline (1 g, 8.25 mmol) and glycolic acid (628 mg, 8.25 mmol) were stirred at 130 °C for 5.5 h. The mixture was then cooled to room temperature and dissolved in ethyl acetate (50 mL). The organic phase was washed with ammonium chloride (NH_4Cl, 1N, 50 mL), aqueous saturated solution of sodium bicarbonate ($NaHCO_3$, 50 ml) and brine (NaCl, 50 mL). The organic phase was dried under magnesium sulfate ($MgSO_4$) and the solvent was removed under reduced pressure. Yield 81% (brown solid, 1.2 g, 6.70 mmol); ^{1}H NMR (400 MHz, DMSO-d_6) ppm 9.42 (s, 1 H), 7.30 (s, 2 H), 6.70 (s, 1 H), 5.62 (t, *J*=5.9 Hz, 1 H), 3.96 (d, *J*=5.8 Hz, 2 H), 2.23 (s, 6 H); ^{13}C NMR (101 MHz, DMSO-*d*6) □ ppm 170.6, 138.3, 137.6, 124.9, 117.3, 61.8, 21.1; UPLC-MS (ESI) (B) RT 0.99 min, *m/z* 180 $[M+H]^+$ (>95%).

*N-(3,5-dimethylphenyl)-2-(quinolin-4-yloxy)acetamide (**13a**).* 4-Chloroquinoline (100 mg, 0.61 mmol), *N*-(3,5-dimethylphenyl)-2-hydroxyacetamide (110 mg, 0.61 mmol), *N,N,N′,N′*-tetramethylethylenediamine (7.1 mg, 0.06 mmol), copper(I) iodide (11.6 mg, 0.06 mmol) and cesium carbonate (398 mg, 1.22 mmol) dissolved in anhydrous *N,N*-dimethylformamide (DMF) (5 mL) were placed in a high pressure tube under nitrogen atmosphere. The reaction mixture was stirred at 95 °C overnight, cooled down and filtered using a nylon syringe filter. The filtrate was evaporated and the residue was dissolved in

ethyl acetate (50 mL) and washed with brine (30 mL x 3). The organic layer was dried over magnesium sulfate ($MgSO_4$) and evaporated under reduced pressure. The residue was purified by HPLC using acetonitrile/water plus 0.1% ammonium bicarbonate as eluent. Yield 21% (off-white solid, 40 mg, 0.13 mmol); mp 162-163 ºC; TLC, R_f 0.40 (ethyl acetate); ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 10.12 (s, 1 H), 8.73 (d, *J*=5.1 Hz, 1 H), 8.30 (d, *J*=7.6 Hz, 1 H), 7.97 (d, *J*=8.3 Hz, 1 H), 7.77 (ddd, *J*=8.3, 6.9, 1.4 Hz, 1 H), 7.61 (ddd, *J*=8.3, 6.9, 1.4 Hz, 1 H), 7.25 (s, 2 H), 6.96 (d, *J*=5.3 Hz, 1 H), 6.74 (s, 1 H), 5.02 (s, 2 H), 2.24 (s, 6 H); ^{13}C NMR (101 MHz, DMSO-d_6) δ ppm 165.3, 160.2, 151.4, 148.8, 138.1, 137.7, 129.8, 128.5, 125.7, 125.3, 121.9, 120.6, 117.4, 101.8, 67.2, 21.0; UPLC-MS (ESI) (B), RT 1.18 min, m/z 307 $[M+H]^+$ (>95%); HRMS (ES) *m/z* calculated for $C_{19}H_{19}N_2O_2$ $[M + H]^+$: 307.1441; found: 307.1440.

*N-(3,5-dimethylphenyl)-2-((6-methoxyquinolin-4-yl)oxy)acetamide (**13b**).* 4-chloro-6-methoxyquinoline (100 mg, 0.52 mmol), *N,N,N′,N′*-tetramethylethylenediamine (6 mg, 0.05 mmol), copper(I) iodide (9.8 mg, 0.05 mmol) and cesium carbonate (337 mg, 1.03 mmol) were placed in anhydrous *N,N*-dimethylformamide (DMF) (5 mL) in a high pressure tube under nitrogen atmosphere. The reaction mixture was stirred at 95 °C overnight. The reaction mixture was allowed to cool down and it was filtered through a nylon syringe filter. The filtrate was evaporated and the residue was dissolved in ethyl acetate (50 mL) and washed with brine (50 mL x 3). The organic layer was dried over sodium sulfate (Na_2SO_4) and evaporated under reduced pressure. The mixture was purified by silica gel column chromatography using cyclohexane/ethyl acetate as eluent and followed by HPLC using acetonitrile/water plus 0.1% ammonium bicarbonate as eluent. Yield 4% (off-white solid, 7.2 mg, 0.02 mmol); mp 156-157 °C; TLC: R_f 0.48 (ethyl acetate); ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 10.13 (s, 1 H), 8.58 (d, *J*=5.1 Hz, 1 H), 7.88 (d, *J*=9.1 Hz, 1 H), 7.55 (d, *J*=3.0 Hz, 1 H), 7.41 (dd, *J*=9.1, 2.8 Hz, 1 H), 7.25 (s, 2 H), 6.91 (d, *J*=5.3 Hz, 1 H), 6.73 (s, 1 H), 5.02

(s, 2 H), 3.92 (s, 3 H), 2.24 (s, 6 H); ^{13}C NMR (101 MHz, DMSO-d_6) δ ppm 165.4, 159.3, 156.8, 148.7, 144.7, 138.2, 137.8, 130.2, 125.2, 121.9, 121.3, 117.3, 101.9, 99.9, 67.1, 55.5, 21.1; UPLC-MS (ESI) (B), RT 1.18 min, *m/z* 337 $[M+H]^+$ (>95%); HRMS (ESI) *m/z* calculated for $C_{20}H_{21}N_2O_3$ $[M+H]^+$: 337.1547; found: 337.1540.

*6-Methoxy-2-methyl-1,5-naphthyridin-4-ol (**16**).* In a round bottom flask, 5-amino-2-methoxypyridine (1 g, 8.06 mmol) and ethyl acetoacetate (1 mL, 8.06 mmol) were stirred at 130 °C for 3h (to form the corresponding imine). The reaction vessel was equipped with a short distillation apparatus. Dowtherm A (10 mL) was added to the reaction mixture and heated at 250 °C for 1 h. The reaction mixture was cooled down and poured into *n*-heptane (50 mL). The formed sticky precipitate was washed with *n*-heptane (20 mL x 3) and ethyl acetate (20 mL x 3) and the solid was filtered off. It was purified by silica gel column chromatography using ethyl acetate/methanol as eluent. Yield 19% (grey solid, 290 mg, 1.53 mmol); TLC, R_f 0.20 (ethyl acetate/methanol/triethylamine, 90/10/0.01); ^{1}H NMR (400 MHz, DMSO) δ 11.63 (s, 1H), 7.86 (d, *J* = 8.9 Hz, 1H), 7.12 (d, *J* = 8.9 Hz, 1H), 6.05 (s, 1H), 3.91 (s, 3H), 2.32 (s, 3H). UPLC-MS (A) (ESI) RT 0.29 min, *m/z* 191.3 $[M+H]^+$ (>95%).

*N-(3,5-dimethylphenyl)-2-((6-methoxy-2-methyl-1,5-naphthyridin-4-yl)oxy)acetamide (**17**).* The title compound was prepared according to the general method A, using 6-methoxy-2-methyl-1,5-naphthyridin-4-ol (265 mg, 1.39 mmol), 2-bromo-*N*-(3,5- dimethylphenyl) acetamide (337 mg, 1.39 mmol) and potassium carbonate (577 mg, 4.18 mmol) in anhydrous DMF (13 mL) and the reaction time was 18 h. Yield 72% (white solid, 354 mg, 1.01 mmol); TLC, R_f 0.43 (ethyl acetate); ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 10.06 (br. s., 1 H), 8.13 (d, *J*=9.1 Hz, 1 H), 7.24 (s, 2 H), 7.21 (d, *J*=9.1 Hz, 1 H), 7.06 (s, 1 H), 6.73 (s, 1 H), 5.03 (s, 2 H), 4.01 (s, 3 H), 2.55 (s, 3 H), 2.23 (s, 6 H); ^{13}C NMR (101 MHz, DMSO-d_6) δ ppm 165.6, 160.4, 158.6, 157.2, 141.5, 139.6, 138.2, 137.8, 132.2, 125.2, 117.1, 116.1, 106.7, 67.3, 53.4, 24.8, 21.1; UPLC-MS (ESI) (A), RT 1.54 min, *m/z* 352.4 $[M+H]^+$ (>95%).

*Quinazolin-4-ol (**20a**).* A 50 mL round bottom flask was charged with 2-aminobenzoicacid (1 g, 7.29 mmol) and formamide (4 mL, 101 mmol) and heated for 7 h at 150 °C. Then, the reaction mixture was cooled down to room temperature and a white precipitate was formed, filtered, washed with water to remove the excess of formamide and dried. Yield 56% (white solid, 595 mg, 4.07 mmol); TLC, R_f 0.48 (ethyl acetate), R_f 0.83 (Ethyl acetate/Methanol, 90/10); ^{1}H NMR (400 MHz, MeOD-d_4) ppm 8.22 (dd, *J*=8.2, 1.4 Hz, 1 H), 8.09 (s, 1 H), 7.83 (ddd, *J*=8.3, 7.2, 1.4 Hz, 1 H), 7.70 (d, *J*=8.3 Hz, 1 H), 7.55 (ddd, *J*=8.0, 7.1, 1.3 Hz, 1 H); UPLC-MS (ESI) (A), RT 0.46 min, *m/z* 147.3 $[M+H]^+$ (>95%).

*6-Methoxyquinazolin-4-ol (**20b**).* A 50 mL round bottom flask was charged with 2-amino-5-methoxybenzoicacid (500 mg, 2.99 mmol) and formamide (3 mL, 75 mmol) and heated at 150 °C for 7 h. Then, the reaction mixture was cooled down to room temperature and the precipitate was filtered, washed with water to remove the excess of formamide and dried. Yield 65% (light brown solid; 343 mg, 1.95 mmol); TLC, R_f 0.66 (ethyl acetate/methanol, 90/10); ^{1}H NMR (400 MHz, DMSO) δ 12.19 (s, 0.4H), 7.98 (s, 1H), 7.62 (d, *J* = 8.9 Hz, 1H), 7.50 (d, *J* = 3.0 Hz, 1H), 7.41 (dd, *J* = 8.9, 3.0 Hz, 1H), 4.04 (s, 0.6H), 3.86 (s, 3H); ^{13}C NMR (101 MHz, DMSO) δ 157.8, 143.2, 143.1, 143.0, 129.0, 123.8, 123.5, 105.9, 55.6; UPLC-MS (ESI) (A), RT 1.11 min, m/z 177.4 $[M+H]^+$ (>95%).

*6-Methoxy-2-methylquinazolin-4-ol (**20c**).* <u>*Method A:*</u> A 50 mL round bottom flask was charged with 2-amino-5-methoxybenzoicacid (500 mg, 2.99 mmol) and acetamide (2.297 g, 38.9 mmol) and heated at 150 °C for 29 h and at 165 °C for additional 25 h. Afterwards, the reaction mixture was cooled down to room temperature and water was added. A dark brown precipitate was formed which was filtered, washed with water and purified by reverse phase column chromatography using methanol/water as eluent. <u>*Method B:*</u> A microwave vial (20 mL) was charged with 2-amino-5-methoxybenzoic acid (0.7 g, 4.19 mmol) and acetamide (4.95 g, 84 mmol). The reaction mixture was then irradiated for 12 h

at 165 °C. The obtained solid was washed with water (50 ml x 3). It was purified by an amine column using ethyl acetate/ethanol as eluent. Yield 14/35% (A/B), (off-white solid, 77/280 mg (A/B), 0.41/1.47 mmol (A/B)); ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 12.15 (br. s., 1 H), 7.52 (d, *J*=8.8 Hz, 1 H), 7.46 (d, *J*=2.8 Hz, 1 H), 7.37 (dd, *J*=8.8, 3.0 Hz, 1 H), 3.85 (s, 3 H), 2.32 (s, 3 H); ^{13}C NMR (101 MHz, DMSO-d_6) δ ppm 161.5, 157.1, 151.8, 143.4, 128.2, 123.7, 121.3, 105.7, 55.5, 21.2; UPLC-MS (ESI) (B), RT 1.11 min, *m/z* 191.1 $[M+H]^+$ (>95%).

*N-(3,5-dimethylphenyl)-2-(4-oxoquinazolin-3(4H)-yl)acetamide (**21a**).* The title compound was prepared according to the general method A, using quinazolin-4-ol (500 mg, 3.42 mmol), 2-bromo-*N*-(3,5-dimethylphenyl)acetamide (828 mg, 3.42 mmol) and potassium carbonate (1.419 g, 10.26 mmol) in anhydrous DMF (38 mL) and the reaction time was 3 h. Yield 95% (white solid, 1.004 g, 3.27 mmol); TLC, R_f 0.65 (ethyl acetate/methanol, 90/10); ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 10.31 (s, 1 H), 8.36 (s, 1 H), 8.15 (dd, *J*=8.0, 1.1 Hz, 1 H), 7.83 - 7.89 (m, 1 H), 7.69 - 7.74 (m, 1 H), 7.53 - 7.61 (m, 1 H), 7.21 (s, 2 H), 6.71 (s, 1 H), 4.85 (s, 2 H), 2.22 (s, 6 H); ^{13}C NMR (101 MHz, DMSO-d_6) δ ppm 165.3, 160.3, 148.6, 148.1, 138.5, 137.8, 134.5, 127.2, 127.1, 126.0, 125.1, 121.4, 116.8, 48.8, 21.1; UPLC-MS (ESI) (A), RT 1.75 min, *m/z* 308.5 $[M+H]^+$ (>95%).

*N-(3,5-dimethylphenyl)-2-(6-methoxy-4-oxoquinazolin-3(4H)-yl)acetamide (**21b**).* The title compound was prepared according to the general method A, using 6-methoxyquinazolin-4-ol (300 mg, 1.70 mmol), 2-bromo-*N*-(3,5-dimethylphenyl) acetamide (412 mg, 1.70 mmol) and potassium carbonate (706 mg, 5.11 mmol) in anhydrous DMF (19 mL) and the reaction time was 3 h. Yield 93 % (light grey solid, 533 mg, 1.58 mmol); TLC, R_f 0.72 (ethyl acetate); ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 10.29 (s, 1 H), 8.25 (s, 1 H), 7.67 (d, *J*=9.1 Hz, 1 H), 7.51 (d, *J*=2.8 Hz, 1 H), 7.46 (dd, *J*=8.8, 2.8 Hz, 1 H), 7.21 (s, 2 H), 6.71 (s, 1 H), 4.84 (s, 2 H), 3.87 (s, 3 H), 2.22 (s, 6 H); ^{13}C NMR (101 MHz, DMSO-d_6) δ ppm 165.3, 160.1, 158.0,

146.4, 142.6, 138.5, 137.8, 128.9, 125.1, 123.9, 122.3, 116.8, 105.9, 55.7, 48.8, 21.1; UPLC-MS (ESI) (A), RT 1.80 min, *m/z* 338.5 $[M+H]^+$ (>95%).

N-(3,5-dimethylphenyl)-2-(6-methoxy-2-methyl-4-oxoquinazolin-3(4H)-yl)acetamide ***(21c)****.* The title compound was prepared according to the general method A, using 6-methoxy-2-methylquinazolin-4-ol (77 mg, 0.41 mmol), 2-bromo-*N*-(3,5-dimethylphenyl) acetamide (98 mg, 0,41 mmol) and potassium carbonate (168 mg, 1.22 mmol) in anhydrous DMF (4.5 mL) and the reaction time was 3 h. Yield: 84% (white solid, 119 mg, 0.34 mmol); TLC, R_f 0.49 (ethyl acetate); ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 10.30 (s, 1 H), 7.57 (d, *J*=8.8 Hz, 1 H), 7.45 (d, *J*=2.8 Hz, 1 H), 7.41 (dd, *J*=8.6, 2.9 Hz, 1 H), 7.20 (s, 2 H), 6.71 (s, 1 H), 4.93 (s, 2 H), 3.85 (s, 3 H), 2.22 (s, 6 H); ^{13}C NMR (101 MHz, DMSO-d_6) δ ppm 165.4, 161.0, 157.4, 153.0, 141.7, 138.5, 137.8, 128.3, 125.1, 124.1, 120.4, 116.9, 106.0, 55.6, 47.0, 22.7, 21.1; UPLC-MS (ESI) (A), RT 1.81 min, *m/z* 352.5 $[M+H]^+$ (>95%).

4-Chloro-6-methoxy-2-methylquinazoline ***(22)****.* 6-methoxy-2-methylquinazolin-4-ol (233 mg, 1.23 mmol) was suspended in phosphorus oxychloride ($POCl_3$) (10 mL, 108 mmol) and the reaction mixture was stirred under reflux for 2.5 days during which the suspension turned into a reddish brown solution. The mixture was allowed to cool down to room temperature and phosphorus oxychloride was evaporated under reduced pressure. Then, the residue was partioned between aqueous solution of sodium bicarbonate ($NaHCO_3$, 5%, 100 mL) and ethyl acetate (100 mL). The organic layers was washed with aqueous solution of sodium bicarbonate ($NaHCO_3$, 5%, 100 mL) and water (100 mL). Then, the organic layer was dried over sodium sulfate (Na_2SO_4) and evaporated under reduced pressure. The residue was purified by silica gel column chromatography using cyclohexane/ethyl acetate as eluent. Yield 56% (white solid, 142 mg, 0681 mmol); ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 7.92 (d, *J*=9.1 Hz, 1 H), 7.70 (dd, *J*=9.1, 2.8 Hz, 1 H), 7.42 (d, *J*=2.8 Hz, 1 H), 3.96 (s, 3 H), 2.71 (s, 3 H); UPLC-MS (ESI) (B); RT 3.88 min, *m/z* 209 $[M+H]^+$ (>95%).

*N-(3,5-dimethylphenyl)-2-((6-methoxy-2-methylquinazolin-4-yl)oxy)acetamide (**23**). N*-(3,5-dimethylphenyl)-2-hydroxyacetamide (60.1 mg, 0.34 mmol) and sodium hydride (NaH), 60% dispersion in mineral oil (26.8 mg, 0.67 mmol) were suspended in anhydrous tetrahydrofuran (THF) (4 mL) and left stirring at room temperature for 1 h, before 4-chloro-6-methoxy-2-methylquinazoline (70 mg, 0.34 mmol) was added to the mixture. Then, the reaction mixture was stirred at room temperature for 2.5 h. The product was purified by silica gel column chromatography using cyclohexane/ethyl acetate as eluent, followed by HPLC using acetonitrile/water plus 0.1% ammonium bicarbonate as eluent. Yield 14% (white solid, 17 mg, 0.05 mmol); mp 180-181 ºC; TLC, R_f 0.40 (cyclohexane /ethyl acetate, 50/50), 0.39 (DCM/methanol: 90/10); ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 10.10 (s, 1 H), 7.78 (d, *J*=9.1 Hz, 1 H), 7.56 (dd, *J*=9.1, 2.8 Hz, 1 H), 7.48 (d, *J*=2.8 Hz, 1 H), 7.22 (s, 2 H), 6.71 (s, 1 H), 5.19 (s, 2 H), 3.92 (s, 3 H), 2.54 (s, 3 H), 2.22 (s, 6 H); ^{13}C NMR (101 MHz, DMSO-d_6) δ ppm 165.5, 164.6, 160.1, 157.2, 146.7, 138.4, 137.7, 128.4, 125.7, 125.0, 117.1, 114.3, 101.4, 64.5, 55.7, 25.7 (d, *J*=1.5 Hz), 21.0; UPLC-MS (ESI) (B), RT 1.22 min, *m/z* 352 $[M+H]^+$ (>95%); HRMS (ES) *m/z* calcd for $C_{20}H_{22}N_3O_3$ [M+H]: 352.1656; found: 352.1649.

4.2. Biological assays

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. Where 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.

HepG2 cytotoxicity assay. HepG2 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 HepG2 (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.

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 by Ballell *et al.* [45].

Supporting Information

Additional data (^{1}H-NMR, ^{13}C-NMR, HSQC, HMBC, NOE and COSY spectra) for the final compounds.

Acknowledgements

This research was funded by a Marie Curie Intra-European Grant within the 7th European Community Framework Programme (FP7-PEOPLE-2012-ITN) under the European Industrial Doctorates Scheme of the Marie Curie Initial Training Networks actions with grant agreement No 316773 and it is part of the OpenMedChem project. The authors gratefully acknowledge the many scientists from GSK DDW, involved in the research.

References

[1] T.-L. Ho, Hard and Soft Acids and Bases Principle in Organic Chemistry, Elsevier, 2012.

[2] J. Frank, A.R. Katritzky, Tautomeric pyridines. Part XV. Pyridone–hydroxypyridine equilibria in solvents of differing polarity, J. Chem. Soc., Perkin Trans. 2. 584 (1976) 1428–1431. doi:10.1039/P29760001428.

[3] D.L. Comins, G. Jianhua, N- vs. O-alkylation in the mitsunobu reaction of 2-pyridone, Tetrahedron Lett. 35 (1994) 968. doi:10.1016/S0040-4039(00)79939-4.

[4] M.C. Torhan, N.P. Peet, J.D. Williams, A comparison of N- versus O-alkylation of substituted 2-pyridones under Mitsunobu conditions, Tetrahedron Lett. 54 (2013) 3926–3928. doi:10.1016/j.tetlet.2013.05.054.

[5] M. Hori, H. Ohtaka, Effects of a 2-substituent on the Ratio of N-and O-Alkylation of 4(3H)-Quinazolinones, Chem. Pharm. Bull. 41 (1993) 1114–1117. doi:http://doi.org/10.1248/cpb.41.1114.

[6] R.G. Pearson, Hard and Soft Acids and Bases, J. Am. Chem. Soc. 85 (1963) 3533–3539. doi:10.1021/ja00905a001.

[7] S.A. Shevelev, Dual Reactivity of Ambident Anions, Russ. Chem. Rev. 39 (1970) 844–858. doi:10.1070/RC1970v039n10ABEH002047.

[8] G. Klopman, Chemical reactivity and the concept of charge- and frontier-controlled reactions, J. Am. Chem. Soc. 90 (1968) 223–234. doi:10.1021/ja01004a002.

[9] L. Salem, Intermolecular orbital theory of the interaction between conjugated systems. I. General theory, J. Am. Chem. Soc. 90 (1968) 543–552. doi:10.1021/ja01005a001.

[10] R. Gompper, H.-U. Wagner, The Concept of Allopolarization. Effects of Substituents on the Reactions of Ambifunctional Anions, Angew. Chemie Int. Ed. English. 15 (1976) 321–333. doi:10.1002/anie.197603211.

[11] H. Mayr, M. Breugst, A.R. Ofial, Farewell to the HSAB treatment of ambident reactivity, Angew. Chemie - Int. Ed. 50 (2011) 6470–6505. doi:10.1002/anie.201007100.

[12] M. Breugst, H. Zipse, J.P. Guthrie, H. Mayr, Marcus analysis of ambident reactivity, Angew. Chemie - Int. Ed. 49 (2010) 5165–5169. doi:10.1002/anie.201001574.

[13] A. Koul, E. Arnoult, N. Lounis, J. Guillemont, K. Andries, The challenge of new drug discovery for tuberculosis., Nature. 469 (2011) 483–490. doi:10.1038/nature09657.

[14] A. Marella, O.P. Tanwar, R. Saha, M.R. Ali, S. Srivastava, M. Akhter, M. Shaquiquzzaman, M.M. Alam, Quinoline: A versatile heterocyclic., Saudi Pharm. J. SPJ

Off. Publ. Saudi Pharm. Soc. 21 (2013) 1–12. doi:10.1016/j.jsps.2012.03.002.

[15] A.A. Boteva, O.P. Krasnykh, The methods of synthesis, modification, and biological activity of 4-quinolones (review), Chem. Heterocycl. Compd. 45 (2009) 757–785. doi:10.1007/s10593-009-0360-1.

[16] R. Rajput, A.P. Mishra, a Review on Biological Activity of Quinazolinones, Acad. Sci. Int. J. Pharm. Pharm. Sci. 4 (2012) 2–6.

[17] E. Pitta, M.K. Rogacki, O. Balabon, S. Huss, F. Cunningham, E.M. Lopez-Roman, J. Joossens, K. Augustyns, L. Ballell, R.H. Bates, P. Van der Veken, Searching for new leads for Tuberculosis: Design, synthesis and biological evaluation of novel 2-quinolin-4-yloxyacetamides, J. Med. Chem. (2016), *published online* **DOI:** 10.1021/acs.jmedchem.6b00245.

[18] R.J. Young, D.V.S.S. Green, C.N. Luscombe, A.P. Hill, Getting physical in drug discovery II: the impact of chromatographic hydrophobicity measurements and aromaticity, Drug Discov. Today. 16 (2011) 822–830. doi:10.1016/j.drudis.2011.06.001.

[19] A.P. Hill, R.J. Young, Getting physical in drug discovery: a contemporary perspective on solubility and hydrophobicity, Drug Discov. Today. 15 (2010) 648–655. doi:10.1016/j.drudis.2010.05.016.

[20] Y. Sawada, H. Kayakiri, Y. Abe, T. Mizutani, N. Inamura, M. Asano, C. Hatori, I. Aramori, T. Oku, H. Tanaka, Discovery of the First Non-Peptide Full Agonists for the Human Bradykinin B 2 Receptor Incorporating 4-(2-Picolyloxy)quinoline and 1-(2-Picolyl)benzimidazole Frameworks, J. Med. Chem. 47 (2004) 2853–2863. doi:10.1021/jm030468n.

[21] E. Morris, S. Glasson, J. Xiang, Method for treating ADAMTS-5-associated disease, US20060004066 A1, 2006.

[22] Y. Kurasawa, K. Yoshida, N. Yamazaki, K. Iwamoto, Y. Hamamoto, E. Kaji, K. Sasaki, Y. Zamami, One-Pot Three-Component Mild Synthesis of 2-Aryl-3-(9-alkylcarbazol-3-yl)thiazolin-4-ones, J. Heterocycl. Chem. 49 (2012) 1458 –1461. doi:10.1002/jhet.

[23] R.H. Reitsema, The Chemistry of 4-Hydroxyquinolines, Chem. Rev. 43 (1948) 43–68. doi:10.1021/cr60134a002.

[24] R. Morgentin, G. Pasquet, P. Boutron, F. Jung, M. Lamorlette, M. Maudet, P. Plé, Strategic studies in the syntheses of novel 6,7-substituted quinolones and 7- or 6-substituted 1,6- and 1,7-naphthyridones, Tetrahedron. 64 (2008) 2772–2782. doi:10.1016/j.tet.2008.01.055.

[25] T.-C. Wang, Y. Chen, C. Tzeng, S. Liou, Y. Chang, C. Teng, Antiplatelet α-Methylidene-γ-butyrolactones: Synthesis and evaluation of quinoline, flavone, and xanthone derivatives, Helv. Chim. Acta. 79 (1996) 1620–1626. doi:10.1002/hlca.19960790612.

[26] T. Oyama, E. Negishi, H. Onigahara, N. Kusano, Y. Miyoshi, M. Mita, M. Nakazono, S. Ohtsuki, A. Ojida, W. Lindner, K. Hamase, Design and synthesis of a novel pre-column derivatization reagent with a 6-methoxy-4-quinolone moiety for fluorescence and tandem mass spectrometric detection and its application to chiral amino acid analysis, J. Pharm. Biomed. Anal. 116 (2015) 71–79. doi:10.1016/j.jpba.2015.05.039.

[27] S.K. Maurya, D.R. Gollapalli, S. Kirubakaran, M. Zhang, C.R. Johnson, N.N. Benjamin, L. Hedstrom, G.D. Cuny, Triazole inhibitors of Cryptosporidium parvum Inosine 5'-Monophosphate Dehydrogenase, J. Med. Chem. 52 (2009) 4623–4630. doi:10.1021/jm900410u.

[28] W. Zhang, Z. Li, M. Zhou, F. Wu, X. Hou, H. Luo, H. Liu, X. Han, G. Yan, Z. Ding, R. Li, Synthesis and biological evaluation of 4-(1,2,3-triazol-1-yl)coumarin derivatives as potential antitumor agents, Bioorg. Med. Chem. Lett. 24 (2014) 799–807.

doi:10.1016/j.bmcl.2013.12.095.

[29] J.M. Hung, H.J. Arabshahi, E. Leung, J. Reynisson, D. Barker, Synthesis and cytotoxicity of thieno[2,3-b]pyridine and furo[2,3-b]pyridine derivatives, Eur. J. Med. Chem. 86 (2014) 420–437. doi:10.1016/j.ejmech.2014.09.001.

[30] Q. Zhou, B. Zhang, T. Du, H. Gu, Y. Ye, H. Jiang, R. Chen, Copper-catalyzed highly regioselective 2-aryloxylation of 2,x-dihalopyridines, Tetrahedron. 69 (2013) 327–333. doi:10.1016/j.tet.2012.10.025.

[31] J.-C. Brouet, S. Gu, N.P. Peet, J.D. Williams, A Survey of Solvents for the Conrad-Limpach Synthesis of 4-Hydroxyquinolones., Synth. Commun. 39 (2009) 5193–5196. doi:10.1080/00397910802542044.

[32] V. Kouznetsov, L. Mendez, C. Gomez, Recent Progress in the Synthesis of Quinolines, Curr. Org. Chem. 9 (2005) 141–161. doi:10.2174/1385272053369196.

[33] F.-R. Alexandre, A. Berecibar, T. Besson, Microwave-assisted Niementowski reaction. Back to the roots, Tetrahedron Lett. 43 (2002) 3911–3913. doi:10.1016/S0040-4039(02)00619-6.

[34] M. Špulák, Z. Novák, K. Palát, J. Kuneš, J. Pourová, M. Pour, The unambiguous synthesis and NMR assignment of 4-alkoxy and 3-alkylquinazolines, Tetrahedron. 69 (2013) 1705–1711. doi:10.1016/j.tet.2012.12.031.

[35] S.B. Bodendiek, C. Mahieux, W. Hänsel, H. Wulff, 4-Phenoxybutoxy-substituted heterocycles - A structure-activity relationship study of blockers of the lymphocyte potassium channel Kv1.3, Eur. J. Med. Chem. 44 (2009) 1838–1852. doi:10.1016/j.ejmech.2008.10.033.

[36] B.R. Ullman, T. Aja, N. Chen, J.L. Diaz, X. Gu, J. Herrmann, V.J. Kalish, D.S. Karanewsky, L. Kodandapani, J.J. Krebs, S.D. Linton, S.P. Meduna, K. Nalley, E.D. Robinson, S.P.

Roggo, R.O. Sayers, A. Schmitz, R.J. Ternansky, K.J. Tomaselli, J.C. Wu, Structure-activity relationships within a series of caspase inhibitors. Part 2: Heterocyclic warheads, Bioorganic Med. Chem. Lett. 15 (2005) 3632–3636. doi:10.1016/j.bmcl.2005.05.036.

[37] H. Peng, G. Kumaravel, G. Yao, L. Sha, J. Wang, H. Van Vlijmen, T. Bohnert, C. Huang, C.B. Vu, C.L. Ensinger, H. Chang, T.M. Engber, E.T. Whalley, R.C. Petter, Novel Bicyclic Piperazine Derivatives of Triazolotriazine and Triazolopyrimidines as Highly Potent and Selective Adenosine A 2 A Receptor Antagonists, J. Med. Chem. 47 (2004) 6218–6229. doi:10.1021/jm0494321.

[38] V.G. Gharat, Laxmikant Atmaram, Banerjee, Abhisek, Khairatkar-joshi, Neelima, Kattige, Bicyclic compounds as mPGES-1 inhibitors, US9,006,257B2, 2015.

[39] S.R. Laplante, F. Bilodeau, N. Aubry, J.R. Gillard, J. O'Meara, R. Coulombe, N- versus O-alkylation: Utilizing NMR methods to establish reliable primary structure determinations for drug discovery, Bioorganic Med. Chem. Lett. 23 (2013) 4663–4668. doi:10.1016/j.bmcl.2013.06.007.

[40] R.M. Silverstein, F.X. Webster, D.J. Kiemle, D.L. Bryce, Spectrometric Identification of Organic Compounds, 8th ed., Willey, New York, 2015.

[41] G. Bodenhausen, D.J. Ruben, Natural abundance nitrogen-15 NMR by enhanced heteronuclear spectroscopy, Chem. Phys. Lett. 69 (1980) 185–189. doi:10.1016/0009-2614(80)80041-8.

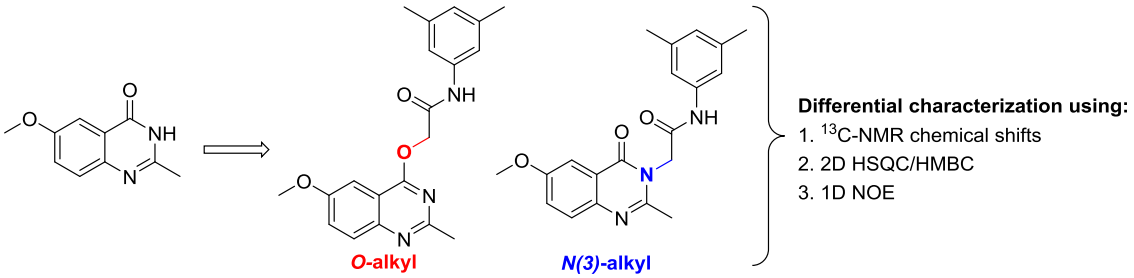
[42] W.F. Reynolds, S. McLean, L.-L. Tay, M. Yu, R.G. Enriquez, D.M. Estwick, K.O. Pascoe, Comparison of13C Resolution and Sensitivity of HSQC and HMQC Sequences and Application of HSQC-Based Sequences to the Total1H and13C Spectral Assignment of Clionasterol, Magn. Reson. Chem. 35 (1997) 455–462. doi:10.1002/(SICI)1097-458X(199707)35:7<455::AID-OMR116>3.0.CO;2-6.

[43] A. Bax, M.F. Summers, 1H and 13C Assignments from Sensitivity-Enhanced Detection of Heteronuclear Multiple-Bond Connectivity by 2D Multiple Quantum NMR, J. Am. Chem. Soc. 108 (1986) 2093–2094. doi:10.1021/ja00268a061.

[44] M.F. Summers, L.G. Marzilli, A. Bax, Complete proton and carbon-13 assignments of coenzyme B12 through the use of new two-dimensional NMR experiments, J. Am. Chem. Soc. 108 (1986) 4285–4294. doi:10.1021/ja00275a008.

[45] L.L. Ballell, R.H. Bates, R.J. Young, D. Alvarez-Gomez, E. Alvarez-Ruiz, V. Barroso, D. Blanco, B. Crespo, J. Escribano, R. Gonzalez, S. Lozano, S. Huss, A. Santos-Villarejo, J.J. Martin-Plaza, A. Mendoza, M.J.M.J. Rebollo-Lopez, M. Remuinan-Blanco, J.L.J.L. Lavandera, E. Perez-Herran, F.J. Gamo-Benito, J.F. Garcia-Bustos, D. Barros, J.P. Castro, N. Cammack, R. González, S. Lozano, S. Huss, A. Santos-Villarejo, J.J. Martín-Plaza, A. Mendoza, M.J.M.J. Rebollo-Lopez, M. Remuiñan-Blanco, J.L.J.L. Lavandera, E. Pérez-Herran, F.J. Gamo-Benito, J.F. García-Bustos, D. Barros, J.P. Castro, N. Cammack, Fueling Open-Source Drug Discovery: 177 Small-Molecule Leads against Tuberculosis, ChemMedChem. 8 (2013) 313–321. doi:10.1002/cmdc.201200428.

GRAPHICAL ABSTRACT



Activity *M. tuberculosis:* MIC = 1.25 uM MIC >250 uM