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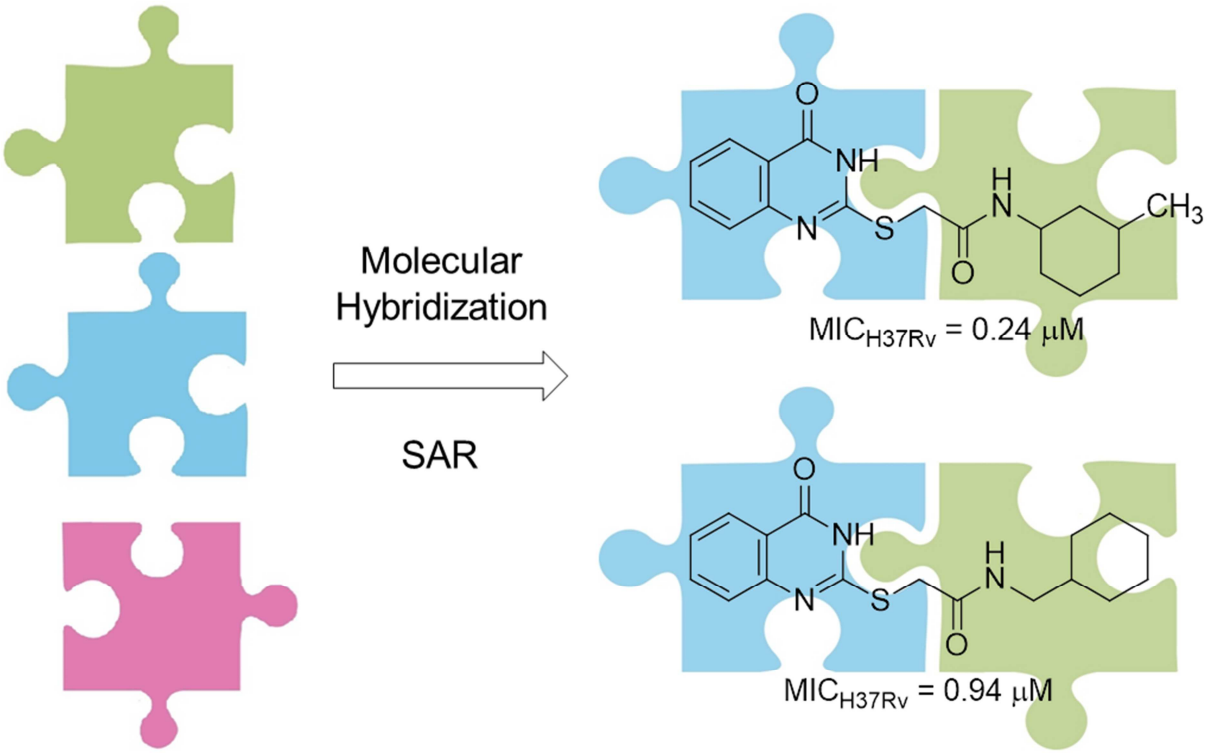

1*H*-Benzo[*d*]imidazoles and 3,4-dihydroquinazolin-4-ones: design, synthesis and antitubercular activity

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Abstract

Using a classical hybridization approach, a series of 1*H*-benzo[*d*]imidazoles and 3,4-dihydroquinazolin-4-ones were synthesized (39 examples) and evaluated as inhibitors of *Mycobacterium tuberculosis* growth. Chemical modification studies yielded potent antitubercular agents with minimum inhibitory concentration (MIC) values as low as 0.24 µM against *M. tuberculosis* H37Rv strain. Further, the synthesized compounds were active against four drug-resistant strains containing different levels of resistance for the first line drugs. These molecules were devoid of apparent toxicity to HepG2, HaCat, and Vero cells with IC_{50s} >30 µM. Viability in mammalian cell cultures was evaluated using MTT and neutral red assays. In addition, some 3,4-dihydroquinazolin-4-ones showed low risk of cardiac toxicity, no signals of neurotoxicity or morphological alteration in zebrafish (*Danio rerio*) toxicity models. 3,4-Dihydroquinazolin-4-ones **9q** and **9w** were considered the lead compounds of these series of molecules with MIC values of 0.24 µM and 0.94 µM against *M. tuberculosis* H37Rv, respectively. Taken together, these data indicate that this class of compounds may furnish candidates for future development of novel anti-TB drugs.

Keywords: *Mycobacterium tuberculosis*, tuberculosis, molecular hybridization, drug-resistant strains, SAR, cardiotoxicity.

1. Introduction

Human tuberculosis (TB) is an infectious disease caused mainly by *Mycobacterium tuberculosis* (Mtb), and has been responsible for the deaths of thousands of people annually [1]. Only in 2015, 9.6 million new cases of the disease and 1.8 million deaths were reported by the World Health Organization (WHO) worldwide [2]. The emergence of multidrug-resistant TB (MDR-TB) and extensively drug-resistant TB (XDR-TB), HIV coinfection, and the elevated number of individuals infected with latent or dormant bacilli have contributed to complicate this scenario [1, 2]. The recommended treatment includes two months of isoniazid (INH), rifampicin (RIF), ethambutol (ETH) and pyrazinamide (PZA), followed by four more months of INH and RIF [3, 4]. Although it has a cure rate of up to 95%, the regime suffers with increasing number of cases of individuals infected with drug-resistant strains [3]. In these cases, the treatment can to be extended and requires the use of second-line drugs that are, in general, more expensive and toxic [5]. Furthermore, the low levels of compliance with treatment, adverse effects, toxicity and impossibility of co-administration with some antiretroviral drugs have limited the use of this therapeutic strategy [6].

Within this context, there is an urgent need to obtain new therapeutic alternatives for tuberculosis treatment; if possible, with innovative mechanisms of action capable of overcoming the drug resistance concern. Although the approval of new drugs such as bedaquiline and delamanid [7] for treatment of drug-resistant TB has brought some hope, the adaptive capacity of Mtb has already led to the emergence of resistant strains for these drugs, evidencing the continued need for new options [8].

As part of our ongoing research we have studied the antimycobacterial activity of 2-quinolin-4-yloxy)acetamides **1** (**Figure 1**) and their derivatives, with some encouraging *in vitro* results [9, 10]. The compounds have been active against resistant and non-resistant Mtb strains and have exhibited selective inhibition of bacillus growth. In addition, lead molecule **1** (R = 4-propyl; **Figure 1**) showed good membrane permeability, reasonable stability in intrinsic clearance analysis, and synergistic *in vitro* effect with first line drug rifampin [10]. Recently, the menaquinol cytochrome c oxidoreductase (bc1 complex) was proposed as a molecular target of this chemical class by using whole-genome sequencing [11], corroborating other findings already described in the literature [12]. It is important to note that although endowed with significant *in vitro* results, initial data have indicated that 2-quinolin-4-yloxy)acetamides present low bioavailability when orally administered to mice (unpublished results). This fact has prompt us to evaluate new derivative structures with possible activity against Mtb. In this sense, SAR studies have shown that acetamide moiety attached to the quinoline nuclei is part of the molecules' pharmacophore, which is prone to be used in molecular hybridization-based approaches (**Figure 1**). In line with this purpose, 1*H*-benzo[*d*]imidazole and 3,4-dihydroquinazolin-4-one were used as molecular scaffolds to evaluate the possibility of obtaining new anti-TB drug candidates by hybridization between the titled heterocycles and acetamide group (**Figure 1**). 1*H*-Benzo[*d*]imidazole and 3,4-dihydroquinazolin-4-one have been obtained as part of the structure of compounds endowed with selective anti-TB activity. Our hypothesis was that the presence of acetamide group attached to these heterocycles could provide novel compounds of optimized activity. Lansoprazole sulfate (**2**) (**Figure 1**), the active metabolite from the drug lanzoprazole, have exhibited significant activity against intracellular and in-broth cultures of Mtb with IC_{50} of 0.59 µM and 0.46 µM, respectively [13]. Interestingly, whole-genome sequencing of resistant

strains to LPZS revealed a unique nucleotide mutation in the b subunit of bc_1 cytochrome [13]. 3,4-Dihydroquinazolin-4-ones **3** have also been described as *in vitro* inhibitors of Mtb growth with Minimal Inhibitory Concentration (MIC) as low as 4.76 µM (**Figure 1**). These compounds were described to inhibit the *Mycobacterium tuberculosis* enoyl acyl carrier protein reductase, a validated molecular target for TB drug development [14].

Therefore, in an attempt to obtain new compounds with activity against drug-susceptible and drug-resistant Mtb strains, new series of hybridized 1*H*-benzo[*d*]imidazoles and 3,4-dihydroquinazolin-4-ones were synthesized and assayed against *M. tuberculosis* H37Rv. First, the basic structural requirements for potency of compounds (SAR) were evaluated. Thereafter, the most active structures against *M. tuberculosis* H37Rv were tested against a panel of clinically isolated drug-resistant strains, and the viability of HepG2, HaCat, and Vero cells after exposure to the compounds was determined. Finally, cardiotoxicity, neurotoxicity and possible morphological alterations by exposure to the compounds using zebrafish (*Danio rerio*) models were also evaluated.

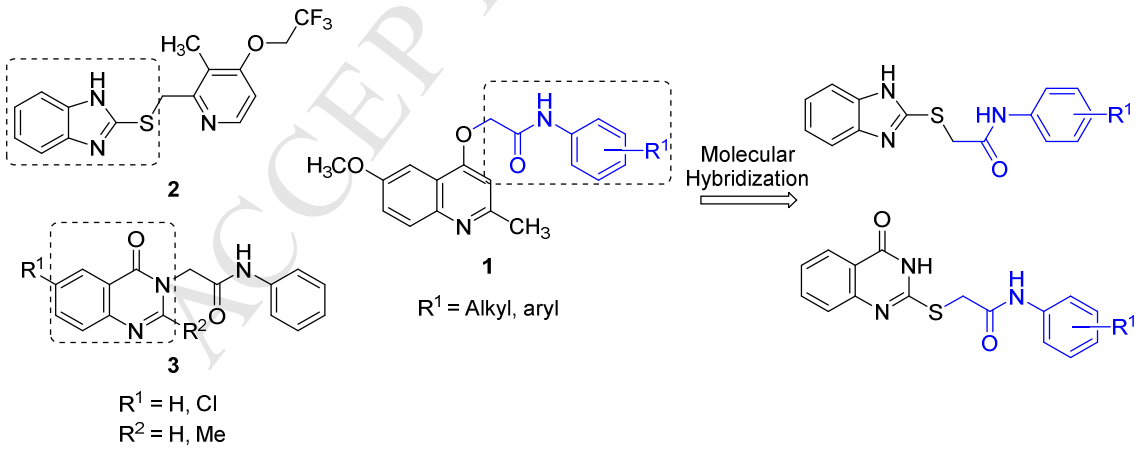
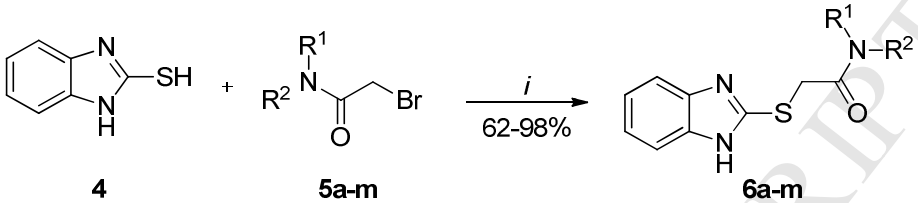


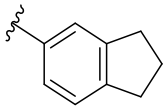
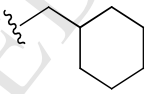
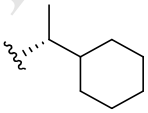
Figure 1. Molecular hybridization using acetamide moieties from 2-(quinolin-4-yloxy)acetamides with 1*H*-benzo[*d*]imidazole and 3,4-dihydroquinazolin-4-one scaffolds.

2. Results and Discussion

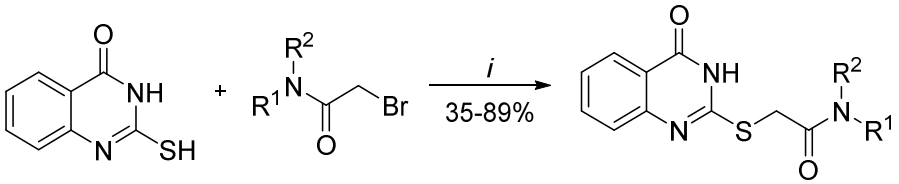
The synthesis of the designed compounds was performed in two synthetic steps. First, bromoacetamides **5** and **8** were obtained in an acylation reaction between primary or secondary amines and bromoacetyl chloride according to already-reported protocols [9, 10]. It is important to mention that the substituents were chosen based on the best antimycobacterial results presented by the 2-(quinolin-4-yloxy)acetamides, including published [9, 10] and unpublished data. The second step was accomplished through a second-order nucleophilic substitution reaction (S_N2). The 1*H*-benzo[*d*]imidazoles **6a**–**m** were obtained from reaction of 2-mercaptobenzoimidazole (**4**) and bromoacetamides **5a**–**m** using potassium carbonate (K_2CO_3) as base, according to a previously described method [15]. The reactants were stirred for 4 h at 40 °C leading to products with 62–98% yields (**Scheme 1**). On the other hand, the synthesis of 3,4-dihydroquinazolin-4-ones **9a**–**z** was accomplished by reaction of 2-mercaptoquinazolin-4(3*H*)-one (**7**) and bromoacetamides **5** and **8** in the presence of diisopropylethylamine (DIPEA) using dimethylformamide (DMF) as solvent. The reaction mixtures were stirred for 16 h at 0–25 °C to afford the desired products **9a**–**z** with 35−89% yields (**Scheme 2**). Spectroscopic and spectrometric data were obtained in agreement with the proposed structures (Supporting Information).



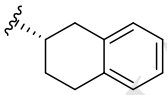
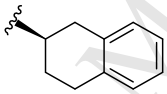
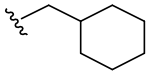
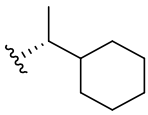
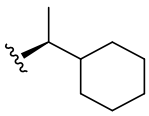
4 **5a-m** **6a-m**

Comp. 5,6	R^1	R^2	Yield (%)
a	2-MeO-C_6H_4	H	83
b	4-pentyl-C_6H_4	H	96
c	4-heptyl-C_6H_4	H	88
d	2-naphthyl	H	88
e		H	92
f	cyclohexyl	H	96
g	2-methylcyclohexyl	H	98
h	3-methylcyclohexyl	H	89
i	4-methylcyclohexyl	H	78
j	*trans*-4-methylcyclohexyl	H	76
k	Bn	H	73
l		H	65
m		H	62

Scheme 1. Reagents and conditions: *i* = K_2CO_3, CH_3CN, 40 °C, 4 h.



7 **5,8** **9a-z**

Comp. 5,8,9	R^1	R^2	Yield (%)
a	Ph	H	86
b	4-MeO-C_6H_4	H	81
c	4-Me-C_6H_4	H	69
d	4-F-C_6H_4	H	68
e	4-Cl-C_6H_4	H	81
f	4-Br-C_6H_4	H	89
g	4-I-C_6H_4	H	86
h	4-O_2N-C_6H_4	H	50
i	4-propyl-C_6H_4	H	75
j	4-pentyl-C_6H_4	H	88
k	2-naphthyl	H	81
l		H	37
m		H	80
n	cyclohexyl	H	65
o	cyclohexyl	Me	52
p	2-methylcyclohexyl	H	55
q	3-methylcyclohexyl	H	35
r	4-methylcyclohexyl	H	45
s	*trans*-4-methylcyclohexyl	H	59
t	cyclopentyl	H	60
u	cycloheptyl	H	76
v	Bn	H	41
w		H	46
x		H	59
y		H	82
z	CH_2-$(CH_2)_3$-CH_2		72

Scheme 2. Reagents and conditions: *i* = DIPEA, DMF, 0–25 °C, 16 h.

The synthesized compounds **6a**–**m** and **9a**–**z** were tested in whole-cell assay against *M. tuberculosis* H37Rv, using the first-line drug isoniazid as reference [16, 17]. The 1*H*-benzo[*d*]imidazoles **6a**–**m** presented only moderate activity against the bacillus under the tested conditions (**Table 1**). Considering the data shown, one can conclude that cycloalkyl substituents exhibited better activity than did aromatic groups. In addition, the extent of the chain using a carbon with cycloalkyl or aromatic substituents did not lead to higher activity. The most active compound **6j** inhibited the Mtb growth with an MIC of 16.5 µM (5 µg/mL). This compound shows the 4-methyl group in a *trans* position relative to the amidic nitrogen attached to the cyclohexane ring. Interestingly, when a mixture of *cis* and *trans* isomers was used the activity of the compound **6i** was nearly 2.5-fold less than 1*H*-benzo[*d*]imidazole **6j**. This finding demonstrates a possible stereochemical preference for increasing antimycobacterial activity of this class of compounds. Changing the methyl group to the 2- or 3-position of the cyclohexane ring in the compounds **6g** and **6h** did not maintain the activity, with MIC values >33 µM (>10 µg/mL). It is noteworthy that the lipophilicities of 1*H*-benzo[*d*]imidazoles **6g**–**j** are the same, with CLogP of 3.81, denoting that structural factors, rather than the physicochemical properties, appear to be linked to the activity of the molecules. The importance of the 4-methyl group attached to the cyclohexane ring can be inferred by the result obtained for compounds **6f–h**, which were ineffective at the highest tested concentration (MIC >31.3 µM; >10 µg/mL). Finally, chain extension using a methylene group in the compounds **6k**–**m** and the presence of aromatic substituents on the molecules **6a**–**e** did not result in better activities when compared to cyclohexyl derivatives (**Table 1**).

Table 1. ClogP values and *in vitro* activities of the 1*H*-benzo[*d*]imidazoles **6a**–**m** against *M. tuberculosis* H37Rv.

Entry	ClogP[a]	MIC H37Rv	
		µM	µg/mL
6a	2.73	>31.9	>10
6b	5.86	>70.7	>25
6c	3.85	>65.5	>25
6d	4.42	>75.0	>25
6e	4.81	>29.6	>10
6f	3.29	>31.3	>10
6g	3.81	>33.0	>10
6h	3.81	>33.0	>10
6i	3.81	41.2	12.5
6j	3.81	16.5	5
6k	3.14	>33.6	>10
6l	3.91	82.4	25
6m	4.22	>31.5	>10
INH	-0.67	2.9	0.3

[a]ClogP calculated by ChemBioDraw Ultra, version 13.0.0.3015. INH, isoniazid.

In the second round of obtaining new drug candidates to treat tuberculosis, the antimycobacterial activity of 3,4-dihydroquinazolin-4-ones **9a**–**z** against *M. tuberculosis* H37Rv strain was determined (**Table 2**). Once more, the cycloalkyl substituents presented the best inhibition activities on bacillus growth, with MICs in the submicromolar range. Substituents containing aromatic groups, which had produced highly potent compounds when present in the 2-(quinolin-4-yloxy)acetamides, led to products with moderate or no activity at the highest concentrations assayed. As expected, ClogP values of the 3,4-dihydroquinazolin-4-ones **9** were reduced when compared to the analogs containing the quinoline scaffold. ClogP values were obtained ranging from 1.38 to 4.63 for the synthesized compounds (**Table 2**). Variation using electron-donating, electron-withdrawing or alkyl groups attached at the 4-position of the phenyl group in molecules

9a–**j** yielded structures with moderate or no activity against Mtb. Increasing the molecular volume by using a 2-naphthyl group in the 3,4-dihydroquinazolin-4-one **9k** also failed to produce satisfactory results (MIC >27.7 µM; >10 µg/mL). Afterwards, the importance of the planarity of the naphthyl group was evaluated by the insertion of the tetrahydronaphthyl groups in the **9l** and **9m** structures. Whereas the stereoisomer of *S* configuration (**9l**) exhibited an MIC >27.4 µM (>10 µg/mL), the *R* isomer (**9m**) was able to inhibit the bacillus growth with an MIC of 17.1 µM (6.3 µg/mL). The apparent stereospecificity of the molecules will be rationalized when subsequent studies reveal the molecular target responsible for the antimycobacterial activity. This moderate MIC value with tetrahydronaphthyl group in **9m** prompted us to investigate its molecular simplification by the removal of the phenyl group and evaluation of the cyclohexyl group. Indeed, 3,4-dihydroquinazolin-4-one **9n** exhibited an MIC of 0.97 µM (0.31 µg/mL), which was approximately 18-fold more potent than tetrahydronaphthyl-containing compound **9m**. Moreover, this compound was almost 3-fold more potent than isoniazid drug (MIC = 2.9 µM; 0.3 µg/mL). These findings corroborated data already described in the literature [18]. Interestingly, the secondary amide seems to be crucial for the activity of the 3,4-dihydroquinazolin-4-ones, as substitution of hydrogen for a methyl abolished completely the antimycobacterial activity of compound **9o** (MIC >75.4 µM; >25 µg/mL). Amidic hydrogen may be involved in hydrogen bond(s) with a putative molecular target, stabilizing the protein-ligand complex, or may be responsible for the correct conformation of the structure through intramolecular stabilization. Following SAR evaluation, the presence of methyl at the 2-position of the cyclohexyl ring did not significantly alter the activity of compounds, as 3,4-dihydroquinazolin-4-one **9p** showed an MIC of 0.94 µM (0.31 µg/mL). By contrast, 3-methyl-, 4-methyl-, and *trans*-4-methylcyclohexyl substituents yielded molecules with an MIC of 0.24

µM (0.08 µg/ml), a potency increase of more than 4-fold compared to the non-substituted **9n**. Reduction in molecular volume by using a cyclopentyl group (**9t**) reduced the antitubercular potency. Cyclopentyl-substituted **9t** exhibited an MIC of 2.07 µM (0.63 µg/mL), which was 2.1-fold less active than cyclohexyl-based compound **9n**. The use of a cycloheptyl substituent again yielded a structure with an MIC in the submicromolar range. 3,4-Dihydroquinazolin-4-one **9u** presented an MIC of 0.48 µM (0.16 µg/mL). In the same manner as for the 1*H*-benzo[*d*]imidazoles series, the 3,4-dihydroquinazolin-4-one side chain was extended with an additional methylene. First, using a benzyl group (**9v**) the MIC obtained was 4.8 µM (1.6 µg/mL), which was more promising than phenyl-substituted **9a**. By contrast, the presence of methylene separating the amidic nitrogen from the cyclohexyl ring did not alter the potency of compound **9w** (MIC = 0.94 µM; 0.31 µg/mL) compared to **9n** (MIC = 0.97 µM; 0.31 µg/mL). Another interesting observation was that the presence of an additional methyl group creating stereogenic centers abolished the activity of the 3,4-dihydroquinazolin-4-ones **9x** and **9y** (MICs >28.9 µM; >10 µg/mL). Finally, piperidinyl-containing compound **9z** was devoid of activity at the evaluated concentration (MIC >33.0 µM; >10 µg/mL), evidencing, once more, the necessity of the secondary amide for the potent activity of the 3,4-dihydroquinazolin-4-ones.

227 **Table 2.** ClogP values and *in vitro* activities of the 3,4-dihydroquinazolin-4-ones **9a**-**z** against *M.*

228 *tuberculosis* H37Rv and clinical resistant isolates.

Entry	ClogP[a]	MIC H37Rv		MIC CDCT10		MIC CDCT16		MIC CDCT27		MIC CDCT28	
		µM	µg/mL	µM	µg/mL	µM	µg/mL	µM	µg/mL	µM	µg/mL
9a	2.02	>32.1	>10	-	-	-	-	-	-	-	-
9b	2.09	>29.3	>10	-	-	-	-	-	-	-	-
9c	2.52	>30.7	>10	-	-	-	-	-	-	-	-
9d	2.42	>30.4	>10	-	-	-	-	-	-	-	-
9e	2.99	28.9	10	-	-	-	-	-	-	-	-
9f	3.14	>25.6	>10	-	-	-	-	-	-	-	-
9g	3.40	>22.9	>10	-	-	-	-	-	-	-	-
9h	2.31	>28.1	>10	-	-	-	-	-	-	-	-
9i	3.57	>28.3	>10	-	-	-	-	-	-	-	-
9j	4.63	26.2	25	-	-	-	-	-	-	-	-
9k	3.19	>27.7	>10	-	-	-	-	-	-	-	-
9l	2.58	>27.4	>10	-	-	-	-	-	-	-	-
9m	2.58	17.1	6.3	-	-	-	-	-	-	-	-
9n	2.07	0.97	0.31	0.50	0.16	0.97	0.31	0.25	0.08	0.5	0.16
9o	2.27	>75.4	>25	-	-	-	-	-	-	-	-
9p	2.58	0.94	0.31	0.95	0.31	3.9	1.3	0.96	0.31	1.9	0.63
9q	2.58	0.24	0.08	0.94	0.31	0.9	0.31	0.24	0.08	0.93	0.31
9r	2.58	0.24	0.08	0.24	0.08	0.48	0.16	0.12	0.04	0.24	0.08
9s	2.58	0.24	0.08	0.12	0.04	0.24	0.08	0.12	0.04	0.12	0.04
9t	1.51	2.07	0.63	-	-	-	-	-	-	-	-
9u	2.62	0.48	0.16	0.48	0.16	0.93	0.31	0.24	0.08	0.48	0.16
9v	1.92	4.8	1.6	-	-	-	-	-	-	-	-
9w	2.68	0.94	0.31	0.93	0.31	0.93	0.31	0.48	0.16	0.93	0.31
9x	2.99	>28.9	>10	-	-	-	-	-	-	-	-
9y	2.99	>28.9	>10	-	-	-	-	-	-	-	-
9z	1.38	>33.0	>10	-	-	-	-	-	-	-	-
INH	-0.67	2.9	0.3	45.6	6.3	>729.2	>100	182.3	25	2.84	0.39

229 [a]ClogP calculated by ChemBioDraw Ultra, version 13.0.0.3015. INH, isoniazid.

230

231 3,4-Dihydroquinazolin-4-ones with MIC values lower than 1 µM (**9n, 9p–s**, **9u** and **9w**) were

232 selected for further evaluation of their inhibitory activity against a panel of clinical isolate strains

233 (**Table 2**). The CDCT10 and CDCT16 strains are described as multidrug-resistant clinical

isolates. CDCT10 presents resistance to drugs such as isoniazid, rifampin and ethambutol, whereas CDCT16 strain exhibits resistance to isoniazid, rifampin, ethambutol and streptomycin. Targeted sequencing from CDCT10 strain has revealed mutations in the *rpoB* and *katG* genes. Also using targeted sequencing, mutations in the *rpoB*, *katG* and *inhA* regulatory region C(-15)T were observed for CDCT16 strain. Additionally, CDCT27 was also evaluated; this drug-resistant strain shows resistance to drugs such as isoniazid and ethambutol. CDCT27 has displayed mutations in the *katG* gene. Finally, clinical isolate CDCT28 does not present resistance to the first-line drugs, and targeted sequencing has also presented mutation in the *rpoB* gene. Notably, the selected compounds exhibited identical activity or were even more potent against CDCT10, CDCT27, and CDCT28 strains than against *M. tuberculosis* H37Rv strain (**Table 2**). By contrast, 3,4-dihydroquinazolin-4-ones **9p**–**r** and **9u** increased MIC values when assayed against CDCT16 strain. Only compounds **9n** and **9w** did not alter MIC values against this strain compared to those presented for *M. tuberculosis* H37Rv. Although these results may suggest the participation of the *inhA* gene product in the activity elicited by the compounds, the mutations in the clinical isolate strains were obtained by target sequencing, and alterations elsewhere in the genome cannot be excluded. Thus, inferences about the mechanism of action of the compounds based on modifications in MIC values for these strains should be made with caution, and further studies are needed to clarify this point. It is noteworthy that this class of compounds has been described to target the membrane-bound type-II NADH dehydrogenase NdhA based on whole-genome sequencing of resistant strains [19].

Cellular viability was carried out after incubation with the test compounds using the neutral red uptake assay [20] and MTT method [10] (**Table 3**). Exposing the HepG2, HaCat, and Vero cell lineages to 3,4-dihydroquinazolin-4-ones **9n**, **9p**–**s**, **9u** and **9w** for 72 h did not significantly

affect the cell viability [21]. The assayed concentration was at least 32 times higher than the MICs of the synthesized compounds against *M. tuberculosis* H37Rv. These results suggest a possible low toxicity of the compounds to mammalian cells and a likely high degree of selectivity for Mtb.

Table 3. Percentage of cell viability of HepG2, HaCat, and Vero cell lineages after exposition to 3,4-dihydroquinazolin-4-ones **9n**, **9p**–**s**, **9u** and **9w**.

Entry	% of cell viability ± SEM[a]					
	HepG2		HaCat		Vero	
	MTT	Neutral red	MTT	Neutral red	MTT	Neutral red
9n	86 ± 6	95 ± 4	100 ± 8	97 ± 3	90 ± 12	92 ± 4
9p	94 ± 5	101 ± 3	100 ± 8	99 ± 12	102 ± 7	95 ± 12
9q	89 ± 6	95 ± 3	94 ± 9	88 ± 7	89 ± 7	95 ± 5
9r	89 ± 3	96 ± 3	95 ± 8	94 ± 3	84 ± 4	95 ± 2
9s	83 ± 7	97 ± 2	97 ± 2	97 ± 4	88 ± 12	93 ± 6
9u	99 ± 3	105 ± 5	100 ± 10	88 ± 6	91 ± 4	94 ± 5
9w	79 ± 3	97 ± 3	98 ± 8	88 ± 3	89 ± 5	97 ± 3

[a]Data are expressed as the mean cell viability ± SEM for each compound, tested at 10 µg/mL. Results were obtained from mean values of triplicates of three independent experiments.

The promising and selective activity showed by the compounds prompted us to investigate other *in vivo* toxicological parameters such as cardiotoxicity, neurotoxicity and morphological alterations, using zebrafish (*Danio rerio*) models [22–24]. In particular, there are possible cardiac side effects under study attributed to the new antitubercular drug bedaquiline [25].

Zebrafish embryos at 2 dpf (days post-fertilization) exposed to single dose of bedaquiline (72 µM) have presented significant alterations of the cardiac functions such as heart rate, stroke volume, cardiac output and fractional shortening [26]. Therefore, cardiac risk assessment for novel compounds should ideally be evaluated in the early drug discovery stages and the method using zebrafish has proved to be a suitable protocol [27]. The 3,4-dihydroquinazolin-4-ones **9n**, **9p**–**s**, **9u** and **9w** were evaluated for the heartbeat rate in viable embryos at 2 and 5 dpf using concentrations of 3, 15 and 20 µM (**Table 4**). Except for compounds **9p** and **9r**, the molecules did not change the heartbeat rates in tested animals at 2 dpf at 3 µM concentration. By contrast, at the highest dose assayed (20 µM) six of the seven structures tested induced changes in the heartbeat rate of animals at 2 dpf. Notably, 3,4-dihydroquinazolin-4-one **9q** did not alter heartbeat rates in any of the concentrations tested. Considering animals at 5 dpf, only compound **9u** was able significantly to alter the rate of heartbeats. This finding indicates an apparent cardiac safety of compounds **9n**, **9p**–**s**, and **9w** in animals at 5 dpf.

Table 4. Cardiac evaluation of 3,4-dihydroquinazolin-4-ones **9n**, **9p**–**s**, **9u** and **9w** in viable embryos at 2 and 5 dpf (days post-fertilization).

Zebrafish heart rate (Mean ± SD/min) – Embryos 2 dpf					
Entry	Control	1% DMSO	3 µM	15 µM	20 µM
9n	141.6 ± 15.8	147.3 ± 16.1	149.5 ± 16.3	155.0 ± 14.8**	155.7 ± 14.1**
9p	141.6 ± 15.8	147.3 ± 16.1	156.3 ± 14.8**	151.7 ± 14.2	153.9 ± 16.1*
9q	146.3 ± 9.3	148.8 ± 9.6	151.2 ± 7.1	151.7 ± 9.4	152.2 ± 8.5
9r	128.7 ± 21.7	134.4 ± 11.7	122.3 ± 9.6$^{\#\#}$	127.2 ± 6.6	146.4 ± 10.9**** / $^{\#\#}$
9s	128.7 ± 21.7	134.4 ± 11.7	135.5 ± 7.3	134.0 ± 6.1	150.2 ± 8.2**** / $^{\#\#\#}$

9u	138.3 ± 10.7	139.9 ± 12.4	139.6 ± 15.0	136.5 ± 16.4	148.4 ± 10.8*
9w	138.3 ± 10.7	139.9 ± 12.4	140.7 ± 12.2	143.2 ± 14.1	152.6 ± 8.8**** / $^{###}$

Zebrafish heart rate (Mean ± SD/min) – Embryos 5 dpf

Entry	Control	1% DMSO	3 µM	15 µM	20 µM
9n	158.2 ± 12.7	157.4 ± 9.7	162.8 ± 10.5	163.0 ± 8.4	158.4 ± 9.9
9p	156.7 ± 16.3	157.4 ± 9.7	162.6 ± 8.0	163.4 ± 7.2	160.6 ± 12.3
9q	155.9 ± 12.5	159.1 ± 9.7	157.2 ±15.3	157.4 ± 9.3	160.9 ± 11.4
9r	148.1 ± 7.4	148.1 ± 11.3	147.7 ± 14.1	150.3 ± 13.5	152.2 ± 10.6
9s	148.1 ± 7.4	148.1 ± 11.3	149.2 ± 13.5	157.4 ± 18.6	153.8 ± 13.2
9u	153.2 ± 8.2	154.5 ± 6.8	160.1 ± 8.4*	160.4 ± 7.7*	153. 8 ± 11.1
9w	153.2 ± 8.2	154.5 ± 6.8	157.8 ± 9.0	158.9 ± 11.9	152.5 ± 11.0

$^{*}P < 0.5$ compared with control group (Tukey post-test). $^{**}P < 0.01$ compared with control group (Tukey post-test). $^{****}P < 0.0001$ compared with control group (Tukey post-test). $^{\#\#}P < 0.01$ compared with the 1% DMSO group (Tukey post-test). $^{\#\#\#}P < 0.001$ compared with the 1% DMSO group.

Furthermore, the distance traveled by the animals was used as a parameter to evaluate neurological impairment after exposure to the compounds. None of the evaluated compounds **9n**, **9p**–**s**, **9u**, and **9w** altered the locomotor activity of the animals (data not shown). Morphological evaluation considered parameters such as body length, ocular distance, and surface area of the eyes. Except for compound **9s** which altered the body length of the larvae at 20 µM concentration, none of the compounds in any of the tested concentrations (3, 15 and 20 µM) shown modifications on the morphological parameters (data not shown). Finally, the larvae

299 survival rate was not altered by exposure to 3,4-dihydroquinazolin-4-ones **9n**, **9p**–**s**, **9u**, and **9w**
300 in the experimental conditions used (data not shown).
301 Aiming at further *in vivo* effectiveness trials in rodents and pharmaceutical formulation studies
302 for oral administration, the stability of 3,4-dihydroquinazolin-4-ones **9n**, **9p**–**s**, **9u**, and **9w** in
303 aqueous medium was determined (Supporting information). The experiments were carried out
304 using 10% DMSO as co-solvent in PBS at 25 °C and 37 °C for up to 48 h. At room temperature
305 (25 °C), only compound **9s** showed chemical instability. After 24 h, only 26% of the 3,4-
306 dihydroquinazolin-4-one **9s** could be recovered. The other products remained stable over 48 h.
307 Elevation of temperature to 37 °C appeared to be crucial for chemical instability of compound
308 **9r**. After 6 h of incubation less than 70% of **9r** was recovered. Once more, the 3,4-
309 dihydroquinazolin-4-one **9s** presented instability under the experimental conditions tested. In
310 contrast, 3,4-dihydroquinazolin-4-ones **9n**, **9p**–**q**, **9u**, and **9w** were stable in aqueous medium for
311 48 h at 37 ° C in the evaluated conditions.
312
313 **3. Conclusion**
314
315 In summary, herein was shown the synthesis of new series of hybridized 1*H*-
316 benzo[*d*]imidazoles and 3,4-dihydroquinazolin-4-ones and their *in vitro* antitubercular activity.
317 The simplicity of the route, easily accessible reactants and reagents, reasonable yields and high
318 purity make the synthetic protocols attractive. In addition, the synthesized compounds showed
319 potent and selective activity against drug-sensitive and drug-resistant Mtb strains, with no
320 apparent cytotoxicity to mammalian cells. The submicromolar antitubercular activity elicited by
321 3,4-dihydroquinazolin-4-ones, coupled with a preliminary outcome of low risk of cardiotoxicity
322 and neurotoxicity, suggests that this class of compounds may furnish candidates for future

development of novel anti-TB drugs. Considering the data described so far, 3,4-dihydroquinazolin-4-ones **9q** and **9w** are considered the lead compounds of this series of synthesized molecules. New structural modifications of the 3,4-dihydroquinazolin-4-ones and pharmaceutical formulation studies are in progress and these data will be reported to the scientific community soon.

4. Experimental Section

4.1 Synthesis and structure: apparatus and analysis

The commercially available reactants and solvents were obtained from commercial suppliers and were used without additional purification. The reactions were monitored by thin-layer chromatography (TLC) with Merck TLC Silica gel 60 F_{254}. The melting points were measured using a Microquímica MQAPF-302 apparatus. ^{1}H and ^{13}C NMR spectra were acquired on a Avance III HD Bruker spectrometer (Pontifical Catholic University of Rio Grande do Sul). Chemical shifts (δ) were expressed in parts per million (ppm) relative to DMSO-d_6, which was used as the solvent, and to TMS, which was used as internal standard. High-resolution mass spectra (HRMS) were obtained for all the compounds on an LTQ Orbitrap Discovery mass spectrometer (Thermo Fisher Scientific, Bremer, Germany). This system combines an LTQ XL linear ion-trap mass spectrometer and an Orbitrap mass analyzer. The analyses were performed through the direct infusion of the sample in MeOH/H_2O (1:1) with 0.1% formic acid (flow rate 10 µL/min) in a positive-ion mode using electrospray ionization (ESI). For elemental composition, calculations used the specific tool included in the Qual Browser module of Xcalibur (Thermo Fisher Scientific, release 2.0.7) software. Compound purity was determined

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

4.2 General procedure for the synthesis of 1*H*-benzo-[*d*]-imidazoles **6a**-**m**

The synthesis of these compounds was adapted from previously described methodology [15]. The appropriately substituted bromoacetamide (1 mmol) was added to a mixture of 1*H*-benzimidazole-2-thiol (1 mmol), potassium carbonate (K_2CO_3, 2 mmol) in acetonitrile (10 mL). The reaction mixture was stirred at 40 °C (oil bath) for 4 h. The precipitated solid was filtered off, washed with chloroform (3 × 20 mL) and dried under reduced pressure to afford the products in good purity.

4.2.1. 2-((1*H*-Benzo[*d*]imidazol-2-yl)thio)-*N*-(2-methoxyphenyl)acetamide (**6a**): Yield 83%; m.p.: 127.5 – 129.3 °C; HPLC 93% (t_R = 15.90); ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 3.67 (s, 3 H), 4.22 (s, 2 H), 6.88 (td, *J* = 8.2, 1.3 Hz, 1 H), 6.97 (td, J=8.2, 1.3 Hz, 1 H), 7.02 (dd, *J* = 8.2, 1.3 Hz), 7.13-7.17 (m, 2 H), 7.48-7.50 (m, 2 H), 8.10 (d, *J* = 8.1, 1 H), 9.94 (s, 1 H). ^{13}C NMR (101 MHz, DMSO-d_6) δ ppm 35.4, 55.6, 111.0, 120.3, 121.5, 124.1, 127.3, 148.7, 150.0, 166.8; FTMS (ESI) m/z 314.0954 $[M+H]^+$; calcd for $C_{16}H_{15}N_3O_2S$: 314.0958.

4.2.2. 2-((1*H*-Benzo[*d*]imidazol-2-yl)thio)-*N*-(4-pentylphenyl)acetamide (**6b**): Yield 96%; m.p.: 103.5 – 104.7 °C; HPLC 94% (t_R = 19.10); ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 0.83 (t, *J* =7.0 Hz, 3 H), 1.25 (m, 4 H), 1.51 (m, 2 H), 2.49 (m, 2 H), 4.21 (s, 2 H), 7.09-7.11 (m, 4 H), 7.43-7.48 (m, 4 H), 10.58 (s, 1H). ^{13}C NMR (101 MHz, DMSO-d_6) δ ppm 13.8, 21.9, 30.6, 30.7,

34.4, 36.1, 109.4, 113.8, 118.9, 121.0, 128.4, 136.6, 137.3, 140.0, 150.5, 166.1; FTMS (ESI) m/z 354.1630 $[M+H]^+$; calcd for $C_{20}H_{23}N_3OS$: 354.1635.

4.2.3. 2-((1*H*-Benzo[*d*]imidazol-2-yl)thio)-*N*-(4-heptylphenyl)acetamide (**6c**): Yield 88%; m.p.: 142.6 – 143.7 °C; HPLC 93% (t_R = 19.68); ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 0.84 (t, *J* = 6.8 Hz, 4 H), 1.23 (d, *J* = 6.1 Hz, 8 H), 1.51 (m, 2 H), 2.50 (m, 2 H), 4.13 (s, 2 H), 7.03-7.06 (m, 2 H), 7.10 (d, *J* = 8.6 Hz, 2 H), 7.42-7.44 (m, 2 H), 7.48 (d, *J* = 8.6 Hz, 2 H). ^{13}C NMR (101 MHz, DMSO-d_6) δ ppm 13.8, 22.0, 28.4, 30.9, 31.2, 34.5, 36.1, 109.4, 113.8, 118.8, 120.2, 122.0, 128.4, 132.6, 136.8, 137.2, 141.3, 151.9, 166.7; FTMS (ESI) m/z 382.1977 $[M+H]^+$; calcd for $C_{22}H_{27}N_3OS$: 382.1948.

4.2.4. 2-((1*H*-Benzo[*d*]imidazol-2-yl)thio)-*N*-(naphthalen-2-yl)acetamide (**6d**): Yield 88%; m.p.: 101.7 – 102.2 °C; HPLC 92% (t_R = 16.61); ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 4.33 (s, 2 H), 7.10-7.14 (m, 2 H), 7.37-7.43 (td, *J* = 7.5, 1.5 Hz, 1 H), 7.44-7.47 (m, 3 H), 7.56-7.59 (dd, *J* = 8.8, 2.2 Hz, 1 H), 7.81 (t, *J* = 8.8, 2 H), 7.86 (d, *J* = 9.0 Hz, 1 H), 8.29 (d, *J* = 1.7 Hz, 1 H), 10.73 (s, 1 H). ^{13}C NMR (101 MHz, DMSO-d_6) δ ppm 36.2, 115.1, 119.6, 121.4, 124.6, 126.4, 127.2, 127.4, 128.4, 129.7, 133.3, 136.4, 149.8, 166.4; FTMS (ESI) m/z 334.1005 $[M+H]^+$; calcd for $C_{19}H_{15}N_3OS$: 334.1009.

4.2.5. 2-((1*H*-Benzo[*d*]imidazol-2-yl)thio)-*N*-(2,3-dihydro-1*H*-inden-5-yl)acetamide (**6e**): Yield 92%; m.p.: 89.7 – 90.6 °C; HPLC 90% (t_R = 16.71); ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 1.97 (q, *J* = 7.4, Hz, 2 H), 2.79 (dt, *J* = 10.7, 7.4 Hz, 4 H), 4.23 (s, 2 H), 7.09-7.13 (m, 3 H), 7.26 (dd, *J* = 8.1, 1.5 Hz, 1 H), 7.42-7.46 (m, 2 H), 7.49 (s, 1 H) 10.40 (s, 1 H). ^{13}C NMR (101 MHz, DMSO-d_6) δ ppm 25.0, 31.7, 32.4, 36.1, 115.2, 117.1, 121.3, 124.1, 137.0, 138.6, 144.1, 149.9, 165.8; FTMS (ESI) m/z 324.1161 $[M+H]^+$; calcd for $C_{19}H_{19}N_3OS$: 324.1165.

4.2.6. 2-((1*H*-Benzo[*d*]imidazol-2-yl)thio)-*N*-cyclohexylacetamide (**6f**): Yield 96%; m.p.: 132.9 – 134.0 °C; HPLC 90% (t_R = 16.59); ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 1.08-1.25 (m, 5 H, 3'-H, 4'-H and 5'H), 1.45-1.48 (m, 1 H, 5'-H), 1.59-1.69 (m, 4 H, 2'-H and 6'-H), 3.54 (m, 1 H, 1'-H), 3.87 (s, 2 H, 8-H), 6.98-7.02 (m, 2 H, 5-H and 6-H), 7.35-7.39 (m, 2 H, 4-H and 7-H), 8.87 (s, 1 H, H-N). ^{13}C NMR (101 MHz, DMSO-d_6) δ ppm 23.9, 25.2, 31.9, 35.1 (9-C), 47.5 (1'-C), 113.8 (4-C and 7-C), 120.1 (5-C and 6-C), 141.5 (3a-C and 7a-C), 152.1 (2-C), 167.3 (10-C); FTMS (ESI) m/z 290.1314 $[M+H]^+$; calcd for $C_{15}H_{19}N_3OS$: 290.1322.

4.2.7. 2-((1*H*-Benzo[*d*]imidazol-2-yl)thio)-*N*-(2-methylcyclohexyl)acetamide (**6g**): Yield 98%; m.p.: 114.8 – 115.5 °C; HPLC 91% (t_R = 16.97 and 17.44); ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 0.74-0.75 (d, *J* = 6.8 Hz, 3 H), 0.79-0.80 (d, *J* = 6.6 Hz, 3 H), 0.84-0.86 (m, 1H), 0.96-1.02 (m, 1 H), 1.12-1.40 (m, 7 H), 1.58 (d, *J* = 11.7 Hz, 2 H), 1.64-1.75 (m, 4 H), 3.18-3.26 (m, 2 H), 3.88-4.03 (m, 4 H), 7.03-7.05 (m, 2 H), 7.06-7.09 (m, 2 H), 7.38-7.42 (m, 4 H), 8.41 (d, *J* = 4.4 Hz, 1 H), 8.88 (d, *J* = 4.4 Hz, 1 H). ^{13}C NMR (101 MHz, DMSO-d_6) δ ppm 18.8, 24.9, 25.2, 28.9, 29.7, 32.6, 33.7, 33.7, 34.9, 35.1, 37.1, 48.8, 53.7, 113.7, 120.3, 120.8, 140.2, 150.7, 166.8, 168.1; FTMS (ESI) m/z 304.1467 $[M+H]^+$; calcd for $C_{16}H_{21}N_3OS$: 304.1478.

4.2.8. 2-((1*H*-Benzo[*d*]imidazol-2-yl)thio)-*N*-(3-methylcyclohexyl)acetamide (**6h**): Yield 89%; m.p.: 128.7 – 129.4 °C; HPLC 90% (t_R = 17.16 and 17.48); ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 0.70-0.98 (m, 5 H), 1.01-1.27 (m, 3 H), 1.37-1.58 (m, 4 H), 1.65 (d, *J* = 9.5 Hz, 2 H), 1.68-

1.77 (m, 2 H), 3.47-3.57 (m, 1 H), 3.98 (s, 2 H), 7.09-7.13 (m, 2 H) 7.41-7.46 (m, 2 H), 8.24 (d, J = 4.4 Hz, 1 H), 8.44 (d, J = 4.4 Hz, 1 H). ^{13}C NMR (101 MHz, DMSO-d_6) δ ppm 20.0, 21.6, 22.2, 24.3, 26.4, 29.8, 31.2, 31.8, 33.4, 33.8, 35.0, 35.3, 38.1, 41.0, 44.5, 48.2, 113.7, 121.2, 150.0, 166.3, 166.9; FTMS (ESI) m/z 304.1467 $[M+H]^+$; calcd for $C_{16}H_{21}N_3OS$: 304.1478.

4.2.9. 2-((1*H*-Benzo[*d*]imidazol-2-yl)thio)-*N*-(4-methylcyclohexyl)acetamide (**6i**): Yield 78%; m.p.: 98.2 – 99.0 °C; HPLC 96% (t_R = 17.12 and 17.41); ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 0.66 (d, J = 6.1 Hz, 3 H_{cis}), 0.84 (d, J = 6.4 Hz, 3 H_{trans}), 0.88-0.97 (m, 2 H_{trans}), 1.08-1.17 (m, 4 $H_{cis/trans}$), 1.26-1.44 (m, 7 $H_{cis/trans}$), 1.53-1.58 (m, 2 H_{cis}), 1.63 (dd, J = 12.5, 2.7 Hz, 2 H_{trans}), 1.77 (dd, J = 12.5, 2.7 Hz, 2 H_{trans}), 3.3-3.4 (m, 2 $H_{cis/trans}$), 3.8 (s, 2 H_{cis}), 3.9 (s, 2 H_{trans}), 6.96-7.05 (m, 2 H_{cis}), 7.02-7.05 (m, 2 H_{trans}), 7.35-7.41 (m, 4 $H_{cis/trans}$), 8.68 (s, 1 H_{trans}), 9.13 (d, J = 4.4 Hz, 1 H_{cis}). ^{13}C NMR (101 MHz, DMSO-d_6) δ ppm 21.4$_{cis}$, 22.0$_{trans}$, 29.0$_{cis}$, 29.1$_{cis}$, 30.8$_{cis}$, 31.3$_{trans}$, 32.0$_{trans}$, 33.3$_{trans}$, 34.7$_{cis}$, 35.1$_{trans}$, 44.2$_{cis}$, 48.1$_{trans}$, 113.7$_{trans}$, 113.9$_{cis}$, 119.6$_{cis}$, 120.3$_{trans}$, 141.0$_{trans}$, 142.2$_{cis}$, 151.6$_{trans}$, 153.1$_{cis}$, 167.0$_{trans}$, 168.0$_{cis}$; FTMS (ESI) m/z 304.1467 $[M+H]^+$; calcd for $C_{16}H_{21}N_3OS$: 304.1478.

4.2.10. 2-((1*H*-Benzo[*d*]imidazol-2-yl)thio)-*N*-(*trans*-4-methylcyclohexyl)acetamide (**6j**): Yield 76%; m.p.: 144.5 – 145.2 °C; HPLC 91% (t_R = 17.17); ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 0.84 (d, J = 6.4 Hz, 3 H), 0.88-0.98 (m, 2 H), 1.10-1.18 (m, 2 H), 1.22-1.39 (m, 1 H), 1.63 (d, J = 12.7 Hz, 2 H), 1.76 (d, J = 9.5 Hz, 2 H), 3.43 (m, 1 H), 3.92 (s, 2 H), 7.05-7.07 (m, 2 H), 7.40-7.41 (m, 2 H), 8.55 (s, 1 H). ^{13}C NMR (101 MHz, DMSO-d_6) δ ppm 22.0, 31.3, 32.0, 33.3, 35.2, 48.1, 113.8, 120.5, 120.6, 140.7, 151.2, 166.9; FTMS (ESI) m/z 304.1496 $[M+H]^+$; calcd for $C_{16}H_{21}N_3OS$: 304.1478.

4.2.11. 2-((1*H*-Benzo[*d*]imidazol-2-yl)thio)-*N*-benzylacetamide (**6k**): Yield 73%; m.p.: 175.5 – 177.0 °C; HPLC 91% (t_R = 15.98); ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 4.10 (s, 2 H), 4.32 (d, J = 6.1 Hz, 2 H), 7.12-7.19 (m, 2 H), 7.21-7.25 (m, 5 H), 7.44-7.46 (m, 2 H), 8.82 (t, J = 5.4 Hz, 1 H). ^{13}C NMR (101 MHz, DMSO-d_6) δ ppm 34.9, 42.4, 121.3, 126.6, 127.0, 128.1, 138.9, 149.7, 167.3; FTMS (ESI) m/z 298.1029 $[M+H]^+$; calcd for $C_{16}H_{15}N_3OS$: 298.1009.

4.2.12. 2-((1*H*-Benzo[*d*]imidazol-2-yl)thio)-*N*-(cyclohexylmethyl)acetamide (**6l**): Yield 65%; m.p.: 166.7 – 167.1 °C; HPLC 90% (t_R = 17.21); ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 0.77-0.88 (m, 2 H), 1.10-1.14 (m, 4 H), 1.34-1.35 (m, 1 H), 1.55-1.69 (m, 6 H), 2.94 (ddd, J = 17.1, 10.8, 6.6 Hz, 2 H), 4.00 (s, 2 H), 7.09-7,14 (m, 2 H), 7.41-7.45 (m, 2 H), 8.32 (s, 1 H). ^{13}C NMR (101 MHz, DMSO-d_6) δ ppm 25.3, 25.9, 30.1, 34.9, 37.3, 45.1, 113.7, 121.2, 139.6, 149.9, 167.3; FTMS (ESI) m/z 304.1474 $[M+H]^+$; calcd for $C_{16}H_{21}N_3OS$: 304.1478.

4.2.13. (*S*)-2-((1*H*-Benzo[*d*]imidazol-2-yl)thio)-*N*-(1-cyclohexylethyl)acetamide (**6m**): Yield 62%; m.p.: 139.7 – 140.2 °C; HPLC 92% (t_R = 17.59); ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 0.81-1.10 (m, 7 H), 1.22-1.26 (m, 1 H), 1.52-1.60 (m, 5 H), 3.55-3.60 (m, 1 H), 3.82-3.99 (m, 2 H), 7.02 (dd, J = 5.9, 3.2 Hz, 2 H), 7.37 (dd, J = 6.1, 3.2 Hz, 2 H), 8.56 (s, 1 H). ^{13}C NMR (101 MHz, DMSO-d_6) δ ppm 17.4, 25.7, 25.8, 28.2, 28.6, 35.0, 42.3, 48.9, 113.7, 120.3, 141.0, 151.5, 167.3; FTMS (ESI) m/z 318.1621 $[M+H]^+$; calcd for $C_{17}H_{23}N_3OS$: 318.1635.

4.3 General procedure for the synthesis of 3,4-dihydroquinazolin-4-ones **9a**-**z**

2-Mercaptoquinazolin-4(3*H*)-one (1 mmol) was diluted in *N*,*N*-dimethylformamide (5 mL) with the addition of diisopropylethylamine (DIPEA, 1.1 mmol) under an argon atmosphere at 0 °C. The solution was stirred for 5 min and bromacetamine (1.1 mmol) was added. After 30 min, the temperature was elevated to 25 °C. The reaction mixture was stirred for additional 18 h and the precipitated product filtered off, washed with water and dried under vacuum. The products were obtained in satisfactory purity without the need for additional purification.

4.3.1. 2-((4-Oxo-3,4-dihydroquinazolin-2-yl)thio)-*N*-phenylacetamide (**9a**): Yield 86%; m.p.: 233.5 – 234.3 °C; HPLC 99% (t_R = 16.51 min); ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 4.17 (s, 2 H, 9-H), 7.04 (t, *J* = 7.3 Hz, 1 H, 4'-H), 7.30 (t, *J* = 7.3 Hz, 2 H, 3'-H), 7.40 (td, *J* = 8.1, 1.5 Hz, 1 H, 6-H), 7.47 (d, *J* = 8.1 Hz, 1 H, 8-H), 7.59 (d, *J* = 7.6 Hz, 2 H, 2'-H), 7.73 (td, *J* = 7.6, 1.6 Hz, 1 H, 7-H), 8.02 (dd, *J* = 8.1, 1Hz, 1 H, 5-H), 10.34 (s, 1 H, NH), 12.68 (s, 1 H, NH, 3-H). ^{13}C NMR (101 MHz, DMSO-d_6) δ ppm 35.1 (9-C), 119.1 (2'-C), 119.9 (4a-C), 123.4 (4'-C), 125.6 (5-C), 125.9(8-C), 128.7 (3'-C), 134.6 (7-C), 138.8 (1'-C), 148.2 (8a-C), 155.2 (2-C), 161.0 (4-C), 165.8 (10-C); FTMS (ESI) m/z 312.0797 $[M+H]^+$; calcd for $C_{16}H_{13}N_3O_2S$: 312.0801.

4.3.2. *N*-(4-Methoxyphenyl)-2-((4-oxo-3,4-dihydroquinazolin-2-yl)thio)acetamide (**9b**): Yield 81%; m.p.: 214.1 – 216.9 °C; HPLC 95% (t_R = 16.34 min); ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 3.70 (s, 3 H), 4.14 (s, 2 H), 6.87 (d, *J* = 9.0 Hz, 2 H), 7.38-7.42 (m, 1 H), 7.47-7.50 (m, 3 H), 7.74 (td, *J* = 8.3, 1.5 Hz, 1 H), 8.02 (dd, *J* = 7.8, 1.5 Hz, 1 H), 10.20 (s, 1 H), 12.66 (s, 1 H). ^{13}C NMR (101 MHz, DMSO-d_6) δ ppm 35.0, 55.1, 113.9, 119.9, 120.7, 125.7, 125.8, 126.0, 132.0, 134.6, 148.2, 155.3, 155.3, 161.0, 165.3; FTMS (ESI) m/z 342.0873 $[M+H]^+$; calcd for $C_{17}H_{15}N_3O_3S$: 342.0907.

4.3.3. 2-((4-Oxo-3,4-dihydroquinazolin-2-yl)thio)-*N*-(*p*-tolyl)acetamide (**9c**): Yield 69%; m.p.: 247.9 – 250.1 °C; HPLC 96% (t_R = 17.00 min); ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 2.23 (s, 3 H), 4.15 (s, 2 H), 7.10 (d, *J* = 8.1 Hz, 2 H), 7.40 (td, *J* = 8.0, 1.5 Hz, 1 H), 7.47 (d, *J* = 8.6 Hz, 3 H), 7.73 (td, *J* = 8.4, 1.5 Hz, 1 H), 8.02 (dd, *J* = 7.9, 1.5 Hz, 1 H), 10.35 (s, 1 H), 12.67 (s, 1 H). ^{13}C NMR (101 MHz, DMSO-d_6) δ ppm 20.4, 35.1, 119.1, 119.9, 125.7, 125.8, 126.0, 129.1, 132.3, 134.6, 136.4, 148.2, 155.2, 161.0, 165.6; FTMS (ESI) m/z 326.0927 $[M+H]^+$; calcd for $C_{17}H_{15}N_3O_2S$: 326.0958.

4.3.4. *N*-(4-Fluorophenyl)-2-((4-oxo-3,4-dihydroquinazolin-2-yl)thio)acetamide (**9d**): Yield 68%; m.p.: 243.6 – 244.8 °C; HPLC 97% (t_R = 16.62 min); ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 4.16 (s, 2 H), 7.11-7.17 (m, 2 H), 7.40 (td, *J* = 8.1, 1.5 Hz, 1 H), 7.46 (d, *J* = 8.1 Hz, 1 H), 7.58-7.63 (m, 2 H), 7.73 (td, *J* = 7.6, 1.6 Hz, 1 H), 8.02 (dd, *J* = 8.1, 1.5 Hz, 1 H), 10.40 (s, 1 H),

12.67 (s, 1 H). ^{13}C NMR (101 MHz, DMSO-d_6) δ ppm 35.0, 115.3 (d, $^{2}J_{CF}$ = 22.0 Hz), 119.9, 120.9 (d, $^{3}J_{CF}$ = 8.0 Hz), 125.7, 125.8, 126.0, 134.6, 135.2 (d, $^{4}J_{CF}$ = 2.9 Hz), 148.2, 155.2, 156.8 (d, $^{1}J_{CF}$ = 239.8 Hz), 161.0, 165.8; FTMS (ESI) m/z 330.0674 $[M+H]^+$; calcd for $C_{16}H_{12}FN_3O_2S$: 330.0707.

4.3.5. *N*-(4-Chlorophenyl)-2-((4-oxo-3,4-dihydroquinazolin-2-yl)thio)acetamide (**9e**): Yield 81%; m.p.: 262.7 – 264.1 °C; HPLC 93% (t_R = 17.21 min); ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 4.18 (s, 2 H), 7.37 (d, *J* = 8.0, 2 H), 7.41 (td, *J* = 8.1, 1.1 Hz, 1 H), 7.46 (d, *J* = 8 Hz,1 H), 7.63 (d, *J* = 8.0, 2 H), 7.75 (td, *J* = 8.0, 1.1 Hz, 1 H), 8.03 (dd, *J* = 7.8, 1.5 Hz, 1 H), 10.49 (s, 1 H), 12.68 (s, 1 H). ^{13}C NMR (101 MHz, DMSO-d_6) δ ppm 35.1, 119.9, 120.7, 125.7, 126.0, 127.0, 128.7, 134.6, 137.9, 140.4, 148.1, 155.3, 161.1, 166.0; FTMS (ESI) m/z 346.0403 $[M+H]^+$; calcd for $C_{16}H_{13}ClN_3O_2S$: 346.0412.

4.3.6. *N*-(4-bromophenyl)-2-((4-oxo-3,4-dihydroquinazolin-2-yl)thio)acetamide (**9f**): Yield 89%; m.p.: 236.2 – 238.1 °C; HPLC 92% (t_R = 17.26 min); ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 4.17 (s, 2 H), 7.40 (td, *J* = 8.1, 1.5 Hz, 1 H), 7.44 (d, *J* = 8.1 Hz, 1 H), 7.48 (d, *J* = 8.8 Hz, 2 H), 7.57 (d, *J* = 8.8 Hz, 2 H), 7.73 (td, *J* = 7.6, 1.6 Hz, 1 H), 8.02 (dd, *J* = 7.8, 1.5 Hz, 1 H), 10.48 (s, 1 H), 12.67 (s, 1 H). ^{13}C NMR (101 MHz, DMSO-d_6) δ ppm 35.1, 114.9, 119.8, 121.0, 125.6, 126.0, 131.5, 134.6, 138.2, 148.0, 155.2, 161.0, 166.0; FTMS (ESI) m/z 391.9843 $[M+H]^+$; calcd for $C_{16}H_{12}BrN_3O_2S$: 391.9886.

4.3.7. *N*-(4-Iodophenyl)-2-((4-oxo-3,4-dihydroquinazolin-2-yl)thio)acetamide (**9g**): Yield 86%; m.p.: 233.5 – 235.5 °C; HPLC 91% (t_R = 17.44 min); ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 4.17 (s, 2 H), 7.39-7.47 (m, 4 H), 7.64 (d, *J* = 7.6 Hz, 2 H), 7.74 (td, *J* = 8.5, 1.5 Hz, 1 H), 8.03 (dd, *J* = 8.1, 1.5 Hz,1 H), 10.36 (s, 1 H), 12.58 (s, 1 H). ^{13}C NMR (101 MHz, DMSO-d_6) δ ppm 34.9, 86.6, 119.8, 121.3, 125.5, 125.8, 134.4, 137.2, 138.5, 147.9, 155.1, 160.9, 165.9.; FTMS (ESI) m/z 437.9723 $[M+H]^+$; calcd for$C_{16}H_{12}IN_3O_2S$: 437.9768.

4.3.8. *N*-(4-Nitrophenyl)-2-((4-oxo-3,4-dihydroquinazolin-2-yl)thio)acetamide (**9h**): Yield 50%; m.p.: 235.8 – 238.1°C; HPLC 95% (t_R = 16.75 min); ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 4.26 (s, 2 H), 7.40-7.44 (m, 2 H), 7.74 (td, *J* = 7.6, 1.6 Hz, 1 H), 7.88 (d, *J* = 9.7 Hz, 2 H), 8.04 (dd, *J* = 7.8, 1.5 Hz, 1 H), 8.25 (d, *J* = 9.7 Hz, 2 H), 11.00 (s, 1 H), 12.73 (s, 1 H). ^{13}C NMR (101 MHz, DMSO-d_6) δ ppm 35.3, 118.8, 119.9, 125.0, 125.7, 126.1, 134.6, 142.3, 145.0, 148.1, 155.1, 161.0, 167.0; FTMS (ESI) m/z 357.0617 $[M+H]^+$; calcd for $C_{16}H_{12}N_4O_4S$: 357.0652.

4.3.9. 2-((4-Oxo-3,4-dihydroquinazolin-2-yl)thio)-*N*-(4-propylphenyl)acetamide (**9i**): Yield 75%; m.p.: 209.8 – 212.1 °C; HPLC 95% (t_R = 17.83 min); ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 0.87 (t, *J* = 8.1 Hz, 3 H), 1.51-1.60 (m, 2 H), 2.47-2.52 (m, 2 H), 4.19 (s, 2 H), 7.13 (d, *J* = 7.6 Hz, 2 H), 7.42 (t, *J* = 7.6 Hz, 1 H), 7.51 (d, *J* = 6.8 Hz, 3 H), 7.76 (t, *J* = 7.6 Hz, 1 H), 8.05 (d, *J* = 7.8 Hz, 1 H), 10.29 (s, 1 H), 12.70 (s, 1 H). ^{13}C NMR (101 MHz, DMSO-d_6) δ ppm 13.5, 24.0, 35.1, 36.6, 119.2, 119.9, 125.7, 125.8, 126.0, 128.5, 134.6, 136.6, 137.2, 148.2, 155.3, 161.0, 165.6; FTMS (ESI) m/z 354.1236 $[M+H]^+$; calcd for $C_{19}H_{19}N_3O_2S$: 354.1271.

4.3.10. 2-((4-Oxo-3,4-dihydroquinazolin-2-yl)thio)-*N*-(4-pentylphenyl)acetamide (**9j**): Yield 88%; m.p.: 218.2 – 219.6 °C; HPLC 94% (t_R = 18.52 min); ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 0.85 (t, *J* = 8.0 Hz, 3 H), 1.20-1.28 (m, 4 H), 1.51-1.57 (m, 2 H), 2.49-2.54 (m, 2 H), 4.17

(s, 2 H), 7.12 (d, *J* = 8.3 Hz, 2 H), 7.42 (t, *J* = 7.9, 1 H), 7.48-7.50 (m, 3 H), 7.75 (td, *J* = 7.6, 1.6 Hz, 1 H), 8.04 (dd, *J* = 7.9, 1.3 Hz, 1 H), 10.27 (s, 1 H), 12.69 (s, 1 H). ^{13}C NMR (101 MHz, DMSO-d_6) δ ppm 13.8, 21.9, 30.6, 30.8, 34.5, 35.1, 119.2, 119.9, 125.7, 125.8, 126.0, 128.4, 134.6, 136.5, 137.4, 148.2, 155.2, 161.0, 165.6; FTMS (ESI) m/z 382.1546 $[M+H]^+$; calcd for $C_{21}H_{23}N_3O_2S$: 382.1584.

4.3.11. *N*-(Naphthalen-2-yl)-2-((4-oxo-3,4-dihydroquinazolin-2-yl)thio)acetamide (**9k**): Yield 81%; m.p.: 219.3 – 221.5 °C; HPLC 95% (t_R = 17,37 min); ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 4.25 (s, 2 H), 7.36-7.43 (m, 3 H), 7.47 (t, *J* = 7.3 Hz, 2 H), 7.62 (d, *J* = 8.8 Hz, 1 H), 7.72 (t, *J* = 8.2 Hz, 1 H), 7.80 (dd, *J* = 11.1, 8.2 Hz, 1 H), 7.86 (d, *J* = 8.8 Hz, 1 H), 8.03 (d, *J* = 8.1 Hz, 1 H), 8.29 (s, 1 H), 10.57 (s, 1 H), 12.70 (s, 1 H). ^{13}C NMR (101 MHz, DMSO-d_6) δ ppm 35.2, 115.3, 119.8, 119.9, 124.6, 125.7, 125.8, 126.0, 126.4, 127.3, 127.4, 128.4, 129.8, 133.4, 134.6, 136.5, 148.2, 155.3, 161.1, 166.1; FTMS (ESI) m/z 362.0942 $[M+H]^+$; calcd for$C_{20}H_{15}N_3O_2S$: 362.0958.

4.3.12. (*S*)-2-((4-Oxo-3,4-dihydroquinazolin-2-yl)thio)-*N*-(1,2,3,4-tetrahydronaphthalen-2-yl)acetamide (**9l**): Yield 37%; m.p.: 244.1 – 245.0 °C; HPLC 93% (t_R = 17.31 min); ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 1.67-1.73 (m, 2 H), 1.87-1.91 (m, 2 H), 2.67-2.78 (m, 2 H), 3.95-4.04 (m, 2 H), 4.94-5.00 (m, 1 H), 7.01 (t, *J* = 7.3 Hz, 1 H), 7.08 (d, *J* = 7.1 Hz, 1 H), 7.11-7.17 (m, 2 H), 7.41-7.46 (m, 2 H), 7.77 (t, *J* = 7.7 Hz, 1 H), 8.04 (d, *J* = 7.8 Hz, 1 H), 8.60 (d, *J* = 8.8 Hz, 1 H), 12,66 (s, 1H). ^{13}C NMR (101 MHz, DMSO-d_6) δ ppm 19.9, 28.6, 29.6, 33.9, 46.9, 119.9, 125.6, 125.6, 125.8, 125.9, 126.6, 128.0, 128.6, 134.4, 136.9, 137.1, 148.2, 155.3, 161.0, 166.3; FTMS (ESI) m/z 366.1269 $[M+H]^+$; calcd for $C_{20}H_{19}N_3O_2S$: 366.1271.

4.3.13. (*R*)-2-((4-Oxo-3,4-dihydroquinazolin-2-yl)thio)-*N*-(1,2,3,4-tetrahydronaphthalen-2-yl)acetamide (**9m**): Yield 80%; m.p.: 241.2 – 242.7 °C; HPLC 96% (t_R = 17.28 min); 1H NMR (400 MHz, DMSO-d_6) δ ppm 1.66-1.72 (m, 2 H), 1.85-1.92 (m, 2 H), 2.64-2.73 (m, 2 H), 3.94-4.03 (m, 2 H), 4.96-5.00 (m, 1 H), 6.98-7.16 (m, 4 H), 7.40-7.44 (m, 2 H), 7.76 (t, *J* = 7.1 Hz, 1 H), 8.03 (d, *J* = 7.8 Hz, 1 H), 8.58 (d, *J* = 8.6 Hz, 1 H), 12.66 (s, 1 H). ^{13}C NMR (101 MHz, DMSO-d_6) δ ppm 19.9, 28.6, 29.6, 33.9, 46.9, 119.9, 125.6, 125.6, 125.8, 125.9, 126.6, 128.0, 128.6, 134.4, 136.9, 137.1, 148.2, 155.3, 161.0, 166.3.; FTMS (ESI) m/z 366.1268 $[M+H]^+$; calcd for $C_{20}H_{19}N_3O_2S$: 366.1271.

4.3.14. *N*-Cyclohexyl-2-((4-oxo-3,4-dihydroquinazolin-2-yl)thio)acetamide (**9n**): Yield 65%; m.p.: 219.0 – 221.3 °C; HPLC 91% (t_R = 16.17 min); ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 1.08-1.30 (m, 5 H), 1.52 (m, 1 H), 1.65-1.74 (m, 4 H), 3.50-3.60 (m, 1 H, 1'-H), 3.92 (s, 2 H, 9-H), 7.42 (td, *J* = 8.1, 1.5 Hz, 1 H, 6-H), 7.50 (d, *J* = 8.1 Hz, 1 H, 8-H), 7.77 (td, *J* = 7.6, 1.6 Hz, 1 H, 7-H), 8.03 (dd, *J* = 7.8, 1.5 Hz, 1 H, 5-H), 8.13 (d, *J* = 7.6 Hz, 1 H, HN), 12.65 (s, 1 H, NH, 3-H). ^{13}C NMR (101 MHz, DMSO-d_6) δ ppm 24.3, 25.1, 32.2, 34.1, 47.9, 119.9, 125.6, 125.7, 125.9, 134.5, 148.2, 155.3, 161.0, 165.8; FTMS (ESI) m/z 318.1277 $[M+H]^+$; calcd for $C_{16}H_{19}N_3O_2S$: 318.1271.

4.3.15. *N*-Cyclohexyl-*N*-methyl-2-((4-oxo-3,4-dihydroquinazolin-2-yl)thio)acetamide (**9o**): Yield 52%; m.p.: 184.2 – 185.2 °C; HPLC 97% (t_R = 17.43 min); ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 1.07-1.11 (m, 2 H), 1.25-1.35 (m, 4 H), 1.43-1.47 (m, 5 H), 1.48-1.57 (m, 4 H), 1.72-1.78 (m, 5 H), 2.74 and 3.01 (s, 2x NCH_3), 3.78 and 4.21 (m, 2x CH), 4.23 and 4.34 (s, 2x CH_2),

7.40-7.48 (m, 4x ArH), 7.74-7.79 (m, 2x ArH), 8.04 (d, *J* = 8.1, 2x ArH), 12.61 (s, 1 H, 2x HN). ^{13}C NMR (101 MHz, DMSO-*d*$_6$) δ ppm 24.7, 24.9, 25.1, 25.2, 27.2, 29.1, 29.6, 30.2, 33.1, 34.0, 52.5, 56.1, 119.9, 125.5, 125.6, 125.7, 125.8, 125.9, 126.0, 134.5, 134.6, 148.2, 148.3, 155.2, 155.5, 161.0, 166.3; FTMS (ESI) m/z 332.1419 $[M+H]^+$; calcd for $C_{17}H_{20}N_3O_2S$: 332.1427.

4.3.16. *N*-(2-Methylcyclohexyl)-2-((4-oxo-3,4-dihydroquinazolin-2-yl)thio)acetamide (**9p**): Yield 55%; m.p.: 230.2 – 232.8 °C; HPLC 91% (t_R = 17.03 and 17.17 min); ^{1}H NMR (400 MHz, DMSO-*d*$_6$) δ ppm 0.81 (*J* = 6.6 Hz, 3 H), 0,93-1.02 (m, 1 H), 1.10-1,39 (m, 4 H), 1.59 (d, *J* = 11.2 Hz, 1 H), 1.69 (q, *J* = 13 Hz, 2 H), 3.23-3.29 (m, 1 H), 3.89-4.01 (m, 2 H), 7.42 (t, *J* = 7.5 Hz, 1 H), 7.50 (d, *J* = 8.1 Hz, 1 H), 7.77 (t, *J* = 7.6 Hz, 1 H), 8.04 (d, *J* = 8.1 Hz, 2 H), 12.66 (s, 1 H). ^{13}C NMR (101 MHz, DMSO-*d*$_6$) δ ppm 19.0, 25.0, 25.3, 32.7, 33.8, 34.1, 37.2, 49.2, 53.8, 119.9, 125.6, 125.8, 126.0, 134.5, 148.3, 155.4, 161.0, 166.1; FTMS (ESI) m/z 332.1414 $[M+H]^+$; calcd for $C_{17}H_{21}N_3O_2S$: 332.1427.

4.3.17. *N*-(3-Methylcyclohexyl)-2-((4-oxo-3,4-dihydroquinazolin-2-yl)thio)acetamide (**9q**): Yield 35%; m.p.: 219.2 – 223.2 °C; HPLC 94% (t_R = 17.24 and 17.47 min); 1H NMR (400 MHz, DMSO-*d*$_6$) δ ppm 0.71-0.90 (m, 7 H), 1.00-1.10 (m, 1 H), 1.20-1.29 (m, 2 H), 1.35-1.51 (m, 2 H), 1.58 (d, *J* = 12.5 Hz, 1 H), 1.75 (d, *J* = 11.7 Hz, 2 H), 3.52-3.54 (m, 1 H), 3.91 (s, 2 H), 7.42 (t, *J* = 8.1, 1 H), 7.50 (d, *J* = 8.1 Hz, 1 H), 7.77 (t, *J* = 7.7 Hz, 1 H), 8.04 (d, *J* = 8.1 Hz, 1 H), 8.12 (d, *J* = 7.1 Hz, 1 H), 12.64 (s, 1 H). ^{13}C NMR (101 MHz, DMSO-*d*$_6$) δ ppm 22.2, 24.4, 31.3, 31.9, 33.8, 34.2, 41.2, 48.3, 120.0, 125.6, 125.8, 126.0, 134.4, 148.3, 155.3, 161.0, 165.9; FTMS (ESI) m/z 332.1415 $[M+H]^+$; calcd for $C_{17}H_{21}N_3O_2S$: 332.1427.

4.3.18. *N*-(4-Methylcyclohexyl)-2-((4-oxo-3,4-dihydroquinazolin-2-yl)thio)acetamide (**9r**): Yield 45%; m.p.: 199.2 – 200.8 °C; HPLC 90% (t_R = 17.23 min); ^{1}H NMR (400 MHz, DMSO-*d*$_6$) δ ppm 0.80 (d, *J* = 6.6 Hz, 3 H), 0.84 (d, *J* = 6.6 Hz, 1 H), 0.89-0.99 (m, 1H), 1.14-1.23 (m, 4 H), 1.39-1.48 (m, 6 H), 1.55-1.65 (m, 3 H), 1.77 (d, *J* = 11.0 Hz, 1 H), 3.5 (m, 1 H)4.2 (s, 2 H), 7.3-7.4 (m, 3 H), 7.4 (t, *J* = 7.3 Hz, 2 H), 7.6 (d, *J* = 8.8 Hz, 1 H), 7.7 (t, *J* = 8.2 Hz, 1 H), 7.8 (dd, *J* = 11.1, 8.2 Hz, 1 H), 7.9 (d, *J* = 8.8 Hz, 1 H), 8.0 (d, *J* = 8.1 Hz, 1 H), 8.3 (s, 1 H), 10.6 (s, 1 H), 12.7 (s, 1 H). ^{13}C NMR (101 MHz, DMSO-*d*$_6$) δ ppm 35.2, 115.3, 119.8, 119.9, 124.6, 125.7, 125.8, 126.0, 126.4, 127.3, 127.4, 128.4, 129.8, 133.4, 134.6, 136.5, 148.2, 155.3, 161.1, 166.1; FTMS (ESI) m/z 332.1414 $[M+H]^+$; calcd for $C_{17}H_{21}N_3O_2S$: 332.1427.

4.3.19. *N*-(*trans*-4-Methylcyclohexyl)-2-((4-oxo-3,4-dihydroquinazolin-2-yl)thio)acetamide (**9s**): Yield 59%; m.p.: 223.4 – 225.8 °C; HPLC 91% (t_R = 17.29 min); ^{1}H NMR (400 MHz, DMSO-*d*$_6$) δ ppm 0.85 (d, *J* = 6.6 Hz, 3 H), 0.92-0.99 (m, 2 H), 1.13-1.23 (m, 2 H), 1.26-1.35 (m, 1 H), 1.65 (d, *J* = 11.7 Hz, 2 H), 1.76 (d, *J* = 11.0 Hz, 2 H), 3.47-3.50 (m, 1 H), 3.91 (s, 2 H), 7.42 (t, *J* = 8.1 Hz, 1 H), 7.49 (d, *J* = 8.1 Hz, 1 H), 7.77 (td, *J* = 7.7, 1.5 Hz, 1 H), 8.03 (dd, *J* = 8.1, 1.5 Hz, 1 H), 8.11 (d, *J* = 7.1 Hz, 1 H), 12.65 (s, 1 H). ^{13}C NMR (101 MHz, DMSO-*d*$_6$) δ ppm 22.0, 31.3, 32.1, 33.4, 34.1, 48.2, 119.9, 125.6, 125.8, 125.9, 134.5, 148.3, 155.3, 161.0, 165.9; FTMS (ESI) m/z 332.1433 $[M+H]^+$; calcd for $C_{17}H_{21}N_3O_2S$: 332.1427.

4.3.20. *N*-Cyclopentyl-2-((4-oxo-3,4-dihydroquinazolin-2-yl)thio)acetamide (**9t**): Yield 60%; m.p.: 214.5 – 242.9 °C; HPLC 98% (t_R = 16.38 min); ^{1}H NMR (400 MHz, DMSO-*d*$_6$) δ ppm 1.38-1.41 (m, 2 H), 1.47-1.51 (m, 2 H), 1.57-1.64 (m, 2 H), 1.73-1.81 (m, 2 H), 3.90 (s, 2 H), 3.95-4.03 (m, 1 H), 7.41 (td, *J* = 8.1, 1.5 Hz, 1 H), 7.48 (d, *J* = 8.1 Hz, 1 H), 7.76 (td, *J* = 8.4, 1.5

Hz, 1 H), 8.02 (dd, *J* = 8.1, 1.5 Hz, 1 H), 8.20 (d, *J* = 7.1 Hz, 1 H), 12.64 (s, 1 H). ^{13}C NMR (101 MHz, DMSO-d_6) δ ppm 23.3, 32.1, 34.0, 50.6, 119.9, 125.6, 125.7, 125.9, 134.5, 148.2, 155.3, 161.0, 166.2; FTMS (ESI) m/z 304.1119 $[M+H]^+$; calcd for $C_{15}H_{17}N_3O_2S$: 304.1114.

4.3.21. *N*-Cycloheptyl-2-((4-oxo-3,4-dihydroquinazolin-2-yl)thio)acetamide (**9u**): Yield 76%; m.p.: 223.0 – 223.3 °C; HPLC 97% (t_R = 17.26 min); ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 1.33-1.55 (m, 10 H), 1.75-1.16 (m, 2 H), 3.72-3.75 (m, 1 H), 3.91 (s, 2 H), 7.42 (td, *J* = 8.0, 1.2 Hz, 1 H), 7.49 (d, *J* = 8.1 Hz, 1 H), 7.77 (td, *J* = 8.4, 1.5 Hz, 1 H), 8.01 (dd, *J* = 7.8, 1.5 Hz, 1 H), 8.16 (d, *J* = 7.8 Hz, 1 H), 12.65 (s, 1 H). ^{13}C NMR (101 MHz, DMSO-d_6) δ ppm 23.6, 27.7, 34.0, 50.0, 119.9, 125.6, 125.7, 125.9, 134.5, 148.2, 155.3, 161.0, 165.5; FTMS (ESI) m/z 332.1432 $[M+H]^+$; calcd for $C_{17}H_{21}N_3O_2S$: 332.1427.

4.3.22. *N*-Benzyl-2-((4-oxo-3,4-dihydroquinazolin-2-yl)thio)acetamide (**9v**): Yield 41%; m.p.: 188.8 – 191.6 °C; HPLC 96% (t_R = 16.32 min); ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 4.03 (s, 2 H), 4.31 (d, *J* = 6.1 Hz, 2 H), 7.18-7.26 (m, 5 H), 7.41-7.48 (m, 2 H), 7.77 (td, *J* = 7.7, 1.5 Hz, 1 H), 8.04 (dd, *J* = 7.8, 1.5 Hz, 1 H), 8.71 (t, *J* = 5.7 Hz, 1 H), 12.64 (s, 1 H). ^{13}C NMR (101 MHz, DMSO-d_6) δ ppm 33.7, 42.4, 119.9, 125.6, 125.9, 126.6, 126.9, 128.1, 134.5, 139.0, 148.2, 155.1, 161.0, 166.9; FTMS (ESI) m/z 326.0924 $[M+H]^+$; calcd for $C_{17}H_{15}N_3O_2S$: 326.0958.

4.3.23. *N*-(Cyclohexylmethyl)-2-((4-oxo-3,4-dihydroquinazolin-2-yl)thio)acetamide (**9w**): Yield 46%; m.p.: 231.0 – 231.9 °C; HPLC 97% (t_R = 17.20 min); ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 0.78-0.87 (m, 2 H), 1.05-1.10 (m, 3 H), 1.36-1.40 (m, 1 H), 1.57-1.64 (m, 5 H), 2.93 (t, *J* = 6.4 Hz, 2 H), 3.93 (s, 2 H), 7.42 (td, *J* = 8.1, 1.5 Hz, 1 H), 7.50 (d, *J* = 8.1 Hz, 1 H), 7.76 (td, *J* = 7.7, 1.5 Hz, 1 H), 8.03 (dd, *J* = 7.8, 1.2 Hz, 1 H), 8.15 (t, *J* = 7.8 Hz, 1 H), 12.64 (s, 1 H). ^{13}C NMR (101 MHz, DMSO-d_6) δ ppm 25.2, 25.8, 30.2, 33.8, 37.4, 45.1, 119.9, 125.6, 125.8, 125.9, 134.4, 148.2, 155.2, 161.0, 166.7; FTMS (ESI) m/z 332.1432 $[M+H]^+$; calcd for $C_{17}H_{21}N_3O_2S$: 332.1427.

4.3.24. (*S*)-*N*-(1-Cyclohexylethyl)-2-((4-oxo-3,4-dihydroquinazolin-2-yl)thio)acetamide (**9x**): Yield 59%; m.p.: 232.5 – 236.7 °C; HPLC 91% (t_R = 17.42 min); ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 0.84-1.13 (m, 7 H), 1.01 (d, *J* = 6.8 Hz, 3 H), 1.22-1.26 (m, 1 H), 1.54-1.69 (m, 6 H), 3.58-3.67 (m, 1H), 3.89 (d, *J* = 14.9 Hz, 1 H), 3.98 (d, *J* = 14.9 Hz, 1 H), 7.42 (t, *J* = 7.5 Hz, 1 H), 7.51 (d, *J* = 8.1 Hz, 1 H), 7.77 (t, *J* = 7.1 Hz, 1 H), 7.97 (d, *J* = 7.8 Hz, 1 H), 8.04 (d, *J* = 8.6 Hz, 1 H), 12.65 (s, 1 H). ^{13}C NMR (101 MHz, DMSO-d_6) δ ppm 17.5, 18.4, 25.6, 25.8, 28.5, 28.7, 34.0, 42.4, 49.0, 56.0, 119.5, 119.9, 125.6, 125.8, 125.9, 134.5, 148.2, 155.3, 161.0, 166.1; FTMS (ESI) m/z 346.1577 $[M+H]^+$; calcd for $C_{18}H_{23}N_3O_2S$: 346.1584.

4.3.25. (*R*)-N-(1-Cyclohexylethyl)-2-((4-oxo-3,4-dihydroquinazolin-2-yl)thio)acetamide (**9y**): Yield 82%, m.p.:217.2 – 217.9 °C; HPLC 91% (t_R= 17.81); ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 0.83-0.95 (m, 2 H), 1.00 (d, *J* = 6.8 Hz, 3 H), 1.03-1.16 (m, 2 H), 1.23-1.29 (m, 1 H), 1.53-1.69 (m, 5 H), 3.57-3.64 (m, 1 H), 3.89 (d, *J* =14.9 Hz, 1 H), 3.98 (d, *J* = 14.9 Hz, 1 H), 7.42 (t, *J* = 7.5 Hz, 1 H), 7.50 (d, *J* = 8.1 Hz, 1 H), 7.77 (t, *J* = 7.6 Hz, 1 H), 7.97 (d, *J* = 7.8 Hz, 1 H), 8.04 (d, *J* = 8.6 Hz, 1 H), 12.66 (s, 1 H). ^{13}C NMR (101 MHz, DMSO-d_6) δ ppm 17.5, 25.6, 25.8, 28.5, 28.7, 34.0, 42.4, 49.0, 119.9, 125.6, 125.8, 125.9, 134.5, 148.2, 155.3, 161.0, 166.0; FTMS (ESI) m/z 346.1606 $[M+H]^+$; calcd for $C_{18}H_{23}N_3O_2S$: 346.1584.

4.3.26. 2-((2-Oxo-2-(piperidin-1-yl)ethyl)thio)quinazolin-4(3*H*)-one (**9z**): Yield 72%; m.p.: 118.5 – 121.0 °C; HPLC 98% (t_R = 16.34 min); ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 1.46 (m, 2 H), 1.62 (m, 4 H), 3.55 (m, 2 H), 3.51-3.56 (m, 2 H), 4.29 (s, 2 H), 7.42 (t, *J* = 7.5 Hz, 1 H), 7.50 (d, *J* = 8.1 Hz, 1 H), 7.76 (t, *J* = 7.2 Hz, 1 H), 8.04 (d, *J* = 7.6 Hz, 1 H), 12,62 (s, 1H). ^{13}C NMR (101 MHz, DMSO-d_6) δ ppm 23.8, 25.2, 26.0, 33.3, 42.7, 46.6, 119.9, 125.6, 125.8, 126.0, 134.5, 148.2, 155.4, 161.0, 165.0; FTMS (ESI) m/z 304.1117 $[M+H]^+$; calcd for $C_{15}H_{17}N_3O_2S$: 304.1140.

4.4 *Mycobacterium tuberculosis* inhibition assay

The measurement of the minimum inhibitory concentration (MIC) for each tested compound was performed in 96-well U-bottom polystyrene microplates. Isoniazid (positive control) and the compound solutions were prepared at 1 mg/mL concentration in dimethylsulfoxide (DMSO). They were diluted in Middlebrook 7H9 medium containing 10% ADC (albumin, dextrose, catalase) to a concentration between 10 and 50 µg/mL of each compound containing 5% DMSO. Serial two-fold dilutions of each drug in 100 µL of Middlebrook 7H9 medium containing 10% ADC were prepared directly in 96-well plates at concentration ranges of 25.0 to 0.05 µg/mL, 10.0 to 0.02 µg/mL, or 0.02 to 0.0004 µg/mL. Growth controls containing no antibiotics, and sterile controls without inoculation, were included. The MIC was determined for *M. tuberculosis* H37Rv ATCC 27294 reference strain (American Type Culture Collection, Rockville, Md.) and for the clinical isolates CDCT10 (1009/09), CDCT16 (630/08), CDCT27 (0128/09) and CDCT28 (1051/10). Mycobacterium strains were grown in Middlebrook 7H9 containing 10% OADC (oleic acid, albumin, dextrose and catalase) and 0.05% Tween 80. The cells were vortexed with sterile glass beads (4 mm) for 5 min to disrupt any clumps and were allowed to settle for 20 min. The supernatant was measured spectrophotometrically at an absorbance of 600 nm. The Mtb suspensions were divided into aliquots and stored at –20 °C. Each suspension was appropriately diluted in Middlebrook 7H9 broth containing 10% ADC to achieve an optical density at 600 nm of 0.006, and 100 µL was

added to each well of the plate except the sterile controls. A 2.5% DMSO final concentration was maintained in each well. The plates were covered, sealed with parafilm, and incubated at 37 °C. After 7 days of incubation, 60 µL of 0.01% resazurin solution was added to each well, and the samples were incubated for an addition 48 h at 37 °C [16, 17]. A change in color from blue to pink indicated bacterial growth, and the MIC was defined as the lowest drug concentration that prevented the color change. Three tests were performed independently, and the MIC values reported here were observed in at least two experiments, or they were the highest values observed among the three assays.

4.5 Cytotoxicity investigation

Cellular viability determination after incubation with the test compounds was performed by using two different methods: the neutral red uptake assay [20] and the MTT method [10]. Briefly, HepG2, HaCat, and Vero cells were grown in DMEM media (Dulbecco's Modified Eagle Medium) supplemented with 10% inactivated fetal bovine serum and 1% antibiotic (penicillin–streptomycin). Cells were seeded at 2×10^3 (HepG2 or HaCat) or 1×10^3 cells/well (Vero) in a 96-well microtiter plate and incubated for 24 h. The medium was carefully aspirated and replaced with 90 µL DMEM, and 10 µL of the different treatment drugs, resulting in a final concentration of 10 µg/mL (DMSO 2%, v/v). Test compounds were incubated with the cell lines for 72 h at 37 °C under 5% CO_2. For the MTT assay, the cultures were incubated with MTT reagent (1 mg/mL) for 3 h. The formazan crystals were dried in room temperature for at least 24 h and dissolved in DMSO. The absorbance was measured at 595 nm (Spectra Max M2e, Molecular Devices, USA). The precipitated purple formazan crystals were directly proportional to the number of live cells with active mitochondria.

For the neutral red assay, after 72 h of incubation with the compounds, cells were washed with PBS before the addition of 250 μL of neutral red dye solution (25 μg/mL, Sigma) prepared in serum-free medium. The plate was incubated for additional 3 h at 37 °C under 5% CO_2. After incubation, cells were washed with PBS followed by incubation with 100 μL of a desorb solution ($CH_3COOH/EtOH/H_2O$, 1:50:49) for 30 min with gentle shaking to extract neutral red from the viable cells. The absorbance was analyzed at 540 nm using a microtiter plate reader. The percentage of cell viability for treated groups was reported considering the control wells (DMSO 2%-treated) as 100% of cell viability: cell viability (%) = (absorbance of treated wells/absorbance of control wells) x 100. Data were expressed as mean of cell viability ± standard error of mean of three independent experiments performed in triplicate. The statistical analysis was accomplished by one-way analysis of variance, followed by Bonferroni's post-test, using GraphPad Prism 5.0 software (San Diego, CA, USA). Differences were considered significant at the 95% level of confidence.

4.6 Treatment and Zebrafish embryo maintenance

Zebrafish embryos (AB strain) were obtained from natural mating of adult *Danio rerio* bred and maintained in an automated re-circulating tank system (Tecniplast, Italy) [22]. After spawning, viable embryos were collected and maintained in sterile petri dishes, kept in an incubator with light-dark cycle of 14–10 h and controlled temperature (28 °C) [22]. At 2 hpf (hours post-fertilization) embryos were treated with different concentrations (3.0, 15.0 and 20.0 μM) of 3,4-dihydroquinazolin-4-ones **9n**, **9p**-**s**, **9u** and **9w** until 5 dpf. All compounds were diluted in 100% DMSO for stock solutions and diluted in fish water (Reverse Osmosis equilibrated with Instant Ocean Salt) to final concentrations of 3.0, 15.0 and 20.0 μM (diluted in

760 1% DMSO). Since compounds were diluted first in DMSO, there were two control groups for
761 each treatment: one with fish water only and the other with 1% DMSO. Survival and hatching
762 efficiency were monitored daily under a stereomicroscope (Nikon, Melville, USA). All protocols
763 were approved by the Institutional Animal Care Committee from Pontifical Catholic University
764 of Rio Grande do Sul (CEUA-PUCRS, permit number 7249).
765
766 4.6.1 Morphological evaluation
767 Morphological evaluation larvae were monitored daily and registered at 2 and 5 dpf under
768 a stereomicroscope (3×) (n = 30) The body length (µm), ocular distance (µm) and surface area of
769 the eyes (μm^2) was measured after photographical registration using the software NIS-Elements
770 D 3.2 for Windows, supplied by Nikon Instruments Inc. (Melville, USA). The body length was
771 considered as the distance from the center of an eye to the tip of the tail bud. The ocular distance
772 was assumed to be the distance between the inner edge between the two eyes, and the size of the
773 eyes was assumed as the surface area of the eyes [23].
774
775 4.6.2 Cardiotoxicity and cardiac evaluation
776 Animals were analyzed for heartbeat rate at 2 and 5 dpf under a stereomicroscope (n =
777 30). Treated larvae and controls were placed in petri dishes with mineral water and their heart
778 rate was monitored for 60 s. For all procedures, temperature was kept stable at 28 ºC [24].
779
780 4.6.3 Neurotoxicity and exploratory behavior evaluation.
781 Five-day-old larvae were placed individually in a 24-well plate filled with 3 mL of water
782 or respective solution treatment for exploratory performance analysis during a 5 min session

following 1 min acclimation [28]. The performance was video-recorded for automated analysis using EthoVision XT software (version 11.5, Noldus), which is able to track the swimming activity of the animals at a rate of 15 positions per second. Total distance travelled (cm) was considered the parameter of exploration of a new environment [23].

4.6.4 Statistical analysis for Zebrafish assays

Survival and hatching rate throughout the five days of experimental treatment was analyzed by Kaplan-Meier test. Data of heartbeat rate, morphological evaluation and exploratory behavior were evaluated using one-way ANOVA followed by post-hoc Tukey's test.

Supporting Information

^{1}H and ^{13}C NMR spectra of compounds, aqueous stability investigation, and MIC values from three independent experiments (μg/mL). This material is available free of charge and can be obtained via the Internet.

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Notes

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Highlights:

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

Activity against drug-susceptible and drug-resistant *Mycobacterium tuberculosis* strains

Compounds devoid of apparent toxicity to HepG2, HaCat, and Vero cells

Low risk of cardiac toxicity, no signals of neurotoxicity or morphological alteration in zebrafish models.