UNIVERSIDADE FEDERAL DO RIO GRANDE DO SUL

Bacharel em Farmácia

KARINA DE VARGAS MARTINEZ

SÍNTESE DE DERIVADOS HETEROCÍCLICOS COMO POTENCIAIS CANDIDATOS A FÁRMACOS TUBERCULOSTÁTICOS

PORTO ALEGRE – RS

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Trabalho de Conclusão de Curso apresentado como requisito parcial para a obtenção do grau de Bacharel em Farmácia, sob orientação do Prof. Dr. Saulo Fernandes de Andrade e co-orientação da Ma. Débora Assumpção Rocha.

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Porto Alegre, 2021

BANCA EXAMINADORA

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Isadora Serraglio Fortes

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vida é fugaz. Aprendi que a minha família é tudo que tenho e que somente eles vão estar comigo até que não estejam mais. Aprendi que o tempo voa e não espera. Aprendi que o luto machuca, mas revela. Aprendi a dualidade da existência. Aprendi que tudo aquilo que dói também contribui para a expansão do próprio ser. Aprendi que amar nunca é demais e que a intensidade da própria sensibilidade não é sinônimo de fraqueza. Aprendi que abraçar as próprias falhas também significa amor-próprio. Aprendi, principalmente, a absorver e a reconstruir. E não acho que aprendi tudo que deveria e nem acho que verbalizei aqui tudo que sinto. Estas palavras são apenas um esboço da minha última versão que vos fala. E que venham as próximas.

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SYNTHESIS OF HETEROCYCLIC DERIVATIVES AS POTENTIAL CANDIDATES TO TUBERCULOSTATIC DRUGS

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ABSTRACT

Tuberculosis is a chronic infectious and curable disease caused by the ancient microorganism *Mycobacterium tuberculosis.* Despite advances in technology, huge scientific knowledge, improvement in quality of life and development of vaccines and drug treatment, the incidence and resistance of Tuberculosis (TB) is increasing worldwide. The World Health Organization (WHO) has been engaged in developing effective strategies and mass monitoring aimed at controlling and eradicating the disease. In this scope, the need to develop new specific antimicrobial drugs has been highlighted. Quinazolines derivatives has shown to be a promising class with several biological and microbiological activities, including activities against *Mycobacterium*. In another line of research developed in our group, compound **PH100**, a quinazolinic derivative, was synthesized and demonstrated antibacterial and antibiofilm activities against *Staphylococcus aureus* and *Staphylococcus epidermidis.* Due to these antibacterial activity findings, a scientific research has started to explore and identify a possible activity against *M. tuberculosis*. In this work, five quinazolinic derivatives (**PH100**, **3a**, **3b**, **3c** and **3d**) were synthesized and characterized. A biological evaluation through an inhibition assay using *M. tuberculosis* H37Rv strains to identify potential anti-TB action in these compounds was performed.

KEYWORDS: tuberculosis, *Mycobaterium sp.*, *Mycobacterium tuberculosis,* heterocycle derivatives.

1. INTRODUCTION

Mycobacterium tuberculosis is an aerobic or microaerophile rod-shaped bacteria belonging to *Actinomycetales* order, *Mycobacteriaceae* family, *Mycobacterium* gender and *M. tuberculosis* species[1]. This gender presents a distinctive morphological characteristic which is responsible for its alcohol-acid resistance and its consequent uses of alternatives staining methods. The composition of the bacillus cell wall, which includes mycolic acid and other complex lipids, provides slow growth and greater resistance to antimicrobials[2].

M. tuberculosis was first identified by Robert Koch in 1882 and denominated as Koch bacillus[3,4]. It is the etiological agent of TB, a curable and preventable chronic infectious disease[5,6] which notification is compulsory, and all its cases should be reported to WHO[6]. Evidence proves that TB infects humans since 3000 B.C, where traces were found in mummies of ancient Egypt[7]. The infection mainly affects lung tissue[8] but can also disseminate through the bloodstream and affect extrapulmonary tissues[9]. The increase of extrapulmonary TB occurrence is related to immune system impairment[10]. Moreover, the transmission can be horizontal through aerosol particles which contains active bacillus[8] and can be vertical, a rare and severe form of contamination transmitted from the mother to the fetus[11]. The affected tissues determine the signs and symptoms which includes at the beginning of disease day fever, weakness, anorexia, chest pain, night sweat, cough and respiratory insufficiency. As the disease advances, symptoms as hemoptysis and persistent cough can be reported[6, 8, 12, 13].

M. tuberculosis identification improved the development of public policies, drug treatments and vaccines currently known[3, 4]. The technological progress in the 20th century has provided the advancement of new therapeutic strategies nowadays used in anti-TB therapy. The current therapeutic scheme in use was developed in 1970[12] and the first vaccine BCG tests were accomplished during the World War I in 1921[14].

Due to the high rate and development of pathology, WHO declared TB as urgency state in 1993[6]. Considered an important health public problem worldwide, mainly in emerging countries, TB is among the top 10 causes of death in the world[6]. Statistics from 2019 appraised the bacillus infects about 10 million people in a global range, and it has a range of 500 new cases per 100.000 population per year[6]. Brazil is on the list of the 30 countries with the highest positive number of cases for TB and it is also on the list of the 30 countries with the highest numbers of coinfection TB-HIV[6]. Besides that, Brazil also presents reports of tuberculosis

resistant to the international therapeutic scheme recommended[6]. In 2019, there were approximately 500.000 cases of resistant TB worldwide, and 78% of these cases were resistant to more than one drug from therapeutic scheme. Studies estimate that 3.3% of new cases and 17.7% of previously treated cases present microbial resistance[6].

TB is a completely curable disease, however, even after several years of research, the therapeutic scheme still presents challenges[5, 6, 8, 12, 13, 14]. Some of the main difficulties are side effects, drug interactions, long-term treatment, microbial resistance, aggravating of other comorbidities and discontinue rates[5, 6, 12, 13, 14]. Due to the increase in cases around the world and the problems involved in drug treatment, WHO has developed and has implemented, since the beginning of the 1990s, a program called Directly Observed Treatment (DOT)[6, 15, 16, 17]. The program covers political and financial support, development and standardization of diagnostic methods and continuous monitoring of treatment progress[6, 15, 16, 17]. In addition, it ensures direct observation of medication intake and builds a relationship of trust between the patient and health professionals, and contributes to decentralization of public policies and increases treatment adherence[6, 15, 16, 17].

The currently international basic therapeutic regimen of tuberculosis consists of four medicines: Rifampicin (Rifaldin®), Isoniazid (Fluodrazin®), Pyrazinamide (Pirazinon®) and Ethambutol (Myambutol®)[6, 16, 17, 18]. The treatment takes at least 6 months[6, 16, 17, 18] and has several adverse effects, including breathing difficulties, extreme vertigo or collapse, vision disturbances, skin rashes, diarrhea, non-usual bleeding, hepatotoxicity, pancreatitis and psychiatric disorders[18]. Long-term treatment, several side effects and bacterial resistance contributes to therapeutic abandonment and highlights the needs for new anti-TB drugs[5, 8, 19]. To effectively eradicate the disease, it is necessary substantial investment in social actions, public policies and scientific knowledge to enable great dominance over the etiologic agent and the epidemiology of infection[17, 20].

In addition to the political and social challenges that limit the development of new anti-TB drugs, some morphological characteristics inherent to the microorganism are also important factors. The presence of mycolic acid and other polysaccharides, as arabinoglycan and peptideoglycan, in the cell wall of the microorganism are determinants for greater resistance to antimicrobials[2]. These components confer high lipophilicity and low permeability to the membrane, thus contributing to its resistance mechanism against the host's immune system and against anti-TB drugs[2].

When exploring new molecules with potential anti-TB action, quinazolines stand out as a promising class of compounds which has already conquered space in therapeutic scenario. Drugs as Afatinib (Giotrif ®), used in lung cancer treatment[21], Methaqualone (Quaalude ®), a central nervous system depressant[22], and Lapatinib (Tykerb®), used in breast cancer treatment[23], are some examples of its efficiency. Quinazolinic derivatives showed notorious activities in pre-clinical studies as anticancer[24], antifungal[24], anti-inflammatory[24], antileishmanial[25], antibiolfim[26] and antibacterial[24, 27], including studies against *Mycobacterium* gender [24, 26] and *M. Tuberculosis* species [19,24, 27, 28]. The protein targets were explored to clarify the antimicrobial activity of quinazolinic derivatives. Lu, W. *et al* studies identified inhibitory activity of quinazolinic compounds against Acetohydroxyacid synthase (AHAS), an important bacterial enzyme involved at biosynthetic route of branched-amino acids[29]. The dihydrofolate reductase (DHFR) enzyme, another important bacterial protein responsible for nucleic acid synthesis, has also been shown to be inhibited by a new class of derivatives of diaminoquinazoline[30]. Gawad, J. and Bonde, C. identified an interesting bioactivity of a novel series of quinazoline-2-carboxamide derivatives against decarprenyl-phosphoryl-b-D-ribose-2-epimerase (DprE1) enzyme[31]. DprE1 is accountable for Arabinose biosynthesis, an essential monosaccharide in *Mycobacterium* cell wall[31].

In another line of research developed in our group, compound **PH100**, a quinazolinic derivative, was identified. This compound was synthesized and its microbial activity was evaluated. **PH100** demonstrated promising antibacterial and antibiofilm activities against *Staphylococcus aureus* and *Staphylococcus epidermidis*[32]. Based on these findings extracted from the scientific literature and the antibacterial activity previously identified by our research group, we designed a new series of quinazolinic derivatives, using the **PH100** as prototype, aiming to investigate and evaluate their activities against *Mycobacterium Tuberculosis.*

2. CHEMISTRY

In this work, the synthesis of five quinazolinic derivatives was performed, using **PH100** as the prototype of the series. The synthesis followed the same pattern described in Van Horn *et al* 2014[25]. Using 2,4-dichloroquinazoline (**1**) as the starting material of the route, it was necessary to accomplish an Aromatic Nucleophilic Substitution reaction (ANSr) to obtain, in two steps, **PH100** and compounds **3a-c** and, in a single step, compound **3d** (Fig. 1).

The reaction starts with compound **1** undergoing a nucleophilic attack in position 4 by the amine corresponding to the R_1 of each compound (**PH100** and **3a-c**). The chemical

mechanism goes through an amine addition step at position 4 followed by a chloride elimination step, resulting in the synthesis of 4-amino-2-chloro-quinazoline (**2**).

To obtain **PH100**, **3a** and **3b**, intermediate **2** was treated directly with the appropriate amine for each compound in ethanol, accomplishing the substitution in R_2, which synthesis mechanism is similar to the substitution with R_1 described above. On the other hand, compound **3c** was obtained by performing a Buchwald-Hartwig amination to couple the substituent on compound **2**[33] previously catalyzed by $Pd(OAc)_2$. The complex formed by oxidative addition undergoes deprotonation of the amine, with the aid of appropriate base. At the same time, the reductive elimination of Pd and consequent compound **3c** formation occurs.

At last, compound **3d** was obtained in a single step starting from **1** in an ANSr with 2 equivalents of its substituent. The compound **1** was treated directly with the appropriate amine and follows the same mechanism described in the first step, with electron reallocation and elimination of chloride.

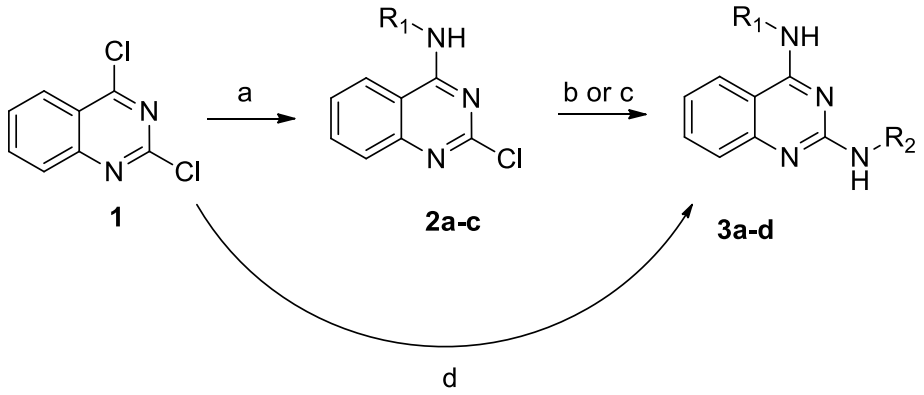
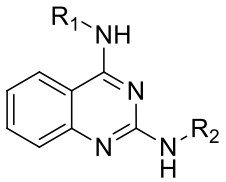


Figure 1. Synthetic procedures to obtain **PH100** and **3a-d**. *Reagents and conditions:* a) appropriate amine, sodium acetate, THF:H_2O, 65 ºC, 62%; b) appropriate amine, EtOH, 120 ºC, 84-88%; c) appropriate amine, $Pd(OAc)_2$, appropriate phosphine, appropriate base, DMF, 140 ºC, 41%; d) aniline, EtOH, 120 ºC, 18 h, 38%.

3. BIOLOGICAL EVALUATION

All synthesized compounds were evaluated against *M. tuberculosis* strain H37Rv ATCC 27294 using isoniazid (INH) as positive control. The antimicrobial evaluation is expressed by MIC - the Minimum Inhibitory Concentration, which prevents microbial growth. The results are listed at Table 1.



3a-d

Figure 2. Quinazolinic general structure of synthesized derivatives.

Table 1. Biological Evaluation against *M. tuberculosis* strain H37Rv.

Compound	MIC (µg/mL)
PH100	5
3a	>40
3b	2.5
3c	>40
3d	>20
Isoniazid	0.31

According to Table 1, the prototype **PH100** presented a MIC of 5 µg/mL. Compound **3d**, an analogue of **PH100**, had a loss in its activity and showed a MIC of 20 µg/mL. Compound **3a** and **3c** presented MIC higher than 40 µg/mL. Compound **3b** proved to be the most active of the series, with a MIC of 2.5 µg/mL. Designed as another analogue to its prototype **PH100**, **3b** has been shown to interact with the binding site more efficiently, resulting in a 2-fold increase in inhibition activity.

4. BIOAVAILABILITY

As a theoretical-practical basis to planning and performing the structural modifications presented in this study, Lipinski's “Rule of 5” was applied. It is a didactic approach widely used in research and development of new drugs which predicts oral bioavailability[34]. Based on Lipinski *et. al.* findings, a molecule will present good absorption or permeation if it has 5 or less hydrogen bond donors, 10 or less hydrogen bond acceptors, molecular weight less than 500 g/mol and the calculated LogP (CLogP) less than 5[34].

Another parameter evaluated was the Topological Polar Surface Area (TPSA), an important factor in transport across membranes and consequent bioavailability[35]. TPSA provides the result of the total area of polar atoms in a molecule and it is closely associated in

intestinal absorption[36]. It is assumed that a TPSA's value less than 140 Å represents a promising transport across membranes[36].

Table 2 exposes the results for each Lipinski parameter, bioavailability and TPSA evaluated using the SwissADME free web tool. This software is widely used to easily predict the *in silico* properties of compounds, including pharmacokinetic parameters, druglike properties, chemical-biological compatibility and physical-chemical characteristic[37]. The web tool operates using several Computer-Aided Drug Designs (CADD), which includes appliance of ligand-based drug design, molecular mechanics, biological target forecast, bioisosteric replacement and molecular coupling[37].

Table 2. Structural Evaluation using SwissADME.

Compound	HBD	HBA	Molecular weight (g/mol)	LogP	Bioavailability Score	TPSA (Å)
PH100	2	2	326.39	4.16	0.55	49.84
3a	3	3	308.38	2.96	0.55	70.07
3b	2	3	330.36	4.51	0.55	49.84
3c	3	5	407.47	2.47	0.55	99.61
3d	2	2	312.37	4.23	0.55	49.84

According to Table 2, every synthesized and studied derivative attended to "Rule of 5", resulting in a score of 0.55 for bioavailability, which describes a probability that a compound will have >10% bioavailability in pre-clinical studies in rats[38]. All derivatives also presented a TPSA value within specified range, predicting an efficient transport across membranes. These data support the fact quinazolinic derivatives are a promising class of therapeutic drugs and its scientific research should be encouraged.

5. DISCUSSION

Although the number of derivatives and the physico-chemical constants and properties evaluated are limited, some qualitative trends are illustrated below. Further complementary studies will determine the definitive SAR.

Compounds **3a** and **3c** showed to be less active against *M. tuberculosis*, evidencing that lipophilicity can be a determining factor for the antimicrobial activity of the molecule. In

addition to the highest MIC of series, compounds **3a** and **3c** are also the least lipophilic, with a LogP of 2.96 and 2.47, respectively. The TPSA parameter can also explain the lower activity of theses derivatives. Presenting the highest values of TPSA, 70.07 Å for **3a** and 99.61 Å for **3c**, this physical-chemical characteristic may be related to an inefficient connection with the binding-site.

Derivative **3b** proved to be 2-fold more potent than its **PH100** prototype, presenting the lower MIC of the series. **PH100** and **3b** proved that high lipophilicity, represented by LogP 4.16 and 4.51 respectively, is directly associated with inhibitory activity. Besides that, both derivatives also evidenced that lower values of TPSA can be positively related with antimicrobial activity through greater interaction with the active site of the microorganism.

Compound **3d** can be considered an outlier of the series. Even presenting a high LogP and a low TPSA value, the derivative showed a lower inhibitory capacity when compared with **PH100** and **3b**. As described before, Lipinski's rule is a tool of great pharmacokinetic relevance which predicts bioavailability, but does not ensure activity by itself. These data can be explained by several factors associated to the interactions between the microorganism and the compound, which are not covered by the scope of this rule.

Drawing a parallel between the results obtained in this work and in the literature findings, it is possible to infer that the derivatives here explored, mainly **PH100** and **3b**, showed up an effectiveness comparable to other quinazolinic compounds already studied. When Lu, W. *et al* investigated antituberculosis activity targeting acetohydroxamic synthase, they found MIC values, using the same H37Rv, equivalent to those found in this present study, where the most effective derivative had a MIC of 2.5 mg/L[29]. Gawad, J. and Bonde, C. investigation presented a potential DprE1 inhibition of a quinazoline-carboxamide derivative series with a MIC range between 0.96 µM/ml to 3.40 µM/ml, also for H37Rv[31], corroborating once again with the results exposed in this study. Despite Li, X. *et al* evaluated the activity of diaminoquinazolines derivatives against DHFR enzyme present in *S. aureus, E. faecalis, H. influnezae* and *E. coli*[30], it is known that this protein also composes *Mycobacterium tuberculosis* molecular structure and it is the molecular target for Isoniazide[39]. Assuming this similarity and the broad antibacterial activity, it is possible to support the results determined by our research group with the findings of Li, X *et al*, which the MIC range found by the author was between 0.03 µg/mL and >64 µg/mL[30].

6. CONCLUSION

Even after several years of *M. tuberculosis* identification and isolation, Tuberculosis is still a public health problem worldwide. Development of medicines, vaccines, methods of diagnosis, continuous mass monitoring and commitment from governmental and non-governmental institutions has contributed to improve the disease control and patient's quality and life expectancy. Nevertheless, TB remains among the top 10 causes of death in the world. The epidemiological data highlighted in this work show a long journey that must still be taken to reduce or eradicate the disease. The expansion of antibiotic arsenal as well as the improvement and investment in public health policies are crucial to reduce transmission and consequent deaths caused by the disease.

After exploring the quinazolinic compounds, it was possible to identify new derivatives with tuberculostatic activity. A series of 5 compounds was synthesized and submitted to microbiology evaluation to appraise its MIC against *M. tuberculosis*. Compounds **3a** and **3c**, which have similarity of being the least lipophilic in the series, presented a MIC >40 µg/mL. Derivative **3d**, despite having good molecular characteristics and bioavailability, showed a MIC >20 µg/mL, proving itself to be an outlier. Compound **3b** and **PH100**, the prototype of the series, were the most active derivatives. With MIC of 2.5 µg/mL and 5 µg/mL, respectively, both derivatives demonstrated to be promising antituberculostatic agents. Assessing its molecular characteristics using the SwissADME tool, it was possible to infer that high lipophilicity and lower values of TPSA are associated with inhibitory activity and it may be related with a better interaction with the active site of the microorganism.

The preliminary results produced and discussed in this article represent the beginning of a novel quinazolinic series to be deeply investigated. These promising outcomes are important to provide better acquaintance about this class, its effects on *M. tuberculosis* and on human body (through the assessment of its bioavailability).

7. PERSPECTIVES

Once quinazolinic compounds with promising activity against *M. tuberculosis* were identified, the need for a thorough evaluation of their derivatives is required. With the results of this study, the future perspective is to develop a new series based on the most promising results achieved on this initial study, increase the discussion about their structure-activity

relationship and improve their chemical structures in order to identify new derivatives with similar or even more potent inhibitory activity.

8. EXPERIMENTAL SECTION

8.1 General

In this work, solvents and reagents were used without any purification, obtained commercially from Sigma-Aldrich, Synth, Fluka and Merck.

8.2 Chemistry

8.2.1 General procedure to obtain **N**-substituted-2-chloroquinazolin-4-amine (**2**)

In the first step sodium acetate (0.226 g, 2.76 mmol) and amine (2.76 mmol) were added to a solution of 2,4-dichloroquinazoline **1** (0.5 g, 2.51 mmol) in THF/H_2O (3:1) (15 mL). The reaction was kept under stirring for 6 h at 65 °C. Completed this period, the mixture was cooled and diluted with ethyl acetate (40 mL) and water (40mL). Using a separatory funnel, the organic layer was segregate and washed with water (2x40 mL). To remove residual water, Na_2SO_4 was added. After, the organic layer was filtered, distilled under reduced pressure and the residue was purified through silica gel column chromatography using as eluent a solution of cyclohexane:ethyl acetate.

2a-c obtained with 62% yield.

8.2.2 General procedure for the preparation of quinazolinic compounds (**PH100-3a-b**)

First, the appropriate amine (1.5 eq.) was added under stirring to a previously synthesized solution of **N**-substituted-2-chloroquinazolin-4-amine (1 eq.) in 2 mL of 0.4 M ethanol and heated to 120 °C for 3 h. In the sequence, after cooling the solution, the ethanol was evaporated, and the product was resuspended in 20 mL of ethyl acetate and 20 mL of a saturated aqueous solution of $NaHCO_3$. Using a separatory funnel, the organic layer was segregated and washed with 20 mL of water. To remove residual water, Na_2SO_4 was added to the organic layer. After, it was filtered, distilled under reduced pressure and the residue was purified through silica gel column chromatography using as eluent a solution of cyclohexane:ethyl acetate.

PH100 obtained as 0.178 g of a yellow solid, 81% yield.

3a obtained as 0.024 g of a yellow oil, 84% yield.

3b obtained as 0.0265 g of a white solid, 88% yield.

8.2.3 General procedure for the preparation of quinazolinic compound **3c**

Using a solution of *N*-substituted-2-chloroquinazolin-4-amine (1 eq.) in toluene or DMF at 0.06 M, palladium acetate (II) (0.007 eq), appropriate phosphine (0.007 eq), appropriate amine (1.5 eq) and appropriate base (2 eq) were added, with stirring. The reaction was set to maintain 140 °C and N_2 atmosphere for 18 h. After completing this period, it was cooled and diluted with ethyl acetate (20 mL). The organic layer was segregated and washed with 20 mL of water. To remove residual water, Na_2SO_4 was added to the organic layer, then it was filtered and concentrated under reduced pressure. The product synthesized was purified by silica gel column chromatography using as eluent a solution of cyclohexane:ethyl acetate.

3c obtained as 0.0217 g of a white solid, 63% yield.

8.2.4 General procedure for the preparation of quinazolinic compound **3d**

To a stirred solution of 2.4-dichloroquinazoline **1** (0.5 g, 2.51 mmol) in THF/H_2O (3:1) (15 mL), the appropriate amine (2 eq.) was added. The reaction was kept under stirring for 6 h at 65 °C. After the 6 h reaction ended, the mixture was cooled and diluted with ethyl acetate (40 mL) and water (40 mL). In the sequence, the organic layer was segregated and washed twice with 40 mL of water. To remove residual water, Na_2SO_4 was added, then it was filtered and concentrated under reduced pressure. The residue formed was purified through silica gel column chromatography using as eluent a solution of cyclohexane:ethyl acetate.

3d obtained as 0.0589 g of a white solid, 38 % yield.

8.3 Biological Evaluation

M. tuberculosis inhibition assay was performed as described in Macchi *et al* 2018[40]. The minimum inhibitory concentration (MIC) was determined in triplicate using *M. tuberculosis* H37Rv ATCC 27294 strains (American Type Culture Collection, Rockville, Md.) by broth microdilution method. Its results were interpreted by color changes, assuming that the blue color indicates absence of microbial growth and the pink color reveals the presence of bacterial

proliferation[41]. The method aims to quantitatively assess the susceptibility of the microorganism to antibiotics and it is mainly used for multiresistant bacteria[41, 42].

The strains were grown in Middlebrook 7H9 broth containing 10% OADC (oleic acid, albumin, dextrose and catalase) and 0.05% tween 80. After growth, mycobacterial suspension was vortexed for 5 min, rested for 20 min, and the supernatants formed were spectrophotometrically evaluated at an absorbance of 600nm. Ultimately, microbial suspensions were stored at -20 °C[40].

The inhibition assay was accomplished in 96 well U-bottom polystyrene microplates. Isoniazid (INH), employed as positive control, was prepared in concentrations of 1mg/mL in neat dimethylsulfoxide (DMSO), as well as **PH100**, **3a**, **3b**, **3c**, and **3d** test solutions. Each solution was diluted in Middlebrook 7H9 broth with 10% ADC to a concentration between 10 and 50 µg/mL with 5% DMSO. In sequence were performed a duplicate serial dilution, directly in microplates, of each compound in 100 µL of Middlebrook broth with 10% ADC. Concentrations ranges were set between 25.0 and 0.05 µg /mL, 10.0 and 0.02 µg /mL, or 0.02 and 0.0004 µg /mL[40].

Each microbial suspension, previously prepared and stored, were accurately diluted in Middlebrook 7H9 medium 10% ADC until reaching an optical density of 0.006 at 600nm. After, 100 µL of this preparation were aliquoted, transferred to microplate wells which contained the test solutions, except sterile control wells, and had its DMSO concentration set at 2.5%. The microplates were covered, sealed, and incubated at 37 °C for 7 days. Elapsed this period, 60 µL of 0.01% resazurin solution, a cell viability indicator responsible for broth color change[41], was added to each microwell and then returned to the 37 °C incubator for another 48 h. The assay was completed after evaluating the broth changes its color. Growth controls and sterility controls were included in the procedure[40].

9. DECLARATION OF COMPETING INTEREST

The author declares that there is no competing financial, conflict of interests nor personal relationships that influence the publication of this paper.

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12. REFERENCES

1. Opromolla, D.V.A.; Baptista, I.M.F.; Nocões de hansenologia. **Bauru: Centro de Estudos Dr. Reynaldo Quagliato.** 2000. ID: lil-642153.
2. Silva, J. M. I. The composition of the bacillus cell wall, which includes mycolic acid and other complex lipids, provides slow growth and greater resistance to antimicrobials. **UFRJ/COPPE**. Rio de Janeiro. 2013.
3. Malaquias, A.G. The protagonist microbe: notes on the communication of bacteriology in the Gazeta Médica da Bahia journal, nineteenth century. **Hist. cienc. Saude – Manguinhos.** 23, 7-14. 2016. Doi:10.1590/S0104-59702016005000015
4. Barberis , N.L. Bragazzi , L. Galluzzo , M. Martini. The history of tuberculosis: from the first historical records to the isolation of Koch's bacillus. **J. Prev. Med. Hyg.** 58, 9-12. 2017.
5. Pereira, A.M. Georreferenciamento e Genotipagem de *Mycobacterium tuberculosis* isolados de pacientes atendidos na cidade de Goiânia – GO pelo método de MIRU-VNTR. **Biblioteca Digital de Teses e Dissertações UFG**. 2012. CDU: 616.24-002.5(817.3)
6. Global tuberculosis report 2020, World health organization, Available from: https://www.who.int/tb/global-report-2020 (accessed in February 2021).
7. Donoghue, H.D.; Lee, O.Y.C.; Minnikin, D.E.; Besra, G.S.; Taylor, J.H; Spigelman, *M. Tuberculosis* in Dr Granville's mummy: a molecular re-examination of the earliest known Egyptian mummy to be scientifically examined and given a medical diagnosis. **Proc. R. Soc. B.** 277, 51–56. 2009. DOI:10.1098/rspb.2009.1484.
8. Ducati, R.G.; Ruffino-Netto, A.; Basso, L.A; Santos, D.S. The resumption of consumption: A review on tuberculosis. **Mem Inst Oswaldo Cruz**. 101, 697-714. 2006. DOI: 10.1590/S0074-02762006000700001.
9. Barros, P.G.; Pinto, M.L.; Silva, T.C.; Silva, E.L.; Ribeiro, T.M.; Figueiredo, M. Epidemiological Profile of Extra-pulmonary Tuberculosis cases in a municipality of Paraiba state. **Cad. saúde colet. [online].** 2014. DOI: 10.1590/1414-462X201400040007.
10. Ferraz, J.C.; Melo, F.B.S.;Albuquerque, M.F.P.M; Montenegro, S.M.L.; Abath, F.G.C. Immune factors and immunoregulation in tuberculosis. Braz J Med Biol Res. 39, 1387-1397. 2006. DOI: 10.1590/S0100-879X2006001100002
11. Bianchi, M.S.; Gonçalves, C.V.; D'avila, N.E.M.; Tornatore, M.; Martins, D. Tuberculose congênita: relato de um caso. **Vittalle.** 21, 15-22. 2009.
12. Rabahi, M.F.; Júnior, J.L.S.; Ferreira, A.C.G.; Silva, D.G.S.T.; Conde, M.B. Tratamento da tuberculose. **J Bras Pneumol**. 43(5):472-486, 2017. DOI: 10.1590/s1806-37562016000000388
13. Silva, M.E.N.S.; Lima, D.S.; Santos, J.E.; Monteiro A.C.F.; Torquato, A.M.M; Freire, V.A.; Ribeiro, D.B.C.R; Feitosa, A.C.S; Teixeira, A.B. General aspects of tuberculosis: an update on the etiologic agent and treatment. **Rev. Bras. Anal. Clin.** 44, 332-338. 2018. DOI: 10.21877/2448-3877.201800717
14. Daniel, T.M. The history of tuberculosis. **J. Prev. Med.** 100, 1862-1870. 2017. DOI: 10.1016/j.rmed.2006.08.006.
15. Maciel, E.L.N.; Guidoni, L.M.; Favero, J.L.; Hadad,D.J.; Molino, L.P.; Jonhson, J.L.; Dietze. R. Adverse effects of the new tuberculosis treatment regimen recommended by the

Brazilian Ministry of Health. **J. bras. pneumol.** 36. 2010. DOI: 10.1590/S1806-37132010000200012.
16. Junges, J.R.; Burille, A.; Tedesco, J. Directly Observed Therapy for treating tuberculosis: critical analysis of decentralization. **Interface.** 24. 2020. DOI: 10.1590/interface.190160.
17. Manual of recommendations for tuberculosis control in Brazil. Brasília: Ministry of Health, 2011.
18. Farmanguinhos Rifampicin + Isoniazide + Pyrazinamide + Ethambutol®. [**leaflet**]. Lupin LTD, India.
19. Gatadi, S.; Gour, J.; Shukla, M.; Kaul, G.; Dasgupta, A.; Madhavi, Y.V.; Chopra, S.; Nanduri, S. Synthesis and evaluation of new quinazolin-4(3H)-one derivatives as potent antibacterial agents against multidrug resistant *Staphylococcus aureus* and *Mycobacterium tuberculosis*. **Eur J Med Chem.** 175, 287-308, 2019.
20. National Plan to End Tuberculosis as a Public Health Problem. Brasília: Ministry of Health, 2017.
21. Giotrif®. [**leaflet**]. Boehringer Ingelheim Pharma GmbH & Co. KG, Germany.
22. Drugs.com [Internet]. Quaalude Information from Drugs.com; c2000-2021 [Updated: 03 March 2021, Cited: 21 March 2021 https://www.drugs.com/illicit/quaaludes.html.
23. Tykerb®. [**leaflet**]. Glaxo Operations UK Limited, United Kingdom.
24. Asif, M. Chemical Characteristics, Synthetic Methods, and Biological Potential of Quinazoline and Quinazolinone Derivatives. **Inter J of Med Chem.** 2014, 395637. 2014. doi:10.1155/2014/395637
25. Van Horn, K.S.; Zhu, X.; Pandharkar, T.; Yang, S.; Vesely, B.; Vanaerschot, M.; Dujardin, J.; Rijal, S.; Kyle, D. E.; Wang, M.Z.; Karl A. Werbovetz, K.A.; Manetsch, R. Antileishmanial Activity of a Series of N2,N4 Disubstituted Quinazoline-2,4-diamines. **J. Med. Chem.** 57, 5141–5156, 2014.
26. Karlie E. Cox, K.E.; Melander, C. Anti-biofilm activity of quinazoline derivatives against Mycobacterium smegmatis. **Med. Chem. Comm.** 10, 1177-1179. 2019. DOI: 10.1039/c9md00156e
27. Odingo, J.; O'Malley, T.; Kesicki, E. A.; Alling, T.; Bailey, M. A.; Early, J.; Ollinger, J.; Dalai, S.; Kumar, N.; Singh, R. V.; Hipskind, P. A.; Cramer, J. W.; Ioerger, T.; Sacchettini, J.; Vickers, R.; Parish, T. Synthesis and evaluation of the 2,4-diaminoquinazoline series as anti-tubercular agents. **Bioorg Med Chem.** 22, 6965–6979, 2014.
28. Asquith, C. R. M.; Fleck, N.; Torrice, C. D.; Crona, D. J.; Grundner, C.; Zuercher, W. J. Anti-tubercular activity of novel 4-anilinoquinolines and 4-anilinoquinazolines. **Bioorg Med Chem. Let.** 29, 2695–2699, 2019.
29. Lu, W.; Baig, I.A.; Sun, H.; Cui, C.; Guo, R.; Jung, I.; Wang, D.; Dong, M.; Yoon, M.; Wang, J. Synthesis, crystal structure and biological evaluation of substituted quinazolinone benzoates as novel antituberculosis agents targeting acetohydroxyacid synthase. **Eur J Med Chem.** 94, 298-305, 2015.
30. Li, X.; Hilgers, M.; Cunningham, M.; Chen, Z.; Trzoss, M.; Zhang, J.; Kohnen, L.; Lam, T.; Creighton, C.; Kedar, G.C.; Nelson, K.; Kwan, B.; Stidham, M.; Brown-Driver, V.; Shaw, K. J.; Finn, J. Structure-based design of new DHFR-based antibacterial agents: 7-aryl-2,4-diaminoquinazolines. **Bioorganic & Medicinal Chemistry Letters**. 21 (18), 5171-5176, 2011. DOI: 10.1016/j.bmcl.2011.07.059
31. Gawad, J.; Bonde, C. Design, synthesis and biological evaluation of novel 6-(trifluoromethyl)-N-(4-oxothiazolidin-3-yl)quinazoline-2-carboxamide derivatives as a potential DprE1 inhibitors. **Journal of Molecular Structure.** 1217, 2020. DOI: 10.1016/j.molstruc.2020.128394.

32. dos Reis, S.V.; Ribeiro, N.S.; Rocha, D.A.; Fortes, I.S.; Trentin D.S.; Andrade, S.F.; Macedo, A.J. N^4-benzyl-N^2-phenylquinazoline-2,4-diamine compound presents antibacterial and antibiofilm Effect Against *Staphylococcus aureus* and *Staphylococcus epidermidis*. **Chem Biol Drug Des.** 2020. doi: 10.1111/cbdd.13745. Online ahead of print.
33. Zhang, G.; Wang, X.; Li, C.; Yin, D. Palladium-catalyzed cross-coupling of Electron-deficient heteroaromatic amines with heteroaryl halides. **Synthetic Communications.** 43, 456–463, 2013.
34. Lipinski, C.A.; Lombardo, F.; Dominy, B.W.; Feeney, P.J. Experimental and computational approaches to estimate solubility and permeability in drug discovery and development settings. **Advanced Drug Delivery Reviews**. 23, 3–25.1, 1997. DOI:10.1016/S0169-409X(00)00129-0.
35. Fernandes, J.; Gattass, C. R. Topological Polar Surface Area Defines Substrate Transport by Multidrug Resistance Associated Protein 1 (MRP1/ABCC1). **J. Med. Chem**. 52, 1214–1218, 2009.
36. Palm, K., Stenberg, P., Luthman, K. *et al*. Polar Molecular Surface Properties Predict the Intestinal Absorption of Drugs in Humans. **Pharm Res**. 14, 568–571, 1997. DOI: 10.1023/A:1012188625088
37. Daiana, A.; Michielin, O.; Zoete, V. SwissADME: a free web tool to evaluate pharmacokinetics, druglikeness and medicinal chemistry friendliness of small molecules. **Nature**. 7:42717, 2017. DOI: 10.1038/srep42717
38. Martin, Y. C. A Bioavailability Score. **J. Med. Chem.** 48, 3164-3170, 2005. DOI: 10.1021/jm0492002.
39. Argyrou, A.; Vetting, M. W.; Aladegbami, B.; Blanchard, J.S. *Mycobacterium tuberculosis* dihydrofolate reductase is a target for isoniazid. **Nature Structural & Molecular Biology.** 13, 408-413, 2006. DOI: 10.1038/nsmb1089
40. Macchi, F.S.; Pissinate, K.; Villela, A.D.; Abbadi, B.L.; Rodrigues-junior, V.; Nabinger, A.D.; Altenhofen, S.; Sperotto, N.; Dadda, A.S.; Subtil, F.T.; Freitas, T.F.; Rauber, A.P.E.; Borsoi, A.F.; Bonan, C.D.; Bizarro, C.V.; Basso, L.A.; Santos, D.S.; Machado, P. 1H-Benzo[d]imidazoles and 3,4-dihydroquinazolin-4-ones: Design, synthesis and antitubercular activity. **Eur J Med Chem.** 155, 153-164, 2018. DOI: 10.1016/j.ejmech.2018.06.005
41. Borra, R.C.; Lotufo, M.A.; Gagioti, S.M.; Barros, F.M.; Andrade, A.M. A simple method to measure cell viability in proliferation and cytotoxicity assays. **Braz Oral Res.** 23(3), 255-62, 2009. DOI: 10.1590/S1806-83242009000300006
42. Methods for diluition antimicrobial susceptibility test for bacteria that grow aerobically. 7th ed. **CLSI approved standard M7–A7**. Pennsylvania; 2006.

13. ATTACHMENT

The characterization results of synthesized compounds were not described in this work due to the secrecy of the molecules and to preserve the originality of the study.