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Research paper

Synthesis and anti-*Mycobacterium tuberculosis* activity of imide-β-carboline and carbomethoxy-β-carboline derivatives

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ABSTRACT

A series of methyl β-carboline carboxylates (**2a-g**) and of imide-β-carboline derivatives containing the phthalimide (**4a-g**), maleimide (**5b**, **g**) and succinimide (**6b, e, g**) moiety were synthesized, and evaluated for their activity against *Mycobacterium tuberculosis* H_{37}Rv. The most active β-carboline derivatives against the reference strain were assayed for their cytotoxicity and the activity against resistant *M. tuberculosis* clinical isolates. Farther, structure-activity relationship (SAR) studies were carried out using the three and four-dimensional approaches for starting to understand the way of β-carboline activity in *M. tuberculosis*. All 19 β-carboline derivatives were assayed, firstly, by determining the minimum inhibitory concentration (MIC) using resazurin microtiter assay plate (REMA) in *M. tuberculosis* H_{37}Rv. Then, five derivatives (**2c**, **4a**, **4e**, **4g**, **6g**), which showed MIC $\leq$ 125 μg/mL, were assayed in nine resistant *M. tuberculosis* clinical isolates (five MDR, three isoniazid monoresistant and one isoniazid plus streptomycin resistant). The MIC values against the resistant clinical isolates ranged from 31.25 to >250 μg/mL. All five derivatives were non-cytotoxic to the VERO cell line, determined by 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide, at the tested concentration (selectivity index ranged from <1.74 to 14.4). Our study demonstrated that (**2c**) and (**6g**) derivatives had better anti-*M. tuberculosis* activity, especially against resistant clinical isolates, what makes them scaffold candidates for further investigations about their anti-tuberculosis activity. The SAR study conducted with the 19 β-carboline derivatives showed the importance of steric effects for the synthesized β-carbolines against *M tuberculosis*, and these models can be used for future proposition of new derivatives, increasing the chances of obtaining potentially anti-tuberculosis compounds.

1. Introduction

Tuberculosis still remains a serious public health problem, and is responsible for the major number of deaths by infectious diseases worldwide. In 2018, it was estimated that approximately 10 million people developed tuberculosis, and 1.2 million died due to the

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disease [1].

The chemotherapeutic agents for treating patients with tuberculosis have been the same since the 70's. The current treatment consists in 2 months of a combination of isoniazid (INH), rifampicin (RIF), ethambutol (EMB), and pyrazinamide (PZA), known as the attack phase, which is intended to reduce the patient bacterial load. In the sequence, there is the maintenance phase, composed of 4 months of INH and RIF, to avoid disease recurrence or reactivation. It is a time-consuming treatment with adverse reactions and drug-drug interactions, what contributes to the lack of patients adhesion to complete the treatment, and, consequently, increase of *Mycobacterium tuberculosis* resistance [2].

The increase of resistance added of multidrug-resistant (MDR-tuberculosis), extensively drug-resistant (XDR-tuberculosis), and the recent cases of totally-resistant strains (TDR-tuberculosis) are a threat to disease control, which demands urgent development of new and more effective drugs. One of the newest anti-tuberculosis drug, bedaquiline, was approved in the market to treat resistant disease cases, however resistant bacilli lineages to this drug have already been reported [2].

The search for new natural or synthetic compounds that inhibit the *in vitro* and *in vivo M. tuberculosis* growth is of paramount importance for choosing potential candidates for new anti-tuberculosis drugs.

Following this line of thinking, the well-known natural or synthetic compounds of the β-carboline class, have been studied for their biological activities [3]. The β-carboline alkaloids were primarily isolated from *Peganum harmala,* an African plant from Zygophillaceae family, which is used in traditional medicine [3] and has several biological activities, including antimicrobial [4,5], antiviral [6], antiparasite [7,8], and antiproliferative [3,9,10]. Besides that, some reports have shown their activities against *Mycobacterium intracellulare* [11], *Mycobacterium smegmatis* [12], and also in *M. tuberculosis* H_{37}Rv [12–14]. In *M. tuberculosis* H_{37}Rv, these compounds displayed significant activity with minimum inhibitory concentration (MIC) below 6.25 μg/mL, and selectivity towards the bacillus [14].

Previous studies of our research group demonstrated that 1-substituted-phenyl-β-carbolines, with an amino or guanidinium group-terminated side chain at C-3, showed *in vitro* activity against *M. tuberculosis* H_{37}Rv (ATCC 27294), with MIC values ranging from 24.9 to 58.3 μg/mL [15].

Besides β-carbolines, cyclic imides containing the phthalimide, maleimide or succinimide subunits, have been described as growth inhibitors of *M. tuberculosis* [16–20]. In these studies, a series of phthalimide derivatives evaluated against *M. tuberculosis* H_{37}Rv, showed to be effective against the bacillus with minimum inhibitory concentration (MIC) ranging from 3.9 to 12.5 μg/mL [17,18,20], and were considered new lead compounds to be studied for the treatment of susceptible and multidrug resistant tuberculosis [20].

Taking into account, the anti-*M. tuberculosis* activity showed by some β-carbolines and cyclic imide derivatives in the literature, in the present study, we synthetized and evaluated the activity of other novel imide-β-carboline derivatives against *M. tuberculosis* H_{37}Rv. Intermediates methyl β-carboline-3- carboxylates were also assayed for their anti-tuberculosis activities. In addition, some of the β-carboline derivatives with higher anti-*M. tuberculosis* H_{37}Rv activity were assayed for their cytotoxicity and the activity against resistant *M. tuberculosis* clinical isolates. Structure-activity relationship (SAR) studies were carried out in order to increase the understanding about the mechanism of action of β-carboline derivatives as anti-*M. tuberculosis* compounds.

2. Results and discussion

2.1. Chemistry

The methodology for the synthesis of 1-substitutedphenyl imide-β-carboline derivatives **4**–**6** containing a phthalimide (**4**), maleimide (**5**), and succinimide (**6**) moieties at C-3 is outlined in Scheme 1. The 1-substituted β-carboline-3-carbohydrazides **3a**-**g**, intermediates for the preparation of compounds **4**–**6**, were prepared by the Pictet-Spengler reaction of L-tryptophan methyl ester **1a** with aromatic aldehydes containing electron donating and electron withdrawing groups, followed by oxidation of 1,2,3,4-tetrahydro-β-carbolines obtained with sulfur, in xylene, to give the corresponding methyl β-carboline-3-carboxylates **2a**-**g** [9]. The treatment of **2a**-**g** with hydrazine hydrate, as described in our previous study, afforded the carbohydrazides **3a-g** [9].

The β-carboline-3-N-(1,3-dioxo-1,3-dihydro-isoindol-2-yl)-carboxamides (**4a-g**) were obtained from the reaction of carbohydrazides **3a**-**g** with phthalic anhydride, under reflux in xylene, according the methodology described by Roston et al. [21]. The formation of **4a-g** was evidenced by the presence of aromatic hydrogens at δ_H 7.75–8.07, in the ^{1}H NMR spectra, and of the additional signals at δ_C 129.4–129.5 (C-2a''/C-6a''), 123.8–123.9 (C-3''/C-6''), 135.3–135.5 (C-4''/C-5'') and δ_C 165.3–165.5 (C=O), in the ^{13}C NMR spectra, which were assigned to the phthalimide moiety.

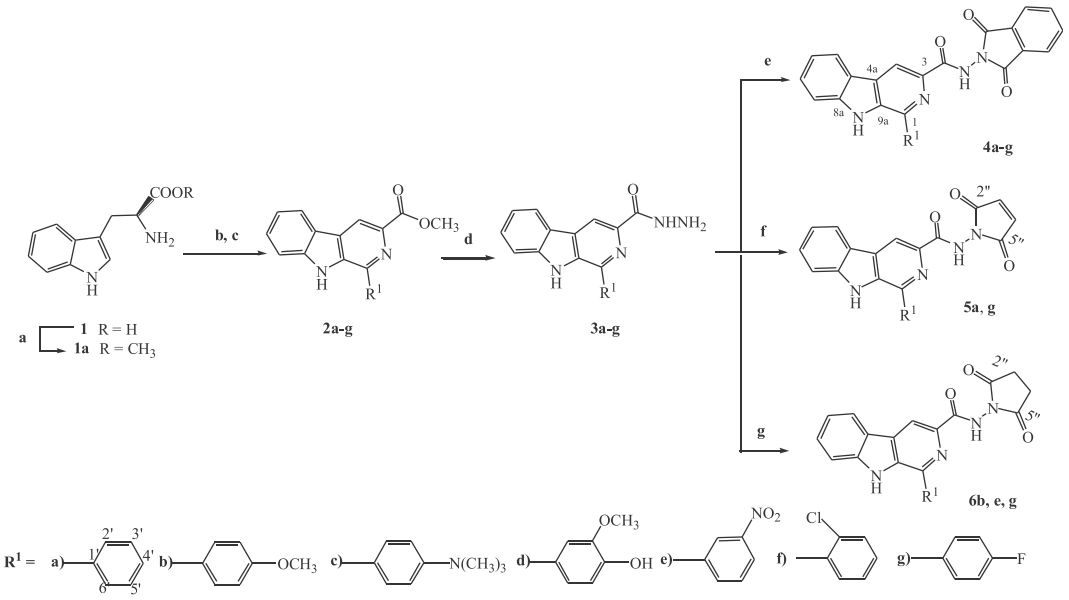
To prepare the β-carboline-3-*N*-(2,5-dioxo-2,5-dihydro-pirrol-1-yl)-carboxamides **5a** and **5g**, containing the phenyl and 4-fluorophenyl groups, respectively, at the position-1 of the β-carboline nucleus, the carbohydrazides **3a** and **3g** were treated with maleic anhydride in acetic acid and anhydrous sodium acetate, under reflux [21]. The formation of maleimides **5a** and **5g** was confirmed by the signals in the ^{1}H and ^{13}C NMR spectra corresponding to the maleimide moiety at δ_H 7.23–7.27 (brs, H-3''/H-4''), δ_C 133.8–133.9 (CH, C-3''/C-4'') and δ_C 168.2–168.3 (C=O).

The preparation of β-carboline-3-*N*-(2,5-dioxo-pyrrolidin-1-yl)-carboxamides (**6**) was firstly attempted by the reaction of **3a-g** with succinic anhydride, under anhydrous toluene reflux, as described by Brosse et al. [22]. However, in this condition only the formation of the non-cyclized intermediates was observed for all carbohydrazides **3a**-**g**. The synthesis of **6b**, **6e** and **6g** was possible by adding catalytic amount of *p*-toluenesulphonic acid in the reaction of the corresponding starting carbohydrazides with succinic anhydride. The formation of compounds **6b**, **6e** and **6g** was confirmed by the presence of signals of succinimide moiety at δ_H 2.84–2.89, relative to hydrogens H-3'' and H-4'', and at 26.2–26.3 and δ_C 174.3–174.4 relative to C-3'' and C-4'', and to the imide carbonyl group, respectively.

2.2. Biological assays

Our research efforts have focused on the development of β-carboline alkaloids derivatives with anti-*M. tuberculosis* activity. Herein, the present study is innovative in describing the *in vitro* activity of new β-carboline derivatives, which can be considered as potential scaffolds for future development of anti-tuberculosis agents.

Five β-carboline derivatives (**2c**, **4a**, **4e**, **4g**, **6g**), from the 19 studied, displayed anti-*M. tuberculosis* H_{37}Rv activity (MIC 125 μg/mL) (Table 1). After the screening in the reference strain *M. tuberculosis* H_{37}Rv, we assayed the activity of all five β-carboline derivatives against nine resistant *M. tuberculosis* clinical isolates (five MDR, three INH monoresistant and one INH and SM resistant) (Table 2). The use of MDR isolates in our study, with resistance to INH (MIC values ranging from 2 to 8 μg/mL) and RIF (MIC values ranging from 2 to 32 μg/mL), previously determined by REMA, can



Scheme 1. Reagents and Conditions: a) CH_3OH, H_2SO_4, reflux, 48 h; b) Aldehyde (R^1CHO), TFA, CH_2Cl_2, rt, 48 h; c) Sulfur, xylene, 48 h. d) $NH_2NH_2 \cdot H_2O$, EtOH, reflux, 48 h; e) phthalic anhydride, xylene, reflux, 24 h; f) maleic anhydride, AcONa, acetic acid, reflux, 24 h; g) succinic anhydride, TsOH, dry toluene, reflux, 48 h.

bring further understanding on the activity of these derivatives in strains that cause tuberculosis that are difficult to treat, and cause a form of the disease that brings global distress.

From the five β-carboline derivatives tested against resistant clinical isolates, the succinimide-β-carboline **6g** and the methyl β-carboline carboxylate **2c** showed MIC 31.25 μg/mL against two and four MDR clinical isolates, respectively.

The MIC values (31.25 μg/mL) against resistant clinical isolates are really interesting and are similar to some values found for the susceptible reference strain $H_{37}Rv$ with different β-carboline derivatives [13,14]. In the literature, some synthetic β-carboline derivatives have shown excellent activity against *M. tuberculosis* $H_{37}Rv$, such as manadomanzamine A and manadomanzamine B (MIC 1.9 μg/mL and 1.5 μg/mL, respectively) [23], nostocarboline and nine dimmers of nostocarboline (MIC ranging from 2.5 to > 10 μg/mL) [11], sixteen diarylpiperazine (MIC ranging from 1.5 to > 50 μg/mL) [14], and natural derivatives as manzamine alkaloids (MIC ranging from 0.4 to 30.2 μg/mL) [13]. However, none of the cited studies above tested the β-carboline derivatives in resistant *M. tuberculosis* clinical isolates as performed in the present study.

Some β-carboline derivatives were also tested against non-tuberculous mycobacteria (NTM) in other studies. We can name the Wahba et al. study [11], in which the amidation of β-carboline compounds resulted in potent activity against *M. intracellulare*. The nostocarboline and its nine dimmers derivatives above mentioned also showed to have activity against *M. smegmatis* (MIC ranged from 12.5 to > 100 μg/mL) [12].

The five compounds that displayed better anti-*M. tuberculosis* activity (**2c**, **4a**, **4e**, **4g**, **6g**), among the tested β-carboline derivatives were evaluated for their cytotoxicity in VERO cell lines (Table 2). All of them were non-cytotoxic to the VERO cell line at the tested concentration and the selectivity index (SI) ranged from <1.74 to 14.4.

According to the literature, compounds that present high lipophilicity tend to have better activity against *M. tuberculosis*, which can be explained by the high amount of mycolic acids in the mycobacteria cell wall structure. Applying the cLogP value for correlating to lipophilicity, in which cLogP value increases linearly when the permeability of a compound increases [24], Fernandes et al. observed that the most active anti-tuberculosis tested compounds that have been described in the literature demonstrated cLogP values ranging from 2 to 6 [25]. In respect to this characteristic, the compounds studied in the present study, **2c, 4a**, **4e**, **4g** and **6g**, showed cLogP values as 4.28, 4.88, 4.84, 5.04 and 1.90, respectively.

In this regard it is important to mention, a weak *in vitro* activity of a given compound compared to another one does not mean it will presents weak activity *in vivo* against a specific microorganism. Some of these compounds may show higher activity *in vivo*, by their metabolic transformation into a more active intermediate compound, as occurs with INH and PZA. Also, β-carbolines interactions, or even an adjuvant effect with old anti-tuberculosis drugs, and the interaction with the immune system of the patient should be considered for further investigation with these molecules [26].

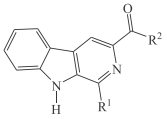
2.3. Structure-activity relationship (SAR)

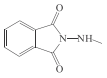
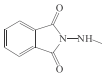
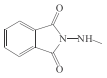
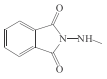
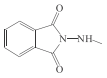
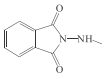
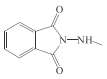
In an attempt to understand how these new β-carbolines interact with *M. tuberculosis*, SAR studies were carried out based on the MIC values obtained, increasing the chances of obtaining potentially anti-tuberculosis compounds.

The 3D and 4D SAR studies resulted in two qualitative models. The use of PLS-DA method in the Pirouette 4 program allowed the prediction equations to be submitted to cross-validation processes. The 3D model (**1**) was formed by 8 molecular interaction field (MIF) descriptors (equation (1)) and 3 latent variables that coded 63.45% of the information (LV1: 14.29%, LV2: 31.11%, LV3: 18.04%). The 4D model (**2**) is formed by 5 descriptors that originate 3 latent variables and accumulate 82.30% of the information (LV1: 30.08%, LV2: 20.39%, LV3: 31.83%). The obtained results for the adjustment (*R2*) and internal prediction ability (Q^2_{LOO}) indicate that both explain and predict information at the levels recommended in the literature [27–29].

$$\text{Class} = -0.220\times(150\_\text{TIP-TIP}) - 0.425\times(169\_\text{TIP-TIP}) + 0.345\times(176\_\text{TIP-TIP}) + 0.243\times(325\_\text{DRY-TIP}) - 0.205\times(403\_\text{O-TIP}) + 0.474\times(439\_\text{N1-TIP}) - 0.408\times(459\_\text{N1-}$$

Table 1
Molar Mass and Minimal Inhibitory Concentration (MIC) of β-carboline derivatives against *Mycobacterium tuberculosis* $H_{37}Rv$.



Compounds	R^1	R^2	Molar Mass	MIC μg/mL	MIC μM
2a	Ph	OCH_3	302.11	>250	>827.51
2b	4-OMe-Ph	OCH_3	432.12	250	578.54
2c	4-Me_2N-Ph	OCH_3	345,15	125	362.16
2d	3-OMe, 4-OH-Ph	OCH_3	348.11	250	718.16
2e	3-NO_2-Ph	OCH_3	347.09	250	720.27
2f	2-Cl-Ph	OCH_3	336.07	>250	>743.89
2g	4-F-Ph	OCH_3	320.10	>250	>781.01
4a	Ph		432.12	125	289.27
4b	4-OMe-Ph		462.31	>250	>540.76
4c	4-Me_2N-Ph		475.16	>250	>526.14
4d	3-OMe, 4-OH-Ph		478.13	250	522.87
4e	3-NO_2-Ph		477.11	125	261.99
4f	2-Cl-Ph		466.08	250	536.39
4g	4-F-Ph		450.11	125	277.71
5a	Ph		382.11	250	654.26
5g	4-F-Ph		400.10	250	624.84
6b	4-OMe-Ph		414.13	>250	>603.68
6e	3-NO_2-Ph		429.11	>250	>582.60
6g	4-F-Ph		402.11	125	310.86

TIP) +0.290×(463_N1-TIP) $R^2 = 0.936$; *RMSEC* = 0.133; $Q^2_{LOO} = 0.878$; *RMSECV* = 0.169 (1)

Class = 0.770×(14_21_12_NH3_LJ)+0.509×(16_11_13_NH3_C)+ 0.337×(14_7_15_NH3_LJ)+0.005×(22_18_10_NH3_LJ)+ 0.046×(11_7_12_NH3_LJ) $R^2 = 0.816$; *RMSEC* = 0.213; $Q^2_{LOO} = 0.674$; *RMSECV* = 0.256 (2)

In both models the equations are presented in their auto scaled forms, which allow a proper interpretation of the models in relation to the most and least important descriptors as to the presence or absence of activity. In model 1 (more information of each descriptor is available in Table 3), descriptors with information encoded by the steric probe (TIP) were observed, being present in all descriptors, with 3 totally steric descriptors (150_TIP-TIP, 169_TIP-TIP and 176_TIP-TIP).

Despite the two most important descriptors (459_N1-TIP and 439_N1-TIP) also present important contributions for hydrogen

Table 2
Susceptibility, Mycobacterial Interpersed Repetitive Unit (MIRU), Spoligotyping, *katG* and *inhA* mutations, Selectivity index (SI) determined in VERO epithelial cells and Minimum Inhibitory Concentration (MIC) of β-carboline derivatives against *Mycobacterium tuberculosis*H_{37}Rv, and resistant clinical isolates.

Strain/ isolates	INH MIC µg/ mL	MIRU	Spoligotyping	Mutations		β-carboline derivatives													
						2c			4a			4e			4 g			6 g		
				KatG	*inhA*	MIC µg/mL (µM)	CC_{50}	SI	MIC µg/mL (µM)	CC_{50}	SI	MIC µg/mL (µM)	CC_{50}	SI	MIC µg/mL (µM)	CC_{50}	SI	MIC µg/mL (µM)	CC_{50}	SI
H_{37}Rv	0.06	133226134333233422423624 8	77777747776077 1	Wt	Wt	125 (362.16)	435	3.48	125 (289.27)	900	7.20	125 (261.99)	650	5.2	125 (277.71)	762.5	6.1	125 (310.86)	270	2.16
3614	4	224225163321224434132227	67773607760771	Wt	Ile21Thr	31.25 (90.54)	435	13.49	62.50 (144.64)	900	14.4	125 (261.99)	650	5.2	62.50 (138.85)	762.5	12.2	62.50 (155.43)	270	4.32
71A	4	225313153323323232437377	77777777772077 1	Ser315Thr	Wt	>250 (>724.32)	435	<1.74	125 (289.27)	900	7.2	>250 (>523.99)	650	<2.6	125 (277.71)	762.5	6.1	125 (310.86)	270	2.16
19	2	224327153324221334211244	77617760776077 1	Ser315Thr	Wt	31.25 (90.55)	435	13.92	62.50 (144.64)	900	14.4	125 (261.99)	650	5.2	62.500 (138.85)	762.5	12.2	62.50 (155.43)	270	4.32
73A	4	224225123321224424132226	67773607760771	Ser315Thr	Wt	31.25 (90.54)	435	13.92	62.50 (144.64)	900	14.4	125 (261.99)	650	5.2	62.50 (138.85)	762.5	12.2	31.25 (77.72)	270	8.64
91	2	224225143321224424132224	67773607760771	Ser315Thr	Wt	62.50 (180.36)	435	6.96	125 (289.27)	900	7.20	>250 (>523.99)	650	<2.6	125 (277.71)	762.5	6.1	125 (310.86)	270	2.16
BRF45	4	224326133323223464525211	77617740000017 1	Ser315Thr	Wt	31.25 (90.54)	435	13.92	62.5 (144.64)	900	14.4	125 (261.99)	650	5.2	62.50 (138.85)	762.5	12.2	31.25 (77.72)	270	8.64
BRF57	4	124226143424224235334132	77777607760731	Ser315Thr	Wt	125 (362.16)	435	3.48	125 (289.27)	900	7.2	>250 (>523.99)	650	<2.6	125 (277.71)	762.5	6.1	125 (310.86)	270	2.16
BRF14	8	123326143324224233424132	77777607760731	Ser315Thr	Wt	250 (724.32)	435	1.74	125 (289.27)	900	7.2	250 (523.99)	650	2.6	62.50 (138.85)	762.5	12.2	125 (310.86)	270	2.16
BRF16	8	123326143324224234424132	77777607760731	Ser315Thr	Wt	125 (362.16)	435	3.48	125 (289.27)	900	7.2	125 (261.99)	650	5.2	62.50 (138.85)	762.5		62.50	270	4.32

R: resistant; INH: isoniazid; EMB: ethambutol; SM: streptomycin; ETH: ethionamide; RMP: rifampicin; Wt: wild type.

Table 3
Characteristics of the selected descriptors for Model 1.

Sign	Descriptor	Field	Distance range (angstrons)
+	150	TIP-TIP	3.60–4.00
–	169	TIP-TIP	11.20–11.60
+	176	TIP-TIP	14.00–14.40
+	325	DRY-TIP	17.20–17.60
–	403	O-TIP	10.80–11.20
+	439	N1-TIP	6.40–6.80
–	459	N1-TIP	14.40–14.80
+	463	N1-TIP	16.00–16.40

bonds, the steric characteristic may be considered more important. However, the importance of hydrophobicity appears in the descriptor 325_DRY-TIP, which favors the activity, as already indicated in the literature.

The graphs obtained by PLS-DA analysis (Fig. 1) showed that these descriptors actually present an excellent discriminatory power, with active and inactive compounds grouping separately into two quadrants defined by LV1.

Fig. 2 shows the descriptors formed around an active compound (2b). It can be clearly observed that the steric components forming the model (green spheres) are located at the ends of the structures. For the descriptor DRY-TIP pharmacophore distances are not well defined as for the others, but it is observed that the hydrophobicity (yellow dots) is defined by the central part of the structure.

It is interesting to note that these results are in agreement with several other 3D-QSAR studies already performed with several compounds that act on the H_{37}Rv strain. The model obtained by Pan et al. [30] for a set of quinoxaline 1,4-di-N-oxide derivatives showed a steric contribution of 52.3% of the information.

Shah et al. [31] obtained a model for 2,3-dideoxy hex-2-enopyranosid-4-uloses with 66.5% steric information. Patel et al. [32] obtained a model for 4- (adamantan-1-yl) quinolone derivatives where the steric contribution corresponded to 60.40% of the model. Also, Wang et al. [33] observed that bulky electropositive groups tended to render nitrofuranyl methyl N-heterocycles derivatives more active against the strain studied, in addition to the anti-tuberculosis also having a positive relationship with cLogP.

Another interesting fact observed is that in all the reported studies the 3D-QSAR CoMFA program was used. Thus, it can be proposed that the results observed for the anti-tuberculosis activity are a specific characteristic of the agents against the studied strain, and it is not a dependent effect of the algorithms for calculating the descriptors of a specific program.

The discriminatory power of the selected descriptors for model 2 showed to be lower (Fig. 3), with the quadrants defined jointly by LV1 and LV2. The adjustment and prediction ability were also inferior to model 1, but the model is still suitable for classification purposes. The two most important descriptors are those closest to the conformational ensemble profiles (Fig. 4). The descriptor named 14_21_12_NH3_LJ is a Lennard-Jones descriptor, while the descriptor 16_11_13_NH3_C is a Coulomb descriptor.

However, although all the coefficients of the model were positive, Coulomb's descriptor values were negative for all analyzed derivatives, indicating that the increased polarization of the molecules can cause loss of activity. This strengthens the interpretation performed for model 1, thus gaining prominence the importance of steric effects and liposolubility in the discrimination.

The results observed also strengthens the hypothesis that the steric effect is an important characteristic for this type of molecules, independent of the program or methodology used to generate the descriptors, since the MIF descriptors are obtained based on a set of different conformations for each molecule, not just a single

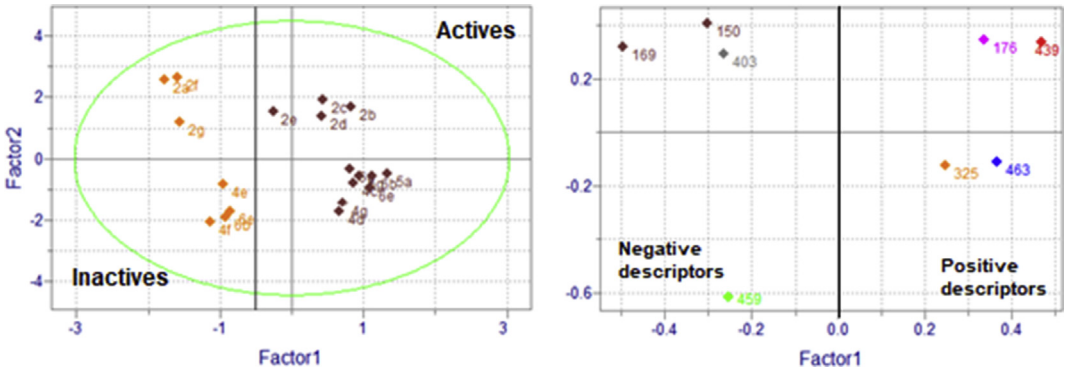


Fig. 1. Plot of the loading and score vectors of 3D model obtained by PLS-DA approach.

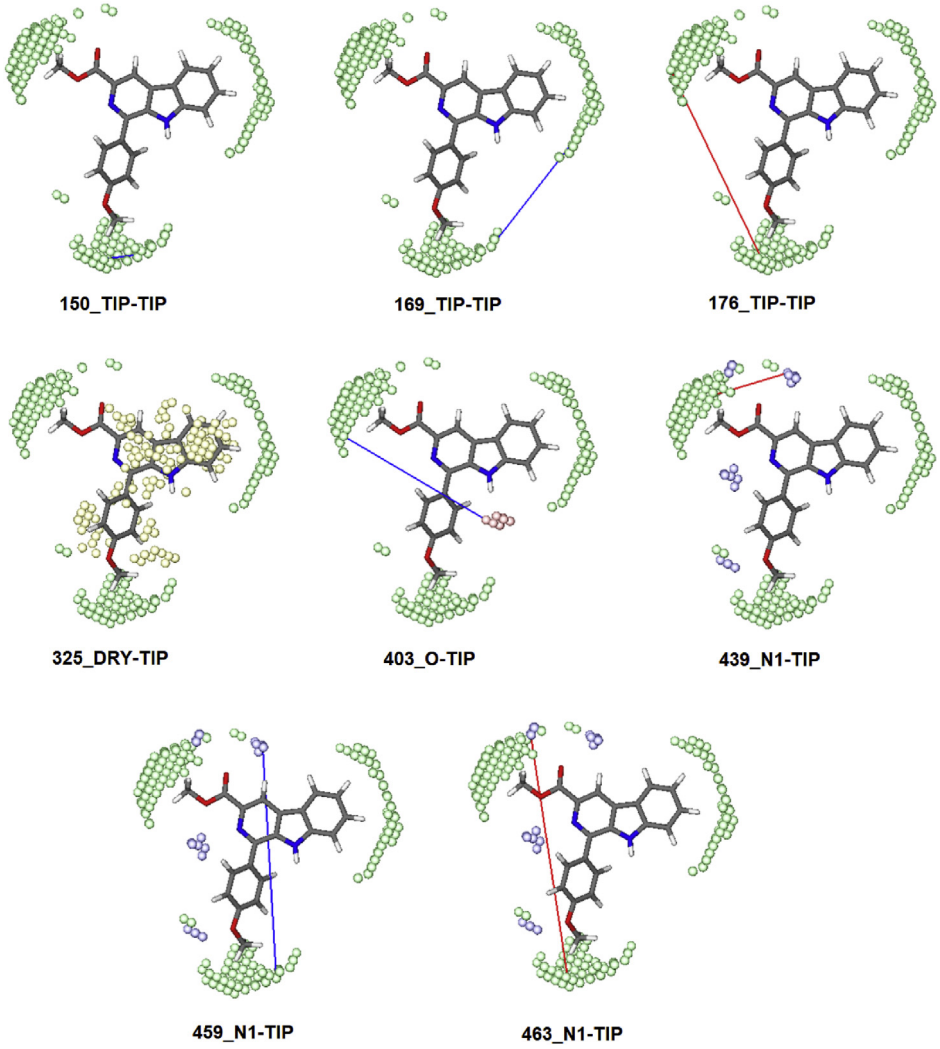


Fig. 2. GRIND selected descriptors of Model 1 associated with an active compound 2b. Red lines: positive descriptors; blue lines: negative descriptors. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

optimized geometry, as commonly performed in 3D-QSAR.

3. Conclusion

The present study demonstrated that five of the β-carboline derivatives tested showed anti-*M. tuberculosis* activity, especially against resistant clinical isolates, what makes them scaffold candidates for further investigations about their anti-tuberculosis activity. The SAR studies performed using 3D and 4D methodologies indicated that the activity was influenced mainly by the steric characteristics (size and shape) and lipophilicity of the molecules, meeting the previous information available in the literature. These

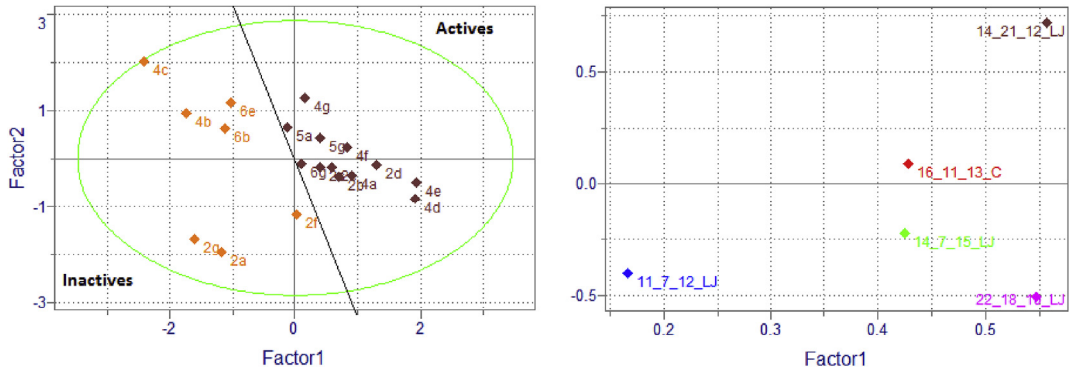


Fig. 3. Plot of the loading and score vectors of 4D model obtained by PLS-DA approach. In this model, all descriptors have positive signs.

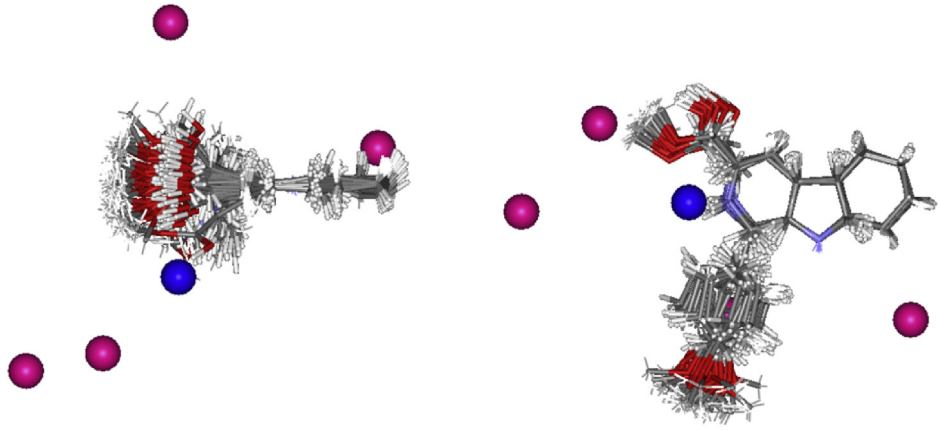


Fig. 4. Full 4D model in three-dimensional space surrounding the CEP of one active compound 2b. Blue: Coulomb descriptor; pink: Lennard-Jones descriptor. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

models can be used for future proposition of new derivatives, increasing the chances of obtaining potentially anti-tuberculosis compounds.

4. Experimental

4.1. General methods

All reagents were purchased from commercial suppliers. The reactions were monitored by thin layer chromatography conducted on Whatman TLC plates (Silica Gel 60 F_{254}). NMR spectra were recorded in an in Varian spectrometer model Mercury plus BB at 300.0 MHz (for ^{1}H) and 75.5 MHz (for ^{13}C) with TMS and deuterated solvents, dimethyl sulfoxide (DMSO-d_6) as internal standard. EI-MS spectra were recorded in a Thermoelectron Corporation Focus-DSQ II spectrometer. Melting points were determined in Microquímica apparatus model MQAPF-301, and were not corrected.

4.2. Synthesis

4.2.1. General procedure for the synthesis of 1-(substituted-phenyl)-β-carboline-3- N-(1,3-dioxo-1,3-dihydro-isoindol-2-yl)-carboxamides (**4a-g**)

A solution of carbohydrazides **3a-g** (2.0 mmol) and phthalic anhydride (3.0 mmol) in xylene was refluxed for 24 h under stirring. Then, the reaction mixture was cooled, and the resulting solid was filtered off and washed with cold ethanol to furnish the phthalimides **4a-g**.

*4.2.1.1. 1-(Phenyl)-β-carboline-3- N-(1,3-dioxo-1,3-dihydro-isoindol-2-yl)-carboxamide (**4a**).* Yield: 85%; mp: 262.4–265.3; IR (KBr) ν_{max}: 3377 (NH), 1622 (C=N), 1703 and 1739 (C=O), 1460–1595 (C=C) cm^{-1}; ^{1}H NMR (300 MHz, DMSO-d_6): δ 8.91 (s, 1H, H-4), 8.46 (d, J = 7.2 Hz, 1H, H-5), 7.33 (t, J = 7.2 Hz, 1H, H-6), 7.51–7.75 (m, 5H, H-7, H-8, H-3′, H-4′ and H-5′), 8.25 (d, J = 7.2 Hz, 2H, H-2′and H-6′), 8.02–8.06 (m, 2H, H-3″ and H-6″), 7.97–8.01 (m, 2H, H-4″ and H-5″), 11.10 (s, 1H, NH), 12.00 (s, 1H, 9-NH); ^{13}C NMR (75.5 MHz): δ 141.5 (C, C-1), 134.7 (C, C-3), 114.4 (CH, C-4), 129.7 (C, C-4a), 121.3 (C, C-4b), 122.1 (CH, C-5), 120.4 (CH, C-6), 128.7 (CH, C-7, C-2′ and C-6′), 112.8 (CH, C-8), 141.2 (C, C-8a), 137.3 (C, C-9a), 129.1 (C, C-1′), 128.5 (CH, C-3′, C-4′and C-5′), 129.5 (C, C-2a" and C-6a"), 123.8 (CH, C-3″ and C-6″), 135.3 (C-4″ and C-5″), 164.1 (C, C=O), 165.3 (C, C=O). EI-MS: calcd for $C_{26}H_{16}N_4O_3$ 432.122, found: 432.956 [$M^{+\cdot}$].

*4.2.1.2. 1-(4-Methoxyphenyl)-β-carboline-3-N-(1,3-dioxo-1,3-dihydro-isoindol-2-yl)-carboxamide (**4b**).* Yield: 89%; mp: > 280.0 (decomp.); IR (KBr) ν_{max}: 3290 (NH), 1625 (C=N), 1670 and 1735 (C=O), 1440–1566 (C=C) cm^{-1}; ^{1}H NMR (300 MHz, DMSO-d_6): δ 8.85 (s, 1H, H-4), 8.45 (d, J = 7.8 Hz, 1H, H-5), 7.31–7.36 (m, 1H, H-6), 7.60–7.65 (m, 1H, H-7), 7.74 (d, J = 7.8 Hz, 1H, H-8), 8.25 (d, J = 7.8 Hz, 2H, H-2′and H-6′), 7.22 (d, J = 7.8, 2H, H-3′ and H-5′), 3.90 (s, 3H, OCH_3), 8.02–8.06 (m, 2H, H-3″ and H-6″), 7.97–8.01 (m, 2H, H-4″ and H-5″), 11.08 (s, 1H, NH), 12.96 (s, 1H, 9-NH); ^{13}C NMR (75.5 MHz): δ 141.5 (C, C-1), 134.5 (C, C-3), 113.9 (CH, C-4), 129.5 (C, C-4a), 121.3 (C, C-4b), 122.2 (CH, C-5), 120.5 (CH, C-6), 128.7 (CH, C-7), 112.8 (CH, C-8), 141.2 (C, C-8a), 137.2 (C, C-9a), 129.5 (C, C-1′), 130.4 (CH, C-2′ and C-6′), 114.2 (CH, C-3′ and C-5′), 160.1 (C, C-4′),

55.4 (O−CH_3), 129.5 (C, C-2a'' and C-6a''), 123.9 (CH, C-3″ and C-6″), 135.5 (C-4″ and C-5″), 164.2 (C, C=O), 165.5 (C, C=O).). EI-MS: calcd for $C_{27}H_{18}N_4O_4$ 462.133, found: 462.951 [$M^{+\cdot}$].

*4.2.1.3. 1-(4-Dimethylaminophenyl)-β-carboline-3-N-(1,3-dioxo-1,3-dihydro-isoindol-2-yl)-carboxamide (**4c**).* Yield: 84%; mp: 297.6−299.8; IR (KBr) ν_{max}: 3377 (NH), 1608 (C=N), 1668 and 1745 (C=O), 1458−1560 (C=C) cm^{-1}; ^1H NMR (300 MHz, DMSO-d_6): δ 8.89 (s, 1H, H-4), 8.31 (d, J = 8.1 Hz, 1H, H-5), 7.34 (t, J = 8.1 Hz, 1H, H-6), 8.31 (d, J = 8.1 Hz, 1H, H-7), 7.72 (d, J = 8.1 Hz, 1H, H-8), 8.45 (d, J = 8.1 Hz, 2H, H-2′and H-6′), 7.63 (d, J = 8.1, 2H, H-3′ and H-5′), 3.44 (s, 1H, N(CH_3)$_2$), 7.99−8.03 (m, 2H, H-3″ and H-6″), 7.99−8.03 (m, 2H, H-4″ and H-5″), 11.12 (s, 1H, NH), 12.05 (s, 1H, 9-NH); ^{13}C NMR (75.5 MHz): δ 141.5 (C, C-1), 137.3 (C, C-3), 114.4 (CH, C-4), 129.8 (C, C-4a), 121.1 (C, C-4b), 130.0 (CH, C-5 and C-7), 120.5 (CH, C-6), 112.7 (CH, C-8), 142.7 (C, C-8a), 134.7 (C, C-9a), 133.3 (C, C-1′), 122.2 (CH, C-2′ and C-6′), 121.2 (CH, C-3′ and C-5′), 162.0 (C, C-4′), 129.5 (C, C-2a'' and C-6a''), 123.8 (CH, C-3″ and C-6″), 135.4 (C-4″ and C-5″), 164.1 (C, C=O), 165.3 (C, C=O). EI-MS: calcd for $C_{28}H_{21}N_5O_3$ 475.164, found: 475.0 [$M^{+\cdot}$].

*4.2.1.4. 1-(3-Methoxy-4-hydroxyphenyl)- β-carboline-3-N-(1,3-dioxo-1,3-dihydro-isoindol-2-yl)-carboxamide (**4d**).* Yield: 83%; mp: 210.8−212.4; IR (KBr) ν_{max}: 3319 (NH), 1623 (C=N), 1666 and 1743 (C=O), 1463−1595 (C=C) cm^{-1}; ^1H NMR (300 MHz, DMSO-d_6): δ 8.82 (s, 1H, H-4), 8.42 (d, J = 7.8 Hz, 1H, H-5), 7.32 (t, J = 7.8 Hz, 1H, H-6), 7.62 (t, J = 7.8 Hz, 1H, H-7), 7.70−773 (m, 2H, H-8 and H-5′), 7.04 (d, J = 7.8 Hz, 1H, H-2′), 7.63 (d, J = 7.8, 1H, H-6′), 8.01−8.05 (m, 2H, H-3″ and H-6″), 3.85 (s, 1H, OCH_3), 9.43 (s, 1H, OH), 10.90 (s, 1H, NH), 12.00 (s, 1H, 9-NH); ^{13}C NMR (75.5 MHz): δ 141.4 (C, C-1), 134.5 (C, C-3), 113.7 (CH, C-4), 129.3 (C, C-4a), 121.3 (C, C-4b), 122.0 (CH, C-5), 120.3 (CH, C-6), 128.7 (CH, C-7), 112.7 (CH, C-8), 141.4 (C, C-8a), 141.9 (C, C-9a), 128.5 (C, C-1′), 115.5 (CH, C-2′), 147.9 (CH, C-3′), 147.8 (CH, C-4′), 113.1 (CH, C-5′), 121.7 (C, C-6′), 129.5 (C, C-2a'' and C-6a''), 123.8 (CH, C-3″ and C-6″), 135.4 (C-4″ and C-5″), 164.2 (C, C=O), 165.4 (C, C=O). EI-MS: calcd for $C_{26}H_{16}N_4O_3$ 448.117, found: 448.963 [$M^{+\cdot}$].

*4.2.1.5. 1-(3-Nitrophenyl)-β-carboline-3-N-(1,3-dioxo-1,3-dihydro-isoindol-2-yl)-carboxamide (**4e**).* Yield: 80%; mp: > 280.0 (decomp.); IR (KBr) ν_{max}: 3369 (NH), 1623 (C=N), 1706 and 1733 (C=O), 1444−1560 (C=C) cm^{-1}; ^1H NMR (300 MHz, DMSO-d_6): δ 8.99 (s, 1H, H-4), 8.50 (d, J = 7.8 Hz, 1H, H-5), 7.38 (t, J = 7.8 Hz, 1H, H-6), 7.69 (t, J = 7.8 Hz, 1H, H-7), 7.73 (d, J = 7.8 Hz, 1H, H-8), 8.30 (brs, 1H, H-2′), 8.43 (dd, J = 7.8 and 1.8 Hz, 1H, H-4′), 7.96 (t, J = 7.8 Hz, 1H, H-5′), 8.67 (d, J = 7.8 Hz, 1H, H-6′), 7.97−8.07 (m, 4H, H-3′, H-4″, H-5″ and H-6″), 11.24 (s, 1H, NH), 12.19 (s, 1H, 9-NH); ^{13}C NMR (75.5 MHz): δ 141.6 (C, C-1), 138.5 (C, C-3), 115.3 (CH, C-4), 129.5 (C, C-4a), 121.4 (C, C-4b), 122.4 (CH, C-5), 120.6 (CH, C-6), 129.1 (CH, C-7), 112.6 (CH, C-8), 138.9 (C, C-8a), 129.4 (C, C-1′), 123.7 (CH, C-2′), 148.3 (CH, C-3′), 123.8 (C, C-4′), 130.2 (CH, C-5′), 135.4 (CH, C-6′), 129.4 (C, C-2a'' and C-6a''), 123.8 (CH, C-3″ and C-6″), 135.3 (C-4″ and C-5″), 163.9 (C, C=O), 165.3 (C, C=O). EI-MS: calcd for $C_{26}H_{15}N_5O_5$ 477.107, found: 477.921 [$M^{+\cdot}$].

*4.2.1.6. 1-(2-Chlorophenyl)-β-carboline-3-N-(1,3-dioxo-1,3-dihydro-isoindol-2-yl)-carboxamide (**4f**).* Yield: 90%; mp: 194.9−196.3; IR (KBr) ν_{max}: 3269 (NH), 1623 (C=N), 1664 and 1737 (C=O), 1460−1598 (C=C) cm^{-1}; ^1H NMR (300 MHz, DMSO-d_6): δ 8.98 (s, 1H, H-4), 8.48 (d, J = 7.8 Hz, 1H, H-5), 7.31−7.37 (d, J = 7.8 Hz, 1H, H-6), 7.58−7.67 (m, 1H, H-7 e H-8), 7.72−7.78 (m, 2H, H-3′and H-4′), 7.58−7.67 (m, 2H, H-5′ and H-6′), 7.95−8.04 (m, 2H, H-3″ and H-6″) 7.95−8.04 (m, 2H, H-4″ and H-5″), 10.95 (s, 1H, NH), 11.85 (s, 1H, 9-NH); ^{13}C NMR (75.5 MHz): δ 141.4 (C, C-1), 136.2 (C, C-3), 115.2 (CH, C-4), 129.5 (C, C-4a), 121.0 (C, C-4b), 122.4 (CH, C-5), 120.4 (CH, C-6), 127.5 (CH, C-7), 112.5 (CH, C-8), 140.5 (C, C-8a), 136.9 (C, C-9a), 132.6 (C, C-1′), 129.5 (C, C-2′), 129.6 (CH, C-3′), 132.2 (CH, C-4′), 128.9 (CH, C-5′), 130.8 (CH, C-6′), 129.5 (C, C-2a'' and C-6a''), 123.8 (C-3″ and C-6″), 135.3 (CH, C-4″ and C-5″), 164.0 (C, C=O), 165.3 (C, C=O). EI-MS: calcd for $C_{26}H_{15}ClN_4O_3$ 466.083, found: 466.002 [$M^{+\cdot}$].

*4.2.1.7. 1-(4-Fluorophenyl)-β-carboline-3-N-(1,3-dioxo-1,3-dihydro-isoindol-2-yl)-carboxamide (**4g**).* Yield: 90%; mp: > 280.0 (decomp.); IR (KBr) ν_{max}: 3330 (NH), 1623 (C=N), 1706 and 1733 (C=O), 1444−1562 (C=C) cm^{-1}; ^1H NMR (300 MHz, DMSO-d_6): δ 8.89 (s, 1H, H-4), 8.45 (d, J = 7.8 Hz, 1H, H-5), 7.33 (t, J = 7.8 Hz, 1H, H-6), 7.62 (t, J = 7.8 Hz, 1H, H-7), 7.72 (d, J = 7.8 Hz, 1H, H-8), 8.30 (dd, J = 9.0 and 8.7 Hz, 2H, H-2′and H-6′), 7.49 (dd, J = 9.0 and 8.7 Hz, 2H, H-3′ and H-5′), 9.88 (s, 1H, OH), 8.02−8.06 (m, 2H, H-3″ and H-6″), 7.97−8.01 (m, 2H, H-4″ and H-5″), 11.13 (s, 1H, NH), 12.03 (s, 1H, 9-NH); ^{13}C NMR (75.5 MHz): δ 141.5 (C, C-1), 137.3 (C, C-3), 114.5 (CH, C-4), 129.8 (C, C-4a), 121.1 (C, C-4b), 122.2 (CH, C-5), 120.5 (CH, C-6), 128.9 (CH, C-7), 112.7 (CH, C-8), 140.2 (C, C-8a), 133.6 (C, C-1′), 131.2 (CH, C-2′ and C-6′), 115.6 (CH, C-3′ and C-5′), 162.7 (C, C-4′), 129.5 (C, C-2a'' and C-6a''), 123.8 (CH, C-3″ and C-6″), 135.4 (C-4″ and C-5″), 164.1 (C, C=O), 165.4 (C, C=O). HRMS-ESI: calcd for 450.421, found: 449.924. EI-MS: calcd for $C_{26}H_{15}FN_4O_3$ 450.113, found: 450.948 [$M^{+\cdot}$].

*4.2.2. General procedure for synthesis 1-(substituted-phenyl)-β-carboline-3-N-(2,5-dioxo-2,5-dihydro-pirrol-1-yl)-carboxamides (**5a** and **g**)*

To a solution of the carbohydrazides **3a** and **3g** (2.0 mmol) and maleic anhydride (2.0 mmol) in acetic acid (20 mL), sodium acetate (2.5 mmol) was added. The resulting solution was refluxed for 24 h, then treated with cold-water. The solid formed was filtered off and washed with cold water to furnish the products **5a** and **5g**, respectively.

*4.2.2.1. 1-(Phenyl)-β-carboline-3- N-(2,5-dioxo-2,5-dihydro-pyrrol-1-yl)-carboxamide (**5a**).* Yield: 70%; mp: 187.5−189.2; IR (KBr) ν_{max}: 3337 (NH), 1626 (C=N), 1697 and 1730 (C=O), 1460−1595 (C=C) cm^{-1}; ^1H NMR (300 MHz, DMSO-d_6): δ 8.87 (s, 1H, H-4), 8.45 (d, J = 7.5 Hz, 1H, H-5), 7.33 (t, J = 7.5 Hz, 1H, H-6), 7.55−7.73 (m, 5H, H-7, H-8 and H-4′), 8.22 (d, J = 7.5 Hz, 2H, H-2′and H-6′), 8.22 (d, J = 7.22 Hz, 2H, H-3′ and H-4′), 7.23 (brs, 2H, H-3″ and H-4″), 10.90 (s, 1H, NH), 12.00 (s, 1H, 9-NH); ^{13}C NMR (75.5 MHz): δ 141.6 (C, C-1), 134.7 (C, C-3), 114.4 (CH, C-4), 129.7 (C, C-4a), 121.2 (C, C-4b), 122.2 (CH, C-5), 120.5 (CH, C-6), 128.8 (CH, C-7 and C-4′), 112.8 (CH, C-8), 141.2 (C, C-8a), 137.1 (C, C-9a), 129.1 (C, C-1′), 128.9 (CH, C-2′ and C-6′), 129.5 (CH, C-3′, C-4′and C-5′), 133.9 (C, C-3″ and C-4″), 164.2 (C, C=O), 168.3 (C, C=O). EI-MS: calcd for $C_{22}H_{14}N_4O_3$: 382.107, found: 382.927 [$M^{+\cdot}$].

*4.2.2.2. 1-(4-Fluorophenyl)-β-carboline-3- N-(2,5-dioxo-2,5-dihydro-pyrrol-1-yl)-carboxamide (**5g**).* Yield: 80%; mp: 182.5−184.5; IR (KBr) ν_{max}: 3350 (NH), 1623 (C=N), 1687 and 1730 (C=O), 1461−1510 (C=C) cm^{-1}; ^1H NMR (300 MHz, DMSO-d_6): δ 8.87 (s, 1H, H-4), 8.45 (d, J = 8.0 Hz, 1H, H-5), 7.34 (t, J = 8.0 Hz, 1H, H-6), 7.63 (t, J = 8.0 Hz, 1H, H-7), 7.72 (d, J = 8.0 Hz, 1H, H-8), 8.30 (dd, J = 8,7 and 8.4 Hz, 2H, H-2′ and H-6′), 7.49 (dd, J = 8.7 and 8.4, 2H, H-3′ and H-5′) 7.27 (*brs*, H-3″ and H-4″, 10.91 (s, 1H, NH), 12.01 (s, 1H, 9-NH); ^{13}C NMR (75.5 MHz): δ 141.5 (C, C-1), 134.5 (C, C-3), 114.3 (CH, C-4), 129.8 (C, C-4a), 121.1 (C, C-4b), 122.1 (CH, C-5), 120.5 (CH, C-6), 128.8 (CH, C-7), 112.7 (CH, C-8), 140.1 (C, C-8a), 137.2 (C, C-9a), 133.5 (J = 2.9 Hz, C, C-1′), 131.2 (J = 8.3, CH, C-2′ and C-6′), 115.5 (J = 21.9, CH, C-3′ and C-5′), 162.7 (J = 246.9, C, C-4′), 133.8 (C, C-3″ and C-4″), 164.1 (C, C=O), 168.2 (C, C=O). EI-MS: calcd for $C_{22}H_{13}FN_4O_3$: 400.097, found: 400.945 [$M^{+\cdot}$].

*4.2.3. General procedure for synthesis of 1-(substituted-phenyl)-β-carboline-3-N-(2,5-dioxo-pirrolidin-1-yl)-carboxamides (**6b, e, g**)*

To a solution of the carbohydrazides **3b**, **3e** and **3g** (2.0 mmol) and succinic anhydride (2.0 mmol) in anhydrous toluene, a catalytic amount of p-toluene sulphonic acid was added. The resulting solution was refluxed for 48 h with a Dean-Stark apparatus in order to remove the water formed during the reaction. Then, the reaction mixture was cooled, and the resulting solid was filtered off, and washed with 15% aqueous Na_2CO_3 and water to furnish the products **6b**, **6e** and **6g**, respectively.

*4.2.3.1. 1-(4-Methoxyphenyl)-β-carboline-3-N-(2,5-dioxo-pirrolidin-1-yl)-carboxamide (**6b**).* Yield: 80%; mp: 241.9–243.4; IR (KBr) ν_{max}: 3330 (NH), 1623 (C=N), 1695 and 1728 (C=O), 1461–1593 (C=C) cm^{-1}; ^1H NMR (300 MHz, DMSO-d_6): δ 8.81 (s, 1H, H-4), 8.43 (d, J = 7.3 Hz, 1H, H-5), 7.33 (t, J = 7.3 Hz, 1H, H-6), 7.61 (t, J = 7.3 Hz, 1H, H-7), 7.72 (d, J = 7.3 Hz, 1H, H-8), 8.20 (d, J = 7.3 Hz, 2H, H-2′and H-6′), 7.20 (d, J = 7.3 Hz, 2H, H-3′and H5′), 2.88 (brs, 2H, H-3″ and H-4″), 10.83 (s, 1H, NH), 11.94 (s, 1H, 9-NH); ^{13}C NMR (75.5 MHz): δ 141.5 (C, C-1), 134.8 (C, C-3), 113.7 (CH, C-4), 129.6 (C, C-4a), 121.1 (C, C-4b), 122.1 (CH, C-5), 120.2 (CH, C-6), 128.7 (CH, C-7), 112.8 (CH, C-8), 141.1 (C, C-8a), 137.4 (C, C-9a), 129.5 (C, C-1′), 130.3 (CH, C-2′ and C-6′), 114.1 (CH, C-3′ and C-5′), 160.1 (CH, C-4′), 55.4 (OCH_3), 26.3 (C, C-3″ and C-4″), 163.4 (C, C=O), 174.4 (C, C=O). EI-MS: calcd for $C_{23}H_{18}N_4O_4$: 414.133, found: 414.047 [$M^{+\cdot}$].

*4.2.3.2. 1-(3-Nitrophenyl)-β-carboline-3- N-(2,5-dioxo-pirrolidin-1-yl)-carboxamide (**6e**).* Yield: 40%; mp: 199.6–199.5; IR (KBr) ν_{max}: 3373 (NH), 1623 (C=N), 1701 and 1712 (C=O), 1460–1593 (C=C) cm^{-1}; ^1H NMR (300 MHz, DMSO-d_6): δ 8.93 (s, 1H, H-4), 8.46 (d, J = 8.1 Hz, 1H, H-5), 7.34 (t, J = 8.1 Hz, 1H, H-6), 7.63 (t, J = 8.1 Hz, 1H, H-7), 7.70 (d, J = 8.1 Hz, 1H, H-8), 8.94 (s, 1H, H-2′), 8.40 (dd, J = 8.1 and 2.1 Hz, 1H, H), 7.92 (t, J = 8.1 Hz, 1H, H5′), 8.64 (d, J = 8.1 Hz, 1H, H-6′), 2.84 (brs, 2H, H-3″ and H-4″), 11.00 (s, 1H, NH); ^{13}C NMR (75.5 MHz): δ 141.6 (C, C-1), 137.5 (C, C-3), 115.1 (CH, C-4), 130.3 (C, C-4a), 121.1 (C, C-4b), 122.4 (CH, C-5), 120.6 (CH, C-6), 129.1 (CH, C-7), 112.8 (CH, C-8), 138.7 (C, C-8a), 138.6 (C, C-9a), 135.5 (J = 3.3 HZ, C, C-1′), 123.7 (CH, C-2′), 148.3 (C, C-3′), 123.7 (CH, C-4′), 130.3 (CH, C-5′), 135.5 (CH, C-6′), 26.3 (C, C-3″ and C-4″), 163.3 (C, C=O), 174.4 (C, C=O).). EI-MS: calcd for $C_{22}H_{15}N_5O_5$: 429.107, found: 429.010 [$M^{+\cdot}$].

*4.2.3.3. 1-(4-Fluorophenyl)-β-carboline-3-N-(2,5-dioxo-pirrolidin-1-yl)-carboxamide (**6g**).* Yield: 79%; mp: 281.3–283.6; IR (KBr) ν_{max}: 3348 and 3236 (NH), 1623 (C=N), 1699 and 1731 (C=O), 1461–1593 (C=C) cm^{-1}; ^1H NMR (300 MHz, DMSO-d_6): δ 8.72 (s, 1H, H-4), 8.46 (d, J = 8.0 Hz, 1H, H-5), 7.35 (t, J = 8.0 Hz, 1H, H-6), 7.63 (t, J = 8.0 Hz, 1H, H-7), 7.72 (d, J = 8.0 Hz, 1H, H-8), 8.29 (dd, J = 8.7 and 8.7 Hz, 2H, H-2′and H-6′), 7.490 (dd, J = 8.7 and 8.7 Hz, 2H, H-3′and H5′), 2.89 (brs, 2H, H-3″ and H-4″), 10.88 (s, 1H, NH), 12.01 (s, 1H, 9-NH); ^{13}C NMR (75.5 MHz): δ 141.5 (C, C-1), 134.5 (C, C-3), 114.2 (CH, C-4), 129.8 (C, C-4a), 121.1 (C, C-4b), 122.2 (CH, C-5), 120.5 (CH, C-6), 128.8 (CH, C-7), 112.7 (CH, C-8), 140.1 (C, C-8a), 137.4 (C, C-9a), 135.5 (J = 3.3 HZ, C, C-1′), 1311 (J = 8.8 Hz, CH, C-2′ and C-6′), 115.5 (J = 21.6 Hz, CH, C-3′ and C-5′), 162.6 (J = 246.6 Hz, C, C-4′), 26.3 (C, C-3″ and C-4″), 163.3 (C, C=O), 174.3 (C, C=O). EI-MS: calcd for $C_{22}H_{15}FN_4O_3$: 402.113, found: 402.035 [$M^{+\cdot}$].

4.3. Biological assays

4.3.1. Mycobacterium tuberculosis reference strain and clinical isolates

The pan-susceptible *M. tuberculosis* $H_{37}Rv$ (ATCC 27294) reference strain was used for screening the β-carboline derivatives compounds activities (Table 1). The most anti-tuberculosis active compounds were then assayed against nine resistant *M. tuberculosis* clinical isolates (five MDR, three INH monoresistant and one INH and streptomycin (SM) resistant). All resistant clinical isolates used in the study were selected from the mycobacterial collection from the laboratory of Medical Bacteriology, Department of Clinical Analysis and Biomedicine, State University of Maringá, Paraná, Brazil. The isolates were previously biochemically identified as *M. tuberculosis* complex and the drug susceptibility testing carried out by Löwenstein-Jensen proportion method [33] and genotyped by spoligotyping [34] and Mycobacterial Interspersed Repetitive Unit - Variable Number Tandem Repeat (MIRU-VNTR) (Table 2) [35].

The reference strain and resistant clinical isolates were grown in Middlebrook 7H9 broth medium (Difco Laboratories, Detroit, USA), added of 0.2% glycerol and supplemented with oleic acid, albumin, dextrose and catalase (OADC) enrichment (BBL/Becton-Dickinson, Sparks, MD, USA) (7H9-OADC) for 15 days at 35 °C. Standardized bacilli inoculums, with turbidity equivalent to McFarland scale 1 (3.0×10^8 CFU/mL), were prepared and diluted 1:20 in 7H9-OADC. Ethical approval was not required for the study.

4.3.2. Minimum inhibitory concentration (MIC)

Anti-*M. tuberculosis* activities of 19 new β-carboline derivatives were determined by resazurin microtiter assay plate (REMA) [36] in three independent assays at different days. Firstly, β-carboline derivatives stock solutions were prepared in dimethyl sulfoxide (Sigma-Aldrich, St Louis, MO, USA). The derivatives stock solutions were diluted in 7H9-OADC to 1000 μg/mL. New dilutions of all derivatives were carried out in 96-well sterile microplates and 100 μL of previously standardized mycobacterial inoculum were added to obtain final derivatives concentrations from 0.98 to 250 μg/mL. The microplates were incubated for 7 days at 35 °C in normal atmosphere. After the incubation time, 30 μL of freshly prepared 0.01% resazurin solution (Acros, Morris Plains, NJ, USA) were added to each well. The microplates were incubated for additional 24 h at 35 °C for subsequent visual reading. A color change from blue to pink, indicated mycobacterial growth, and the MIC was defined as the lowest concentration of the derivatives that prevented color change. Medium, β-carboline derivatives sterility and mycobacterial growth with and without 2.5% (v/v) DMSO controls were included in all assays. Isoniazid (Sigma-Aldrich, St Louis, MO, USA) was used as the reference drug in all assays.

4.3.3. Cytotoxicity

The cytotoxicity of β-carboline derivatives was determined in three independent assays at different days, using VERO epithelial cells (ATCC CCL81). The cells were cultured in 10% Fetal Bovine Serum (FBS) Dulbecco's Modified Eagle's Medium (DMEM) and incubated at 37 °C under 5% CO_2 until confluent growth. In a transparent 96-well microplate, 5×10^4 cell/well were added, and incubated at 37 °C under 5% CO_2 for 24 h. After, the medium was removed and different concentrations of the β-carboline derivatives (1.95–1000 μg/mL), diluted in DMEM, were added to the microplate wells. All medium was removed after 24-h incubation at 37 °C under 5% CO_2, and the microplate wells washed twice with phosphate-buffered saline (PBS). Then, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) was diluted in PBS (2 mg/mL), and 50 μL were added to each microplate well. The microplate was incubated at 37 °C under 5% CO_2 for 4 h. After, MTT was removed and 150 μL of DMSO was added to the microplate wells, and absorbance was determined at 550 nm. The CC_{50} value was defined as the highest drug concentration that 50% of the cells are viable in comparison to the control. The selectivity index (SI) was calculated by the CC_{50}/MIC ratio against the reference strain $H_{37}Rv$ and clinical isolates.

4.3.4. Three and four-dimensional structure-activity relationships studies

In order to improve understanding on the way of β-carboline derivatives action in *M. tuberculosis*, structure-activity relationship (SAR) studies were carried out using the three and four-dimensional approaches. Thus, three-dimensional structures were built in HyperChem 7 (Hyper Co.), and calculations of B3LYP/6−311++g (d,p) approach were carried in Gaussian 09 (Gaussian Inc.). Using these structures, field descriptors of each derivative were obtained in Pentacle (Molecular Discovery Ltd), which uses the GRIND approach for calculating 3D field descriptors in an alignment-independent way. The used descriptors were computed using the AMANDA/MACC2 algorithms [26−28,37].

A qualitative SAR study based in the approach used by Ermondi et al. [38] was carried out. Compounds with MIC >250 μg/mL were defined as inactives, and were set as −1; on the other hand, derivatives showing MIC ≤250 μg/mL were set as actives, 1. The field descriptors used were based in distances at angstroms between different regions of a molecule: hydrophobic-hydrophobic groups (DRY-DRY), hydrogen bond acceptor-hydrogen bond acceptor groups (O−O), hydrogen bond donor-hydrogen bond donor groups (N1−N1), shape-shape groups (TIP-TIP), and the combinations between these (DRY-O, DRY-N1, DRY-TIP, O−N1, O-TIP, and N1-TIP). The set of obtained descriptors was reduced using the Fractional Factorial Design (FDD) variable selection, available in the same program [26−28,37]. This selection was carried out by the consecutive construction of regression models by Partial Least Squares (PLS) with a smaller number of descriptors, but with improved statistical quality. Next, the selected descriptors were exported and a new selection of variables was performed using the Ordered Predictors Selection (OPS) [39] method, implemented in the QSAR Modeling [40] program, in order to obtain a model with a lower number of descriptors, but more significant and easier to interpret.

Using the activities as defined for the 3D-QSAR study, a 4D-QSAR study was carried out by LQTA-QSAR methodology [41], employing the free software web-4D-QSAR [42]. This approach was based on the generation of a conformational ensemble profile for each compound, followed by the calculation of 3D descriptors, which were an average of the interaction experienced by a probe, NH_3^+ in this study, in each grid point for all conformations. Coulomb and Lennard-Jones interactions were calculated for each derivative tested. The matrix with the descriptors generated by LQTAgrid module in web-4D-QSAR was treated using digital filters for molecular field interactions [43] followed by a variable reduction with the elimination of descriptors with the absolute value of the correlation coefficient between the descriptor and biological activity lower than 0.3. Then, the resulting matrix was also submitted to the OPS method [39].

The quality of the obtained models was assessed on its coefficient of determination (R^2), the root mean square error of calibration (*RMSEC*), the *F*-test, the coefficient of determination of cross-validation (Q^2_{LOO}), and the root mean square error of cross-validation (*RMSECV*). In all steps of variable selection and construction of models, descriptors were autoscaled, the most recommended data pre-processing approach for QSAR studies [44].

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Ethical approval

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Declaration of competing interest

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

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Appendix A. Supplementary data

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