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Microwave induced three-component synthesis and antimycobacterial activity of benzopyrazolo[3,4-*b*]quinolindiones

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ABSTRACT

Three series of novel 4-arylbenzo[*h*]pyrazolo[3,4-*b*]quinolin-5,6-diones **4**, **7** and **9** have been efficiently obtained in good yields by three-component microwave assisted reaction between aminopyrazoles **1** or **6** (both 1-phenyl and 1-H substituted), 2-hydroxynaphthoquinone **2** and benzaldehydes **3**. Compounds **4**, **7** and **9** have been evaluated against fifteen *Mycobacterium* spp strains, and six of them have shown antimycobacterial activity. The highest inhibitory activity with MIC ≤2 μg/mL for three of these compounds (**4a**, **4b** and **4g**) was related with their highest lipophilicity and lesser polarity within these series.

1. Introduction

Human tuberculosis (TB) is a contagious–infectious disease mainly caused by *Mycobacterium tuberculosis*, which is an aerobic pathogenic bacterium that establishes its infection usually in the lungs [1]. About one-third of the world's population is currently infected with *M. tuberculosis*; 10% of those infected will develop clinical opportunistic disease in inmunocompromised patients, such those infected by the human immunodeficiency virus (HIV) [1]. Although there are medical regimens for treating TB, they are far from ideal [2]. In addition, there have been reported an increasing number of multidrug-resistant strains (MDR-TB) and extensively drug-resistant (XDR-TB) strains and HIV epidemics. These two factors make necessary the development of new drug that could improve the understanding of drug action molecular mechanisms and drug resistance, and accordingly could provide significant insights for the development of new generation of drug for the treatment of TB [2].

Numerous tri- and tetracyclic planar nitrogen-containing heterocycles, such as amsacrine, the benzo[*c*]phenanthridines, the ellipticines, intoplicine and coralyne are well known as topoisomerase inhibitors and have been investigated as potential anticancer agents [3]. The benzoquinolines such as acridines or the isomeric phenanthridine are important scaffolds in antitumor agents, as well as their benzo-fused derivatives benzophenanthridines, representing an important alkaloid family.

Between them, the benzoquinolinediones have exhibited the best overall activity against both bacteria and fungi. Particularly noteworthy is the significant antifungal activity, which is comparable to the activity of the standard antifungal antibiotic amphotericin B [4]. These derivatives are a very important class of compounds for the production of pharmaceuticals, herbicides, dyes, etc. [5]. There are several classical methods for the synthesis of these compounds from various starting materials, but still need to improve the reaction efficiency employing readily available starting materials.

We are interested in pyrazolo[3,4-*b*]quinoline derivatives, which are used as pharmaceutical agents [6] and their derivatives

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have shown antitubercular activity [7], as inhibitors of oncogenic Ras [8]. Additionally, pyrazolo[3,4-*b*]quinoline derivatives exhibit a high fluorescence in both solution and solid state under exposure to the white light, which make them appropriates in the design of electroluminescent materials, like organic light emitting diodes (OLEDs) and as a dopant in the multiplayer OLED fabrication [9]. In the past several decades, three general strategies for the synthesis of pyrazolo[3,4-*b*]quinolines have been developed: i) by the Friedländer condensation reaction of 2-aminobenzophenones and pyrazolin-5-ones [10], but the availability of 2-aminobenzo phenones limits the scope of this reaction; ii) by cyclo-condensation of 4-arylidenepyrazolin-5-ones with anilines [11] or 5-*N*-arylpyrazoles with aromatic aldehydes [12]; and iii) by a three-component one-pot reaction of aromatic aldehydes, 5-amino-3-methyl-1-phenylpyrazole and dimedone under thermal [13] or microwave irradiation condition [14].

In this way, multicomponent reactions have shown great importance for its high levels of brevity and diversity, allowing time-saving in one-pot operations, and the combination of three or more reagents to get flexible building blocks [15]. The structure of the reaction products can be diversified by systematic variation of each starting material that could be either commercially available or easily prepared [16].

Microwave assisted organic synthesis (MAOS) has also emerged as a powerful tool for high-throughput procedures. This can improve the yield and purity of the final compounds in short reaction times through the precise control of parameters such as power irradiation, pressure and temperature [17].

We have concentrated much of our recent efforts in the preparation of pyrazolo[3,4-*b*]quinolines as bioactive compounds, and have already described simple and efficient procedures to prepare them in a three-component reaction starting from 5-amino pyrazoles **1** [18]. In an extension to get benzo-fused derivatives of those compounds, that can increase their biological potential, we are reporting here the synthesis of 4-arylphenylbenzo[*h*]pyrazolo [3,4-*b*]quinolin-5,6-diones, which have been tested as anti-mycobacterial agents.

2. Results and discussion

2.1. Chemistry

For the preparation of the new tetracyclic pyrazolic derivatives **4**, we have carried out a microwave irradiation on an equimolar mixture of 5-amino-3-methyl-1-phenylpyrazole **1**, 2-hydroxynaph thoquinone **2** and benzaldehydes **3** during 3 min. From this approach the new 4-aryl-3-methyl-1-phenylbenzo[*h*]pyrazolo[3,4-*b*]quinolin-5,6-diones 4 were isolated (Scheme 1) in an easy way and in good yields (80–85%) as stable crystalline solids (see Experimental section).

In all cases this three-component reaction led regioselectively to non-linear tetracyclic compounds **4** (but not **5**) and their structures were characterized by spectroscopic and analytical methods.

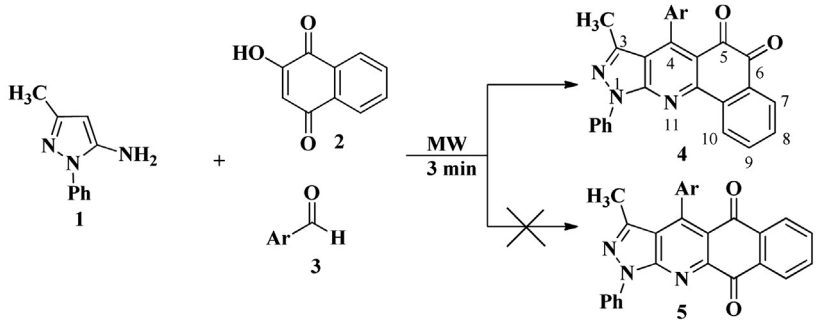
The NMR spectroscopic data were consistent with the proposed structures **4**. Regarding to the tetracyclic system, for example compound **4a** exhibits a ^{1}H NMR spectrum with a singlet at 1.93 ppm corresponding to CH_3-group of pyrazolic ring at position 3. At 7.60, 7.83, 8.15 and 8.81 ppm appear four signals, each one integrating for one proton, assigned to H-7, H-8, H-9 and H-10 respectively, corresponding to protons of the benzo-moiety.

The whole carbon skeleton was assigned from ^{13}C NMR spectra, combined with DEPT and bidimensional experiments (COSY, HSQC and HMBC). Compound **4a** showed in its ^{13}C NMR spectrum at 14.2 ppm the signal for the methyl group and at 179.9 and 180.5 ppm the signals for C-5 and C-6 carbonyl groups. The signals for C11a and C-10b appeared at higher δ values, 150.6 and 153.7 ppm. In contrast, carbon atoms C-3a and C-4a appeared unusually at low chemical shifts, 116.9 and 119.8 ppm respectively. Regarding to the mass spectra, all products **4** exhibited similar behavior in their fragmentation pattern, showing the molecular ion peak along with a typical loss of the aryl group at position 4 as the base peak.

The regioselective formation of the non-linear (angular) tetracyclic structure **4** instead of a linear one **5** was unambiguously confirmed by X-ray diffraction experiments [19] (Fig. 1).

It is worth mentioning that compounds **4a**–**c**,**e**,**g**,**h** have previously been synthesized elsewhere in solution but with reaction times between 10 and 26 min (61–91% [20]) and 60–90 min (87–91% [21]). In order to verify the versatility of the above described method, we carried out, the three-component reaction between the 5-amino-1-*NH*-pyrazole **6**, 2-hydroxynaphthoquinone **2** and benzaldehydes **3** under the same reaction conditions. In this case, the non-linear 4,11-dihydrobenzo[*h*]pyrazolo[3,4-*b*]quinolin-5,6-diones **7** were also obtained, in a regioselective manner, also in good isolated yields (Scheme 2). Although, formation of the regioisomer **8** during the cyclization process was also expected owing to the presence of an additional nucleophilic center (1-NH) in aminopyrazole **6** [10,15]; the exclusive formation of compounds **7** was confirmed by spectroscopic analysis. Possible formation of benzopyrazoloquinazolines **8** was finally excluded based on NMR examination, mainly because of the absence of signals for H-3 (H-4 in precursors **6**) in their ^{1}H NMR spectra.

Thus, ^{1}H NMR spectra of compounds **7a**-**f** showed three singlets, which corresponding to the protons H-4 (between 5.29 and 5.70 ppm) and two deuterium exchangeable protons 1-NH (between 12.02 and 12.90 ppm), 11-NH (around 10.07–10.38 ppm)



Ar = **4a** C_6H_5, **4b** 4-$CH_3C_6H_4$, **4c** 4-$CH_3OC_6H_4$, **4d** 3,4,5-tri-$CH_3OC_6H_2$, **4e** 4-ClC_6H_4, **4f** 4-BrC_6H_4, **4g** 4-FC_6H_4, **4h** 4-$O_2NC_6H_4$

Scheme 1.

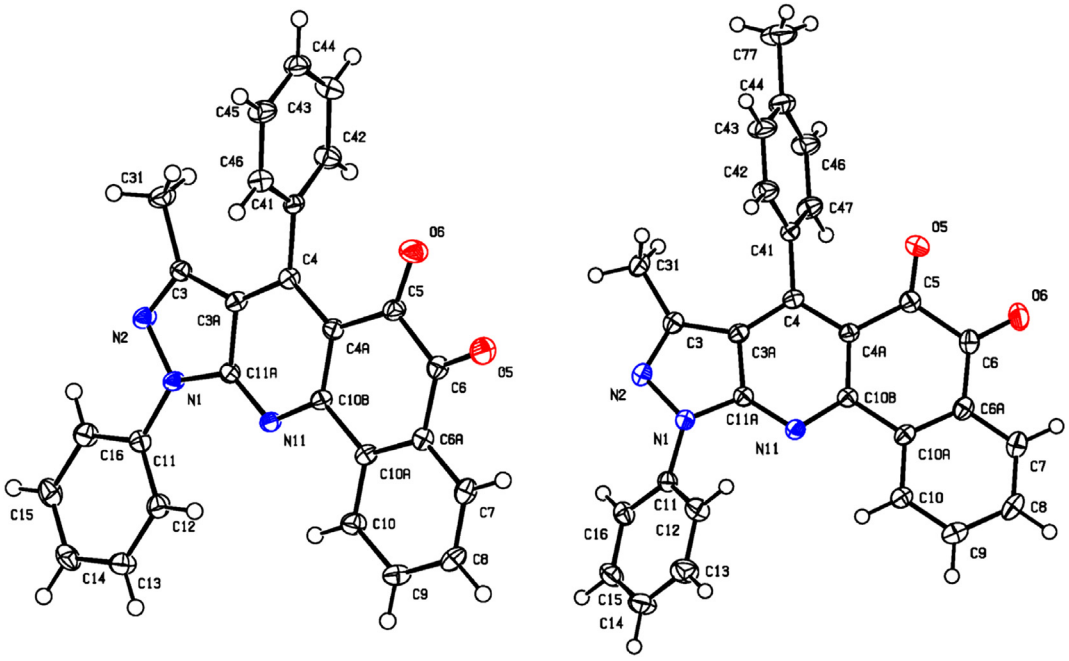


Fig. 1. ORTEP drawing for **4a** and **4b**.

respectively, and signals corresponding to aromatic protons found between 6.69 and 8.75 ppm. Type of carbon atoms was determined by ^{13}C NMR (DEPT-135 experiment) spectroscopy, and it was consistent with the proposed structure **7**. Structures of compounds **7** were thoroughly confirmed by NMR and HR-MS.

It is important to remark that in some cases the reaction led to the formation of aromatized 3,4-diaryl-1*H*-benzo[*h*]pyrazolo[3,4-*b*]quinoline-5,6-diones **9a**–**g**, depending on the type of substituent in the Ar-ring (Scheme 3). We assume that the reaction proceeded through the initial formation of dihydroderivatives type **7**, which were then aromatized under the reaction conditions. Under inert atmosphere this reaction also led to the formation of the aromatized products **9**.

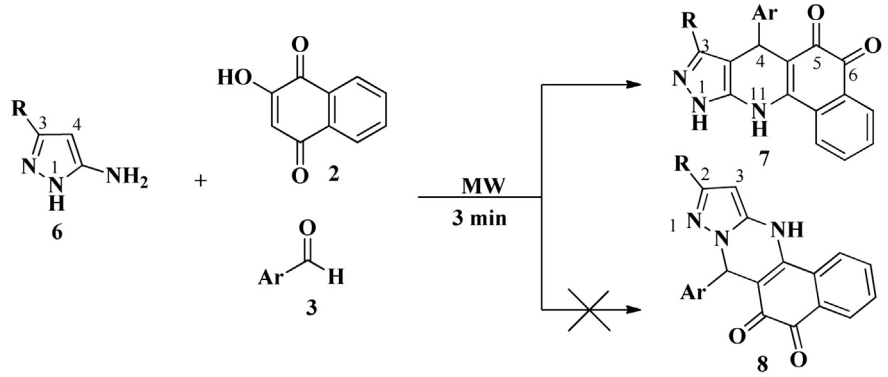
The structure of benzopyrazolo[3,4-*b*]quinolines **9a**–**g** was confirmed by NMR and HR-MS.

2.2. Antimycobacterial screening

Results of the antimycobacterial studies of thirteen selected pyrazolo[3,4-*b*]quinolines derivatives **4**, **7**, **9** are consigned in Table 1. Six of such compounds (i.e. **4a**, **4b**, **4g**, **7d**, **7f** and **9b**) exhibited antimycobacterial activity; three of them **4a**, **4b** and **4g** showed the highest antitubercular activity (MIC between 2 and 4 μg/mL for *M. tuberculosis* species) and the remaining **7d**, **7f** and **9b** showed moderate activity (MIC between 8 and 16 μg/mL).

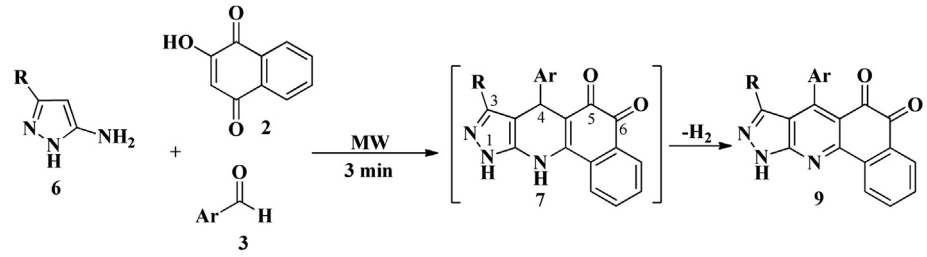
The antimicrobial activity of our compounds might be associated with the fact, that the pyrazolo[3,4-*b*]quinolines have previously shown to be bactericidal with an *in vitro* significant inhibitory activity on bacterial serine/threonine protein kinases [22], because these kinases are necessary for an adequate response to environmental stimuli and functionality of these enzymes [23]. Owing to they are essential in nutrition and cell division in mycobacteria, kinases have emerged as novel therapeutic target eukaryotic-like [24], and their inhibitors become into important candidates for new anti-bacterial drugs development, with the ability to avoid resistance mechanisms [23–25].

In order to correlate a qualitative structure–activity relationship described above, with quantitative parameters, we calculated the log *P* for several benzopyrazolo[3,4-*b*]quinolinediones (**4**, **7** and **9**), and attempted to find a relationship between these values and their



7a R = Ar = C_6H_5; **7b** R = C_6H_5; Ar = 4-$CH_3C_6H_4$; **7c** R = C_6H_5, Ar = 4-$CH_3OC_6H_4$;
7d R = 4-$CH_3C_6H_4$, Ar = C_6H_5; **7e** R = 4-ClC_6H_4; Ar = C_6H_5; **7f** R = 4-$O_2NC_6H_4$, Ar = C_6H_5

Scheme 2.



9a R = 4-$CH_3C_6H_4$, Ar = 4-$O_2NC_6H_4$; **9b** R = 4-ClC_6H_4, Ar = 4-$CH_3OC_6H_4$; **9c** R = Ar = 4-ClC_6H_4;
9d R = 4-ClC_6H_4,Ar = 4-$O_2NC_6H_4$; **9e** R = 4-$O_2NC_6H_4$, Ar = 4-$CH_3OC_6H_4$; **9f** R = 4-$O_2NC_6H_4$, Ar = 4-ClC_6H_4;
9g R = Ar = 4-$O_2NC_6H_4$

Scheme 3.

MICs (in μg/mL). For this study was selected *MTB*2556, the culture cell that was inhibited by a larger number of compounds (Table 2).

For the calculation of log *P* and dipole we used quantum mechanical at semiempirical level using Mopac, with the parametric method 3 (PM3). The molecular modeling were prepared using CS Chem-Office Software version 9.0 (Cambridge software) [26]. The models were minimized until the root mean square (RMS) gradient value reached a value smaller than 0.0001 kcal/mol. The lowest energy structure was used for each molecule to calculate log *P* values and dipole, as lipophilicity measures. Antimicrobial activity against *Mycobacterium* species depends on how efficiently solutes cross the cell wall that constitutes an efficient permeability barrier. Hydrophobic cell wall, principally in the form of very long-chain fatty acids (mycolic acids), obviously acts as a barrier to penetration of hydrophilic solutes, this fact causes that mycobacteria would be often more susceptible to less-polar compounds [25]. The derivatives (**4a**, **4g** and **9b**) showed appropriate values for log *P* and dipole agree with their antimicrobial activity. In contrast, the **7f** derivative showed the lowest lipophilicity in comparison with the other reported compounds. However the log *P* and dipole values for compound **7f** disagree with its antimicrobial activity, what can be attributed to the presence of the nitro group in the molecule. On the

Table 1
The *in vitro* activity (MIC μg/mL) of pyrazolo[3,4-*b*]quinolinedione derivatives against fifteen *Mycobacterium* spp.

Compound	*Mycobacterium* spp[a]							
	H37Rv	35837	35838	35822	35820	MTB2556	MTB4000	UT544
MIC (μg/mL)								
4a	≤2	≤2	≤2	≤2	≤2	≤2	≤2	≤2
4b	4	4	4	≤2	≤2	≤2	≤2	≤2
4d	>32	>32	>32	>32	>32	>32	>32	>32
4e	>32	>32	>32	>32	>32	>32	>32	>32
4g	4	4	4	≤2	≤2	≤2	≤2	≤2
7a	>32	>32	>32	>32	>32	>32	>32	>32
7b	>32	>32	>32	>32	>32	>32	>32	>32
7c	>32	>32	>32	>32	>32	>32	>32	>32
7d	16	16	4	16	16	16	16	16
7e	>32	>32	>32	>32	>32	>32	>32	>32
7f	32	>32	16	16	16	8	16	8
9a	>32	>32	>32	>32	>32	>32	>32	>32
9b	>32	>32	16	>32	16	8	16	8
Isoniazid	0.25	0.25	0.25	>0.5	0.25	>0.5	>0.5	>0.5
Rifampicin	0.125	0.125	>0.5	0.125	0.125	>0.5	>0.5	>0.5
	MTB411	MTB985	MNT1407	MNT1073	MNT1100	MNT1193	MNT1408	
4a	4	≤2	>32	>32	>32	>32	>32	
4b	4	≤2	>32	>32	>32	>32	>32	
4d	>32	>32	>32	>32	>32	>32	>32	
4e	>32	>32	>32	>32	>32	>32	>32	
4g	4	≤2	>32	>32	>32	>32	>32	
7a	>32	>32	>32	>32	>32	>32	>32	
7b	>32	>32	>32	>32	>32	>32	>32	
7c	>32	>32	>32	>32	>32	>32	>32	
7d	16	16	>32	>32	>32	>32	>32	
7e	>32	>32	>32	>32	>32	>32	>32	
7f	16	8	>32	>32	>32	>32	>32	
9a	>32	>32	>32	>32	>32	>32	>32	
9b	16	16	>32	>32	>32	>32	>32	
Isoniazid	>0.5	>0.5	>0.5	>0.5	>0.5	>0.5	>0.5	
Rifampicin	>0.5	>0.5	0.125	>0.5	>0.5	>0.5	>0.5	

[a] *M. tuberculosis* H37Rv (27294) susceptible to all five first-line antituberculosis drugs (streptomycin, isoniazid, rifampicin, ethambutol and pirazynamide), H37Rv (35837) ethambutol-resistant, H37Rv (35838) rifampicin resistant, H37Rv (35822) isoniazid-resistant and H37Rv (35820) streptomycin-resistant,; MTB2556, MTB4000, UT544, MTB411, MTB985 (*M. tuberculosis* clinical isolates Beijing genotype, isoniazid and rifampicin resistant); MNT 1407 (*M. chelonae* clinical isolate), MNT1073 (*M. fortuitum* clinical isolate), MNT1100 (*M. szulgai* clinical isolate), MNT1193 (*M. scrofulaceum* clinical isolate) and MNT1408 (*M. intracellulare* clinical isolate).

Table 2
The *in vitro* activity (MIC μg/mL) of compounds **4**, **7** and **9** assayed against the *MTB*2556 and their calculated log *P* and dipole.

Compound	Log *P*	Dipole (D)	*MTB*2556
4a	6.68	3076	≤2
4b	6.17	2801	≤2
4d	5.30	5788	>32
4e	6.24	4525	>32
4g	6.84	4927	≤2
7a	4.31	9524	>32
7b	4.80	9527	>32
7c	4.18	9451	>32
7d	4.80	9508	16
7e	4.87	9726	>32
7f	2.53	9966	8
9a	5.24	8425	>32
9b	5.91	3031	8

other hand, the remaining compounds exhibited low values of log *P* and dipole, therefore less antimycobacterial activities (Fig. 2).

3. Conclusion

We are herein describing the synthesis and antimycobacterial activity of novel series of 4-arylbenzopyrazolo[3,4-*b*]quinolindiones **4**, **7** and **9**. These new compounds were obtained in a straightforward manner *via* a three-component approach assisted by microwave irradiation. These 4-arylbenzopyrazolo[3,4-*b*]quinolindiones have been tested against fifteen *Mycobacterium*, and six of them have shown antimycobacterial activity. The highest inhibitory activity with MIC ≤2 μg/mL for compounds **4a**, **4b** and **4g** was related with their higher lipophilicity and lesser polarity within these series.

4. Experimental

Melting points were determined on a Büchi melting point apparatus and are uncorrected. IR spectra were recorded on a Shimadzu FTIR 8400 spectrophotometer in KBr disks. ^{1}H and ^{13}C NMR spectra were recorded on a Bruker Avance 400 spectrophotometer operating at 400 MHz and 100 MHz respectively, using DMSO-d_6 and $CDCl_3$ as solvents and tetramethylsilane as internal standard. Mass spectra were run on a SHIMADZU-GCMS 2010-DI-2010 spectrometer (equipped with a direct inlet probe) operating at 70 eV. High Resolution Mass Spectra (HRMS) by electron impact were recorded on a Micromass AutoSpec-Ultima, magnetic sector mass spectrometer at 70 eV. The elemental analyses were obtained using a LECO CHNS-900 elemental analyzer and the values are within ±0.4% of the theoretical values. Microwave experiments were carried out on a focused microwave reactor (300 W CEM Discover). Silica gel aluminum plates (Merck 60 F_{254}) were used for analytical TLC. All chemicals including the starting 2-hydroxynaphthoquinone **2** and benzaldehydes **3** were purchased from Aldrich, Fluka and Acros (analytical reagent grades) and were used without further purification. The key amines **1** and **6** were obtained according to a procedure previously described by us [27].

4.1. Chemistry

4.1.1. General procedure for the synthesis of 4-aryl-3-methyl-1-phenylbenzo[h]pyrazolo[3,4-b]quinolin-5,6-diones *4a–h*, 3,4-diaryl-4,11-dihydro-1H-benzo[h]pyrazolo[3,4-b]quinolin(1H,11H)-5,6-diones *7a–f* and 3,4-diaryl-1H-benzo[h]pyrazolo[3,4-b]quinolin-5,6-diones *9a–g*

A mixture containing 1.0 mmol of 5-amino-3-methyl-1-phenylpyrazole **1** or 5-amino-3-arylpyrazole **6**, 2-hydroxynaphtho quinone **2** (1.0 mmol) and the appropriate aldehyde **3** (1.0 mmol) was exposed to microwave irradiation for 3 min (120 °C and 200 W). The solids obtained were purified by recrystallization from ethanol.

*4.1.1.1. 3-Methyl-1,4-diphenylbenzo[h]pyrazolo[3,4-b]quinolin-5,6-dione **4a**.* Yellow solid, mp 272–273 °C (85%) (272–273 °C (83%) [20]). ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm): 1.93 (s, 3H, CH_3), 7.25–730 (m, 2H, H_m Ar), 7.39 (t, 1H, H_p NPh, $J = 8.1$), 7.50–7.54 (m, 3H, H_p Ar, H_o Ar), 7.60 (m, 3H, H-8, H_m NPh), 7.83 (t, 1H, H-9, $J = 8.1$), 8.15 (d, 1H, H-7, $J = 7.9$ Hz), 8.31 (d, 2H, H_o NPh, $J = 8.0$ Hz), 8.81 (d, 1H, H-10, $J = 8.1$ Hz). ^{13}C NMR (100 MHz, $CDCl_3$) δ (ppm): 14.2 (CH_3), 117.0 (C-3a), 119.8 (C-4a), 121.3 (C_o NPh), 126.5 (C_p NPh), 127.0 (C_m NPh), 127.2 (C-10), 128.4 (C_o, C_p Ar), 129.0 (C-7), 129.1 (C_m Ar), 131.3 (C-8), 131.7 (C-6a), 132.6 (C-4), 135.9 (C-9), 137.4 (C-10a), 138.7 (C_i NPh), 146.5 (C-3), 150.6 (C-11a), 152.2 (C_i Ar), 153.7 (C-10b), 179.9 (C-5), 180.5 (C-6). EI MS (70 eV): *m/z* (%): 415 (M^+, 16), 386 (100). Anal. Calcd for $C_{27}H_{17}N_3O_2$: C:76.40, H: 4.27, N: 9.90, found: C: 76.07, H: 3.96, N: 9.66.

*4.1.1.2. 3-Methyl-1-phenyl-4-(4-tolyl)benzo[h]pyrazolo[3,4-b]quinolin-5,6-dione **4b**.* Red solid, mp 273–274 °C (85%) (276–277 °C (81–84%) [20,21]). ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm): 1.97 (s, 3H, CH_3),

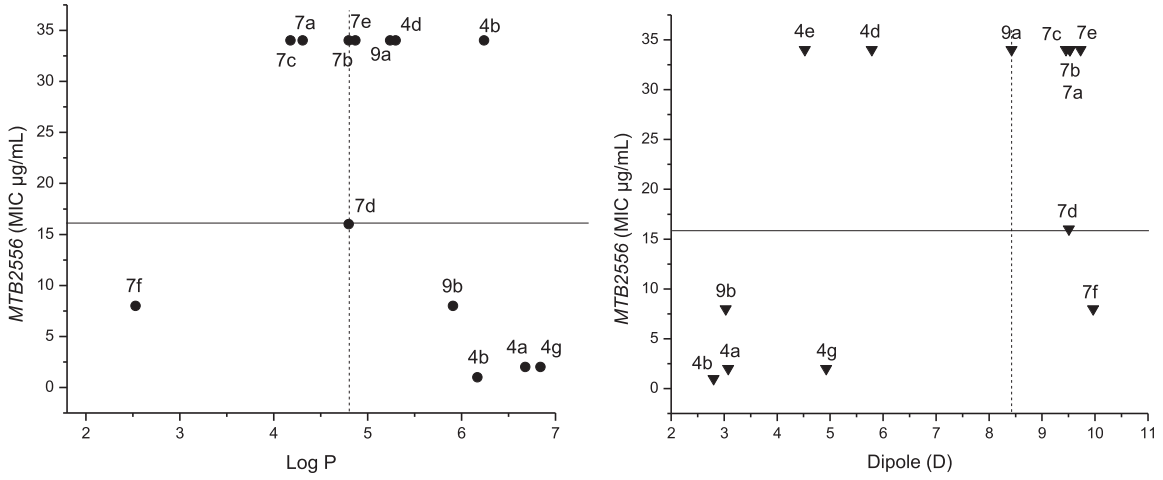


Fig. 2. Log *P* vs. MIC and dipole vs. MIC of benzopyrazolo[3,4-*b*]quinolindiones derivatives against *MTB*2556 culture cell.

2.48 (s, 3H, CH_3 Ar) 7.17 (d, 2H, H_m Ar, $J = 7.2$ Hz), 7.32 (d, 2H, H_o Ar, $J = 7.2$ Hz), 7.38 (t, 1H, H_p NPh, $J = 8.0$ Hz), 7.59 (m, 3H, H-8, H_m NPh), 7.82 (t, 1H, H-9, $J = 8.0$ Hz), 8.13 (d, 1H, H-7, $J = 8.1$ Hz), 8.31 (d, 2H, H_o NPh, $J = 8.0$ Hz), 8.79 (d, 1H, H-10, $J = 8.1$). ^{13}C NMR (100 MHz, $CDCl_3$) δ (ppm): 14.3 (CH_3), 21.4 (CH_3 Ph), 117.1 (C-3a), 120.0 (C-4a) 120.2 (C_p Ar), 121.3 (C_o NPh), 126.5 (C_p NPh), 127.0 (C_m Ar), 127.2 (C-10), 128.9 (C-7), 129.0 (C_o Ar), 129.1 (C_m NPh), 131.2 (C-8), 131.6 (C-6a), 132.8 (C-4) 135.8 (C-9), 137.4 (C-10a), 138.7 (C_i NPh), 146.6 (C-3), 150.5 (C-11a), 152.6 (C_i Ar) 153.7 (C-10b), 179.9 (C-5), 180.5 (C-6). EI MS (70 eV): *m/z* (%): 431/429 (M^{+2}/M^+, 63/24), 400 (100), 386 (95). Anal. Calcd for $C_{28}H_{19}N_3O_2$: C: 77.23, H: 4.55, N: 9.65, found: C: 77.43, H: 4.55, N: 9.65.

4.1.1.3. 3-Methyl-4-(4-methoxyphenyl)-1-phenylbenzo[h]pyrazolo [3,4-b]quinolin-5,6-dione ***4c***. Red solid, mp 280–281 °C (80%) (279–281 °C, 83% [20]). ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm): 2.00 (s, 3H, CH_3), 3.91 (s, 3H, OCH_3), 7.50 (d, 2H, H_m Ar, $J = 8.7$ Hz), 7.22 (d, 2H, H_o Ar, $J = 8.7$ Hz), 7.38 (t, 1H, H_p NPh, $J = 7.7$ Hz), 7.59 (m, 3H, H-8, H_m Nph), 7.82 (t, 1H, H-9 $J = 8.3$ Hz), 8.13 (d, 1H, H-7, $J = 8.3$ Hz), 8.30 (d, 2H, H_o NPh, $J = 7.7$ Hz), 8.78 (d, 1H, H-10, $J = 8.3$ Hz). ^{13}C NMR (100 MHz, $CDCl_3$) δ (ppm): 14.3 (CH_3), 56.3 (OCH_3), 113.9 (C_m Ar), 117.3 (C-3a), 120.2 (C-4a), 121.3 (C_o NPh), 126.5 (C_p NPh), 127.2 (C-10), 128.6 (C_o Ar), 128.9 (C-7), 129.1 (C_m NPh), 131.2 (C-8), 129.3 (C-6a), 131.6 (C-4) 135.9 (C-9), 137.4 (C-10a), 138.7 (C_i NPh), 146.6 (C-3), 150.5 (C-11a), 152.4 (C_i Ar) 153.7 (C-10b), 159.9 (C_p Ar), 179.9 (C-5), 180.5 (C-6). EI MS (70 eV): *m/z* (%): 445 (M^+, 22), 416 (100), 386 (26). HR-MS calcd for $C_{28}H_{19}N_3O_3$ 445.1426, found 445.1425.

4.1.1.4. 3-Methyl-4-(3,4,5-trimethoxyphenyl)-1-phenylbenzo[h]pyrazolo[3,4-b]quinolin-5,6-dione ***4d***. Yellow solid, mp 314–315 °C (85%). ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm): 2.07 (s, 3H, CH_3), 3.86 (s, 6H, OCH_{3m} Ar), 3.97 (s, 3H, OCH_{3p} Ar), 6.51 (s, 2H, H_o Ar), 7.40 (t, 1H, H_p NPh, $J = 7.9$ Hz), 7.60 (m, 3H, H-8, H_m NPh), 7.83 (t, 1H, H-9, $J = 7.8$ Hz), 8.15 (d, 1H, H-7, $J = 7.8$ Hz), 8.31 (d, 2H, H_o NPh, $J = 8.0$ Hz), 8.80 (d, 1H, H10, $J = 7.8$ Hz). ^{13}C NMR (100 MHz, $CDCl_3$) δ (ppm): 14.2 (CH_3), 56.3 (OCH_{3m}), 61.1 (OCH_{3p}), 104.6 (C_o Ar), 117.0 (C-3a), 120.2 (C-4a), 121.4 (C_o NPh), 126.6 (C_p NPh), 127.3 (C-10), 129.1 (C-7), 129.2 (C_m NPh), 131.2 (C-6a), 131.4 (C-8), 131.6 (C-4) 136.0 (C-9), 137.4 (C-10a), 138.3 (C_p Ar), 138.6 (C_i NPh) 146.6 (C-3), 150.6 (C-11a), 152.0 (C_i Ar), 153.5 (C_m Ar), 153.7 (C-10b), 179.6 (C-5), 180.4 (C-6). EI MS (70 eV): *m/z* (%): 505 (M^+, 93), 476 (47.7), 462 (57), 462 (76), 446 (100), 430 (36). HR-MS calcd for $C_{30}H_{23}N_3O_5$ 505.1638, found 505.1634. Anal. Calcd for $C_{30}H_{23}N_3O_5$: C: 70.03, H: 4.70, N: 8.17, found: C: 70.39, H: 4.53, N: 8.18.

4.1.1.5. 4-(4-Chlorophenyl)-1-phenyl-3-methylbenzo[h]pyrazolo[3,4-b]quinolin-5,6-dione ***4e***. Brown solid, mp 328–329 °C (85%) (248–250 °C (85%) [21]). ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm): 1.91 (s, 3H, CH_3), 7.40 (d, 2H, H_o Ar, $J = 8.3$ Hz), 7.42 (t, 1H, H_p NPh, $J = 7.9$ Hz), 7.57 (d, 2H, H_m Ar, $J = 8.3$ Hz), 7.63 (m, 2H, H_m NPh), 7.68 (t, 1H, H-8, $J = 7.6$ Hz), 7.92 (t, 1H, H-9, $J = 7.6$ Hz), 8.03 (d, 1H, H-7, $J = 7.6$ Hz), 8.27 (d, 2H, H_o NPh, $J = 8.0$ Hz), 8.73 (d, 1H, H-10, $J = 7.5$ Hz). ^{13}C NMR (100 MHz, $CDCl_3$) δ (ppm): 14.3 (CH_3), 116.6 (C-3a), 120.9 (C-4a), 121.5 (C_o NPh), 126.9 (C_p NPh), 127.1 (C-10), 128.5 (C_m Ar), 128.6 (C-7), 129.7 (C_m NPh), 129.9 (C_o Ar), 131.8 (C-8), 132.4 (C_p Ar), 133.6 (C-6a), 135.5 (C-4) 136.8 (C-9), 137.2 (C-10a), 138.9 (C_i NPh), 145.9 (C-3), 150.0 (C-11a), 150.6 (C_i Ar) 153.8 (C-10b), 179.7 (C-5), 180.2 (C-6). EI MS (70 eV): *m/z*: 451/449 (M^{+2}/M^+, 8/26), 420 (100), 386 (17). HR-MS calcd for $C_{27}H_{16}ClN_3O_2$ 449.0931 found 449.0930. Anal. Calcd for $C_{27}H_{16}ClN_3O_2$: C, 72.08; H, 3.58; N, 9.34. found: C, 71.60; H, 3.46; N, 8.86.

4.1.1.6. 4-(4-Bromophenyl)-3-methyl-1-phenylbenzo[h]pyrazolo[3,4-b]quinolin-5,6-dione ***4f***. Yellow solid, mp 263–264 °C (80%). ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm): 1.99 (s, 3H, CH_3), 7.18 (d, 2H, H_m Ar, $J = 8.3$ Hz), 7.40 (t, 1H, H_p NPh, $J = 7.8$ Hz), 7.60 (m, 3H, H-8, H_m NPh), 7.66 (d, 2H, H_o Ar, $J = 8.3$ Hz), 7.83 (t, 1H, H-9, $J = 7.9$ Hz), 8.14 (d, 1H, H-7, $J = 7.9$ Hz), 8.30 (d, 2H, H_o NPh, $J = 7.8$ Hz), 8.78 (d, 1H, H-10 $J = 7.9$ Hz). ^{13}C NMR (100 MHz, $CDCl_3$) δ (ppm): 14.5 (CH_3), 116.7 (C-3a), 119.7 (C-4a), 121.4 (C_o NPh), 122.7 (C_p Ar), 126.7 (C_p NPh), 127.2 (C-10), 128.8 (C_m NPh), 129.1 (C-7), 129.2 (C_m Ar), 131.4 (C-8), 131.6 (C_o Ar), 133.3 (C-6a), 134.8 (C-4), 136.0 (C-9), 137.1 (C-10a), 138.6 (C_i NPh), 146.2 (C-3), 150.5 (C-11a), 150.7 (C_i Ar) 153.8 (C-10b), 179.9 (C-5), 180.3 (C-6). EI MS (70 eV): *m/z* (%): 495/493 (M^{+2}/M^+, 32/30), 466 (100), 356 (52), 77 (15). Anal. Calcd for $C_{27}H_{16}BrN_3O_2$: C: 64.43, H: 3.40, N: 8.35, found: C: 64.85, H: 3.13, N: 8.08.

4.1.1.7. 4-(4-Fluorophenyl)-3-methyl-1-phenylbenzo[h]pyrazolo[3,4-b]quinolin-5,6-dione ***4g***. Yellow solid, mp 289–290 °C (85%) (283–284 °C (85%) [20,21]). ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm): 1.97 (s, 3H, CH_3), 7.21 (d, 2H, H_m Ar, $J = 9.5$ Hz), 7.28 (d, 2H, H_o Ar, $J = 9.5$ Hz), 7.40 (t, 1H, H_p Ar $J = 7.6$, Hz), 7.60 (m, 3H, H-8, H_m NPh), 7.83 (t, 1H, H-9, $J = 8.4$ Hz), 8.14 (d, 1H, H-7, $J = 8.4$ Hz), 8.30 (d, 2H, H_o NPh, $J = 7.6$ Hz), 8.79 (d, 1H, H-10, $J = 8.4$ Hz). ^{13}C NMR (100 MHz, $CDCl_3$) δ (ppm): 14.5 (CH_3), 116.7 (C-3a), 119.7 (C-4a), 121.4 (C_o NPh), 122.7 (C_p Ar), 126.7 (C_p NPh), 127.2 (C-10), 128.8 (C_m NPh), 129.1 (C-7), 129.2 (C_m Ar), 131.4 (C-8), 131.6 (C_o Ar), 133.4 (C-6a), 134.8 (C-4), 136.0 (C-9), 137.1 (C-10a), 136.6 (C_i NPh), 146.2 (C-3), 150.5 (C-11a), 150.7 (C_i Ar) 153.8 (C-10b), 179.9 (C-5), 180.3 (C-6). EI MS (70 eV): *m/z* (%): 433 (M^+, 11), 405 (100). Anal. Calcd for $C_{27}H_{16}FN_3O_2$: C: 74.82, H: 3.72, N: 9.69, found: C: 74.62, H: 3.69, N: 9.45.

4.1.1.8. 3-Methyl-4-(4-nitrophenyl)-1-phenylbenzo[h]pyrazolo[3,4-b]quinolin-5,6-dione ***4h***. Yellow solid, mp 338–340 °C (80%) (334–336 °C (83%) [21]). ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm): 1.94 (s, 3H, CH_3), 7.42 (t, 1H, H_p NPh, $J = 7.8$ Hz), 7.50 (d, 2H, H_m Ar, $J = 8.1$ Hz), 7.61 (m, 3H, H-8, H_m NPh), 7.86 (t, 1H, H-9, $J = 8.0$ Hz), 7.15 (d, 1H, H-7, $J = 8.0$ Hz), 8.29 (d, 2H, H_o NPh, $J = 7.8$ Hz), 8.40 (d, 2H, H_o Ar $J = 8.1$ Hz), 8.80 (d, 1H, H-10, $J = 8.0$ Hz). ^{13}C NMR (100 MHz, $CDCl_3$) δ (ppm): 14.5 (CH_3), 116.7 (C-3a), 119.7 (C-4a), 121.4 (C_o NPh), 122.7 (C_p Ar), 126.7 (C_p NPh), 127.2 (C-10), 128.8 (C_m Ar), 129.1 (C-7), 129.2 (C_m NPh), 131.4 (C-8), 131.6 (C_o Ar), 133.8 (C-6a), 134.8 (C-4), 136.0 (C-9), 136.6 (C_i NPh), 137.1 (C-10a), 146.2 (C-3), 150.5 (C-11a), 150.7 (C_i Ar) 153.8 (C-10b), 179.9 (C-5), 180.3 (C-6). EI MS (70 eV): *m/z* %: 460 (M^+, 27), 431 (100), 415 (69), 385 (98), 77 (22). Anal. Calcd for $C_{27}H_{16}N_4O_4$: C: 67.78, H: 3.79, N: 11.71, found: C: 67.99, H: 3.64, N: 11.46.

4.1.1.9. 3,4-Diphenyl-4,11-dihydro-1H-benzo[h]pyrazolo[3,4-b]quinolin(1H,11H)-5,6-dione ***7a***. Red solid, mp 315–317 °C (80%). ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm): 5.70 (s, 1H, H-4), 7.00 (t, 1H, H_p Ar, $J = 7.2$ Hz), 7.12 (t, 2H, H_m Ar, $J = 7.2$ Hz), 7.19 (d, 2H, H_o Ar, $J = 7.2$ Hz), 7.32 (t, 1H, H_p R, $J = 7.4$ Hz), 7.41 (t, 2H, H_m R, $J = 7.4$ Hz), 7.54 (d, 2H, H_o R, $J = 7.4$ Hz), 7.73 (t, 1H, H-8, $J = 7.5$ Hz), 7.79 (t, 1H, H-9, $J = 7.5$ Hz), 7.90 (d, 1H, H-7, $J = 7.5$ Hz), 7.99 (d, 1H, H-10, $J = 7.5$ Hz), 10.38 (s, 1H, NH-11), 12.83 (s, 1H, NH-1). ^{13}C NMR (100 MHz, $CDCl_3$) δ (ppm): 35.4 (C-4), 113.4 (C-4a), 114.5 (C-3a), 125.6 (C-10), 125.7 (C-7), 126.2 (C_o R), 127.8 (C_p R), 128.1 (C_m R), 128.8 (C_o Ar), 130.2 (C_p Ar), 130.6 (C_i R), 132.4 (C_m Ar), 132.7 (C-8), 133.0 (C_i Ar), 134.1 (C-6a), 134.8 (C-3), 136.0 (C-9), 137.2 (C-10a), 146.4 (C-11a), 157.4 (C-10b), 180.4 (C-5), 180.7 (C-6). EI MS (70 eV): *m/z* (%): 403 (M^+, 24), 326 (100). HR-MS calcd for $C_{26}H_{17}N_3O_2$ 403.1321, found 403.1319.

4.1.1.10. 3-Phenyl-4-(4-tolyl)-4,11-dihydro-1H-benzo[h]pyrazolo[3,4-b]quinolin(1H,11H)-5,6-dione ***7b***. Purple solid, mp 315–317 °C (80%). ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm): 2.11 (s, 3H, CH_3), 5.66 (s, 1H, H-4), 6.90–6.95 (m, 3H, H_p R, H_m Ar), 7.08 (d, 2H, H_o Ar, $J = 7.2$ Hz), 7.41 (t, 2H, H_m R, $J = 7.4$ Hz), 7.56 (d, 2H, H_o R, $J = 7.4$ Hz),

7.72 (t, 1H, H-8, $J = 7.5$ Hz), 7.78 (t, 1H, H-9, $J = 7.5$ Hz), 7.89 (d, 1H, H-7, $J = 7.5$ Hz), 7.98 (d, 1H, H-10, $J = 7.5$ Hz), 10,34 (s, 1H, NH-11), 12.90 (s, 1H, NH-1). ^{13}C NMR (100 MHz, $CDCl_3$) δ (ppm): 21.1 (CH_3), 35.4 (C-4), 113.4 C-4a), 114.5 (C-3a), 125.6 (C-10), 125.7 (C-7), 126.2 (C_o R), 127.8 (C_p R), 128.1 (C_m R), 128.8 (C_o Ar), 130.1 (C_p Ar), 130.7 (C_i R), 132.4 (C_m Ar), 132.7 (C-8), 133.0 (C_i Ar), 134.10 (C-6a), 134.7 (C-3), 136.0 (C-9), 137.2 (C-10a), 146.4 (C-11a), 157.5 (C-10b), 180.4 (C-5), 180.7 (C-6). EI MS (70 eV): *m/z* (%): 417 (M^+, 15), 326 (100). Anal. Calcd for $C_{27}H_{19}N_3O_2$: C: 77.68, H: 4.59, N: 10.07, found: C: 77.59, H: 4.53, N: 10.15.

4.1.1.11. 4-(4-Methoxyphenyl)-3-phenyl-4,11-dihydro-1H-benzo[h] pyrazolo[3,4-b]quinolin(1H,11H)-5,6-dione ***7c***. Purple solid, mp 313 °C (70%). ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm): 3.61 (s, 3H, CH_3), 5.67 (s, 1H, H-4), 6.71 (d, 2H, H_m Ar, $J = 8.8$ Hz), 7.12 (d, 2H, H_o Ar, $J = 8.8$ Hz), 7.33 (t, 1H, H_p R, $J = 8.3$ Hz), 7.43 (t, 2H, H_m R, $J = 8.3$ Hz), 7.58 (d, 2H, H_o R, $J = 8.3$ Hz), 7.73 (t, 1H, H-8, $J = 7.3$ Hz), 7.79 (t, 1H, H-9 $J = 7.3$ Hz), 7.91 (d, 1H, H-7, $J = 7.3$ Hz), 7.99 (d, 1H, H-10, $J = 7.3$ Hz), 10.34 (s, 1H, NH-11), 12.85 (s, 1H, NH-1). ^{13}C NMR (100 MHz, $CDCl_3$) δ (ppm): 35.6 (C-4), 55.3 (OCH_3), 113.8 (C_m Ar), 114.2 (C-4a), 115.8 (C-3a), 125.9 (C_p R), 125.9 (C-7), 126.2 (C-10), 126.5 (C_o R), 128.7 (C_o Ar), 128.6 (C_i Ar), 129.3 (C_m R), 130.6 (C_i R), 132.9 (C-6a), 133.1 (C-8), 134.2 (C-3), 135.2 (C-10), 139.0 (C-9), 140.3 (C-10a), 146.8 (C-11a), 156.9 (C-10b), 157.9 (C_p Ar), 170.8 (C-5), 181.2 (C-6). EI MS (70 eV): *m/z* (%): 433 (M^+, 58), 326 (100). HR-MS calcd for $C_{27}H_{19}N_3O_3$ 433.1426, found 433.1424.

4.1.1.12. 4-Phenyl-3-(4-tolyl)-4,11-dihydro-1H-benzo[h]pyrazolo[3,4-b]quinolin(1H,11H)-5,6-dione ***7d***. Red solid, mp > 320 °C (70%). ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm): 2.28 (s, 3H, CH_3), 5.63 (s, 1H, H-4), 7.02 (t, 1H, H_p Ar, $J = 7.9$ Hz), 7.09–7.15 (m, 4H, H_m Ar, H_o Ar), 7.20 (d, 2H, H_m R, $J = 7.9$ Hz), 7.50 (d, 2H, H_o R, $J = 7.9$ Hz), 7.66 (t, 1H, H-8, $J = 7.5$ Hz), 7.82 (t, 1H, H-9, $J = 7.5$ Hz), 8.00 (d, 1H, H-7, $J = 7.5$ Hz), 8.52 (d, 1H, H-10, $J = 7.5$ Hz), 10.01 (broad s, 1H, NH-11), 12.50 (broad s, 1H, NH-1). ^{13}C NMR (100 MHz, $CDCl_3$) δ (ppm): 20.6 (CH_3), 35.0 (C-4), 115.3 (C-3a), 119.6 (C-4a), 125.5 (C-10), 125.7 (C_p Ar), 125.6 (C-7), 125.9 (C_o Ar), 128.8 (C_m Ar), 129.3 (C_m R), 130.2 (C-8) 132.5 (C_p R), 132.6 (C_i Ar), 134.1 (C-3), 134.7 (C_i R), 137.5 (C-6a), 138.6 (C_o R), 139.8 (C-9), 140.0 (C-10a), 155.2 (C-11a), 158.3 (C-10b), 180.3 (C-5), 180.5 (C-6). Anal. calcd for $C_{27}H_{19}N_3O_2$: C: 77.68, H: 4.59, N: 10.07, found: C: 77.53, H: 4.49, N: 10.21.

4.1.1.13. 3-(4-Chlorophenyl)-4-phenyl-4,11-dihydro-1H-benzo[h]pyrazolo[3,4-b]quinolin(1H,11H)-5,6-dione ***7e***. Purple solid, mp > 300 °C (d) (75%). ^{1}H NMR (400 MHz DMSO-d_6) δ (ppm): 5.70 (s, 1H, H-4), 7.00 (t, 1H, H_p R, $J = 7.3$ Hz), 7.12 (t, 2H, H_m R, $J = 7.3$ Hz), 7.19 (d, 2H, H_o R, $J = 7.3$ Hz), 7.47 (d, 2H, H_o Ar, $J = 8.4$ Hz), 7.57 (d, 2H, H_m Ar, $J = 8.4$ Hz), 7.71 (t, 1H, H-8, $J = 7.5$ Hz), 7.77 (t, 1H, H-9, $J = 7.5$ Hz), 7.88 (d, 1H, H-7, $J = 7.5$ Hz), 7.98 (d, 1H, H-10, $J = 7.5$ Hz), 10.38 (s, 1H, NH-11), 12.89 (s, 1H, NH-1). ^{13}C NMR (100 MHz, DMSO-d_6) δ (ppm): 35.4 (C-4), 113.5 (C-3a), 126.7 (C-10), 127.7 (C_m Ar), 127.9 (C_m R), 128.6 (C-7), 130.3 (C_o Ar), 130.9 (C_o R), 131.7 (C-8), 133.0 (C-6a), 133.2 (C_p R), 135.3 (C-3), 136.0 (C-9), 137.1 (C-10a), 132.5 (C_i Ar), 147.1 (C_i R), 149.3 (C_p Ar), 152.8 (C-11a), 153.3 (C-10b), 179.8 (C-5), 180.0 (C-6). EI MS (70 eV): *m/z*: 439/437 (M^{+2}/M^+, 14/38), 360 (100), 210 (22). Anal. calcd for $C_{26}H_{16}N_3O_2$: C: 71.32, H: 3.68, N: 9.60, found: C: 77.49, H: 3.57, N: 9.72.

4.1.1.14. 3-(4-Nitrophenyl)-4-phenyl-4,11-dihydro-1H-benzo[h]pyrazolo[3,4-b]quinolin(1H,11H)-5,6-dione ***7f***. Red solid, mp 260–261 °C (70%). ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm): 5.61 (s, 1H, H4), 7.01 (m, 3H, H_m Ar, H_p Ar), 7.09 (d, 2H, H_o Ar $J = 7.9$ Hz), 7.17 (d, 2H, H_o R, $J = 8.3$ Hz), 7.67 (t, 1H, H-8, $J = 8.0$ Hz), 7.83 (d, 2H, H_m R, $J = 8.3$ Hz), 8.14 (t, 1H, H-9, $J = 8.0$ Hz), 8.30 (d, 1H, H-7, $J = 8.0$ Hz), 8.75 (d, 1H, H-10, $J = 8.1$ Hz), 10.11 (broad s, 1H, NH-11), 12.50 (broad s, 1H, NH-1). Compound ***7f*** is barely soluble in dimethyl sulfoxide or any other solvent normally used for NMR spectroscopy; thus, made the registration of a high resolution ^{13}C NMR spectrum impossible. EI MS (70 eV): *m/z* (%): 446 (M^+, 39), 417 (99), 371 (100), 186 (59). HR-MS calcd for $C_{27}H_{16}N_4O_5$ 446.1015, found 446.1011.

4.1.1.15. 4-(4-Nitrophenyl)-3-(4-tolyl)-1H-benzo[h]pyrazolo[3,4-b] quinolin-5,6-dione ***9a***. Yellow solid, mp > 350 °C (75%). ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm): 2.17 (s, 3H, CH_3), 6.74 (d, 2H, H_m Ar, $J = 7.8$ Hz), 6.81 (d, 2H, H_o Ar, $J = 7.8$ Hz), 7.31 (d, 2H, H_o R, $J = 8.0$ Hz), 7.68 (t, 1H, H-8, $J = 7.9$ Hz), 7.91 (m, 3H, H-9, H_m R), 8.02 (d, 1H, H-7, $J = 7.9$ Hz), 8.75 (d, 1H, H-10, $J = 8.1$ Hz), 14.34 (s, 1H, NH-1). ^{13}C NMR (100 MHz, DMSO-d_6) δ (ppm): 21.1 (CH_3), 113.2 (C-3a), 120.0 (C-4a), 122.9 (C_m Ar), 126.7 (C-10), 128.3 (C_o Ar), 128.6 (C-7), 129.2 (C_m R), 129.8 (C-6a), 129.9 (C_o R), 131.2 (C_p R), 131.8 (C-8), 132.3 (C_i R), 132.3 (C-4), 136.0 (C-9), 137.1 (C-10a), 137.5 (C_p Ar), 144.0 (C_i Ar), 148.1 (C-11a), 152.7 (C_p Ar), 153.2 (C-10b), 179.4 (C-5), 179.6 (C-6). EI MS (70 eV): *m/z* (%):460 (M^+, 11), 431 (32), 417 (100), 313 (61). HR-MS calcd for $C_{27}H_{16}N_4O_4$ 460.1170, found 460.1170.

4.1.1.16. 3-(4-Chlorophenyl)-4-(4-methoxyphenyl)-1H-benzo[h]pyrazolo[3,4-b]quinolin-5,6-dione ***9b***. Purple solid, mp 270 °C (d) (70%). ^{1}H NMR (400 MHz DMSO-d_6) δ (ppm): 3.74 (s, 3H, OCH_3), 6.66 (d, 2H, H_m Ar, $J = 8.8$), 6.88 (d, 2H, H_o R, $J = 8.5$), 6.97 (d, 2H, H_o Ar, $J = 8.8$), 7.08 (d, 2H, H_m R, $J = 8.5$), 7.67 (t, 1H, H-8, $J = 7.6$), 7.92 (t, 1H, H-9, $J = 7.6$), 8.01 (d, 1H, H-7, $J = 7.6$), 8.75 (d, 1H, H-10, $J = 7.6$), 14.35 (s, 1H, NH-1). ^{13}C NMR (100 MHz, DMSO-d_6) δ (ppm): 55.2 (OCH_3), 113.0 (C_o Ar), 113.3 (C-3a), 120.2 (C-4a), 126.3 (C-10), 127.2 (C_m Ar), 127.8 (C_p Ar), 128.0 (C-7), 128.1 (C-8), 129.6 (C_m R), 130.4 (C_o R) 131.2 (C_i Ar), 131.5 (C_p R), 131.7 (C3), 132.2 (C-6a), 132.7 (C-4), 133.2 (C_i R), 135.5 (C-9), 136.9 (C-10a), 152.9 (C-11a), 159.2 (C-10b), 179.7 (C-5), 179.9 (C-6). HR-MS calcd for $C_{27}H_{16}ClN_3O_2$ 465.0880, found 465.0901.

4.1.1.17. 3,4-Di(4-chlorophenyl)-1H-benzo[h]pyrazolo[3,4-b]quinolin-5,6-dione ***9c***. Brown solid, mp > 300 °C (d) (70%). ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm): 6.90 (d, 2H, H_m R, $J = 7.5$ Hz), 7.07 (d, 2H, H_m Ar, $J = 7.9$ Hz), 7.11 (d, 2H, H_o Ar, $J = 7.9$ Hz), 7.17 (d, 2H, H_o R, $J = 7.5$ Hz), 7.67 (t, 1H, H-8, $J = 7.5$ Hz), 7.91 (t, 1H, H-9, $J = 7.5$ Hz), 8.01 (d, 1H, H-7, $J = 7.5$ Hz), 8.74 (d, 1H, H-10, $J = 7.5$ Hz), 14.41 (s, 1H, NH-1). ^{13}C NMR (100 MHz, DMSO-d_6) δ (ppm): 113.5 (C-3a), 120.4 (C-4a), 126.7 (C-10), 127.7 (C_o Ar), 127.9 (C_o R), 128.6 (C-7), 130.3 (C_m Ar), 130.9 (C_m R), 131.6 (C-4), 131.7 (C-8), 133.0 (C-6a), 133.2 (C_p R), 133.2 (C_i Ar), 135.3 (C-3), 136.0 (C-9), 137.2 (C-10a), 147.1 (C_i R), 149.3 (C_p Ar), 152.8 (C-11a), 153.4 (C-10b), 179.8 (C-5), 180.0 (C-6). EI MS (70 eV): *m/z* (%):473/471/469 (M^{+4}/M^{+2}/M^+, 27/68/100), 434 (30). HR-MS calcd for $C_{26}H_{13}N_3O_2$ 469.0385 found 469.0365.

4.1.1.18. 3-(4-Chlorophenyl)-4-(4-nitrophenyl)-1H-benzo[h]pyrazolo [3,4-b]quinolin-5,6-dione ***9d***. Yellow solid, mp > 350 °C (75%). ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm): 6.95 (d, 2H, H_o R, $J = 8.3$ Hz), 7.08 (d, 2H, H_m R, $J = 8.3$ Hz), 7.37 (d, 2H, H_o Ar), 7.70 (t, 1H, H-8, $J = 7.6$ Hz), 7.90–7.98 (m, 3H, H-9, H_m Ar), 8.05 (d, 1H, H-7, $J = 7.6$ Hz), 8.80 (d, 1H, H-10, $J = 7.7$ Hz), 14.27 (s, 1H, NH-1). ^{13}C NMR (100 MHz, DMSO-d_6) δ (ppm): 114.5 (C-3a), 119.8 (C-4a), 122.5 (C_m Ar), 126.2 (C-10), 127.3 (C_m R), 128.1 (C-7), 129.5 (C_o Ar), 130.6 (C_o R), 131.1 (C8), 131.3 (C_i Ar), 132.8 (C3), 132.8 (C6a), 135.5 (C9), 136.5 (C10a), 138.2 (C-4) 143.5 (C_p R), 146.4 (C_i R), 146.7 (C_p Ar), 152.3 (C-11a), 152.9 (C-10b), 178.9 (C-5), 179.04 (C-6). EI MS (70 eV): *m/z* (%):480 (M^+, 12), 451 (100), 417 (78), 370 (44). HR-MS calcd for $C_{26}H_{13}ClN_4O_4$ 480.0625, found 480.0618.

4.1.1.19. 4-(4-Methoxyphenyl)-3-(4-nitrophenyl)-1H-benzo[h]pyrazolo[3,4-b]quinolin-5,6-dione ***9e***. Red solid, mp > 300 °C (80%). ^{1}H

NMR (400 MHz, DMSO-d_6) δ (ppm): 3.68 (s, 3H, OCH_3), 6.66 (d, 2H, H_m Ar, $J = 8.4$), 6.99 (d, 2H, H_o Ar, $J = 8.4$), 7.14 (d, 2H, H_o R, $J = 8.3$), 7.69 (t, 1H, H-8, $J = 8$), 7.78 (d, 2H, H_m R, $J = 8.3$), 7.92 (t, 1H, H-9, $J = 8$), 8.01 (d, 1H, H-7, $J = 8$), 8.76 (d, 1H, H-10, $J = 8.1$), 14.49 (s, 1H, NH-1). ^{13}C NMR (100 MHz, DMSO-d_6) δ (ppm): 54.9 (OCH_3), 112.9 (C_m Ar), 120.0 (C-4a), 113.6 (C-3a), 121.9 (C_m R), 126.2 (C-10), 127.8 (C_p Ar), 128.1 (C-3), 128.3 (C-7), 129.5 (C_o Ar), 129.7 (C_o R), 130.0 (C-6a), 131.0 (C-8), 131.5 (C-4), 135.4 (C-9), 136.7 (C_i R), 137.5 (C-10a), 146.1 (C_p R), 152.7 (C-11a), 153.0 (C_i Ar), 159.3 (C-10b), 179.6 (C-5), 179.8 (C-6). EI MS (70 eV): m/z (%):476 (M^+, 26), 447 (100), 401 (42). HR-MS calcd for $C_{27}H_{16}N_4O_5$ 476.1121, found 476.1205.

4.1.1.20. 4-(4-Chlorophenyl)-3-(4-nitrophenyl)-1H-benzo[h]pyrazolo [3,4-b]quinolin-5,6-dione ***9f****.* Brown solid, mp > 350 °C (85%). ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm): 7.11 (d, 2H, H_o Ar, $J = 8.6$ Hz), 7.15–7.21 (m, 4H, H_m Ar, H_o R), 7.71 (t, 1H, H-8, $J = 7.6$ Hz), 7.91–7.98 (m, 3H, H-9, H_m R), 8.05 (d, 1H, H-7, $J = 7.6$ Hz), 8.77 (d, 1H, H-10, $J = 7.7$ Hz), 14.65 (s, 1H, NH). ^{13}C NMR (100 MHz, DMSO-d_6) δ (ppm): 113.9 (C-3a), 120.7 (C-4a), 122.8 (C_m R), 126.8 (C-10), 128.0 (C_m Ar), 128.6 (C-7), 130.5 (C_o Ar), 130.5 (C_o R), 131.8 (C-8), 132.3 (C_i Ar), 133.4 (C-3), 135.6 (C-6a), 136.0 (C-9), 137.1 (C-10a), 139.7 (C-4) 146.2 (C_p R), 146.8 (C_i R), 149.0 (C_p Ar), 152.8 (C-11a), 153.6 (C-10b), 179.6 (C-5), 179.8 (C-6). EI MS (70 eV): m/z (%):480 (M^+, 27), 451 (100), 435 (36), 405 (54), 370 (25). HR-MS calcd for $C_{26}H_{13}ClN_4O_4$ 480.0625, found 480.0625.

4.1.1.21. 3,4-Di(4-nitrophenyl)-1H-benzo[h]pyrazolo[3,4-b]quinolin-5,6-dione ***9g****.* Purple solid, mp > 300 °C (70%). ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm): 7.21 (d, 2H, H_m R, $J = 8.6$ Hz), 7.40 (d, 2H, H_o Ar, $J = 8.8$ Hz), 7.71 (t, 1H, H-8, $J = 7.7$ Hz), 7.87 (d, 2H, H_m Ar, $J = 8.8$ Hz), 7.91–7.98 (m, 3H, H-9, H_o R), 8.06 (d, 1H, H-7, $J = 7.7$ Hz), 8.81 (d, 1H, H-10, $J = 7.7$ Hz), 14.41 (s, 1H, NH-1). ^{13}C NMR (100 MHz, DMSO-d_6) δ (ppm): 112.9 (C-3a), 120.0 (C-4a), 122.2 (C_m R), 122.6 (C_m Ar), 126.2 (C-10), 128.1 (C-7), 129.5 (C_o R), 130.1 (C_o Ar), 131.4 (C-8), 131.9 (C-4), 135.5 (C-6a), 136.5 (C-9), 138.9 (C-10a), 143.5 (C_p Ar), 145.5 (C_p R), 146.5 (C_i R), 146.8 (C-3), 147.2 (C-11a), 152.3 (C_i Ar), 153.1 (C-10b), 178.8 (C-5), 178.9 (C-6). EI MS (70 eV): m/z (%):491 (M^+, 13), 462 (92), 446 (78), 416 (100), 370 (56). HR-MS calcd for $C_{26}H_{13}N_5O_6$ 491.0866, found 491.1022.

4.2. Antimycobacterial evaluation

Mycobacterium species were obtained from the Laboratorio Micobacterias, Instituto Nacional de Salud, Bogotá, Colombia, *M. tuberculosis* H37Rv (ATCC 27294), their resistant variants (ATCC 35837 ethambutol resistant, ATCC 35838 rifampin resistant, ATCC 35822 isoniazid resistant and ATCC 35820 streptomycin resistant), five strains of *M. tuberculosis* Beijing genotype belong to Colombia National Study of Drug resistance and five clinical isolates from humans with mesotherapy associated outbreak caused by non-tuberculous mycobacteria (NTM): *Mycobacterium chelonae*, *Mycobacterium fortuitum*, *Mycobacterium intracellulare*, *Mycobacterium scrofulaceum* and *Mycobacterium szulgai*, were used. Anti-mycobacterial activity of the derivatives dissolved in DMSO (Sigma, New Jersey, USA) was evaluated following the MTT colorimetric microdilution protocol, described by Caviedes et al. with modifications [28,29]. For standard tests, the MIC values of rifampin and isoniazid, (Sigma, New Jersey, USA) were determined each time. The minimum inhibitory concentration (MIC) of each molecule corresponded to the lowest concentration at which the bacteria tested did not show growth. Susceptibility testing was performed 3 times.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.ejmech.2013.12.037.

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