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Synthesis biological evaluation and SAR study of novel pyrazole analogues as inhibitors of *Mycobacterium tuberculosis*: Part 2. Synthesis of rigid pyrazolones.

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Abstract – Two series of novel rigid pyrazolone derivatives were synthesized and evaluated as inhibitors of *Mycobacterium tuberculosis* (MTB), the causative agent of tuberculosis. Two of these compounds showed high activity against MTB (MIC = 4 μg/mL). The newly synthesized pyrazolones were also computationally investigated to analyze their fit properties to the pharmacophoric model for antitubercular compounds previously built by us. Results are in agreement with that previously found by us for a class of pyrazole analogues and confirm the fundamental role of the *p*-chlorophenyl moiety at C4 for the antimycobacterial activity.

1. Introduction

On 2008 falled the 125th anniversary of Robert Koch's discovery of the bacillus *Mycobacterium tuberculosis* (MTB),[1] the etiological agent of the well known respiratory disease Tuberculosis (TB). Despite MTB was identified more than one century ago and many efficient drugs were discovered during this time to eradicate the disease, TB still remains one of the leading cause of worldwide illness and death. About 9.2 million new cases and 1.7 million deaths from TB occurred in 2006, of which 0.7 million cases and 0.2 million deaths were in HIV-positive people.[2] Moreover the emergence of multiple drug resistant (MDR-TB) strains and, more recently, extensively drug resistant (XDR-TB) strains makes the discovery and the development of new drugs a priority.[3]

In the last years, our work was focused into the discovery and the synthesis of new antimycobacterial compounds having pyrazole structure. Computational as well as synthetic studies led us to identify pyrazoles **1-4** as the best hit compounds with MIC values ranging between 4 and 12 μg/mL (Figure 1).[4-5] Structure-activity realtionship (SAR) studies revealed that the presence of the *p*-chlorobenzoyl moiety at the C4 of the pyrazole ring of **1-4** is fundamental for the

antimycobacterial activity of these compounds. These pyrazoles bearing a benzoyl moiety at C4 exist under certain conditions[6] in one or more tautomeric forms and also in an hydrogen bond stabilized form (Structure A, Figure 1). In particular, an in-depth investigation by Holzer and co-workers revealed that 5-hydroxypyrazoles such as **1-4** might exist in the chelated form **A**, because of the stabilization by intramolecular hydrogen bond which prevents the free rotation of C4-C6 bond.[6] On this basis, we assumed that the antimycobacterial activity of **1-4** could be partly related to the constrained conformation **A** where the *p*-Cl-phenyl ring is fixed in a "syn" relative position with respect to the C3-methyl group. Accordingly, we planned the synthesis of a series of pyrazolones with general structure **B** and **C** (Figure 1). Pyrazolones **B** could be considered as rigid derivatives of pyrazoles **1-4** with a "syn" conformation. The introduction of secondary amine moieties on pyrazolones **B** and **C** was settled on the basis of recent results on the synthesis of antimycobaterial pyrrole compounds.[7] It is known that the thiomorpholine or *N*-methylpiperazine moieties on pyrrole derivatives, such as the active BM212, play a crucial role in the inhibition of MTB. Hence, the intrduction of a secondary amine coud play a dual role, leading to compounds with constrained conformation and with a potential improvement of their antimycobacterial activity. Finally, the synthesis of a series of pyrazolones having general structure **C** was planned to support the hypothesis that the *p*-chlorophenyl moiety at C4 could have a fundamental role for the antimycobacterial activity.
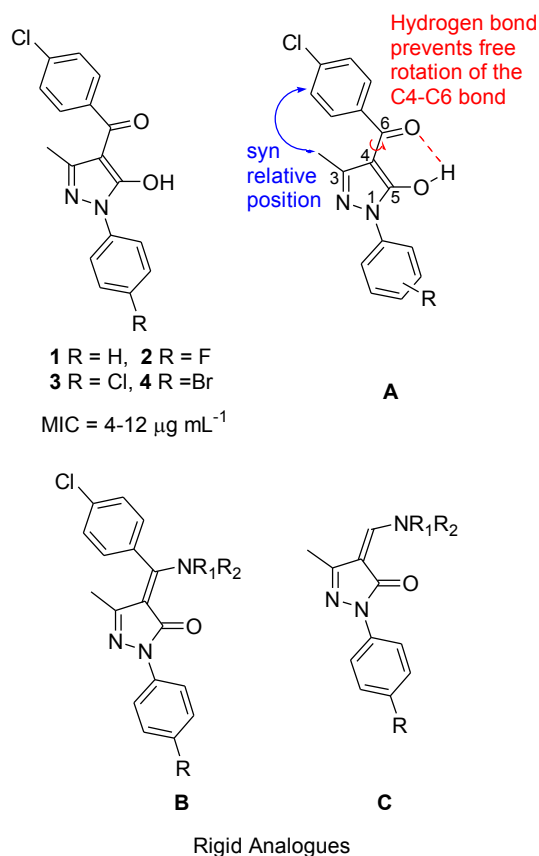

1 R = H, **2** R = F
3 R = Cl, **4** R =Br

MIC = 4-12 μg mL^{-1}

Rigid Analogues

2. Chemistry

We first focused our attention on the synthesis of derivatives with general structure **B**. Pyrazoles **1** and **3,** chosen as synthetic precursors, were synthesized according to reported procedures.[4-5] Reaction of **1** and **3** with different secondary amines led to desired pyrazolones **5a-k**. The reactions were performed in DMF or DME under microwave irradiation at 160 °C and were completed in only 10 minutes (Scheme 1).
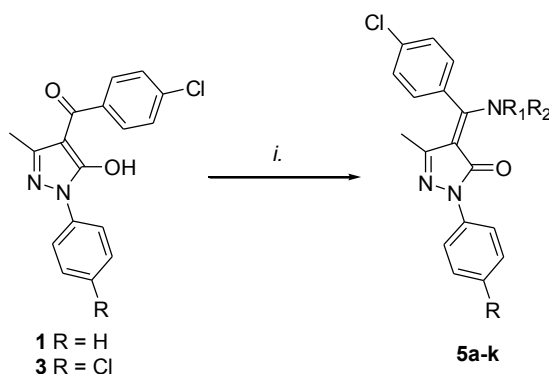
Scheme 1. Reagents and conditions: *i.* Secondary amines (1.2 eq), DMF (for **5a-h**) or DME (for **5i-k**), μW, 160 °C, 10 min.

A second series of pyrazolone derivatives with general structure **C** was synthesized. Commercially available pyrazolone **6** was reacted under the Vilsmeir conditions with POCl$_3$ in DMF at 80 °C. When the reaction was stopped with NaOH 30% solution, **7** was isolated as the only product. On the other hand, when Vilsmeir reaction was quenched with distilled H$_2$O and stirred in aqueous medium for 48 h, desired aldehyde **8** was obtained in 92 % yield. Aldehyde **8** was then reacted with several secondary amines at 100 °C under microwave irradiation affording desired compounds **9a-g** in good yields (Scheme 2). Stereochemistry of **5a-k** and **9a-g** was determined by NOESY experiments. NOE-cross couplings are illustrated in Figure 2.
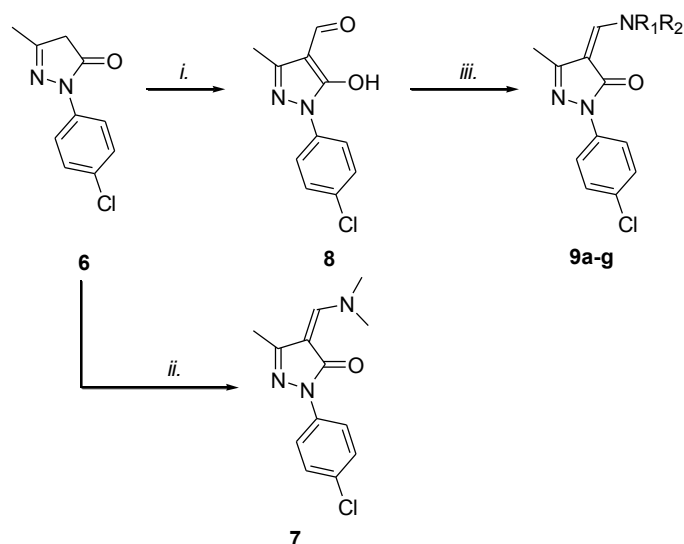
Scheme 2. Reagents and conditions: *i.* a) POCl$_3$, DMF, 2 h, 80 °C then b) H$_2$O, rt, 48 h. *ii.* a) POCl$_3$, DMF, 2 h, 80 °C then b) NaOH 30%. *iii.* R$_1$R$_2$NH, μW, 100 °C, 5 min.

Figure 2. Observed NOE cross-couplings

3. Results and discussion.

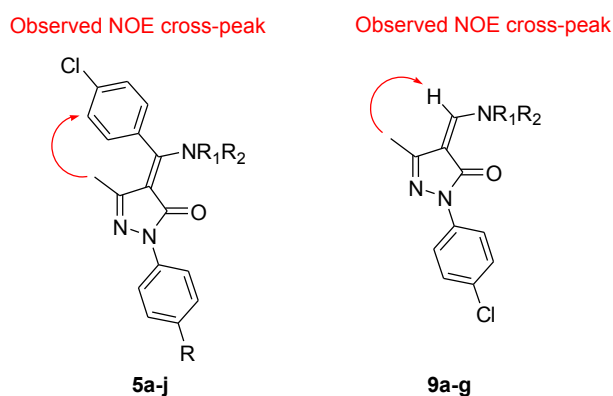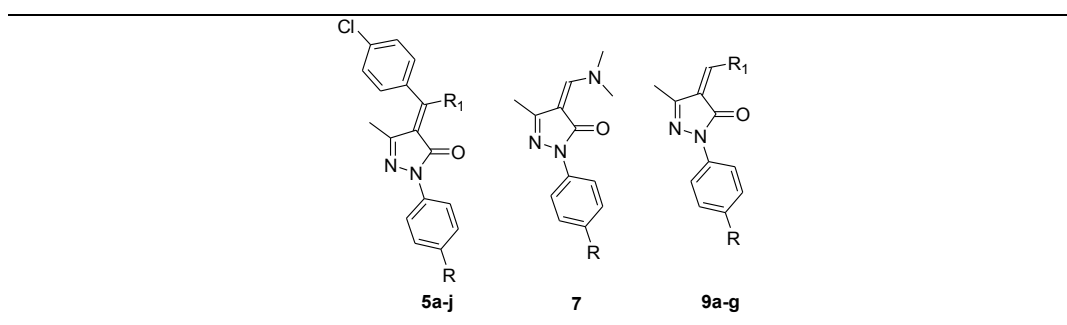Componds **5a-k**, **7** and **9a-g** were assayed for their inhibitory activity toward *M. tuberculosis* H37Rv (ATCC27294). The minimum inhibitory concentration (MIC expressed as μg mL^{-1}) was determined for each compound. Resulting data were reported in Table 1.

Table 1. Schematic representation and MIC values for **5**, **7** and **9**.

Compound	R	R₁	MIC (µg mL⁻¹) M. tuberculosis
5a	H	thiomorpholine	8
5b	H	N-Me-piperazine	8
5c	H	morpholine	16
5d	H	N(Me)₂	32
5e	Cl	thiomorpholine	8
5f	Cl	N-Me-piperazine	4
5g	Cl	morpholine	4
5h	Cl	N(Me)₂	16
5i	Cl	piperidine	>64
5j	Cl	4-Me-piperidine	64
5k	Cl	PrNMe	64
7	Cl		>64
9a	Cl	N-Ph-piperazine	64
9b	Cl	N-Me-piperazine	64
9c	Cl	morpholine	64
9d	Cl	N-Ac-piperazine	64
9e	Cl	NH-Ph	64
9f	Cl	N-(2-hydroxyethyl)-piperazine	64
9g	Cl	N-(2-furoyl)-piperazine	32

The presence of a chlorine atom on the N1-phenyl ring (**5e-h**) caused an improvement of activity with respect to non-halogenated **5a-d**. In particular, **5f-g**, bearing N-Me-piperazine and morpholine moieties proved to be very active with MIC = 4 µg/mL. On the other hand, the presence of a piperidine or Me-piperidine moieties (**5i-k**) resulted to be detrimental for activity. This result suggests the relevant role of an additional heteroatom on the cycloalkyl amine ring. Compounds **9a-g** resulted inactive against MTB. On the basis of these latter biological data, it seems evident that the p-chlorophenyl ring is crucial for antimycobacterial activity.

The cytotoxicity of compounds **5a-k**, **7** and **9a-g** toward VERO cells was also assayed. The active compounds **5a-b** and **5e-f** showed significant cytotoxicity (CC_{50} = 5.71 µg/mL for **5a**, 5.58 µg/mL for **5b**, 4.86 µg/mL for **5e**, 5.49 µg/mL for **5f**). The active compound **5g** showed a slightly better

cytotoxic profile with CC_{50} = 13.29 μg/mL. On the contrary the inactive compounds **9a-g** proved to be no cytotoxic with CC_{50} > 125 μg/mL.

4. Computational studies.

The new pyrazolone derivatives were analyzed for their ability to fit a pharmacophoric model for antimycobacterial compounds, consisting in two hydrophobics, two aromatic ring features and a hydrogen bond acceptor group. Superposition of one of the most active compounds (**5g**) to the model (Figure 3A) showed a full complementarity between chemical groups of **5g** and the pharmacophoric features. In fact, the RA1-HY1 system was matched by the *p*-chlorophenyl moiety at N1. The correspondence between the chlorine atom at R and HY1 accounted for the difference in activity found between R-chlorinated and unchlorinated analogues. In general, MIC values of chloro derivatives (**5f-i**) were better than those of the corresponding non halogenated analogues **5a-d**, lacking a group able to fit HY1. Moreover, the oxygen atom of the morpholine ring of **5g** was the hydrogen bond acceptor group interacting with HBA. Also this contact was suggested to be very important for activity. In fact, compounds with a reduced ability (the thiomorpholino derivative **5e**) or completely unable to fit HBA (**5h-k**) were all characterized by activity values lower that that found for **5f-g** and **7**, whose amino nitrogen atom is located at a ~2.8 Å distance from HBA. Finally, the additional *p*-chlorophenyl moiety at C4 matched RA2, while the methyl group at C3 was the hydrophobic group filling HY2.

Superposition of compounds **9** to the model showed an interaction pattern similar to that found for compounds **5** (Figure 3B). However, the lack of the *p*-chlorophenyl moiety at C4 made such compounds unable to fit RA2, thus accounting for their low antimycobacterial activity.

Such results were in agreement with that found by us for a class of pyrazole derivatives and suggesting that the *p*-chlorophenyl moiety at N1 was very important for activity, in addition to the ability of compounds to make a hydrogen bond contact coded by the HBA feature of the model.
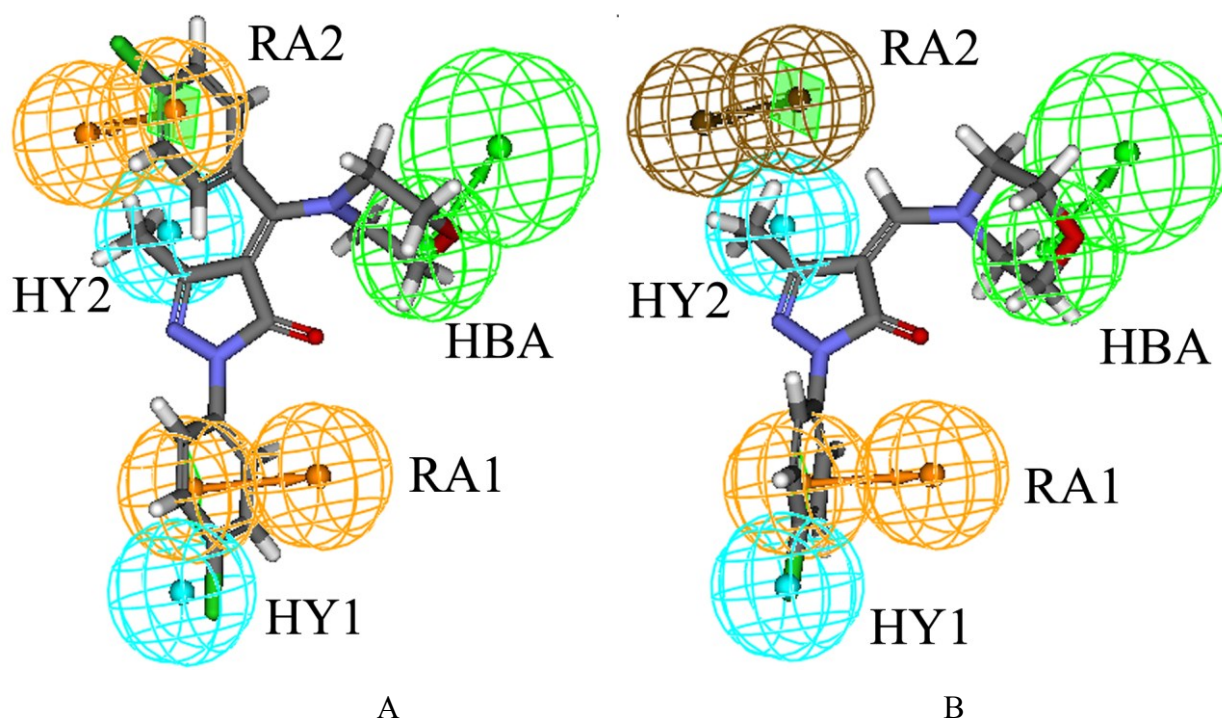
Figure 3. Superposition of **5g** (A) and **9a** (B) into the model for antimycobacterial compounds. Pharmacophoric features are color coded: orange for aromatic rings (RA); green for hydrogen bond acceptor groups (HBA); cyan for hydrophobic regions (HY). The aromatic ring feature RA2 not mapped by **9a** is in brown.

5. Experimental

Reagents were obtained from commercial suppliers and used without further purification. *N,N*-dimethylformamide (DMF) and dimethoxyethane (DME) were purchased anhydrous (Aldrich), dichloromethane was dried over CaH_2 prior to use. Anhydrous reactions were run under a positive pressure of dry N_2. Merck silica gel 60 was used for flash chromatography (23–400 mesh). ^1H NMR and ^{13}C NMR spectra were measured at 200 MHz on a Bruker AC200F spectrometer and at 400 MHz on a Bruker Avance DPX400. Chemical shifts were reported relative to $CDCl_3$ at δ 7.24 ppm and to tetramethylsilane at δ 0.00 ppm.

5.1. HPLC and MS analysis

The purity of compounds was assessed by reverse-phase liquid chromatography and a mass spectrometer (Agilent series 1100 LC/ MSD) with a UV detector at k = 254 nm and with an electrospray ionization source (ESI). All the solvents were of HPLC grade (Fluka). Mass spectral (MS) data were obtained using an Agilent 1100 LC/ MSD VL system (G1946C) with a 0.4 mL/min flow rate using a binary solvent system of 95:5 methyl alcohol/water. UV detection was monitored

at 254 nm. Mass spectra were acquired in positive mode scanning over the mass range of 50–1500. The following ion source parameters were used: drying gas flow, 9 mL/min; nebulize pressure, 40 psig; drying gas temperature, 350 °C.

5.2. Microwave irradiation experiments

Microwave irradiation was conducted using a CEM Discover Synthesis Unit (CEM Corp., Matthews, NC). The machine consists of a continuous focused microwave power delivery system with operator selectable power output (0 to 300 W). The temperature of the contents of the vessels was monitored using a calibrated infrared temperature sensor mounted under the reaction vessel. All experiments were performed using the stirring option whereby the contents of the vessels are stirred by means of rotating magnetic plate located below the floor of the microwave cavity and a Teflon-coated magnetic stir bar in the vessel.

5.3. Synthesis of pyrazolones 5a–k.

Compounds **1** or **3** (1 eq./mol) were dissolved in anhydrous DMF or DME in a sealed vessel equipped with magnetic stirring bar. The appropriate amine was added (1.2 eq./mol) to the solution and the vessel placed in the microwave oven and heated (160 °C , 10 min.) under microwave irradiation. After cooling, a saturated solution of NH_4Cl was added and the aqueous phase was extracted with AcOEt (3x 10 mL). The combined organic layers were washed with a saturated solution of NH_4Cl and H_2O, dried over anhydrous Na_2SO_4, filtered and evaporated to dryness to afford crude compounds **5a–k**, which were purified by flash chromatography (CH_2Cl_2/MeOH 99:1 for compounds **5b**, **5f**, **5i-k**; petroleum ether/ EtOAc 8:2 for compounds **5a**, **5c**, **5e**, **5g**) to afford the final products (25-70 % yield).

5a: Yield: 70 %. ^{1}H NMR ($CDCl_3$): δ (ppm) 7.91 (2H, d, J = 8.04 Hz, Ph), 7.44 (2H, d, J = 8.35 Hz, Ph), 7.32-7.28 (4H, m, Ph), 7.04 (1H, t, J = 7.30 Hz, Ph), 4.12 (2H, m, CH_2NCH_2), 3.55 (2H, m, CH_2NCH_2), 2.96 (2H, m, CH_2SCH_2), 2.64 (2H, m, CH_2SCH_2), 1.29 (3H, s, CH_3). ^{13}C NMR ($CDCl_3$): δ (ppm) 165.43, 161.66, 149.02, 139.40, 138.60, 133.70, 131.80, 131.60, 129.90, 129.50, 129.20, 129.15, 124.10, 123.90, 119.20, 104.02, 57.73, 57.71, 28.81. MS: m/z 398-400 (M+1)$^+$; 420-422 (M+Na)$^+$; 817-819. Anal. for $C_{21}H_{20}ClN_3OS$ Calcd.%: C, 63.39; H, 5.07; N, 10,56; Found: C, 63.58; H, 5.08; N, 10.59.

5b: Yield: 60 %. ^{1}H NMR ($CDCl_3$): δ (ppm) 7.91 (2H, d, J = 7.71 Hz, Ph), 7.41 (2H, d, J = 7.52 Hz, Ph), 7.27-7.26 (4H, m, Ph), 7.01 (1H, t, J = 7.00 Hz, Ph), 3.92 (2H, m, CH_2NCH_2), 3.35 (2H, m, CH_2NCH_2), 2.65 (2H, m, $CH_2N(CH_3)CH_2$), 2.43 (2H, m, $CH_2N(CH_3)CH_2$), 2.27 (3H, s, NCH_3), 1.28 (3H, s, CH_3). ^{13}C NMR ($CDCl_3$): δ (ppm) 164.56, 161.81, 148.95, 139.52, 138.34, 133.54,

131.80, 129.45, 128.74, 128.71, 128.67, 128.61, 128.57, 123.90, 119.31, 102.95, 55.96, 55.86, 55.09, 51.10, 55.67, 15.81. MS: m/z 395-397 $(M+1)^+$; 417-419 $(M+Na)^+$; 433-435 $(M+K)^+$, 811-813 $(2M+Na)^+$. Anal. for $C_{22}H_{23}ClN_4O$ Calcd.%: C, 66.91; H, 5.87; N, 14.19; Found: C, 67.11; H, 5.71; N, 14.23.

5c Yield: 30 %. ^{1}H NMR (CDCl$_3$): δ (ppm) 7.95 (2H, d, J = 8.05 Hz, Ph), 7.48 (2H, d, J = 8.05 Hz, Ph), 7.36-7.32 (4H, m, Ph), 7.08 (1H, t, J = 7.30 Hz, Ph), 4.02 (2H, m, CH_2OCH$_2$), 3.96 (2H, m, CH$_2$OCH_2), 3.74 (2H, m, CH_2NCH$_2$), 3.38 (2H, m, CH$_2$NCH_2), 1.35 (3H, s, CH_3). ^{13}C NMR (CDCl$_3$): δ (ppm) 164.39, 161.74, 148.92, 139.44, 138.54, 133.16, 131.85, 129.60, 128.66, 124.02, 119.29, 103.10, 67.68, 67.35, 55.60, 51.72, 15.83. MS: m/z 382-384 $(M+1)^+$; 404-406 $(M+Na)^+$; 785-787 $(2M+Na)^+$. Anal. for $C_{21}H_{20}ClN_3O_2$ Calcd.%: C, 66.05; H, 5.28; N, 11; Found: C, 66.76; H, 5.30; N, 11.04.

5d: Yield: 25 %. ^{1}H NMR (CDCl$_3$): δ (ppm) 8.01 (2H, d, J = 8.04 Hz, Ph), 7.48 (2H, d, J = 8.04 Hz, Ph), 7.37-7.34 (4H, m, Ph), 7.09 (1H, t, J = 7.29 Hz, Ph), 3.58 (3H, s, CH_3NCH$_3$), 3.08 (3H, s, CH$_3$NCH_3), 1.37 (3H, s, CH$_3$). ^{13}C NMR (CDCl$_3$): δ (ppm) 165.64, 162.00, 148.51, 139.65, 138.32, 133.45, 131.96, 129.37, 128.60, 123.76, 119.12, 102.44, 46.76, 43.51, 15.69. MS: m/z 340-342 $(M+1)^+$; 362-364 $(M+Na)^+$; 701-703 $(2M+Na)^+$. Anal. for $C_{19}H_{18}ClN_3O$ Calcd.%: C, 67.15; H, 5.34; N, 12.37; Found: C, 67.28; H, 5.35; N, 12.39.

5e: Yield: 50 %. ^{1}H NMR (CDCl$_3$): δ (ppm) 7.91 (2H, d, J = 8.53 Hz, Ph), 7.44 (2H, d, J = 8.53 Hz, Ph), 7.29-7.23 (4H, m, Ph), 4.10 (2H, m, CH_2NCH$_2$), 3.55 (2H, m, CH$_2$NCH_2), 2.95 (2H, m, CH_2SCH$_2$), 2.63 (2H, m, CH$_2$SCH_2), 1.27 (3H, s, CH_3). ^{13}C NMR (CDCl$_3$): δ (ppm) 165.67, 161.61, 149.41, 138.71, 138.06, 133.57, 132.21, 132.12, 131.80, 130.05, 129.75, 129.65, 129.19, 128.83, 120.05, 103.77, 57.76, 53.78, 28.66. MS: m/z 432-434 $(M+1)^+$; 454-456 $(M+Na)^+$; 470-472 $(M+K)^+$; 887-885 $(2M+Na)^+$. Anal. for $C_{21}H_{19}Cl_2N_3OS$ Calcd.%: C, 58.34; H, 4.43; N, 9.72; Found: C, 58.46; H, 4.44; N, 9.74.

5f: Yield: 50 %. ^{1}H NMR (CDCl$_3$): δ (ppm) 7.96 (2H, d, J = 8.63 Hz, Ph), 7.46 (2H, d, J = 8.63 Hz, Ph), 7.33-7.26 (4H, m, Ph), 3.96 (2H, m, CH_2NCH$_2$), 3.41 (2H, m, CH$_2$NCH_2), 2.69 (2H, m, CH_2N(CH$_3$)CH$_2$), 2.49 (2H, m, CH$_2$N(CH$_3$)CH_2), 2.33 (3H, s, NCH_3), 1.32 (3H, s, CH_3). ^{13}C NMR (CDCl$_3$): δ (ppm) 164.75, 161.77, 149.33, 138.49, 138.20, 133.41, 131.77, 129.53, 128.63, 120.12, 102.74, 55.85, 55.66, 55.17, 51.21, 45.81, 15.78. MS: m/z 429-431 $(M+1)^+$; 451-453 $(M+Na)^+$; 881-879 $(2M+Na)^+$. Anal. for $C_{22}H_{22}Cl_2N_4O$ Calcd.%: C, 61.54; H, 5.16; N, 13.05; Found: C, 61.68; H, 5.18; N, 13.10.

5g: Yield: 50 %. ^{1}H NMR (CDCl$_3$): δ (ppm) 7.94 (2H, d, J = 8.50 Hz, Ph), 7.48 (2H, d, J = 8.50 Hz, Ph), 7.32-7.26 (4H, m, Ph), 4.00 (2H, m, CH_2OCH$_2$), 3.95 (2H, m, CH$_2$OCH_2), 3.73 (2H, m, CH_2NCH$_2$), 3.37 (2H, m, CH$_2$NCH_2), 1.34 (3H, s, CH_3). ^{13}C NMR (CDCl$_3$): δ (ppm) 164.77,

161.65, 149.34, 138.72, 138.05, 132.94, 131.83, 129.67, 128.88, 128.63, 120.21, 102.82, 67.59, 67.40, 55.66, 51.79, 15.76. MS: m/z 416-418 (M+1)$^+$; 438-440 (M+Na)$^+$; 855-853 (2M+Na)$^+$. Anal. for $C_{21}H_{19}Cl_2N_3O_2$ Calcd.%: C, 60.59; H, 4.60; N, 10.09; Found: C, 60.83; H, 4.62; N, 10.13.

5h: Yield: 26 %. ^{1}H NMR (CDCl$_3$): δ (ppm) 7.99 (2H, d, J = 8.50 Hz, Ph), 7.47 (2H, d, J = 8.50 Hz, Ph), 7.34-7.24 (4H, m, Ph), 3.57 (3H, s, *CH$_3$*NCH$_3$), 3.07 (3H, s, CH$_3$N*CH$_3$*), 1.35 (3H, s, CH$_3$). ^{13}C NMR (CDCl$_3$): δ (ppm) 165.99, 161.90, 148.91, 138.49, 138.27, 133.23, 131.92, 129.41, 128.63, 128.56, 120.08, 102.18, 46.75, 43.58, 15.62. MS: m/z 374-376 (M+1)$^+$; 396-398 (M+Na)$^+$; 412-414 (M+K)$^+$. Anal. for $C_{19}H_{17}Cl_2N_3O$ Calcd.%: C, 60.97; H, 4.58; N, 11.23; Found: C, 61.15; H, 4.59; N, 11.26.

5i: Yield: 55%. ^{1}H NMR (CDCl$_3$): δ (ppm) 8.02 (2H, d, J = 8.53 Hz, Ph), 7.51 (2H, d, J = 8.53 Hz, Ph), 7.39 (2H, d, J = 8.53 Hz, Ph), 7.33 (2H, d, J = 8.53 Hz, Ph), 3.95 (2H, m, CH$_2$N*CH$_2$*), 3.40 (2H, m, *CH$_2$*NCH$_2$), 1.88-1.74 (6H, m, *CH$_2$CH$_2$CH$_2$*), 1.38 (3H, s, *CH$_3$*). ^{13}C-NMR (CDCl$_3$): δ (ppm) 165.18, 161.80, 149.39, 138.45, 133.95, 131.55, 129.23, 128.82, 128.44, 119.89, 102.56, 56.54, 52.50, 29.69, 27.46, 27.24, 23.54. MS: m/z 414-416 (M+1)$^+$. Anal. for $C_{22}H_{21}Cl_2N_3O$ Calcd.%: C, 63.77; H, 5.11; N, 10,14; Found: C, 62.37; H, 5.13; N, 10.19.

5j: Yield: 56%. ^{1}H NMR (CDCl$_3$): δ (ppm) 7.99 (2H, d, J = 8.85 Hz, Ph), 7.46 (2H, d, J = 8.83 Hz, Ph), 7.36 (2H, d, J = 8.63 Hz, Ph), 7.28 (2H, d, J = 8.63 Hz, Ph), 3.95 (2H, m, CH$_2$N*CH$_2$*), 3.48 (1H, m, *CH$_2$*NCH$_2$), 3.23 (1H, m, *CH$_2$*NCH$_2$), 1.87 (4H, m, *CH$_2$*CH(CH$_3$)*CH$_2$*), 1.49-1.46 (1H, m, *CH*CH$_3$), 1.33 (3H, s, *CH$_3$*), 1.01 (3H, d, J = 5.76 Hz, *CH$_3$*). ^{13}C-NMR (CDCl$_3$): δ (ppm) 165.18, 161.82, 149.36, 138.34, 133.37, 129.95, 129.18, 128.47, 120.01, 102.63, 55.65, 51.83, 35.39, 29.86, 21.35, 15.67. MS: m/z 428-430 (M+1)$^+$; 450-452 (M+Na)$^+$. Anal. for $C_{23}H_{24}Cl_2N_3O$ Calcd.%: C, 64.49; H, 5.41; N, 9.81; Found: C, 64.37; H, 5.35; N, 9.97.

5k: Yield: 56 %. ^{1}H NMR (CDCl$_3$): δ (ppm) 7.97 (2H, d, J = 8.73 Hz, Ph), 7.46 (2H, d, J = 8.46 Hz, Ph), 7.36-7.30 (4H, m, Ph), 3.56 (3H, s, N-*CH$_3$*), 3.38-3.34 (2H, m, CH$_3$CH$_2$*CH$_2$*NCH$_3$), 1.69-1.66 (2H, m, CH$_3$*CH$_2$*CH$_2$), 1.36 (3H, s, *CH$_3$*), 0.81 (3H, t, J = 7.32, CH$_2$*CH$_3$*). ^{13}C-NMR (CDCl$_3$): δ (ppm) 165.55, 161.74, 152.73, 148.84, 138.22, 133.74, 131.87, 129.37, 128.49, 127.95, 119.96, 102.74, 60.20, 57.54, 44.11, 40.99, 29.64, 21.94, 21.24, 15.66, 11.13, 10.47. MS: m/z 402-404 (M+1)$^+$; 424-426 (M+Na)$^+$. Anal. for $C_{21}H_{21}Cl_2N_3O$ Calcd.%: C, 62.69; H, 5.26; N, 10.44; Found: C, 62.71; H, 5.30; N, 10.47.

5.4. Synthesis of aldehyde 8.

To a stirred solution of compound **6** (1equiv/mol.) in DMF, 0.7 eq./mol of POCl$_3$ and 2 eq./mol of DMF were added. The mixtures was heated at 80 °C for 2 h. After cooling, distilled H$_2$O was added and the resulting solution was stirred for 48 h. The desiesred product **8** was obtained as a yellow

precipitate which was separated by filtration and used in the next step without any further purification.

8: Yield: 92%. ^{1}H NMR (CDCl$_3$): δ (ppm) 10.26 (1H, bs, O*H*), 9.33 (1H, s, *CH*O), 7.67 (2H, bd, Ph), 7.31 (2H, bd, Ph), 2.34 (3H, s, *CH$_3$*). ^{13}C-NMR (CDCl$_3$): δ (ppm) 159.60, 149.56, 135.30, 132.07, 129.45, 128.93, 128.32, 121.86, 105.99, 13.12. MS: m/z 235 [M-H]$^-$. Anal. for C$_{11}$H$_9$ClN$_2$O$_2$ Calcd.%: C, 55.83; H, 3.83; N, 11.84; Found: C, 56.01; H, 4.03; N, 12.04.

5.5. Synthesis of pyrazolones 9a–g.

Compound **8** (0.42 mmol, 1 eq./mol) and the appropriate amine (4 eq./ mol) were irradiated under microwave at 100 ° C for 5 min. The crude products **9a-g** were then directly crystallized using AcOEt 100%.

9a: Yield: 70%. ^{1}H NMR (CDCl$_3$): δ (ppm) 7.95 (2H, d, *J* = 8.30 Hz, Ph), 7.34-7.27 (4H, m, Ph), 7.04 (1H, s, C=CH-piperazine), 6.96 (2H, d, *J* = 8.32 Hz, Ph), 4.93 (2H, m, *CH$_2$*NCH$_2$), 3.78 (2H, m, CH$_2$N*CH$_2$*), 3.38 (4H, m, *CH$_2$*N(Ph)*CH$_2$*), 2.22 (3H, s, *CH$_3$*). ^{13}C-NMR (CDCl$_3$): δ (ppm) 162.25, 150.82, 150.26, 149.35, 138.06, 129.48, 129.44, 129.40, 129.35, 128.99, 128.59, 128.55, 121.16, 121.12, 120.40, 120.36, 116.92, 116.87, 99.46, 56.29, 51.45, 50.70, 50.64, 50.60, 49.82, 13.56. MS: m/z 381 (M+1)$^+$. Anal. for C$_{21}$H$_{21}$ClN$_4$O Calcd.%: C, 66.22; H, 5.56; N, 14.71; Found: C, 66.34; H, 5.71; N, 14.83.

9b: Yield: 60%. ^{1}H NMR (CDCl$_3$): δ (ppm) 7.94 (2H, d, *J* = 7.18 Hz, Ph), 7.29 (2H, d, *J* = 7.18 Hz, Ph), 6.93 (1H, s, C=*CH*-piperazine), 4.74 (2H, m, *CH$_2$*NCH$_2$), 3.58 (2H, s, CH$_2$N*CH$_2$*), 2.58 (4H, m, *CH$_2$*N*CH$_2$*), 2.33 (3H, s, N*CH$_3$*), 2.16 (3H, s, *CH$_3$*). ^{13}C-NMR (CDCl$_3$): δ (ppm) 162.03, 150.79, 138.17, 128.82, 128.49, 128.37, 99.13, 56.38, 55.38, 54.97, 51.53, 45.65, 13.43. MS: m/z 319 (M+1)$^+$. Anal. for C$_{16}$H$_{19}$ClN$_4$O Calcd.%: C, 60.28; H, 6.01; N, 17.57; Found: C, 60.33; H, 5.07; N, 17.63.

9c: Yield: 53%. ^{1}H NMR (CDCl$_3$): δ (ppm) 7.93 (2H, d, *J* = 7.64 Hz, Ph), 7.31 (2H, d, *J* = 7.64 Hz, Ph), 6.94 (1H, s, C=*CH*-morpholine), 4.80 (2H, m, *CH$_2$*NCH$_2$), 3.86 (4H, m, *CH$_2$*O*CH$_2$*), 3.58 (2H, m, *CH$_2$*NCH$_2$), 2.19 (3H, s, *CH$_3$*). ^{13}C-NMR (CDCl$_3$): δ (ppm) 161.74, 148.29, 138.04, 128.52, 128.47, 120.37, 67.07, 56.15, 52.34, 13.41. MS: m/z 312 (M+1)$^+$; 334 (M+Na)$^+$. Anal. for C$_{15}$H$_{16}$ClN$_3$O$_2$ Calcd.%: C, 58.92; H, 5.27; N, 13.74; Found: C, 59.02; H, 5.35; N, 14.02.

9d: Yield: 30%. ^{1}H NMR (CDCl$_3$): δ (ppm) 7.93 (2H, d, *J* = 8.48 Hz, Ph), 7.33 (2H, d, *J* = 8.48 Hz, Ph), 7.01 (1H, s, C=*CH*-piperazine, 4.78 (2H, m, *CH$_2$*NCH$_2$), 3.83 (2H, m, CH$_2$N*CH$_2$*), 3.70 (2H, m, *CH$_2$*N(CH$_3$)CH$_2$), 3.59 (2H, m, CH$_2$N(CH$_3$)*CH$_2$*), 2.21 (3H, s, *CH$_3$*), 2.17 (3H, s, CO*CH$_3$*). ^{13}C-NMR (CDCl$_3$): δ (ppm) 152.75, 149.40, 137.87, 129.19, 128.56, 120.39, 55.79, 51.49, 41.86, 21.21,

13.43. MS: m/z 369 (M+Na)$^+$. Anal for C$_{17}$H$_{19}$ClN$_4$O$_2$ Calcd.%: C, 58.87; H, 5.52; N, 16.15; Found: C, 58.93; H, 5.54; N, 16.32.

9e: Yield: 48%. ^{1}H NMR (CDCl$_3$): δ (ppm) 11.42 (1H, bs, *NH*), 8.0 (2H, d, *J* = 8.16 Hz, Ph), 7.89 (1H, s, *CH*=NHPh), 7.41 (2H, d, *J* = 7.18 Hz, Ph), 7.35 (2H, d, *J* = 8.16 Hz, Ph), 7.25-7.20 (3H, m, Ph), 2.29 (3H, s, *CH$_3$*). ^{13}C-NMR (CDCl$_3$): δ (ppm) 161.15, 148.22, 142.85, 138.47, 137.57, 130.06, 129.14, 128.72, 125.84, 119.79, 117.31, 102.94, 12.60. MS: m/z 306 (M+1)$^+$; 328 (M+Na)$^+$. Anal. for C$_{17}$H$_{16}$ClN$_3$O Calcd.%: C, 65.07; H, 5.14; N, 13.39; Found: C, 66.01; H, 5.24; N, 13.77.

9f: Yield: 37%. ^{1}H NMR (CDCl$_3$): δ (ppm) 7.93 (2H, d, *J* = 8.10 Hz, Ph), 7.31 (2H, d, *J* = 8.10 Hz, Ph), 6.96 (1H, s, C=*CH*-piperazine, 4.78 (2H, m, *CH$_2$*NCH$_2$), 4.78 (2H, m, CH$_2$N*CH$_2$*), 3.68 (2H, m, *CH$_2$*NCH$_2$), 3.64 (2H, m, *CH$_2$*OH), 2.74 (4H, m, CH$_2$N(CH$_2$CH$_2$OH)CH$_2$), 2.64 (2H, m, N*CH$_2$* CH$_2$OH), 2.19 (3H, s, *CH$_3$*). ^{13}C-NMR (CDCl$_3$): δ (ppm) 162.04, 150.75, 149.41, 130.08, 128.97, 128.56, 120.40, 59.17, 58.08, 56.39, 53.43, 53.0, 51.58, 13.42. MS: m/z 349 (M+1)$^+$. Anal. for C$_{17}$H$_{21}$ClN$_4$O$_2$ Calcd.%: C, 58.53; H, 6.07; N, 16.06; Found: C, 58.62; H, 6.11; N, 16.12.

9g: Yield: 74%. ^{1}H NMR (CDCl$_3$): δ (ppm) 7.93 (2H, d, *J* = 8.56 Hz, Ph), 7.50 (1H, s, *H$_3$*-Furane), 7.31 (2H, d, *J* = 8.56 Hz, Ph), 7.09 (1H, s, C=*CH*-Piperazine), 6.98 (1H, s, *H$_4$*-Furane), 6.51 (1H, s, *H$_5$*-Furane), 4.80 (2H, m, *CH$_2$*NCH$_2$), 4.05 (2H, m, CH$_2$N(CO)*CH$_2$*), 3.96 (2H, m, *CH$_2$*N(CO)CH$_2$), 3.62 (2H, m, CH$_2$N*CH$_2$*), 2.19 (3H, s, *CH$_3$*). ^{13}C-NMR (CDCl$_3$): δ (ppm) 163.02, 150.73, 149.44, 147.30, 144.22, 137.96, 129.10, 128.52, 120.35, 117.71, 111.65, 100.04, 55.92, 51.81, 29.66, 13.42. MS: m/z 399 (M+1)$^+$. Anal. for. C$_{20}$H$_{19}$ClN$_4$O$_3$ Calcd.%: C, 60.23; H, 4.80; N, 14.05; Found: C, 60.35; H, 5.12; N, 14.09.

6. Microbiological assays

6.1. Mycobacterial strain

M. tuberculosis H37Rv ATCC 27294 was used in this study. It was maintained on Löwenstein-Jensen (bioMérieux, Marcy l'Étoile, France) agar slants until needed.

6.2. Antimicrobial susceptibility testing

MICs were determined by a standard twofold agar dilution method. Briefly, 1 mL of Middlebrook 7H11 agar (Becton Dickinson BBL, Sparks, MD) supplemented with 10% oleic acid-albumin-dextrose-catalase enrichment containing the testing compounds in 24-multiwell plates at concentrations ranging between 0.0312 and 64 µg/mL was inoculated with 10 µL of a suspension containing *M. tuberculosis* H37Rv 1.5×10^5 cfu/mL grown on Middlebrook 7H9 broth (Difco Laboratories, Detroit, MI) supplemented with 10% albumin-dextrose-catalase enrichment. Final

inoculum was 1.5×10^3 per well and was obtained as described previously.[8] Plates were incubated for 21–28 days and MICs were read as minimal concentrations of compounds completely inhibiting visible growth of mycobacteria.

7. Computational details

Computational analysis was performed by means of the Catalyst software package, version 4.10, following a protocol previously described.[5]

8. Acknowledgements

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