



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

Daniele Castagnolo^a, Fabrizio Manetti^a, Marco Radi^a, Beatrice Bechi^a, Mafalda Pagano^a, Alessandro De Logu^b, Rita Meleddu^b, Manuela Saddi^b, Maurizio Botta^{a,*}

^a Università degli Studi di Siena, via A. De Gasperi 2, 53100 Siena, Italy

^b Sezione di Microbiologia Medica, Dipartimento di Scienze e Tecnologie Biomediche, Università di Cagliari, Viale Sant'Ignazio 38, 09123 Cagliari, Italy

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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 a high activity against MTB (MIC = 4 µg/mL). The newly synthesized pyrazolones were also computationally investigated to analyze if their properties fit the pharmacophoric model for antitubercular compounds previously built by us. The results are in agreement with those reported by us previously for a class of pyrazole analogues and confirm the fundamental role of the *p*-chlorophenyl moiety at C4 in the antimycobacterial activity.

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1. Introduction

In 2008 fell 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 being identified more than one century ago and with many efficient drugs being discovered during that time to eradicate the disease, TB still remains one of the leading causes 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 on 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 (Fig. 1).^{4,5} Structure–activity relationship (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 conditions⁶ in one tautomeric form or more tautomeric forms and also in a hydrogen bond-stabilized form

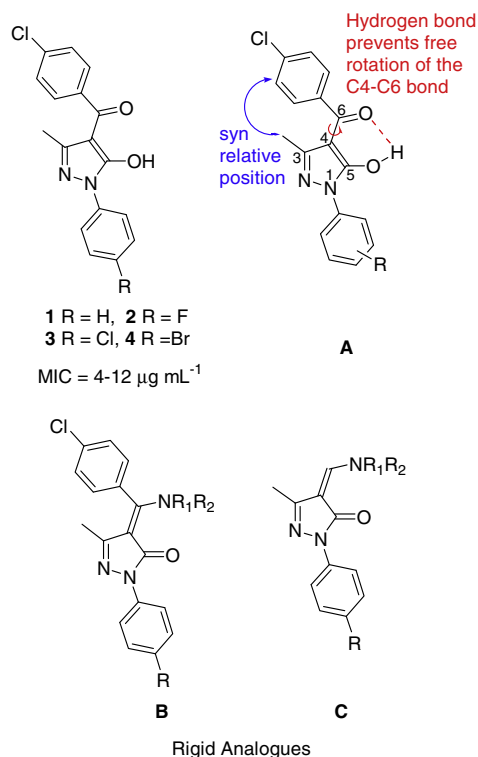


Figure 1. Pyrazoles and pyrazolones derivatives.

* Corresponding author.

E-mail address: botta@unisi.it (M. Botta).

(Structure A, Fig. 1). In particular, an in-depth investigation by Holzer et al. 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 structures **B** and **C** (Fig. 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 the recent results obtained from the synthesis of antimycobacterial 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 introduction of a secondary amine could play a dual role, leading to compounds with constrained conformation and with a potential improvement in their antimycobacterial activity. Finally, the synthesis of a series of pyrazolones having the general structure **C** was planned to support the hypothesis that the *p*-chlorophenyl moiety at C4 could have a fundamental role in the antimycobacterial activity.

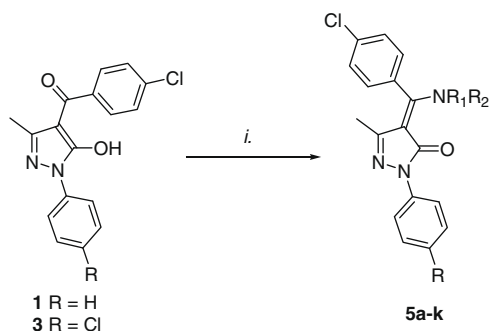
2. Chemistry

We first focused our attention on the synthesis of derivatives with the general structure **B**. Pyrazoles **1** and **3**, chosen as synthetic precursors, were synthesized according to the reported procedures.^{4,5} Reaction of **1** and **3** with different secondary amines led to the desired pyrazolones **5a–k**. The reactions were performed in DMF or DME under microwave irradiation at 160 °C and were completed in only 10 min (Scheme 1).

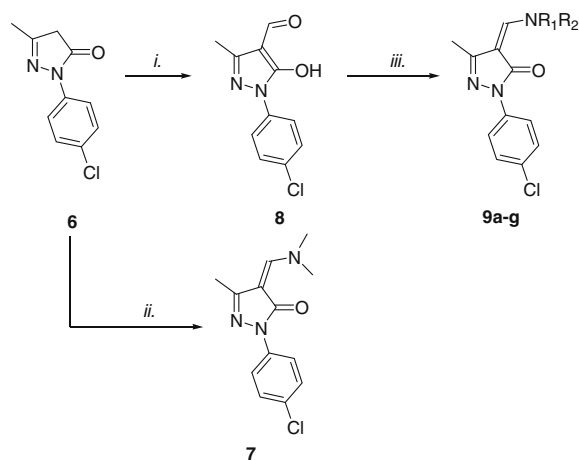
A second series of pyrazolone derivatives with the general structure **C** were synthesized. Commercially available pyrazolone **6** was reacted under the Vilsmeier conditions with POCl₃ in DMF at 80 °C. When the reaction was stopped with NaOH 30% solution, **7** was isolated as the only product. On the other hand, when Vilsmeier reaction was quenched with distilled H₂O and the reaction mixture was 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.

3. Results and discussion

Compounds **5a–k**, **7**, and **9a–g** were assayed for their inhibitory activity toward *M. tuberculosis* H37Rv (ATCC27294). The minimum



Scheme 1. Reagents and conditions: (i) secondary amines (1.2 equiv), DMF (for **5a–h**) or DME (for **5i–k**), μ W, 160 °C, 10 min.



Scheme 2. Reagents and conditions: (i) (a) POCl₃, DMF, 2 h, 80 °C then (b) H₂O, rt, 48 h; (ii) (a) POCl₃, DMF, 2 h, 80 °C then (b) NaOH 30%; (iii) R₁R₂NH, μ W, 100 °C, 5 min.

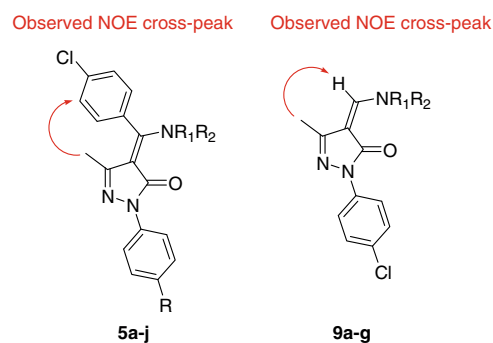


Figure 2. Observed NOE-cross couplings.

inhibitory concentration (MIC expressed as $\mu\text{g mL}^{-1}$) was determined for each compound. The resulting data are reported in Table 1.

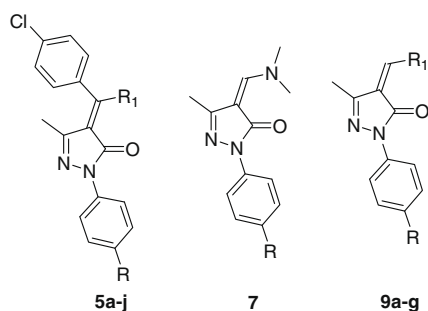
The presence of a chlorine atom on the N1-phenyl ring (**5e–h**) caused an improvement in the activity with respect to non-halogenated **5a–d**. In particular, **5f–g**, bearing *N*-Me-piperazine and morpholine moieties proved to be very active with MIC = 4 $\mu\text{g/mL}$. On the other hand, the presence of piperidine or Me-piperidine moieties (**5i–k**) resulted to be detrimental for the 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 the 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 $\mu\text{g/mL}$ for **5a**, 5.58 $\mu\text{g/mL}$ for **5b**, 4.86 $\mu\text{g/mL}$ for **5e**, and 5.49 $\mu\text{g/mL}$ for **5f**). The active compound **5g** showed a slightly better cytotoxic profile with CC_{50} = 13.29 $\mu\text{g/mL}$. On the contrary the inactive compounds **9a–g** proved to be non-cytotoxic with CC_{50} > 125 $\mu\text{g/mL}$.

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**) on the model (Fig. 3A) showed a full com-

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



| Compound | R | R ₁ | MIC (μg mL ⁻¹) <i>M. tuberculosis</i> |
|-----------|----|---------------------------------------|---|
| 5a | H | Thiomorpholine | 8 |
| 5b | H | <i>N</i> -Me-piperazine | 8 |
| 5c | H | Morpholine | 16 |
| 5d | H | <i>N</i> (Me) ₂ | 32 |
| 5e | Cl | Thiomorpholine | 8 |
| 5f | Cl | <i>N</i> -Me-piperazine | 4 |
| 5g | Cl | Morpholine | 4 |
| 5h | Cl | <i>N</i> (Me) ₂ | 16 |
| 5i | Cl | Piperidine | >64 |
| 5j | Cl | 4-Me-piperidine | 64 |
| 5k | Cl | PrNMe | 64 |
| 7 | Cl | | >64 |
| 9a | Cl | <i>N</i> -Ph-piperazine | 64 |
| 9b | Cl | <i>N</i> -Me-piperazine | 64 |
| 9c | Cl | Morpholine | 64 |
| 9d | Cl | <i>N</i> -Ac-piperazine | 64 |
| 9e | Cl | NH-Ph | 64 |
| 9f | Cl | <i>N</i> -(2-Hydroxyethyl)-piperazine | 64 |
| 9g | Cl | <i>N</i> -(2-Furoyl)-piperazine | 32 |

plementarity 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 thio-

morpholino derivative **5e**) or completely unable to fit HBA (**5h–k**) were all characterized by activity values lower than those 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** on the model showed an interaction pattern similar to that found for compounds **5** (Fig. 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 those reported by us for a class of pyrazole derivatives, 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.

5. Experimental

Reagents were obtained from commercial suppliers and used without further purification. *N,N*-Dimethylformamide (DMF) and dimethoxyethane (DME) were purchased in an anhydrous form (Aldrich), dichloromethane was dried over CaH₂ prior to use. Anhydrous reactions were run under a positive pressure of dry N₂. Merck Silica Gel 60 was used for flash chromatography (23–400 mesh). ¹H NMR and ¹³C 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 CDCl₃ at δ 7.24 ppm and to tetramethylsilane at δ 0.00 ppm.

5.1. HPLC and MS analysis

The purity of the 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; nebulizer pressure, 40 psig; and drying gas temperature, 350 °C.

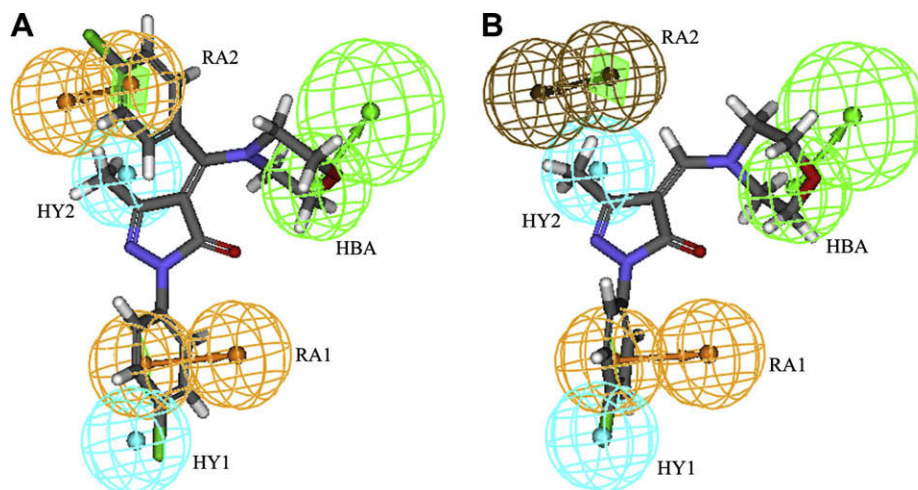


Figure 3. Superposition of **5g** (A) and **9a** (B) on the model for antimycobacterial compounds. Pharmacophoric features are color coded: orange for aromatic rings (RA); green for hydrogen bond acceptor groups (HBA); and cyan for hydrophobic regions (HY). The aromatic ring feature RA2 not mapped by **9a** is in brown.

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 (0–300 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 were stirred by means of a 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

Compound **1** or **3** (1 equiv/mol) was dissolved in anhydrous DMF or DME in a sealed vessel equipped with a magnetic stirring bar. The appropriate amine was added (1.2 equiv/mol) to the solution and the vessel was placed in a 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 ($3 \times 10 \text{ mL}$). 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 ($\text{CH}_2\text{Cl}_2/\text{MeOH}$ 99:1 for compounds **5b**, **5f**, and **5i–k**; petroleum ether/ EtOAc 8:2 for compounds **5a**, **5c**, **5e**, and **5g**) to afford the final products (25–70% yield).

Compound 5a: Yield: 70%. ^1H NMR (CDCl_3): δ (ppm) 7.91 (2H, d, $J = 8.04 \text{ Hz}$, Ph), 7.44 (2H, d, $J = 8.35 \text{ Hz}$, Ph), 7.32–7.28 (4H, m, Ph), 7.04 (1H, t, $J = 7.30 \text{ Hz}$, 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 ($\text{M}+1$) $^+$; 420–422 ($\text{M}+\text{Na}$) $^+$; 817–819. Anal. Calcd for $\text{C}_{21}\text{H}_{20}\text{ClN}_3\text{OS}$: C, 63.39; H, 5.07; N, 10.56. Found: C, 63.58; H, 5.08; N, 10.59.

Compound 5b: Yield: 60%. ^1H NMR (CDCl_3): δ (ppm) 7.91 (2H, d, $J = 7.71 \text{ Hz}$, Ph), 7.41 (2H, d, $J = 7.52 \text{ Hz}$, Ph), 7.27–7.26 (4H, m, Ph), 7.01 (1H, t, $J = 7.00 \text{ Hz}$, Ph), 3.92 (2H, m, CH_2NCH_2), 3.35 (2H, m, CH_2NCH_2), 2.65 (2H, m, $\text{CH}_2\text{N}(\text{CH}_3)\text{CH}_2$), 2.43 (2H, m, $\text{CH}_2\text{N}(\text{CH}_3)\text{CH}_2$), 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 ($\text{M}+1$) $^+$; 417–419 ($\text{M}+\text{Na}$) $^+$; 433–435 ($\text{M}+\text{K}$) $^+$, 811–813 ($2\text{M}+\text{Na}$) $^+$. Anal. Calcd for $\text{C}_{22}\text{H}_{23}\text{ClN}_4\text{O}$: C, 66.91; H, 5.87; N, 14.19. Found: C, 67.11; H, 5.71; N, 14.23.

Compound 5c: Yield: 30%. ^1H NMR (CDCl_3): δ (ppm) 7.95 (2H, d, $J = 8.05 \text{ Hz}$, Ph), 7.48 (2H, d, $J = 8.05 \text{ Hz}$, Ph), 7.36–7.32 (4H, m, Ph), 7.08 (1H, t, $J = 7.30 \text{ Hz}$, Ph), 4.02 (2H, m, CH_2OCH_2), 3.96 (2H, m, CH_2OCH_2), 3.74 (2H, m, CH_2NCH_2), 3.38 (2H, m, CH_2NCH_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 ($\text{M}+1$) $^+$; 404–406 ($\text{M}+\text{Na}$) $^+$; 785–787 ($2\text{M}+\text{Na}$) $^+$. Anal. Calcd for $\text{C}_{21}\text{H}_{20}\text{ClN}_3\text{O}_2$: C, 66.05; H, 5.28; N, 11. Found: C, 66.76; H, 5.30; N, 11.04.

Compound 5d: Yield: 25%. ^1H NMR (CDCl_3): δ (ppm) 8.01 (2H, d, $J = 8.04 \text{ Hz}$, Ph), 7.48 (2H, d, $J = 8.04 \text{ Hz}$, Ph), 7.37–7.34 (4H, m, Ph), 7.09 (1H, t, $J = 7.29 \text{ Hz}$, Ph), 3.58 (3H, s, CH_3NCH_3), 3.08 (3H, s, CH_3NCH_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 ($\text{M}+1$) $^+$; 362–364 ($\text{M}+\text{Na}$) $^+$; 701–703 ($2\text{M}+\text{Na}$) $^+$. Anal. Calcd for

$\text{C}_{19}\text{H}_{18}\text{ClN}_3\text{O}$: C, 67.15; H, 5.34; N, 12.37. Found: C, 67.28; H, 5.35; N, 12.39.

Compound 5e: Yield: 50%. ^1H NMR (CDCl_3): δ (ppm) 7.91 (2H, d, $J = 8.53 \text{ Hz}$, Ph), 7.44 (2H, d, $J = 8.53 \text{ Hz}$, Ph), 7.29–7.23 (4H, m, Ph), 4.10 (2H, m, CH_2NCH_2), 3.55 (2H, m, CH_2NCH_2), 2.95 (2H, m, CH_2SCH_2), 2.63 (2H, m, CH_2SCH_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 ($\text{M}+1$) $^+$; 454–456 ($\text{M}+\text{Na}$) $^+$; 470–472 ($\text{M}+\text{K}$) $^+$; 887–885 ($2\text{M}+\text{Na}$) $^+$. Anal. Calcd for $\text{C}_{21}\text{H}_{19}\text{Cl}_2\text{N}_3\text{OS}$: C, 58.34; H, 4.43; N, 9.72. Found: C, 58.46; H, 4.44; N, 9.74.

Compound 5f: Yield: 50%. ^1H NMR (CDCl_3): δ (ppm) 7.96 (2H, d, $J = 8.63 \text{ Hz}$, Ph), 7.46 (2H, d, $J = 8.63 \text{ Hz}$, Ph), 7.33–7.26 (4H, m, Ph), 3.96 (2H, m, CH_2NCH_2), 3.41 (2H, m, CH_2NCH_2), 2.69 (2H, m, $\text{CH}_2\text{N}(\text{CH}_3)\text{CH}_2$), 2.49 (2H, m, $\text{CH}_2\text{N}(\text{CH}_3)\text{CH}_2$), 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 ($\text{M}+1$) $^+$; 451–453 ($\text{M}+\text{Na}$) $^+$; 881–879 ($2\text{M}+\text{Na}$) $^+$. Anal. Calcd for $\text{C}_{22}\text{H}_{22}\text{Cl}_2\text{N}_4\text{O}$: C, 61.54; H, 5.16; N, 13.05. Found: C, 61.68; H, 5.18; N, 13.10.

Compound 5g: Yield: 50%. ^1H NMR (CDCl_3): δ (ppm) 7.94 (2H, d, $J = 8.50 \text{ Hz}$, Ph), 7.48 (2H, d, $J = 8.50 \text{ Hz}$, Ph), 7.32–7.26 (4H, m, Ph), 4.00 (2H, m, CH_2OCH_2), 3.95 (2H, m, CH_2OCH_2), 3.73 (2H, m, CH_2NCH_2), 3.37 (2H, m, CH_2NCH_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 ($\text{M}+1$) $^+$; 438–440 ($\text{M}+\text{Na}$) $^+$; 855–853 ($2\text{M}+\text{Na}$) $^+$. Anal. Calcd for $\text{C}_{21}\text{H}_{19}\text{Cl}_2\text{N}_3\text{O}_2$: C, 60.59; H, 4.60; N, 10.09. Found: C, 60.83; H, 4.62; N, 10.13.

Compound 5h: Yield: 26%. ^1H NMR (CDCl_3): δ (ppm) 7.99 (2H, d, $J = 8.50 \text{ Hz}$, Ph), 7.47 (2H, d, $J = 8.50 \text{ Hz}$, Ph), 7.34–7.24 (4H, m, Ph), 3.57 (3H, s, CH_3NCH_3), 3.07 (3H, s, CH_3NCH_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 ($\text{M}+1$) $^+$; 396–398 ($\text{M}+\text{Na}$) $^+$; 412–414 ($\text{M}+\text{K}$) $^+$. Anal. Calcd for $\text{C}_{19}\text{H}_{17}\text{Cl}_2\text{N}_3\text{O}$: C, 60.97; H, 4.58; N, 11.23. Found: C, 61.15; H, 4.59; N, 11.26.

Compound 5i: Yield: 55%. ^1H NMR (CDCl_3): δ (ppm) 8.02 (2H, d, $J = 8.53 \text{ Hz}$, Ph), 7.51 (2H, d, $J = 8.53 \text{ Hz}$, Ph), 7.39 (2H, d, $J = 8.53 \text{ Hz}$, Ph), 7.33 (2H, d, $J = 8.53 \text{ Hz}$, Ph), 3.95 (2H, m, CH_2NCH_2), 3.40 (2H, m, CH_2NCH_2), 1.88–1.74 (6H, m, $\text{CH}_2\text{CH}_2\text{CH}_2$), 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 ($\text{M}+1$) $^+$. Anal. Calcd for $\text{C}_{22}\text{H}_{21}\text{Cl}_2\text{N}_3\text{O}$: C, 63.77; H, 5.11; N, 10.14. Found: C, 62.37; H, 5.13; N, 10.19.

Compound 5j: Yield: 56%. ^1H NMR (CDCl_3): δ (ppm) 7.99 (2H, d, $J = 8.85 \text{ Hz}$, Ph), 7.46 (2H, d, $J = 8.83 \text{ Hz}$, Ph), 7.36 (2H, d, $J = 8.63 \text{ Hz}$, Ph), 7.28 (2H, d, $J = 8.63 \text{ Hz}$, Ph), 3.95 (2H, m, CH_2NCH_2), 3.48 (1H, m, CH_2NCH_2), 3.23 (1H, m, CH_2NCH_2), 1.87 (4H, m, $\text{CH}_2\text{CH}(\text{CH}_3)\text{CH}_2$), 1.49–1.46 (1H, m, CHCH_3), 1.33 (3H, s, CH_3), 1.01 (3H, d, $J = 5.76 \text{ Hz}$, 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 ($\text{M}+1$) $^+$; 450–452 ($\text{M}+\text{Na}$) $^+$. Anal. Calcd for $\text{C}_{23}\text{H}_{24}\text{Cl}_2\text{N}_3\text{O}$: C, 64.49; H, 5.41; N, 9.81. Found: C, 64.37; H, 5.35; N, 9.97.

Compound 5k: Yield: 56%. ^1H NMR (CDCl_3): δ (ppm) 7.97 (2H, d, $J = 8.73 \text{ Hz}$, Ph), 7.46 (2H, d, $J = 8.46 \text{ Hz}$, Ph), 7.36–7.30 (4H, m, Ph), 3.56 (3H, s, N-CH_3), 3.38–3.34 (2H, m, $\text{CH}_3\text{CH}_2\text{CH}_2\text{NCH}_3$), 1.69–1.66 (2H, m, $\text{CH}_3\text{CH}_2\text{CH}_2$), 1.36 (3H, s, CH_3), 0.81 (3H, t, $J = 7.32$, CH_2CH_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 ($\text{M}+1$) $^+$; 424–426 ($\text{M}+\text{Na}$) $^+$. Anal. Calcd for

C₂₁H₂₁Cl₂N₃O: 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** (1 equiv/mol) in DMF, 0.7 equiv/mol of POCl₃ and 2 equiv/mol of DMF were added. The mixtures were heated at 80 °C for 2 h. After cooling, distilled H₂O was added and the resulting solution was stirred for 48 h. The desired product **8** was obtained as a yellow precipitate which was separated by filtration and used in the next step without any further purification.

Compound 8: Yield: 92%. ¹H NMR (CDCl₃): δ (ppm) 10.26 (1H, br s, OH), 9.33 (1H, s, CHO), 7.67 (2H, br d, Ph), 7.31 (2H, br d, Ph), 2.34 (3H, s, CH₃). ¹³C NMR (CDCl₃): 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. Calcd for C₁₁H₉ClN₂O₂: 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 equiv/mol) and the appropriate amine (4 equiv/mol) were irradiated under microwave at 100 °C for 5 min. The crude products **9a–g** were then directly crystallized using AcOEt 100%.

Compound 9a: Yield: 70%. ¹H NMR (CDCl₃): 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, CH₂NCH₂), 3.78 (2H, m, CH₂NCH₂), 3.38 (4H, m, CH₂N(Ph)CH₂), 2.22 (3H, s, CH₃). ¹³C NMR (CDCl₃): 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. Calcd for C₂₁H₂₁ClN₄O: C, 66.22; H, 5.56; N, 14.71. Found: C, 66.34; H, 5.71; N, 14.83.

Compound 9b: Yield: 60%. ¹H NMR (CDCl₃): 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, CH₂NCH₂), 3.58 (2H, s, CH₂NCH₂), 2.58 (4H, m, CH₂NCH₂), 2.33 (3H, s, NCH₃), 2.16 (3H, s, CH₃). ¹³C NMR (CDCl₃): 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. Calcd for C₁₆H₁₉ClN₄O: C, 60.28; H, 6.01; N, 17.57. Found: C, 60.33; H, 5.07; N, 17.63.

Compound 9c: Yield: 53%. ¹H NMR (CDCl₃): 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, CH₂NCH₂), 3.86 (4H, m, CH₂OCH₂), 3.58 (2H, m, CH₂NCH₂), 2.19 (3H, s, CH₃). ¹³C NMR (CDCl₃): 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. Calcd for C₁₅H₁₆ClN₃O₂: C, 58.92; H, 5.27; N, 13.74. Found: C, 59.02; H, 5.35; N, 14.02.

Compound 9d: Yield: 30%. ¹H NMR (CDCl₃): 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, CH₂NCH₂), 3.83 (2H, m, CH₂NCH₂), 3.70 (2H, m, CH₂N(CH₃)CH₂), 3.59 (2H, m, CH₂N(CH₃)CH₂), 2.21 (3H, s, CH₃), 2.17 (3H, s, COCH₃). ¹³C NMR (CDCl₃): 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. Calcd for C₁₇H₁₉ClN₄O₂: C, 58.87; H, 5.52; N, 16.15. Found: C, 58.93; H, 5.54; N, 16.32.

Compound 9e: Yield: 48%. ¹H NMR (CDCl₃): δ (ppm) 11.42 (1H, br s, NH), 8.0 (2H, d, *J* = 8.16 Hz, Ph), 7.89 (1H, s, CH=NPh), 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, CH₃). ¹³C NMR (CDCl₃): 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. Calcd for C₁₇H₁₆ClN₃O: C, 65.07; H, 5.14; N, 13.39. Found: C, 66.01; H, 5.24; N, 13.77.

Compound 9f: Yield: 37%. ¹H NMR (CDCl₃): 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, CH₂NCH₂), 4.78 (2H, m, CH₂NCH₂), 3.68 (2H, m, CH₂NCH₂), 3.64 (2H, m, CH₂OH), 2.74 (4H, m, CH₂N(CH₂CH₂OH)CH₂), 2.64 (2H, m, NCH₂CH₂OH), 2.19 (3H, s, CH₃). ¹³C NMR (CDCl₃): 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. Calcd for C₁₇H₂₁ClN₄O₂: C, 58.53; H, 6.07; N, 16.06. Found: C, 58.62; H, 6.11; N, 16.12.

Compound 9g: Yield: 74%. ¹H NMR (CDCl₃): δ (ppm) 7.93 (2H, d, *J* = 8.56 Hz, Ph), 7.50 (1H, s, H₃-furane), 7.31 (2H, d, *J* = 8.56 Hz, Ph), 7.09 (1H, s, C=CH-piperazine), 6.98 (1H, s, H₄-furane), 6.51 (1H, s, H₅-furane), 4.80 (2H, m, CH₂NCH₂), 4.05 (2H, m, CH₂N(CO)CH₂), 3.96 (2H, m, CH₂N(CO)CH₂), 3.62 (2H, m, CH₂NCH₂), 2.19 (3H, s, CH₃). ¹³C NMR (CDCl₃): 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. Calcd for C₂₀H₁₉ClN₄O₃: 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 × 10⁵ cfu/mL grown in Middlebrook 7H9 broth (Difco Laboratories, Detroit, MI) supplemented with 10% albumin–dextrose–catalase enrichment. Final inoculum was 1.5 × 10³ 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 described previously.⁵

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