***Supplementary Information***

**for**

**X-ray single-crystal analysis, pharmaco-toxicological profile and enoyl-ACP reductase inhibiting activity of leading sulfonyl hydrazone derivatives with potent antimycobacterial activity**

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 **Table S1.** Spectral characteristics of **3a** and **3b**.

|  |  |
| --- | --- |
| Compound | Spectral characteristics |
| *N’*-[(E)-(4-nitrophenyl)methylidene]benzenesulfonohydrazide, **3a** | Yellow solid. Yield: 92%; m.p.166–167 ºC. 1H NMR (600 MHz, DMSO-d6) δ 12.01 (s, 1H, NH), 8.23 (td, J = 2.1, 9.3 Hz, 2H, H-3 and H-5), 8.03 (s, 1H, CH=N), 7.90 (td, J = 1.5, 6.7 Hz, 2H, H-o), 7.83 (td, J = 1.4, 8.9 Hz, 2H, H-2 and H-6), 7.68 (tt, J = 1.6, 7.4 Hz, 1H, H-p), 7.62 (tt, J = 1.5, 7.5 Hz, 2H, H-m). 13C NMR (151 MHz, DMSO-d6) δ 147.90 (C-p), 144.62 (CH=N), 139.78 (C-i), 138.85 (C-i), 133.32 (C-p), 129.42 (C-m), 127.76 (C-2 and C-6), 127.15 (C-o), 124.09 (C-3 and C-05). HRMS (ESI) m/z: calcd: [M+H]+ 306.054302. Found: [M+H]+ 306.0535. |
| *N’*-[(1E,2E)-3-phenylprop-2-en-1-ylidene]benzenesulfonohydrazide, **3b** | White solid. Yield: 85%;m.p. 168–170 ºC. 1H NMR (600 MHz, DMSO-d6) δ 11.50 (s, 1H, NH), 7.83–7.85 (m, 2H, H-o), 7.73 (d, J = 9.2 Hz, 1H, H-1), 7.66–7.68 (m, 1H, H-p), 7.60–7.63 (m, 2H, H-m), 7.54–7.56 (m, 2H, H-o), 7.33–7.36 (m, 2H, H-m), 7.28–7.31 (m, 1H, H-p), 6.95 (d, J = 16.1 Hz, 1H, H-3), 6.84 (dd, J = 9.2, 16.1 Hz, 1H, H-2). 13C NMR (151 MHz, DMSO-d6) δ 149.48 (C-1), 139.38 (C-3), 139.14 (C-i), 135.65 (C-i), 133.06 (C-p), 129.32 (C-m), 128.94 (C-p), 128.80 (C-m), 127.15 (C-o), 127.12 (C-o), 124.68 (C-2). HRMS (ESI) m/z: calcd: [M+H]+ 287.084874. Found:[M+H]+ 287.0842.  |

**Table S2.** Crystal data and structure refinement for **3b**.

|  |  |
| --- | --- |
| Identification code | **3b** |
| Empirical formula | C15H14N2O2S |
| Formula weight | 286.34 |
| Temperature/K | 298.00 |
| Crystal system | monoclinic |
| Space group | *P*21*/c* |
| a/Å | 17.0965(7) |
| b/Å | 5.3584(2) |
| c/Å | 15.3372(6) |
| α/° | 90 |
| β/° | 98.3530(10) |
| γ/° | 90 |
| Volume/Å3 | 1390.13(9) |
| Z | 4 |
| ρcalcg/cm3 | 1.368 |
| μ/mm‑1 | 0.235 |
| F(000) | 600.0 |
| Crystal size/mm3 | 0.3 × 0.2 × 0.15 |
| Radiation | MoKα (λ = 0.71073) |
| 2Θ range for data collection/° | 4.816 to 52.74 |
| Index ranges | -21 ≤ h ≤ 21, -6 ≤ k ≤ 6, -19 ≤ l ≤ 19 |
| Reflections collected | 37815 |
| Independent reflections | 2858 [Rint = 0.0531, Rsigma = 0.0284] |
| Data/restraints/parameters | 2858/0/185 |
| Goodness-of-fit on F2 | 1.092 |
| Final R indexes [I>=2σ (I)] | R1 = 0.0358, wR2 = 0.0903 |
| Final R indexes [all data] | R1 = 0.0397, wR2 = 0.0936 |
| Largest diff. peak/hole / e Å-3 | 0.47/-0.22 |
| CCDC | 2356559 |

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# **Table S3.** Bond Lengths for **3b**

| **Atom** | **Atom** | **Length/Å** |   | **Atom** | **Atom** | **Length/Å** |
| --- | --- | --- | --- | --- | --- | --- |
| S1 | O3 | 1.4297(11) |   | C10 | C15 | 1.388(2) |
| S1 | O2 | 1.4225(11) |   | C7 | C8 | 1.436(2) |
| S1 | N1 | 1.6262(13) |   | C2 | C3 | 1.383(2) |
| S1 | C1 | 1.7630(15) |   | C8 | C9 | 1.329(2) |
| N2 | N1 | 1.3921(17) |   | C4 | C3 | 1.376(3) |
| N2 | C7 | 1.2758(19) |   | C4 | C5 | 1.374(2) |
| C1 | C6 | 1.379(2) |   | C11 | C12 | 1.381(2) |
| C1 | C2 | 1.382(2) |   | C15 | C14 | 1.382(2) |
| C6 | C5 | 1.383(2) |   | C13 | C12 | 1.365(3) |
| C10 | C9 | 1.465(2) |   | C13 | C14 | 1.378(3) |
| C10 | C11 | 1.391(2) |   |   |   |  |

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# **Table S4**. Bond Angles for **3b**

| **Atom** |  | **Atom** | **Atom** | **Angle/˚** |   | **Atom** | **Atom** | **Atom** | **Angle/˚** |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| O3 |  | S1 | N1 | 107.82(7) |   | C15 | C10 | C11 | 117.79(15) |
| O3 |  | S1 | C1 | 107.38(7) |   | N2 | C7 | C8 | 121.56(15) |
| O2 |  | S1 | O3 | 119.68(7) |   | C1 | C2 | C3 | 119.31(15) |
| O2 |  | S1 | N1 | 103.97(7) |   | C9 | C8 | C7 | 121.68(16) |
| O2 |  | S1 | C1 | 109.56(7) |   | C8 | C9 | C10 | 127.91(16) |
| N1 |  | S1 | C1 | 107.89(7) |   | C5 | C4 | C3 | 120.19(16) |
| C7 |  | N2 | N1 | 114.36(13) |   | C4 | C3 | C2 | 120.09(16) |
| N2 |  | N1 | S1 | 117.46(10) |   | C12 | C11 | C10 | 121.11(17) |
| C6 |  | C1 | S1 | 120.17(11) |   | C4 | C5 | C6 | 120.45(16) |
| C6 |  | C1 | C2 | 120.87(14) |   | C14 | C15 | C10 | 120.77(16) |
| C2 |  | C1 | S1 | 118.96(11) |   | C12 | C13 | C14 | 119.75(16) |
| C1 |  | C6 | C5 | 119.07(15) |   | C13 | C12 | C11 | 120.24(17) |
| C11 |  | C10 | C9 | 119.32(15) |   | C13 | C14 | C15 | 120.33(18) |
| C15 |  | C10 | C9 | 122.90(15) |   |   |   |   |  |

**Table S5**. Torsion Angles for **3b**

| **A** | **B** | **C** | **D** | **Angle/˚** |   | **A** | **B** | **C** | **D** | **Angle/˚** |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| S1 | C1 | C6 | C5 | 179.24(12) |   | C10 | C11 | C12 | C13 | 1.1(3) |
| S1 | C1 | C2 | C3 | -179.89(12) |   | C10 | C15 | C14 | C13 | -0.3(3) |
| O3 | S1 | N1 | N2 | 57.09(13) |   | C7 | N2 | N1 | S1 | 173.69(11) |
| O3 | S1 | C1 | C6 | -10.97(14) |   | C7 | C8 | C9 | C10 | 177.78(15) |
| O3 | S1 | C1 | C2 | 168.75(11) |   | C2 | C1 | C6 | C5 | -0.5(2) |
| O2 | S1 | N1 | N2 | -174.89(11) |   | C9 | C10 | C11 | C12 | 178.04(15) |
| O2 | S1 | C1 | C6 | -142.42(12) |   | C9 | C10 | C15 | C14 | -178.42(16) |
| O2 | S1 | C1 | C2 | 37.30(13) |   | C3 | C4 | C5 | C6 | -0.2(3) |
| N2 | C7 | C8 | C9 | -177.43(16) |   | C11 | C10 | C9 | C8 | -172.08(17) |
| N1 | S1 | C1 | C6 | 105.01(13) |   | C11 | C10 | C15 | C14 | 1.5(3) |
| N1 | S1 | C1 | C2 | -75.27(13) |   | C5 | C4 | C3 | C2 | -0.4(3) |
| N1 | N2 | C7 | C8 | 178.07(13) |   | C15 | C10 | C9 | C8 | 7.9(3) |
| C1 | S1 | N1 | N2 | -58.60(13) |   | C15 | C10 | C11 | C12 | -1.9(2) |
| C1 | C6 | C5 | C4 | 0.7(2) |   | C12 | C13 | C14 | C15 | -0.6(3) |
| C1 | C2 | C3 | C4 | 0.6(3) |   | C14 | C13 | C12 | C11 | 0.2(3) |
| C6 | C1 | C2 | C3 | -0.2(2) |   |   |   |   |   |  |

**Table S6.** Hydrogen bonds and weak interactions **3b**

| **D** | **H** | **A** | **d(D-H)/Å** | **d(H-A)/Å** | **d(D-A)/Å** | **D-H-A/°** |
| --- | --- | --- | --- | --- | --- | --- |
| N1 | H1 | O3i | 0.826(8) | 2.19(2) | 2.9845(2) | 162.2(2) |
| C7 | H7 | O3i | 0.93 | 2.51 | 3.273(2) | 139.3 |
| C3 | H3 | O2ii | 0.93 | 2.64(3) | 3.549(3) | 164.8 |
| Symmetry operation: (i) *x*,*1+y*, *z*; (ii) *2-x*,*y – 1/2* , *3/2 – z*. |

**Table S7.** Hematological parameters (HP) after 14 day administration of INH, **3a** at doses of 45 mg/kg and 90 mg/kg and **3b** at doses of 65 mg/kg and 130 mg/kg. Results are presented as mean ± SD (n=6). The significance of the data was assessed using the nonparametric Mann–Whitney U test. Values of p ≤ 0.05 were considered statistically significant and are marked with \*. Abbreviations: white blood cells (WBC), lymphocytes (LYM), hemoglobin (HgB), hematocrit (HCT), platelets (PLT).

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
| HP | Control | INH 50 mg/kg  | 3a 45 mg/kg | 3a 90 mg/kg | 3b 65 mg/kg | 3b 130 mg/kg | Ref. values |
| WBC, x103/ µl | 10.53 ± 1.18 | 10.85 ± 0.90 | 5.28 ± 0.82 | 5.98 ± 0.60  | 5.18 ± 0.39 | 4.88 ± 0.62 | 2,9 - 15,3 |
| LYM, x103/ ul | 8.35 ± 0.83 | 7.25 ± 0.96 | 6.18 ± 0.56 | 7.48 ± 0.84 | 6.63 ± 0.90 | 7.83 ± 0.50 | 5,6 - 10,4 |
| HgB, g/L | 160.25 ± 8.18 | 150.25 ± 3.86 | 143.25 ± 7.80 | 138.75 ± 5.80 | 151.25 ± 2.63 | 141.25 ± 8.54 | 120 - 160 |
| HCT, % | 46.98 ± 1.32 | 43.83 ± 0.17 | 40 ± 1.21 | 40.65 ± 1.54 | 43.13 ± 0.26 | 42.55 ± 2.63 | 36 - 52 |
| PLT, x103/ul | 683.25 ± 73.08 | 580.75 ± 35.7 | 934.75 ± 144.60 | 801.5 ± 243.49 | 913.5 ± 40.42 | 859.75 ± 106.02 | 127 - 939 |

**Table S8.** Biochemical parameters (BP) of serum and plasma from experimental animals after 14 days of administration of INH and sulfonyl hydrazone **3a** at doses of 45 mg/kg and 90 mg/kg and **3b** at doses of 65 mg/kg and 130 mg/kg. Results are presented as mean±SD (n=6). The significance of the data was assessed using the nonparametric Mann–Whitney U test. Values of p ≤0.05 were considered statistically significant and are marked with \*. Abbreviations: GLU (glucose level); CREAT (creatinine); TP (total protein); ALB (albumin); ASAT (aspartate aminotransferase); ALAT (alanine aminotransferase)*.*

|  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **BP** | **Controls** | **INH50 mg/kg** | **3a 45 mg/kg (serum)**  | **3a 45 mg/kg (plasma)** | **3a 90mg/kg (serum)**  | **3a 90 mg/kg (plasma)** | **3b 65 mg/kg (serum)**  | **3b65 mg/kg (plasma)** | **3b 130 mg/kg (serum)**  | **3b 130 mg/kg (plasma)** | **Ref. values** |
| GLU, mmol/L | 6.3 | 10.36 | 10.52 | 11.43 | 9.04 | 11.24 | 10.49 | 9.19 | 11.13 | 11.36 | 4,2 – 11,6 (1) |
| UREA, mmol/L | 7.2 | 9.08 | 7.64 | 8.38 | 8.27 | 10.35 | 9.22 | 9.37 | 12.08 | 11.32 | 3.8 – 12.3(1) |
| CREAT, µmol/L | 86.9 | 24.1 | 35.1 | 35.6 | 37.1 | 35.8 | 39.3 | 36.8 | 33.9 | 37 | 35 – 53(1) |
| TP, g/L | 60 | 71.4 | 56.1 | 65.7 | 54 | 62.3 | 58.8 | 60.7 | 62.9 | 55.5 | 53 – 63(1) |
| ALB, g/L | 28 | 33.7 | 32.8 | 35.7 | 35.4 | 37.6 | 37.6 | 36.8 | 33.9 | 36.1 | 26 – 39(2) |
| ASAT, U/L | 296.2 | 288.7 | 243.4 | 232.2 | 266.7 | 311.1 | 259 | 261.4 | 319 | 324.8 | 57 – 329(3) |
| ALAT, U/L | 70.7 | 90.5 | 63.1 | 48.4 | 95 | 90.8 | 85.5 | 61.6 | 139.3 | 100.9 | 7 – 227 (3) |
| AMYL, U/L | 1550 | 1342 | 1620 | 1565.7 | 1627 | 1671.1 | 1682 | 1513.9 | 1619.2 | 1522.1 | 1512–3084 (4) |
| URIC ACID, mmol/L | 245.9 | 226 | 160 | 292 | N.D. | 165 | 134 | N.D. | 189 | 320 | 0.1 – 760 (5) |

The protein–ligand interactions (PLI) diagrams of the sulfonyl hydrazones **3a** and **3b** in the ligand-binding domains of both receptors, 2X22 and 4TZK, were obtained using the “Ligand Interactions” tool of MOE at the maximum distance of 4.5 Å between the heavy atoms of the ligands and receptors. Figure S1\_A presents the PLI diagrams of both compounds in the receptors 2X22 (compound **3a** in left and compound **3b** in right), while, by analogy, Figure S1\_B presents the PLIs in the receptor 4TZK.

**A: Receptor 2X22**

  **Compound 3a Compound 3b**

**B: Receptor 4TZK**

  **Compound 3a Compound 3b**

**Figure S1**. Interaction diagrams of the ligand-binding domains of *M. tuberculosis* InhA with the compounds **3a** (left) and **3b** (right) in both domains: **A) 2X22; B) 4TZK**.

As seen from the left subplots of **Figure S1**, the compound **3a** repeats one out of three interactions of the native ligand of the complex 2X22 (6), namely arene-H interaction with Met161, and the only one PLI of the native ligand of the complex 4TZK (6), namely H-bond with Tyr158. As seen from the right subplots of **Figure S1**, the compound **3b** demonstrates the reproduction of two out of three interactions of the native ligand of the complex 2X22 (6), namely H-bond wit with Tyr158S1 and arene-H interaction with Met161, while in the receptor 4TZK it demonstrates only one newly appeared arene-H interaction with Phe97.

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