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NOVEL SCHIFF BASES OF INDOLINE-2,3-DIONE AND NALIDIXIC ACID HYDRAZIDE: SYNTHESIS, *IN VITRO* ANTIMYCOBACTERIAL AND *IN SILICO* MYCOBACTERIUM TUBERCULOSIS (*MTB*) DNA GYRASE INHIBITORY ACTIVITY

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A novel series of Schiff bases **5b-g** was synthesized by the reaction of *N*-substitutedbenzylisatin **3b-g** with nalidixic acid hydrazide **4** in order to optimize the antimycobacterial activity of our lead Schiff base **5a**. Antimycobacterial (anti-*mtb*) activity of the synthesized hydrazones was investigated against four Mycobacterium strains: *M. intercellulari*, *M. xenopi*, *M. cheleneo* and *M. smegmatis*. It was found that *para*-substitution, with electron withdrawing group, of benzyl moiety in *N*-benzylisatins resulted in 7 fold enhancement of the anti-mtb activity as shown with compounds **5b, 5d** and **5f** (MIC 0.09 μg/ml) with *p*-chloro, fluoro and nitro substituents respectively. *In silico* docking study of these hydrazones with mtb-DNA gyrase revealed that there is parallelism between the antimycobacterial activity of these hydrazones and docking with the active site of the mtb-DNA gyrase B subunit.

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1. Introduction

Tuberculosis (TB) is the most prevalent infectious disease worldwide and a leading killer caused by a single infectious agent, that is, Mycobacterium tuberculosis (*mtb*). According to World Health Organization (WHO), *mtb* currently infects over 2 billion people worldwide, almost one-third of the world's population, with 30 million new cases reported each year. This intracellular infection accounts for at least 3 million deaths annually, a life lost to TB every 15 second [1].

The gravity of the situation is worsened by the emergence of multidrug-resistant (MDR)- and extensively drug-resistant (XDR)- *mtb* strains that are also unresponsive to the drugs used to treat TB, and frequent co-infection with HIV further complicates patient care and prognosis [2,3]. Thus there is an urgency to develop new drugs and strategies to fight against TB or a tragedy may occur.

Hydrazones are attractive target compounds for new drug development due to their synthetic and biological versatility including anti-TB activities [4-8]. Furthermore, there are significant reasons for investigating Schiff base derivatives of indolin-2,3-dione (isatin) for example the recent reported remarkable anti-TB activity [9-12]. Moreover, it was recently reported that indolin-2-one structural scaffold as potent DNA gyrase inhibitors [13-15]. DNA gyrase is a bacterial enzyme that catalyzes the introduction of negative supercoils in a closed-circular DNA using the energy of the ATP hydrolysis. Since it is found only in prokaryotes and is vital for their survival, it has become an attractive target for antibacterial agents.

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Encouraged by the above and in continuation to our synthetic work on Schiff bases of isatin [8, 10-12, 16] the current work describes synthesis and anti-*mtb* of Schiff bases of *N*-substituted-benzylisatin with nalidixic acid hydrazide. The aim of the work is to optimize the anti-*mtb* activity of our recently reported Schiff bases of 1-benzylisatin and nalidixic acid hydrazide that revealed potent anti-*mtb* with MIC 0.625 μg/ml [10]. Furthermore, *in silico* interactions the synthesized hydrazones within the *mtb*-DNA gyrase active site could provide valuable information for their possible mode of action.

2. Experimental

2.1. Materials and equipments

Isatin **1** and substituted benzyl chlorides (bromides) **2a-f** were obtained commercially. Compounds **3a-g** [16, 17], nalidixic acid hydrazide **4** and the corresponding hydrazone **5a** [10] were synthesized according to the reported literature. All other chemicals used were of commercially available reagent grade and were used without further purification. Melting points were determined on a Gallenkamp melting point apparatus, and are uncorrected. NMR Spectra were scanned in DMSO-d_6 on a Bruker NMR spectrophotometer operating at 500 MHz for 1H and 125.76 MHz for ^{13}C at the Research Center, College of Pharmacy, King Saud University (Riyadh, Saudi Arabia). Chemical shifts are expressed in δ-values (ppm) relative to TMS as an internal standard. Coupling constants (*J*) are expressed in Hz. D_2O was added to confirm the exchangeable protons. Mass spectra were measured on a Varian MAT CH-5 spectrometer (70 eV) or in Agilent Triple Quadrupole 6410 QQQ LC/MS with ESI (Electrospray ionization) source. Homology modeling of *M. tuberculosis* DNA gyrase subunit B was carried out by SWISS-MODEL [18-20], based on the crystal structure of the 43 K ATPase domain of *Thermus thermophilus* gyrase B in complex with novobiocin (1KIJ.pdb, 2.30 ˚A resolution, 43% identity among 420 aligned residues) [21].

2.2. Chemistry

2.2.1. General procedure for the synthesis of N-substituted isatines 3a-g

A flask equipped with a magnetic stirring bar is charged with DMF (100 ml) and potassium carbonate (1.8 g, 13 mmol). The mixture stirred at room temperature for 5 minutes then isatin **1** (1.47 g, 10 mmol) was added. Stirring is continued for 45 minutes then the appropriate substituted benzyl chloride (bromide) **2a-g** (11 mmol) was then added. Stirring was continued at 80 ^{o}C for 12 h, the mixture was diluted with water (200 ml). The mixture is extracted with three portions (100-ml) of diethyl ether. The combined organic layers were washed with water (3x 50ml), dried over calcium chloride, and the solvent is removed at slightly reduced pressure to yield compounds **3a-g** in 60-75% yield [16, 17].

*1-Benzylindoline-2,3-dione (**3a**).* Mp: 129-130 °C; ^{1}H–NMR (DMSO-d_6): 4.92 (2H, s, N$\underline{CH_2}$-ph), 6.98 (1H, d, J= 9.5, C_7H), 7.12 (1H, t, J= 8.5, C_5H), 7.28–7.39 (5H, m, phenyl protons), 7.43 (1H, d, J= 10, C_4H), 7.58 (1H, t, J= 6.5, C_6H).; ^{13}C–NMR (DMSO-d_6): 46.19 (N$\underline{CH_2}$ ph), 111.56 (C_7), 118.17 (C_{3a}), 123.83 (C_5), 124.96 (C_4), 127.36, 127.83, 128.97, 129.13, 134.32, 135.32 (phenyl carbons), 138.48 (C_6), 151.29 (C_{7a}), 158.79 (C_2), 183.57 (C_3). MS ESI: 237.0 (M^+), 238.0 (M^++1).

*1-(4-Chlorobenzyl)indoline-2,3-dione (**3b**).* Mp: 150-151 °C; ^{1}H–NMR ($CDCl_3$) δ: 5.30 (2H, s, N-$\underline{CH_2}$), 6.77 (1H, d, *J* = 8.0 Hz, isatin C_4H), 7.14 (1H, t, *J* = 7.5 Hz, isatin C_5H), 7.3 (2H, d, *J* = 8.5 Hz, ArH), 7.36 (2H, d, *J* = 8.0 Hz, ArH), 7.53 (1H, t, *J* = 7.5 Hz, isatin C_6H), 7.65 (1H, d, *J* = 7.0 Hz, isatin C_7H); MS ESI: 272.0 (M^++1).

*1-(2-Fluorobenzyl)indoline-2,3-dione (**3c**).* Mp: 138-139 °C; ^{1}H–NMR ($CDCl_3$) δ: 5.02 (2H, s, N-$\underline{CH_2}$), 6.93 (1H, d, *J* = 8.0 Hz, isatin C_4H), 7.11-7.15 (3H, m, isatin C_5H and 2 ArH), 7.31-7.34 (1H, m, ArH), 7.39 (1H, t, *J* = 7.0 Hz, ArH), 7.55 (1H, t, *J* = 7.5 Hz, isatin C_6H), 7.64 (1H, d, *J* = 7.5 Hz, isatin C_7H); MS ESI: 255.8 (M^+).

*1-(4-Fluorobenzyl)indoline-2,3-dione (**3d**).* Mp: 121-122 °C; ^{1}H–NMR ($CDCl_3$) δ: 4.93 (2H, s, N-$\underline{CH_2}$), 6.79 (1H, d, *J* = 8.0 Hz, isatin C_4H), 7.07 (2H, m, ArH), 7.14 (1H, t, *J* = 7.5 Hz, isatin C_5H),

7.35 (2H, d, ArH), 7.53 (1H, t, *J* = 7.5 Hz, isatin C_6H), 7.65 (1H, d, *J* = 7.0 Hz, isatin C_7H); ^{13}C–NMR ($CDCl_3$) δ: 43.39 (N$\underline{CH_2}$), 110.81, 115.98, 116.15, 117.72, 124.01, 125.56, 129.24, 129.30, 130.34, 138.32, 150.50, 158.23, 161.53, 163.50, 183.07; MS ESI: 255.8 (M^+).

*1-(3-Nitrobenzyl)indoline-2,3-dione (**3e**).* Mp: 175-177 °C; ^1H–NMR ($CDCl_3$) δ: 5.06 (2H, s, N-$\underline{CH_2}$), 6.79 (1H, d, *J* = 8.0 Hz, isatin C_4H), 7.19 (1H, t, *J* = 7.5 Hz, isatin C_5H), 7.55-7.61 (2H, m, isatin C_6H and ArH), 7.69-7.73 (2H, isatin C_7H and ArH), 8.22-8.25 (2H, m, ArH); MS ESI: 282.8 (M^+).

*1-(4-Nitrobenzyl)indoline-2,3-dione (**3f**).* Mp: 165-166 °C; ^1H–NMR ($CDCl_3$) δ: 5.06 (2H, s, N-$\underline{CH_2}$), 6.74 (1H, d, *J* = 8.0 Hz, isatin C_4H), 7.18 (1H, t, *J* = 7.5 Hz, isatin C_5H), 7.54-7.55 (3H, m, isatin C_6H and ArH), 7.69 (1H, d, *J* = 7.0 Hz, isatin C_7H) 8.26 (2H, d, *J* = 8.0 Hz, ArH); MS ESI: 282.8 (M^+).

*1-(3-Methoxybenzyl)indoline-2,3-dione (**3g**).* Mp: 99-100 °C; ^1H–NMR ($CDCl_3$) δ: 3.80 (3H, s, -O$\underline{CH_3}$), 4.93 (2H, s, N-$\underline{CH_2}$), 6.79-6.94 (3H, m, isatin C_4H and 2 ArH), 7.11 (1H, t, *J* = 7.0 Hz, isatin C_5H), 7.28-7.30 (2H, m, ArH), 7.51 (1H, t, *J* = 7.5 Hz, isatin C_6H), 7.63 (1H, d, *J* = 8.0 Hz, isatin C_7H); MS ESI: 268.0 (M^++1).

2.2.2 General procedure for the synthesis of nalidixic hydrazones 5a-g

To a mixture of the appropriate isatin derivative **3a-g** (1 mmol) and nalidixic acid hydrazide **4** (0.246 g, 1 mmol) in ethanol (25 ml) a few drops of glacial acetic acid were added. The reaction mixture was refluxed for 4-6 h, and then cooled to room temperature. The precipitate was filtered and dried. The crude product was recrystallized from EtOH/DMF to obtain hydrazones **5a-g** in 72-85% yield.

*(Z)-N'-(1-benzyl-2-oxoindolin-3-ylidene)-1-ethyl-7-methyl-4-oxo-1,4-dihydro-1,8-naphthyridine-3-carbohydrazide (**5a**)* [16]. Mp: 292 – 294 °C; ^1H–NMR ($CDCl_3$) δ: 1.58 (3H, t, J= 6.5, $N_1'CH_2\underline{CH_3}$), 2.73 (3H, s, $C_7'\underline{CH_3}$), 4.63 (2H, q, J= 7.5, $N_1'\underline{CH_2}CH_3$), 5.08 (2H, s, $N_1\underline{CH_2}$ph), 6.76 (1H, d, J= 8, C_7H), 7.12 (1H, t, J= 7.5, C_5H), 7.29–7.39 (7H, m, C_4H, C_6H and phenyl protons), 7.93 (1H, d, J= 7.5, $C_6'H$), 8.88 (1H, d, J= 8.5, $C_5'H$), 9.07 (1H, s, $C_2'H$), 15.42 (1H, s, CO$\underline{NH}$); 15.38 ($N_1'CH_2\underline{CH_3}$), 25.19 ($C_7'\underline{CH_3}$), 43.44 ($N_1\underline{CH_2}$ph), 47.07 ($N_1'\underline{CH_2}CH_3$), 110.03 (C_7), 110.94 (C_5'), 120.01 (C_{3a}), 120.37 (C_6'), 121.43 (C_{4a}'), 121.92 (C_5), 122.27 (C_4), 127.39, 127.83 128.93, 132.68, 135.73, 135.84 (phenyl carbons), 131.39 (C_3'), 136.45 (C_3), 137.69 (C_6), 144.03 (C_2'), 148.48 (C_{8a}'), 149.37 (C_{7a}), 163.12 (C_2), 163.72 (C_7'), 164.24 (C_9'), 176.55 (C_4'); MS ESI: 465.8 (M^+),466.9 (M^++1).

*(Z)-N'-(1-(4-Chlorobenzyl)-2-oxoindolin-3-ylidene)-1-ethyl-7-methyl-4-oxo-1,4-dihydro-1,8-naphthyridine-3-carbohydrazide (**5b**).* Mp: 336 – 337 °C; ^1H–NMR ($CDCl_3$) δ: 1.49 (3H, t, *J* = 6.5 Hz, $N_1'CH_2\underline{CH_3}$), 2.64 (3H, s, $C_7'\underline{CH_3}$), 4.54 (2H, q, *J* = 7.0 Hz, $N_1'\underline{CH_2}CH_3$), 4.94 (2H, s, $N_1\underline{CH_2}$), 6.63 (1H, d, *J* = 8.0 Hz, C_4H), 7.04 (1H, t, *J* = 7.5 Hz, C_5H), 7.19-7.26 (7H, m, C_6H, C_7H and 5 ArH), 7.84 (1H, d, *J* = 7.5 Hz, $C_6'H$), 8.78 (1H, d, *J* = 8.0 Hz, $C_5'H$), 8.98 (1H, s, $C_2'H$), 15.32 (1H, s, CO$\underline{NH}$); MS ESI: 500.1 (M^++1).

*(Z)-1-ethyl-N'-(1-(2-fluorobenzyl)-2-oxoindolin-3-ylidene)-7-methyl-4-oxo-1,4-dihydro-1,8-naphthyridine-3-carbohydrazide (**5c**).* Mp: 360 – 361 °C; ^1H–NMR ($CDCl_3$) δ: 1.58 (3H, t, *J* = 7.0 Hz, $N_1'CH_2\underline{CH_3}$), 2.74 (3H, s, $C_7'\underline{CH_3}$), 4.63 (2H, q, *J* = 7.0 Hz, $N_1'\underline{CH_2}CH_3$), 5.12 (2H, s, $N_1\underline{CH_2}$), 6.85 (1H, d, *J* = 8.0 Hz, C_4H), 7.07-7.15 (3H, m, isatin C_5H and 2 ArH), 7.29-7.41 (4H, m, C_6H, C_7H and 2 ArH), 7.93 (1H, d, *J* = 7.0 Hz, $C_6'H$), 8.88 (1H, d, *J* = 8.0 Hz, $C_5'H$), 9.10 (1H, s, $C_2'H$), 15.41 (1H, s, CO$\underline{NH}$); ^{13}C–NMR ($CDCl_3$) δ: 15.34 ($N_1'CH_2\underline{CH_3}$), 25.21 ($C_7'\underline{CH_3}$), 47.17 ($N_1'\underline{CH_2}CH_3$), 63.44 ($N_1\underline{CH_2}$), 109.32, 112.05, 115.36, 115.53, 120.54, 120.62, 121.59, 122.03, 122.41, 122.52, 123.19, 124.66, 124.85, 129.53, 129.59, 129.74, 131.21, 136.89, 137.03, 142.64, 148.66, 148.82, 159.56, 160.37, 161.52, 163.36, 163.47, 176.47; MS ESI: 484.1 (M^++1).

*(Z)-1-ethyl-N'-(1-(4-fluorobenzyl)-2-oxoindolin-3-ylidene)-7-methyl-4-oxo-1,4-dihydro-1,8-naphthyridine-3-carbohydrazide (**5d**).* Mp: 309 – 310 °C; ^1H–NMR ($CDCl_3$) δ: 1.49 (3H, t, *J* = 7.0 Hz, $N_1'CH_2\underline{CH_3}$), 2.64 (3H, s, $C_7'\underline{CH_3}$), 4.55 (2H, q, *J* = 6.5 Hz, $N_1'\underline{CH_2}CH_3$), 4.95 (2H, s, $N_1\underline{CH_2}$), 6.66 (1H, d, *J* = 7.5 Hz, C_4H), 6.92-7.0 (2H, m, ArH), 7.03 (1H, t, *J* = 7.5 Hz, isatin C_5H), 7.19-7.27 (4H, m, C_6H, C_7H and 2 ArH), 7.84 (1H, d, *J* = 7.0 Hz, $C_6'H$), 8.78 (1H, d, *J* = 7.0 Hz, $C_5'H$), 8.98 (1H, s, $C_2'H$), 15.33 (1H, s, CO$\underline{NH}$); MS ESI: 484.0 (M^++1).

*(Z)-1-Ethyl-7-methyl-N'-(1-(3-nitrobenzyl)-2-oxoindolin-3-ylidene)-4-oxo-1,4-dihydro-1,8-naphthyridine-3-carbohydrazide (**5e**).* Mp: 299 – 302 °C; ^{1}H–NMR ($CDCl_3$) δ: 1.49 (3H, t, *J* = 7.0 Hz, $N_{1'}CH_2\underline{CH_3}$), 2.64 (3H, s, $C_{7'}\underline{CH_3}$), 4.55 (2H, q, *J* = 6.5 Hz, $N_{1'}\underline{CH_2}CH_3$), 5.08 (2H, s, $N_1\underline{CH_2}$), 6.66 (1H, d, *J* = 7.5 Hz, C_4H), 7.07 (1H, t, *J* = 7.5 Hz, C_5H), 7.44 (1H, t, *J* = 7.5 Hz, C_6H), 7.62 (1H, t, *J* = 7.0 Hz, C_7H), 7.88 (1H, d, *J* = 7.0 Hz, $C_{6'}H$), 8.08 (1H, d, *J* = 7.0 Hz, ArH), 8.16 (1H, s, ArH), 8.77 (1H, d, *J* = 8.0 Hz, $C_{5'}H$), 8.99 (1H, s, $C_{2'}H$), 15.33 (1H, s, CO<u>NH</u>); MS ESI: 511.0 (M^++1).

(Z)-1-Ethyl-7-methyl-N'-(1-(4-nitrobenzyl)-2-oxoindolin-3-ylidene)-4-oxo-1,4-dihydro-1,8-naphthyridine-3-carbohydrazide (5f). Mp: 313 – 315 °C; ^{1}H–NMR ($CDCl_3$) δ: 1.59 (3H, t, *J* = 7.0 Hz, $N_{1'}CH_2\underline{CH_3}$), 2.73 (3H, s, $C_{7'}\underline{CH_3}$), 4.64 (2H, q, *J* = 6.5 Hz, $N_{1'}\underline{CH_2}CH_3$), 5.14 (2H, s, $N_1\underline{CH_2}$), 6.70 (1H, d, *J* = 7.5 Hz, C_4H), 7.17-7.36 (3H, m, C_5H, C_6H and C_7H), 7.54 (2H, d, *J* = 8.0 Hz, ArH), 7.97 (1H, d, *J* = 6.5 Hz, $C_{6'}H$), 8.22 (2H, d, *J* = 8.5 Hz, ArH), 8.86 (1H, d, *J* = 8.0 Hz, $C_{5'}H$), 9.08 (1H, s, $C_{2'}H$), 15.42 (1H, s, CO<u>NH</u>); MS ESI: 511.0 (M^++1).

(Z)-1-Ethyl-N'-(1-(3-methoxybenzyl)-2-oxoindolin-3-ylidene)-7-methyl-4-oxo-1,4-dihydro-1,8-naphthyridine-3-carbohydrazide (5g). Mp: 288 – 290 °C; ^{1}H–NMR ($CDCl_3$) δ: 1.49 (3H, t, *J* = 7.0 Hz, $N_{1'}CH_2\underline{CH_3}$), 2.63 (3H, s, $C_{7'}\underline{CH_3}$), 3.70 (3H, s, -O$\underline{CH_3}$), 4.54 (2H, q, *J* = 6.5 Hz, $N_{1'}\underline{CH_2}CH_3$), 4.95 (2H, s, $N_1\underline{CH_2}$), 6.66-7.26 (8H, m, isatin H and ArH), 7.84 (1H, d, *J* = 8.0 Hz, $C_{6'}H$), 8.78 (1H, d, *J* = 9.0 Hz, $C_{5'}H$), 8.98 (1H, s, $C_{2'}H$), 15.33 (1H, s, CO<u>NH</u>); MS ESI: 495.9 (M^++1).

2.3. Evaluation of antimycobacterial activity of the synthesized hydrazones

Antimycobacterial activity was performed at the research center, College of Pharmacy, King Saud University, Saudi Arabia. The tested Mycobacterium tuberculosis strains are *M. intercellulari* (ATCC 35743), *M.xenopi* (ATCC 14470), *M. cheleneoi* (ATCC 35751) *and M. smegmatis* (ATCC 35797) using Rist and Grosset proportion method (agar dilution method) [16].

The Schiff bases **5a-g** and INH were dissolved in DMSO at a concentration of 1 mg/ml. The appropriate aliquot of each solution was diluted with 10% molten agar to give concentrations of 100 μg/ml. The agar and the compound solution were mixed thoroughly and the mixture was poured into Petri-dishes on a level surface to result in an agar depth of 3 to 4 mm and allowed to harden. The incula were prepared by growing overnight culture in Muller-Hinton broth. The cultures were diluted 1:100. Tested organisms were streaked in a radial pattern and plates were incubated at 35 ˚C for 48 hr to check the growth of the tested strains at this single concentration. Active compounds were further diluted and tested by the same way to determine the minimum inhibitory concentration (MIC) of these compounds. Experiment using the tested strains in a medium free of investigated compounds was also carried out and the results are given in Table 1.

2.4. Calculation of log P values

The log P values of the synthesized compounds were computed with a routine method called calculated log P (C log P) contained in a PC-software package (McLogP 2.0, BioByte Corp., CA, USA). A representation of the molecular structure where hydrogens are omitted or, suppressed (SMILES notation), is entered into the program, which computes the log P based on the fragment method developed by Leo [22].

2.5. Molecular modeling

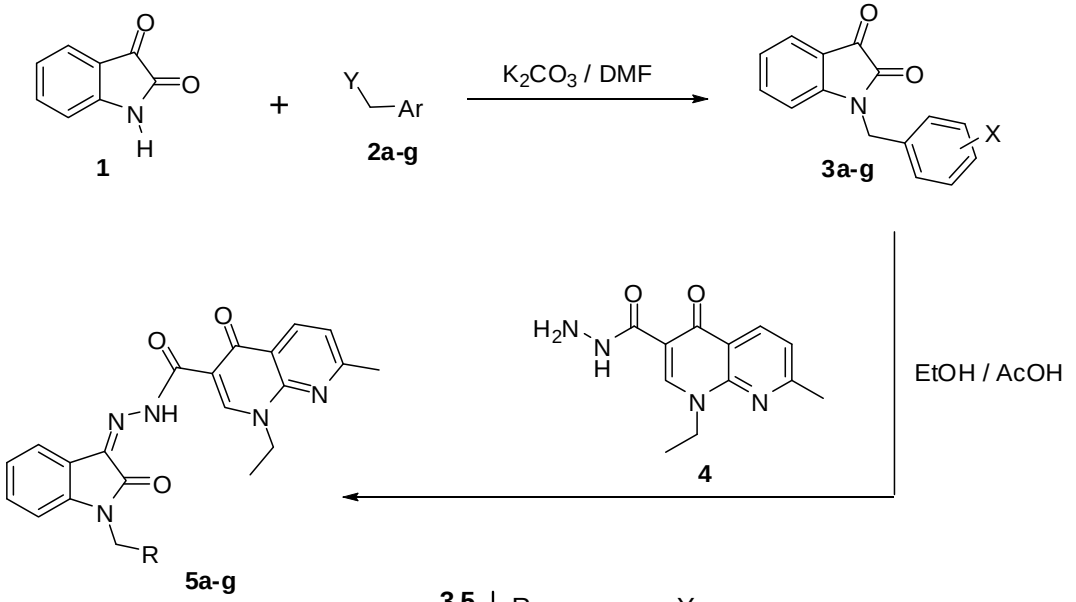
The sequence of *M. tuberculosis* DNA gyrase subunit B was (access number P0C5C5) was aligned with the 1kijB.pdb sequence structure using BLAST followed by HH Search and subsequently building the building model based on 1kijB (16-427) was successful. During the modeling process, the position of crystallized novobiocin and a water molecule, which was believed to be responsible for key interactions, were kept in their original positions for rebuilding the binding cavity in the specific and original size. The parameters for model selection include, minimal number of uncovered target residues after BLAST to run HHSEARCH of 50, minimal number of uncovered target residues to model an additional template of 25 and automated mode of SMR-pipeline. The homology modeling-obtained model of mtb-DNA gyrase subunit B was used

for the preparation of the input receptor files within Dock6.4 program. The newly synthesized active compounds were subjected for flexible docking inside the binding site of the model using default dock6 parameters using Grid score including van der Waals and electrostatic target-ligend interaction and internal energy of the ligend-binding conformation.

3. Results and discussion

3.1. Chemistry

The designed Schiff's bases were obtained through 2 steps reaction as described in scheme 1. The first step involved reaction of isatin **1** with substituted benzyl chloride or bromide **2a-g** in DMF using 1.5 equivalent of K_2CO_3 at 80°C for 12 h to afford compounds **3a-g** in 60-75% yield [16], scheme 1. The ^{1}H NMR spectra of these *N*-substitutedbenzylisatins **3a-g** were consistent with their assumed structures and characterized by the disappearance of isatin NH and the appearance benzyl–CH_2- protons around δ 4.93-5.3 in addition to the aromatic protons of the phenyl moiety.



3,5	R	Y
a	Ph	Cl
b	4-Cl-C_6H_4	Cl
c	2-F-C_6H_4	Br
d	4-F-C_6H_4	Br
e	3-NO_2-C_6H_4	Cl
f	4-NO_2-C_6H_4	Br
g	3-MeO-C_6H_4	Br

Scheme 1: Synthesis of N-substitutedbenzylisatins 3a-g and nalidixic acid hydrazones 5a-g

The second step is the condensation of the 1-substituted isatins **3a-g** with nalidixic acid carbohydrazide **4**. The later prepared according to a reported procedures [10]. The prepared hydrazones **5a-g** were obtained in 72-85% yield and their structures were confirmed on the bases of spectral methods of analyses (NMR and Mass spectrometry). All spectral data are in accordance with the assumed structures. The ^{1}H NMR spectra of hydrazones revealed the appearance of hydrazone NH signal around δ 15.32-15.42 beside to the characteristics signals of nalidixic acid. The mass spectra of compounds **3a-g** and **5a-g** showed the mass peaks of $[M^+]$ and $[M+1]^+$.

3.2. Antimycobacterial activity

The synthesized hydrazones **5a-g** were evaluated for their anti-*mtb* activity *in vitro* against four Mycobacterium strains: *M. intercellulari* (ATCC 35743), *M. xenopi* (ATCC 14470), *M. cheleneo* (ATCC 35751) and *M. smegmatis* (ATCC 35797) according to the protocol described in the experimental section using Rist and Grosset proportion method (agar dilution method) [23]. Isoniazid (INH) was used as a reference drug and control experiments were done using a growth media free from drugs or the tested compounds. Results of the *in vitro* anti-*mtb* activity of the tested compounds along with the standard drug for comparison are given in Table 1.

Table 1: Lipophilicity and antimycobacterial activity of the synthesized compounds.

Compound No.	*Clog P*	MIC (μg/ml)			
		M. intercellulari	***M. xenopi***	***M. cheleneoi***	***M. smegmatis***
5a	2.77	0.625	0.625	0.625	0.625
5b	3.49	0.09	0.09	0.09	0.09
5c	2.92	>100	>100	>100	>100
5d	2.92	0.09	0.09	0.09	0.09
5e	2.52	>100	>100	>100	>100
5f	2.52	0.09	0.09	0.09	0.09
5g	2.69	>100	>100	>100	>100
INH	-0.67	12.5	12.5	12.5	12.5

The data of the anti-*mtb* screening of compounds **5c**, **5e** and **5g** revealed no activity on the tested strains up to concentration of 100μg/ml.

The active compounds, **5b**, **5d** and **5f** were found to be almost 139 times more potent than the first line antitubercular drug INH and 7 folds more potent than the lead compound **5a** under the investigation conditions. Interestingly, the *para*-substituents, with electron withdrawing groups on the benzyl moiety, are with positive Hammett constant.

3.3. Relation between lipophilicity and antimycobacterial activity

The lipophilicity is a well-known physico-chemical factor affecting biological activities, characterizing the distribution process of compound in the human organism and being a key factor of both pharmacokinetic and pharmacodynamic properties of drug molecules (plasma protein binding, blood–brain barrier (BBB) penetration, and penetration through cell membranes) [24-26]. Correlation between lipophilicity and anti-TB activity was also reported as lipophilicity of drug molecules may render them more capable of penetrating various biomembranes consequently improving their permeation properties toward microbial cell membrane [27-29]. Lipophilicity of the synthesized compounds expressed in the term of their Clog P values, is shown in table 1. Computation of the log P was based on the fragment method developed by Leo contained in a PC-software package [22].

It was found that there is no evident relation between the anti-TB activity of the tested compounds and their lipophilicity. Clearly the lipophilicity has an influence on the activity, but it does not solely determine the anti-mtb activity of these compounds. However, compared with INH there is a significant enhancement in the lipophilicity.

3.4. Molecular modeling study

In order to investigate the possible interactions between our newly synthesized compounds and the active site of the *Mycobacterium* DNA gyrase B subunit, homology modeling and a docking process were undertaken. For homology modeling of the DNA gyrase subunit B of *M. tuberculosis*, the crystal structure of the gyrase B 43 K ATPase domain complex with the potent inhibitor novobiocin (1KIJ.pdb) [21], was selected as the template. Its “open” conformation of the active site in the absence of ATP is unique, as it clearly demonstrates large conformational changes during inhibition processes. This particular template has been selected based not only on

BLAST-p alignment but also on the structural similarity between our synthesised compounds and co-crystallized novobiocin.

During homology modeling, after the heavy atoms were modeled and all hydrogen atoms were added, the protein coordinates were minimized using the AMBER94 force field [30]. The pair-wise percentage residue identity was determined as 42.548 between 2 chains, where the pair-wise RMSD values for Cα atoms of the superimposed model and template was 0.586 ˚A. In brief, the structure constitute a compact single domain with an 8-stranded beta sheet and 6-alpha-helices and random coils, Figure 1. The RMSD value difference of 0.67˚A of the pose from the non-restricted redocking of the novobiocin structure itself also confirmed the approach, Figure 2.

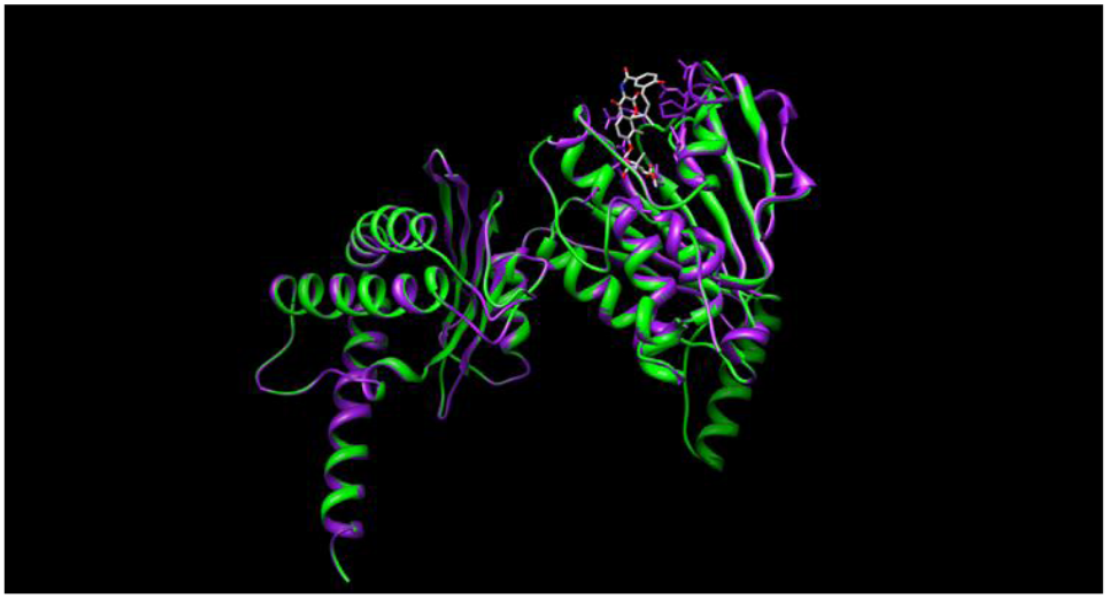


Fig. 1: The backbone structure of M. tuberculosis DNA gyrase subunit B (P0C5C5) (colored green) is overlayed onto that of 1kijB.pdb (colored magenta) showing bound ligand, novobiocin, (colored grey).

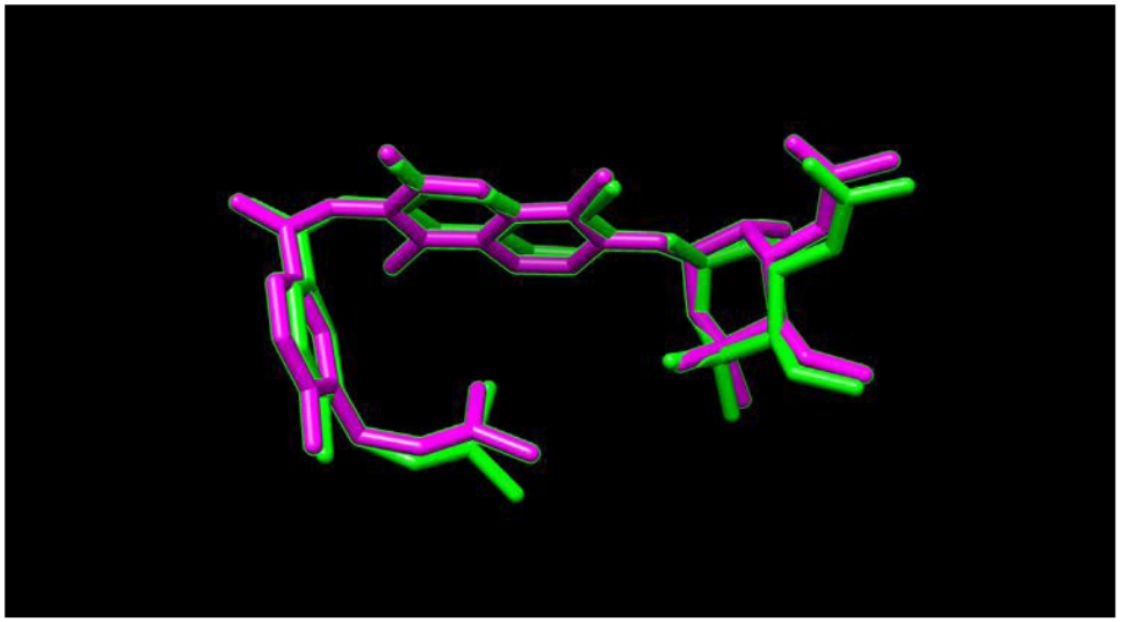


Fig. 2: Superimposition of the co-crystallized Novobiocin (from 1kijB.pdb, colored magenta) and the re-docked Novobiocin structure (colored magenta).

The binding site includes hydrophobic pocket delineated by the side chains of Ala 59, Val 98, Val 99, Phe 109, Val 125, Val 123, Ile 84, and Asn 52 and the orifice of the binding bocket is enriched with hydrogen bond donor groups represented by Arg 82, Arg 141 and Lys 108. The docking poses belonging to our newly synthesised compounds with *para*-substituted benzyl on istatin nitrogen atom (anti-mtb active compounds), Figure 3a,b, suggested that hydrogen bonding between the carbonyl oxygen of the hydrazide or that of isatin and the amino group of Arg 141 or Lys 108, respectively, stabilized the seventh-position methyl group over the 1,8-naphthyridine ring system with hydrophobic interactions with Val 123 and Ala 53 whereas *N*-ethyl, carbons were stabilized between Phe 109 and Ile 84 with the same kind of interactions. The substituted 1,8-naphthyridine structures were generally oriented in the hydrophobic pocket surrounded by the side chains of Val 99, Val 125, Val 123, Ile 84, and Asn 52. The *para*-substituted benzyl moiety is positioned in parallel orientation between its pi system and Arg 141 with hydrophobic interaction between its group in the *para*-position and Ala 87 side chain. If there is a hydrogen accepting group in the 5- or 6-position of isatin then there is a chance of a hydrogen bond existing with that group and the Lys 108 side chain amino group.

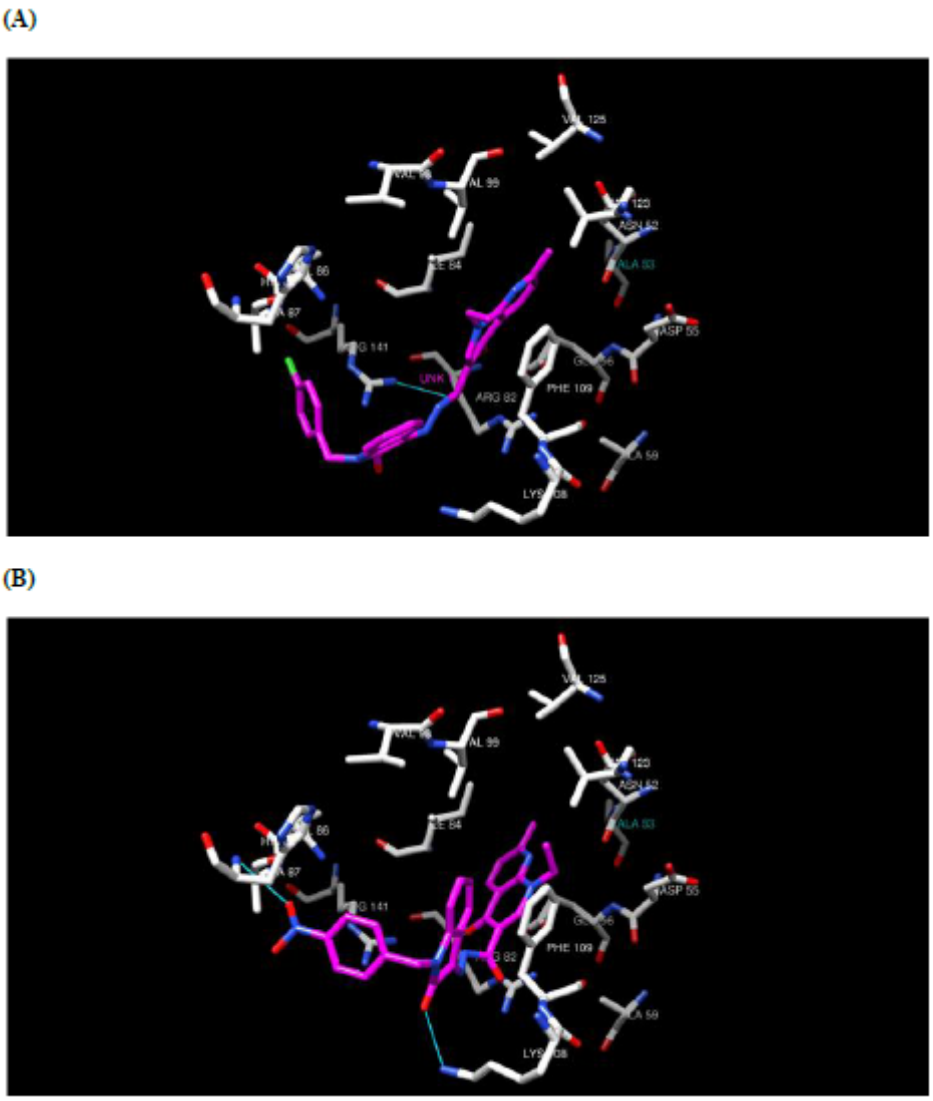


Fig. 3: M. tuberculosis DNA gyrase subunit B (P0C5C5) homology modelled: the docked compound 5b (A), 5f (B). Hydrogen bond is displayed in cyan.

The docking poses belonging to our newly synthesized compounds with *ortho*- or *meta*-substituted benzyl moiety on istatin nitrogen atom (anti-*mtb* inactive compounds **5c, 5e** and **5g**) failed to be oriented inside the proposed binding pocket.

Although the generated docking poses illustrated a parallelism between MIC values against *M. tuberculosis* and compound interactions with the surrounding environment regarding side chain changes in general, further molecular modeling studies for this computationally estimated crystal structure of the complex, under physiological conditions, would be the best option for verification.

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