Antimycobacterial Activity, In Silico ADME Evaluation, and Docking Study of Novel Thiazolidinedione and Imidazolidinone Conjugates

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Abstract—A series of new thiazolidine-2,4-dione and hydantoin derivatives were synthesized by Knoevenagel condensation. The compounds were identified by their melting points, \textsuperscript{1}H NMR, \textsuperscript{13}C NMR, and HR-MS spectral data. The in vitro antimycobacterial activity was evaluated against reference strain \textit{Mycobacterium tuberculosis} H37Rv and compared to that of the first-line antituberculosis drugs isoniazid (INH) and ethambutol (EMB). The new compounds showed promising antimycobacterial activity (MIC ranging from 0.75 to 1.54 μM) and low cytotoxicity in the human embryonic kidney cell line HEK-293T (IC\textsubscript{50} > 200 μM). The most potent compounds (\textit{IIIa}) and (\textit{V}) could be promising drug candidates for further development. In silico ADME screening revealed the biological potential of all synthesized compounds using SwissADME online biological activity prediction software. Molecular docking studies were carried out as well to confirm the groove mode of binding and receptor-complex interactions.

Keywords: thiazolidine-2,4-dione, hydantoin derivatives, antimycobacterial agents, ADME, molecular docking

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INTRODUCTION

Despite the availability of effective treatment, prevention and control, the widespread transmission of resistant variants of \textit{Mycobacterium tuberculosis}, which does not respond to any commercial drugs, remains a serious health problem. Consequently, the multidrug-resistant form (MDR-TB) which does not respond to treatment both with rifampicin and with isoniazid—the two most effective anti-tuberculosis drugs—requires urgent need of new anti-mycobacterial agents and makes their chemical, microbiological, molecular and pharmacological investigation more and more urgent. Moreover, considering the high incidence of AIDS in some geographical regions such as Africa, Asia and some East European countries, MDR-TB is one of the main priorities of the World Health Organization. Although the approval of new drugs such as bedaquiline and delamanid [1] for treatment of drug-resistant TB has brought some hope, the adaptive capacity of Mtb has already led to the emergence of resistant strains for these drugs, evidencing the continuing necessity of new options [2]. Thus, the constantly expanding and growing knowledge of the pathogenesis of tuberculosis, as well as the mechanisms of emergence of drug resistance in its various forms, require a constant search for new therapeutic approaches. However, the final victory over this socially significant disease seems to be still a long way off, which means that in the coming years, the development of new potential candidates for tuberculosis treatment will continue to be up-to-date.

Imidazolidinones (hydantoins) [3] and 2,4-thiazolidinediones [4–6] derivatives have been thoroughly researched in various areas and have proven to possess promising activities [7, 8]. Hydantoin-containing compounds exhibit a broad spectrum of pharmacological and biological activities [4] such as antitubercular [9–11], anti-inflammatory [12, 13], antidiabetic [14, 15], antimicrobial [16–18], anticonvulsant [19, 20], as well as antimycobacterial [8] ones. Clinically approved drugs such as phenytoin, mephenytoin, fosphenytoin, nitrofurantoin, dantrium, nilutamide and enzalutamide are representative pharmacological compounds. Major achievements in the field of thiazolidinedione derivatives are related to 2,4-thiazolidinedione, rhodanine (2-thioxo-4-thiazolidinone), 2-alkyl(aryl)-substituted,
and 2-\textit{R}-amino(imo)-substituted 4-thiazolidinone subtypes as sources of antimicrobial [21–24], antidiabetic [23, 25–28], anti-inflammatory [29] and anticancer lead compounds and drug candidates [23, 30–37]. Finding numerous antimycobacterial agents with 5-arylidenic aromatic hydantoins and 2,4-thiazolidinedione systems [8, 24, 38–46] we purpose to design new molecules containing 1,3-thiazolidine-2,4-dione and 2,4-imidazolidinodione scaffolds and an indole fragment. The evaluation of the ADME properties (absorption, distribution, metabolism and excretion) which are crucial for the clinical success of the molecules could help in optimizing chemical compounds with a certain pharmacological or biological activity.

In continuation of our efforts working on coumarin [47, 48] and indole [49] based hydrazide–hydrazones as well as 2-aryl-[1]benzopyrano[4,3-c]pyrazol-4(1\textit{H})-ones [50], in this study we intend to design potent and non-toxic 2,4-imidazolidinodione and 2,4-thiazolidinedione derivatives with indole fragment and to compare them with chromone containing 2,4-imidazolidinodione and 2,4-thiazolidinedione derivatives, as well as to the first-line antituberculosis drugs – isoniazid (INH) and ethambutol (EMB). Thus, we synthesized a series of new derivatives having antimycobacterial activity by Knoevenagel condensation of the substituted indol-3-carbaldehyde with 2,4-imidazolidinodione and 2,4-thiazolidinedione rings. The in silico ADME screening, pharmacokinetics and drug-likeness profiles of the synthesized compounds were determined and discussed based on the detailed computational data. Molecular docking studies were also carried out to confirm the groove mode of binding and receptor-complex interactions.

RESULTS AND DISCUSSION

Chemistry

In this work, we replace chromone scaffold with an indole heterocycle to test the possibility of increasing the antimycobacterial activity of hydantoin or imidazolidine-2,4-dione derivatives activity by replacing 5-arylidenic moiety. The synthetic route used for the preparation of the title compounds is presented in Scheme 1. The Knoevenagel condensation of the active methylene at the fifth position of 2,4-thiazolidinedione (IIa) or hydantoin (IIb) with substituted indole-3-carbaldehydes (Ia, b) is an efficient way to obtain 5-arylidenyl 2,4-thiazolidinedione or hydantoin derivatives (IIla–d). The reactions were carried out in abs. ethanol under base conditions in the presence of a catalytic amount of piperidine at moderate temperature. The compounds’ structure was elucidated based on FTIR, \textit{1H} NMR, \textit{13C} NMR, and HRMS data. The IR spectra of all the compounds showed 1500–1578 cm\textsuperscript{−1} (NH str.), 1735–1770 cm\textsuperscript{−1} (C=O str.), 1600–1475 cm\textsuperscript{−1} (C=C str.), 1144 and 690 (C=S of thiazolidinodione ring), which confirmed the formation of thiazolidinodione derivatives. The \textit{1H} NMR spectra showed protons of CH=N group of all the newly synthesized compounds as singlets at 8.11–8.19 ppm. The protons of NH group of the thiazolidine-2,4-dione and hydantoin ring appeared in the 8.20–9.93 ppm region for compounds (IIla, b) and in the 12.09–12.19 ppm region for compounds (IIlc, d) as singlets. The \textit{13C} NMR spectra were consistent with the presence of the two C=O signals at 155.2–168.9 ppm and 169.4–185.4 ppm and with the presence of the C=O vinylic signal at 132.4–135.4. The synthesis of 2-methyl-2\textit{H}-chromene-3-carbaldehyde (IV) and 2-phenyl-2\textit{H}-chromene-3-carbaldehyde (VI) was carried out through the domino oxime-Michael/aldol condensation reactions. The preparation of 2-methyl-2\textit{H}-chromene-3-carbaldehyde (IV) was conducted in 1,4-dioxane under reflux [20]. For synthesis 2-phenyl-2\textit{H}-chromene-3-carbaldehyde (VI) was used pyrrolidine as a catalyst according to the literature [23]. The compound 5-[(2-methyl-2\textit{H}-chromen-3-yl)methylidene]-1,3-thiazolidine-2,4-dione (V) was synthesized by Knoevenagel condensation of 2-methyl-2\textit{H}-chromene-3-carbaldehyde (IV) and 1,3-thiazolidine-2,4-dione (IIa) (Scheme 1). Several conditions were tested (sodium acetate in acetic acid, sodium acetate in DMF and piperidine in ethanol/methanol under thermal conditions) for the preparation of 2-phenylchromene—derivative (VII), but the best result was obtained by refluxing ethanol and in the presence of a catalytic amount of piperidine. As outlined in Scheme 1, when 2-phenyl-2\textit{H}-chromene-3-carbaldehyde (VI) reacted with hydantoin (IIb) under the selected conditions, dehydration did not occur and the final product is intermediate alcohol 5-[hydroxy(2-phenyl-2\textit{H}-chromen-3-yl)methyl]imidazolidine-2,4-dione (VII). Our efforts to obtain a Knoevenagel product (VII) after the condensation reaction under the conditions described above failed. The detailed results of \textit{1H} NMR and \textit{13C} NMR spectral studies and MS are presented in the experimental part.

Antimycobacterial Activity Assessment

The antimycobacterial activities of compounds (IIla–d) were assessed against \textit{M. tuberculosis} H37Rv using the Resazurin microtiter assay (REMA) [51–53] and compared to that of the first-line anti-tuberculosis drugs – isoniazid and ethambutol, (Table 1). The newly synthesized compounds demonstrated good activity at minimum inhibitory concentrations (MIC) against a referent strain \textit{M. tuberculosis} H37Rv ranging from 0.75 to 1.54 \textmu M. All of the compounds from the series were more active than the first-line anti-tuberculosis drugs isoniazid and ethambutol, reinforcing the pharmacophoric contribution of imidazolidinodione moiety to the mechanism of action against the \textit{M. tuberculosis}.

The cytotoxicity of selected promising agents was evaluated in the human embryonal kidney cell line
HEK-293 cells (Table 1). The new indole containing imidazolidinones and hydantoin derivatives showed low cytotoxicity. Amongst the tested compounds (IIIa) was found to be the most potent molecule with a good selectivity index (SI) value of 266.48. The two hydantoin derivatives (IIIa) and (IIIb), are more active than thiazolidinedione analogs and were nearly equipotent but compound (V) and (VII) comprising a chromene ring remain the most active with MIC of 0.36 and were 0.297 μM and 6 times more potent than the standard drugs isoniazid and ethambutol, respectively. It should be emphasized that even for analogs with increased lipophilicity, no appreciable cytotoxicity was found throughout the series of modifications, indicating a promising safety profile of the investigated series.

Relying on the obtained results, it may be assumed that the modifications introduced to the imidazolidine-2,4-dione derivatives may result in the synthesis of new compounds with a broad biological activity spectrum.

**ADME Screening Results**

The synthesized compounds were subjected to in silico ADME screening. The specific molecular and physicochemical properties were analysed, compared to the available drugs used for treating TB (Table 2), namely: molecular weight (MW), topological polar surface area (TPSA), hydrogen bond acceptors (HBA), hydrogen bond donors (HBD), Moriguchi’s logP (MlogP) and water solubility.

All of the synthesized compounds (IIIa–d, and VII) have molecular weight from 306 to 350 g/mol. For MW, the qualifying range is between 160 and 480. The higher MW is associated with poor bioavailability, poor fraction absorption and higher bond fraction [54]. TPSA is used to predict the absorption of the compounds through the cell membrane. Too low TPSA (<75 Å²) may lead to an increase in the toxic risk, particularly if lipophilicity is high (log P > 4). On the other hand, a high TPSA (>140 Å²) means that the molecules cannot pass through the membrane easily, which is significant for compounds designed for oral use. In our assessment, the TPSA values vary within small boundaries between 73 and 96 Å². It indicates that the synthesized compounds are moderately soluble and can be absorbed by the cell membrane.

The value of Moriguchi’s log P is used to evaluate the lipophilicity of compounds; a higher log P value indicates low level of lipophilicity [55]. All synthesized compounds revealed low MlogP values corresponding to potential ability to pass the biological membranes. The chemical properties from Table 2 can be used to determine the compounds drug-likeness. The results suggest that all of the synthesized compounds have a high theoretical oral bioavailability according to the Lipinski’s “Rule of Five” (MW < 500, HBD < 5, HBA < 10, MlogP < 4.15).
Table 1. Antimycobacterial activity, *in vitro* cytotoxicity and selectivity index of 5-arylidenyl 2,4-thiazolidinedione and hydantoin derivatives

| Compd. | Formula | MIC, μM<sup>1</sup> | IC<sub>50</sub>, μM | SI<sup>4</sup> | logP<sup>5</sup> |
|--------|---------|---------------------|-------------------|--------|---------------|
| (IIIa) | ![Chemical Structure](attachment://IIIa.png) | 0.7505 | >200 | 266.48 | 3.217 |
| (IIIb) | ![Chemical Structure](attachment://IIIb.png) | 0.8167 | >200 | 244.88 | 2.271 |
| (IIIc) | ![Chemical Structure](attachment://IIIc.png) | 1.4281 | >200 | 140.04 | 4.495 |
| (IIIId) | ![Chemical Structure](attachment://IIIId.png) | 1.5475 | >200 | 129.24 | 3.5490 |
| (V) | ![Chemical Structure](attachment://V.png) | 0.360 | NT | NT | 3.139 |
| (VII) | ![Chemical Structure](attachment://VII.png) | 0.297 | NT | NT | 2.095 |
| EMB<sup>2</sup> | ![Chemical Structure](attachment://EMB.png) | 2.0024 | – | – | 2.46 |
| INH<sup>3</sup> | ![Chemical Structure](attachment://INH.png) | 1.8234 | – | – | –0.8 |

<sup>1</sup>MIC (in μM) was defined as the lowest concentration resulting into a complete inhibition of the bacterial growth and reproduction;  
<sup>2</sup>EMB-2HCl (ethambutol dihydrochloride) – reference compound and  
<sup>3</sup>Isoniazid—reference compound;  
<sup>4</sup>SI ratio = IC<sub>50</sub>/MIC;  
<sup>5</sup>logP was calculated using Molecular Operating Environment of Chemical Computing Group (MOE, version 2016.08, https://www.chemcomp.com/MOE-Molecular_Operating_Environment.htm).
Additional pharmacokinetic properties including gastrointestinal absorption (GI absorption), P-glycoprotein (PGP) and Blood brain barrier permeability were calculated. The compounds were checked for inhibition of isoforms of cytochrome P450 (CYP) family—CYP1A2, CYP2D6, CYP2C19 and CYP3A4. The Lipinski, Ghose and Veber rules were applied to assess drug-likeness to predict whether a compound was likely to be a bioactive according to some important parameters such as MW, MlogP, number of HPA and HBD. The results are presented in Table 3.

According to the pharmacokinetic properties, all compounds show high gastrointestinal absorption, have no BBB permeability and only (IIIa) and (VII) are predicted as P-gp substrates, hence most of the synthesized compounds could spread through the human body to suppress the deployment of *M. tuberculosis*. The Lipinski, Ghose and Veber rules were applied to assess drug-likeness to predict whether a compound was likely to be a bioactive according to some important parameters such as MW, MlogP, number of HPA and HBD. The results are presented in Table 3.

The molecular screening has revealed that most of the compounds have characteristics of CYP1A2 inhibitors except compound (VII) but only (IIIa) shows inhibition to CYP2D6. Similar results are observed towards the CYP2C19 and CYP3A4 inhibition—only a few of the compounds (IIIb), (V), (VII) are predicted as non-inhibitors. The drug-likeness has been predicted on the basis of the selected Lipinski, Ghose and Veber rules and bioavailability score (Table 3). The Lipinski’s “Rule of Five” states that the absorption or permeation of a molecule is more likely when the molecular weight is under 500 g/mol, the value of logP is lower than 5, and the molecule has 5 H-donor and 10 H-acceptor atoms at most. Ghose filter [57] defines drug-likeness constraints as follows: calculated logP is between –0.4 and 5.6, MW is between 160 and 480, molar refractivity is between 40 and 130, and the total number of atoms is between 20 and 70. Veber [58] defines drug-likeness constraints as Rotatable bond count ≤10 and polar surface area (PSA ≤ 140). The bioavailability score was implemented without changes [51]. The screening process has shown that all compounds pass the criteria of drug-likeness assessment (Table 3).

### Table 2. Chemical properties of the compounds

| Compd. | MW1, g/mol | TPSA2, Å2 | HBA3 | HBD4 | Rotable bounds | Moriguchi’s logP | Water solubility |
|--------|------------|-----------|------|------|----------------|-----------------|-----------------|
| (IIIa) | 333.34 | 83.22 | 3 | 3 | 4 | 1.24 | Moderately soluble |
| (IIIb) | 306.11 | 73.99 | 2 | 3 | 1 | 1.33 | Soluble |
| (IIIc) | 350.39 | 96.49 | 3 | 2 | 4 | 2.05 | Moderately soluble |
| (IIId) | 323.17 | 87.26 | 2 | 2 | 1 | 1.76 | Moderately soluble |
| (V)    | 273.31 | 80.70 | 3 | 1 | 1 | 1.70 | Soluble |
| (VII)  | 336.34 | 87.66 | 4 | 3 | 3 | 1.53 | Moderately soluble |
| INH    | 137.14 | 68.01 | 3 | 2 | 2 | –0.47 | Very soluble |
| EMB    | 204.31 | 64.52 | 4 | 4 | 9 | 0.18 | Very soluble |

1Molecular weight; 2topological polar surface area (TPSA); 3hydrogen bond acceptors; 4hydrogen bond donors.

### Table 3. Pharmacokinetics and drug-likeness prediction

| Compd. | GI absorption | BBB perm. | P-gp | CYP1A2 Inhib. | CYP2D6 Inhib. | CYP2C19 Inhib. | CYP3A4 Inhib. | log Kp, cm/s | Lipinski | Ghose | Veber | bio. Score |
|--------|---------------|----------|------|----------------|---------------|----------------|---------------|-------------|----------|-------|-------|------------|
| (IIIa) | High          | No       | Yes  | Yes            | Yes           | Yes            | Yes           | –6.44       | Yes      | Yes   | Yes   | 0.55      |
| (IIIb) | High          | No       | No   | No             | No            | No             | No            | –6.82       | Yes      | Yes   | Yes   | 0.55      |
| (IIIc) | High          | No       | Yes  | No             | Yes           | Yes            | No            | –5.75       | Yes      | Yes   | Yes   | 0.55      |
| (IIId) | High          | No       | No   | No             | Yes           | Yes            | No            | –6.13       | Yes      | Yes   | Yes   | 0.55      |
| (V)    | High          | No       | Yes  | No             | No            | No             | No            | –6.21       | Yes      | Yes   | Yes   | 0.55      |
| (VII)  | High          | No       | No   | No             | No            | No             | No            | –6.96       | Yes      | Yes   | Yes   | 0.55      |
| INH    | High          | No       | No   | No             | No            | No             | No            | –7.63       | Yes      | No: 3 viol. | Yes   | 0.55 |
| EMB    | High          | No       | No   | No             | No            | No             | No            | –7.60       | Yes      | Yes   | Yes   | 0.55      |

Molecular Docking Elucidation

Mycobacteria have a unique cell wall consisting of mycolic acids, very long-chain lipids that provide pro-
One of the most active compound (IIIa) repeats one of the interactions of the native ligand TLM as Fig. 2 shows; namely with His311, while demonstrating three newly appeared interactions—with Asp273, Glu322 and Thr313. Compound (V) demonstrates one newly appeared interaction, while two of the residues involved in interactions with TLM—Cys171 and His311—are too close, but still in a non-binding distance.

This study shows that the tested compounds probably inhibit mycolic acid biosynthesis by targeting KasA, an essential member of the FAS–II complex in M. tuberculosis. The compounds possessed in vitro activity comparable to that of INH, and presumably, it targeted the same cyclic pathway producing long-chain mycolic acids. We can conclude that the new indole and coumarin based imidazolidine-2,4-diones and thiazolidinediones are efficient antimycobacterial agents and that the information about their structure may be instrumental for the conventional rational drug design, as well as for the most recently established method—fragment-based drug discovery.

EXPERIMENTAL

Synthesis

The melting points were determined using a Buchi 535 apparatus. The IR spectra were recorded on a Nicolet iS10 FT-IR spectrometer from Thermo Scientific (USA) using an ATR technique. The NMR experiments were carried out on a Bruker Avance II+ 600 MHz NMR spectrometer in DMSO-d6. The IR spectra were recorded using an electrospray ionization module Ion Max® (ThermoFisher, Germany) operating in positive mode. All reagents were purchased from E. Merck (Darmstadt, Germany) and Aldrich (Milwaukee, MI, USA).

General Synthetic Procedure for Compounds (IIIA–d)

5-Benzyloxyindol-3-carbaldehyde (Ia) (1 mmol) or 5-bromoindol-3-carbaldehyde (Ib) (1 mmol) was dissolved in 10 mL of absolute ethanol. Anhydrous piperidine (100 mg), (1 mmol) and 1,3-imidazolidine-2,4-dione (IIa) or 1,3-thiazolidine-2,4-dione (IIb) were added to the solution. The mixture was heated at 70°C and stirred for 24 hours. After that the reaction mixture was cooled, the resulting crystals filtered off and dried.

Synthesis of (5Z)-5-(5-benzyloxy-3-indolymethylene)-1,3-imidazolidine-2,4-dione (IIIA) C19H15N3O3 (333.3407) Yield 172 mg (52%), mp 186–187°C, pale...
yellow crystals. FTIR $\nu_{\text{max}}$ 3414 (NH-indol); 3230 (NH-hyd); 1775, 1685 (2 C=O); 1620 (C=C); 1595, 1500, 1460 (benzene) cm$^{-1}$; $^1$H NMR (500 MHz, DMSO-d$_6$): 9.97 (s, 1H, NH-hyd); 8.20 (s, 1H, NH-hyd); 8.19 (s, 1H, CH-8 H-vinylic); 7.67 (s, 1H, NH-indole); 7.39 (d, 1H, CH-7)); 7.37 (s, 1H, CH-2); 6.94 (s, 1H, CH-4); 6.85 (d, 1H, CH-6); 7.46—7.38 (m, 5H, benzene); 5.10 (s, 2H, CH$_2$). $^{13}$C NMR (125 MHz, DMSO-d$_6$): 185.4 (C=O); 155.2 (C=O); 132.4 (C-8 vinylic); 139.0 (C-5); 132.4 (C-8); 131.3 (C7a); 128.1

Fig. 1. PLI diagram of the ligand-binding domain of *M. tuberculosis* KasA with thiolactomycin (TLM) (PDB ID 2WGE).

Fig. 2. PLI diagrams of the ligand-binding domain of *M. tuberculosis* KasA with compounds (IIIa) (left) and (V) (right) (the legend is equal to the presented one in Fig. 1).
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(C-4); 125.4 (C-6); 118.5 (C-2); 114.3 (C-3); 113.6 (C-7); 137.9 (C-15); 128.4 (C-16 and C-20); 128.0 (C-17 and C-19); 104.5 (C-9). HRESIMS m/z: found [M + H]+ 334.11813 (calcd. [M + H]+: 334.118618).

Synthesis of (5Z)-5-(5-bromo-3-indolylmethylene)-1,3-imidazolidine-2,4-dione (IIIb) C_{12}H_{8}BrN_{3}O_{2} (306.11482) Yield 157 mg (62%); mp 236–237 °C. FTIR ν max 3412 (NH-indol); 3238 (NH-hyd); 1770, 1685 (2 C=O); 1620 (C=C); 1590, 1510, 1450 (benzene) cm–1; 1H NMR (500 MHz, DMSO-d6): 9.93 (s, 1H, NH-3, hyd); 8.34 (s, 1H, NH-hyd); 8.11 (s, 1H, CH-8, H-vinylic); 8.05 (s, 1H, CH-4); 7.70 (s, 1H, NH-indole); 7.37(s, 1H, CH-2); 7.39 (d, 1H, CH-6); 7.28 (d, 1H, CH-7); 6.72 (s, 1H, CH-2). 13C NMR (125 MHz, DMSO-d6): 174.3 (C=O); 165.8 (C=O); 137.9 (); 135.0 (C-8 vinylic); 128.2 (C-6); 128.4 (C-16, C-20); 125.3 (); 124.7 (); 121.2 (); 114.2 (C-2); 113.2 (C-3); 108.7 (C-7); 101.6 (C-9 thiazolidine). HRES-IMS m/z: found [M + H]+ 305.98744 (calcd. [M + H]+: 305.987258).

Synthesis of (5Z)-5-(5-benzyloxy-3-indolylmethylen)-1,3-thiazolidine-2,4-dione (IIIc) C_{19}H_{14}N_{2}O_{3}S (350.39106) Yield 222 mg (63%); mp 190–191 °C, yellow crystals. FTIR ν max 3420 (NH-indol); 3221 (NH-hyd); 1750, 1680 (2 C=O); 1620 (C=C); 1605, 1490, 1450 (benzene) cm –1; 1H NMR (500 MHz, DMSO-d6): 12.09 (s, 1H, NH-hyd); 8.19 (s, 1H, CH-8, H-vinylic); 7.67 (s, 1H, NH-indole); 7.37 (s, 1H, CH-2); 7.30 (d, 1H, CH-6); 7.37 (s, 1H, CH-2); 6.72 (s, 1H, CH-2). 13C NMR (125 MHz, DMSO-d6): 165.8; 174.3 (2 C=O); 135.0 (C-8 vinylic); 134.8 (7a) 108.7 (C-7), 113.2 (C-3), 114.2 (C-2), 124.5 (C-3a); 128.0 (C-17, C-19); 128.2 (C-4,C-6, C-18); 128.4 (C-16, C-20); 132.4 (C-8); 131.3 (C-5), 135.0 (C-8); 137.9 (C-15); 128.4; 128.1; 128.0 (benzene C atoms C-16, C-12, C-17, C-19, C-18); 104.5 (C-9); 101.6 (C-9); 70.0 (C-14, methylene). HRESIMS m/z: found [M + H]+ 351.07932 (calcd. [M + H]+ 351.079789).

Synthesis of (5Z)-5-[(5-bromo-1H-indol-3-yl)methylidene]-1,3-thiazolidine-2,4-dione (IIId) C_{12}H_{7}BrN_{2}O_{2}S (323.16518) Yield 201 mg (62%); mp 300 °C (dec.), yellow crystals. FTIR ν max 3422 (NH-indol); 3222 (NH-hyd); 1750, 1680 (2 C=O); 1620 (C=C); 1600, 1500. 1458 (benzene) cm–1; 1H NMR (500 MHz, DMSO-d6): 12.19 (bs, 1H, NH-hyd); 8.11 (s, 1H, CH-8, H-vinylic); 7.71 (s, 1H, NH-indole), 7.98 (s, 1H, CH-4); 7.44 (d, 1H, J = 8 Hz, CH-6); 7.33 (d, 1H, CH-7); 6.72 (s, 1H, CH-2). 13C NMR (125 MHz, DMSO-d6): 169.4; 168.9 (2 C=O); 135.4 (C-8 vinylic); 135.3 (C-7a); 129.8 (C-5); 124.3 (C-3a); 128.4 (C-16, C-20); 125.3 (;) 124.7 (;) 121.2 (;) 114.2 (C-2); 113.2 (C-3); 108.7 (C-7); 101.6 (C-9 thiazolidine). HRES-IMS m/z: found [M + H]+ 322.9484 (calcd. [M + H]+: 322.948429).

2-Methyl-2H-chromene-3-carbaldehyde (IV) was obtained according to the literature [47]: To a mixture of 2-hydroxybenzaldehyde (0.85 g, 0.8 mL, 7 mmol) and potassium carbonate (0.97 g, 7 mmol) in 1,4-dioxane (12.5 mL) crotonaldehyde (0.5 mL) was added dropwise under vigorous stirring. The mixture was heated at 100 °C for 48 h (monitored by TLC) and allowed to cool. The reaction was quenched with water and extracted several times with ether. The combined ether extracts were dried (Na_2SO_4) and evaporated to give raw compound as a yellow oil. The product was purified by silica gel column chromatography (hexane-EtOAc mixture 10 : 1) to give 2-methyl-2H-chromene-3-carbaldehyde (IV). C_{11}H_{10}O_{2} (174.1959) Yield 0.70 g (57%), yellow oil. HRESIMS m/z: found [M + H]+ 175.0753 (calcd. [M + H]+ 175.07527).

2-Phenyl-2H-chromene-3-carbaldehyde (VI) was synthesized according to the literature [62] with modifications. In this example, 3.19 mL (3.3 g, 25 mmol) cinnamaldehyde, 3.05 mL (3.05 g, 25 mmol) salicylal-
dehydrate, benzoic acid (0.61 g, 5 mmol) and pyrrolidine (0.41 mL, 5 mmol) were dissolved in 100 mL toluene. The mixture was stirred vigorously at 25°C for 20 hours. The reaction was monitored by thin-layer chromatography. The solution was filtered and toluene evaporated on a rotary evaporator at elevated temperature. The residue was dissolved in 5 mL of CHCl₃ and 5 mL hexane, and filtered with eluent hexane-EtOAc mixture (10 : 1) over 15 g silica gel. After eluent chromatography, yellow crystals of the final compound (VI) were obtained. C₁₄H₁₁NO₃S (273.30704) Yield 60%, 3.36 g; yellow crystals, mp 75–77°C; lit. mp 75–76°C.

(Z)-5-[2-Methyl-2H-chromen-3-yl)methylidene]-1,3-thiazolidine-2,4-dione (V) was synthesized according to the literature [48]. C₁₉H₁₇NO₅S (336.34134) Yield 0.551 g (44%); mp 214–215°C. FTIR νmax cm⁻¹: 3350, 1734, 1673, 1607, 1573 cm–1; 1H NMR (600 MHz, DMSO-d₆): δ (ppm) = 2.30 (CH₃), 2.31 (CH₃), 2.36 (CH₃), 2.47 (CH₃), 4.98 (s, 3H, CONH), 5.25 (q, J = 6.5 Hz, 1H, CH₂), 6.85 (d, J = 8.0 Hz, 1H, H-5), 6.94 (d, J = 1.0, 7.5 Hz, 1H, H-7), 7.03 (s, 1H, H-4), 7.23–7.26 (m, 1H, H-8), 7.27 (dd, J = 1.4, 7.6 Hz, 1H, H-6), 7.29 (s, 1H, CH H-vinylic), 12.59 (s, 1H, NH). ¹³C NMR (150 MHz, DMSO-d₆): δ (ppm) = 9.93 (CH₃), 71.30 (C-2), 116.45 (C-9), 121.32 (C-8), 121.38 (C-7), 122.61 (C-5), 128.36 (o-Ph), 128.63 (m-Ph + p-Ph), 129.70 (CH CH NH), 129.72 (CH CH NH), 130.41 (C-4), 131.59 (C-8), 131.93 (C-3), 131.95 (C-4), 134.45 (C-3), 135.98 (i-Ph), 151.95 (C-10), 158.10 (NHCONH), 173.61 (CONH). HRESIMS m/z: found [M + H]+ 274.05334 (calcd. [M + H]+ 274.05324).

5-[Hydroxy(2-phenyl-2H-1-benzopyran-3-yl)methyl]imidazolidine-2,4-dione (VII) was synthesized according to the literature [48]. C₁₉H₁₆N₂O₄ (336.34134) Yield 0.551 g (54%); yellow solid; mp 250–251°C. FTIR νmax cm⁻¹: 3350, 1734, 1673, 1607, 1573 cm–1; ¹H NMR (600 MHz, DMSO-d₆): δ (ppm) = 2.30 (CH₃), 2.31 (CH₃), 2.36 (CH₃), 2.47 (CH₃), 4.98 (s, 3H, CONH), 5.25 (q, J = 6.5 Hz, 1H, CH₂), 6.85 (d, J = 8.0 Hz, 1H, H-5), 6.94 (d, J = 1.0, 7.5 Hz, 1H, H-7), 7.03 (s, 1H, H-4), 7.23–7.26 (m, 1H, H-8), 7.27 (dd, J = 1.4, 7.6 Hz, 1H, H-6), 7.29 (s, 1H, CH H-vinylic), 12.59 (s, 1H, NH). ¹³C NMR (150 MHz, DMSO-d₆): δ (ppm) = 9.93 (CH₃), 71.30 (C-2), 116.45 (C-9), 121.32 (C-8), 121.38 (C-7), 122.61 (C-5), 128.36 (o-Ph), 128.77 (CH), 130.41 (C-4), 131.93 (C-8), 131.95 (C-3), 135.98 (i-Ph), 151.95 (C-10), 158.10 (NHCONH), 173.61 (CONH). HRESIMS m/z: found [M + H]+ 274.05334 (calcd. [M + H]+ 274.05324).

**Antimycobacterial Activity Test**

The resazurin microtiter assay (REMA) [51–53] is a colorimetric method (the results are a color reaction) for determining the minimum inhibitory concentration (MIC). Briefly, the inoculum was prepared from fresh LJ medium resuspended in 7H-9-S medium (7H9 broth, 0.1% casitone, 0.5% glycerol, supplemented oleic acid, albumin, dextrose, and catalase [OADC], Becton Dickinson), adjusted to a McFarland tube no. 1, and diluted 1 : 20; 100 μL was used as inoculum. Each compound was dissolved in DMSO and each drug stock solution was thawed and diluted in 7H-9-S. The range concentrations tested for compounds and reference drugs INH and EMB were: 0.125–4.0 μg/mL. Serial two-fold dilutions of each drug were prepared directly in a sterile 96-well microtiter plate (Becton Dickinson) using 100 μL 7H-9-S. A growth control containing no antibiotic and a sterile control was also prepared on each plate. Sterile water was added to all perimeter wells to avoid evaporation during the incubation. The plate was covered, sealed in plastic bags and incubated at 37°C in a normal atmosphere. After 7 days incubation, 30 μL of resazurin solution was added to each well, and the plate was reincubated overnight. A change in colour from blue (oxidised state) to pink (reduced) indicated the growth of bacteria, and the MIC (in μM) was defined as the lowest concentration of drug that prevented this change in colour.

**In Vitro Cytotoxicity Test**

The cytotoxicity of selected promising agents was evaluated in the human embryonal kidney cell line HEK-293 cells [63, 64]. They were obtained from the German Collection of Microorganisms and Cell Cultures (DSMZ GmbH, Braunschweig, Germany). The cells were grown in a controlled environment—cell culture flasks at 37°C in an incubator 'BB 16-Function Line' Heraeus (Kendro, Hanau, Germany) with humidified atmosphere and 5% CO₂. The cells were reset by tripipensation and supplementation with a fresh medium two times a week. The cell lines were maintained in 90% RPMI-1640 + 10% FBS. The cell viability was assessed using the standard MTT-dye reduction assay. In brief exponentially growing cells were seeded in 96-well flat-bottomed microplates (100 μL/well) at a density of 2 × 10⁴ cells per mL. After 24 h incubation at 37°C they were treated with the tested compounds for 72 h. For each concentration a set of at least 8 wells were used. After the exposure period 10 μL MTT solution (10 mg/mL in PBS) aliquots were added to each well. Thereafter the microplates were incubated for 4 h at 37°C and the MTT-formazan crystals formed were dissolved through addition of 100 μL/well 5% formic acid (in 2-propanol). The absorption was measured using a Beckman Coulter DTX-800 multimode microplate reader at 580 nm. Cell survival fractions were calculated as a percentage of the untreated control. IC₅₀ values were derived from the concentration-response curves, using non-linear regression analysis (Curve-fit, GraphPad Prizm Software package).
Selectivity Index (SI) Calculation

The data were obtained, dividing the IC_{50} value by the MIC value (SI ratio = IC_{50}/MIC).

In Silico ADME Screening, Pharmacokinetics and Drug-Likeness Simulation

The newly synthesized compounds were subjected to an in silico ADME screening to predict their chemical properties, pharmacokinetics and drug-likeness, compared to INH and EMB. The online tool SwissADME [65] of the Swiss Institute of Bioinformatics (https://www.sib.swiss) was used to perform the screening. First, the 2D structural models were drawn through the sketcher Marvin JS (version 16.4.18, 2016, https://chemaxon.com) and simplified molecular-input line-entry system (SMILES) by JChem Web Services (version 14.9.29, 2013, https://chemaxon.com) was used to present each of the compounds. In the next step, the molecular and physicochemical descriptors were calculated and pharmacokinetic profiles of the compounds were designed on the basis of the multiple linear regression, binary classification and support vector machine algorithms performed over the large data sets of known inhibitors/non-inhibitors, as well as on substrates/non-substrates [66]. Several computational rule-based filters were applied to assess the drug-likeness of the new compounds with respect to their bioavailability. That aimed at excluding the molecules incompatible with the acceptable pharmacokinetic profile from the available chemical libraries.

Docking Study

A docking study was performed using the Molecular Operating Environment (MOE, version 2016.08) of Chemical Computing Group (https://www.chemcomp.com/MOE-Molecular_Operating_Environment.htm). The crystal structure of β-Ketoacyl-ACP Synthase (KasA) protein—ligand complex was extracted from Protein Data Bank (http://www.rcsb.org/, PDB ID 2WGE). In this crystal structure, KasA receptor was crystallized bound to thiolactomycin (TLM). The correct ionization states of the protein structure were assigned before its processing to docking, and the missing hydrogen atoms were positioned with the tool “Protonate 3D” of MOE. The molecular docking was performed in “Docking” module in MOE. During the docking process, water molecules and the ligands in the crystal structures were removed, except TLM. No changes were made in the default settings of the docking procedure. The top 30 poses ranked by London dG were kept, further refined by induced fit applying GBVI/WSA dG scoring function and finally, only the best 5 poses were recorded.

CONCLUSION

Novel hydantoin and thiazolidinedione derivatives were synthesized in good yields by Knoevenagel condensation. The structures were assigned based on the spectral data. Assay results show the target molecules to have anti-TB activity, as well as that the hydantoin derivatives to be more active than thiazolidinedione analogues. The compounds have weak cytotoxicity against the human embryonic kidney cell line HEK-293, indicating that they are selective for Mtb. The in silico ADME screening reveals high oral bioavailability and high gastrointestinal absorption of the synthesized compounds. All of the synthesized compounds are lipophilic and do not penetrate the blood-brain barrier (BBB). Despite the fact that most of them appear to be potential inhibitors of some isoforms of Cytochrome P450 enzymes, compounds (IIIa) and (V) show promising results. In addition, all compounds exactly comply with the applied drug-likeness rules. Therefore, these newly synthesized compounds could be considered as a starting point for further optimization in order to identify more effective structures for tuberculosis treatment.

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COMPLIANCE WITH ETHICAL STANDARDS

This article does not contain any studies involving human participants performed by any of the authors and does not contain any studies involving animals performed by any of the authors.

Conflict of Interests

The authors declare no conflict of interest.

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