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Synthesis and evaluation of chromenyl barbiturates and thiobarbiturates as potential antitubercular agents

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ABSTRACT

A novel series of barbiturate and thiobarbiturate analogs of 2-benzoyl-3-methyl-5-oxo-5*H*-furo[3,2-*g*]chromene-6-carbaldehydes (**3a**–**g** and **4a**–**d**, respectively) and 6-methyl-4,8-dioxo-4,8-dihydropyrano[3,2-*g*]chromenes (**7a**–**c**), were synthesized and evaluated for their antitubercular activities against *Mycobacterium tuberculosis* H37RV, and cytotoxicity (CC_{50}) in the VERO cell MABA assay. The results indicate that the furanochromene series of compounds (**3a**–**g** and **4a**–**d**) showed only weak to moderate antitubercular activity. However, the pyranochromene analog **7b** showed good antitubercular activity (IC_{90}: 5.9 μg/mL) and cytotoxicity (CC_{50}: 14.27 μg/mL). The antitubercular activity of **7b** was superior to the antituberculosis drug, pyrazinamide (PZA; IC_{90}: >20 μg/mL). Analog **7b** was considered to be a lead compound for subsequent structural optimization.

For over many centuries, tuberculosis (TB) remains the leading respiratory infectious disease in the world.[1] The culprit bacterium, *Mycobacterium tuberculosis* causes the death of 2–3 million people and inflicts suffering on almost 8 million people annually. Oxygen heterocycles such as chromene-2-ones, chromene-4-ones and their derivatives are well documented in the literature as important pharmacophores that possess a wide range of biological activities, such as anti-inflammatory,[2] antibacterial[3] and anticancer activities.[4,5] Recently, Prado[6–8] and Luke[9] reported the synthesis and antimycobacterial activity of some novel furochromenes (Fig. 1, structures A and B), and in the literature, pyrimidine-2,4,6-triones are generally reported as biologically active agents.[10–12]

In view of the marked biological activity of both oxygen heterocycles and pyrimidine-2,4,6-triones, we have focused on the design of novel structural entities that incorporate substituted furochromene-5-one and pyrano-chromene-2,6-dione moieties, and barbituric acid and thiobarbituric acid moieties into a single molecular scaffold, to evaluate the potential additive effects of these heterocyclic systems on biological activity, especially with regard to antitubercular activity.

A series of simple and substituted 5-((2-benzoyl-3-methyl-5-oxo-5*H*-furo[3,2-*g*]chromen-6-yl)methylene)pyrimidine-2,4,6(1*H*,3*H*,5*H*)-triones (**3a**–**g**), 5-((2-benzoyl-3-methyl-5-oxo-5*H*-furo[3,2-*g*]chromen-6-yl)methyl-ene)-2-thioxodihydropyrimidine-4,6(1*H*,5*H*)-diones (**4a**–**d**), 5-(6-methyl-4,8-dioxo-4,8-dihydropyrano[3,2-*g*]chr-omen-3-yl)methylene)pyrimidine-2,4,6(1*H*,3*H*,5*H*)triones (**7a**–**b**) and 5-(6-methyl-4,8-dioxo-4,8-dihydropyrano [3,2-*g*]chromen-3-yl)methylene)-2-thioxodihydropyrimi-dine-4,6(1*H*,5*H*)-dione (**7c**) were synthesized and evaluated for in vitro antitubercular activity against *M. tuberculosis* and for cytotoxicity in the VERO cell assay.

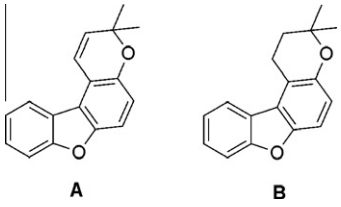

Figure 1. Structures of antimycobacterial furochromene derivatives **A** and **B**.

In the synthesis of the furochromene analogs, reaction of substituted 2-benzoyl-3-methyl-5-acetyl-6-hydroxy benzofurans[13] (**1**) with DMF/$POCl_3$ under Vilsmeier–Haack conditions afforded the substituted 2-benzoyl-3-methyl-5-oxo-5*H*-furo[3,2-*g*]chromene-6-carboxaldeh-ydes (**2a**–**g**) in 90% yield. Knoevenagel condensation of each of the resulting products with barbituric acid or thiobarbituric acid under reflux conditions in ethanol furnished furochromenes **3a**–**g**, or **4a**–**d**, respectively, in 85–90% yield (Scheme 1).

For the synthesis of the pyranochromenes, 6-acetyl-7-hydroxy-4-methyl/4,8-dimethylcoumarin (**5a**, R = H; **5b**, R = CH_3) was transformed into the corresponding 6-pyrano[3,2-g]chromene-3

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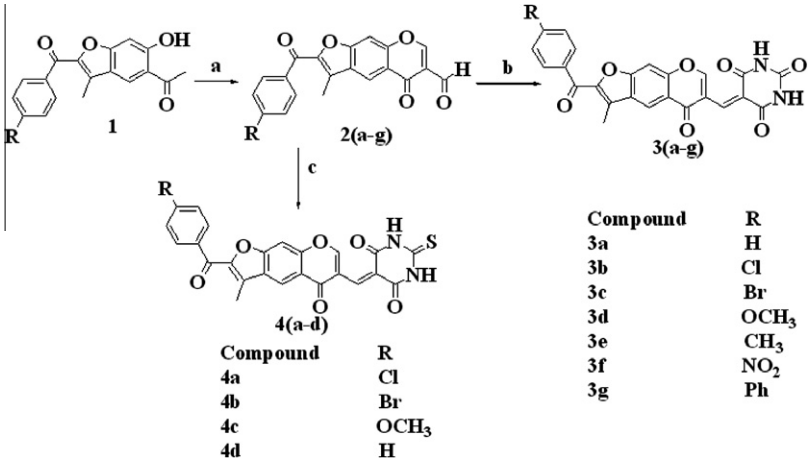


Scheme 1. Reagents and conditions; (a) DMF/$POCl_3$, 0–5 °C; (b) barbituric acid, EtOH, reflux, 4–5 h; (c) thiobarbituric acid, EtOH, reflux, 4–5 h.

-carbaldehyde analog (**6a**, R = H; **6b**, R = CH_3) by employing Vilsmeier–Haack reaction conditions. Knoevenagel condensation of **6a**/**6b** with barbituric acid or thiobarbituric acid, afforded the 5-(6-methyl-4,8-dioxo-4,8-dihydropyrano[3,2-g]chromen-3-yl)methylene)pyrimidine-2,4,6(1*H*,3*H*,5*H*)-triones **7a**/**7b** and 5-(6-methyl-4,8-dioxo-4,8-dihydropyran[3,2-*g*]chromen-3-yl)methylene)-2-thioxodihydropyrim-idine-4,6(1*H*,5*H*)-dione, **7c** (Scheme 2). All the synthesized compounds were characterized by ^{1}H NMR and ^{13}C NMR spectral analysis.[14]

Analogs **3a**–**g**, **4a**–**d**, and **7a**–**c** were evaluated against *M. tuberculosis* H37RV (ATCC27294) in BACTEC 12B medium using a broth microdilution assay, the Microplate Alamar Blue Assay (MABA).[15] The primary antitubercular screening was performed in accordance with the protocol utilized by the Tuberculosis Antimicrobial Acquisition and Coordinating Facility (TAACF) at Southern Research Institute. Rifampin was used as control drug in these tests. Compounds demonstrating a percent inhibition of bacterial growth $\geqslant$90% in the primary screen were tested against *M. tuberculosis* H37RV to determine the actual minimum inhibitory concentration (MIC) in the MABA. The MIC was defined as the lowest concentration effecting a reduction in fluorescence of 90%, relative to control. This value was determined from the dose–response curve as the IC_{90} using a curve-fitting program. Compounds with IC_{90} values <10 μg/mL were considered active antitubercular agents.

Analogs **3d**, **3g**, **4c**, **4d**, **7a**, and **7c** did not exhibit activity in the MABA assay, while analogs **3b**, **3c**, **3e**, and **4a** showed some inhibitory activity against *M. tuberculosis* with MIC values (IC_{90}) of 93.04, 55.35, 84.69, and 63.80 μg/mL, respectively. Analog **7b** was identified as the most potent inhibitor against *M. tuberculosis*, with a MIC value (IC_{90}) of 5.90 μg/mL in the MABA assay and its antitubercular activity was superior to that of the antitubercular drug, pyrazinamide (PZA; IC_{90}: >20 μg/mL).

Table 2
IC_{90}, CC_{50}, and selectivity index (SI) values for compound **7b**

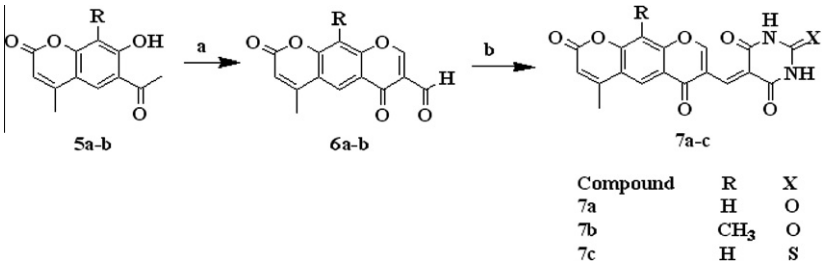
Compd	MABA:H37RV IC_{90} (μg/mL)	CTG:VERO CC_{50} (μg/mL)	SI
7b	5.90	14.27	2.41

Table 1
Antimycobacterial activity and IC_{50} and IC_{90} values against *Mycobacterium tuberculosis* H37RV in the MABA assay for compounds **3a**–**3g**, **4a**–**4d**, and **7a**–**7c**

Compound	IC_{50} (μg/mL)	IC_{90} (μg/mL)
3b	65.07	93.04
3c	38.52	55.35
3d	>100	>100
3e	55.20	84.69
3g	>100	>100
4a	42.04	63.80
4c	>100	>100
4d	>100	>100
7a	>100	>100
7b	4.71	5.90
7c	>100	>100
PZA[a]	—	>20

[a] PZA: Pyrazinamide.

Analog **7b** was further evaluated for cell cytotoxicity (CC_{50}) in the VERO cell cytotoxicity assay (Table 2) Cytotoxicity was determined from dose–response curves as a CC_{50} value, using a curve-fitting program. Concurrent with the determination of MICs, active compounds were tested for cytotoxicity in VERO cells at a concentration of 10 μM, the MIC for *M. tuberculosis* H37RV (Table 1). The selectivity index (SI) is defined as the ratio of the measured CC_{50} in VERO cells to the MIC from the MABA assay (Table 2), and is a determinant of whether an analog is suitable for subsequent



Scheme 2. Reagents and conditions: (a) DMF/$POCl_3$, 0–5 °C; (b) barbituric acid or thiobarbituric acid, EtOH, reflux, 4–5 h.

in vivo evaluation in a mouse functional assay. In this respect, the MIC and SI values for advancing compounds to in vivo evaluation should be 6.25 and 10 (occasionally lower) μg/mL, respectively. In the case of analog **7b**, the CC_{50} value was 14.27 μg/mL, while the MIC (IC_{90}) was 5.90 μg/mL, affording an SI value of 2.41. Thus, the pyranochromene **7b** was found to be a potent anti-tubercular agent in the MABA and VERO assays.

Interestingly, the inactivity of the structurally related analogs **7a** and **7c** indicates that the presence of the C8-methyl group in **7b** may be critical for antitubercular activity. Thus, compound **7b** was considered to be a lead analog for subsequent optimization in the search for novel antitubercular agents.

Acknowledgments

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14. Analytical data for the representative compounds: (**2a**): M.F.: $C_{20}H_{12}O_5$; yellow solid, mp 185–187 °C; Mass (ESI): M^{+1} 333 (100%). ^{1}H NMR (400 MHz, DMSO-d_6): 1.42 (3H, s), 7.25 (1H, s), 7.5–8.07 (5H, m), 8.55 (1H, s), 8.71 (1H, s), 10.4 (1H, s): (**3a**): M.F.: $C_{24}H_{14}N_2O_7$; yellow solid, mp >260 °C; Mass (ESI): M^{+1} 443 (60%); ^{1}H NMR (400 MHz, DMSO-d_6): δ 2.6 (s, 3H), 7.62–8.03 (m, 5H), 8.22 (s, 1H), 8.48 (s, 1H), 8.67 (s, 1H), 9.81 (s, 1H), 11.40 (s, 1H, NH), 11.49 (s, 1H, NH). ^{13}C NMR (100 MHz, DMSO-d_6): δ 9.6, 101.9, 119.3, 120.0, 120.2, 126.8, 127.9, 128.9, 129.9, 132.1, 136.2, 144.1, 149.4, 150, 150.5, 155.1, 155.9, 162.4, 162.9, 163.5, 174.7, 183.6. IR (KBr): 3410, 3077, 1744, 1711, 1686, 1648, 1617, 1476, 1346, 1041 cm^{-1}.
Compound (**7b**): M.F.: $C_{19}H_{12}N_2O_7$: brown solid, mp >260 °C; Mass (ESI): M^{+1} 381(80%); ^{1}H NMR (400 MHz, DMSO-d_6): 1.4 (3H, s), 2.2 (3H, s), 7.25 (1H, s), 7.49 (1H, s), 8.58 (1H, s), 9.87 (1H, s), 11.43 (1H, s, NH), 11.45 (1H, s, NH). ^{13}C NMR (100 MHz), DMSO-d_6): 12.7, 18.6, 110.3, 111.8, 112.7, 113.4, 114.2, 115.8, 126.1, 151.8, 152.2, 153, 153.7, 159.3, 160.6, 162.7, 174IR (KBr) cm^{-1}: 3420, 1746, 1708, 1689, 1679, 1668, 1469, 1044 cm^{-1}. (**4a**): M.F.: $C_{24}H_{13}ClN_2O_6S$: yellow solid, mp >260 °C; Mass ESI M^{+1} 493 (100%); ^{1}H NMR (400 MHz, DMSO-d_6): δ 2.64(s, 3H), 7.14–8.0 (m, 4H), 8.23(s, 1H) 8.5 (s, 1H), 8.6 (s, 1H), 9.8 (s, 1H), 12.45 (s, 1H, NH), 12.49 (s, 1H, NH). ^{13}C NMR (100 MHz, DMSO-d_6): δ 9.58, 101.9, 116.3, 119.3, 119.9, 125.3, 128.1, 128.3, 129.1,129.3, 131.1, 144.9, 150.1, 154.9, 155.8, 160.2, 161.2, 163.4, 174.6, 178.4, 183, 188.2. IR (KBr): 3431, 1711, 1686, 1623, 1617, 1475, 1345, 1167, 1045 cm^{-1}.
15. *Microbiology Assays*: All compounds were evaluated for antimycobacterial activity against *Mycobacterium tuberculosis* H37RV. Antitubercular activities of the compounds were performed by the Center of Tuberculosis Antimicrobial Acquisition and Coordinating Facility (TAACF) at Southern Research Institute. Compounds were tested for in vitro antitubercular activity against *M. tuberculosis* H37RV (ATCC 27294) at 6.25 μg/mL, in BACTEC 12B medium using a broth microdilution assay, the Microplate Alamar Blue Assay (MABA). Compounds exhibiting fluorescence were tested in the BACTEC 460 Radiometric System.[16,17] Compounds were tested in 10 twofold dilutions, typically from 100 μg/mL to 0.19 μg/mL. The IC_{90} is defined as the concentration effecting a reduction in fluorescence of 90% relative to controls. This value is determined from the dose–response curve using a curve-fitting program. Any IC_{90} value of $\leqslant$10 μg/mL is considered 'active' for antitubercular activity. The Minimum Inhibitory Concentration (MIC) is defined as the lowest concentration effecting a reduction in fluorescence of 90% relative to controls. Also the cytotoxicity (CC_{50}) values of compounds against cultured VERO cells, as well as the selectivity index (SI), defined as IC_{90}/MIC, were determined. After 72-h exposure, viability was assessed using Promega's Cell Titer Glo Luminescent Cell Viability Assay, a homogenous method of determining the number of viable cells in culture based on quantization of the ATP present. Cytotoxicity was determined from the dose–response curve as the CC_{50} using a curve-fitting program. The CC_{50} value was divided by the IC_{90} value to calculate a selectivity index (SI) value.
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