



Design, characterization, in vitro antibacterial, antitubercular evaluation and structure–activity relationships of new hydrazinyl thiazolyl coumarin derivatives

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Abstract Herein, we describe the synthesis of 11 new thiazolyl coumarin derivatives and evaluation of their potential role as antibacterial and antituberculosis agents. The structures of the synthesized compounds were established by extensive spectroscopic studies (Fourier transform infrared spectroscopy, ¹H-nuclear magnetic resonance, ¹³C-nuclear magnetic resonance, 2D-nuclear magnetic resonance and liquid chromatography–mass spectrometry) and elemental analysis. All synthesized compounds were assayed for their in vitro antibacterial activity against a few gram positive and gram negative bacteria and anti-tuberculosis activity against *Mycobacterium tuberculosis*

H37Rv ATCC 25618 by using colorimetric microdilution assay method. Nine derivatives showed moderate antibacterial and anti-tuberculosis activities against all the tested strains. The highest activity against all the pathogens including *Mycobacterium tuberculosis* was observed by compound **7c** with MIC values ranging between 31.25–62.5 µg/mL, indicating that coumarin skeleton could indeed provide useful scaffold for the development of new anti-microbial drugs.

Keywords Anti-bacterial activity · 3-Bromoacetyl coumarin · *Mycobacterium tuberculosis* · Thiazolyl coumarin · Structure-activity relationships

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Introduction

Coumarin belongs to biologically active class of compounds as they show quite diverse biological activities. There are a number of reports which show that natural and synthetic coumarins have drawn considerable attention due to their numerous therapeutic applications, as well as precursors in medicinal drug synthesis. Coumarin compounds possessing anti-bacterial (Vazquez-Rodriguez et al. 2015; Azelmat et al. 2015), antifungal (Karatas et al. 2015; Patil et al. 2015; Shaikh et al. 2016), anticoagulant (Abdelgadir and Van Staden 2013), antituberculosis (Arshad et al. 2011), anti-inflammatory (Abdelgadir and Van Staden 2013), antitumor (Nocentini et al. 2015; Elhalim and Ibrahim 2015) and anti-human immunodeficiency virus (HIV) (Matsuda et al. 2015) activities. The biological activities of the synthesized coumarin derivatives are effectively influenced by its substitution. These substitution in the coumarin

nucleus are quite important for predicting structure–activity relationship (SAR) analysis in the design and development of new coumarin derivatives with remarkable biological properties.

Microbial infections are one of the major health concerns incurring insurmountable morbidity causing millions of death worldwide. Both, gram positive and gram negative bacteria can cause acute and chronic diseases (Bjarnsholt 2013). Gram positive bacteria such as *Staphylococcus aureus* and *Streptococcus pneumoniae* are mainly responsible for nosocomial diseases, whereas Gram-negative bacteria such as *Escherichia coli*, *Enterobacter aerogenes* and *Salmonella typhi* can cause various systemic infections. One of the deadly contagious, airborne diseases is tuberculosis (TB), which is caused by *Mycobacterium tuberculosis*. The 2015 World Health Organization annual global TB report estimates higher global totals for new TB cases than in previous years. Worldwide, 9.6 million people were estimated to have fallen ill with TB in 2014 among which 1.5 million died from the disease. TB now ranks alongside HIV as a leading cause of death worldwide (WHO 2015). The increasing incidence of TB is alarming due to its persistent occurrence in cohort with acquired immunodeficiency syndrome (Arya 2011; Deribew et al. 2013). Even though TB is treatable, the treatment regimen entails the intake of a combination of 2–4 first line drugs over a long period of 6–9 months (Joshi 2011). TB treatment is further complicated by the rapid emergence of multi-drug resistant (MDR) and extensively drug resistant (XDR) *M. tuberculosis* strains (Kharb et al. 2011; Atre 2015; Elmi et al. 2016; Prakash et al. 2016). Treatment of MDRTB and XDRTB requires the use of second-line drugs, which are more toxic, less effective, and expensive over a much longer period. Hence, the proper use of second-line drugs must be ensured to cure existing MDRTB, so as to reduce the risk of its transmission and to prevent XDRTB (Prasad and Srivastava 2013). Considering the WHO report (Muthukrishnan et al. 2011), which declared bacterial infections as a major health concern, there is an urgent need to develop inexpensive new drugs to cure these diseases and ease the global burden.

Materials and methods

Chemistry

Melting points of all the synthesized compounds were determined by Stuart Scientific SMP-1 (UK) melting point apparatus and were uncorrected. Infrared (IR) spectra of all the synthesized compounds were recorded on Perkin-Elmer System 2000 FT-IR spectrometer (England, UK) using KBr disc method and scanned in the measurement range of

400–4000 cm^{-1} . ^1H and ^{13}C spectra of all the synthesized compounds were recorded on Bruker Avance spectrometer operated at 500 MHz for ^1H -NMR and 125 MHz for ^{13}C -NMR, respectively. Chemical shifts were reported in ppm (δ). The 2D spectra (correlation spectroscopy (COSY), heteronuclear multiple-quantum correlation (HMQC), and heteronuclear multiple bond correlation (HMBC) of the new compounds were recorded on a Bruker Avance 500 spectrometer at 500 MHz containing tetramethylsilane as the internal standard and DMSO- d_6 as solvent. Mass spectra were conducted on an Agilent Technologies 6224 TOF LC/MS spectrometer. The measurements were carried out in positive mode. Elemental analyses for all the new synthesized compounds were performed on a Perkin Elmer series II, 2400 CHN analyzer. The chemicals salicylaldehyde, ethylacetoacetate, piperidine, thiosemicarbazide and bromine were purchased from sigma-aldrich for synthesis of 3-acetyl coumarins **3a–g**, 3-bromoacetyl coumarins **6a, b** and coumarin thiosemicarbazones **5a–g**, according to the reported literature procedure (Gursoy and Karali 2003; Hasanen 2012).

General procedure for the synthesis of 3-acetyl coumarins (**3a–g**) and 3-bromoacetyl coumarins (**6a, b**)

Mixture of various salicylaldehyde (**1a–g**) (0.20 mol) and ethyl acetoacetate **2** (0.25 mol) was cooled and maintained at 0–5 °C. Few drops of piperidine was added drop wise with continuous stirring. The reaction mixture was left overnight, resulting in the formation of yellow colored solid which was washed by ether and recrystallized by ethanol/ CHCl_3 1:3 mixture, to afford pure 3-acetylcoumarins (**3a–g**) as fine yellow needles in good yields. Various 3-acetyl coumarins (**3a–g**) (0.15 mol) were dissolved in alcohol-free chloroform (150 mL) and a solution of bromine (0.15 mol) in alcohol free chloroform (20.0 mL) was added drop-wise from an equilibrating funnel, with constant stirring at 0–5 °C. After 4–5 h, a dark yellow solid separated. The reaction mixture was heated for 15 min on water bath and CHCl_3 was removed by rotary evaporator. The solid obtained was washed by ether. Purification by recrystallization from glacial acetic acid gave 3-bromoacetylcoumarins (**6a, b**) as white shiny needles in good yields.

General procedure for the synthesis of coumarin thiosemicarbazones (**5a–g**)

The different coumarin thiosemicarbazones (**5a–g**), were synthesized according to the literature procedure with slight modification (Arshad et al. 2011). Thiosemicarbazide (**4**) (2.8 mmol) was added to a stirred solution of various 3-acetyl-2H-chromen-2-ones (**3a–g**) (2.8 mmol) in methanol. The resulting solution was refluxed for 4 h in the presence

of catalytic amount of glacial acetic acid. The precipitates formed were filtered and washed with ethanol. Recrystallization from ethanol/ethylacetate (2:1) afforded coumarin thiosemicarbazones (**5a–g**), in good yields as yellow needles.

General procedure for the synthesis of thiazolyl coumarin derivatives (**7a–k**)

Solution of different 3-bromoacetyl coumarins (**6a, b**) (2.5 mmol) and various coumarin thiosemicarbazones (**5a–g**) (2.5 mmol) in chloroform/ethanol (2:1) (15.0 mL) mixture was refluxed for 3 h at 80 °C. Initially, clear solution was formed followed by the deposition of thick yellow precipitates. The reaction mixture was cooled and treated with ammonium hydroxide (5.0%). The solid obtained was collected, washed by distilled water and dried. Purification and recrystallization from $\text{CHCl}_3/\text{EtOH}$ (1:3) gave the desired compounds (**7a–k**) in pure form as yellow solid in good to moderate yields.

(*E*)-8-Methoxy-3(2-(2-(1-(2-oxo-2*H*-chromen-3-yl)ethylidene)hydrazinyl)thiazol-4-yl)-2*H*-chromen-2-one (**7a**). Yellow solid; Yield: 71.3%; m.p. 257–259 °C; IR (KBr, cm^{-1}) ν_{max} : 3359.86 (N–H), 1731.95 (O–C=O), 1604.90 (C=N); ^1H NMR (500 MHz, $\text{DMSO}-d_6$): δ_{H} 11.45 (1H, s, N–H), 8.58 (1H, s, H-4'), 8.15 (1H, s, H-4), 7.82 (1H, d, $J = 7.5$ Hz, H-5), 7.79 (1H, s, H-10), 7.63 (1H, t, $J = 8.5$ Hz, H-7'), 7.46 (1H, d, $J = 8.0$ Hz, H-7), 7.40 (2H, t, $J = 7.0$ Hz, H-6, H-6'), 7.32 (2H, t, $J = 6.5$ Hz, H-5', H-8'), 3.95 (3H, s, OCH₃), 2.30 (3H, s, CH₃); ^{13}C NMR (125 MHz, $\text{DMSO}-d_6$): δ_{C} 168.23 (C-11), 157.75 (C-2'), 158.21 (C-2), 154.25 (C-8a'), 147.23 (C-7'), 142.44 (C-8a), 140.11 (C-9), 141.34 (C-4'), 136.27 (C-5'), 130.30 (C-4), 122.70 (C-6), 122.22 (C-3), 120.10 (C-14), 120.19 (C-7), 112.42 (C-6'), 113.13 (C-5), 111.22 (C-4a'), 110.56 (C-10), 107.26 (C-4a), 105.65 (C-3'), 101.72 (C-8'), 58.21 (OCH₃), 19.23 (CH₃); MS: (+ESI) (m/z): 460.10 (M+H)⁺; Anal. Calcd. for $\text{C}_{24}\text{H}_{17}\text{O}_5\text{N}_3\text{S}$ (459.47): C, 62.74; H, 3.70; N, 9.15. Found: C, 62.70; H, 3.65; N, 9.10%.

(*E*)-6-Bromo-3-(1-(2-(4-(8-methoxy-2-oxo-2*H*-chromen-3-yl)thiazol-2-yl)hydrazono)ethyl)-2*H*-chromen-2-one (**7b**). Yellow solid; Yield: 72.4%; m.p. 248–250 °C; IR (KBr, cm^{-1}) ν_{max} : 3247.62 (N–H), 1729.93 (O–C=O), 1578.11 (C=N); ^1H NMR (500 MHz, $\text{DMSO}-d_6$): δ_{H} 10.26 (1H, s, N–H), 8.43 (1H, s, H-4), 8.23 (1H, d, $J = 2.5$ Hz, H-5'), 8.06 (1H, s, H-4'), 7.90 (1H, dd, $J = 9.0, 2.5$ Hz, H-7'), 7.67 (1H, s, H-10), 7.77–7.84 (2H, m, H-5, H-7), 7.35 (2H, m, H-6, H-8'), 3.87 (3H, s, OCH₃), 2.22 (3H, s, CH₃); ^{13}C NMR (125 MHz, $\text{DMSO}-d_6$): δ_{C} 169.32 (C-11), 158.35 (C-2'), 158.22 (C-2), 154.26 (C-8a'), 147.22 (C-7'), 143.47 (C-8a), 140.11 (C-9), 141.64 (C-4'), 137.27 (C-5'), 132.50 (C-4), 124.70 (C-6), 122.22 (C-3), 120.42 (C-14), 120.22 (C-7), 113.92 (C-6'), 113.13 (C-5), 111.23 (C-4a'), 110.54 (C-10),

107.25 (C-4a), 105.62 (C-3'), 102.43 (C-8'), 57.22 (OCH₃), 17.24 (CH₃); MS: (+ESI) (m/z): 538.01(M+H)⁺; Anal. Calcd. for $\text{C}_{24}\text{H}_{16}\text{O}_5\text{N}_3\text{SBr}$ (538.37): C, 53.54; H, 2.97; N, 7.81. Found: C, 53.61; H, 3.00; N, 7.85%.

(*E*)-7-Hydroxy-3-(1-(2-(4-(8-methoxy-2-oxo-2*H*-chromen-3-yl)thiazol-2-yl)hydrazono)ethyl)-2*H*-chromen-2-one (**7c**). Yellow solid; Yield: 72.3%; m.p. 260–262 °C; IR (KBr, cm^{-1}) ν_{max} : 3400 (O–H), 3292.28 (N–H), 1689.76 (O–C=O), 1615.12 (C=N); ^1H NMR (500 MHz, $\text{DMSO}-d_6$): δ_{H} 11.13 (1H, s, N–H), 10.54 (1H, s, O–H), 8.55 (1H, s, H-4), 8.15 (1H, s, H-4'), 7.76 (1H, s, H-10), 7.64 (1H, d, $J = 8.5$ Hz, H-5'), 7.29–7.32 (3H, m, H-5, H-6, H-7), 6.83 (1H, dd, $J = 8.5, 2.0$ Hz, H-6'), 6.75 (1H, d, $J = 1.5$ Hz, H-8'), 3.98 (3H, s, OCH₃), 2.32 (3H, s, CH₃); ^{13}C NMR (125 MHz, $\text{DMSO}-d_6$): δ_{C} 169.34 (C-11), 159.90 (C-2'), 158.88 (C-2), 155.96 (C-7'), 146.96 (C-8a), 143.76 (C-9), 142.41 (C-8a'), 141.65 (C-4'), 138.71 (C-4), 130.68 (C-5'), 125.07 (C-6), 122.27 (C-14), 121.35 (C-3), 120.42 (C-7), 114.84 (C-5), 114.13 (C-6'), 111.93 (C-4a'), 111.55 (C-10), 109.51 (C-4a), 103.87 (C-3'), 102.56 (C-8), 102.38 (C-8'), 57.06 (OCH₃), 16.54 (CH₃); MS: (+ESI) (m/z): 476.10 (M+H)⁺; Anal. Calcd. for $\text{C}_{24}\text{H}_{17}\text{O}_6\text{N}_3\text{S}$ (475.47): C, 60.63; H, 3.60; N, 8.84. Found: C, 60.67; H, 3.64; N, 8.80%.

(*E*)-8-Methoxy-3(2-(2-(1-(6-methoxy-2-oxo-2*H*-chromen-3-yl)ethylidene)hydrazinyl)thiazol-4-yl)-2*H*-chromen-2-one (**7d**). Yellow solid; Yield: 68.0%; m.p. 267–269 °C; IR (KBr, cm^{-1}) ν_{max} : 3431.26 (N–H), 1724.30 (O–C=O), 1606.98 (C=N); ^1H NMR (500 MHz, $\text{DMSO}-d_6$): δ_{H} 11.41 (1H, s, N–H), 8.49 (1H, s, H-4), 8.10 (1H, s, H-4'), 7.76 (1H, s, H-10), 7.39–7.18 (6H, m, H-5, H-5', H-6, H-7, H-7', H-8'), 3.92 (3H, s, OCH₃), 3.81 (3H, s, OCH₃'), 2.27 (3H, s, CH₃); ^{13}C NMR (125 MHz, $\text{DMSO}-d_6$): δ_{C} 170.22 (C-11), 160.35 (C-2'), 159.11 (C-2), 154.24 (C-7'), 146.21 (C-8a'), 143.40 (C-8a), 141.25 (C-9), 141.11 (C-4'), 136.24 (C-5'), 130.11 (C-4), 122.65 (C-3), 122.20 (C-6), 120.11 (C-7), 120.18 (C-14), 112.32 (C-6'), 113.11 (C-5), 111.11 (C-4a'), 110.31 (C-10), 107.24 (C-4a), 104.56 (C-3'), 100.22 (C-8'), 56.01 (OCH₃), 54.65 (OCH₃'), 20.13 (CH₃); MS: (+ESI) (m/z): 490.12 (M+H)⁺; Anal. Calcd. for $\text{C}_{25}\text{H}_{19}\text{O}_6\text{N}_3\text{S}$ (489.50): C, 61.34; H, 3.91; N, 8.58. Found: C, 61.39; H, 3.96; N, 8.54%.

(*E*)-8-Methoxy-3(2-(2-(1-(7-methoxy-2-oxo-2*H*-chromen-3-yl)ethylidene)hydrazinyl)thiazol-4-yl)-2*H*-chromen-2-one (**7e**). Yellow solid; Yield: 73.8%; m.p. 250–252 °C; IR (KBr, cm^{-1}) ν_{max} : 3424.20 (N–H), 1730.68 (O–C=O), 1575.68 (C=N); ^1H NMR (500 MHz, $\text{DMSO}-d_6$): δ_{H} 11.35 (1H, s, N–H), 8.55 (1H, s, H-4), 8.15 (1H, s, H-4'), 7.76 (1H, s, H-10), 7.35–7.33 (2H, m, H-5', H-7), 7.31–7.30 (2H, m, H-5, H-6'), 7.03 (1H, d, $J = 2.0$ Hz, H-8'), 6.98 (1H, dd, $J = 9.0, 2.0$ Hz, H-6), 3.92 (3H, s, OCH₃), 3.89 (3H, s, OCH₃'), 2.56 (3H, s, CH₃); ^{13}C NMR (125 MHz, $\text{DMSO}-d_6$): δ_{C} 169.12 (C-11), 163.45 (C-2'), 160.01 (C-2), 156.26 (C-8a'), 148.20 (C-7'), 146.20 (C-8a), 143.20 (C-9), 142.21

(C-4'), 137.39 (C-4), 134.16 (C-5'), 125.55 (C-6), 123.26 (C-3), 124.17 (C-7), 123.10 (C-14), 116.32 (C-6'), 115.43 (C-5), 111.14 (C-4a'), 109.71 (C-10), 107.20 (C-3'), 104.36 (C-4a), 102.11 (C-8'), 58.41 (OCH₃), 57.23 (OCH₃'), 22.70 (CH₃); MS (+ESI) (*m/z*): 490.11 (M+H)⁺; Anal. Calcd. for C₂₅H₁₉O₆N₃S (489.50): C, 61.34; H, 3.91; N, 8.58. Found: C, 61.30; H, 3.87; N, 8.62%.

(*E*)-8-Methoxy-3-(2-(2-(1-(8-methoxy-2-oxo-2*H*-chromen-3-yl)ethylidene)hydrazinyl)thiazol-4-yl)-2*H*-chromen-2-one (**7f**). Yellow solid; Yield: 68.0%; m.p. 255–257 °C; IR (KBr, cm⁻¹) ν_{\max} : 3422.83 (N–H), 1733.31 (O–C=O), 1625.03 (C=N); ¹H NMR (500 MHz, DMSO-*d*₆): δ_{H} 11.45 (1H, s, N–H), 8.56 (1H, s, H-4), 8.17 (1H, s, H-4'), 7.80 (1H, s, H-10), 7.40 (1H, dd, *J* = 6.5, 2.5 Hz, H-5'), 7.35–7.31 (5H, m, H-5, H-6, H-6', H-7, H-7'), 3.97 (3H, s, OCH₃), 3.86 (3H, s, OCH₃'), 2.30 (3H, s, CH₃); ¹³C NMR (125 MHz, DMSO-*d*₆): δ_{C} 170.13 (C-11), 165.25 (C-2), 163.21 (C-2'), 157.20 (C-8a'), 146.70 (C-7'), 144.62 (C-8a), 142.32 (C-9), 142.21 (C-4'), 138.38 (C-4), 134.15 (C-5'), 122.22 (C-6), 121.26 (C-3), 121.19 (C-7), 121.10 (C-14), 118.92 (C-6'), 113.01 (C-5), 100.10 (C-4a'), 106.62 (C-10), 107.43 (C-3'), 103.30 (C-4a), 101.01 (C-8'), 56.32 (OCH₃), 55.32 (OCH₃'), 24.33 (CH₃); MS: (+ESI) (*m/z*): 490.10 (M+H)⁺; Anal. Calcd. for C₂₅H₁₉O₆N₃S (489.50): C, 61.34; H, 3.91; N, 8.58. Found: C, 61.32; H, 3.95; N, 8.61%.

(*E*)-8-methoxy-3-(2-(2-(1-(6-nitro-2oxo-2*H*-chromen-3-yl)ethylidene)hydrazinyl)thiazol-4-yl)-2*H*-chromen-2-one (**7g**). Yellow solid; Yield: 61.9%; m.p. 267–269 °C; IR (KBr, cm⁻¹) ν_{\max} : 3245.34 (N–H), 1723.63 (O–C=O), 1618.09 (C=N); ¹H NMR (500 MHz, DMSO-*d*₆): δ_{H} 10.25 (1H, s, N–H), 8.48 (1H, s, H-4), 8.39 (1H, d, *J* = 2.5 Hz, H-5'), 7.98 (1H, s, H-4'), 7.67 (1H, dd, *J* = 9.0, 2.5 Hz, H-7'), 7.45 (1H, s, H-10), 7.34–7.39 (2H, m, H-5, H-7), 7.28–7.32 (2H, m, H-6, H-8'), 3.01 (3H, s, OCH₃), 2.11 (3H, s, CH₃); ¹³C NMR (125 MHz, DMSO-*d*₆): δ_{C} 169.32 (C-11), 158.32 (C-2'), 158.35 (C-2), 153.29 (C-8a'), 147.82 (C-7'), 142.27 (C-8a), 141.21 (C-9), 141.11 (C-4'), 136.36 (C-5'), 133.45 (C-4), 123.87 (C-6), 123.11 (C-3), 121.34 (C-14), 120.24 (C-7), 112.09 (C-6'), 113.56 (C-5), 111.11 (C-4a'), 110.76 (C-10), 106.32 (C-4a), 104.21 (C-3'), 103.79 (C-8'), 55.11 (OCH₃), 18.90 (CH₃); MS (+ESI) (*m/z*): 505.10 (M+H)⁺; Anal. Calcd. for C₂₄H₁₆O₇N₄S (504.47): C, 57.14; H, 3.20; N, 11.11. Found: C, 57.19; H, 3.25; N, 11.15%.

(*E*)-6-Methoxy-3-(1-(2-(4-(2-oxo-2*H*-chromen-3-yl)thiazol-2-yl)hydrazono)ethyl)-2*H*-chrom-en-2-one (**7h**). Yellow solid; Yield: 69.6%; m.p. 270–272 °C; IR (KBr, cm⁻¹) ν_{\max} : 3437.57 (N–H), 1724.77 (O–C=O), 1576.92 (C=N); ¹H NMR (500 MHz, DMSO-*d*₆): δ_{H} 11.43 (1H, s, N–H), 8.55 (1H, s, H-4), 8.13 (1H, s, H-4'), 7.80 (1H, s, H-5'), 7.77 (1H, s, H-10), 7.62 (1H, t, *J* = 6.0 Hz, H-7), 7.43 (1H, d, *J* = 8.0 Hz, H-8'), 7.39 (2H, dd, *J* = 6.5, 2.0 Hz, H-5, H-7'), 7.36 (1H, t, *J* = 8.5 Hz, H-6), 7.20 (1H, dd, *J* = 8.5, 2.5

Hz, H-8), 3.83 (3H, s, OCH₃'), 2.72 (3H, s, CH₃); ¹³C NMR (125 MHz, DMSO-*d*₆): δ_{C} 169.24 (C-11), 159.61 (C-2), 159.15 (C-2'), 156.50 (C-8a), 152.95 (C-8a'), 148.38 (C-9), 146.10 (C-6'), 144.60 (C-4), 140.95 (C-4'), 138.60 (C-7), 132.04 (C-5'), 120.05 (C-7'), 127.20 (C-3), 125.14 (C-3'), 121.27 (C-4a'), 120.42 (C-8), 119.91 (C-14), 119.74 (C-4a), 117.42 (C-5), 116.31 (C-8'), 111.97 (C-6), 111.62 (C-10), 56.52 (OCH₃'), 16.49 (CH₃); MS: (+ESI) (*m/z*): 460.10 (M+H)⁺; Anal. Calcd. for C₂₄H₁₇O₅N₃S (459.47): C, 62.74; H, 3.73; N, 9.15. Found: C, 62.70; H, 3.77; N, 9.19%.

(*E*)-7-Methoxy-3-(1-(2-(4-(2-oxo-2*H*-chromen-3-yl)thiazol-2-yl)hydrazono)ethyl)-2*H*-chrom-en-2-one (**7i**). Yellow solid; Yield: 69.6%; m.p. 262–264 °C; IR (KBr, cm⁻¹) ν_{\max} : 3437.75 (N–H), 1725.27 (O–C=O), 1565.01 (C=N); ¹H NMR (500 MHz, DMSO-*d*₆): δ_{H} 11.43 (1H, s, N–H), 8.14 (1H, s, H-4), 8.83 (1H, s, H-4'), 7.78 (1H, s, H-5'), 7.79 (1H, s, H-10), 7.62 (1H, t, *J* = 8.5 Hz, H-7), 7.47–55 (3H, m, H-6, H-6', H-8'), 7.39 (2H, d, *J* = 6.5 Hz, H-5, H-8), 3.93 (3H, s, OCH₃'), 2.35 (3H, s, CH₃); ¹³C NMR (125 MHz, DMSO-*d*₆): δ_{C} 168.22 (C-11), 159.61 (C-2), 159.15 (C-2'), 156.22 (C-8a), 151.05 (C-8a'), 146.23 (C-9), 146.10 (C-6'), 144.31 (C-4), 139.39 (C-4'), 137.48 (C-7), 134.06 (C-5'), 124.48 (C-7'), 127.23 (C-3), 124.14 (C-3'), 120.07 (C-4a'), 121.40 (C-8), 117.91 (C-14), 117.04 (C-4a), 116.23 (C-5), 116.31 (C-8'), 113.76 (C-6), 111.43 (C-10), 51.11 (OCH₃'), 18.81 (CH₃); MS: (+ESI) (*m/z*): 460.09 (M+H)⁺; Anal. Calcd. for C₂₄H₁₇O₅N₃S (459.47): C, 62.74; H, 3.73; N, 9.15. Found: C, 62.78; H, 3.78; N, 9.20%.

(*E*)-8-Methoxy-3-(1-(2-(4-(2-oxo-2*H*-chromen-3-yl)thiazol-2-yl)hydrazono)ethyl)-2*H*-chrom-en-2-one (**7j**). Yellow solid; Yield: 70.4%; m.p. 258–260 °C; IR (KBr, cm⁻¹) ν_{\max} : 3438.52 (N–H), 1723.93 (O–C=O), 1605.61 (C=N); ¹H NMR (500 MHz, DMSO-*d*₆): δ_{H} 11.45 (1H, s, N–H), 8.58 (1H, s, H-4), 8.15 (1H, s, H-4'), 7.82 (1H, d, *J* = 7.5 Hz, H-5'), 7.79 (1H, s, H-10), 7.63 (1H, t, *J* = 8.5 Hz, H-7), 7.46 (1H, d, *J* = 8.0 Hz, H-7'), 7.40 (2H, t, *J* = 7.0 Hz, H-6, H-6'), 7.32 (2H, t, *J* = 6.5 Hz, H-5, H-8), 3.95 (3H, s, OCH₃'), 2.35 (3H, s, CH₃); ¹³C NMR (125 MHz, DMSO-*d*₆): δ_{C} 170.20 (C-11), 157.41 (C-2), 158.10 (C-2'), 157.56 (C-8a), 152.01 (C-8a'), 147.81 (C-9), 146.22 (C-6'), 144.63 (C-4), 143.25 (C-4'), 137.22 (C-7), 134.13 (C-5'), 122.45 (C-7'), 128.11 (C-3), 126.13 (C-3'), 123.20 (C-4a'), 122.22 (C-8), 120.02 (C-14), 120.74 (C-4a), 120.32 (C-5), 119.21 (C-8'), 114.02 (C-6), 112.32 (C-10), 58.11 (OCH₃'), 20.21 (CH₃); MS: (+ESI) (*m/z*): 460.08 (M+H)⁺; Anal. Calcd. for C₂₄H₁₇O₅N₃S (459.47): C, 62.74; H, 3.73; N, 9.15. Found: C, 62.78; H, 3.78; N, 9.20%.

(*E*)-6-Nitro-3-(1-(2-(4-(2-oxo-2*H*-chromen-3-yl)thiazol-2-yl)hydrazono)ethyl)-2*H*-chromen-2-one (**7k**). Yellow solid; Yield: 70.6%; m.p. 271–273 °C; IR (KBr, cm⁻¹) ν_{\max} : 3432.43 (N–H), 1722.54 (O–C=O), 1617.26 (C=N); ¹H NMR (500 MHz, DMSO-*d*₆): δ_{H} 11.40 (1H, s, N–H), 8.45 (1H, s, H-4), 8.11 (1H, s, H-4'), 7.98 (1H, s, H-5'),

7.87 (1H, s, H-10), 7.33 (1H, t, $J = 6.0$ Hz, H-7), 7.40 (1H, d, $J = 8.0$ Hz, H-8'), 7.23 (2H, dd, $J = 6.5, 2.0$ Hz, H-5, H-7'), 7.14 (1H, t, $J = 8.5$ Hz, H-6), 7.10 (1H, dd, $J = 8.5, 2.5$ Hz, H-8), 2.72 (3H, s, CH₃); ¹³C NMR (125 MHz, DMSO-*d*₆): δ_C 169.23 (C-11), 159.63 (C-2), 159.18 (C-2'), 156.55 (C-8a), 152.98 (C-8a'), 148.32 (C-9), 146.11 (C-6'), 144.65 (C-4), 140.87 (C-4'), 138.89 (C-7), 132.07 (C-5'), 120.78 (C-7'), 127.22 (C-3), 125.11 (C-3'), 121.26 (C-4a'), 120.32 (C-8), 119.98 (C-14), 119.76 (C-4a), 117.22 (C-5), 116.11 (C-8'), 111.90 (C-6), 111.22 (C-10), 16.43 (CH₃); MS: (+ESI) (*m/z*): 475.05 (M+H)⁺; Anal. Calcd. for C₂₃H₁₄O₆N₄S (474.44): C, 58.23; H, 2.95; N, 11.81. Found: C, 58.27; H, 2.98; N, 11.86%.

Pharmacology

In vitro evaluation of antibacterial activity

All the synthesized compounds (**7a–k**) were evaluated for their in vitro antibacterial activity against two gram positive bacteria (*Streptococcus pneumoniae* and *S. aureus*) and three Gram-negative bacteria (*E. coli*, *Enterobacter aerogenes* and *Salmonella typhi*) using colorimetric microdilution assay. The standard drugs used were streptomycin, kanamycin, and vancomycin in order to compare the minimum inhibitory concentration (MIC) values (Table 1). The results clearly showed that different coumarins derivatives exhibited different degree of inhibition against the tested bacterial strains ranging in between 31.25–125 µg/mL. The highest activity against all the pathogens was

observed by compound **7c** with MIC values of 31.25–62.5 µg/mL followed by compound **7b**. The inhibitory activities of these two compounds especially against *S. typhi*, *S. pneumoniae*, and *S. aureus* were comparable and even better than that of standard drug, kanamycin. Activity against all strains was also exhibited by compounds **7i**, **7j**, and **7k** which was in the range of 62.5–125 µg/mL. According to the recent review, pure compounds are considered to have good anti-bacterial activity worthy of further investigation for drug development if the MIC values are in good range (Arshad et al. 2011). Since, the test compounds in this study exhibited good to moderate activity range, therefore they could be accepted to possess antimicrobial properties. However, the observed anti-bacterial profile of these compounds suggested that the introduction of hydroxyl group and halogen, especially bromine in coumarin skeleton for the compound **7b** and **7c** enhanced the activity against all the tested strains compared to other substituents. Further improvement on the substitution pattern is being carried out to increase their potential as anti-bacterial agents.

In vitro anti-TB activity

The anti-TB potential of all the compounds (**7a–k**) were evaluated against *M. tuberculosis*, H37Rv strain ATCC 25618. All test compounds except **7g** and **7k** exhibited anti-TB activity with the highest chosen concentration level of 50 µg/mL. The indication that the introduction of bromine and hydroxyl group enhanced the anti-TB activity was supported by the result observed for **7b** and **7c**. It was also

Table 1 In Vitro anti-bacterial and anti-tuberculosis activities of compounds **7a–k** and their MIC values in µg/mL

Compounds	<i>E. coli</i>	<i>E. aerogenes</i>	<i>S. typhi</i>	<i>S. pneumoniae</i>	<i>S. aureus</i>	<i>M. tuberculosis</i>
7a	125	125	62.5	62.5	62.5	50
7b	125	125	62.5	62.5	62.5	50
7c	62.5	62.5	62.5	31.25	31.25	50
7d	125	125	125	125	125	50
7e	125	125	125	125	125	50
7f	125	125	125	125	125	50
7g	125	125	125	125	125	*
7h	125	125	62.5	62.5	125	50
7i	125	125	62.5	62.5	125	50
7j	125	125	62.5	62.5	125	50
7k	62.5	62.5	62.5	62.5	125	*
Streptomycin	31.25	62.5	62.5	31.25	31.25	–
Kanamycin	62.5	62.5	62.5	62.5	125	–
Vancomycin	250	125	125	31.25	31.25	–
Isoniazid	–	–	–	–	–	0.0781

* No inhibition observed even at the highest test concentration of 50 µg/mL

– Not applicable

apparent from the results that the introduction of methoxy group too could have exerted considerable anti-TB activity as shown by compounds **7c**, **7d**, **7e**, **7h**, **7i**, and **7j** with MIC values of 50 $\mu\text{g/mL}$. Compounds **7g** and **7k** did not exhibit anti-TB activity, indicating that the presence of nitro group had no inhibitory effects on the tubercle cells even at the highest concentration range of 50 $\mu\text{g/mL}$. Compared to the control drug isoniazid, which exhibited exquisite activity with MIC value of 0.0781 $\mu\text{g/mL}$, the active compounds fared moderately. With regards to anti-TB activity, compounds with MIC values of 1.5 $\mu\text{g/mL}$ are considered promising (Ma et al. 2005). Even so, such compounds may not be drugs per se if they are toxic, insoluble or pharmacokinetically limited. More importantly, the structural skeletons of the compounds could be useful as templates for the development of new anti-microbial agents. Hence, the structural skeleton of compounds **7b** and **7c** could also provide useful template for the development of new anti-TB drugs.

SAR analysis

The structural similarity in term of substituents, of several of the antibiotics (streptomycin, vancomycin and kanamycin) and the recent synthesized coumarin hybrids have permitted a systematic comparison of the SAR relationships. The number and site of attachment of the thiazole ring, halogen, methyl, methoxy, hydroxyl, and amino substituents profoundly effect the ability of these compounds to inhibit in vitro bacterial infection (Hummelova et al. 2014). The SAR reveals that introduction of halogen and hydroxyl into the coumarin scaffold increases the potential to treat bacterial infections. The physiochemical properties such as lipophilicity or hydrophobicity might be concerned with their activities. Halogens are very reactive due to their high electronegativity and high effective nuclear charge. Therefore, sufficient quantities of halogens can be lethal to micro organisms. In general, halogen substituents (Cl, Br, I), increases the lipophilicity or hydrophobicity of the molecules, making the molecule bigger, more polarized and accordingly increasing the London dispersion forces, which are responsible for the interaction of the lipophilic substance to themselves or with others. Hydroxyl groups (OH), found in alcohols, are quite polar and therefore hydrophilic (water loving). However, their carbon chain portion is non-polar hence making them hydrophobic, overall more nonpolar and therefore less soluble in the polar water as the carbon chain grows. Since, the methoxy group (OCH_3) has little influence on the molecular hydrophobicity, hence its effects to biological activities is moderate. Nitro functional groups (NO_2) being hydrophilic forms strong hydrogen bonds with water molecules, despite of their high polarities arising due

to large dipole moments. Therefore, these compounds are hydroneutral, with hydrophilicity between hydrophilic and hydrophobic (Mannhold et al. 2008). Hence, it could be concluded that more hydrophobic is the substituent, the more effective is its antibacterial property. Based on the above facts we can justify the SAR of our compounds in bacterial inhibition. The antibacterial activities of compounds **7b** and **7c** with Br and OH functional groups respectively, was 4–8 times stronger than that of the standards streptomycin, kanamycin and vancomycin. This is due to the fact that Br and OH groups being more lipophilic/hydrophobic, tend to react with the pathogens the most as compared to other chosen compounds. Introduction of these groups anywhere in the coumarin skeleton will enhance the antibacterial and anti-TB properties likewise. Compounds **7d**, **7e**, **7f**, **7i**, and **7j** with moderately hydrophobic OCH_3 group, were also interestingly found to inhibit tested bacterial strain. In addition to these it was noticed that **7g** and **7k**, bearing hydrophilic NO_2 group, were relatively lower in their anti-bacterial activities. In this context the activity of the coumarin hybrids can be controlled by changing the pharmacophores with respect to the attached substituent and these structural modifications are mainly well carried out by the reaction between various coumarin thiosemicarbazone derivatives with α -bromo-3-acetyl coumarin derivatives.

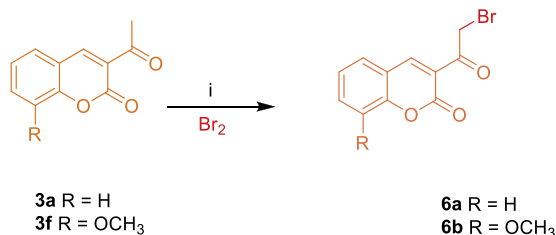
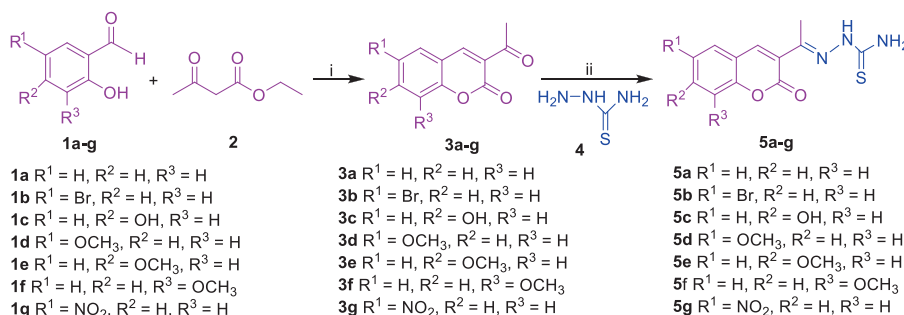
Results and discussion

Chemistry

The first step of the synthesis involved formation of 3-acetylcoumarins (**3a–g**), obtained by the condensation reaction between corresponding salicylaldehyde derivatives (**1a–g**) and ethylacetoacetate (**2**) at 0–5 $^\circ\text{C}$ in the presence of catalytic amount of piperidine, which were further reacted with thiosemicarbazide to get coumarin thiosemicarbazone derivatives (**5a–g**) (Scheme 1). The other precursors, 3-(2-bromoacetyl)-2*H*-chromen-2-one (**6a, b**) were synthesized by brominating 3-acetyl coumarins (**3a, f**) (Scheme 2). Synthesis of series of titled compounds (**7a–k**) were accomplished by Hantzsch cyclization which involves the cyclocondensation of coumarin thiosemicarbazones (**5a–g**) and α -bromo-3-acetyl coumarins (**6a, b**) in $\text{CHCl}_3/\text{EtOH}$ (2:1) mixture for 3 h at 80 $^\circ\text{C}$, followed by treatment of reaction mixture with 5% NH_4OH . Purification by recrystallization gave pure thiazolyl coumarin derivatives in good yield (Scheme 3).

The structures of all the pure compounds were characterized by spectroscopic techniques IR, $^1\text{H-NMR}$, $^{13}\text{C-NMR}$, LC-MS and elemental analysis, which was within range of $\pm 0.4\%$. The structure of the representative compound (**7c**) was further confirmed by 2D NMR (COSY,

Scheme 1 Synthesis of coumarin thiosemicarbazones (**5a–g**). Reagent and reaction conditions: (i) piperidine, 0–5 °C; (ii) CH₃COOH, CH₃OH, reflux



Scheme 2 Synthesis of 3-bromocoumarin (**6a, b**). Reagent and reaction conditions: (i) Br₂, CHCl₃, 0–5 °C

HMQC, and HMBC). This gave the confirmation of exact configuration of the compounds. The IR spectroscopic analysis of representative compound (**7c**), featured a broad band at 3400.0 cm⁻¹ and a sharp band at 3292.28 cm⁻¹ due to O–H and NH stretching. The bands at 1689.76 and 1615.12 cm⁻¹ corresponded to lactone group (O=C=O) and C=N stretching, respectively. The ¹H-NMR spectroscopic technique revealed the presence of one broad singlet at the most downfield value 11.13 ppm for the proton attached to nitrogen atom next to the imine nitrogen. Broad signal at 10.54 ppm represented OH group. The diagnostic resonance at 8.31 ppm indicated the presence of coumarin H-4 and a multiplet at 7.30 ppm integrating to 3 protons indicated the presence of H-5, H-6 and H-7. Besides this, a doublet at 7.77 ($J = 7.5$ Hz) was assigned to H-5' due to its *ortho* coupling with H-6'. This confirms the presence of other coumarin nucleus in the structure. This was further confirmed, by a doublet of doublet appearing at 6.83 ppm ($J = 8.5, 2.0$ Hz) for H-6' due to its *ortho* coupling with H-5' and *meta* coupling with H-8'. Moreover, a doublet at 6.75 ppm ($J = 1.5$ Hz) was assigned to H-8' due to its *meta* coupling with H-6'. Most importantly, the absence of two broad signals at 8.35 and 7.91 ppm for the primary amine of coumarin thiosemicarbazone (**5c**) and appearance of singlet at 7.76 ppm for the thiazole proton (H-10) confirmed the formation of thiazole ring in the expected compound. The most upfield signal at 3.98 ppm integrating to three protons was assigned to the methoxy group of the coumarin nucleus. The ¹³C-NMR spectroscopic analysis of (**7c**)

showed all the expected signals for 24 carbons. Signals at 169.34, 143.45, and 111.55 ppm indicated the presence of thiazole ring carbons C-11, C-9 and C-10 in the structure which was further confirmed by ¹H-¹³C HMQC. The ¹H-¹H COSY spectra of **7c** showed very clear contours for the coupling of H-5' (δ_H 7.64) with H-6' (δ_H 6.83). The HMQC spectra showed that out of 24 carbons, 13 were quaternary, 9 were methines and the remaining 2 were methoxy and methyl carbons. The spectra showed clear coupling of H-4 (δ_H 8.55) with C-4 (δ_C 138.71), H-5 (δ_H 7.30) with C-5 (δ_C 114.84), H-6 (δ_H 7.30) with C-6 (δ_C 125.07), H-7 (δ_H 7.30) with C-7 (δ_C 120.42) in one of the coumarin nucleus and of H-4' (δ_H 8.15) with C-4' (δ_C 138.71), H-5' (δ_H 7.64) with C-5' (δ_C 130.68), H-6' (δ_H 6.83) with C-6' (δ_C 114.13), H-8' (δ_H 6.75) with C-8' (δ_C 102.38) in the other coumarin nucleus. Moreover, correlations were also observed for the methyl group H-13 (δ_H 2.32) with C-13 (δ_C 16.71) and methoxy group H-15 (δ_H 3.98) with C-15 (δ_C 56.97). In the thiazole moiety direct correlations of H-10 (δ_H 7.76) with C-10 (δ_C 111.55) confirmed thiazole ring in the structure. In ¹H-¹³C HMBC spectra of **7c**, structural identity was confirmed by long range correlation of C-H (Fig. 1). Taking into account the important HMBC's correlation, it was clearly found that H-10 (δ_H 7.76) showed cross peaks with C-11 (δ_C 169.34) and C-9 (δ_C 143.76), which confirmed the formation of thiazole ring. Furthermore, H-4 (δ_H 8.55) was found to give cross peaks with C-2 (δ_C 158.88) and C-8a (δ_C 146.96), which confirmed the presence of one coumarin nucleus in the structure. It also showed cross peaks with C-3 (δ_C 121.35) and C-9 (δ_C 143.76), which confirmed that thiazole ring was attached to C-3 of the coumarin. Besides this, the methyl proton H-13 (δ_H 2.32) was found to give cross peak with C-14 (δ_C 122.27). Moreover, H-5 (δ_H 7.30) was found to give cross peaks with C-5 (δ_C 114.84), C-7 (δ_C 120.42) and C-8a (δ_C 146.96). H-6 (δ_H 7.30) and H-7 (δ_H 7.30) were also found to show correlation with C-8a (δ_C 146.96). Moreover, H-4' (δ_H 8.15) showed cross peaks with C-5' (δ_C 130.68) and C-2' (δ_C 159.90). In addition, H-8' (δ_H 6.75) showed cross peaks with C-6' (δ_C 114.13), C-7' (δ_C 155.96) and C-4a' (δ_C 111.93), which further confirms, the presence of the other coumarin nucleus in the **7c** structure.

Scheme 3 Synthesis of thiazolyl coumarin derivatives (**7a–k**).

Reagent and reaction conditions:

(i) $\text{CHCl}_3/\text{EtOH}$ (2:1), reflux, NH_4OH (5%)

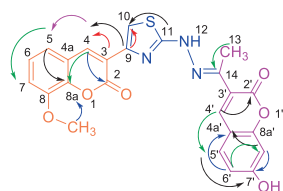
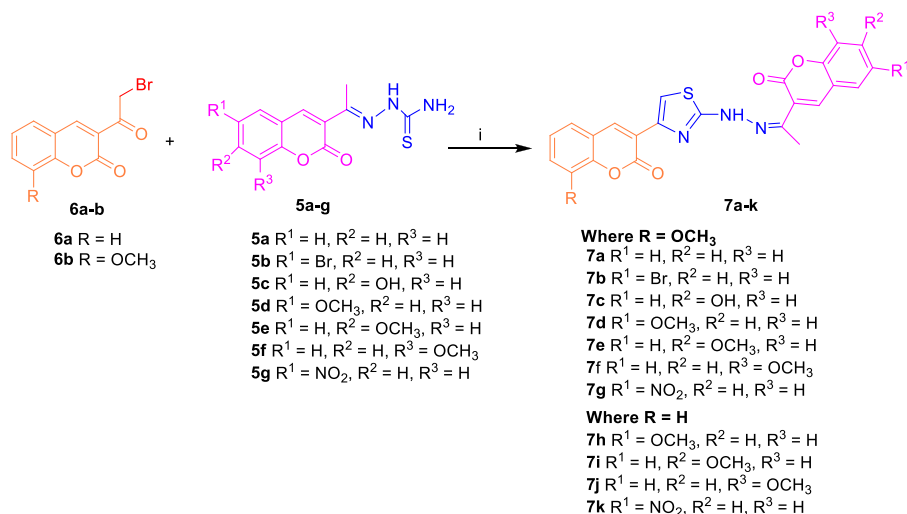


Fig. 1 Possible ^1H - ^{13}C HMBC correlations of **7c**

Similarly, the structures of the other compounds of this series have been established.

Pharmacology

In vitro evaluation of antibacterial activity

All the pure compounds were screened for in vitro antibacterial activity against two gram positive bacteria (*S. pneumoniae* and *S. aureus*) and three gram negative bacteria (*E. coli*, *E. aerogenes*, and *S. typhi*) by colorimetric microdilution method (Ghosh et al. 2013; Mahato et al. 2016) with slight modifications. Each compound was tested in triplicate twice in sterile 96-well microtiter plates. The MIC was determined in $\mu\text{g}/\text{mL}$. The test organisms were freshly grown and incubated for 48 h at 37 °C. The 2-day old bacterial cultures were emulsified in 5 mL of Muller Hinton broth (MHB) and incubated again at 37 °C overnight to attain log growth phase. The turbidity of each inoculum was then adjusted to McFarland standard no. 0.5 by further addition of MHB to give inoculum concentration 1.5×10^8 CFU/mL. A volume of 100 μL of MHB was added into all the wells of the microtiter plate except the first column A. Then, 200 μL working solution of each test compound was

transferred to column A in triplicate of each plate. Serial 2-fold dilution was made starting from column A, ranging 3.91–250 $\mu\text{g}/\text{mL}$ and the excess 100 μL of the mixture was dispensed off from the wells in the last column. Each well was then inoculated with 100 μL of each bacterial inoculum. Each plate was sealed with parafilm and incubated at 37 °C for 24 h. A volume of 50 μL of 3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2H-tetrazolium bromide (MTT) (0.2 mg/mL dissolved in distilled water) was added into each well and the plates were again incubated for 30 min at 37 °C. Streptomycin, kanamycin and vancomycin were used as positive controls, whereas DMSO was used as the negative control. The color change of MTT after 30 min from yellow to purple indicated the presence of active bacterial cells. The MIC was interpreted as the lowest concentration of compounds that prevented the color change.

In vitro evaluation of anti-TB activity

A well characterized H37Rv ATCC 25618 virulent strain of *M. tuberculosis* was used for the screening of in vitro anti-TB activity of the compounds by colorimetric microdilution with minor modifications (Kumar et al. 2014). The MIC was also measured in $\mu\text{g}/\text{mL}$. The mycobacterial inoculum was prepared from a 10-day fresh culture on Middlebrook 7H9 agar plate supplemented with oleic, albumin, dextrose, and catalase (OADC) and emulsified in Middlebrook 7H9 broth supplemented with, albumin, dextrose, and catalase (ADC). The inoculum turbidity was adjusted to McFarland standard no. 1 to give a concentration of approximately 3×10^8 CFU/mL. This suspension was further diluted in Middlebrook 7H9 broth supplemented with OADC in a 1:20 ratio. Initially, 200 μL sterile distilled water was added into all outer walls of a 96-well microtiter plate to prevent

dehydration of the culture medium and then 100 μL of Middlebrook 7H9 broth supplemented with OADC was added into all the wells except the first column A. Then, 100 μL working solution of each test compound prepared in 10% DMSO, was transferred into each well in column A. Serial 2-fold dilution was made starting from column A, ranging between 0.195–50 $\mu\text{g}/\text{mL}$ and the final 100 μL of the mixture was dispensed off from the wells in the last row. Each well was then inoculated with 100 μL of *M. tuberculosis* inoculum. Isoniazid, a standard drug for TB was used as a positive control, whereas DMSO was used as a negative control in this assay. Each compound and drug was tested in triplicate twice. Each plate was sealed with parafilm and incubated for 5 days at 37 °C in 8% CO_2 . On the fifth day, 50 μL of tetrazolium-tween 80 mixtures (1.5 mL of 1 mg/mL MTT in absolute ethanol and 1.5 mL of 10% Tween-80) was added into all the wells and incubated again for 24 h at 37 °C. On the following day, the MIC due to color change by MTT from yellow to purple in the presence of active microorganism was reported.

Conclusion

We have described an efficient procedure for the synthesis of new thiazolyl coumarin derivatives **7a–k**. All newly synthesized compounds were screened for in vitro antibacterial activity against various gram positive and gram negative bacteria (*E. coli*, *E. aerogenes*, *S. typhi*, *S. pneumoniae*, and *S. aureus*) and in vitro anti-TB against *M. tuberculosis* and the results were complied. The introduction of hydroxyl group and bromine in the coumarin skeleton for compounds **7b** and **7c** probably enhanced their activity against all bacterial strains due to hydrophobic or lipophilic nature of its substituents. **7a** also showed good activity due to the presence of moderately lipophilic methoxy substituent. The present communication serves as a combination of synthesis and biological activity screening which indicates that coumarin skeletons could provide useful scaffolds or templates for the development of new anti-microbial drugs.

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Compliance with ethical standards

Conflict of interest The authors declare no competing interest.

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