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Original article

Synthesis and antimicrobial properties of some new thiazolyl coumarin derivatives

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ABSTRACT

Two novel series of hydrazinyl thiazolyl coumarin derivatives have been synthesized and fully characterized by IR, ^{1}H NMR, ^{13}C NMR, elemental analysis and mass spectral data. The structures of some compounds were further confirmed by X-ray crystallography. All of these derivatives, **10a**–**d** and **15a**–**h**, were screened *in vitro* for antimicrobial activity against various bacteria species including *Mycobacterium tuberculosis* and *Candida albicans*. The compounds **10c**, **10d** and **15e** exhibited very good activities against all of the tested microbial strains.

1. Introduction

A large number of naturally occurring compounds contain heterocyclic rings as an important part of their structure. Such compounds including alkaloids, flavonoids, coumarins and terpenoids are widely used as medicines. Among them, coumarin and its derivatives possess remarkable activities against bacteria [1], fungi [2], tumours [3], viruses [4] and especially against HIV protease [5]. They also act as anti-coagulants [6], free radical scavengers [7], lipoxygenase [8] and cyclooxygenase [9] inhibitors.

Compounds containing thiazole rings have remarkable medicinal value due to their potential chemotherapeutic [10], fungicidal [11], antiviral [12] and pesticidal [13] properties. In addition, 2-aminothiazole derivatives are reported to exhibit significant biological activities such as anti-tuberculosis [14], anti-inflammatory [15], enzyme inhibition [16] and anti-tumour activities [17]. They have also found broad application in the treatment of allergies [18], schizophrenia [19] and hypertension [20]. Furthermore, coumarin derivatives having various substituted thiazole rings at carbon-3 exhibit promising biological activities [21]. Recently, Siddiqui and his co-workers reported the synthesis of some new coumarin-incorporated thiazolyl semicarbazones with good anticonvulsant activity [22], while analgesic and anti-inflammatory activities of thiazolyl coumarins [23] are also known. Some thiazolyl coumarin analogues are found to have potential as anticancer and antimicrobial agents [24]. On the basis of all of this evidence, we set out to prepare a new series of biologically active agents containing both of these two important pharmacophores. This study reports the synthesis, characterization, and *in vitro* antimicrobial activities of these new thiazolyl coumarin derivatives.

2. Results and discussion

2.1. Chemistry

Two new series of coumarin-thiazole derivatives, **10a**–**d** and **15a**–**h**, which incorporated three important pharmacophores (coumarin ring, thiazole heterocycle and imine group), were synthesized by the condensation of thiosemicarbazones with 3-bromoacetylcoumarin (**4**) [25]. The first step of the synthesis involved the preparation of acetylcoumarin (**3**) and 3-bromoacetylcoumarin (**4**) as shown in Scheme 1. In the first series, a thiosemicarbazone subset (**7a**,**b**, **9a**,**b**) was synthesized by treating thiosemicarbazide (**5**) with a range of substituted ketones in the presence of a catalytic amount of glacial acetic acid.

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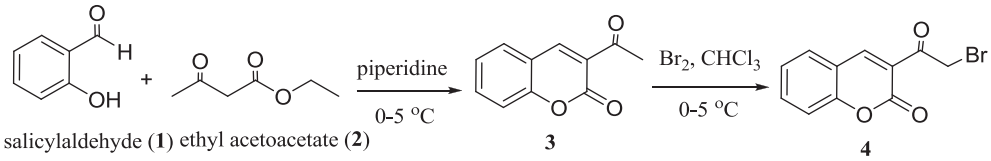


Scheme 1. Synthesis of acetylcoumarin (**3**) and 3-bromoacetylcoumarin (**4**).

Purification by recrystallization gave the thiosemicarbazides (**7a**,**b**, **9a**,**b**) in good yield (75–80%) in a single crystalline form. X-ray crystallographic analysis of **9a** (Fig. 1) provided further confirmation of structure [26]. Condensation of subset (**7a**,**b**, **9a**,**b**) with 3-bromoacetylcoumarin (**4**) in a solvent mixture of $CHCl_3$–EtOH (2:1) (Scheme 2), followed by basification with ammonium hydroxide, gave the first library of coumarin derivatives **10a**–**d** in good (70–80%) yield after purification by recrystallization. Structure elucidation was confirmed by spectroscopic and elemental analysis, as well as X-ray crystallographic evidence for **10a** [27] and **10b** [28] (Figs. 2 and 3). Interestingly, infrared spectroscopic analysis of coumarins **10a**–**d** revealed the presence of lactone (C=O) and imine (C=N) functional groups with absorptions at ν 1690–1718 and 1609–1638 cm^{-1}, respectively [29], as well as other characteristic absorption bands. ^1H NMR spectroscopic analysis revealed diagnostic resonances for the coumarin (4-H) and thiazole methine protons at δ_H 8.52–8.59 and 7.76–8.08 ppm, respectively [30]. ^{13}C NMR spectroscopic analysis also confirmed structural identity, with resonances observed at δ_C 169.1–170.3 (thiazole 2-C), 159.6–162.6 (C=O), and 153.2–156.3 (C=N) ppm. Elemental analyses of coumarins **10a**–**d** were within the range ±0.4% and fully supported structural assignment.

A second series of coumarin derivatives **15a**–**h** was prepared by a related route (Scheme 3). A range of differentially substituted aldehydes was treated with thiosemicarbazide (**5**) in hot ethanol to give the arylthiosemicarbazone, subsets **12a**–**f** and **14a**,**b**. Hantzsch cyclocondensation with 3-bromoacetylcoumarin (**4**) in $CHCl_3$–EtOH (2:1) at reflux, followed by treatment with ammonium hydroxide (5%) gave coumarin derivaties **15a**–**h** in very good yield (70–85%). The structure of this series of derivatives was established on the basis of spectroscopic data and mass spectrometric data. IR spectroscopic analysis revealed the same diagnostic absorption bands, whereas ^1H NMR spectroscopic analysis confirmed the presence of both coumarin and thiazole heterocycle methine resonances. In addition, high resolution mass spectrometry (EI) showed accurate molecular ion peaks for all derivatives. Furthermore, one of the library members, 2*H*-chromen-2-one **15b** hemihydrate, was obtained as bright yellow needles and was analyzed by X-ray crystallography, which revealed its existence as a dimer (Fig. 4) along with a water molecule of crystallization [31].

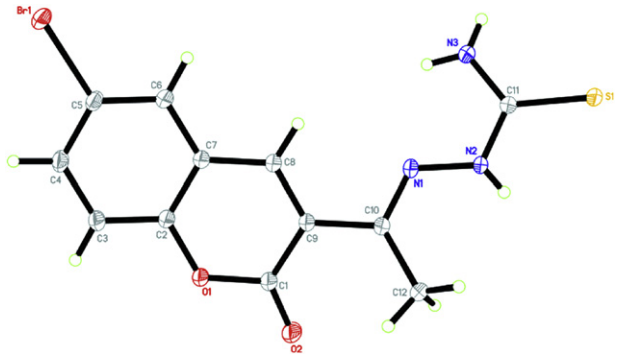


Fig. 1. ORTEP diagram of (*E*)-1-[1-(6-bromo-2-oxo-2*H*-chromen-3-yl)ethylidene] thiosemicarbazide (**9a**).

2.2. In vitro antibacterial and anti-fungal activity

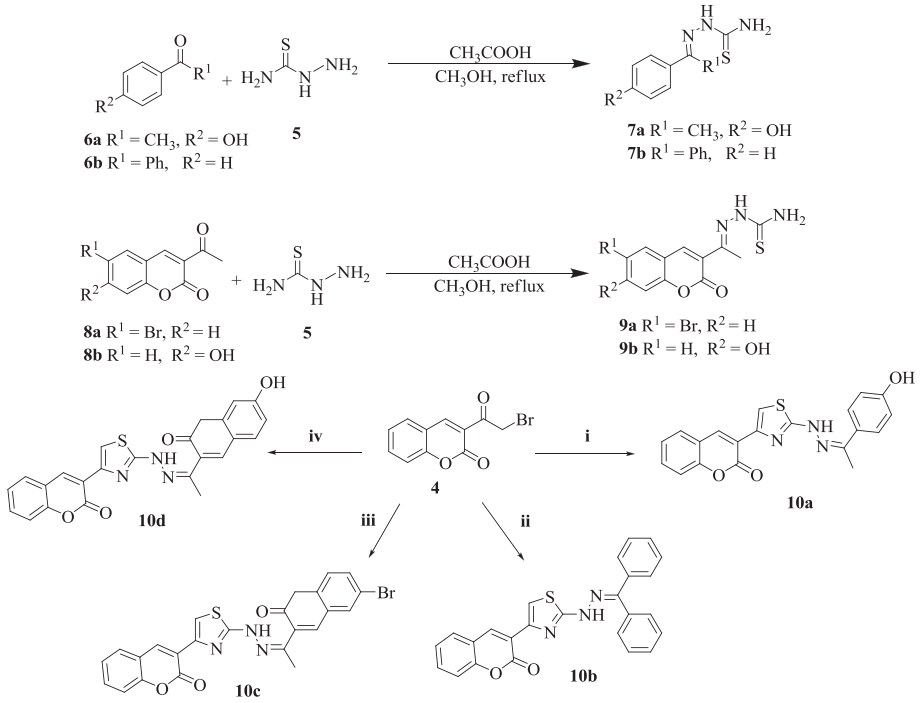
All of the synthesized compounds were evaluated for *in vitro* antimicrobial and anti-tuberculosis activity. Antibacterial activity was tested against Gram-positive and Gram-negative bacteria, including *Mycobacterium tuberculosis*, using established drugs as standards. Both series of coumarin derivatives, **10a**–**d** and **15a**–**h**, were screened for antimicrobial activity using the broth microdilution method against various microbial strains, including Gram-positive (*Staphylococcus aureus*, *Streptococcus pyogenes*) and Gram negative (*Haemphilus influenzae*) bacteria. Tetracycline and gentamicin were used as standards in order to validate methodology and also for comparison of minimum inhibition concentration (MIC) values (Table 1). It was apparent that different coumarins exhibited appropriate MIC values against different strains of bacteria. The bis-coumarin **10d** showed significant inhibition against *S. aureus* with a MIC value (17 μM) less than that of tetracycline. A number of derivatives, **10c**, **15d**, **15e** and **15h**, also exhibited MIC values comparable to those of the standards. Good activity against *S. pyogenes* was exhibited by a number of the compounds tested (**10c**, **10d**, **15e** and **15h**) with MIC values of 62–75 μM. Exceptional activity against *H. influenzae* was observed by two compounds, **10c** and **15e**, with MIC values of 15 μM and 17 μM, respectively. In addition, two other compounds, **10d** and **15a**, also exhibited significant activity against the same pathogen with MIC values of 35 and 43 μM, respectively.

The *in vitro* anti-fungal activity of coumarins **10a**–**d** and **15a**–**h** was studied against *Candida albicans* using the drug fluconazole as a reference standard. Only two compounds, **10c** (31 μM) and **15e** (35 μM) were found to have promising activity, whilst the anti-fungal activity of the other compounds was in the range of 86–331 μM. The observed anti-fungal activity profile suggested that the presence of bromine had enhanced the activity.

2.3. Anti-tuberculosis activity

The H37Rv strain of *Mycobacterium tuberculosis* was used to evaluate the anti-tuberculosis potential of these coumarin derivatives. Even though, the observed activities exhibited by the tested compounds were lower than that of the standard drug isoniazid, they demonstrated the ability to inhibit the growth of mycobacterium with minimum inhibition concentration (MIC) values from 15 to 344 μM. Significant activity was shown by compound **10c** with the lowest MIC value of 15 μM. In addition, **10d** and **15e** also exhibited good activity, both with a MIC of 17 μM.

Based on the results shown in Table 1, the introduction of a hydroxyl group at the *ortho* position probably enhances antibacterial activity. This is also supported by the comparison of MIC values for **15g** and **15h**. It was also apparent that *para*-hydroxylation reduced activity, as was evident from the MIC of compounds **10a**, **15a** and **15c**. Compound **15a**, having a hydroxyl group at the *ortho* position, exhibited significant antimicrobial activity, whereas, in the case of **10a** and **15c**, a *para*-hydroxyl group reduced activity considerably. The results also indicated that the presence of two bulky imine substituents, as observed in **10b**, decreased activity,



Scheme 2. Synthesis of the hydrazinyl thiazolyl coumarin derivatives (**10a**–**d**). Reagents and reaction conditions: (i) $C_9H_{19}N_3OS$ (**7a**), EtOH–$CHCl_3$ (1:2), reflux; NH_4OH (5%); (ii) $C_{14}H_{13}N_3S$ (**7b**), EtOH–$CHCl_3$ (1:2), reflux; NH_4OH (5%); (iii) $C_{12}H_{10}BrN_3O_2S$ (**9a**), EtOH–$CHCl_3$ (1:2), reflux; NH_4OH (5%); (iv) $C_9H_{19}N_3OS$ (**9b**), EtOH–$CHCl_3$ (1:2), reflux; NH_4OH (5%).

possibly as a consequence of a steric clash caused by the bulky phenyl groups. In addition, the introduction of bromine in the structure dramatically increased the activity of the compounds, as observed for **10c** and **15e**. These compounds have showed maximum activity against all microbial strains.

3. Conclusion

The synthesis, structure elucidation and biological evaluation of twelve novel hydrazinyl thiazolyl coumarin derivatives have been reported. All the compounds were purified by recrystallization and obtained in good overall yield. The spectroscopic properties of compounds **10a**–**d** and **15a**–**h** fully supported the proposed structures. Study of their antimicrobial properties revealed that all compounds have the ability to inhibit the growth of different strains of bacteria and fungi, to a different extent. However, inhibitors **10c**, **10d** and **15e** showed significant activity against all microbial strains. It was found that the antimicrobial activity of analogues was enhanced by bromide and hydroxyl groups. Furthermore, a hydroxyl group at the *ortho* position of a benzylidene imine was more effective than *meta* and *para* substitution patterns. All these results can be useful for future efforts to synthesize and evaluate coumarin derivatives in order to enhance their antimicrobial properties.

4. Experimental

4.1. General

Melting points were determined by using Stuart Scientific SMP1 melting point apparatus and were uncorrected. The infrared (IR) spectra were recorded on Perkin Elmer system 2000 FTIR spectrophotometer using KBr disc method. ^1H and ^{13}C spectra were recorded on a Bruker 400 MHz spectrometer at 25 °C using tetramethylsilane as an internal standard and DMSO-d_6 as the solvent. Chemical shifts were reported in ppm (δ). Splitting patterns were assigned as: s for singlet, br s for broad singlet, d for doublet, t for triplet, td for triplet of doublets, dd for doublet of doublets, ddd for doublet of doublet of doublets and m for multiplet. HREIMS spectra were obtained using a Micro TOF-Q spectrometer. Elemental analyses were performed on a Perkin Elmer series II, 2400 CHN analyzer and experimental data were within ±0.4% of the theoretical values. X-ray crystallographic data were recorded on a Bruker SMART APEX 2CCD area-detector diffractometer. All of the chemicals, including thiosemicarbazide, ethyl acetoacetate, aldehydes and ketones,

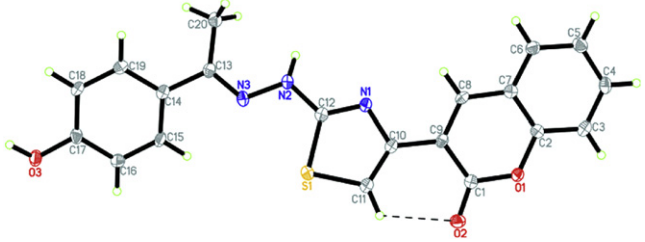


Fig. 2. ORTEP diagram of (*E*)-3-(2-{2-[1-(4-hydroxyphenyl)ethylidene]hydrazin-1-yl}-1,3-thiazol-4-yl)-2*H*-chromen-2-one (**10a**).

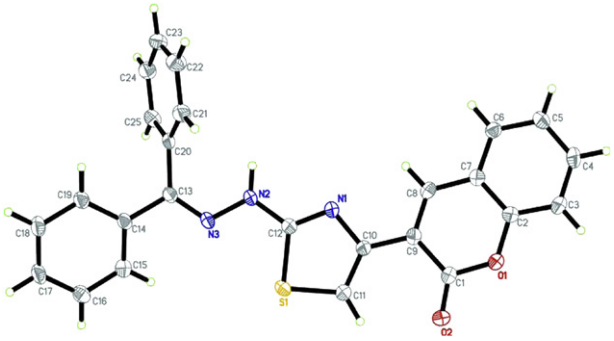
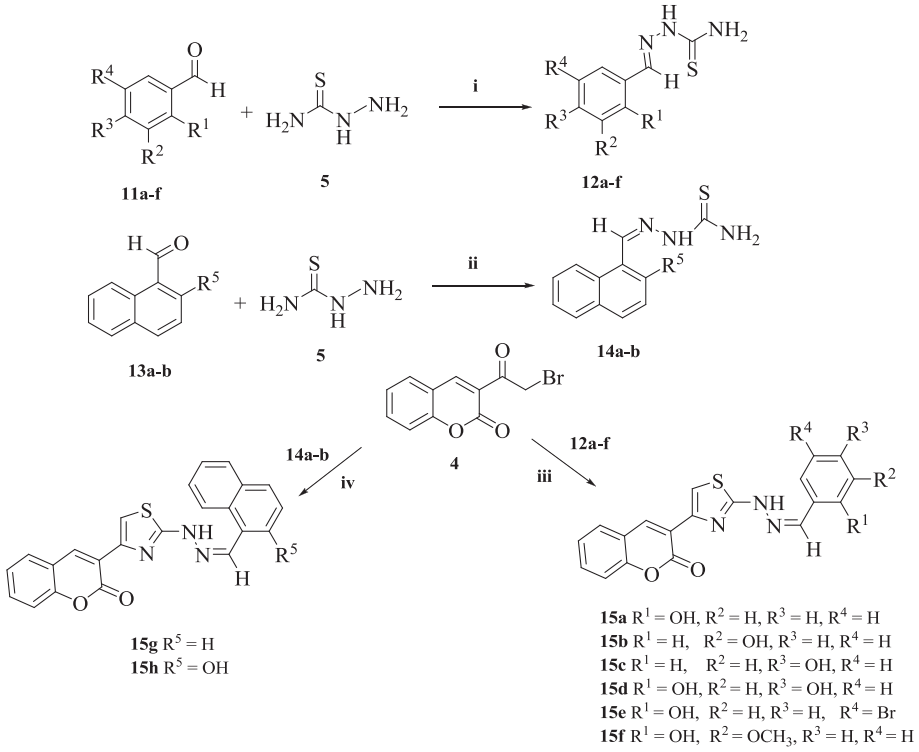


Fig. 3. ORTEP diagram of 3-{2-[2-(diphenylmethylene)hydrazinyl]-thiazol-4-yl}-2*H*-chromen-2-one (**10b**).



Scheme 3. Synthesis of the hydrazinyl thiazolyl coumarin derivatives (**15a**–**h**). Reagents and reaction conditions: (i) & (ii) CH_3OH, reflux; (iii) & (iv) $CHCl_3$:EtOH (2:1), reflux; NH_4OH (5%).

except 6-bromo-3-(1-(2-(4-(2-oxo-2*H*-chromen-3-yl)thiazol-2-yl) hydrazono)ethyl)-2*H*-chromen-2-one (**8a**) and 7-hydroxy-3-(1-(2-(4-(2-oxo-2*H*-chromen-3-yl)thiazol-2-yl)hydrazono)ethyl)-2*H*-chromen-2-one (**8b**), used in this study were purchased from Sigma–Aldrich.

4.2. Synthesis of 3-acetylcoumarin (*3*) and 3-bromoacetylcoumarin (*4*)

3-Acetylcoumarin (**3**) and 3-bromoacetylcoumarin (**4**) were prepared by the methods reported in the literature [32]. A mixture of salicylaldehyde (**1**) (25 mL, 0.204 mol) and ethyl acetoacetate (**2**) (33.3 mL, 0.255 mol) was cooled and maintained at 0–5 °C. Piperidine was added dropwise to this mixture while stirring. The reaction mixture was left overnight, resulting in the formation of a yellow coloured solid. Purification by recrystallization (EtOH) gave 3-acetylcoumarin (**3**) (30.7 g, 80%) as fine yellow needles, mp 121–125 °C (Lit. mp [22] 125–128 °C).

3-Acetylcoumarin (**3**) (28.2 g, 0.15 mol) was dissolved in alcohol free chloroform (150 mL) and a solution of bromine (7.69 mL, 0.15 mol) in chloroform (20 mL) was added dropwise from a dropping 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 and $CHCl_3$ was removed using a rotary evaporator. Purification by recrystallization (glacial acetic acid) gave 3-bromoacetylcoumarin (**4**) (29.8 g, 75%) as light yellow or off white needles, mp 162–165 °C (Lit. mp [22] 160–163 °C) as shown in Scheme 1.

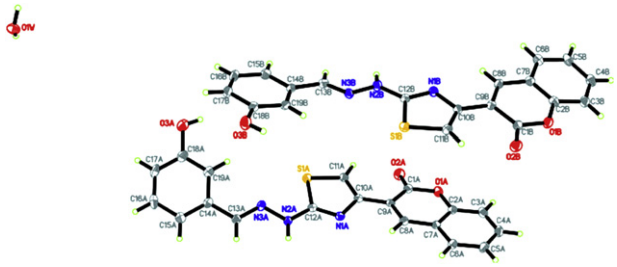


Fig. 4. ORTEP diagram of 3-{2-[2-(3-hydroxybenzylidene)hydrazin-1-yl]-1,3-thiazol-4-yl}-2*H*-chromen-2-one hemihydrates (**15b**).

4.3. General procedure for the synthesis of substituted arylideneth-iosemicabazide analogues (*7a*,*b*, *9a*,*b*) from ketones

The different thiosemicarbazones were synthesized by treating appropriate ketones with thiosemicarbazide (**5**) as reported in literature with some modifications [33]. The methanol solution of

Table 1
In vitro antimicrobial activity (μM) of hydrazinyl thiazolyl coumarin derivatives against different microbial species.

Compounds	MIC in μM				
	S. aureus	*S. pyogenes*	*H. influenzae*	*C. albicans*	*M. tuberculosis*
10a	165	165	331	331	>663
10b	295	295	147	147	147
10c	62	62	15	31	15
10d	17	72	35	140	17
15a	172	344	43	86	43
15b	172	344	172	172	172
15c	344	172	344	172	344
15d	82	329	329	165	329
15e	35	17	17	35	17
15f	159	159	79	79	79
15g	157	314	314	157	157
15h	75	75	151	151	75
Gentamicin	–	–	13	–	–
Tetracycline	70	14	35	–	–
Isoniazid	–	–	–	–	1.3
Fluconazole	–	–	–	10	–

Gram-positive bacterial strains: *S. aureus*; *S. pyogenes*; *M. tuberculosis*, Gram-negative bacteria strain: *H. influenzae*, Fungus strain: *C. albicans*.

thiosemicarbazide (**5**) (5.00 mmol) was added to a solution of appropriate ketones (5.00 mmol) in hot methanol (10 mL) with stirring. The resulting solution was refluxed for 4–6 h in the presence of glacial acetic acid (Scheme 2). The compounds (**7a**,**b**, **9a**,**b**) were purified by recrystallization from ethanol or a mixture of chloroform:ethanol.

Two ketones, 3-acetyl-6-bromo-2*H*-chromen-2-one (**8a**) and 3-acetyl-7-hydroxy-2*H*-chromen-2-one (**8b**) were prepared in the laboratory by the reaction of appropriate aldehydes with ethyl acetoacetate [34,35].

*4.4. General method for the synthesis of first series of thiazolyl coumarin derivatives (**10a**–**d**)*

Title compounds (**10a**–**d**) were obtained by the cyclocondensation of substituted thiosemicarbazide compounds (**7a**,**b**, **9a**,**b**) with 3-bromoacetylcoumarin (**4**). A solution of 3-bromoacetylcoumarin (2.5 mmol) and substituted thiosemicarbazone (2.5 mmol) in chloroform–ethanol (2:1) mixture was refluxed for 1–3 h at 60 °C to obtain dense yellow or brown precipitates (Scheme 2). The reaction mixture was cooled in ice bath and basified with ammonium hydroxide to pH 7–8. The title compounds were recrystallized from ethanol–chloroform or ethanol–ethyl acetate.

*4.4.1. (E)-3-(2-(2-(1-(4-Hydroxyphenyl)ethylidene)hydrazinyl) thiazol-4-yl)-2H-chromen-2-one (**10a**)*

Brownish yellow needle like crystals. Recrystallized from $CHCl_3$–EtOH (1:2). Yield: 80%; mp: 272–274 °C; IR KBr (cm^{-1}): 3424 (NH), 3290 (OH), 1690 (O–C=O), 1615 (C=N); ^1H NMR (δ, ppm, 400 MHz, DMSO-d_6): 11.21 (1H, br s), 9.72 (1H, br s), 8.57 (1H, s), 7.82 (1H, dd, J = 7.5, 1.3 Hz), 7.76 (1H, s), 7.65 (2H, dd, J = 8.8, 2.5 Hz), 7.60 (1H, ddd, J = 8.2, 7.5, 1.3 Hz), 7.45 (1H, d, J = 8.2 Hz), 7.39 (1H, td, J = 7.5, 0.9 Hz), 6.82 (2H, dd, J = 8.8, 2.5 Hz), 2.28 (3H, s); ^{13}C NMR (δ, ppm, 100 MHz, DMSO-d_6): 170.3, 159.6, 159.2, 153.2, 148.2, 144.9, 138.8, 132.5, 129.6, 129.6, 128.2, 128.1, 125.5, 121.6, 120.1, 116.8, 116.8, 116.1, 111.6, 14.82; Anal. Calcd. for $C_{20}H_{15}N_3O_3S$: C, 63.65; H, 4.01; N, 11.13. Found: C, 63.48; H, 3.78; N, 11.05.

*4.4.2. 3-(2-(2-(Diphenylmethylene)hydrazinyl)thiazol-4-yl)-2H-chromen-2-one (**10b**)*

Dark brown feather like crystals. Recrystallized from $CHCl_3$–EtOH (3:1). Yield: 78%; mp: 206–208 °C; IR KBr (cm^{-1}): 3421 (NH), 1716 (O–C=O), 1609 (C=N); ^1H NMR (δ, ppm, 400 MHz, DMSO-d_6): 10.58 (1H, br s), 8.52 (1H, s), 7.80 (1H, s), 7.74 (1H, dd, J = 7.4, 1.2 Hz), 7.61 (1H, ddd, J = 8.1, 7.4, 1.2 Hz), 7.59 (3H, td, J = 8.2, 2.0 Hz), 7.48 (4H, dd, J = 8.2, 2.0 Hz), 7.43 (1H, d, J = 8.1 Hz), 7.41 (1H, t, J = 7.4 Hz), 7.36 (3H, t, J = 8.2 Hz); ^{13}C NMR (δ, ppm, 100 MHz, DMSO-d_6): 169.1, 159.6, 153.2, 149.8, 144.9, 139.2, 138.1, 138.0, 133.4, 132.6, 130.3, 130.2, 130.0, 129.6, 129.5, 129.4, 128.6, 128.6, 127.5, 125.7, 125.6, 124.8, 120.0, 116.8, 112.0; Anal. Calcd for $C_{25}H_{17}N_3O_2S$: C, 70.90; H, 4.05; N, 9.92. Found: C, 70.62; H, 3.69; N, 9.69.

*4.4.3. 6-Bromo-3-(1-(2-(4-(2-oxo-2H-chromen-3-yl) thiazol-2-yl) hydrazono)ethyl)-2H-chromen-2-one (**10c**)*

Yellow solid. Recrystallized from EtOAc–EtOH (3:1). Yield: 71%; mp: 217–220 °C; IR KBr (cm^{-1}): 3412 (NH), 1718 (O–C=O), 1638 (C=N); ^1H NMR (δ, ppm, 400 MHz, DMSO-d_6): 11.52 (1H, br s), 8.59 (1H, s), 8.16 (2H, s), 7.83 (1H, dd, J = 7.7, 1.2 Hz), 7.82 (1H, s), 7.78 (1H, dd, J = 8.6, 2.4 Hz), 7.64 (1H, ddd, J = 8.2, 7.7, 1.2 Hz), 7.46 (1H, d, J = 8.6 Hz), 7.42 (1H, d, J = 8.8 Hz), 7.41 (1H, t, J = 8.4 Hz), 2.27 (3H, s); ^{13}C NMR (δ, ppm, 100 MHz, DMSO-d_6): 169.5, 159.6, 159.6, 153.2, 153.2, 145.8, 144.9, 140.3, 139.1, 135.3, 132.6, 131.9, 129.7, 128.3, 125.7, 121.7, 121.5, 120.0, 118.0, 117.2, 116.8, 114.8, 17.0; Anal. Calcd for $C_{23}H_{14}BrN_3O_4S$: C, 54.34; H, 2.78; N, 8.27. Found: C, 54.08; H, 2.43; N, 7.98.

*4.4.4. 7-Hydroxy-3-(1-(2-(4-(2-oxo-2H-chromen-3-yl)thiazol-2-yl)hydrazono)ethyl)-2H-chromen-2-one (**10d**)*

Brownish yellow solid. Recrystallized from $CHCl_3$–EtOH (2:1). Yield: 76%; mp: 250 °C decompose; IR KBr (cm^{-1}): 3474 (NH), 3414 (OH), 1698 (O–C=O), 1618 (C=N); ^1H NMR (δ, ppm, 400 MHz, DMSO-d_6): 11.37 (1H, br s), 10.67 (1H, br s), 8.59 (1H, s), 8.08 (1H, s), 7.84 (1H, dd, J = 7.6, 1.3 Hz), 7.69 (1H, s), 7.69 (1H, d, J = 8.5 Hz), 7.64 (1H, ddd, J = 8.3, 7.6, 1.3 Hz), 7.47 (1H, d, J = 8.3 Hz), 7.40 (1H, td, J = 7.6, 1.0 Hz), 6.83 (1H, dd, J = 8.5, 2.2 Hz), 6.76 (1H, d, J = 2.2 Hz), 2.27 (3H, s); ^{13}C NMR (δ, ppm, 100 MHz, DMSO-d_6): 169.7, 162.6, 160.4, 156.3, 153.2, 146.9, 144.9, 142.3, 146.1, 139.0, 132.6, 131.5, 129.6, 125.6, 122.6, 121.5, 120.0, 116.8, 114.5, 112.2, 112.0, 102.7, 17.2; Anal. Calcd for $C_{23}H_{15}N_3O_5S$: C, 62.02; H, 3.39; N, 9.43. Found: C, 61.82; H, 3.09; N, 9.07.

*4.5. General procedure for the synthesis of (substituted arylidene) thiosemicabazide compounds (**12a**–**f** and **14a**,**b**) from various substituted aldehydes*

Thiosemicarbazide (**5**) (5.00 mmol) was slowly added to a solution of substituted aldehyde in hot absolute ethanol (10 mL) while stirring (Scheme 2). The resulting solution was refluxed for 2–4 h and then cooled in ice bath for 45 min to get white to pale yellow precipitates. These precipitates were filtered and recrystallized from ethanol or a mixture of chloroform:ethanol [36].

*4.6. General method for the synthesis of second series of thiazolyl coumarin derivatives (**15a**–**h**)*

A solution of 3-bromoacetylcoumarin (**4**) (2.5 mmol) and substituted thiosemicarbazide compounds (**12a**–**f** and **14a**,**b**) (2.5 mmol) in chloroform–ethanol (2:1) mixture was refluxed for 1–4 h at 80 °C (Scheme 2). Initially, clear solution was formed followed by the deposition of thick yellow precipitates. The reaction mixture was cooled and treated with ammonium hydroxide (5%). The title compounds (**15a**–**h**) were purified and recrystallized from the mixture of ethanol: ethyl acetate or ethanol: chloroform.

*4.6.1. 3-(2-(2-(2-Hydroxybenzylidene)hydrazinyl)thiazol-4-yl)-2H-chromen-2-one (**15a**)*

Yellow solid. Recrystallized from $CHCl_3$–EtOH (1:3). Yield: 70%; mp: 270–272 °C; IR KBr (cm^{-1}): 3420 (NH), 3212 (OH), 1699 (O–C=O), 1603 (C=N); ^1H NMR (δ, ppm, 400 MHz, DMSO-d_6): 12.15 (1H, br s), 10.12 (1H, br s), 8.55 (1H, s), 8.37 (1H, s), 7.86 (1H, dd, J = 7.5, 1.2 Hz), 7.77 (1H, s), 7.65 (1H, dd, J = 8.0, 1.1 Hz), 7.64 (1H, ddd, J = 8.2, 7.5, 1.2 Hz), 7.46 (1H, d, J = 8.2 Hz), 7.41 (1H, t, J = 7.5 Hz), 7.23 (1H, td, J = 8.0, 1.1 Hz), 6.91 (1H, d, J = 8.0 Hz), 6.89 (1H, t, J = 8.0 Hz); ^{13}C NMR (δ, ppm, 100 MHz, DMSO-d_6): 168.3, 159.6, 156.8, 153.2, 144.9, 140.7, 139.1, 132.6, 131.5, 129.7, 127.1, 125.6, 121.4, 120.9, 120.4, 120.0, 117.0, 116.8, 111.3; MS (ESI): m/z (100%) = 364.0750 (MH^+). $C_{19}H_{14}N_3O_3S$ (MH^+) requires 364.0750.

*4.6.2. 3-(2-(2-(3-Hydroxybenzylidene)hydrazinyl)thiazol-4-yl)-2H-chromen-2-one (**15b**)*

Shiny bright yellow crystals. Recrystallized from EtOAc–EtOH (1:2). Yield: 72%; mp: 254–256 °C; IR KBr (cm^{-1}): 3365 (NH), 3262 (OH), 1698 (O–C=O), 1602 (C=N); ^1H NMR (δ, ppm, 400 MHz, DMSO-d_6): 12.15 (1H, br s), 9.62 (1H, br s), 8.60 (1H, s), 7.98 (1H, s), 7.86 (1H, dd, J = 7.6, 0.9 Hz), 7.78 (1H, s), 7.63 (1H, ddd, J = 8.2, 7.6, 0.9 Hz), 7.46 (1H, d, J = 8.2 Hz), 7.42 (1H, t, J = 7.6 Hz), 7.23 (1H, t, J = 7.8 Hz), 7.12 (1H, s), 7.05 (1H, d, J = 7.8 Hz), 6.80 (1H, dd, J = 7.8, 2.4 Hz); ^{13}C NMR (δ, ppm, 100 MHz, DMSO-d_6): 168.6, 159.6, 158.5, 153.2, 144.8, 142.8, 139.0, 136.4, 132.6, 130.8, 129.7, 125.6, 121.4,

120.1, 118.9, 117.7, 116.8, 114.5, 112.8; MS (ESI): *m/z* (100%) = 364.0750 (MH^+). $C_{19}H_{14}N_3O_3S$ (MH^+) requires 364.0750.

4.6.3. 3-(2-(2-(4-Hydroxybenzylidene)hydrazinyl)thiazol-4-yl)-2H-chromen-2-one (**15c**)

Dark brown small crystals. Recrystallized from $CHCl_3$–EtOH (1:3). Yield: 80%; mp: 249–250 °C; IR KBr (cm^{-1}): 3424 (NH), 3212 (OH), 1706 (O–C=O), 1605 (C=N); ^1H NMR (δ, ppm, 400 MHz, DMSO-d_6): 12.05 (1H, br s), 10.12 (1H, br s), 8.53 (1H, s), 7.97 (1H, s), 7.84 (1H, dd, *J* = 7.6, 1.0 Hz), 7.74 (1H, s), 7.62 (1H, ddd, *J* = 8.2, 7.6, 1.0 Hz), 7.51 (2H, d, *J* = 8.6 Hz), 7.48 (1H, d, *J* = 8.2 Hz), 7.38 (1H, t, *J* = 7.6 Hz), 6.82 (2H, d, *J* = 8.6 Hz); ^{13}C NMR (δ, ppm, 100 MHz, DMSO-d_6): 168.7, 159.7, 159.6, 153.2, 144.8, 143.1, 138.9, 132.5, 129.7, 128.9, 126.2, 126.2, 125.6, 121.4, 120.1, 116.7, 116.6, 116.6, 111.1; MS (ESI): *m/z* (100%) = 364.0750 (MH^+). $C_{19}H_{14}N_3O_3S$ (MH^+) requires 364.0750.

4.6.4. 3-(2-(2-(2,4-Dihydroxybenzylidene)hydrazinyl)thiazol-4-yl)-2H-chromen-2-one (**15d**)

Yellow fluffy solid. Recrystallized from EtOH. Yield: 72%; mp: 271–274 °C; IR KBr (cm^{-1}): 3466 (NH), 3270 (OH), 1697 (O–C=O), 1608 (C=N); ^1H NMR (δ, ppm, 400 MHz, DMSO-d_6): 11.95 (1H, br s), 10.15 (1H, br s), 9.84 (1H, br s), 8.54 (1H, s), 8.25 (1H, s), 7.86 (1H, dd, *J* = 7.4, 1.2 Hz), 7.74 (1H, s), 7.63 (1H, ddd, *J* = 8.5, 7.4, 1.2 Hz), 7.47 (1H, d, *J* = 8.6 Hz), 7.41 (1H, s), 7.39 (1H, t, *J* = 7.4 Hz), 6.34 (2H, dd, *J* = 7.5, 2.1 Hz); ^{13}C NMR (δ, ppm, 100 MHz, DMSO-d_6): 168.3, 161.0, 159.6, 158.6, 153.2, 142.4, 139.0, 138.3, 132.5, 129.7, 129.2, 125.6, 121.4, 120.1, 116.8, 112.4, 110.8, 108.8, 103.4; MS (ESI): *m/z* (100%) = 380.0700 (MH^+). $C_{19}H_{14}N_3O_4S$ (MH^+) requires 380.0700.

4.6.5. 3-(2-(2-(2-Hydroxy-5-bromobenzylidene)hydrazinyl) thiazol-4-yl)-2H-chromen-2-one (**15e**)

Bright yellow fluffy solid. Recrystallized from EtOAc–EtOH (3:1). Yield: 78%; mp: 296–298 °C; IR KBr (cm^{-1}): 3430 (NH), 3260 (OH), 1701 (O–C=O), 1583 (C=N); ^1H NMR (δ, ppm, 400 MHz, DMSO-d_6): 12.27 (1H, br s), 10.42 (1H, br s), 8.54 (1H, s), 8.29 (1H, s), 7.85 (1H, dd, *J* = 7.5, 1.1 Hz), 7.78 (1H, s), 7.77 (1H, d, *J* = 2.5 Hz), 7.65 (1H, ddd, *J* = 8.3, 7.5, 1.1 Hz), 7.47 (1H, d, *J* = 8.3 Hz), 7.40 (1H, t, *J* = 7.5 Hz), 7.36 (1H, dd, *J* = 8.5, 2.5 Hz), 6.85 (1H, d, *J* = 8.5 Hz); ^{13}C NMR (δ, ppm, 100 MHz, DMSO-d_6): 168.3, 159.6, 155.9, 153.2, 139.1, 138.1, 133.6, 132.6, 129.7, 128.3, 125.6, 123.6, 121.4, 121.4, 120.0, 119.3, 116.8, 111.7, 111.5; MS (ESI): *m/z* (100%) = 463.9696 (MNa^+). $C_{19}H_{12}BrN_3NaO_3S$ (MNa^+) requires 463.9675.

4.6.6. 3-(2-(2-(2-Hydroxy-3-methoxybenzylidene)hydrazinyl) thiazol-4-yl)-2H-chromen-2-one (**15f**)

Brown shiny solid. Recrystallized from EtOAc–EtOH (2:1). Yield: 70%; mp: 266 °C; IR KBr (cm^{-1}): 3445 (NH), 3239 (OH), 1704 (O–C=O), 1600 (C=N); ^1H NMR (δ, ppm, 400 MHz, DMSO-d_6): 12.17 (1H, br s), 9.45 (1H, br s), 8.55 (1H, s), 8.39 (1H, s), 7.86 (1H, dd, *J* = 7.5, 0.9 Hz), 7.77 (1H, s), 7.63 (1H, ddd, *J* = 8.3, 7.5, 0.9 Hz), 7.46 (1H, d, *J* = 8.3 Hz), 7.42 (1H, t, *J* = 7.5 Hz), 7.27 (1H, d, *J* = 8.0 Hz), 6.98 (1H, d, *J* = 8.0 Hz), 6.84 (1H, t, *J* = 8.0 Hz), 3.83 (3H, s); ^{13}C NMR (δ, ppm, 100 MHz, DMSO-d_6): 168.4, 159.6, 153.2, 148.9, 146.3, 140.5, 139.1, 132.6, 129.7, 125.6, 123.3, 121.4, 121.4, 120.1, 120.1, 118.5, 116.8, 113.4, 111.3, 56.7; MS (ESI): *m/z* (100%) = 392.0696 ([M–H^+]). $C_{20}H_{14}N_3O_4S$ ([M–H^+]) requires 392.0700.

4.6.7. 3-(2-(2-(1-Naphthylidene)hydrazinyl)thiazol-4-yl)-2H-chromen-2-one (**15g**)

Dark yellow powder. Recrystallized from EtOAc–EtOH (3:1). Yield: 70%; mp: 262–265 °C (Lit. mp and yield [37] 240–242 °C, 70%); IR KBr (cm^{-1}): 3424 (NH), 1702 (O–C=O), 1594 (C=N); ^1H NMR (δ, ppm, 400 MHz, DMSO-d_6): 12.31 (1H, br s), 8.77 (1H, d, *J* = 8.5 Hz), 8.71 (1H, s), 8.58 (1H, s), 8.01 (2H, t, *J* = 7.8 Hz), 7.87 (2H, d, *J* = 7.6 Hz), 7.82 (1H, s), 7.69 (1H, ddd, *J* = 8.1, 7.5, 0.9 Hz), 7.64 (1H, t, *J* = 8.5 Hz), 7.62 (2H, dd, *J* = 7.8, 1.8 Hz), 7.46 (1H, d, *J* = 8.1 Hz), 7.41 (1H, t, *J* = 7.5 Hz); ^{13}C NMR (δ, ppm, 100 MHz, DMSO-d_6): 168.5, 159.6, 153.2, 145.0, 142.7, 139.1, 134.5, 132.6, 130.8, 130.7, 130.3, 129.7, 128.1, 128.0, 127.1, 126.8, 126.5, 125.6, 124.9, 121.4, 120.1, 116.7, 111.6; MS (ESI): *m/z* (100%) = 420.0778 (MNa^+). $C_{23}H_{15}N_3NaO_2S$ (MNa^+) requires 420.0777.

4.6.8. 3-(2-(2-(2-Hydroxy-1-naphthylidene)hydrazinyl)thiazol-4-yl)-2H-chromen-2-one (**15h**)

Brown shiny powder. Recrystallized from EtOAc–EtOH (2:1). Yield: 75%; mp: 272–274 °C; IR KBr (cm^{-1}): 3439 (NH), 3207 (NH), 1705 (O–C=O), 1602 (C=N); ^1H NMR (δ, ppm, 400 MHz, DMSO-d_6): 12.26 (1H, br s), 10.95 (1H, br s), 8.99 (1H, s), 8.78 (1H, d, *J* = 8.6 Hz), 8.59 (1H, s), 7.89–7.84 (3H, m), 7.82 (1H, s), 7.65 (1H, ddd, *J* = 8.2, 7.5, 1.5 Hz), 7.58 (1H, t, *J* = 7.5 Hz), 7.47 (1H, d, *J* = 8.2 Hz), 7.41 (2H, ddd, *J* = 8.2, 7.2, 4.0 Hz), 7.24 (1H, d, *J* = 8.4 Hz); ^{13}C NMR (δ, ppm, 100 MHz, DMSO-d_6): 168.0, 159.6, 157.3, 153.2, 145.2, 145.1, 139.2, 132.9, 132.6, 131.9, 129.8, 129.7, 129.1, 128.6, 125.6, 124.3, 124.0, 121.4, 120.1, 119.1, 116.8, 111.2, 111.1; MS (ESI): *m/z* (100%) = 436.0739 (MNa^+). $C_{23}H_{15}N_3NaO_3S$ (MNa^+) requires 436.0726.

4.7. Biological activities

4.7.1. In vitro antibacterial and anti-fungal activity

Antimicrobial activity of all synthesized compounds was assessed against two Gram-positive bacteria (*S. aureus* ATCC 25923, *S. pyogenes* ATCC 19615), one Gram-negative bacterium (*H. influenzae* ATCC 10211) and a fungal strain (*C. albicans* ATCC 10231). The minimum inhibition concentration (MIC) of the compounds was determined by broth micro-dilution method [38]. The microbial suspensions were prepared in Muller-Hinton broth from test organisms sub-cultured on nutrient agar and incubated at 37 °C for 48 h. The turbidity of the microbial suspensions was adjusted to McFarland standard number 0.5. All compounds were dissolved in DMSO and further diluted with water to prepare the working solution of 500 μg/mL concentration. Serial two-fold dilutions were made in the range of 3.91–250 μg/mL in 96 well micro-titre plates and inoculated with 100 μL of the respective microbial strains. Two standard drugs, gentamicin and tetracycline were used as positive controls while DMSO was used as a negative control in this assay. The micro-titre plates were then sealed with parafilm and incubated at 37 °C for 24 h. After incubation, MTT [3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide] (50 μL, 0.2 mg/mL dissolved in sterilized distilled water) was added to each well and plates were incubated again at 37 °C for 30 min. The colour of MTT changed from yellow to purple in the presence of biologically active microorganisms. The MIC was determined as the lowest concentration of compounds required to prevent the colour changes from yellow to purple.

4.7.2. Anti-tuberculosis activity

A well characterized virulent strain, *M. tuberculosis* H37Rv ATCC 27294 was used for the screening of anti-tuberculosis activity. The mycobacterium inoculum was prepared from a log phase culture in Middle brook 7H9 broth (Difco, USA) supplemented with albumin, dextrose, and catalase (ADC) (Difco, USA), and its turbidity was adjusted to McFarland standard no. 1 (approximately 3×10^7 CFU/mL). The bacterial suspension was further diluted 1:20 in Middle brook 7H9 broth supplemented with OADC. The anti-tuberculosis activity was evaluated by a colourimetric tetrazolium micro plate assay (TEMA) as reported in the literature [39] with some modifications. First, sterile distilled water (200 μL) was added to all outer wells of a sterile 96-well micro plate (TPP, Germany). One hundred micro litre of Middle brook 7H9 broth supplemented oleic acid,

albumin, dextrose and catalase (OADC) (Difco, USA) was added into the wells in columns F to B, rows 2 to 11. Then, 100 μL of each compound's working solution (500 μg/mL) was added into the wells in columns G and F, rows 2 to11 in triplicates. Two-fold serial dilutions of the compounds were made within the range of 3.91–250 μg/mL 100 μL of *M. tuberculosis* inoculum was added into the wells in columns G to B, rows 2 to 11. The wells in column B served as inoculum-growth controls with no test compounds. Isoniazid, a standard drug for tuberculosis was used as a positive control. The micro-titre plates were then sealed with parafilm and incubated at 37 °C in 8% CO_2 for 5 days. On day 5, 50 μL of tetrazolium-Tween 80 mixture (1.5 mL of 1 mg/mL MTT in absolute ethanol and 1.5 mL of 10% Tween 80) was added into well B2 and incubated again for 24 h. If well B2 turned purple, the reagent mixture was added into all wells and MIC were recorded on the following day.

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