Synthesis and *in Vitro* Study of Novel Bis-[3-(2-arylmethylidenimino-1,3-thiazol-4-yl)-4-hydroxy-*2H*-chromen-2-one-6-yl]methane and Bis-[3-(2-arylidenhydrazo-1,3-thiazol-4-yl)-4-hydroxy-*2H*-chromen-2-one-6-yl]methane as Potential Antimicrobial Agents

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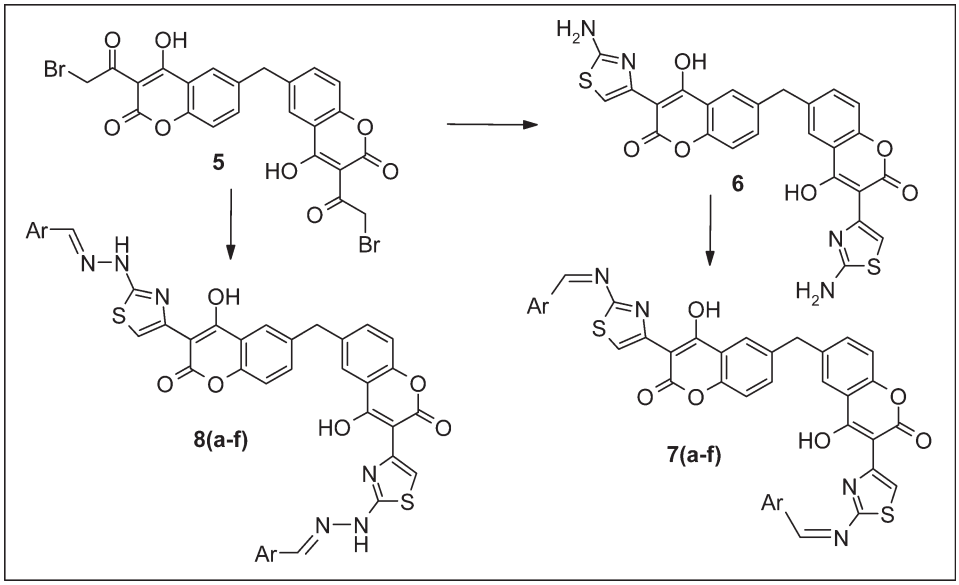

A series of novel bis-[3-(2-arylmethylidenimino-1,3-thiazol-4-yl)-4-hydroxy-*2H*-chromen-2-one-6-yl]methane **7a-f** and bis-[3-(2-arylidenhydrazo-1,3-thiazol-4-yl)-4-hydroxy-*2H*-chromen-2-one-6-yl]-methane **8a-f** were synthesized in good yields from bis-[3-(2-bromoacetyl-4-hydroxy-*2H*-chromen-2-one-6-yl]methane **5**. The chemical structures of the newly synthesized compounds were elucidated by their IR, ^{1}H NMR, MS, and elemental analyses. Further, all the compounds were screened for their antimicrobial activity against Gram-positive, Gram-negative bacteria, and fungi. Among the synthesized compounds, **7c**, **7e,** and **8c** were found to be the most active against almost all the test bacteria. The compound **8c** displayed notable antibacterial activity against *Bacillus subtilis* (ATCC 6633), *Staphylococcus aureus* (ATCC6538p), *Micrococcus luteus* (IFC 12708), *Proteus vulgaris* (ATCC 3851) and *Salmonella typhimurium* (ATCC 14028), equal to that of ampicillin. Similarly these compounds also showed potent antifungal effect against *Candida albicans* (ATCC 10231), *Aspergillus fumigatus* (HIC 6094), *Trichophyton rubrum* (IFO 9185), and *Trichophyton mentagrophytes* (IFO 40996).

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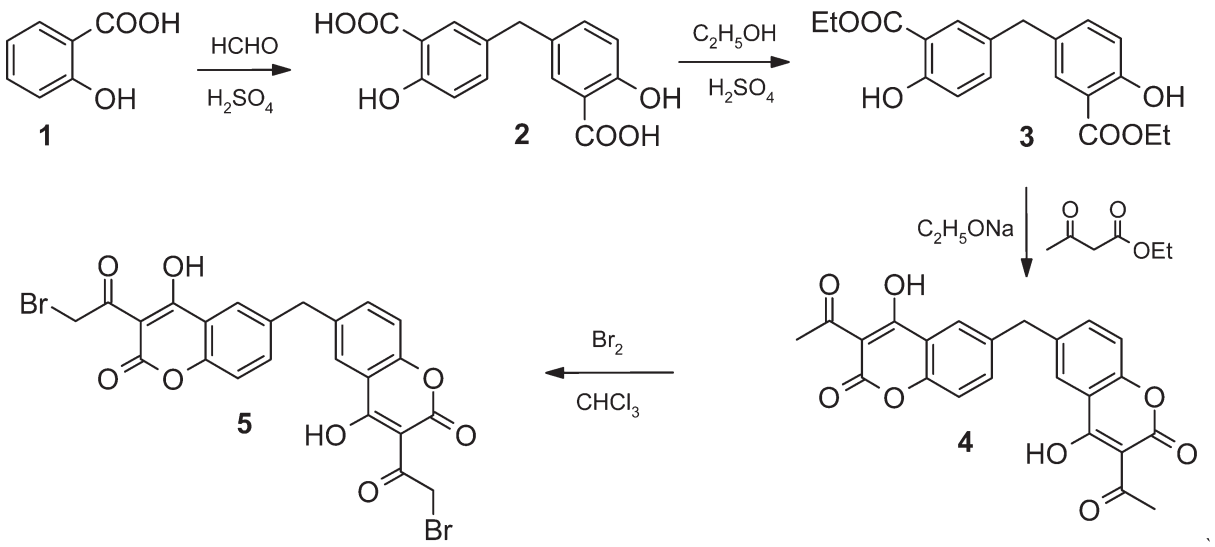
INTRODUCTION

The treatment of infectious diseases still remains an important and challenging problem because of a combination of factors including emerging infectious diseases and the increasing number of multidrug resistant microbial pathogens. Despite a large number of antibiotics and chemotherapeutics available for medical use, at the same time the emergence of old and new antibiotic resistance created in the last decades revealed a substantial medical need for new classes of antimicrobial agents. There is a real perceived need for the discovery of new compounds endowed with antimicrobial activity, possibly acting through mechanism of action, which are distinct from those of well-known classes of antibacterial agents to which many clinically relevant pathogens are now resistant. Similarly in the recent decades, an increased incidence of fungal infections has been observed as a consequence of the growing number of immunocompromised patients and the frequent use of antibacterial and cytotoxic drugs. For many fungal

Scheme 1



infections, polyenes, such as amphotericin B, represent the standard therapy. Polyenes bind to membrane sterols, leading to membrane permeability, leakage, and cell death. However, the clinical use of amphotericin B is limited by a high frequency of renal toxicity, and several adverse effects [1]. Although the various molecules were designed and synthesized for the above aim and to reduce the adverse effects, it was demonstrated that azoles and thiazoles, such as fluconazole, which act on ergosterol biosynthesis, offer several advantages in terms of decreased toxicity after oral or intravenous [2] administration and are often employed in the treatment of fungal infections, therefore the thiazoles, azoles, and their derivatives could be considered as possible antimicrobial agents [3].

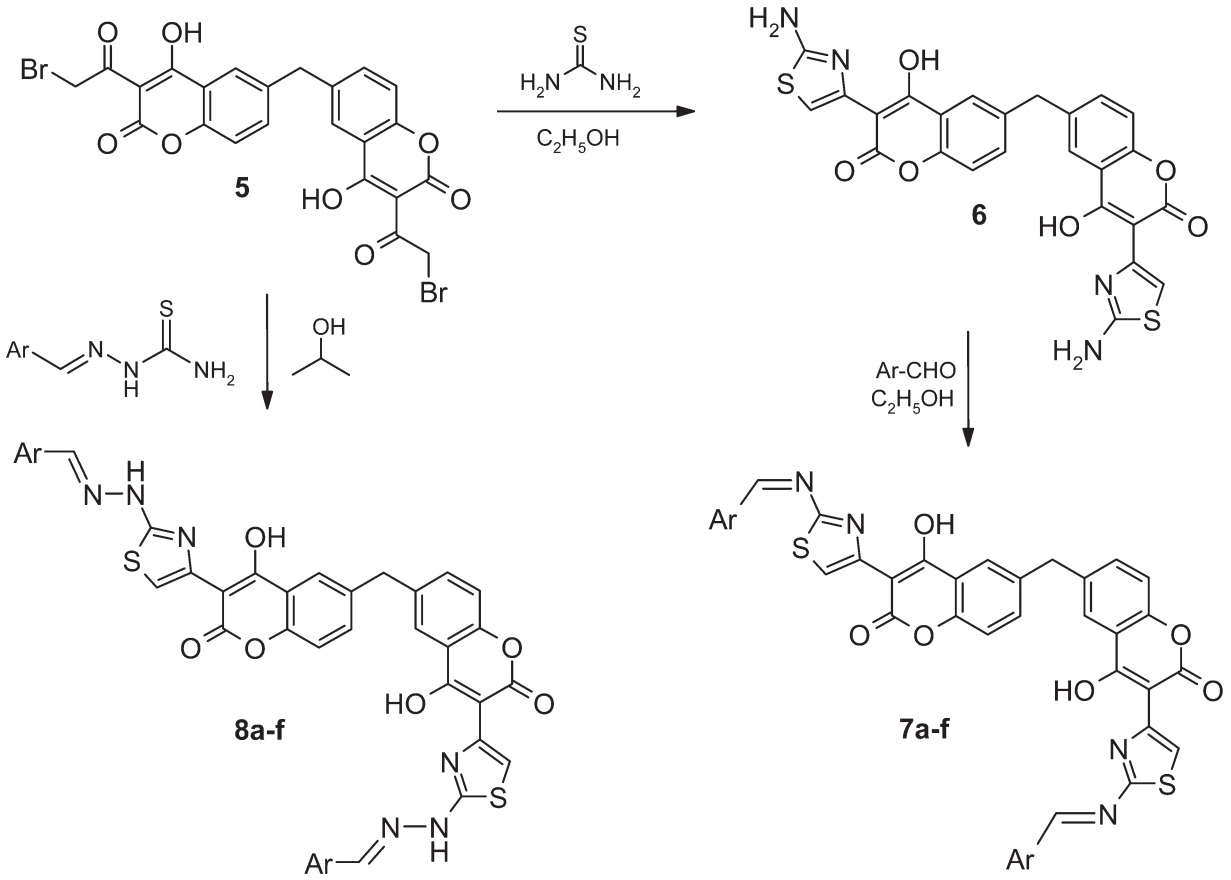
The thiazole nucleus also appears frequently in the structure of various natural products and biologically active compounds, notably thiamine (vitamin-B), antibiotics such as penicillin, micrococcin [4], troglitazone [5], and many metabolic products of fungi and primitive marine animals, including 2-(aminoalkyl)thiazole-4-carboxylic acids [6]. Similarly coumarins are a class of compounds with biological activity [7], such as analgesics [8], anticoagulant [9], specific inhibitors of α-chymotripsin [10], human leukocyte elastase [11], diuretics [12], platelet aggregation [13], anticancer [14], inhibitor of HIV-1 protease [15], and antibacterial agents [16]. Following the successful introduction of antimicrobial agents, inspired by the biological profile of thiazole and coumarin and their increasing importance in pharmaceutical and biological fields, and in continuation of our research on biologically active heterocycles [17] considering the scope to introduce thiazolyl moiety into the coumarin ring, it was thought worthwhile to undertake the synthesis of the title compounds with the view to obtain certain new chemical entities with both active pharmacophores in a single molecular frame work for the potential intensified biological activities.

In this article, we wish to report the synthesis of novel bis-[3-(2-arylmethylidenimino-1,3-thiazol-4-yl)-4-hydroxy-2*H*-chromen-2-one-6-yl]methane **7** in good yields, from bis-[3-(2-amino-1,3-thiazol-4-yl)-4-hydroxy-2*H*-chromen-2-one-6-yl]methane **6**, which in tern is synthesized by the reaction of bis-[3-(2-bromoacetyl-4-hydroxy-*2H*-chromen-2-one-6-yl]methane **5** with thiourea, and also the synthesis of novel bis-[3-(2-arylidenhydrazo-1,3-thiazol-4-yl)-4-hydroxy-*2H*-chromen-2-one-6-yl]methane **8** in good yields, from compound **5** and arylthiosemicarbazone (Schemes 1 and 2). The antibacterial and antifungal activities of the compounds **7a-f** and **8a-f** have also been evaluated.

RESULTS AND DISCUSSION

The key intermediate **5**, required for the synthesis of the title compounds was prepared according to the procedure outlined in the Scheme 1. Condensation of the salicylic acid **1** and formaldehyde in the presence of sulfuric acid gave bis-(4-hydroxy-3-carboxyphenyl)methane **2** in good yield [18]. Compound **2** was then reacted with ethyl alcohol in presence of catalytic amount of sulfuric acid to afford bis-(4-hydroxy-3-carbethoxyphenyl)methane **3** in good yield [17b], subsequently cyclocondensation of compound **3** with ethylacetoacetate in presence of sodium ethoxide gave bis-(3-acetyl-4-hydroxy-2*H*-chromen-2-one-6-yl)methane **4** [17b]. α-Bromination of compound **4** with bromine in chloroform

Scheme 2



7/8: Ar = **a**) C_6H_5; **b**) 4-NO_2-C_6H_4; **c**) 4-Br-C_6H_4; **d**) 4-MeO-C_6H_4; **e**) 3,4-(O-CH_2-O)C_6H_3; **f**) 2-furyl

at low temperature afforded bis-[3-(2-bromoacetyl-4-hydroxy-2*H*-chromen-2-one-6-yl] methane **5** in 85% yield. The cyclocondensation of compound **5** with thiourea in refluxing ethanol gave bis-[3-(2-amino-1,3-thiazol-4-yl)-4-hydroxy-2*H*-chromen-2-one-6-yl]methane **6** in 78% yield. Subsequently condensation of compound **6** with various aromatic aldehydes in refluxing ethanol gave the title compound bis-[3-(2-arylmethylideniminо-1,3-thiazol-4-yl)-4-hydroxy-2*H*-chromen-2-one-6-yl]methane **7a-f** in good to excellent yields (Scheme 2).

Further, the intermediate **5** was treated with the various arylthiosemicarbazone (prepared by the reaction of aromatic aldehydes with thiosemicarbazide in presence of sodium acetate in acetic acid) in refluxing isopropyl alcohol to give the other title compound bis-[3-(2-aryliden-hydrazo-1,3-thiazol-4-yl)-4-hydroxy-2*H*-chromen-2-one-6-yl]methane **8a-f** in good yields (Scheme 2). The structures of all the synthesized compounds were confirmed by their IR, ^{1}H NMR, MS, and elemental analyses and further screened for their antibacterial and antifungal activities.

Antibacterial activity. The *in vitro* antibacterial activity of the newly prepared compounds was screened against three representative gram-positive bacteria *viz*. *Bacillus subtilis* (ATCC 6633), *Staphylococcus aureus* (ATCC 6538p), and *Micrococcus luteus* (IFC 12708), three Gram-negative bacteria *viz*. *Proteus vulgaris* (ATCC 3851), *Salmonella typhimurium* (ATCC 14028), and *Escherichia coli* (ATCC 25922) by the broth dilution method, recommended by National Committee for Clinical Laboratory Standards (NCCLS) [19]. Bacteria were grown over night in Luria Bertani (LB) broth at 37°C, harvested by centrifugation, and then washed twice with sterile distilled water. Stock solutions of the series of compounds were prepared in DMSO. Each stock solution was diluted with standard method broth (Difco) to prepare serial two-fold dilutions in the range of 50–0.8 μg/mL. Ten microtiters of the broth containing about 10^5 colony forming units (cfu)/mL of test bacteria was added to each well of 96-well microtiter plate. Culture plates were incubated for 24 h at 37°C, and the growth was monitored visually and

Table 1

Antibacterial activity of compounds **7a-f** and **8a-f**.

Compound	Minimum inhibitory concentration (MIC, μg/mL)					
	B. subtilis	*S. aureus*	*M. luteus*	*P. vulgaris*	*S. typhimurium*	*E. coli*
7a	25.0	25.0	—	—	25.0	—
7b	12.5	12.5	12.5	12.5	12.5	25.0
7c	3.12	6.25	1.56	1.56	3.12	6.25
7d	12.5	12.5	6.25	—	12.5	25.0
7e	6.25	6.25	3.12	12.5	12.5	12.5
7f	25.0	12.5	3.12	12.5	6.25	25.0
8a	25.0	25.0	50.0	—	50.0	—
8b	6.25	12.5	12.5	25.0	12.5	25.0
8c	1.56	1.56	1.56	1.56	1.56	12.5
8d	12.5	12.5	12.5	12.5	12.5	—
8e	3.12	3.12	3.12	6.25	3.12	6.25
8f	12.5	12.5	6.25	6.25	12.5	12.5
Ampicillin	1.56	1.56	1.56	3.12	3.12	12.5

—Indicates bacteria are resistant to the compound >50 μg/mL concentration. Standard deviation 0.05.

spectrophotometrically. Amphicillin was used as a standard drug, the lowest concentration (highest dilution) required to arrest the growth of bacteria was regarded as minimum inhibitory concentration (MIC, μg/mL), was determined for all the compounds and compared with the control. The MIC values of the compounds screened are given in Table 1.

The investigation of antibacterial screening data revealed that all the tested compounds exhibited interesting biological activity however, with a degree of variation. Compound **8c** is highly active against all the microorganisms employed, except *E. coli*, at 1.56 μg/mL concentration, it is almost equal to the standard. Compound **7c** is also highly active but only against *M.luteus* and *P. vulgaris* at the same concentration as **8c**. The compound **8e** also showed good antibacterial activity against *B.subtilis*, *S. aureus*, *M. Luteus*, and *S. typhimurium*.

Compound **7a** is almost inactive towards *M. luteus*, *P. vulgaris*, and *E. coli*. The remaining compounds showed moderate to good activity.

Antifungal activity. The newly prepared compounds were screened for their antifungal activity against four fungal organism *viz*. *Candida albicans* (ATCC 10231), *Aspergillus fumigatus* (HIC 6094), *Trichophyton rubrum* (IFO 9185), and *Trichophyton mentagrophytes* (IFO 40996). *C. albicans* was grown for 48 h at 28°C in YPD broth (1% yeast extract, 2% peptone, and 2% dextrose), harvested by centrifugation and then washed twice with sterile distilled water. *A. fumigatus*, *T. rubrum*, and *T. mentagrophytes* were plated in potato dextrose agar (PDA) (Difco) and incubated at 28°C for two weeks. Spores were washed three times with sterile distilled water and resuspended in distilled water to obtain an initial inoculum size of 10^5 spores/mL. Each test compound was dissolved in DMSO and diluted with potato dextrose broth (Difco) to prepare serial two-fold dilutions in the range of 100 to 0.8 μg/mL. Ten microtiters of the broth containing about 10^3 (for yeast) and 10^4 (for filamentous fungi) cells/mL of test fungi was added to each well of a 96-well microtiter plate. Culture plates were incubated for 48 ~ 72 h at 28°C. The fungal activity of each compound was compared with standard drug Amphotericin B. Minimum inhibitory concentration (MIC, μg/mL) were measured and compared with controls; the MIC values of the compounds screened are given in Table 2.

The antifungal screening data showed only moderate activity of the test compounds. Among the screened compounds, compound **7c** is highly active against *T. rubrum*, *T. mentagrophytes*, compound **7e** is also active against only *C. albicans* and compound **8c** is highly active against *C. albicans*, *T. mentagrophytes*, the activity of these compounds are almost equal to the standard, it is interesting to note that compounds **7e** and **8c** showed good antifungal activity toward *C. albicans* at the concentration of 3.12 μg/mL, it is less than the concentration of standard.

In conclusion, a series of novel bis-[3-(2-arylmethylidenimino-1,3-thiazol-4-yl)-4-hydroxy-*2H*-chromen-2-one-6-yl]methane **7** and novel bis-[3-(2-arylidenhydrazo-1,3-thiazol-4-yl)-4-hydroxy-*2H*-chromen-2-one-6-yl]methane **8** has been designed and synthesized. The antimicrobial activity of these compounds was evaluated against various Gram-positive, Gram-negative bacteria and fungi. Among the synthesized compounds, **7c**, **7e,** and **8c** showed good activity against bacteria and fungi

Table 2

Antifungal activity of compounds **7a-f** and **8a-f**.

Compound	Minimum inhibitory concentration (MIC, μg/mL)			
	C. albicans	*A. fumigatus*	*T. rubrum*	*T. mentagropytes*
7a	—	—	—	25.0
7b	25.0	25.0	25.0	25.0
7c	12.5	6.25	3.12	3.12
7d	25.0	12.5	6.25	12.5
7e	3.12	12.5	12.5	25.0
7f	50.0	25.0	12.5	12.5
8a	25.0	25.0	12.5	25.0
8b	6.25	12.5	25.0	12.5
8c	3.12	6.25	6.25	3.12
8d	50.0	50.0	50.0	25.0
8e	12.5	25.0	6.25	25.0
8f	25.0	6.25	6.25	12.5
Amphotericin B	6.25	3.12	3.12	3.12

—Indicates fungi are resistant to the compound >50 μg/mL concentration. Standard deviation 0.05.

and emerged as potential molecules for further development.

EXPERIMENTAL

Research chemicals were purchased from either Aldrich Company or Fluka and used without further purification, or were prepared according to the procedure described in the literature. Reactions were monitored by thin layer chromatography (TLC) on silica gel plates (60 F_{254}; Merck) visualizing with ultraviolet light or iodine vapors. The yields of the products reported here are unoptimized. Melting points were determined on a Fisher-Johns apparatus and are uncorrected. IR spectra were recorded on a Perkin-Elmer FTIR 5000 spectrometer, using KBr pellets. ^{1}H NMR spectra were recorded on a Varian Gemini spectrometer, operating at 300 MHz. Chemical shifts (δ) are reported in parts per million down field from tetramethylsilane. Mass spectra were obtained on a VG micro mass 7070H spectrometer operating at 70 eV. Elemental analyses were performed on a Perkin-Elmer 240 CHN elemental analyzer.

Synthesis of bis-[3-(2-bromoacetyl-4-hydroxy-2*H*-chromen- 2-one-6-yl] methane (5). To a stirred solution of **4** (1 mmol) in chloroform (20 mL), was added drop wise a solution of bromine (3 mmol) dissolved in chloroform (20 mL) at 0–5°C. The mixture was further stirred for 4 h. After completion of the reaction, monitored by TLC, the mixture was then poured into ice cold water. Crude product was collected by filtration, washed with water, dried, and recrystallized from ethanol to give pure compound **5** in 85% of yield as red solid, mp 208–210°C; IR (KBr): ν 3480, 1728, 1680, 1587, 1180, 586 cm^{-1}; ^{1}H NMR (DMSO-d_6): δ 15.07 (2H, s, OH), 7.50 (2H, s, ArH), 7.18 (2H, d, J = 8.62 Hz, ArH), 7.14 (2H, d, J = 8.62 Hz, ArH), 4.21 (4H, s, CH_2Br), 3.98 (2H, s, CH_2); MS: m/z 578 (M^+). Anal. calcd for $C_{23}H_{14}Br_2O_8$: C, 47.78; H, 2.44. Found: C, 47.72; H, 2.46.

Synthesis of bis-[3-(2-amino-1,3-thiazol-4-yl)-4-hydroxy-2*H*-chromen-2-one-6-yl]methane (6). To a stirred solution of thiourea (3 mmol) in 1-propanol (20 mL), was added drop wise a solution of **5** (1 mmol) in 1-propanol (20 mL) over a period of 30 min. The mixture was refluxed for 3 h, and then pyridine (5 mL) was added and continued reflux for 5 h. After completion of the reaction, monitored by TLC, the solvent was removed *in vacuo*. The crude product was dried and crystallized from ethanol to give compound **6** in 78% of yield as brown solid, mp 224-26°C; IR (KBr): ν 3480-3275, 1720, 1614, 1582, 758, 638 cm^{-1}; ^{1}H NMR (DMSO-d_6): δ 7.78 (2H, s, ArH), 7.63 (2H, s, ArH), 7.40 (4H, bs, NH_2), 7.32 (2H, s, OH), 7.23 (2H, d, J = 8.72 Hz, ArH), 7.14 (2H, d, J = 8.72 Hz, ArH), 3.98 (2H, s, CH_2); MS: m/z 532 (M^+). Anal. calcd for $C_{25}H_{16}N_4O_6S_2$: C, 56.39; H, 3.03; N, 1052. Found: C, 55.82; H, 3.09; N, 10.44.

Synthesis of bis-[3-(2-arylmethylidenimino-1,3-thiazol-4-yl)-4-hydroxy-2*H*-chromen-2-one-6-yl]methane (7a-f). A mixture of aromatic aldehyde (2 mmol) and compound **6** (1 mmol) in ethanol (20 mL) was refluxed for 1 h. After completion of the reaction (monitored by TLC), the mixture was cooled and the solvent evaporated. The formed crude product was washed with cold aq. ethanol and then the product was purified by recrystallization from ethanol to afford pure compound **7**.

Bis-[3-(2-phenylmethylidenimino-1,3-thiazol-4-yl)-4-hydroxy-2*H*-chromen-2-one-6-yl]methane (7a). This compound was obtained as yellow solid; Yield 81%; Mp 254-56°C; IR (KBr): ν 3410, 3062, 1720, 1615, 1580, 1471, 1180, 638 cm^{-1}; ^{1}H NMR (DMSO-d_6): δ 11.07 (2H, s, OH), 8.47 (2H, s, ArH), 8.26 (2H, s, CH), 7.62 (2H, s, ArH), 7.38 (4H, d J = 7.2 Hz, ArH), 7.30 (4H, d, J = 7.2 Hz, ArH), 7.19 (2H, d, J = 8.72 Hz, ArH), 7.17 (4H, s, ArH), 3.99 (2H, s CH_2); MS: m/z 532 (M^+). Anal. calcd. for $C_{39}H_{24}N_4O_6S_2$: C, 66.09; H, 3.41; N, 7.90. Found: C, 66.00; H, 3.36; N, 7.92.

Bis-[3-(2-(4-nitrophenyl)methylidenimino-1,3-thiazol-4-yl)-4-hydroxy-2*H*-chromen-2-one-6-yl]methane (7b). This compound was obtained as brown solid; Yield 76%; Mp 247-49°C;

IR (KBr): ν 3410, 3065, 1728, 1612, 1580, 1472, 1345, 1180, 638 cm^{-1}; ^1H NMR (DMSO-d_6): δ 11.72 (2H, s, OH), 8.46 (2H, s, ArH), 8.28 (2H, s, CH), 8.24 (4H, d, J = 8.5 Hz, ArH), 7.72 (4H, d, J = 8.5 Hz, ArH), 7.62 (2H, s, ArH), 7.20 (2H, d, J = 8.72 Hz, ArH), 7.17 (2H, d, J = 8.7 Hz, ArH), 4.00 (2H, s CH_2); MS: m/z 799 (M^+ +1). Anal. calcd. for $C_{39}H_{22}N_6O_{10}S_2$: C, 58.65; H, 2.78; N, 10.52. Found: C, 58.60; H, 2.80; N, 10.46.

Bis-[3-(2-(4-bromophenyl)methylidenimino-1,3-thiazol-4-yl)-4-hydroxy-2*H*-chromen-2-one-6-yl]methane (7c). This compound was obtained as yellow solid; Yield 75%; Mp 223-25°C; IR (KBr): ν 3410, 3065, 1720, 1616, 1582, 1474, 1180, 638, 586 cm^{-1}; ^1H NMR (DMSO-d_6): δ 11.72 (2H, s, OH), 8.46 (2H, s, ArH), 8.28 (2H, s, CH), 7.62 (2H, s, ArH), 7.49 (4H, d, J = 8.5 Hz, ArH), 7.38 (4H, d, J = 8.3 Hz, ArH), 7.20 (2H, d, J = 8.72 Hz, ArH), 7.17 (2H, d, J = 8.7 Hz, ArH), 4.00 (2H, s CH_2); MS: m/z 866 (M^+). Anal. calcd. for $C_{39}H_{22}Br_2N_4O_6S_2$: C, 54.06; H, 2.56; N, 6.47. Found: C, 54.00; H, 2.51; N, 6.50.

Bis-[3-(2-(4-methoxyphenylmethylidenimino-1,3-thiazol-4-yl)-4-hydroxy-2*H*-chromen-2-one-6-yl]methane (7d). This compound was obtained as red solid; Yield 74%; Mp 252-54°C; IR (KBr): ν 3410, 3065, 1728, 1615, 1584, 1472, 1250, 1180, 638 cm^{-1}; ^1H NMR (DMSO-d_6): δ 11.42 (2H, s, OH), 8.49 (2H, s, ArH), 8.34 (2H, s, CH), 7.62 (2H, s, ArH), 7.21 (2H, d, J = 8.52 Hz, ArH), 7.14 (2H, d, J = 8.52 Hz, ArH), 7.09 (4H, d, J = 8.51 Hz, ArH), 6.72 (4H, d, J = 8.51 Hz, ArH), 4.00 (2H, s CH_2), 3.79 (6H, s, OCH_3); MS: m/z 768 (M^+). Anal. calcd. for $C_{41}H_{28}N_4O_8S_2$: C, 64.05; H, 3.67; N, 7.29. Found: C, 64.09; H, 3.64; N, 7.22.

Bis-[3-(2-(3,4-methylendioxyphenyl)methylidenimino-1,3-thiazol-4-yl)-4-hydroxy-2*H*-chromen-2-one-6-yl]methane (7e). This compound was obtained as black solid; Yield 76%; Mp 262-64°C; IR (KBr): ν 3415, 3062, 1728, 1615, 1584, 638 cm^{-1}; ^1H NMR (DMSO-d_6): δ 11.59 (2H, s, OH), 8.52 (2H, s, ArH), 8.30 (2H, s, CH), 7.62 (2H, s, ArH), 7.20-7.18 (2H, m, ArH), 7.12 (2H, d, J = 8.52 Hz, ArH), 6.87 (2H, d, J = 7.56 Hz, ArH), 6.80 (2H, d, J = 7.56 Hz, ArH), 5.81 (4H, s, CH_2), 4.00 (2H, s, CH_2); MS: m/z 796 (M^+). Anal. calcd. for $C_{41}H_{24}N_4O_{10}S_2$: C, 61.81; H, 3.04; N, 7.03. Found: C, 61.76; H, 2.99; N, 7.08.

Bis-[3-(2-(2-furyl)methylidenimino-1,3-thiazol-4-yl)-4-hydroxy-2*H*-chromen-2-one-6-yl]methane (7f). This compound was obtained as black solid; Yield 82%; Mp 271-73 °C; IR (KBr): ν 3400, 3065, 1728, 1615, 1584, 1130, 1070, 638 cm^{-1}; ^1H NMR (DMSO-d_6): δ 11.78 (2H, s, OH), 8.52 (2H, s, ArH), 8.12 (2H, s, CH), 7.62 (2H, s, ArH), 7.52 (2H, d, J = 3.2 Hz, ArH), 7.19 (2H, d, J = 7.52 Hz, ArH), 7.17 (2H, d, J = 7.52 Hz, ArH), 6.76 (2H, d, J = 1.56 Hz, ArH), 6.47 (2H, m, ArH), 4.00 (2H, s, CH_2); MS: m/z 688 (M^+). Anal. calcd. for $C_{35}H_{20}N_4O_8S_2$: C, 61.04; H, 2.93; N, 8.14. Found: C, 61.00; H, 3.05; N, 8.11.

Bis-[3-(2-arylidenhydrazo-1,3-thiazol-4-yl)-4-hydroxy-2*H*-chromen-2-one-6-yl]methane (8a-f). A mixture of arylthiosemicarbazone (2 mmol) and compound **5** (1 m mol) in isopropylalcohol (20 mL) was refluxed for 1.5 h. When the foaming product was formed, the mixture was allowed to cool and the solid filtered, purified by recrystallization from aq. ethanol afforded pure compound **8**.

Bis-[3-(2-phenylidenhydrazo-1,3-thiazol-4-yl)-4-hydroxy-2*H*-chromen-2-one-6-yl]methane (8a). This compound was obtained as yellow solid; Yield 76%; Mp 222-24°C; IR (KBr): ν 3400-3300, 3065, 1720, 1614, 1580, 1471, 1180, 638 cm^{-1}; ^1H NMR (DMSO-d_6): δ 11.20 (2H, s, OH), 9.52 (2H, s, NH), 8.19 (2H, s, CH), 7.67 (2H, s, ArH), 7.56 (2H, s, ArH), 7.35-7.25 (10H, m, ArH), 7.23 (2H, d, J = 8.7 Hz, ArH), 7.10 (2H, d, J = 8.7 Hz, ArH), 4.00 (2H, s, CH_2); MS: m/z 738 (M^+). Anal. calcd. for $C_{39}H_{26}N_6O_6S_2$: C, 63.41; H, 3.55; N, 11.38. Found: C, 63.45; H, 3.49; N, 11.29.

Bis-[3-(2-(4-nitrophenyliden)hydrazo-1,3-thiazol-4-yl)-4-hydroxy-2*H*-chromen-2-one-6-yl]methane (8b). This compound was obtained as red solid; Yield 72%; Mp 272-74°C; IR (KBr): ν 3400-3300, 3065, 1720, 1614, 1580, 1471, 1365, 1180, 638 cm^{-1}; ^1H NMR (DMSO-d_6): δ 11.20 (2H, s, OH), 9.52 (2H, s, NH), 8.30 (2H, d, J = 8.4 Hz, ArH), 8.21 (2H, s, CH), 7.81 (2H, d, J = 8.4 Hz, ArH), 7.67 (2H, s, ArH), 7.52 (2H, s, ArH), 7.23 (2H, d, J = 8.7 Hz, ArH), 7.12 (2H, d, J = 8.7 Hz, ArH), 4.00 (2H, s, CH_2); MS: m/z 828 (M^+). Anal. calcd. for $C_{39}H_{24}N_8O_{10}S_2$: C, 56.52; H, 2.92; N, 13.52. Found: C, 56.46; H, 2.98; N, 13.46.

Bis-[3-(2-(4-bromophenyliden)hydrazo-1,3-thiazol-4-yl)-4-hydroxy-2*H*-chromen-2-one-6-yl]methane (8c). This compound was obtained as brown solid; Yield 70%; Mp 239-41°C; IR (KBr): ν 3400-3300, 3062, 1720, 1615, 1580, 1471, 1180, 638, 586 cm^{-1}; ^1H NMR (DMSO-d_6): δ 11.20 (2H, s, OH), 9.52 (2H, s, NH), 8.21 (2H, s, CH), 7.67 (2H, s, ArH), 7.52 (2H, s, ArH), 7.42 (4H, d, J = 8.3 Hz, ArH), 7.38 (4H, d, J = 8.3 Hz, ArH), 7.23 (2H, d, J = 8.7 Hz, ArH), 7.12 (2H, d, J = 8.7 Hz, ArH), 4.00 (2H, s, CH_2); MS: m/z 896 (M^+). Anal. calcd. for $C_{39}H_{24}Br_2N_6O_6S_2$: C, 52.25; H, 2.70; N, 9.37. Found: C, 52.20; H, 2.72; N, 9.31.

Bis-[3-(2-(4-methoxyphenyliden)hydrazo-1,3-thiazol-4-yl)-4-hydroxy-2*H*-chromen-2-one-6-yl]methane (8d). This compound was obtained as brown solid; Yield 79%; Mp 238-40°C; IR (KBr): ν 3400-3300, 3062, 1728, 1619, 1580, 1471, 1270, 1180, 640 cm^{-1}; ^1H NMR (DMSO-d_6): δ 11.20 (2H, s, OH), 9.52 (2H, s, NH), 8.21 (2H, s, CH), 7.67 (2H, s, ArH), 7.52 (2H, s, ArH), 7.23 (6H, m, ArH), 7.12 (2H, d, J = 8.7 Hz, ArH), 6.67 (4H, d, J = 8.5 Hz, ArH), 4.00 (2H, s, CH_2), 3.76 (6H, s, OCH_3); MS: m/z 798 (M^+). Anal. calcd. for $C_{41}H_{30}N_6O_8S_2$: C, 61.65; H, 3.79; N, 10.52. Found: C, 61.70; H, 3.71; N, 10.46.

Bis-[3-(2-(3,4-methylendioxyphenyliden)hydrazo-1,3-thiazol-4-yl)-4-hydroxy-2*H*-chromen-2-one-6-yl]methane (8e). This compound was obtained as black solid; Yield 70%; Mp 248-50 °C; IR (KBr): ν 3400-3300, 3065, 1720, 1620, 1580, 1471, 1365, 1180, 637 cm^{-1}; ^1H NMR (DMSO-d_6): δ 11.20 (2H, s, OH), 9.52 (2H, s, NH), 8.19 (2H, s, CH), 7.67 (2H, s, ArH), 7.52 (2H, s, ArH), 7.24 (2H, s, ArH), 7.17 (2H, d, J = 8.5 Hz, ArH), 7.12 (2H, d, J = 8.5 Hz, ArH), 7.09-6.98 (4H, m, ArH), 5.84 (4H, s, CH_2), 4.00 (2H, s, CH_2); MS: m/z 826 (M^+). *Anal*. calcd. for $C_{41}H_{26}N_6O_{10}S_2$: C, 59.56; H, 3.17; N, 10.16. Found: C, 59.46; H, 3.14; N, 10.20.

Bis-[3-(2-(2-furyliden)hydrazo-1,3-thiazol-4-yl)-4-hydroxy-2*H*-chromen-2-one-6-yl]methane (8f). This compound was obtained as black solid; Yield 79%; Mp 286-88°C; IR (KBr): ν 3400, 3065, 1720, 1620, 1180, 1030, 637 cm^{-1}; ^1H NMR (DMSO-d_6): δ 11.20 (2H, s, OH), 9.52 (2H, s, NH), 7.72 (2H, s, CH), 7.67 (4H, m, ArH), 7.52 (2H, s, ArH), 7.17 (2H, d, J = 8.5 Hz, ArH), 7.12 (2H, d, J = 8.5 Hz, ArH), 6.78-6.60 (4H, m, ArH), 4.00 (2H, s, CH_2); MS: m/z 718 (M^+). Anal. calcd. for $C_{35}H_{22}N_6O_8S_2$: C, 58.49; H, 3.09; N, 11.69. Found: C, 58.41; H, 3.10; N, 11.61.

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