International Journal of Pharmacy and Pharmaceutical Sciences

ISSN- 0975-1491 Vol 8, Issue 4, 2016

Original Article

SYNTHESIS AND BIOLOGICAL EVALUATION OF NOVEL COUMARIN DERIVATIVES AS POTENTIAL ANTIMICROBIALs AGENTS

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Received: 09 Jan 2016 Revised and Accepted: 11 Feb 2016

ABSTRACT

Objective: Synthesize new series of 7-hydroxy-4-methylcoumarin and 7-alkoxy-4-methylcoumarin derivatives featuring thiosemicarbazone or thiazolidin-4-one moieties and to evaluate their antimicrobial activity against two strains of Gram-positive bacteria *(Staphylococcus aureus* and *Bacillus subtilis)*, two Gram-negative bacteria *(Escherichia Coli* and *Pseudomonas aeruginosa)*, and *Candida albicans*.

Methods: Preparation of the new coumarin derivatives was done by adopting Pechmann condensation and attaching different isothiocyanates to give coumarin-thiosemicarbazone hybrids. Thiosemicarbazones were cyclized into thiazolidine-4-ones using chloroacetic acid or diethyl bromo malonate.

Results: Compounds VIb, Xb, XIVb, and XVc gave the highest inhibition zones (>20 mm) against *Staphylococcus aureus.* Their MIC (minimum inhibitory concentration) values ranging from 0.19-0.36 µg/ml were better than the reference drug tobramycin with MIC= 2µg/ml.

Conclusion: The newly synthesized compounds with the 7-hydroxyl group showed better antimicrobial activity than those with the 7-alkoxy groups.

Keywords: Coumarin, Thiosemicarbazones, Thiazolidin-4-ones, Antimicrobial activity

INTRODUCTION

Coumarins are a class of naturally occurring compounds, found in variable levels throughout the plant kingdom. Some important coumarins were isolated from microorganisms such as novobiocin 1 from Streptomyces species. They are used by plants as pesticides to protect themselves from predators [1, 2]. Applications of coumarins range from additives in food, perfumes, and cosmetics, to the preparation of insecticides, optical brighteners, and tunable laser dyes [3]. Today coumarins are very important in the pharmaceutical field due to their wide occurrence, and versatile pharmacological activity associated with low toxicity profile such as antimicrobial, anticoagulant, antioxidant, and anticancer activities [1-4]. Coumarin itself was reported to have an immunostimulatory activity on macrophages and other cells of the immune system. This results in the use of coumarin in chronic infections such as chronic brucellosis, mycoplasmosis, toxoplasmosis, and Q fever [1].

Novobiocin 1 and clorobiocin 2 are DNA-gyrase inhibitors having a strong activity against Gram-positive bacteria especially methicillin-resistant strains of *Staphylococcus aureus* (MRSA) [fig. 1]. But due to limitations regarding solubility, toxicity, and development of resistance, efforts were dedicated to designing an effective, orally bioavailable antimicrobial agents bearing coumarin nucleus [4]. Over the past decades, thiosemicarbazones attracted researchers for thorough investigation due to their diverse biological activity. They were known to have antiviral [5], antibacterial [6], anti-tuberculosis [7], anti-Trypanosoma cruzi [8] and antineoplastic activities [9]. This wide range of pharmacological activities was attributed to the strong chelating ability of thiosemicarbazones ligand to biologically important metals like iron, copper, nickel, and to their reductive capacities [10]. In 2011, Patil *et al.* reported the synthesis of new coumarin-8-yl-thiosemicarbazones 3, 4 that possessed potential antibacterial activity against *S. aureus*, *S. typhi*, and *E. coli* [11]. Also, thiosemicarbazones act as key intermediates in the preparation of important compounds that in turn have a potential antimicrobial activity such as thiazolidin-4-one derivatives. Thiazolidine-4-one derivative 5 possessed comparable activity to ampicillin and chloramphenicol at a concentration 25 µg/ml [12].

Also, 4-methylcoumarin-thiazolidine-4-one hybrids 6 and 7 were reported to exhibit good antimicrobial activity; the former compound had comparable activity to ciprofloxacin and griseofulvin at 10 µg/ml [13, 14] and the later possessed potent antifungal activity with MIC value of 0.10 µg/ml [15] (fig. 1).

Thus, the purpose of this work was to study the effect of hybridizing 7-hydroxy-4-methylcoumarin and their 7-alkoxy analogs with different N4-substituted thiosemicarbazone that were cyclized into the C5-substituted-thiazolidine-4-one ring (fig. 2). The antimicrobial activity of new compounds VI-XVII was evaluated.

MATERIALS AND METHODS

Starting materials and reagents were purchased from Sigma-Aldrich and were used without further purification. Melting points were determined using Electrothermal capillary melting point apparatus 9100 and were uncorrected. IR spectra were recorded on a Shimadzu FT-IR Affinity-1 Spectrophotometer, using KBr discs at MUST University. ^{1}H-NMR and ^{13}C-NMR spectra were recorded in δ scale given in ppm and performed on a JEOL ECA 300, 400 MHz spectrometer using $CDCl_3$ or DMSO as stated, using TMS as an internal standard at Cairo University.

Mass spectra were performed on Shimadzu Qp-2010 plus (70 eV) spectrometer at Cairo and Azhar University. Elemental analysis was performed at Azhar University. The microorganisms were purchased from Microbiological Resources Centre (MIRCEN), Faculty of agriculture, Ain-Shams University.

Synthesis of 8-Acetyl-7-alkoxy-4-methylcoumarin (IIIa-c): General Procedures: The 7-hydroxy Compound II (2.18 g., 0.010 mol) was stirred in dry acetone with anhydrous K_2CO_3 (1.5 g., 0.011 mol) for one hour, then the appropriate alkyl halide (ethyl iodide for IIIa, allyl bromide for IIIb, butyl bromide for IIIc) (0.050 mol) was added to the solution. The reaction mixture was refluxed for 8 h, concentrated and poured onto ice cold water. The solid formed was filtered and recrystallized from ethanol.

8-Acetyl-7-ethoxy-4-methylcoumarin (IIIa): **Yield:** 98%; m. p: 123-124 ˚C; IR (ῡ max, cm^{-1}): 3084 (CH, Ar), 2980 (CH, aliphatic), 1728 (CH_3-C-C=O), 1705 (C=O, α–pyrone), 1598 (C=C, Ar).

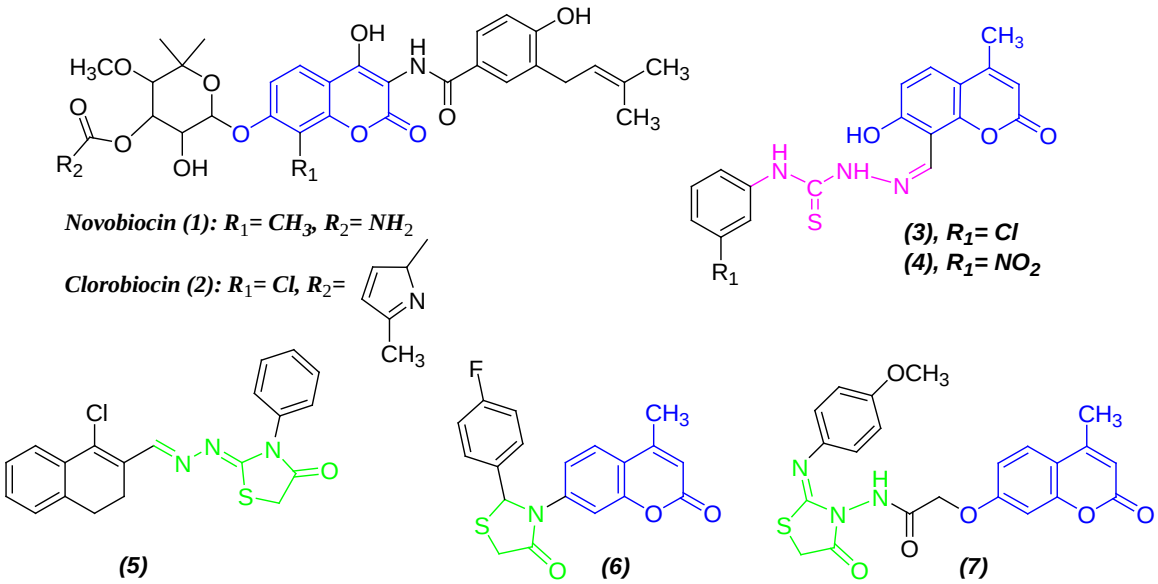


Fig. 1: Some reported lead antimicrobials having the main pharmacophores under investigation

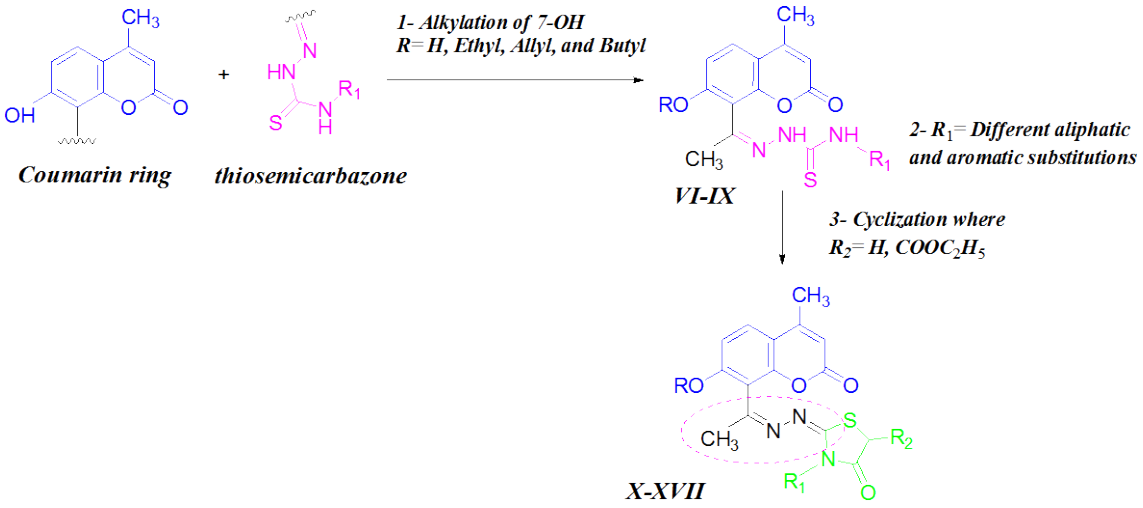


Fig. 2: Design strategy for the new compounds VI-XVII

8-Acetyl-7-allyloxy-4-methylchromen-2-one (IIIb): **Yield:** 97%; m. p: 118-120 ˚C; IR (ῡ max, cm^{-1}): 3088 (CH, Ar), 2991 (CH, aliphatic), 1724 (CH_3-C-C=O), 1703 (C=O, α–pyrone), 1598 (C=C, Ar and allyl); MS (m/z): 258.

8-Acetyl-7-butoxy-4-methylcoumarin (IIIc): Yield: 97%; m. p: 89-90 ˚C; IR (ῡ max, cm^{-1}): 3084 (CH, Ar), 2987 (CH, aliphatic), 1716 (CH_3-C-C=O), 1703 (C=O, α–pyrone), 1597 (C=C, Ar).

Synthesis of 8-Acetyl-7-substituted-4-methylcoumarin-hydrazones (IV and Va-Vc): **General Procedures:** 8-Acetyl-7-substituted-4-methylcoumarins II and IIIa-c (0.010 mol) were dissolved in 25 ml ethanol, poured onto hydrazine hydrate 99% (0.55 ml, 0.011 mole) and heated under reflux for 2 h. Light yellow crystals of the hydrazones were separated, collected by filtration and washed with water.

8-Acetyl-7-hydroxy-4-methylcoumarin-hydrazone (IV): Yield= 68%; m. p: 205-208 ˚C; IR (ῡ max, cm^{-1}): 3468 (OH), 3381 and 3381 (NH_2), 2926 (CH, aliphatic), 1697 (C=O, α–pyrone), 1558 (C=C, Ar).

8-Acetyl-7-ethoxy-4-methylcoumarin-hydrazone (Va): **Yield=** 92%; m. p: 138-140 ˚C; IR (ῡ max, cm^{-1}): 3412 and 3234 (NH_2), 3051 (CH, Ar), 2981 (CH, aliphatic), 1708 (C=O, α–pyrone), 1597 (C=C, Ar).

8-Acetyl-7-allyloxy-4-methylcoumarin-hydrazone (Vb): **Yield=** 99%; m. p: 84-86 ˚C; IR (ῡ max, cm^{-1}): 3379 and 3226 (NH_2), 3088 (CH, Ar), 2968 (CH, aliphatic), 1724 (C=O, α–pyrone), 1597 (C=C, Ar); MS (m/z):247.

8-Acetyl-7-butoxy-4-methylcoumarin-hydrazone (Vc): **Yield=** 60%; m. p: 146-148 ˚C; IR (ῡ max, cm^{-1}): 3390 (NH), 3084 (CH, Ar), 2987 (CH, aliphatic), 1707 (C=O, α–pyrone), 1622 (C=N, imine),

1597 (C=C, Ar); Anal. Calc: C, 66.65; H, 6.99; N, 9.72; Found: C, 66.91; H, 7.12; N, 9.89.

Synthesis of thiosemicarbazones (VI-IX): General Procedures: The hydrazones IV and Va-Vc (0.005 mol) were dissolved in the minimal amount of dimethyl formamide diluted with 20 ml ethanol then the appropriate isothiocyanate derivative (0.005 mol) was added. The solution was refluxed for 8 h then diluted with iced cold water. A crystalline solid was separated, collected, and recrystallize from ethanol.

8-Acetyl-7-hydroxy-4-methylcoumarin-4-benzyl thiosemicarbazone (VIa): Yield= 74.9%; m. p: 220-222 ºC; IR (ῡ max, cm^{-1}): 3466 (OH), 3406 and 3292 (2NH), 3040 (CH, Ar), 1720 (C=O, α–pyrone), 1616 (C=N imine), 1597 (C=C, Ar); ^{1}HNMR (400 MHz, $CDCl_3$): δ= 1.54 (s, 3H, N=C-CH_3), 2.45 (s, 3H,-C=C-CH_3), 4.56 (d, 2H,-CH_2-Ph, *J*=4 Hz), 6.16 (s, 1H, 3-H), 6.94 (d, 1H, 6-H, *J*= 8 Hz), 7.29-7.32 (m, 5H, Ar), 7.52 (d, 1H, 5-H, *J*=8 Hz), 6.12 (s, 1H, OH, D_2O exchangeable), 9.42 and 7.97 (s, 2H, 2NH, D_2O exchangeable); MS (m/z): 381, 383; Anal calcd. C, 62.97; H, 5.02; N, 11.02; found: C, 63.14; H, 5.09; N, 11.17.

8-Acetyl-7-hydroxy-4-methylcoumarin-4-benzoyl thiosemicarbazone (VIb): **Yield**= 73%; m. p: 220-223 ˚C; IR (ῡ max, cm^{-1}): 3547 (OH), 3234 and 3473 (2NH), 3120 (CH, Ar), 1728 and 1662 (2C=O), 1635 (C=N imine), 1598 (C=C, Ar); ^{1}HNMR (300 MHz, $CDCl_3$): δ= 2.4 (s, 3H, N=C-CH_3), 2.7 (s, 3H, C4-CH_3), 6.15 (s,1H, H-3 of coumarin), 7.03 (d, 1H, C6-H of coumarin, *J*= 9 Hz), 7.59 (t, t, 2H, C3-H, C5-H of phenyl, *J*=6 Hz), 7.68 (d, d, 2H, C2-H,C6-H of phenyl, *J*=6 Hz), 7.93 (d, 1H, C6-H of coumarin), 9.29 and 13.85 (s, 2H, 2NH, D_2O exchangeable); MS (m/z): 395; Anal calcd. C, 60.75; H, 4.33; N, 10.63; found: C, 60.89; H, 4.38; N, 10.79.

8-Acetyl-7-ethoxy-4-methylcoumarin-4-cyclohexyl thiosemicarbazone (VIIa): **Yield**= 92%; m. p: 190-191 ˚C; IR (ῡ max, cm^{-1}): 3244 and 3142 (2NH), 3014 (CH, Ar), 2931 (CH, aliphatic), 1728 (C=O), 1629 (C=N imine), 1597 (C=C, Ar); ^{1}HNMR (300 MHz, $CDCl_3$): δ= 1.47 (t, 3H, CH_3-, *J*=6 Hz), 2.31 (s,3H, N=C-CH_3), 2.37-2.43 (m, 6H,C3-2H,C4-2H, and C5-2H of cyclohexyl), 2.71 (s, 3H, C4-CH_3), 2.82-2.89 (m, 4H, C2-2H, C6-2H of cyclohexyl), 3.22 (m, 1H, C1-H of cyclohexyl), 4.23 (q, 2H,-CH_2-O, *J*=6 Hz), 5.99 (s,1H, NH, D_2O exchangeable), 6.17 (s,1H, C3-H), 6.94 (d,1H, C6-H, *J*=9 Hz), 7.64 (d,1H, C5-H, *J*=9); MS (m/z): 401; Anal calcd. C, 62.82; H, 6.78; N, 10.47; found: C, 63.04; H, 6.86; N, 10.61.

8-Acetyl-7-ethoxy-4-methylcoumarin-4-phenyl thiosemicarbazone (**VIIb**): **Yield**= 98%; m. p: 110-112 ˚C; IR (ῡ max, cm^{-1}): 3444 and 3460 (2NH), 3055 (CH, Ar), 2981 (CH, aliphatic), 1734 (C=O), 1597 (C=C, Ar), 1174 (C-O ether); ^{1}HNMR (300 MHz, $CDCl_3$): δ= 1.45 (t, 3H, CH_3-, *J*=6 Hz), 2.36 (s, 3H, N=C-CH_3), 2.42,(s, 3H, 4-CH_3), 4.22 (m, 2H,-CH_2-O, *J*=6 Hz), 6.20 (s, 1H, C3-H), 6.95 (d, 1H, C6-H, *J*=9 Hz), 7.32 (t, 1H, C4-H of phenyl), 7.37 (d, 2H, C2-H, C6-H of phenyl, *J*=9 Hz), 7.65 (d, 2H, C3-H, C5-H of phenyl, *J*=9 Hz), 7.69 (d,1H, C5-H), 9.84 (s,1H, NH, D_2O exchangeable), 10.46 (s,1H, OH, D_2O exchangeable); ^{13}CNMR (400 MHz, $CDCl_3$): δ= 14.6 (CH_3-), 18.6 (CH_3-), 23.2 (CH_3-), 32.6 (C5 of thiazolidin-4-one), 64.5 (-CH_2-O), 107.9 (C3 of coumarin), 108.3 (C8 of coumarin), 112.0 (C6 of coumarin), 117.3 (C10 of coumarin), 125.0 (C2, C6 of phenyl), 127.3 (C4 of phenyl), 128.2 (C3, C5 of phenyl), 129.1 (C5 of coumarin), 134.5 (C1 of phenyl), 151.9 (C9 of coumarin), 152.1 (C4 of coumarin), 157.4 (C7 of coumarin), 159.2 (C2 of coumarin), 160.3 (C2 of thiazolidin-4-one), 161.1 (-C=N-), 171.5 (-C=O of thiazolidin-4-one); MS (m/z): 395; Anal calcd. C, 63.78; H, 5.35; N, 10.63; found: C, 63.97; H, 5.42; N, 10.88.

8-Acetyl-7-ethoxy-4-methylcoumarin-4-(4-methoxyphenyl) thiosemicarbazone (VIIc): Yield= 99%; m. p: 206-208 ˚C; IR (ῡ max, cm^{-1}): 3319 and 3278 (2NH), 3062 (CH, Ar), 2837-2981 (CH, aliphatic), 1722 (C=O), 1595 (C=C, Ar), 1182 (C-O ether); ^{1}HNMR (300 MHz, $CDCl_3$): δ= 1.45 (t, 3H, CH_3-, *J*=8 Hz), 2.35 (s, 3H, CH_3-), 2.45 (s, 3H, C4-CH_3), 3.83 (s, 3H, CH_3-O-Ph), 4.17-4.24 (m, 2H,-CH_2-O, *J*=8 Hz), 6.20 (s, 1H, 3-H), 6.88-6.97 (m, 4H, Ar), 7.48 (d, 1H, C6-H, *J*=8 Hz), 7.67 (d,1H, C5-H, *J*=8 Hz), 8.27 and 9.19 (s,2H, 2NH, D_2O exchangeable); MS (m/z): 426; Anal calcd. C, 62.10; H, 5.45; N, 9.88; found: C, 62.42; H, 5.51; N, 10.03.

8-Acetyl-7-allyloxy-4-methylcoumarin-4-cyclohexyl thiosemicarbazone (VIIIa): Yield= 96%; m. p: 206-208 ˚C; IR (ῡ max, cm^{-1}): 3354 and 3244 (2NH), 3053 (CH, Ar), 2962 (CH, aliphatic), 1724 (C=O), 1597 (C=C, Ar); ^{1}HNMR (400 MHz, $CDCl_3$): δ= 1.59 (s, 3H, N=C-CH_3), 1.88-1.92 (m, C3-H, C4-H, and C5-H of cyclohexyl), 2.11-2.17 (m C2-H, C6-H of cyclohexyl), 2.37 (m, 1H, C1-H of cyclohexyl), 2.42 (s, 3H, C4-CH_3), 4.77 (d, 2H,–CH_2-O, *J*= 8 Hz), 5.23 and 5.31 (d, d, 2H, CH_2=, *J*=8 Hz), 5.96-6.00 (m, 1H, =CH–), 6.12 (s, 1H, C3-H), 6.92 (d, 1H, C6-H, *J*=8 Hz), 7.53 (d, 1H, C5-H, *J*=8 Hz); MS (m/z): 413; Anal calcd. C, 63.90; H, 5.92; N, 10.16; found: C, 64.08; H, 5.97; N, 10.31.

8-Acetyl-7-allyloxy-4-methylcoumarin-4-benzyl thiosemicarbazone (VIIIb): Yield= 84%; m. p: 102-104˚C; IR (ῡ max, cm^{-1}): 3419 and 3367 (2NH), 3084 (CH, Ar), 2980 (CH, aliphatic), 1732 (C=O), 1598 (C=C, Ar); ^{1}HNMR (400 MHz, $CDCl_3$): δ= 2.26 (s, 3H, N=C-CH_3), 2.43 (s, 3H, C4-CH_3), 4.50 (d, 2H,-CH_2-O-, *J*= 8 Hz), 4.76 (d, 2H, Ph-CH_2-), 5.33 and 5.36 (d, d, 2H, CH_2=, *J*=8 Hz), 5.95-6.04 (m, 1H, =CH–), 6.20 (s, 1H, C3-H), 6.95 (d, 1H, C6-H, *J*=8 Hz), 7.25-7.40 (m, 5H, Ar), 7.54 (d, 1H, C5-H, *J*=8 Hz), 8.2 and 8.8 (s, 2H, 2NH, D_2O exchangeable); ^{13}CNMR (400 MHz, $CDCl_3$): δ= 18.7 (CH_3-), 23.5 (-CH_3), 48.4 (-CH_2-ph), 69.7 (-CH_2-O-), 109.0 (C3 of coumarin), 110.0 (C8 of coumarin), 112.3 (C6 of coumarin), 114.7 (CH_2=), 118.7 (C10 of coumarin), 125.7 (C4 of phenyl), 127.4 (C2, C6 of phenyl), 127.9 (C3, C5 of phenyl), 128.7 (C5 of coumarin), 131.6 (=CH-), 137.5 (C1 of phenyl), 142.9 (C9 of coumarin), 151.1 (C4 of coumarin), 152.1 (-C=N-), 157.1 (C2 of coumarin), 159.8 (C7 of coumarin), 177.6 (-C=S); MS (m/z): 421; Anal calcd: C, 65.54; H, 5.5; N, 9. 97; found: C, 65.73; H, 5.54; N, 10.08.

8-Acetyl-7-allyloxy-4-methylcoumarin-4-(4-methoxyphenyl) thiosemicarbazone (VIIIc): Yield= 95%; m. p: 186-187 ºC; IR (ῡ max, cm^{-1}): 3319 and 3278 (2NH), 3032, (CH, Ar), 2970 (CH, aliphatic), 1720 (C=O), 1597 (C=C, Ar); ^{1}HNMR (400 MHz, $CDCl_3$): δ= 2.29 (s, 3H,-N=C-CH_3), 2.43 (s, 3H, C4-CH_3), 3.81 (s, 3H, CH_3-O-Ph-), 4.69 (d, 2H,-CH_2-O-, *J*= 8 Hz), 5.37 (d, 2H, CH_2=, *J*=8 Hz), 6.02 (m, 1H, =CH–), 6.18 (s, 1H, C3-H), 6.88-6.97 (m, 4H, phenyl), 7.47-7.51 (d, 2H, C5-H, C6-H, *J*=8 Hz), 8.8 and 9.10 (s, 2H, 2NH, D_2O exchangeable); MS (m/z): 437, 438 (M+1); Anal calcd. C 63.14; H 5.30; N 9.60; found: C 63.29; H 5.32; N 9.67.

8-Acetyl-7-butoxy-4-methylcoumarin-4-ethyl thiosemicarbazone (IXa): Yield= 99%; m. p: 170-169 ˚C; IR (ῡ max, cm^{-1}): 3448 and 3220 (2NH), 2954 (CH, aliphatic), 1732 (C=O), 1598 (C=C, Ar); ^{1}HNMR (400 MHz, $CDCl_3$): δ= 0.98 (t, 3H, CH_3-, *J*= 8 Hz), 1.25 (t, 3H, CH_3-, *J*= 8 Hz), 1.47 (m, 2H,-CH_2-, *J*= 8 Hz), 1.78 (m, 2H,-CH_2-, *J*= 8 Hz), 2.22 (s, 3H,-N=C-CH_3), 2.43 (s, 3H, C4-CH_3), 3.72 (q, 2H,-CH_2-N-, *J*= 8 Hz), 4.09 (t, 2H,-CH_2-O-, *J*= 8 Hz), 6.17 (s, 1H, C3-H), 6.92 (d,1H, C6-H), 7.58 (d, H, C5-H, *J*= 8 Hz), 8.64 (s, 1H, NH, D_2O exchangeable); MS (m/z): 375; Anal calcd. C, 60.78, H, 6.71, N, 11.19; found: C, 60.91; H, 6.82; N, 11.31.

8-Acetyl-7-butoxy-4-methylcoumarin-4-benzyl thiosemicarbazone (IXb): Yield= 99%; m. p: 140-142 ˚C; IR (ῡ max, cm^{-1}): 3419 and 3253 (2NH), 3088 (CH, Ar), 2960 (CH, aliphatic), 1732 (C=O), 1602 (C=N imine), 1550 (C=C, Ar); ^{1}HNMR (400 MHz, $CDCl_3$): δ= 0.96 (t, 3H, CH_3-, *J*= 8 Hz), 1.44 (m, 2H,-CH_2-, *J*= 8 Hz), 1.74 (m, 2H,-CH_2-, *J*= 8 Hz), 2.24 (s, 3H, N=C-CH_3), 2.40 (s, 3H, C4-CH_3), 4.05 (t, 2H,-CH_2-O, *J*= 8 Hz), 4.92 (s, 2H,-CH_2-ph), 6.15 (s, 1H, C3-H), 6.88 (d,1H, C6-H, *J*= 8 Hz), 7.26-7.37 (5H, phenyl), 7.55 (d, H, C5-H, *J*= 8 Hz), 8.79 (s, 1H, NH, D_2O exchangeable) MS (m/z): 437; Anal calcd. C, 65.88; H, 6.22; N, 9.60; found: C, 65.98; H, 6.28; N, 9.72.

Synthesis of thiazolidine-4-ones (X-XIII): General procedures: The thiosemicarbazones **VI-IX** (0.005 mol) were reacted with chloroacetic acid (0.00505 mol, 0.618 g) in freshly fused sodium acetate (0.00505 mol, 0.414 g) and 30 ml ethanol. The solution was refluxed for 8 h, concentrated, and diluted with ice cold water. A crystalline solid was separated, collected, and recrystallized from ethanol.

3-Benzyl-2-{[1-(7-hydroxy-4-methylcoumarin-8-yl)-ethylidene] -hydrazono}-thiazolidin-4-one (Xa): Yield= 67%; m. p: 144-146 ˚C; IR (ῡ max, cm^{-1}): 3444 (OH), 3089 (CH, Ar), 2924 (CH, aliphatic), 1720 and1687 (2C=O), 1629 (C=N imine), 1597 (C=C, Ar); ^{1}HNMR (400 MHz, $CDCl_3$): δ= 1.56 (s, 3H, 4-CH_3), 2.46 (s, 3H, N=C-CH_3), 2.85 (s, 2H, S-CH_2-CO), 4.57 (s, 2H, Benzyl CH_2), 6.16 (s, 1H, C3-H), 6.95 (d,1H, C6-H), 7.35 (m, 5H, Ar), 7.62 (d, 2H, 4, C5-H, *J*= 8 Hz); MS

(m/z): 421; Anal calcd. C 62.69; H 4.54; N 9.97; found: C 62.78; H 4.51, N 10.08.

3-Benzoyl-2-{[1-(7-hydroxy-4-methylcoumarin-8-yl)-ethylidene]-hydrazono}-thiazolidin-4-one (Xb): Yield= 75%; m. p: 128-130 °C; IR (ṽ max, cm^{-1}): 3446 (OH), 3066 (CH, Ar), 2980 (CH, aliphatic), 1728 and1670 (2C=O), 1624 (C=N imine), 1598 (C=C, Ar); ^{1}HNMR (400 MHz, $CDCl_3$): δ= 2.44 (s, 3H, 4-CH_3), 2.84 (s, 3H, N=C-CH_3), 3.83 (s, 2H, S-CH_2-CO), 6.19 (s, 1H, C3-H), 6.95 (d,1H, C6-H, *J*=8 Hz), 7.50-7.62 (m, 3H, C3, C4, C5-H of phenyl), 7.70 (d,1H, C5-H, *J*=8 Hz); MS (m/z): 435; Anal calcd: C 60.68; H 3.93; N 9.65; found: C, 60.74; H, 3.96; N, 9.77.

3-Cyclohexyl-2-{[1-(7-ethoxy-4-methylcoumarin-8-yl)-ethylidene]-hydrazono}-thiazolidin-4-one (XIa): Yield= 56%; m. p: 246-247 °C; IR (ṽ max, cm^{-1}): 3059 (CH, Ar), 2978 (CH, aliphatic), 1716 (C=O), 1620 (C=N imine), 1597 (C=C, Ar); ^{1}HNMR (400 MHz, DMSO): δ= 1.08 (t, 3H, CH_3-CH_2-O), 2.07 (s, 3H, 4-CH_3), 2.16 (s, 3H, N=C-CH_3), 2.27 (t, 4H, 2andC6-H of cyclohexyl), 3.65 (m, 1H, C1-H of cyclohexyl), 3.68 (s, 2H, S-CH_2-CO), 3.93 (t, 2H, CH3-CH_2-O, *J*= 8 Hz), 5.98 (s, 1H, C3-H), 6.91 (d,1H, C6-H, *J*=8 Hz), 7.52 (d, 2H, 4, C5-H, *J*=8 Hz); MS (m/z): 441; Anal calcd: C, 62.56; H, 6.16; N, 9.52; found: C, 62.67; H, 6.30; N, 9.61.

2-{[1-(7-Ethoxy-4-methylcoumarin-8-yl)-ethylidene]-hydrazono}-3-phenyl-thiazolidin-4-one (XIb): Yield= 89%; m. p: 257-260 °C; IR (ṽ max, cm^{-1}): 3064 (CH, Ar), 2980 (CH, aliphatic), 1732 and 1720 (2C=O), 1597 (C=C, Ar); ^{1}HNMR (400 MHz, $CDCl_3$): δ= 1.32 (t, 3H, CH_3-CH_2-O, *J*=8 Hz), 2.23 (s, 3H, C4-CH_3), 2.39 (s, 3H, N=C-CH_3), 3.92 (m, 4H, S-CH_2-CO and CH3-CH_2-O), 6.10 (s, 1H, C3-H), 6.71 (d,1H, C6-H, *J*=8 Hz), 6.95 (d, 2H, C2, C6-H of phenyl, *J*= Hz), 7.11 (m, 3H, C3,4,C5-H of phenyl), 7.41 (d, 2H, 4, C5-H, *J*=8 Hz); MS (m/z): 435; Anal calcd. C, 63.43; H, 4.86; N, 9.65; found: C 63.65; H 4.92; N 9.78.

2-{[1-(7-Ethoxy-4-methylcoumarin-8-yl)-ethylidene]-hydrazono}-3-(4-methoxy-phenyl)-thiazolidin-4-one (XIc): **Yield**= 89%; m. p: 170-172 °C; IR (ṽ max, cm^{-1}): 2980 (CH, aliphatic), 1718 (C=O), 1624 (C=N imine), 1598 (C=C); ^{1}HNMR (400 MHz, $CDCl_3$): δ= 1.34 (t, 3H, CH_3-CH_2-O, *J*=8 Hz), 2.19 (s, 3H, 4-CH_3), 2.41 (s, 3H, N=C-CH_3), 3.75 (s, 3H, CH_3-O-Ph), 3.88 (s, 2H, S-CH_2-CO), 4.05 (q, 2H, CH_3-CH_2-O), 6.10 (s, 1H, C3-H), 6.87 (d,1H, C6-H, *J*=8 Hz), 6.89-7.39 (m, 5H, Ar), 7.55 (d, 2H, 4, C5-H, *J*=8 Hz),; MS (m/z): 465; Anal calcd: C, 64.13; H, 5.16; N, 9.35; found: C, 64.28; H, 5.20; N, 9.43.

3-Cyclohexyl-2-{[1-(7-allyloxy-4-methylcoumarin-8-yl)-ethylidene]-hydrazono}-thiazolidin-4-one (XIIa): Yield= 91%; m. p: 114-116 °C; IR (ṽ max, cm^{-1}): 3080 (CH, Ar and allyl), 2962 (CH, aliphatic), 1720 (C=O), 1618 (C=N imine), 1598 (C=C, Ar, allyl); ^{1}HNMR (400 MHz, $CDCl_3$): δ= 2.10 (s, 3H, C4-CH_3), 2.17 (s, 3H, N=C-CH_3), 3.45 (s, 2H, S-CH_2-C=O), 3.74-3.82 (m, 1H, C1-H of cyclohexyl), 4.40 (d, 2H, CH_2=CH-CH_2-O-, *J*= 8 Hz), 5.01-5.15 (d, d, 2H, CH_2=CH-CH_2-O-, *J*=8 Hz), 5.72-5.76 (m, 1H, CH_2=CH–CH_2-O-), 6.69 (d, 1H, C6-H, *J*=8 Hz), 7.31 (d, 1H, C5-H, *J*=8 Hz); MS (m/z): 453; Anal calcd.: C, 63.56; H, 6.00; N, 9. 26; found, C, 63.78; H, 6.13; N, 9.49.

3-Benzyl-2-{[1-(7-allyloxy-4-methylcoumarin-8-yl)-ethylidene]-hydrazono}-thiazolidin-4-one (XIIb): **Yield**= 81%; m. p: 182-184 °C; IR (ṽ max, cm^{-1}): 3005-3086 (CH, Ar and allyl), 2981 (CH, aliphatic), 1728 and 1710 (2C=O), 1622 (C=N imine), 1600 (C=C, Ar, allyl); ^{1}HNMR (400 MHz, $CDCl_3$): δ= 2.35 (s, 3H, C4-CH_3), 2.41 (s, 3H, N=C-CH_3), 3.77 (s, 2H, S-CH_2-C=O), 4.56 (d, 2H, CH_2=CH-CH_2-O-, *J*= 8 Hz), 4.68 (d, 2H, Ph-CH_2-NH-), 5.31 and 5.37 (d, d, 2H, CH_2=CH-CH_2-O-, *J*=8 Hz), 5.92-6.05 (m, 1H, CH_2=CH–CH_2-O-), 6.16 (s, 1H, C3-H), 6.88-7.56 (m, 5H, Ar); MS (m/z): 461; Anal calcd. C, 65.06; H, 5.02; N, 9.10; found: C, 65.24; H, 5.11; N, 9.31.

3-(4-Methoxy-phenyl)-2-{[1-(7-allyloxy-4-methylcoumarin-8-yl)-ethylidene]-hydrazono}-thiazolidin-4-one (XIIc): **Yield**= 97%; m. p: 200-202 °C; IR (ṽ max, cm^{-1}): 3057 (CH, Ar and allyl), 2966 (CH, aliphatic), 1724 (C=O), 1616 (C=N imine), 1597 (C=C, Ar, allyl); ^{1}HNMR (400 MHz, $CDCl_3$): δ= 2.3 (s, 3H, C4-CH_3), 2.40 (s, 3H, N=C-CH_3), 3.74 (s, 3H, methoxy), 3.89 (s, 2H, S-CH_2-C=O), 4.50 (d, 2H, CH_2=CH-CH_2-O-, *J*=8 Hz), 5.20-5.31 (d, d, 2H, CH_2=CH-CH_2-O-), 5.85-5.94 (m, 1H, CH_2=CH–CH_2), 6.09 (s, 1H, C3-H), 6.75 (d, 2H, C3 and C5 of phenyl, *J*= 8 Hz), 6.72 (d, 1H, C6-H, *J*=8 Hz), 6.85 (d, 2H, C2 and C6 of phenyl, *J*= 8 Hz), 7.42 (d, 1H, C5-H, *J*=8 Hz); MS (m/z): 477; Anal calcd. C, 62.88; H, 4.85; N, 8.80; found: C, 63.01; H, 4.89; N, 8.92.

2-{[1-(7-Butoxy-4-methylcoumarin-8-yl)-ethylidene]-hydrazono}-3-ethyl-thiazolidin-4-one (XIIIa): **Yield**= 99%; m. p: 201-203 °C; IR (ṽ max, cm^{-1}): 3080 (CH, Ar), 2953 (CH, aliphatic), 1737 and 1722 (2C=O), 1571 (C=C, Ar); ^{1}HNMR (400 MHz, $CDCl_3$): δ= 0.96 (t, 3H, CH_3-$(CH_2)_3$-O, *J*= 8 Hz), 1.34 (t, 3H, CH_3-CH_2-N, *J*= 8 Hz), 1.48 (m, 2H, CH_3-CH_2-$(CH_2)_2$-O, *J*= 8 Hz), 1.79 (m, 2H, CH_3-CH_2-CH_2-CH_2-O, *J*= 8 Hz), 2.36 (s, 3H, C4-CH_3), 2.41 (s, 3H,-N=C-CH_3), 3.73 (s, 2H, S-CH_2-C=O), 3.94 (t,2H, CH_3-CH_2-N-, *J*= 8 Hz), 4.09 (t, 2H, CH_3-CH_2-CH_2-CH_2-O, *J*= 8 Hz), 6.14 (s, 1H, C3-H), 6.90 (d,1H, C6-H, *J*= 8 Hz), 7.54 (d, H, C5-H, *J*= 8 Hz); MS (m/z): 415; Anal calcd. C, 60.70; H, 6.06; N, 10.11; found: C, 60.37; H, 5.38; N, 10.69.

3-Benzyl-2-{[1-(7-butoxy-4-methylcoumarin-8-yl)-ethylidene]-hydrazono}-thiazolidin-4-one (XIIIb): **Yield**= 91%; m. p: 180-182 °C; IR (ṽ max, cm^{-1}): 3082 (CH, Ar), 2939 (CH, aliphatic), 1720 (C=O), 1589 (C=C, Ar); ^{1}HNMR (400 MHz, $CDCl_3$): δ= 0.91 (t, 3H, CH_3-$(CH_2)_3$-O, *J*= 8 Hz), 1.39 (m, 2H, CH_3-CH_2-$(CH_2)_2$-O, *J*= 8 Hz), 1.68 (m, 2H, CH_3-CH_2-CH_2-CH_2-O, *J*= 8 Hz), 2.39 (s, 3H, C4-CH_3), 2.43 (s, 3H, N=C-CH_3), 3.76 (s, 2H, S-CH_2-C=O), 3.91-3.95 (t, 2H, CH_3-CH_2-CH_2-CH_2-O, *J*= 8 Hz), 4.54 (s, 2H, CH_2-N-), 6.12 (s, 1H, C3-H), 6.90 (d,1H, C6-H, *J*= 8 Hz), 7.05 (m, 3H, C2,6,4 of phenyl), 7.15 (t, 2H, C3,5 of phenyl), 7.55 (d, H, C5-H); MS (m/z): 474; Anal calcd. C, 65.39; H, 5.70; N, 8.80; found: C, 65.62; H, 5.78; N, 8.91.

Synthesis of thiazolidine-4-ones (XIV-XVII): General procedures: The thiosemicarbazones V-VIII (0.005 mol) were reacted with diethyl bromo malonate (0.00505 mol, 1.207 g) in freshly fused sodium acetate (0.00505 mol, 0.41400 g) and 30 ml ethanol. The solution was refluxed for 8 h, concentrated, and diluted with ice cold water. A crystalline solid was separated, collected, and recrystallized from ethanol.

3-Benzyl-2-{[1-(7-hydroxy-4-methylcoumarin-8-yl)-ethylidene]-hydrazono}-thiazolidin-4-one-5-carboxylic acid ethyl ester (XIVa): Yield= 91%; m. p: 137 °C; IR (ṽ max, cm^{-1}): 3088 (CH, Ar), 2981 (CH, aliphatic), 1737 and 1724 (2C=O), 1595 (C=C, Ar); ^{1}HNMR (400 MHz, $CDCl_3$): δ= 2.37 (s, 3H, C4-CH_3), 2.45 (t, 3H, CH_3-), 2.79 (s, 3H,-N=C-CH_3), 4.42-4.91 (m, 4H, 2CH_2-of benzyl and ethyl), 5.1 (s, 2H, S-CH_2-C=O), 6.30 (s, 1H, C3-H), 6.95 (d,1H, C6-H, *J*= 8 Hz), 7.28-7.75 (m, 6H, Ar); MS (m/z): 493; Anal calcd. C, 60.84; H, 4.70; N, 8.51; found: C, 60.47; H, 4.89; N, 8.91.

3-Benzoyl-2-{[1-(7-hydroxy-4-methylcoumarin-8-yl)-ethylidene]-hydrazono}-thiazolidin-4-one-5-carboxylic acid ethyl ester (XIVb): Yield= 94%; m. p: 135 °C; IR (ṽ max, cm^{-1}): 3066 (CH, Ar), 2933 (CH, aliphatic), 1732 and 1724 (2C=O), 1598 (C=C, Ar); ^{1}HNMR (400 MHz, $CDCl_3$): δ= 2.44 (t, 3H, CH_3-), 2.84 (s, 3H, C4-CH_3), 2.98 (s, 3H, N=C-CH_3), 4.23-4.39 (m, 4H, 2CH_2-of benzyl and ethyl), 6.19 (s, 1H, C3-H), 6.94 (d,1H, C6-H, *J*= 8 Hz), 7.54-8.09 (m, 6H, Ar), 7.61 (d, H, C5-H); MS (m/z): 508; Anal calcd. C, 59.16; H, 4.17; N, 8.28; found: C, 58.73; H, 4.37; N, 8.63.

3-Cyclohexyl-2-{[1-(7-ethoxy-4-methylcoumarin-8-yl)-ethylidene]-hydrazono}-thiazolidin-4-one-5-carboxylic acid ethyl ester (XVa): Yield= 85%; m. p: 247-248 °C; IR (ṽ max, cm^{-1}): 3055 (CH, Ar), 2976 (CH, aliphatic), 1720 (C=O), 1598 (C=C, Ar); ^{1}HNMR (400 MHz, $CDCl_3$): δ= 1.22-1.36 (t, 6H, 2CH_3-), 2.00-2.43 (m, 10H, cyclohexyl), 2.28 (s, 3H, C4-CH_3), 2.44 (s, 3H,-N=C-CH_3), 4.01 (t, 2H,-CH_2-O), 4.12-4.28 (m, 3H, S-CH-C=O of thiazolidine and-CH_2-O of ethyl ester), 6.21 (s, 1H, C3-H), 6.99 (d,1H, C6-H), 7.63 (d, H, C5-H); MS (m/z): 514; Anal calcd. C, 60.80; H, 6.08; N, 8.18; found: C, 60.55; H, 6.37; N, 8.61.

2-{[1-(7-Ethoxy-4-methylcoumarin-8-yl)-ethylidene]-hydrazono}-4-oxo-3-phenyl-thiazolidine-5-carboxylic acid ethyl ester (XVb): **Yield**= 86%; m. p: 196-198 °C; IR (ṽ max, cm^{-1}): 2983 (CH, aliphatic), 1745 and 1730 (2C=O), 1597 (C=C, Ar); ^{1}HNMR (400 MHz, $CDCl_3$): δ= 1.23-1.38 (m, 6H, 2CH_3-), 2.21 (s, 3H, C4-CH_3), 2.41 (s, 3H,-N=C-CH_3), 3.85-3.91 (m, 2H,-O-CH_2-), 4.19-4.41 (m, 2H,-O-CH_2-), 4.67 (s, 1H, S-CH-C=O), 6.11 (s, 1H, C3-H), 6.68 (s, 1H, C6-H), 7.05-7.15 (m, 2H, C3-H, C5-H of phenyl), 7.39 (m, 2H, C2-H, C6-H of phenyl), 7.54 (s, 1H, C5-H); MS (m/z): 507; Anal calcd. C, 61.53; H, 4.96; N, 8.28; found: C, 61.32; H, 5.19; N, 8.73.

2-{[1-(7-Ethoxy-4-methylcoumarin-8-yl)-ethylidene]-hydrazono}-3-(4-methoxy-phenyl)-thiazolidin-4-one-5-carboxylic acid ethyl ester (XVc): **Yield**= 75%; m. p: 217-218 °C; IR ($\tilde{\nu}$ max, cm^{-1}): 3062 (CH, Ar), 2981 (CH, aliphatic), 1716 (C=O), 1595 (C=C, Ar); ^{1}HNMR (400 MHz, $CDCl_3$): δ= 1.18-1.35 (m, 6H, $2CH_3$-), 2.28 (s, 3H, C4-CH_3), 2.41 (s, 3H, N=C-CH_3), 3.31 (s, 3H,-O-CH_3), 3.73 (q, 2H,-O-CH_2-), 4.25 (q, 2H, CO-O-CH_2-), 4.63 (s, 1H, S-CH-C=O), 6.24 (s, 1H, C3-H), 6.85 (d, 2H, C3-H, C5-H of phenyl), 6.90 (s, 1H, C6-H), 7.42 (d, 2H, C3-H, C5-H of phenyl), 7.75 (s, 1H, C5-H); MS (m/z): 537; Anal calcd. C, 60.32; H, 5.06; N, 7.82; found: C, 59.95; H, 5.30; N, 8.19.

2-{[1-(7-Allyloxy-4-methylcoumarin-8-yl)-ethylidene]-hydrazono}-3-cyclohexyl-4-oxo-thiazolidine-5-carboxylic acid ethyl ester (XVIa): Yield= 80%; m. p: 80-82 °C; IR ($\tilde{\nu}$ max, cm^{-1}): 3055 (CH, Ar and allyl), 2962 (CH, aliphatic), 1737 and1724 (2C=O), 1620 (C=N imine), 1597 (C=C, Ar, allyl); ^{1}HNMR (400 MHz, $CDCl_3$): δ= 1.19-1.42 (m, 9H, C3-2H, C4-2H, C5-2H of cyclohexyl and-CH_3 of ethyl), 1.77-1.87 (m, 4H, C2-2H, C6-2H of cyclohexyl), 2.34 (s, 3H, C4-CH_3), 2.42 (s, 3H, N=C-CH_3), 3.40 (s, 1H, C1-H of cyclohexyl), 4.06-4.09 (m, 2H,-CH_2-O), 4.56 (s, 1H, S-CH-C=O), 4.67 (d, 2H,-CH_2-O), 5.22-5.42 (m, 2H, CH_2=), 5.97-6.01 (m, 1H, =CH-), 6.15 (s, 1H, C3-H), 6.91 (d, 1H, C-6, *J*=8 Hz), 7.55 (d, 1H, C5-H, *J*=8 Hz); MS (m/z): 523; Anal calcd. C, 61.70; H, 5.94; N, 7.99; found C, 61.94; H, 6.01; N, 8.14.

2-{[1-(7-Allyloxy-4-methylcoumarin-8-yl)-ethylidene]-hydrazono}-3-4-methoxyphenyl-thiazolidin-4-one-5-carboxylic acid ethyl ester (XVIc): Yield= 87%; m. p: 188 °C; IR ($\tilde{\nu}$ max, cm^{-1}): 3066 (CH, Ar and allyl), 2968 (CH, aliphatic), 1730 and 1718 (C=O), 1598 (C=C, Ar, allyl); ^{1}HNMR (400 MHz, $CDCl_3$): δ= 2.35 (s, 3H, C4-CH_3), 2.44 (s, 3H, N=C-CH_3), 3.83 (s, 3H, CH_3-O-Ph), 4.05-4.12 (m, 3H,-CH_2-O and S-CH-C=O), 4.72 (d, 2H,-CH_2-O), 5.35-5.40 (d, d, 2H, CH_2=), 5.99-6.04 (m, 1H, =CH–), 6.17 (s, 1H, C3-H), 6.92-6.98 (m, 3H, C3-H, C5-H of phenyl and C6-H), 7.49 (d, 2H, C2 and C6 of phenyl, *J*= 8 Hz), 7.66 (d, 1H, C5-H, *J*=8 Hz); MS (m/z): 550; Anal calcd. C, 61.19; H, 4.95; N, 7.65; found: C, 61.09; H, 5.21; N, 8.08.

2-{[1-(7-Butoxy-4-methylcoumarin-8-yl)-ethylidene]-hydrazono}-3-ethyl-thiazolidin-4-one-5-carboxylic acid ethyl ester (XVIIa): Yield= 85%; m. p: 224-225 °C; IR ($\tilde{\nu}$ max, cm^{-1}): 2958 (CH, aliphatic), 1732 (C=O), 1627 (C=N, imine), 1593 (C=C, Ar); ^{1}HNMR (400 MHz, $CDCl_3$): δ= 0.96 (t, 3H, CH_3-, *J*= 8 Hz), 1.35-1.38 (m, 3H, CH_3-), 1.43-1.52 (m, 2H,-CH_2-), 1.75-1.82 (m, 2H,-CH_2-), 2.37 (s, 3H, C4-CH_3), 2.40 (s, 3H, N=C-CH_3), 3.50-3.53 (m, 2H,-CH_2-N), 4.01 (t, 2H,-CH_2-O), 4.06-H, *J*= 8 Hz), 7.54 (d, 1H, C5-H, *J*= 8 Hz); MS (m/z): 485, 487; Anal calcd. C, 59.12; H, 6.00; N, 8.62; found: C, 58.75; H, 6.26; N, 9.03. 4.10 (m, 2H,-CH_2-O), 4.18 (s, 2H, S-CH-C=O), 6.13 (s, 1H, C3-H), 6.90 (d,1H, C6-

3-Benzyl-2-{[1-(7-butoxy-4-methylcoumarin-8-yl)-ethylidene]-hydrazono}-thiazolidin-4-one-5-carboxylic acid ethyl ester (XVIIb): **Yield**= 93%; m. p: charring; IR ($\tilde{\nu}$ max, cm^{-1}): 3032 (CH, Ar), 2958 (CH, aliphatic), 1724 (C=O), 1627 (C=N, imine), 1597 (C=C, Ar); ^{1}HNMR (400 MHz, $CDCl_3$): δ= 0.95 (t, 3H, CH_3-, *J*= 8 Hz), 1.39-1.48 (m, 2H,-CH_2-), 1.75-1.79 (m, 2H,-CH_2-), 2.32 (s, 3H, C4-CH_3), 2.40 (s, 3H, N=C-CH_3), 3.74 (t, 2H,-CH_2-O, *J*= 8 Hz), 4.04-4.07 (m, 3H,-CH_2-N-and S-CH-), 5.09 (s, 2H, CH_2-), 6.12 (s, 1H, C3-H), 6.88 (d,1H, C6-H, *J*= 8 Hz), 7.30-7.37 (m, 3H, C2-H,C6-H, and C4-H of phenyl), 7.48 (t, 2H, C3-H, C5-H of phenyl), 7.53 (d, H, C5-H); MS (m/z): 549; Anal calcd. C, 63.37; H, 5.68; N, 7.65; found: C, 62.97; H, 5.89; N, 7.94.

Antimicrobial activity

Sensitivity test

The agar disc plate method using Hi-Media agar medium was employed to study the antimicrobial activity of the synthesized compounds with tobramycin as the reference drug. The prepared compounds were examined against two strains of Gram-positive (*Staphylococcus aureus* ATCC 25923 and *Bacillus subtilis* ATCC 14579), Gram-negative bacteria (*Pseudomonas aeruginosa* ATCC 27853 and *Escherichia coli* ATCC 25922) and *Candida albicans* ATCC 10231). Each test compound (50 mg) was dissolved in DMSO (dimethyl sulphoxide) (0.5 ml, 100 mg/ml), which was used as a sample solution. 6 mm discs were impregnated with 100 mg/ml solution of the test compound were placed on the solidified nutrient agar medium that had been inoculated with the respective microorganism and the Petri dishes were subsequently incubated at 37 °C for 48 h. Tobramycin was used as reference drugs and DMSO as a negative control. Zones of inhibition produced by each compound were measured in millimetres [16].

Minimum inhibitory concentration test (MIC)

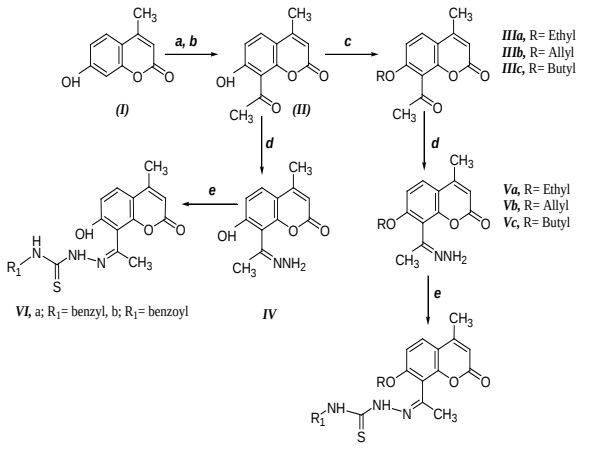
The agar cup plate method using Hi-Media agar medium was employed to study the antibacterial activity against Staphylococcus aureus. Each test compound (50 mg) was dissolved in dimethyl sulphoxide (100 mg/ml), which was used as a stock solution to carry out two-fold dilution technique. The sample size for all the compounds was fixed at 0.1 ml. Using a sterilized cork borer, cups were scooped out of Agar medium contained in a Petri dish which was previously inoculated with the microorganisms. The test compound solution (0.1 ml) was added to the cups, and the Petri dishes were subsequently incubated at 37 °C for 48 h. MIC was defined as the lowest compound concentration preventing visible bacterial growth [16].

RESULTS AND DISCUSSION

Chemistry

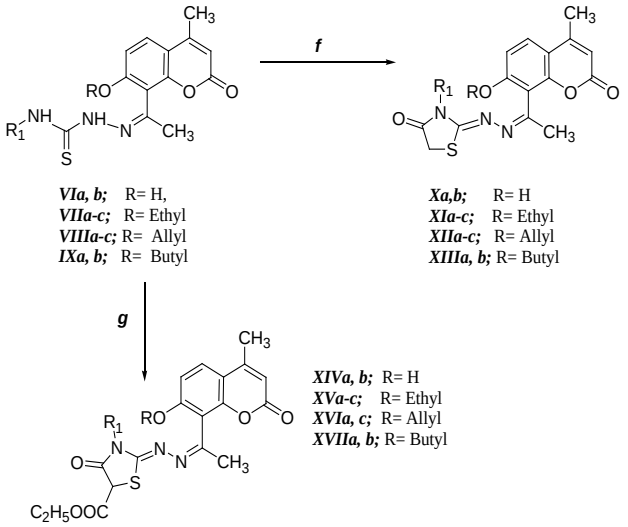
The starting compound 7-hydroxy-4-methylcoumarin (I) was prepared as reported in the literature via Pechmann-Duisburg reaction [17]. Then the 8-acetylcoumarin derivative (II) was prepared by acetylation of the 7-hydroxy group with acetic anhydride [18] followed by Fries rearrangement using anhydrous $AlCl_3$ [19]. To study the effect of alkylation of the 7-hydroxyl group on antimicrobial activity, the 7-ethoxy (IIIa), 7-allyloxy (IIIb), and 7-butyloxy (IIIc) derivatives were prepared using the appropriate alkyl halide in dry acetone [20]. The 7-hydroxy (II) and the 7-alkoxy derivatives (IIIa-IIIc) were then treated with hydrazine hydrate to yield the hydrazones (IV and Va-Vc) [21, 22], that reacted with different isothiocyanates to give coumarin-thiosemicarbazones (VI-IX) in good yields [23]. Coumarin-thiazolidine-4-ones (X-XIII) were formed by cyclizing the thiosemicarbazone (VI-IX) with chloroacetic acid, freshly fused sodium acetate in absolute ethanol, while the thiazolidin-4-one-5-carboxylix acid ethyl ester derivatives (XIV-XVII) were prepared from intermediates (VI-IX) through cyclization with diethyl bromo malonate in refluxing absolute ethanol in the presence of fused sodium acetate [24].

The synthetic pathways are outlined in scheme 1 and 2. The structures of the synthesized compounds were confirmed by spectral data and elemental analysis, and they were in full agreement with the proposed structures.



VII, R= Ethyl , a; R_1= cyclohexyl, b; R_1= phenyl, c; R_1= p.methoxyphenyl
VIII, R= Allyl, a; R_1= cyclohexyl, b; R_1= benzyl, c; R_1= p.methoxyphenyl
IX, R= Butyl , a; R_1= ethyl, b; R_1= benzyl

Scheme 1: Reagent and conditions: a) acetic anhydride, reflux; b) Aluminum Chloride, fusion, 2 h, from 120 to 175 °C; c) appropriate Alkyl halide, anhydrous K_2CO_3 in dry acetone, and reflux; d) Hydrazine hydrate, ethanol, and reflux; e) appropriate isothiocyanate derivatives, ethanol, and reflux



Scheme 2: Reagent and conditions: f) Chloroacetic acid and fused sodium acetate, ethanol, and reflux; g) diethyl bromo malonate and fused sodium acetate, ethanol, and reflux

Antimicrobial activity

The results (table 1) showed that compounds VIb, Xb, XIVb, XVa and XVc possessed strong inhibitory activity against *S. aureus* compared to the reference leads listed in table 2. While, compounds VIIa, VIIIa, IXa, IXb, and XIVa have moderate activity compared to the reference compound tobramycin. Compounds XIII and XIVb have fair to moderate activity against *B. subtilis*. The synthesized compounds had no activity on the Gram-negative strains used. The active compounds Xb, XIVb, XVa, and XVc showed activity against *C. albicans* beside the antibacterial activity which was better than lead 7 that had only antifungal activity with MIC value of 0.1µg/ml.

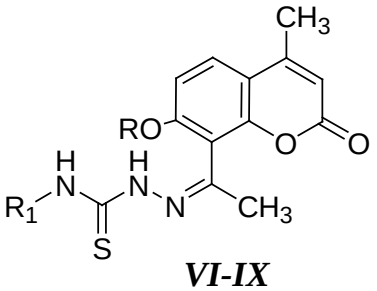
Compounds VIb, Xb, XIVb, and XVc, showed better inhibitory activity in terms of lower MIC values (0.19-0.36 µg/ml) than the lead drug bearing the same nucleus novobiocin 1 (MIC= 1 µg/ml). Novobiocin is known for its potent inhibitory activity against *S. aureus* especially methicillin-resistant strains through DNA-gyrase inhibition. Thus, the newly synthesized compounds represent promising antibacterial agents, especially against this strain.

By comparing the minimum inhibitory concentration (MIC) (table 3) of the active compounds VIb, Xb, XIVb, and XVc to tobramycin (MIC= 2 µg/ml) and novobiocin (MIC= 1 µg/ml), they gave better values ranging from 0.195-0.390 µg/ml; which were also better values reported for lead 5 (MIC= 25 µg/ml) and lead 6 (MIC= 10 µg/ml).

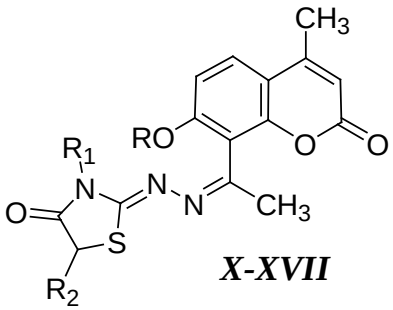
Among the thiosemicarbazone series VI-IX, the 7-hydroxy derivative VIb with benzoyl group at N4 of thiosemicarbazone was the most active with a zone of inhibition value of 35 mm and MIC value of 0.195µg/ml. This result showed the importance of the free hydroxyl group at this position. On the other hand, the 7-alkoxy derivatives VIIa, VIIIa, and IXa were moderately active when compared to their aromatic analogs VIIb, VIIc, VIIIb, and VIIIc.

In thiazolidine-4-one series X-XIII, compound Xb had stronger activity against *S. aureus* with MIC value of 0.390 µg/ml and showed weak activity against *C. albicans*, which may be attributed to the presence of thiazolidine-4-one ring when compared to its precursor compound VIb. Also, compound XIIIa showed slight activity against *B. subtilis*. While, in the thiazolidine-4-one-5-carboxylic acid ethyl ester series XIV-XVII, compounds XIVb, XVa, XVc had strong antibacterial activity when compared to their analogs (Xb), (VIIa, XIa), and (VIIc, XIc) respectively. Compound XIVa possessed moderate activity over compound VIa, and Xa which may be attributed to the 5-carboxylic acid ethyl ester on the thiazolidine-4-one ring. In summary, it was evidenced that the presence of free 7-hydroxyl group on 4-methycoumarin ring was very important for activity. The hybridization with thiosemicarbazone substituted with benzoyl moiety as in compound VIb or thiazolidine-4-one-5-carboxylic acid ethyl ester as in compound XIVb gave rise to promising antimicrobial agents.

Table 1: The mean* of zone of inhibition (mm) of the active compounds against gram-positive bacteria



VI-IX

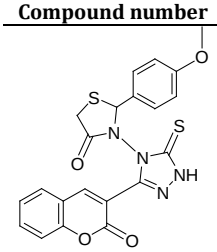
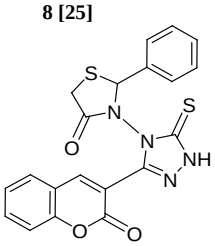
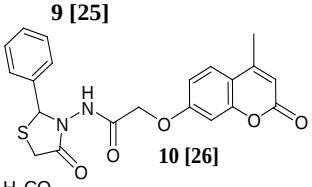
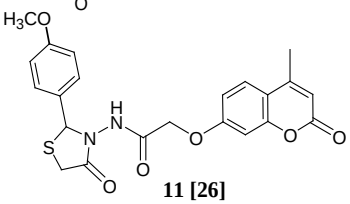


X-XVII

Compound number	R	R_1	R_2	*S. aureus*	*B. subtilis*	*C. albicans*
Tobramycin				21 mm	ND	ND
VIb	H	Benzoyl		35 mm	-	-
VIIa	Ethyl	Cyclohexyl		10 mm	-	-
VIIIa	Allyl	Cyclohexyl		14 mm	-	-
IXa	Butyl	Ethyl		14 mm	ND	ND
IXb	Butyl	Benzyl		10 mm	ND	ND
Xb	H	Benzoyl	H	30 mm	-	8 mm
XIIIa	Butyl	Ethyl	H	-	8 mm	-
XIVa	H	Benzyl	$COOC_2H_5$	13 mm	-	-
XIVb	H	Benzoyl	$COOC_2H_5$	30 mm	12 mm	14 mm
XVa	Ethyl	Cyclohexyl	$COOC_2H_5$	19 mm	-	9 mm
XVc	Ethyl	*P.* methoxyphenyl	$COOC_2H_5$	22 mm	-	11 mm

*Presented are the mean of 3 separate experiments; Errors are in the range±10% of the reported values. Inactive: inhibition zone<5 mm; slightly active: inhibition zone = 5-10 mm; moderately active: inhibition zone = 10-15 mm; highly active: inhibition zone>15 mm, **ND; not determined, *** *S. aureus: Staphylococcus aureus, B. subtilis: Bacillus subtilis, C. albicans: Candida albicans*

Table 2: Comparison of the activity of the new active compounds to previously reported leads against *Staphylococcus aureus*

Compound number	Zone of inhibition (mm)	% inhibition*
 **8 [25]**	15 mm	71%
 **9 [25]**	23 mm	109%
 **10 [26]**	18 mm	85%
 **11 [26]**	18 mm	85%
VIb (this work)	35 mm	166%
Xb (this work)	30 mm	142%
XIVb (this work)	30 mm	142%
XVc (this work)	22 mm	104%

* % Inhibition= zone of inhibition of compounds in mm/mm of tobramycin*100 [25]

Table 3: The mean* MIC (µg/ml) values of the active compounds VIb, Xb, XIVb, and XVc and the comparison of these values to the reported MIC values of compounds having similar pharmacophores

Compound number	Minimum inhibitory concentration MIC (µg/ml)
Tobramycin	2
Novobiocin	1 [27]
5	25 [12]
6	10 [13, 14]
7	0.1 [15]
VIb	0.195*
Xb	0.390*
XIVb	0.195*
XVc	0.390*

* Presented are the mean of 3 separate experiments; errors are in the range±10% of the reported values. MIC mean values for compounds with a zone of inhibition>20 mm was determined.

CONCLUSION

The novel series of 4-methylcoumarin bearing thiosemicarbazone moiety (VI-IX) and the series having thiazolidine-4-one (X-XVII) were synthesized, and their antimicrobial activity was evaluated. These novel coumarin derivatives showed potential activity against Gram-positive *Staphylococcus aureus* especially these with a free 7-hydroxy group (VIb, Xb, XIVb); that emphasize the importance of this position for antibacterial activity and compounds XIVa, XVa, and XVc with 5-carboxylic acid ethyl ester group on thiazolodin-4-one ring showed enhanced potency than their parent compounds. Hence, it can be concluded that these novel compounds are potential antibacterial agents better than the reference compounds and represent promising leads for further optimization and clinical studies.

CONFLICT OF INTERESTS

Declared none

REFERENCES

1. Jain PK, Himanshu J. Coumarin: chemical and pharmacological profile. J Appl Pharm Sci 2012;2:236-40.
2. Vahid V, Farhad H. Microwave assisted convenient one-pot synthesis of coumarin derivatives via pechmann condensation catalyzed by FeF3 under solvent-free conditions and

antimicrobial activities of the products. Molecules 2014;19:13093-103.

3. Varughese MA, Ramakrishna PB, Shriniwas DS. Bismuth (III) nitrate pentahydrate—a mild and inexpensive reagent for the synthesis of coumarins under mild conditions. Tetrahedron Lett 2005;46:6957–9.
4. Borges F, Roleira F, Milhazes N, Santana L, Uriarte E. Simple coumarins and analogues in medicinal chemistry: occurrence, synthesis, and biological activity. Curr Med Chem 2005; 12:887-916.
5. Heiner GG, Fatima, Russell PK, Haase AT, Ahmad N, Mohammed N, *et al.* Field trials of methisazone as a prophylactic agent against smallpox. Am J Epidemiol 1971;94:435-49.
6. Sau DK, Butcher RJ, Chaudhuri S, Saha N. Spectroscopic, structural and antibacterial properties of copper(II) complexes with bio-relevant 5-methyl-3-formylpyrazole N-benzyl-N-methyl thiosemicarbazone Mol Cell Biochem 2003;253:21-9.
7. Pavan FR, da S Maia P, Leite SR, Deflon VM, Batista AA, Sato DN, *et al.* Thiosemicarbazones, semicarbazones, dithiocarbazates and hydrazide/hydrazones: anti–mycobacterium tuberculosis activity and cytotoxicity. Eur J Med Chem 2010;45:1898-905.
8. Magalhaes Moreira DR, De Oliveira AD, Teixeira de Moraes Gomes PA, De Simone CA, Villela FS, Ferreira RS, *et al.* Conformational restriction of aryl thiosemicarbazones produces potent and selective anti-Trypanosoma cruzi compounds which induce apoptotic parasite death. Eur J Med Chem 2014;75:467-78.
9. Afrasiabi Z, Sinn E, Chen JN, Ma YF, Rheingold AL, Zakharov LN, *et al.* Appended 1,2-naphthoquinones as anticancer agents 1: synthesis, structural, spectral and antitumor activities of ortho-naphthoquinone thiosemicarbazone and its transition metal complexes. Inorg Chim Act A 2004;357:271-8.
10. Sankaraperumal A, Karthikeyan J, Nityananda A, Lakshmi SR. Nickel (II) complex of *p*-[N, N-bis(2-chloroethyl) amino] benzaldehyde-4-methyl-thiosemicarbazone: Synthesis, characterization, and biological application. Polyhedron 2013; 50:264-9.
11. Patil S, Unki S, Kulkarni A, Naik V, Badami P. Co(II), Ni(II) and Cu(II) complexes with coumarin-8-yl Schiff-bases: spectroscopic, *in vitro* antimicrobial, DNA cleavage and fluorescence studies. Spectrochim Acta Part A 2011;79:1128-36.
12. Bondock S, Khalifa W, Fadda AA. Synthesis and antimicrobial evaluation of some new thiazole, thiazolidinone and thiazoline derivatives starting from 1-chloro-3, 4-dihydronaphthalene-2-carboxaldehyde. Eur J Med Chem 2007;42:948-54.
13. Sandhu S, Bansal Y, Silakari O, Bansal G. Coumarin hybrids as novel therapeutic agents. Bioorg Med Chem 2014;15:3806-14.
14. Ronad PM, Noolvi MN, Sapkal S, Dharbhamulla S, and Maddi VS. Synthesis and antimicrobial activity of 7-(2-substituted phenylthiazolidinyl)-benzopyran-2-one derivatives. Eur J Med Chem 2010;45:85-9.
15. Bojan Š, Maja M, Milan C, Lars G. 4-Methyl-7-hydroxycoumarin antifungal and antioxidant activity enhancement by substitution with thiosemicarbazide and thiazolidinone moieties. Food Chem 2013;139:488–95.
16. Ali P, Jyotsna M, Vandana T, Javed S, Rajendra D, Moulay HY, *et al.* Pharmacophores modeling in terms of prediction of theoretical physicochemical properties and verification by experimental correlations of novel coumarin derivatives produced via Betti's protocol. Eur J Med Chem 2010;45:4370-8.
17. Pechmann HV, Duisberg C. Uber die verbindungen der phenole mit acetessigather. Ber Dtsch Chem Ges 1883;16**:**2119–28.
18. Chakraborti A, Gulhane R, Shivani. Copper (II) tetrafluoroborate-catalyzed acetylation of phenols, Thiols, Alcohols-, and amines. Synthesis 2004;1:111-5.
19. Valery F Traven. New synthetic routes to furocoumarins and their analogs: a review. Molecules 2004;9:50-66.
20. Alexander Williamson. "XLV. Theory of etherification". Philos Mag 1850;37**:**350-6.
21. Smith PAS, Most EE Jr. Quaternary hydrazones and their rearrangement. J Org Chem 1957;22**:**358-62.
22. Newkome GR, Fishel DL. Synthesis of simple hydrazones of carbonyl compounds by an exchange reaction. J Org Chem 1966;31:677-81.
23. Mohamad MM, Hussein. Synthesis, photosensitizing and antimicrobial studies on coumarin thiosemicarbazones. Al-Azhar J Pharm Sci 2009;39-55.
24. Shiva PS, Surendra SP, Krishna R, Virgil IS. Chemistry and biological activity of thiazolidinones. Chem Rev 1981;81:175-203.
25. Mashooq AB, Nadeem S, Suroor AK, Mohamed IM. Synthesis of triazolothiazolidinone derivatives of coumarin with antimicrobial activity. Acta Pol Pharm 2009;66:625-32.
26. Naceur H, Abdullah SA, Ridha B, Alary F. Synthesis and characterization of new thiazolidinones containing coumarin moieties and their antibacterial and antioxidant activities. Molecules 2012;17:9321-34.
27. Salmon SA, Watts JL, Aarestrup FM, Pankey JW, Yancey RJ Jr. Minimum inhibitory concentrations of selected antimicrobial agents against organisms isolated from the mammary glands of dairy heifers in New Zealand and Denmark. J Dairy Sci 1998;81:570-8.