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Synthesis, Characterization and Primary Antituberculosis Activity Evaluation of 4-(3-Coumarinyl)-3-benzyl-4-thiazolin-2-one Benzylidenehydrazones

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In this study a new series of 4-(3-coumarinyl)-3-benzyl-4-thiazolin-2-one benzylidenehydrazones **3a-t** was synthesized. Structures of the title compounds were elucidated by elemental analyses and spectrometric data (IR, ^{1}H-NMR, ^{13}C-NMR and EIMS). **3a-t** were evaluated for antituberculosis activity against *Mycobacterium tuberculosis* H37Rv in BACTEC 12B medium using the BACTEC 460 radiometric system.

Key Words: Coumarinylthiazolines, synthesis, antituberculosis activity.

Introduction

Coumarin derivatives constitute an important class of heterocyclic compounds with anticoagulant (e.g., warfarin, acenocoumarol)[1,2], anticoagulant rodenticide (e.g., brodifacoum, bromadiolone)[3], insecticide (e.g., coumaphos)[4] and antibacterial (e.g., novobiocin, clorobiocin)[5,6] pharmacological properties. The cytotoxic activities of coumarin and its known metabolite 7-hydroxycoumarin were tested in several human tumor cell lines. Both compounds inhibited cell proliferation of a gastric carcinoma cell line, a colon-carcinoma cell line, a hepatoma-derived cell line and a lymphoblastic cell line[7]. On the other hand, the iminothiazoline derivatives have been reported to exhibit antibacterial and antifungal activities[8,9]. Our previously reported works on the synthesis of 4-thiazolinylarylidene hydrazones indicated that most of the compounds showed high antituberculosis activity[10,11]. In this study we report the synthesis, structural determination and in vitro antituberculosis activity of the new 4-(3-coumarinyl)-3-benzyl-4-thiazolin-2-one benzylidenehydrazones.

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Experimental

Melting points were estimated with a Büchi 530 melting point apparatus in open capillaries and are uncorrected. Elemental analyses were performed on a Carlo Erba 1106 elemental analyzer. IR spectra were recorded on KBr disks, using a Perkin-Elmer Model 1600 FT-IR spectrometer. ^{1}H-NMR and ^{13}C-NMR spectra were obtained on a Bruker AC 200 (200 MHz and 50.3 MHz) spectrophotometer. EIMS were determined on a VG Zab Spec (70 eV) mass spectrometer

Synthesis of 3-(ω-bromoacetyl)coumarins (1a,b)[12,13]

To a cold mixture of salicylaldehyde (0.10 mol) and ethylacetoacetate (0.10 mol) was added 1 g of piperidine by rapid shaking. The solid separated was filtered and washed with ethanol. Crystallization of the solid from water gave pure 3-acetylcoumarin. A solution of bromine (4 g) in chloroform was added by shaking to a solution of 3-acetylcoumarin (0.025 mol) in chloroform. The mixture was heated under reflux for 1 h and cooled. The solid separated was washed with ether and crystallized from ethanol-chloroform (2:1).

Synthesis of 1-substituted benzylidene-4-benzylthiosemicarbazides (2a-l)[14]

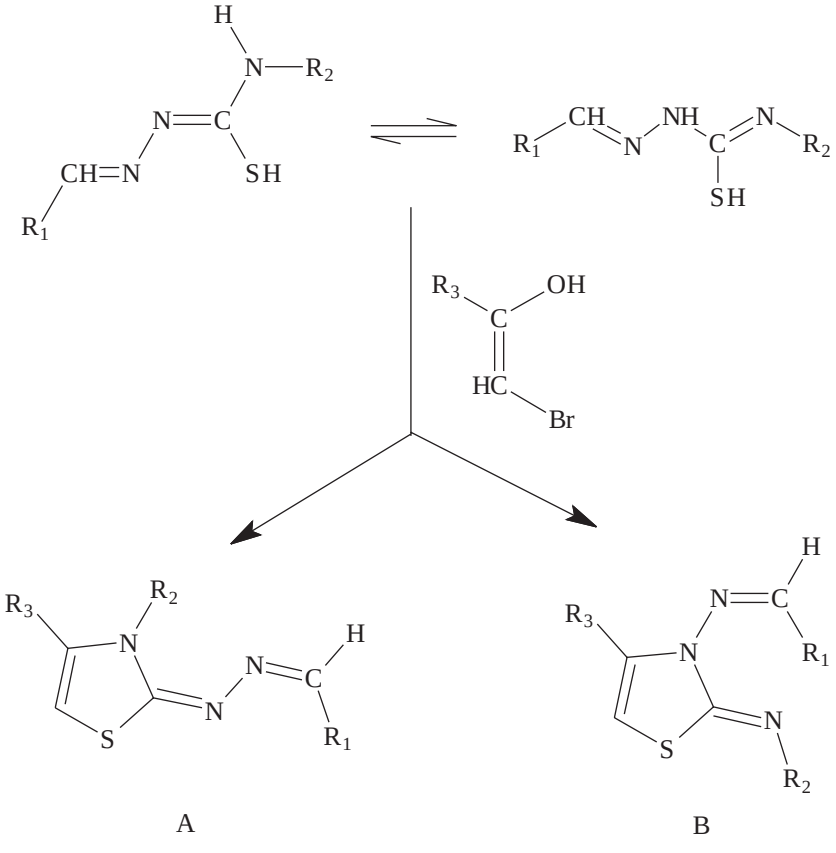
A solution of 4-benzylthiosemicarbazide (0.02 mol) in ethanol was added to a boiling solution of substituted benzaldehyde (0.02 mol) in ethanol. The mixture was refluxed for 1 h. The solid separated was filtered and crystallized from ethanol or ethanol-chloroform (2:1).

Synthesis of 4-(3-coumarinyl)-3-benzyl-4-thiazolin-2-one benzylidenehydrazones (3a-t)

A solution of 3-(ω-bromoacetyl)coumarin (**1a**, **1b**) (0.0025 mol) and 1-substituted benzylidene-4-benzylthiosemicarbazide (**2a-l**) (0.0025 mol) in chloroform-ethanol (2:1) was refluxed for 2 h and allowed to stand overnight. The crystals thus obtained were filtered, and then crystallized from ethanol or ethanol-chloroform (2:1).

Results and Discussion

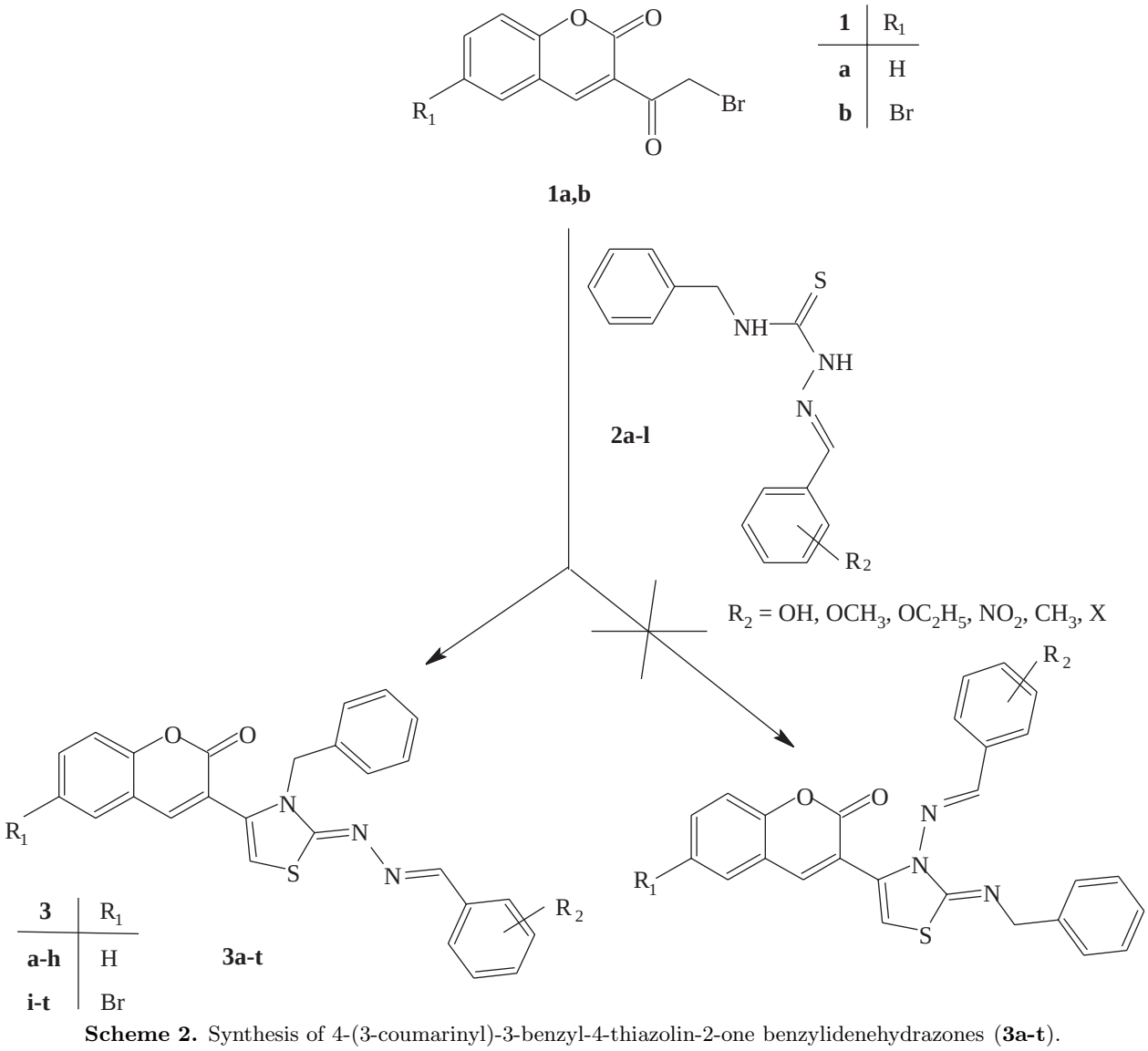
It is known that the thiosemicarbazones with α-haloketones give different products depending on reaction conditions. Literature surveys show that this reaction in neutral medium results in the formation of 4-thiazolin-2-ylidene hydrazone. In thiazole cyclization the ene-thiol form determines the isomeric structures that are to be formed. The ene-thiol formation involves the NH group adjacent to the more electron-withdrawing moiety[15] (Scheme 1). Tautomerization is easier at the N^2 position than it is at the N^4 position in our compounds and the anticipated structure is A in the scheme.



Scheme 1. The isomeric structures (A/B).

An independent proof of structure A of 4-(3-coumarinyl)-3-benzyl-4-thiazolin-2-one 4-methylbenzylidenehydrazone was also achieved by single crystal X-ray diffraction analysis[16]. In view of these observations, the reaction of 3-(ω-bromoacetyl)coumarin **1a,b**[12,13] with 1-substituted-benzylidene-4-benzylthiosemicarbazides **2a-l**[14] in neutral medium resulted in the formation of 4-(3-coumarinyl)-3-benzyl-4-thiazolin-2-one benzylidenehydrazones **3a-t**, as bases (**3a**, **3d-h**, **3k** and **3n-t**) or as HBr salts (**3b**, **3c**, **3i**, **3j**, **3l** and **3m**). The structures of **3a-t** were established by elemental analysis and spectrometric data (IR, ^{1}H-NMR, ^{13}C-NMR and EIMS) (Scheme 2) (Tables 1 and 2).

In the IR spectra of **3a-t** the lactone C=O bands of the coumarin ring were observed in the 1740-1706 cm^{-1} region[17,18]. The spectra of the compounds with HBr salts (**3b**, **3c**, **3i**, **3j**, **3l** and **3m**) showed the NH$^+$ stretching bands in the 2743-2605 cm^{-1}region. The ^{1}H-NMR spectra of **3b-d**, **3k-m** and **3o** showed singlets at 6.27- 6.86 ppm and 7.55-8.48 ppm due to thiazoline 5-H and N=CH, respectively[19,20]. Coumarin 4-H on the β-carbon of an α, β-unsaturated carbonyl group is highly deshielded due to the polarization caused by the electron attracting carbonyl function. Therefore, coumarin 4-H resonated at 8.15-9.30 ppm[21]. In the APT ^{13}C-NMR spectra of **3k** CH_3, NCH_2, thiazoline C-5, thiazoline C-4, coumarin C-4, N=CH, thiazoline C-2 and coumarin C-2 resonances in the 21.51, 49.49, 118.36, 139.65, 141.58, 152.23, 158.59 and 169.40 ppm, respectively, were observed[22–24]. In the EIMS spectra of **3b-d**, **3k-m** and **3o** molecular ions, which were also the base peak (except **3o**), were observed. Fragments corresponding to 3-benzyl-4-(3-coumarinyl)-2-imino-4-thiazoline (m/z 333 or m/z 411, 413) and substituted-benzylideneimine moieties were formed by the cleavage of the N-N bond[19].



Scheme 2. Synthesis of 4-(3-coumarinyl)-3-benzyl-4-thiazolin-2-one benzylidenehydrazones (**3a-t**).

3a-t were evaluated for in vitro antituberculous activity against *Mycobacterium tuberculosis* H37Rv using the BACTEC 460 radiometric system[10,11]. Rifampin was used as the standard in the tests. Only R_1 = Br and R_2 = 2-OH and 5-NO_2 substituted compound **3o** exhibited 11% inhibition in the primary screening that was conducted at 6.25 μg/ml in BACTEC 12B medium.

Table 1. Formulas, physical constants and elemental analysis of **3a-t**.

Comp.	R_1	R_2	Yield (%)	m.p. °C	Formula (M.W.)	Elemental Analyses (Calc./Found.) C	H	N
3a	H	2-OCH_3	88	199-200	$C_{27}H_{21}N_3O_3S.H_2O$ (485.57)	66.78 66.22	4.77 5.13	8.65 9.27
3b	H	4-OCH_3	85	217	$C_{27}H_{21}N_3O_3S.HBr\ 1^1/_2H_2O$ (575.50)	56.35 56.13	4.37 3.69	7.30 7.19
3c	H	3-OC_2H_5 4-OH	89	189-191	$C_{28}H_{23}N_3O_4S.HBr$ (578.48)	58.13 58.33	4.18 3.90	7.26 7.36
3d	H	2-OH 5-NO_2	72	264-266	$C_{26}H_{18}N_4O_5S.^1/_2H_2O$ (507.53)	61.53 61.20	3.77 3.17	11.03 10.71
3e	H	2-OH 5-Br	85	198-199	$C_{26}H_{18}BrN_3O_3S.1^1/_2H_2O$ (559.45)	55.82 55.64	3.78 3.28	7.51 7.27
3f	H	4-F	77	179	$C_{26}H_{18}FN_3O_2S.H_2O$ (473.53)	65.94 65.45	4.25 3.72	8.87 8.50
3g	H	4-Br	90	208-210	$C_{26}H_{18}BrN_3O_2S$ (516.42)	60.47 60.01	3.51 3.64	8.13 7.92
3h	H	3-Cl 4-Cl	72	172-176	$C_{26}H_{17}Cl_2N_3O_2S.H_2O$ (524.43)	59.54 59.83	3.65 3.64	8.01 7.45
3i	Br	2-OCH_3	81	211-217	$C_{27}H_{20}BrN_3O_3S.HBr$ (627.36)	51.69 51.46	3.37 3.08	6.69 6.55
3j	Br	4-OCH_3	87	222-226	$C_{27}H_{20}BrN_3O_3S.HBr$ (627.36)	51.69 51.33	3.37 3.29	6.69 6.55
3k	Br	4-CH_3	82	225-229	$C_{27}H_{20}BrN_3O_2S.2H_2O$ (566.49)	57.24 57.09	4.27 3.49	7.41 7.27
3l	Br	3-OCH_3 4-OH	84	242-243	$C_{27}H_{20}BrN_3O_4S.HBr^1/_2H_2O$ (652.38)	49.71 49.30	3.39 3.23	6.44 6.31
3m	Br	3-OC_2H_5 4-OH	81	221-222	$C_{28}H_{22}BrN_3O_4S.HBr$ (657.38)	51.15 51.39	3.52 3.54	6.39 6.37
3n	Br	3-OCH_3 4-OCH_3	76	194-199	$C_{28}H_{22}BrN_3O_4S.^1/_2H_2O$ (585.48)	57.44 51.39	3.95 3.53	7.17 7.16
3o	Br	2-OH 5-NO_2	89	288-289	$C_{26}H_{17}BrN_4O_5S$ (577.42)	54.08 53.70	2.96 2.59	9.70 9.12
3p	Br	2-OH 5- Br	78	234	$C_{26}H_{17}Br_2N_3O_3S$ (611.31)	51.08 50.59	2.80 2.50	6.87 6.63
3q	Br	4-F	83	181	$C_{26}H_{17}BrFN_3O_2S.1^1/_2H_2O$ (561.44)	55.62 55.54	3.59 2.88	7.48 7.34
3r	Br	4-Cl	81	208-209	$C_{26}H_{17}BrClN_3O_2S.^1/_2H_2O$ (559.87)	55.77 55.96	3.24 2.84	7.50 7.27
3s	Br	4-Br	75	222-224	$C_{26}H_{17}Br_2N_3O_2S$ (595.31)	52.45 52.99	2.87 2.92	7.05 6.95
3t	Br	3-Cl 4-Cl	89	158-62	$C_{26}H_{16}BrCl_2N_3O_2S$ (585.32)	53.35 52.82	2.75 2.63	7.17 6.97

Table 2. NMR and EIMS data of **3b-d**, **3k-m** and **3o**.

Comp.	NMR (δ, ppm)	EIMS (70 ev) m/z (%)
3b	^{1}H-NMR ($CDCl_3$): 3.86 (s, 3H, OCH_3), 5.99 (s, 2H, NCH_2), 6.86 (s, 1H, thiazoline 5-H), 6.91-7.68 (m, 13H, aromatic), 7.72 (s, 1H, N=CH), 9.30 (s, 1H, coumarin 4-H).	468 (MH^+, 49), 467 (M^+, 100), 376 (67), 348 (25), 334 (34), 333 (35), 317 (20), 244 (18), 229 (15), 224 (40), 210 (22), 178 (10), 172 (28), 134 (15), 91 (56).
3c	^{1}H-NMR ($CDCl_3$): 1.47 (t, J: 6.9 Hz, 3H, $OCH_2C\underline{H}_3$), 4.15 (q, J: 6.9 Hz, 2H, $OC\underline{H}_2CH_3$), 5.97 (s, 2H, NCH_2), 6.85 (s, 1H, thiazoline 5-H), 6.93-7.68 (m, 13H, aromatic and OH), 7.55 (s, 1H, N=CH), 9.23 (s, 1H, coumarin 4-H).	498 (MH^+, 32), 497 (M^+, 100), 406 (53), 377 (11), 334 (61), 333 (34), 254 (27), 244 (17), 240 (14), 229 (14), 172 (22), 166 (22), 164 (7), 150 (11), 106 (11), 91 (77).
3d	^{1}H-NMR ($CDCl_3$): 5.35 (s, 2H, NCH_2), 6.41 (s, 1H, thiazoline 5-H), 7.05-8.13 (m, 13H, aromatic), 8.22 (s, 1H, N=CH), 8.62 (s, 1H, coumarin 4-H).	499 (MH^+, 49), 498 (M^+, 100), 407 (40), 390 (27), 360 (21), 334 (45), 333 (50), 258 (14), 255 (31), 244 (26), 229 (16), 172 (25), 164 (3), 92 (16), 91 (91).
3k	^{1}H-NMR ($CDCl_3$): 2.38 (s, 3H, CH_3), 5.23 (s, 2H, NCH_2), 6.27 (s, 1H, thiazoline 5-H), 7.00-7.64 (m, 12H, aromatic), 7.66 (s, 1H, N=CH), 8.33 (s, 1H, coumarin 4-H). ^{13}C-NMR ($CDCl_3$): 21.51 (CH_3), 49.49 (NCH_2), 118.36 (thiazoline C-5), 139.65 (thiazoline C-4), 141.58 (coumarin C-4), 152.23 (N=CH), 152.58 (coumarin C-8a), 158.59 (thiazoline C-2), 169.40 (coumarin C-2), 105.56-136.85 (other aromatic carbons).	530 [(MH^+, 45 (532, 43)], 529 [M^+, 95 (531, 100)], 439 [17 (441, 16)], 438 [53 (440, 55)], 412 [52 (414, 39)], 411 [34 (413, 40)], 410 [16 (412, 52)], 395 [12 (397, 13)], 322 [16 (324, 15)], 307 [12 (309, 13)], 252 (17), 208 (54), 198 (21), 194 (32), 162 (10), 118 (13), 90 (79).
3l	^{1}H-NMR (DMSO-d_6): 3.80 (s, 3H, OCH_3), 5.09 (s, 2H, NCH_2), 6.74 (s, 1H, thiazoline 5-H), 6.79-7.94 (m, 11H, aromatic), 8.11 (s, 1H, N=CH), 8.16 (s, 1H, coumarin 4-H).	562 [MH^+, 31 (564, 30)], 561 [M^+, 90 (563, 100)], 470 [46 (472, 48)], 455 [8 (457, 9)], 440 [4 (442, 4)], 427 [8 (429, 8)], 412 [28 (414, 24)], 368 (13), 313 (9), 262 (10), 240 (41), 236 (11), 226 (21), 150 (7), 135 (8), 123 (9), 111 (9), 109 (9), 97 (15), 94 (14), 91 (64).
3m	^{1}H-NMR (DMSO-d_6): 1.35 (t, J: 6.9 Hz, 3H, $OCH_2C\underline{H}_3$), 4.04 (q, J: 6.8 Hz, 2H, $OC\underline{H}_2CH_3$), 5.10 (s, 2H, NCH_2), 6.77 (s, 1H, thiazoline 5-H), 6.80-8.03 (m, 12H, aromatic and OH), 8.11 (s, 1H, N=CH), 8.15 (s, 1H, coumarin 4-H).	576 [MH^+, 35 (578, 34)], 575 [M^+, 97 (577, 100)], 484 [50 (486, 54)], 456 [10 (458, 10)], 412 [37 (414, 34)], 322 [18 (324, 17)], 307 [9 (309, 9)], 254 (40), 240 (19), 165 (3), 91 (44).
3o	^{1}H-NMR ($CDCl_3$): 5.47 (s, 2H, NCH_2), 6.51 (s, 1H, thiazoline 5-H), 7.07-8.28 (m, 12H, aromatic), 8.48 (s, 1H, N=CH), 8.83 (s, 1H, coumarin 4-H).	577 [MH^+, 15 (579, 15)], 576 [M^+, 45 (578, 48)], 485 [9 (487, 9)], 412 [28 (414, 25)], 411 [18 (413, 21)], 322 [8 (324, 8)], 255 (16), 164 (2), 91 (100).

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