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Synthesis and antitubercular activity of 4-(3-coumarinyl)-3-cyclohexyl-4-thiazolin-2-one benzylidenehydrazones

N. Karalı*, A. Kocabalkanlı, A. Gürsoy, Ö. Ateş

Department of Pharmaceutical Chemistry, Faculty of Pharmacy, University of İstanbul, 34452 Beyazit-Istanbul, Turkey

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Abstract

In this study, a new series of 4-(3-coumarinyl)-3-cyclohexyl-4-thiazoline-2-one benzylidene hydrazones (**3a**–**p**) were synthesized. Structures of the title compounds were determined by analytical and spectral methods. **3a**–**p** were evaluated for antituberculosis activity against *Mycobacterium tuberculosis* H37Rv.

Keywords: Coumarins; Thiazolines; Benzylidene hydrazones; Antitubercular activity

1. Introduction

Heterocycles containing the coumarin ring system involve some novel pharmacologically active compounds such as dicumarol, warfarin, mercumatilin, novobiocin. On the other hand, literature surveys show that thiazolinylhydrazones exhibit antitubercular [1] and antimicrobial [2] activities. Our previously reported works on the synthesis of 4-thiazolinylarylidenehydrazones [3–7] indicated that cyclohexyl substitution on 3-position of the thiazoline led to the highest antituberculosis activity. Encouraged by these findings, a series of 4-(3-coumarinyl)-3-cyclohexyl-4-thiazoline-2-one benzylidenehydrazones (**3a**–**p**) were synthesized by condensation of 3-(ω-bromoacetyl)coumarins (**1a** and **1b**) with 1-substituted benzylidene-4-cyclohexylthiosemicarbazides (**2a**–**j**). The structures of these new compounds were determined by analytical and spectral (UV, IR, ^{1}H NMR, EIMS) methods. Compounds **3a**–**p** were tested for antituberculosis activity against *Mycobacterium tuberculosis* H37Rv at the single concentration 6.25 μg ml^{-1}. Most of the compounds showed some inhibition, yet the inhibitions being less than 90% indicated that the compounds were all considered to be inactive.

2. Material and methods

2.1. Chemical studies

Melting points were estimated with a Büchi 530 melting point apparatus (Flawil, Switzerland) in open capillaries and are uncorrected. Elemental analyses were performed on a Carlo Erba 1106 elemental analyzer. UV spectra in ethanol were taken on a Shimadzu Model UV1601 UV spectrophotometer. IR spectra were recorded on KBr discs, using a Perkin–Elmer Model 1600 FT-IR spectrophotometer. ^{1}H NMR spectra were obtained on a Bruker AC 200 (200 MHz) spectrophotometer using DMSO-d_6. EIMS were determined on a VG Zab Spec (70 eV) mass spectrometer. Starting materials were purchased from E. Merck.

2.2. Synthesis of 4-(3-coumarinyl)-3-cyclohexyl-4-thiazolin-2-one benzylidenehydrazones (3a–p)

A solution of 3-(ω-bromoacetyl)coumarins (**1a** and **1b)** (0.0025 mol) and 1-substituted benzylidene-4-substituted thiosemicarbazides (**2a**–**j**) (0.0025 mol) in chloroform–ethanol (2:1) was refluxed for 2 h and allowed to stand overnight. The crystals were filtered, dried and purified by crystallization from ethanol or ethanol–chloroform (2:1).

* Corresponding author
E-mail address: nilgunkarali@yahoo.com (N. Karalı).

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2.3. In vitro evaluation of antituberculosis activity

Primary screen was conducted at 6.25 μg ml^{-1} against *M. tuberculosis* H37Rv (ATCC 27294) in BACTEC 12B medium using a broth microdilution assay the Microplate Alamar Blue Assay (MABA). Our compounds which effected $<90\%$ inhibition at this concentration were not evaluated further.

2.4. BACTEC radiometric method of susceptibility test

Inocula for susceptibility testing were either from a positive BACTEC isolation vial with a growth index (GI) of 500 or more, or a suspension of organisms isolated earlier on a conventional medium.

The culture was well mixed with a syringe and 0.1 ml of a positive BACTEC culture was added to each of the vials containing the test compounds (6.25 μg ml^{-1}). The standard vials contain rifampin (0.25 μg ml^{-1}). A control vial was inoculated with a 1:100 dilution of the culture. A suspension equivalent to a Mc Farland No. 1 standard was prepared in the same manner as a BACTEC positive vial when growth from a solid medium was used.

Each vial was tested immediately on a BACTEC instrument to provide CO_2 in the headspace.

The vials were incubated at 37 °C and tested daily with a BACTEC instrument. When the GI in the control read at least 30, the increase in the GI (ΔGI) from the previous day in the control was compared

Table 1
Physical constants of **3a–p**

Comp.	R_1	R_2	Yield (%)	M.p. (°C)	Formula (M.W.)	Analyses (calc./found)			
						C	H	N	S
3a	H	4-CH_3	81	236–240	$C_{26}H_{25}N_3O_2S{\cdot}H_2O$ (461.59)	67.66 67.89	5.89 5.34	9.10 9.36	6.95 6.44
3b	H	3-OCH_3 4-OCH_3	81	156–162	$C_{27}H_{27}N_3O_4S{\cdot}1/2H_2O$ (498.61)	65.04 65.07	5.66 5.28	8.43 8.63	6.43 6.04
3c	H	2-OH 5-NO_2	98	271–276	$C_{25}H_{22}N_4O_5S{\cdot}HBr\ 1/2H_2O$ (580.47)	51.73 51.57	4.17 3.68	9.65 9.08	5.52 4.88
3d	H	4-F	98	225–228	$C_{25}H_{22}FN_3O_2S{\cdot}1/2H_2O$ (456.55)	65.77 65.21	5.08 4.90	9.20 9.20	7.02 6.58
3e	H	4-Br	93	188–193	$C_{25}H_{22}BrN_3O_2S$ (508.44)	59.06 59.30	4.36 4.55	8.26 8.17	6.31 5.94
3f	H	3-Cl 4-Cl	82	192–195	$C_{25}H_{21}Cl_2N_3O_2S{\cdot}HBr$ (579.35)	51.83 51.62	3.83 4.62	7.25 7.09	5.54 5.26
3g	Br	2-OCH_3	83	206	$C_{26}H_{24}BrN_3O_3S{\cdot}H_2O$ (556.49)	56.12 56.68	4.71 4.95	7.55 7.79	5.76 5.26
3h	Br	4-OCH_3	74	153–159	$C_{26}H_{24}BrN_3O_3S{\cdot}H_2O$ (556.49)	56.12 56.19	4.71 4.32	7.55 7.54	5.76 5.48
3i	Br	4-CH_3	94	221–222	$C_{26}H_{24}BrN_3O_2S{\cdot}1/2H_2O$ (531.48)	58.76 59.37	4.74 5.36	7.91 8.03	6.03 5.50
3j	Br	3-OCH_3 4-OH	99	247–248	$C_{26}H_{24}BrN_3O_4S{\cdot}HBr$ (635.39)	49.15 49.60	3.97 4.48	6.61 6.70	5.05 4.89
3k	Br	3-OCH_3 4-OCH_3	99	239–240	$C_{27}H_{26}BrN_3O_4S{\cdot}HBr$ (649.41)	49.94 50.44	4.19 4.93	6.47 6.64	4.94 4.58
3l	Br	2-OCH_3 5-OCH_3	97	231	$C_{27}H_{26}BrN_3O_4S{\cdot}HBr\ H_2O$ (667.43)	48.59 48.45	4.38 4.49	6.30 6.42	4.80 4.73
3m	Br	2-OH 5-NO_2	96	288–289	$C_{25}H_{21}BrN_4O_5S{\cdot}1/2H_2O$ (578.45)	51.91 51.91	3.83 4.18	9.69 9.62	5.54 5.37
3n	Br	4-F	99	162–165	$C_{25}H_{21}BrFN_3O_2S{\cdot}H_2O$ (544.45)	55.15 55.37	4.26 4.51	7.72 7.76	5.89 5.57
3o	Br	4-Br	84	205–210	$C_{25}H_{21}Br_2N_3O_2S{\cdot}1/2H_2O$ (596.35)	50.35 50.27	3.72 3.66	7.05 7.01	5.38 5.06
3p	Br	3-Cl 4-Cl	76	245–246	$C_{25}H_{20}BrCl_2N_3O_2S$ (577.34)	52.01 51.77	3.49 2.76	7.28 7.03	5.55 5.35

Table 2
UV and IR spectral data of compounds **3a**–**p**

Comp.	UV (λ_{max}, EtOH, ε, nm)	IR (KBr, cm^{-1})	Comp.	UV (λ_{max}, EtOH, ε, nm)	IR (KBr, cm^{-1})
3a	365 (23 125) 285 (17 991) 253 (19 848)	1724 (CO) 1566 (CN)	**3i**	365 (21 568) 281 (15 920) 252 (18 367)	1736 (CO) 1566 (CN)
3b	370 (22 391) 278 (18 599)	1729 (CO) 1563 (CN)	**3j**	365 (24 426) 279 (19 490) 224 (35 892)	1737 (CO) 1570 (CN)
3c	391 (21 266) 293 (15 976) 240 (13 931)	1716 (CO) 1562 (CN)	**3k**	368 (23 727) 276 (19 371) 224 (35 432)	1728 (CO) 1569 (CN)
3d	371 (16 128) 285 (13 149) 250 (13 012)	1716 (CO) 1564 (CN)	**3l**	383 (23 471) 280 (16 304) 225 (36 995)	1725 (CO) 1572 (CN)
3e	387 (24 976) 282 (18 006) 259 (22 793)	1729 (CO) 1565 (CN)	**3m**	390 (11 477) 284 (8690) 231 (14 514)	1727 (CO) 1558 (CN)
3f	393 (25 610) 283 (18 302) 261 (21 733)	1710 (CO) 1553 (CN)	**3n**	365 (22 123) 283 (18 166) 249 (19 414)	1727 (CO) 1558 (CN)
3g	376 (22 566) 280 (16 848) 258 (17 460)	1735 (CO) 1573 (CN)	**3o**	383 (23 618) 259 (23 618) 231 (26 128)	1727 (CO) 1576 (CN)
3h	365 (25 982) 272 (22 445)	1732 (CO) 1573 (CN)	**3p**	395 (23 314) 261 (21 169)	1747 (CO) 1556 (CN)

with that in the drug vial. The following formula was used to interpret the results:

$$\Delta GI \text{ control} > \Delta GI \text{ drug} = \text{susceptible}$$

$$\Delta GI \text{ control} < \Delta GI \text{ drug} = \text{resistant}$$

If a clear susceptibility pattern (the difference of ΔGI of control and the drug bottle) was not seen at the time of the control GI is 30. The vials were read for 1 or 2 additional days to establish a definite pattern of GI differences.

3. Results and discussion

In thiazole cyclization the key intermediate ene-thiol form determines the isomeric structures to be obtained; two isomers (A/B) are possible depending upon the nitrogen atom involved in tautomerism. Tautomerism always involves nitrogen atom bearing the electron attracting residue [8]. Thiosemicarbazones with α-haloketones react in neutral medium to give structure A [9]. Thus, the reaction of 3-(ω-bromoacetyl)coumarin **1a**,**b** with 1-substituted-benzylidene-4-substituted thiosemicarbazides **2a**–**j** in ethanolic medium was resulted in 4-(3-coumarinyl)-3-cyclohexyl-4-thiazoline-2-one benzylidenehydrazones **3a**–**p**, as bases (**3a**, **3b**, **3d**, **3e**, **3g**–**i** and **3m**–**p**) or as HBr salts (**3c** and **3j**–**l**). The structures of **3a**–**p** were confirmed by elemental analysis and spectral data (UV, IR, ^{1}H NMR and EIMS) (Tables 1–3, Scheme 1).

The UV spectra of **3a**–**p** showed two absorption bands at 259–285 and 364–395 nm regions due to coumarin and benzylidene hydrazone moieties, respectively [10].

In the IR spectra of **3a**–**p** were observed the lactone C=O and C=N bands in the 1754–1710 and 1579–1552 cm^{-1} regions that confirmed the coumarin structure [11]. In the spectra of the compounds with hydrobromide acid salts (**3c**, **3f** and **3j**–**l**) were found bands resulting from NH^+ stretching in 2701–2600 cm^{-1} region.

Observation of two singlets assigned to the thiazoline 5-H (δ 5.67–6.48 ppm) and N=CH (δ 7.61–8.23 ppm) in the ^{1}H NMR of **3a**, **3b**, **3d**, **3g**, **3i** and **3n** suggested the presence of thiazolylhydrazone structure [12]. Coumarin 4-H on the β-carbon of an α,β-unsaturated carbonyl group (δ 8.19–8.37 ppm) is highly deshielded due to the polarization caused by the electron attracting carbonyl function [13].

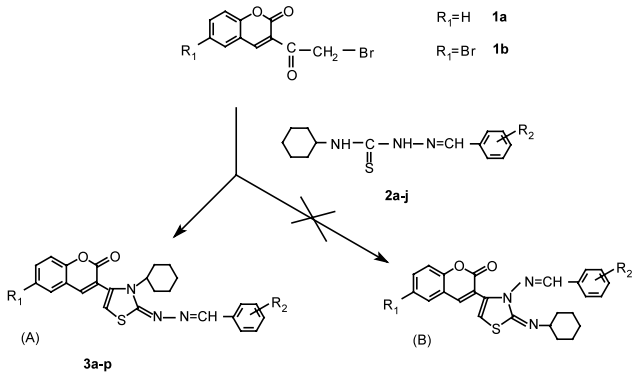
Molecular ions of different intensity observed in the EIMS spectra of **3a**, **3b**, **3d**, **3g**, **3i** and **3n** confirmed their molecular weights. The fragments corresponding to 3-substituted-4-(3-coumarinyl)-2-imino-4-thiazoline and substituted benzylideneimine moieties formed by N–N bond rupture were consistent with the assigned

Table 3
^{1}H NMR and EIMS data of compounds **3a**, **3b**, **3d**, **3g**, **3i** and **3n**

Comp.	^{1}H NMR (DMSO-d_6, δ, ppm)	EIMS (70 eV) m/z (%)
3a	1.20–2.68 (m, 10H, cyclohexyl $C_{2,3,4,5,6}$–H), 2.37 (s, 3H, CH_3), 3.55 (br.s, 1H, cyclohexyl C_1–H), 6.13 (s, 1H, thiazoline C_5-H), 7.84–7.16 (m, 8H, aromatic), 7.87 (s, 1H, N=CH), 8.37 (s, 1H, coumarin C_4–H)	444 [MH$^+$, 21], 443 [M^+, 68], 365 (14), 362 (17), 361 (64), 360 (19), 333 (9), 325 (3), 245 (16), 244 (100), 230 (35), 211 (9), 174 (8), 172 (19), 118 (8), 104 (21), 69 (8), 57 (12)
3b	1.19–2.72 (m, 10H, cyclohexyl $C_{2,3,4,5,6}$–H), 3.50 (br.s, 1H, cyclohexyl C_1–H), 3.91, 3.95 (2 s, 2X3H, 2 OCH_3), 6.07 (s, 1H, thiazoline C_5–H), 6.83–7.65 (m, 7H, aromatic), 7.83 (s, 1H, N=CH), 8.29 (s, 1H, coumarin C_4–H)	490 [MH$^+$, 25], 489 [M^+, 71], 408 (15), 407 (56), 406 (11), 325 (3), 246 (12), 245 (20), 244 (100), 230 (31), 211 (11), 172 (13), 150 (49), 135 (13), 107 (11), 79 (6), 77 (7), 57 (3)
3d	1.19–2.72 (m, 10H, cyclohexyl $C_{2,3,4,5,6}$–H), 3.52 (br.s, 1H, cyclohexyl C_1–H), 6.09 (s, 1H, thiazoline C_5–H), 7.01–7.75 (m, 8H, aromatic), 7.84 (s, 1H, N=CH), 8.31 (s, 1H, coumarin C_4–H)	448 [MH$^+$, 44], 447 [M^+, 93], 368 (25), 367 (15), 366 (41), 365 (100), 364 (32), 337 (19), 336 (8), 325 (6), 313 (10), 270 (11), 262 (13), 246 (10), 245 (24), 244 (97), 236 (15), 231 (12), 230 (67), 211 (15), 174 (17), 173 (13), 172 (43), 171 (12), 159 (9), 147 (11), 123 (9), 122 (11), 121 (9), 115 (10), 111 (10), 109 (14), 108 (38), 98 (13), 97 (18), 96 (11), 95 (19), 85 (11), 84 (23), 83 (23), 82 (11), 81 (19), 71 (15), 69 (20), 67 (15), 57 (22)
3g	1.11–2.61 (m, 10H, cyclohexyl $C_{2,3,4,5,6}$–H), 3.61 (s, 1H, cyclohexyl C_1–H), 3.77 (s, 1H, OCH_3), 5.67 (s, 1H, thiazoline C_5–H), 6.77–7.62 (m, 7H, aromatic), 7.78 (s, 1H, N=CH), 8.31 (s, 1H, coumarin C_4–H)	538 [MH$^+$, 18 (540, 18)], 537 [M^+, 54 (539, 60)], 455 [16 (457, 18)], 425 [18 (427, 16)], 424 [71 (426, 69)], 403 [3 (405, 3)], 322 [96 (324, 100)], 250 [10 (252, 13)], 216 (9), 198 (8), 134 (10), 120 (10), 119 (33), 92 (11), 91 (30), 83 (11), 77 (10), 69 (9), 57 (10)
3i	0.94–3.36 (m, 10H, cyclohexyl $C_{2,3,4,5,6}$–H), 2.26 (s, 3H, CH_3), 4.04 (m, 1H, cyclohexyl C_1–H), 5.97 (s, 1H, thiazoline C_5–H), 6.95–7.58 (m, 7H, aromatic), 7.61 (s, 1H, N=CH), 8.19 (s, 1H, coumarin C_4–H)	522 [MH$^+$, 23 (524, 23)] 521 [M^+, 73 (523, 73)], 440 [35 (442, 21)], 439 [65 (441, 72)], 438 [19 (440, 35)], 411 [8 (413, 9)], 403 [3 (405, 3)], 323 [20 (325, 17)], 322 [97 (324, 100)], 308 [29 (310, 28)], 252 [21 (254, 9)], 251 [27 (253, 27)], 250 [13 (252, 21)], 198 [12 (200, 9)], 120 (10), 119 (10), 118 (37), 105 (14), 104 (87), 103 (16), 91 (23), 83 (13), 78 (15), 67 (8), 57 (5)
3n	1.02–2.62 (m, 10H, cyclohexyl $C_{2,3,4,5,6}$–H), 3.63 (m, 1H, cyclohexyl C_1–H), 6.48 (s, 1H, thiazoline C_5–H), 7.20–8.05 (m, 7H, aromatic), 8.23 (s, 1H, N=CH), 8.31 (s, 1H, coumarin C_4–H)	526 [MH$^+$, 24 (528, 24)], 525 [M^+, 78 (527, 80)], 444 [41 (446, 26)], 443 [94 (445, 100)], 442 [17 (444, 41)], 415 [11 (417, 10)], 403 [3 (405, 3)], 323 [20 (325, 16)], 322 [93 (324, 90)], 308 [40 (310, 39)], 252 [23 (254, 8)], 251 [14 (253, 13)], 250 [16 (252, 21)], 122 (14), 108 (59), 95 (11), 83 (11), 67 (6), 57 (5)

Table 4
Primary antituberculosis activity screen results of **3a–p**

Comp.	MIC (μg ml^{-1})	Inhibition (%)	Comp.	MIC (μg ml^{-1})	Inhibition (%)
3a	>6.25	0	**3i**	>6.25	5
3b	>6.25	1	**3j**	>6.25	13
3c	>6.25	24	**3k**	>6.25	2
3d	>6.25	0	**3l**	>6.25	3
3e	>6.25	0	**3m**	>6.25	0
3f	>6.25	42	**3n**	>6.25	11
3g	>6.25	3	**3o**	>6.25	6
3h	>6.25	58	**3p**	>6.25	4



Scheme 1.

structures [12]. The fragments formed by loss of cyclohexane from the 3-substituted-4-(3-coumarinyl)-2-imino-4-thiazoline moiety were the base peaks in all compounds other than compound **3n**. Further fragments peculiar to the coumarin and thiazoline moieties were also observed in the spectra of these compounds [14]. Compounds **3a**–**p** were tested for antituberculosis activity against *M. tuberculosis* H37Rv and found to exhibit varying degrees of inhibition in the *in vitro* primary screening that was conducted at 6.25 μg ml^{-1} against *M. tuberculosis* H37Rv. Most of the compounds showed some inhibition, yet the inhibitions being less than 90% indicated that the compounds were all considered to be inactive (Table 4).

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