4-(3-Coumarinyl)-4-thiazolin-2-one Benzylidene-hydrazones with Antituberculosis Activity

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Summary

In this study a new series of 4-(3-coumarinyl)-4-thiazoline-2-one benzylidenehydrazones **(3a–v)** were synthesized by condensation of 3-(ω-bromoacetyl)coumarins **(1a** and **1b)** with 1-substituted benzylidene-4-substituted thiosemicarbazides **(2a–l)**. Structures of the title compounds were elucidated by elemental analyses and spectrometric data (UV, IR, ^{1}H-NMR and EIMS). These new compounds and some previously reported compounds **(1a–d)** were evaluated for antituberculosis activity against Mycobacterium tuberculosis H37Rv. The compounds exhibited varying degrees of inhibition in the in vitro primary screening that was conducted at 12 μg/ml against M. tuberculosis H37Rv in BACTEC 12B medium using the BACTEC 460 radiometric system. **1b, 3b, 3e, 3h, 3o** and **3p** demonstrating activity in the primary screen were re-tested at lower concentrations against M. tuberculosis H37Rv to determine the actual minimum inhibitory concentration (MIC) in CABTEC 460 and Alamar Blue assay (MABA). The most active compound was found to be **1b**. The structure-activity relationships of the derivatives were investigated.

Zusammenfassung

4-(3-Coumarinyl)-4-thiazolin-2-on-benzylidenhydrazone mit antituberkulöser Aktivität

In dieser Arbeit wurde durch Kondensation von 3-(ω-Bromoacetyl)coumarinen **(1a** und **1b)** mit 1-substituierten Benzyliden-4-substituierten Thiosemicarbaziden **(2a–l)** eine neue Reihe von 4-(3-Coumarinyl)-4-thiazolin-2-on-benzylidenhydrazonen **(3a–v)** synthetisiert. Die Strukturen der Titelsubstanzen wurden mittels Elementaranalyse und spektroskopischen Methoden (UV, IR, ^{1}H-NMR und EIMS) aufgeklärt. Die neuen Substanzen und einige bereits veröffentlichte **(1a–d)** wurden gegen Mycobacterium tuberculosis H37Rv getestet. Dazu wurden das radiometrische System BACTEC 460 und BACTEC 12B Medium verwendet. In der primären Testphase zeigten die Verbindungen in einer Konzentration von 12 μg/ml verschieden starke Aktivität gegen M. tuberculosis H37Rv. In der primären Testphase zeigten **1b, 3b, 3e, 3h, 3o** und **3p** eine Wirkung. Sie wurden hinsichtlich der MHK-Werte untersucht, wobei die Testmethoden CABTEC 460 und Alamar Blue Assay (MABA) verwendet wurden. **1b** erwies sich als aktivste Verbindung. Die Struktur-Wirkungs-Beziehungen wurden ermittelt.

Key words Antituberculosis drugs · Coumarinylthiazolinylhydrazones, antituberculosis activity, structure-activity relationship, synthesis

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1. Introduction

Compounds carrying the coumarin skeleton play important roles in therapy. Among developed compounds of the coumarin series are the anticoagulant drugs such as dicumarol [1], warfarin [2] and acenocoumarol [3]. Chromonar is a coronary vasodilator [4]. The methoxypropylhydroxymercumate derivative mercumatilin exhibits diuretic activity [5]. Moreover, novobiocin is an antibiotic containing the coumarin moiety that it has a fairly broad range of activity against gram-positive and some gram-negative bacteria [6]. On the other hand, thiazolinylhydrazones have been reported to exhibit antitubercular [7], antibacterial and antifungal activities [8].

Encouraged by these findings and in continuation of our work on the synthesis of 4-thiazolinylarylidenhydrazones [9–12], we report here the synthesis

of the new coumarinyl-4-thiazolinylbenzylidenehydrazones, evaluation of in vitro antituberculosis activity and structure-activity relationship of these new compounds and some previously reported compounds.

2. Material and methods

2.1. Chemistry

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 (Milano, Italy). IR spectra were recorded on KBr (BDH, Poole, England) discs, using a Perkin-Elmer Model 1600 FT-IR spectrometer (Norwalk, CT, USA). ^{1}H-NMR spectra were obtained on Bruker AC 200 (200 MHz) (Rheinstetten, Germany) spectrophotometer using DMSO-d_6 (E. Merck, Darmstadt, Germany). EIMS were determined on a VG Zab Spec (70 eV) mass spectrometer (Manchester, England). Starting materials were purchased from E. Merck.

2.1.1 Synthesis of 4-(3-coumarinyl)-4-thiazolin-2-one benzylidenehydrazones (3a–v)

A solution of 3-(ω-bromoacetyl)coumarins (**1a** and **1b**) (0.0025 mol) and 1-substituted benzylidene-4-substituted thiosemicarbazides (**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, then crystallized from ethanol or ethanol-chloroform (2:1).

Spectral data of **3a**: UV (λ_{max}, EtOH, ε, nm): 373 (24785), 283 (17816), 253 (20614). IR (KBr, cm^{-1}): 2717 (NH^+), 1717 (C = O), 1582 (C = N). ^{1}H-NMR (DMSO-d_6, δ, ppm): 1.46 (t, J: 6.9 Hz, 3H, CH_2CH_3), 4.49 (q, J: 6.9 Hz, 2H, CH_2CH_3), 6.78 (s, 1H, thiazoline 5-H), 7.36–7.70 (m, 8H, aromatic), 8.05 (s, 1H, N = CH), 9.26 (s, 1H, coumarin 4-H). EIMS (70 eV) m/z (%): 409 [M^+, 100 (411, 55)], 380 [8 (382, 3)], 317 (13), 271 (35), 258 (44), 244 (47), 243 (14), 230 (31), 212 (19), 201 (12), 174 (20), 172 (29), 166 [33 (168, 14)], 152 [9 (154, 3)], 145 (13), 142 (13), 139 [11 (141, 4)], 124 [18 (126, 8)], 111 [13 (113, 8)], 102 (15), 89 (40), 75 (14).

Spectral data of **3e**: UV (λ_{max}, EtOH, ε, nm): 360 (32604), 280 (23303). IR (KBr, cm^{-1}): 3238 (OH), 2726 (NH^+), 1725 (C = O), 1584 (C = N). ^{1}H-NMR (DMSO-d_6, δ, ppm): 1.36 (t, J: 6.9 Hz, 3H, OCH_2CH_3), 4.07 (q, J: 6.9 Hz, 2H, OCH_2CH_3), 4.22 (br. s, 1H, NH^+), 4.59 (d, J: 3.3 Hz, 2H, CH_2-CH = CH_2), 4.97, 5.08 (dd, J: 17.1, 10.4 Hz, CH_2-CH = CH_2), 5.74–5.90 (m, 1H, CH_2-CH = CH_2), 6.75 (s, 1H, thiazoline 5-H), 6.82–7.82 (m, 8H, aromatic, OH), 8.21 (s, 1H, N = CH), 8.30 (s, 1H, coumarin 4-H). EIMS (70 eV) m/z (%): 447 (M^+, 59), 418 (13), 284 (69), 283 (34), 269 (19), 258 (19), 257 (100), 256 (39), 244 (13), 174 (15), 172 (35), 171 (17), 165 (15), 163 (16), 149 (24), 145 (19), 137 (21), 136 (27), 135 (44), 129 (20), 121 (18), 115 (22), 109 (18), 107 (20), 106 (20), 105 (34), 99 (24), 98 (28), 97 (43), 95 (23), 91 (50), 85 (25), 84 (27), 83 (52), 81 (37), 79 (28), 78 (26), 77 (45), 73 (41), 71 (39), 70 (34), 69 (51), 67 (33), 63 (29).

Spectral data of **3g**: UV (λ_{max}, EtOH, ε, nm): 374 (27170), 283 (20218). IR (KBr, cm^{-1}): 3243 (OH), 2739 (NH^+), 1714 (C = O), 1585 (C = N). ^{1}H-NMR (DMSO-d_6, δ, ppm): 0.79 (t, J: 7.3 Hz, 3H, $CH_2CH_2CH_2CH_3$), 1.20 (sex., J: 7.4 Hz, 2H, $CH_2CH_2CH_2CH_3$), 1.65 (p, J: 7.3 Hz, 2H, $CH_2CH_2CH_2CH_3$), 3.86 (t, J: 7.4 Hz, 2H, $CH_2CH_2CH_2CH_3$), 4.19 (s, 1H, NH^+), 6.88 (s, 1H, thiazoline 5-H), 6.90–7.88 (m, 8H, aromatic, OH), 8.41 (s, 1H, N = CH), 8.61 (s, 1H, coumarin 4-H).

Spectral data of **3i**: UV (λ_{max}, EtOH, ε, nm): 383 (28989), 282 (22831), 256 (26155). IR (KBr, cm^{-1}): 2658 (NH^+), 1708 (C = O), 1567 (C = N). ^{1}H-NMR (DMSO-d_6, δ, ppm): 1.12–2.63 (m, 11H, cyclohexyl), 6.55 (s, 1H, thiazoline 5-H), 7.41–7.83 (m, 8H, aromatic), 8.31 (s, 1H, N = CH), 8.32 (s, 1H, coumarin 4-H). EIMS (70 eV) m/z (%): 463 [M^+, 30 (465, 13)], 446 (20), 393 (41), 381 [30 (383, 12)], 244 (31), 230 (13), 184 (42), 156 (30), 149 (19), 132 (23), 128 (34), 99 (19), 97 (17), 88 (42), 87 (25), 86 (53), 85 (21), 84 (18), 83 (34), 73 (18), 71 (36), 70 (31), 69 (38), 67 (15), 57 (100), 56 (27).

Spectral data of **3n**: UV (λ_{max}, EtOH, ε, nm): 367 (24025), 280 (18266). IR (KBr, cm^{-1}): 2695 (NH^+), 1725 (C = O), 1582 (C = N). ^{1}H-NMR (DMSO-d_6, δ, ppm): 4.46 (d, J: 4.9 Hz, 2H, CH_2-CH = CH_2), 4.97, 5.08 (dd, J: 17.2, 10.3 Hz, 2H, CH_2-CH = CH_2), 5.80–5.87 (m, 1H, CH_2-CH = CH_2), 6.69 (s, 1H, thiazoline 5-H), 7.46–8.06 (m, 7H, aromatic), 8.24 (s, 1H, N = CH), 8.30 (s, 1H, coumarin 4-H). EIMS (70 eV) m/z (%): 499 [M^+, 73 (501, 100), (503, 33)], 395 [19 (397, 20)], 362 [28 (364, 24)], 361 [57 (363, 51)], 347 [32 (349, 36)], 335 [37 (337, 38)], 321 [10 (323, 10)], 250 [21 (252, 24], 198 (31), 178 (18), 164 (14), 138 [22 (140, 10)], 129 (25), 111 [23 (113, 19)].

Spectral data of **3r**: UV (λ_{max}, EtOH, ε, nm): 373 (30423), 280 (22168), 225 (54323). IR (KBr, cm^{-1}): 3431 (OH), 1726 (C = O), 1578 (C = N). ^{1}H-NMR (DMSO-d_6, δ, ppm): 0.86 (t, J: 7.2 Hz, 3H, $CH_2CH_2CH_2CH_3$), 1.25 (sex., J: 7.4 Hz, 2H, $CH_2CH_2CH_2CH_3$), 1.71 (p, J: 7.2 Hz, 2H, $CH_2CH_2CH_2CH_3$), 3.82 (t, J: 7.4 Hz, 2H, $CH_2CH_2CH_2CH_3$), 6.23 (s, 1H, thiazoline 5-H), 6.87–7.73 (m, 6H, aromatic), 7.79 (s, 1H, N = CH), 8.33 (s, 1H, coumarin 4-H), 11.41 (s, 1H, bonded OH). EIMS (70 eV) m/z (%): 575 [M^+, 13 (577, 27), (579, 15)], 377 [7 (379, 8)], 322 [28 (324, 29)], 207 (49), 198 [15 (200, 13)], 171 (18), 149 (23), 145 (16), 135 (30), 133 (19), 129 (25), 121 (19), 119 (22),115 (24), 111 (18), 109 (18), 107 (21), 105 (41), 98 (31), 97 (39), 95 (28), 91 (50), 85 (24), 84 (25), 83 (44), 82 (25), 81 (33), 79 (27), 77 (44), 73 (37), 71 (37), 69 (56), 67 (41), 65 (22), 60 (36), 57 (76), 56 (49), 55 (100).

Spectral data of **3v**: UV (λ_{max}, EtOH, ε, nm): 365 (30742), 276 (25235), 222 (47353). IR (KBr, cm^{-1}): 3251 (OH), 2669 (NH^+), 1731 (C = O), 1582 (C = N). ^{1}H-NMR (DMSO-d_6, δ, ppm): 1.17–2.64 (m, 11H, cyclohexyl), 1.37 (t, J: 6.9 Hz, 3H, OCH_2CH_3), 4.07 (q, J: 7.0 Hz, 2H, OCH_2CH_3), 6.41 (s, 1H, thiazoline 5-H), 6.80–8.18 (m, 7H, aromatic, OH), 8.21 (s, 1H, N = CH), 8.26 (s, 1H, coumarin 4-H). EIMS (70 eV) m/z (%): 567 [M^+, 67 (569, 70)], 486 [23 (488, 15)], 485 [49 (487, 54)], 484 [10 (486, 23)], 418 (33), 409 (13), 403 [9 (405, 10)], 390 (17), 321 [18 (323, 17)], 322 [100 (324, 98)], 308 [28 (310, 27)], 164 (6), 150 (65), 137 (18), 136 (20), 122 (15), 91 (22), 83 (20), 57 (21), 56 (20), 55 (63).

2.2. In vitro evaluation of antituberculosis activity

A primary screening was conducted at 12.5 μg/ml (or molar equivalent of highest molecular weight compound in a series of congeners) against Mycobacterium tuberculosis H37Rv in BACTEC 12B medium using the BACTEC 460 radiometric system. Compounds effecting < 90 % inhibition in the primary screen (MIC > 12.5 μg/ml) were not generally evaluated further. Compounds demonstrating at least 90 % inhibition in the primary screen were re-tested at lower concentrations against M. tuberculosis H37Rv to determine the actual minimum inhibitory concentration (MIC) in the CABTEC 460 and a broth microdilution Alamar Blue assay (MABA). The MIC was defined as the lowest concentration inhibiting 90 % of the inoculum in the CABTEC 460 and effecting a reduction in 90 % fluorescence of relative to controls in the MABA.

2.2.1. BACTEC radiometric method of susceptibility testing

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 (12.5 µg/ml). The standard vials contain rifampin (0.25 µg/ml). 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 GI (Δ GI) from the previous day in the control was compared with that in the drug vial. The following formula was used to interpret the results:

Δ GI control > Δ GI drug = susceptible

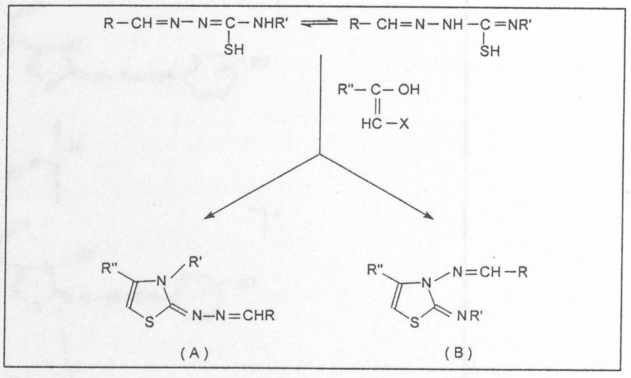
Δ GI control < Δ GI drug = resistant

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

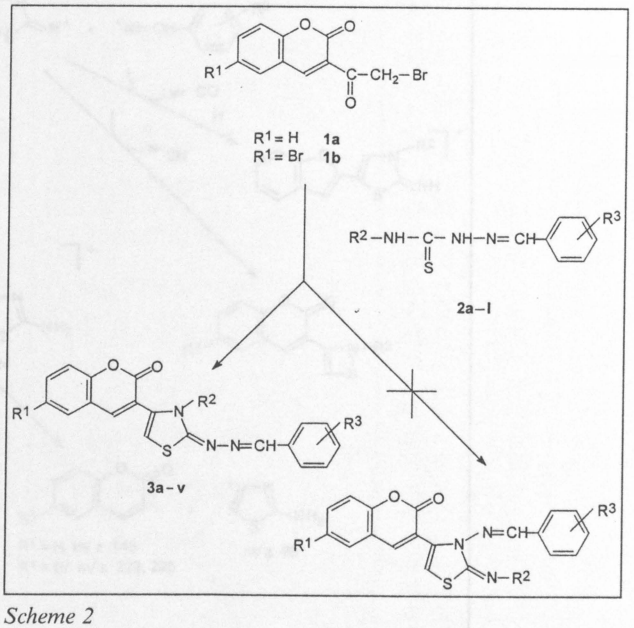
3. Results and discussion

3.1. Chemistry

It is known that the thiosemicarbazones with α-haloketones give different products dependently on conditions of reaction [13]. Literature surveys show that this reaction in neutral medium results in the formation of 4-thiazolin-2-ylidene hydrazone [14, 15]. In thiazole cyclization the ene-thiol form determines the isomeric structures which may be considered. The ene-thiol formation involves the NH group adjacent to the more electron-withdrawing moiety [16] (Scheme 1). So that tautomerization is easier at N^2 position than it is at N^4 position in our compounds and anticipated structure is A.



Scheme 1



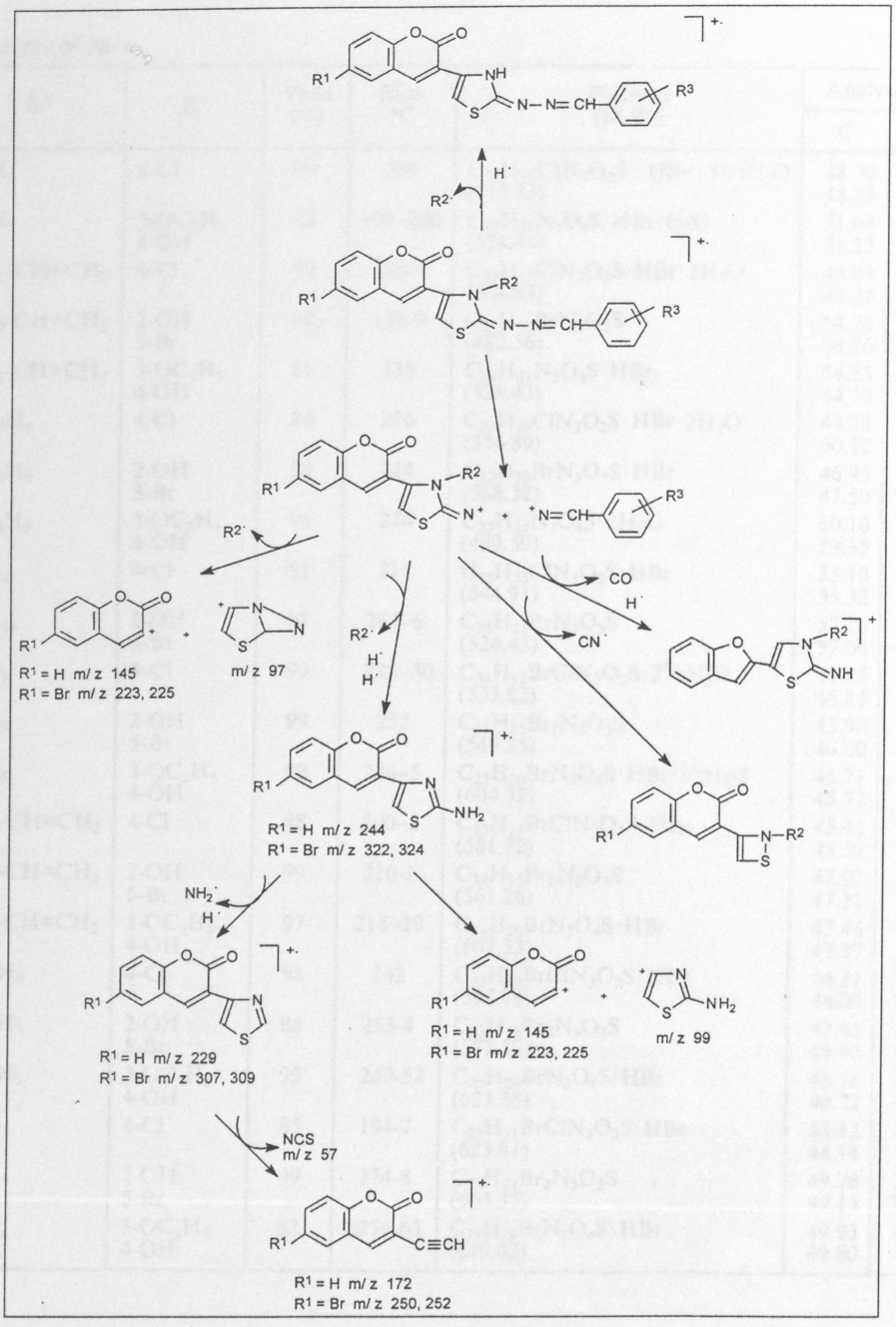
Scheme 2

In view of these observations, the reaction of 3-(ω-bromoacetyl)coumarin **1a, b** with 1-substituted benzylidene-4-substituted thiosemicarbazides **2a–l** in neutral medium resulted in the formation of 4-(3-coumarinyl)-4-thiazoline-2-one benzylidenehydrazones **3a–v** (Scheme 2). The structures of the synthesized compounds were established by elemental analysis and spectrometric data (UV, IR, ^{1}H-NMR and EIMS) (Table 1).

The UV spectra of **3a–v** displayed two absorption bands at 276–283 and 360–384 nm regions due to coumarin and benzylidene hydrazone moieties, respectively [17, 18]. In the IR spectra of **3a–v** the lactone C = O band is observed in the 1731–1714 cm^{-1} region confirming the coumarin structure [19, 20]. In the spectra of the compounds with hydrobromide acide salts (except **3d**, **3h**, **3r** and **3u**) bands resulting from NH^+ stretching in the 2743–2606 cm^{-1} region were found. The thiazolylhydrazone structure of **3a–v** was supported by its ^{1}H-NMR spectrum which included singlets at 6.23–6.78 ppm and 7.79–8.21 ppm due to the thiazoline 5-H and N = CH, respectively [21, 22]. 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 as a singlet at 8.26–9.26 ppm [23]. The absorption bands associated with other functional groups displayed peaks in the expected regions. Molecular ions of different intensity observed in the EIMS spectra of **3a**, **3e**, **3r** and **3v** 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 structures [21, 24]. The fragments formed by loss of R^2 or CN from the 3-substituted-4-(3-coumarinyl)-2-imino-4-thiazoline moiety were the base peaks in most cases. Further fragments peculiar to the coumarin and thiazoline moieties were also observed in the spectra of these compounds [25, 26].

3.2. Activity of hydrazonoindolinones against Mycobacterium tuberculosis H37Rv

4-(3-Coumarinyl)-4-thiazoline-2-one benzylidenehydrazones **1a–d** which were previously reported [10] were evaluated for in vitro antituberculous activity against Mycobacterium tuberculosis H37Rv using the BACTEC 460 radiometric system [27].



Scheme 3

Rifampicin was used as the standard in the tests. As can be seen in Table 2, R^2 ethyl and cyclohexyl substituted compounds **1a–c** exhibited varying degrees of inhibition in the primary screening that was conducted at 12 µg/ml in BACTEC 12B medium whereas the R^2 phenyl substituted compound **1d** was inactive against M. tuberculosis H37Rv in the same conditions.

These preliminary results indicate that R^2 alkyl and cycloalkyl substituted coumarinyl-4-thiazolinylbenzylidenehydrazones have antituberculosis potential and their structural modification may lead to new derivatives with enhanced activity. In the light of these results and in continuation of our work on coumarinyl-4-thiazolinylbenzylidenehydrazones, new R^2 alkyl and cycloalkyl substituted derivatives **(3a–v)** were synthesized and tested for in vitro antituberculosis activity against M. tuberculosis H37Rv to construct a structure-activity relationship. All the compounds tested (except **3r**) showed varying degrees of inhibition (Table 3).

1b showing 98 % inhibition in primary screen at 12 µg/ml was re-tested at lower concentrations against M. tuberculosis H37Rv to determine the actual minumum inhibitory concentration (MIC) in the CABTEC 460 and broth microdilution Alamar Blue assay (MABA). The MIC of **1b** was found to be 12.5 and 6.25 µg/ml, respectively. New R^2 alkyl and cycloalkyl substituted coumarinyl-4-thiazolinylbenzylidenehydrazones **3b**, **3e**, **3h**, **3o**, and **3p**

Table 1: Physical constants of **3a–v**.

Comp.	R^1	R^2	R^3	Yield (%)	M.p. °C	Formula (M.W.)	Analysis (calc./found)		
							C	H	N
3a	H	C_2H_5	4-Cl	99	209	$C_{21}H_{16}ClN_3O_2S \cdot HBr \cdot 1\frac{1}{2}H_2O$ (517.83)	48.70 48.53	3.89 3.29	8.11 7.47
3b	H	C_2H_5	3-OC_2H_5 4-OH	88	199–200	$C_{23}H_{21}N_3O_4S \cdot HBr \cdot H_2O$ (534.44)	51.68 51.22	4.52 4.09	7.86 7.36
3c	H	CH_2-CH=CH_2	4-Cl	99	198-9	$C_{22}H_{16}ClN_3O_2S \cdot HBr \cdot 2H_2O$ (538.85)	49.03 49.55	3.92 3.64	7.79 7.66
3d	H	CH_2-CH=CH_2	2-OH 5-Br	88	198-9	$C_{22}H_{16}BrN_3O_3S$ (482.36)	54.78 54.26	3.34 3.22	8.71 8.30
3e	H	CH_2-CH=CH_2	3-OC_2H_5 4-OH	81	239	$C_{24}H_{21}N_3O_4S \cdot HBr$ (528.43)	54.55 54.10	4.19 4.06	7.95 7.60
3f	H	n-C_4H_9	4-Cl	84	226	$C_{23}H_{20}ClN_3O_2S \cdot HBr \cdot 2H_2O$ (554.89)	49.78 50.12	4.54 3.88	7.57 7.01
3g	H	n-C_4H_9	2-OH 5-Br	80	244	$C_{23}H_{20}BrN_3O_3S \cdot HBr$ (588.32)	46.95 47.50	3.77 3.38	7.14 6.40
3h	H	n-C_4H_9	3-OC_2H_5 4-OH	96	274	$C_{25}H_{25}N_3O_4S \cdot 2H_2O$ (499.59)	60.10 59.65	5.85 5.17	8.41 8.14
3i	H	C_6H_{11}	4-Cl	81	211	$C_{25}H_{22}ClN_3O_2S \cdot HBr$ (544.91)	55.10 55.32	4.25 3.96	7.71 7.02
3j	H	C_6H_{11}	2-OH 5-Br	97	263–6	$C_{25}H_{22}BrN_3O_3S$ (524.45)	57.25 57.06	4.22 4.38	8.01 7.90
3k	Br	C_2H_5	4-Cl	99	228–30	$C_{21}H_{15}BrClN_3O_2S \cdot 2\frac{1}{2}H_2O$ (533.82)	47.25 46.83	3.77 3.00	7.87 7.30
3l	Br	C_2H_5	2-OH 5-Br	99	252	$C_{21}H_{15}Br_2N_3O_3S$ (549.25)	45.92 46.00	2.75 2.58	7.65 7.59
3m	Br	C_2H_5	3-OC_2H_5 4-OH	99	224–5	$C_{23}H_{20}BrN_3O_4S \cdot HBr \cdot \frac{1}{2}H_2O$ (604.32)	45.71 45.72	3.67 3.61	6.95 6.81
3n	Br	CH_2-CH=CH_2	4-Cl	88	200–2	$C_{22}H_{15}BrClN_3O_2S \cdot HBr$ (581.72)	45.42 45.50	2.77 2.92	7.22 6.80
3o	Br	CH_2-CH=CH_2	2-OH 5-Br	99	210-1	$C_{22}H_{15}Br_2N_3O_3S$ (561.26)	47.07 47.32	2.69 2.70	7.48 7.38
3p	Br	CH_2-CH=CH_2	3-OC_2H_5 4-OH	97	218–20	$C_{24}H_{20}BrN_3O_4S \cdot HBr$ (607.33)	47.46 47.57	3.48 3.56	6.91 6.95
3q	Br	n-C_4H_9	4-Cl	98	242	$C_{23}H_{19}BrClN_3O_2S \cdot HBr$ (597.76)	46.21 46.00	3.37 3.53	7.02 6.50
3r	Br	n-C_4H_9	2-OH 5-Br	85	253-4	$C_{23}H_{19}Br_2N_3O_3S$ (577.30)	47.85 48.00	3.31 3.18	7.27 6.95
3s	Br	n-C_4H_9	3-OC_2H_5 4-OH	99	249-52	$C_{25}H_{24}BrN_3O_4S \cdot HBr$ (623.38)	48.16 48.72	4.04 4.12	6.74 6.75
3t	Br	C_6H_{11}	4-Cl	85	194-7	$C_{25}H_{21}BrClN_3O_2S \cdot HBr$ (623.81)	48.13 48.14	3.55 3.36	6.73 6.23
3u	Br	C_6H_{11}	2-OH 5-Br	99	274-8	$C_{25}H_{21}Br_2N_3O_3S$ (603.35)	49.76 49.15	3.50 3.53	6.96 6.58
3v	Br	C_6H_{11}	3-OC_2H_5 4-OH	82	259-63	$C_{27}H_{26}BrN_3O_4S \cdot HBr$ (649.42)	49.93 49.60	4.19 4.09	6.47 6.97

showing > 90 % inhibition in the primary screen at 12 µg/ml were re-tested at lower concentrations against M. tuberculosis H37Rv to determine the actual minumum inhibitory concentration (MIC)

Table 2: Primary antituberculosis activity screen results of **1a–d**[a].

Comp.	R^1	R^2	R^3	MIC (µg/ml)	Inhibition %
1a	H	C_2H_5	2-OH 5-Br	> 12.5	83
1b	H	C_6H_{11}	3-OC_2H_5 4-OH	< 12.5	98
1c	H	C_6H_{11}	2-NO_2	> 12.5	12
1d	H	C_6H_5	2-OH 5-Br	> 12.5	0

[a] MIC of rifampicin: 0.25 µg/ml, 97 % inhibition.

in the CABTEC 460 or broth microdilution Alamar Blue assay (MABA). MIC values of these compounds were found to be 12.5 or > 12.5 µg/ml (Table 4).

As can be seen in Tables 2 and 3 the most active compounds were R^3 3-ethoxy and 4-hydroxy substituted derivatives **1b**, **3b**, **3e**, **3h** and **3p**. The activity of these compounds was decreased by elongation of the alkyl chain in R^2 alkyl substituted derivatives probably due to steric hindrance or to a change in the lipophilicity of compounds. Furthermore, R^1 bromine substituted compounds **3m**, **3p**, **3s** and **3v** were less active than **3b**, **3e**, **3h** and **1b** which were unsubstituted possibly because of inductive effect of the bromine. In R^3 chlorine substituted derivatives, the activity was generally increased by elongation of the alkyl chain and R^1-bromine substitution whereas R^2 butyl and cyclohexyl substituted compounds **3f** and **3i** were more

Table 3: Primary antituberculosis activity screen results of **3a-v**[a)].

Comp.	MIC (μg/ml)	Inhibition (%)
3a	> 12.5	24
3b	< 12.5	97
3c	> 12.5	36
3d	> 12.5	83
3e	< 12.5	96
3f	> 12.5	81
3g	> 12.5	3
3h	< 12.5	94
3i	> 12.5	75
3j	> 12.5	83
3k	> 12.5	63
3l	> 12.5	81
3m	> 12.5	86
3n	> 12.5	71
3o	> 12.5	91
3p	> 12.5	91
3q	> 12.5	71
3r	> 12.5	–
3s	> 12.5	69
3t	> 12.5	40
3u	> 12.5	20
3v	> 12.5	89

[a)] MIC of rifampicin: 0.125 μg/ml, 97 % inhibition.

Table 4: Level II antituberculosis activity assay results of **1b**, **3b**, **3e**, **3h**, **3o**, and **3p**.

Compound	Assay	MIC (μg/ml)	Rifampicin MIC (μg/ml)
1b	MABA	6.25	0.06
	CABTEC	12.5	0.5
3b	CABTEC	> 12.5	0.125
3e	CABTEC	> 12.5	0.125
3h	CABTEC	12.5	0.125
3o	CABTEC	> 12.5	0.125
3p	MABA	> 12.5	0.03

active than **3q** and **3t** with R^1bromine substitution. Among R^3 2-hydroxy and 5-bromine substituted derivatives, the R^2 ethyl, allyl and cyclohexyl substituted compounds **1a**, **3d** and **3j** showed 83 % inhibition in the primary screen. The activity was decreased by R^1 bromine substitution in the R^2 ethyl and cyclohexyl substituted compounds (**3l** and **3u**) whereas R^1 bromine and R^2 allyl substituted compound **3o** exhibited 91 % inhibition. Moreover R^2 butyl substituted compounds **3g** and **3r** were inactive against M. tuberculosis H37Rv.

These preliminary results show that the most active compound was R^2 cyclohexyl and R^3 3-ethoxy and 4-hydroxy substituted derivative **1b**. Nevertheless, all of the R^3 3-ethoxy and 4-hydroxy substituted compounds showed > 90 % inhibition in the primary screen. The activity of all the compounds tested was generally decreased by R^1 bromine substitution whereas R^1 bromine and R^3 chlorine substituted compounds were more active than other entries without R^1 bromine substitution. The increase in the antituberculosis activity may be related to a change in the lipophilicity of the compounds.

4. References

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