

## Synthesis and antifungal activity of new carbamodithioic acid esters derived from 3-acetylcoumarin

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### Introduction

Coumarin derivatives have been reported to possess a wide variety of pharmacological activities such as anticoagulant, fungicidal, tuberculostatic and diuretic [1, 2]. On the other hand, antifungal and antibacterial activities of *N*-substituted and *N,N*-disubstituted carbamodithioic acid esters are well known [3, 4]. Previous publications from our laboratory have dealt with the synthesis of a series of 4-[(*N,N*-disubstituted thiocarbamoylthio)acyl]antipyridines and 4-[(*N,N*-disubstituted thiocarbamoylthio)acetamido]antipyridines, some of which are endowed with significant antifungal activity [5, 6]. In view of this observation we synthesized new 3-[(*N,N*-disubstituted thiocarbamoylthio)acetyl]coumarins and tested them for antifungal activity.

### Chemistry

The reaction of 3-( $\omega$ -bromoacetyl)coumarins (**1** or **2**) [7] with potassium salts of dithiocarbamic acids (**3a–k**) which were obtained by literature methods [8, 9] afforded in ethanolic medium 3-[(*N,N*-disubstituted thiocarbamoylthio)acetyl]coumarin derivatives (**4a–k** and **5a–c**) (scheme 1). Analytical and spectral data [IR, <sup>1</sup>H-NMR, CIMS (CH<sub>4</sub>), EIMS (70 eV)] confirmed the structures of **4a–k** and **5a–c** (table I).

IR spectra of **4a–k** and **5a–c** showed C=O lacton stretching of the coumarin residue around 1744–1715 cm<sup>-1</sup>,  $\alpha,\beta$ -unsaturated keton C=O stretching in the region of 1695–1653 cm<sup>-1</sup> and thiocarbonyl group C=S stretching at about 1255–1228 cm<sup>-1</sup> [10–12]. In the <sup>1</sup>H-NMR spectra, the C4-H proton of the coumarin moiety and the COCH<sub>2</sub> protons appeared at about 8.48–8.68 and 4.81–4.90 ppm, respectively. Aromatic, (CH<sub>3</sub>)<sub>2</sub>N, (C<sub>2</sub>H<sub>5</sub>)<sub>2</sub>N, pyrrolidine, piperidine, piperazine

and morpholine protons were observed in accordance with the literature [13, 14]. The compounds **4c,d,f,i,k** and **5a** showed quasi-molecular (MH<sup>+</sup>, CIMS) and

Table I. Some characteristics of compounds **4a–k** and **5a–c**.

Compound	Formula (MW)	Mp (°C)	Yield (%)
<b>4a</b>	C <sub>14</sub> H <sub>13</sub> NO <sub>3</sub> S <sub>2</sub> (307.38)	168–170	85
<b>4b</b>	C <sub>16</sub> H <sub>17</sub> NO <sub>3</sub> S <sub>2</sub> (335.45)	129–130	72
<b>4c</b>	C <sub>16</sub> H <sub>15</sub> NO <sub>3</sub> S <sub>2</sub> (333.43)	187–189	100
<b>4d</b>	C <sub>17</sub> H <sub>17</sub> NO <sub>3</sub> S <sub>2</sub> (347.46)	179–180	89
<b>4e</b>	C <sub>18</sub> H <sub>19</sub> NO <sub>3</sub> S <sub>2</sub> (361.48)	105–108	90
<b>4f</b>	C <sub>18</sub> H <sub>19</sub> NO <sub>3</sub> S <sub>2</sub> (361.48)	133–134	92
<b>4g</b>	C <sub>19</sub> H <sub>21</sub> NO <sub>3</sub> S <sub>2</sub> (375.51)	169–172	83
<b>4h</b>	C <sub>24</sub> H <sub>23</sub> NO <sub>3</sub> S <sub>2</sub> (437.57)	165–166	90
<b>4i</b>	C <sub>22</sub> H <sub>20</sub> N <sub>2</sub> O <sub>3</sub> S <sub>2</sub> (424.53)	181–182	100
<b>4j</b>	C <sub>23</sub> H <sub>22</sub> N <sub>2</sub> O <sub>3</sub> S <sub>2</sub> (438.56)	144	100
<b>4k</b>	C <sub>16</sub> H <sub>15</sub> NO <sub>4</sub> S <sub>2</sub> (349.43)	163	86
<b>5a</b>	C <sub>16</sub> H <sub>14</sub> BrNO <sub>3</sub> S <sub>2</sub> (412.33)	186–188	93
<b>5b</b>	C <sub>22</sub> H <sub>19</sub> BrN <sub>2</sub> O <sub>3</sub> S <sub>2</sub> (503.43)	210	68
<b>5c</b>	C <sub>16</sub> H <sub>14</sub> BrNO <sub>4</sub> S <sub>2</sub> H <sub>2</sub> O (446.35)	207–209	69

**Table II.** MIC values ( $\mu\text{g/mL}$ ) of **4** and **5**.

Compound	Fungi						
	A	B	C	D	E	F	G
<b>4a</b>	25	25	25	25	>25	25	25
<b>4b</b>	25	25	>25	25	>25	25	25
<b>4c</b>	12.5	25	>25	25	>25	25	25
<b>4d</b>	25	25	>25	12.5	>25	25	25
<b>4e</b>	25	25	>25	25	25	25	25
<b>4f</b>	25	25	>25	25	25	25	25
<b>4g</b>	25	25	>25	25	25	25	25
<b>4h</b>	25	25	25	25	25	12.5	25
<b>4i</b>	25	25	25	25	25	12.5	25
<b>4j</b>	25	25	25	25	25	12.5	>25
<b>4k</b>	25	25	>25	25	25	25	25
<b>5a</b>	>25	6.2	>25	25	25	25	25
<b>5b</b>	25	12.5	25	25	25	12.5	6.2
<b>5c</b>	25	6.2	25	25	25	12.5	>25
Miconazole	0.2	0.2	0.2	0.2	0.2	0.2	0.2
Clotrimazole	0.2	0.2	0.2	0.2	0.2	0.2	0.2

A = *Trichophyton tonsurans* NCPF 245, B = *Microsporium gypseum* NCPF 580, C = *Trichophyton mentagrophytes* var. *erinacei* ATCC 375, D = *Microsporium audouinii*, E = *Microsporium canis*, F = *Trichophyton violaceum*, G = *Trichophyton mentagrophytes*.

molecular ( $\text{M}^+$ , EIMS) ions which confirmed their molecular weights. Most of the fragments [14–16] were common to both CIMS and EIMS, only some variations in intensity were observed.

## Pharmacology

Antifungal activities were investigated against *Trichophyton tonsurans* NCPF 245, *Microsporium gypseum* NCPF 580, *Trichophyton mentagrophytes* var. *erinacei* ATCC 375, *Microsporium audouinii*, *Microsporium canis*, *Trichophyton violaceum* and *Trichophyton mentagrophytes* using the microdilution method [17].

## Results and discussion

The antifungal activity of the compounds was evaluated against seven representative fungi and compared with that of clotrimazole and miconazole. None of them showed comparable activity to the standards against tested fungi (table II).

## Experimental protocols

### Chemistry

Melting points were estimated with a Buchi 530 apparatus and are uncorrected. IR (KBr) and  $^1\text{H-NMR}$  ( $[d_6]$ DMSO or  $\text{CDCl}_3$ ) spectra were recorded on Perkin-Elmer 1600 and Bruker AC 200 (200 MHz) instruments, respectively. CIMS( $\text{CH}_4$ ) were recorded at the Sittingbourne Research Centre, UK. EIMS were determined on a VG Zab Spec (70 eV) instrument. Elemental analyses were performed on a Carlo Erba 1106 apparatus.

### General procedure for the synthesis of **3a–k**

KOH (10 mmol) was dissolved in  $\text{C}_2\text{H}_5\text{OH}$  (100 mL) with constant stirring. After addition of the secondary amine (10 mmol) the mixture was cooled in an ice bath and  $\text{CS}_2$  (10 mmol) was added dropwise with stirring. The reaction mixture thus obtained was further agitated for 1 h at room temperature; after evaporation of the solvent under reduced pressure and consequent addition of dry ether until precipitation reached completion, filtration afforded **3a–k** which were either recrystallized from  $\text{C}_2\text{H}_5\text{OH}$  or used without further purification.

### General procedure for the synthesis of **4a–k** and **5a–c**

To an ethanolic solution of **1** or **2** (10 mmol), **3a–k** (10 mmol) was added and the reaction mixture refluxed for 1 h. After cooling the solution was evaporated to dryness under reduced pressure and the products were washed with water and purified by recrystallization from ethanol.

3-[(1-Pyrrolidinylthiocarbamoylthio)acetyl]coumarin **4c**. IR (cm<sup>-1</sup>): 1720 (C=O ring), 1690 (C=O), 1250 (C=S); <sup>1</sup>H-NMR δ (ppm): 8.50 (s, 1H, coum C4-H), 7.66 (d, 1H, *J* = 7 Hz, coum C5-H), 7.64 (t, 1H, *J* = 8 Hz, coum C7-H), 7.37 (t, 1H, *J* = 8 Hz, coum C6-H), 7.33 (d, 1H, *J* = 7 Hz, coum C8-H), 4.82 (s, 2H, COCH<sub>2</sub>), 3.86, 3.74 (2t, *J* = 7 Hz, 2H each, pyr H2, H5), 2.11–1.98 (m, 4H, pyr H3, H4); CIMS (CH<sub>4</sub>) *m/z* (rel int %): 334 (MH<sup>+</sup>, 27), 263 (1), 219 (20), 203 (8), 189 (100), 171 (5), 162 (4), 148 (30), 147 (17).

6-Bromo-3-[(1-pyrrolidinylthiocarbamoylthio)acetyl]coumarin **5a**. IR (cm<sup>-1</sup>): 1728 (C=O ring), 1694 (C=O), 1246 (C=S); <sup>1</sup>H-NMR δ (ppm): 8.38 (s, 1H, coum C4-H), 7.77 (s, 1H, coum C5-H), 7.73 (dd, 1H, *J*<sub>5,7</sub> = 1.5 Hz, *J*<sub>7,8</sub> = 9 Hz, coum C7-H), 7.27 (d, 1H, *J* = 9 Hz, coum C8-H), 4.76 (s, 2H, COCH<sub>2</sub>), 3.85, 3.72 (2t, *J* = 7 Hz, 2H each, pyr H2, H5), 2.13–1.94 (m, 4H, pyr H3, H4); EIMS *m/z* (rel int %): 413 (M + 2, 12), 411 (M<sup>+</sup>, 12), 380 (15), 378 (14), 298 (2), 296 (2), 282 (1), 280 (1), 268 (6), 266 (6), 267 (7), 265 (5), 253 (22), 251 (22), 225 (3), 223 (2), 211 (5), 209 (6), 181 (13), 178 (3), 169 (15), 167 (14), 114 (100), 72 (93), 70 (49).

#### Antifungal activity

All the compounds to be tested were dissolved in DMSO at a concentration of 4000 μg/mL and the final concentration was reduced to 200 μg/mL with sterile distilled water. No effect of DMSO (5%) was observed upon growth of dermatophytes. The dermatophyte strains which were grown on slant medium of Sabouraud (Difco) were transferred to 3.5 mL nutrient broth (NB, Diagnostic Pasteur) and incubated for three to five days at 25 °C. At the end of the incubation period these strains were transferred into screwcapped bottles containing sterilized beads, and shaken for 4–5 min in a vortex (IKA-VF, Germany). The suspensions of the cultures were adjusted to have an absorbance degree of 0.6 at 450 nm in the spectrophotometer. Eight different dilutions between 25–0.2 μg/mL were prepared in microplates by serial dilutions from top to bottom. Then all the wells except the 12th wells (positive control) were filled with 10 μL of the standardized strains. These plates were incubated at 25 °C for five or six days.

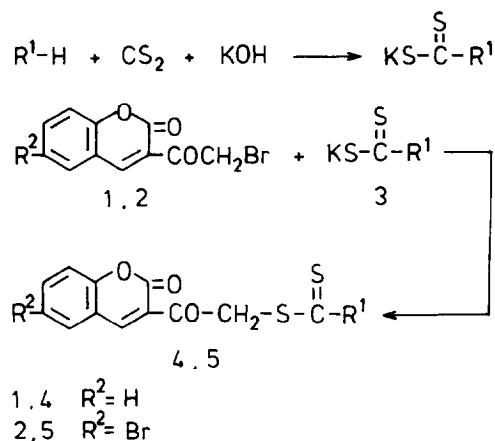
The minimum concentration at which no growth was observed was taken as the MIC value. It should be noted, however, that these techniques leave a variable number of broken hyphae, and therefore even an identical optical density of such hyphal suspensions could lead to a considerable variation in the number of viable cells; this would obviously prevent proper standardization of the inoculum [17].

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#### References

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4	R <sup>1</sup>
a	-N(CH <sub>3</sub> ) <sub>2</sub>
b	-N(C <sub>2</sub> H <sub>5</sub> ) <sub>2</sub>
c, 5a	-N<img alt="pyrrolidine ring" data-bbox="645 450 685 475"/>
d	-N<img alt="piperidine ring" data-bbox="645 485 705 510"/>
e	-N<img alt="piperidine ring with methyl group at position 2" data-bbox="645 520 735 555"/>
f	-N<img alt="piperidine ring with methyl group at position 3" data-bbox="645 565 745 600"/>
g	-N<img alt="piperidine ring with methyl groups at positions 2 and 3" data-bbox="645 605 705 650"/>
h	-N<img alt="piperidine ring with benzyl group at position 2" data-bbox="645 655 775 685"/>
i, 5b	-N<img alt="piperazine ring with benzyl group at position 2" data-bbox="645 695 755 725"/>
j	-N<img alt="piperazine ring with benzyl group at position 4" data-bbox="645 735 785 765"/>
k, 5c	-N<img alt="morpholine ring" data-bbox="645 775 710 805"/>

#### Scheme 1.

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