Synthesis of New 7-Substituted 4-Methylcoumarin Derivatives of Antimicrobial Activity

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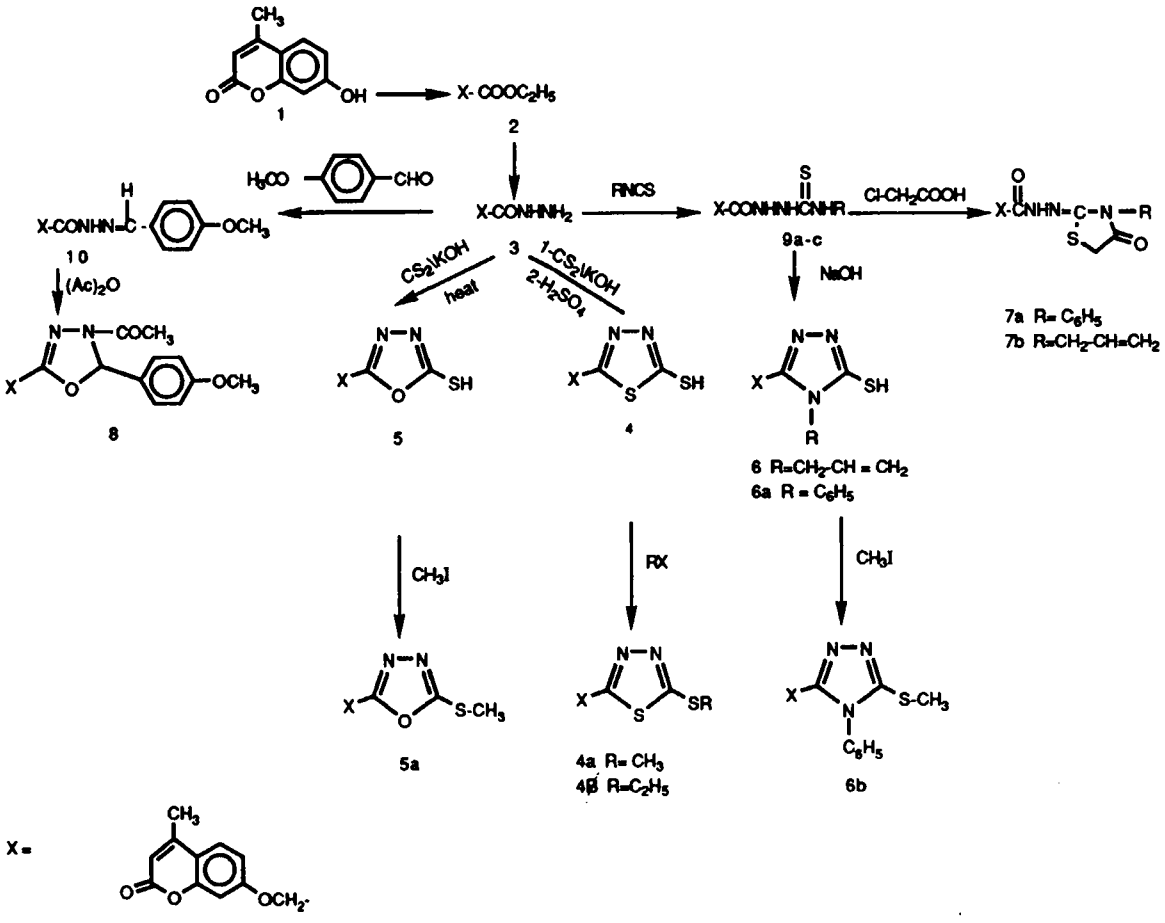
New cyclic derivatives derived from 4-methyl-7-coumarinyloxyacetic acid hydrazide have been synthesized. Some representative examples were screened for antimicrobial activity.

Synthese neuer 7-substituierter 4-Methylcumarin-Derivate mit antimikrobieller Wirkung

Einige neue C-7-substituierte 4-Methylcumarinyloxy-essigsäurehydrazide wurden synthetisiert. Die antibakteriellen Aktivitäten einiger repräsentativer Verbindungen wurden geprüft.

A number of coumarin derivatives have been reported to exert most notably antimicrobial[1-4] as well as antifungal[5] activity. Moreover, the antibiotic novobiocin belongs to the hydroxy coumarin series. On the other hand, several 1,3,4-thiadiazole[6-7] derivatives and their bioisosteres 1,3,4-oxadiazoles[8,11] 1,2,4-triazoles[12,14] have antibacterial and antifungal activity. In addition, 4-oxothiazolidine[15] and oxadiazoline[16,17] derivatives exhibit pronounced antimicrobial activity.

These observations prompted us to synthesize the cyclic derivatives thiadiazole **4**, oxadiazole **5**, triazole **6**, 4-oxothiazolidine **7**, and oxadiazoline **8** from 4-methyl-7-couma-



Scheme 1

  0365-6233/94/0404-0211 $ 5.00 + .25/0

rinyloxy acetic acid hydrazide (**3**). The 5-mercapto group of **4, 5**, and **6** was alkylated to investigate the effect of increasing the partition coefficient on the biological activity of these compounds.

The new compounds were prepared as depicted in Scheme 1. The acid hydrazide **3** required as starting material was prepared from the corresponding ester **2** according to lit.[18,19]. The thiadiazole derivative **4** was synthesized from **3** by reaction with CS_2 and KOH below 10°C to form the intermediate potassium dithiocarbazate which was cyclodehydrated to **4** with conc. H_2SO_4. The oxadiazole derivative **5** was obtained by reaction of **3** with CS_2 and KOH in refluxing ethanol. The triazole derivatives **6** and **6a** were prepared through the initial conversion of **3** into the corresponding thiosemicarbazides **9a** and **9b** and subsequent cyclization with alkali. *S*-Alkylation of **4, 5**, and **6a** was accomplished by treatment with methyl or ethyl iodide to give **4a, 4b, 5a**, and **6b**, respectively. The 4-oxothiazolidine derivatives **7a** and **7b** were obtained from the thiosemicarbazides **9a** and **9b** and chloroacetic acid in the presence of anhydrous sodium acetate. Reaction of **3** with *p*-methoxybenzaldehyde in acetic acid affords the hydrazone **10** which on treatment with acetic anhydride and sodium acetate underwent acetylation with concomitant cyclization to give the corresponding oxadiazoline derivative **8.**

Compounds **2, 3, 4, 4b, 5, 6b, 7b, 9a, 9c,** and **10** were tested for antimicrobial activity using the agar diffusion method[20] against representatives of gram positive bacteria (*Bacillus subtilis, Staphylococcus aureus*, and *Sarcina lutea*), gram negative bacteria (*Escherichia coli, Pseudomonasaeruginose,* and *Proteus vulgaris*) and yeast (*Candida albicans*).

Compounds **2, 3, 4, 4b, 7b, 9a,** and **9c** showed activity against *Pseudomonas aeruginosa*. The thiosemicarbazide **9c** and the thiadiazole **4** showed in addition a good activity against *Bacillus subtilis*. The compounds were inactive against *Staphylococcus aureus, Sarcina lutea, Escherichia coli,* and *Proteus Vulgaris*. Only compound **2** exhibits antifungal activity. Alkylation of the SH group in **4** and **6b** did not improve the antimicrobial activity.

Experimental Part

Microanalysis: Microanalytical Centre, Cairo University. - IR spectra: Shimadzu IR 435. - ^{1}H-NMR spectra: Jeol Fx 90Q 90 MHz. - Melting points: Uncorrected, Griffin melting point apparatus

7-[(5-Mercapto-1,3,4-thiadiazol-2-yl)methoxy]-4-methylcoumarin (**4**)

To a stirred ice-cooled mixture of **3** (2.48 g, 0.01 mol) in absol. EtOH (50 ml) was gradually added KOH (0.56 g, 0.01 mol) in absol. EtOH (20 ml). The clear solution was kept below 10°C and CS_2 (1 g, 0.013 mol) was added dropwise with stirring. The precipitate was washed with dry Et_2O. The resulting potassium dithiocarbazate (0.01 mol) was added portionwise to ice cold conc. H_2SO_4 (7 ml) while stirring. The reaction mixture was left overnight and gradually added to crushed ice. The separated solid was washed with water, dried, and crystallized from EtOH. Mp. 250°C, yield 80%. $C_{13}H_{10}N_2O_3S_2$ (306.4) Calcd. C 51.0 H 3.26 N 9.2 S 20.9 Found C 51.4 H 3.7 N 9.5 S 20.6. - ^{1}H-NMR ([D$_6$]DMSO): δ (ppm) = 2.35 (s, 3H, CH_3), 4 (br. s, 1H, SH), 4.75 (s, 2H, OCH_2), 6.2 (s, 1H, 3-H), 7.0 (d, J = 5 Hz, 1H, 6-H), 7.15 (s, 1H, 8-H), 7.7 (d, J = 5 Hz, 1H, 5-H), 9.15 (s, 1H, NH).

7-[(5-Thiomethyl or thioethyl-1,3,4-thiadiazol-2-yl)methoxy]-4-methyl coumarin (**4a**) *and* (**4b**)

A mixture of **4** (3.1 g, 0.01 mol), CH_3I or C_2H_5I (0.02 mol) and anhydrous K_2CO_3 (2.8 g, 0.02 mol) in dry acetone (100 ml) was refluxed while stirring for 24 h. The mixture was filtered while hot, the solvent was removed *in vacuo* and the residue was crystallized from EtOH

4a: Mp. 155°C, yield 47%. - $C_{14}H_{12}N_2O_3S_2$ (320.4) Calcd. C 52.5 H 3.75 N 8.8 Found C 52.7 H 4.3 N 9.2.

4b: Mp. 170°C, yield 54%. - $C_{15}H_{14}N_2O_3S_2$ (334.4) Calcd. C 53.9 H 4.19 N 8.4 Found C 53.9 H 4.2 N 8.7. - ^{1}H-NMR ([D$_6$]DMSO): δ (ppm) = 1.2 (t, J = 7 Hz, 3H, $CH_2\underline{CH_3}$), 2.4 (s, 3H, CH_3), 3.4 (q, J = 7H, 2H, $\underline{CH_2}CH_3$), 5.6 (s, 2H, OCH_2), 6.15 (s, 1H, 3-H), 7 (d, J = 5 Hz, 1H, 6-H), 7.2 (s, 1H, 8-H), 7.6 (d, J = 5 Hz, 1H, 5-H).

7-[(5-Mercapto-1,3,4-oxadiazol-2-yl)methoxy]-4-methyl coumarin (**5**)

To a solution of KOH (0.56 g, 0.01 mol) in EtOH (50 ml) were added **3** (2.48 g, 0.01 mol) and CS_2 (1 g, 0.013 mol), and the mixture was refluxed for 48 h while stirring. The solvent was removed *in vacuo* and the residue was dissolved in water and acidified with dil.HCl. The precipitate was washed with water and crystallized from EtOH. Mp. 250°C, yield 80%. - $C_{13}H_{10}N_2O_4S$ (290.3) Calcd. C 53.8 H 3.44 N 9.7 Found C 54.0 H 3.4 N 9.3. - ^{1}H-NMR ([D$_6$]DMSO): δ (ppm) = 2.25 (s, 3H, CH_3), 4.2 (br. s, 1H, SH), 5.2 (s, 2H, OCH_2), 6.2 (s, 1H, 3-H), 7 (d, J = 5 Hz, 1H, 6-H), 7.2 (s, 1H, 8-H), 7.7 (d, J = 5 Hz, 1H, 5-H).

7-[(5-Thiomethyl-1,3,4-oxadiazol-2-yl)methoxy]-4-methyl coumarin (**5a**)

A mixture of **5** (2.9 g, 0.01 mol), CH_3I (2.8 g, 0.02 mol) and anhydrous K_2CO_3 (2.8 g, 0.02 mol) in dry acetone (100 ml) was treated as described for **4a**. Mp. 230°C., yield 50%. - $C_{14}H_{12}N_2O_4S$ (304.3) Calcd. C 55.3 H 3.94 N 9.2 Found C 56.0 H 4.5 N 9.4. - IR (KBr): 1730 (C=O), 1620 (C=C, C=N) cm^{-1}. - ^{1}H-NMR ([D$_6$]DMSO): δ (ppm) = 2.9 (s, 3H, C-CH_3), 3.45 (s, 3H, S-CH_3), 6 (s, 2H, OCH_2), 6.7 (s, 1H, 3-H), 7.6 (m, 2H, 6-H, 8-H), 8.3 (d, J = 5 Hz, 1H, 5-H).

N^4-Substituted-N^1-(4-methyl-7-coumarinyloxymethylcarbonyl)thiosemicarbazides **9a-c**

To a solution of **3** (0.01 mol) in hot absol. EtOH was added the appropriate alkyl or aryl isothiocyanate (0.01 mol). The mixture was refluxed while stirring for 3 h. The separated solid was washed with EtOH and crystallized.

9a (R=CH_2-CH=CH_2): Mp. 205°C (DMF), yield 96%. - $C_{16}H_{17}N_3O_4S$ (347.4) Calcd. C 55.3 H 4.89 N 12.1 S 9.2 Found C 55.0 H 5.0 N 12.4 S 9.2.

9b (R=C_6H_5): Mp. 175°C, (DMF), yield, 83%. - $C_{19}H_{17}N_3O_4S$ (383.4) Calcd. C 59.5 H 4.43 N 10.9 Found C 59.3 H 4.5 N 10.9. - ^{1}H-NMR ([D$_6$]DMSO): δ (ppm) = 2.4 (s, 3H, CH_3), 5.4 (s, 2H, OCH_2), 5.7 (s, 1H, NH), 6.2 (s, 1H, 3-H), 6.95 (s, 1H, NH), 7.15 (d, J = 5 Hz, 1H, 6-H), 7.7 (d, J = 5 Hz, 1H, 5-H), 8.35 (s, 6H, 5H aromat., 8-H), 9.4 (s, 1H, NH).

9c (R=C_6H_{11}): Mp. 155°C (DMF), yield 96%. - $C_{19}H_{23}N_3O_4S$ (389.5) Calcd. C 58.6 H 5.91 N 10.8 S 8.2 Found C 57.9 H 5.9 N 11.0 S 8.4. - IR (KBr): 3540, 3320 (NH); 1700 (C=O); 1610 (C=C); 1040 cm^{-1} (N-C=S).

7-[(4-Substituted-5-mercapto-1,2,4-triazol-3-yl)methoxy]-4-methylcoumarin **6** *and* **6a**

A solution of **9a** and **9b** (1.4 g, 0.004 mol) in 2N NaOH (12 ml) was refluxed for 2 h. The mixture was cooled, acidified with dil. HCl and the separated solid was crystallized from EtOH.

6: Mp. 200°C, yield 91%. - $C_{16}H_{15}N_3O_3S$ (329.4) Calcd. C 58.4 H 4.55 N 12.8 S 9.7 Found C 58.2 H 5.0 N 12.7 S 9.2.

6a: Mp. 160°C, yield 92%. - $C_{19}H_{15}N_3O_3S$ (365.4) Calcd. C 62.5 H 4.10 N 11.5 Found C 62.6 H 4.9 N 11.8.

*7-[(4-Phenyl-5-thiomethyl-1,2,4-triazol-3-yl)methoxy)-4-methylcoumarin (**6b**)*

A mixture of **6a** (3.7 g, 0.01 mol), CH_3I (2.8 g, 0.02 mol) and anhydrous K_2CO_3 (2.8 g, 0.02 mol) in dry acetone (100 ml) was treated as described for **4a**. - Mp. 150°C (EtOH). - $C_{20}H_{17}N_3O_3S$ (379.4) Calcd. C 63.3 H 4.48 N 11.1 Found C 62.8 H 4.6 N 11.2. - IR (KBr): 1720 (C=O); 1620 cm^{-1} (C=C, C=N). - ^{1}H-NMR ([D$_6$]DMSO): δ (ppm) = 2.9 (s, 3H, C-CH_3), 3.7 (s, 3H, S-CH_3), 5.6 (s, 2H, OCH_2), 6.7 (s, 1H, 3-H), 7.3-7.6 (m, 5H, phenyl), 7.7-7.9 (m, 2H, 6-H, 8-H), 8.2 (d, J = 5 Hz, 1H, 5-H).

*7-[N^2[(3-Substituted-4-oxothiazolidin-2-ylidenyl)hydrazinocarbonyl]-methoxy]-4-methyl coumarin **7a** and **7b***

A mixture of **9a** and **9b** (0.01 mol), chloroacetic acid (0.01 mol) and anhydrous sodium acetate (0.5 g) in absol. EtOH (50 ml) was refluxed while stirring for 24 h. The solution was filtered, cooled and left to crystallize.

7a: Mp. 205°C (EtOH), yield 55%. - $C_{18}H_{17}N_3O_5S$ (387.4) Calcd. C 55.8 H 4.39 N 10.9 S 8.3 Found C 56.2 H 4.5 N 10.9 S 8.0. - ^{1}H-NMR ([D$_6$]DMSO): δ (ppm) = 2.9 (s, 3H, CH_3), 4.6 (s, 2H, CH_2), 5.8 (s, 2H, OCH_2), 6.65 (s, 1H, 3-H), 7.4 (m, 2H, 6-H, 8-H), 7.9-8.2 (m, 6H, phenyl, 5-H), 13.8 (s, 1H, NH).

7b: Mp. 200°C (EtOH), yield 45%. - $C_{21}H_{17}N_3O_5S$ (423.5) Calcd. C 59.6 H 4.01 N 9.9 Found C 59.8 H 3.5 N 9.5. - IR (KBr): 3100 (NH); 1750 (C=O thiazolidinone); 1680 (C=O coumarin); 1620 cm^{-1} (C=N, C=C).

*7-(p-Methoxybenzylidenehydrazinocarbonylmethoxy)-4-methylcoumarin (**10**)*

A mixture of **3** (2.6 g, 0.01 mol) and *p*-methoxybenzaldehyde (1.49, 0.01 mol) in acetic acid (20 ml) was refluxed for 6 h. The solvent was removed *in vacuo* and the residue was crystallized from EtOH. Mp. 190°C, yield 69%. - $C_{20}H_{18}N_2O_5$ (366.4) Calcd. C 65.6 H 4.91 N 7.7 Found C 65.8 H 4.8 N 8.3. - IR (KBr): 3300 (NH), 1720, 1680 (C=O), 1580 cm^{-1} (C=C, C=N).

*7-[[4-Acetyl-5-(p-methoxyphenyl-Δ^2-1,3,4-oxadiazolin-2-yl]methoxy]-4-methylcoumarin (**8**)*

A mixture of **10** (3.7 g, 0.01 mol), acetic anhydride (15 ml) and anhydrous sodium acetate (0.1 g) was refluxed for 1 h. The solution was poured into ice cold water and the solid was crystallized from EtOH. Mp. 170°C, yield 30%. - $C_{21}H_{19}N_2O_6$ (395.4) Calcd. C 63.8 H 4.81 N 7.1 Found C 64.0 H 4.6 N 7.1. - IR (KBr): 1740 (C=O); 1620 cm^{-1} (C=C, C=N). - ^{1}H-NMR ([D$_6$]DMSO): δ (ppm) = 2.1 (s, 3H, $COCH_3$), 2.4 (s, 3H, CH_3), 3.9 (s, 3H, OCH_3), 4.9 (s, 2H, OCH_2), 6.2 (s, 1H, 3-H), 7 (m, 6H, phenyl, 6-H, 8-H), 7.8 (s, 1H, 5-H).

Antimicrobial activity

Test organisms: *Bacillus subtilis* ATCC 6633, *Sarcina lutea* ATCC 9341, *Staphylococcus aureus, Escherichia coli, Pseudomonas aeruginosa, Proteus vulgaris,* and *Candida albicans* are clinical isolates from the Department of Microbiology, Faculty of Pharmacy, Cairo University.

Culture media: Nutrient broth, *Sabouraud's* broth, nutrient agar and *Sabouraud's* agar are products of Oxoide LTD, England.

Method: Agar plate disc diffusion technique.

Standards of 6 mm in diameter sterilized Whatman filter paper discs were impregnated with 10 mg/ml solution of the test compound dissolved in DMF (200 µg/disc) and allowed to air dry. The discs were applied to the surface of nutrient agar plates seeded with the test organism (each plate contains 15 ml of the agar medium previously seeded with 0.2 ml of 18 h growth culture in liquid media for each organism). The inoculated plates were incubated at 37°C for 48 h and the inhibition zone was measured in mm around each disc. Discs impregnated with DMF were used as a control. The antibacterial reference tetracycline and the antifungal reference amphotericin B were assayed concurrently. Activity against *B. subtilis* (inhibition zone in mm): **4**: 10 mm; **9c**: 15 mm, Tetracyclin: 40 mm.

Against *P. aeruginosa:* **2, 3, 4, 4b, 7a, 9a, 9c**: 10 mm each; Tetracyclin: 20 mm.

Against *C. albicans:* **2**: 15 mm, Amphotericin B: 30 mm.

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