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5-(Ethoxymethylene)thiazolidine-2,4-dione Derivatives: Reactions and Biological Activities

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The 5-(ethoxymethylene)-4-thioxothiazolidin-2-ones **2a–c** were synthesized and reacted with acrylonitrile, ethyl acrylate, β-nitrostyrene, *N*-phenylmaleimide and malononitrile under different conditions, to yield the cycloaddition products **3–14**. The antibacterial and antifungal activities of the new products were tested.

Reaktionen mit 5-Ethoxymethylenthiazolidin-2,4-dion-Derivaten und ihre biologische Wirksamkeit

Die 5-Ethoxymethylen-4-thioxo-2-one **2a–c** wurden synthetisiert und mit Acrylnitril, Ethylacrylat, β-Nitrostyrol, N-Phenylmaleinsäureimid und Malonodinitril unter verschiedenen Bedingungen umgesetzt. Die Wirkungen der neuen Verbindungen gegen Bakterien und Pilze wurde geprüft.

As a part of our studies directed towards the synthesis of new compounds of biological potentialities, containing thiopyrano-thiazole ring system[1–3], we report here the results of cycloaddition of some dienophiles with the ethoxymethylene derivatives of 4-thioxo-thiazolidine-2-ones.

The 5-ethoxymethylene derivatives **2a–c** were synthesized by the reaction of 2-thiazolidinone-4-thiones **1a–c** with ethylorthoformate in acetic anhydride.

Treatment of the coloured **2a–c** with acrylonitrile in toluene at room temp. afforded the colourless 1:1 adducts **3a–c**. Structure **3** was established from elemental and IR data. Besides, the ^{1}H-NMR data of **3c** can be interpreted in terms of 6-cyano-3-phenyl-7-ethoxy-5,6-dihydrothiopyrano[2,3-d]-thiazolidin-2-one. The formation of the adduct **3c** is a conclusive evidence for 2 + 4 cycloaddition rather than N-cyanoethylation reaction represented by structure 4.

On the other hand, when the above reaction was carried out in refluxing acetic acid, **5a–c** were obtained via ethoxy group elimination. Similar ethoxy group elimination has been reported by acid treatment[4]. Moreover, when **3a–c** were refluxed in acetic acid, **5a–c** were obtained.

The reaction of **2a–c** with ethyl acrylate and/or β-nitrostyrene in toluene, at room temp., afforded the 7-ethoxy-5,6-dihydrothiopyrano-[2,3-d]-thiazolidin-2-ones **6a–c** and **7a–c**, respectively. When the above reaction was carried out in refluxing acetic acid, ethoxy group elimination took place to yield compounds **8a–c** and **9a–c**, respectively; which are also obtained by refluxing **6** and/or **7** in acetic acid.

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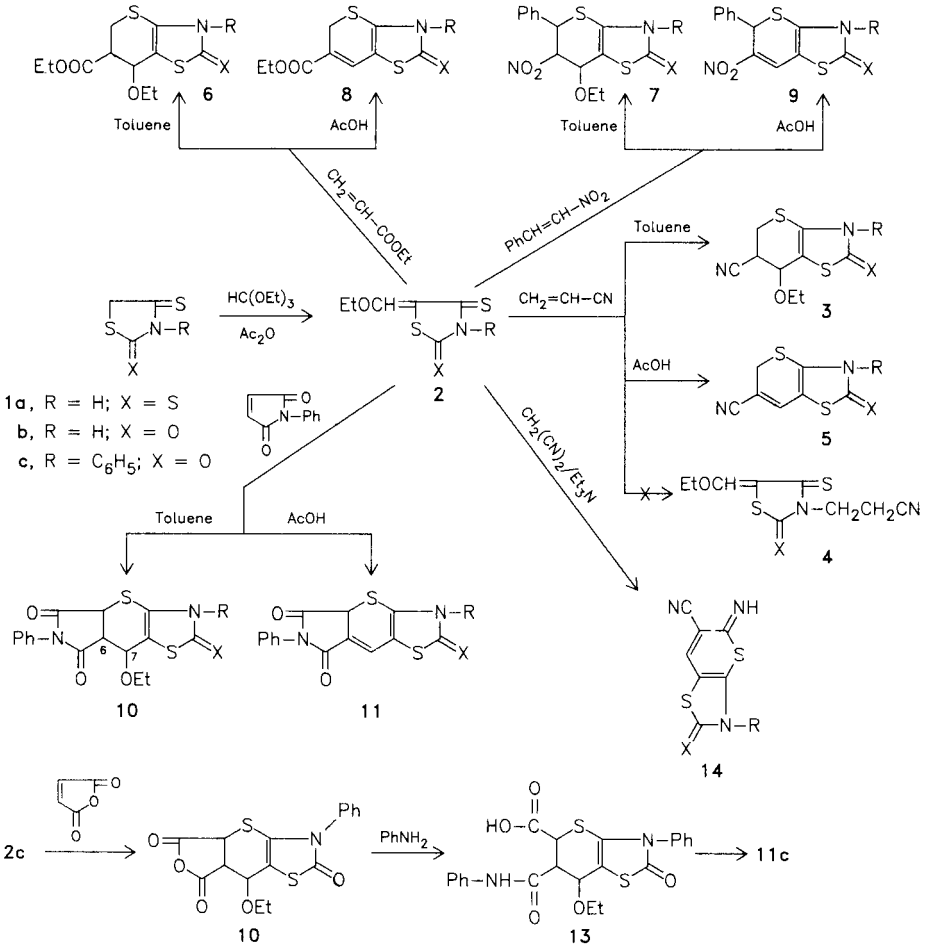
When **2a–c** were subjected to the action of N-phenylmaleimide in toluene at room temp., the colourless adducts **10a–c** were obtained. The gross structure of **10** was assigned on the basis of its elemental and spectral data. The ^{1}H-NMR data of **10c** can be readily interpreted in terms of N,3-diphenyl-7-ethoxy-5,6-dihydrothiopyrano-[2,3-d]-thiazolidin-2-one-5,6-dicarboximide. The stereochemical assignment is based on proton coupling constants, H-6 was observed to be vicinally coupled with H-7 (J = 9 Hz) and H-5 (J = 9 Hz) but had no long-range coupling. This led to the assignment of exo-stereochemistry to the imide ring.

Refluxing **10a–c** in acetic acid resulted in the elimination of the ethoxy group and the formation of compounds **11a–c**.

The reaction of **2c** with maleic anhydride afforded the colourless adduct **12**, which reacted with aniline to give the amide **13**, as inferred from their elemental and spectral data. On heating **13** in acetic acid containing anhydrous sodium acetate, **11c** was obtained; this provided another support to the assigned structures of **10** and **11**.



The reactivity of the 5-ethoxymethylene group of the α,β-unsaturated system was further studied toward the action of active methylene compounds. Thus, treatment of **2a–c** with malononitrile, at room temp., in absol. EtOH, and in the presence of few drops of triethylamine afforded dark coloured products **14a–c**. The reaction pathway took place via nucleophilic attack of the active malononitrile anion on the double bond of the α,β-unsaturated thiocarbonyl system, followed by cyclization and ethoxy group elimination to afford **14a–c**. The structure of **14a–c** is based on elemental and spectral data, besides their synthesis via the reaction between **1a–c** and ethoxymethylenemalononitrile in absol. EtOH and in the presence of triethylamine.

Biological Results:

Results of antibacterial and antifungal tests are shown in Table 1; it reveals that most of the compounds have significant activity. The maximum overall activity were found for compounds **2b**, **2c**, **6b**, and **8c**.

Tab. 1: *Antibacterial and antifungal activities in vitro*

Organisms	2a	2b	2c	3a	3b	3c	5a	5c	6b	6c	8c	9a	10b	10c
E. Coli	–	–	–	–	–	–	–	+	–	–	+	–	–	–
Bacillus subtilis	–	–	–	–	–	–	–	–	–	–	+++	–	–	–
Mycobacterium sp.	++	++	++	–	–	–	–	+	+++	++	+++	–	–	+++
Pseudomonas lachrymans	++	+++	+++	–	–	+	–	–	+++	+++	++++	–	–	–
Erwinia carotovora var. *Carotovora*	–	–	–	–	+	–	–	–	–	–	–	–		–
Erwinia carotovora var. *citrullis*	–	–	++	–	–	–	–	–	–	–	+++	–	–	–
Erwinia toxica	–	–	–	–	++	–	–	++	–	–	+++	–	–	–
Penicillium simplex	–	–	+++	–	–	–	–	–	+++	–	–	–	–	–
Mucor hiemalis	–	–	+	–	–	–	–	–	++	–	–	–	–	–
Phoma leveillei	–	–	++	–	–	–	–	–	++	–	–	–	–	–
Ulocladium chartorium		–	++	–	++	–	–	–	++	++	++	–	–	–
Alternaria sp.	–	–	–	–	–	–	–	–	+	–	–	–	–	–
Trichoderma harzianum	–	–	–	–	–	–	–	–	++	–	–	–	–	–
Aspergillus oryzae		+	++	–	–				+	+	–	–	–	–

Inhibition zone around the disc (each disc contains 100 μg): + = < 1 mm, ++ = < 4 mm, +++ = < 8 mm, ++++ = < 12 mm.

Experimental

Melting points are uncorrected. – IR spectra: KBr discs, Pye-Unicam SP 1100 spectrophotometer. – ^{1}H-NMR spectra: Varian EM-390, 90 MHz spectrometer in DMSO-d6, TMS as internal indicator; and chemical shifts in δ ppm.

Preparation of 5-Ethoxymethylene-4-thioxo-thiazolidin -2-one derivatives **(2a–c).**

Equimolar amounts (0.01 mole) of each of **1a–c** and ethylorthoformate in acetic anhydride were stirred on a steam bath for 1 h, and left overnight to afford the coloured 5-ethoxymethylene derivatives **2a–c**. These solid products were recrystallized from acetic acid.

5-Ethoxymethylene-2,4-dithioxo-thiazolidine **(2a)**: 70 %; m. p. 180° (acetic acid). – $C_6H_7NS_3O$ (205.1) Calc. C 35.1 H 3.41 N 6.8 S 46.8 Found C 35.3 H 3.40 N 6.8 S 46.6. – IR: 3225 (NH); 3050 (-HC=C); 2960–2400 (CH-CH-); 1600 (C=C) and 1175 (C=S).

5-Ethoxymethylene-4-thioxo-thiazolidin-2-one **(2b)**: 60 %; m. p. 150° (acetic acid). – $C_6H_7NS_2O_2$ (189.1) Calc. C 38.0 H 3.70 N 7.4 S 33.8 Found C 38.0 H 3.70 N 7.3 S 33.8.

5-Ethoxymethylene-4-thioxo-3-phenyl-thiazolidin-2-one **(2c)**: 70 %; m. p. 155° (acetic acid). – $C_{12}H_{11}NS_2O_2$ (265.2) Calc. C 5.43 H 4.15 S 24.1 Found C 54.0 H 4.10 S 24.1.

General procedure for the reaction of **2a–c** *with acrylonitrile, ethyl acrylate, β-nitrostyrene, N-phenylmaleimide and maleic anhydride to give* **3a–c, 6a–c, 7a–c, 10a–c** *and* **12**.

A solution of equimolar amounts (0.01 mole) of each of **2a–c** and acrylonitrile, ethyl acrylate, β-nitrostyrene, N-phenylmaleimide or maleic anhydride in toluene (50 ml) was stirred at room temp. for 2 h and then left overnight. The white solid so formed was filtered off and crystallized from EtOH. The colourless products **3a–c, 6a–c, 7a–c, 10a–c** and **12** are listed below.

6-Cyano-7-ethoxy-5,6-dihydrothiopyrano-[2,3-d]-thiazolidine-2-thione **(3a)**: 50 %; m. p. 183°. – $C_9H_{10}N_2S_3O$ (258.2) Calc. C 41.8 H 3.87 N 10.8 Found C 41.6 H 3.81 N 10.7.

6-Cyano-7-ethoxy-5,6-dihydrothiopyrano-[2,3-d]-thiazolidin-2-one **(3b)**: 65 %; m. p. 175°. – $C_9H_{10}N_2S_2O_2$ (242.2) Calc. C 44.6 H 4.13 S 26.4 Found C 44.3 H 4.02 S 26.2. – IR: 3200 (NH); 2220 (C≡N) and 1680 (C=O). – ^{1}H-NMR: 10.3 (br, 1H, NH); 4.6 (d, 2H, C-5); 3.5 (m, 1H, C-7); 3.75 (m, 1H, C-6); 3.9 (q, 2H, $C\underline{H}_2CH_3$) and 1.22 (t, 3H, $CH_2C\underline{H}_3$).

6-Cyano-3-phenyl-7-ethoxy-5,6-dihydrothiopyrano-[2,3-d]-thiazolidin-2-one **(3c)**: 60 %; m. p. 177°. – $C_{15}H_{14}N_2S_2O_2$ (318.3) Calc. C 56.6 H 4.40 N 8.8 S 20.1 Found C 56.3 H 4.38 N 8.5 S 20.0. – IR: 2220 (C≡N) and 1660 (C=O). – ^{1}H-NMR: 7.66–7.55 (m, 5H, $C_6\underline{H}_5$); 4.6 (d, 2H, C-5); 3.45 (m, 1H, C-7); 3.7 (m, 1H, C-6); 3.9 (q, 2H, $C\underline{H}_2CH_3$) and 1.22 (t, 3H, $CH_2C\underline{H}_3$).

6-Carbethoxy-7-ethoxy-5,6-dihydrothiopyrano-[2,3-d]-thiazolidine-2-thione **(6a)**: 65 %; m. p. 140°. – $C_{11}H_{15}NS_3O_3$ (305.3) Calc. C 43.2 H 4.91 S 31.4 Found C 43.2 H 4.91 S 31.2.

6-Carbethoxy-7-ethoxy-5,6-dihydrothiopyrano-[2,3-d]-thiazolidin-2-one **(6b)**: 75 %; m. p. 146°. – $C_{11}H_{15}NS_2O_4$ (289.2) Calc. C 45.6 H 5.19 S 22.1 Found C 45.6 H 5.16 S 22.1. – IR: 3200 (NH); 1710 and 1680 (ester and ring C=O). – ^{1}H-NMR: 10.3 (NH); 4.6 (d, 2H, C-5); 3.5 (m, 1H, C-7); 3.9–3.6 (m, 1H, C-6); 4.4 (q, 4H, two $C\underline{H}_2CH_3$) and 1.4 (t, 6H, two $CH_2C\underline{H}_3$).

6-Carbethoxy-3-phenyl-7-ethoxy-5,6-dihydrothiopyrano-[2,3-d]-thiazolidin-2-one **(6c):** 60 %; m. p. 136°. – $C_{17}H_{19}NS_2O_4$ (365.3) Calc. C 55.9 H 5.20 N 3.8 S 17.5 Found C 55.6 H 5.10 N 3.8 S 17.3.

6-Nitro-5-phenyl-7-ethoxy-5,6-dihydrothiopyrano-[2,3-d]-thiazolidine-2-thione **(7a)**: 60 %; m. p. 176°. – $C_{14}H_{14}N_2S_3O_3$ (354.3) Calc. C 47.4 H 3.95 Found C 47.4 H 3.85.

6-Nitro-5-phenyl-7-ethoxy-5,6-dihydrothiopyrano-[2,3-d]-thiazolidin-2-one **(7b)**: 65 %; m. p. 175°. – $C_{14}H_{14}N_2S_2O_4$ (338.3) Calc. C 49.7 H 4.14 Found C 49.5 H 4.10.

6-Nitro-3,5-diphenyl-7-ethoxy-5,6-dihdrothiopyrano-[2,3-d]-thiazolidin-2-one **(7c)**: 60 %; m. p. 173° $C_{20}H_{18}N_2S_2O_4$ (414.4) Calc. C 57.9 H 4.34 S 15.4 Found C 57.7 H 4.30 S 15.3.

N-Phenyl-7-ethoxy-5,6-dihydrothiopyrano-[2,3-d]-thiazolidine-2-thioxo-5,6-dicarboximide **(10a)**: 60 %; m. p. > 300°. – $C_{16}H_{14}N_2S_3O_3$ (378.3) Calc. C 50.8 H 3.73 N 7.40 S 25.6 Found C 50.6 H 3.65 N 7.4 S 25.3.

N-Phenyl-7-ethoxy-5,6-dihydrothiopyrano-[2,3-d]-thiazolidin-2-one-5,6-dicarboximide **(10b)**: 65 %; m. p. 208°. – $C_{16}H_{14}N_2S_2O_4$ (362.3) Calc. C 53.0 H 3.90 S 17.6 Found C 53.0 H 3.85 S 17.6.

N,3-Diphenyl-7-ethoxy-5,6-dihydrothiopyrano-[2,3-d]-thiazolidin-2-one-5,6-dicarboximide **(10c)**: 70 %; m. p. 194°. – $C_{22}H_{18}N_2S_2O_4$ (438.4) Calc. C 60.3 H 4.14 S 14.6 Found C 60.2 H 4.00 S 14.4. – IR: 1740 and 1680 (amide and ring C=O). – ^{1}H-NMR: 7.6–7.1 (m, 10 H, $2C_6\underline{H}_5$); 5.1 (d, 1H, J = 9 Hz, C-7); 4.3–4 (dd, 1H, J = 6; 9 Hz, C-6); 3.9 (d, 1H, J = 6 Hz, C-5); 3.6 (q, 2H, $C\underline{H}_2CH_3$) and 1.3 (t, 3H, $CH_2C\underline{H}_3$).

3-Phenyl-7-ethoxy-5,6-dihydrothiopyrano-[2,3-d]-thiazolidin-2-one-5,6-dicarboxylic anhydride **(12)**: 70 %; m. p. 186°. – $C_{16}H_{13}NS_2O_5$ (363.3) Calc. C 52.9 H 3.61 N 3.9 S 17.6 Found C 52.7 H 3.61 N 3.9 S 17.6. – IR: 1870 and 1770 (anhydride C=O) and 1680 (ring C=O).

General procedure for the reaction of **2a–c** *with acrylonitrile, ethyl acrylate, β-nitrostyrene and N-phenylmaleimide to give* **5a–c**, **8a–c**, **9a–c** *and* **11a–c**

A solution of equimolar amounts (0.01 mole) of each of **2a–c** and acrylonitrile, ethyl acrylate, β-nitrostyrene or N-phenylmaleimide in galcial acetic acid (50 ml) was refluxed for 1 h, then left at room temp. overnight. The coloured products so obtained were filtered off and recrystallized from glacial acetic acid. The products **5a–c**, **8a–c**, **9a–c** and **11a–c** are listed below.

6-Cyano-5H-thiopyrano-[2,3-d]-thiazolidine-2-thione **(5a)**: 40 %; m. p. > 300°. – $C_7H_4N_2S_3$ (212.1) Calc. C 39.6 H 1.90 Found C 39.4 H 1.81.

6-Cyano-5H-thiopyrano-[2,3-d]-thiazolidin-2-one **(5b)**: 50 %; m. p. 213°. – $C_7H_4N_2S_2O$ (196.1) Calc. C 42.8 H 2.06 S 32.6 Found C 42.7 H 2.00 S 32.4. – IR: 3200 (NH); 2180 (C≡N) and 1670 (C=O). – ^{1}H-NMR: 10.4 (s, 1H, NH); 7.14 (s, 1H, C-7) and 4.6 (s, 2H, C-5).

6-Cyano-3-phenyl-5H-thiopyrano-[2,3-d]-thiazolidin-2-one **(5c)**: 55 %; m. . 184°. – $C_{13}H_8N_2S_2O$ (272.2) Calc. C 57.3 H 2.96 S 23.5 Found C 57.2 H 2.91 S 23.2 – IR: 2200 (C≡N) and 1680 (C=O). – ^{1}H-NMR: 7.6 (m, 5H, C_6H_5); 7.0 (s, 1H, C-7) and 3.85 (s, 2H, C-5).

6-Carbethoxy-5H-thiopyrano-[2,3-d]-thiazolidine-2-thione **(8a)**: 70 %; m. p. 164°. $C_9H_9NS_3O_2$ (259.2) Calc. C 41.7 H 3.50 N 5.4 S 37.0 Found C 41.6 H 3.41 N 5.3 S 36.9.

6-Carbethoxy-5H-thiopyrano-[2,3-d]-thiazolidin-2-one **(8b)**: 68 %; m. p. 171°. $C_9H_9NS_2O_3$ (243.2) Calc. C 44.4 H 3.73 S 26.3 Found C 44.1 H 3.70 S 26.0. – IR: 3200 (NH); 1700 and 1680 (ester and ring C=O). – ^{1}H-NMR: 10.8 (s, 1H, NH); 7.3 (s, 1H, C-7); 4.35 (q, 2H, CH_2CH_3); 4.0 (s, 2H, C-5); 1.45 (t, 3H, CH_2CH_3).

6-Carbethoxy-3-phenyl-5H-thiopyrano-[2,3-d]-thiazolidin-2-one **(8c)**: 70 %; m. p. 118°. $C_{15}H_{13}NS_2O_3$ (319.3) Calc. C 56.4 H 4.10 N 4.4 S 20.0 Found C 56.4 H 4.00 N 4.3 S 20.0. – IR: 1680 and 1650 (ester and ring C=O). – ^{1}H-NMR: 7.5–7.4 (m, 5H, C_6H_5); 7.3 (s, 1H, C-7); 4.33 (q, 2H, CH_2CH_3); 3.85 (s, 2H, C-5) and 1.33 (t, 3H, CH_2CH_3).

6-Nitro-5-phenyl-5H-thiopyrano-[2,3-d]-thiazolidine-2-thione **(9a)**: 55 %; m. p. 145°. – $C_{12}H_8N_2S_3O_2$ (308.2) Calc. C 46.8 H 2.62 S 31.1 Found C 46.7 H 2.60 S 31.0.

6-Nitro-5-phenyl-5H-thiopyrano-[2,3-d]-thiazolidin-2-one **(9b)**: 50 %; m. p. 186°. – $C_{12}H_8N_2S_2O_3$ (292.2) Calc. C 49.3 H 2.76 S 21.9 Found C 48.9 H 2.70 S 21.7.

6-Nitro-3,5-diphenyl-5H-thiopyrano-[2,3-d]-thiazolidin-2-one **(9c)**: 75 %; m. p. 234. – $C_{18}H_{12}N_2S_2O_3$ (368.3) Calc. C 58.7 H 3.28 N 7.6 S 17.3 Found C 58.3 H 3.16 N 7.55 S 17.2.

N-Phenyl-5H-thiopyrano-[2,3-d]-thiazolidine-2-thioxo-5,6-dicarboximide **(11a)**: 65 %; m. p. > 300°. $C_{14}H_8N_2S_3O_2$ (323.2) Calc. C 50.6 H 2.49 S 28.9 Found C 50.3 H 2.40 S 28.8.

N-Phenyl-5H-thiopyrano-[2,3-d]-thiazolidin-2-one-5,6-dicarboximide **(11b)**: 70 %; m. p. 210°. $C_{14}H_8N_2S_2O_3$ (316.2) Calc. C 53.2 H 2.55 S 20.2 Found C 53.0 H 2.50 S 20.1.

N,3-Diphenyl-5H-thiopyrano-[2,3-d]-thiazolidin-2-one-5,6-dicarboximide **(11c)**: 65 %; m. p. 215°. – $C_{20}H_{12}N_2S_2O_3$ (392.3) Calc. C 61.2 H 3.06 N 7.1 S 16.3 Found C 61.1 H 3.01 N 7.1 S 16.1. – ^{1}H-NMR: 7.5–7.3 (m, 10 H, 2 C_6H_5); 6.9 (s, 1H, C-7) and 3.9 (s, 1H, C-5).

Conversion of **3**, **6**, **7** *and* **10** *to* **5**, **8**, **9** *and* **11**

A solution of 0.01 mole of each of **3a–c**, **6a–c**, **7a–c** and/or **10a–c** in glacial acetic acid (50 ml) was refluxed for 1 h, then left at room temp. overnight. The solid products so obtained were recrystallized from glacial acetic acid to afford **5a–c**, **8a–c**, **9a–c** and **11a–c**, respectively (mp. and mixed mp.).

Reaction of the anhydride **12** *with aniline to afford the amide* **13**

To a stirred solution of **12** (0.01 mole) in benzene (50 ml), was added dropwise a solution of aniline (0.01 mole) in benzene (10 ml). The precipitated solid so formed was recrystallized from benzene/petroleum ether to afford yellow crystals of **13**, 40 %; m. p. 90°. $C_{22}H_{20}N_2S_2O_5$ (456.4) Calc. C 57.9 H 4.42

N 6.1 S 14.0 Found C 57.7 H 4.38 N 6.0 S 14.0. IR: 1700–1680 (br, C=O amide and carboxylic acid) and 3200–2860 (br, OH and NH).

Cyclization of the amide **13** *to* **11c**

To a suspension of **13** (0.01 mole) in glacial acetic acid (50 %) was added fused sodium acetate (1.0 g). The reaction mixture was refluxed for 1 h, left to cool then poured into water. The solid product so formed was washed with water and recrystallized from acetic acid to afford **11c** (mp. and mixed mp.).

Reaction of **2a–c** *with malononitrile to give* **14a–c**

Equimolar amounts (0.01 mole) of each of **2a–c** and malononitrile were dissolved in absol. EtOH (40 ml) and few drops of triethylamine were added. The solution was kept at room temp. overnigh with stirring. The coloured solid products that separated were recrystallized from EtOH to afford **14a–c**, listed below.

6-Cyano-5-imino-5H-thiopyrano-[2,3-d]-thiazolidine-2-thione **(14a)**: 50 %; m.p. > 300°. – $C_7H_3N_3S_3$ (225.1). Calc. C 37.3 H 1.34 S 42.6 Found C 37.1 H 1.32 S 42.6.

6-Cyano-5-imino-5H-thiopyrano-[2,3-d]-thiazolidin-2-one **(14b)**: 60 %; m. p. > 300°. – $C_7H_3N_3S_2O$ (209.1) Calc. C 40.2 H 1.45 S 30.6 Found C 40.1 H 1.40 S 30.5. – IR: 3300, 3100 (imino and ring NH); 2220 (C≡N) and 1660 (C=O). – ^{1}H-NMR: 9.7–9.2 (br, 2H, ring and imino H) and 8.2 (s, 1H, C-7).

6-Cyano-3-phenyl-5-imino-5H-thiopyrano-[2,3-d]-thiazolidin-2-one **(14c)**. 55 %; m. p. 260°. – $C_{13}H_7N_3S_2O$ (285.2) Calc. C 54.7 H 2.47 N 14.7 S 22.4 Found C 54.3 H 2.42 N 14.5 S 22.2.

Reaction of ethoxymethylenemalononitrile with thiazolidinone derivatives **1a–c** *to afford* **14a–c**

Equimolar amounts (0.01 mole) of ethoxymethylenemalononitrile and each of the thiazolidinones **1a–c** were dissolved in absol. EtOH (40 ml) then a few drops of triethylamine were added. The solution was kept overnight at room temp. with stirring. The solid product that separated was recrystallized from EtOH to afford **14a–c** (mp. and mixed mp.).

Method used in biological tests

The method used for bacteria was beef-peptone agar composed of the following ingredients (g/l): beef extract 3, peptone 5, NaCl 3, distilled water 1 L. The PH was adjusted to 7.0.

The *Dox's* medium was used for the fungal tests and composed of the following ingredients (g/l): sucrose 1, $NaNO_3$ 2, KH_2PO_4 1, KCl 0.5, $MgSO_4$ 0.5, $FeSO_4$ 0.001, distilled water 1 L, agar 20. The PH was adjusted to 7.0.

Test of antimicrobial action

A sterile petri dish containing the proper medium was inoculated by 1 ml of a heavy microbial suspension or of spores. Then the discs containing 100 µg of each compound were placed on the surface of the agar. The plates were incubated at 28° for 24 h in case of bacteria and 4 d in case of fungi. The inhibition zones around the disc were measured in mm.

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