Synthesis and Antibacterial Activity of 7-Ethyl-3-methyl-4,7-dihydro-4-oxoisothiazolo[5,4-*b*]pyridine-5-carboxylic Acid

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The synthesis of the quinolone, 7-ethyl-3-methyl-4,7-dihydro-4-oxoisothiazolo[5,4-*b*]pyridine-5-carboxylic acid **4** was accomplished utilizing the Gould-Jacobs dependent route. The compound had very weak *in vitro* activity as compared to nalidixic acid versus *E. coli, P. aeruginosa, K. pnuemoniae, S. aureus* and *P. mirabilis.*

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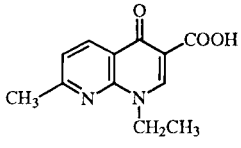
Introduction.

Following the discovery in 1962 of the antibacterial utility of nalidixic acid **1** [1], much research has been done to find similar compounds with increased activity, oral efficacy and a broader spectrum [2]. Nalidixic acid analogs (generally referred to as quinolones or pyridonecarboxylic acid antibacterials) are compounds which contain the 1,4-dihydro-4-oxo-3-pyridinecarboxylic acid moiety substituted at the one position with an aromatic or heteroaromatic ring fused at the 5,6-position. Most quinolones have a six membered ring fused at the 5,6-position of the 1-substituted 1,4-dihydro-4-oxo-3-pyridinecarboxylic acid moiety [2] including a highly active group of compounds called fluoroquinolones which are receiving great recognition [3,4].

Quinolones having a five membered heterocyclic ring system at the 5,6-position have received some attention and some active compounds have been obtained [2,5-12], especially those containing the thieno[2,3-*b*]pyridine and thieno[3,2-*b*]pyridine systems represented in **2** and **3** [5,6]. Some quinolone compounds containing these systems have shown significant activity. Compound **2** (R = CH_3) is reported to have activity similar to nalidixic acid [5].



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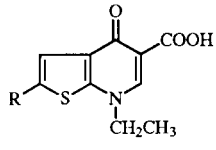

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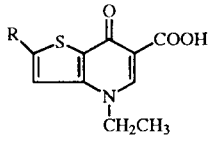

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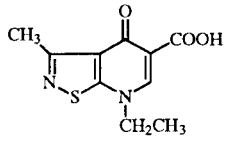

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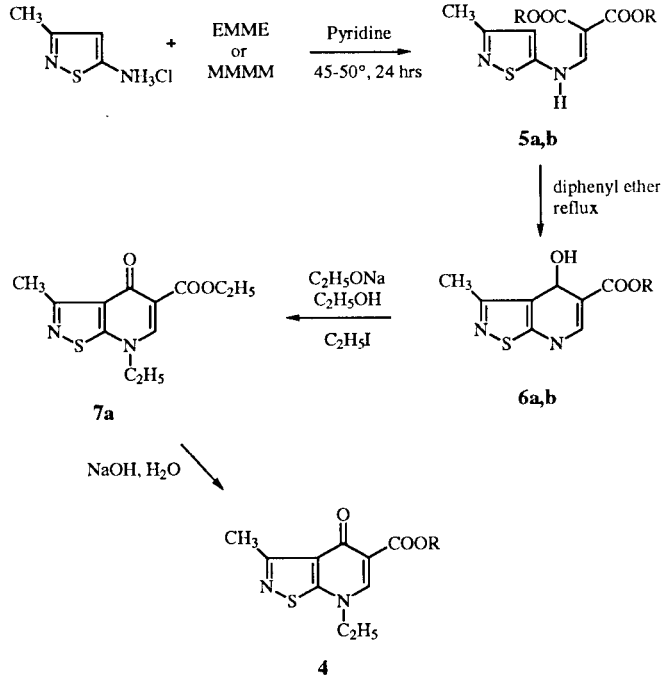
With the purpose of obtaining a compound more active than nalidixic acid, we synthesized the title compound which introduces a nitrogen into the thiophene ring of compound **2** next to the sulfur atom while moving the methyl group to the adjacent position on the thiophene ring.

Scheme 1



EMME = Diethyl ethoxymethylenemalonate; MMMM = Dimethyl methoxymethylenemalonate; for a, R = ethyl; for b, R = methyl.

Chemistry.

The synthesis of compound **4** was accomplished by a four step process as shown in Scheme 1 which follows the Gould-Jacobs route [13]. The method of Khan and Guarconi [14] was adapted for the synthesis of compounds **5a** and **5b** which were each obtained in 66% yield by reacting 5-amino-3-methylisoxazole hydrochloride with diethyl ethoxymethylenemalonate or dimethyl methoxymethylenemalonate respectively in pyridine at 45-50° for 24 hours. Attempts to obtain compounds **5a** and **5b** using sodium methoxide/methanol or sodium ethoxide/ethanol as base/solvent systems failed to give the products. Thermal cyclization of compound **5a** and **5b**

with boiling diphenyl ether gave **6a** and **6b**, both in 85% yield. The route using dimethyl methoxymethylenemalonate was performed in order to compare it to the one using diethyl ethoxymethylenemalonate and was pursued as far as the cyclized product. Reaction of **6a** with sodium ethoxide/ethanol followed by ethyl iodide resulted in **7a** in 87% yield. Compound **6a** is capable of *O*-alkylation as well as *N*-alkylation. The site of alkylation was determined by infrared spectroscopy as compounds **7a** showed two carbonyl absorptions (1678 and 1638 cm^{-1}) indicating that alkylation took place on the nitrogen. Treatment of **7a** with 1*M* sodium hydroxide followed by neutralization with 1*M* hydrochloric acid gave **4** in 84% yield.

Microbiological Results.

Determination of the minimal inhibitory concentration (MIC) was performed by two fold serial dilution using Mueller Hinton liquid media. Compound **4** showed very weak activity as compared to nalidixic acid *in vitro* against *E. coli* ATCC 25922, *P. aeruginosa* ATCC 27853, *K. pnuemoniae* ATCC 13883, *S. aureus* ATCC 25923 and *P. vulgaris* ATCC 13315. The highest activity was against *p. vulgaris* at 83 μg/ml.

In view of the weak activity of the title compound **4**, further research on this system was discontinued.

EXPERIMENTAL

Melting points were determined in open melting point capillaries using an Electrothermal Melting Point apparatus and are uncorrected. The proton nmr spectra were determined on a Brucker Aspect 2000 nmr spectrometer. Chemical shifts are expressed in ppm relative to tetramethylsilane (TMS). The infrared spectra were determined with a Perkin-Elmer 1605 FTIR spectrometer.

Diethyl *N*-(3-Methyl-5-isothiazoyl)aminomethylenemalonate (**5a**).

A mixture of 5-amino-3-methylisothiazole hydrochloride (10g, 0.065 mole) and diethyl ethoxymethylenemalonate (14 g, 0.065 mole) in 200 ml of pyridine, contained in a 500 ml Erlenmyer flask attached to a rotary evaporator, was heated at 45-50° for 24 hours. The pyridine was removed at the rotary evaporator. Water was added to the residue and the insoluble material was collected by filtration and dried (12.3 g, 66% yield). The product **5a** was recrystallized from ethanol, mp 82-83°; ir (potassium bromide): 3172, 2988, 1682, 1648, 1592, 1548, 1448, 1404, 1387, 1348, 1270, 1222, 1154, 1092, 1030, 1018, 964, 838, 794 cm^{-1}; ^1H nmr (deuterated chloroform): δ 1.33 (t, 6H, $2CH_3CH_2$), 2.41 (s, 3H, CH_3), 4.27 (q, 4H, $2CH_2CH_3$), 6.60 (s, 1H, Ar CH), 8.10 (d, 1H, =C*H*-NH).

Anal. Calcd. for $C_{12}H_{16}N_2O_4S$: C, 50.69; H, 5.67; N, 9.85; S, 11.28. Found: C, 50.78; H, 5.89; N, 9.82; S, 11.60

Dimethyl *N*-(3-Methyl-5-isothiazoyl)aminomethylenemalonate (**5b**).

A mixture of 5-amino-3-methylisothiazole hydrochloride (20 g, 0.133 mole) and dimethyl methoxymethylenemalonate (23 g, 0.133 mole) in 400 ml of pyridine, contained in a 1000 ml flask attached to a rotary evaporator, was heated at 45-50° for 24 hours. The pyridine was removed at the rotary evaporator. Water was added to the residue and the insoluble material was collected by filtration and dried (22.5 g, 66% yield). Product **5b** was recrystallized from methanol, mp 112-114°; ir (potassium bromide): 3184, 2958, 1738, 1678, 1616, 1552, 1448, 1430, 1394, 1310, 1238, 1206, 1180, 990, 972, 938, 826, 798 cm^{-1}; ^1H nmr (deuterated chloroform): δ 2.47 (s, 3H, CH_3), 3.85 (s, 3H, CH_3), 3.95 (s, 3H, CH_3), 6.79 (s, 1H, Ar CH), 8.15 (d, 1H, =C*H*-NH).

Anal. Calcd. for $C_{10}H_{12}N_2O_4S$: C, 46.87; H, 4.72; N, 10.93. Found: C, 46.80; H, 4.81; N, 10.81.

Ethyl 3-Methyl-4-hydroxyisothiazolo[5,4-*b*]pyridine-5-carboxylate (**6a**).

Compound **5a** (4.80 g, 0.031 mole) was added to refluxing diphenyl ether (100 ml) contained in a 500 ml Erlenmyer flask heated by a hot plate. The mixture was maintained at reflux for 15 minutes. The mixture was cooled and hexane (100 ml) was added. The product **6a** was collected by filtration, washed with hexane and dried (6.3 g, 85% yield). The product was recrystallized from *N,N*-dimethylformamide, mp 280-283°; ir (potassium bromide): 3474, 3008, 1696, 1606, 1532, 1350, 1282, 1206, 1184, 1102, 1024, 804, 776, 624, 546 cm^{-1}; ^1H nmr (trifluoroacetic acid): δ 1.38 (t, 3H, CH_3CH_2), 2.92 (s, 3H, CH_3), 4.54 (q, 2H, CH_2CH_3), 9.25 (s, 1H, Ar CH).

Anal. Calcd. for $C_{10}H_{10}N_2O_3S$: C, 50.41; H, 4.23; N, 11.77. Found: C, 50.67; H, 4.36; N, 11.82.

Methyl 3-Methyl-4-hydroxyisothiazolo[5,4-*b*]pyridine-5-carboxylate (**6b**).

Compound **5b** (3.0 g, 0.012 mole) was added to refluxing diphenyl ether (60 ml) contained in a 500 ml Erlenmyer flask heated by a hot plate. The mixture was maintained at reflux for 15 minutes. The mixture was cooled and hexane (60 ml) was added. The product **6b** was collected by filtration, washed with hexane and dried (2.2 g, 85% yield). The product was recrystallized from *N,N*-dimethylformamide, mp 262-265°; ir (potassium bromide): 3436, 3014, 1696, 1606, 1586, 1538, 1442, 1356, 1300, 1206, 1164, 1106, 866, 804, 690, 628 and 548 cm^{-1}; ^1H nmr (trifluoroacetic acid): δ 3.26 (s, 3H, CH_3), 4.42 (s, 3H, CH_3), 9.60 (s, 1H, Ar CH).

Anal. Calcd. for $C_9H_8N_2O_3S$: C, 48.21; H, 3.60; N, 12.50. Found: C, 48.29; H, 3.65; N, 12.38.

Ethyl 7-Ethyl-3-methyl-4,7-dihydro-4-oxothiazolo[5,4-*b*]pyridine-5-carboxylate (**7a**).

Compound **6a** (4.80 g, 0.02 mole) was added to a solution of sodium (0.58 g, 0.025 mole) in absolute ethanol (100 ml) contained in a 250 ml round bottom flask equipped for reflux. The mixture was refluxed for 30 minutes. Ethyl iodide (5 ml, 0.05 mole) was added and the mixture was refluxed for 24 hours. After the reflux period, the solvent was removed at the rotary evaporator. Chloroform was added to the residue and the solution was washed with water and dried. The chloroform was removed at the rotary evaporator to obtain the product (4.5 g, 87% yield). Product **7a** was recrystallized from ethyl acetate, mp 115-117°; ir (potassium bromide): 3056, 2990, 1678, 1638, 1498, 1384, 1360, 1312, 1280, 1230, 1174, 1112, 1020, 804 cm^{-1}; ^1H nmr (carbon tetrachloride): δ 1.34 (t, 3H, CH_3CH_2), 1.60 (t, 3H, CH_3CH_2), 2.85 (s, 3H, CH_3), 4.02 (q, 2H,

CH_2CH_3), 4.40 (q, 2H, CH_2CH_3), 8.26 (s, 1H, Ar CH).

Anal. Calcd. for $C_{12}H_{14}N_2O_3S$: C, 54.12; H, 5.30; N, 10.52. Found: C, 54.14; H, 5.26; N, 10.58.

7-Ethyl-3-methyl-4,7-dihydro-4-oxoisothiazolo[5,4-*b*]pyridine-5-carboxylic Acid (**4**).

Compound **7a** (2.38 g, 0.009 mole) was added to a 250 ml round bottom flask equipped for reflux and 20 ml of 1*M* sodium hydroxide was added. The mixture was heated at reflux until all solid dissolved. The solution was filtered, cooled and the filtrate was neutralized with 1*M* hydrochloric acid to give a solid precipitate. The solid was collected by filtration and dried (1.8 g, 84% yield). The product **4** was recrystallized from *N,N*-dimethylformamide, mp 200-202°; ir (potassium bromide): 3058, 2998, 1720, 1648, 1604, 1552, 1520, 1496, 1478, 1454, 1384, 1358, 1298, 1256, 1224, 1168, 1092, 956, 810, 782, 722, 494; ^{1}H nmr (trifluoroacetic acid): δ 1.69 (t, 3H, CH_3CH_2), 2.93 (s, 3H, CH_3), 4.60 (q, 2H, CH_2CH_3), 9.22 (s, 1H, Ar CH).

Anal. Calcd. for $C_{10}H_{10}N_2O_3S$: C, 50.41; H, 4.23; N, 11.77; S, 13.43. Found: C, 50.27; H, 4.26; N, 11.80; S, 13.73.

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