Synthesis of 4,7-Dihydro-4-oxoisoxazolo[5,4-*b*]pyridine-5-carboxylic Acid Derivatives as Potential Antimicrobial Agents

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Received March 4, 1987

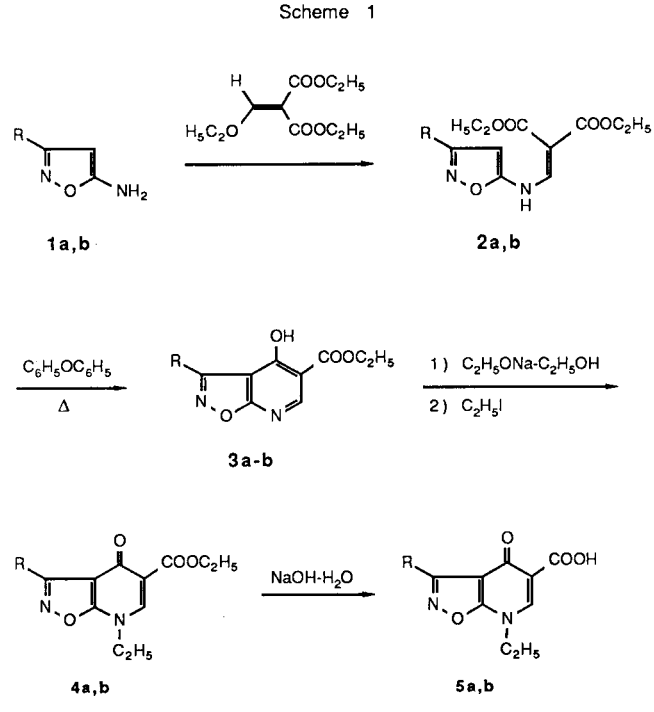
Synthetic approaches to 3-substituted-7-ethyl-4,7-dihydro-4-oxoisoxazolo[5,4-*b*]pyridine-5-carboxylic acid derivatives from 3-substituted-5-aminoisoxazoles are reported.

J. Heterocyclic Chem., **25**, 231 (1988).

During the last few years a great deal of synthetic effort has been spent, since the introduction of nalidixic acid as an antimicrobial agent in the treatment of urinary tract infections [1], in order to obtain analogs that might possess a broader spectrum of activity and an increased potency. Most of these compounds can be described as 1-substituted-1,4-dihydro-4-oxo-3-pyridinecarboxylic acid bearing an aromatic or heteroaromatic ring fused in the 5,6 position [2].

Our recent interest in the isoxazole chemistry [3] and the proved capacity of this ring in replacing the phenyl nucleus in compounds endowed with β-adrenergic activity [4], prompted us to synthesize a series of 7-ethyl-4,7-dihydro-4-oxoisoxazolo[5,4-*b*]pyridine-5-carboxylic acids (Scheme 1) in order to evaluate their possible antibacterial activity.

Scheme 1



Compound **1a-5a** R = C_6H_5
Compound **1b-5b** R = CH_3

Chemistry.

In 1972 Denzel and Höhn [5] reported the synthesis of ethyl 4-ethoxy-3-methylisoxazolo[5,4-*b*]pyridine-5-carboxylate obtained by *O*-alkylation of 4-hydroxy derivative **3b** with ethyl iodide and potassium carbonate in dimethylformamide. In an attempt to synthesize the *N*-alkyl derivative **4b** we tested an alternative route (Scheme 2).

Alkylation of the open intermediate **2b** [5] was easily achieved by means of ethyl iodide and potassium carbonate in dimethylformamide to give compound **6**. The second step provided the cyclization of compound **6** to **4b** but, utilizing the known procedures [6a-e] no trace of the desired compound was found.

So, in order to obtain the desired *N*-ethylisoxazolo-[5,4-*b*]pyridine-5-carboxylic acids **5a,b** we followed the synthetic route shown in Scheme 1.

3-Substituted-5-aminoisoxazoles **1a,b** [7,8] were condensed with diethyl ethoxymethylene malonate to yield compounds **2a** and **2b** respectively. The cyclization of **2a,b** was achieved in good yield by refluxing them in diphenyl ether for 10 minutes to give compounds **3a,b**. The alkylation of compounds **3a,b** was the crucial step of this

Scheme 2

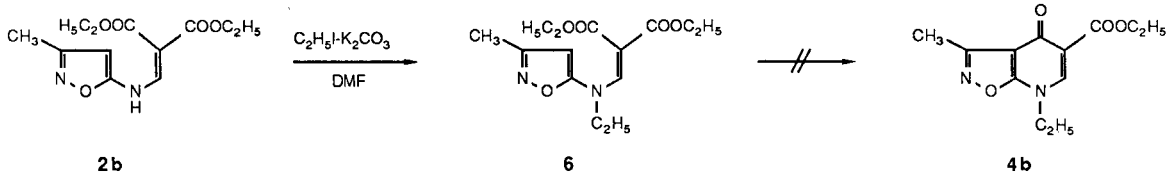


Table 1

Analytical Data of New Compounds

Compound	IR (Potassium bromide) (C=O) cm^{-1}	^{1}H-NMR (DMSO-d_6) δ (ppm)	Molecular Formula	Analysis, % Calcd./Found C	H	N
2a	1680, 1650, 1620	1.29 (t, 6H), 4.24 (q, 2H), 4.31 (q, 2H), 6.72 (s, 1H), 7.50-8.20 (m, 5H), 8.40 (d, 1H), 11.30 (d, 1H)	$C_{17}H_{18}N_2O_5$	61.81 61.94	5.49 5.41	8.48 8.71
3a	1680	1.39 (t, 3H), 4.47 (q, 2H), 7.50-8.30 (m, 5H), 8.95 (s, 1H), 9.80-10.20 (broad s, 1H)	$C_{15}H_{12}N_2O_4$	63.38 63.10	4.25 4.20	9.86 9.96
4a	1680, 1650	1.30 (t, 3H), 1.46 (t, 3H), 4.27 (q, 2H), 4.33 (q, 2H), 7.40-8.50 (m, 5H), 8.59 (s, 1H)	$C_{17}H_{16}N_2O_4$	65.38 65.33	5.16 5.23	8.97 8.87
4b	1690, 1660	1.27 (t, 3H), 1.40 (t, 3H), 2.48 (s, 3H), 4.25 (q, 4H), 8.50 (s, 1H)	$C_{12}H_{14}N_2O_4$	57.59 57.29	5.64 5.64	11.19 11.09
5a	1730, 1640	1.52 (t, 3H), 4.49 (q, 2H), 7.50-8.50 (m, 5H), 9.06 (s, 1H), 9.93 (s, 1H)	$C_{15}H_{12}N_2O_4$	63.38 63.21	4.25 4.20	9.86 9.80
5b	1710, 1650	1.44 (t, 3H), 2.58 (s, 3H), 4.43 (q, 2H), 9.00 (s, 1H), 9.96 (s, 1H)	$C_{10}H_{10}N_2O_4$	54.05 54.23	4.54 4.41	12.61 12.68
6	1710, 1610	1.17 (t, 3H), 1.23 (t, 6H), 2.17 (s, 3H), 3.72 (q, 2H), 4.15 (q, 2H), 4.18 (q, 2H), 6.02 (s, 1H), 7.76 (s, 1H)	$C_{14}H_{20}N_2O_5$	56.75 56.87	6.80 6.64	9.45 9.41

synthetic approach: the tendence towards *O*-alkylation of these compounds shown in aprotic dipolar medium could be avoided adopting a solvent able to give a hydrogen bond with the β-ketoester moiety of these derivatives.

In fact the *N*-alkylation of compounds **3a,b**, utilizing sodium ethoxide in ethanol followed by ethyl iodide, resulted in **4a** and **4b** in good yield.

The site of alkylation was assigned on the basis of infrared and ^{1}H-nmr data. As expected compounds **4a,b** showed two typical carbonyl absorptions each in the region from 1700 to 1640 cm^{-1}; in the ^{1}H-nmr spectra, the vinyl proton at the 6-position appeared as a singlet at 8.59 and 8.50 ppm compared to the singlet at 8.95 and 8.89 of precursors **3a,b**. Furthermore analytical data of compound **4b** were significantly different from those reported for the *O*-ethylated compound [5].

The desired carboxylic acids **5a,b** were easily obtained by hydrolysis of the corresponding ethyl esters with aqueous sodium hydroxide at reflux.

The new isoxazolopyridines **5a,b** were tested *in vitro* against a variety from Gram-negative and Gram-positive bacterial strains but none of the tested compounds showed significant antimicrobial activity. In view of the microbiological results this class of compounds was not further developed.

EXPERIMENTAL

Melting points were determined on a Büchi SMP-20 apparatus and are uncorrected. The ir spectra were obtained with Pye Unicam SP3-200 spectrophotometer; ^{1}H-nmr spectra were determined with a Varian EM-360L spectrometer.

Diethyl *N*-(3-Phenyl-5-isoxazolyl)aminomethylenemalonate (**2a**).

A stirred mixture of 3-phenyl-5-aminoisoxazole (**1a**) [7] (1.60 g, 0.01 mole) and diethyl ethoxymethylenemalonate (2.16 g, 0.01 mole) was heated for 4 hours at 130°. The obtained clear solution was cooled at 80° and ethanol (10 ml) was added. After cooling at room temperature the precipitate was collected and crystallized from ethanol (45 ml) to give pure **2a** (2.30 g, 70%, mp 140-142°).

Ethyl 3-Phenyl-4-hydroxyisoxazolo[5,4-*b*]pyridine-5-carboxylate (**3a**).

Compound **2a** (26.5 g, 0.08 mole) was added to refluxing diphenyl ether (100 ml) and the mixture was vigorously refluxed for 10 minutes. The cooled reaction mixture was poured into 5% aqueous sodium hydroxide (600 ml) and diphenylether was removed by washing with ethyl acetate. The aqueous solution was acidified with concentrated hydrochloric acid and the precipitate was collected, washed with water, dried and crystallized from acetonitrile (180 ml) to give pure **3a** (12.7 g, 56%, mp 157-158°).

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

Compound **3a** (2.80 g, 0.01 mole) was added to a stirred solution of 0.2*N* ethanolic sodium ethoxide (50 ml) and the mixture was refluxed for 30 minutes. Ethyl iodide (3.1 g, 0.02 mole) was slowly added to the resulting solution and refluxing was continued for further 24 hours. After removal of the solvent the residue was taken up with chloroform (100 ml); the organic solution was washed with water and dried. The crude product, obtained after evaporation of the solvent, was chromatographed on a silica gel column by eluting with 10% ethyl acetate in chloroform to give **4a** (2.2 g, 71%). The analytical sample was obtained by crystallization from ethyl acetate (mp 167-168°).

By employing this procedure compound **4b** (63%, mp 126-127° from ethyl acetate) was obtained from **3b** [5].

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

Compound **4a** (1.50 g, 5 mmoles) was suspended in water (150 ml) and treated with aqueous sodium hydroxide (20 ml of a 2% solution). The suspension was refluxed with stirring until a homogeneous solution was achieved (10 minutes). The resulting solution was cooled and acidified with 6*N* hydrochloric acid. The white precipitate was collected, washed with water and crystallized from acetonitrile (30 ml) to give pure **5a** (1.29 g, 91%, mp 190-192°).

By employing this procedure compound **5b** (88%, mp 211-212° from dimethylformamide) was obtained from **4b**.

Diethyl *N*-(3-Methyl-5-isoxazolyl)ethylaminomethylenemalonate (**6**).

To a stirred mixture of compound **2b** [5] (2.68 g, 0.01 mole) and potassium carbonate (2.76 g, 0.02 mole) in dimethylformamide (25 ml) ethyl iodide (3.12 g, 0.02 mole) was slowly added and the reaction mixture was stirred at room temperature overnight. After evaporation of the solvent the residue was taken up in water (30 ml) and extracted with ethyl acetate (50 ml). The organic layer was washed with water, dried and evaporated to give an oil that solidified on standing. Crystallization from *n*-hexane (30 ml) gave pure **6** (2.50 g, 84%, mp 56-57°).

Acknowledgements.

We thank A. Pilotto for his expert technical assistance and E. Albini for the microbiological evaluation.

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