

Nalidixic Acid Prodrugs: Amides from amino acid ester and nalidixic acid

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Abstract □ Amides from amino acid ester and nalidixic acid were synthesized. The solubility characteristics and partition coefficient of the compounds were studied. The hydrolysis of the compounds was studied in the simulated gastric fluid and simulated intestinal fluid. Some compounds showed better antibacterial activity than nalidixic acid.

Keywords □ Nalidixic acid, amide, amino acid ester, partition coefficient

Nalidixic acid (1-ethyl-7-methyl-4-oxo-1,4-dihydro-1,8-naphthyridine-3-carboxylic acid) is an antimicrobial agent. Most widely used against infections due to gram negative organisms and specially in the urinary tract infections¹. It has a high melting point (225-231°C) and has a relatively poor water and lipid solubility.

The present work aims at the preparation and evaluation of some amide derivatives of nalidixic acid which would be non-irritable, readily absorbable, rapidly showing its effects and has got a greater half life in body.

The objective of this study was to investigate: a) whether amide derivatives of nalidixic acid would behave as prodrugs, b) to what extent the physical properties of the prodrugs vary with structure, c) to what degree the *in vitro* cleavage rates vary with structure and d) to seek prodrug derivatives of nalidixic acid which would be non-irritant, readily absorbed and rapidly hydrolyzed to release free drug.

EXPERIMENTAL METHODS

General procedure for the synthesis

The compounds were synthesized in the following steps: Methyl ester hydrochloride of amino acid utilizing the process of Ronald², methyl ester hydrochlorides of L-alanine, L-phenylalanine and tryptophan were synthesized. Amino acid (0.05 mol) was added to a solution of thionyl chloride (0.05 mol in methanol) and the reaction mixture was then

stirred under reflux for 4 hours. The solvent was removed under reduced pressure and the residue was triturated with several 20 ml portions of cold ether at 0°C. The crude product was recrystallized from hot methanol (25 ml) by slow addition of ether (150-200 ml) followed by cooling at 0°C. L-alanine methyl ester hydrochloride (79% yield, mp. 153-155°) L-phenylalanine methyl ester hydrochloride (81% yield, mp. 158-163°) DL-glycine methyl ester hydrochloride (83% yield, mp. 162-164°) and DL-tryptophan methyl ester hydrochloride (76% yield mp. 201°-204°) were thus synthesized.

Nalidixic acid chloride

Nalidixic acid 40 g (0.17 mol) was dissolved in 300 ml of chloroform and then the solution was added to 23.8 g (0.2 mol) of thionyl chloride. The contents were refluxed for 4 hours at 60°-70°C. The solvent and excess of thionyl chloride were distilled off under reduced pressure to give the pure nalidixic acid chloride in the solid form.

Amides from amino acid ester hydrochloride and nalidixic acid chloride

The procedure based on modified Schotten-Baumen reaction³ was adapted. Amino acid methyl ester hydrochloride (0.025 mol) was added slowly to 50 ml ice cold potassium carbonate solution (10%), and the reaction mixture was stirred for 30 minutes at room temperature. To this solution nalidixic acid chloride (0.025 mol) was added gradually and finally

Table I. Characteristics of amide derivatives of nalidixic acid

S. No.	Compound	R	Molecular formula*	Yield %	Melting point °C
1	1		C ₂₂ H ₂₃ N ₃ O ₄	85.12	161-163
2	2		C ₁₅ H ₁₇ N ₃ O ₄	85.90	185-187
3	3		C ₂₄ H ₂₃ N ₄ O ₄	86.40	164-167
4	4		C ₁₅ H ₁₉ N ₃ O ₄	87.23	184-187

*All compounds were analysed for C,H and N. All values were within $\pm 0.4\%$ of the theoretical value. The IR spectra in KBr phase showed the presence of amide linkage in all compounds. The m.p. was determined in open capillaries and are uncorrected.

Table II. Physical data for amide derivatives of nalidixic acid

Compound (prodrug)	Solubility in		Partition coefficient benzene/0.1 N HCl	Fractional distribution f.d.	Availability factor
	0.1 N HCl mg/ml	benzene mg/ml			
1	0.7	12.5	17.85	0.9469	16.84
2	1.1	27.0	24.54	0.9608	34.83
3	0.9	32.0	35.55	0.9726	30.23
4	1.4	45.0	32.14	0.9698	42.77

Fractional distribution (f.d.) of the compound in benzene/water system calculated by dividing the benzene solubility by the sum of the benzene and water solubility.

Availability factor = f.d. \times molar aqueous solubility $\times 10^4$

the mixture was stirred vigorously for 2 hours at 10°C. The compounds thus obtained were washed with 0.5% cold sodium hydroxide solution and recrystallized from chloroform. The physical data of these compounds are listed in Table I and II.

EVALUATION OF PRODRUGS

Solubility studies

The saturation solubilities of the compounds at

37°C were determined in 0.1 N HCl and benzene (spectral grade) in rotating bottles using an assembly reported earlier⁴. HCl (0.1 N) was employed in order to ascertain that the compounds remained completely unionized in the aqueous medium and also to reduce the degree of hydrolysis of the compounds during equilibration. The aqueous samples were rotated for 6 hours and the benzene samples for 16 hours. Excess compound was removed by filtration through a sintered glass funnel and sam-

Table III. *In vitro* hydrolysis of prodrugs in simulated gastric and intestinal fluid

S.No.	Time	% Release of prodrug 1		% Release of prodrug 2		% Release of prodrug 3		% Release of prodrug 4	
		S.G.F.	I.F.	S.G.F.	I.F.	S.G.F.	I.F.	S.G.F.	I.F.
1	0.5	12.6	16.8	8.5	14.5	12.4	12.5	11.0	15.0
2	1.0	23.0	30.0	21.5	31.0	19.6	26.2	21.2	29.5
3	1.5	33.28	40.0	29.06	39.5	27.0	40.8	38.2	43.0
4	2.0	39.5	52.4	34.5	48.4	34.2	50.2	36.8	54.6
5	2.5	45.1	59.0	39.24	61.6	40.0	56.0	44.0	64.0
6	3.0	50.0	66.2	44.26	68.0	47.2	61.0	51.0	70.8
7	3.5	55.5	75.5	48.52	71.0	52.4	62.8	57.8	76.4
8	4.0	59.2	77.0	50.5	72.6	54.0	64.2	60.4	78.8

S.G.F.=Simulated gastric fluid, I.F.=Intestinal fluid.

ples were analysed.

Determination of partition coefficient

The partition coefficient of each compound between benzene and 0.1 N HCl was determined using a method reported earlier⁵. The partition coefficients can be regulated of the unionized compounds as HCl (0.1 N) was used as the aqueous phase.

Availability factors

The availability factors which are products of the molar aqueous solubilities and a factor related to the lipid/water distribution characteristics of the compounds were determined⁶.

In vitro hydrolysis rates

Half lives for hydrolysis of the compounds at 37°C in 0.1 N phosphate buffer with trypsin (0.1%) and in simulated gastric fluid (pH 1.2) were determined. The results are shown in Table III.

Anti-bacterial activity

All the compounds were studied for their antibacterial activity against *E. coli* and *S. typhi* using agar diffusion with cup method⁷. The results are shown in Table IV.

RESULTS

The amide derivatives of nalidixic acid were less soluble in 0.1 N HCl and have greater solubility in benzene. Compounds 2, 3 and 4 were found to have greater solubility in 0.1 N HCl than 1 showing the presence of highest crystal lattice energy

in compound 1.

All the compounds had a melting point lower than nalidixic acid (mp. 225-231°). The higher melting point of nalidixic acid may probably be ascribed to intramolecular hydrogen bonding⁸. By introducing the amide linkage such hydrogen bonding is no longer possible which helps to decrease the crystal lattice forces present in the acid. Partition coefficients and lipid solubility of the compounds was higher as compounds to the parent drug.

DISCUSSION

The results of antibacterial activity are shown in Table IV, all the compounds showed antibacterial activity at 0.1% w/v concentration. The antibacterial activity at 0.1% w/v concentration is significant. The increase in the concentration of the active compounds increase the activity. Compound 3 was found to be more potent than nalidixic acid, where as other three compounds showed less activity than nalidixic acid.

This study was concerned primarily with establishing the principles rather than a determination of synthesized compounds. The amide linkage which was employed to activity profiles of create derivative of nalidixic acid improved the physico-chemical parameters of the compounds. It seems fair to assume that the compounds reported here would behave as nalidixic acid prodrugs.

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Table IV. Antibacterial activity

Micro-organism	Average diameter of zone of inhibition (mm)											
	Compound 1			Compound 2			Compound 3			Compound 4		
	a	b	c	a	b	c	a	b	c	a	b	c
<i>S. typhi</i>	10.5	13.0	12.5	10.0	12.0	13.0	12.0	14.0	13.0	11.0	12.5	13.0
<i>E. coli</i>	11.0	13.5	13.0	—	11.5	12.5	13.0	15.0	13.0	10.5	12.5	13.0

a: 0.1% w/v solution of compound in DMF

b: 0.2% w/v solution of compound in DMF

c: 0.2% w/v solution of nalidixic acid

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