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Preliminary communication

1,2-Dihydropyridine derivatives as potential antiulcer agents

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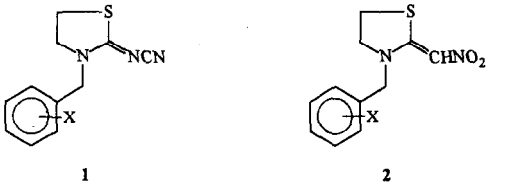
Summary — Some 1-benzyl-2-cyanimino- and 1-benzyl-2-nitroimino-1,2-dihydropyridines were synthesized as new potential antiulcer agents. Some of these compounds exhibited significant antisecretory activity in the Shay test, like 1-(2-chlorobenzyl)-2-cyanimino-1,2-dihydropyridine (**3c**, ED_{50} = 1.8 mg/kg *po*), and some also had a cytoprotective effect, like 1-benzyl-2-cyanimino-1,2-dihydropyridine (**3a**, ED_{50} = 10.0 mg/kg *po*), in a modified Robert test, depending on the substitution of the benzyl group.

synthesis / 1,2-dihydropyridine / antisecretory activity / cytoprotective effect / gastric and duodenal ulcer

Introduction

Gastric and duodenal ulcers are commonly occurring diseases. Although several efficient drugs are available for their treatment with different mechanisms of action (histamine-H_2 receptor antagonists, H^+/K^+-ATP-asc inhibitors, anticholinergics, cytoprotectants, etc) there is always a need for new agents with even better efficacy and safety profiles. The targets of our anti-ulcer research are new compounds with both antisecretory and cytoprotective activities. By our standards a compound is regarded as effective and worthy of more detailed investigations if its ED_{50} values are < 10 mg/kg *po* both in the Shay test [1] and in the modified Robert test [2].

1,3-Thiazolidine derivatives, synthesized and tested by our research division (fig 1; **1** and **2**) displayed potent antisecretory and cytoprotective properties [3]. Since most of them were toxic, we tried to modify their structures, employing the concept of 'bioisosterism' [4], in the hope that their toxicity could be diminished while retaining their therapeutic effects. Accordingly, the *S* atom in the thiazolidine ring was substituted by a –CH=CH– moiety in order to obtain 1,2-dihydropyridines **3** and **4** [5].



RGH-5901 (X = H)

Shay ED_{50}: 5.3 mg/kg p.o.

Robert ED_{50}: 2.8 mg/kg p.o.

RGH-5981 (X = H)

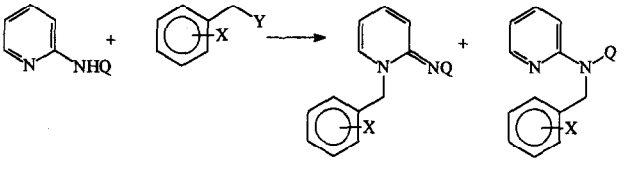
ED_{50}: 21.0 mg/kg p.o.

ED_{50}: 15.0 mg/kg p.o.

Fig 1. 1,3-Thiazolidine derivatives with significant antisecretory and cytoprotective effects.

Chemistry

Starting from the readily available 2-pyridinamine we prepared **5a** [6] and **5b** [7]. The benzylation of these derivatives with substituted benzyl halogenides led to mixtures of 2 isomeric products **3** + **6** and **4** + **7**, respectively (scheme 1). 1,2-Dihydropyridine derivatives **3** and **4**, which were found to be the biologically active isomers, were separated exploiting their weaker



5a Q = CN
5b Q = NO_2

3 Q = CN
4 Q = NO_2

6 Q = CN
7 Q = NO_2

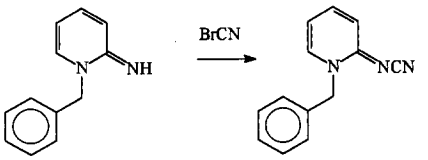
Scheme 1. The reaction of 2-pyridinamine derivatives with substituted benzyl halogenides.

solubilities in apolar organic solvents. Their structures were assigned by spectroscopic methods (UV, IR, ^{1}H-NMR), the UV and IR spectra showing characteristic differences between the isomers (table II). The structure of **3a** was confirmed by independent synthesis involving the reaction of the known **8** [8] with cyanogen bromide (scheme 2).

The selection of the substituents on the benzyl group was based on previous results in the thiazolidine series.

The benzylation reactions were performed: a) in refluxing acetonitrile in the presence of *N,N*-diisopropylethylamine; and/or b) in refluxing acetone with K_2CO_3 (in the case of benzyl chlorides, equimolar KI was added) (table I).

Since **6** (X=H) and **7** (X=H) were less active than their 1,2-dihydropyridine isomers in the Shay test, the substituted analogs were not prepared.



8 **3a**

Scheme 2. Verification of the structure **3a** by synthesis.

Pharmacological results and discussion

Pharmacological results obtained in the Shay and Robert tests are summarized in table III. Our original aim, *ie* to prepare compounds with gastric acid secretion inhibitory and cytoprotective effects with ED_{50}s < 10 mg/kg, was fulfilled only in the case of **3a**. Substituents on the benzyl ring generally decreased the activity in at least one of the tests. The acute toxicity studies showed that the most effective compounds of general structure **3** had a LD_{50} between 500–1000 mg/kg *po* in rats.

Nevertheless, the isosteric replacement of the *S* atom in thiazolidines **1** and **2** can be regarded as a successful modification, since the new 1,2-dihydropyridines have more or less the same activities. Thus, **3** and **4** can be considered as bioisosters of **1** and **2**.

As far as the difference in the toxicity of the bioisosteres is concerned, only longer-term studies would determine whether the 1,2-dihydropyridines are safer than the thiazolidines.

Experimental protocols

Chemistry

Melting points were determined in a Büchi 535 melting point apparatus and are uncorrected. IR spectra were obtained using KBr discs on a Nicolet 20 DXC FT-IR spectrophotometer. UV spectra were recorded in ethanolic solutions on a Varian DMS-200 instrument. ^{1}H-NMR spectra were taken on a Varian EM-360 spectrometer using tetramethylsilane as internal standard. All new compounds were analysed for C, H, N, and the values found were within 0.4% of the theoretical values.

Table I. Physicochemical properties of 2-pyridinamine derivatives.

Compd	*X*	*Y*	*Method*	*Yield (%)*	*mp (°C)*	*Solvent* [a]	*Anal (C, H, N)*
3a	H	Br	**a**	53	147–148	A	$C_{13}H_{11}N_3$
	H	Br	**b**	53	147–148	A	
6a	H	Br	**a**	18	65–66	A	$C_{13}H_{11}N_3$
3b	2-CH_3	Br	**b**	66	152–154	B	$C_{14}H_{13}N_3$
3c	2-Cl	Cl	**b**	80	167–168	A	$C_{13}H_{10}ClN_3$
3d	4-Cl	Cl	**b**	56	168–170	C	$C_{13}H_{10}ClN_3$
3e	4-NO_2	Br	**b**	28	208–210	A	$C_{13}H_{10}N_4O_2$
3f	2,6-di-Cl	Br	**b**	39	219–220	A	$C_{13}H_9Cl_2N_3$
4a	H	Br	**a**	62	125–126	D	$C_{12}H_{11}N_3O_2$
7a.HCl	H	Br	**a**	12	115–116	D	$C_{12}H_{12}ClN_3O_2$
4b	2-F	Cl	**a**	29	165–166	A	$C_{12}H_{10}FN_3O_2$
4c	2-Cl	Cl	**a**	9	185–186	B	$C_{12}H_{10}ClN_3O_2$
4d	4-Cl	Cl	**a**	49	164–165	A	$C_{12}H_{10}ClN_3O_2$
4e	4-Br	Br	**a**	43	184–185	B	$C_{12}H_{10}BrN_3O_2$
4f	4-NO_2	Br	**a**	60	224–225	B	$C_{12}H_{10}N_4O_4$

[a]Recrystallization solvent: A = ethanol, B = acetonitrile, C = 2-propanol, D = ethyl acetate.

Table II. Spectral data of 2-pyridinamine derivatives.

Compd	*IR [ν (cm⁻¹)]*				*UV [λ_{max}(nm), ε]*		
	C≡N	*C=N*	*NO_2*				
3a	2160	1636			338 (7290)	262 (15 700)	
6a	2228					278 (4385)	231 (14353)
3b	2158	1640			336 (9270)	262 (19 300)	
3c	2163	1642			337 (6170)	262 (12 400)	
3d	2160	1642			337 (6820)	262 (14 300)	218 (15 000)
3e	2162	1640			338 (7940)	262 (25 700)	
3f	2161	1639			336 (8520)	263 (15 700)	
4a		1625	1563	1234	349 (18 400)	284 (5130)	
7a.HCl			1562	1267		260 (7060)	
4b		1626	1563	1237	349 (18 300)	283 (4920)	
4c		1626	1563	1241	350 (18 100)	283 (4620)	
4d		1633	1564	1232	349 (18 300)	284 (5000)	220 (18 900)
4e		1633	1565	1238	349 (18 300)	283 (5000)	
4f		1628	1565	1234	352 (15 800)	274 (11 600)	

*1-Benzyl-2-cyanimino-1,2-dihydropyridine **3a***

a. To a suspension of 12 g (0.1 mol) 2-cyanaminopyridine **5a** in 100 ml acetonitrile, 20 ml (0.114 mol) *N,N*-diisopropylethylamine and 12 ml (0.1 mol) benzyl bromide were added. The reaction mixture was refluxed for 4 h. The solvent was evaporated under reduced pressure, and the residue was triturated in succession with water and with ether, collecting by filtration the insoluble **3a** and keeping the filtrate.

^{1}H-NMR ($CDCl_3$): 5.28 (s, 2H), 6.50 (td, 1H), 7.32 (s, 5H), 7.30–7.65 (m, 3H).

From the ethereal filtrate the isomer **6** (X=H) was isolated by column chromatography on silica gel using a 95:5 mixture of chloroform/methanol as eluent.

^{1}H-NMR ($CDCl_3$): 5.00 (s, 2H), 6.70–7.80 (m, 8H), 8.3 (m, 1H).

b. To a solution of 6.0 g (0.050 mol) 2-cyanaminopyridine **5a** in 100 ml acetone 7.0 g (0.050 mol) K_2CO_3 and 6.5 ml (0.055 mol) benzyl bromide were added. The reaction mixture was vigorously stirred and refluxed for 2.5 h. The inorganic salts were filtered off, and the filtrate was evaporated under reduced pressure. The residue was triturated with 10 ml ether, then filtered off and washed with 5 ml ether. **3b–3f** were prepared by the same procedure.

c. To a suspension of 5.3 g (0.020 mol) 1-benzyl-2-aminopyridinium bromide (**8.HBr**) in 20 ml ether, 25 ml 1 N aqueous NaOH was added with stirring. After dissolution of the solid, the phases were separated and the aqueous layer was extracted with 20 ml ether. The combined organic extracts were dried on Na_2SO_4, concentrated to ≈ 20 ml volume, and treated with a solution of 1.1 g (0.010 mol) cyanogen bromide in 5 ml ether, added dropwise at room temperature. After 30 min stirring, the crystalline solid was filtered off, washed with ether and then water.

Yield: 0.91 g (44%) of **3a**; mp: 147–148°C (ethanol), found identical (mixed mp, TLC and IR spectra) with the compound prepared by procedure **a**.

*1-Benzyl-2-nitroimino-1,2-dihydropyridine **4a***

a. To a suspension of 14 g (0.1 mol) 2-nitroaminopyridine **5b** in 100 ml acetonitrile, 20 ml (0.114 mol) *N,N*-diisopropylethylamine and 12 ml (0.1 mol) benzyl bromide were added and the reaction mixture was refluxed for 2.5 h. The solvent was

Table III. Pharmacological properties of 2-pyridinamine derivatives.

Compd	*Shay test (acid output)*		*Robert test (haemorrhage)*	
	% Inhibition at 25 mg/kg po	*ED_{50} mg/kg po*	*% Inhibition at 10 mg/kg po*	*ED_{50} mg/kg po*
3a	98	3.3	42	10.0
6a	20		24	
3b	83	11.7	66	11.0
3c	87	1.8	16	
3d	69		20	
3e	36		17	
3f	21		0	
4a	71	13.6	0	
7a.HCl	0		41	
4b	76		29	
4c	52		33	
4d	45		16	
4e	17		33	
4f	14		32	
Cimetidine		50.0		200

evaporated under reduced pressure and the residue was triturated in succession with water and hexane. The insoluble **4a** was collected by filtration, leaving **7** (X=H) in the filtrate.

^{1}H-NMR ($CDCl_3$): 5.50 (s, 2H), 6.80 (td, 1H), 7.25 (s, 5H), 7.75 (td, 1H), 7.85 (dd, 1H), 8.30 (dd, 1H).

From the filtrate, the isomer **7** (X=H) was isolated by column chromatography on silica gel using a 2:1 mixture of toluene/acetone as eluent, followed by hydrochloride salt formation.

^{1}H-NMR (DMSO–d_6): 5.55 (s, 2H), 7.40 (s, 5H), 7.40 (td, 1H), 7.70 (d, 1H), 8.10 (td, 1H), 8.75 (dd, 1H). **4b–4f** were prepared by the same procedure.

Pharmacology

General procedures

Female RG Wistar rats weighing between 120–150 g were used. Before each experiment the animals were fasted for 24 h but received water *ad libitum*. The test compounds, which were suspended in 1–2 drops of Tween 80 and then diluted with physiological saline (0.9% w/v NaCl) solution, were administered orally (*po*) in a vol of 5 ml/kg body weight (bw) to groups of 5 rats. Doses for studying the 1,2-dihydropyridine antisecretory and cytoprotective activities were 25 mg/kg and 10 mg/kg, respectively. The control animals were treated with the vehicle and cimetidine was used as reference drug. Dose–response studies were performed only when antisecretory and/or cytoprotective effects raised our hopes of obtaining reasonably low ED_{50}s.

Statistics

Antisecretory and cytoprotective effects are expressed as differences in percentage between the control and the treated group values. For statistical evaluation, the Student's paired *t*-test was used. A $p < 0.05$ was considered significant.

Effect on gastric secretion

The antisecretory activity was examined in pylorus-ligated rats as described by Shay *et al* [1]. Test compounds were administered 30 min before pylorus ligation. Animals were killed 4 h after surgery and gastric contents collected. The volume and acid output of the gastric juice were measured. ED_{50} values were defined as the drug concentrations needed to decrease the gastric secretion by 50%.

Cytoprotection against acidified ethanol

Cytoprotective effect was studied by a slightly modified method of Robert [2]. Acidified ethanol (a mixture of 1 ml concentrated HCl and 50 ml absolute ethanol) given intragastrically was used as a strong irritant in a dose of 5 mg/kg bw. The test compound was administered orally 30 min before the acidified ethanol challenge. One h after the exposure to the irritant the animals were killed and the longitudinal haemorrhagic lesions on the stomach were assayed by length. The ED_{50} values were defined as the drug concentration that gave 50 % decrease in the ulcer index which was the mean value in mm of the measured lesions.

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