3. Xerogel dressings preparation

The dressings [3] were prepared by dissolving Eudragit E and methylcellulose in a mixture of acetone and water with the addition of 1,2-propylene glycol. The obtained mixture was used to prepare xerogels by evaporating the solvent on a glass plate covered with white wax. The composition of the dressing is included in the chart.

4. Measurement of xerogel dressings adhesiveness

The adhesiveness was determined with Műnzel apparatus [3]. Xerogel dressings swelled in water were placed between the plates of the apparatus and the power necessary to separate them was measured in g.

5. Measurement of xerogel dressings wash out rate

5.1. In vitro wash out

The speed of dressings wash out in water and in artificial gastric juice was measured with a pharmacopeal apparatus for tests of therapeutic substances in pills [3].

5.2. In vivo wash out

Dressings were placed on gums after a meal and their performance was observed visually. The exact time of their wash out is difficult to determine thus the results may only be given approximately.

6. Measurement of Kunitz' protease inhibitor liberation rate

The measurements were carried out with the apparatus of Olszewski and Kubis [1], designed for measuring drug liberation rate from ointments. For 180 min, 2 ml portions of eluate were sampled in 30 min intervals. Amounts of inhibitor liberated were determined spectrophometrically by measurement of absorbance of particular eluates at 260 nm, with water as the reference.

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Activity of calvatic acid and its analogs against *Helicobacter pylori*

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Calvatic acid is an interesting antibiotic with a diazene N-oxide structure [1, 2]. Many of its derivatives display potent antibacterial, antifungal and antitumor properties [2–6]. In this communication we report the results of a study showing that calvatic acid and its analogues can display potent antimicrobial activity against *Helicobacter pylori*, the microorganism associated with a variety of gastric disorders and particularly with gastric ulcers [7]. *H. pylori* infections are usually treated using double or triple-therapy based on the combination of broad-spectrum antibiotics with inhibitors of acid secretion, such as H_2-antagonists or proton pump inhibitors. Several problems are associated with this complex therapy and great interest is devoted to novel agents suitable for a single-therapy treatment [8].

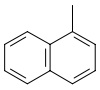
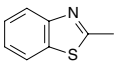
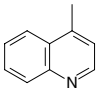
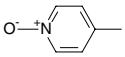
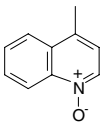
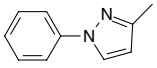
Calvatic acid and its analogues were evaluated for their antimicrobial activity against 19 clinical and NCTC 11637 *H. pylori* strains. Two of the used strains (NCTC 11637 and 102R) were metronidazole resistant. Metronidazole was taken as reference. The minimal inhibitory concentrations MIC_{50} and MIC_{90}, namely the minimal concentration able to inhibit 50% and 90% of the used strains respectively, were evaluated using the agar dilution method. The results are reported in the Table.

Calvatic acid (**1**) triggers potent inhibitory action against all strains tested, including the metronidazole resistant ones. The values of MIC_{50} and MIC_{90} are close and about 15 and 250 times, respectively, lower than those of their reference values. When the experiments were repeated in the presence of variable concentrations of cysteine, a strong decrease or disappearance of the activity was observed, depending on the amount of cysteine used. Such behaviour is in keeping with the known ability of **1** to react in physiological conditions with −SH groups [9, 10]. This reactivity, which was thought to be responsible for a number of biological properties of the antibiotic, could be also involved in its antimicrobial response. Action mechanism apart, the active form of calvatic acid, which is a rather strong acid with pKa = 3.2 [2], should be the ionised one. The small dimension and high polarity of this form should allow its permeation through the pores of the microorganisms membrane.

Shift of the −COOH group either to *m*- or to *o*-position reduces the activity, but the level of the antimicrobial action remains high, in particular in the *m*-analogue **2**. When other groups with different physicochemical properties are substituted for the −COOH in **1**, a modification of the anti *H. pylori* activity occurs. The most active compounds we found are the unsubstituted product **4** and the *m*-CH_3 derivative **9** which appear to be about 4 times more active than calvatic acid. The products are very active also against the two metronidazole resistant strains. A relevant decrease in the antimicrobial action occurs when the electron attracting substituents −CN and $-NO_2$. (**10**, **11**) are inserted in *p*-position.

Derivatives obtained by modification of −N(O)=NCN function are less active than calvatic acid. In fact when in

Table 1: Minimal inhibitory concentrations (MIC) in μg/ml against 20 *H. pylori* strains, including two metronidazole resistant strains

Compd.	$R-Ar-\overset{(O)_n}{\overset{\uparrow}{N}}=N-X$				MIC (μg/ml)				
					All strains			Metronidazole resistant	
	R	Ar	n	X	Range	MIC_{50}	MIC_{90}	NCTC 11637	102R
1	*p*-COOH	Ph	1	−CN	<0.0039–0.062	0.016	0.031	0.0078	0.031
2	*m*-COOH	Ph	1	−CN	0.25–1	0.5	0.5	0.25	1
3	*o*-COOH	Ph	1	−CN	0.5–8	4	4	2	8
4	*p*-H	Ph	1	−CN	<0.0039–0.125	0.0039	0.0078	<0.0039	0.0078
5	*m*-CN	Ph	1	−CN	<0.0039–0.5	0.0039	0.016	0.0039	0.016
6	*m*-NO_2	Ph	1	−CN	0.031–1	0.062	0.125	0.062	0.125
7	*m*-Cl	Ph	1	−CN	<0.0039–0.125	0.0039	0.125	<0.0039	<0.0039
8	*m*-CF_3	Ph	1	−CN	<0.0039–0.062	0.016	0.062	0.062	0.031
9	*m*-CH_3	Ph	1	−CN	<0.0039–0.0078	0.0078	0.0078	0.062	0.0078
10	*p*-CN	Ph	1	−CN	0.062–2	0.5	2	2	1
11	*p*-NO_2	Ph	1	−CN	0.25–8	4	8	8	8
12	*p*-Cl	Ph	1	−CN	<0.0039–0.25	0.0078	0.031	<0.0039	0.016
13	*p*-OCH_3	Ph	1	−CN	0.0078–0.125	0.016	0.031	0.0078	0.031
14	*p*-CH_3	Ph	1	−CN	<0.0039–0.125	0.016	0.031	0.016	0.031
15	*p*-$N(CH_3)_2$	Ph	1	−CN	<0.0039–0.125	0.016	0.016	0.016	0.0078
16	*p*-COOH	Ph	0	−CN	2–16	16	16	16	16
17	*p*-COOH	Ph	1	−Ph	1–8	4	8	8	8
18	*p*-COOH	Ph	1	−$CONH_2$	4–>32	32	>32	>32	>32
19	*p*-COOH	Ph	1	−COOEt	2–16	8	16	8	16
20	*p*-COOH	Ph	1	−SO_2Ph	8–32	16	16	16	32
21	*p*-H	Ph	1	−$CONH_2$	8–>32	>32	>32	>32	>32
22	*p*-H	Ph	1	−COOEt	16–>32	16	>32	>32	32
23	*p*-H	Ph	1	−SO_2Ph	<0.0039–0.25	0.125	0.25	32	0.125
24			1	−CN	0.0078–0.062	0.0078	0.062	0.062	0.031
25			1	−CN	4–32	4	32	16	16
26			1	−CN	16–>32	32	>32	32	>32
27			1	−CN	2–32	16	32	16	16
28			1	−CN	0.25–8	2	8	2	4
29			1	−CN	16–16	16	16	16	16
30			1	−CN	0.016–>32	2	>32	4	2
31			1	−CN	<0.0039–0.25	0.031	0.25	0.125	0.031
Metronidazole					0.031–16	0.25	8	16	8

The MIC of all compounds including metronidazole, are reported in equimolar concentrations to **1** (see experimental part).

the antibiotic **1** the oxygen of the N → O moiety is suppressed (**16**) a strong decrease in the activity occurred. Decrease in activity was also observed as a consequence of the substitution in **1** and **4** of C_6H_5, $CONH_2$, COOEt, $SO_2C_6H_5$ groups for the CN one (**17**–**23**). The only product that retains a good level of antimicrobial action is the unsubstituted benzenesulfonyl analogue **23**. These findings indicate that the −N(O)=NCN function has a high degree of specificity in inducing anti-*H. pylori* activity.

Substitution of the naphthalene ring for the benzene one in **4** affords the potent anti-*H. pylori* agent **24**. By contrast the use of other ring system is detrimental for the activity. In fact, insertion of a −N(O)=NCN group at the 2-positions of pyridine, pyrimidine, benzothiazole, (**25, 26, 27**), 4-positions of quinoline, pyridine N-oxide, quinoline N-oxide (**28, 29, 30**), decreases the activity. Among the heterocyclic system used, only 1-phenylpyrazol-3-yl is appropriate for the generation of a potent anti *H. pylori* agent (**31**).

Preliminary results of the *in vivo* activity determination show that calvatic acid **1** is able to induce 67% *H. felis* elimination rate by treating infected mice (n = 3) with a standard dose (50 mg/kg, t.i.d., by gavage) for 4 days. Elimination rate was determined 24 h after the last admin-

istration by a urease assay. However, compound **1** after oral administration (50 mg/kg, t.i.d., by gavage) for 4 days was not effective in eradicating *H. felis* from the mouse stomach, as demonstrated by an urease biopsy test. Under the same experimental conditions, metronidazole displayed a rate of 100% in elimination and eradication of *H. felis* when administered at 10 mg/kg and 50 mg/kg, respectively.

The *in vivo* activity determination of the most representative compounds of the series is in progress.

Experimental

1. Chemistry

Compounds **1** [5]**, 2**, **3**, **18–20** [5], **4**, **12**, **15**, **22, 23**, **25, 31** [11], **5**, **8–10**, **14** [12], **6**, **7**, **11**, **13** [3], **16** [10], **21, 24, 26, 28–30** [6], **17** [13] were synthesised according to methods reported in literature. The new product **27** (m.p. 171–172 °C) was synthesised from the corresponding 2-nitrosobenzothiazole according to the general procedure we described in a previous paper [11].

2. Antibacterial activity

MICs of all the compounds and of metronidazole taken as reference were determined using the agar dilution method according to the procedure previously reported [14]. The substances were dissolved in dimethylsulfoxide (DMSO) and serial double dilutions were performed for calvatic acid **1** ranging from 128 to 0.0039 μg/ml. All remaining compounds including metronidazole, employed in equimolar concentrations to **1**, were diluted in agar medium in serial double dilutions.

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