contributed to the dramatic T1 relaxivity changes. Gd–DTPA alone did not provide any meaningful enhancement of the signal, even when a 600-fold excess was used in the experiment.

In conclusion, we have developed new types of imaging probes that have the potential to be used for in vivo imaging of blood coagulation. The specific peptide derived from α2AP labeled with various reporter groups can be covalently attached to fibrin by blood coagulation factor FX13 through transglutamination. Theoretically, the same approach could be applied to other transglutaminases, which are widely found in generic tissue stabilization and also contribute to a variety of diseases, such as cancer, neurodegenerative diseases, and celiac disease.[17, 18]

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A New Furoxan NO-Donor Rabeprazole Derivative and Related Compounds

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drug design · furoxans · medicinal chemistry · nitric oxide · rabeprazole

The design of hybrid molecules by combining appropriate pharmacophoric groups with NO-releasing moieties is a promising approach to the production of new drugs with interesting potential for treating a variety of diseases.[1–3] Our research group has been active in this field and we have designed several such products.[1] These compounds include NO-donor nonsteroidal antiinflammatory drugs (NSAIDs)[4, 5] and NO-donor H_2-receptor antagonists.[6] We pursued this line because nitric oxide ($NO^{\bullet}$) has protective effects on the gastric mucosa through a number of mechanisms, such as promotion of mucus secretion, increased mucosal blood flow and decreased adherence of neutrophils to the gastric vascular endothelium.[7] In confirmation of this idea, we herein describe the new hybrid **12**, obtained by joining the 4-alkoxy-3-phenylsulfonylfuroxan substructure to rabeprazole (**1**; Scheme 1). It is known that furoxans are able to release NO at physiological pH, in the presence of thiol cofactors. The mechanism of this release appears to be complex and may

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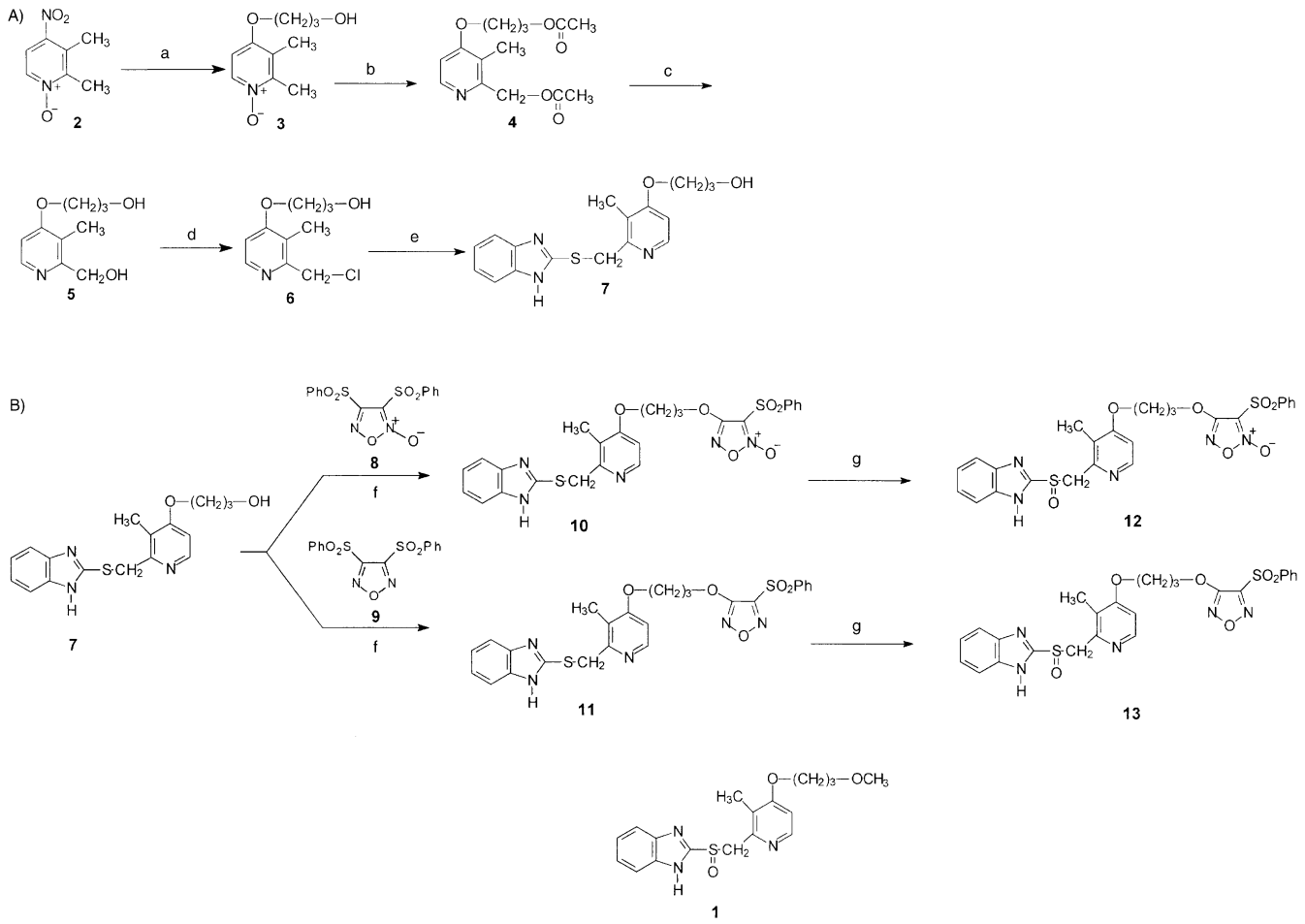

Scheme 1. *Synthesis of furoxan and furazan rabeprazole derivatives* ***12*** *and* ***13****, respectively. a) HO(CH$_2$)$_3$OH, K$_2$CO$_3$, 105 °C; b) (CH$_3$CO)$_2$O, 100 °C; c) NaOH, EtOH, H$_2$O, RT; d) CH$_2$Cl$_2$, SOCl$_2$, − 10 °C; e) EtOH, NaOH, 2-mercaptobenzimidazole; f) dry THF, 50% NaOH, RT; g) MCPBA, − 45 °C, CH$_2$Cl$_2$. THF, tetrahydrofuran; MCPBA,* m*-chloroperoxybenzoic acid.*

involve more than one redox form of NO.[8] Rabeprazole is a potent inhibitor of the H^+/K^+-ATPase enzyme (the gastric proton pump; ATP, adenosine triphosphate).[9, 10] Proton pump inhibitors (PPIs) have proved to be a major therapeutic advance in a range of acid – peptic diseases, which include gastric and duodenal ulcers, gastroesophageal reflux (GERD) and Zollinger – Ellison syndrome,[11] as well as in preventing NSAID-induced gastropathy[12] and in eradicating *Helicobacter pylori* as part of combination regimens.[13] Rabeprazole is one of the most recently marketed PPIs and it has been reported to have slightly different pharmacokinetics[14] to those of the other members of this group of compounds (higher pK_a, faster conversion in the activated form and faster dissociation from the enzyme). Like all other PPIs, rabeprazole displays reasonable anti-*H. pylori* activity and inhibits both bacterial urease activity and motility.[15, 16] It has recently been reported that nitric oxide in the gastric mucosa may influence conversion of *H. pylori* from the virulent spiral to the coccoid dormient form.[17] Thus, the hybrid **12** is an interesting model that may be expected to be endowed with both NO- and rabeprazole-dependent activities. We report herein the synthesis and in vivo antisecretory and gastroprotective properties of **12**, as well as those of the furazan analogue **13**, which is unable to release NO and was taken as a control. The anti-*H. pylori* activities of **12** and **13** and of their synthetic intermediate sulfides **10** and **11** are also discussed.

Benzimidazole derivative **7** was used as the starting material for the synthesis of the final furoxan and furazan products **12** and **13** (Scheme 1). Treatment of **7** dissolved in dry THF with bis(phenylsulfonyl)furoxan (**8**) or bis(phenylsulfonyl)furazan (**9**) at room temperature in the presence of aqueous 50% (w/w) NaOH afforded the expected sulfides **10** and **11**. It is known that, under similar conditions, **8** reacts with EtOH to give 4-ethoxy-3-(phenylsulfonyl)furoxan.[18] Based on this observation, **10** was expected to be the product of the reaction of **7** with **8**. ^{13}C NMR spectra confirm this hypothesis: the signals at 158.8 ppm and 110.3 ppm are characteristic for C(4) and C(3), respectively, in the 4-alkoxy-3-(phenylsulfonyl)-substituted furoxan ring. Sulfide derivatives **10** and **11** were oxidised by treatment with *m*-chloroperoxybenzoic acid to give the final products, which were isolated as sodium salts.

In vitro NO release from the two furoxans **10** and **12** was indirectly evaluated by detecting nitrite (Griess reaction) produced in buffered solution (pH 7.4) at 37 °C under the action of an excess of cysteine (% NO_2^- (mol mol^{-1}): **10**, 53 ± 1; **12**, 49 ± 2)

by following a procedure described elsewhere.[4] No nitrite production was observed in the absence of cysteine. The two derivatives displayed similar capacities to produce nitrite under the chosen experimental conditions.

The effect of the final derivatives **12** and **13**, and of rabeprazole (**1**; sodium salt, taken as a reference), on histamine-induced acid secretion was evaluated in vivo (intravenous (i.v.) administration) on anaesthetised rats with lumen-perfused stomachs. The results are reported as absolute values in microequiv HCl $kg^{-1} min^{-1}$. The inhibitory effect of the different compounds is expressed as percentage inhibition of the stimulated acid secretion (plateau response), arbitrarily taken as 100%. ID_{50} values (dose producing 50% of the maximum possible effect) were derived from the inhibitory dose–response curves and are reported with 95% confidence limits (CLs). The lumen-perfused stomach of the anaesthetised rat secreted acid spontaneously at rates of 0.2–2.0 μequiv HCl $kg^{-1} min^{-1}$. Histamine (20 μmol $kg^{-1} h^{-1}$) caused an increase in acid secretion, which reached a maximum after 60–80 min and remained constant over several hours. Intravenous injection of rabeprazole (**1**; sodium salt, 0.1–1 μmol kg^{-1}), compound **13** (1–10 μmol kg^{-1}) or compound **12** (1–30 μmol kg^{-1}), administered at the acid secretion plateau caused dose-dependent inhibition of the acid production induced by histamine. Compound **12** was approximately fifty times less potent than rabeprazole and three times less potent than **13** (Table 1 and Figure 1). Maximum inhibitory responses occurred within 40–60 min after drug injection (data not shown).

Table 1. *Effect of rabeprazole (**1**; sodium salt), **12** or **13** on histamine-induced acid secretion.*

	ID_{50} [μmol kg^{-1}]	(CL 95%)
1	0.15	(0.07–0.30)
12	6.83	(0.66–7.07)
13	2.07	(0.81–7.07)

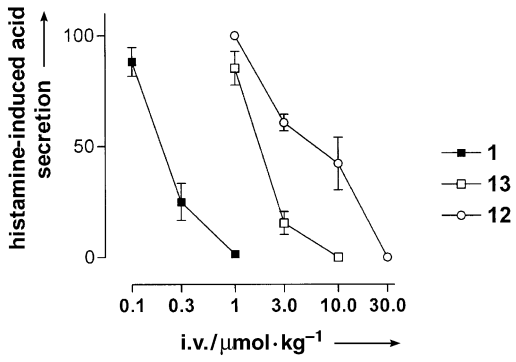

Figure 1. *Effects of intravenous (i.v.) injections of rabeprazole (**1**; sodium salt; ■), **13** (□) or **12** (○) on the gastric-acid-secretion plateau reached with histamine (20 μmol $kg^{-1} h^{-1}$) stimulation, arbitrarily taken as 100. In control experiments the drug vehicle (dimethylsulfoxide) was administered alone. The values plotted are the mean values ± standard error of the mean (SEM) measured on 6–8 rats.*

Results obtained for the acid secretion model confirm the antisecretory activity of rabeprazole already observed in previous studies.[9] Substitution of the methyl group in the rabeprazole molecule with a furazan or furoxan moiety significantly reduced the antisecretory potency of the molecule but not its efficacy. ID_{50} values for compounds **13** and **12** were between 15 and 45 times higher, respectively, than that for rabeprazole (Table 1). The reduction of potency seems to be greater for the furoxan derivative than in the furazan, which suggests that the NO-donor property could further impair the antisecretory activity of the proton pump inhibitor. Data reported in the literature concerning the effects of NO on acid secretion are contradictory: some studies on rats[19] have reported inhibitory effects for NO donors whereas stimulatory effects have been observed in isolated mouse stomach.[20]

The gastroprotective activity of rabeprazole (**1**; sodium salt) and the related compounds **12** and **13** against lesions induced by indomethacin was evaluated in conscious rats (intragastric (i.g.) administration). Indomethacin produced multiple hemorrhagic lesions in the glandular portion of the stomach, with a lesion index (see the Supporting Information for details) of 41.9 ± 5.7 (Figure 2). Intragastric administration of rabeprazole (**1**; sodium salt, 3–30 μmol kg^{-1}) significantly reduced the lesion index and gave a maximum inhibition of 75% (Figure 2). The

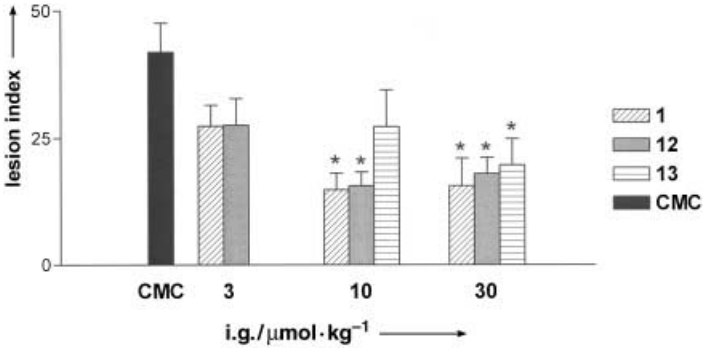

Figure 2. *Effect of intragastric (i.g.) administration of rabeprazole (**1**; sodium salt, 3–30 μmol kg^{-1}), **12** (3–30 μmol kg^{-1}) or **13** (10–30 μmol kg^{-1}) on gastric mucosal lesions induced by indomethacin (20 mg kg^{-1} i.g.) in conscious rats. Control rats received vehicle (1% carboxymethyl cellulose, CMC) in equivalent volumes. The values plotted are the mean values ± SEM measured on 7–12 rats. *, $P < 0.05$ with respect to the control group; determined by one-way analysis of variance (ANOVA) followed by a Newman–Keuls test.*

rabeprazole analogue endowed with NO-donor properties (Compound **12**) induced similar gastroprotective activity at equivalent doses (3–30 μmol kg^{-1}); in contrast, the furazan analogue (Compound **13**), which does not release NO, only induced a significant reduction of acute damage by indomethacin at doses three times higher than those required for the other two compounds (Figure 2). While different in potency, the compounds were similar in the magnitude of the protective effect exerted.

The gastroprotective experiments indicate that rabeprazole can prevent the damaging effect of indomethacin and behaves similarly to other proton pump inhibitors such as omeprazole

and lansoprazole.[21] Rabeprazole was recently reported to have protective activity against ethanol-induced gastric mucosal damage, produced by an NO-mediated increase in mucin content.[22] The furazan analogue of rabeprazole, **13**, was less potent in preventing indomethacin-induced lesions than the parent drug, possibly because of the lower antisecretory activity of **13**; it is well known that NSAID-induced gastric lesions are highly dependent on the presence of acid in the gastric mucosa.[23] In contrast, the NO-donor analogue showed gastroprotective activity at doses similar to those needed with rabeprazole, while being approximately 50 times less active as a gastric antisecretagogue. This result suggests the NO-donor property of **12** might contribute to its protective effect on the gastric mucosa.

The anti-*H. pylori* activity of the final products **12** and **13**, the intermediates **10** and **11**, and of rabeprazole (**1**; sodium salt) was evaluated in vitro against 14 clinical strains of *H. pylori* and the reference strain NCTC 11637. Five of these strains were resistant to metronidazole, which was taken as a reference compound. The minimal inhibitory concentrations (MICs) were determined for each compound by using the agar dilution method. From these values, MIC_{50} and MIC_{90}, namely the minimal concentrations inhibiting 50% and 90% of the strains used, were calculated. MICs were expressed as $\mu g\,mL^{-1}$ metronidazole equivalent.

Table 2 shows that all the derivatives tested display an inhibitory activity against *H. pylori* ranging from potent to moderate. The compounds can be ranked according to this activity: **10** $\approx$ **12** $>$ rabeprazole $>$ **13** $\geq$ **11** $>$ metronidazole. Interestingly, all the synthesised compounds are active against all the metronidazole-resistant strains tested, in particular the two furoxans **10** and **12** (Table 3). The anti-*H. pylori* properties of rabeprazole are thus potentiated in its furoxan analogues **12** and **10** (a potential prodrug of **12**) and slightly decreased in the corresponding furazans **13** and **11** (a potential prodrug of **13**). Additional interest in these derivatives stems from the fact that *H. pylori* seropositivity appears to be an independent risk factor for stroke of atherothrombotic origin,[24] and from the antiaggregatory and vasodilating properties of furoxans.

Table 2. *MICs needed to inhibit 50% (MIC_{50}) and 90% (MIC_{90}) of all the* H. pylori *strains considered.*

	MIC [$\mu g\,mL^{-1}$]		
	Range	MIC_{50}	MIC_{90}
metronidazole	0.5 – 32	1	> 32
10	< 0.0039 – 0.125	0.031	0.062
11	1 – 8	2	4
12	< 0.0039 – 0.125	0.015	0.125
13	0.25 – 2	0.5	2
1 (sodium salt)	0.062 – 0.5	0.125	0.5

Table 3. *MICs of rabeprazole **1** (sodium salt) and its analogues **10 – 13** against metronidazole-resistant strains.*

metronidazole resistant strain	MIC [$\mu g\,mL^{-1}$] metronidazole	**1**	**10**	**11**	**12**	**13**
NCTC 11637	> 32	0.5	0.062	8	0.062	2
107 R	16	0.125	0.062	4	0.125	1
VILLA R	> 32	0.125	0.015	2	0.015	2
77	> 32	0.125	0.125	4	0.125	1
110 R	> 32	0.125	0.0078	2	< 0.0039	1

In conclusion, rabeprazole possesses both gastric antisecretory and protective activity against damaging agents, as already observed with other pump inhibitors.[21] In addition, this compound is a potent anti-*H. pylori* agent and acts against a number of metronidazole-resistant strains. Chemical modification of the rabeprazole moiety by introduction of a furazan or furoxan group significantly reduces its antisecretory potency but not its efficacy; however, addition of the furoxan group, which releases NO, gives the molecule a protective activity comparable to that of the parent rabeprazole. This effect could lead to a better balance between antisecretory (PPI-dependent) and protective (NO-dependent) activities in the hybrid derivative that would favour gastroprotection over acid suppression.

In addition, the anti-*H. pylori* activity of these products against metronidazole-resistant strains makes them a new interesting class of rabeprazole derivatives worthy of further development.

The syntheses of all the products described in this work and the optimisation of the preparation of intermediates **2** – **7**, which is only described in patent literatures, as well as the procedures for the biochemical and pharmacological characterisation of the rabeprazole derivatives **10** – **13** are discussed in the Supporting Information.

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Aminoacyl Adenylate Substrate Analogues for the Inhibition of Adenylation Domains of Nonribosomal Peptide Synthetases

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adenylation domain · enzymes · inhibitors · nonribosomal peptide synthetases · synthetases

Microorganisms are well-known producers of bioactive peptides, which have become important elements of modern medicine. The sphere of activity of these compounds is vast and ranges from antibiotic to immunosuppressive, cytostatic to toxic effects. For instance, among these peptides are important antimicrobial agents such as the heptapeptide vancomycin, and immunosuppressive substances such as the cyclic undecapeptide cyclosporine.[1, 2] Both of these compounds are produced by nonribosomal peptide synthetases (NRPS), which are complex enzymes organized in modules that catalyze multistep reactions.[3, 4] A module needed for the incorporation of one amino acid into the final product comprises an adenylation (A) domain for substrate recognition, a 4′-phosphopantetheine-dependent carrier protein that holds the activated substrate and the growing chain, and a condensation (C) domain for peptide bond formation. Additionally, the activated substrate may also be altered by NRPS-associated optional domains that epimerize, methylate, or *N*-formylate, or by postsynthetic enzymes that glycosylate or halogenate the substrate; these alterations make the final products not easily accessible through chemical synthesis.[5] A domains activate, in addition to proteinogenic amino acids, a number of nonproteinogenic amino acids as well as carboxy acid. These domains specifically activate one substrate as the corresponding acyl adenylate at the expense of adenosine triphosphate (ATP).[6–8] Two crystal structures of A domains have been solved. The crystal structure of the gramicidin S-synthetase A (GrsA) phenylalanine-activating A domain from *Bacillus brevis* led to the elucidation of the so-called "nonribosomal code," which allows the prediction of the specificity of an A domain.[9, 10] This prediction was limited to amino-acid-activating A domains until the structure of dihydroxybenzoate-adenosine monophosphate ligase (DhbE) was solved recently, which further extended the code to include carboxy-acid-activating A domains.[11] Although the NRPS A domains exhibit the same enzymatic activity as other adenylate-forming enzymes, such as aminoacyl tRNA synthetases of ribosomal protein synthesis, these enzymes are not related on either the sequence or the structural level.[12–15] Instead, A domains are structurally related to the luciferase from *Photinus pyralis*.[16] We present herein the application of the first A domain inhibitors. Not only did these acyl adenylate analogues prove to be excellent inhibitors, their modification with linkers, which allows binding to a matrix, enlarges the number of possible applications.

Aminoacyl tRNA synthetases have successfully been inhibited by the aminoacyl analogues 5′-*O*-[*N*-(aminoacyl)-sulfamoyl] adenosine.[17] For instance, the alanyl-tRNA-synthetase from *Escherichia coli* can be inhibited by the alanyl derivative of this compound.[18] Following this approach, we synthesized the corresponding 5′-*O*-[*N*-(phenylalanyl)-sulfamoyl] adenosine (**1**) and 5′-*O*-[*N*-(leucyl)-sulfamoyl] adenosine (**2**) to test whether these analogues also inhibit A domains based on the similarity in enzymatic activity of such domains. Two A domains were chosen to test the functionality of the analogues **1** and **2**. We chose the GrsA A domain (PheA) because it has been crystallized in the presence of the substrates adenosine monophosphate and phenylalanine, which yielded a detailed insight into the mechanism of binding and catalysis of this domain. The second A domain, LeuA, is the excised A domain of the surfactin synthetase C (SrfA-C) from *Bacillus subtilis*. The corresponding gene fragments of the A domains, namely *PheA* and *LeuA*, were cloned (Figure 1) and the proteins were produced as strep-tag II fusions in *E. coli* and subsequently purified by streptactin affinity chromatography.

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