Synthesis and Biological Activity of Photoactive Derivatives of Erythromycin

M. Angeles Arévalo, Francisco Tejedor, Florentino Polo, and J. P. G. Ballesta*

Centro de Biología Molecular, CSIC and UAM, Canto Blanco, 28049 Madrid, Spain. *Received May 31, 1988*

Five photoactive derivatives of erythromycin have been synthesized by linking to 9(*S*)-aminoerythromycin either an aryl azide or a *p*-nitrophenyl ether. One derivative is an amide formed by reaction with (5-azido-2-formylphenoxy)acetic acid. Three derivatives are also amides, synthesized with 4-(*p*-nitroguaiacoxy)butanoic acid as a photoreactive group either directly or by interposing an amino acid (glycine or tyrosine). The last derivative is the product of the aldehyde condensation of aminoerythromycin with 10-(*p*-nitroguaiacoxy)decanal. Two of these derivatives can easily be made radioactive for affinity labeling studies either by reduction with [^{3}H]borohydride (aryl azide derivative) or by ^{125}I iodination (4-(*p*-nitroguaiacoxy)tyrosyl derivative). Although affected to different extents, the five erythromycin derivatives are biologically active and bind to the erythromycin-specific site on the bacterial ribosomes. In addition, the introduction of these groups changes the erythromycin inhibition pattern of peptide bond model reactions.

The macrolide antibiotics that inhibit protein synthesis block ribosome activity by binding to the large ribosomal subunit.[1,2] However, when their mode of action is studied in detail, they can be divided in two groups showing clear differences in their inhibitory patterns. Thus, while most macrolides having a 14-atom lactone ring, like erythromycin, are poor inhibitors of most peptide bond model reactions, those with a 16-membered ring, like spiramycin, efficiently block these types of assays.[1,2]

An affinity labeling study of the ribosomal binding site of both types of antibiotics was initiated to analyze the structural reasons for this difference. A number of naturally occurring photoactive macrolides of the 16-membered ring group, such as carbomycin, niddamycin, and tylosin, have been used.[3-5] However, no intrinsically photoactive 14-membered macrolide antibiotic was available. Therefore, as part of this project, the synthesis of different photoactive erythromycin derivatives was approached by linking to the antibiotic molecule groups of broad reactivity, keeping in mind the possibility of reaction with proteins as well as nucleic acids.

The aromatic azides and the *p*-nitrophenol ethers[6,7] were chosen and have been linked to the erythromycin through hydrocarbon chains of different types. The derivatives were also designed considering the possibility of radioactive labeling of their molecules.

A number of reports have been published describing the preparation of different erythromycin derivatives, many of them obtained from erythromycylamine, an amino derivative of the drug.[8-10] The data obtained indicated that modifications in this group can be performed without drastically affecting the activity of the drug. In some cases the derivatives described were susceptible to photoactivation,[9] but no report has been published, to our knowledge, on their use of affinity labeling studies probably due to the difficulty in preparing radioactively labeled ones.

In this report, we describe the synthesis and biological activity of five photoactive derivatives of erythromycin, using erythromycylamine as a substrate. Four of them carry a *p*-nitrophenyl ether as a photoactive group while the fifth derivative carries an aromatic azide. All of them, although less efficient than the original drug, are biologically active and in some cases are able to inhibit model peptide bond forming reactions which are insensitive to erythromycin. Their use in affinity labeling experiments is the subject of a different report.[11]

Results

Synthesis of N-Substituted Erythromycin Derivatives. Five derivatives, compounds **5**, **7**, **9**, **10**, and **16** (Schemes I and II), have been prepared by linking two different photoreactive groups, namely a *p*-nitrophenyl ether and an aromatic azide, to erythromycylamine. The groups have been introduced by condensation with either the carboxylic acids or the aldehyde of the appropriate compound to yield an amide bond or a Schiff base at the amino group in position 9 of erythromycylamine as outlined in Schemes I and II. The joining chain has a different length and characteristics in the five derivatives going from a simple aliphatic chain in derivative **10** to a peptide chain, including an amino acid residue, in derivatives **7** and **9**.

Competition for Binding to Ribosomes. The affinity for the ribosomes as well as the binding specificity of the five erythromycin photoactive derivatives was tested by checking their capacity to compete with ^{14}C-labeled, unmodified drug for interaction with the particles. The results are summarized in Figure 1 and indicate that the introduction of the photoreactive group affects the affinity of erythromycin for the ribosomes to a different extent. Nonetheless, all the derivatives are still considerably active and their interaction with the particles seems to be specific, since all of them compete for binding with erythromycin. From the competition curves (Figure 1) it can be deduced that the ribosome affinity of compound **7**, the less active derivative, is reduced by less than 1 order while compound **10** shows approximately half the affinity of erythromycin.

Bacterial Growth Inhibition Activity of Derivatives. The capacity of the erythromycin derivatives to inhibit the growth of Gram-positive (*Bacillus subtilis*) and Gram-negative (*Escherichia coli*) bacteria was tested in liquid medium. As Figure 2 shows, the results correspond reasonably well with the affinity of the derivatives for the ribosome, although there are some discrepancies probably

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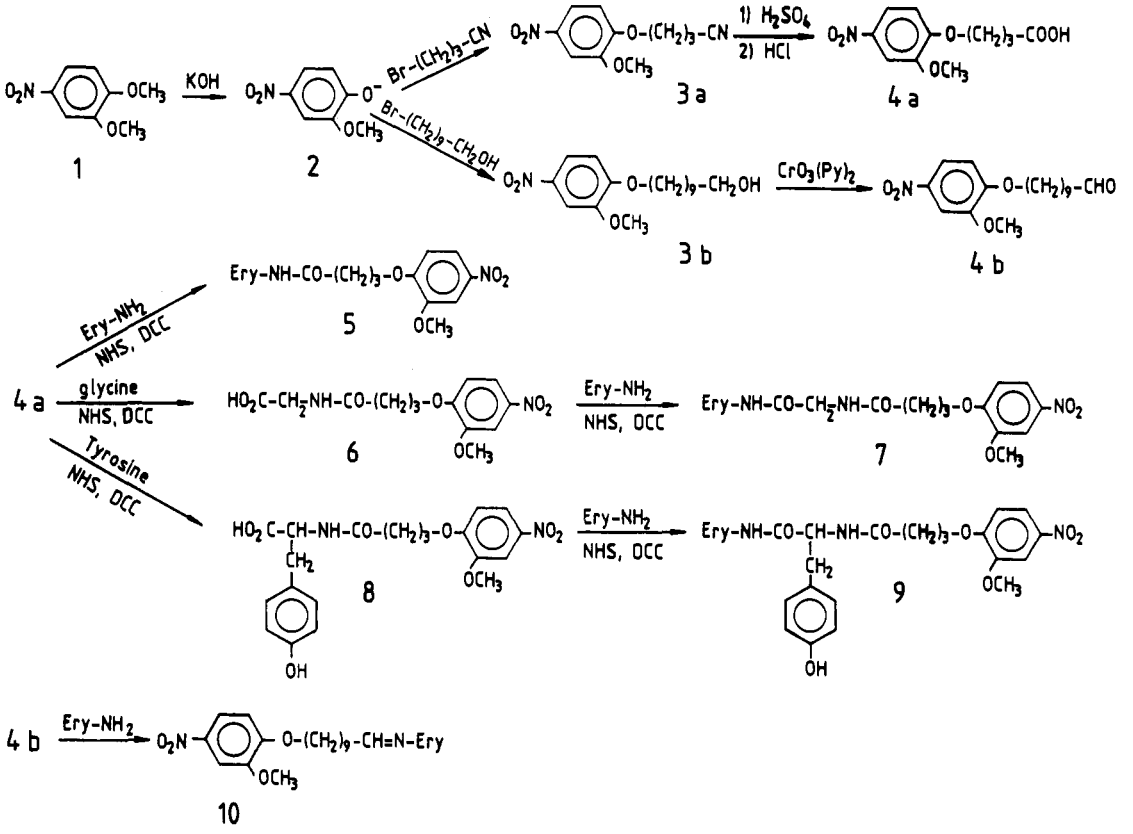
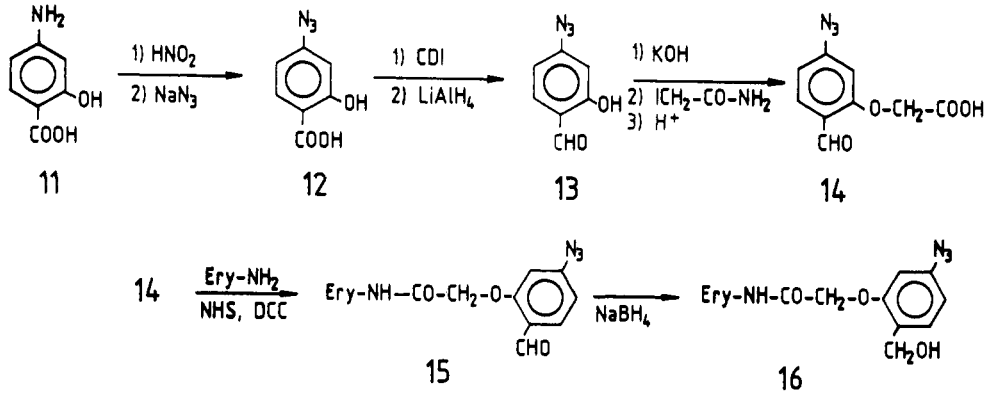
Scheme I



Scheme II



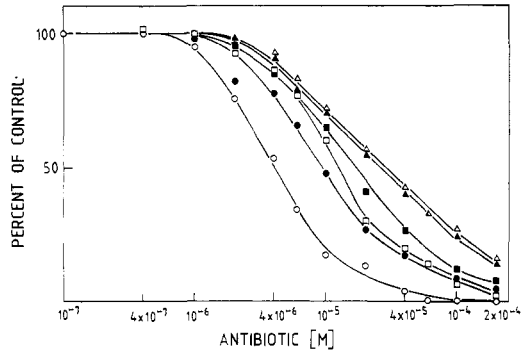


Figure 1. Competition of erythromycin derivatives with radioactive erythromycin for binding to bacterial ribosomes. 70S ribosomes from *E. coli* were incubated with [*N-methyl*-^{14}C]-erythromycin in the presence of an increasing concentration of unlabeled erythromycin (○), derivative **5** (▲), derivative **7** (△), derivative **9** (□), derivative **10** (●), and derivative **16** (■). The bound radioactivity was tested by filtration through nitrocellulose filters.

due to permeability problems caused by the presence of a rather bulky group in the modified drug molecules.

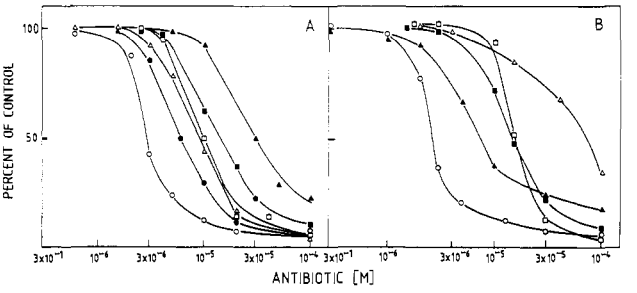


Figure 2. Inhibition of *E. coli* (A) and *B. subtilis* (B) growth in liquid cultures by erythromycin (○) and derivatives **5** (▲), **7** (△), **9** (□), **10** (○), and **16** (■).

Thus, in *B. subtilis* inhibition, derivative **5** is surprisingly active and derivative **16** is less active than expected, considering their respective competition curves (Figure 1). Similarly, derivative **7** inhibits *E. coli* growth more efficiently than its affinity for the ribosome seems to predict.

Inhibition of Peptide Bond Forming Model Reactions. Erythromycin is a poor inhibitor of most peptide bond forming model reactions.[1,2] This drug has no effect,

Table I. Inhibition of Peptide Bond Formation by Erythromycin Derivatives

antibiotic	inhibition: puromycin reaction 10^{-6} M	inhibition: puromycin reaction 10^{-5} M	inhibition: fragment reaction: 10^{-5} M
erythromycin	0	3	0
erythromycylamine	1	4	0
derivative **5**	13	25	11
derivative **7**	18	30	24
derivative **9**	18	27	15
derivative **10**	7	14	0
derivative **16**	15	23	0
spiramycin	53	85	48

at concentrations that otherwise inhibit protein synthesis, on the standard puromycin reaction and the "fragment reaction" using *N*-acetylphenylalanine-tRNA as substrate.[1,2] The photoactive derivatives inhibit these two reactions to a different extent, although they are less active than well-known inhibitors such as spiramycin (Table I).

Discussion

We have prepared five new photoactive derivatives of erythromycin, four of them carrying a *p*-nitrophenoxy group that has some advantages over other photoreactive radicals,[7] and another one having the more classical arylazido group.

Since our erythromycin derivatives were meant for affinity labeling studies, it was important to make them susceptible to easy radioactive labeling; something that is not possible in the case of other previously reported erythromycin analogues, most of which carry nitro arylazido groups that are difficult to label.[9] We have tried to resolve this problem by including in some of our derivatives groups that are easy to label. Thus, derivative **9** is susceptible to iodination in the tyrosine group present in the linking chain. Similarly, the alcohol in the aromatic azide ring of derivative **16** can be labeled by reduction of the aldehyde group present in the precursor compound with [^{3}H]borohydride (Scheme II). Derivative **7** can be labeled by using commercially available radioactive glycine in the synthesis process. In fact, our synthetic scheme allows the preparation of a variety of labeled erythromycin derivatives using the appropriate amino acids.

As reported for other erythromycin derivatives modified at the same position,[8,9] our modified drugs are biologically active, but to a lesser extent than the unmodified antibiotic. We have not found stimulation of the drug activity in any of our derivatives, as was reported in one case.[10]

Our results confirm, therefore, that the presence of rather large chemical residues in the atom 9 of the erythromycin lactone ring does not drastically hinder the activity of the drug. It is interesting to note that the least affected derivative is the one carrying the larger linking chain (**10**), suggesting that the proximity of the photoactive group to the lactone ring affects the drug activity. Supporting this is the fact that derivatives **5** and **16**, having shorter linking chains, are the less active in most biological tests. The proximity of modifying groups on the biological activity of an antibiotic has a similar effect in the case of streptomycin, too.[19]

Perhaps the most striking effect of the presence of a bulky group in the erythromycin derivatives studied is the change in their capacity to inhibit the peptide bond forming reactions.

Erythromycin is a poor inhibitor of the classical puromycin reaction and the so-called "fragment reaction" unless specific aminoacyl-tRNAs are used as a substrate.[1,21] However, the five derivatives block the reaction of puromycin with *N*-acetylphenylalanine-tRNA and three of them also affect the "fragment reaction". The failure of two derivatives to inhibit the last assay might be due to the presence of 33% ethanol in this reaction, which has been shown to affect the interaction of sparsomycin derivatives with the ribosomes differently, depending on the modifying group in their molecules.[18] It is interesting to note that the derivatives prepared are able to inhibit these erythromycin-insensitive model reactions. The inhibition of these reactions by the erythromycin derivatives is low compared with that of the typical peptide bond inhibitors, such as the 16-membered macrolide spiramycin. Considering, however, the lower affinity of these compounds for the ribosome, as shown by the erythromycin competition experiment, the effect produced on these reactions can be considered important.

The different mode of action of the 16- and 14-membered lactone macrolides have been explained on the basis of the different size of the sugar residues that modify the lactone ring in both cases.[21] Thus, while the 16-membered antibiotics carry a disaccharide, the erythromycin-like drugs have only monosaccharides. According to this hypothesis both antibiotics, although binding to the same ribosomal site, would affect the activity of the ribosome differently, according to the size of their lateral modifying groups. Our results, showing that the presence of a rather bulky lateral group affects the peptide bond inhibition pattern of the erythromycin derivatives, are compatible with this hypothesis, although the structural data available from affinity labeling studies indicate that at least some of the components of the ribosomal binding site of 16-membered[3-5] and 14-membered macrolides[11] are different.

Experimental Section

All the chemicals used were of analytical grade and were obtained from either Merck or Aldrich. Aminoerythromycin was a gift of Abbott Laboratories, North Chicago, IL, and Lilly Research Laboratories, Indianapolis, IN. [*N*-*methyl*-^{14}C]-erythromycin was a gift of Dr. R. E. McMahon (Lilly Research Laboratories). The IR spectra were obtained with a Perkin-Elmer Model 325 using KBr, the NMR spectra were recorded on a Bruker Model MW 360, and the visible–UV spectra were recorded on a Perkin-Elmer Model 554. Analyses were performed at the Organic Chemistry Department, Universidad Autónoma, with a Perkin-Elmer Elemental Analyzer 2400 CHN.

TLC was performed on precoated silica gel plates obtained from Merck with the following eluants: (1) ethyl acetate–ethanol (1:1), (2) ethyl acetate–ethanol (3:1), (3) ethyl acetate–hexane (1:1), (4) ethyl acetate–ethanol–25% NH_4OH (3:3:0.2), (5) ethyl acetate–ethanol–7% NH_4OH (3:3:0.5), (6) ethyl acetate–ethanol–7% NH_4OH (4:1:1), (7) ethyl acetate–ethanol–hexane (2:2:2), (8) ethyl acetate–methanol–25% NH_4OH (8:1:0.5), (9) ethyl acetate–methanol–7% NH_4OH (5:2:1), (10) $CHCl_3$–methanol–7% NH_4OH (2:2:1).

Synthesis of Erythromycin Derivatives. The five erythromycin derivatives were prepared from 9(*S*)-aminoerythromycin according to the reactions described in Schemes I and II.

I. Synthesis of Potassium 4-Nitroguaiacoxide (2). **2** was prepared according to Pollekoff.[12]

II. Synthesis of 4-(*p*-Nitroguaiacoxy)butanenitrile (3a). This compound was synthesized following Jelenc.[7]

III. Synthesis of 4-(*p*-Nitroguaiacoxy)butanoic Acid (4a). Compound **3a** (1 g) was dissolved in 5 mL of concentrated H_2SO_4 and left at room temperature for 48 h. NaOH (5 N) was added until the solution was slightly basic and the precipitated compound was collected by filtration, washed with cold water, and dried. This compound (1 g) was taken in 10 mL of 1 N HCl and heated under reflux for 15 h. The hot solution was filtered, and compound **4a** crystallized from it upon cooling. IR (KBr) in cm^{-1}: 3090, 3050

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(ar CH), 2800–2500 (COOH st), 1710 (C=O), 1510 (NO_2, as. st), 1345 (NO_2, sy st), 1270–1250 (ar COC-al, as. st). ^1H NMR (DMSO-d_6) δ (ppm): 12.07 (1 H, carboxylic); (aromatic ring) 7.78 (dd, 1 H, H-5, J_{35} = 2.6 Hz. J_{56} = 8.8 Hz), 7.63 (d, 1 H, H-3), 7.07 (d, 1 H, H-6); 4.03 (t, 2 H, OCH_2, $J_{\beta\gamma}$ = 6.3 Hz), 3.79 (s, 3 H, OCH_3), 1.91 (m, 2 H, H-β). UV (ethanol) in nm: 336 (ϵ 9775), 305 (ϵ 7200), 238 (ϵ 11 525), 211 (ϵ 12 500).

IV. Synthesis of 4-(Nitroguaiacoxy)erythromycin Derivatives 5, 7, and 9. The *N*-hydroxysuccinimide ester of **4a**, a common step for the synthesis of these derivatives, was prepared according to the method described by Anderson et al.[13]

IVa. Synthesis of 9(*S*)-[*N*-[4-(*p*-Nitroguaiacoxy)-butyryl]amino]erythromycin (5). The *N*-hydroxysuccinimide ester of **4a** (0.2 mmol) in 0.5 mL of ethyl ether was added to 50 mg of 9(*S*)-aminoerythromycin and the reaction was allowed to proceed at room temperature in the dark for 5 h. The extent of the reaction was checked by TLC in silica gel (eluant 3) and the product was purified by chromatography on a silica gel column using the same eluant. The purity of the compound was checked by TLC on silica gel using eluants 5 and 7. In both cases, a single ninhydrin-negative spot was detected by UV irradiation and H_2SO_4 treatment (R_f 0.22 and 0.32 in eluants 5 and 7, respectively). IR (KBr) in cm^{-1}: 3550–3300 (OH st, NH st), 1730 (C=O, lactone), 1655 (C=O st, amide I), 1510 (NO_2, as. st), 1335 (NO_2, sy st), 1270, 1250 (ar COC-al, as. st), 1180, 1170 (al COC-al, as. st). UV (ethanol): like compound **4a**. Anal. ($C_{48}H_{81}N_3O_{17}$) C, H, N.

IVb. Synthesis of 9(*S*)-[[*N*-[4-(*p*-Nitroguaiacoxy)-butyryl]glycyl]amino]erythromycin (7). The *N*-hydroxysuccinimide ester of **4a** (0.14 mmol) in 0.4 mL of 1,2-dimethoxyethane was mixed with 0.128 μmol of glycine in 0.1 mL of 5% $NaHCO_3$ and the mixture was allowed to react with continuous agitation in the dark at room temperature for 48 h. The reaction product was extracted with ethyl ether after acidification with trifluoroacetic acid, and the product was then dried with anhydrous Na_2SO_4 and evaporated. This product (10 mg, 61 μmol) and 7 mg (61 μmol) of *N*-hydroxysuccinimide in 0.5 mL of dimethoxyethane were added with strong agitation to 15.7 mg (76 μmol) of *N*,*N*-dicyclohexylcarbodiimide, and they were left at room temperature for 15 h with continuous shaking. After removal of the precipitated dicyclohexylurea by filtration, the solution was added to 39.3 mg (5.4 μmol) of 9(*S*)-aminoerythromycin and allowed to react with continuous agitation in the dark at room temperature for 10 h. The reaction product was purified by preparative TLC on silica gel, using eluant 4. The purity of the product was checked by TLC on silica gel. A single ninhydrin-negative spot was detected by UV irradiation and H_2SO_4 treatment (R_f 0.28 and 0.42 in eluant 4 and 10, respectively). IR (KBr) in cm^{-1}: 3600–3200 (OH st, NH st), 1730 (C=O, lactone), 1655, 1640 (C=O st, amide I), 1510 (NO_2, as. st), 1335 (NO_2, sy st), 1270, 1250 (ar COC-al, as. st), 1180, 1160, (al COC-al, as. st). UV (ethanol): like compound **4a**. Anal. ($C_{50}H_{84}N_4O_{18}$) C, H, N.

IVc. Synthesis of 9(*S*)-[[*N*-[4-(*p*-Nitroguaiacoxy)-butyryl]tyrosyl]amino]erythromycin (9). The *N*-hydroxysuccinimide ester of **4a** (0.3 mmol) in 0.8 mL of 1,2-dimethoxyethane was added to 0.2 mL of 5% $NaHCO_3$ containing 0.3 mmol of tyrosine, the mixture was left in the dark for 48 h with continuous agitation at room temperature, and the reaction product was extracted with ethyl ether after acidification with trifluoroacetic acid. This product (25 mg, 60 μmol) and 6.9 mg (60 μmol) of *N*-hydroxysuccinimide in 0.5 mL of 1,2-dimethoxyethane were added to 14.9 mg (72 μmol) of *N*,*N*-dicyclohexylcarbodiimide, and the reaction was allowed to proceed in the dark at room temperature for 48 h. After removal of the precipitated dicyclohexylurea, the filtrated solution was added to 39.7 mg (5.4 μmol) of 9(*S*)-aminoerythromycin and the mixture was allowed to react for 10 h at room temperature in the dark. The reaction was followed by TLC and the final product was purified by preparative TLC chromatography in silica gel using eluant 4. A chromatographically homogeneous product (28 mg), showing a single ninhydrin-negative, UV-absorbing, and H_2SO_4-sensitive spot by TLC, was obtained (R_f 0.35 and 0.49 in eluant 4 and 10, respectively). IR (KBr) in cm^{-1}: 3600–3200 (OH st, NH st), 1730 (C=O, lactone), 1655, 1645 (C=O st, amide I), 1510 (NO_2, as. st), 1335 (NO_2, sy st), 1270, 1255 (ar COC-al, as. st), 1175, 1155 (al COC-al as. st). UV (ethanol): like compound **4a**. Anal. ($C_{57}H_{90}N_4O_{19}$) C, H, N.

V. Synthesis of 10-(*p*-Nitroguaiacoxy)-1-decanol (3b). 10-Bromo-1-decanol (10 mmol) was added to 10 mmol of the 4-nitroguaiacol potassium salt, prepared as described,[14] in 15 mL of dimethylformamide with vigorous shaking. The mixture was heated at 120 °C for 3 h, resulting in the disappearance of the reddish color and the precipitation of KBr. The mixture was added slowly to 150 mL of 60 mM Na_2CO_3 with vigorous agitation and this was maintained for 1 h, resulting in the precipitation of a yellow compound that was collected by filtration and recrystallized from ethanol–water solutions. IR (KBr) in cm^{-1}: 3540 (OH st), 2920 (CH st); 2840 (CH st, alkoxy), 1585 (C=C st, ar), 1510 (NO_2, sy st), 1275, 1260 (ar COC-al, as. st). ^1H NMR ($CDCl_3$) in δ (ppm): (aromatic ring) 7.90 (dd, 1 H, H-5, $J_{3,5}$ = 2.6 Hz, $J_{5,6}$ = 8.8 Hz), 7.74 (d, 1 H, H-3), 6.89 (d, 1 H, H-6), 4.10 (t, 2 H, $PhOCH_2$, $J_{9,10}$ = 6.6 Hz), 3.95 (s, 3 H, OCH_3), 3.64 (t, 2 H, CH_2OH, 6.2 Hz), 1.88–1.31 (m, 16 H, alkyl chain). UV (ethanol): like **4a**.

VI. Synthesis of 10-(*p*-Nitroguaiacoxy)decanal (4b). Compound **3b** (5 mmol) in 2 mL of dichloromethane was added to a solution of 20 mmol of $CrO_3 \cdot (Py)_2$, prepared as described,[14] and shaken for 5 h at room temperature. The heavy precipitate of CrO_2 was filtered and washed with 5 mL of chloroform, and the filtered solution, together with the washings, were shaken in a decantation funnel first with 0.2 M carbonate buffer, pH 10, and then 0.5 N HCl and finally 0.2 M $NaCO_3H$ and water. Then, water traces were removed with Na_2SO_4 and the solution was finally evaporated under vacuum. The final product was then purified by chromatography with a silica gel column using ethyl acetate and hexane (1:1). IR (KBr) in cm^{-1}: 2920 (CH st), 2840 (CH st, alkoxy), 1720 (C=O st, aldehyde), 1585 (C=C st ar), 1510 (NO_2, as. st), 1350, 1335 (NO_2, sy st), 1275, 1260 (ar COC-al, as. st). ^1H NMR ($CDCl_3$) in δ (ppm): 9.76 (t, 1 H, C*H*O, *J* 1.8 Hz), (aromatic ring) 7.89 (dd, 1 H, H-5, $J_{3,5}$ = 2.6 Hz, $J_{5,6}$ = 8.8 Hz), 7.74 (d, 1 H, H-3), 6.89 (d, 1 H, H-6); 4.10 (t, 2 H, $PhOCH_2$, $J_{9,10}$ = 6.6 Hz), 3.94 (s, 3 H, OCH_3), 2.42 (m, 2 H, CH_2CHO), 1.878–1.311 (m, 14 H, alkyl chain). UV (ethanol): like **4a**.

VII. Synthesis of Derivative 9(*S*)-[*N*-[10-(*p*-Nitroguaiacoxy)decanylidene]amino]erythromycin (10). 9(*S*)-Aminoerythromycin (100 mg, 0.14 mmol) and 50.5 mg (0.15 mmol) of **4b** in 1 mL of 0.2% acetic acid in ethanol–chloroform (1:1) were allowed to react for 60 h at room temperature in the dark. The reaction was followed by TLC and the final product was purified by preparative TLC with silica gel using eluant 3. The isolated product was checked by TLC using eluants 1 and 9. In both systems a single ninhydrin-negative spot (R_f 0.17 and 0.54, respectively) was detected by UV and H_2SO_4 treatment. Anal. ($C_{54}H_{93}N_3O_{16}$) C, H, N.

VIII. Synthesis of 4-Azidosalicylic Acid (Compound 12). This compound was prepared according to the procedure of Smith and Brown.[15]

IX. Synthesis of 4-Azidosalicylaldehyde (13). Compound **13** was prepared as described previously by Maassen et al.[16]

X. Synthesis of (5-Azido-2-formylphenoxy)acetic Acid (14). NaOH (56 mg, 1 mmol) in 1 mL of water was added to 163 mg (1 mmol) of 4-azidosalicylaldehyde (**13**) dissolved in 25 mL of ethanol. The solution was dried under vacuum and the residue was dissolved, together with 370 mg (2 mmol) of iodoacetamide, in 3 mL of dimethylformamide. It was kept at 60 °C with continuous agitation for 1 h. Then, 20 mL of 25% HCl were added and the solution was heated at 100 °C for 30 min with agitation. After cooling, **14** crystallized as a red product chromatographically pure by TLC (R_f 0.25 in eluant 2). IR (KBr) in cm^{-1}: 3100–2500 (COOH st), 2100 ($N{=}N^+{=}N^-$, as. st), 1720 (C=O st, carboxylic acid), 1710 (C=O st, aldehyde), 1585 (C=C st, ar), 1270 (ar COC, as. st). UV (ethanol) in nm: 320 (aldehyde, ϵ 9000), 284 (azide, ϵ 15 600). ^1H NMR (DMSO-d_6), δ (ppm): 10.3 (s, 1 H, CHO), (aromatic ring) 7.7 (d, 1 H, H-3, $J_{3,4}$ = 9.0 Hz), 6.8 (m, 2 H, H-4, H-6); 4.9 (s, 2 H, OCH_2).

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XI. Synthesis of 9(*S*)-[*N*-[(5-Azido-2-formylphenoxy)acetyl]amino]erythromycin (15). (5-Azido-2-formylphenoxy)acetic acid (14) (11 mg) was mixed with 6 mg of *N*-hydroxysuccinimide and 11 mg of *N*,*N*-dicyclohexylcarbodiimide in 0.5 mL of 1,2-dimethoxyethane, and the solution was left in the dark for 3 h at room temperature and then at 0 °C overnight with continuous agitation. After removal of the precipitated dicyclohexylurea, 40 mg of 9(*S*)-aminoerythromycin in 0.2 mL of 1,2-dimethoxyethane was added and the reaction was allowed to proceed in the dark at 0 °C overnight. The extent of the reaction was followed by TLC using eluant 8 and the final product was purified by preparative TLC using the same eluant. The product gave a single ninhydrin-negative, UV-absorbing, and H_2SO_4-sensitive spot when checked by analytical TLC (R_f 0.36 in eluant 8 and 2). IR (KBr) in cm^{-1}: 3600–3100 (OH st, NH st); 2100 (N=N⁺=N⁻, as. st), 1730 (C=O st, lactone), 715 (C=O st, aldehyde), 1655 (C=O st, amide I), 1280 (ar COC, as. st), 1175–1160 (al COC-al, as. st). UV (ethanol): like 14.

XII. Reduction of 15 to 9(*S*)-[*N*-[[5-Azido-2-(hydroxymethyl)phenoxy]acetyl]amino]erythromycin (16). Derivative 15 (8.5 mg) in 0.2 mL of ethanol was made to react at 0 °C with 0.085 mL of $NaBH_4$ (2 mg/mL in ethanol), which was added drop by drop with continuous agitation followed by addition of 0.085 mL of ethanol. The solution was left at 0 °C and the reaction was followed by UV spectroscopy until the 320-nm aldehyde-absorbing band disappears. After 3 h, the reaction was over and 0.8 mL of 5% $NaCO_3H$, pH 8.8, was added and the mixture was kept in ice for 10 min. A chromatographically homogeneous product (R_f 0.41; eluant 8) was extracted with chloroform. Its UV spectrum showed the absence of the aldehyde band and the presence of azide absorption. Anal. ($C_{46}H_{77}N_5O_{15}$) C, H, N.

Biological Tests. Ribosomes were prepared from *E. coli* MRE600 following standard methods as previously reported.[3]

Binding and competition studies were carried out by filtration on nitrocellulose filters as described elsewhere.[17]

Basically, the ribosomes (1 mg/mL) in 60 mM NH_4Cl, 8 mM $MgCl_2$, 10 mM Tris-HCl, pH 7.4, and 4 mM β-mercaptoethanol were incubated at 30 °C for 15 min in the presence of 0.1 μM [^{14}C]erythromycin and then the competing antibiotic was added at the concentration indicated; after additional incubation for 20 min, the samples were filtered on nitrocellulose filters that were washed twice with 5 mL of the same buffer, dried, and counted. Growth inhibition was tested in 1 mL of culture growing exponentially at 37 °C, to which the appropriate concentration of antibiotic was added. After incubation for 1 h, growth was stopped by addition of 1 mL of 1 mg/mL sodium azide and the optical density of the culture (A_{600}) was checked.

The "puromycin reaction" and the "fragment reaction" were performed with [^{3}H]-*N*-acetylphenylalanine-tRNA as a substrate under the conditions described previously.[18]

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Registry No. **2**, 100200-99-5; **3a**, 121329-76-8; **3b**, 121329-82-6; **4a**, 121329-77-9; **4a** (*N*-hydroxysuccinimide ester), 111621-31-9; **4b**, 121329-83-7; **5**, 121329-78-0; **6**, 121329-79-1; **7**, 121329-80-4; **8**, 111621-32-0; **9**, 121329-81-5; **10**, 121329-84-8; **13**, 66761-28-2; 14, 85819-05-2; **15**, 121329-85-9; **16**, 121329-86-0; Ery-NH_2, 26116-56-3; H-Gly-OH, 56-40-6; H-Tyr-OH, 60-18-4; $Br(CH_2)_{10}OH$, 53463-68-6; ICH_2CONH_2, 144-48-9.

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Synthesis and Biological Activity of 3-Substituted Imidazo[1,2-*a*]pyridines as Antiulcer Agents

John E. Starrett, Jr.,* Thomas A. Montzka, Alfred R. Crosswell, and Robert L. Cavanagh

Pharmaceutical Research and Development Division, Bristol-Myers Company, 5 Research Parkway, Wallingford, Connecticut 06492. *Received February 7, 1989*

New imidazo[1,2-*a*]pyridines substituted at the 3-position have been synthesized as potential antisecretory and cytoprotective antiulcer agents. The synthetic routes began with cyclization of aminopyridines **5a,b** and chloro ketones **6a,b** to give imidazo[1,2-*a*]pyridines **7–9**. The side chain at the 3-position was elaborated to give primary amines **12a–c**, which were treated with either butoxyaminocyclobutenedione **13** or methoxyaminothiadiazole 1-oxide (**15**) to give **14a,b** and **16a–c**, respectively. Thiadiazole 1-oxides **16a–c** were converted to thiadiazoles **19a–c** in a two-step process which involved extrusion of the sulfoxide in **16a–c** to afford diimidamides **17a–c**, which were subsequently treated with thiobisphthalimide (**18**). None of the compounds displayed significant antisecretory activity in the gastric fistula rat model, but several demonstrated good cytoprotective properties in both the EtOH and HCl models. 8-(Benzyloxy)-3-[1-[[2-[(4-amino-1,2,5-thiadiazol-3-yl)amino]ethyl]thio]ethyl]-2-methylimidazo[1,2-*a*]pyridine (**19c**) showed comparable cytoprotective activity to SCH-28080 (**4**).

The commercial success of ranitidine (**1**) and cimetidine (**2**) (see Chart I) in the treatment of peptic ulcer disease has resulted in a search for other drugs to combat this commonly occurring malady. As a result of this effect, several new compounds have been identified which act via a number of different mechanisms of action. These mechanisms include inhibition of acid secretion (H_2-receptor antagonists, H^+/K^+ ATPase inhibitors, anticholinergics, and prostaglandins), cytoprotection (prostaglandins), and mucosal coating (sucralfate).[1] Most of these agents are reported to be active from a single mechanism

(1) For recent summaries on agents being used in the treatment of peptic ulcer disease, see: Garay, G. L.; Muchowski, J. M. *Annu. Rep. Med. Chem.* **1985**, *20*, 93 and Bauer, R. F.; Collins, P. W.; Jones, P. H. *Annu. Rep. Med. Chem.* **1987**, *22*, 191.

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