Eur J Med Chem (1997) 32, 995–1000

Short communication

Prodrugs – Part 2. Acylbenzoate esters of metronidazole†

K Bowden*, J Izadi

Department of Biological and Chemical Sciences, Central Campus, University of Essex, Wivenhoe Park, Colchester, Essex CO4 3SQ, UK

(Received 13 April 1997; accepted 21 July 1997)

Summary — The design and synthesis of a series of chain and cyclic acylbenzoate esters of metronidazole are described. The esters are designed to be both lipophilic and reactive in their hydrolysis reactions. The alkaline hydrolyses of the chain 2-acylbenzoates are relatively rapid, employing an intramolecular catalytic route, while the reactions of the 4-formylbenzoate and cyclic 2-formylbenzoate are also relatively rapid using the normal pathway. The anti-bacterial activity of the esters are comparable to that of metronidazole.

prodrugs / metronidazole

Introduction

Prodrugs have been designed as reversible derivatives of a drug to eliminate a variety of undesirable properties of the drug, such as poor absorption, unwanted side-effects, etc [2]. The linkage employed in forming a prodrug have been various, but the formation of esters has been common [3]. Esters can be hydrolysed either by enzymes or non-enzymatically to liberate the parent drug. Metronidazole (1-(2-hydroxyethyl)-2-methyl-5-nitroimidazole) **1** is an important drug, particularly useful as an anti-bacterial and protozoan agent [4]. There are problems relating to the low aqueous solubility, toxicity and poor absorption characteristics of the drug [5]. Certain esters and hemi-esters of metronidazole have been prepared [6]. However, phosphate and amino acid esters of metronidazole have been studied in order to increase aqueous solubility [7–9].

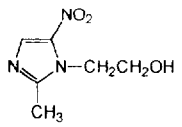
Ester hydrolysis can be facilitated to a remarkable and tuneable degree by intramolecular catalysis. Thus, neighbouring-group participation by suitably orientated carbonyl groups can expedite the alkaline hydrolysis of esters [10, 11]. Furthermore, the alkaline hydrolysis of many cyclic (pseudo) esters of formyl carboxylic acids are especially rapid due to the lactonic structure of these esters [12]. Esters of metronidazole can be designed to be relatively lipophilic and show markedly improved absorption if used, for instance, topically. However, esters of metronidazole studied previously [6] are relatively stable under physiological conditions and novel esters would be required to hydrolyse relatively rapidly under these conditions.

The present report comprises a study of the design, synthesis and hydrolysis of novel esters of metronidazole, together with preliminary studies of their biological activity.

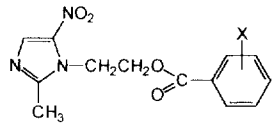
Design

Two series of metronidazole esters have been designed in which the ester function is intended to suffer relatively rapid alkaline hydrolysis. The first series is composed of acylbenzoate esters of metronidazole (**2b–g**). The 2-acylbenzoates (**2b**, **2d–g**) are all chain (normal) esters. The second series is composed of the cyclic (pseudo) 2-formylbenzoate esters **3**, ie, the 3-alkoxyphthalides. The esters **2b**, **2d–g** and **3b** are expected to hydrolyse relatively rapidly employing an intramolecular catalytic route [10], while the esters **2c** and **3a** should also hydrolyse relatively rapidly by a normal $B_{AC}2$ pathway [12]. The esters will also be considerably more lipophilic than metronidazole with a calculated increase in π, relative to metronidazole, of 2.13 (**2a**), 1.48 (**2b**,**c**), 3.38 (**2d**), 0.91 (**2e**), 1.48 (**2f**), 0.83 (**2g**), 1.83 (**3a**) and 1.18 (**3b**) [13]. Thus, they fulfil both features required in the design above.

†Part 1: see [1]
*Correspondence and reprints

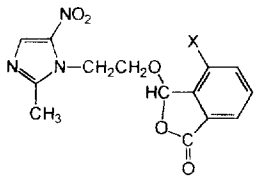


1



2

2a X = H
2b X = 2-CHO
2c X = 4-CHO
2d X = 2-C(O)C(O)Ph
2e X = 2-C(O)CH_3
2f X = 2-C(O)CF_3
2g X = 2,6-$(CHO)_2$



3

3a X = H
3b X = CHO

Synthesis

Two synthetic methods were employed in these studies. The first was a coupling process between the carboxylic acid and metronidazole using diethyl azodicarboxylate (DEAD) and triphenyl phosphine, see [14]. This was successfully employed for the synthesis of **2a–f**. The second was by conversion of the carboxylic acid into the acid chloride using thionyl chloride, followed by reaction of the acid chloride with metronidazole in the presence of triethylamine, see [15]. These gave mixtures of the chain (normal) and, mainly, the cyclic (pseudo) esters, **2b/3a** and **2g/3b**, which could be separated by chromatography and recrystallisation. The physical properties and methods of preparation of the esters of metronidazole are shown in table I.

Alkaline hydrolysis

The alkaline hydrolysis of all the esters resulted in the quantitative formation of metronidazole and the respective carboxylate anion. The reactions were found to be first-order both in the ester substrate and in the hydroxide anion. The rate coefficients, k_2, for the alkaline hydrolysis of the esters in 70% (v/v) dioxane–water at 30.0 °C and, for the less reactive esters, at 60.0 °C are shown in table II. The activation parameters are shown in table III. The latter have been obtained by a least-mean-squares treatment of log k_2 versus $1/T$ [16]. The acylbenzoate esters of metronidazole are all significantly more reactive than the reference benzoate ester **2a**. The 4-formylbenzoate **2c** and the cyclic 2-formylbenzoate **3a** have the increased reactivity expected from the electron-withdrawing formyl substituent and the lactonic carbonyl group, respectively. The relative rates and activation parameters for the latter two esters clearly indicate a normal $B_{AC}2$ pathway for alkaline hydrolysis [10]. However, those esters with a proximate acyl group have even faster rates of reaction. Two of the criteria noted for the observation of intramolecular catalysis by neighbouring keto or formyl groups can be employed in this study [10, 11]. The first is the rate enhancement above that expected for 'normal' unassisted ester hydrolysis. The latter has been estimated on the basis of the known polar and steric effects on the alkaline hydrolysis of esters [10]. The rate ratios, relative to that of the benzoate ester, can be corrected for the expected values to give the enhanced rate ratio, r_c. The latter for the benzoate esters studied here can be estimated to be ca 54 (**2b**), 37 (**2d**), 110 (**2e**), 22 (**2f**) and 420 (**2g**). The second is the combination of very small enthalpies of activation, $\Delta H^{\ddagger}$ and large negative entropies of activation, $\Delta S^{\ddagger}$, which appear to be associated with facile intramolecular catalysis by keto or formyl groups. The acylbenzoate esters **2e** and **2f** demonstrate this behaviour, as does the cyclic ester **3b**. Thus, it appears that the benzoate esters **2b**, **2d–g**, as well as the cyclic ester **3b**, react by addition of the hydroxide anion to the neighbouring carbonyl group, followed by intramolecular nucleophilic attack. The reaction pathway for hydrolysis involving intramolecular catalysis is shown in the scheme. From the solvent effect on the alkaline hydrolysis of methyl 2-acylbenzoates [17], it can be estimated that the rate of alkaline hydrolysis of the esters in water will be about twice that in 70% aqueous dioxane. Thus, at pH 7.3 in water at 37 °C, for **2g**, **2d**, **2b**, **2f**, and **2e**, $t_{1/2}$ for alkaline hydrolysis can be calculated to be ca 10 min, 3 h, 6 h, 15 h and 16 h, respectively, with all the other esters having $t_{1/2} > 100$ h.

Antibacterial activities

The antibacterial activities of the esters are shown in table IV, together with that of metronidazole. The activities of *all* the esters against a range of anaerobic

Table I. Preparation of 2-(2-methyl-5-nitroimidazol-1-yl)ethyl benzoates **2** and phthalides **3**.

No	*Subst*	*Method*[a]	*Mp (°C)*	*Recryst solvent*	*Anal*	*Ref*
Benzoates (**2**)						
2a	H	A	105[b]	Et_2O/CH_2Cl_2	C, H, N	[6]
2b	2-CHO	A	121–123	Et_2O	C, H, N	
2c	4-CHO	A	127–128	Et_2O/CH_2Cl_2	C, H, N	
2d	2-C(O)C(O)Ph	A	140–142	EtOAc	C, H, N	
2e	2-C(O)CH_3	A	118–120	Et_2O	C, H, N	
2f	2-C(O)CF_3	A	144–146	Et_2O/hexane	C, H, F, N	
2g	2,6-$(CHO)_2$	B	163	Et_2O/EtOAc	C, H, N	
Phthalides (**3**)						
3a	H	B	132	Et_2O/hexane	C, H, N	
3b	CHO	B	165–166	Et_2O/EtOAc	C, H, N	

[a]A,B = see *Experimental protocols* for details; [b]Lit: Mp 102 °C [6].

Table II. Rate coefficients (k_2) for the alkaline hydrolysis of 2-(2-methyl-5-nitroimidazol-1-yl)ethyl benzoates **2** and phthalides **3** in 70% (v/v) dioxane–water[a].

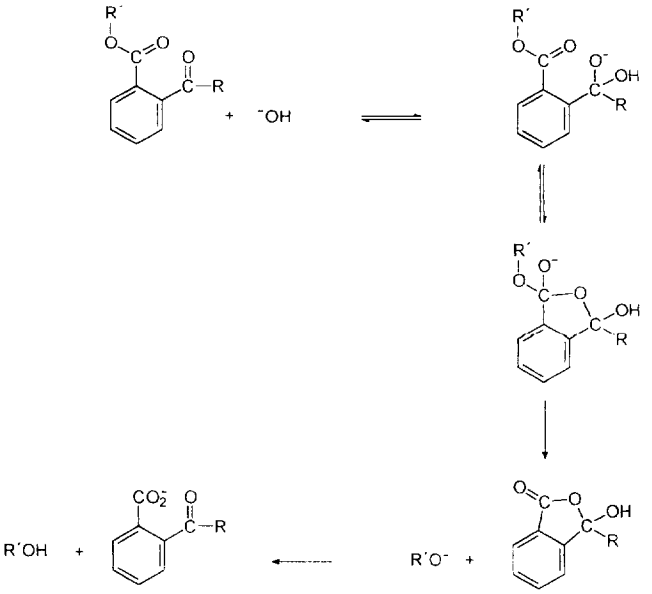
No	*Subst*	*k_2/dm^3 mol^{-1} s^{-1}*		*λ/nm*[b]
		at 30.0 °C	*at 60.0 °C*	
Benzoates (**2**)				
2a	H	0.0772	0.559	235
2b	2-CHO	20.8		233
2c	4-CHO	1.08	5.61	283
2d	2-C(O)C(O)Ph	42.6		300
2e	2-C(O)CH_3	8.25	17.9	240
2f	2-C(O)CF_3	8.54	19.1	232
2g	2,6-$(CHO)_2$	7.96		232
Phthalides (**3**)				
3a	H	0.900	5.82	257
3b	CHO	1.26	3.59	232

[a]Rate coefficients were reproducible to within ± 3%; [b]wavelength used to monitor alkaline hydrolysis.

Table III. Activation parameters for the alkaline hydrolysis of 2-(2-methyl-5-nitroimidazol-1-yl)ethyl benzoates **2** and phthalides **3** in 70% (v/v) dioxane–water at 30.0 °C[a].

No	*Subst*	$\Delta H^{\ddagger}$/ *kcal mol*$^{-1}$[b]	$\Delta S^{\ddagger}$/ *cal mol*$^{-1}$ *K*$^{-1}$[b]
Benzoates (**2**)			
2a	H	12.6	–22
2c	4-CHO	10.5	–24
2e	2-C(O)CH_3	4.6	–39
2p	2-C(O)CF_3	4.8	–39
Pthalides (**3**)			
3a	H	11.2	–22
3b	CHO	6.4	–37

[a]The uncertainties are considered to be ± 300 cal mol^{-1} for $\Delta H^{\ddagger}$ and ± 2 cal mol^{-1} for $\Delta S^{\ddagger}$; [b]1 cal = 4.184 J.



Scheme 1.

Table IV. Anti-bacterial activity of benzoates **2** and phthalides **3**; MIC (serial dilution assay)/10^{-6} mol dm^{-3}.

No	*Peptococcus asaccharolyticus 488*	*C perfringens IP615*	*C septicum IP Sebald*	*B fragilis ATCC 5285*	*B thetalotaomicron ATCC 29741*
2a	3.6	7.3	1.8	7.3	7.3
2b	1.7	3.3	0.83	6.6	13
2c	1.7	1.7	0.83	3.3	6.6
2d	2.5	4.9	0.61	4.9	9.8
2e	3.2	6.3	0.79	6.3	6.3
2f	1.3	2.7	0.32	5.4	11
2g	3.0	3.0	1.5	6.0	24
3a	3.3	3.3	0.40	3.3	6.6
3b	3.0	6.0	1.5	12	12
Metronidazole	2.9	2.9	0.70	5.8	23

bacteria were very comparable with that of metronidazole. As the assay is at pH 7.3 at 37 °C and is incubated under anaerobiosis for 48 h, hydrolysis of the esters will occur and the estimates of the extent stated above will apply. Thus, in the main, the esters **2b**, **2d–g** will be acting as metronidazole; but the esters **2a**, **2c**, **3a** and **3b** could be acting as themselves, assuming only alkaline hydrolysis. However, the latter esters have an antibacterial activity comparable to that of metronidazole itself and it could be that enzymic hydrolysis also occurs under these conditions to complete hydrolysis to metronidazole for *all* esters.

Prodrug utility

These esters have potential as topical prodrugs for metronidazole as they are both relatively lipophilic and labile to hydrolysis under physiological conditions. Further studies will be required to test their efficacy.

Experimental protocols

Chemistry

The structures of all compounds prepared were confirmed by spectroscopy and microanalysis. ^{1}H- and ^{13}C-NMR spectra were recorded at ambient temperature using a Jeol EX270, 270 MHz multinuclear FT spectrometer, while infrared spectra were obtained using a Perkin-Elmer 1600 FTIR spectrophotometer. Preparative column and flash chromatography were used in the purification, together with Prep LC/system 500A HPLC and chromatotron methods. All organic solutions were dried over anhydrous magnesium sulphate. Metronidazole was supplied pure as a gift from Rhône-Poulenc Rorer.

2-(2-Methyl-5-nitroimidazol-1-yl)ethyl benzoate ***2a*** *(Method A)*
A solution of metronidazole (7.70 g, 0.045 mol) and triphenylphosphine (7.86 g, 0.03 mol) in anhydrous acetone (100 mL) funnel was added dropwise to a solution of benzoic acid (3.66 g, 0.03 mol) and DEAD (5.22 g, 0.03 mol) in anhydrous diethyl ether (200 mL). The mixture was stirred at room temperature for 18 h and then evaporated under reduced pressure to yield a semi-solid product which was dissolved in chloroform. Purification by flash chromatography (chloroform/diethyl ether, 85:15/silica) gave **2a** which was recrystallised from ethyl acetate as a yellow crystalline powder, 2.97 g (36%); Mp 105 °C (Lit Mp 102 °C) [6]. Anal $C_{13}H_{13}N_3O_4$ (C, H, N). ^{1}H-NMR ($CDCl_3$): 2.49 (3H, s, CH_3), 4.66–4.70 (4H, m, CH_2), 7.41–7.96 (5H, m, arom *H*) 7.89 (1H, s, imidazole *H*). ^{13}C-NMR: 11 signals consistent with structure; 165.98(CO), 150.89(nitro C), 138.54(C), 131.96(C), 129.49(C), 128.62(C), 128.59(C), 62.83(CH_2), 45.25(CH_2), 14.32(CH_3).

2-(2-Methyl-5-nitroimidazol-1-yl)ethyl 2-formylbenzoate ***2b***
Using the method described for **2a**, employing metronidazole (7.70 g, 0.045 mol) and triphenylphosphine (7.86 g, 0.03 mol) in anhydrous acetone (100 mL) with 2-formylbenzoic acid (4.50 g, 0.03 mol) and DEAD (5.22 g, 0.03 mol) in anhydrous acetone (30 mL), purification by flash chromatography (diethyl ether/silica) and recrystallisation from diethyl ether, gave **2b** as a pale yellow crystalline powder, 5.46 g (60%); Mp 121–123 °C. Anal $C_{14}H_{13}N_3O_5$ (C, H, N). ^{1}H-NMR ($CDCl_3$): 2.40 (3H, s, CH_3), 4.66 (4H, m, CH_2), 7.58–7.84 (4H, m, arom *H*), 7.86 (1H, s, imidazole *H*), 10.34 (1H, s, formyl *H*). ^{13}C-NMR: 14 signals consistent with structure; 191.34(formyl CO), 165.69(ester CO), 150.71(nitro C), 138.44(C), 136.81(C), 133.03(C), 132.64(C), 130.69(C), 129.61(C), 128.99(C), 128.02(C), 63.46(CH_2), 44.77(CH_2), 14.01(CH_3).

2-(2-Methyl-5-nitroimidazol-1-yl)ethyl 4-formylbenzoate ***2c***
Using the method described for **2a**, employing metronidazole (7.70 g, 0.045 mol) and triphenylphosphine (7.86 g, 0.045 mol) in anhydrous acetone (100 mL) with 4-formylbenzoic acid (4.50 g, 0.03 mol) and DEAD (5.22 g, 0.03 mol) in anhydrous acetone (50 mL) and diethyl ether (250 mL), purification by flash chromatography (chloroform/ethyl acetate, 80:20/silica) and recrystallisation from diethyl ether/dichloromethane, gave a pale yellow crystalline powder, 7.27 g (80%); Mp 127–128 °C. Anal $C_{14}H_{13}N_3O_5$ (C, H, N). ^{1}H-NMR ($CDCl_3$): 2.40 (3H, s, CH_3), 4.64 (4H, m, CH_2), 7.80–8.20 (4H, m, arom *H*), 8.04 (1H, s, imidazole *H*), 10.12 (1H, s, formyl *H*). ^{13}C-NMR: 12 signals consistent with structure, including 192.61 (formyl CO).

2-(2-Methyl-5-nitroimidazol-2-yl)ethyl 2-(2-phenyloxyacetyl)-benzoate ***2d***
Using the method described for **2a**, employing metronidazole (2.57 g, 0.015 mol) and triphenylphosphine (2.62 g, 0.01 mol) in anhydrous acetone (50 mL) with 2-carboxybenzil (2.54 g, 0.01 mol) and DEAD (1.74 g, 0.01 mol) in anhydrous acetone (15 mL) and diethyl ether (15 mL), purification by flash chromatography (chloroform/diethyl ether, 85:15/silica) and recrystallisation from ethyl acetate, gave a yellow crystalline powder, 1.83 g (45%); Mp 140–142 °C. Anal $C_{21}H_{17}N_3O_6$ (C, H, N). ^{1}H-NMR ($CDCl_3$): 2.28 (H, s, CH_3), 4.40–4.47 (4H, m, CH_2), 7.40–8.09 (9H, m, arom *H*), 7.83 (1H, s, imidazole *H*). ^{13}C-NMR: 19 signals consistent with structure, including 193.02 (benzoyl CO), 188.93 (benzoyl CO).

2-(2-Methyl-5-nitroimidazol-1-yl)ethyl 2-acetylbenzoate ***2e***
Using the method described for **2a**, employing metronidazole (12.84 g, 0.075 mol) and triphenylphosphine (13.11 g, 0.05 mol) in anhydrous acetone (150 mL) with 2-acetylbenzoic acid (8.20 g, 0.05 mol) and DEAD (8.70 g, 0.05 mol) in anhydrous diethyl ether (300 mL), purification by flash chromatography (chloroform/diethyl ether/ethyl acetate, 70:20:10/silica) and recrystallisation from diethyl ether, gave a pale yellow crystalline powder, 11.41 g (72%); Mp 118–120 °C. Anal $C_{15}H_{15}N_3O_5$ (C, H, N). ^{1}H-NMR ($CDCl_3$): 2.37 (3H, s, CH_3), 2.41 (3H, s, acetyl CH_3), 4.55–4.59 (4H, m, CH_2), 7.45–7.53 (4H, m, arom *H*), 7.85 (1H, s, imidazole *H*). ^{13}C-NMR: 15 signals consistent with structure, including 201.29 (acetyl CO).

2-(2-Methyl-5-nitroimidazol-1-yl)ethyl 2-(trifluoroacetyl)benzoate ***2f***
Using the method described for **2a**, employing metronidazole (2.57 g, 0.015 mol) and triphenylphosphine (2.62 g, 0.01 mol) in anhydrous acetone (50 mL) with 2-(trifluoroacetyl)benzoic acid (2.18 g, 0.01 mol) and DEAD (1.74 g, 0.01 mol) in anhydrous acetone (25 mL) and diethyl ether (25 mL), purification by flash chromatography (chloroform/dichloromethane/diethyl ether, 60.20:20/silica) and recrystallisation from diethyl ether/hexane, gave a colourless crystalline powder, 1.78 g (48%); Mp 144–146 °C. Anal $C_{15}H_{12}F_3N_3O_5$ (C, H, F, N).

^{1}H-NMR ($(CD_3)_2CO$): 2.44 (3H, s, CH_3), 3.80–4.52 (4H, m, CH_2), 7.40–8.08 (4H, m, arom *H*), 7.96 (1H, s, imidazole *H*). ^{13}C-NMR: 15 signals (one as q) consistent with structure, including 165.95 (trifluoroacetyl CO).

1,3-Dihydro-1-(2-methyl-5-nitroimidazole-1-yl)ethoxy-4-isobenzofuran ***3a*** *(Method B)*

2-Formylbenzoic acid (15.0 g, 0.1 mol) and thionyl chloride (25 mL) in anhydrous toluene (75 mL) were refluxed for 6 h. Evaporation of the mixture gave the crude acid chloride which was mixed with metronidazole (17.1 g, 0.1 mol) and anhydrous triethylamine (12.1 g, 0.13 mol), dioxane (150 mL) and chloroform (200 mL) before refluxing for 12 h. After evaporation of the solvent, the product was dissolved in chloroform. The latter was subjected to flash chromatography (ethyl acetate/silica). Examination by NMR spectroscopy indicated the product to be a mixture of **3a** and **2b**. Purification by column chromatography (ethyl acetate/hexane, 80:20/alumina) gave **3a** which was recrystallised from ethyl acetate/hexane as a pale yellow crystalline powder, 10.61 g (35%); Mp 132 °C. Anal $C_4H_{13}N_3O_5$ (C, H, N). ^{1}H-NMR ($(CD_3)_2SO$): 2.37 (3H, s, CH_3), 4.09–4.15 (2H, t, CH_2, J = 7 Hz), 4.52–4.59 (2H, t, CH_2, J = 7 Hz), 6.58 (1H, s, C*H*), 7.41–7.82 (4H, m, arom *H*), 8.03 (1H, s, imidazole *H*). ^{13}C-NMR: 14 signals consistent with structure, including 167.82 (lactone CO), 102.07 (quart C); **2b** could also be obtained by this method as a byproduct, 2.12 g (7%).

2-(2-Methyl-5-nitroimidazole-1-yl)ethyl 2,6-diformylbenzoate ***2g*** *and 1,3-dihydro-1-(2-(2-methyl-5-nitroimidazol-1-yl)-ethoxy)-4-isobenzofurancarboxaldehyde* ***3b***

Using the method described for **3a**, employing 2,6-diformylbenzoic acid (1.78 g, 0.01 mol) with metronidazole (1.88 g, 0.011 mol) and anhydrous triethylamine (1.21 g, 0.013 mol) and chlorobenzene (100 mL) refluxing for 24 h. After evaporation of the solvent under reduced pressure, the product was dissolved in chloroform. The latter was subjected to flash chromatography (ethyl acetate/silica). Examination by NMR spectroscopy indicated the product to be a mixture of **3b** and **2g** in the ratio 85:15 as a pale yellow crystalline powder, 2.25 g (68%). The separation of the tautomers was achieved with difficulty using the chromatotron (ethyl acetate/silica). **2g** as a pale yellow crystalline powder, Mp 163 °C, recrystallised from ethyl acetate/diethyl ether. Anal $C_{15}H_{13}N_3O_6$ (C, H, N). ^{1}H-NMR ($CDCl_3$): 2.43 (3H, s, CH_3), 4.14–4.34 (4H, m, CH_2), 7.28–8.19 (3H, m, arom *H*), 8.00 (1H, s, imidazole *H*), 9.99 (2H, s, formyl *H*). ^{13}C-NMR: 12 signals consistent with structure, including 189.28 (formyl CO). **3b** as a pale yellow crystalline powder, Mp 165–166 °C, recrystallised from ethyl acetate/diethyl ether. Anal $C_{15}H_{13}N_3O_6$ (C, H, N). ^{1}H-NMR ($CDCl_3$): 2.43 (3H, s, CH_3), 4.51–4.78 (4H, m, CH_2), 6.35 (1H, s, C*H*), 7.28–8.19 (3H, m, arom *H*), 8.00 (1H, s, imidazole *H*), 10.99 (1H, s, formyl *H*). ^{13}C-NMR: 15 signals consistent with structure, including 188.26 (formyl CO), 166.73 (lactone CO), 101.83 (quart C).

Inorganic salts were of analytical grade and were used without further purification. The solvents for the kinetic studies were purified by standard procedures [18].

Alkaline hydrolysis of esters

The products of the alkaline hydrolysis of the esters were found to be metronidazole and the respective carboxylic acid, as the anion, in quantitative yield in all cases. These were further confirmed spectrophotometrically by comparison of the spectrum of metronidazole and the respective carboxylic acid in base with that of the reaction product. Rate coefficients for the alkaline hydrolysis of the esters were determined spectrophotometrically by use of a Perkin-Elmer lambda 5 UV-VIS spectrometer. A Haake thermostated water-circulating bath was used to control the temperature of the cell to ± 0.05 °C. The procedure was described previously [1, 19]. The reactions were followed at the wavelengths shown in table II.

Biological methods

The esters were studied in vitro against a series of anaerobic bacteria. The minimum inhibitory concentrations (MIC) against selected bacteria are shown in table IV. The inhibitory properties of the esters were determined by a 2-fold serial dilution method: for each compound a series of solutions was prepared whose concentrations differed by a factor 2; each solution was added to a fixed amount of a previously prepared test culture, and the mixture incubated at 37 °C for 48 h under anaerobiosis. The antibacterial activity was then expressed as its MIC in units of 10^{-6} mol dm^{-3}.

Acknowledgments

We wish to thank NV Harris and MJ Ashton of Rhône-Poulenc Rorer, Dagenham, for their advice and arranging for the biological tests.

References

1 Bowden K, Huntington AP, Powell SL (1997) *Eur J Med Chem* 32, 987–993
2 Sinkula AA, Yalkowsky SH (1975) *J Pharm Sci* 64, 181–210
3 Bundgaard H (1985) In: *Design of Prodrugs* (Bundgaard H, ed) Elsevier, Amsterdam, ch 1
4 Baines EJ (1978) *J Antimicrol Chemother* 4 (Suppl C), 97–111
5 Amidon GL (1981) *Drugs Pharm Sci* 12, 183–211
6 Cosar C, Crisan C, Horclois R, Jacob RRM, Robert J, Tchelitcheff S, Vaupré R (1966) *Arzneimitt Forsch* 16, 23–29
7 Cho MJ , Kurtz RR, Lewis C, Machkovech SM, Houser DJ (1982) *J Pharm Sci* 71, 410–414
8 Bundgaard H, Larsen C, Thorbek P (1984) *Int J Pharm* 18, 67–77
9 Bundgaard H, Larsen C, Arnold E (1984) *Int J Pharm* 18, 79–87
10 Bowden K (1993) *Adv Phys Org Chem* 28, 171–206
11 Bowden K (1995) *Chem Soc Rev* 24, 431–436
12 Anvia F, Bowden K, El Kaissi FM, Saez V (1990) *J Chem Soc Perkin Trans II*, 1809–1814
13 Hansch C, Leo A, Hoekman D (1995) *Exploring QSAR Hydrophobic, Electronic and Steric Constants*, American Chemical Society, Washington
14 Mitsunoba O (1981) *Synthesis* 1–28
15 Blankley CJ, Sauter FJ, House HO (1973) *Org Syn Coll* 5, 128–263
16 Maskill H (1985) *The Physical Basis of Organic Chemistry*, Oxford University Press, Oxford
17 Bowden K, Taylor GR (1971) *J Chem Soc B* 149–156
18 Perrin DD, Armarego WLF (1988) *Purificaiton of Laboratory Chemicals*, 3rd ed, Pergamon, Oxford
19 Bowden K, Last AM (1973) *J Chem Soc Perkin Trans II*, 345–351