ELSEVIER

Il Farmaco 53 (1998) 58–61

IL FARMACO

Multifunctional derivatives of metronidazole

Keith Bowden *, Jamshid Izadi

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

Received 11 April 1997; accepted 5 May 1997

Abstract

The design and synthesis of a series of multifunctional metronidazole derivatives are described. The main series are multi-esters having two or more metronidazole groups linked together by aryl or alkyl systems. A second system is two nitro-2-methylimidazole groups joined by a dimethylene link. The third is polymeric 2-(2-methyl-5-nitroimidazol-1-yl)ethyl acrylate. The triester, metronidazole trimesate, is exceptionally active as an antibacterial compound, which appears to be associated with a rigid, three-point attachment.

Keywords: Metronidazole; Nitroimidazoles

1. Introduction

A drug can be designed to have more than one of the same biologically-active moiety to give a multifunctional system. This modification can increase the activity of a drug by enhanced delivery or by a novel activity arising from bridging. The extreme form of such multifunctional drugs can be considered to be polymeric drug systems [1,2]. Such systems can act either as a carrier of drugs (prodrug) or an independent drug.

Metronidazole, 1-(2-hydroxyethyl)-2-methyl-5-nitroimidazole (**1**), is known to be a powerful antiprotozoal and antibacterial drug [3]. There are problems relating to the low aqueous solubility, toxicity and poor absorption. Esters have been studied as prodrugs to modify such problems. The parent drug can be liberated by enzymes or non-enzymatically. Some esters and hemi-esters of metronidazole have been prepared [4]. However, phosphate and amino acid esters of metronidazole have been studied to improve water solubility of metronidazole [5–7].

A recent study of 2-acylbenzoates of metronidazole has demonstrated that hydrolysis employing intramolecular catalysis can facilitate the hydrolysis reaction to a remarkable and tuneable degree [8].

The present report details a study of the design, synthesis and antibacterial activity of a series of multifunctional metronidazole esters and related derivatives.

2. Chemistry

The first model is that of esters of metronidazole which were designed on the basis of having two or more metronidazole groups linked together by aryl or alkyl spacing systems having di-, tri- or tetra-carboxylic groups. These are shown in Scheme 1 as the phthalate **2b**, terephthalate **2c**, trimesate **2d**, pyromellitate **2c**, succinate **3a**, glutarate **3b**, adipate **3c** and suberate **3d**. The second model is that of a dimethylene link between two nitro-2-methylimidazole groups, which are shown as 1-(2-methyl-4-nitroimidazol-1-yl)-2-(2-methyl-5-nitroimidazol-1-yl)ethane (**4a**) and 1,2-di(2-methyl-5-nitroimidazol-1-yl)ethane (**4b**). The third model is polymeric 2-(2-methyl-5-nitroimidazol-1-yl)ethyl acrylate (**6**), formed from the corresponding monomer **5**.

The synthesis of the simple esters was either by reaction of the corresponding carboxylic acid chloride with metronidazole, in the presence of triethylamine [9], or by coupling the carboxylic acid and metronidazole using diethyl azodicarboxylate (DEAD) and triphenyl phosphine [10].

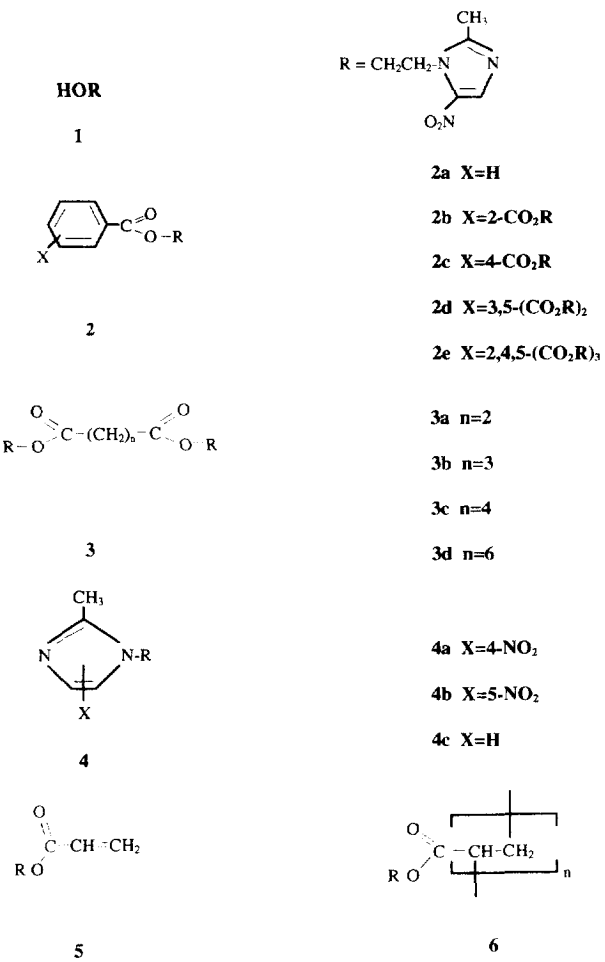
3. Experimental

3.1. Chemistry

Melting points were determined using a Kofler melting point apparatus and are uncorrected. The structures of all the compounds were confirmed by IR and ^{1}H and ^{13}C NMR spectra. IR spectra were determined using a Zeiss Specord

* Corresponding author.

0014-827X/98/$19.00
PII S0014-827X(97)00007-4



Scheme 1.

M-80 spectrophotometer. NMR spectra were recorded at ambient temperatures on $CDCl_3$ or $(CD_3)_2SO$ solutions using a JEOL EX270 FT spectrometer with $(CH_3)_4Si$ as internal reference. All compounds were analysed for C, H and N and gave results within ±0.3% of the calculated values. Preparative column chromatography was performed using Still's method of flash chromatography [11]; the stationary phase used normally was Sorbsil C60 silica gel with chloroform/ethyl acetate as the eluent. All organic solutions were dried over anhydrous magnesium sulfate.

3.1.1. General method for the synthesis of esters 2 and 3 using DEAD and triphenylphosphine

A solution of metronidazole (0.045*n* mol (*n* equals the number of carboxylic acid groups per molecule)) and triphenylphosphine (0.03*n* mol) in anhydrous acetone (100 ml) in a separating funnel was added dropwise to a solution of the carboxylic acid (0.03*n* mol) and DEAD (0.03*n* mol) in anhydrous diethyl ether (200 ml) in a flask fitted with an air-bleed over 1 h. The mixture was stirred at ambient for 18 h and then evaporated under reduced pressure to yield a semi-solid product which was dissolved in chloroform. The product was purified by flash chromatography (chloroform/diethyl ether/silica). The solid or oil so obtained were then recrystallised to give the product described in Table 1.

This method for **2** and **3** gave yields of between 32 and 80% and was generally superior to the following method.

3.1.2. General method for the synthesis of esters 2 and 3 using the carboxylic acid chloride in the presence of triethylamine

The carboxylic acid chloride (prepared from the carboxylic acid (0.05 mol) and excess $SOCl_2$ or PCl_5 and used without further purification) was mixed with metronidazole and triethylamine (both 0.06*n* mol (*n* equals the number of carboxylic acid groups per molecule)) in dry dioxan (200 ml) and $CHCl_3$ (200 ml). After stirring at ambient for 30 min, the mixture was refluxed under a gentle stream of nitrogen for 4 h. The solvent was evaporated under reduced pres-

Table 1
Metronidazole esters and derivatives

Compound	Formula	Anal. [a]	M.p. (°C)	Lit. m.p. (°C)	Lit. ref.	Recryst. solvent
2a	$C_{13}H_{13}N_3O_4$	C,H,N	105–106	102	[4]	CH_2Cl_2/diethyl ether
2b	$C_{20}H_{20}N_6O_8$	C,H,N	126–128	130	[4]	$CHCl_3$/heptane
2c	$C_{20}H_{20}N_6O_8$	C,H,N	233–235			$CHCl_3/CH_2Cl_2$
2d	$C_{27}H_{27}N_9O_{12}$	C,H,N	63–65			$CHCl_3$/heptane
2e	$C_{34}H_{34}N_{12}O_{16}$	C,H,N	50–52			$CHCl_3$/pet.ether (b.p. 60–80°C)
3a	$C_{16}H_{20}N_6O_8$	C,H,N	123–125	129	[4]	$CHCl_3$/pet.ether (b.p. 40–60°C)
3b	$C_{17}H_{22}N_6O_8$	C,H,N	93–95			$CHCl_3$/pet.ether (b.p. 40–60°C)
3c	$C_{18}H_{24}N_6O_8$	C,H,N	93–95			$CHCl_3$/pet.ether (b.p. 40–60°C)
3d	$C_{20}H_{28}N_6O_8$	C,H,N	88–89			$CHCl_3$/pet.ether (b.p. 40–60°C)
4a	$C_{10}H_{12}N_6O_4$	C,H,N	259–260	264–265, 257	[14,15]	$(CH_3)_2SO$
4b	$C_{10}H_{12}N_6O_4$	C,H,N	>270	>250	[16]	$(CH_3)_2SO$
5	$C_9H_{11}N_3O_4$	C,H,N	oil			
6	$[C_9H_{11}N_3O_4]_n$	C,H,N	[b]			$CHCl_3$

[a] Analytical results were within ±0.3% of calculated values.
[b] No distinct m.p. (see text).

sure. The residue was taken up in chloroform and filtered. The filtrate was washed with water (200 ml) and twice with 0.5 mol dm^{-3} aqueous sodium hydrogen carbonate (200 ml) and with water (200 ml). After drying and filtering, the solution was evaporated under reduced pressure. The product was then purified by flash chromatography (chloroform/ethyl acetate/silica). The solid or oil so obtained was then recrystallised to give the product described in Table 1.

This method for **2** and **3** gave yield of between 3 and 50% and was generally inferior to the previous method.

3.1.3. Polymer of 2-(2-methyl-5-nitroimidazol-1-yl)ethyl acrylate

After a number of unsuccessful attempts using other methods, the compound was obtained as a polymer using the dicyclohexylcarbodiimide (DCC) coupling method [12].

Acrylic acid (7.2 g, 0.1 mol) and metronidazole (8.5 g, 0.05 mol) were dissolved in dry pyridine (100 ml). *p*-Toluenesulfonic acid (0.9 g), hydroquinone (1.4 g) and DCC (24.8 g, 0.12 mol) were added to the solution, which was stirred at ambient for 48 h. Acetic acid (10 ml) was added and the mixture kept overnight at 4°C. The mixture was filtered and the filtrate was evaporated under reduced pressure. The residue was dissolved in $CHCl_3$ and filtered. The filtrate was washed thrice with 0.5 mol dm^3 aqueous HCl (3×200 ml) and with water (100 ml), before being dried and filtered. The filtrate was evaporated under reduced pressure and the residue was dissolved in the minimum amount of ethyl acetate before being subjected to preparative column chromatography using silica gel, eluting with chloroform/ethyl acetate. The acrylate monomer was obtained as a yellow oil whose structure was confirmed as usual. After a few days, the oil solidified to form a waxy solid whose melting point was indistinct. The structure of the solid was confirmed as the polymer in the usual manner, but the molecular weight was not determined.

*3.1.4. 1,2-Di(2-methyl nitroimidazol-1-yl)ethanes **4a** and **4b***

The successful route to these compounds involved reaction of metronidazole tosylate with 2-methylimidazole, followed by nitration.

2-Methylimidazole (7.6 g, 0.092 mol) was mixed with metronidazole tosylate [13] (15 g, 0.046 mol) in dry DMF (100 ml) and the mixture was stirred at 150°C for 22 h. The solvent was evaporated under reduced pressure and the residue was dissolved in the minimum of chloroform, before being subject to preparative column chromatography using activated neutral alumina, with chloroform as the eluent. The product, 1-(2-methylimidazol-1yl)-2-(2-methyl-4-nitroimidazol-1-yl)ethane (**4c**), was then recrystallised as a cream-coloured crystalline solid (2 g, 10%), m.p. 112–115°C, whose structure was confirmed in the usual manner.

The ethane **4c** (2.5 g, 0.01 mol) was dissolved in 98% sulfuric acid (10 ml). The solution was heated at 130°C and 70% nitric acid (4 ml) was added dropwise. The solution was allowed to cool to ambient and stirred for 1.5 h, before pouring onto ice. The pH of the resulting mixture was adjusted to between 6 and 7 by addition of 30% aqueous ammonia. The solid precipitate was filtered off and dried. Any unreacted starting material was removed by refluxing with chloroform. The product (1 g, 40%) was a mixture of **4a** and **4b**. Both were almost insoluble in most standard solvents, except dimethyl sulfoxide. The mixture was separated by fractional recrystallisation with the symmetric isomer **4b** being least soluble. Both **4a**, m.p. 259–260°C (lit. [14,15], m.p. 264–265°C. 257°C) 0.38 g, 15%), and **4b**, m.p. > 270°C (lit. [16] m.p. > 250°C) (0.62 g, 25%) were obtained as cream-coloured crystalline solids.

3.2. Biology

The esters were studied in vitro against a series of anaerobic bacteria. The minimum inhibitory concentrations (MIC) against selected bacteria are shown in Table 2. The inhibitory properties of the esters were determined by a two-fold serial dilution method: for each compound a series of solutions was prepared whose concentrations differed by a factor of 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}.

4. Results and discussion

The anti-bacterial activities are shown in Table 2. In terms of the MIC values in units of 10^{-6} mol dm^{-3}, the ester **2d** has a very significantly greater activity than metronidazole and the other compounds. If the results are 'normalised' in terms of nitro-2-methylimidazole groups, the compounds **2b, 3a, 3b, 3c, 3d, 4a** and **4b** all have comparable activity to metronidazole; whereas the compounds **2a, 2c, 2e** and **6** are less active than metronidazole. However, the multi-esters would not be significantly hydrolysed under the pH of the test conditions. A comparison of the activity of the triester **2d** with that of the diesters, **2a** to **2c** and **3a** to **3d** and the tetraester **2e** clearly indicates that the very significant increase in activity is associated with the stereochemistry of the trimesate ester, i.e. 1,3,5-benzene system. Metronidazole, as well as other 5-nitroimidazoles, appears to be, in effect, an antibacterial prodrug for a reduced form which is the active drug. The latter reacts with DNA, causing strand breakage and cell death [17]. The reduced nitroimidazoles bind to specific residues in the DNA. The reduced triester **2d** could effectively bridge certain critical residues, giving rise to its greater activity. Unfortunately the latter increased activity parallels increased potential mutagenic activity compared to that of metronidazole [18].

Table 2
In vitro antimicrobial activity of metronidazole esters and derivatives

Compound	MIC (serial dilution assay) (10^{-6} mol dm^{-3})				
	Peptococcus asaccharolyticus 488	*C. perfringens* IP615	*C. septicum* IP Sebald	*B. fragilis* ATCC 25285	*B. thetaiotamicron* ATCC 29741
Metronidazole	0.70	1.5	0.70	2.9	23
2a	3.6	7.3	1.8	7.3	7.3
2b	0.25	1.1	0.25	2.1	
2c	0.85	14	3.4	>27	>27
2d	0.0060	0.0030	0.045	0.090	0.090
2e	0.29	4.6	0.58	17	
3a	0.59	1.2	0.28	1.2	
3b	0.27	0.57	0.27	0.57	
3c	0.13	0.55	0.27	0.55	
3d	0.52	2.1	0.52	2.1	
4a	0.88	7.2	3.5	0.88	3.5
4b	0.88	7.2	1.8	0.44	0.88
6 [a]	4.4	8.9	2.2	8.9	18

[a] Calculated as monomer.

Acknowledgements

We wish to thank Drs N.V. Harris and M.J. Ashton of Rhône-Poulenc Rorer, Dagenham, for their help and for arranging for the biological tests.

References

[1] S.W. Kim, R.V. Peterson, J. Feijen, Polymeric drug delivery systems, Drug Design 10 (1980) 193.
[2] C.M. Samour, in: L.G. Donaruma, O. Vogl (Eds.), Polymeric Drugs, Academic Press, New York, 1978, p. 161.
[3] E.J. Baines, Metronidazole: its past, present and future, J. Antimicrob. Chemother. 4 (Suppl. C) (1978) 97.
[4] C. Cosar, C. Crissan, R. Horclois, R.M. Jacob, J. Robert, S. Tchelitcheff, R. Vaupré, Nitroimidazoles, Arzneium.-Forsch. 16 (1966) 23.
[5] M.J. Cho, R.R. Kurtz, C. Lewis, S.M. Machkovech, D.J. Houser, Metronidazole phosphate — a water-soluble prodrug for parenteral solutions of metronidazole, J. Pharm. Sci. 71 (1982) 410.
[6] H. Bundgaard, C. Larsen, P. Thorbek, Prodrugs as drug delivery systems. XXVI. Preparation and enzymic hydrolysis of various water-soluble amino acid esters of metronidazole, Int. J. Pharm. 18 (1984) 67.
[7] H. Bundgaard, C. Larsen, E. Arnold, Prodrugs as drug delivery systems. XXVII. Chemical stability and bioavailability of a water-soluble prodrug of metronidazole for parenteral adminstration, Int. J. Pharm. 18 (1984) 79.
[8] K. Bowden, J. Izadi, Prodrugs. Part 2. Acylbenzoate esters of metronidazole, Eur. J. Med. Chem. 32 (1997) in press.
[9] C.J. Blankley, F.J. Sauter, H.O. House, Crotyl diazoacetate, Org. Syn. Coll. 5 (1973) 258.
[10] O. Mitsunoba, The use of diethyl azodicarboxylate and triphenylphosphine in synthesis and transformation of natural products, Synthesis. (1981) 1.
[11] W.C. Still, M. Kahn, A. Mitra, Rapid chromatographic technique for preparative separations with moderate resolution, J. Org. Chem. 43 (1978) 2923.
[12] K. Holmberg, B. Hansen, Ester synthesis with dicyclohexylcarbodiimide improved by acid catalysts, Acta Chem. Scand. B 33 (1979) 410.
[13] Lilly Industries Ltd., Fr. Demande 2 015 181 (24 Apr. 1970), Antiparasitic imidazoles (Chem. Abstr. 74 (1971) 53790s).
[14] V. Sunjic, T. Fajoliga, N. Blazevic, F. Kajfez, 1,1'-Bis(2-methyl-4(5)-nitroimidazoles), Acta Pharm. Jugoslav. 19 (1969) 65 (Chem. Abstr. 72 (1970) 66866e).
[15] E. Winkelmann, W. Raether, U. Gebert, A. Sinharay, Chemotherapeutically active nitro compounds. 4. 5-Nitroimidazoles. Part 1, Arzneim. Forsch. 27 (1977) 2251.
[16] Z. Zhang, M. Yen, X. Lei, Studies on chemotherapy of filariasis. II. Synthesis of 5-nitroimidazole derivatives, Yaoxue Xuebao 16 (1981) 548 (Chem. Abstr. 97 (1982) 72290v).
[17] D.I. Edwards, Nitroimidazole drugs-action and resistance mechanisms. II. Mechanisms of resistance, J. Antimicrob. Chemother. 31 (1993) 9.
[18] M.J. Ashton, personal communication.