International Journal of Pharmaceutics, 102 (1994) 231–239

IJP 03396

N-Alkoxycarbonyl prodrugs of mebendazole with increased water solubility

Lise Sylvest Nielsen [a], Frank Sløk [b] and Hans Bundgaard [c,1]

[a] *Department of Pharmaceutics,* [b] *Department of Organic Chemistry and* [c] *Department of Pharmaceutical Chemistry, Royal Danish School of Pharmacy, 2 Universitetsparken, DK-2100 Copenhagen (Denmark)*

(Received 14 June 1993)
(Accepted 13 August 1993)

Key words: Mebendazole; Prodrug; *N*-Alkoxycarbonyl derivative; Solubility; Lipophilicity

Summary

Bioreversible derivatization of mebendazole to improve its poor water solubility, which is attributed to its low peroral bioavailability, was performed by *N*-acylation of the benzimidazole moiety with various chloroformates. The *N*-alkoxycarbonyl derivatives were readily hydrolyzed to mebendazole in rabbit liver homogenate and in human and rabbit plasma. The pH-rate profiles for the hydrolysis of the derivatives were derived at 37°C, and the lipophilicity of the compounds was assessed by partition experiments in octanol-aqueous buffer systems. The water solubility of the *N*-alkoxycarbonyl derivatives was up to 16-times higher than that of the parent drug. The results obtained suggest that *N*-alkoxycarbonyl derivatives of benzimidazole anthelmintics may be useful to improve the peroral bioavailability.

Introduction

Mebendazole, albendazole, and other benzimidazole carbamates are active against many gastrointestinal and tissue-stage helminths (Van den Bossche et al., 1982). These benzimidazoles are in general characterized by having a very poor solubility in water (Van den Bossche et al., 1982). This property is most likely the main reason for the poor oral absorption of mebendazole and most of the other compounds (Münst et al., 1980; Dawson et al., 1982, 1985a,b; Marriner et al., 1986; McKellar and Scott, 1990). The very low aqueous solubility makes the compounds less suitable for the treatment of systemic infections like alveolar and cystic echinococcosis (hydatide disease) which are caused by infection with the larval stage of the cestodes *Echinococcus multilocularis* and *E. granulosus*, respectively (Thompson, 1986).

Several reports have indicated the potential usefulness of the benzimidazole carbamates, especially mebendazole and albendazole, in treating human echinococcosis (Witassek et al., 1981; Schantz et al., 1982; Davis et al., 1986, 1989), but because of their poor and variable oral bioavailability inefficient tissue and plasma concentrations are generally obtained (Braithwaite et al., 1982).

Correspondence to: L.S. Nielsen, A/S DUMEX (DUMEX Ltd), Dept of Pharmaceutical Development, 11 Dalslundsgade, DK-2300 Copenhagen S, Denmark.
[1] Deceased 1992.

Studies have previously been undertaken in our laboratory to overcome or diminish this delivery problem by the prodrug approach. It was shown with thiabendazole, used as a model compound for the benzimidazole carbamates, that it was possible to obtain prodrugs possessing a higher water solubility than the parent drug and with an adequate lipophilicity in regard to oral absorption (Nielsen et al., 1992). In the present work, a series of *N*-alkoxycarbonyl derivatives (Scheme 1) of the widely used mebendazole (**I**) was prepared. Their physicochemical properties, chemical and enzymatic stability were investigated. In contrast to thiabendazole, mebendazole and related compounds are asymmetrically substituted at position 5 in the benzene ring of the benzimidazole moiety. Thus, acylation of the benzimidazole nitrogens in mebendazole may result in the formation of two isomeric derivatives (Scheme 1) (Viswanathan and Sidhaye, 1985).

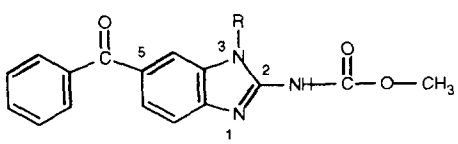
Materials and Methods

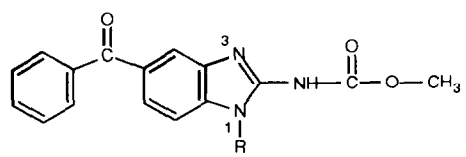
Apparatus

High-performance liquid chromatography (HPLC) was performed with a Hitachi system consisting of a Model L-6000 constant flow pump equipped with a Model 4000 UV detector, a Model As-2000 Auto Sampler, and a Rheodyne 7125 20 μl loop injection valve. Separations were performed on a deactivated reversed-phase Lichrocart Lichrospher 100 RP-8 column (125 × 8 mm, 5 μm particles) (E. Merck, Germany) equipped with a precolumn packed with Perisorb RP-8 (30–40 μm particles) (E. Merck, Germany). A silica saturation column placed, between the pump and the injection device, was packed with LiChroprep SI 60 (15–25 μm particles) (E. Merck, Germany). Readings of pH were carried out on a Radiometer PHM83 Autocal instrument at the temperature of study. Melting points were taken in capillary tubes and were not corrected. ^{1}H-NMR spectra were run on a Bruker AC-200F instrument. Elemental analyses were performed at the Microanalytical Laboratory, Leo Pharmaceutical Products, Ballerup, Denmark.

Synthesis of the mebendazole derivatives **II–IV**

The *N*-alkoxycarbonyl derivatives **II–IV** were prepared by reacting mebendazole (obtained from Sigma Chemical Co., St. Louis) with the appropriate chloroformate in dichloromethane in the presence of NaOH. The chloroformate (6 ml, 88 mmol) was added over 5 min to a stirred suspension of mebendazole (10 g, 34 mmol) in a mixture of dichloromethane (300 ml) and 1 N NaOH (70 ml, 70 mmol). The reaction mixture was stirred at





I R = H

IIa R = $-C(=O)-OCH_3$

IIIa R = $-C(=O)-OCH_2CH_3$

IVa R = $-C(=O)-OCH_2CH_2CH_3$

IIb R = $-C(=O)-OCH_3$

IVb R = $-C(=O)-OCH_2CH_2CH_3$

Scheme 1.

TABLE 1

Physical and analytical data of various N-alkoxycarbonyl derivatives of mebendazole

Compound	m.p. (°C)	Formula		Analysis (%)	
				Calc.	Found
II [a]	156–157	$C_{18}H_{15}N_3O_5$	C	61.19	60.09
			H	4.28	4.30
			N	11.89	11.84
IIIa	165–166	$C_{19}H_{16}N_3O_5$	C	62.12	61.90
			H	4.66	4.67
			N	11.44	11.31
IV [b]	113–114	$C_{20}H_{19}N_3O_5$	C	62.98	62.83
			H	5.02	5.11
			N	11.02	10.84

[a] The ^{1}H-NMR spectra of **II** showed it to be a 50:50 mixture of **IIa** and **IIb**, respectively.

[b] The ^{1}H-NMR spectra of **IV** showed it to be a 40:60 mixture of **IVa** and **IVb**, respectively.

room temperature overnight. The dichloromethane phase was separated from precipitated (undissolved) mebendazole by filtration, then washed with water, dried over anhydrous sodium sulphate and evaporated in vacuo. The residue (oil) obtained was dissolved in dichloromethane and ethyl acetate-petroleum ether. The crystalline precipitate formed, upon cooling to −10°C, was filtered off and recrystallized from dichloromethane and ethyl acetate-petroleum ether. The yields of the *N*-alkoxycarbonyl derivatives were 30–40%. Physical and analytical data for the derivatives are given in Table 1.

Structure determination (by ^{1}H-NMR)

To determine the structure of compounds **II–IV**, a ^{1}H-NMR assignment was carried out with compound **III** using a NOESY experiment. The spectra showed NOE between the ethyl group and the aromatic proton at 8.27 ppm, while no NOE was observed between the ethyl group and the protons at 7.46–7.83 ppm. Furthermore, a NOESY experiment was carried out on the mixture of **IIa** and **IIb**. The spectra showed NOE between only one of the methoxycarbonyl groups (at 4.17 ppm) and the aromatic proton at 8.27 ppm. No NOE was observed between the other methoxycarbonyl group (at 4.21 ppm) and the aromatic protons at 7.46–7.82 ppm or from either of the methoxycarbonyl groups and the proton at 8.07 ppm. From these NOESY experiments, it can be concluded that the structure of the compounds, where R is ethyl, is **IIIa** in agreement with the structure assignment of the corresponding 3-amino compounds (Ravindranathan et al., 1987). ^{1}H-NMR spectral data are listed in Table 2.

Hydrolysis in aqueous solutions

The hydrolysis of the mebendazole derivatives was studied in aqueous buffer solutions at constant temperature (±0.2°C). The buffers used were hydrochloric acid, acetate, phosphate, borate and carbonate buffers. The total buffer concentration was generally 0.02 M, and a constant ionic strength (μ) of 0.5 was maintained for each buffer by adding a calculated amount of potassium chloride. The rates of degradation were followed by using isocratic reversed-phase HPLC procedures capable of separating the derivatives from mebendazole. The chromatographic column was eluted with mobile phase systems consisting of mixtures of 0.05 M phosphoric acid (pH 2.5)

TABLE 2

^{1}H-NMR spectral data of compounds II–IV [a]

Compound	H_4	$NHCOOCH_3$	$NHCOOCH_3$	R
IIa	8.27 (bs)	10.03 (bs)	3.92 (S)	4.17 (S)
IIb	8.07 (bs)	9.88 (bs)	3.90 (S)	4.21 (S)
IIIa	8.27 (bs)	10.02 (bs)	3.92 (S)	4.59 (q); 1.47 (t)
IVa	8.27 (bs)	10.04 (bs)	3.92 (S)	4.48 (t); 1.85 (m); 0.99 (t)
IVb	8.07 (bs)	9.89 (bs)	3.90 (S)	4.53 (t); 1.96 (m); 1.14 (t)

[a] In DMSO-d_6. The chemical shifts of the 5-benzoyl group and H_6 and H_7 (δ): 7.71–7.83 (4H,m) and 7.46–7.60 (3H,m).

and methanol. The concentration of methanol (50–65% v/v) was adjusted for each compound to give an appropriate retention time (3–8 min). The flow rate was 1.0 ml min^{-1}, and the column effluent was monitored at 251 nm. Quantitation of the compounds was carried out by measuring the peak heights in relation to those of standards chromatographed under the same conditions.

The reactions were initiated by adding 50–200 μl of a stock solution of the compounds in acetonitrile to 5 ml of preheated buffer solution in screw-capped test tubes to give an initial concentration of about 5×10^{-5} M. The solutions were kept in a water-bath at a constant temperature, and at appropriate intervals samples were withdrawn and chromatographed immediately. Pseudo-first-order rate constants for the degradation were determined from the slopes of linear plots of the logarithm of residual derivative against time.

Stability in various biological media

The hydrolysis of the derivatives **II–IV** was studied at 37°C in human and rabbit plasma diluted to 80% with 0.05 M phosphate buffer of pH 7.4 as well as in 2.5% rabbit liver homogenate. The liver homogenate was prepared as previously described (Buur and Bundgaard, 1985).

The initial concentration of the derivatives was in the range of $0.5–1 \times 10^{-4}$ M. The mixtures were kept in a water bath at 37°C, and at appropriate intervals samples of 250 μl of the reaction solution were withdrawn and deproteinized by addition of 500 μl of acetonitrile. After mixing and centrifugation for 3 min at 10000 rpm, 20 μl of the clear supernatant was analyzed by HPLC as described above.

Determination of solubility and partition coefficients

The aqueous solubility of the *N*-alkoxycarbonyl derivatives in 0.02 M acetate buffer of pH 5.0 was determined at room temperature ($\sim$ 22°C) by adding excess amounts of the compounds to the buffer in screw-capped test-tubes. The suspensions were placed in an ultrasonic water bath for 15 min and then rotated on a mechanical spindle for 20–25 h. It was ensured that saturation equilibrium was established. The mixtures were filtered, and an aliquot of the filtrate was diluted with buffer and analyzed by HPLC.

The apparent partition coefficients of mebendazole and the *N*-alkoxycarbonyl derivatives were determined in an octanol-buffer system at 20–22°C. The aqueous phase was a 0.02 M acetate buffer solution of pH 5.0. The buffer solution and octanol were mutually saturated at 20–22°C before use. The compounds were dissolved in the aqueous buffer phase at a concentration of 10^{-4}–2×10^{-4} M, and the octanol-water mixtures were shaken for about 2 h to reach a distribution equilibrium. The volumes of each phase were chosen so that the solute concentration in the aqueous phase, before and after distribution, could readily be measured using the above-mentioned HPLC procedure. At distribution equilibrium, the two phases were separated by centrifugation for about 5 min. The partition coefficients (P) were calculated from Eqn 1:

$$P = \left(\frac{C_i - C_w}{C_w}\right)\left(\frac{V_w}{V_o}\right) \quad (1)$$

where C_i and C_w represent the solute concentrations in the aqueous buffer phase before and after distribution, respectively, V_w is the volume of the aqueous phase, and V_o denotes the volume of the octanol phase. For each compound, determinations were carried out in triplicate, and the P values thereby obtained were reproducible to within $\pm 10\%$.

Results and Discussion

The various *N*-alkoxycarbonyl derivatives (**II–IV**) of mebendazole were prepared by reacting mebendazole with excess of the appropriate chloroformates. Since mebendazole exists in two tautomeric forms, the N(1)-H-tautomer and the N(3)-H-tautomer (shown as **I**), acylation of the benzimidazole nitrogens may occur either at N(1) or at N(3). HPLC analysis of compound **II** and **III** revealed only one major peak, accounting for

more than 95%, but two peaks of almost equal size were seen for compound **IV** indicating two isomers (**IVa** and **IVb**), consistent with the NMR spectrum. However, the NMR spectrum of the *N*-methoxycarbonyl derivative (**II**) showed that it also consisted of two isomers (**IIa** and **IIb**) in the proportion 1:1. On the other hand, the NMR spectrum of the *N*-ethoxycarbonyl derivative (**III**) showed that acylation had only occurred at position N(3) resulting in **IIIa**. Viswanathan and Sidhaye (1985) have previously reported that both isomers in most cases are obtained by acylation with alkyl chloroformates. Further support for the assignment of the position of substitution in compound **IIIa** was provided by NMR spectroscopy (see Structure determination).

Kinetics of hydrolysis

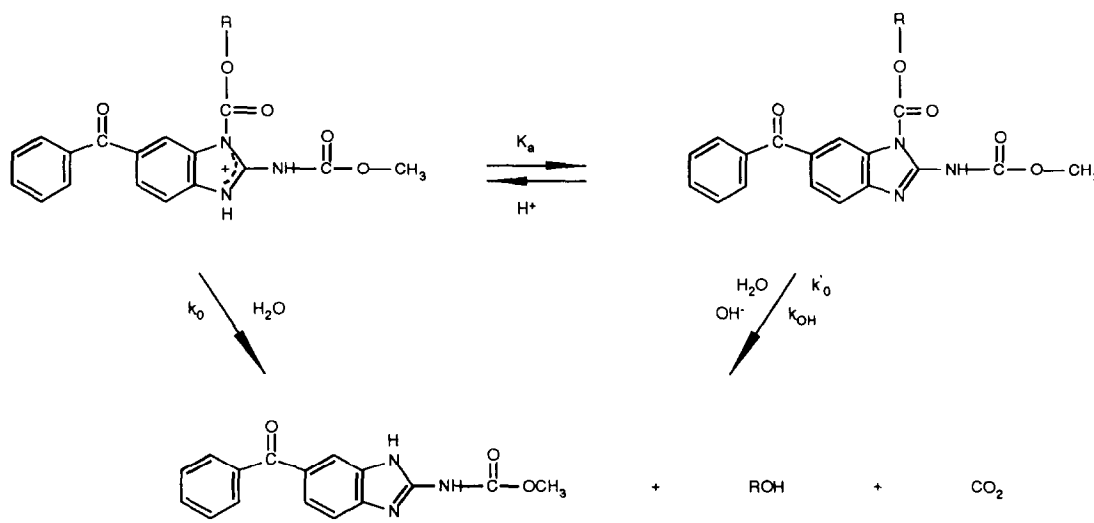
The chemical stability of the derivatives (**II–IV**) in aqueous solution was examined in detail as a function of pH and temperature. At constant pH and temperature, the hydrolysis of the compounds followed strict first-order kinetics and proceeded with the quantitative formation of mebendazole. At the buffer concentration used (0.02 M), no significant buffer catalysis was observed. The hydrolysis of the two isomeric forms of the *N*-propoxycarbonyl derivative (**IVa** and **IVb**) proceeded approximately with the same rate of hydrolysis (Table 3 and Fig. 1). This finding suggests that hydrolysis of the two isomeric forms of the *N*-methoxycarbonyl derivative (**IIa** and **IIb**) may also proceed with the same rate.

TABLE 3

pK_a values and rate data for the hydrolysis of various N-alkoxycarbonyl derivatives of mebendazole

Compound	pK_a	k_o (min^{-1})	k'_o (min^{-1})	k_{OH} ($M^{-1}\ min^{-1}$)
IIa,b	1.5	2.5×10^{-2}	2.5×10^{-4}	6000
IIIa	1.5	1.2×10^{-2}	1.2×10^{-4}	2700
IVa	1.4	1.1×10^{-2}	7.0×10^{-5}	2500
IVb	1.4	1.2×10^{-2}	8.0×10^{-5}	3000

The influence of pH on the rates of hydrolysis of the *N*-alkoxycarbonyl derivatives of mebendazole at 37°C is shown in Fig. 1 where the logarithm of the observed pseudo-first-order rate constants (k_{obs}) is plotted against pH. The rate of hydrolysis of the *N*-alkoxycarbonyl derivatives (**II–IV**) increases sharply above and below pH 4–6, whereas it approaches a plateau below pH 1. This is consistent with the pH-rate profile previously found for the *N*-ethoxycarbonyl derivative of thiabendazole (Nielsen et al., 1992). The shape of the pH-rate profile is similar to that of *N*-acetylimidazole (Jencks and Carriulo, 1959) as well as to those of *N*-ethoxycarbonyl imidazole



Scheme 2.

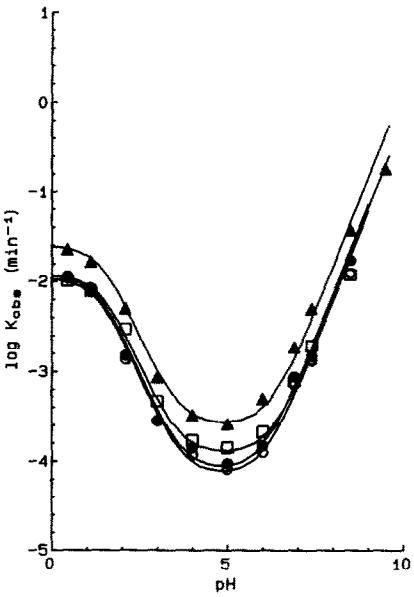


Fig. 1. The pH-rate profiles for hydrolysis of compound **II** (▲), compound **IIIa** (□) and compound **IVa** (○) and **IVb** (●) in aqueous solution ($\mu = 0.5$) at 37°C.

(Melchior and Fahrney, 1970) and other imidazole carbamates (Klixbüll and Bundgaard, 1983; Bundgaard and Møss, 1990; Buur and Bundgaard, 1991). The hydrolysis of *N*-acetylimidazole has been demonstrated to involve a water-catalyzed or spontaneous hydrolysis of the acetylimidazolium cation and a spontaneous or water-catalyzed as well as a hydroxide ion-catalyzed reaction of unprotonated acetylimidazole (Jencks and Carriulo, 1959; Wolfenden and Jenks, 1961). Accordingly, the reactions depicted in Scheme 2 are suggested to account for the hydrolysis of the *N*-alkoxycarbonyl derivatives of mebendazole, and the following rate expression may be formulated:

$$k_{obs} = k_o \frac{a_H}{a_H + K_a} + k'_o \frac{K_a}{a_H + K_a} + k_{OH} a_{OH} \frac{K_a}{a_H + K_a} \quad (2)$$

where a_H and a_{OH} refer to the hydrogen ion and hydroxide ion activity, respectively, K_a is the apparent ionization constant of the protonated benzimidazole group, $a_H/(a_H + K_a)$ and $K_a/(a_H + K_a)$ are the fractions of the derivative in protonated and unprotonated form, respectively, and k_o, k'_o, and k_{OH} are rate constants referring to the reactions shown in Scheme 2. The values of a_H and a_{OH} were calculated from the measured pH at 37°C according to the following equations (Harned and Harmer, 1933):

$$\log a_H = 0.14 - \text{pH} \quad (3)$$

$$\log a_{OH} = \text{pH} - 13.62 \quad (4)$$

The values of the specific rate and ionization constants for compounds (**II–IV**) listed in Table 3 were obtained from the pH-rate profile and Eqn 2.

The *N*-alkoxycarbonylation is seen to result in a marked depression of the basicity. This parallels the behaviour of similar carbamate derivatives of imidazole-containing compounds (Melchior and Fahrney, 1970; Bundgaard and Møss, 1990; Buur and Bundgaard, 1991; Nielsen et al., 1992).

At pH 2.0 and 37°C, the pseudo-first-order rate constant for the hydrolysis of the *N*-methoxycarbonyl derivative (**II**), the most labile derivative, was determined to be 6×10^{-3} min^{-1}, corresponding to a half-life of 2 h. However, its poor stability in the stomach does not appear to represent a problem regarding oral absorption, because it is suggested that the poor availability of mebendazole is due to its very low solubility and not to the absorption of mebendazole itself (Dawson et al., 1982, 1985a,b).

The effect of temperature (37–60°C), on the stability of compounds **II–IV** was studied in 0.02 M acetate buffer of pH 5.0, at which pH the compounds showed maximal stability. The rate constants obtained were plotted according to the Arrhenius equation (Eqn 5) (Fig. 2):

$$\log k = \log A - \frac{E_a}{2.303RT} \quad (5)$$

where A is the frequency factor, E_a denotes the apparent energy of activation, R is the gas constant and T is the absolute temperature (in K).

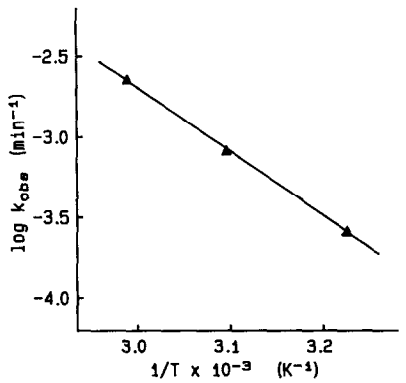


Fig. 2. Arrhenius plot of the rate of hydrolysis of the *N*-methoxycarbonyl derivative (**II**) in 0.02 M acetate buffer solution of pH 5.0.

From such plots, the Arrhenius parameters A and E_a were obtained and are listed in Table 4. On the basis of these data, it is possible to estimate the time for 10% degradation at pH 5.0 and at various temperatures. The results of such calculations are shown in Table 4.

Enzymatic hydrolysis of the derivatives

The susceptibility of the *N*-alkoxycarbonyl derivatives, to undergo a potential enzymatic hydrolysis, was studied in vitro at 37°C in a phosphate buffer of pH 7.4 containing either 80% human plasma, 80% rabbit plasma or 2.5% rabbit liver homogenate. The hydrolysis followed strict first-order kinetics and proceeded in all cases to give mebendazole in quantitative amounts. The observed half-lives for hydrolysis in the biological media are listed in Table 5 together with the half-lives of hydrolysis in pure buffer solutions of pH 7.4. As appears from the data, both plasma and rabbit liver enzymes markedly increase the rate of hydrolysis. Similar observations were reported for the *N*-alkoxycarbonyl derivatives of thiabendazole (Nielsen et al., 1992). These observations indicate that *N*-alkoxycarbonyl derivatives of benzimidazoles may be expected to be readily cleaved in vivo.

TABLE 4

Energies of activation (E_a) and frequency factors (A) for hydrolysis of various N-alkoxycarbonyl derivatives of mebendazole in aqueous solution at pH 5.0 ($\mu = 0.5$) and predicted shelf-lives ($t_{10\%}$) of such solutions at 5° and 21°C

Compound	E_a (kcal mol^{-1})	log A (A in min^{-1})	$t_{10\%}$ (h)	
			21°C	5°C
IIa,b	76.2	9.24	34	206
IIIa	65.1	7.10	50	233
IVa	66.7	7.13	92	440
IVb	66.7	8.18	87	478

TABLE 5

Half-lives ($t_{1/2}$) for the hydrolysis of various N-alkoxycarbonyl derivatives of mebendazole (II–IV) in aqueous buffer, 80% human and rabbit plasma and in 2.5% rabbit liver homogenate (pH 7.4) at 37°C

Compound	$t_{1/2}$ (min)			
	Buffer	Human plasma	Rabbit plasma	Rabbit liver
IIa,b	139	1.3	0.6	6.1
IIIa	347	2.9	2.1	5.5
IVa	517	5.3	1.6	4.4
IVb	462	4.4	1.4	6.8

Lipophilicity and aqueous solubility

The lipophilicity of the derivatives and of mebendazole was assessed by measuring the partition coefficients (P) between octanol and 0.02 M phosphate buffer of pH 5.0. At this pH, the derivatives **II–IV** and mebendazole are present in their free base forms. The log P values obtained are shown in Table 6. The difference in the log P

TABLE 6

Melting points (m.p.), aqueous solubilities (S) and partitions coefficients (P) of mebendazole (I) and the N-alkoxycarbonyl derivatives (II–IV)

Compound	m.p. (°C)	S [a] (mg/ml)	S (M)	log P [b]
I	289–290	5×10^{-4} [c]	1.7×10^{-6}	2.83
IIa,b	156–157	7×10^{-3}	1.9×10^{-5}	2.47
IIIa	165–166	1×10^{-2}	2.7×10^{-5}	2.93
IVa,b	113–114	5×10^{-3}	1.3×10^{-5}	3.43

[a] Solubility in 0.02 M acetate buffer solution of pH 5.0 at 21°C.
[b] Between octanol and 0.02 M acetate buffer of pH 5.0 at 21°C.
[c] From Allan and Watson (1983).

values for the derivatives **II–IV** is as expected on the basis of the π substituent values (Hansch and Leo, 1979).

The water solubilities of the compounds are also listed in Table 6. It is apparent that despite their increased lipophilicity the derivatives **III** and **IV** also possess a higher aqueous solubility relative to mebendazole.

The higher solubility of the derivatives **II–IV** relative to mebendazole can be ascribed to a decreased crystal lattice energy achieved by removing an NH- proton in the benzimidazole moiety by *N*-alkoxycarbonylation and is manifested in the pronounced melting point decrease (cf. Table 6) (Yalkowsky and Morozowich, 1980).

Conclusions

The results of the present study show that *N*-alkoxycarbonylation of the benzimidazole moiety in mebendazole may be a useful prodrug approach to obtain derivatives possessing increased water solubilities with retainment of appropriate lipophilicities. The *N*-alkoxycarbonyl derivatives possess an adequate stability with regard to oral administration, and are readily cleaved enzymatically and quantitatively to the parent drug. The potential utility of such a prodrug type, to improve the oral bioavailability of benzimidazole anthelmintics, has recently been demonstrated with derivatives **II–IV** given to rabbits (to be published elsewhere).

Acknowledgement

The collaboration and discussions of the NMR data with Dr Erik Falch, Department of Organic Chemistry, Royal Danish School of Pharmacy are gratefully acknowledged.

References

Allan, R.J. and Watson, T.R., The metabolic and pharmacokinetic disposition of mebendazole in the rat. *Eur. J. Drug Metab. Pharmacol.*, 8 (1983) 373–381.

Binderup, E. and Hansen, E.T., Chlorosulfates as reagents in the synthesis of carboxylic acid esters under phase-transfer conditions. *Synth. Commun.*, 14 (1984) 857–864.

Braithwaite, P.A., Roberts, M.S., Allan, R.J. and Watson T.R., Clinical pharmacokinetics of high dose mebendazole in patients treated for cystic hydatid disease. *Eur. J. Clin. Pharmacol.*, 22 (1982) 161–169.

Bundgaard, H. and Møss, J., Prodrugs of peptides: 6. Bioreversible derivatives of thyrotropin-releasing hormone (TRH) with increased lipophilicity and resistance to cleavage by the TRH-specific serum enzyme. *Pharm. Res.*, 7 (1990) 885–892.

Buur, A. and Bundgaard, H., Prodrugs of 5-fluorouracil: III. Hydrolysis kinetics in aqueous solution and biological media, lipophilicity and solubility of various 1-carbamoyl derivatives of 5-fluorouracil. *Int. J. Pharm.*, 23 (1985) 209–222.

Buur, A. and Bundgaard, H., Prodrugs of cimetidine with increased lipophilicity: *N*-acyloxymethyl and *N*-alkoxycarbonyl derivatives. *Acta Pharm. Nord.*, 3 (1991) 51–56.

Davis, A., Dixon, H. and Pawlowski, Z.S., Multicentre clinical trials of benzimidazole carbamates in human cystic echinococcosis (phase 2). *Bull. WHO*, 67 (1989) 503–508.

Davis, A., Pawlowski, Z.S. and Dixon, H., Multicentre clinical trials of benzimidazole carbamates in human echinococcosis. *Bull. WHO*, 64 (1986) 383–388.

Dawson, M., Allan, R.J. and Watson, T.R., The pharmacokinetics and bioavailability of mebendazole in man: a pilot study using [^{3}H]-mebendazole. *Br. J. Clin. Pharmacol.*, 14 (1982) 453–455.

Dawson, M., Braithwaite, P.A., Roberts, M.S. and Watson, T.R., The pharmacokinetics and bioavailability of a tracer dose of [^{3}H]-mebendazole in man. *Br. J. Clin. Pharmacol.*, 19 (1985a) 79–86.

Dawson, M. and Watson, T.R., The effect of dose form on the bioavailability of mebendazole in man. *Br. J. Clin. Pharmacol.*, 19 (1985b) 87–90.

Hansch, C. and Leo, A., *Substituent Constants for Correlation Analysis in Chemistry and Biology*, Wiley, New York, 1979.

Harned, H.S. and Harmer, W.J., The ionization constant of water and the dissociation of water in potassium chloride solutions from electromotive forces of cells without liquid junctions. *J. Am. Chem. Soc.*, 55 (1933) 2194–2206.

Jencks, W.P. and Carriulo, J., Imidazole catalysis: II. Acyl transfer and the reactions of acetyl imidazole with water and oxygen anions. *J. Biol. Chem.*, 234 (1959) 1272–1279.

Klixbüll, U. and Bundgaard, H., Prodrugs as drug delivery systems: XXIX. Imidazole-1-carboxylic acid esters of hydrocortisone and testosterone. *Arch. Pharm. Chem. Sci. Ed.*, 11 (1983) 101–110.

Marriner, S.E., Morris, D.L. and Bogan, J.A., Pharmacokinetics of albendazole in man. *Eur. J. Clin. Pharmacol.*, 30 (1986) 705–708.

McKellar, Q.A. and Scott, E.W., The benzimidazole anthelmintic agents – a review. *J. Vet. Pharmacol. Ther.*, 13 (1990) 223–247.

Melchior, W.B., Jr and Fahrney, D., Ethoxyformylation of proteins. Reaction of ethoxyformic anhydride with α-chymotrypsin, pepsin, and pancreatic ribonuclease at pH 4. *Biochemistry,* 9 (1970) 251–258.

Münst, G., Karlaganis, G. and Bircher, J., Plasma concentrations of mebendazole during treatment of echinococcosis. *Eur. J. Clin. Pharmacol.,* 17 (1980) 375–378.

Nielsen, L.S., Bundgaard, H. and Falch, E., Prodrugs of thiabendazole with increased water-solubility. *Acta Pharm. Nord.,* 4 (1992) 43–49.

Ravindranathan, T., Wakharkar, R.D. and Landge, A.B., Synthesis of mebendazole and enviroxime. Acylation of 2-amino-5-benzoylbenzimidazole. *Org. Prep. Proced. Int.,* 19 (1987) 9–16.

Schantz, P.M., Van den Bossche, H. and Eckert, J., Chemotherapy for larval echinococcosis in animals and humans: report of a workshop. *Z. Parasitenkd.,* 67 (1982) 5–26.

Thompson, R.C.A., *The Biology of Echinococcus and Hydatide Disease,* George Allen and Unwin, London, 1986.

Van den Bossche, H., Rochette, F. and Hörig, C., Mebendazole and related anthelmintics. *Adv. Pharmacol. Chemother.,* 19 (1982) 67–128.

Viswanathan, N. and Sidhaye, A.R. 1-Alkoxycarbonylbenzimidazole-2-carbamates. *Indian J. Chem.,* 24B (1985) 948–951.

Witassek, F., Burkhardt, B., Eckert, J. and Bircher, J., Chemotherapy of alveolar echinococcosis. Comparison of plasma mebendazole concentrations in animals and man. *Eur. J. Clin. Pharmacol.,* 20 (1981) 427–433.

Wolfenden, R. and Jencks, W.P., Acetyl transfer reactions of 1-acetyl-3-methyl-imidazolium chloride. *J. Am. Chem. Soc.,* 83 (1961) 4390–4393.

Yalkowsky, H. and Morozowich, W., A physical chemical basis for the design of orally active prodrugs. In Ariëns, E.J. (Ed.), *Drug Design,* Vol. IX, Academic Press, New York, 1980, pp. 121–185.