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Synthesis and Hydrolytic Stability Studies of Albendazole Carrier Prodrugs

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Abstract—Three *N*-acyl (**2**, **3**, and **4**), two *N*-alkoxycarbonyl (**5** and **6**), and one *N*-acyloxymethyl (**7**) derivatives of albendazole (**1**) have been prepared and assessed as potential prodrugs. The determination of the aqueous solubility and partition coefficient, as well as the conversion of these derivatives to **1** in buffer solution, human plasma, and pig liver esterase were determined.

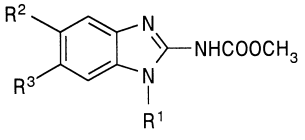
Introduction

Albendazole (**1**) is a potent, broad spectrum anthelmintic agent used mainly in the treatment of intestinal helminthiosis.[1] Also, it has been demonstrated that **1** is effective against some tissue-dwelling infestations, such as trichinellosis, hydatid disease and neurocysticercosis.[2] However, high doses and long treatment are required in the latter cases, due mainly to the poor solubility and absorption of **1**.[2,3] In previous studies various *N*-acyl, *N*-alkoxycarbonyl, and *N*-acyloxymethyl derivatives of other benzimidazole carbamates were evaluated as possible prodrug form with enhanced solubility properties.[4,5] In order to increase the bioavailability of **1** by the prodrug approach, we have synthesized three *N*-acyl (**2**, **3**, and **4**), two *N*-alkoxycarbonyl (**5** and **6**), and one *N*-acyloxymethyl (**7**) derivatives of this compound (Table 1). In this work, aqueous solubility, partition coefficients and stability in buffer solution, human plasma, and pig liver esterase of derivatives synthesized were measured.

Chemistry

Compounds **2**–**4** were prepared by known procedures.[4] Treatment of a suspension of **1** (Smith & Kline, Mexico) and Na_2CO_3 in dichloromethane, with an equivalent of ethyl chloroformate for 12 h, led to a mixture of **5** and **6**. After workup and recrystallization of the residue from isopropyl ether, **5** could be obtained. Compound **6** was isolated by evaporating the mother liquor and washing the residue with ethyl acetate–petroleum ether. For the synthesis of **7**, commercial **8** was treated with di-*tert*-butyldicarbonate to give **9**, which could be converted to **10** according to the procedure of Binderup et al.[6] A solution of **10** in DMF was then added to a previous 2 h reaction mixture of **1** and NaH in DMF. After 12 h of reaction, water was added, and the suspension was filtered to give 1-[4-(*tert*-butoxy-carbonylaminomethyl)benzoyloxymethyl]-albendazole, as a mixture of two isomers. Compound **11** was obtained by recrystallization of the residue from benzene; a second portion remained in the mother liquor together with the other

Table 1. Synthesized derivatives of albendazole (**1**–**7**)



Compd	R^1	R^2	R^3
1	H	$S(CH_2)_2CH_3$	H
2	$COCH_3$	$S(CH_2)_2CH_3$	H
3	$COCH_2CH_3$	$S(CH_2)_2CH_3$	H
4	$COCH_2CH_2CH_3$	$S(CH_2)_2CH_3$	H
5	$COOCH_2CH_3$	$S(CH_2)_2CH_3$	H
6	$COOCH_2CH_3$	H	$S(CH_2)_2CH_3$
7	$CH_2OOCC_6H_4NH_2$ 2HCl	$S(CH_2)_2CH_3$	H

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isomer, but their separation was not successful. Compound **11** was dissolved in ethyl acetate and a 3 N solution of hydrochloric acid in ethanol was added. The mixture was stirred for 3 h at room temperature. The precipitate was collected and recrystallized from methanol–ether to give the dihydrochloride of **7** (Scheme 1). The structures of **2**–**7** were established by IR, ^{1}H NMR, ^{13}C NMR, and MS data.[7–12]

Determination of apparent partition coefficients and aqueous solubility

The apparent partition coefficients of **1**–**7** were determined using HPLC retention times, as previously described by Thomas et al.[13] In this case, the reference compounds (benzaldehyde, benzene, toluene, chlorobenzene, xylene, fluorene and anthracene) were detected at 255 nm, whereas **1**–**7** were detected at 310 nm. The log of the capacity factor (K) in each mobile phase composition was calculated using the equation:

$$\log K = \log[(t_r - t_0)/t_0]$$

where t_0 refers to the column 'dead volume' (elution time of methanol, a nonretained compound). The log of the predicted capacity factor at 0% of methanol ($\log K_0$) was determined for each standard by linear regression analysis of log K versus percent methanol. Calculation of log K_0 values of **1**–**7** was then done allowing predicted P_0/w values to be calculated using the regression equation obtained for the standards of known P_0/w.

All solubility studies were performed in 0.02 M phosphate buffer of pH 6.0 at 25 °C by adding an excess amount of the compounds to the buffer in screw-capped test tubes.[5] It was ensured that saturation equilibrium was established with exception of compounds **2**, **3**, and **4**, which were evaluated after 15 min due to stability problems (>25% degradation). The pH values of the solution were recorded prior to their filtration through 0.45 mm pore size Millipore filters. The solutions were analyzed for compound content using a UV spectrophotometric assay.

Hydrolysis in aqueous solutions

All rate studies were performed in aqueous buffer solution at 37±0.2 °C. The buffers used were hydrochloric acid, acetate, phosphate, and borate buffers. The reactions were initiated by adding 100 μL of a stock solution of the prodrugs in methanol to 10 mL of preheated buffer solution in screw-capped test tubes; the final concentration being about 4×10^{-4} M (1% methanol).[14] At appropriate intervals, 10 μL of samples were taken and chromatographed immediately. Appearance of **1** was followed by HPLC as previously described by Hurtado et al.[15]

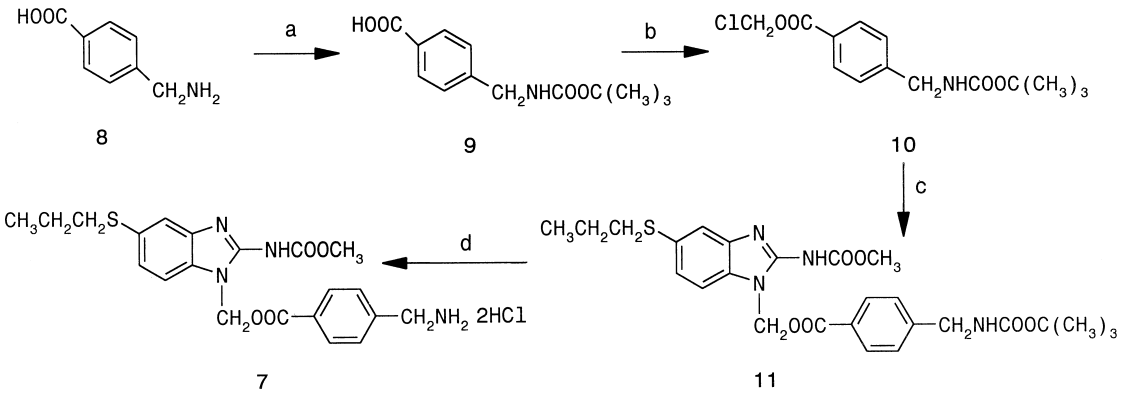
Hydrolysis in biological media

Compounds **1**–**7** were incubated at 37 °C in 80% human plasma; at an initial concentration of 4×10^{-4} M (1% methanol). At various time points, samples of plasma were withdrawn and extracted with a Sep-Pak C18 cartridge. Appearance of **1** was followed by HPLC as previously described.[15] Enzymatic hydrolysis was conducted similarly with pig liver esterase (Sigma Co.) instead of plasma.

Results and Discussion

Prodrugs **2**–**6** were prepared by one-step reaction and obtained in low yields (30%) due to difficulties that appeared during the separation of each isomer. In the case of **2**–**4**, it was not possible to obtain the two isomers due to instability of one of them.

In the ^{1}H NMR spectrum for **2**, the H-7 absorption is shifted down-field from that in **1** by about 1.0 ppm in DMSO, because of the orientation of the acetyl carbonyl group. Since the ^{1}H NMR spectra of **3**, **4**, and **5** exhibit similar shifts of their H-7 absorption, they must also be 1/5 disubstituted. In **6**, H-7 appears as a doublet at 7.75 (J = 2 Hz). Differentiation between **5** and **6** could be made from the coupling constant of the signal for H-7, the proton deshielded by N^1-$COOC_2H_5$ group (Table 2).[16] Compounds **2**–**4**, with –COR group, and **5** and **6** with –$COOC_2H_5$ group, would be expected to exist in



Scheme 1. (a) $O[CO_2C(CH_3)_3]_2$, *tert*-BuOH; (b) $ClCH_2OSO_2Cl$, CH_2Cl_2, H_2O, $[CH_3(CH_2)_3]_4N(HSO_4)$, $NaHCO_3$; (c) **1**, DMF; (d) HCl/EtOH.

Table 2. Chemical shifts in H-7 and H-4 for compounds **1**–**6**

Compd	H-7 (ppm) (Hz)	H-4 (ppm) (Hz)
1	7.33 ($J=10$)	7.42 ($J=2$)
2	7.97 ($J=10$)	7.41 ($J=2$)
3	8.01 ($J=9$)	7.43 ($J=2$)
4	7.99 ($J=9$)	7.42 ($J=2$)
5	7.70 ($J=10$)	7.53 ($J=2$)
6	7.75 ($J=2$)	7.51 ($J=10$)

Table 3. Melting points, log *P*, and solubility for compounds **1**–**7**

Compd	mp (°C)	log P[a]	S[b] (mM)[a] [λ_{max} (nm)]
1	205	3.07±0.36	16.2±5.36 (295)
2	128	2.82±0.28	58.2±2.33 (332)
3	145	3.17±0.26	42.9±5.22 (328)
4	138	3.92±0.31	17.4±1.27 (334)
5	75	3.92±0.31	85.3±4.32 (317)
6	89	3.19±0.57	83.6±5.43 (312)
7[c]	nd[d]	2.91±0.42	114±8.34 (322)

[a]Mean±standard deviation.
[b]S, solubility pH 6.
[c]Dihydrochloride salt.
[d]nd, not determined.

the *endo* conformation which relieves the dipole–dipole interaction between this group and the $NHCOOCH_3$ in the 2-position.[17] The N^1-substitution position in **7** was assigned by ^1H NOESY spectroscopy.[18]

The melting points, log *P* and solubility of **1**–**7** are shown in Table 3.

Compound **7** was the most soluble of all compounds (8-fold higher solubility than **1**). In relation to **5** and **6**, their higher solubility could be ascribed to a decreased crystal lattice energy achieved by removing the NH-proton in **1**, and manifested in the pronounced melting point decrease (>100 °C).[4] Compounds **2**–**4** did not show significant stability in aqueous solution, for this reason they were not used in further studies. The difference in the log P values for the derivatives **2**–**6** is as expected on the basis of the π substituents values.[19]

The hydrolysis of **5**, **6**, and **7** at 37 °C in the two buffer solutions tested, pig liver esterase, and human plasma displayed first-order kinetics to give **1**. Order rate constants observed (k_{obs}) were calculated from slopes of linear plots of the logarithm% formed **1** versus time. The k_{obs} and half-lives ($t_{1/2}=0.693/k_{obs}$) for the hydrolysis are listed in Tables 4 and 5.

As appears from data, plasma and esterase markedly increase the rate of hydrolysis in relation to buffer solutions. This indicates that **5**, **6**, and **7** may be expected to be readily cleaved in vivo. It is of interest to note that the rates of biological hydrolysis observed for **5** and **6** are considerably higher than that of **7**. The position of the ethoxycarbonyl group, in **5** and **6**, has only a minor influence in the hydrolysis in plasma, esterase, and buffer solution at pH 7.4.

The influence of pH on the hydrolysis rate was studied for the compound **7** at 37 °C. The k_{obs} obtained are shown in Table 6.

Table 4. k_{obs} for the hydrolysis of prodrugs **5**–**7**

	k_{obs} (10^{-3} min^{-1})[a]			
Compd	pH 5.0	pH 7.4	Plasma	Esterase
5	2.19±0.59	5.73±1.08	83.29±10.24	100.14±29.35
6	4.21±0.93	5.78±0.95	96.33±23.12	99.14±34.56
7	3.01±1.15	5.62±2.08	12.43±5.68	15.29±3.34

[a]Mean±standard deviation.

Table 5. $t_{1/2}$ for the hydrolysis of prodrugs **5**–**7**

	$t_{1/2}$ (min)			
Compd	pH 5.0	pH 7.4	Plasma	Esterase
5	316.43	120.94	8.32	6.92
6	164.60	119.89	7.19	6.99
7	230.23	123.30	55.75	45.32

Table 6. Influence of pH on the hydrolysis rate for compound **7**

pH	k_{obs} (min^{-1})[a]	pH	k_{obs} (min^{-1})[a]
1	0.3203±0.1305	6.	0.0036±0.0015
2	0.2826±0.0680	7.4	0.0056±0.0020
3	0.0323±0.0057	8	1.8066±0.6651
4	0.0087±0.0010	9	8.0010±1.1861
5	0.0030±0.0011		

[a]Mean±standard deviation.

The logarithms of k_{obs} were plotted against pH. The pH-rate profile was U-shaped indicating the occurrence of apparent specific acid and base catalysis as well as a spontaneous or water-catalyzed reaction according to the following rate expression: $k_{obs}=k_0+k_H a_H+k_{OH}a_{OH}$, where a_H and a_{OH} refer to hydrogen ion and hydroxide ion activity, respectively.[5] The latter was calculated from the measured pH at 37 °C according to the following equation: $\log a_H = pH - 13.62$.[20] Values of the second-order rate constants for the apparent specific acid (k_H) and specific base (k_{OH}) catalyzed decomposition were determined from the straight line portion of the pH–rate profile at low and high pH values, respectively. The value of the apparent first-order rate constant for spontaneous decomposition (k_0) was obtained from the plateau region of the pH-rate profile. The values of the rate constants were $k_H=2.11\ M^{-1}\ min^{-1}$, $k_0=3.65\times10^{-3}\ min^{-1}$, $k_{OH}=37.45\ M^{-1}\ min^{-1}$. Compound **7** is most stable at weakly acidic pH. Studies are in progress to determine the permeability of **5**–**7** into Caco-2 cells.

Acknowledgements

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6. Binderup, E.; Hansen, E. T. *Synth. Commun.* **1984**, *14*, 857.
7. Methyl 1-acetyl-5-(propylthio)-2-benzimidazole-carbamate (**2**). IR (KBr) ν 3312, 2962, 1718, 1646, 1612, 1320, 1284, 1248, 1188, 1116 cm^{-1}; ^1H NMR (DMSO-d_6) δ 0.9 (t, 3H, $CH_3CH_2CH_2S$–), 1.59 (m, 2H, $CH_3CH_2CH_2S$), 2.78 (s, 3H, CH_3CO–), 2.9 (t, 2H, $CH_3CH_2CH_2S$–), 3.7 (s, 3H, –OCH_3), 7.15 (dd, $J=2$ Hz, $J=10$ Hz, 1H, H-6), 7.4 (d, $J=2$ Hz, 1H, H-4), 7.99 (d, $J=10$ Hz, 1H, H-7), 12.0 (s, 1H, exchangeable, –N*H*CO–) ppm; M(EI) *m*/*z* 307 (M^+, 20%), 265 (100%). Anal. calcd $C_{14}H_{17}N_3O_3S$: C, 54.66; H, 5.53; N, 13.66; found: C, 54.66; H, 5.53; N, 13.66.
8. Methyl 1-propyl-5-(propylthio)-2-benzimidazole-carbamate (**3**). IR (KBr) ν 3312, 2964, 1722, 1648, 1602, 1310, 1276, 1152, 1114 cm^{-1}; ^1H NMR (DMSO-d_6) δ 1.0 (t, 3H, $CH_3CH_2CH_2S$–), 1.15 (t, 3H, CH_3CH_2CO–), 1.6 (m, 2H, $CH_3CH_2CH_2S$–), 2.9 (t, 2H, $CH_3CH_2CH_2S$–), 3.3 (q, 2H, CH_3CH_2CO–), 3.65 (s, 3H, –OCH_3), 7.15 (dd, $J=2$ Hz, $J=9$ Hz, 1H, H-6), 7.41 (d, $J=2$ Hz, 1H, H-4), 7.95 (d, $J=9$ Hz, 1H, H-7), 12.1 (br, 1H, –N*H*CO–) ppm; M(EI) *m*/*z* 321 (M^+, 13%), 265 (100%). Anal. calcd $C_{15}H_{19}N_3O_3S$: C, 56.05; H, 5.96; N, 13.08; found: C, 56.66; H, 5.74; N, 13.46.
9. Methyl 1-butyl-5-(propylthio)-2-benzimidazole-carbamate (**4**). IR (KBr) ν 3334, 2958, 1716, 1632, 1588, 1270, 1098 cm^{-1}; ^1H NMR (DMSO-d_6) δ 1.0 (t, 3H, $CH_3CH_2CH_2S$–), 1.2 (t, 3H, $CH_3CH_2CH_2CO$–), 1.65 (m, 4H, $CH_3CH_2CH_2S$–, $CH_3CH_2CH_2CO$–), 2.85 (t, 2H, $CH_3CH_2CH_2S$–), 3.3 (t, 3H, $CH_3CH_2CH_2CO$–), 7.15 (dd, $J=2$ Hz, $J=9$ Hz, 1H, H-6), 7.41 (d, $J=2$ Hz, 1H, H-4), 8.01 (d, $J=9$ Hz, 1H, H-7), 12.1 (br, 1H, –N*H*CO–) ppm; M(EI) *m*/*z* 335 (M^+, 11%), 265 (100%). Anal. calcd $C_{16}H_{21}N_3O_3S$: C, 57.29; H, 6.32; N, 12.54; found: C, 57.19; H, 5.94; N, 12.46.
10. Methyl 1-ethoxycarbonyl-5-(propylthio)-2-benzimidazolecarbamate (**5**). IR (KBr) ν 3300, 2988, 1774, 1720, 1590, 1544, 1372, 1328, 1288, 1218, 1164 cm^{-1}; ^1H NMR (DMSO-d_6) δ 0.95 (t, 3H, $CH_3CH_2CH_2S$–), 1.37 (t, 3H, –OCH_2CH_3), 1.55 (m, 2H, $CH_3CH_2CH_2S$–), 2.92 (t, 2H, $CH_3CH_2CH_2S$–), 3.71 (s, 3H, –OCH_3), 4.45 (q, 2H, –OCH_2CH_3), 7.26 (dd, $J=1.8$ Hz, $J=9$ Hz, 1H, H-6), 7.53 (d, $J=1.8$ Hz, 1H, H-4), 7.70 (d, $J=9$ Hz, 1H, H-7), 10.31 (s, 1H, exchangeable, N*H*CO–); ^{13}C NMR (DMSO-d_6) δ 13.05, 13.72, 21.90, 35.19, 52.72, 64.47, 114.54, 118.88, 125.06, 129.30, 131.83, 141.05, 145.05, 149.69, 152.89 ppm; M(EI) *m*/*z* 337 (M^+, 100%). Anal. calcd $C_{15}H_{19}N_3O_4S$: C, 53.40; H, 5.68; N, 12.46; found: C, 53.38; H, 5.61; N, 12.41.
11. Methyl 1-ethoxycarbonyl-6-(propylthio)-2-benzimidazolecarbamate (**6**). IR (KBr) ν 3314, 2960, 1764, 1736, 1536, 1374, 1334, 1288, 1220, 1186 cm^{-1}; ^1H NMR (DMSO-d_6) δ 0.95 (t, 3H, $CH_3CH_2CH_2S$–), 1.37 (t, 3H, OCH_2CH_3), 1.57 (m, 2H, $CH_3CH_2CH_2S$–), 2.92 (t, 2H, $CH_3CH_2CH_2S$–), 3.71 (s, 3H, OCH_3), 4.45 (q, 2H, –OCH_2CH_3), 7.29 (dd, $J=2$ Hz, $J=9$ Hz, 1H, H-5), 7.51 (d, $J=9$ Hz, 1H, H-4), 7.75 (d, $J=2$ Hz, 1H, H-7), 10.32 (s, 1H, exchangeable, N*H*CO–); ^{13}C NMR (DMSO-d_6) δ 12.98, 13.66, 21.90, 35.59, 52.69, 64.47, 114.57, 119.03, 125.89, 131.21, 131.49, 138.87, 144.43, 149.57, 153.113 ppm; M(EI) *m*/*z* 337 (M^+, 100%). Anal. calcd $C_{15}H_{19}N_3O_4S$: C, 53.40; H, 5.68; N, 12.46; found: C, 53.41; H, 5.63; N, 12.60.
12. Methyl 1-[(4-aminomethyl)benzoyloxy-5-(propylthio)-2-benzimidazolecarbamate dihydrochloride (**7**). IR (KBr) ν 3432, 2958, 2606, 1768, 1730, 1614, 1454, 1252, 1228, 1076 cm^{-1}; ^1H NMR ($CDCl_3$/MeOD) δ 1.038 (t, 3H, $CH_3CH_2CH_2S$–), 1.68 (m, 2H, $CH_3CH_2CH_2S$–), 2.97 (t, 2H, $CH_3CH_2CH_2S$–), 4.03 (s, 3H, –OCH_3), 4.17 (s, 2H, –CH_2NH_2HCl), 6.62 (s, 2H, –CH_2OOC–), 7.48 (dd, $J=2$ Hz, $J=10$ Hz, 1H, H-6), 7.62 (d, $J=10$ Hz, 2H, H-2′, H-6′), 7.67 (d, $J=2$ Hz, 1H, H-4), 7.77 (d, $J=10$ Hz, 1H, H-7), 8.09 (d, $J=10$ Hz, 2H, H-3′, H-6′); ^{13}C NMR ($CDCl_3$/MeOD) δ 13.38, 22.60, 36.41, 43.11, 54.91, 66.04, 112.44, 114.02, 127.11, 127.54, 128.79, 129.55, 129.75, 131.15, 136.94, 139.57, 145.17, 153.36, 165.45 ppm. Anal. calcd $C_{21}H_{26}N_4O_4SCl_2$: C, 50.39; H, 5.24; N, 11.20; found: C, 50.23; H, 5.16; N, 11.04.
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