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PREPARATION OF N-METHOXYCARBONYL-N′-[2-NITRO-4(5)-PROPYL-THIOPHENYL]THIOUREA AS PRODRUGS OF ALBENDAZOLE.

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Abstract: N-methoxycarbonyl-N′-(2-nitro-4-propylthiophenyl)thiourea and N-methoxycarbonyl-N′-(2-nitro-5-propylthiophenyl)thiourea, prodrugs of albendazole, have been synthesized. The biotransformation in rats, after oral administration of these prodrugs to albendazole and albendazole sulphoxide, is described.
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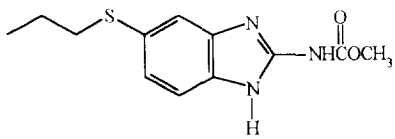
Introduction. In the chemotherapy of many human and veterinary parasitic diseases scientists have widely used benzimidazoles,[1] among which albendazole (ABZ, **1**) has been successfully used in the treatment of enteral helminthic infections[2,3] as well as some tissue-dwelling parasitosis, such as trichinellosis,[4] echinococcosis[5] and neurocysticercosis.[2,6] Despite its anthelmintic activity, albendazole has poor oral bioavailability as a result of its low aqueous solubility (ca. 0.5 mg/L)[7] and its extensive first pass metabolism in the small intestine[8] and the liver,[9] where it is rapidly transformed into its main metabolite–albendazole sulphoxide derivative (ricobendazole).[2,8,9] In the plasma of men, dogs, rats, cattle, and sheep,[10,11] albendazole is undetectable and the levels of albendazole sulphoxide have shown great intraindividual variability;[12] therefore, high doses and long treatments are necessary to reach therapeutic effects.



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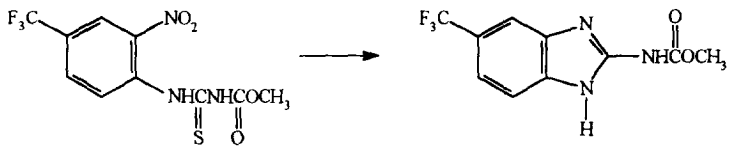
An approach to increase the oral bioavailability of benzimidazoles is the production of prodrugs (pro-benzimidazoles) with higher solubility and adequate lipophilicity.[13,14] In a previous report, Walchshofer et al.[15] demonstrated that N-methoxycarbonyl-N′-[2-nitro-4-trifluoromethylphenyl]thiourea undergo nitroreduction and cyclization in gerbils to give their corresponding benzimidazole-2-carbamate (Figure 1). In addition, this compound shows anthelmintic activity against *Echinococcus multilocularis*.[15]



Prodrug Drug

Figure 1

This observation stimulated us to design prodrug **2** (Figure 2), based on an analogy to the phenyl thiourea described above. On the other hand, prodrug **3** was designed in order to find out if there is any preference in the reduction of the nitro group and hence cyclization to give albendazole sulphoxide. In this paper, we report the synthesis of phenyl thioureas **2** and **3** and the bioconversion of these compounds into ABZ and albendazole sulphoxide in rats.

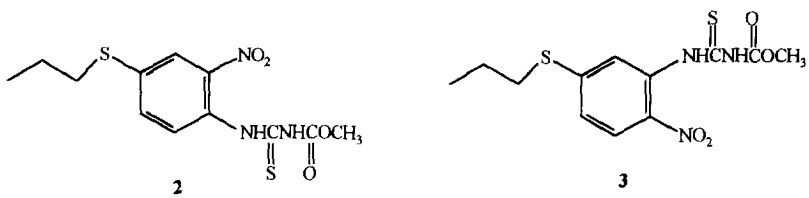


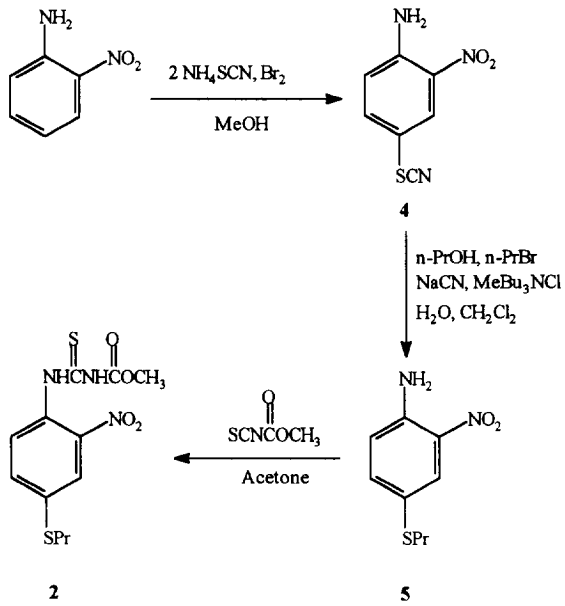
Figure 2

Chemistry. The routes used to synthesize prodrugs **2** and **3** are depicted in Schemes 1 and 2.

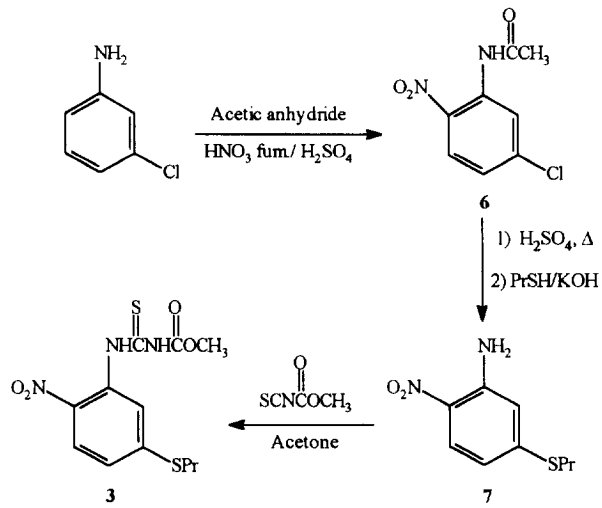
Preparation of N-Methoxycarbonyl-N′-(2-Nitro-4-Propylthiophenyl)Thiourea (2). The synthesis began with the heating of a mixture of 2-nitroaniline, NH_4SCN, and Br_2 in methanol under a nitrogen atmosphere at reflux for an extended period of time.[16] This procedure gave an excellent yield of **4**. The reaction of **4** with a mixture of n-PrOH, n-PrBr, NaCN, $MeBu_3NCl$, H_2O, and CH_2Cl_2 gave **5**.[16] The methoxycarbonyl isothiocyanate, [15] formed in situ from potassium thiocyanate and methyl chloroformate in acetone, was allowed to react with **5** to produce the phenyl thiourea **2**.

Preparation of N-Methoxycarbonyl-N′-(2-Nitro-5-Propylthiophenyl)Thiourea (3). The synthesis of this prodrug began with the nitration of 3-chloroaniline by using fuming nitric acid and acetic anhydride to give **6**.[17] The reaction of **6** in hot H_2SO_4 gave 3-chloro-6-nitroaniline, which was reacted with PrSH/NaOH in ethylene glycol to give **7**.[17] The methoxycarbonyl isothiocyanate, [15] formed as above, was allowed to react with **7** to produce the phenyl thiourea **3**. Both prodrugs, **2** and **3**, were recrystallized from EtOH-H_2O.

Scheme 1.



Scheme 2.



Biological Assay. ABZ was administered as a commercial suspension (Zentel), and the solutions of prodrug **2** and prodrug **3** were freshly prepared in ethanol, Tween 80, water (50:25:25) at room temperature. Doses of 0.037 mmol/Kg of each compound were given orally to Sprague Dawley rats. Blood samples were obtained

from the external jugular of 2 rat/group, each from 2 h to 12 h post-dose. The plasma was separated by centrifugation and extracted with a Sep-Pak C18 cartridge. Samples were analyzed by HPLC, as previously described by Hurtado et al.[18]

Results and Discussion

The mp, IR, and ^{1}H NMR data of compounds **4**, **5**, **6**, and 7 were consistent with those reported in the literature.[16,17] The structures of prodrugs **2** and **3** were established by IR, MS, ^{1}H NMR, and ^{13}C NMR. Elementary analyses were within ± 0.4% of calculated values.

Table 1. Physical data of the prodrugs **2** and **3**.

Prodrug	Yield (%)	mp (°C)	Spectroscopic data
2	35	121-122	IR (KBr) ν:3170, 1732, 1566, 1048, cm^{-1}. ^{1}H NMR (DMSO-d_6) δ: 1(t, 3H, -C*H*$_3$), 1.7 (m, 2H, -C*H*$_2$-), 3.1 (t, 3H, -C*H*$_2$S), 3.7 (s, 3H, C*H*$_3$O), 7.6 (dd, 1H, *H*-C5), 7.8 (d, 1H, *H*-C6), 7.9 (d, 1H, *H*-C3), 11.6 (s, 1H, -N*H*), 11.7 (s, 1H, N*H*), ppm. ^{13}C NMR (DMSO-d_6) δ: 13.14 (CH_3-), 21.66 (-CH_2-), 33.56(-CH_2S-), 53.19 (CH_3O-), 122.29 (C6), 129.14 (C1), 130.50 (C3), 131.71 (C5), 137.40 (C4), 144.45 (C2), 153.85 (C=O carbamate), 180.137 (C=S thiourea). MS (CI, CH_4), m/z: 330 (M^++1) (100%). Anal. ($C_{12}H_{15}N_3O_4S_2$) C, H, N.
3	48	122	IR (KBr) ν:3186, 1736, 1578, 1038, cm^{-1}. ^{1}H NMR (DMSO-d_6) δ: 1.0 (t, 3H, C*H*$_3$), 1.7 (m, 2H, -C*H*$_2$-), 3.1 (t, 2H, -C*H*$_2$S), 3.8 (s, 3H, C*H*$_3$O-), 7.3 (dd, 1H, *H*-C4), 7.9 (d, 1H, *H*-C6), 8.0 (d, 1H, *H*-C3), 11.6 (s, 1H, -N*H*-), 12.1 (s, 1H, -N*H*-), ppm. ^{13}C NMR (DMSO-d_6) δ: 13.117 (CH_3-), 21.67 (-CH_2-), 32.66 (CH_2S-), 53.14 (CH_3O-), 124.03 (C6), 125.5 (C4, C3), 132.97 (C1), 139.50 (C2), 145.96 (C5), 153.67 (C=O carbamate), 179.80 (C=S thiourea). MS (CI, CH_4), m/z: 330 (M^++1) (100%). Anal. ($C_{12}H_{15}N_3O_4S_2$) C, H, N.

Compound **4** was prepared in good yield (90%) according to the procedure described in the literature.[16] However, compound **5** was isolated in low yield (47%) due to difficulties that appeared during the purification by recrystallization from hexane of this low-melting compound. The last compound of this series, prodrug **2**, gave low yields (37%), probably due to the diminished nucleophilic character of the amino group conjugated to the *o*-nitro group. Another problem encountered was the formation of a less polar secondary product which was eliminated during the recrystallization. In an effort to increase the yield of prodrug **2** the solvent and temperature of the reaction were changed, but the same results were obtained.

Compound **6** was prepared with an excellent yield in the one-step reaction by using fuming nitric acid in acetic anhydride without isolation of the intermediate amide. However, **6** had to be hydrolized with hot concentrated

H_2SO_4 before the nucleophilic substitution with 1-propanethiolate in ethylene glycol. Prodrug **3** was obtained in low yield (30%) due to the same difficulties presented in the preparation of **2**.

In relation to the biotransformation studies, all rats survived after the administration of prodrugs, and no signs of toxicity were observed. It was evident by HPLC that prodrugs **2** and **3** suffered bioconversion to ABZ and its metabolite (Table 2).

Table 2. Bioconversion of prodrugs in rats.

	1		**2**		**3**	
Time (h)	ABZ-S (μg/mL)	ABZ (μg/mL)	ABZ-S (μg/mL)	ABZ (μg/mL)	ABZ-S (μg/mL)	ABZ (μg/mL)
2	0.5930	0.055	0.1802	d	nd	nd
2	0.2450	d	0.1088	d	nd	nd
4	0.3084	d	0.0927	nd	0.0960	nd
4	0.1935	d	0.0958	nd	nd	nd
6	0.2875	d	nd	d	nd	0.1500
6	0.4081	d	0.1633	nd	nd	0.7450
8	0.2803	d	nd	d	d	0.0792
8	0.1113	d	nd	d	nd	nd
10	0.2696	d	nd	d	nd	d
10	0.1297	d	nd	d	0.0889	d
12	3.4802	0.0980	0.2704	d	0.1622	nd
12	0.5919	d	0.2704	d	nd	nd

ABZ: albendazole; ABZ-S: albendazole sulphoxide; d = detectable; nd = not detectable.

Although prodrugs **2** and **3** did not reach an equivalent concentration of ABZ and ABZ sulphoxide as ABZ itself, prodrug **2** was more efficiently biotransformed than prodrug **3**. Also, the production of ABZ and its metabolite from prodrug **2** resembles ABZ itself. In addition, a great variability in the amount of ABZ detected/ ABZ sulphoxide in plasma samples taken at the same time from animals treated with the prodrugs was evident. However, the same variability was observed with ABZ itself. The data obtained in this study show that prodrug **3** produced ABZ after 6 h and albendazole sulphoxide after 10 h. This suggests that the reduction of the nitro group is disminished by the electron-donating capacity of the propylthio group in the para position, effect that is not exerted in prodrug **2**. It is likely then, that the biotransformation of prodrugs **2** and **3** is initiated with the reduction of the nitro group by the intestinal microflora followed by intramolecular cyclization of the molecules. Studies are currently being carried out in order to evaluate the anthelmintic activity of these prodrugs.

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