Highly Water-Soluble Prodrugs of Anthelmintic Benzimidazole Carbamates: Synthesis, Pharmacodynamics, and Pharmacokinetics

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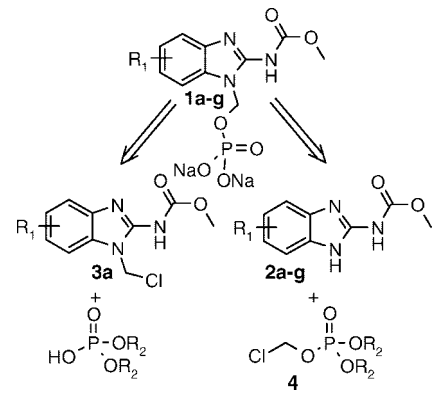
Abstract: Highly water-soluble prodrugs **1a**−**g** of anthelmintic benzimidazole carbamates **2a**−**g** were synthesized. These prodrugs combine high aqueous solubility and stability with high lability in the presence of alkaline phosphatases. The veterinary utility of **1a** was shown by a pharmacodynamic and pharmacokinetic study performed in swine. Comparable anthelmintic efficacy was observed with prodrug **1a** or the parent fenbendazole **2a**. The pharmacokinetic results showed that **2a** is better absorbed when derived from **1a** than when applied as such.

Parasitic infection in animals is a major cause of animal suffering, loss in production, and in extreme cases even death. Among the parasitic diseases, a survey has shown that infection by endo parasitic helminths is perceived as the most important when compared to infections by ectoparasites such as ticks, mange mites, flies, or lice.[1] The following figures help to get an understanding on the economic impact of helminthiasis on livestock production. Losses induced by gastrointestinal parasites to sheep production in Australia were estimated at $175 millions[2] in 1995 and about $350 millions for ruminants in the U.S.[3] The most common intestinal nematode parasite of pig worldwide, *Ascaris suum*, was solely responsible for condemnation of livers valued at an estimated $17.5 million in Eastern Europe in 1999, while an estimated loss of $60.1 million for extra feed needed to attain the desired bodyweight was incurred in the U.S. for the same period.[4] The development of potent chemotherapies against veterinary relevant helminths started about 40 years ago[5] with the discovery of Levamisole. Since then, even better drugs have been introduced on the market, and two classes of compounds have gained prevalence in the field: the macrocyclic lactones and the benzimidazoles.[6] The benzimidazoles are characterized by a broad spectrum of activity associated with a wide safety margin combined with ovicidal larvicidal and adulticidal effects. The most effective representatives of the group are fenbendazole **2a** (Scheme 1, R_1 = PhS) and albendazole **2c** (Scheme 1, R_1 = *n*-PrS) because of their particularly long in vivo half-lives. The anthelmintic benzimidazoles are generally poorly soluble in water and are given orally as suspension, paste, powder, or intraruminal bolus.[7] Among them, 2-aminobenzimidazole carbamates have particularly low aqueous solubility probably because of the presence of an intramolecular hydrogen bond interaction between the benzimidazole NH and the carbonyl of the carbamate moiety. This characteristic has been until now a major obstacle for the development of more convenient application forms, such as via the drinking water.

Scheme 1. Retrosynthetic approaches to **1**



In cases where the discovery phase of a drug is completed, prodrug design constitutes a method of choice to influence the physicochemical properties of a drug.[8] With this approach, some efforts have been dedicated to the development of water-soluble anthelmintic benzimidazole prodrugs. In the majority of cases, the prodrug strategy relies on the attachment of promoieties to the benzimidazole core which included acyl groups,[9,10] alkoxycarbonyl moieties,[11,12] or Mannich bases.[13,14] However, since these approaches imply a chemically driven conversion of the prodrug into the drug, the optimal conversion conditions of the prodrugs are often too close to those where the prodrug should remain stable. This probably explains why none of these prodrugs combined all prerequisites for veterinary use: a high solubility in water for the preparation of a concentrate prior to dilution and distribution to the animals, a sufficient aqueous stability during the application (hours) in a pH range from 5 to 9,[15] and a rapid conversion into the corresponding drug at the site of action (typically the gastrointestinal tract). In the 1990s, Stella and co-workers developed a prodrug concept for derivatizing tertiary amines using a phosphonooxymethyl group as promoiety,[16,17] which was later extended to the derivatization of hydroxy functions.[18] In this concept, the release of the parent drug involves a two-step process. Following a phosphatase-catalyzed hydrolytic dephosphorylation step, the hydroxymethyl intermediate obtained spontaneously decomposes into the parent drug and formaldehyde.

In the present report we describe the synthesis of phosphonooxymethyl prodrugs derived from water-insoluble benzimidazole carbamates. Furthermore, we describe the investigation of the enzymatic in vitro conversion of one of the prodrugs and the comparison of the pharmacodynamic and pharmacokinetic profiles of this prodrug and of the parent drug in swine after oral application. Since established protocols allowing the large scale production of compounds **2a**−**g** are available, synthetic accesses based on the substitution of the parent drugs were privileged for the preparation of the phosphonooxymethyl prodrugs **1a**−**g**. Therefore, the synthetic strategies envisaged were based on the preparation of intermediates **3** followed by the addition of a phosphorus nucleophile or on a nucleophilic attack of alkali benzimidazole salts on the chloromethylphosphate triester **4** (Scheme 1).

Our initial efforts focused on preparation of intermediates **3** via hydroxymethylbenzimidazoles usually obtained by condensation with formaldehyde[19] and were unsuccessful when applied to 2-aminobenzimidazole carbamates. Finally, chloromethylbenzimidazole **3a** was obtained in one step by reacting **2a** in

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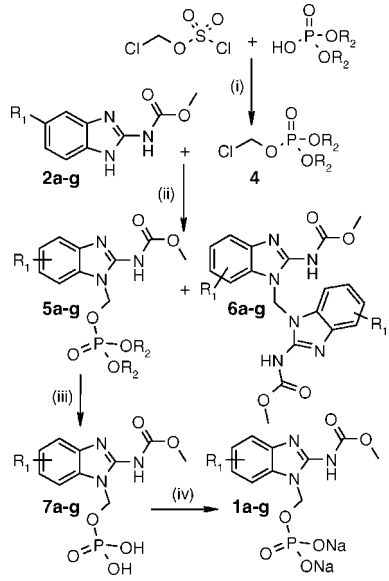
Scheme 2. Synthesis of **1a**−**g**[a]



[a] Reagents: (i) $NaHCO_3$, *n*-Bu_4NHSO_4, CH_2Cl_2−H_2O, 0 °C to room temp; (ii) NaH, DMF, room temp; (iii) HCl, dioxane, room temp; (iv) MeNa, MeOH, room temp.

the presence of a large excess of formaldehyde and phosphor trichloride. Because of the low stability of this intermediate, it was directly engaged in the next step without isolation. Unfortunately, nucleophilic substitution attempts performed with a wide range of phosphorus nucleophiles, optionally in the presence of Lewis acids or of phase transfer catalysts, resulted in the absence of reaction or in the degradation of the starting material. The introduction of the phosphate promoiety on the benzimidazole via an electrophilic phosphate reagent was then studied. To this end chloromethyl phosphate triester **4** was prepared according to a literature protocol.[20] During this work, we found that the incomplete conversion of di-*tert*-butyl phosphate due to the hydrolysis of chloromethyl chlorosulfate can be improved by adding a second crop of electrophile after 20 h reaction time or by using iodochloromethane as an alternative electrophile.[21] Condensations of the obtained electrophile **4** with 2-aminobenzimidazole carbamates **2a**−**g** were then performed by adding the electrophile to solutions of the alkali benzimidazole salts obtained by the addition of 3 equiv of base. Under these conditions the desired phosphate triesters **5a**−**g** were successfully obtained, generally in the presence of varying amounts of methylene-bis-benzimidazoles **6a**−**g**. Although the need of a third equivalent of the base is still not fully understood, it proved to be critical to the reaction. The use of less base resulted in lower conversion rates, while an increase in the stoichiometry favored the formation of larger amounts of compounds **6a**−**g**. Similarly, we observed that the temperature has a strong influence on the reaction course. In general, higher temperatures resulted in the isolation of a larger amount of dimers **6** and lower temperatures led to lower conversion. The intermediates **5a**−**g** were obtained as a nearly 1 to 1 mixture of regioisomers resulting from the undifferentiated substitution of both benzimidazole nitrogen atoms. The presence of the N-2′ substituted isomer was not detected (Scheme 2).

In the case of derivative **5a**, the two regioisomers formed were separated by high-performance liquid chromatography (HPLC), and their structures could be successfully assigned by spectroscopic analysis on the basis of ^1H, ^{13}C, H−H COSY, H−H NOESY, H−C HSQC, and H−C HMBC NMR experiments. The signals of the protons H−C1′ were easily attributed

Scheme 3. ^1H NMR Signal Assignments for **5a**

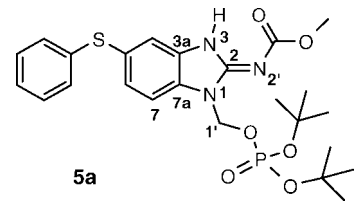


Table 1. Aqueous Solubility and Stability of **1a**−**g**

compd	R_1	aqueous solubility[a] (mM)	aqueous stability[b] (h)
1a	PhS	130	>8
1b	PhSO	>50	>8
1c	*n*-PrS	>50	>8
1d	PhCO	>50	>8
1e	4F-PhCO	>50	>8
1f	4F-$PhSO_3$	>50	>8
1g	*n*-Bu	>50	>8

[a] Determined at pH 7.4. [b] >95% UV−HPLC, determined at pH 5 and 9.

to both isomers. Since H−C1′ exhibited long-range proton−carbon correlations to C2 and C7a but not to the carbon of the carbamate carbonyl, the formation of the N-2′ substituted derivative could be excluded. Second, nuclear Overhauser correlations were observed between H-C7 and H-C1′ allowing the distinction between N-1 and N-3 substituted regioisomers. Interestingly, the remaining proton attached to one of the nitrogen atoms in regioisomer N-1 displayed a long-range carbon−proton coupling to C7a. This observation can only be rationalized by assuming that this proton is located at the benzimidazole N-3, thus implying an exocyclic double bond and supporting our hypothesis on the presence of an intramolecular hydrogen bond in unsubstituted benzimidazole carbamates **2**. No similar correlation was observed in the case of the other regioisomer (Scheme 3).

The deprotection of the phosphate di-*tert*-butyl diesters **5a**−**g** was then accomplished under acidic conditions to afford the desired phosphates **7a**−**g**, which were finally converted into the corresponding disodium salts **1a**−**g** by addition of sodium methoxide. These transformations had no effect on the ratio of the two regioisomers. Aqueous solubility of the prodrugs **1a**−**g** was then ensured at 50 mM corresponding to the targeted conditions, and the stability was determined at pH 5 and 9. For all compounds, the desired stability (>95% after 8 h at room temperature) was achieved (Table 1). In addition, aqueous solubility of 130 mM was determined for **1a**, thus representing a 195.000-fold increase when compared to the parent drug fenbendazole **2a**.

Under these conditions prodrugs **1a**−**g** also showed high stability for at least 8 h because no conversion into **2a**−**g** was observed during this period. In the following part of our work, the in vitro conversion of prodrugs **1** was studied. To this end, **1a** was used as a representative example. The lability of this prodrug was studied in the presence of chicken or porcine alkaline phosphatases by varying the enzyme concentrations, incubation times, and conditions in pH (Figures 1 and 2). As expected, an increase in pH promoted higher conversion rates. However, prolonging the incubation time from 15 to 30 min resulted only in a moderate increase of the conversions (Figure 2). From these results we concluded that under physiologically relevant pH conditions, both enzymes achieve complete conversion of **1a** into **2a** within either 15 or 30 min when concentrated at 25 or 10 mU/mL respectively.

The alkaline phosphatase being a membrane-bound protein of the microvillar enterocytes,[22] incubations were conducted in

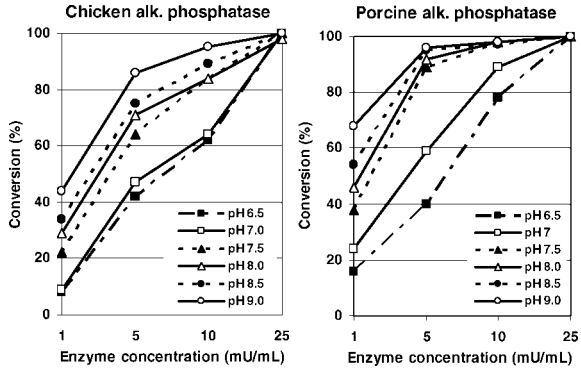


Figure 1. Conversion of prodrug **1a** to **2a** in the presence of chicken or porcine alkaline phosphatase after 15 min of incubation time.

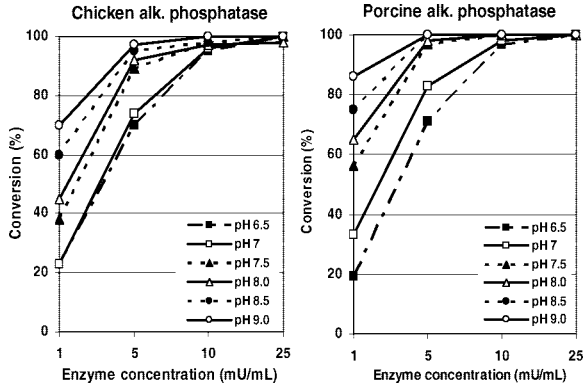


Figure 2. Conversion of prodrug **1a** to **2a** in the presence of chicken or porcine alkaline phosphatase after 30 min of incubation time.

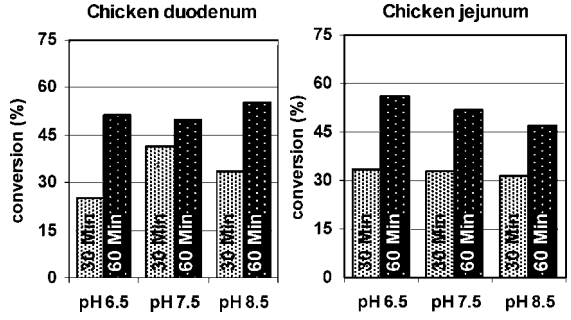


Figure 3. Conversion of prodrug **1a** to **2a** after incubation over chicken intestine mucosa disks.

the presence of chicken and swine duodenum and jejunum mucosa disks to mimic in vivo conditions in an in vitro setting (Figures 3 and 4).

Conversion of **1a** into **2a** was observed under every experimental condition studied. In the case of porcine duodenum and jejunum mucosa disks, particularly high conversions were observed already after 30 min of incubation time (for example, 80% and 70%, respectively, at pH 6.5, Figure 4).

These results prompted us to perform a combined pharmacodynamic and pharmacokinetic study in swine with **1a** and the parent drug fenbendazole **2a**.[23] Both compounds were applied orally as aqueous solution or as a suspension at single doses of 6.8 and 5.0 mg/kg body weight for **1a** and **2a**, respectively. The anthelmintic efficacy against the parasite *Oesophagostomum dentatum* was assessed by coproscopic examination of egg shedding and post mortem by determination of the worm burden. The pharmacokinetic profiles of fenbendazole **2a** (FBZ[a]) and of its metabolites fenbendazole sulfoxide (FBZ-SO[a]) and sulfone (FBZ-SO_2[a]) were determined (plasma concentration of **1a** was not measured). In terms of anthelmintic efficacy, identical results were obtained with the prodrug **1a** and for fenbendazole **2a**. In both cases, 6–7 days after treatment, complete fecal egg shedding reduction and complete reduction of worm burden were observed.

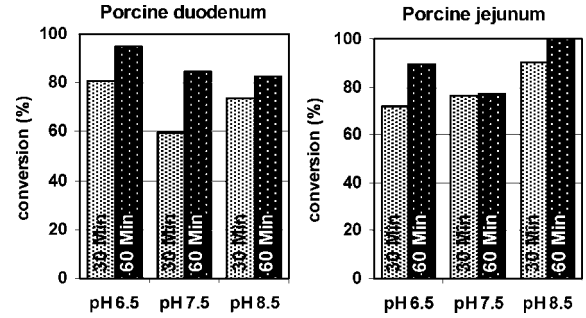


Figure 4. Conversion of prodrug **1a** to **2a** after incubation over porcine intestine mucosa disks.

Table 2. Pharmacokinetic Parameters after Oral Application of 6.8 mg/kg **1a** and of 5.0 mg/kg **2a** to Swine

	FBZ		FBZ-SO		FBZ-SO_2	
	1a	**2a**	**1a**	**2a**	**1a**	**2a**
C_{max} (μg/mL)	0.14	0.05	1.41	1.09	0.78	0.38
T_{max}(h)	6.5	5.4	15	17	36	34
AUC (μg·h/mL)	1.77	0.31	58.77	35.00	31.15	12.73

The pharmacokinetic results showed that **2a** is significantly better absorbed into the bloodstream when derived from the prodrug **1a** than when applied as such (Table 2, Figure 5).[24]

C_{max} and AUC values for FBZ and its active metabolite FBZ-SO were significantly higher after application of the prodrug **1a** than after application of the parent drug **2a**. In the case of **1a**, maximal plasma concentrations of 0.14 and 1.41 ng/mL were reached for FBZ and FBZ-SO, respectively, whereas only 0.05 and 1.09 μg/mL were measured for **2a**. Similarly, areas under curves of 0.31 and 35 μg·h/mL were calculated for FBZ and FBZ-SO after treatment with **2a**, while 1.77 and 58.8 μg·h/mL were reached when the prodrug **1a** was applied. Finally, concerning the T_{max} values, no significant differences were noticed among all treatment groups. We thus concluded that **2a** derived from the metabolism of the prodrug **1a** is absorbed to a markedly higher extent but follows the same curve profile in comparison to **2a** administered as such in powder form. Since it is a common understanding that the intestinal absorption of a drug is a function of the dissolution rate, which is in turn dependent on the particle size,[25] we believe that the metabolism of prodrug **1a** generating molecular particles of **2a** at the mucosa surface constitutes the principal driver of the observed higher bioavailability. Achievement of local drug supersaturation in the neighborhood of the intestinal mucosa following enzymatic conversion or increased solubility of the parent drug in the presence of the prodrug has been also cited in the literature as other potential factors contributing to a better oral absorption of drugs derived from phosphate prodrugs.[26]

In conclusion, we have shown that *N*-phosphonooxymethyl prodrugs **1a**−**g** can be synthesized in a straightforward way from

[a] Abbreviations: FBZ, fenbenbazole; FBZ-SO, fenbendazole sulfoxide; FBZ-SO_2, fenbendazole sulfone.

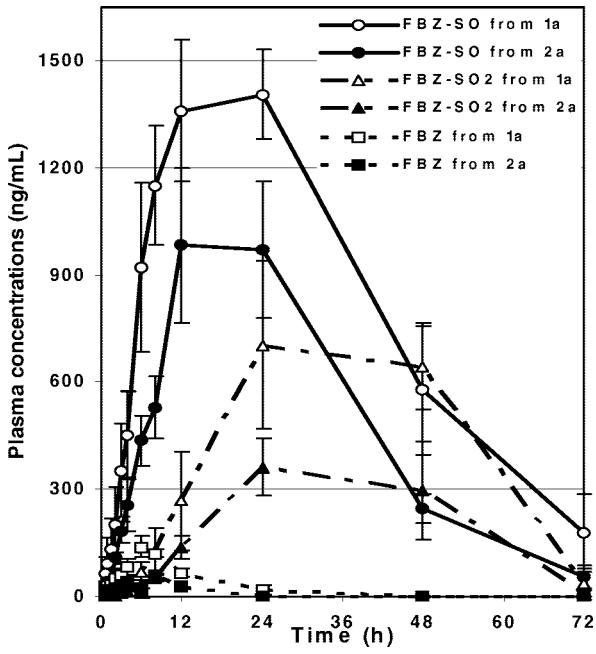


Figure 5. Pharmacokinetic profile after oral application of 6.8 mg/kg **1a** and of 5.0 mg/kg **2a** to swine.

the parent anthelmintic benzimidazole carbamates **2a**−**g**. These prodrugs combine high solubility and stability in water and are efficiently cleaved by intestinal alkaline phosphatases, making them suitable for convenient veterinary application forms such as via the drinking water. An in vivo study performed in swine with **1a** has shown that the application of the prodrug results in at least a comparable level of anthelmintic efficacy when compared to the parent drug **2a**, thus attesting for the therapeutic potential of the compounds developed. Furthermore, a comparative pharmacokinetic study has revealed that higher plasma concentrations of fenbendazole and its active metabolite fenbendazole sulfoxide are obtained when the prodrug **1a** is applied. This observation opens the opportunity to apply lower doses using prodrugs **1** than usually recommended for the anthelmintic benzimidazoles **2**. Since the link between the plasma concentration of fenbendazole and its metabolites and the efficacy is still not fully understood, further studies are currently ongoing to verify this hypothesis. The application of prodrugs **1a**−**g** to other target animal species and via other modes of application is also being investigated and will be reported in a separate paper in due course.

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Supporting Information Available: Experimental details for the preparation of **4** and **1a**−**g**, their corresponding analytical information, and details of the materials and methods used for the characterization of the **5a** regioisomers. This material is available free of charge via the Internet at http://pubs.acs.org.

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