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A highly water soluble benzimidazole derivative useful for the treatment of fasciolosis

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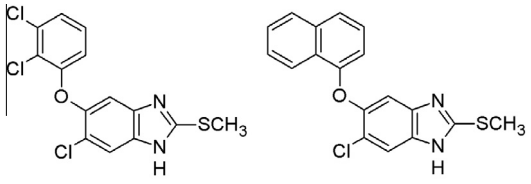
ABSTRACT

This study describes the synthesis of compound (**7**), a highly hydrosoluble phosphonooxymethyl prodrug of compound alpha (**4**). Compound (**7**) improved the aqueous solubility of its precursor compound (**4**) by 50,000 times and it is stable at neutral pH. The prodrug showed faciolicidal activity when evaluated in vitro against excysted *Fasciola hepatica* metacercariae. The in vivo evaluation of (**7**) was carried out via oral, intramuscular and subcutaneous administration in sheep artificially infected with *F. hepatica* metacercariae. At an intramuscular dose of 4 mg/kg, the activity of (**7**) was similar to that of compound alpha (**4**) at an oral dose of 15 mg/kg.

The trematode *Fasciola hepatica* is the causative agent of fasciolosis, a foodborne zoonotic disease affecting grazing animals and humans worldwide.[1,2] In humans, fasciolosis is a re-emerging disease with estimates of 2.4–17 million people infected worldwide.[2] *F. hepatica* also causes economic losses of over 3 billion US dollars worldwide per annum due to livestock susceptibility to other infections, reduction in host fecundity, mortality, decrease in production of meat, milk and wool and market recall of infected livers.[3] Some fasciolicides used to control these infections include halogenated phenols, salicylanilides, benzimidazoles, sulfonamides and phenoxyalkanes, but not all are active in all life stages of *F. hepatica*.[4] Of these, triclabendazole (TCBZ, 5-chloro-2-(methylthio)-6-(2,3-dichlorophenoxy)-1*H*-benzimidazole) has been the drug of choice for the treatment of infection by *F. hepatica*.[4] However, resistance to TCBZ in this parasite has been reported worldwide.[5–10] Because of this evolving resistance, there have been increased efforts in recent years to identify new highly effective compounds against *F. hepatica* at all stages of development. One molecule in particular, 'compound alpha' [5-chloro-2-(methylthio)-6-(1-naphthyloxy)-1*H*-benzimidazole], is an experimental fasciolicidal agent that is a bioisostere of TCBZ[11,12] (Fig. 1).

Compound alpha (**4**) has shown a range of activity against *F. hepatica*, similar to that of TCBZ.[13–17] However, no resistant strains have been detected so far for compound alpha, fact that could be of advantage as compared with the drug of choice. However, despite the efficacy of TCBZ and compound alpha, these are poorly soluble in water and need to be administered orally as suspensions, pastes, powders, or as intraruminal boli.[18] Compound alpha in particular is administered in suspension at a dose of 15 mg/kg in artificially infected sheep[11] and the reported absorption rate constant (ka) and delay in absorption denote a slow appearance of the compound in plasma. This could be explained by the drug's low solubility or an association between the compound (**4**) and particulate matter of the gastrointestinal tract, which retards its rate of



TCBZ **4**

Figure 1. Structures of triclabendazole (**TCBZ**) and compound alpha (**4**).

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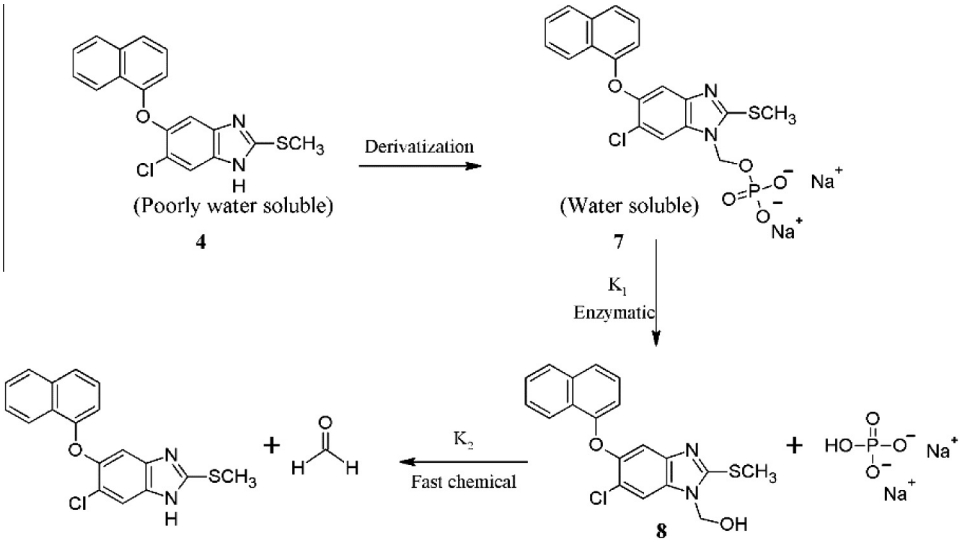


Figure 2. Illustration of the prodrug strategy utilized.

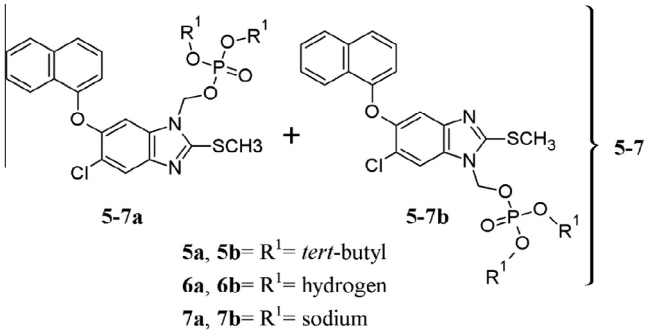


Figure 3. Mixture of regioisomers.

passage and prolongs the duration of absorption.[19,20] No less important is the veterinarýs comment about the convenience of administering compound alpha intramuscularly, rather than an oral suspension.

This poor water solubility continues to be a major obstacle in the use of other administration routes for compound alpha.[20] One approach to solve this issue would be to synthesize a hydrosoluble prodrug that when administered via intramuscular route would chemically or enzymatically regenerate compound alpha, also avoiding the first-pass effect and lowering the dose required and the time necessary for absorption.

The design of prodrugs is a widely used method to modify the physical and chemical properties of compounds such as the solubility.[21] Here, the formation of a phosphate ester serves as a strategy for improving aqueous solubility of drugs intended for oral or parenteral administration.[21–23] In the 1990s, Stella and co-workers developed a prodrug approach for derivatizing tertiary

Table 1
Aqueous solubility and chemical stability of prodrug **7**

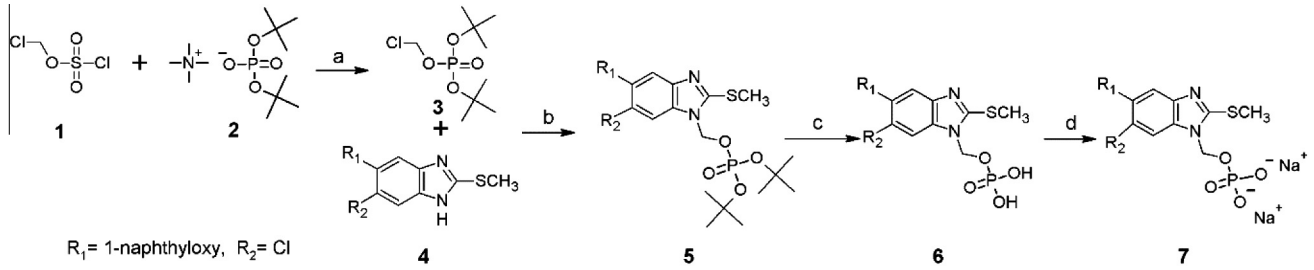
Compound	Aqueous solubility[a] (mg/mL) pH 7	Aqueous stability[b] (h) pH 7
7	13.0	>26
Compound alpha	2.6×10^{-4}	>26
TCBZ[c]	2.0×10^{-4}	>24

[a] Determined at 25 °C.
[b] >95% by UV-HPLC determined at room temperature.
[c] Solubility of TCBZ reported in literature.[33]

Table 2
Percentage of mortality of *F. hepatica* in vitro after treatment with compound **7**

Compound and concentration (mg/L)	Efficacy (%)		
	24 h Mean ± SD	48 h Mean ± SD	72 h Mean ± SD
Compound **7** (50)	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0
Compound **7** (10)	95.8 ± 0.1	95.8 ± 0.1	100.0 ± 0.1
TCBZ (50)[a]	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0
TCBZ (10)[a]	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0
Control[b]	0.0	0.0	0.0

[a] (TCBZ) triclabendazole (Fasinex®-Novartis) as a reference control.
[b] Untreated control.



Scheme 1. General procedure for the synthesis of prodrug **7**. Reagents and conditions: (a) $NaHCO_3$, *n*-Bu_4NHSO_4, CH_2Cl_2–H_2O, 0 °C to room temp; (b) NaH, DMF, room temp; (c) HCl 4 M in dioxane, room temp; (d) NaOH, MeOH/H_2O, room temp.

Table 3
Efficacy of compound **7** expressed as percentage of reduction of *F. hepatica* flukes and eggs in experimentally infected sheep

Group and route of administration ($n = 6$)	Dose[a] (mg/kg/bw)	Mean ± SD		Efficacy (%)	
		Egg reduction	Fluke reduction	Egg reduction	Fluke reduction
1 (Oral)	12	3.6 ± 1.5	12.3 ± 2.3	95.1	83.3
2 (Intramuscular)	4	35.7 ± 5.6	9.0 ± 1.2	44.9	87.8
3 (Subcutaneous)	4	23.0 ± 4.3	28.5 ± 3.4	65.8	61.4
4 (Control group)	Untreated	76.3 ± 8.9	74.0 ± 9.2	0	0

[a] Body weight.

amines using a phosphonooxymethyl group as a promoiety.[24,25] This was later extended to the derivatization of hydroxyl functions.[26] In this concept, the release of the parent drug involves a two-step process. Following an initial alkaline phosphatase hydrolytic dephosphorylation, the hydroxymethyl intermediate (**8**) obtained, spontaneously decomposes into the parent drug and formaldehyde (Fig. 2).[27,25]

In the current study, we present the synthesis, water solubility, chemical stability, in vitro and in vivo evaluations of the novel prodrug (**7**), a phosphate salt present as a mixture of regioisomers **7a** and **7b** (this applies also for compounds **5** and **6** shown in Figure 3). Prodrug **7** was designed using a phosphonooxymethyl group as a moiety to increase solubility and bioavailability of the initial compound alpha, favoring a parenteral administration at a reduced dose.

Prodrug **7** was synthesized according to the synthetic pathway described in Scheme 1 from compound alpha (**4**), which was prepared by methods previously described by our research group.[11] The phosphate group was introduced using di-*tert*-butyl chloromethyl phosphate (**3**) prepared from chloromethyl chlorosulfate[28] (**1**) and tetramethylammonium di-*tert*-butyl hydrogen phosphate (**2**), an approach adapted from methods reported in the literature.[29] Tetramethylammonium di-*tert*-butyl hydrogen phosphate was synthesized using di-*tert*-butyl hydrogen phosphate, also an adapted method from those reported in the literature.[30]

In the substitution reaction between **3** and **4**, a 1 to 1 mixture of regioisomers **5a** and **5b** was obtained. Compound (**4**) was treated with sodium hydride in DMF and then with **3** to give a 1 to 1 mixture of two regioisomers of **5**, resulting in the *N*-substituted product as intended. **5a** was successfully isolated and characterized by ^{1}H NMR, ^{13}C NMR, H-C HSQC, H-H COSY and NOE-1D. In this step, the excess NaH was eliminated and the resinous product recovered was subjected to hydrolysis to obtain **6**. This was the limiting step in the synthesis of prodrug **7**, resulting in a 70% yield. The de-protection of the di-*tert*-butyl chloromethyl phosphate (**5**) was then accomplished under acidic conditions to afford the desired phosphates (**6**), which were finally converted into the corresponding disodium salt (**7**) by addition of sodium hydroxide.[31] These transformations had no effect on the ratio of the two regioisomers. The structures of the new compounds were established from spectroscopic and spectrometric data.

The hydrosolubility of prodrug **7** was determined according to the procedure of Yalkowski et al.[32] The aqueous solubility of compound alpha, determined as 2.6×10^{-4} mg/mL, was substantially increased in prodrug **7** with the introduction of the phosphonooxymethyl group, the latter with an observed solubility of 13.0 mg/mL at pH 7 (Table 1), demonstrating a 50,000 fold increase in the solubility of the precursor compound **4**. This result is consistent with previous reports that the prodrug design of compounds, such as **7**, is useful for increasing the aqueous solubility of nonpolar drugs.[21–26]

In formulating a prodrug, the compound should also demonstrate adequate chemical stability, especially in parenteral dosage forms. Compound **7** showed desired stability (>95% after 26 h) at neutral pH, which is ideal for the formulation from a physiological standpoint of view.

Compound **7** was tested in vitro against recently excysted metacercariae.[34] It was evaluated at concentrations of 10 and 50 mg/L, see Table 2. At 50 mg/L and 24 h, compound **7** demonstrated a fasciolicidal efficacy of 100%, at a concentration of 10 mg/L this efficacy was 95.83% at 24 and 48 h, obtaining 100% efficacy until 72 hours post-treatment. These results compare with the drug of choice Triclabendazole (Fasinex®, Novartis) as the reference control. However, the fasciolicidal activity of compound **7** in the in vitro tests at 10 mg/L suggests that the parasite does not express alkaline phosphatase that can release the active compound from the prodrug and that this is hydrolyzed chemically with time.

Compound **7** was evaluated *in sheep* against adult 10 week-old *F. hepatica* using three different routes of administration (Table 3). Results obtained show an efficacy of 87.8% at an intramuscular dose of 4 mg/kg (Table 3). In previous studies the fasciolicidal activity of compound alpha (**4**) was also determined, demonstrating an 86.9% efficacy against mature and juvenile fasciolas at an oral dose of 15 mg/kg.[11] It is evident then, that the fasciolicidal efficacy of prodrug **7**, at a lower dose via intramuscular injection, is comparable with respect to orally administered compound alpha (**4**), both demonstrating similar effects.

In summary, we have synthesized a new prodrug (**7**) as a phosphate disodium salt, which shows a 50,000 fold increase in aqueous solubility compared to its precursor compound alpha (**4**). Compound **7** was stable at neutral pH, ideal for parenteral formulation. Evaluations of compound **7** in vitro and in vivo showed fasciolicidal activity. The in vivo results prove that **7** offers a considerable reduction in dose administered compared to compound alpha (**4**) while achieving similar effects via parenteral administration.

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bmcl.2014.10.017.

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