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

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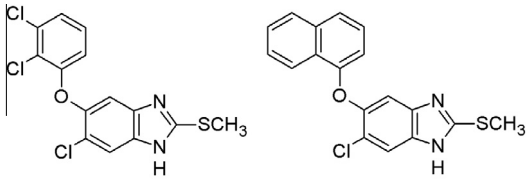
ABSTRACT

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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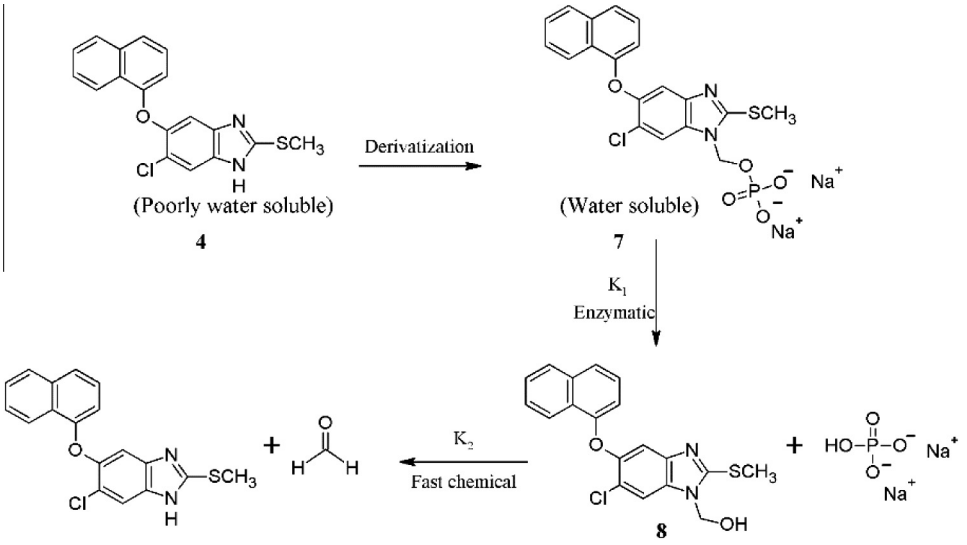


Figure 2. Illustration of the prodrug strategy utilized.

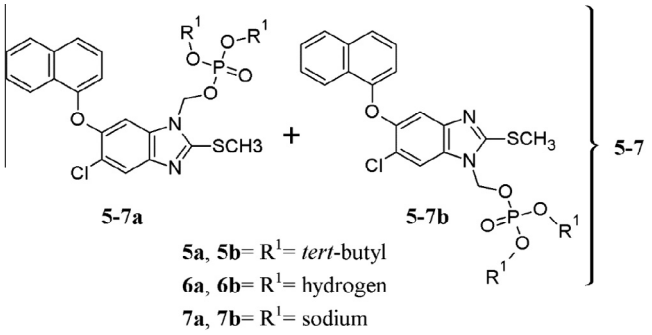


Figure 3. Mixture of regioisomers.

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**

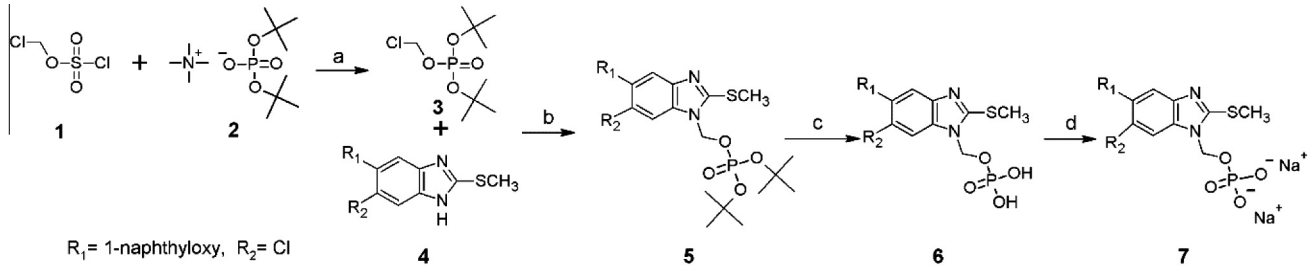
Compound	Aqueous solubility[a] (mg/mL) pH 7	Aqueous stability[b] (h) pH 7
7	13.0	>26
Compound alpha	2.6×10^{-4}	>26
TCBZ[c]	2.0×10^{-4}	>24

[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.