Experimental Parasitology 153 (2015) 75–80

Contents lists available at ScienceDirect

Experimental Parasitology

journal homepage: www.elsevier.com/locate/yexpr

Full length article

Development of novel valerolactam-benzimidazole hybrids anthelmintic derivatives: Diffusion and biotransformation studies in helminth parasites

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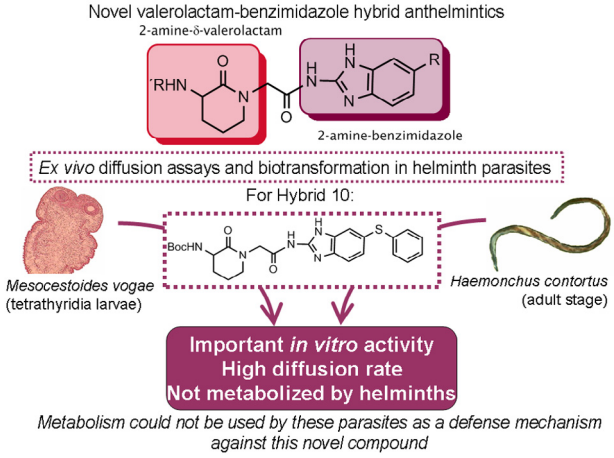
HIGHLIGHTS

- Further studies of novel benzimidazole bioactive hybrids are presented.
- Diffusion in *H. contortus* (susceptible/from sheep farms) and *M. vogae* is presented.
- Sulphoxidation drug metabolism was measured in both target parasites.
- Oxidation was more relevant in *H. contortus* parasites from sheep farms.
- New hybrid compound 10 was not oxidized and showed a high diffusion rate.

GRAPHICAL ABSTRACT



ARTICLE INFO

Article history:
Received 17 October 2014
Received in revised form 18 March 2015
Accepted 20 March 2015
Available online 25 March 2015

ABSTRACT

In the search for new anthelmintics able to overcome the resistance problem against all available drugs in livestock, the synthesis of novel valerolactam-benzimidazole hybrid compounds was reported. This allowed us to obtain these *in vitro* and *in vivo* bioactive compounds using *Nippostrongylus brasiliensis* rat model by integrating physiology-based assays and *ex vivo* diffusion studies. In order to further study those novel hybrid molecules, *Haemonchus contortus* (a sheep gastrointestinal nematode of interest) and *Mesocestoides vogae* tetrathyridia (a useful system to study the efficacy of anthelmintic drugs against cestoda) were used as parasite models to compare the *ex vivo* patterns of diffusion and biotransformation of benzimidazoles and their valerolactam-benzimidazole hybrid derivatives. On average, a nine-fold higher intraparasitic concentration of compounds was found in *M. vogae* compared with *H.contortus*, with

Abbreviations: BZ, benzimidazoles; ABZ, albendazole; FLU, flubendazole; FEB, febendazole; ABZ SX, albendazole sulfoxide; FEB SX, febendazole sulfoxide.

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http://dx.doi.org/10.1016/j.exppara.2015.03.013

Keywords:
Drug resistance
Nematodes
Cestodes
Valerolactam-benzimidazole hybrids
Haemonchus contortus
Mesocestoides vogae

similarities regarding the order of entry of compounds, highlighting febendazole (FEB) and its hybrid compound 10, while valerolactam compound 2 practically did not penetrate the parasites. Interestingly, sulphoxidation drug metabolism was observed and measured, revealing percentages of oxidation of 8.2% and 14.5% for albendazole (ABZ) and febendazole respectively in *M. vogae*, while this effect was more relevant in *H. contortus* parasite. More importantly, significant differences were observed between anthelmintic-susceptible adult parasites (*Hc S*) and those from sheep farms (*Hc U*). In fact, the percentages of oxidation of FEB and the hybrid compound 8 were higher in *Hc U* (25.5%, 54.1%, respectively) than in *Hc S* (8.8%, 38.2%). Interestingly, sulphoxidation of hybrid compound 10 was neither observed in *M. vogae* nor in *H. contortus* parasites, suggesting that increased drug metabolism (oxidation reactions) could not be used by these parasites as a defense mechanism against this novel drug.

1. Introduction

Parasitic helminth infections are a major issue causing serious health and economic problems for livestock worldwide (Neiuwhoff and Bishop, 2005). The Nematoda class, such as *Haemonchus contortus* (one of the most prevalent in sheep), is the single most important constraint to sheep production, causing significant economic losses (Waller, 2006). Since chemotherapy remains the most accessible means to fight helminth parasites, continued heavy reliance on anthelmintic drugs has led to the development of resistance in many helminth isolates. As a consequence of the prevalence of multiple-resistant parasites, it is not uncommon to find sheep farms where animals show resistance to most common available anthelmintic drugs (Skrebsky et al., 2010; Torres-Acosta et al., 2012). In particular, the vast majority of parasites from sheep farms (>80%) in Uruguay were resistant to the main anthelmintic groups used in sheep (benzimidazoles, imidazothiazoles, macrocyclic lactones) (Bonino and Mederos, 2003).

In this context, it is necessary to invest in the search for new anthelmintics with novel biological pathways, which will make it possible to overcome the resistance problem (Geary et al., 2004).

Drug resistance can arise in different ways such as changes of the sites for binding of drugs, detoxifying processes, and increased drug efflux by membrane transporters (James et al., 2009). Although the full mechanism of resistance development has not been thoroughly elucidated yet, it is probable that additional mechanisms of resistance already exist, especially in multi-resistant isolates. Furthermore, an increased drug metabolism produced by the action of xenobiotic metabolizing enzymes is a possible way to facilitate drug resistance. In this regard, enhanced S-oxidative metabolism in triclabendazole-resistant *Fasciola hepatica* was shown (Alvarez et al., 2007). Benzimidazole-2-carbamate (BZ) derivatives are among the most widely used antihelmintic drugs with a broad spectrum of action (including nematode and some cestode helminths) and efficacy, but their intensive and inadequate use has contributed to the development of resistance. In fact, BZ inhibit the microtubule polymerization pathway through binding selectively to the β-tubulin subunit where mutations that led to drug resistance have been identified (von Samson-Himmelstjerna et al., 2009). However, in a recent communication, interest in these molecules was reconsidered. In fact, based on possible tools described at molecular level (docking and dynamics) for BZ derivative optimization, these findings have been suggested as useful to design more potent and selective drugs (Aguayo-Ortiz et al., 2013).

In this context, we have recently reported (Munguía et al., 2013) the design and preparation of hybrid molecules with a dual mode of action (Meunier, 2008) to create efficient new anthelmintic drugs. Novel valerolactam-benzimidazole hybrids were synthesized based on the fusion of two active fragments (Fig. 1). This strategy was used to improve physicochemical properties regarding *in vitro* bioactive valerolactam moiety (compound 2, Munguía et al., 2013), together with the *ex vivo* ability of compounds to diffuse into the target parasite studied (the rat parasitic nematode *Nippostrongylus brasiliensis* four-stage). In that report we have showed the usefulness of diffusion studies jointly with *in vitro* physiology-based assays to search for anthelmintics.

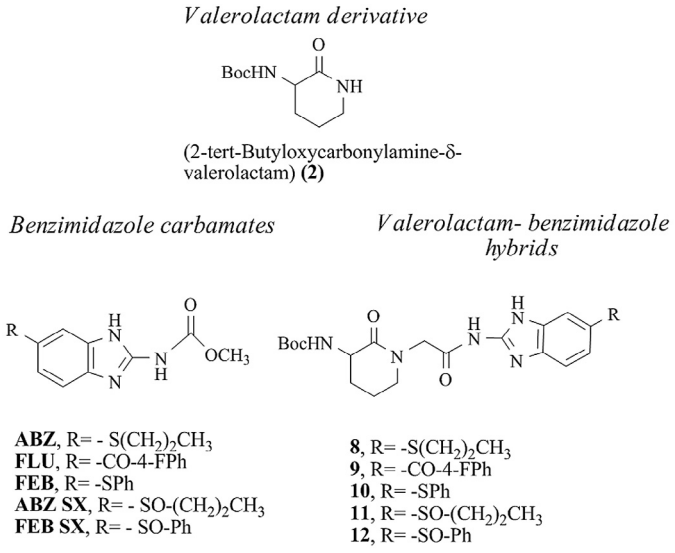

Fig. 1. Chemical structure of compounds.

In this work, *H. contortus*, a sheep gastrointestinal nematode parasite of interest, and *Mesocestoides vogae* tetrathyridia (syn. corti, Cestoda: Cyclophyllidea), a useful system to study the efficacy of anthelmintic drugs against cestodes (Saldaña et al., 2001, 2003), were used as parasite models to compare the *ex vivo* patterns of diffusion of different BZ anthelmintics, their corresponding novel hybrids molecule derivatives, and valerolactam compound 2 (Fig. 1). Also, as increased drug metabolism could be an additional mechanism of resistance, we specifically explored sulphoxidation drug metabolism. We focused on the study of oxidized metabolites produced by means of comparative diffusion assays of those compounds: an anthelmintic-susceptible isolate of *H. contortus* adult parasites (*Hc S*) and those recovered from sheep farms (*Hc U*), which were used under the assumption that they will probably be resistant to BZ anthelmintics (Bonino and Mederos, 2003).

2. Materials and methods

2.1. Chemicals

Albendazole (ABZ), flubendazole (FLU) and febendazole (FEB) were kindly supplied by Laboratorio Uruguay S.A. (LUSA). Phenacetin was used as internal standard (IS) as previously reported (Domínguez et al., 1995). The new series of hybrid compounds valerolactam-benzimidazole (compounds 8, 9 and 10) and valerolactam compound 2 were synthesized according to our recent findings (Munguía et al., 2013). Sulphoxide metabolites of ABZ, FEB, and hybrid compounds 8 and 10 (ABZ SX, FEB SX, 11, and 12, respectively) were synthesized and characterized as described in the Supplementary data. Chemical structures are shown in Fig. 1.