MINISTÉRIO DA EDUCAÇÃO

UNIVERSIDADE FEDERAL DO RIO GRANDE

PROGRAMA DE PÓS-GRADUAÇÃO EM CIÊNCIAS DA SAÚDE

AVALIAÇÃO DA ASSOCIAÇÃO DE COMPOSTOS SINTÉTICOS AO ALBENDAZOL CONTRA *Toxocara canis*: ESTUDOS *in vitro, in silico* E *in vivo*.

Victória Pires Panassolo

RIO GRANDE, 2026

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Tese apresentada ao Programa de Pós-Graduação em Ciências da Saúde da Universidade Federal do Rio Grande, como requisito parcial para obtenção do título de Doutora em Ciências da Saúde.

Orientadores: Profª. Drª Luciana Farias da Costa de Avila
Prof. Dr. Carlos James Scaini (*in memoriam*)
Coorientadora: Drª Débora Carvalho Rodrigues

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ATA DA SESSÃO DE DEFESA ABERTA DE TESE DE DOUTORADO EM CIÊNCIAS DA SAÚDE

A banca examinadora, designada pela Portaria nº 493/2026 de dois de março de dois mil e vinte e seis, em sessão presidida e registrada pela orientadora, Profa. Dra. Luciana Farias da Costa de Avila, reuniu-se no dia vinte e três de março de dois mil e vinte e seis, às nove horas e trinta minutos, na sala 700 FaMed/FURG (Prédio novo), para avaliar a Tese de Doutorado do Programa de Pós-Graduação em Ciências da Saúde, intitulada: "AVALIAÇÃO DA ASSOCIAÇÃO DE COMPOSTOS SINTÉTICOS AO ALBENDAZOL CONTRA *Toxocara canis*: ESTUDOS *in vitro, in silico* E *in vivo*." da doutoranda Victória Pires Panassolo. Para o início dos trabalhos, o Senhor Presidente procedeu à abertura oficial da sessão, com a apresentação dos membros da banca examinadora. A seguir, prestou esclarecimentos sobre a dinâmica de funcionamento da sessão, concedendo o tempo de até 50 (cinquenta) minutos para a apresentação da tese pela doutoranda, que iniciou às 09 horas e 36 minutos e terminou às 10 horas e 16 minutos. Após a apresentação, passou a palavra aos membros da banca examinadora, para que procedessem à arguição e apresentassem suas críticas e sugestões. Ao término dessa etapa de avaliação, de acordo com os membros da banca examinadora, a tese de doutorado avaliada foi APROVADA.

Rio Grande, 23 de Março de 2026.

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Victória Pires Panassolo - Doutoranda FURG

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Resumo

PANASSOLO, V.P. **AVALIAÇÃO DA ASSOCIAÇÃO DE COMPOSTOS SINTÉTICOS AO ALBENDAZOL CONTRA *Toxocara canis*: ESTUDOS *in vitro, in silico* E *in vivo*.** Orientadora: Luciana Farias da Costa de Avila. 2026. 131 p. Tese (Doutorado em Ciências da Saúde) – Faculdade de Medicina, Universidade Federal do Rio Grande, Rio Grande, 2026.

A toxocaríase é uma zoonose parasitária de distribuição mundial causada pelas larvas de *Toxocara canis*, considerada um importante problema de saúde pública. No ser humano, a infecção ocorre pela ingestão de ovos embrionados presentes no ambiente ou pelo consumo de carne ou vísceras cruas ou malcozidas de hospedeiros paratênicos contendo larvas, resultando em migração larval e manifestações clínicas que variam de assintomáticas a graves, incluindo acometimento hepático, ocular e neurológico. O tratamento baseia-se no uso de benzimidazóis, especialmente o albendazol; entretanto, sua eficácia variável em helmintíases teciduais evidencia a necessidade de novas abordagens terapêuticas. Este estudo avaliou a atividade antiparasitária de compostos sintéticos derivados de hidroxietilamina e de anilina, isoladamente e em associação ao albendazol, contra larvas de *Toxocara canis*. Foram realizados ensaios *in vitro* para determinação da atividade larvicida, análises de citotoxicidade e estudos *in silico* de propriedades farmacocinéticas e toxicológicas, além de um modelo experimental *in vivo* em camundongos infectados com *T. canis* para avaliar o efeito de derivados de anilina na carga parasitária tecidual. Os resultados demonstraram atividade larvicida dos compostos contra *T. canis*, com baixa citotoxicidade e propriedades farmacocinéticas compatíveis com biodisponibilidade oral. A associação com albendazol evidenciou efeitos aditivos e sinérgicos (FICI entre 0,75–0,625 e 0,35–0,325). Em modelo murino, a terapia combinada reduziu a carga larval em 66,9%, superior ao albendazol (52,1%) e ao composto isolado (POHA) (47,2%). Em conjunto, os achados indicam que os compostos sintéticos derivados de hidroxietilamina e anilina representam plataformas promissoras para o desenvolvimento de novos candidatos antiparasitários, reforçando o potencial da associação com albendazol no controle da toxocaríase.

Palavras chave: Toxocaríase; Anti-helmíntico; Tratamento; Benzimidazóis; Compostos sintéticos; Compostos anilínicos; Hidroxietilamina.

ODS contemplados: Saúde e Bem-estar

Abstract

PANASSOLO, V. P. **EVALUATION OF THE ASSOCIATION OF SYNTHETIC COMPOUNDS WITH ALBENDAZOLE AGAINST *Toxocara canis: in vitro, in silico*, AND *in vivo* STUDIES**. Supervisor: Luciana Farias da Costa de Avila. 2026. 131p. Doctoral thesis (Doctorate of Health Sciences) – Faculty of Medicine, Federal University of Rio Grande, Rio Grande, 2026.

Toxocariasis is a parasitic zoonosis with worldwide distribution caused by the larvae of *Toxocara canis*, and is considered an important public health problem. In humans, infection occurs through the ingestion of embryonated eggs present in the environment or through the consumption of raw or undercooked meat or viscera from paratenic hosts containing larvae, resulting in larval migration and clinical manifestations that range from asymptomatic to severe, including hepatic, ocular, and neurological involvement. Treatment is based on the use of benzimidazoles, particularly albendazole; however, its variable efficacy in tissue helminthiases highlights the need for new therapeutic approaches. This study evaluated the antiparasitic activity of synthetic compounds derived from hydroxyethylamine and aniline, alone and in association with albendazole, against *Toxocara canis* larvae. In vitro assays were performed to determine larvicidal activity, along with cytotoxicity analyses and in silico studies of pharmacokinetic and toxicological properties, in addition to an in vivo experimental model using mice infected with *T. canis* to assess the effect of aniline derivatives on tissue parasite burden. The results demonstrated larvicidal activity of the compounds against *T. canis*, with low cytotoxicity and pharmacokinetic properties compatible with oral bioavailability. The association with albendazole showed additive and synergistic effects (FICI ranging from 0.75–0.625 and 0.35–0.325). In the murine model, combination therapy reduced larval burden by 66.9%, which was higher than that observed for albendazole alone (52.1%) and the compound alone (POHA) (47.2%). Taken together, these findings indicate that synthetic compounds derived from hydroxyethylamine and aniline represent promising platforms for the development of new antiparasitic candidates, reinforcing the potential of their association with albendazole for the control of toxocariasis.

Key-words: Toxocariasis;; Aniline compounds; Hydroxyethylamine; Antiparasitic activity. **ODS Covered:** Good Health and Well-being.

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Manuscrito 1

1. INTRODUÇÃO

A toxocaríase é uma zoonose parasitária de distribuição global causada principalmente pelas larvas de *Toxocara canis*, nematódeo intestinal de cães e gatos. Considerada uma das helmintíases zoonóticas mais comuns no mundo, a doença apresenta ampla distribuição geográfica e está frequentemente associada a condições sanitárias inadequadas e à alta densidade de hospedeiros definitivos no ambiente (Rostami et al. 2019; Lopez-Alamillo et al. 2025). A infecção humana ocorre de forma acidental, principalmente pela ingestão de ovos embrionados presentes no solo, água ou alimentos contaminados e ingestão de carne crua contendo as larvas do helminto em seus tecidos, representando um importante problema de saúde pública, especialmente em regiões tropicais e subtropicais (Macpherson et al. 2013).Após a ingestão dos ovos infectantes, as larvas eclodem no intestino delgado e penetram na parede intestinal, alcançando a circulação sistêmica. A partir desse momento, inicia-se um processo de migração larval para diferentes tecidos do hospedeiro, incluindo fígado, pulmões, sistema nervoso central, músculos e olhos (Ma et al. 2018). Diferentemente do que ocorre nos hospedeiros definitivos, nos quais o parasito completa seu ciclo evolutivo no intestino, no ser humano as larvas não se desenvolvem até a fase adulta, permanecendo em migração ou encapsuladas nos tecidos por períodos prolongados. Essa condição pode desencadear respostas inflamatórias e imunológicas responsáveis pelas diferentes manifestações clínicas da toxocaríase (Ma et al. 2018; Lopez-Alamillo et al. 2025).As manifestações clínicas da doença variam de acordo com o órgão acometido, a intensidade da infecção e a resposta imunológica do hospedeiro. Entre as principais formas clínicas destacam-se a síndrome da larva *migrans* visceral, caracterizada por febre, hepatomegalia e eosinofilia; a toxocaríase ocular, associada a lesões inflamatórias que podem comprometer a visão; e a neurotoxocaríase, na qual a migração larval pode afetar o sistema nervoso central. Além dessas apresentações, também são descritas formas subclínicas ou menos específicas da infecção, como a toxocaríase comum e a toxocaríase oculta, que podem apresentar sintomas inespecíficos e dificultar o diagnóstico clínico (Ma et al. 2018; Lopez-Alamillo et al. 2025).

O tratamento da toxocaríase baseia-se principalmente no uso de benzimidazóis, sendo o albendazol o fármaco mais amplamente utilizado (Magnaval et al. 2022).

Apesar disso, o albendazol apresenta limitações terapêuticas em determinadas situações clínicas, especialmente em helmintíases teciduais, nas quais a erradicação completa das larvas nem sempre é alcançada (Horiuchi et al. 2005; Leonardi et al. 2009; Barrera et al. 2010; Beshay et al. 2020; Mengarda et al. 2023; Salama et al. 2023; Rodrigues et al. 2024; Rodrigues et al. 2025). Além disso, fatores farmacocinéticos, como variações na absorção e no metabolismo do fármaco, podem influenciar a resposta terapêutica entre indivíduos (Whittaker et al. 2022).

Diante dessas limitações, a busca por novas alternativas terapêuticas tem estimulado a investigação de compostos sintéticos com potencial atividade antiparasitária. A química medicinal desempenha papel fundamental nesse processo, permitindo a exploração de diferentes núcleos estruturais e modificações moleculares capazes de modular propriedades físico-químicas e biológicas relevantes para o desenvolvimento de novos candidatos a fármacos (Ferreira et al. 2018). Diversas classes de compostos sintéticos vêm sendo investigadas quanto ao seu potencial antiparasitário, incluindo derivados heterocíclicos, sulfonamidas, quinonas e outras estruturas com capacidade de interação com diferentes alvos moleculares do parasito (Mata-Santos et al. 2016; Ortiz-Pérez et al. 2021; Soto-Sánchez et al. 2021; Mengarda et al. 2023; Kuzminac et al. 2023; Rodrigues et al. 2024; Rodrigues et al. 2025).

Além da identificação de novos compostos bioativos, outra abordagem promissora consiste na associação de fármacos com mecanismos de ação distintos. Estratégias de terapia combinada podem resultar em efeitos sinérgicos ou aditivos, ampliar a atuação sobre múltiplos alvos biológicos e permitir a redução das doses individuais necessárias, contribuindo para melhorar a eficácia terapêutica e reduzir potenciais efeitos adversos (Sheng et al. 2018; El Hassouni et al. 2019; Guieu et al. 2021; Wang et al. 2021; Li et al. 2024; Hashemi et al. 2026). Nesse sentido, a associação de compostos sintéticos com fármacos já estabelecidos, como o albendazol, tem sido investigada como estratégia para potencializar a atividade antiparasitária e otimizar o tratamento de diferentes helmintíases. Entretanto, essa abordagem requer avaliação cuidadosa da segurança, pois combinações farmacológicas podem resultar em interações medicamentosas e aumento da toxicidade.

Considerando a relevância da toxocaríase como zoonose negligenciada e as limitações terapêuticas associadas ao uso do albendazol, torna-se necessário ampliar o

conhecimento sobre novas abordagens terapêuticas e o potencial de compostos sintéticos no desenvolvimento de estratégias mais eficazes de tratamento da doença.

Nesse contexto, a revisão bibliográfica a seguir apresenta os principais aspectos relacionados à toxocaríase humana, às limitações terapêuticas do albendazol e ao potencial de compostos sintéticos como estratégia para otimização do tratamento. A presente tese teve como hipótese que a associação de compostos sintéticos derivados de hidroxietilamina e anilina ao albendazol poderia potencializar a atividade antiparasitária contra *Toxocara canis*. Para isso, foram realizados ensaios *in vitro* de atividade larvicida e citotoxicidade, análises *in silico* de propriedades farmacocinéticas e toxicológicas e avaliação *in vivo* em modelo murino de toxocaríase. A tese está organizada em um artigo e um manuscrito: o primeiro avaliando derivados de hidroxietilamina em associação ao albendazol e o segundo investigando derivados de anilina por meio de abordagens *in vitro*, *in silico* e *in vivo.*

2. REVISÃO BIBLIOGRÁFICA

2.1 Toxocaríase humana

A toxocaríase humana é uma zoonose parasitária negligenciada de distribuição global. (Chen et al. 2018 causada pela migração e permanência de larvas de helmintos em tecidos e órçãos de seres humanos (Beaver, 1959). O principal agente etiológico dessa parasitose tecidual é o nematódeo *Toxocara canis*, parasito do intestino delgado de cães, embora a espécie *T. cati,* parasito intestinal de gatos, também seja um importante agente causal dessa parasitose (Chen et al. 2018).

A infecção em humanos apresenta soroprevalência global estimada em cerca de 19%, correspondendo a aproximadamente 1,4 bilhão de pessoas infectadas ou expostas a *Toxocara* spp. A prevalência varia entre as regiões, com maiores taxas na África (37,7%) e no Sudeste Asiático (34,1%), seguidas pelo Pacífico Ocidental (24,2%) e pelas Américas (22,8%), enquanto valores menores são observados na Europa (10,5%) e no Mediterrâneo Oriental (8,2%) (Rostami et al., 2019; Ma et al., 2020; Strube et al., 2020). No Brasil, a soroprevalência varia amplamente entre 4,2% e 82,7%, dependendo da região e da população estudada, evidenciando a ampla distribuição e relevância epidemiológica da toxocaríase no país (Fialho & Correa, 2016; Schoenardie et al., 2013; Santarém et al., 2023).

Os seres humanos (hospedeiros acidentais e paratênicos) adquirem a infecção por *Toxocara* spp. pela ingestão dos ovos embrionados presentes em mãos, fômites, solo, hortaliças e água ou pelo consumo de carne e vísceras malcozidas de ruminantes, suínos e aves (hospedeiros paratênicos) contendo a larva infectante (Holland, 2017). No organismo humano, as larvas migram pela circulação sistêmica e podem se alojar no fígado, pulmões, coração, encéfalo, rins, olhos e musculatura esquelética (Smith et al. 2009).

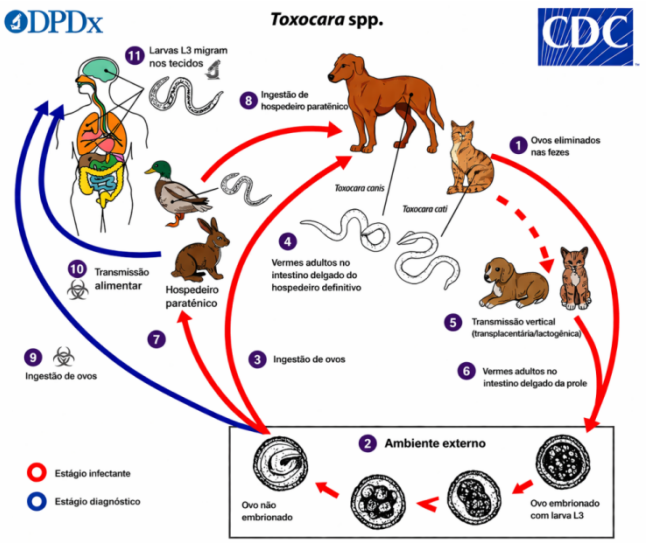

Figura 1 – Ciclo biológico de *Toxocara canis*. Ovos eliminados nas fezes tornam-se infectantes no ambiente e são ingeridos por hospedeiros definitivos ou paratênicos. Em cães, ocorre desenvolvimento de vermes adultos no intestino; em humanos, as larvas migram por tecidos sem completar o ciclo. (Adaptado de Centers for Disease Control and Prevention (CDC), DPDx.).

As manifestações da toxocaríase são variáveis e inespecíficas, influenciadas pela localização das larvas de *Toxocara* spp. e pela resposta imunológica do hospedeiro (Carvalho & Rocha, 2011). O espectro clínico é amplo, podendo ser assintomático como ocorre na maioria dos casos, ou apresentar formas leves como na toxocaríase oculta e na toxocaríase comum (Rubinsky-Elefant et al. 2010) ou formas graves, como em alguns casos de larva *migrans* visceral ou toxocaríase visceral, larva *migrans* ocular ou toxocaríase ocular (TO) e neurotoxocaríase (NT) (Smith et al. 2009; Rubinsky-Elefant et al. 2010; Meliou et al. 2020).

O perfil do paciente com toxocaríase visceral são crianças de 2 a 7 anos, geralmente com histórico de geofagia e contato com cães. Essa forma clínica está associada à migração larval para o fígado e pulmões, resultando em febre, dor abdominal, hepatomegalia, sintomas respiratórios inferiores, como tosse, dispneia e broncoespasmo (Fragoso et al. 2011; Chieffi et al. 2021). Além disso, são frequentes leucocitose, eosinofilia sanguínea acentuada e hipergamaglobulinemia (Bede et al. 2008; Ma et al. 2018). O fígado é o órgão mais frequentemente acometido nessa forma clínica, podendo desenvolver granulomas típicos com células gigantes multinucleadas e células epitelióides ao redor de detritos necróticos ou material eosinofílico amorfo (Musso et al. 2007). Erupções cutâneas também podem estar presentes (Gavignet et al.

2008), incluindo prurido crônico, urticária, eczema e vasculite. Outras manifestações são miocardite (Lampejo et al. 2021), síndrome nefrótica (Shetty & Aviles, 1999) e artrite (Smith et al. 2009).

A toxocaríase ocular é geralmente unilateral e afeta principalmente crianças maiores, adolescentes e adultos jovens. Os principais sintomas e sinais clínicos incluem perda de visão progressiva, leucocória, vitreíte e estrabismo. A toxocaríase ocular apresenta três padrões clássicos: granuloma posterior submacular ou peripapilar com estrias retinianas, granuloma periférico e endoftalmite (Cunningham Jr. & Zierhut, 2021). Já, a neurotoxocaríase é uma forma rara, podendo ser grave, geralmente, observada em adultos. Essa condição pode se manifestar como meningite, encefalite, meningoencefalite, mielite ou vasculite cerebral, podendo em alguns casos ser letal. Entre os sintomas relatados estão demência, epilepsia, paresia, tetraparesia e outras complicações neurológicas severas (Deshayes et al. 2016; Nicoletti et al. 2020).

A toxocaríase oculta é associada à febre, cefaleia, distúrbios comportamentais e do sono, tosse, anorexia, dor abdominal, hepatomegalia, náusea e vômito, com ou sem eosinofilia. A toxocaríase comum, que acomete principalmente adultos, caracteriza-se por dispneia crônica, astenia, erupções cutâneas, prurido e dor abdominal, geralmente acompanhados de eosinofilia (Ma et al. 2018; Auer et al. 2020). Ambas representam variações leves do espectro clínico da toxocaríase em crianças e adultos, respectivamente (Rubinsky-Elefant et al. 2010). A diversidade de manifestações clínicas, associada à inespecificidade dos sinais e sintomas, contribui para desafios diagnósticos e terapêuticos, reforçando a necessidade de estratégias de manejo adequadas e de intervenções farmacológicas eficazes.

2.2 Tratamento da toxocaríase humana

Os anti-helmínticos da classe dos benzimidazólicos, amplamente utilizados no tratamento de helmintíases intestinais como ascaridíase, tricuríase e ancilostomíase (Chen et al. 2018), também são indicados para a toxocaríase humana. Fármacos como albendazol e mebendazol atuam pela inibição da polimerização da β-tubulina, comprometendo o metabolismo energético das larvas (Chai et al. 2021). No entanto, diferentemente das infecções intestinais, em que o parasito permanece no lúmen e é diretamente exposto ao fármaco, a toxocaríase é uma parasitose tecidual, com larvas localizadas em órgãos como fígado, pulmões e sistema nervoso central. Nesses casos, a

eficácia dos benzimidazólicos é limitada devido à baixa biodisponibilidade sistêmica e à reduzida penetração tecidual, resultando em concentrações insuficientes nos sítios de infecção (Magnaval et al. 2022).

O albendazol é considerado o anti-helmíntico de primeira escolha para o tratamento da toxocaríase humana, principalmente por apresentar melhor absorção quando comparado a outros benzimidazóis, como mebendazol e tiabendazol. A posologia recomendada varia de 10 a 15 mg/kg/dia, geralmente por 14 dias (Hotez & Wilkins, 2009). Além desses fármacos, a dietilcarbamazina também pode ser utilizada como alternativa terapêutica. Entretanto, conforme destacado por Magnaval et al. (2022), há escassez de ensaios clínicos randomizados bem delineados que avaliem de forma consistente a eficácia desses medicamentos, incluindo o próprio albendazol. Os estudos disponíveis apresentam heterogeneidade quanto ao delineamento (observacional ou randomizado), às doses administradas e à duração do tratamento, dificultando a padronização de protocolos terapêuticos. De modo geral, o manejo da toxocaríase baseia-se no uso de anti-helmínticos, podendo ser associado a anti-inflamatórios esteroidais ou não esteroidais para controle das manifestações inflamatórias (Fillaux & Magnaval, 2013; Ranasuriya et al. 2014). Independentemente da forma clínica, é fundamental o monitoramento de possíveis efeitos adversos, especialmente em pacientes alérgicos, gestantes, lactantes, crianças com menos de 15 kg e idosos (Chen et al. 2018).

No tratamento da toxocaríase ocular, o protocolo padrão consiste na associação de albendazol a corticosteroides sistêmicos e/ou tópicos, com o objetivo de controlar a inflamação e prevenir complicações como fibrose e dano estrutural permanente (Othman, 2012). Nos casos em que já há alterações anatômicas estabelecidas, como descolamento de retina ou formação de granulomas, pode ser necessária intervenção cirúrgica (Ahn, 2014). Na neurotoxocaríase, a abordagem terapêutica também se baseia na combinação de anti-helmínticos e corticosteroides. O albendazol administrado por pelo menos três semanas é considerado a opção preferencial (Ma et al. 2018), sendo os corticosteroides recomendados para reduzir a resposta inflamatória e as reações de hipersensibilidade desencadeadas pela degeneração larval no sistema nervoso central (Goffette, 2007; Fan et al. 2015).

2.2.1 Albendazol

O albendazol tem como princípio ativo [metil-5-(propil-tio)-1H-benzimidazol-2-il] carbamato. Desde a década de 1990, esse fármaco faz parte da lista de medicamentos essenciais da Organização Mundial da Saúde (OMS) (OMS, 1995). Esse fármaco pertence à classe dos benzimidazóis, os quais são formados pela fusão de um anel benzênico e um anel imidazol, sendo este um heterocíclico aromático de cinco membros planar com dois átomos de nitrogênio **(Figura 1).** Os benzimidazóis despertam crescente interesse científico devido à sua ampla gama de atividades farmacológicas, derivados contendo o núcleo benzimidazólico demonstram efeitos antibacterianos, antivirais, antitumorais, anti-inflamatórios, antiparasitários e antifúngicos (Brishty et al. 2021).

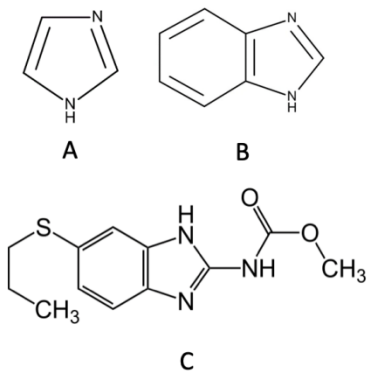

Figura 2: Estrutura química: A: imidazol; B: benzimidazol; C: albendazol (Santos, 2021), com modificações.

Com destaque para sua atividade anti-helmíntica (Singh & Silakari, 2018), os benzimidazóis são considerados a primeira classe química de anti-helmínticos modernos (Aronson, 2016). Esses compostos atuam principalmente interferindo na polimerização da tubulina dos parasitos, comprometendo processos celulares essenciais e levando à morte do helminto. Além da eficácia contra diversos helmintos de importância médica e veterinária, alguns benzimidazóis também apresentam atividade contra protozoários, como *Giardia lamblia* (Martínez-Espinosa et al., 2015).

A absorção do albendazol no trato gastrointestinal é limitada, geralmente não ultrapassando 5% (Whittaker et al. 2022), o que pode resultar em baixas concentrações sistêmicas e teciduais do fármaco, comprometendo sua eficácia terapêutica, especialmente em helmintíases teciduais como a toxocaríase (Magnaval et al., 2022; Ma

et al., 2018). No entanto, a ingestão concomitante de alimentos gordurosos pode aumentar sua biodisponibilidade (Ochoa et al. 2021). O albendazol é considerado um pró-fármaco, uma vez que sua atividade farmacológica depende da biotransformação hepática em seu metabólito ativo, o sulfóxido de albendazol. Após a absorção, o fármaco passa por extenso metabolismo de primeira passagem no fígado, sendo rapidamente convertido em sulfóxido de albendazol, seu principal metabólito ativo (Moser et al. 2017). Esse processo ocorre por oxidação da cadeia lateral, catalisada por enzimas hepáticas. O sulfóxido de albendazol atinge concentrações plasmáticas médias de 300 ng/mL e aproximadamente 70% desse metabólito se liga às proteínas plasmáticas (Ochoa et al. 2021). Sua meia-vida varia entre 4 e 15 horas, com pico plasmático entre 2 e 3 horas. Distribui-se amplamente nos tecidos e sua eliminação ocorre majoritariamente pela urina dentro de 24 horas, com uma pequena fração excretada pela bile (Hardman et al. 2001).

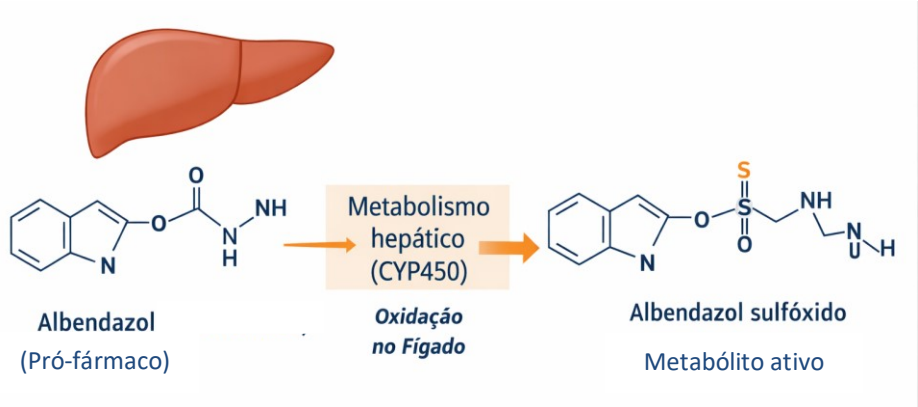

Figura 3. Metabolismo hepático do albendazol mostrando a conversão do pró-fármaco albendazol em albendazol sulfóxido, seu principal metabólito ativo, mediado por enzimas do citocromo P450 Panassolo (2026) com base em Moser et al. (2017) e Whittaker et al. (2022).

Os benzimidazóis são anti-helmínticos de amplo espectro que se ligam à β-tubulina, inibindo a polimerização dos microtúbulos, comprometendo processos essenciais como divisão celular, transporte intracelular e absorção de nutrientes, levando à redução da produção de ATP e morte do parasito.. Assim, ocorre declínio no nível energético do helminto e resulta na redução da formação de adenosina trifosfato (ATP), necessário para a sobrevivência e reprodução das formas adultas, e a motilidade das larvas e sua a capacidade de realização de migração pelos tecidos (LiverTox, 2021; Jacob et al. 2022).

2.2.2 Limitações terapêuticas e resistência aos benzimidazóis

A Organização Mundial da Saúde e a Organização Pan-Americana da Saúde (OMS/OPAS) estabelecem diretrizes para o controle de helmintos transmitidos pelo solo, como *Ascaris lumbricoides*, *Trichuris trichiura* e ancilostomídeos, na América Latina e no Caribe. Essas organizações recomendam estratégias de desparasitação por quimioterapia preventiva em populações de risco, geralmente baseadas na administração periódica de benzimidazóis, como albendazol e mebendazol. O uso repetido desses fármacos, especialmente em programas de administração em massa, pode exercer pressão seletiva sobre as populações parasitárias, favorecendo a sobrevivência de indivíduos com mutações na β-tubulina associadas à redução da sensibilidade ao tratamento. Esse processo tem sido relacionado ao surgimento e à disseminação de resistência aos benzimidazóis, o que gera preocupação quanto à manutenção da eficácia desses anti-helmínticos (Curico et al., 2022). Nos animais de produção, como por exemplo os ovinos, os benzimidazóis têm sido usados há décadas para tratar infecções por nematódeos gastrointestinais, e o fenômeno de resistência a esses anti-helmínticos é amplamente reconhecido (Kaplan, 2004; Furtado et al. 2016). Nos casos de resistência, têm sido relatados polimorfismos de nucleotídeo único (PNU) no gene da β-tubulina isotipo-1. Essas alterações estão envolvidas no desenvolvimento da resistência aos benzimidazóis em nematódeos gastrointestinais de ruminantes, especialmente tricostrongilídeos de ovinos, e podem ser utilizadas como marcadores genéticos para detecção de resistência (Von Samson-Himmelstjerna et al., 2009; Munguía et al., 2018). Entretanto, apesar de os benzimidazóis constituírem o tratamento de escolha para diversas helmintíases, sua baixa absorção e limitada biodisponibilidade tecidual reduzem significativamente a eficácia contra parasitos com localização extraintestinal, como ocorre na toxocaríase. Além disso, o avanço da resistência aos anti-helmínticos observada em nematódeos de animais de produção acende um alerta para possíveis mecanismos semelhantes em outras espécies parasitárias, reforçando a necessidade de investigar novos compostos ou estratégias que aumentem a biodisponibilidade, como modificações estruturais ou otimização farmacocinética, visando maior eficácia larvicida.

2.3 Estratégias de associação farmacológica no controle de helmintíases

A terapia combinada em doenças parasitárias é definida como o uso simultâneo de dois ou mais fármacos, que podem atuar por mecanismos de ação distintos ou complementares, com o objetivo de aumentar a eficácia terapêutica, reduzir a toxicidade, encurtar o tempo de tratamento e retardar o desenvolvimento de resistência. Essa estratégia torna-se especialmente relevante em cenários nos quais a monoterapia apresenta eficácia limitada ou risco de seleção de cepas resistentes, como observado em diversas helmintíases e na leishmaniose visceral (van Griensven et al. 2024, Su et al. 2026). Em infecções por helmintos, a associação de benzimidazóis, como o albendazol, com lactonas macrocíclicas, como ivermectina ou moxidectina, tem demonstrado, em ensaio clínico randomizado, taxas de cura superiores às observadas com o uso isolado de cada agente, especialmente em casos de *Trichuris trichiura* (Schnoz et al., 2025). De forma semelhante, na leishmaniose visceral, revisões recentes têm descrito o uso de esquemas combinando antimoniais com paromomicina ou miltefosina com o objetivo de otimizar a resposta terapêutica e melhorar o perfil de segurança (van Griensven et al., 2024). Assim, a escolha das associações farmacológicas baseia-se em evidências clínicas e revisões sistemáticas, visando maximizar o benefício terapêutico e minimizar eventos adversos (Su et al. 2026).

A interação entre fármacos em terapias combinadas pode resultar em sinergismo, aditividade, indiferença ou antagonismo, conforme o efeito observado em relação aos efeitos individuais. O sinergismo ocorre quando a combinação supera a soma das atividades isoladas, enquanto a aditividade corresponde ao efeito esperado pela soma simples e o antagonismo à redução da eficácia combinada (Francisca et al. 2025; Fayd'Herbe et al. 2026). A análise dessas interações é frequentemente realizada pelo método *checkerboard*, que permite o cálculo do índice de concentração inibitória fracionada (FICI). Esse índice é obtido pela soma das frações das concentrações inibitórias utilizadas na combinação e classifica a interação como sinérgica ($\leq$ 0,5), aditiva ($>$ 0,5–1,0), indiferente ($>$ 1,0–4,0) ou antagonista ($>$ 4,0), segundo critérios estabelecidos na literatura (Meletiadis et al. 2010; Talawanich et al. 2015). Apesar de possíveis variações metodológicas, o FICI permanece como parâmetro amplamente utilizado na avaliação *in vitro de* combinações farmacológicas.

A combinação de albendazol com outros antiparasitários tem sido proposta para superar limitações da monoterapia, ampliar o espectro de ação e reduzir o risco de resistência. O fármaco apresenta eficácia limitada contra helmintos como *Trichuris*

trichiura e atividade reduzida frente a *Strongyloides stercoralis* (Speich et al. 2015; Krolewiecki et al. 2025), além de relatos de polimorfismos no gene da β-tubulina associados à possível resistência (Diawara et al. 2013; Walker et al. 2021; Grau-Pujol et al. 2022).

Enquanto a combinação albendazol-praziquantel mostrou superior atividade na neurocisticercose (Garcia et al. 2014; Garcia et al. 2016; White et al. 2018), com perfil de segurança semelhante ao da monoterapia (Hürlimann et al. 2022; Farhan et al. 2025). Considerando ainda sua baixa biodisponibilidade e eficácia variável em parasitos de localização tecidual, a associação com novos compostos sintéticos surge como estratégia para potencializar a atividade larvicida, sendo a avaliação *in vitro* por meio do método *checkerboard* uma etapa inicial para investigar possíveis efeitos sinérgicos ou aditivos e fundamentar estudos posteriores *in vivo*.

2.4 Compostos sintéticos com potencial antiparasitário

O processo de descoberta de novos fármacos tem início quando uma doença ou condição clínica carece de terapias eficazes (Scavone et al. 2019). Nessa etapa, a indústria farmacêutica e grupos de pesquisa acadêmica desenvolvem estratégias voltadas à identificação de moléculas com propriedades farmacológicas desejáveis. Essas abordagens podem basear-se tanto na modulação de alvos biológicos específicos quanto na triagem fenotípica de bibliotecas de compostos, seguida da identificação dos mecanismos de ação. Essas novas entidades químicas podem ser obtidas tanto a partir de fontes naturais quanto por meio de síntese química (Swinney et al., 2011). No entanto, o número de novos antiparasitários introduzidos nas últimas décadas permanece limitado; entre 1981 e 2019, aproximadamente 20 fármacos com atividade antiparasitária foram aprovados, incluindo compostos naturais, semissintéticos e sintéticos, o que evidencia a necessidade de identificação de novas entidades químicas com potencial terapêutico (Newman & Cragg, 2020). No contexto da química medicinal, os compostos sintéticos são definidos como novas entidades químicas obtidas por síntese química, planejadas para apresentar atividades biológicas específicas (Boström et al. 2018). A síntese química constitui uma das ferramentas mais poderosas da química moderna, permitindo a criação de moléculas bioativas com estrutura e função controladas (Nicolaou et al. 2014). O domínio das etapas de síntese possibilita o

desenho racional de compostos capazes de interagir seletivamente com alvos biológicos, o que favorece a eficácia terapêutica e reduz potenciais efeitos adversos (Fischer et al. 2019). A obtenção desses compostos por síntese química permite o controle estrutural necessário para a investigação de suas propriedades biológicas e farmacológicas (Federsel et al. 2023).

Na parasitologia, os derivados sintéticos assumem papel crucial no desenvolvimento de agentes antiparasitários mais eficazes e seletivos. Obtidos pela modificação estrutural de moléculas naturais ou pelo planejamento estrutural de novas entidades químicas, esses compostos buscam aprimorar propriedades farmacocinéticas, reduzir toxicidade e contornar mecanismos de resistência observados em parasitas de relevância médica e veterinária. Um exemplo dessa estratégia é o desenvolvimento da moxidectina, derivado semissintético relacionado à ivermectina, que apresenta maior lipofilicidade, meia-vida prolongada e melhor distribuição tecidual, resultando em perfil farmacocinético mais favorável (Prichard et al., 2012). Diversas classes químicas de origem sintética, como os benzimidazóis, nitroimidazóis, sulfonamidas, quinolinas e naftoquinonas (Ortiz-Pérez et al. 2021), exemplificam sua ampla aplicação terapêutica. A otimização estrutural desses compostos, aliada a estudos *in silico*, *in vitro* e *in vivo*, tem revelado moléculas promissoras com atividade frente a helmintíases, como *Toxocara canis*, reforçando a importância da química sintética na busca por novas alternativas terapêuticas contra doenças parasitárias negligenciadas (Mata-Santos et al. 2016; Ortiz-Pérez et al. 2021; Rodrigues et al. 2024; Rodrigues et al. 2025a; Rodrigues et al. 2025b).

2.4.1 Derivados de hidroxietilamina como farmacóforo promissor

Na química medicinal, o farmacóforo é definido como o arranjo tridimensional mínimo de características moleculares essenciais, como grupos doadores e aceitadores de ligações de hidrogênio, centros de carga e regiões hidrofóbicas, necessárias para a interação de um composto com um alvo biológico e consequente atividade farmacológica (Dror et al. 2004; Pirhadi et al. 2013; Vuorinen & Schuster, 2015). Trata-se de um padrão abstrato de propriedades químicas e espaciais, independente de uma estrutura molecular específica, que representa os requisitos mínimos para o reconhecimento molecular (Pirhadi et al. 2013; Vuorinen & Schuster, 2015). A

identificação e aplicação de modelos farmacofóricos constituem ferramentas centrais no desenvolvimento de fármacos, sendo amplamente empregadas na triagem virtual, otimização de compostos líderes e exploração de novas classes químicas com potencial atividade biológica (Pirhadi et al. 2013; Güner et al. 2014; Caporuscio & Tafi, 2011; Giordano et al. 2022).

A hidroxietilamina apresenta estrutura química composta por uma cadeia etil substituída por um grupo hidroxila (–OH) em um dos carbonos e uma amina primária (–NH_2) no carbono adjacente, correspondendo à fórmula estrutural HO–CH_2–CH_2–NH_2 e fórmula molecular C_2H_7NO (Figura 2). A presença concomitante desses grupos funcionais confere elevada polaridade e significativo momento dipolar à molécula, possibilitando a formação de ligações de hidrogênio tanto como doadora quanto como aceitadora, além de interações dipolo-dipolo em meio aquoso, favorecendo sua solubilidade e comportamento intermolecular em sistemas biológicos (Simond et al. 2014; Dach et al. 2025). Do ponto de vista farmacológico, essa unidade estrutural é frequentemente empregada como isóstero de estado de transição em inibidores enzimáticos, especialmente em inibidores de proteases, devido à sua similaridade com intermediários formados durante a hidrólise de ligações peptídicas (Dohnálek et al. 2002; Kale et al. 2011).

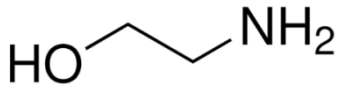

Figura 4. Estrutura química da 2-hidroxietilamina, apresentando um grupo hidroxila (–OH) e um grupo amino (–NH_2) ligados por uma cadeia etilênica.

A relevância de arcabouços contendo unidades amínicas e alcoólicas pode ser ilustrada pelo etambutol (EMB), fármaco antimicobacteriano de primeira linha amplamente utilizado no tratamento da tuberculose (NATIONAL LIBRARY OF MEDICINE, 2024). Estruturalmente, o EMB corresponde ao composto 2,2'-etilenodiiminodi-1-butanol, apresentando duas unidades amínicas e grupos hidroxila em sua cadeia, características determinantes para sua interação com alvos biológicos (Zhao et al. 2022 O mecanismo de ação do etambutol consiste na inibição das arabinosiltransferases EmbA, EmbB e EmbC, enzimas essenciais para a biossíntese do arabinogalactano, um componente estrutural da parede celular de *Mycobacterium*

tuberculosis. O etambutol liga-se ao sítio ativo dessas enzimas, bloqueando a polimerização do arabinano e comprometendo a integridade da parede celular, resultando em efeito bacteriostático (Zhang et al. 2020). A exploração de análogos estruturais do EMB ao longo das últimas décadas, incluindo o derivado SQ109, evidencia como modificações em arcabouços amina-álcool podem impactar potência e propriedades farmacológicas (Jia et al. 2005).

Esses exemplos reforçam que a combinação estratégica de funções amínicas e alcoólicas constitui um motivo estrutural relevante na química medicinal, passível de modulação para diferentes alvos biológicos. Nesse contexto, a hibridização molecular, estratégia que consiste na combinação de dois ou mais fragmentos farmacofóricos em uma única entidade química, permite gerar derivados com propriedades otimizadas, como maior afinidade, seletividade modificada e potencial ação *multitarget* (Pedrosa et al. 2017; Ivasiv et al. 2019). A modificação estrutural desses farmacóforos contribui para o aprimoramento de propriedades farmacodinâmicas e farmacocinéticas e para o desenvolvimento de novos candidatos a fármacos (Caporuscio & Tafi, 2011; Giordano et al. 2022; Talevi, 2024).

Entre os grupos explorados em estratégias de hibridização, as sulfonamidas destacam-se por sua versatilidade estrutural e capacidade de estabelecer interações polares por meio do grupo –SO_2NH–, favorecendo sua aplicação no planejamento de derivados sintéticos com potencial atividade biológica (Culletta et al. 2023). A incorporação desse grupo funcional a diferentes farmacóforos tem sido explorada para modular propriedades farmacocinéticas e farmacodinâmicas. Nesse contexto, a associação da unidade hidroxietilamina a núcleos sulfonamídicos heteroaromáticos fundamenta a concepção estrutural dos compostos avaliados neste estudo, propostos para investigar seu potencial biológico e sua possível associação com o albendazol no controle de *Toxocara canis*.

2.4.2 Núcleo anilínico e derivados orto-hidroxilados como plataforma estrutural

A anilina é formada por um anel benzênico ligado a um grupo amino primário (–NH_2), sendo classificada como uma amina aromática, em contraste com as aminas alifáticas (Lv et al. 2018) apresentadas na Figura 3. A interação entre o par de elétrons não ligantes do nitrogênio e o sistema eletrônico do anel aromático promove deslocalização eletrônica, reduzindo sua basicidade em comparação às aminas alifáticas e influenciando sua reatividade química. Essas características determinam seu

comportamento em reações como oxidação e substituição eletrofílica aromática, além de serem moduladas pela presença e natureza de substituintes no anel benzênico (Stasyuk et al., 2016). Na química medicinal, o núcleo anilínico é amplamente utilizado como *scaffold* estrutural versátil, pois a introdução de diferentes grupos no anel aromático permite modular propriedades farmacocinéticas e atividade biológica de diversos compostos (Chrobak et al. 2024).

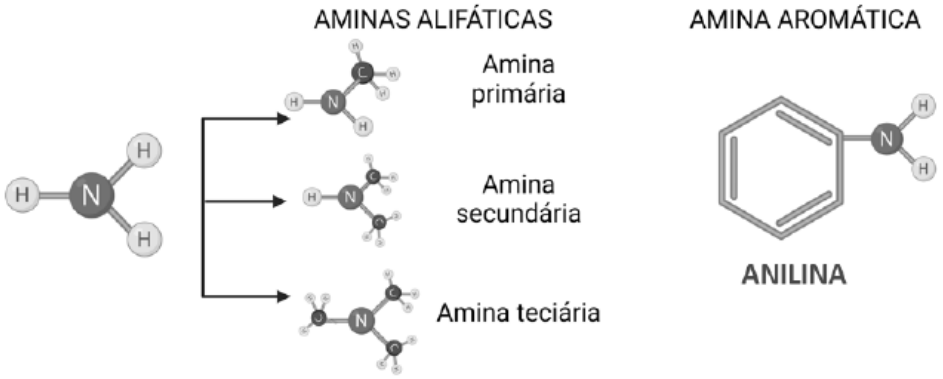

Figura 5. Classificação dos grupos orgânicos amina em aminas alifáticas (primária, secundária e terciária) e amina aromática (anilina). Fonte: Kramer et al. (2023).

Contudo, a anilina não substituída apresenta perfil toxicológico relevante, principalmente associado à bioativação metabólica por N-hidroxilação, processo que leva à formação de metabólitos reativos, como a fenilhidroxilamina (Kalgutkar et al. 2015). Esses intermediários estão relacionados à indução de metemoglobinemia e a eventos de estresse oxidativo, podendo desencadear alterações hematológicas e danos celulares decorrentes da interação com macromoléculas biológicas (Möller et al. 2017; Koenig et al. 2018). Além disso, evidências indicam que parte dos efeitos adversos observados pode estar associada a mecanismos indiretos envolvendo sobrecarga oxidativa e alterações redox persistentes (Koenig et al. 2018).

Tais modificações podem alterar a densidade eletrônica do anel aromático, influenciar a estabilidade dos intermediários formados durante o metabolismo e limitar a geração de espécies eletrofílicas associadas à toxicidade sistêmica (Zhang et al. 2020; Asai et al. 2021). Além disso, ajustes estruturais direcionados podem modular propriedades físico-químicas, como lipofilicidade e padrão de interação intermolecular, impactando diretamente o perfil farmacocinético das moléculas candidatas (Zhang et al. 2020). Essas abordagens buscam, portanto, equilibrar eficácia biológica e segurança

toxicológica no contexto do planejamento racional de novos compostos (Asai et al. 2021).

A hidroxilação em posição orto (C2) no anel anilínico pode modificar de maneira significativa o perfil metabólico e toxicológico das anilinas. A introdução de um grupo hidroxila nessa posição tende a dificultar a N-hidroxilação catalisada por enzimas do citocromo P450, principal via responsável pela formação de metabólitos reativos associados à hematotoxicidade, como a metemoglobinemia (Miura et al. 2021). Esse efeito é atribuído tanto ao impedimento estérico quanto à alteração da distribuição eletrônica promovida pelo grupo hidroxila, que reduz a acessibilidade do grupo amino à bioativação metabólica e, consequentemente, a formação de intermediários eletrofílicos potencialmente mutagênicos (Miura et al. 2021; Zhang et al. 2020).

Enquanto a anilina não substituída é predominantemente convertida em fenilhidroxilamina, associada à anemia hemolítica, estresse oxidativo e lesão hepatocelular (Möller et al. 2017; Jin et al. 2022), as anilinas orto-hidroxiladas tendem a redirecionar o metabolismo para a formação de aminofenóis com menor potencial de bioativação tóxica (Zhang et al. 2020; Miura et al. 2021). Além do impacto metabólico, a presença da hidroxila em posição orto favorece a formação de ligações de hidrogênio e pode influenciar a interação com alvos enzimáticos, contribuindo para propriedades biológicas relevantes e ampliando seu potencial como *scaffold* farmacológico (Ghiazza et al. 2023).

Modificações estruturais em aminas aromáticas, incluindo hidroxilação e metilação de grupos fenólicos, representam estratégias frequentemente empregadas na química medicinal para modular propriedades fisico-químicas, como polaridade, permeabilidade de membrana e estabilidade metabólica. Essas modificações podem influenciar tanto a atividade biológica quanto o perfil de toxicidade de moléculas bioativas (Sun et al. 2018; Schönherr & Cernak, 2022). Nesse contexto, a avaliação de derivados de anilina estruturalmente relacionados pode contribuir para a identificação de esqueletos moleculares promissores no desenvolvimento de novos agentes antiparasitários.

2.4.3 Potencial antiparasitário de derivados sulfonamídicos e anilínicos

As sulfonamidas exercem atividade antiparasitária principalmente por inibição competitiva da enzima diidropteroato sintase (DHPS), atuando como análogos do ácido para-aminobenzoico (PABA) e bloqueando a biossíntese de folato, essencial para a replicação e sobrevivência de diversos protozoários (Bilbao-Ramos et al. 2012; Mishra et al. 2025). Além desse mecanismo clássico, alguns derivados também têm sido associados à geração de espécies reativas de oxigênio (ROS) e danos ao DNA, especialmente em *Leishmania* spp. e *Trypanosoma cruzi* (Bilbao-Ramos et al. 2012; Hackler et al. 2017). Clinicamente, sulfonamidas como sulfadiazina (utilizada no tratamento da toxoplasmose), sulfadoxina (em associação com pirimetamina para malária) e sulfametoxazol (com trimetoprima para *Pneumocystis jirovecii*) ilustram sua relevância terapêutica (Figura 6).

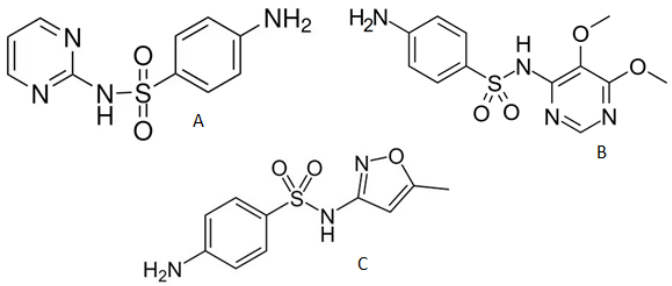

Figura 6. Estruturas químicas de sulfonamidas de importância clínica: (A) sulfadiazina, (B) sulfadoxina e (C) sulfametoxazol (Mishra et al. 2025; DailyMed, 2026 com modificações).

Embora o uso clínico contra helmintos seja mais limitado, estudos experimentais têm demonstrado atividade contra diferentes espécies de nematódeos, incluindo *Ancylostoma ceylanicum*, *Trichuris muris* e *Caenorhabditis elegans*, com impacto sobre motilidade, fecundidade e viabilidade em ensaios *in vitro* e *in vivo* (Elfawal et al. 2019). Triagens fenotípicas também indicaram potencial atividade contra nematódeos intestinais e filariais, como *Ascaris suum* e *Heligmosomoides bakeri*, especialmente no contexto de reposicionamento de fármacos (Hu et al. 2013; Tyagi et al. 2019). Entre os cestódeos, destaca-se *Echinococcus granulosus*, no qual sulfonamidas demonstraram redução da viabilidade e da síntese de DNA em protoescolices (Panesso et al. 2023). Esses achados reforçam o potencial desse núcleo estrutural na prospecção de novos agentes anti-helmínticos (Panesso et al. 2023).

Derivados anilínicos têm mostrado atividade contra protozoários como *Leishmania* spp. e *Trypanosoma cruzi*, atuando por diferentes mecanismos. Esses compostos podem interferir na formação dos microtúbulos, estruturas importantes para a divisão celular do parasita, além de induzir a produção de espécies reativas de oxigênio (ROS), causando dano mitocondrial e levando à morte celular. Também podem inibir enzimas essenciais para o metabolismo redox, como a pteridina redutase (PTR1) e a superóxido dismutase dependente de ferro (Fe-SOD), comprometendo a sobrevivência do parasito (Galiana-Roselló et al. 2013; Freitas et al. 2024). Estudos de modelagem molecular indicam ainda que esses derivados podem interagir diretamente com a PTR1 em *Leishmania* spp., reforçando seu potencial como inibidores enzimáticos (do Carmo et al. 2023; Gomes et al. 2026).

Do ponto de vista estrutural, diferentes *scaffolds* anilínicos têm sido explorados, incluindo N-benzenossulfonamidas, hidrazonas aril-anilínicas, anilino-benzimidazóis, anilino-fenanthrolinas, anilino-pirazinas, anilinas halogenadas e arilalquilaminas. Diversos compostos dessas classes demonstraram atividade potente e seletiva contra *Leishmania* spp. e *Trypanosoma* spp. em modelos *in vitro* e *in vivo* (Galiana-Roselló et al. 2013; Iniguez et al. 2015; Freitas et al. 2024).

Além dos protozoários, há evidências experimentais de atividade de derivados anilínicos contra helmintos. Compostos como diaminas e indóis aril-anilínicos apresentaram efeito sobre nematódeos como *Teladorsagia circumcincta* e *Haemonchus contortus*, com redução da motilidade larval, interferência na eclosão de ovos e possível inibição do complexo mitocondrial II (Valderas-García et al. 2021; Kadlecová et al. 2025). Esses achados ampliam o espectro biológico do núcleo anilínico e reforçam seu potencial na prospecção de novos agentes antiparasitários.

2.4.4 Associação de compostos sintéticos ao albendazol como estratégia de otimização terapêutica

A associação de fármacos com mecanismos de ação distintos constitui estratégia consolidada para otimização terapêutica em doenças complexas, incluindo infecções parasitárias. Essa abordagem pode promover efeitos sinérgicos ou aditivos, ampliar a

atuação sobre múltiplos alvos biológicos e permitir a redução das doses individuais, com impacto positivo sobre eficácia, segurança e resistência (van Hasselt et al. 2018; Narwal et al. 2025; Gilron et al. 2013). A racionalização dessas combinações, baseada em princípios farmacodinâmicos e farmacocinéticos, é essencial para maximizar benefícios clínicos e minimizar riscos.

Apesar de amplamente empregado no tratamento de helmintíases, o albendazol apresenta limitações terapêuticas em determinados contextos. Sua eficácia é variável frente a algumas espécies, como *Trichuris trichiura*, além de desempenho limitado contra helmintos filariais e trematódeos (Moser et al. 2017; Sisay et al. 2024; Gray et al. 2026). Em helmintíases teciduais, como a toxocaríase visceral, a erradicação completa das larvas é frequentemente incompleta, e reações inflamatórias decorrentes da morte parasitária podem exigir manejo adjuvante (Ma et al. 2018; Magnaval et al. 2022).

Adicionalmente, variações interindividuais na exposição ao sulfóxido de albendazol, seu metabólito ativo, podem influenciar a resposta terapêutica, sendo afetadas por fatores como estado nutricional e uso concomitante de outros medicamentos (Whittaker et al. 2022). Em cenários de uso repetido em larga escala, também têm sido descritas reduções na eficácia associadas à pressão seletiva e a polimorfismos na β-tubulina, reforçando a necessidade de estratégias que ampliem o desempenho do fármaco (Sisay et al. 2024).

Nesse contexto, estudos *in vitro* demonstram que a associação de albendazol com compostos sintéticos de diferentes classes químicas pode potencializar sua atividade antiparasitária. Derivados de imidazo[1,2-a]pirimidinas apresentaram sinergismo contra *Giardia lamblia* (Velázquez-Olvera et al. 2016), enquanto a combinação com monoterpenos sintéticos mostrou efeito sinérgico contra ovos de *Haemonchus contortus*, com alterações ultraestruturais e biofísicas observadas por microscopia de força atômica, sugerindo ação combinada sobre a superfície do parasito (Silva et al. 2021). Esses dados fundamentam a associação com compostos sintéticos como estratégia de otimização da atividade do albendazol. A partir dessa perspectiva, a investigação de compostos sintéticos estruturalmente distintos em associação ao albendazol representa uma abordagem promissora para ampliar a eficácia terapêutica frente a helmintíases teciduais. Assim, a avaliação de novos derivados químicos com

potencial atividade antiparasitária pode contribuir para o desenvolvimento de estratégias terapêuticas mais eficazes no controle da toxocaríase.

3. OBJETIVOS

3.1 Objetivo Geral

Avaliar o potencial de compostos sintéticos derivados de hidroxietilamina e anilina, isoladamente e em associação com o albendazol, como estratégia de otimização terapêutica no controle de *Toxocara canis*.

3.2 Objetivos Específicos

- Avaliar a atividade larvicida *in vitro* dos derivados de hidroxietilamina e dos derivados de anilina frente a larvas de *Toxocara canis*;
- Determinar o potencial de citotoxicidade dos compostos sintéticos em células de mamíferos;
- Investigar a interação entre os compostos sintéticos e o albendazol por meio do ensaio *checkerboard*, com interpretação dos resultados baseada no índice de concentração inibitória fracionada (FICI).
- Avaliar propriedades farmacocinéticas preditivas e perfil toxicológico dos compostos sintéticos por meio de análises *in silico*;
- Avaliar o efeito da administração dos compostos sintéticos, isoladamente e em associação com o albendazol, sobre a intensidade da infecção em modelo murino de toxocaríase visceral.

4. HIPÓTESE

A associação de compostos sintéticos derivados de hidroxietilamina e dos derivados de anilina ao albendazol aumentam a atividade antiparasitária contra *Toxocara canis*.

5. ARTIGO 1

Additive larvicidal activity of albendazole combined with a hydroxyethylamine-derived compound against *Toxocara canis* larvae: an *in vitro* and *in silico* study

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Abstract

Human toxocariasis is a neglected zoonotic helminthiasis characterized by larval migration through tissues such as the liver, lungs, central nervous system, and eyes. Current pharmacological treatment presents variable efficacy, highlighting the need for improved therapeutic strategies. This study evaluated the larvicidal activity of albendazole combined with heteroaromatic sulfonamide compounds derived from hydroxyethylamine against *Toxocara canis*, using *in vitro assays*, *in silico* analyses, and a *checkerboard design*. Three synthetic compounds were initially screened for larvicidal activity and cytotoxicity. All induced 100% larval unviability at 1 mg/mL; however, only HIDROXI 1 maintained complete larvicidal activity at 0.5 mg/mL, while exhibiting an acceptable cytotoxicity profile. *In silico* analyses indicated

physicochemical properties consistent with favorable oral bioavailability and compliance with Lipinski's Rule of Five. Albendazole alone induced complete larval unviability only at 40 mg/mL. In the *checkerboard* assay, the combination of HIDROXI 1 (0.25 mg/mL) with albendazole (10 or 5 mg/mL) resulted in 100% and 99% larval unviability, respectively, indicating an additive interaction. These findings demonstrate that HIDROXI 1 exhibits significant *in vitro* larvicidal activity and enhances the effect of albendazole at lower concentrations, supporting further investigation of this combination as a potential therapeutic approach for toxocariasis.

Keywords: Anthelmintic activity; Parasitic diseases; Helminth infections; Drug combination;

1. INTRODUCTION

Human toxocariasis is characterized by the migration of *Toxocara canis* larvae through different tissues, including the liver, lungs, central nervous system, and eyes, resulting in nonspecific clinical manifestations whose severity depends on parasite burden, larval localization, and the host immune response (Meliou et al. 2020). This condition results from a neglected parasitic zoonosis with worldwide distribution, in which humans act as accidental hosts for the intestinal parasite of dogs (Chen et al. 2018). Transmission occurs mainly through the ingestion of embryonated eggs present in contaminated soil, water, and food, as well as through the ingestion of larvae encysted in raw or undercooked meat or viscera from paratenic hosts, such as pigs, cattle, and poultry (Magnaval et al. 2022).

The treatment of toxocariasis is based on the use of benzimidazole-class anthelmintics, widely used for intestinal helminthiasis such as ascariasis, trichuriasis, and hookworm infection (Chen et al. 2018). However, in *Toxocara* spp. infection, in which there is tissue damage, the efficacy of these drugs is limited, in particular due

855 to their low bioavailability and reduced action on migratory larvae, around 45-80%
856 (Magnaval et al. 2001; Magnaval et al. 2022). Considering the challenges in the
857 treatment of toxocariasis, several studies have investigated new bioactive compounds
858 against *Toxocara canis*, including heteroaromatic derivatives such as camphor
859 analogues, nitrofurans, and naphthoquinones, which have demonstrated larvicidal
860 activity in experimental models (Mata-Santos et al. 2016a; Rodrigues et al. 2025a;
861 2025b; 2025c).

862 Sulfonamides are a widely used class of compounds with recognized
863 antibacterial activity resulting from the inhibition of folic acid synthesis by acting as
864 competitive analogues of para-aminobenzoic acid (PABA) (Krátký et al. 2012). In
865 addition to this application, compounds containing sulfonamide rings have
866 significant antiparasitic activity, especially against protozoa, particularly *Toxoplasma*
867 *gondii*, both in established therapeutic regimens and in the development of new
868 derivatives with promising activity (Feliciano-Alfonso et al. 2021; Aljohani et al.
869 2022; Tambat et al. 2024). Sulfonamides are also used in the treatment of
870 opportunistic intestinal coccidiosis, such as *Cystoisospora belli* and *Cyclospora*
871 *cayetanensis*, through the combination of sulfamethoxazole-trimethoprim (Smith &
872 Paton, 2002; Lucas et al. 2012), in addition to being part of well-established
873 regimens against *Plasmodium falciparum*, such as the sulfadoxine-pyrimethamine
874 combination (Hyde, 2005).

875 Although several studies have investigated new bioactive compounds
876 against *Toxocara canis*, the potential interaction between synthetic sulfonamide
877 derivatives and albendazole has not yet been explored. Accordingly, the present study
878 aimed to investigate the larvicidal activity of three hydroxyethylamine-derived
879 sulfonamide compounds against *T. canis* larvae, as well as their cytotoxicity profile and

predicted bioavailability through *in silico* analyses. In addition, the interaction between the most promising compound and albendazole was evaluated using a checkerboard assay to explore the effects of this pharmacological association on larvicidal activity.

MATERIALS AND METHODS

2.1. Obtaining *Toxocara canis* eggs and larvae

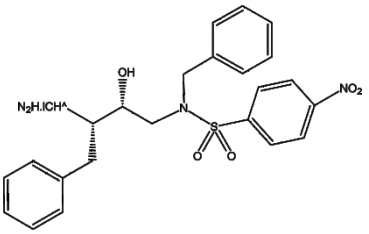
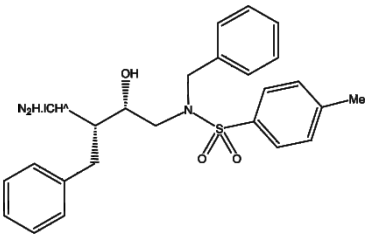
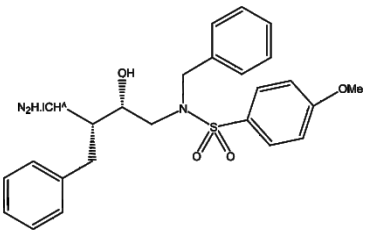
T. canis eggs were collected from adult females of the parasite, recovered from young dogs naturally infected and treated with the anthelmintic pyrantel pamoate (15 mg/kg). These were incubated in a 2% formalin solution at a temperature of 28°C, with humidity above 80% and under daily oxygenation, for a period of 30 days (Avila et al. 2013). The larvae were extracted by manual agitation and collected using a Baermann device, and kept in RPMI-1640 medium (Gibco®), supplemented with 25 mM HEPES (Sigma®), 1% glucose, and PSF antibiotic-antimycotic solution (penicillin, streptomycin, and amphotericin B) (Gibco®), at 37°C in an atmosphere with 5% CO_2 (Savigny, 1975).

2.2. Hydroxyethylamine derivatives

Three hydroxyethylamine-derived compounds presenting structural similarity to the antimycobacterial drug ethambutol were synthesized at the Institute of Drug Technology (Farmanguinhos), Oswaldo Cruz Foundation (Fiocruz), according to the methodology described by Facchinetti et al. (2015) (Table 1). The spectroscopic identification data of the evaluated compounds (^{1}H NMR, ^{13}C NMR, IR, MS and elemental analysis) are provided in the Supplementary Material. Reagents and solvents commonly used in organic synthesis were obtained from commercial suppliers and used without further purification. The compounds were prepared from a solution of tert-butyl((2S,3R)-N-[4-(N-benzyl-4-R-phenylsulfonamido)-3-hydroxy-1-phenylbutan-2-

yl)carbamates (3.0 mmol) in ethanol, which was treated with hydrogen chloride gas. After the reaction, the volatiles were removed by evaporation, and the crude product was crystallized in diethyl ether, yielding an average yield of 92.3%, according to the methodology described by Facchinetti et al. (2015). For biological assays, the synthesized compounds were solubilized in 2.5% dimethyl sulfoxide (DMSO) and sterile distilled water, obtaining a final concentration of 1 mg/mL, according to Mata-Santos et al. (2015).

Table 1. Chemical structure of hydroxyethylamine-derived compounds tested *in vitro* on *Toxocara canis* larvae (Facchinetti et al. 2015).

Identification	Molecular structure	IUPAC name
HIDROXI 1		N-((*2R*,*3S*)-3-amino-2-hydroxy-4-phenylbutyl)-N-benzyl-4 nitrobenzenesulfonamide hydrochloride
HIDROXI 2		N-((*2R*,*3S*)-3-amino-2-hydroxy-4-phenylbutyl)-N-benzyl-4 methylbenzenesulfonamide hydrochloride
HIDROXI 3		N-((*2R*,*3S*)-3-amino-2-hydroxy-4-phenylbutyl)-N-benzyl-4 methoxybenzenesulfonamide hydrochloride

2.3. *In vitro* screening tests on *Toxocara canis*

The larvicidal assays were performed in duplicate due to the limited number of viable larvae obtained from the Baermann recovery procedure, a limitation commonly

reported in in vitro screening assays involving *Toxocara* larvae (Rodrigues et al. 2025; Rodrigues et al. 2024). At an initial concentration of 1 mg/mL in 96-well microculture plates (Cral®) containing 100 *Toxocara canis* larvae per well in RPMI-1640 medium. Live larvae and dead larvae obtained by heat shock, a physical method previously described as effective for the inactivation of *T. canis* eggs and larvae, were used as controls. The plates were incubated for 48 h at 37°C in an atmosphere containing 5% CO_2. After this period, Trypan Blue (0.4%) was added, and larval viability was assessed by optical microscopy (10× objective). Distended, immobile larvae stained with Trypan Blue were considered non-viable, according to criteria described by Mata-Santos et al. (2015). For classification purposes, total larvicidal activity was considered to be ≥ 99% larval unviability, adopting this threshold to minimize the impact of low-magnitude technical variations inherent in experimental handling, such as pipetting and microscopic reading. To determine the minimum larvicidal concentration (MLC), compounds that showed total larvicidal activity were subjected to serial dilutions (1 mg/mL to 0.05 mg/mL). The anthelmintic albendazole was also subjected to this evaluation, at concentrations ranging from 40 mg/mL to 5 mg/mL, in a test performed under the same conditions mentioned above. The 50% lethal concentrations (LC50) and 90% lethal concentrations (LC90) of the compounds against *T. canis* larvae were estimated from the results obtained in the CLM test. For the calculations, linear interpolation was used between adjacent experimental points that delimit the mortality intervals close to 50% and 90%. This procedure provides an approximate estimate suitable for preliminary analyses and has been widely applied in dose–response screening approaches based on limited experimental points (Baharith et al., 2005).

2.4. *In vitro* cytotoxicity assay

For the *in vitro* cytotoxicity assay, murine macrophages of the J774.A1 strain

(1 × 10^5 cells/100 µL) were used, cultured in DMEM medium supplemented with 10% fetal bovine serum and PSF antibiotic-antimycotic solution (Gibco®). The three compounds were evaluated in triplicate at the same concentrations used in the MLC tests (1 to 0.05 mg/mL). For result assessment, 0.01% resazurin was added, and after 24 h of incubation at 37°C in an atmosphere containing 5% CO_2, fluorescence was measured at 620 nm using a BioTek™ ELx800™ microplate reader. Results were expressed as percentage of cell viability relative to the control, as described by Ansar Ahmed et al. (1994).

2.5 Bioavailability *in silico*

After initial screening, the compound selected based on the lowest minimum larvicidal concentration (MLC) associated with an adequate cytotoxicity profile was then subjected to structural characterization and *in silico* evaluation. The selected compound was designed using the ChemBioDraw Ultra 12.1 chemical editor (Halford, 2015), and its bioavailability was evaluated using the SwissADME platform (www.swissadme.ch), following the criteria established by Lipinski's "Rule of Five," which considers four parameters: molecular weight ≤ 500, miLogP ≤ 5, number of hydrogen donors ≤ 5, and number of hydrogen acceptors ≤ 10 (Lipinski, 2004).

2.6 *In vitro* tests of the albendazole-compound association

To evaluate the association of albendazole with the compound selected in the screening tests, a *checkerboard* assay was conducted, following the methodology of Bellio et al. (2021). Duplicate 96-well microculture plates containing 100 *T. canis* larvae in RPMI-1640 medium were used, which were incubated at 37°C with 5%

CO_2 for 48 hours, according to the methodology previously described by Mata-Santos et al. (2015). To evaluate the larvicidal activity of the combination, serial dilutions of the compound were used starting from its minimum larvicidal concentration (MLC), combined with albendazole at concentrations of 10 and 5 mg/mL, to determine the minimum larvicidal concentration associated with albendazole (MLC-ABZ).

The interaction between albendazole and the selected compound was quantified using the fractional inhibitory concentration index (FICI). The fractional inhibitory concentrations (FIC) were calculated as follows: $FIC_{HIDROXI\ 1} = (MLC_{HIDROXI\ 1\ +ABZ})/(MLC_{HIDROXI\ 1})$; $FIC_{ABZ} = (MLC_{ABZ+\ HIDROXI\ 1})/(MLC_{ABZ})$; $FICI = FIC_{HIDROXI\ 1} + FIC_{ABZ}$.

FICI values ≤ 0.5 were interpreted as synergistic, $0.5 < FICI \leq 1$ as an additive effect, $1 < FICI \leq 4$ as indifferent, and $FICI > 4$ as antagonistic, according to the criteria proposed by Meletiadis et al. (2010). Similar approaches using the FICI to characterize drug interactions in checkerboard assays have been widely reported in the literature (Hall et al. 1983; Chauzy et al. 2019; Antonelli et al. 2022).

2.7 Ethics statement

Adult worms were obtained from naturally infected dogs treated as part of routine veterinary care. No experimental infection of animals or human subjects was performed in this study. Therefore, ethical approval was not required.

2.8 Data analysis

Linear interpolation was used to estimate the 50% lethal concentrations (LC50) and 90% lethal concentrations (LC90) of the compounds against *Toxocara canis* larvae from the dose–response data obtained in the MLC assay.

For the cytotoxicity assay, optical density values obtained in technical triplicates were used to generate dose–response curves for IC_{50} determination.

3. RESULTS

In *in vitro* screening tests, the three compounds demonstrated larvicidal activity, causing 100% larval unviability at a concentration of 1 mg/mL. In the assay to determine the minimum larvicidal concentration (MLC), only compound HIDROXI 1 showed 100% larvicidal activity at a concentration of 0.5 mg/mL (Table 2).

Table 2. Percentage of *in vitro* larvicidal activity of hydroxyethylamine derivatives (HIDROXI) at different concentrations on *Toxocara canis* larvae after 48 hours of exposure.

Compound (Code)	% larvicidal activity				
	1 mg/mL	0.5 mg/mL	0.25 mg/mL	0.125 mg/mL	0.05 mg/mL
HIDROXI 1	100	100	57.1	13.1	18.8
HIDROXI 2	100	93.3	50	28.6	10.5
HIDROXI 3	100	81.8	66.6	64.4	17.8

*Live control: average unviability of 11.6%; dead control: 100% of larvae were unviable.

Table 3 shows the MLC of albendazole alone, confirming larvicidal activity in 100% of the larvae only at a concentration of 40 mg/mL. Based on the mortality data obtained in the MLC assay, the estimated LC50 and LC90 values were 0.22 mg/mL and 0.48 mg/mL for HIDROXI 1, 0.25 mg/mL and 0.47 mg/mL for HIDROXI 2, and 0.14 mg/mL and 0.43 mg/mL for HIDROXI 3, respectively.

Table 3. Percentage of *in vitro* larvicidal activity of the anthelmintic albendazole, at different concentrations, on *Toxocara canis* larvae after 48 hours of exposure.

	% larvicidal activity			
Albendazole	40 mg/mL	20 mg/mL	10 mg/mL	5 mg/mL
	100	65.0	16.4	7.4

*Live control: average unviability of 3.6%; dead control: 100% of larvae were unviable.

Regarding cytotoxicity, compound HIDROXI 1 showed 78% cell viability at a concentration of 0.25 mg/mL after 24 hours of exposure to J774.A1 macrophage cells. At the same concentration, compound HIDROXI 2 showed 96% cell viability, while HIDROXI 3 showed 94% cell viability (Table 4), however, at this concentration the compounds HIDROXI 2 and HIDROXI 3 presented 28.6% and 66.6% activity against *T. canis*, respectively, which disqualified them from further analysis according to the established criteria.

Table 4. Cytotoxicity test after 24 hours of exposure to different concentrations of compounds HIDROXI 1, HIDROXI 2, and HIDROXI 3.

	Cell viability				
Compound	**1 mg/mL**	**0.5 mg/mL**	**0.25 mg/mL**	**0.125 mg/mL**	**0.05 mg/mL**
HIDROXI 1	51	57	78	89	97
HIDROXI 2	37	94	96	100	100
HIDROXI 3	88	87	94	99	100

In silico predictions indicated that compound HIDROXI 1 has properties compatible with good oral bioavailability (Table 5) and compliance with Lipinski's

1029 Rule of Five (Lipinski, 2004).

1030 **Table 5**. *In silico* pharmacokinetic parameters of the compound N-((*2R*,*3S*)-3-
1031 amino-2-hydroxy-4-phenylbutyl)-N-benzyl-4 nitrobenzenesulfonamide
1032 hydrochloride (HIDROXI 1) and albendazole.

Compound	Molecular weight (g/mol)	miLogP	H-bond donors	H-bond acceptors
HIDROXI 1	490.98	1.95	2	7
Albendazole	265.33	1.62	2	3

1033

1034 Considering that the selected compound, HIDROXI 1, showed 100%
1035 larvicidal activity at concentrations of 1 mg/mL and 0.5 mg/mL, the evaluation of the
1036 combination with albendazole was conducted at concentrations where the activity of
1037 the isolated compound was partial or absent. Thus, the *checkerboard* assay was used
1038 to investigate whether the combination with albendazole could enhance the
1039 larvicidal activity of HIDROXI 1 under these conditions (Table 6). It was observed
1040 that, at a concentration of 0.25 mg/mL, the combination of HIDROXI 1 with
1041 albendazole (10 mg/mL or 5 mg/mL) resulted in 100% and 99% larval unviability,
1042 respectively.

1043 The interaction between the compounds was further evaluated using the
1044 fractional inhibitory concentration index (FICI). The combination of HIDROXI 1 at
1045 0.25 mg/mL with albendazole at 10 mg/mL resulted in a FICI value of 0.75, while
1046 the combination of the same concentration with albendazole at 5 mg/mL resulted in a
1047 FICI value of 0.625. According to the criteria proposed by Meletiadis et al. (2010),
1048 FICI values between 0.5 and 1.0 indicate an additive interaction.

1049

1050 **Table 6.** Percentage of *in vitro* larvicidal activity on *Toxocara canis* larvae of

the compound *N*-((*2R*,*3S*)-3-amino-2-hydroxy-4-phenylbutyl)-N-benzyl-4 nitrobenzenesulfonamide hydrochloride (HIDROXI 1) in combination with the anthelmintic albendazole, at different concentrations.

Compound	% *in vitro* activity				
	1 mg/mL	0.5 mg/mL	0.25 mg/mL	0.125 mg/mL	0.05 mg/mL
HIDROXI 1 + ABZ 10 mg/mL	100	100	100	82	56.1
HIDROXI 1 + ABZ 5 mg/mL	100	100	99.4	86.8	29.5
ABZ control 1 (10 mg/mL)	16.4	-	-	-	-
ABZ control 2 (5 mg/mL)	7.4	-	-	-	-

*Live control: average unviability of 3.6%; dead control by thermal shock: 100% of larvae were unviable.

4. DISCUSSION

In this study, all compounds showed total larvicidal activity (100%) at a concentration of 1 mg/mL *in vitro*, indicating antiparasitic potential. In screening studies, this result represents a starting point for evaluating the degree of larvicidal activity, making it necessary to determine the minimum larvicidal concentration (MLC) to differentiate the tested compounds (Pink et al. 2005; Mengarda et al., 2023). In this context, the compound HIDROXI 1 stood out for having the lowest MLC (0.5 mg/mL).

Studies conducted with other synthetic compounds revealed larvicidal activity *in vitro,* as well as in experimental models of toxocariasis (Mata-Santos et al. 2015, 2016; Rodrigues et al. 2024, 2025a–c). Considering the therapeutic limitations of albendazole in controlling tissue larvae (Magnaval et al. 2022), these results support the investigation of combinatorial strategies aimed at enhancing the larvicidal effect.

In this context, alternative strategies to the development of new molecules, such as drug repositioning, have been successfully explored in the control of different helminthiasis. Several pharmacological classes originally indicated for other diseases, including antimalarials, antibiotics, antiprotozoals, anticancer agents, and veterinary antiparasitics, have already demonstrated activity against *Schistosoma* sp., liver flukes, geohelminths, and filariae (Panic et al. 2014).

Taken together, these results support the approach adopted in this study, which evaluated the increased efficacy of a well-established anthelmintic through its association with a bioactive synthetic compound. In addition to the identification of new compounds, the evaluation of pharmacological associations represents a complementary strategy to enhance the efficacy of established drugs.

The combination of albendazole and the compound HIDROXI 1 was evaluated using the *checkerboard* assay, an approach widely used for the quantitative analysis of pharmacological interactions, allowing the classification of effects such as synergism, additivity, or antagonism (Meletiadis et al. 2010; Bellio et al. 2021). Although more frequently applied in studies with bacteria and fungi (Guerra et al. 2012; Duarte et al. 2012; Rosato et al. 2018; Costa et al. 2019; Azzam et al. 2020), these observations indicate that the *checkerboard* assay may also be useful for broadening the understanding of combinatorial strategies in parasite control.

Albendazole is the main choice for treating human toxocariasis (Magnaval et al. 2022). The therapeutic efficacy of albendazole in human toxocariasis is considered moderate, mainly due to limitations related to bioavailability and tissue distribution (Magnaval et al. 2022). The combination of albendazole with HIDROXI 1 resulted in increased larvicidal activity compared with each compound used alone. This effect was consistent with an additive interaction, as indicated by the calculated FICI values. This type of interaction is defined when the combined effect is compatible with the sum of the individual effects, with no evidence of additional potentiation, and it is not possible to infer the mechanisms involved from these data (Tallarida, 2001; Gupta et al. 2002; Co et al. 2009; Gorka et al. 2013; Ritz et al. 2021).

Consistent with these results, evidence from different clinical contexts supports the potential benefits of combination therapies involving albendazole. For example, the association with ivermectin has shown superior efficacy compared with albendazole alone in the treatment of *Trichuris trichiura* and hookworm infections (Krolewiecki et al. 2025). In a clinical trial conducted in Uganda, combination therapy achieved cure rates of 31.3% for *T. trichiura*, compared with 12.3% for monotherapy, and reduced parasite load by 91.4% versus 52.7%, respectively (Palmeirim et al. 2024). Similarly, in the treatment of neurocysticercosis, the combination of albendazole and praziquantel resulted in complete lesion resolution in 62% of patients, compared with 26.3% observed with albendazole alone (Singh et al. 2022; Dewi et al. 2024).

Despite these advances, there are still no reports describing the association of albendazole with synthetic compounds from other bioactive chemical classes to enhance its anthelmintic activity. This gap reinforces the importance of investigating

new pharmacological combinations aimed at improving the efficacy of existing anthelmintic therapies.

The superior activity observed for HIDROXI 1, compared to the other analogues evaluated, may be related to specific chemical-structural characteristics of the compound. Although they all share the same structural core derived from hydroxyethylamine and the sulfonamide group, HIDROXI 1 is distinguished by the presence of a nitro group in the aromatic ring. The nitro group (NO_2) confers electronic and polarity properties that may favor interactions with proteins and is widely exploited in the development of molecules with antimicrobial, antifungal, antitubercular, anti-inflammatory, and antitumor activities (Noriega et al. 2022).

In addition, it has been described that nitro groups can undergo bioreduction in biological systems, in which the action of nitroreductases leads to the formation of reactive intermediates, such as nitroso, hydroxylamine, and amine, as well as the generation of reactive oxygen species, capable of causing DNA damage and cytotoxic effects (Nepali et al. 2019; Penning et al. 2022). These redox activation processes have been associated with the cell death of microorganisms (Rice et al. 2021; Noriega et al. 2022; Penning et al. 2022), which may help explain the higher larvicidal activity observed for HIDROXI 1 and justify its selection for association trials with albendazole.

Consistently, different classes of synthetic compounds have shown activity against relevant parasites: pyrimidine derivatives have already demonstrated action against *Toxoplasma gondii*, *Trypanosoma brucei, and Leishmania infantum* (Gangjee et al. 1997, 1999; Lopes et al. 2022; Torchelsen et al. 2024), and heterocyclic analogues of ethambutol have been described as active against *Strongyloides*

venezuelensis, *Schistosoma mansoni*, and protozoa such *as Trichomonas vaginalis*, and *Giardia lamblia*, in addition to *Leishmania* spp. and *Trypanosoma brucei* (Giordani et al. 2011; Fernandes et al. 2013; Olmo et al. 2012; Legarda-Ceballos et al. 2016). Collectively, these results reinforce support the idea that structural modifications of known chemical nuclei may generate compounds with antiparasitic activity.

In addition to biological activity, pharmacokinetic properties are important in the characterization of candidate bioactive compounds. According to Lipinski's Rule of Five (RO5), oral bioavailability can be estimated based on physicochemical parameters such as lipophilicity and the number of hydrogen bond donors and acceptors (Lipinski, 2004). The results indicated moderate lipophilicity, adequate membrane permeability, and compliance with RO5 parameters (Daina et al. 2017), suggesting a favorable profile for oral absorption. Together with the larvicidal activity observed in vitro, these findings reinforce the potential of HIDROXI 1, particularly when associated with albendazole (Tallarida, 2001; Meletiadis et al. 2010; Ritz et al. 2021).

Finally, the cellular safety of the evaluated compounds was considered. HIDROXI 1 showed mild cytotoxicity (78% cell viability) at a CLM of 0.25, indicating limited effects on mammalian cell integrity and suggesting a favorable selectivity profile. Although HIDROXI 3 showed slightly higher cell viability, HIDROXI 1 was prioritized due to its greater larvicidal potency, reflected by the lowest minimum larvicidal concentration (MLC), while still presenting a cytotoxicity profile considered acceptable for early antiparasitic screening studies. (Pink et al. 2005; Butt et al. 2023). Similar findings were reported for ethambutol analogues evaluated by Linhares et al. (2023), in which aminoalkanol and alkane-1,2-diamine

derivatives showed low cytotoxicity and favorable selectivity indices.

However, cytotoxicity was evaluated using a single murine macrophage cell line (J774.A1), which represents a limitation of the present study. Although this cell line is commonly used in preliminary screening assays for antiparasitic compounds (Silva et al. 2018; Tietze et al. 2025), the use of a single cellular model limits a broader assessment of the compound's safety profile. Therefore, these results should be interpreted with caution, and future studies including additional mammalian cell lines may provide a more comprehensive evaluation of the cellular safety of this compound.

In summary, HIDROXI 1 demonstrated promising larvicidal activity with limited cytotoxic effects under the evaluated conditions. The additive interaction observed when combined with albendazole reinforces the relevance of exploring pharmacological associations to improve the efficacy of existing anthelmintic therapies. The obtained results highlight the potential of synthetic compounds as complementary strategies to enhance the activity of albendazole and may contribute to overcoming limitations related to its bioavailability and tissue distribution.

5. CONCLUSION

This study demonstrated significant *in vitro* larvicidal activity and predicted *in silico* oral bioavailability of the synthetic hydroxyethylamine-derived compound N-((2R,3S)-3-amino-2-hydroxy-4-phenylbutyl)-N-benzyl-4-nitrobenzenesulfonamide hydrochloride (HIDROXI 1), structurally related to the antimycobacterial drug ethambutol. The checkerboard assay revealed an additive interaction between HIDROXI 1 and albendazole, suggesting that this compound may enhance the antiparasitic activity of the anthelmintic against *Toxocara canis*

larvae. These findings indicate that the combination of albendazole and HIDROXI 1 represents a promising strategy to overcome limitations associated with the isolated use of albendazole. However, further preclinical *in vivo* studies are required to confirm the safety and efficacy of this combination in murine visceral toxocariasis.

DECLARATIONS

Ethics statement

Adult worms were obtained from naturally infected dogs treated with pyrantel pamoate according to standard veterinary procedures. No experimental infection of vertebrate animals or human subjects was performed in this study.

Conflict of interest

The authors declare that they have no competing interests.

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Data availability

The datasets generated and/or analyzed during the current study are available from the corresponding author on reasonable request.

Author contributions (CRediT statement)

Victória Pires Panassolo: Conceptualization, Methodology, Investigation, Formal analysis, Writing – original draft.

Andrezza Medeiros Faria: Methodology, Investigation.

Carolina Netto de Oliveira da Cunha: Methodology, Investigation.

Lourdes Helena Rodrigues Martins: Methodology, Investigation.

Julia Gonçalves Britto: Methodology, Investigation.

Victor Facchinetti Luz: Resources, Methodology (compound synthesis).

Cláudia Regina Brandão: Resources, Methodology (compound synthesis).

Marcus Vinícius Nora de Souza: Resources, Methodology (compound synthesis), Validation, Writing – review and editing.

Daniela Fernandes Ramos: Conceptualization, Methodology, Supervision.

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Luciana Farias da Costa de Avila: Supervision, Conceptualization, Writing – review and editing.

Declaration of generative AI and AI-assisted technologies in the manuscript preparation process

During the preparation of this work, the authors used ChatGPT (OpenAI) to assist in English-language editing and structural refinement of the manuscript. After using this tool, the authors reviewed, revised, and validated all content and take full responsibility for the accuracy and integrity of the published article.

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6. MANUSCRITO 1

Aniline derivative enhances albendazole activity against *Toxocara canis*: *in vitro*, *in silico* and *in vivo* evidences

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Abstract

Human toxocariasis is a zoonotic helminth infection caused by larvae of *Toxocara canis*, characterized by tissue migration and limited therapeutic options. Albendazole is the drug most commonly used for treatment. However, its low oral bioavailability and variable efficacy highlight the need for improved therapeutic strategies. In this context, the present study evaluated the antiparasitic potential of the aniline derivative 2-hydroxyaniline (POHA) against *Toxocara canis* through *in vitro***,** *in silico***,** and *in vivo* approaches, as well as its interaction with albendazole. In *vitro* assays demonstrated that POHA exhibited larvicidal activity against *T. canis*, with a minimum larvicidal concentration (MLC) of 0.125 mg/mL. However, checkerboard assays revealed a synergistic interaction between POHA and albendazole (FICI $\leq$ 0.5), indicating potentiation of the antiparasitic effect *in vitro* (MLC POHA = 0.0125 mg/mL and MLC ABZ = 10 mg/mL). Cytotoxicity assays in Vero cells showed acceptable cell viability at 0.0125 mg/mL. *In silico* analyses predicted physicochemical properties compatible with oral bioavailability and suggested a favorable safety profile. In a murine model of experimental toxocariasis, treatment with POHA (0.4 mg/kg) reduced larval burden by 47.2%, albendazole (10 mg/kg) reduced larval burden by 52.1%, and the combination therapy reduced larval burden by 66.9% compared with the control group. Additionally, *O*-methylated aniline derivatives were evaluated to assess their bioactivity and toxicity profile in comparison with 2-hydroxyaniline (POHA), maintaining high larvicidal activity while exhibiting lower cytotoxicity. Together, these findings support the potential of aniline compounds as adjuvant molecules capable of enhancing albendazole efficacy and reinforce the relevance of combination strategies for improving antiparasitic activity against *T. canis.*

Key-words: Toxocariasis; Drug Synergism; Anthelmintics; Disease Models, Animal; Larva *migrans* visceral.

Introduction

Human toxocariasis is recognized as one of the most frequently reported helminthic zoonoses worldwide (Magnaval et al. 2001; Nicoletti, 2013), exerting a significant impact on global public health (Zibaei and Sadjjadi, 2017). This infection is caused by the larvae of nematodes of the genus *Toxocara*, mainly *Toxocara canis* and *Toxocara cati*, intestinal helminths of dogs and cats, respectively. Toxocariasis can trigger different clinical manifestations in humans, including visceral larva migrans, neurotoxocariasis, ocular toxocariasis, and covert toxocariasis (Ma et al. 2018). Humans acquire the infection primarily through the ingestion of embryonated eggs containing third-stage (L3) larvae present in contaminated soil, water, or vegetables (Bowman et al. 2020), as well as through the consumption of raw or undercooked meat from paratenic hosts containing encysted larvae (Healy et al. 2022).

Despite its clinical and epidemiological relevance, the therapeutic options available for human toxocariasis remain limited. Anthelmintics of the benzimidazole class are widely used in the treatment of intestinal helminth infections (Chen et al. 2018). However, in toxocariasis, a tissue-dwelling parasitosis, the efficacy of these drugs is often compromised. Albendazole ([methyl-5-(propyl-thio)-1H-benzimidazole-2-yl]carbamate) is considered the drug of choice due to its greater absorption and tissue distribution compared with other benzimidazoles, such as mebendazole and thiabendazole. Nevertheless, its gastrointestinal absorption generally does not exceed 5%, limiting systemic bioavailability and contributing to variable therapeutic responses, particularly in infections characterized by tissue migration of larvae (Hardman et al. 2001; Magnaval et al. 2022).

In view of these limitations, innovative strategies aimed at both the identification

of new drug candidates and the repositioning of existing drugs have been increasingly explored (Pink et al. 2005; Magnaval et al. 2022). In this context, experimental murine models of toxocariasis have become essential tools for understanding infection dynamics and evaluating the efficacy of new therapeutic approaches against this parasitosis (Satou et al. 2005; Mata-Santos et al. 2015; Walcher et al. 2017; Salama et al. 2023; Rodrigues et al. 2025a,b). At the same time, the prospection of bioactive compounds has received increasing attention, particularly heterocyclic compounds whose antiparasitic activity has been widely investigated through *in vitro*, *in vivo*, and *in silico* assays in different experimental models, including studies demonstrating activity against *Toxocara canis* (Mata-Santos et al. 2016a; 2016b; Walcher et al. 2017; 2023; Ortiz-Pérez et al. 2021; Rodrigues et al. 2024; 2025a; 2025b).

Among synthetic compounds with bioactive potential, camphor (1,7,7-trimethylbicyclo[2.2.1]heptan-2-one), a natural bicyclic monoterpene traditionally used in topical pharmaceutical formulations, cosmetics, perfumery, and hygiene products, has been recognized in recent decades as an important building block in organic synthesis (Alves and Victor, 2010). Its rigid and chiral skeleton, associated with a reactive carbonyl group, favors the production of a wide range of derivatives, including hydrazones, imines, hydrazides, and amines, which can be explored both as chiral auxiliaries in asymmetric synthesis and as drug candidates. In addition to its classical use as a chiral auxiliary and model in carbocation rearrangement studies, interest has grown in exploring the camphor nucleus as a platform for the development of new bioactive molecules.

In this context, several series of camphor derivatives have been synthesized with a focus on therapeutic applications, particularly against infectious agents. In our research group, we have systematically explored the derivatization of the carbonyl

group of camphor to obtain hydrazones, aromatic imines, and nitroimine intermediates through condensation reactions with hydrazines and anilines, followed by subsequent chemical transformations. These studies enabled the establishment of robust synthetic routes with satisfactory yields and reproducible reaction conditions, facilitating the generation of structurally related molecules suitable for biological evaluation and structure–activity relationship (SAR) studies (Rodrigues et al. 2025a). The biological results obtained with these derivatives indicate that small structural modifications in the aromatic ring linked to camphor can significantly alter the activity profile. For example, in assays against *Mycobacterium tuberculosis*, compounds containing phenolic groups in the ortho position relative to the amino group showed more pronounced antimycobacterial activity than analogs lacking this substitution pattern. This observation suggests that functional groups capable of acting as coordination sites, such as ortho-hydroxyl groups, may play an important role in molecular recognition and/or interference with processes essential for microbial survival (Silva et al. 2016).

The introduction of phenolic groups in the ortho position has also been confirmed in more recent studies conducted by our group focusing on the control of helminths. Among these compounds, the derivative (E)-2-((1,7,7-trimethylbicyclo[2.2.1]heptan-2-ylidene)amino)phenol (compound 1) stands out (Figure 1). Data obtained by our group *in vitro* and *in vivo* models of toxocariasis indicate that this derivative presents a promising activity and safety profile, characterized by high larvicidal efficacy at low concentrations, low cytotoxicity in host cells, and physicochemical properties compatible with good oral bioavailability (Rodrigues et al. 2025a). Based on these findings, the objective of the present study was to evaluate the isolated aniline derivative 2-hydroxyaniline, used in the synthesis of compound 1 is illustraded in Fig. 1. In addition, considering the promising biological activity of this

aniline and its relative cytotoxicity, other *O*-methylated aniline derivatives were evaluated *in vitro* in order to investigate whether structural methylation could reduce cytotoxicity while maintaining or improving antiparasitic activity.

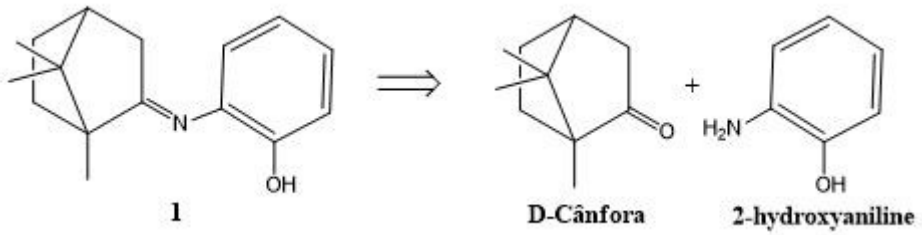


Figure 1. Schematic representation of the derivation of (E)-2-((1,7,7-trimethylbicyclo[2.2.1]heptan-2-ylidene)amino)phenol (compound 1) from D-camphor and 2-hydroxyaniline.

2. Material and Methods

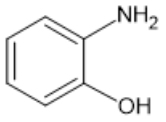
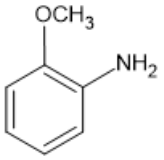
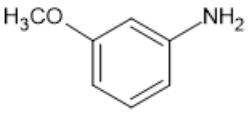
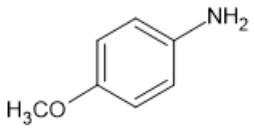
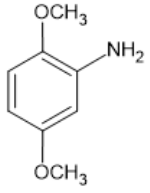
2.1. Obtaining eggs and larvae of *Toxocara canis*

Adult females of *T. canis* were recovered from naturally infected young dogs after anthelmintic treatment with pyrantel pamoate (15 mg/kg) and used as a source for obtaining parasite eggs. The collected eggs were incubated in a 2% formalin solution at 28 °C under controlled humidity conditions (>80%) with daily oxygenation for 30 days, according to a previously described protocol (Avila et al. 2013). At the end of the incubation period, the larvae were released by mechanical agitation and recovered using the Baermann apparatus. The larvae were then stored in RPMI-1640 medium (Gibco®) supplemented with 25 mM HEPES (Sigma®), 1% glucose, and PSF antibiotic-antimycotic solution (penicillin, streptomycin, and amphotericin B) (Gibco®), and maintained at 37 °C in an atmosphere containing 5% CO_2, according to the classical methodology described by Savigny (1975), with modifications.

2.2. Anilines

Aniline derivatives (1–5) were purchased from Sigma-Aldrich (St. Louis, MO, USA). Their chemical structures and physicochemical properties are presented in Table 1. The compounds were solubilized in 2.5% DMSO and sterile distilled water for the biological assays, reaching a final concentration of 1 mg/mL, according to the methodology described by Mata-Santos et al. (2015).

Table 1. Chemical structures of 2-hydroxyaniline and O-methylated aniline derivatives evaluated against *Toxocara canis* larvae.

Identification		Molecular structure	IUPAC Name
1	POHA		*2-hydroxyaniline*
2	POMA		*2-methoxyaniline*
3	PMMA		*3-methoxyaniline*
4	PPMA		*4-methoxyaniline*
5	P2.5DMA		*2,5-dimethoxyaniline*

2.3. *In vitro* screening tests on *Toxocara canis*

The compound was tested in duplicate at an initial concentration of 1 mg/mL in 96-well microculture plates (CRAL®) containing 100 *T. canis* larvae per well in RPMI-1640 medium. Live larvae and dead larvae obtained by thermal shock were used as controls, a physical method previously described as effective for the inactivation of eggs and larvae of *T. canis* (Overgaauw and Van Knapen, 1993). The plates were incubated for 48 h at 37 °C in an atmosphere containing 5% CO_2. After this period, 0.4% trypan blue viability indicator was added and, after 30 min, larval viability was evaluated by light microscopy (10× objective). Larvae that were distended, immobile, and stained by the indicator were considered non-viable according to criteria described by Mata-Santos et al. (2015). To determine the minimum larvicidal concentration (MLC), the compound was subjected to serial dilutions (1 mg/mL to 0.005 mg/mL). Albendazole was also evaluated at concentrations ranging from 40 mg/mL to 5 mg/mL under the same experimental conditions to determine its MLC for subsequent checkerboard assays.

2.4. *In vitro* cytotoxicity test

In vitro cytotoxicity assays were conducted using Vero cells (BCRJ 0245) at a density of 3.4×10^5 cells/mL cultured in DMEM supplemented with 10% fetal bovine serum and PSF antibiotic–antimycotic solution (Gibco®). The compounds were evaluated in triplicate at concentrations ranging from 1 to 0.005 mg/mL. To determine cell viability, 0.01% resazurin was added and, after incubation for 24 h at 37 °C in an atmosphere containing 5% CO_2, fluorescence was measured at 620 nm using a BioTek™ ELx800™ reader. From the optical density readings, cell viability was calculated and expressed as the percentage of viable cells relative to the control (Ahmed et al. 1994). The cytotoxic profile of the compound was evaluated across the tested

concentrations, and the inhibitory concentration capable of reducing cell viability by 50% (IC_{50}) was estimated from the dose–response curve.

2.5 Prediction of *in silico* toxicity

The toxicity was evaluated using *in silico* predictive models available on the ProTox-3.0 platform. This platform applies machine-learning algorithms to predict toxicity parameters such as toxicity class and median lethal dose (LD_{50}).

2.6. *In silico* bioavailability

After the *in vitro* larvicidal screening and cytotoxicity assays, the compounds were subjected to structural characterization and *in silico* evaluation. The chemical structure were generated using ChemBioDraw Ultra 12.1 (Halford, 2015), and bioavailability analysis was performed using the SwissADME platform (www.swissadme.ch) based on Lipinski's Rule of Five.

2.7. *In vitro* tests of the albendazole-compound combination

To determine the interaction between albendazole and compound POHA, a checkerboard assay was performed (Odds, 2003). For this purpose, 2-hydroxyaniline (POHA) was tested at concentrations of 0.005, 0.0125, and 0.025 mg/mL in combination with albendazole at concentrations of 5 and 10 mg/mL. The selection of the compound concentrations used in the combinations considered previous cytotoxicity results, prioritizing concentrations with lower cytotoxic effects in the cell model. The interaction was assessed by determining the fractional inhibitory concentration (FIC) values for each compound in combination, and subsequently calculating the fractional inhibitory concentration index (FICI) as: $FIC_{POHA} = (CLM_{POHA+ABZ})/(CLM_{POHA})$, $FIC_{ABZ} = (CLM_{ABZ+POHA})/(CLM_{ABZ})$, $FICI = FIC_{POHA} + FIC_{ABZ}$.

FICI values ≤ 0.5 were interpreted as synergy; 0.5 < FICI ≤ 1 as an additive effect; 1 < FICI ≤ 4 as indifferent; and FICI > 4 as antagonism (Odds, 2003).

2.8 *In vivo* test of compound alone and in association with albendazole in a murine model of experimental toxocariasis

Female mice, between 7 and 8 weeks of age, from the Bioterium of the Federal University of Santa Maria (UFSM) were used. The animals were kept in mini-isolators (Tecniplast®), in an environment with controlled temperature of 22 ± 1 °C, under a light/dark cycle of 12/12 h, with *ad libitum* access to feed and water without antibiotics. The *in vivo* experiments were conducted with animals distributed in four experimental groups (n = 5): Group 1 (animals infected with *T. canis* eggs and treated with the compound POHA associated with ABZ), Group 2 (animals infected with *T. canis* eggs, treated only with the compound POHA), Group 3 (animals infected with *T. canis* eggs, treated only with ABZ) and Group 4 (animals infected with *T. canis* eggs, treated with phosphate-buffered saline solution, at 1% 0.15 M and pH 7.2 – PBS) as control of the experimental design. Treatments were administered via intragastric route once daily, with POHA at 0.4 mg/kg and ABZ at 10 mg/kg.

All mice were inoculated intragastrically (IG) with 300 embryonated *T. canis* eggs on day zero of the experiment, as described by Rubinsky-Elefant et al. (2010) and Mata-Santos et al. (2016a). Ten days after infection, treatment was initiated by the IG route once daily for five consecutive days. The compound was administered at a dose of 0.4 mg/kg, defined based on the MLC (0.0125 mg/mL) associated with the safety observed in cytotoxicity tests, while albendazole was administered at a dose of 10 mg/kg.

At the end of the treatment period, animals were euthanized, and larvae were

recovered using the tissue digestion technique. Samples of brain, liver, lungs, kidneys, heart, and skeletal muscle (carcass) were subjected to digestion in a solution containing 1% pepsin and 1% hydrochloric acid under agitation in a water bath at 45 °C overnight, according to Xi and Jin (1998). The recovery and quantification of *T. canis* larvae were performed by optical microscopy (Nikon®) at magnifications of 100× and 400×. Statistical analysis was conducted using the unpaired Student's t-test for comparison between independent groups, adopting a significance level of 5% ($p < 0.05$). The study was approved by the Research Ethics Committee of the Federal University of Rio Grande (Protocol No. P 034/2020).

2.9 Evaluation of *O*-methylated aniline derivatives

Based on the results obtained with 2-hydroxyaniline (POHA), *O*-methylated derivatives (POMA, PMMA, PPMA, and P2,5DMA) were further evaluated to investigate whether methylation of the chemical structure could maintain or enhance their biological activity. For this purpose, *in vitro* assays were performed to determine larvicidal activity against *Toxocara canis* larvae, along with cytotoxicity evaluation and *in silico* predictions of toxicity and bioavailability, following the same methodology previously described.

3. RESULTS

In the *in vitro* assay, the compound POHA demonstrated 100% larvicidal activity against *T. canis* at a concentration of 1 mg/mL. The minimum larvicidal concentration (MLC) was determined at 0.125 mg/mL (Table 2).

Table 2. Percentage of *in vitro* larvicidal activity of POHA at different concentrations against *T. canis* larvae after 48 h of exposure.

	Larvicidal activity (%)					
	1 mg/mL	0,5 mg/mL	0.25 mg/mL	0.125 mg/mL	0.05 mg/mL	0.025 mg/mL
POHA	100	100	100	100	86,9	53,8

*Live control showed 4.8% larval non-viability, whereas the dead control showed 100% non-viability.

The MLC of albendazole was determined separately, confirming complete larval inhibition at a concentration of 40 mg/mL (Table 3).

Table 3. Percentage of *in vitro* larvicidal activity of albendazole at different concentrations against *T. canis* larvae after 48 h of exposure.

	Larvicidal activity (%)			
	40 mg/mL	20 mg/mL	10 mg/mL	5 mg/mL
Albendazole	100	65,0	16,4	7,4

*Live control: average inviability of 3.6%; Dead control: 100% of the larvae were non-viable.

In the in vitro cytotoxicity assay, POHA maintained acceptable cell viability at concentrations of 0.0125 mg/mL and 0.005 mg/mL, with 74% and 90% viability, respectively. The *in silico* assessment of systemic toxicity using the ProTox-3.0 platform predicted an LD_{50} of 800 mg/kg for POHA. In addition, the predicted

pharmacokinetic properties of POHA and albendazole were evaluated according to Lipinski's Rule of Five (RO5) (Table 4).

Table 4. *In silico* evaluation of pharmacokinetic properties of POHA and albendazole based on Lipinski's Rule of Five (RO5).

Compound	Molecular weight (g/mol)	miLogP	H donors	H Acceptors
Albendazole	265,33	2,53	2	3
POHA	109,13	0,79	2	1

*Molecular weight ≤ 500, miLogP ≤ 5, number of hydrogen bond donors ≤ 5 and number of hydrogen bond acceptors ≤ 10.

The *in vitro* assay showed that POHA promoted complete larval non-viability at concentrations of 1, 0.5, 0.25, and 0.125 mg/mL. At concentrations below the MLC (0.025 mg/mL), partial larvicidal activity (53.8%) was observed, indicating a concentration-dependent response. Based on these results, the interaction between POHA and albendazole was evaluated using a checkerboard assay with concentrations below the MLC of the compound (0.025, 0.0125, and 0.005 mg/mL) (Table 5).

The fractional inhibitory concentration index (FICI) values were 0.325 for the combination POHA 0.025 mg/mL + albendazole 5 mg/mL and 0.35 for POHA 0.0125 mg/mL + albendazole 10 mg/mL. According to the established interpretative criteria (FICI $\leq$ 0.5), these results indicate a synergistic interaction between POHA and albendazole under the experimental conditions evaluated.

Based on the results of *in vitro* cytotoxicity assays and the evaluation of the compound's interaction with the anthelmintic albendazole, a concentration of 0.0125 mg/mL was used as a reference to determine the dose administered *in vivo*, using a

murine model of experimental toxocariasis. Treatment with albendazole alone resulted in a 52.1% reduction in larval burden compared to the control group ($p < 0.05$), while treatment with POHA alone reduced larval recovery by 47.2% compared to the control group ($p < 0.05$). The combination of POHA and albendazole resulted in a 66.9% reduction in larval load compared to the control group and differed significantly from treatment with albendazole alone ($p < 0.05$). However, no difference was observed between combination therapy and POHA alone (Figure 1)).

Table 5. Percentage of *in vitro* larvicidal activity of POHA compound in association with the anthelmintic albendazole at different concentrations.

	Non-viable larvae (%) and Standard deviation (SD)		
Compound	0,025 mg/mL	0,0125 mg/mL	0,005 mg/mL
POHA + ABZ 10 mg/mL	96,3 (DP 9)	96,2 (DP 2,5)	83,8 (DP 1,5)
POHA + ABZ 5 mg/Ml	92,6 (DP 6,5)	52,6 (DP 2,5)	28,0 (DP 1)
ABZ Control 1 (10 mg/mL)	16,4 (DP 5,1)	-	-
ABZ 2 Control (5 mg/mL)	7,4 (DP 4,5)	-	-

*Live control: average inviability of 4.4%; dead control by thermal shock: 100% of the larvae were non-viable.

In the *in vitro* screening assay, *O*-methylated aniline derivatives evaluated at a concentration of 1 mg/mL also demonstrated high larvicidal activity against *T. canis* larvae after 48 h of exposure (Table 7).

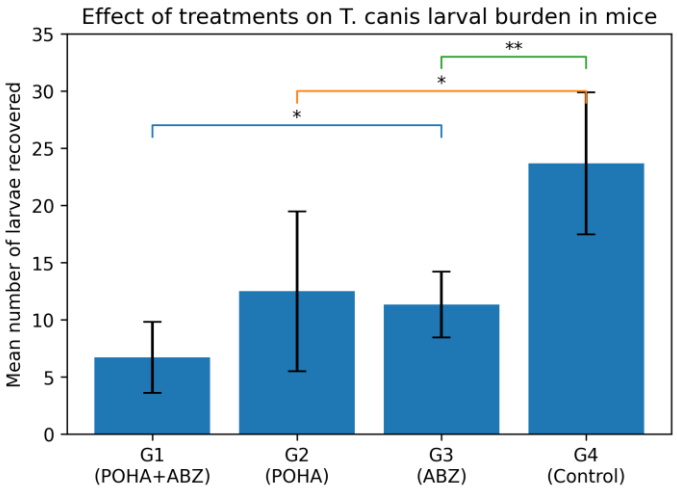


Figure 2. Effect of treatments on *Toxocara canis* larval burden in mice. Data are expressed as mean ± standard deviation of larvae recovered per group. *p < 0.05, **p < 0.01 (unpaired Student's t-test). G1: animals infected with *T. canis* eggs treated with compound + albendazole; G2: infected animals treated only with the compound; G3: infected animals treated with albendazole alone; G4: infected animals treated with experimental control (PBS). Source: Author's own elaboration.

Table 6. Screening test, percentage of *in vitro* larvicidal activity of *O*-methylated compounds, at 1 mg/ml, on *T. canis* larvae after 48 h of exposure.

	POMA	**PMMA**	**PPMA**	**P2,5DMA**
Larvicidal activity				
at 1 mg/ml (%)	99.5	94.03	91.94	94.18

* Live control showed 3.3% of larval non-viability, while the dead control showed 100% of non-viability.

Cytotoxicity assays revealed IC_{50} values ranging from 0.801 mg/mL to >1 mg/mL for the O-methylated aniline derivatives. In addition, in silico toxicity prediction was performed using the ProTox-3.0 platform to estimate LD_{50} values. These parameters are presented in Table 8.

Table 7. Cytotoxicity (IC_{50}) and predicted toxicity (LD_{50}) of O-methylated aniline derivatives.

Molecule	IC_{50}	Predicted LD_{50}
POMA	0.801 mg/mL	1150 mg/kg
PMMA	> 1 mg/mL	350 mg/kg
PPMA	> 1 mg/mL	1320 mg/kg
P2,5DMA	> 1 mg/mL	120 mg/kg

IC_{50}: inhibitory concentration capable of reducing cell viability by 50%.

LD_{50}: predicted median lethal dose for 50% of experimental models.

In addition, the predicted pharmacokinetic properties of the *O*-methylated compounds were analyzed according to Lipinski's Rule of Five (RO5) (Table 9).

Table 8. Predicted pharmacokinetic properties of *O*-methylated aniline derivatives according to Lipinski's Rule of Five (RO5).

Compound	Molecular weight (g/mol)	miLogP	H donors	H Acceptors
POMA	123.15	1.15	1	1
P2,5DMA	153.18	0.87	1	2
PMMA	123.15	1.15	1	1
PPMA	123.15	1.15	1	1

4. Discussion

Human toxocariasis remains a relevant therapeutic challenge, mainly due to the tissue nature of the infection and the difficulty in reaching migratory larvae distributed in different organs with the anthelmintics currently available (Ma et al. 2018; Lopez-

Alamillo et al. 2025). Among the benzimidazole derivatives used in treatment, such as albendazole, mebendazole, and thiabendazole, albendazole is considered the drug of choice for visceral larva *migrans*. However, its limited oral bioavailability, estimated to be lower than 5% (Whittaker et al. 2022), may restrict the amount of drug reaching systemic circulation and, consequently, the larvae located in tissues. In this context, the search for new compounds or pharmacological associations represents a promising strategy for therapeutic optimization (Magnaval et al. 2022; Ma et al. 2018).

The investigation of POHA in the present study originated from previous work conducted by our research group involving camphor-derived compounds. In that study, a camphor derivative containing an ortho-hydroxylated aniline moiety demonstrated promising larvicidal activity against *T. canis* larvae (Rodrigues et al. 2025a). Considering the structural composition of that molecule, it was hypothesized that the aniline fragment could contribute to the observed antiparasitic activity. Based on this premise, the isolated aniline derivative 2-hydroxyaniline (POHA) was evaluated in the present study, both alone and in association with albendazole, through *in vitro*, *in silico*, and *in vivo* approaches. *In vitro* assays demonstrated that POHA exhibited larvicidal activity against *T. canis* larvae at concentrations lower than those required for albendazole under the same experimental conditions. Importantly, this activity was further confirmed in the murine model of experimental toxocariasis, where treatment with POHA resulted in a significant reduction in larval burden.

The activity observed for POHA in the present study is consistent with previous reports describing the antiparasitic potential of aniline derivatives in different experimental models. Dinitroanilines such as oryzalin and trifluralin demonstrated activity against nematodes and protozoa, including *Leishmania* spp. and *Trypanosoma* spp., mainly through interference with the dynamics of parasitic microtubules (Armson

et al. 1990; Mitra and Sept, 2006; George et al. 2007; Roussaki et al. 2023). In addition, aniline-derived sulfanilamides exhibited activity against *Leishmania* spp. and *Trypanosoma cruzi* with favorable selectivity profiles (Galiana-Roselló et al. 2013), while synthesized nitroaromatic anilines showed anti-leishmanial activity associated with low cytotoxicity in mammalian cells (Lopes et al. 2011). Substituted diamines derived from aniline also demonstrated submicromolar activity against *Plasmodium* spp. and *Leishmania* spp., targeting metabolic pathways related to polyamines (Labadie et al. 2004). Together, these studies reinforce the potential of the aniline scaffold as a versatile structural platform for the modulation of different parasitic targets.

After evaluating larvicidal activity, the cytotoxic profile of POHA was investigated. The compound had an IC_{50} of 0.024 mg/mL, indicating that cytotoxic effects occurred at concentrations similar to those associated with larvicidal activity when tested in isolation. However, when combined with albendazole, the observed MLC decreased to 0.0125 mg/mL, at which concentration the compound promoted 74% cell viability. This observation highlights the importance of integrating antiparasitic efficacy with cellular safety during the early stages of drug candidate characterization. Although *in vitro* cell assays represent an important step in preliminary screening, they do not fully reproduce the pharmacokinetic and metabolic complexity observed in whole organisms (Houston and Galetin, 2008). In addition, the toxicological prediction performed using the ProTox 3.0 platform (Banerjee et al. 2018) classified POHA in toxicity class 4 according to the GHS system, with a predicted LD_{50} of 800 mg/kg.

In addition to biological activity, the characterization of pharmacokinetic properties represents an essential step in the evaluation of new therapeutic candidates. Lipinski's Rule of Five (RO5) is widely used as a preliminary predictor of oral bioavailability (Lipinski et al. 2001; Lipinski, 2004). POHA presented physicochemical

parameters within the limits established by RO5, which are associated with a higher probability of adequate membrane permeability and oral bioavailability (Lipinski et al. 2001; Veber et al. 2002). These analyses were performed using computational tools commonly applied for the prediction of ADME properties (Daina et al. 2017). Considering its moderate lipophilicity, which favors a balance between aqueous solubility and passive diffusion through biological membranes (Leeson and Springthorpe, 2007), these data suggest preliminary pharmacokinetic feasibility for POHA.

Combination therapy has been explored as an important strategy for improving the treatment of parasitic diseases. Studies evaluating drug associations have demonstrated improved therapeutic outcomes in infections caused by protozoa and helminths, including *Leishmania* spp., *Trypanosoma cruzi*, *Trichuris muris*, *Schistosoma mansoni*, and *Echinococcus* spp. (Seifert and Croft, 2006; Keiser et al. 2012; El-Lakkany et al. 2011; Strauss et al. 2018). In the present study, the association between POHA and albendazole demonstrated a synergistic interaction in the checkerboard assay (FICI < 0.5) and significantly increased larval non-viability compared with albendazole alone. These findings are consistent with previous experimental studies reporting greater reductions in parasite burden with combination therapies compared with monotherapy (Keiser et al. 2012; Diniz et al. 2018; Enkai et al. 2021; Cajas et al. 2024; Francisca et al. 2025).

In the murine model of experimental toxocariasis, both albendazole and POHA alone significantly reduced larval burden compared with the control group, confirming the biological activity of both treatments. The association between POHA and albendazole resulted in an additional reduction in larval burden compared with albendazole alone, indicating a potential benefit of the combinatorial strategy over

standard monotherapy. This observation is particularly relevant considering that albendazole, although widely used for the treatment of toxocariasis, often fails to completely eliminate migrating larvae (Magnaval et al. 2001; Ma et al. 2018). However, the absence of a significant difference between the combination therapy and POHA alone suggests that the intrinsic antiparasitic activity of POHA contributed substantially to the observed effect. Importantly, the dose used in the *in vivo* model was well below the predicted LD_{50}, and no clinical signs of toxicity were observed in the animals, indicating that the reduction in larval burden was not associated with nonspecific toxic effects.

Based on the promising results obtained with POHA, additional O-methylated aniline derivatives were evaluated to investigate whether methylation of the phenolic group could influence biological activity and cytotoxicity. These derivatives retained high larvicidal activity against *T. canis* larvae and showed low cytotoxicity, with IC_{50} values above the tested concentration range. Methylation of phenolic hydroxyl groups is a common strategy in medicinal chemistry to modulate physicochemical properties and biological activity (Sun et al. 2018; Schönherr et al. 2022; Pinheiro et al. 2023) and may influence molecular polarity, membrane permeability, and metabolic stability (Pérez et al. 2012; Li et al. 2022). These findings indicate that the free phenolic hydroxyl group is not essential for antiparasitic activity and highlight the aniline scaffold as a promising platform for the development of new antiparasitic candidates.

Taken together, these results demonstrate that POHA exhibits significant larvicidal activity against *T. canis in vitro*, presents a preliminary pharmacokinetic profile compatible with potential oral absorption, and promotes a reduction in larval burden in the murine model of experimental toxocariasis. The synergistic interaction observed with albendazole, together with the effects confirmed *in vivo*, highlights the

potential of this combinatorial strategy to improve antiparasitic efficacy. Moreover, the biological activity observed for POHA and its O-methylated derivatives reinforces the relevance of the aniline scaffold as a promising structural platform for the development of new antiparasitic agents and supports further studies aimed at elucidating their mechanisms of action and therapeutic potential.

5. Conclusion

2-Hydroxyaniline (POHA) exhibited significant activity against *Toxocara canis larvae in vitro* and reduced larval burden in a murine model of experimental toxocariasis, while also presenting favorable *in silico* pharmacokinetic predictions. The synergistic interaction observed with albendazole supports the potential of combination strategies to enhance antiparasitic efficacy. Furthermore, O-methylated aniline derivatives maintained high larvicidal activity while showing lower cytotoxicity, reinforcing the relevance of structural modification approaches in the optimization of bioactive molecules. Together, these findings highlight the aniline scaffold as a promising platform for the development of new antiparasitic candidates and support further investigations into their mechanisms of action and therapeutic potential for toxocariasis control.

Statements

In accordance with Brazilian Law No. 11,794/2008 (Arouca Law), Article 2 establishes that the provisions apply to animals belonging to the phylum Chordata, subphylum Vertebrata, subject to current environmental legislation. The Federal University of Rio Grande is accredited by the Ethics Commission on the Use of Animals (CEUA), as provided for in article 8 of the aforementioned law. This study was approved by the Animal Ethics Committee of the Federal University of Rio Grande (CEUA-FURG, protocol P034/2020).

Conflict of Interest Statement

The authors have no conflicts of interest.

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Data availability

The data supporting the findings of this study are available from the corresponding author upon reasonable request.

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Declaration of generative AI and AI-assisted technologies in the manuscript preparation process

During the preparation of this work, the authors used ChatGPT (OpenAI) in order to assist with language editing and improvement of clarity in the English writing of the manuscript. After using this tool, the authors reviewed and edited the content as needed and take full responsibility for the content of the published article.

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7. CONCLUSÕES

Os resultados obtidos neste estudo demonstram que os compostos sintéticos

avaliados apresentam atividade larvicida significativa contra *Toxocara canis*, associada a perfil de citotoxicidade compatível com aplicação biológica e propriedades farmacocinéticas preditivas favoráveis. A investigação das associações com o albendazol evidenciou interações do tipo sinérgica ou aditiva em modelos experimentais *in vitro*, indicando potencial de ampliação da atividade antiparasitária quando comparada ao uso isolado dos compostos. Em modelo murino de toxocaríase visceral, observou-se redução da carga larval nos grupos tratados, reforçando a relevância biológica dessa abordagem. Além disso, a avaliação de derivados estruturalmente relacionados demonstrou que modificações químicas podem manter a atividade larvicida enquanto reduzem a citotoxicidade. Em conjunto, os achados indicam que a associação de compostos sintéticos ao albendazol representa uma estratégia promissora para o desenvolvimento de novas abordagens terapêuticas no controle da toxocaríase.

8. CONSIDERAÇÕES FINAIS

A toxocaríase humana permanece como um desafio terapêutico relevante, sobretudo diante da eficácia variável do albendazol em infecções teciduais e da limitada disponibilidade de alternativas terapêuticas consolidadas. Nesse contexto, a investigação de associações farmacológicas fundamentadas em evidências químicas e biológicas configura uma abordagem alinhada às necessidades contemporâneas da pesquisa em doenças parasitárias negligenciadas.

A integração entre síntese de compostos, avaliação *in vitro*, análises *in silico* e validação em modelo experimental *in vivo* permitiu o desenvolvimento de uma abordagem metodologicamente consistente e abrangente. Os resultados obtidos indicam que a associação de compostos sintéticos ao albendazol pode ampliar a atividade antiparasitária observada em monoterapia, evidenciando o potencial da estratégia combinada frente às limitações terapêuticas atualmente descritas.

Adicionalmente, a avaliação de derivados estruturalmente relacionados demonstrou que modificações químicas no núcleo anilínico, como a metilação do grupo hidroxila fenólico, podem manter elevada atividade larvicida contra *Toxocara canis* ao mesmo tempo em que reduzem a citotoxicidade. Esses achados reforçam a importância das estratégias de modificação estrutural na otimização de propriedades biológicas e

destacam o potencial de *scaffolds* anilínicos como plataformas promissoras para o desenvolvimento de novos candidatos antiparasitários.

Embora a extrapolação para o contexto clínico exija investigações adicionais, incluindo estudos farmacocinéticos mais aprofundados e avaliações ampliadas de segurança, os achados desta pesquisa estabelecem uma base científica relevante para a continuidade e o refinamento dessa linha investigativa.

Assim, os resultados obtidos nesta tese contribuem para o avanço da investigação de novos candidatos antiparasitários e abrem caminho para estudos futuros voltados à elucidação de mecanismos de ação, otimização estrutural e avaliação pré-clínica de compostos com potencial aplicação no controle da toxocaríase.

9. COMUNICAÇÃO CIENTÍFICA

A toxocaríase é uma doença parasitária causada principalmente pelo nematódeo *Toxocara canis*, comum em cães. A infecção humana acontece de forma acidental,

principalmente pelo contato com solo, água ou alimentos contaminados com ovos do parasito. Após entrar no organismo, as larvas podem migrar para diferentes órgãos, como fígado, pulmões, olhos e sistema nervoso, causando desde sintomas leves até manifestações graves. Apesar de ser uma doença frequente em diversos países, incluindo o Brasil, a toxocaríase ainda é considerada negligenciada e apresenta desafios no tratamento. Atualmente, o albendazol é o medicamento mais utilizado contra a toxocaríase. No entanto, sua eficácia pode ser limitada em infecções em que as larvas se encontram nos tecidos do organismo, o que reforça a necessidade de novas alternativas terapêuticas. Esta tese avaliou o potencial de compostos sintéticos derivados de hidroxietilamina e anilina, isoladamente e em associação ao albendazol, contra larvas de *Toxocara canis*. Foram realizados estudos laboratoriais (*in vitro*), análises computacionais (*in silico*) e testes em modelo experimental animal (*in vivo*). Os resultados demonstraram que os compostos avaliados apresentaram atividade antiparasitária promissora, perfil citotóxico favorável e propriedades compatíveis com possível uso oral. Além disso, quando associados ao albendazol, alguns compostos potencializaram a ação do medicamento, permitindo maior redução da viabilidade das larvas em concentrações menores. Em modelo experimental em camundongos, a terapia combinada reduziu significativamente a carga parasitária em comparação ao uso isolado dos tratamentos. Os achados desta pesquisa contribuem para o desenvolvimento de novas estratégias terapêuticas contra a toxocaríase e reforçam a importância da pesquisa científica na busca por tratamentos mais eficazes para doenças parasitárias negligenciadas.

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11. ANEXOS

Anexo 1. Certificado de aprovação da Comissão de Ética em Uso Animal

COMISSÃO DE ÉTICA EM USO ANIMAL
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CEUA

CERTIFICADO Nº P034/2020

Certificamos que o projeto intitulado "Avaliação *in vitro* e *in vivo* de moléculas sintéticas para o tratamento da toxocaríase visceral", protocolo nº 23116.002377/2020-53, sob a responsabilidade de Carlos James Scaini - que envolve a produção, manutenção e/ou utilização de animais pertencentes ao Filo Chordata, subfilo Vertebrata (exceto o homem), para fins de pesquisa – encontra-se de acordo com os preceitos da Lei nº 11.794, de 8 de outubro de 2008, do Decreto nº 6.899, de 15 de julho de 2009, e com as normas editadas pelo Conselho Nacional de Controle da Experimentação Animal (CONCEA), e foi APROVADO pela COMISSÃO DE ÉTICA EM USO ANIMAL DA UNIVERSIDADE FEDERAL DO RIO GRANDE (CEUA-FURG), em reunião de 30 de setembro de 2020 (Ata 010/2020).

A CEUA lembra aos pesquisadores que qualquer alteração no protocolo experimental ou na equipe deve ser encaminhada à comissão para avaliação e aprovação. Um relatório final deve ser enviado à CEUA no término da vigência do seu projeto.

CEUA Nº	Pq009/2020
COLABORADORES AUTORIZADOS A MANIPULAR OS ANIMAIS	Lívia Silveira Munhoz, Lourdes Helena Rodrigues Martins, Márcia Cristiane Feltrin Dias de Souza
VIGÊNCIA DO PROJETO	31/12/2022
ESPÉCIE/ LINHAGEM / RAÇA	Camundongo *Swiss*
NÚMERO DE ANIMAIS	158
PESO/ IDADE	25 a 30 g; 7 a 8 semanas;
SEXO	fêmeas
ORIGEM	Biotério Central da UFSM
ENVIO DO RELATÓRIO PARCIAL	Janeiro 2021; janeiro 2022
ENVIO DO RELATÓRIO FINAL	Janeiro de 2023

Rio Grande, 14 de outubro de 2020.

Alice Teixeira Meirelles Leite
Coordenadora da CEUA-FURG

Controle da CEUA - Parecer(es) prévio(s) relacionado(s) ao processo: P031/2020 Página 1/1

Anexo 2. Primeira página do artigo publicado na revista *Acta Tropica*.

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Additive larvicidal activity of albendazole combined with a hydroxyethylamine-derived compound against *Toxocara canis* larvae: An *in vitro* and *in silico* study

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ABSTRACT

Human toxocariasis is a neglected zoonotic helminthiasis characterized by larval migration through tissues such as the liver, lungs, central nervous system, and eyes. Current pharmacological treatment presents variable efficacy, highlighting the need for improved therapeutic strategies. This study evaluated the larvicidal activity of albendazole combined with heteroaromatic nitroxazole compounds derived from hydroxyethylamine against *Toxocara canis*, using *in vitro* assays, *in silico* analyses, and a checkerboard design. Three synthetic compounds were initially screened for larvicidal activity and cytotoxicity. All induced 100% larval unviability at 1 mg/mL; however, only HIDROXI 1 maintained complete larvicidal activity at 0.5 mg/mL, while exhibiting an acceptable cytotoxicity profile. *In silico* analyses indicated physicochemical properties consistent with favorable oral bioavailability and compliance with Lipinski's Rule of Five. Albendazole alone induced complete larval unviability only at 40 mg/mL. In the checkerboard assay, the combination of HIDROXI 1 (0.25 mg/mL) with albendazole (10 or 5 mg/mL) resulted in 100% and 99% larval unviability, respectively, indicating an additive interaction. These findings indicate that HIDROXI 1 exhibits promising *in vitro* larvicidal activity and may enhance the effect of albendazole at lower concentrations, supporting further investigation of this combination as a potential therapeutic approach for toxocariasis.

1. Introduction

Human toxocariasis is characterized by the migration of *Toxocara canis* larvae through different tissues, including the liver, lungs, central nervous system, and eyes, resulting in nonspecific clinical manifestations whose severity depends on parasite burden, larval localization, and the host immune response (Mellou et al., 2020). This condition results from a neglected parasitic zoonosis with worldwide distribution, in which humans act as accidental hosts for the intestinal parasite of dogs (Chen et al., 2018). Transmission occurs mainly through the ingestion of embryonated eggs present in contaminated soil, water, and food, as well as through the ingestion of larvae encysted in raw or undercooked meat or viscera from paratenic hosts, such as pigs, cattle, and poultry (Magnaval et al., 2022).

The treatment of toxocariasis is based on the use of benzimidazole-class anthelmintics, widely used for intestinal helminthiasis such as ascariasis, trichuriasis, and hookworm infection (Chen et al., 2018). However, in *Toxocara* spp. infection, in which there is tissue damage, the efficacy of these drugs is limited, particular due to their low bioavailability and reduced action on migratory larvae, around 45–80% (Magnaval et al., 2001; Magnaval et al., 2022). Considering the challenges in the treatment of toxocariasis, several studies have investigated new bioactive compounds against *Toxocara canis*, including heteroaromatic derivatives such as camphor analogues, nitrofurans, and

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