

MINISTÉRIO DA EDUCAÇÃO
UNIVERSIDADE FEDERAL DO RIO GRANDE
PROGRAMA DE PÓS-GRADUAÇÃO EM CIÊNCIAS DA SAÚDE

**Estudos pré-clínicos de derivados de cânfora, nitrofurano e pirimidina no
tratamento da toxocaríase visceral**

Débora Carvalho Rodrigues

RIO GRANDE, 2025



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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.

Orientador: Prof. Dr. Carlos James Scaini
Coorientadora: Prof^a. Dr^a. Daniela Fernandes Ramos

RIO GRANDE, 2025

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A banca examinadora, designada pela Portaria nº 599/2025 de quatro de fevereiro de dois mil e vinte e cinco, em sessão presidida e registrada pelo orientador, Prof. Dr. Carlos James Scaini, reuniu-se no dia vinte e quatro de fevereiro de dois mil e vinte e cinco, às 09 horas, por meio de videoconferência (<https://conferenciaweb.rnp.br/sala/daniela-fernandes-ramos-soares-daniela>), para avaliar a Tese de Doutorado do Programa de Pós-Graduação em Ciências da Saúde, intitulada: “Estudos pré-clínicos de derivados de nitrofurano, cânfora e pirimidina no tratamento da toxocaríase visceral” da doutoranda Débora Carvalho Rodrigues. 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 até 50 minutos para a apresentação da tese pela doutoranda, que iniciou às 9 horas e 10 minutos e terminou às 10 horas. 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 foi **APROVADA**.

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Resumo

RODRIGUES, D.C. Estudos pré-clínicos de derivados de cânfora, nitrofurano e pirimidina no tratamento da toxocaríase visceral. Orientador: Carlos James Scaini. 2025. 93 p. Tese (Doutorado em Ciências da Saúde) – Faculdade de Medicina, Universidade Federal do Rio Grande, Rio Grande, 2025.

O tratamento da toxocaríase humana, parasitose tecidual negligenciada de ocorrência global, é baseado no uso de anti-helmínticos benzimidazóis, os quais apresentam ação limitada e há carência de estudos clínicos de avaliação desses fármacos. Assim, a pesquisa e o desenvolvimento de novos anti-helmínticos para toxocaríase são essenciais. O objetivo deste estudo foi selecionar compostos derivados de cânfora, nitrofurano e pirimidinas, em testes pré-clínicos. Ensaio *in vitro* de *screening* e de determinação da concentração larvicida mínima (CLM) foram realizados, em duplicata, em placas de microcultivo, contendo larvas de *Toxocara canis* em meio RPMI-1640 e compostos na concentração inicial de 1 mg/mL e posteriormente na concentração de 0,5 a 0,05 mg/mL. Assim, foram selecionados os compostos, C2 derivado da cânfora, PFUR derivado de nitrofurano e PNAH 8 derivado de pirimidina, os quais apresentaram atividade sobre 100% das larvas na CLM de 0,25 mg/mL, 0,25 mg/mL e 0,5 mg/mL, respectivamente. Nas análises *in silico*, utilizando o software Swiss ADME, os três compostos selecionados apresentaram parâmetros de biodisponibilidade adequados, como importante capacidade de metabolismo e absorção gastrointestinal e adequada solubilidade em água. Além disso, houve ausência de citotoxicidade desses compostos em todas as concentrações testadas. Os compostos com a menor CLM (C2 e PFUR) foram selecionados para os testes *in vivo*, os quais foram realizados com camundongos Swiss com 10 dias e com 30 dias pós-inoculação de 500 ovos de *T. canis* via intragástrica (IG). Os compostos (C2 e PFUR; 10 mg/kg/5d) e os controles (albendazol 40 mg/kg e PBS 0,15M, pH 7,2) foram administrados durante cinco dias pela via IG. O composto C2 reduziu a intensidade de infecção em 75,7% após 10 dias da inoculação e de 54,8% em 30 dias ($p < 0,05$), enquanto que PFUR reduziu a intensidade de infecção em 62,4% após 30 dias da inoculação de ovos. Os resultados desse estudo demonstram que os compostos selecionados têm potencial como candidatos a novos anti-helmínticos para o tratamento da toxocaríase visceral.

Palavras chave: Compostos sintéticos; Parasitose tecidual; Helminíase;

ODS contemplados: Saúde e Bem-estar;

Abstract

RODRIGUES, D. C. Preclinical studies of camphor, nitrofurantoin and pyrimidine derivatives in the treatment of visceral toxocariasis. Supervisor: Carlos James Scaini. 2025. 93 p. Doctoral thesis (Doctor of Health Sciences) - Faculty of Medicine, Federal University of Rio Grande, Rio Grande, 2025.

Treatment of human toxocariasis, a neglected tissue parasitosis of global importance, is based on the use of benzimidazole anthelmintics, which have limited efficacy and there is a lack of clinical trials evaluating these drugs. Therefore, research and development of new anthelmintics for toxocariasis are essential. The aim of this study was to select compounds derived from camphor, nitrofurantoin and pyrimidines for preclinical testing. *In vitro* screening tests and determination of the minimum larvicidal concentration (MLC) were performed, in duplicate, on microculture plates containing *Toxocara canis* larvae in RPMI-1640 medium and the compounds at an initial concentration of 1 mg/mL and then at concentrations of 0.5 to 0.05 mg/mL. The compounds selected were camphor derivative C2, nitrofurantoin derivative PFUR and pyrimidine derivative PNAH 8, which showed activity against 100% of the larvae at 0.25 mg/mL, 0.25 mg/mL and 0.5 mg/mL, respectively. In the *in silico* analyses, using Swiss ADME software, the three selected compounds showed adequate bioavailability parameters, such as a significant capacity for metabolism and gastrointestinal absorption as well as adequate water solubility. In addition, these compounds were non-cytotoxic at any of the concentrations tested. The compounds with the lowest CLM (C2 and PFUR) were selected for *in vivo* testing, which was performed with Swiss mice 10 and 30 days after inoculation with 500 *T. canis* eggs via intragastric (IG) administration. The compounds (C2 and PFUR; 10 mg/kg/5d) and controls (albendazole 40 mg/kg and PBS 0.15M, pH 7.2) were administered via IG for five days. C2 reduced infection intensity by 75.7% at 10 days post inoculation and 54.8% at 30 days post inoculation ($p < 0.05$), while PFUR reduced infection intensity by 62.4% at 30 days post inoculation. The results of this study show that the selected compounds have potential as new anthelmintic candidates for the treatment of visceral toxocariasis.

Key-words: Synthetic compounds; Tissue parasitosis; Helminthiasis;

ODS Covered: Health and Wellness;

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Lista de Abreviaturas

ABZ	Albendazol
ADME	Absorção - distribuição - metabolização - excreção
ATP	Trifosfato adenosina
CAPES	Coordenação de Aperfeiçoamento de Pessoal de Nível Superior
CEUA	Comissão de Ética no Uso de Animais
CNPq	Conselho Nacional de Desenvolvimento Científico e Tecnológico
DEC	Dietilcarbamazina
DNA	Ácido desoxiribonucléico
DMEM	Meio Eagle Modificado de Dulbecco
DMSO	Dimetilssulfóxido
ELISA	Ensaio imunoenzimático direto
Farmanguinhos	Instituto de Tecnologia em Fármacos
Fiocruz	Fundação Oswaldo Cruz
FURG	Universidade Federal do Rio Grande
G7	Grupo dos sete
HEPES	Solução tampão (4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid)
HIV	Vírus da imunodeficiência humana
IC 50	Inviabilidade celular de 50%
IG	Intragástrico
INPI	Instituto Nacional da Propriedade Industrial
IP	Intraperitoneal
J774.A1	Linhagem de macrófagos murinos
LMO	Larva migrans ocular
LMV	Larva migrans visceral
L3	Larva de terceiro estágio
L4	Larva de quarto estágio
MBZ	Mebendazol
MLC	Concentração larvicida mínima
NT	Neurotoxocaríase
NUDEFA	Núcleo de Desenvolvimento de Novos Fármacos
PBS	Solução salina tamponada com fosfato
PSF	Antibiótico-antimicótico (penicilina, estreptomicina e anfotericina B)

RNA	Ácido ribonucléico
RPMI-1640	Meio Memorial Roswell Park Institute 1640
TBZ	Tiabendazol
TC	Toxocaríase oculta
TES	Antígeno de excreção e secreção de larvas de <i>Toxocara canis</i>
TO	Toxocaríase ocular
TV	Toxocaríase visceral
TRPA1	Potencial receptor transiente A1
TRPV1	Potencial receptor transiente V1

1. INTRODUÇÃO

A toxocaríase humana é uma antroponose parasitária de distribuição global (Fakhri et al., 2018; Magnaval et al., 2022), que representa um importante desafio para a saúde pública (Chen et al., 2018), pois acomete cerca de 1,4 bilhão de pessoas em todo o mundo (Ma et al., 2017). Com base na magnitude de sua soroprevalência e sua associação com diversas doenças e manifestações clínicas, a toxocaríase é uma doença negligenciada que deve receber atenção dos sistemas de saúde pública (Fialho e Correa, 2016; Fakhri et al., 2018; Hotez, 2020).

Essa parasitose é causada pela migração e permanência de larvas de *Toxocara* spp. em tecidos de órgãos como fígado, pulmões, coração, rins, encéfalo e na musculatura estriada esquelética (Fan et al., 2015). Esse parasito modula a resposta imunológica do hospedeiro, reduzindo o processo inflamatório e, consequentemente, favorecendo sua própria sobrevivência (Hamilton et al., 2008). A toxocaríase humana apresenta um amplo espectro clínico, que vai desde infecções assintomáticas e subclínicas até condições graves, incluindo distúrbios alérgicos, oftálmicos, neurológicos, além de alterações hepáticas, respiratórias e doenças cardíacas (Kuenzli et al., 2016; Ma et al., 2017; Healy et al., 2022).

Nas últimas quatro décadas, houve um avanço importante sobre o conhecimento da toxocaríase humana, especialmente em relação à epidemiologia, fisiopatologia, clínica e diagnóstico (laboratorial e por imagem), porém os estudos sobre o tratamento dessa parasitose não progrediram na mesma proporção (Magnaval et al., 2022). O tratamento baseia-se na administração dos benzimidazóis albendazol (ABZ), mebendazol (MBZ) e tiabendazol (TBZ), os quais são eficazes para o tratamento de helmintíases intestinais (Fillaux e Magnaval, 2013).

O ABZ e o MBZ são os fármacos de primeira e segunda escolha para o tratamento da toxocaríase, respectivamente (Fillaux e Magnaval, 2013). Tanto o ABZ quanto o MBZ ligam-se à β -tubulina livre, que é o componente proteico essencial dos microtúbulos em nematódeos. Esses medicamentos têm maior afinidade por esse componente do que pela proteína humana, levando à inibição da polimerização da tubulina e à perda de microtúbulos citoplasmáticos (Lacey, 1990). No entanto, na toxocaríase visceral a eficácia desses anti-helmínticos é considerada insatisfatória,

devido a sua difícil absorção em nível tecidual. Além disso, poucos ensaios clínicos randomizados foram desenvolvidos para avaliação desses fármacos sobre a toxocaríase, e os estudos realizados utilizaram doses e tempo de tratamento inferiores aos recomendados (Magnaival et al., 1987; Stürchler et al., 1989; Magnaival et al., 1995), dificultando a comparação e avaliação da eficácia desses anti-helmínticos (Magnaival et al., 2022).

Em função das dificuldades encontradas para o tratamento dessa parasitose tecidual, estudos devem ser desenvolvidos na busca por compostos com potencial para o desenvolvimento de um novo anti-helmíntico (Epe e Kaminsky, 2013). Assim, a realização de testes pré-clínicos é fundamental, e esses, correspondem aos ensaios conduzidos com o objetivo de avaliar a ação direta em testes *in vitro*, a citotoxicidade, efeitos farmacológicos em ensaios *in silício* e *in vivo* em modelos experimentais (Chi, Burrows e Anderson, 2022).

Diante do desafio global imposto pelas doenças parasitárias, incluindo a toxocaríase humana, e da necessidade de terapias mais eficazes e seguras, compostos sintéticos representam uma promissora plataforma para o desenvolvimento de novos agentes terapêuticos, reforçando seu papel central na química medicinal moderna. Além disso, emergem como candidatos promissores, devido à sua ampla versatilidade estrutural e potencial de bioatividade (Peerzada, Gaur e Azam, 2021).

2. REVISÃO BIBLIOGRÁFICA

2.1. *Toxocara* spp.: agente etiológico da toxocaríase humana

O nematódeo *T. canis*, parasito intestinal de cães, é o agente etiológico mais frequentemente associado a toxocaríase humana (Overgaauw e Knapen, 2013), porém *T. cati*, parasito intestinal de gatos, também é relevante na epidemiologia dessa parasitose (Chen et al., 2018). Os principais fatores de risco para essa parasitose incluem renda familiar baixa, desnutrição, higiene pessoal e ambiental precária, saneamento básico inadequado e contato com cães e gatos (Hotez, 2020; Magnaval et al., 2022).

O ciclo biológico do geohelminto *Toxocara canis* é complexo, pode ser direto e envolver apenas o hospedeiro definitivo (cão, gato ou algumas espécies de carnívoros silvestres) ou pode ser indireto e apresentar hospedeiros paratênicos (aves, mamíferos de produção e silvestres), além do hospedeiro definitivo (**Figura 1**).

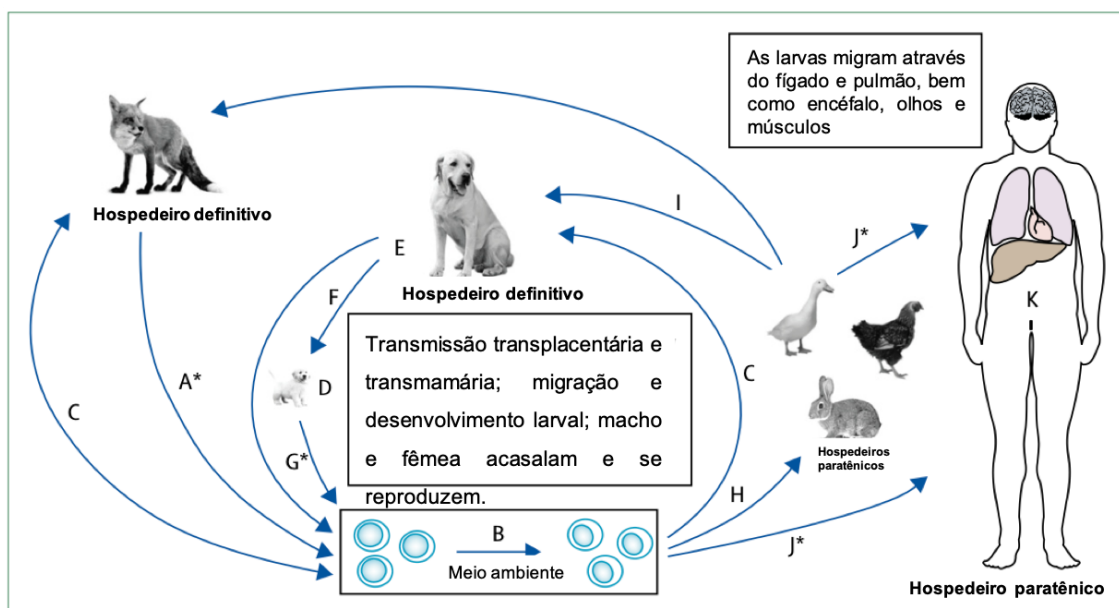


Figura 1 - Ciclo biológico de *Toxocara canis*, principal agente etiológico da toxocaríase humana, conforme Ma et al. (2017) modificado.

Entre os cães (hospedeiros definitivos), a transmissão de *T. canis* pode ocorrer pelas vias: oral, transplacentária e transmamária. Nos cães jovens, as larvas infectantes de terceiro estágio (L3) realizam migração sistêmica, evoluem para quarto estágio (L4) nos pulmões, essas sobem a árvore brônquica atingindo a faringe, são deglutidas e

retornam para o intestino, onde amadurecem sexualmente. As fêmeas adultas realizam a oviposição e os ovos são carregados pelas fezes do hospedeiro, contaminando o ambiente. Nos gatos, larvas de *T. cati* podem ser transmitidas pela via transmamária para filhotes se a genitora for infectada durante a gestação (Miller, 2020). Para que ocorra o desenvolvimento embrionário, os ovos de *T. canis* passam por um período de maturação no solo e onde podem permanecer viáveis por meses ou anos, desde que sejam mantidas condições ambientais adequadas, como temperatura entre 15 e 30°C, umidade relativa superior a 50%, oxigenação e presença de áreas sombreadas (Felix et al., 2020).

Nos seres humanos (hospedeiros paratênicos) a infecção por esse parasito pode ocorrer principalmente pela ingestão de ovos embrionados (contendo a larva infectante), que podem ser levados à boca pelas mãos contaminadas e pelo consumo de hortaliças e água contaminada (Rostami et al., 2019). Outro modo de infecção, também oral, ocorre pelo consumo de carnes ou vísceras malcozidas ou cruas de hospedeiros paratênicos (bovinos, ovinos, suínos, coelhos, cervídeos, equídeos, coelhos, galinhas, avestruzes e patos) que podem alojar larvas infectantes encistadas em seus tecidos (Healy et al., 2021).

Após a ingestão de ovos embrionados ou de larva, essas penetram na parede intestinal e migram para o fígado através da veia porta. Após, migram aos pulmões e posteriormente podem ser carregadas pela circulação arterial para diversos órgãos. Parte das larvas entra num estado de quiescência (hipobiose) e pode permanecer viável durante vários anos, outras ficam retidas no interior de granulomas e são subsequentemente destruídas pela resposta imunológica do hospedeiro contra antígenos larvares solúveis de origem excretora-secretora (Magnaval et al., 2022).

2.2. Toxocaríase humana

A toxocaríase humana é uma parasitose tecidual negligenciada encontrada tanto em países desenvolvidos como em países em desenvolvimento (Fakhri et al. 2018). A soroprevalência global é estimada em 19% em humanos (Ma et al., 2020). Na América Latina e Caribe, um estudo de meta-análise de soroprevalência revela taxa média de 31%, sendo que em 45% dos estudos a soroprevalência foi superior a 50% (Ulloque-Badaracco et al., 2023). Outro estudo de meta-análise mostra que a taxa varia de 2% a

8% em áreas urbanas de países industrializados do Grupo dos Sete (G7), sendo que em áreas rurais desses países pode chegar a 40%. Já em países de clima tropical ou subtropical com alta umidade, a soropositividade para toxocaríase encontrada é de até 80% (Rostami et al., 2019). No Brasil, estudos revelam que a soroprevalência pode atingir taxa de 71,8% (Araujo et al., 2018) e a contaminação ambiental ultrapassar 30% (Ma et al., 2020).

Esses dados sugerem que a toxocaríase constitui papel importante no contexto “One Health”, além de destacá-la, em conjunto com a ascaridíase, tricuriíase, ancilostomíase e estrogiloidíase, como uma das principais geohelminthíases que acomete os humanos (Ma et al., 2020). Vale ressaltar que a toxocaríase humana apresenta ampla distribuição, atingindo principalmente crianças, devido a uma combinação de fatores como hábitos de geofagia e higiene precária (Woodhall e Fiore, 2014), contato com animais de estimação (Felix et al., 2020) e imaturidade imunológica (Overgaauw & Nederland, 1997).

Em hospedeiros paratênicos, como os humanos, as larvas de *Toxocara* não atingem a fase adulta, permanecendo em estágio larvário e migrando pelos tecidos de órgãos e musculatura estriada esquelética, o que ocasiona uma reação inflamatória aguda (Felix et al., 2020). O quadro clínico da toxocaríase pode variar amplamente de acordo com as lesões causadas durante a migração das larvas e isso traz implicações e desafios significativos tanto para o diagnóstico como para o tratamento (Fan et al., 2015). Os quadros clínicos apresentados na toxocaríase podem ser desde assintomático que é o mais frequente, sistêmicos que compreendem as formas visceral (TV) e oculta (TC), e compartimentalizados, como as formas ocular (TO) e a neurológica (NT) (Magnaival et al., 2022).

A TV acomete especialmente crianças entre dois e sete anos de idade e é resultante de sucessivas reinfecções e de elevada intensidade de infecção. O fígado é o órgão mais frequentemente parasitado, podendo ocorrer a formação de granulomas, hepatomegalia e hepatite (Chen et al., 2018). Os sinais clínicos mais frequentes são dores abdominais, febre, tosse, dificuldade respiratória e hipereosinofilia (Magnaival et al., 2022). A TC ocorre geralmente de maneira benigna e auto limitante, durando cerca de três meses. Os sinais clínicos são inespecíficos e variam desde astenia até disfunção e insuficiência pulmonar e dor abdominal em adultos, enquanto nas crianças esse quadro

clínico pode se manifestar com febre, anorexia, cefaleia, náuseas, vômitos, dor abdominal, letargia, dores musculares e sintomas respiratórios (Rostami et al., 2019).

A TO acomete principalmente crianças entre cinco e 10 anos, geralmente apresenta-se de forma unilateral (Macpherson, 2013), podendo estar associada a infecção causada por uma baixa quantidade de larvas de *T. canis* (Fan et al., 2015). A ocorrência mais frequente da TO é a formação de um granuloma no polo posterior do olho e a mais grave é a endoftalmite (Despommier, 2003; Fan et al., 2015). Outras alterações incluem opacidades vítreas, lesões na retina com papiledema, presença da larva do parasito intraocular e retina (Chen et al., 2018).

Outra forma clínica importante é a NT, causada pela invasão de larvas de *T. canis* tanto no encéfalo como na medula espinhal. Essa forma clínica pode manifestar-se, principalmente, em indivíduos de meia idade e apresenta-se mais frequentemente como mielite ou encefalite isoladas e meningoencefalite, com febre inconstante (Deshayes et al., 2016). Além desses, outros sinais clínicos podem ocorrer como encefalopatia com declínio cognitivo, vasculites cerebrais e epilepsia (Finsterer e Auer, 2007). Há ainda discussões na literatura sobre possíveis associações entre a toxocaríase neurológica e o surgimento de doenças neurodegenerativas, incluindo convulsões, esquizofrenia, déficits cognitivos, doença de Parkinson idiopática e até mesmo demência (Rostami et al., 2019).

O diagnóstico da toxocaríase humana é difícil em especial por sua sintomatologia inespecífica (Fillaux e Magnaval, 2013). As técnicas mais indicadas para o diagnóstico são o ensaio imunoenzimático indireto (ELISA) e o Western blotting, geralmente utilizando antígenos excretorios e secreção de larvas de *T. canis* (TES) (Deshayes et al., 2016). A eosinofilia sanguínea, embora não seja específica da toxocaríase, tem sido constantemente associada à TV, geralmente sendo acentuada nessa forma clínica. Já na TC e TO a eosinofilia sanguínea pode ser baixa ou até mesmo estar ausente (Magnaval et al., 2022). Os níveis séricos totais de IgE podem estar elevados nas formas sistêmicas da toxocaríase humana, porém esse mecanismo ocorre na maioria das helmintíases e não pode ser utilizado no diagnóstico diferencial (Fillaux e Magnaval, 2013).

2.3. Tratamento da toxocaríase humana

O tratamento preconizado para a toxocaríase humana inclui o emprego de anti-helmínticos benzimidazólicos (Magnaival et al., 2022). Os benzimidazóis são uma classe de compostos sintéticos com atividade anti-helmíntica de largo espectro e amplamente utilizados para tratar helmintoses intestinais, como a ascaridíase, tricuriase, ancilostomíase. No entanto, quando utilizados no tratamento de parasitoses teciduais, como a toxocaríase humana, não conseguem atingir excelentes taxas de resolução clínica (Chen et al., 2018).

Dentre as opções do grupo dos benzimidazóis, o albendazol (ABZ) é o fármaco de primeira escolha devido à sua disponibilidade, baixo preço, eficácia aceitável e ausência de reações adversas importantes (Magnaival et al., 2022). O mebendazol (MBZ) é o fármaco de segunda escolha em função de sua menor ação extraintestinal (Hotez, 1993; Despommier, 2003). A dietilcarbamazina (DEC) utilizada na terapia da filariose desde 1949 (Magnaival et al., 2001) e o tiabendazole (TBZ) também foram opções para o tratamento dessa parasitose. (Magnaival et al., 1987; Stürchler et al., 1989).

Vale ressaltar que poucos estudos foram realizados para avaliar o tratamento para toxocaríase, sendo que alguns desses estudos foram ensaios clínicos randomizados controlados e outros foram observacionais prospectivos ou retrospectivos (Tabela 1). Assim, além da utilização de diferentes metodologias, nesses estudos foram empregadas dosagens baixas dos anti-helmínticos e o período de tratamento foi inferior ao ideal. Além disso, também há heterogeneidade dos estudos sobre ABZ, e a dose e a duração ideais do tratamento ainda necessitam ser estabelecidas (Magnaival et al., 2022).

Além do tratamento com anti-helmínticos, pode ser necessária a utilização de tratamento de suporte, que pode incluir oxigenioterapia nos casos de comprometimento das vias respiratórias e anti-histamínicos preconizados contra reações alérgicas. Nos casos graves, especialmente quando há inflamação severa ou acometimento ocular e neurológico, os corticosteroides podem ser administrados para reduzir a resposta inflamatória e evitar danos permanentes (Chen et al., 2018; Magnaival et al., 2022).

Tabela 1 - Características dos estudos clínicos de avaliação dos anti-helmínticos albendazol (ABZ) emebendazol (MBZ) para o tratamento da toxocaríase humana e taxa de cura, conforme Magnaval et al. (2022) com modificações.

Autores	Tipo de estudo clínico	ABZ		MBZ	
		Dose	Cura % (n)	Dose	Cura % (n)
Magnaval et al. 1987	Randomizado controlado			25/mg/kg/7d	57% (24)
Stürchler et al. 1989	Randomizado controlado	10/mg/kg/5d	31,6% (6)		
Magnaval et al. 1995	Randomizado controlado			20–25/mg/kg/21d	17,1% (7)
Altcheh et al. 2003	Observational prospectivo	15 mg/kg/15d	100% (16)		
Turrientes et al. 2011	Observational retrospectivo	0–15 mg/kg/5d	100% (4)		
Kim et al. 2017	Observational retrospectivo	800 mg/5-7d	NA		
Krotten et al. 2018	Observational retrospectivo	15 mg/kg/10d x2	71% (47)		
Yoon et al. 2018	Observational prospectivo	800mg / variável	85,1% (120)		
Hombu et al. 2019	Observational prospectivo	15 mg/kg/228d x 2	80,5% (91)		
Phuc et al. 2021	Observational prospectivo	50 mg/kg/5d	78,8% (63)		

Os anti-helmínticos exercem ação antiparasitária por mecanismos de ação distintos, podendo ocorrer desde interferência no metabolismo energético, coordenação neuromuscular e função microtubular, até a alteração na permeabilidade celular do patógeno alvo (Craig e Stitzel, 2003). A atividade de um fármaco não depende apenas da sua ligação ao receptor específico do patógeno alvo (farmacodinâmica), mas também da capacidade de atingir e manter concentrações elevadas em diferentes tecidos durante período suficiente para induzir o seu efeito (Torrado et al., 1996).

Os fármacos benzimidazólicos, em geral, atuam diretamente sobre os microtúbulos dos parasitos adultos, bloqueando a sua capacitação de glicose e por consequência reduzem a formação de trifosfato de adenosina (ATP) necessária para a

sobrevivência e reprodução dos helmintos (Korolkovas, 2009). Já sobre as larvas, esses fármacos exercem seu mecanismo sobre suas funções motoras, ligando-se à β -tubulina livre, componente fundamental dos microtúbulos dos parasitos, levando à inibição da polimerização da tubulina e à perda de microtúbulos citoplasmáticos. Esse efeito acaba por inibir a motilidade das larvas e por consequência impede que realizem a migração pelos tecidos (Frixione e Meza, 2017; Magnaval et al., 2022). Há uma estreita ligação entre o comportamento farmacocinético do anti-helmíntico no hospedeiro e sua eficácia final, uma vez que quando ingerido o medicamento precisa passar por todo o processo de metabolização até que esteja pronto para agir sobre seu alvo. A absorção e metabolização do medicamento no organismo do indivíduo são os principais fatores limitantes para a quantidade de fármaco que chega à circulação sistêmica e posteriormente até o patógeno (Torrado et al., 1996).

Vale ressaltar que, apesar da carência de avaliação dos anti-helmínticos em humanos para toxocaríase humana, foram desenvolvidos recentemente estudos *in vitro* que demonstram potencial de diversos compostos químicos. Entre eles, destacam-se o óleo essencial de própolis vermelha brasileira (Sinott et al., 2019), naftoquinonas naturais e sintéticas (Mata-Santos et al., 2016a; Ortiz-Pérez et al., 2021), uma amida sintética de ácido graxo (Mata-Santos et al., 2016b) e derivados sintéticos de cumarina (Rodrigues et al., 2024). No entanto, é importante que sejam realizados estudos que avaliem atividade *in vivo* e biodisponibilidade como será abordado no presente estudo.

Em vista das limitações encontradas nos tratamentos disponíveis para toxocaríase humana, mostra-se necessário o desenvolvimento de estudos com o foco na descoberta de potenciais novos anti-helmínticos, porém o tempo necessário para realização dessas pesquisas e os altos custos envolvidos acabam se tornando um obstáculo difícil de ser ultrapassado. Uma maneira de superar esses obstáculos seria o reposicionamento de fármacos (Pink et al., 2005) ou por meio de estudos de compostos que apresentem bioatividade sobre outros patógenos, como é o caso dos compostos derivados de cânfora, nitrofurano e pirimidina selecionados para o presente estudo, cuja atividade contra outros agentes foi previamente relatada (Cardoso et al., 2016; Alves et al., 2020; Silva et al., 2016; Pinheiro et al., 2020). Vale ressaltar que a bioprospecção de novos fármacos não deve se limitar somente ao estudo de compostos bioativos, mas também é importante incluir a inovação ao explorando propriedades farmacocinéticas (vias de administração e biodisponibilidade) de tais compostos (Beugnet e Franc, 2012).

2.4. Compostos sintéticos e seu potencial terapêutico

Nas últimas cinco décadas, a bioatividade de diversos grupos de compostos foi amplamente testada contra diferentes micro-organismos e patologias, com a maioria das pesquisas focada no tratamento de infecções bacterianas e neoplasias. Menos de 2% dos compostos químicos testados e aprovados nesse período tiveram sua atividade antiparasitária avaliada (Newman e Cragg, 2020), evidenciando a escassez de estudos voltados para essa área.

Dentre esses diferentes compostos químicos estudados, encontra-se a classe de compostos heteroaromáticos, que estão baseados no anel benzeno, uma estrutura cíclica composta por seis átomos de carbono (C) dispostos em um formato hexagonal, com ligações simples e duplas alternadas. Cada átomo de carbono está ligado a um átomo de hidrogênio (H), resultando na fórmula molecular C_6H_6 , que é a fórmula do benzeno. Essa estrutura é fundamental em química orgânica, pois serve como base para diversas substâncias químicas (Caramori e Oliveira, 2009), como pode ser observado na Figura 2.

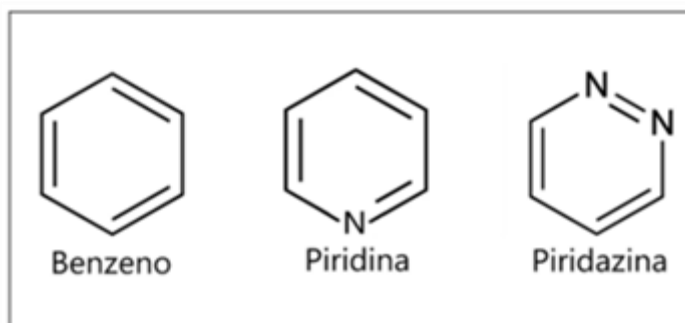


Figura 2. Anel benzeno e seus derivados heteroaromáticos (Merck, 2025a).

Na classe dos compostos heterocíclicos aromáticos, destacam-se os derivados conjugados com nitrogênio, como o triazol, piridinas e pirimidinas, os quais apresentam atividade biológica antiparasitária contra *Entamoeba histolytica* e *Giardia intestinalis* (Saadeh et al., 2009). Além disso, outros compostos heterocíclicos nitrogenados têm apresentando atividades farmacológicas promissoras sobre os protozoários *Trypanosoma cruzi* (Maya et al., 2006), *Entamoeba histolytica*, *Trichomonas vaginalis* (Tenório et al., 2005) e *Giardia muris* (Mahfouz et al., 1998) e sobre as bactérias *Staphylococcus aureus* (Masunari e Tavares, 2007) e *Mycobacterium tuberculosis* (Cardoso et al., 2016).

Um estudo com naftoquinonas revelou que a adição de um grupo amino (Figura 3) triplica sua ação contra cepas de *Mycobacterium tuberculosis* (Halicki et al., 2018). Esse achado reforça os resultados de outras pesquisas, que demonstraram que grupos nitrogenados apresentam bioatividade contra fungos e bactérias Gram-positivas e Gram-negativas (Riffel et al., 2002; Rahmoun et al., 2013).

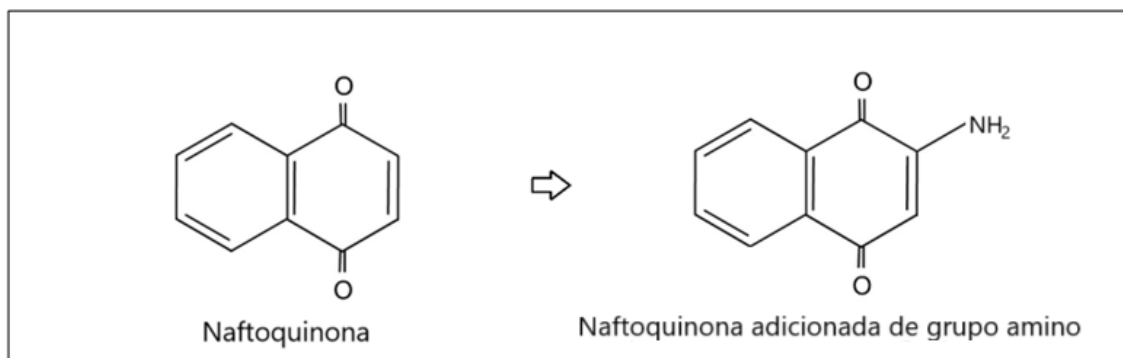


Figura 3. Estrutura molecular da naftoquinona e sua versão adicionada de grupo amino (NH₂) (Halicki et al. 2018).

2.4.1. Compostos de cânfora

Outro grupo de compostos com propriedades biológicas relevantes é o dos terpenóides. Dentro desse, destacam-se as atividades de compostos derivados da cânfora, uma substância cristalina branca, extraída do *Cinnamomum canphora* (canforeiro), *Dryobalanops aromatica* (cânfora-do-bornéu) e *Ocoteaus ambarensis* (família do louro). A cânfora é um monoterpenobicíclicocetônico e sua estrutura (1,7,7-trimetilbiciclo-[2.2.1]-heptan-2-ona) foi proposta em 1893 pelo pesquisador alemão Julius Brecht (Figura 5). Esse composto também pode ser sinteticamente obtido a partir do α -pineno, um monoterpeno natural abundantemente presente no óleo da terebintina (Alves & Victor, 2010).

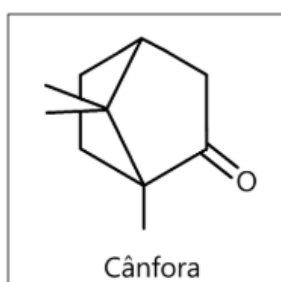


Figura 4. Estrutura molecular da cânfora (Silva et al., 2016).

Terpenóides, como os monoterpenos, constituem um vasto grupo de metabólitos secundários que podem ser isolados de diferentes fontes vegetais, como é o caso da *Artemisia annua*, planta conhecida por ser ativa sobre os protozoários *Plasmodium* spp., *Toxoplasma gondii* e *Leishmania* spp. (Feng et al., 2020). O mecanismo de ação de monoterpenos pode estar relacionado à atividade inibidora da enzima acetilcolinesterase (Howes e Houghton, 2003), o que justifica sua ação também sobre o sistema nervoso central, onde se destacam as atividades sedativa, ansiolítica, antinociceptiva e anticonvulsivante (Passos et al., 2009). Terpenóides e outros metabólitos de *Artemisia annua* já se mostraram ativos em estudos *in vitro* contra helmintos, como *Ecchinococcus* spp. e *Schistosoma* spp. (Lam et al., 2018), em estudo *in vitro* sobre ovos e *in vivo* em ovinos naturalmente infectados com *Haemonchus contortus* (Cala et al., 2014). Além disso, um estudo comparativo *in vitro* foi realizado por Shalaby et al. (2009), no qual a atividade do Artemether, um derivado semissintético da artemisinina, utilizado na terapia antimalária, e os efeitos do anti-helmíntico albendazol foram avaliados sobre *Toxocara canis*, concluindo que ambos são capazes de induzir alterações morfológicas na forma adulta do parasito, como distorção de papilas sensoriais e ruptura cuticular.

A molécula de cânfora apresenta um grupo funcional que permite transformações sintéticas e a transferência de características químicas relevantes para o produto químico final, permitindo assim a preparação de várias estruturas de interesse de maneira rápida, garantindo ainda a inclusão de propriedades ideais de absorção, distribuição, metabolização e excreção (ADME) (Silva et al., 2022). Moléculas de cânfora sintetizadas por Silva et al. (2016) apresentaram atividade *in vitro* frente ao *Mycobacterium tuberculosis* e a maioria das propriedades desse composto foi relacionada a presença do fragmento heptano biciclo[2.2.1] (Figura 6), que confere maior lipofilicidade e arranjo espacial mais favorável à molécula, o que favorece sua bioatividade. No campo da química medicinal, estudos de estrutura-atividade proporcionam a obtenção de compostos mais adequados para avaliação pré-clínica, visto que agilizam o processo de descoberta de novas entidades químicas e permitem o fornecimento de novos medicamentos a um custo menor para o mercado (Silva et al., 2022)

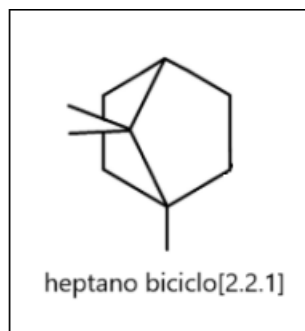


Figura 5. Estrutura molecular do fragmento heptano biciclo[2.2.1] (Silva et al., 2016).

Estudos com cânforas de origem natural também foram realizados demonstrando a bioatividade dessas contra fungos e bactérias. Extratos de cânfora extraídos da planta *Artemisia camphorata* apresentam efeito sobre *Alternaria solani* (Itako et al., 2008) e *Exserohilum turcicum* (Scapin et al., 2010), além de reduzirem o crescimento micelial de *Bipolaris sorokiniana* (Stangarlin et al., 2003). Outros estudos comprovaram a atividade de óleos essenciais canforados contra as bactérias *Staphylococcus aureus*, *Micrococo luteo*, *Escherichia coli*, *Salmonella typhimirium*, *Bacillus cereus*, *Enterococcus faecalis* e sobre as leveduras *Candida albicans*, *C. glabrata*, *C. sake* e *C. tropicalis* (Mighri et al., 2010). Resultados como esses demonstram a vasta bioatividade apresentada por essa classe de compostos e a coloca em uma posição favorável para ensaios sobre outros patógenos.

2.4.2.Compostos de nitrofurano

Os nitrofuranos são compostos heterocíclicos aromáticos que contém nitrogênio e que pertencem a uma família de compostos que ganharam destaque na década de 1940 com sua introdução na terapêutica com amplo espectro de atividade. No entanto, nas duas décadas seguintes, seus efeitos tóxicos foram observados, e com o surgimento de novos agentes antibacterianos, seu uso foi reduzido (Paula et al., 2009).

A atividade biológica dessa classe de compostos é dependente da presença do grupo nitro ligado à molécula e resulta, basicamente, de mudanças na estabilidade e redução desse grupo por nitro redutases de micro-organismos, o que gera intermediários eletrofílicos nitrosos e hidroxilamina que, por sua vez, podem se ligar ao DNA e a outras biomoléculas interferindo no seu metabolismo. Essa característica exclusiva dos nitrofuranos resulta em um perfil de baixa resistência, uma vez que a adaptação dos micro-organismos a esses compostos demanda um elevado custo energético (Gallardo-

Garrido et al., 2020). Esse efeito demonstra a importância das propriedades físico-químicas que um grupo nitrogênio exerce para o desencadeamento e manutenção da atividade biológica de compostos. Além disso, estudos demonstram que compostos heterocíclicos contendo radicais nitrogenados podem induzir o estresse oxidativo em sistemas biológicos e seu mecanismo de ação seria tanto pela produção de radicais livres, que acabam sendo tóxicos aos micro-organismos, como também, induzindo a ocorrência de estresse oxidativo no patógeno por inibir enzimas envolvidas no processo de neutralização desses radicais (Hall et al., 2011).

Os efeitos de nitro compostos estão intimamente ligados à sua capacidade aceptora de elétrons que reduzem seu grupamento nitro desencadeando sua bioatividade (Yan et al., 2005). A maioria dos compostos nitrogenados apresenta como característica a capacidade de redução enzimática, o que resulta em radicais livres que interagem com biomoléculas essenciais para a sobrevivência dos patógenos (Viodé et al., 1999; Paula et al., 2009). Alguns desses compostos apresentam ação contra protozoários *Trypanosoma cruzi* (Hall et al., 2011; Palace Berl et al., 2018) e *Leishmania infantum* (Petri e Silva et al., 2016) e sobre *Mycobacterium tuberculosis* e *M. smegmatis* (Sriram et al., 2010). Além disso, foi relatada a atividade inibitória da enzima transcriptase reversa (RT), presente em vírus do tipo HIV (Teitz et al., 1994), demonstrando o potencial terapêutico extenso que esse grupo de compostos químicos apresentam.

2.4.3. Compostos de pirimidina

Compostos de pirimidina são constituídos por duas diazinas, ou seja, são compostos heterocíclicos constituídos por seis membros em um anel benzênico com dois átomos de nitrogênio nas posições 1 e 3, respectivamente (Figura 7) (Saladino et al., 2002).

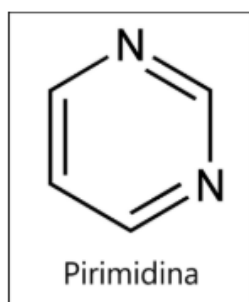


Figura 6. Estrutura molecular da pirimidina (Merck, 2025b).

Nas últimas décadas diversos estudos foram conduzidos com compostos derivados da pirimidina. Nesses, foram obtidos resultados promissores contra diferentes agentes patogênicos, como o efeito antiparasitário sobre *Trypanosoma cruzi* (De Melo et al., 2018), a atividade antibacteriana sobre *Staphylococcus aureus*, *Bacillus subtilis* e *Pseudomonas aeruginosa* (Do Monte et al., 2018), além da atividade contra o vírus da rubéola (Saladino et al., 2002). Estudos conduzidos por Pinheiro et al. (2020) demonstraram ainda que diferentes derivados pirimidínicos apresentam atividade contra *Mycobacterium tuberculosis*, e revelaram que, dentro dos compostos sintetizados pelo grupo, aqueles com fragmento 5-nitrofurano-2-yl produzem maior atividade antimicobacteriana que os demais. Já Hafez et al. (2016) investigaram a atividade biológica de derivados pirazolo[3,4-d]pirimidina, obtendo resultados contra cepas de *Pseudomonas aeruginosa*, *Escherichia coli*, *Streptococcus lactis* e *Staphylococcus aureus*.

Pirimidinas são componentes essenciais do ácido desoxirribonucleico (DNA) e ácido ribonucleico (RNA), sendo um dos principais componentes endógenos do organismo, o que lhes confere diversas propriedades farmacológicas como anticâncer, antiviral, antimicrobiana, anti-inflamatória, analgésica, antioxidante e antimalárica (Zhuang e Ma, 2020). Os derivados de pirimidina (citosina, timina e uracila) são três das cinco nucleobases que compõem o material genético, e como componente essencial dos nucleosídeos, as pirimidinas interagem facilmente com enzimas, material genético, bio-componentes celulares e biopolímeros, o que resulta em maior atividade com menor possibilidade de citotoxicidade (Nadar e Khan, 2022).

As pirimidinas desempenham um papel importante nos projetos de desenvolvimento de medicamentos, pois têm considerável significado farmacológico e químico. Compostos heterocíclicos contendo um núcleo pirimidina são de grande interesse devido ao seu papel significativo como compostos naturais e sintéticos (Nadar e Khan, 2022). Devido à sua boa biocompatibilidade, as estruturas de pirimidina são frequentemente associadas a compostos antibacterianos conhecidos (Zhuang e Ma, 2020), como é o caso de medicamentos à base de sulfas, como a sulfadiazina, sulfamerazina e sulfadimidina (Figura 8), que apresentam atividade antibacteriana superior devido a essa associação.

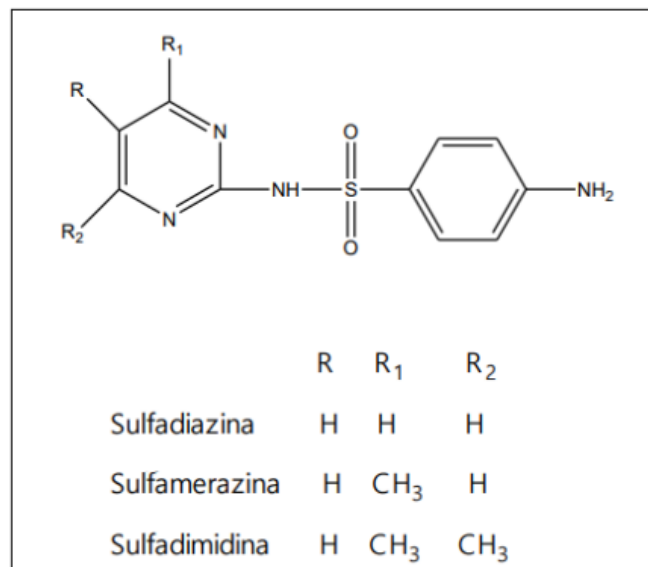


Figura 7. Derivados pirimidínicos sintetizados a partir de sulfas (Patil, 2018).

A trimetoprima é também um agente representativo dessa associação, contendo um grupo pirimidina ativo. Esse fármaco é capaz de inibir o crescimento de bactérias, e funciona de forma sinérgica com algumas sulfonamidas, desempenhando papel importante no alívio da crise de resistência bacteriana (Zhuang e Ma, 2020). Estudos conduzidos por Rani et al. (2016) demonstraram que compostos pirimidínicos possuem antagonismo a bactérias Gram-positivas e Gram-negativas, concluindo que esses derivados apresentam amplo espectro de ação. Outros antibióticos derivados de pirimidinas utilizados atualmente são a Bacimetrimina (5-hidroximetil-2-metoxipirimidina-4-amina), usado em infecções estafilocócicas, e o Gourgetin, um derivado de citosina utilizado no combate a micobactérias e que também apresenta ação antagônica contra bactérias Gram-positivas e Gram-negativas (Patil, 2018).

Devido à presença de um anel heterocíclico básico contendo nitrogênios, as pirimidinas são solúveis em água. Sua solução aquosa é fracamente básica e apresenta baixo risco de nefrotoxicidade, o que as torna opções mais seguras para o tratamento de pacientes com insuficiência renal (Patil, 2018). Esses fatores indicam fortemente que os derivados pirimidínicos possuem potencial para o combate a outras infecções, incluindo aquelas causadas por parasitas.

3.OBJETIVOS

3.1. Objetivo Geral

Avaliar o potencial terapêutico de compostos sintéticos derivados de cânfora, nitrofurano e pirimidinas, através de testes pré-clínicos, visando à seleção de candidatos a novos anti-helmínticos para o tratamento da toxocaríase visceral.

3.2. Objetivos Específicos

- Selecionar compostos sintéticos por meio de triagem *in vitro* e determinação da concentração larvicida mínima com eficácia de 100% (CLM) sobre larvas infectantes de *Toxocara canis*;
- Confirmar *in vivo*, em modelo murino, a inviabilidade das larvas de *Toxocara canis* que foram expostas aos compostos sintéticos derivados de cânfora, nitrofurano e pirimidina selecionados nos testes de triagem *in vitro*;
- Avaliar *in vitro* a citotoxicidade dos compostos sintéticos derivados de cânfora, nitrofurano e pirimidina selecionados;
- Estimar *in silico* a biodisponibilidade dos compostos sintéticos derivados de cânfora, nitrofurano e pirimidina selecionados;
- Avaliar *in vivo* a eficácia dos compostos sintéticos selecionados sobre a intensidade da infecção em modelos murinos;

4. HIPÓTESES

Compostos sintéticos derivados de cânfora, nitrofurano e pirimidina apresentam: baixa ou nenhuma citotoxicidade, atividade larvicida sobre *Toxocara canis*, parâmetros adequados de biodisponibilidade e absorção gastrointestinal e eficácia em reduzir a intensidade de infecção aguda e/ou crônica em camundongos infectados experimentalmente.

5. ARTIGO 1

Synthetic camphor derivative (E)-2-((1,7,7-trimethylbicyclo[2.2.1]heptan-2-ylidene)amino)phenol: a novel anthelmintic drug candidate for visceral toxocariasis

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Abstract

Human toxocariasis is a neglected parasitic disease with a global distribution, treated with current anthelmintics that have low to moderate efficacy, and requires the discovery of novel drugs. Camphor derivatives have antimicrobial properties against various pathogens such as fungi and bacteria. This study aimed to identify a camphor derivative with activity against *Toxocara canis* larvae and evaluate its cytotoxicity, *in silico* bioavailability, and *in vivo* activity in Swiss mice infected with this parasite. Three compounds were tested *in vitro* in duplicate at a concentration of 1.0 to 0.05 mg/mL in a microplate containing 100 *T. canis* larvae in RPMI-1640 medium incubated for 48 h at 37 °C and 5% CO₂. The compound (E)-2-((1,7,7-trimethylbicyclo [2.2.1] heptan-2-ylidene)amino)phenol (C2) presented a minimum larvicidal concentration (MLC) of 0.25 mg/mL and was selected for the subsequent steps. This compound showed 100% cell viability in MLC and adequate bioavailability in computational

models. Two subsequent *in vivo* tests were performed on Swiss mice inoculated with 500 *T. canis* infective eggs through intragastric (IG) intubation, one at 10 days post-inoculation (n=5), and the other at 30 days post-inoculation (n=10). The selected compound (10 mg/kg, via IG) and two controls (albendazole, 40 mg/kg, IG and phosphate buffered saline 0,15M, pH 7,2, via IG) were used for this evaluation. The compound reduced the intensity of infection by 75.7% and 54.8% at 10 and 30 days post inoculation, respectively ($p<0.05$). The results of this study demonstrate that this compound has potential as an anthelmintic candidate for visceral toxocariasis treatment.

Keywords: Toxocara; Parasitic; Synthetic compound; Camphor; Preclinical trial.

Introduction

Human toxocariasis is a neglected tissue parasitic disease with worldwide distribution. Caused by the larval migration of *Toxocara canis* and *T. cati*, intestinal nematodes found in dogs and cats, respectively (Chen et al., 2018). This disease presents a significant challenge to global public health problem (Magnaval et al., 2022). Humans become infected mainly by ingesting embryonated eggs present in the soil and contaminated vegetables or water. Another route of infection is the consumption of undercooked or raw meat or viscera from paratenic hosts such as chickens, cows, ducks, lambs, pigs, ostriches, or rabbits, which may contain encysted infective larvae in their tissues (Chen et al., 2018). In humans, this disease is caused by the migration and residence of *T. canis* larvae in the liver, lungs, heart, kidneys, brain, and skeletal striated muscles (Fan et al., 2015).

Clinical manifestations can range from asymptomatic to severe organ injury and can result in different syndromes. Depending on the type of tissue involved, the infection can be classified as systemic, including visceral larvamigrans syndrome (VLM) and common or covert toxocariasis, or compartmentalized, including ocular and neurological toxocariasis (Chen et al., 2018). Pathological changes in human toxocariasis include hemorrhage, necrosis, and granuloma formation in somatic tissues (Strube et al., 2013).

Treatment varies according to symptoms and clinical form, starting with an anthelmintic approach. Benzimidazole, albendazole, and mebendazole are the first- and second-choice medications, respectively. Additionally, palliative therapies, such as corticosteroids, antihistamines, and oxygen therapies, may be used (Magnaval et al., 2022). The clinical efficacy of these anthelmintics is considered low to moderate, mainly because of their low gastrointestinal absorption and, consequently, low bioavailability after metabolism (Chen et al., 2018) indicating the need for research and development of novel drug candidates.

Mouse is an excellent laboratory host to study experimental infections by *T. canis*, as it tolerates infections for long periods, and the larvae follow the enteric-pulmonary-somatic cycle with signs and symptoms similar to those observed in humans (Nicholas et al., 1984). Thus, murine toxocariasis is to be a suitable model for evaluating chemical compounds, medicines, and probiotics, among others (Mata-Santos et al., 2015; Walcher et al., 2017; Rodrigues et al., 2024).

A wide range of natural products have been the subject of research in biomedical and biological fields, and camphor and its derivatives have been identified. Camphor is a white crystalline substance that can be obtained from *Cinnamomum camphora* and other natural sources such as *Dryobalanops aromatica* and *Ocoteaus ambarensis*. Camphor can also be synthetically obtained from α -pinene, an abundant natural compound present in turpentine oil (Alves and Victor, 2010). This is a secondary metabolite of the plant *Artemisia annua*, which is known to be active against *Plasmodium* spp., *Toxoplasma gondii* and *Leishmania* spp. (Feng et al., 2020). Studies suggest that terpenoids, compounds produced by *Artemisia annua*, may be active against various helminths such as *Haemonchus contortus*, *Ecchinococcus* spp., *Schistosoma* spp. and *Toxocara canis* (Lam et al., 2018). These compounds contain special functional groups that allow suitable synthetic transformations, making it possible to prepare several structures of interest within a short period (Silva et al., 2022).

Several extracts and derivatives of camphor have been studied against a wide range of pathogens; for example, *Artemisia camphorata* has a fungitoxic effect against *Bipolaris sorokiniana* (Stangarlin et al., 2003), *Alternaria solani* (Itako et al., 2008), and *Exserohilum turcicum* (Scapin et al., 2010). Furthermore, camphor essential oils

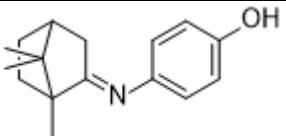
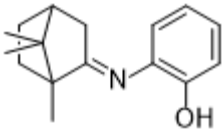
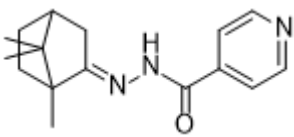
showed antibacterial activity against *Staphylococcus aureus*, *Micrococo luteus*, *Escherichia coli*, *Salmonella typhimurium*, *Bacillus cereus*, *Enterococcus faecalis*, *Candida albicans*, *C. glabrata*, *C. saque*, and *C. tropicalis* (Mighri et al., 2010). This study aimed to select a camphor derivative as a candidate drug for the treatment of visceral toxocariasis using preclinical tests, including *in vitro*, *in silico*, and *in vivo* assays.

Materials and Methods

Camphor derivatives

Arenealdehyde and d-camphor were purchased from commercial sources. All solvents and reagents were used as received without further purification. Three camphor derivatives were prepared using an ultrasonic homogenizer model “Desruptor Eco Sonics” (25 KHz, Eco Sonics, Indaiatuba, Brasil), that was used to carry out the reactions at 90 W, with an average yield of 68% (Silva et al., 2016). Camphor derivatives were produced by the Institute of Drug Technology (Farmanguinhos) of the Oswaldo Cruz Foundation (Fiocruz) and were available as lyophilized samples at the New Drug Development Center (NUDEFA/FURG) (Table 1). All the synthesized compounds were solubilized at 2.5% in dimethyl sulfoxide (DMSO) and sterile distilled water to obtain a concentration of 1 mg/mL (Mata-Santos et al., 2015).

Table 1. Chemical structure of the three camphor derivatives used for the study.

Code	Chemical structure	IUPAC Name
C1		(E)-4-((1,7,7-trimethylbicyclo[2.2.1]heptan-2-ylidene)amino)phenol
C2		(E)-2-((1,7,7-trimethylbicyclo[2.2.1]heptan-2-ylidene)amino)phenol
C3		(E)-N'-(1,7,7-trimethylbicyclo[2.2.1]heptan-2-ylidene)isonicotinohydrazide

Parasite

The *Toxocara canis* eggs were maintained in 2% formalin solution and incubated at 28 °C, humidity above 90%, and oxygenation for 30 days (Avila et al., 2012). The infective larvae (third-stage) (Bowman, 2020) were extracted using a modified Baermann apparatus and maintained in sterile tubes for cultivation with RPMI-1640 medium (Gibco®), supplemented with 25 mM HEPES (Sigma®), 1% glucose, PSF antibiotic-antimycotic (Gibco®) at 37 °C with an atmosphere of 5% CO₂ (Savigny, 1975).

In vitro tests

The *in vitro* screening tests were performed in duplicate at a concentration of 1 mg/mL in a 96-well flat bottom plate, using 100 *T. canis* larvae (third-stage) per well and three controls: one live larva, one dead larva (thermal shock) and one DMSO (2.5%). The microculture plate was maintained for 48 h at 37 °C with 5% CO₂. Subsequently, trypan blue viability indicator was added and the plate was incubated for an additional 30 min. The samples were examined under an optical microscopy (10× objective). Viability was assessed based on the motility of the larvae and the absence of impregnation using a cell viability indicator. The compounds that showed 100% of larvicidal activity in the screening test (1.0 mg/mL) were evaluated at concentrations of 0.5 mg/mL to 0.05 mg/mL to determine their minimum larvicidal concentration (MLC) (Mata-Santos et al., 2015).

To confirm the non-viability of the larvae observed in the *in vitro* tests of the compounds that presented 100% MLC, we followed the methodology described by Mata-Santos et al (2015). Briefly, the contents of the wells of a microculture plate containing larvae exposed to the compounds and unexposed larvae (control) were intraperitoneally inoculated in duplicate into female Swiss mice intraperitoneally (IP). After 48 h of inoculation, the mice were euthanized and tissue digestion of the carcasses, brains, livers, lungs, kidneys, and hearts was performed in a solution of 1% hydrochloric acid and 1% pepsin (Xi and Jin, 1998). The material was then examined under a microscope with a 10× objective.

Cytotoxicity

Compounds that showed larvicidal activity against 100% of the larvae in the MLC test were subjected to cytotoxic assays at the same concentrations of the *in vitro* screening tests to determine the concentration of the compound capable of maintaining 50% cell viability (IC 50). The test was performed using murine macrophages J774.A1 (1.10^5 cells/100 μ L), placed in DMEM medium supplemented with 10% fetal bovine serum. A compound free cell control was used. To read the results, the resazurin cell viability indicator was added at 0.01% and microculture plate was incubated for 6 h. Cell viability was measured by fluorescence at a 620 nm wavelength through a BioTek™ ELx800™ reader (Ansar Ahmed et al., 1994)

In silico bioavailability

The selected compound and albendazole were drawn using the molecular editor Chem Bio Draw Ultra 12.1 (Halford, 2014) and analyzed using the Swiss ADME computer model (www.swissadme.ch) (Daina et al., 2017). To predict adequate parameters of absorption, distribution, metabolism and excretion of compounds by the human body, the analysis was made following the "rule of five" (Lipinski, 2004), which include four parameters and the reference values accepted are equal to or less than multiples of five, being accepted the following values: molecular weight ≤ 500 , $\text{miLogP} \leq 5$ and, number of hydrogen donors ≤ 5 , and hydrogen acceptors ≤ 10 .

Evaluation of murine toxocariasis

Female swiss mice aged 5–7 weeks of age were used for all *in vivo* tests. The mice were randomly divided into three groups, and two experiments were conducted: the first was to evaluate the efficacy of the compound in mice (n=5) 10 days post-inoculation with *T. canis*, and the second was to evaluate the efficacy in mice (n=10) 30 days post-inoculation. The selected compounds and two controls were used for this evaluation. The mice were infected with 500 embryonated eggs of *T. canis* via intragastric (IG) route (Satou et al., 2005). The groups were administered for 5 days with the selected compound (10 mg/kg, IG), albendazole (40 mg/kg, IG), and phosphate buffered saline solution (PBS) (0.15 M and pH 7.2) (Mata-Santos et al., 2015). After 5 days of treatment, all the mice were euthanized. The recovery of *T. canis* larvae was performed

by tissue digestion of organs (liver, lungs, heart, kidneys, brain, and ocular globes) and carcasses. The larvae were examined and quantified using microscopy (10× objective) (Xi and Jin, 1998).

The mice were housed in mini-isolators (Tecniplast®) under a controlled environment of 22±1 °C, with a lightening cycle of 12/12 h light/darkness, provided with food without antibiotics and free access to water. This study was approved by the Ethics Committee in Research of the Federal University of Rio Grande (P034/2020). An invention patent titled ‘Use of synthetic compound derived from camphor as an anthelmintic’ owned by the Federal University of Rio Grande was deposited on April 17, 2024, at the National Institute of Industrial Property – INPI, under the number BR 10 2024 007530 7.

Statistical analysis was performed using analysis of variance (ANOVA) with Tukey's *post-hoc* test, with a significance level of $p \leq 0.05$.

Results

After the *in vitro* screening tests, two compounds (C1 and C2) showed activity against 100% of *T. canis* larvae at a concentration of 1 mg/mL. Both were submitted to the MLC test and the results showed 100% efficacy at 1 mg/mL for C1 and at 0.25 mg/mL for C2 (Table 2), this way, compound (*E*)-2-((1,7,7-trimethylbicyclo[2.2.1]heptan-2-ylidene)amino)phenol (C2) was selected for the next steps. In the cytotoxicity test, compound C2 resulted in 100% cell viability at its MLC (0.25 mg/mL) and an estimated cell viability of 77% at the maximum concentration tested (2 mg/mL). Following the described procedures, a confirmatory test was performed. The larvae exposed to C2 were not viable and did not have the ability to infect mice. This result was corroborated by the controls, as on average, nine larvae were found in the liver and lung tissues.

Table 2. Percentage of non-viable (dead) *Toxocara canis* larvae after exposure to camphor-derived compounds evaluated at different concentrations.

IUPAC Name	Code	% of non-viability <i>in vitro</i>		
		(1 mg/mL)	(0.5 mg/mL)	(0.25 mg/mL)
(<i>E</i>)-4-((1,7,7-trimethylbicyclo[2.2.1]heptan-2-ylidene)amino)phenol	C1	100	89.5	82
(<i>E</i>)-2-((1,7,7-trimethylbicyclo[2.2.1]heptan-2-ylidene)amino)phenol	C2	100	100	100
(<i>E</i>)- <i>N'</i> -(1,7,7-trimethylbicyclo[2.2.1]heptan-2-ylidene)isonicotinohydrazide	C3	16.1	-	-
	Live larvae Control	14.5	9.8	9.8

The results presented in Table 3 reveal the characteristics associated with oral bioavailability and indicate acceptable water solubility and gastrointestinal absorption. The physicochemical properties of (*E*)-2-((1,7,7-trimethylbicyclo[2.2.1]heptan-2-ylidene)amino)phenol (C2) are similar to those of albendazole.

Table 3. Physicochemical properties of the selected *in vitro* camphor derived (*E*)-2-((1,7,7-trimethylbicyclo[2.2.1]heptan-2-ylidene)amino)phenol (C2) and the albendazole.

Parameters	C2	Albendazole
Molecular weight (g/mol)	243,56	265,33
miLogP	4,36	1,62
Hydrogen Donors	1	2
Hydrogen Acceptors	2	3

In both *in vivo* tests, 10 and 30 days post-inoculation with *T. canis*, C2 promoted a significant reduction in infection intensity compared to PBS and showed similar results to albendazole (Table 4).

Table 4. Average recovery of the larvae per group 10 and 30 days post-inoculation with 500 eggs of *Toxocara canis* in Swiss mice.

Group	Identification	Average larvae recovery	Standard deviation	Average reduction
10 days				
	(E)-2-((1,7,7-trimethylbicyclo[2.2.1]heptan-2-ylidene)amino)phenol (C2) (10 mg/kg)	3,4 ^A	2.60	75,7%
	Albendazole (40 mg/kg)	4,4 ^A	4.50	68,6%
	PBS 1%	14 ^B	3.60	-
30 days				
	(E)-2-((1,7,7-trimethylbicyclo[2.2.1]heptan-2-ylidene)amino)phenol (C2) (10 mg/kg)	9,5 ^A	8.01	54,8%
	Albendazole (40 mg/kg)	10,6 ^A	4.27	49,6%
	PBS 1%	21 ^B	13.45	

Discussion

Camphor has been used as a herbal medicine for treating several pathogens and diseases. In a study conducted by Kang et al. (2019) the leaves of *Cinnamomum camphora* showed reduction *in vitro* in the production of inflammatory chemokines and, *in vivo*, was capable of relieving the symptoms of atopic dermatitis. The same extract has already demonstrated activity against coronavirus (SARS-CoV-2) (Malabadi et al., 2021) while essential oils from *Cinnamomum osmophloeum*, another species of the genus *Cinnamomum*, showed antioxidant activity *in vivo* in *Caenorhabditis elegans* (Hsu et al., 2012). Studies conducted by Fazmiya et al. (2022) demonstrated that *C. camphora* can also inhibit transient receptor potential A1 (TRPA1) and desensitize the receptor TRPV1, causing an analgesic action with potential use for different clinical conditions.

In this study, we evaluated the efficacy of a synthetic camphor derivative against murine visceral toxocariasis. In the last decade, major studies *in vitro* have focused on assessing the potential larvicidal activity of many compounds against *Toxocara* spp., such as Brazilian red propolis essential oil (Sinott et al., 2019), natural and synthetic naphthoquinones (Mata-Santos et al., 2016a et al; Ortiz-Pérez et al., 2021), synthetic fatty acid amide (Mata-Santos et al., 2016b), and synthetic coumarin derivatives (Rodrigues et al., 2024). Several extracts and derivatives of camphor have been studied

in fungi (Stangarlin et al., 2003; Itako et al., 2008; Scapin et al., 2010) and bacteria (Mighri et al., 2010). This is the first study to demonstrate the potential of camphor for treating visceral toxocariasis.

The results obtained in this study revealed a larvicidal activity *in vitro* of two camphor derivatives against *T. canis*, (*E*)-4-((1,7,7-trimethylbicyclo[2.2.1]heptan-2-ylidene)amino)phenol (C1) at a concentration of 1 mg/mL and (*E*)-2-((1,7,7-trimethylbicyclo[2.2.1]heptan-2-ylidene)amino)phenol (C2) at 0.25 mg/mL. Considering the low active concentration found for C2, it was selected for subsequent tests and was found to be non-cytotoxic at the concentrations tested. In addition, Silva et al. (2016) who synthesized the compounds used in this study, performed *in vitro* tests against *Mycobacterium tuberculosis* ATCC 27294 and obtained excellent antimycobacterial activity for compound C2.

Benzimidazoles are widely used to treat infections caused by various intestinal parasites. However, when used in tissue parasitosis, such as human toxocariasis, they are unable to achieve excellent rates of clinical resolution, which may be due to their low solubility in aqueous media (Chen et al., 2018). In relation to *in silico* bioavailability, (*E*)-2-((1,7,7-trimethylbicyclo[2.2.1]heptan-2-ylidene)amino)phenol (C2) did not violate the Lipinski's "rule of five" indicating adequate gastrointestinal absorption. In relation to the descriptors evaluated, this compound showed a low molecular weight, indicating its easy transport by proteins, which led to better absorption and metabolization capacity. Moreover, in relation to hydrogen donors and acceptors, this compound may exhibit good permeability through lipophilic membranes. The parameters obtained from the *in silico* analysis also revealed that C2 showed a miLogP higher than that of albendazole, indicating that this compound may present better results when administered with high-fat diets, as albendazole is poorly absorbed in the gastrointestinal tract and should be taken with a fat diet (Magnaval et al., 2022).

The results obtained *in vivo* in the present study confirmed the efficacy of (*E*)-2-((1,7,7-trimethylbicyclo[2.2.1]heptan-2-ylidene)amino)phenol (C2) against *T. canis* infection through a significant reduction in the infection intensity in the experimental models used. When we analyzed and compared the dosages used for the treatment groups, we noticed that despite having similar results in the quantification of larval

recovery, the group treated with C2 received a daily dose of 10 mg/kg, which was four times lower than the dose used for albendazole (40 mg/kg).

After infection, *T. canis* larvae begin to migrate to the liver and lungs via the circulatory system. After the first week, the hepato-pulmonary phase concludes, and the larvae continue with a neurotropic-myotropic phase when the larvae predominate in the brain and muscle (Waindok et al., 2019). In our study, we observed a major reduction in the infection intensity in the group treated 10 days post-inoculation, which revealed that this compound may be more suitable for recent clinical toxocariasis, in which the larvae are still beginning their neurotropic-myotropic phase, and tissues such as the liver, lungs, and heart present a greater number of larvae.

Experiments performed in mice 30 days after *T. canis* infection have provided consistent data on the efficacy of these compounds in reducing or eradicating the infection (Mata-Santos et al., 2016; Barrera et al., 2010). It is important to note that during this period of infection, the nematode larvae have already completed the initial phase of hepato-pulmonary migration and tend to spread throughout the body of the host. Therefore, the observation of a significant reduction in the intensity of infection is valuable data for a preclinical study. However, it is important to carry out studies on the therapeutic activity of the compound in murine models of chronic toxocariasis. Studies have shown that even mild *T. canis* infections in mice lead to changes in the general appearance and activity of the animals, causing disturbances in motility and balance, accompanied by the development of necrosis, hemorrhage and accumulation of foamy cells (Bernardová et al., 2024).

These findings, in addition to the lack of cytotoxicity, reinforce the therapeutic benefits of camphor derivatives in treating visceral toxocariasis. The results of this research have also resulted in a patent for the use of this compound as an antiparasitic, which has been filed with the Brazilian National Institute of Industrial Property (INPI) under the number BR 10 2024 007530 7, demonstrating the potential of this compound in the production of a novel antiparasitic drug candidate for use against visceral toxocariasis.

Conclusion

From this research, it can be concluded that the results obtained with synthetic camphor (E)-2-((1,7,7-trimethylbicyclo[2.2.1]heptan-2-ylidene)amino)phenol (C2) demonstrate its high activity against *T. canis* larvae and in the treatment of murine visceral toxocariasis, in addition to not presenting cytotoxicity and adequate bioavailability. Based on this research and the patent already filed for the use of this compound, it should be considered a candidate antiparasitic therapy for treating visceral toxocariasis.

Declarations

According to Brazilian law no 11.794/2008 (Arouca law), article 2° applies to animals of species classified as phylum Chordata, subphylum Vertebrata, subject to environmental legislation. The Federal University of Rio Grande is accredited by the Commission for Ethics in the Use of Animals (CEUA) in accordance with the provisions of article 8° of the Arouca law. This study was approved by the Ethics Committee in Research of the Federal University of Rio Grande (P034/2020).

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Funding declaration

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Data availability

Invention patent titled 'Use of synthetic compound derived from camphor as an anthelmintic' owned by the Federal University of Rio Grande, deposited on April 17,

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6. Manuscrito 1

Nitrofuran (E)-N'-((5-nitrofuran-2-yl)methylene)furan-2-carbohydrazide: drug candidate for the treatment of visceral toxocariasis

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Abstract

Human toxocariasis is a globally neglected parasitic disease, commonly treated with benzimidazole anthelmintics. However, their efficacy is considered unsatisfactory, requiring the research and development of new drugs. Studies have shown that heterocyclic compounds with nitrogenous molecules are known for their properties of induce oxidative stress on pathogens. This study aimed to evaluate the efficacy of the compound (E)-N'-((5-nitrofuran-2-yl)methylene)furan-2-carbohydrazide (PFUR) against visceral toxocariasis in preclinical tests. The compound PFUR was tested *in vitro*, in duplicate, at a concentration of 1.0 mg/mL to 0.05 mg/mL in a microplate containing 100 *Toxocara canis* larvae in RPMI-1640 medium. This compound showed activity against 100% of the larvae at the minimum larvicidal concentration (MLC) of 0.25 mg/mL. Furthermore, this compound also showed non-cytotoxicity to murine

macrophages and an adequate estimate of oral bioavailability by the “rule of five” in computational models. After, two *in vivo* tests were conducted on Swiss mice. In the groups treated with PFUR (10mg/kg/5d, IG) 10 days and 30 days after inoculation with 500 *T. canis* eggs, there was a reduction of 23% ($p>0.05$) and 62.4% ($p<0.05$) in the intensity of infection, respectively, compared to the PBS control. In both experiments, the PFUR compound presented results similar to those of albendazole (40mg/kg/5d, IG) ($p>0.05$). The results of this study demonstrated the potential of this compound as a candidate for a new anthelmintic.

Keywords: Toxocara; Parasitic disease; Nitrofunan; Anthelmintic.

Introduction

Human toxocariasis affects approximately 1.4 billion people worldwide (Ma et al., 2020) and is considered a neglected tissue parasitosis (Hotez, 2020). This disease is mainly caused by the nematode *Toxocara canis*, an intestinal parasite of dogs. It can be transmitted through soil, water or vegetables contaminated with embryonated eggs or undercooked meat and internal organs from paratenic hosts (cattle, pigs, sheep, chickens, quail, pigeons) (Xu and Han, 2024). In humans, the infective larva migrates through the systemic circulation reaching organs such as the liver, lungs, heart, eyes and central nervous system and skeletal muscles (Ma et al., 2017). Thus, human toxocariasis can present in different clinical forms, including the most common asymptomatic form, as well as visceral *larva migrans* (VLM), ocular *larva migrans* (OLM), covert toxocariasis, and neurotoxocariasis (Auer and Walochnik, 2020).

The treatment of human toxocariasis is based on the use of benzimidazole anthelmintics, which are successfully used in the treatment of intestinal helminthiasis, but have unsatisfactory cure rates in patients with this tissue parasitosis. Furthermore, the efficacy of these anthelmintics has rarely been evaluated in prospective controlled clinical trials, including albendazole, which is the first-choice drug for the treatment of this parasitosis (Magnaval et al., 2022). Among the main difficulties in the treatment of this parasitosis, poor absorption by the gastrointestinal tract stands out due to the low solubility and low bioavailability of these anthelmintics (Rostami et al., 2019).

All these factors point to the need for research and development of new drugs. Studies have shown that heterocyclic compounds containing nitrogen radicals are capable of inducing oxidative stress in pathogens, both through their own production of free radicals that are toxic to pathogenic microorganisms, and through the inhibition of enzymes released by the pathogen that are involved in the process of neutralization of these radicals (Hall et al., 2011). The biological activity of nitrogen compounds is directly related to the presence of the nitro group in the molecule and the changes in molecular stability caused by its interaction with the target, due to its high electron acceptor capacity, which reduces its nitro group and triggers its bioactivity (Yan et al., 2005).

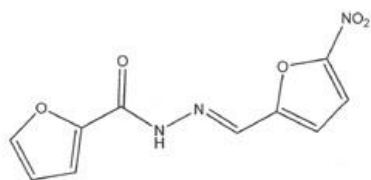
Several studies *in vitro* with nitro compounds have shown promising results in terms of bioactivity against different pathogens, such as *Staphylococcus aureus* (Masunari e Tavares, 2007), *Trypanosoma cruzi* (Maya et al., 2006), *Entamoeba histolytica*, *Trichomonas vaginalis* (Tenório et al., 2005) and *Giardia muris* (Mahfouz et al., 1998). The aim of this study was to evaluate the efficacy of the compound (*E*)-*N'*-((5-nitrofur-2-yl)methylene)furan-2-carbohydrazide through *in vitro* assays, cytotoxicity, *in silico* bioavailability and *in vivo* assays using experimentally infected mice.

Materials and Methods

Nitrofur compound

The nitrofur (*E*)-*N'*-((5-nitrofur-2-yl)methylene)furan-2-carbohydrazide (PFUR) was prepared by reaction between furan-2-ylcarbohydrazide (0.2 g, 1.4 mmol) and 5-nitrofur-2-carbaldehyde (1.6 mmol) in ethanol (Table 1). After 72 hours, the solvent was concentrated under reduced pressure and the residue was purified by washing with cold diethyl ether, resulting in the pure derivative as solids in 84% yield (Cardoso et al., 2016). This compound was produced by the Institute of Pharmaceutical Technology (Farmanguinhos) of the Oswaldo Cruz Foundation (Fiocruz), and available as lyophilized sample at the New Drug Development Center of the Federal University of Rio Grande. The synthesized compound was solubilized at 2.5% in dimethyl sulfoxide (DMSO) and sterile distilled water to obtain a concentration of 1 mg/mL (Mata-Santos et al., 2015).

Table 1. Chemical structure of synthetic nitrofurans evaluated in preclinical assays against visceral toxocariasis (Cardoso et al., 2016).

Compound(Code)	Molecular structure	IUPAC Name
PFUR		(E)-N'-((5-nitrofuran-2-yl)methylene)furan-2-carbohydrazide

Recovery of adults, eggs and larvae of Toxocara canis

Eggs were collected from the uterine tubes of adult *T. canis* and incubated for 30 days under 28 °C of temperature, oxygenation and 90% relative humidity (Avila et al., 2013). The larvae were extracted by using a modified Baermann apparatus and maintained in sterile tubes for cultivation with RPMI-1640 medium (Gibco®), supplemented with 25 mM HEPES (Sigma®), 1% glucose, PSF antibiotic-antimycotic (Gibco®) at 37 °C with an atmosphere of 5% CO₂ (Savigny, 1975).

In vitro tests

The cytotoxicity assay was performed at concentrations of 1 to 0.05 mg/mL to determine the concentration of compound able to maintain 50% of the cell viability (IC₅₀). The cell viability was measured by fluorescence at a wavelength of 620nm using a BioTek™ ELx800™ reader, after incubation of murine macrophages J774.A1 (1.105 cells/100µl), in DMEM medium supplemented with 10% fetal bovine serum, in triplicate, for 24 h at 37 °C with a 5% CO₂ atmosphere, and addition of cell viability indicator resazurin (Ansar Ahmed et al., 1994).

To evaluate the larvicidal activity of the compound, an *in vitro* test was performed in duplicate with an initial concentration of 1 mg/mL that was serially diluted to obtain the concentrations of 0.5 mg/mL, 0.25 mg/mL, 0.125 mg/mL and 0.05 mg/mL. For this test we used 100 *T. canis* larvae in a microculture plate and three controls, one with live larvae, one dead larvae (submitted to thermal shock) and one DMSO 2.5%. After the incubation at 37 °C with 5% CO₂, the viability indicator Trypan blue was added and the material was read by optical microscopy (10x objective), according to their motility and

impregnation by Trypan blue. Thus, the minimum larvicidal concentration (MLC) of the compound was determined (Mata-Santos et al., 2015).

To confirm the non-viability of larvae exposed to the compound PFUR in MLC, the contents of the microculture plate containing 100 larvae were inoculated intraperitoneally (IP) into two female swiss mice (Mata-Santos et al., 2015). As a positive control, 100 live larvae not exposed to the compound were also inoculated into two mice, via IP. Forty-eight hours after inoculation, the mice were euthanized and the carcasses, liver, lungs, heart, kidneys, brain and eyeballs were digested with a solution of 1% hydrochloric acid and 1% pepsin. The tissues were incubated for 24 h at 37 °C (Xi and Jin, 1998) and the larvicidal activity was evaluated by microscopy (10x objective) for the presence or absence of *T. canis* larvae.

In silico bioavailability

We used the Chem Bio Draw Ultra 12.1 editor to draw the molecule of the compound PFUR and the anthelmintic albendazole (Halford 2014). The Swiss ADME computer model (www.swissadme.ch) was used to predict absorption, distribution, metabolism and excretion parameters (Daina et al., 2017). For this analysis we used the four descriptors of the "rule of five" by Lipinski (2004) which accept equal to or less than multiples of five values: molecular weight ≤ 500 ; $\text{miLogP} \leq 5$; number of hydrogen donors ≤ 5 ; number of hydrogen acceptors ≤ 10 (Lipinski, 2004).

Efficacy on murine toxocariasis

Two experiments were conducted to evaluate the efficacy of the PFUR compound in mice 10 days and 30 days after inoculation of 500 embryonated *T. canis* eggs by the intragastric (IG) route (Satou et al., 2005). For this, in both experiments, groups of female Swiss mice of 5 and 7 weeks of age were formed. The mice at 10 days post-inoculation and 30 days post-inoculation of *T. canis* eggs were administered with the compound PFUR (10mg/kg, IG), albendazole - ABZ (40mg/kg, IG) and phosphate buffered saline solution - PBS 1%, 0.15 M, pH 7.2, every 24 hours, for 5 consecutive days (Mata-Santos et al., 2015).

Five days after administration of the compound and controls, the mice were euthanized to perform digestion of organ tissues (livers, lungs, hearts, kidneys, brains

and eyeballs) and carcasses in a solution of 1% hydrochloric acid and 1% pepsin (Xi and Jin, 1998). To evaluate the efficacy of the compound, we quantified the larvae found by microscopy (10x objective). This study was approved by the Research Ethics Committee of the Federal University of Rio Grande (P034/2020).

Statistical analysis

Statistical analysis was performed by ANOVA with Tukey's post-hoc test, considering a significance level of $p \leq 0.05$.

Results

The compound (*E*)-*N'*-((5-nitrofuran-2-yl)methylene)furan-2-carbohydrazide (PFUR) showed activity against 100% of *T. canis* larvae at concentrations of 1 mg/mL, 0.5 mg/mL and 0.25 mg/mL, its minimum larvicidal concentration (MLC). At the other concentrations, the compound showed activity of 86.5% (0.125 mg/mL) and 25% (0.05 mg/mL). The confirmatory test showed that larvae exposed to PFUR *in vitro* were unable to infect mice and were therefore not viable. However, an average of nine larvae were detected in the liver and lungs of control mice.

In the cytotoxicity test, PFUR showed no cytotoxicity against murine macrophages (strain J774.A1), with 100% cell viability at 0.25 mg/mL. Following this test, *in silico* evaluation was performed and the results are presented in Table 2, showing oral bioavailability characteristics and indicating acceptable solubility in aqueous media and gastrointestinal absorption. The physicochemical properties showed that PFUR did not violate Lipinski's (2004) "rule of five" and showed similar results to ABZ.

Table 2. Physicochemical properties of synthetic nitrofuran PFUR and albendazole.

Parameters	PFUR	ABZ
Molecular weight (g/mol)	249,18	265,33
miLogP	1,7	1,62
Hydrogen Donors	1	2
Hydrogen Acceptors	6	3

*Molecular weight ≤ 500 g/mol; miLogP ≤ 5 ; Hydrogen Acceptors ≤ 10 ; Hydrogen Donors ≤ 5 .

In mice treated with PFUR 10 days and 30 days after inoculation of *T. canis* eggs (Table 3), there was a reduction of 23% ($p>0.05$) and 62.4% ($p<0.05$) in the intensity of infection, respectively, compared to the PBS control. In both experiments, the compound did not show results superior to the ABZ control ($p>0.05$).

Table 3. Average recovery of the larvae per group 10 and 30 days post-inoculation with 500 eggs of *Toxocara canis* in Swiss mice.

Group (n)	Identification	Average larvae recovery*	Average reduction**
10 days			
(n=5)	(<i>E</i>)- <i>N'</i> -((5-nitrofuran-2-yl) methylene) furan-2-carbohydrazide (PFUR)	10.8 ^{AB}	23%
	Control ABZ	4,4 ^A	68,6%
	Control PBS	14 ^B	-
30 days			
(n=10)	(<i>E</i>)- <i>N'</i> -((5-nitrofuran-2-yl) methylene) furan-2-carbohydrazide (PFUR)	7.9 ^A	62.4%
	Control ABZ	10,6 ^A	49,6%
	Control PBS	21 ^B	-

*Different capital letters between the lines showed a significant difference ($p<0.05$).

** Average reduction on infection intensity compared to Control PBS.

Discussion

Despite major health advances in recent decades, toxocariasis remains a global public health problem (Chen et al., 2018). Still a few options for anthelmintics are available, and many of the drugs were originally intended for veterinary use (Magnaval et al., 2022). In this study we evaluated the antiparasitic activity and the efficacy of a nitrofurantoin against the nematode *T. canis* in murine visceral toxocariasis. Our *in vivo* tests revealed the efficacy of treatment only with the nitrofurantoin PFUR in mice with 30 days of infection by *T. canis*, since there was a significant reduction in the intensity of infection in the group treated with the PFUR compound when compared to the PBS control ($p<0.05$). This result is relevant since human toxocariasis is characterized as a chronic parasitic disease.

Furthermore, the compound presented similar results to the ABZ control in mice with 10 and 30 days of infection ($p>0.05$), and ABZ is the drug of choice for the treatment of visceral toxocariasis due to its availability, acceptable efficacy and absence of important side effects (Chen et al., 2018). When we analyzed the dosages used for the groups treated with PFUR and ABZ, we noticed that despite presenting similar results in the quantification of larval recovery, the group treated with PFUR received daily doses of 10 mg/kg, four times lower than the dose usually recommended for ABZ (40 mg/kg). This result shows us that this nitrofurantoin outperformed the group treated with the anthelmintic albendazole, which indicates its high anthelmintic activity against *T. canis*.

This group of compounds, with heterocyclic parts conjugated with nitrogen, have already been tested and their activity has been confirmed against *Entamoeba histolytica*, *Giardia intestinalis* trophozoites (Saadeh et al., 2009), *Trypanosoma cruzi* epimastigotes and amastigotes (Maya et al., 2006), *Leishmania infantum* promastigotes and amastigotes (Petri e Silva et al., 2016), *Mycobacterium tuberculosis* (Elsaman et al., 2019) and others. Our *in vitro* tests showed the high larvicidal activity of this nitrofurantoin on *T. canis* larvae, capable of completely inactivating them, a result that was confirmed by inoculating these larvae into mice, which did not become infected. Although there have been reports of side effects with nitrogen-based drugs, the current success of nitro-decorated heterocyclic compounds is due to careful optimization of the molecules synthesized, leading to a reduction in cytotoxicity and an improvement in their efficacy against pathogens (Gallardo-Garrido et al., 2020). In the cytotoxicity test performed for PFUR, we obtained as result no cytotoxicity against murine macrophages (strain J774. A1), with 100% of cell viability at 0.25 mg/mL, its minimum larvicidal concentration.

In our study, nitrofurantoin PFUR also showed acceptable oral bioavailability and satisfactory gastrointestinal solubility and absorption in the *in silico* assay. This compound does not violate Lipinski's (2004) "rule of five" and two parameters stood out in this analysis: molecular weight and miLogP, a descriptor of lipid solubility. Molecular weight not only influences the transport capacity of molecules, but can also decrease solubility and reduce the concentration of the compound present in the intestinal epithelium, thus interfering with gastrointestinal absorption (Rodrigues et al., 2020). MiLogP gives us information about lipid solubility. A moderately lipid-soluble

compound is better absorbed, therefore, good absorption is more likely when the values of this coefficient remain between 1 and 3, since a hydrophilic-lipophilic balance is necessary for absorption (Di and Kerns, 2016). In our study, we obtained a miLogP similar to the anthelmintic albendazole, which allows us to estimate an adequate hydrophilic-lipophilic balance. Albendazole is recommended to be administered with a high-fat diet due to its poor gastrointestinal absorption, which contributes to its low bioavailability (Magnaval et al., 2022), consequently, the parameters similar to ABZ found in our analyses for PFUR show that its oral administration may also be favored by a high-fat diet, which favors the absorption and bioavailability of the compound due to its lipophilicity.

The results of this research also led to the filing of a patent for the use of this compound as an anti-parasitic. The patent has been filed with the National Institute of Industrial Property (INPI) under the number BR 10 2024 007546 3. All of these results, together with the patent, reinforce the therapeutic benefit of this nitrofurantoin and demonstrate the potential of this compound in the production of a new antiparasitic drug candidate for therapy in the treatment of experimental toxocariasis.

Declarations

According to Brazilian law no 11.794/2008 (Arouca law), article 2° applies to animals of species classified as phylum Chordata, subphylum Vertebrata, subject to environmental legislation. The Federal University of Rio Grande is accredited by the Commission for Ethics in the Use of Animals (CEUA) in accordance with the provisions of article 8° of the Arouca law. This study was approved by the Ethics Committee in Research of the Federal University of Rio Grande (P034/2020).

Informed consent

Not applicable.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Data availability

Invention patent titled ‘Use of synthetic nitrofurans as anthelmintics’ owned by the Federal University of Rio Grande, deposited on April 17, 2024, at the National Institute of Industrial Property – INPI, under the number BR 10 2024 007546 3.

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7. Manuscrito 2

***Toxocara canis*: larvicidal activity and bioavailability analysis of 2-pyrimidinyl-hydrazone derivatives**

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Abstract

The treatment of human toxocariasis, a parasitic zoonosis with global distribution, is based on the use of benzimidazole anthelmintic, which have limited efficacy against *Toxocara* spp. larvae at the tissue level. Therefore, innovation and research in to candidates for the development of new drugs for toxocariasis are essential. This study aimed to assess the efficacy of pyrimidine compounds against *Toxocara canis* larvae, as well as their cytotoxicity and bioavailability. The screening test was performed at 1 mg/mL, in duplicate, in a microplate containing 100 *T. canis* larvae in RPMI-1640 medium, and the active compound was then tested at 0.5 to 0.05 mg/mL to determine the minimum larvicidal concentration. The compound, (*E*)-2-(2-((5-nitrofuran-2-yl)methylene)hydrazinyl)pyrimidine (PNAH 8), showed larvicidal activity at 1 mg/mL and 0.5 mg/mL and demonstrated non-cytotoxicity at any tested concentration. *In silico* analysis, employing computational models, indicated that the compound adhered to the “rule of five” descriptors. To confirm the non-viability of the larvae, the tested microplate wells were intraperitoneally inoculated into female Swiss mice, in duplicate,

which confirmed the larvicidal activity of this compound. Furthermore, this compound did not show cytotoxicity and demonstrated adequate bioavailability *in silico*, comparable to albendazole, the first-choice anthelmintic for the treatment of human toxocariasis. These results suggest the potential of this compound for future investigations in preclinical testing.

KEYWORDS: Toxocariasis; Synthetic Pyrimidine; Anthelmintic;

1. Introduction

Human toxocariasis is important neglected zoonosis with global distribution (Chen et al., 2018), that is characterized by the migration and persistence of larvae in organs such as the liver, lungs, heart, eyes and central nervous system and skeletal muscles (Ma et al., 2017). The main etiological agents of this parasitosis is the nematode *Toxocara canis*, an intestinal parasite of dogs (Overgaauw and Knapen, 2013). The nematode modulates the host's immune response, which reduces the inflammatory process and ensures its survival for prolonged periods (Hamilton et al., 2008). This parasitosis present in different clinical forms, including asymptomatic form, visceral, ocular, covert and neurological toxocariasis (Auer and Walochnik, 2020).

Many human intestinal helminthiases are treated with benzimidazoles, such as albendazole and mebendazole. These drugs target the microtubules of adult parasites, inhibiting glucose uptake and reducing the production of adenosine triphosphate (ATP), which is essential for parasite survival (Laxmikeshav et al., 2022). However, their effectiveness in treating human toxocariasis is often limited (Magnaval et al., 2022) due to their low water solubility and poor gastrointestinal absorption. Furthermore, these drugs undergo first-pass metabolism in the liver, which restricts their ability to reach host tissues and effectively act on the larvae (Ma et al., 2017).

Therefore, innovation and research into candidates for the development of new drugs for toxocariasis are essential. In this context, pyrimidine derivatives stand out as highly promising structures in the development of new drugs due to their chemical versatility and selective interaction with molecular targets essential to the metabolism of pathogens. Studies have also demonstrated the activity of pyrimidines against *Leishmania infantum*, promastigote and amastigote forms (Lopes et al., 2022), *Trypanosoma cruzi*, epimastigote and trypomastigote forms (De Melo et al., 2018),

Staphylococcus aureus, *Bacillus subtilis* and *Pseudomonas aeruginosa* (Do Monte et al., 2018) and Rubella virus (Saladino et al., 2002).

It should be pointed out that drug bioprospecting should not be limited to bioactivity studies but should also include the understanding of pharmacokinetic properties, such as bioavailability and routes of administration (Beugnet and Franc, 2012). In this context, the aim of this study was to evaluate the activity of 2-pyrimidinylhydrazone derivatives against *T. canis* larvae and their cytotoxicity *in vitro* as well as their bioavailability *in silico*.

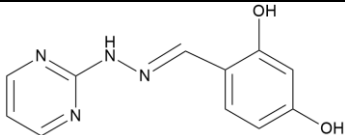
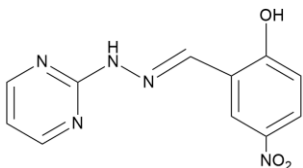
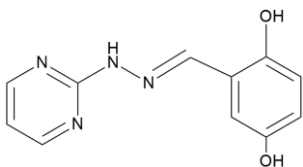
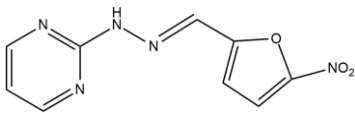
2. Material and methods

2-pyrimidinyl-hydrazone derivatives

Four synthetic compounds derived from 2-pyrimidinyl-hydrazone were produced by the Institute of Technology in Pharmaceuticals (Farmanguinhos) of the Oswaldo Cruz Foundation (Fiocruz) (Table 1). The synthesis of the 2-pyrimidinyl-hydrazone derivatives was carried out by the reaction between 2-hydrazinylpyrimidine (0.04 g, 0.36 mmol) and the corresponding aromatic or heteroaromatic aldehyde (1.0 eq., 0.36 mmol) in ethanol. After rotary evaporation, the product was purified with ethanol and diethyl ether, resulting in the pure derivatives as solids with yields ranging from 27 to 75% (Pinheiro et al., 2020).

These compounds were available as lyophilized samples at the Center for New Drug Development at the Federal University of Rio Grande. All compounds were solubilized at 2.5% in dimethyl sulfoxide (DMSO) and sterile distilled water to obtain a concentration of 1 mg/mL (Mata-Santos et al., 2015).

Table 1. Chemical structure of four 2-pyrimidinylhydrazone derivatives evaluated in larvicidal activity tests against *Toxocara canis* and in cytotoxicity assay and *in silico* bioavailability test (Pinheiro et al., 2020).

Code	Chemical structure	IUPAC Name
PNAH 4		(E)-4-((2-(pyrimidin-2-yl)hydrazono)methyl)benzene-1,3-diol
PNAH 5		(E)-4-nitro-2-((2-(pyrimidin-2-yl)hydrazono)methyl)phenol
PNAH 6		(E)-2-((2-(pyrimidin-2-yl)hydrazono)methyl)benzene-1,4-diol
PNAH 8		(E)-2-(2-((5-nitrofuran-2-yl)methylene)hydrazinyl)pyrimidine

Parasite and in vitro tests

Toxocara canis larvae were extracted from embryonated eggs using a modified Baermann apparatus and maintained in sterile tubes for cultivation with RPMI-1640 medium (Gibco®), supplemented with 25 mM HEPES (Sigma®), 1% glucose, PSF antibiotic-antimycotic (Gibco®) at 37°C with an atmosphere of 5% CO₂ (Savigny, 1975).

An *in vitro* screening test was performed, in duplicate, in a 96-well flat bottom plate with 100 *T. canis* larvae and the compounds at an initial concentration of 1 mg/mL. Three controls were used: one with live larvae, one with dead larvae (by thermal shock) and one with DMSO 2,5%. After 48 h of incubation at 37°C with 5% CO₂, the larvae were evaluated by optical microscopy (10x objective) for mobility and impregnation by Trypan blue. The compounds with 100% of larvicidal activity were subjected to a minimal larvicidal concentration (MLC), test where they were serially diluted to obtain concentrations ranging from 0.5 mg/mL to 0.05 mg/mL (Mata-Santos et al., 2015).

The compound with the lower MLC (0.5 mg/mL) was tested for cytotoxicity to determine the concentration capable of maintaining 50% of cell viability (IC 50). The test was performed at the same concentrations as the screening, using murine macrophages J774.A1 (1.10^5 cells/100 μ l), placed in DMEM medium supplemented with 10% fetal bovine serum. After 24 hours of incubation at 37°C in a 5% CO₂ atmosphere, 0.01% resazurin was added. After 24 hours, the cell viability was measured by fluorescence at a 620 nm wavelength using a BioTek™ ELx800™ reader (Ansar Ahmed et al., 1994).

To confirm larval non-viability, the contents of microplate wells of the compound at MLC were inoculated intraperitoneally (IP) into female Swiss mice, in duplicate, according to the methodology of Mata-Santos et al. (2016). Forty-eight hours post-inoculation, the carcasses, brains, livers, lungs, kidneys and hearts were digested with a solution of 1% hydrochloric acid and 1% pepsin (Xi and Jin, 1998), and an optical microscopy (10x objective) was used to analyze the larvae. The statistical analysis of the confirmatory test was qualitative, and the detection of at least one *T. canis* larva after tissue digestion was considered positive. This study was approved by the Research Ethics Committee of the Federal University of Rio Grande (P034/2020).

In silico bioavailability

The selected compound and the albendazole were drawn using the molecule editor Chem Bio Draw Ultra 12.1 (Halford, 2014), and the analysis was performed using the Swiss ADME computer model (www.swissadme.ch) to predict absorption, distribution, metabolism and excretion parameters (Daina et al., 2017). The analysis was performed according to the four parameters of the Lipinski (2004) "rule of five", where the accepted reference values, equal to or less than multiples of five, are: molecular weight ≤ 500 g/mol, $\text{miLogP} \leq 5$, number of hydrogen donors ≤ 5 and hydrogen acceptors ≤ 10 .

3. Results

In the *in vitro* screening tests, one compound, (*E*)-2-(2-((5-nitrofuranyl)methylene)hydrazinyl)pyrimidine (PNAH 8), showed 100% of larvicidal activity against *T. canis* at a concentration of 1 mg/mL (Table 2). This compound was subjected to an MLC test and showed 100% of activity at 0.5mg/mL. In the confirmatory test, the

larvae exposed to PNAH 8 were not viable and did not have the ability to infect mice. This result corroborated with the controls, in which an average of nine larvae were found in the liver and lung tissues.

Table 2. Effects of the pyrimidine derived compounds (PNAH) against *Toxocara canis* larvae after 48 h of *in vitro* incubation in RPMI-1640 medium, using viable larvae control.

Compound Code	Dead larvae <i>in vitro</i> (%)				
	1 mg/mL	0.5 mg/mL	0.25 mg/mL	0.125 mg/mL	0.05 mg/mL
PNAH 4	28.9	-	-	-	-
PNAH 5	53.2	-	-	-	-
PNAH 6	24.9	-	-	-	-
PNAH 8	100	100	97.6	80	64.1
Control	12.5	9.8	9.8	9.8	9.8

Control: 100 *Toxocara canis* live larvae in RPMI-1640 medium.

(-): concentrations were not tested.

In the cytotoxicity test, PNAH 8 showed 100% of cell viability at all concentrations, demonstrating that it is non-cytotoxic. *In silico* results showed that PNAH 8 did not violate Lipinski's (2004) "rule of five" parameters, has acceptable water solubility and gastrointestinal absorption and shows similar results to the albendazole (ABZ), which may indicate a higher water-solubility of this compound compare to albendazole (Table 3).

Table 3. Physicochemical properties of pyrimidine derived PNAH 8 and albendazole.

Compound	Molecular weight (g/mol)	miLogP	Hydrogen donors	Hydrogen acceptors
ABZ	265.33	1.62	2	3
PNAH 8	233.18	0.6	1	6

4. Discussion

In this study, the compound (*E*)-2-(2-((5-nitrofuran-2-yl)methylene)hydrazinyl)pyrimidine (PNAH 8) showed significant larvicidal activity against *T. canis* larvae. In addition to 100% activity at concentrations of 1 and 0.5 mg/mL, this pyrimidine derivative also showed high larvicidal activity at 0.25 mg/mL

and 0.125, with results of 97.6 and 80% efficacy, respectively. Heterocyclic compounds containing the pyrimidine nucleus are of great interest due to their significant role as both natural and synthetic compounds, exhibiting diverse and valuable biological activities, making them attractive candidates for clinical translation as therapeutic agents in the treatment of various diseases (Nadar and Khan, 2022). Pyrimidine, as an essential component of both DNA and RNA, possesses a wide range of pharmacological properties. Its significant biological potential extends to various drug therapies, including anticancer, antiviral, antimicrobial, anti-inflammatory, analgesic, antioxidant, and antimalarial treatments. As a major endogenous component of the body, pyrimidine derivatives readily interact with enzymes, genetic material, and cellular bio-components (Zhuang and Ma, 2020).

Pyrimidine analogs can be considered prodrugs. However, like their natural counterparts, they must be activated within the cell, which involves a series of metabolic steps, including the sequential phosphorylation of the pyrimidine analogue to its monophosphate, diphosphate and triphosphate forms. Ultimately, these intracellularly phosphorylated metabolites, known as nucleotides, mediate the pharmacological effects of the pyrimidine analogue (Derissen and Beijnen, 2020). As a critical component of nucleosides, pyrimidine readily interacts with biopolymers, increasing activity and decreasing the chance of cytotoxicity (Zhuang and Ma, 2020). Our tests showed that PNAH 8 is non-cytotoxic in its MCL, and analyzing the optical densities of the microplate, we estimated the cell viability of this compound at different concentrations and above 2 mg/mL, the highest concentration tested, the estimated cell viability was 79%.

In the *in silico* evaluation, PNAH 8 had a lower miLogP value than albendazole, which can indicate a high water-solubility that are expected for these compounds, which is an advantage over the conventional anthelmintic. Due to their basic nitrogen heterocyclic ring, pyrimidines are highly soluble in water, and their aqueous solution is weakly basic, which leads to a low risk of nephrotoxicity, making them safer options for treating patients with kidney failure (Patil, 2018). The low water solubility observed for the conventional anthelmintics appears to be directly related to their low bioavailability and their ability to act against the target (Magnaval et al., 2022).

Over time, anthelmintic resistance has evolved and now represents a significant economic challenge to animal industries and a threat to the effective control of human parasites (Jayawardene et al., 2021). Despite the efforts of scientists to combat this problem, it is becoming apparent that the most effective approach is to expand clinical treatment options through the discovery of new chemical entities, particularly compounds with novel mechanisms. Pyrimidines and their analogues are well-known biologically active nitrogen-containing heterocyclic rings, widely used in antibacterial, anti-inflammatory, and antitumor drug applications. Due to their good biocompatibility, pyrimidine structures are often associated with known antibacterial compounds (Zhuang and Ma, 2020). These factors strongly suggest that pyrimidine derivatives have the potential to combat other infections, including those caused by parasites, which has been confirmed by our study. The results of this research have also led to a patent for the use of this compound as an antiparasitic, which has been filed with the National Institute of Industrial Property (INPI) under the number BR 10 2024 007541 2, demonstrating the potential of this compound as a new antiparasitic drug candidate for use against visceral toxocariasis, which needs to be elucidated by further preclinical testing.

5. Conclusion

The results obtained with PNAH 8 showed its larvicidal activity against *T. canis* larvae, absence of cytotoxicity and adequate *in silico* bioavailability. Based on this study it is now feasible to conduct *in vivo* preclinical trials, using murine models.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Data availability

Invention patent titled ‘Use of synthetic compound derived from pyrimidine as an anthelmintic’ owned by the Federal University of Rio Grande, deposited on April 17, 2024, at the National Institute of Industrial Property – INPI, under number BR 10 2024 007541 2.

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8. CONCLUSÕES

A importante atividade larvicida do derivado da cânfora C2 e do derivado de nitrofurano PFUR foi comprovada *in vivo* ao reduzir significativamente a intensidade de infecção, aliada a ausência de citotoxicidade, a disponibilidade oral e absorção gastrointestinal equivalentes ao albendazol, tornam esses compostos promissores como candidatos para o desenvolvimento de novos anti-helmínticos para tratamento da toxocaríase humana.

A atividade larvicida *in vitro* do derivado de pirimidina PNAH 8, associada a ausência de citotoxicidade, a biodisponibilidade oral e absorção gastrointestinal adequadas e a alta solubilidade em água, demonstram que esse composto apresenta importante potencial anti-helmíntico.

Todos esses resultados, aliados as patentes depositadas, reforçam o potencial terapêutico desses derivados da cânfora, nitrofurano e pirimidina como candidatos a novos fármacos para o tratamento da toxocaríase visceral.

9. CONSIDERAÇÕES FINAIS

Os resultados obtidos neste estudo demonstram a eficácia de três compostos, evidenciando seu potencial como agentes antiparasitários, o que motivou o depósito de patentes para o uso desses compostos, consolidando o interesse do grupo em dar continuidade às pesquisas. Os resultados fortalecem a viabilidade de desenvolvimento desses compostos como potenciais candidatos a fármacos antiparasitários, abrindo caminho para futuras investigações pré-clínicas e, possivelmente, para a produção de tratamentos inovadores no controle às parasitoses.

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12. ANEXOS

Anexo 1

Carta de aceite “Journal of Helminthology”

Dear Ms Rodrigues,

Re: Manuscript number: JOH-D-24-00169R1

Title: Synthetic camphor derivative (*E*)-2-((1,7,7-trimethylbicyclo[2.2.1]heptan-2-ylidene)amino)phenol: a novel anthelmintic drug candidate for visceral toxocariasis

I am pleased to inform you that your submission has now been accepted for publication in the Journal of Helminthology. The Deputy Editor has read your revised manuscript and is satisfied that you have addressed his comments/the points raised by the reviewers. I am therefore pleased to accept your paper for publication.

It was accepted on 13 Jan 2025.

Our Production Team will contact you once the proofs of your article are ready for approval. For further information about your article in the Production stages please contact jhlproduction@cambridge.org.

Thank you for submitting your work to the Journal of Helminthology.

Before Cambridge University Press can publish your manuscript, we need a signed licence from you. You will shortly hear from our digital licensing partner Ironclad in order to complete your licensing agreement for publishing this article. Please ensure you check all email folders including your junk and spam folders for receipt.

With kind regards,

Sharon Ryan

Editorial Office

Journal of Helminthology

Anexo 2

Depósito de Patente “Utilização de composto sintético derivado da cânfora como anti-helmíntico”



SERVIÇO PÚBLICO FEDERAL
MINISTÉRIO DO DESENVOLVIMENTO, INDÚSTRIA, COMÉRCIO E SERVIÇOS
INSTITUTO NACIONAL DA PROPRIEDADE INDUSTRIAL

EXAME PRELIMINAR

N.º do Pedido: BR102024007530-7 N.º de Depósito PCT:
Data de Depósito: 17/04/2024

O pedido atende formalmente as disposições legais, especialmente quanto ao Art. 19 da LPI e o Instrução Normativa nº 31/2013, estando apto a ser protocolado.

Condições do Pedido	S	N
Requerimento de depósito com os campos obrigatórios preenchidos	X	
Idioma Português	X	
Relatório Descritivo	X	
Reivindicações	X	
PI e C – Apresenta desenhos citados ou não cita nem apresenta desenhos. MU – Apresenta desenhos.	X	
Resumo	X	
Formatado no padrão exigido	X	
Valor correto de Recolhimento	X	

Rio de Janeiro, 19 de julho de 2024.

Andrea Massad Fonseca Barbosa
Mat. Nº 1466814
DIRPA / COSAP/SEFOR

Anexo 3

Depósito de Patente “Utilização de composto sintético derivado da pirimidina como anti-helmíntico”



SERVIÇO PÚBLICO FEDERAL
MINISTÉRIO DO DESENVOLVIMENTO, INDÚSTRIA, COMÉRCIO E SERVIÇOS
INSTITUTO NACIONAL DA PROPRIEDADE INDUSTRIAL

EXAME PRELIMINAR

N.º do Pedido: BR102024007541-2 **N.º de Depósito PCT:**
Data de Depósito: 17/04/2024

O pedido atende formalmente as disposições legais, especialmente quanto ao Art. 19 da LPI e o Instrução Normativa nº 31/2013, estando apto a ser protocolado.

Condições do Pedido	S	N
Requerimento de depósito com os campos obrigatórios preenchidos	X	
Idioma Português	X	
Relatório Descritivo	X	
Reivindicações	X	
PI e C – Apresenta desenhos citados ou não cita nem apresenta desenhos. MU – Apresenta desenhos.	X	
Resumo	X	
Formatado no padrão exigido	X	
Valor correto de Recolhimento	X	

Rio de Janeiro, 17 de julho de 2024.

Aline Aimée Carneiro de Oliveira
Mat. Nº 1326227
DIRPA / COSAP/SEFOR

Anexo 4

Depósito de Patente “Utilização de composto sintético nitrofurano como anti-helmíntico”



SERVIÇO PÚBLICO FEDERAL
MINISTÉRIO DO DESENVOLVIMENTO, INDÚSTRIA, COMÉRCIO E SERVIÇOS
INSTITUTO NACIONAL DA PROPRIEDADE INDUSTRIAL

EXAME PRELIMINAR

N.º do Pedido: BR102024007546-3 **N.º de Depósito PCT:**
Data de Depósito: 17/04/2024

O pedido atende formalmente as disposições legais, especialmente quanto ao Art. 19 da LPI e o Instrução Normativa nº 31/2013, estando apto a ser protocolado.

Condições do Pedido	S	N
Requerimento de depósito com os campos obrigatórios preenchidos	X	
Idioma Português	X	
Relatório Descritivo	X	
Reivindicações	X	
PI e C – Apresenta desenhos citados ou não cita nem apresenta desenhos. MU – Apresenta desenhos.	X	
Resumo	X	
Formatado no padrão exigido	X	
Valor correto de Recolhimento	X	

Rio de Janeiro, 17 de julho de 2024.

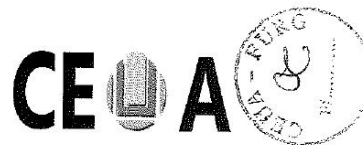
Aline Aimée Carneiro de Oliveira
Mat. Nº 1326227
DIRPA / COSAP/SEFOR

Anexo 5

Certificado de aprovação da Comissão de Ética em Uso Animal

COMISSÃO DE ÉTICA EM USO ANIMAL

Universidade Federal do Rio Grande
Pró-Reitoria de Pesquisa e Pós-Graduação - PROPESP
ceua@furg.br http://www.propesp.furg.br



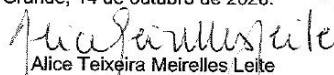
CERTIFICADO Nº P034/2020

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	Livia 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