UNIVERSIDADE FEDERAL DE PERNAMBUCO
CENTRO DE CIÊNCIAS DA SAÚDE
PROGRAMA DE PÓS-GRADUAÇÃO EM CIÊNCIAS FARMACÊUTICAS

"Avaliação biológica de novos ftalil-tiazóis com Potencial atividade contra *Schistosoma mansoni*"

EDNA DE FARIAS SANTIAGO

RECIFE – 2014

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Dedico este trabalho:

Aos meus pais

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“Tudo eu posso Naquele que me fortalece”

Filipenses 4,13

RESUMO

A esquistossomose mansônica é considerada um grave problema de saúde pública, afetando não só o bem estar social do indivíduo como a economia do país. Acredita-se que existam no Brasil no mínimo 2,5 milhões de portadores de esquistossomose mansoni e cerca de 25 milhões de indivíduos expostos aos riscos de contraí-la. O tratamento da esquistossomose é realizado basicamente com uma única droga, o praziquantel, e atualmente, a diminuição da susceptibilidade a este medicamento tem sido relatado em vários estudos. Diante desse novo panorama e da gravidade da doença, faz-se necessário estudo de novas moléculas com atividade esquistossomicida. Dentro deste contexto, o grupo de pesquisa do Laboratório de Planejameto em Química Medicial (LPQM/UFPE) tem sintetizado novas moléculas candidatas a agentes esquistossomicidas, obtidas apartir da ligação de grupos farmacofóricos como as ftalimidas, tiossemicarbazonas e seus bioisósteros cíclicos tiazois e tiazolidinonas, que apresentam um amplo espectro de atividades biológicas. Este estudo tem como objetivo a avaliação da atividade esquistossomicida de uma série de derivados das tiossemicarbazonas: fenoxi-tiossemicarbazonas (LPQM -01, LPQM-02 e LPQM-03), fenil-tiazóis (LPQM-14 e LPQM-17), ftalil-tiossemicarbazona (LPQM-38), ftalil-tiazóis (LPQM-43, LPQM-45 e LPQM-47) e ftalil-tiazolidinona (LPQM-40). Como controle utilizou-se o praziquantel (PZQ), o qual foi avaliado nas mesmas condições que as amostras. Os resultados revelaram que as séries derivadas dos compostos heterocíclos tiazoís apresentaram uma melhor atividade em relação às tiossemicarbazonas, demonstrando potencial terapêutico para o tratamento da esquistossomose mansônica. Dentre os tiazóis, a molécula LPQM-45 se destacou, levando a uma maior taxa de mortalidade em menor tempo e ocasionando alterações como descamação e formação de bolhas no tegumento do verme. Diante destes resultados compostos derivados do LPQM-45 (LPQM-39, LPQM-48, LPQM-37 e LPQM-PM02) foram testados revelando uma atividade esquistossomicida mais acentuada e evidenciando que os compostos heterocíclicos tiazóis podem atuar como protótipo a um novo fármaco esquistossomicida.

Palavras-chaves: Esquistossomose. Moléculas. Heterocíclicos.

ABSTRACT

Schistosomiasis mansoni is considered a serious public health problem, affecting not only the welfare of the individual as the country's economy. It is believed that in Brazil there are at least 2.5 million people with schistosomiasis and about 25 million people at risk of contracting it. Treatment of schistosomiasis is accomplished primarily with a single drug, praziquantel, and currently, decreased susceptibility to this drug has been reported in several studies. Given this new situation and the severity of the disease, it is necessary to study new molecules with activity schistosomicidal. Within this context, the research group of the Laboratory of planning medicinal Chemistry (LPQM/UFPE) has synthesized new molecules candidates for antischistosomal agents, obtained from the connection farmacoforics groups as phthalimides, thiosemicarbazones and their cyclic bioisósteros thiazoles and thiazolidinone, which present a broad spectrum of biological activities. This study aims to evaluate a series of derivatives of thiosemicarbazones: phenoxy-thiosemicarbazones (LPQM-01, LPQM-02 and LPQM-03), phenyl-thiazoles (LPQM-14 and LPQM-17), ftalil-thiosemicarbazone (LPQM-38), phthalil -thiazoles (LPQM-43, LPQM-45 and LPQM-47) and phthalil-thiazolidinone (LPQM-40). The Praziquantel (PZQ), used as control, was evaluated under the same conditions as samples. The results showed that the series derived from heterocyclic compounds thiazoles showed a better activity compared to thiosemicarbazones, demonstrating therapeutic potential for the treatment of schistosomiasis. Among the thiazoles, the LPQM-45 molecule stood out, leading to a higher mortality rate in less time and causing significant changes in the integument of the worm. Given these results, compounds derived from LPQM-45 (LPQM-39, LPQM-48-37 and LPQM LPQM-PM02) were tested and showed improved schistosomicidal activity, demonstrating that the heterocyclic thiazoles exhibit antischistosomal activity and its derivatives can act as prototype for obtaining a new antischistosomal drug.

Keywords: thiosemicarbazones. Thiazoles. ftalil-thiosemicarbazones.

LISTA DE ABREVIATURAS

CP - cisteína protease

DMSO – dimetilsulfóxido

DNA - ácido desoxirribonucleico

GABA - ácido gama-aminobutírico

GR - glutationa redutase

GSH - tripeptídeo glutationa

GSSG - glutationa dissulfido

IL - interleucina

IFN- γ – interferon gama

K11777 - N-metil-piperazina-fenilalanil-homofenilalanil-vinilsulfona fenilvinil sulfona

LPS – lipopolissacarídeo

NADPH - Nicotinamida Adenina Dinucleótido Fosfato reduzida

NK – Células Natural Killer

NO – óxido nítrico

PK- proteína quinase

RTK - proteína tirosina quinase

PZQ – praziquantel

RDR- ribonucleotídeo difosfato redutase

RNA - ácido ribonucleico

RTKS – Receptor Tirosina Quinase

SmPlK- polo-like quinases em *S. mansoni*

SmPNP - purina nucleosídeo fosforilase em *S. mansoni*

SmIR- receptores de insulina *S. mansoni*

TGR - tioredoxina glutationa redutase

TNF-α - fator de necrose tumoral – alfa

Th – Linfócito T auxiliar

Trx - tioredoxina

TrxR - tioredoxina redutase

LISTA DE FIGURAS

Revisão da literatura

Atividade *in vivo* de novos derivados ftalil-tiaazóis contra *S. mansoni* (Apêndice)

Avaliação da atividade esquistossomicida *in vitro* de derivados do protótipo LpQM-45.

LISTA DE TABELAS

Atividade *in vivo* de novos derivados ftalil-tiaazóis contra *S. mansoni.*

Avaliação da atividade esquistossomicida *in vitro* de derivados do protótipo LpQM-45.

SUMÁRIO

1. INTRODUÇÃO

A esquistossomose é uma infecção ocasionada por um trematódeo do gênero *Schistosoma* e ocupa, após a malária, a segunda posição no mundo entre as infecções parasitárias (LESCANO et al., 2004; KEISER et al., 2009). É uma doença crônica prevalente em regiões tropicais e subtropicais, especialmente em comunidades pobres sem acesso a água tratada e saneamento básico estando associada com uma variedade de complicações clínicas que pode levar a morbidade severa (KUNTZ et al., 2007; WHO, 2012).

As três principais espécies de *Schistossoma* que infectam o homem são *S. mansoni*, *S. japonicum* e *S. haematobium* (KEISER et al., 2009, CAFFREY, 2007). A esquistossomose pode se apresentar na forma intestinal e urogenital, sendo forma intestinal ocasionada pelo *S. mansoni* e *S. japonicum* e a forma urogenital ocasionada pelo *S. haematobium* (WHO, 2012).

Na ausência de um controle eficiente do vetor e da falta de saneamento básico adequado, o combate à esquistossomose vem sendo praticamente realizado ao longo dos anos através da quimioterapia. O tratamento da esquistossomose hoje está sendo realizado basicamente com uma única droga, o praziquantel (2 – ciclohexilcarbonil - 1,2,3,6,7,11 b – hexahidro – 4H-pirazino{2,1 -a}isoquinolina – 4 – ona). A mais expressiva alternativa ao praziquantel é a oxamniquine, contudo esta droga apresenta uma comprovada resistência, além de um espectro de ação reduzido, uma produção limitada e de um maior custo efetivo, restringindo a sua potencial utilidade (KUNTZ et al., 2007; CAFFREY et al., 2007; SAYED et al., 2008).

Atualmente, a diminuição da susceptibilidade ao praziquantel tem sido relatada em vários estudos (PICA-MATTOCCIA et al., 2009; MELMAN et al., 2009; DOENHOFF, CIOLI & UTZINGER, 2008); e se considerarmos que não há nenhuma alternativa eficaz para o controle da esquistossomose, é evidente que o risco do desenvolvimento de resistência ao praziquantel irá aumentar nas próximas décadas, sendo uma preocupação para a comunidade médica. Diante disto, o desenvolvimento de novos protótipos de fármacos anti-*S. mansoni* é uma necessidade inquestionável.

Diante da necessidade de obtenção de alternativas para o tratamento da esquistossomose e de outras doenças negligenciadas, têm-se desprendido esforços

dentro da química medicinal, utilizando-se as diversas técnicas de planejamento para obter substâncias com características estruturais melhoradas e com menor toxicidade (BARREIRO et al., 2002). Dentre as estratégias empregadas destaca-se a utilização do princípio do bioisosterismo, através de modificação ou variação molecular, da hibridização molecular e da homologação (BARREIRO & FRAGA, 2008).

Estruturas como as ftalimidas, tiossemicarbazonas e seus bioisósteros cíclicos tiazois e tiazolidinonas, têm sido consideradas estruturas privilegiadas pela química medicinal por apresentarem um amplo espetro de ação (DUARTE, BARREIRO & FRAGA, 2007). Os derivados de ftalimidas, por exemplo, são conhecidos por suas atividades imunomoduladoras, sendo a inibição do Fator de Necrose Tumoral α (TNF-α) com destacada importância por ser considerada mediadora dos processos inflamatórios e tumorais. Tem-se ainda as atividades anti-angiogênica, anti-proliferativa, ativadora de apoptose, das células T e Natural Killer (NK), inibidora de adesão celular entre outras atividades (TEO, 2005; HASHIMOTO, 2002).

As tiossemicarbazonas e seus bioisósteros cíclicos – tiazolidinona e tiazol apresentam-se como moléculas bastante promissoras, demonstrando um amplo perfil farmacológico e constituindo uma importante classe de compostos cujas propriedades têm sido extensivamente estudadas na química medicinal. Estes compostos apresentam, entre outras, atividades antitumorais, antivirais, antifúngicas, antibacterianas e antimaláricas. As tiossemicarbazonas têm sido estudadas como potenciais inibidores das proteases cisteínas (Cys-proteases), em especial aquelas presentes no *Trypanosoma cruzi*, apresentando resultados promissores (ROMEIRO et al., 2009; MALLARI et al., 2009; PORCAL et al., 2008). As Cys-proteases são fundamentais para o metabolismo de muitos parasitos (SAJID & McKERROW, 2002).

Diante do amplo espectro de ação destes grupos farmacofóricos, a equipe de pesquisa do Laboratório de Planejameto em Química Medicinal da Universidade Federal de Pernambuco (LPQM/UFPE) resolveu utilizar estes grupos para a obtenção de moléculas híbridas como fenoxi-tiossemicarbazona, fenil-tiazol, ftalil-tiossemicarbazona, ftalil-tiazol e ftalil-tiazolidinona fazendo uso de ferramentas que são empregadas nas estratégias de planejamento de fármacos, sendo estes compostos avaliados neste trabalho.

Os compostos obtidos pelo LPQM foram avaliados com relação a sua potencial atividade esquistossomicida através da realização de um "screen" *in vitro* onde foram testados dez compostos, sendo este grupo composto por fenoxi-tiossemicarbazonas,

fenil-tiazois, ftalil-tiossemicarbazonas, ftalil-tiazois e ftalil-tiazolidinona. Entre os compostos testados, os ftalil-tiazois obtiveram uma melhor atividade, sendo estes avaliados com relação à atividade imunomoduladora, citotoxicidade e avaliação ultra-estrutural do verme exposto ao melhor composto. O composto que obteve melhor resultado *in vitro*, também foi avaliado através de testes *in vivo* não se obtendo a mesma eficácia.

2. OBJETIVOS

Gerais

Verificar o potencial terapêutico de novos derivados dos grupos farmacofóricos ftalimida, tiossemicarbazona, tiazol e tiazolidinona, candidatos a fármacos esquistossomicidas.

Específicos

- Avaliar a suscetibilidade *in vitro* de vermes adultos de S. mansoni frente às novas moléculas sintetizadas;
- Analisar em culturas de células esplênicas de camundongos a citotoxicidade dessas substâncias;
- Analisar por microscopia ótica e eletrônica as alterações morfológicas dos vermes de S. mansoni submetidos ao tratamento com essas substâncias.
- Avaliar em cultura de macrófago de camundongo a atividade imunomoduladora dessas substâncias.
- Verificar a suscetibilidade *in vivo* de vermes adultos de S. mansoni frente à molécula que apresentou o melhor resultado *in vitro*;
- Determinar, através de contagem de vermes e ovos, a carga parasitária dos animais submetidos à terapia no teste *in vivo*;
- Verificação do estágio de desenvolvimento dos ovos em fragmento do intestino de animais tratados no estudo *in vivo*;
- Verificar se teve alterações na ultra-estrutura de vermes adultos recuperados de animais tratados no estudo *in vivo* através da microscopia eletrônica de transmissão;
- Realizar estudo morfométrico quantitativo dos granulomas no tecido hepático de animais tratados no estudo *in vivo*, fazendo uma comparação entre os grupos.

3. REVISÃO DE LITERATURA

A esquistosomose é considerada um dos maiores problemas de saúde pública em regiões tropicais e subtropicais incluindo África, América central e Améria do Sul, China e sudeste da Ásia (ABDULLA et al., 2007; BERTÃO et al., 2012). Estima-se que mais de 200 milhões de pessoas no mundo estejam infectadas pela esquistossomose, sendo 90% destas pessoas residentes na Africa, com 20 milhões exibindo a forma severa da doença e que 700 milhões de pessoas vivem em local de risco nos 74 países endêmicos (ABDULLA et al., 2009, KUNTZ et al., 2007; SAYED et al., 2008; BERTÃO et al., 2012; WHO, 2012).

Existem seis espécies de *Schistosoma*: *S. mansoni, S. japonicum, S. mekongi, S. intercalatum, S. malayensis e S. haematóbium*. Destas, as que mais acomete o homem são o *S. haematobium* que causa a esquistossomose urinária e o *S. mansoni e S. japonicum* que causam a esquistossome intestinal. A transmissão destas espécies tem sido documentada em 78 países, sendo o *S. haematobium* encontrado basicamente na África e no Oriente médio; o *S. Japonicum* encontrado na China, Idonésia e Filipinas; e o *S. mansoni* encontrado África, oriente médio, Caribe, Venezuela, Suriname e Brasil (WHO, 2013).

A esquistossomose intestinal causada pelo *S. mansoni* acomete cerca de 6 milhões de pessoas no Brasil, sendo a região nordeste a mais endêmica com maior prevalência nos estados de Alagoas, Bahia, Minas Gerais e Pernambuco (BARBOSA & GOMES, 2008).

Em Pernambuco, que ocupa o 3º lugar em prevalência na Região Nordeste, a endemia está presente basicamente em áreas que circundam a faixa litorânea, correspondendo à chamada Zona da Mata Sul e Norte. Dos 185 municípios do Estado, 93 são endêmicos para esquistossomose (SILVA & DOMINGUES, 2011).

3.1. CICLO BIOLÓGICO

O *Schistosoma* tem um ciclo de vida complexo que envolve o caramujo como hospedeiro intermediário e o ser humano como um dos hospedeiros definitivos (Figura 1). A infecção do ser humano ocorre através do contato com a água contendo cercárias liberadas pelo caramujo infectado. As cercárias penetram na pele do hospedeiro definitivo perdendo sua cauda e se transformando em esquistossomulo (RAMIREZ et al., 2007; SAYED, 2008; HOLTFRETER et al, 2011). Os esquistossomulos

permanecem na pele por alguns dias até atingir a circulação sanguínea sendo conduzidos ao pulmão onde maturam para forma pré-adulta, residindo no pulmão por mais alguns dias até finalmente se alojarem no sistema venoso portal hepático onde se tornam adultos macho e fêmea (SAYED, 2008; HOLTFRETER et al, 2011). O tempo requerido para esta migração depende da espécie do *Schistossoma*, mas para o *S. mansoni* é normalmente de sete dias (ABDULLA et al., 2007).

Uma vez no fígado, o parasito sofre rápido crescimento com desenvolvimento e maturação sexual. Após o acasalamento, o parasito migra para a veia mesentérica, no caso de *S. mansoni* e *S. japonicum* ou para o sistema urogenital no caso do *S. haematobium*, onde começam a oviposição. Em torno de 49 dias após a infecção os vermes já estão adultos, acasalados e eliminando ovos (ABDULLA et al., 2007; SAYED et al., 2008). Seis dias após a postura dos ovos, estes chegam à luz intestinal e são eliminados para o exterior pelas fezes, os que não alcançam o lúmen intestinal são levados pela circulação porta e ficam presos na parede intestinal e no fígado, e são circundados por uma resposta granulomatosa do hospedeiro (ALMEIDA, 2009)

A falta de saneamento básico e os hábitos inadequados da população proporcionam a contaminação de fontes de águas com fezes contendo ovos do parasito, os quais, em contato com água doce e em condições ideais de hipotonicidade e temperatura, eclodem eliminando o miracídio que penetra no hospedeiro intermediário, caramujo do gênero *Biomphalaria*. Após a penetração o miracídio sofre reprodução assexuada dando origem a esporocistos. Os mesmos, através da poliembionia originam as cercarias, forma infectante para o hospedeiro vertebrado, sendo eliminadas de seis a oito semanas após a infecção do caramujo. Cada miracídio dar origem de 100 a 300 mil cercárias. Após a eliminação, as cercárias nadam ativamente até o encontro com o hospedeiro definitivo recomeçando o ciclo (CAFFREY, 2007; RAMIREZ et al., 2007).

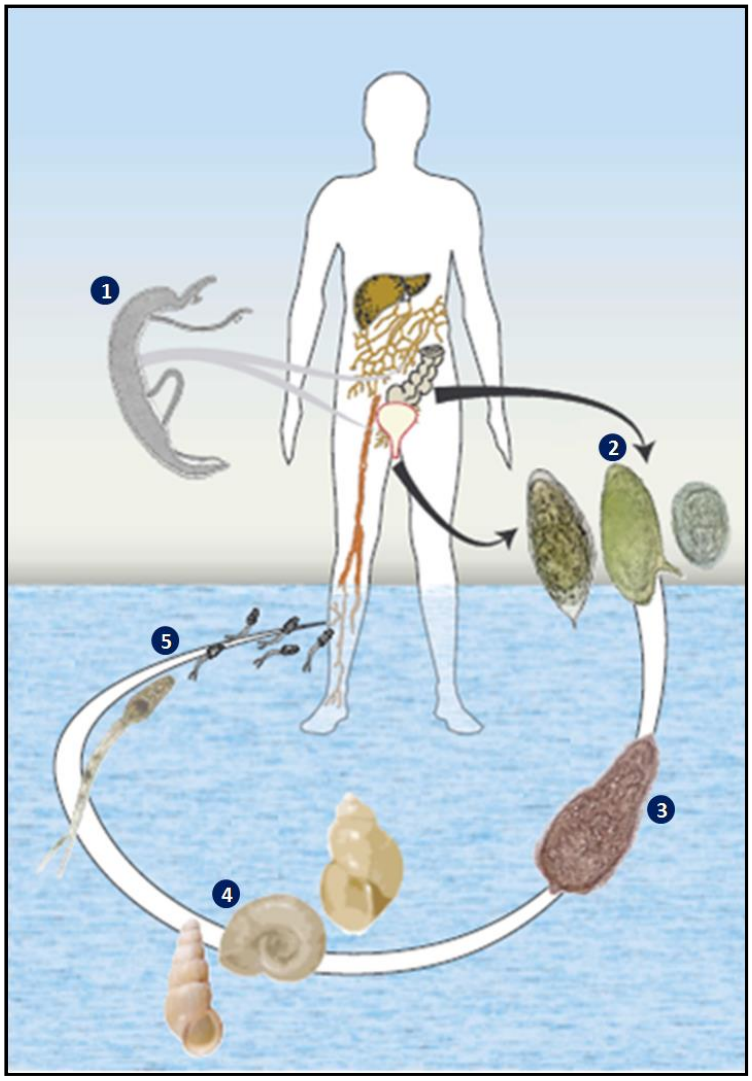

FIGURA 1 – Ciclo de vida da *Schistosoma*. 1: vermes adultos do *S. mansoni*; 2: ovos; 3: Miracídios; 4: molusco do gênero *Biomphalaria glabrata, B. tenagophila* e *B. straminea*; 5: cercárias (GRYSEELS, 2006/adaptada

3.2. PATOGENIA

As consequências patológicas da infecção pelo Schistosoma são oriundas da resposta imunológica do hospedeiro contra os ovos do parasito. Inicialmente ocorre a formação de granulomas ao redor dos ovos depositados pelas fêmeas, podendo levar a oclusão do fluxo sanguíneo, e dependendo da espécie, causar fibrose do fígado e hipertensão portal ou fibrose do trato urinário, obstrução urinária e carcinoma da bexiga (ABDULLA et al., 2007; MORAIS et al., 2008; KRAUTZ-PETERSON, 2009; BERTÃO et al., 2012).

A patogenia da esquistossomose mansoni depende de uma série de fatores como a linhagem do parasito, a carga parasitária, a idade, o estado nutricional e a imunidade do hospedeiro (BUTTERWORTH et al., 1998; ALMEIDA et al., 2009).

A esquistossomose mansônica produz quatro manifestações clínicas: doença aguda e três formas crônicas (intestinal, hepatointestinal e hepatoesplênica). A forma aguda é uma doença febril debilitante (Katayama fever) acompanhada de fraqueza, náusea, vômitos, diarreia, perda de pesso e marcante eusinofilia que pode ocorrer antes do aparecimento dos ovos nas fezes, 4-6 semanas após a infecção (LAMBERTUCCI et al., 2000; PEARCE & MACDONALD 2002). Esta fase normalmente é assintomática em indivíduos residentes em áreas edêmicas e os indivíduos normalmente apresentam níveis elevados de fator de necrose tumoral-alfa (TNF-α) e interleucina (IL-1) e (IL-6) (De JESUS et al., 1993; ALMEIDA, 2009).

Na fase crônica, a forma clínica intestinal é a mais frequentemente encontrada em pacientes infectados e apresenta sintomas brandos como perda de apetite, dispepsia e desconforto abdominal. A forma hepatointestinal é pouco estudada e o indivíduo apresenta hepatomegalia sem esplenomegalia. A forma hepatoesplênica é a forma mais conhecida da doença crônica que acomete 1 a 10% da população infectada, sendo caracterizada pelo aumento considerável do baço e fígado devido à fibrose periportal central e periférica, que pode levar a complicações significativas como hipertensão portal, mielite e hipertensão pulmonar (LAMBERTUCCI et al., 2000; BINA &PRATA, 2003; ALMEIDA, 2009).

A maioria das pessoas que vivem em áreas edêmicas apresentam poucas manifestações patológicas, contudo, uma pequena porcentagem das pessoas infectadas desenvolve fibrose hepática associada à formação de granulomas, com hipertensão portal e varizes esofágicas, apresentando risco de morte (MORAIS et al., 2008).

A resposta imune na esquistossomose tem mostrado um mecanismo células-T-dependente, com uma resposta mediada por linfócito T auxiliar (Th1) no estágio inicial da doença e uma resposta Th2 após a deposição dos ovos (SILVA, 2012)

Na esquistossomose murina, a resposta do tipo Th1, onde ocorre produção de interferon gama (IFN-γ), interleucina (IL) IL-2 e fator de necrose tumoral (TNF-α), têm sido relacionadas à imunidade; enquanto que a resposta do tipo Th2, onde ocorre produção de IL-4, IL-5, IL-10 e IL-13, correlaciona-se com a morbidade induzida pelos ovos do parasito (MORAIS et al., 2008; ALMEIDA, 2009; NEVES et al., 2011). Já na esquistossomose humana, a dicotomia Th1/Th2 na resistência a infecção e morbidade

não é tão evidente e a regulação da imunopatologia da esquistossomose parece ser mais complexa e altamente variável, não existindo um consenso sobre o padrão de produção de citocinas. (ALMEIDA, 2009).

Estudos prévios têm mostrado que o padrão de produção de citocinas muda de acordo com os diferentes estágios da esquistossomose humana, estando os dois perfis de citocinas, tipo 1 e tipo 2, envolvidos na reposta inflamatória granulomatosa, contudo atuando em fases diferentes. (MORAIS et al., 2008).

3.3. IMPACTO SOCIAL DA ESQUISTOSSOMOSE

A esquistossomose apresenta um elevado potencial mórbido, que torna esta doença um grave problema de saúde publica, estando associada a manifestações crônicas e debilitantes podendo levar o indivíduo a uma deficiência cognitiva, estafa e diminuição do crescimento (ABDULLA et al., 2007).

O principal impacto da esquistossomose na saúde do paciente é a morbidade crônica que pode ser resultado de repetidas infecções e desenvolvimento de sequelas não fatais, mas debilitantes (ALMEIDA, 2009).

Análises realizadas sugerem que a morbidade devido à esquistossomose é grosseiramente subestimada, resultando em uma estimativa de 280.000 óbitos anualmente apenas na África subsariana (KUNTZ et al., 2007, SAYED, 2008). Estima-se que a carga global de esquistossomose leva a uma perda na soma de anos potenciais de vida de 1,7 a 4,5 milhões de pessoas, tanto devido a morte prematura como devido aos anos de vida produtiva perdidos em decorrência da morbidade da doença (KEISER et al., 2009), além disto, nas crianças esta doença pode levar a anemia, nanismo e reduzir a aprendizagem, embora esses efeitos possam ser revertidos com tratamento (WHO, 2012).

Outra medida de morbidade a ser considerada é o número de internações e óbitos devido à esquistossomose mansônica, divulgados pelo sistema Único de Saúde (SUS) para o Brasil, com uma média de 820 internações entre o ano de 2000 e 2010 e uma média de 505 mortos neste mesmo período (SVS, 2011).

3.4. TRATAMENTO

O controle da esquistossomose pode ser realizado através da implantação de saneamento básico, fornecimento de água potável, educação em higiene, controle dos

caramujos, desenvolvimento de vacinas e através da quimioterapia (WHO, 2013). A quimioterapia é, atualmente, a arma mais eficaz no controle da endemia levando a uma diminuição significatica da carga parasitária e à regressão da fibrogênese hepática (SAYED, 2011). Baseado nesta evidência, a Organização Mundial de Saúde, em 1985, passou a indicar o tratamento em massa com Praziquantel (PZQ) para controlar da esquistossomose em áreas altamente endêmicas, através de um documento intitulado: "Lutte contre la schistosomiasis" (REY, 1987; ALMEIDA, 2009).

Durante muito tempo, apenas drogas antimoniais eram utilizadas na quimioterapia contra esquistossomose (ALMEIDA, 2009). A quimioterapia "moderna" foi iniciada entre 1917 e 1918 com a utilização do tartarato emético (tartarato de amônio e potássio) administrado a pacientes infectados com *S. haematobium* (OLIVEIRA et al., 2004; KUNTZ et al., 2007). A descoberta desta droga representou um avanço para o tratamento da doença e foi o ponto de partida para a busca de novos compostos sintéticos contra o parasito. O uso deste medicamento persistiu até a segunda Guerra Mundial, época em que a Lucantona foi introduzida na clínica. Um período de intenso progresso ocorreu apartir da década de 1960 com a introdução da emetina, niridazol, hicantona, oxamniquine e metrifolato (OLIVEIRA et al., 2004). Este período culminou em 1977 com a síntese do PZQ, atualmente a droga de escolha, recomendada pela organização mundial de saúde para o tratamento individual e em massa da esquistossomose (OLIVEIRA et al., 2004; ABDULLA et al., 2007).

O oxamniquine apresentou um papel fundamental na terapêutica do *S. mansoni* (RICHTER, 2003). Contudo, o aparecimento de resistência ao oxamniquine associado ao fato de que ele apresenta eficácia apenas contra o *S. mansoni*, fez com que o tratamento da esquistossomose passasse a ser basicamente realizado pelo uso do PZQ, o qual apresenta eficácia contra todas as espécies d*e Schistosoma* que infecta o homem; apresenta-se como uma droga segura, com efeitos colaterais leves e transitórios que desaparecem dentro de 24 horas e ainda possui um baixo custo devido ao aumento de consumo e competição no mercado (KUNTZ et al., 2007; CAFFREY, 2007; SAYED, 2008).

O PZQ (2-ciclohexilcarbonil-1,2,3,6,7,11b-hexahidro-4H-pirazino-(2,1-α)isoquinolina-4-ona) (FIGURA 2) é um pó branco cristalino de sabor amargo, normalmente estável , praticamente insolúvel em água e solúvel em solventes orgânicos. Apresenta-se como uma mistura racêmica sendo a forma Levógiro a única forma ativa contra o *Schistosoma* (DOENHOFF, 2009). É um medicamento bem tolerado,

facilmente administrada na forma de comprimido e relativamente barata (ABDULLA et al., 2007; RAMIREZ et al., 2007; SAYED, 2008).

O uso do PZQ tem sido empregado para o tratamento das esquistossomoses a mais de vinte e cinco anos, demonstrando-se seguro e efetivo com uma única dose oral de 40-60 mg/Kg chegando a cura de 60-90% da população tratada (CAFFREY, 2007). A menor dose é geralmente usada contra *S. mansoni* e *S. haematobium*, enquanto que a maior dose é especialmente recomendada contra *S. japonicum* e *S. mekongi* (ALMEIDA, 2009).

O alvo molecular preciso do PZQ ainda não foi completamente elucidado, contudo, sabe-se que a exposição dos vermes ao PZQ leva a um influxo maciço de cálcio, contração da musculatura e alteração do tegumento. Evidências sugerem também que o PZQ pode atuar sobre os canais de cálcio e sítios de fosforilação da proteína kinase C e que pode está envolvido na ativação dos receptores de adenosina e na ligação com actina (CAFFREY, 2007).

Uma falha notável do PZQ é a falta de eficácia contra as formas imaturas do *Schistosoma* (CAFFREY, 2007). A eficácia do PZQ sobre o parasito é bifásica, sendo eficaz contra a forma larval do parasito até sete dias após a infecção; após este período ocorre uma diminuição da atividade até 28 dias de infecção. Após este período, a atividade do PZQ é lentamente restaurada, sendo totalmente eficaz após 40 dias de infecção. Diante disto, o uso desta droga em regiões de alta transmissão, deve ser repetido quatro a seis semanas após a primeira dose para remover alguns parasitos que tenham se tornado adulto neste período (CAFFREY, 2007; ABDULLA et al., 2007).

O baixo custo do praziquantel e a sua eficácia leva ao uso generalizado com mais de dez milhões de prescrições anuais; além disto, a alta taxa de reinfecção ocasiona a administração anual ou ainda semestral do praziquantel, aumentando ainda mais a utilização desta droga e acelerando a seleção de parasitos resistentes a droga (SAYED et al., 2008; KEISER et al., 2009).

Existe a suspeita de que baixas taxas de cura do praziquantel em um surto de esquistossomose no Senegal (STELMA & TALLA, 1995) e no Egito (ISMAIL et al, 1996) no início dos anos 90 seja parcialmente devido à resistência a droga, embora interpretações alternativas sejam discutidas, os altos níveis de pré-tratamento da infecção e a intensa transmissão da doença resultaram no alojamento de um largo número de formas imaturas do parasito menos sensível a droga (ABDULLA et al., 2007; ALMEIDA, 2009).

Além da preocupação com o surgimento de parasitos resistentes ao PZQ, a baixa eficácia desta droga contra as formas juvenis do parasito intensifica a necessidade do desenvolvimento uma alternativa para o tratamento da esquistossomose (KEISER et al., 2009).

3.5. PERSPECTIVAS DE NOVOS ALVOS BIOLÓGICOS

Potenciais alvos de drogas parasitárias vêm sendo descobertos utilizando-se métodos químicos ou de biologia molecular durante o tratamento quimioterápico, além da supervisão dos fatores bioquímicos e cinéticos encontrados entre o parasito e o seu hospedeiro (PINK et al., 2005).

3.5.1. Vias de Sínteses de Purinas

As vias de síntese de purinas são de grande importância para a sobrevivência dos organismos, pois elas estão relacionadas com as sínteses de ácidos nucléicos, proteínas e outros metabólitos envolvidos nas reações energéticas. Os nucleotídeos purínicos podem ser sintetizados pela via 'de novo' ou podem ser fornecidos pela via de salvação (CASTILHO, 2010).

Os parasitos do *S. mansoni* não possuem enzimas para a via de síntese das bases púricas 'de novo', precisando consequentemente, da via de salvação das purinas para suprir a necessidade de nucleotídeos para a síntese de RNA e DNA (PEREIRA *et al.*, 2005). Uma das principais enzimas participantes desta via de salvação é a Purina Nucleosídeo Fosforilase encontrada no *S. mansoni* (SmPNP). A descoberta dessa enzima como importante componente da via de salvação das purinas tem sido proposto como potencial alvo para a busca de novos agentes esquistossomicidas (PEREIRA et al., 2010).

3.5.2. Tiorredoxina Glutationa Redutase

Os vermes adultos do *S. mansoni* vivem em um ambiente aeróbico, veia mesentérica do hospedeiro humano, portanto deve ter um mecanismo efetivo para manter o balanço redox celular, minimizando os danos causados pelas espécies de

oxigênio reativo produzido pela sua própria respiração aeróbica, assim como pela resposta do sistema imune do hospedeiro (KUTZ et al., 2007; SAYED et al., 2008).

O ser humano apresenta dois sistemas de detoxificação de espécies oxigênio reativas, uma baseada no tripeptídeo glutationa (GSH) e outra baseada na proteína tiorredoxina (Trx). Ambos os sistemas são realizados por flavoenzimas oxidorredutoras por via NADPH. A flavoproteína glutationa redutase (GR) reduz o glutationa dissulfido (GSSG) e conduz o sistema dependente de GSH; enquanto que a flavoproteína tiorredoxina redutase (TrxR) é essencial para o sistema dependente de Trx. (KUTZ et al., 2007; SAYED et al., 2008).

Análises do genoma do *S. mansoni* não identificou homólogos das enzimas GR e TrxR, demonstrando que o parasito não possui essas enzimas, apresentando em seu lugar uma única enzima multifuncional, a Tiorredoxina Glutationa Redutase (TGR) que é responsável pela redução da GSSH e da Trx. A TGR apresenta similaridade estrutural com as enzimas GR e TrxR encontrada os mamíferos; portanto, dada a importância do sistema celular redox e a diferença bioquímica entre o metabolismo redox do *S. mansoni* o do hospedeiro humano, a TGR deve ser um importante alvo terapêutico na busca de novos fármacos (KUTZ et al., 2007; SAYED et al., 2008).

3.5.3. Proteases Cisteínas

As cisteína proteases (CP) são fundamentais para o metabolismo de muitos parasitos, e muitos inibidores desta enzima tem se mostrado eficaz contra protozoários parasitos em ensaios *in vitro* e *in vivo* (ABDULLA et al., 2007). O *Schistosoma* também expressa um certo número de CPs que atuam na digestão, reprodução, síntese e produção de proteína (ABDULLA et al., 2007).

Nos últimos 10 anos, tem-se observado um intenso enfoque da química medicinal concentrado no modelo de pequenas moléculas inibidoras de CPs como novo modelo de agente antiparasitário, tendo como alvo as CPs que estão presentes em protozoários e helmintos (ABDULLA et al., 2007).

Formas jovens e adultas do *Schistossoma* expressam um certo número de Clan CA proteases incluindo catepsinas B_1, F e L, as quais operam sinergicamente no intestino do parasito para degradar proteínas do hospedeiro como fonte de nutrientes (ABDULLA et al., 2007). Inibidores das CP tem mostrado excelentes perspectivas de

atividade contra esquistossomose durante a fase inicial da deposição de ovos, reduzindo a carga parasitária e melhorando a recuperação da doença hepática (BERTÃO, 2012).

3.5.4. Sistema Neuromuscular

O sistema neuromuscular dos vermes adultos do *S. mansoni* é considerado alvo para a descoberta de novos fármacos esquistossomicidas. Apesar de o parasito possuir um sistema de neurotransmissão e controle da atividade motora qualitativamente semelhante ao dos mamíferos, pesquisas detalhadas baseadas na modulação farmacológica dos receptores envolvidos e nas proteínas relacionadas no controle homeostático do cálcio, demonstraram apresentar diferenças entre o parasito e o hospedeiro, sugerindo à possibilidade de se haver a síntese de moléculas relativamente específicas (NOEL, 2008).

Alguns candidatos a potentes alvos esquistossomicidas foram caracterizados, tais como os receptores glutamatérgicos e a via de transmissão GABAérgica nos parasitos do *S. mansoni*, uma vez que há agonistas e/ou antagonistas que podem atuar nesses sistemas alterando a atividade motora desses vermes (NOEL, 2008).

3.5.5. Proteína Quinase

Algumas moléculas transdutoras de sinal como as proteínas quinases (PKs) têm sido isoladas de *Schistosoma* por abordagem de clonagem convencional, apoiada por análises in silico usando os conjuntos de dados de genomas disponíveisou através de simulação computacional (DISSOUS & GREVELDING, 2010).

Entre as PKs de interesse estão as PKs mitogênicas como as “Polo-like kinases” (Plks) que são importantes reguladoras do ciclo celular. Duas Plks de *S. mansoni*, SmPlk1 e SmSak, foram identificadas e caracterizadas. A SmPlk1 é predominantemente expressa pelas células vitelinas e oocistos na fêmea e pelos espermatócitos no macho (DISSOUS & GREVELDING, 2010).

Além das PKs mitogênicas, outras proteínas foram identificadas com potencial papel no precesso de diferenciação celular. Entre elas estão os receptores da proteína tirosina quinase (RTKs) como os receptores SER presente na musculatura e no vitelário e ovário do *Schistosoma* fêmea; o SmVKR1 presente nos oocistos maduros das fêmeas; e os receptores de insulina, SmIR-1 presente no tegumento da membrana basal, nos

músculos e epitélio intestinal de ambos os sexos e SmIR-2, localizado no parênquima (DISSOUS & GREVELDING, 2010).

3.5.6. Canais de Cálcio

Os canais de cálcio são complexos protéicos formados por subunidades moduladoras denominadas β-subunidades (JEZIORSKIA & GREENBERG, 2006), responsáveis pela regulação do fluxo de Ca^{2+} e parecem está relacionados com o mecanismo de ação do agente esquistossomicida praziquantel (TALLIMA & RIDI, 2007; DOENHOFF, CIOLI & UTZINGER, 2008).

Os canais de cálcio são locais essenciais para a entrada de cálcio extracelular e podem ter um papel importante na regulação dos níveis de cálcio no meio intracelular (BLAIR, BENNETT & PAX, 1992).

Sabendo-se que existem diferenças estruturais e farmacológicas entre as β-subunidades dos canais de cálcio existente nos mamíferos e nos vermes do *S. mansoni*, a total elucidação da composição e função desses canais pode transformá-los em possíveis alvos para a obtenção de novos agentes esquistossomicidas (DOENHOFF, CIOLI & UTZINGER, 2008; GREENBERG, 2005).

3.5.7. DNA Topoisomerases

A DNA topoisomerase também atua como possível alvo na descoberta de novos agentes esquistossomicidas (ABDUL-GHANI, LOUTFY & HASSAN, 2009). Fármacos como os compostos acridínicos possuem como mecanismo de ação a capacidade de se intercalar a moléculas de DNA e inibir as enzimas topoisomerases I e II responsáveis pelo processo de replicação celular (YANG et al., 2006). A acridina (10-2-(dietilamino)etil-9-acridanona(2-tiazolin-2-il)hidrazona), também denominada 9-acridanona-hidrazona apresentou efeito esquistossomicida quando utilizados no tratamento de camundongos infectados com *S. mansoni* (COELHO et al., 1995).

3.6. PERSPECTIVAS DE NOVOS FÁRMACOS

Embora não existam programas dedicados à descoberta e desenvolvimento de medicamentos para a esquistosomose por parte das indústrias farmacêuticas ou parcerias

públicas privadas, alguns compostos com promissora atividade anti-*schistosoma* têm sido identificados no meio acadêmico (KEISER et al., 2009; BERTÃO, 2012).

Sayed et al. (2008) demonstrou que o oxadiazol 4-fenil-1,2,5-oxadiazol-3-carbonitrila-2-oxido (fig. 2) foi capaz de reduzir a carga parasitária em animais infectados independentemente da fase de infecção e de melhorar o processo patológico associado a formação de granuloma induzido pelos ovos do *Schistosoma* atuando via produção de NO (óxido nítrico) e inibição de TGR. Este composto apresentou também atividade contra o verme adulto do *S. japonicum* e *S. hamatobium.*

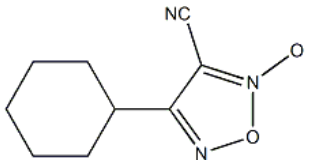

FIGURA 2 - Estrutura química do 4-fenil-1,2,5-oxadiazol-3-carbonitrila-2-oxido

O K11777 (fig. 3), inibidor de várias cisteínas proteases, tem mostrado uma boa atividade contra a esquistossomose durante a fase inicial de deposição dos ovos, reduzindo a carga parasitária e melhorando a recuperação da doença hepática (ABDULLA, 2007).

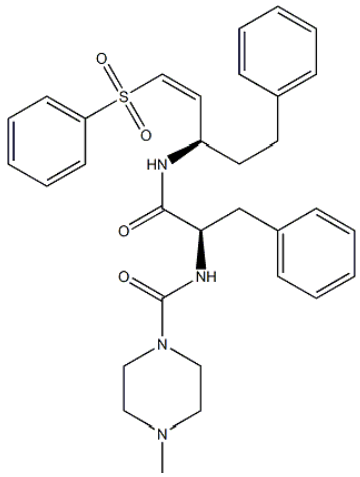

FIGURA 3 - Estrutura química da vinil sulfona K11777 (N-metil-piperazina ureia-fenilalanil-homofenilalanil vinil sulfona-fenil).

Keiser et al. (2009) demonstrou que a mefloquina ((R,S)-(±)-α-(2-piridinil)-2,8-bis(trifluorometil)-4-quinolinametanol) (fig. 4), análogo sintético da quinina utilizado no tratamento e profilaxia da malária, apresenta uma promissora atividade anti-

Schistosoma tanto na fase jovem como na fase adulta do *S. mansoni* e *S. japonicum*, com uma marcada redução da carga parasitária. A mefloquina também exibiu uma alta capacidade de induzir alterações morfológicas na membrana de esquistossômulos e vermes adultos (MANNECK, HAGGENMULLER & KEISER, 2010).

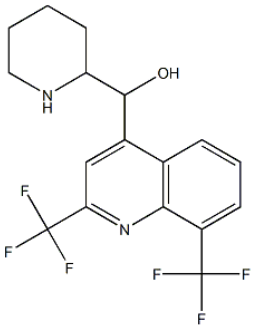

FIGURA 4 - Estrutura química da mefloquina ([(R*,S*)-2,8-bis(trifluoromethyl) quinolin-4-yl]-(2-piperidyl)metanol).

O Artemeter (fig. 5), medicamento utilizado para o tratamento e controle da malária e que está sendo avaliado em combinação com o praziquantel para o tratamento da esquistossome, apresenta atividade contra o *S. mansoni, S. japonicum e S. haematobium*, demonstrando uma considerável atividade conta o estágio larval e uma menor ação contra as formas adultas (SHUHUA et al., 2002; SAYED et al., 2008, KEISER et al., 2009).

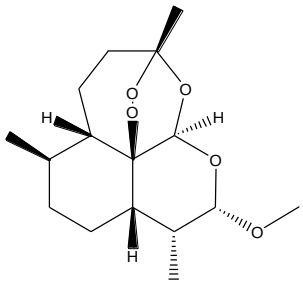

FIGURA 5 - Estrutura química da artemeter (dihidroartemisina metil éter)

As imidazolidinas, moléculas heterocíclicas pentagonais, tem mostrado atividade esquistossomicida em diversos estudos (OLIVEIRA et al., 2004; PITTA et al., 2006; NEVES et al., 2010; NEVES et al., 2011; SILVA et al., 2012). Entre as imidazolidinas estudadas pode-se destacar o LPSF/PT-5 (1-benzil-4-[(4-cloro-fenil)-hidrazono]-5-

tioxo-imidazolidin-2-ona) (fig. 6) que causou 100% de mortalidade num período de 24h com significativas alterações no tegumento do parasito (PITTA et al, 2006).

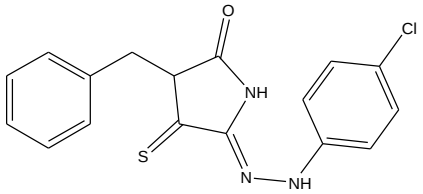

FIGURA 6 - Estrutura química do LPSF/PT-5 (1-benzil-4-[(4-cloro-fenil)-hidrazono]-5-tioxo-imidazolidin-2-ona).

Derivados do grupo químico 9-acridona-hidrazona mostram-se efetivo contra esquistossômulos de *S. mansoni* em camundongo (SULAIMAN et al, 1989). O 9-acridona-hidrazona-tiazol (fig. 7) foi efetivo contra o *S. mansoni* na fase de esquistossômulo, matando todos os parasitos quando administrado na dose de 100mg/Kg, 24h após a penetração das cercárias. Neste mesmo estudo, quando o composto foi administrado a macacos na dose de 25mg/Kg, verme e ovos estavam ausentes no tecido hepático sete dias após a infecção (PEREIRA et al, 1995).

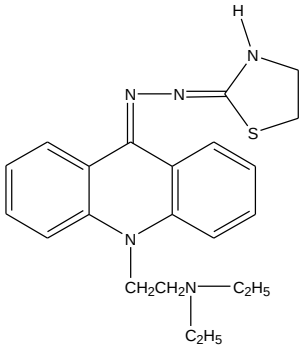

FIGURA 7 - Estrutura química do 9-acridona-hidrazona-tiazol

3.7. FTALIMIDAS

A talidomida (figura 8) é um dos principais representantes da classe ftalimidas. Ela é uma droga não barbitúrica com atividade hipnosedativa e antiemética que foi associada a graves efeitos colaterais com inúmeros casos de defeitos congênitos, fato que culminou com sua retirada abrupta do mercado no início de 1961 (BARTLETT, DREDGE & DALGLEISH, 2004; LENZ, 1988).

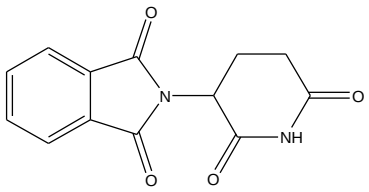

FIGURA 08 - Estrutura química da talidomida

Em 1998 a talidomida retorna ao mercado sendo aprovada pela Food and Drug Administration para o tratamento, a curto prazo, de manifestações cutâneas de eritema nodoso hansênico de moderado a grave, uma complicação da lepra (hanseníase) (HASHIMOTO, 2002). Desde então, a talidomida despertou interesse de diversos grupos de pesquisa e inúmeras propriedades atraentes da talidomida têm sido observadas. Os efeitos farmacológicos benéficos da talidomida incluem: atividade anti-caquexia, anti-tumoral, anti-angiogênica, anti-metastásica, imunorregulador, anti-viral (destacado-se como anti-HIV), anti-parasitária, e efeito hiperglicêmico. A talidomida também tem sido relatada como reguladora da produção de várias citocinas, incluindo o fator de necrose tumoral a (TNF-α), interleucinas (ILs) 2, 4, 5, 6, 10 e 12, e IFN-γ. A regulação dessas citocinas afetam a função e população de células T (HASHIMOTO, 2002).

MULLER e colaboradores demonstraram em 1999, a importância da presença do anel ftalimídico na atividade anti-TNF-α, sugerindo o caráter farmacofórico desta subunidade estrutural da talidomida. Estudo realizado por PESSOA et al (2010) utilizando o anel ftalimídico associado a outros grupos farmacofóricos também demonstrou a atividade imunomoduladora deste farmacóforo.

3.8. TIOSSEMICARBAZONAS

Tiossemicarbazonas, de acordo com BERALDO (2004), apresentam um amplo perfil farmacológico, consequentemente é uma importante classe de compostos, cujas propriedades têm sido extensivamente estudadas na Química Medicinal, particularmente, na Química Medicinal Inorgânica (figura 9).

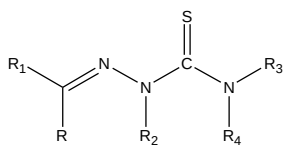

FIGURA 9 - Estrutura química da tiossemicarbazida

As atuações farmacológicas conhecidas para as tiossemicarbazonas são como drogas anticancerígenas e antivirais como demonstrado respectivamente por BROCKMAN et al. (1956) e LEVINSON et al. (1973). Além de antifúngicos, antibacterianos e antimaláricos (WEST et al., 1993).

Entre as razões estruturais que explicariam esse amplo perfil farmacológico está o fato de serem esses compostos bons agentes quelantes, podendo coordenar-se a metais existentes nas estruturas de enzimas, inativando-as. Outro ponto é que o enxofre com caráter de tiol nas tiossemicarbazonas pode se envolver em reações redox dentro do organismo (BERALDO, 2004).

De modo geral, as tiossemicarbazonas apresentam como mecanismos de ação a inibição de enzimas através da coordenação com metais existentes nas estruturas das enzimas, como a ribonucleosídeo difosfato redutase (RDR) e a tioredoxina redutase (TRxR); ligação ao DNA e inibição de sua síntese; inibição da síntese de proteínas; e através do envolvimento de seu átomo de enxofre em reações redox e da complexação com variados metais endógenos (HALL et al., 2009; BERALDO, 2004)

As tiossemicarbazonas têm sido estudadas como potenciais inibidores das proteases cisteínas (Cys-proteases), em especial aquelas presentes no *Trypanosoma cruzi*, apresentando resultados promissores (ROMEIRO, 2009; MALLARI et al., 2009; PORCAL et al., 2008). As Cys-proteases são fundamentais para o metabolismo de muitos parasitos (SAJID & McKERROW, 2002). Os vermes do *S. mansoni* se caracterizam por expressar numerosas Cys-proteases as quais estão relacionadas com sua digestão, reprodução e na síntese de proteínas (CAFREY, 2004).

3.9.TIAZOL E TIAZOLIDINONAS

Durante a década passada, a química combinatória tem fornecido o acesso a bibliotecas químicas baseadas em estruturas privilegiadas. Dentre essas estruturas, os compostos heterocíclicos tem recebido uma atenção especial por pertencer a uma classe de compostos com comprovada utilidade na química medicinal, existindo um grande número de moléculas biologicamente ativas com anel de cinco membros contendo dois heteroátomos (VERMA & SARAF, 2008).

O tiazol é uma classe química, formada por anel de cinco membros, sendo que dois destes são heteroátomos - um átomo de nitrogênio e um átomo de enxofre. Enquanto

que seu análogo tiazolidinona (figura 10), se diferencia pela presença de uma carbonila na posição quatro do anel tiazol.

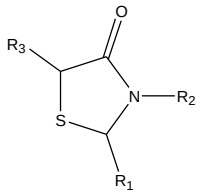

Figura 10 - Estrutura química da 4-Tiazolidinona

Derivados da tiazolidinona são conhecidos por suas várias atividades farmacológicas, como anticonvulsivante, hipnótica, antihelmíntica, antibacteriana, anticancerígena, anti-fúngica, antihistamínica, antiviral, antiinflamatória (VERMA & SARAF, 2008), anti-fibrótica (KON et al., 2002), anti-diabética, anti-arterosclerose (PERGAL et al., 2005), antimicrobiana (BOZDAG-DUNGAR et al., 2007), e antiprotozoária (LIESEN et al., 2008).

4. AVALIAÇÃO DA ATIVIDADE ANTI-*Schistossoma mansoni* DE TIOSSEMICABAZONAS E TIAZÓIS

Foi realizado um screening de dez compostos contendo os grupos farmacofóricos tiossemicarbazona, tiazol, tiazolidinona, hidrazona e ftalimida. Os compostos foram analizados in vitro, sendo avaliada a mortalidade, motilidade e alteração no tegumento dos vermes, assim como a atividade imunomoduladora, IC50 e citotoxicidade dos compostos com melhor atividade. A ultraestrutura dos vermes tratados com o composto de melhor atividade esquistossomicida, 2-(2-(2-(4-(4-methoxyphenyl)thiazol-2-yl)hydrazono)ethyl)isoindoline-1,3-dione (LpQM-45), foi avaliada através da microscopia eletrônica de varredura. Os resultados mostraram que os derivados ftalil-tiazóis apresentaram melhor atividade esquistossomicida.

O referido artigo foi publicado na revista científica Antimicrobial agentes and chemotherapy, v.58, n.1, p.352-363, 2014 (ver em anexo).

5. ATIVIDADE *IN VIVO* DE UM NOVO DERIVADO FTALIL-TIAZOL CONTRA *S. mansoni*

Este estudo descreve a atividade esquistossomicida *in vivo* do composto que apresentou melhor atividade *in vitro,* o 2-(2-(2-(4-(4-methoxyphenyl)thiazol-2-yl)hydrazono)ethyl)isoindoline-1,3-dione (LpQM-45). Animais infectados com *Schistossoma mansoni* foram tratados com uma dose diária de 150 e 25 mg/Kg do composto LPQM-45 durante um período de 5 dias. Após 15 dias do tratamento os animais foram submetidos à eutanásia para recuperação dos vermes e recolhimento de material como intestino e fígado para a realização das análises. A atividade do composto foi avaliada através da determinação da carga parasitária e da carga de ovos, determinação do estágio de desenvolvimento dos ovos, quantificação de tecido fibroso e observação de alterações do tegumento dos vermes recuperados pela microscopia eletrônica de varredura.

O manuscrito será submetido à resvista Memórias do Instituto Oswaldo Cruz (ver em apêndice).

6. AVALIAÇÃO DA ATIVIDADE ESQUISTOSSOMICIDA *in vitro* DE DERIVADOS DO PROTÓTIPO LPQM-45

Compostos heterocíclicos são estruturas que tem recebido atenção especial nos útimos anos por pertencer a uma classe de compostos com comprovada utilidade na química medicinal, existindo um grande número de moléculas biologicamente ativas com anel de cinco membros contendo dois heteroátomos (VERMA & SARAF, 2008).

O tiazol, formado por um anel de cinco membros que contém o nitrogênio e o enxofre como heteroátomos, constitui um desses cíclicos. Ele apresenta um perfil farmacológico amplo fazendo parte da composição de compostos que apresentaram ação antibacteriana (BHARTI et al, 2010), antifúngica (BHARTI et al, 2010), anti-inflamatória (GIRI et al., 2009) e antitubercular (SHIRADKAR, 2007). Os derivados ftalimidicos contitui outra classe de compostos com amplo perfil terapêutico sendo conhecida por suas atividades imunomoduladoras (TEO, 2005; HASHIMOTO, 2002). A junção destes grupos farmacofóricos utilizando a estratégia de hibridação molecular realizada pelo laboratório de planejamento em química medicinal (LPQM) originou moléculas que foram chamadas de ftalil-tiazóis, as quais apresentaram propriedades esquistossomicidas demonstradas no segundo capítulo deste estudo.

Tendo-se como objetivo otimizar a atividade esquistossomicida obtida pelos derivados ftalil-tiazóis, quatro derivados do LPQM-45 (fig. 1), molécula que apresentou melhor atividade no screening inicial (ver capítulo 2), foram avaliados através de estudo *in vitro*. Neste estudo foram as utilizadas técnicas empregadas no capítulo anterior com algumas adaptações, sendo avaliada mortalidade, motilidade e alterações no tegumento dos vermes tratados com esses compostos, assim como determinada a capacidade desses compostos em estimular a produção de NO.

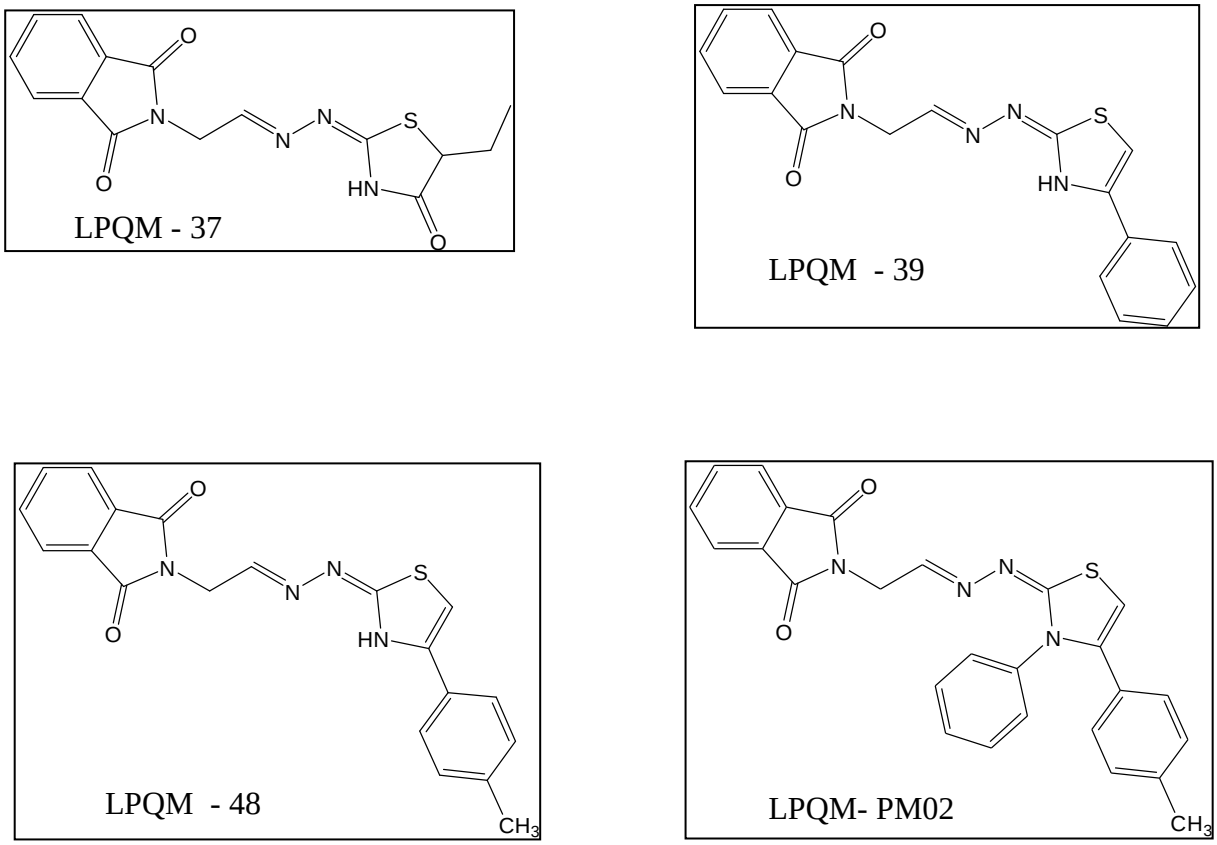

FIGURA 1. Estrutura química dos derivados ftalil-tiazóis utilizados neste estudo.

6.1. METODOLOGIA

Avaliação da suscetibilidade *in vitro* de vermes adultos de *Schistosoma mansoni* frentes aos derivados ftalil-tiazóis

Os parasitos foram removidos dos camundongos através da perfusão do sistema porta-hepático, 60 dias após a infecção; e em seguida lavados em meio RPMI-1640 acrescido de HEPES 20mM pH = 7,5 e suplementado com penicilina (100UI/ml), estreptomicina (100µg/mL) e soro bovino fetal a 10%. Após a lavagem os vermes adultos foram transferidos para placas de cultura de tecidos de vinte e quatro poços contendo 2mL de meio de cultura. Cada dose foi testatada em três poços, o primeiro poço recebeu quatro fêmeas, o segundo poço recebeu quatro machos e o terceiro poço recebeu dois casais de vermes. Após a distribuição dos vermes, estes foram incubados a 37ºC, em atmosfera úmida contendo 5% de CO_2. Após um período de 2 horas de

adaptação ao meio, os derivados ftalil-tiazois foram adicionados nas concentrações de 5 µg/mL, 10 µg/mL, 20 µg/mL, 40 µg/mL, 80 µg/mL e 100µg/mL. O praziquantel a 3 µg/mL e dois controles, um contendo DMSO e meio RPMI 1640 completo e outro contendo apenas meio RPMI 1640 completo, foram testados nas mesmas condições das amostras. Os parasitos foram mantidos em cultura por oito dias sendo monitorados a cada 24 horas para avaliação da atividade motora, da taxa de mortalidade e de alterações do tegumento.

A avaliação da motilidade foi realizada seguindo critérios definidos por RAMIREZ et al (2007), sendo a motilidade medida numa escala de 0-3 (0 = ausência; 1= motilidade mínima, com movimentos ocasionais da cauda e cabeça e ausência dos movimentos intestinais; 2= diminuição da motilidade; 3= motilidade normal). O resultado da motilidade foram utilizados juntamente a observação de alterações no tegumento para a determinação da atividade do composto.

Produção de óxido nítrico

A produção de óxido nítrico (NO) foi estimada apartir da dosagem de nitrito, produto estável da decomposição do NO, em sobrenadante de cultura de macrófagos estimulados com os compostos LPQM-37, LPQM-39, LPQM-48 e LPQM-PM02 na concentração do CC50 durante um período de 24h, 48h e 72h. O meio DMEM suplementado com 10% de soro bovino fetal inativado e o LPS (50ng/mL) foram usados como controle negativo e positivo respectivamente. Os níveis de nitrito foram quantificado pelo método indireto de Griess (DING et al, 1988), utilizando 50µL de cada sobrenadante e igual volume de reagente de Griess (1% sulfanilamida, 0,1% de dicloridrato de N-(1-naftil)-etilenodiamina, 2,5% de ácido fosfórico). Após a mistura, as amostras foram incubadas a temperatura ambiente por dez minutos e a absorbância foi medida a 540 nm (Multiskan FC; Thermo Scientific) sendo os níveis de nitrito em cada tempo determinado por extrapolação de uma curva padrão previamente preparada.

6.2. RESULTADOS E DISCUSSÕES

Efeito dos derivados ftalil-tiazóis na sobrevivência de vermes adultos de *S. mansoni*

Os derivados ftalil-tiazóis 2-((E)-2((E)-(5-ethyl-4oxothiazolidin-2-uli dene)hydrazono)ethyl)isoindoline-1,3-dione (LPQM-37), 2-((E)-2((E)-(4-phenylthiazol-2(3H)-ylidene)hydrazono)ethyl)isoindoline-1,3-dione (LPQM-39), 2-((E)-2((E)-(4-*p*-tolylthiazol-2(3H)-ylidene)hydrazono)ethyl)isoindoline-1,3-dione (LPQM-48) e 2-((E)-2((E)-(3-phenyl-4-*p*-tolythiazol-2(3H)-ylidene)hydrazono)ethyl)isoindoline-1,3-dione (LPQM-PM02) (fig. 1) foram avaliados com relação a sua atividade esquistossomicida demonstrando ação contra o S. mansoni em teste i*n vitro*. As referidas moléculas foram testadas nas concentrações de 5 – 100 µg/mL, onde foi avaliado a motilidade, mortalidade e alterações no tegumento dos vermes expostos aos compostos, assim como determinado citotoxicidade das moléculas.

A fig. 2 faz uma comparação entre as quatro moléculas avaliadas na dose de 100 µg/mL, O PZQ na dose de 3 µg/mL e o controle negativo, mostrando que o LPQM-39 e o LPQM-48 apresentaram melhor atividade em relação aos demais compostos, com 86% e 100% de mortalidade dentro das primeiras 24h de observação. O PZQ ocasionou 100% de mortalidade dentro das primeiras 24h e com o controle negativo os vermes permaneceram ativos até o último tempo do experimento. O composto LPQM-39 e o LPQM-48 ocasionaram mortalidade significativa até a dose de 40 µg/mL (tabela 1), apresentado ausência de oviposição nas doses de 10 – 100 µg/mL e separação dos vermes acasalados desde o primeiro tempo de observação. Os vermes expostos a esses compostos também apresentaram aterações em relação aos vermes expostos ao DMSO e meio de cultura, apresentando uma coloração mais escura e opaca e alguns vermes apresentando bolhas e descamações. A confirmação destas alterações está sendo avaliada pela microscopia eletrônica de varredura que está em andamento.

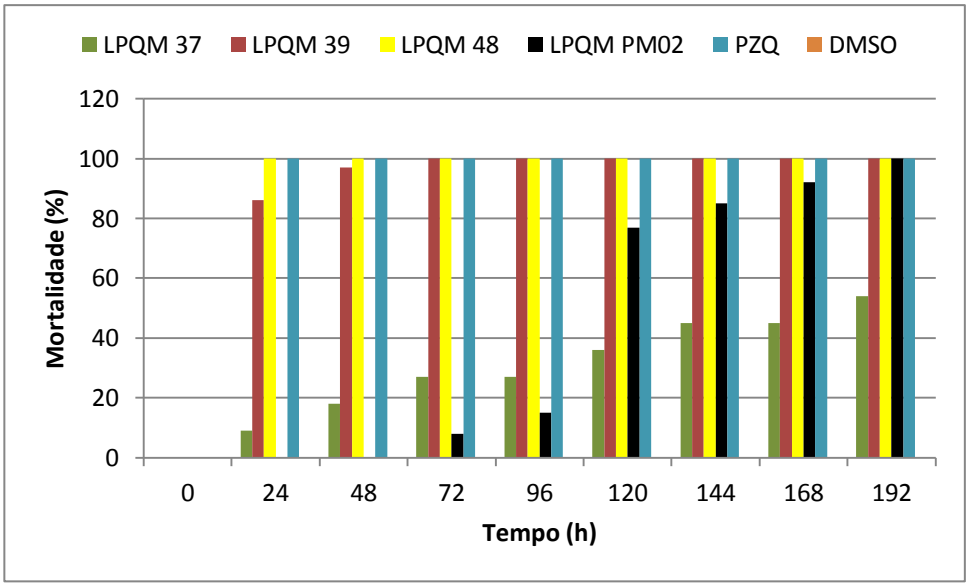

FIGURA 2. Efeito dos derivados ftalil-tiazóis na mortalidade de *S. mansoni* em teste *in vitro* na concentração de 100µg/mL

As moléculas LPQM-PM02 e LPQM-37 causaram 100% e 54% de mortalidade dos vermes respectivamente na dose de 100 µg/mL no último dia de observação (192h) (tabela 1). A molécula LPQM-37 não suprimiu a oviposição enquanto que com o LPQM-PM02 não foi observado oviposição na faixa de concentração de 20 – 100 µg/mL. Em ambos compostos foi observada separação dos vermes acasalados, uma coloração mais escura e opaca dos vermes e a presença de bolhas e descamação em alguns vermes.

TABELA 1. Efeito in vitro dos derivados ftalil-tiazóis contra os vermes adultos de *S. mansoni*

Molécula	Tempo	Mortalidade (%) na concentração ($\mu g.mL^{-1}$) indicada					
		100	**80**	**40**	**20**	**10**	**5**
LPQM 37	**24 h**	9	0	0	0	0	0
	48 h	18	0	0	0	0	0
	72 h	27	0	0	7	0	0
	96 h	27	8	0	7	0	8
	120 h	31	8	8	14	0	8
	144 h	45	8	25	14	15	17
	168 h	45	25	25	21	15	17
	192 h	54	42	25	28	23	25
LPQM 39	**24 h**	86	42	19	0	0	0
	48 h	97	70	72	7	0	0
	72 h	100	93	79	32	0	0
	96 h	100	100	96	54	0	0
	120 h	100	100	96	65	3	4
	144 h	100	100	100	73	4	4
	168 h	100	100	100	73	4	4
	192 h	100	100	100	73	4	4
LPQM 48	**24 h**	100	56	26	0	0	0
	48 h	100	63	48	8	7	0
	72 h	100	78	67	19	15	0
	96 h	100	90	86	46	34	0
	120 h	100	96	88	68	35	0
	144 h	100	96	88	71	49	4
	168 h	100	96	94	75	57	4
	192 h	100	100	100	79	72	8
LPQM PM02	**24 h**	0	0	0	0	0	0
	48 h	0	0	0	0	0	0
	72 h	8	0	0	0	0	0
	96 h	15	0	0	7	0	0
	120 h	77	8	8	7	0	0
	144 h	85	54	23	7	0	0
	168 h	92	54	23	14	0	0
	192 h	100	77	46	14	0	0

Na tabela 2 pode-se observar uma redução significativa da motilidade dos vermes expostos ao LPQM-39 com 120h de exposição, não sendo observado verme ativo nas doses de 20 – 100 µg/mL. O LPQM-48 apresentou redução significativa da motilidade em todas as doses testadas, com ausência total de vermes ativos nas doses de 40 – 100 µg/mL. O LPQM-PM02 reduziu significativamente a motilidade dos vermes nas doses de 20 – 100 µg/mL, com ausência total de vermes ativos nas doses de 40 – 100 µg/mL e o LPQM-37 apresentou redução considerável da motilidade na dose de 100 µg/mL com apenas 38% de vermes ativos.

Os resultados de motilidade juntamente com as alterações na morfologia dos vermes foram utilizados para determinar a atividade do composto seguindo os critérios estabelecidos por Ramirez et al (2007). Segundo esses critérios, o composto LPQM-39 foi considerado ativo nas doses de 20 – 100 µg/mL; o composto LPQM-48 foi considerado ativo nas doses de 10 – 100 µg/mL e parcialmente ativo na dose de 5 µg/mL; o LPQM-PM02 foi considerado ativo na dose de 100 µg/mL e parcialmente ativo na dose de 20 – 80 µg/mL; e o LPQM-37 não foi considerado ativo.

Comparando-se os resultados obtidos neste capítulo com os reultados obtidos no segundo capítulo pode-se obsevar que a presença do grupo fenil ligado ao anel tiazol contribui consideravelmente para a eficácia do composto, tendo em vista que o composto LPQM-37, que não apresenta o fenil ligado ao tiazol não apresentou uma boa atividade esquistossomida. Os resultados obtidos com as moléculas LPQM-39, LPQM-48 e LPQM-PM02 corroboram com os resultados obtidos no capítulo anterior, sugerindo que a eficácia das moléculas varia de acordo com o substituinte na posição 4 do grupo fenil. A presença de outro substituinte no fenil parece influenciar negativamente nos resultados, principalmente se esse substituinte é volumoso como no caso do composto LPQM-PM02.

TABELA 2. Escala de motilidade dos vermes controle e tratados com PZQ e com os derivados ftalil-tiazóis

Grupos	Concentração	Motilidade (%)				Alteração no tegumento	Atividade da droga*
		3	2	1	0		
Controle	***	100	0	0	0	Não	3
PZQ	3µg/mL	0	0	0	100	Sim	1
LPQM-37	100 µg/mL	38	15	15	31	Sim	1
	80 µg/ml	62	31	0	8	Sim	1
	40 µg/mL	67	17	8	8	Sim	1
	20 µg/mL	57	21	7	14	Sim	1
	10 µg/mL	58	24	18	0	Sim	1
	5 µg/mL	57	26	9	8	Sim	1
LPQM 39	100 µg/mL	0	0	0	100	Sim	3
	80 µg/ml	0	0	0	100	Sim	3
	40 µg/mL	0	0	4	96	Sim	3
	20 µg/mL	0	27	8	65	Sim	3
	10 µg/mL	73	23	0	3	Sim	1
	5 µg/mL	72	4	0	4	Sim	1
LPQM 48	100 µg/mL	0	0	0	100	Sim	3
	80 µg/ml	0	0	4	96	Sim	3
	40 µg/mL	0	9	3	88	Sim	3
	20 µg/mL	4	14	15	68	Sim	3
	10 µg/mL	13	34	18	35	Sim	3
	5 µg/mL	5	53	47	0	Sim	2
LPQM PM02	100 µg/mL	0	15	8	77	Sim	3
	80 µg/ml	0	81	11	8	Sim	2
	40 µg/mL	0	77	15	8	Sim	2
	20 µg/mL	29	64	0	7	Sim	2
	10 µg/mL	75	25	0	0	Sim	1
	5 µg/mL	93	7	0	0	Sim	1

* 1- inativa; 2- Moderadamente ativa (motilidade 2-1); 3- Ativa (motolidade 0 ou de 1 combinado com alteração morfológica (RAMIRES ET AL., 2007)

Produção de óxido nítrico

A produção de óxido nítrico foi determinada pela dosagem nitrito em sobrenadande de cultura de macrófago exposto aos compostos LPQM-37, LPQM-39, LPQM-48 e LPQM-PM02 por um período de 48h e 72h. Os resultados obtidos mostraram que os compostos não estimularam a produção de NO em cultura de macrófago

7. CONCLUSÃO E PERSPECTIVAS

Conclusão

Apartir do screening inicial com os derivados fenoxi-tiossemicarbazonas, fenil-tiazóis, ftalil-tiossemicarbazona, ftalil-tiazóis e ftalil-tiazolidinona pode-se concluir que os compostos heterocíclos tiazóis substituídos na posição quatro com um benzil apresentaram uma melhor atividade *in vitro* em relação às tiossemicarbazonas e tiazolidinona, demonstrando um potencial terapêutico, especialmente pela molécula LPQM-45, para o tratamento da esquistossomose mansônica.

Os resultados obtidos no teste *in vivo* com a molécula LPQM-45 demonstra uma moderada eficácia no tratamento. Considerando as barreiras no processo de absorção e nas demais fases farmacocinéticas pode concluir a molécula LPQM-45 possui potencial ação terapêutica requerendo ajustes na formulação e na rota de administração.

Considerando os testes realizados com os derivados ftalil-tiazóis e comparando com os resultados obtidos no primeiro screening, pode concluir que a presença do grupo fenil ligado ao anel tiazol contribui consideravelmente para a eficácia do composto, que esta eficácia varia de acordo com o substituinte na posição 4 do grupo fenil e que a presença de um segundo substituinte no tiazol parece influenciar negativamente nos resultados.

Perspectivas

- Avaliar os resultados obtidos da microscopia eletrônica de trasmissão dos vermes submetidos ao teste *in vitro* com a molécula LPQM-45.

- Realizar avaliação da ultraestrutura dos vermes submetidos ao teste *in vitro* com as moléculas LPQM-39 e LPQM-48 através da microscopia eletrônica de varredura e transmissão, cujos vermes já foram coletados e estão fixados.

- Dosar citocina em sobrenadante de esplenócitos expostos às moléculas LPQM-37, LPQM-39, LPQM-48 e LPQM-PM02.

- Determinar a citotoxicidade dos compostos LPQM-37, LPQM-39, LPQM-48 e LPQM-PM02.

- Determinar a melhor via de solubilização e administração dos compostos acima citados.

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APÊNDICE

In vivo activity of a new phthalyl-thiazole derivative against *S. mansoni*

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ABSTRACT

The control of schistossomiasis, a chronic and debilitating disease, still is carried out by the use of antischistosomal drugs, being Praziquantel (PZQ) the drug of choice for the treatment and control of schistosomiasis in most areas where the disease is endemic. Despite the effectiveness of PZQ, the use of a single drug for treatment of schistosomiasis is dangerous, especially considering that studies have shown that repeated use of this drug in areas of endemicity may cause a temporary reduction in susceptibility in isolates of *Schistosoma mansoni*, therefore, there is a recognized need to develop new schistosomicides drugs. In this context, a number of compounds with promising antischistosomal properties have been identified as sources of new drug prototypes. Among these, derivatives chemical phthalyl-thiazole, especially compound LPQM-45, showed *in vitro* schistosomicidal activity against adults worms of *S. mansoni*. The present work evaluates the *in vivo* effect of compound LPQM-45 against S. *mansoni*. During the assays, parameters such the total worm burden and eggs, the development stages of the eggs, the quantification of granuloma, as well as morphologic

changes of adult worms recovered after treatment were used. The results showed that the compound LPQM-45 presented moderate efficacy with 46% reduction in worm burden at a dose of 150 mg/kg. The additional tests showing no significant difference compared to control. Considering the barriers in the process of absorption and in pharmacokinetics phase, theses results demonstrate that LPQM-45 has potential action achistosomicide, requiring adjustments in the formulation or in route of administration.

INTRODUCTION

Schistosomiasis is a chronic and debilitating disease that affects more than 207 million people (WHO, 2012; ROLLINSON 2002). According to the World Health Organization, schistosomiasis is the cause of more than 200,000 deaths per year in sub-Saharan Africa and this may still be an underestimate (WHO, 2012). There are five species of schistosomes that can infect humans, of which *Schistosoma mansoni*, *S. japonicum* and *S. haematobium* are the most important ones (TAHA, 2007). *S. mansoni* is one of the most common etiological agents of human schistosomiasis and the disease is triggered by the inflammatory granulomatous reaction that occurs during deposition of parasite eggs in the liver and other host tissues (GRYSEELS, 2006).

The use of antischistosomal drugs in the control of schistosomiasis still occupies a leading position being Praziquantel (PZQ) the drug of choice for the treatment and control of schistosomiasis in most areas where the disease is endemic (MORAES, 2011; BERTÃO, 2012; HOLTFRETER, 2011). PZQ is effective against all species of schistosomes infecting humans and is a drug well tolerated, easily administered in tablet form and relatively inexpensive (ABDULA, 2007; UTZINGER, 2003; RAMIREZ et al., 2007; SAYED, 2008). However, an important shortcoming of PZQ is the lack of efficacy against schistosomula, the young developing stages of the parasite, with 3- to 4-week-old (DOENHOFF, 2008; ABDULA 2007; TAHA, 2007; XIAO, 2009). The effectiveness is only gradually regained as worms mature, becoming fully susceptible about 6 to 7 weeks old (DOENHOFF, 2008). This issue might explain the low observed “cure” rates and rapid “reinfection” rates in areas of heavy schistosomiasis transmission

where patients are likely to be infected with juvenile and adult parasites concurrently (XIAO, 2009).

The high rate of reinfection and the use of PZQ in the concept of "preventive chemotherapy" bring about the annual or biannual administration of PZQ, leading to the widespread use of this drug with more than ten million prescriptions annually, thus increasing the possibility of selection parasites resistant to PZQ (SAYED et al., 2008; KEISER et al., 2009). Although there is not yet clear-cut evidence for the existence of praziquantel-resistant schistosome strains, decreased susceptibility to the drug has been reported in several studies (MELMAN et al., 2009). Such facts have encouraged new studies in search of alternative therapies that could either replace or complement the use of PZQ (DOENHOFF et al., 2008; OLIVEIRA, 2012). In this context, a number of compounds with promising antischistosomal properties have been identified as sources of new drug prototypes (XIAO, 2007; ABDULLA, 2007; KEISER, 2009; SAYED, 2008).

Hydrazones, Phthalimides and thiazoles are considered privileged structures as leads in Medicinal Chemistry and are present in various compounds being used for a broad spectrum of activities (WATTS, 1986, PEREIRA, 1995, BERALDO, 2004).

Hydrazones are known for their potential pharmaceutical applications and possess antimicrobial (KIM, 2004), antischistosomal (TAHA, 2007), antifungal (ASATI, 2006), antimalarial (KRISTINA, 2009), herbicidal (SANEMITSU, 2006), antiviral (EIICHI, 2007), antidiabetic (MURUGAN, 2009), and antioxidant (SHIH, 2004) properties; Thiazole derivatives have been used to prepare various drugs that are important for antimicrobial (GOUDA, 2010), antibacterial (BHARTI, 2010; KHALIL, 2009), antifungal (BHARTI, 2010), antiinflammatory (GIRI, 2009), antitubercular (SHIRADKAR, 2007) and antiprotozoals (TAPIA, 2003); and phthalimide derivatives are known for their immunomodulatory activities, inhibiting the cytokine tumor necrosis factor-α (TNF-α), interleukins (IL) -1β, -6, -12, and granulocyte macrophage colony stimulating factor. Phthalimide also activate the Th1 response, by increasing IFN-γ and IL-2, have anti-angiogenic and anti-proliferative properties, activate apoptosis, T cells and NK, and inhibit cell adhesion (TEO, 2005; HASHIMOTO, 2002).

Among these chemical groups, derivatives phthalyl-hydrazone-thiazol showed schitosomicidal activity against adults worms of *S. mansoni in vitro*. In this work, 10 molecules containing these structures, were evaluate for their *in vitro* schistosomicidal activity being evaluate parameters such motility and mortality, oviposition,

morphological changes in the tegument, cytotoxicity, and immunomodulatory activity. These compounds, compound LpQM-45, showed substantial schistosomicidal properties against adult S. mansoni worms, with a significant reduction in motility, severe alterations in the integument and mortality of worms, lower toxicity than the reference drug (PZQ), inhibition oviposition and production of nitric oxide (SANTIAGO, 2014). Taking account these results, the present work aims to evaluate, in a mouse model, the effect of compound 2-(2-(2-(4-(4-methoxyphenyl)thiazol-2-yl)hydrazono)ethyl)isoindoline-1,3-dione LqQM45 against *S. mansoni*. In this way, the total worm burden and eggs was measured, the development stages of the eggs, the quantification of fibrous tissue, as well as morphologic changes of adult worms recovered from mice after treatment.

MATERIALS AND METHODS

Compounds

The compound 2-(2-(2-(4-(4-methoxyphenyl)thiazol-2-yl)hydrazono)ethyl) isoindoline-1,3-dione (LpQM-45) (fig. 1) was obtained by synthesis as described by Pessoa (2010), being chemically characterized by NMR, infrared, mass spectra, elemental analysis, and presenting purity > 95 %. The PZQ (reference number P4668) was purchased from Sigma-Aldrich (St. Louis, USA).

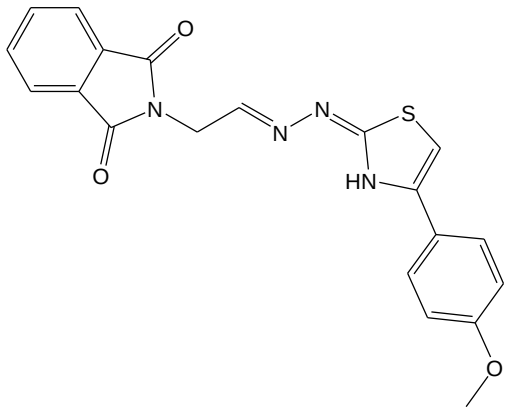

FIGURE 1: chemical structure of the compound 2-(2-(2-(4-(4-methoxyphenyl)thiazol-2-yl)hydrazono)ethyl)isoindoline-1,3-dione (LpQM-45)

Parasites and Hosts

The LE strains (Belo Horizonte, Minas Gerais, Brazil) of *S. mansoni* were used throughout this study. These strains were maintained in the laboratory at the Aggeu Magalhães Research Center (CPqAM) of the *Oswaldo Cruz Foundation (FIOCRUZ/PE/Brazil)*.

Forty female Swiss mice (*Mus musculus*) weighing 20±2 grams were used as the definitive host and were infected transcutaneously with about 80 cercariae (LE strain). The animals were kept in a controlled temperature and light environment and had access to food and water *ad libitum*. Fifty days after infection, a parasitological examination was done from feces of mice to evaluate the positivity of the infection (HOFFMAN et al. 1934).The experiments were approved by the Ethics Committee on Animal Use (CEUA - FIOCRUZ), Process nº 22/2011.

Experimental Treatment

The animals were randomly divided into four groups: Group I treated with 25 mg/Kg of LPQM-45; group II treated with 150 mg/Kg of LPQM-45; group III treated with 250 mg/Kg of PZQ; and group IV untreated. The compounds were dissolved in saline and a solubilizer comprising propylene and Tween 20 (2:1), saccharin and tutti-frutti flavoring, prior to administration. The administration of the doses was done orally, after fifty days of the infection for five consecutive days. The untreated group received vehicle and were submitted to the same testing conditions.

Assessment of Parasitological Criteria

Fifteen days after treatment the animals were euthanized by an overdose of anesthetic (100-200 mg/Kg of ketamine and 5-16 mg/ Kg of xylazine) with perfusion of the portal system for removal of the worms, which were separated in petri dishes containing saline, counted and classified according to sex and vitality (DURVAL & DEWITT, 1967). Worms recovered were subjected to Scanning Electron Microscopy for structural assessment. Fragments of liver and intestine were removed for quantification eggs and histological analysis.

Determination of the effectiveness of treatment

The evaluation of the effectiveness of treatment was determined by reducing the percentage of parasitic load in each group treated. The percent of reduction in worm number was calculated by the method of Fallon et al (1995) as follows: % reduction = C − V/C × 100, where C is the mean number of parasites recovered from infected untreated animals and V is the mean number of parasites recovered from treated animals.

Eggs count in hepatic and intestine tissue

To estimate the number of eggs per gram of hepatic and intestine tissue, One piece of the liver taken from each mouse was weighed and digested separately using 4% potassium hydroxide (KOH) (CHEEVER, 1968), and the eggs found were quantified with the aid of a cellcounting "Sedgewick Rafter" camera (Graticules Limited: model S50, Tonbridge-England).

Percentage egg developmental stages (oogram pattern)

Three fragments of the distal portion of the small intestine per animal were removed and used to evaluate the development and maturation of eggs, as described by Pellegrino et al. (1962). One hundred eggs of each fragment were randomly chosen, evaluated by microscopic examination, and classified according to their developmental stage, being the mean of each stage per animal obtained.

Morphometric evaluation

Samples of liver from each mouse was fixed in 10% formalin solution, included in paraffin blocks, sectioned and stained by Picro-sirius red. Ten microscopical fields from histological liver sections (5µm), that presented large production of conective tissue, were selected and the collagen tissue was labeled and acquired as percentage using Image Processing and Analysis System LEICA Qwin 2.6 (Leica Cambridge, England). The measurement result from each animal was the mean percentual from these ten microscopical fields, and the ultimate result was the mean percentual per group. The system was calibrated for 10x lens magnification using a Leica DM LB2 microscope.

Scanning Electron Microscopy (SEM)

Worms recovered, fifteen days after treatment, were fixed overnight at room temperature with 2.5% glutaraldehyde, 4% formaldehyde and a 0.1M cacodylate buffer at pH 6.8. They were then post-fixed in 2% osmium tetroxide (OsO_4) in a 0.1M cacodylate buffer at pH 6.8 for 60 min in the absence of light at room temperature. The next steps included washing and dehydration in a graded ethanol series for 15 min each. The worms were critical point dried using liquid CO_2, directly sputter-coated with colloidal gold for 1 min and examined under a JEOL- 5600LV microscope.

RESULTS

The compound LPQM-45 presented moderate efficacy in the treatment of mansonic schistosomiasis in mice when used at a dose of 150 mg/kg, reducing the number of adult worms after treatment by 46%, with 37% being represented by female worms. The use of the lower dose, 25 mg/kg was able to cut down on the number of worms by 37%, with 32% being represented by female worms. PZQ, in turn, had efficacy of 100% in both the doses evaluated.

In the eggs count in hepatic tissue, PZQ was effective in reducing the number of eggs ($p<0.01$) compared to the not treated group, while LPQM-45 showed no significant differences ($p>0,05$). In the evaluation of the intestine, no significant reduction was observed between the number of eggs found in animals treated with LPQM-45, with PZQ or not treated (table 01).

TABLE 1. Effect LPQM-45 on collagen content in hepatic tissue and egg load in hepatic and intestinal tissue of *S. mansoni-infeted* mice

Animal groups[#]	Morphometry of tissue collagen (image analysis system)	Tissue egg loads x 10^3	
		Hepatic	Intestinal
I	12.75 ± 6.73	15.26 ± 5.05	22.44 ± 11.50
II	14.48 ± 9.45	22.14 ± 8.88	45.70 ± 25.52 *
III	20.48 ± 4.79	3.45 ± 3.63 **	1.16 ± 1.11
IV	20.02 ± 4.13	16.60 ± 3.61	9.19 ± 5.19

* Significant difference from infected untreated control at $P<0.05$, ** at $P<0.01$, *** at $P<0.001$.
#Group I: SC45 25 mg/Kg; group II: SC45 150 mg/Kg; group III: PZQ 250 mg/Kg; group IV: untreated

The Percentage egg developmental stage is listed in Table 2 and no statistical differences were observed between untreated infected mice and LPQM-45-treated *S. mansoni*-infected animals, being observed a greater number of eggs in the immature stage and reduced number of matures and dead eggs. The animals treated with PZQ showed reduced number of immature eggs and a greater number of mature and dead eggs.

TABLE 2. Effect LPQM-45 on percentage of egg developmental stages in intestinal tissue of *S. mansoni-infected* mice

Animal groups[#]	% egg developmental stages		
	Immature eggs	**Mature eggs**	**Dead eggs**
I	95.50 ± 3.06	2.89 ± 1.55	1.89 ± 1.89
II	96.60 ± 2.88	1.89 ± 1.56	1.44 ± 2.12
III	34.33 ± 53.11 ***	53.11 ± 8.08 ***	12.56 ± 10.17 **
IV	97.67 ± 1.94	1.00 ± 0.87	1.33 ± 1.33

* Significant difference from infected untreated control at $P<0.05$, ** at $P<0.01$, *** at $P<0.001$.
#Group I: SC45 25 mg/Kg; group II: SC45 150 mg/Kg; group III: PZQ 250 mg/Kg; group IV: untreated

In the morphometric evaluation, the collagen tissue was labeled and acquired as percentage, being the collagen percentage of different groups described in Table 1. Although having been observed a lower amount of collagen in the groups treated with LPQM-45, was not observed statistical difference between the treated and control groups.

The tegument surface of male and female *S. mansoni* worms recovered of mice treated with LPQM 45 was examined using scanning electronmicroscopy (SEM). The worms recovered of mice no treated and treated with LPQM 45 presented normal surface membrane topography. The male worms showed numerous tubercles with typical spines, sensory papillae and oral and ventral suckers no abnormality. The female showed parallel fissures, spines and sensory papillae no abnormality. Several male worms treated with 150 mg/Kg of LPQM-45 were presented involved with agglomeration of host cells. The worms of the control group and worms of *in vitro* test did not show this change.

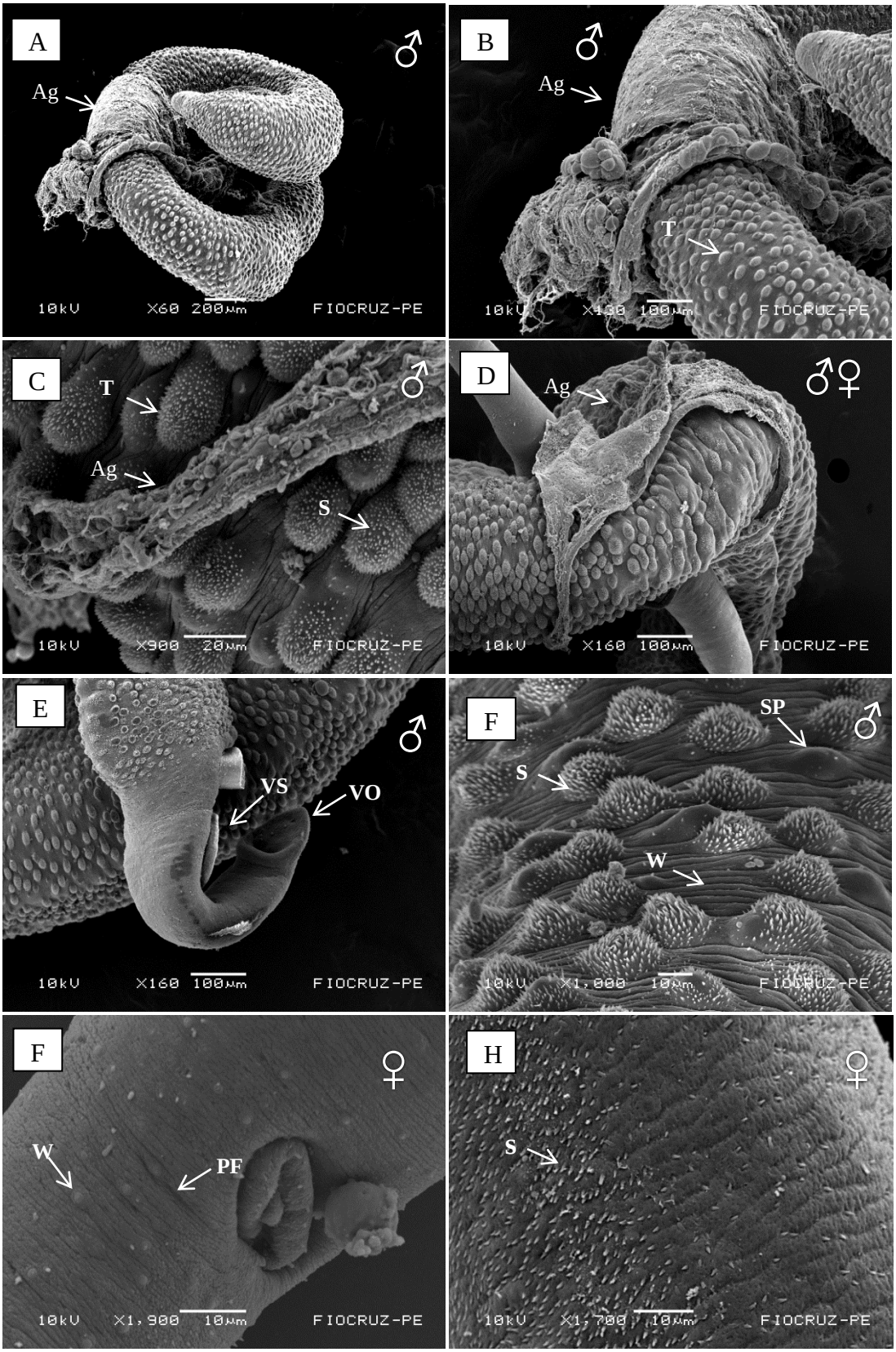

FIG. 2: Scanning electron micrographs of adult *S. mansoni* worms after treatment with 150 mg/Kg and 25 mg/Kg of LpQM-45 over a five day period. A-D: Worms treated with 150 mg/Kg of LpQM-45 showing numerous tubercles (T) and spines (S) no abnormalities and agglomeration of host cells (Ag) involving the male worm. E-H: Worms treated with 150 mg/Kg of LpQM-45 showing oral (OS) and ventral suckers (VS), spines (S), parallel wrinkles (W), sensory papillae (SP) and parallel fissures (PF) no abnormalities.

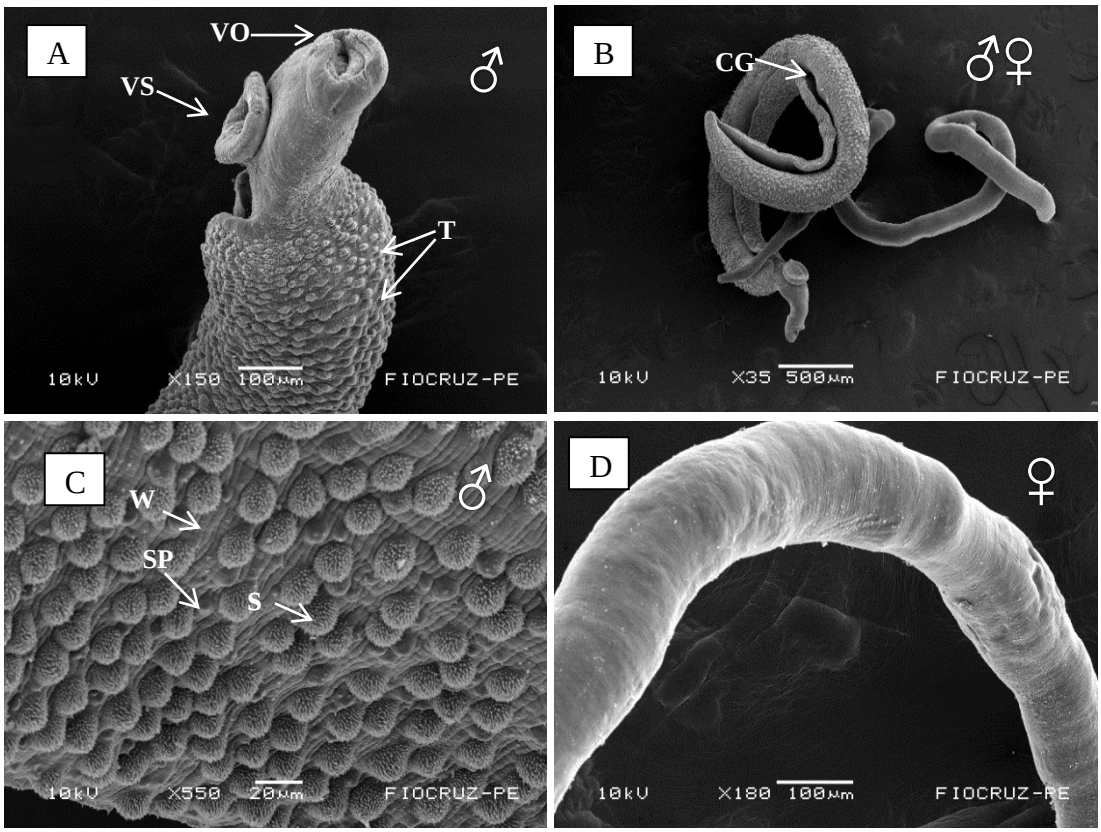

FIG. 3: Scanning electron micrographs of adult *S. mansoni* worms from the control group. A and C – Male worms showing oral (OS) and ventral suckers (VS), numerous tubercles (T) with spines (S), parallel wrinkles (W), sensory papillae (SP) no abnormalities. B – Male and female worms showing gynecophoral canal (GC). D - Female worms showing the integrity of the tegument.

DISCUSSION

Heterocyclic compounds have shown schistosomicidal activity (SOUZA, 2005; PEREIRA, 2010; OLIVEIRA, 2004) demonstrating to be a class of substances with good prospects in this field of study. Recently a screen heterocyclic compounds showed that the 2-(2-(2-(4-(4-methoxyphenyl)thiazol-2-yl)hydrazono)ethyl)isoindoline-1,3-dione (LpQM-45) demonstrated significant in vitro activity causing marked structural changes on the tegument of adult worms of *S. mansoni*. Here, in *in vivo* experiment, the compound LpQM-45 was tested orally and showed a moderate efficacy with 46% reduction in worm burden at a dose of 150 mg/kg compared with control group. Additional tests were realised as quantification of eggs in the liver and intestinal tissue, assessing the degree of maturation of eggs in the intestine and quantification of fibrous tissue in the liver, no significant difference was showing when compared to control.

In vivo study, orally, not having had such promising results regarding the in vitro study, does not prevent the said compound is active for other routes of administration such as intraperitoneal, an exemple is oxadiazoles and cysteine protease inhibitor, K11777 that showed significant activity when administered intraperitoneally.

Similar discrepancies as in this study were also observed in studies which used mefloquine against schistosomula and adult worms of *S. mansoni* (MANNECK et al. 2011). Studies conducted by Silva et al (2012) also showed discrepancies between in vitro and in vivo study, in which the LPSF-PT05, heterocyclic compound of five members, showed excellent in vitro activity, but had no significant activity *in vivo* test when diluted in tween 1% and saline. In an attempt to improve the solubility of the compound was prepared in emulsion oil/water (70:30). No improvement being observed in the activity of the compound, another alternative was to prepare a solid dispersion at 10% in polyethylene glycol (hydrophilic) diluted in water. This third formulation allowed to obtain a significant reduction in worm burden (SILVA, 2012); These results demonstrate the impact that the barriers in the absorption process have on the final outcome of the product and that dosage adjustments, or addition of adjuvants which promote increased solubility may lead to improvement efficacy of the product.

The action of the drug after administration is divided into three phases: pharmaceutical, pharmacokinetics and pharmacodynamics. The pharmacokinetic phase covers the processes of absorption, distribution, metabolism and excretion, causing a profound impact on the pharmacological effect since it determines the concentration and the time spent by the drug at its site of action (ABDEL-RAHMAN , 2004; PEREIRA, 2007). Traditionally, drug research starts from preliminary screening using *in vitro* models. From these studies, *in vivo* models are often employed with the administration of the compound to animals and observation the pharmacological effect. However, many compounds that exhibit good activity *in vitro* may not reproduce these results *in vivo* model. This difference could be associated with pharmacokinetic features of compounds that lead to decreased bioavailability, duration of acting very short or very long, or the presence of active or inactive metabolites (MASIMIREMBWA, 2003; PEREIRA, 2007).

CONCLUSIONS

Therefore, Taking into account the barriers in the process of absorption from oral administration and the other processes involved in pharmacokinetics phase, and on the results obtained in vitro and in vivo, the LpQM-45, undoubtedly, has potential as a schistosomicide. We believe that adjustments in dosing schedule, in the formulation or in route of administration may increase the schistosomicidal activity *in vivo* studies.

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ANEXO

Antimicrobial Agents and Chemotherapy

Evaluation of the Anti-Schistosoma mansoni Activity of Thiosemicarbazones and Thiazoles

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Evaluation of the Anti-*Schistosoma mansoni* Activity of Thiosemicarbazones and Thiazoles

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Schistosomiasis is a chronic and debilitating disease caused by a trematode of the genus *Schistosoma* and affects over 207 million people. Chemotherapy is the only immediate recourse for minimizing the prevalence of this disease and involves predominately the administration of a single drug, praziquantel (PZQ). Although PZQ has proven efficacy, there is a recognized need to develop new drugs as schistosomicides since studies have shown that repeated use of this drug in areas of endemicity may cause a temporary reduction in susceptibility in isolates of *Schistosoma mansoni*. Hydrazones, thiosemicarbazones, phthalimides, and thiazoles are thus regarded as privileged structures used for a broad spectrum of activities and are potential candidates for sources of new drug prototypes. The present study determined the *in vitro* schistosomicidal activity of 10 molecules containing these structures. During the assays, parameters such motility and mortality, oviposition, morphological changes in the tegument, cytotoxicity, and immunomodulatory activity caused by these compounds were evaluated. The results showed that compounds formed of thiazole and phthalimide led to higher mortality of worms, with a significant decline in motility, inhibition of pairing and oviposition, and a mortality rate of 100% starting from 144 h of exposure. These compounds also stimulated the production of nitric oxide and tumor necrosis factor alpha (TNF-α), thereby demonstrating the presence of immunomodulatory activity. The phthalyl thiazole LpQM-45 caused significant ultrastructural alterations, with destruction of the tegument in both male and female worms. According to the present study, phthalyl thiazole compounds possess antischistosomal activities and should form the basis for future experimental and clinical trials.

Schistosomiasis is a chronic and debilitating disease caused by a trematode of the genus *Schistosoma* and is one of the most prevalent and neglected diseases of tropical and subtropical regions. This parasitic disease ranks second after malaria in terms of its public health importance and has a significant economic and social impact. It is estimated that more than 207 million people have been infected worldwide, while 779 million people remain at risk of infection (1–6).

According to the World Health Organization, schistosomiasis is the cause of more than 200,000 deaths per year in sub-Saharan Africa, and this may still be an underestimate (5). *Schistosoma mansoni* is one of the most common etiological agents of human schistosomiasis, and the disease is triggered by the inflammatory granulomatous reaction that occurs during deposition of parasite eggs in the liver and other host tissues (7).

The eggs released during the progression of schistosomiasis produce antigens that induce a stronger Th2 response, leading to the formation of granulomas, whereas the parasite antigen induces a Th1 response, with a predominantly Th1 response being observed in the acute phase that is replaced by a Th2 immune response upon egg antigen production (8–10).

Current schistosomiasis treatment is based on the use of praziquantel (PZQ), a pirazylisoquinoline, which is effective against all *Schistosoma* species infecting humans (11, 12) and has been successfully used over the last 20 years as the drug of choice in most areas where the disease is endemic (3, 13, 14). Even though PZQ the antihelminthic drug of choice and despite its advantages, which include tolerability, safety, efficacy, and low cost, PZQ does not protect individuals from reinfection and is not active against the immature stages of the worm, such as the schistosomula and preadult and juvenile adult stages (13, 15).

Furthermore, the appearance of drug-resistant strains of *Schistosoma* is a constant concern for public health authorities (16–18). Hence, the massive use of PZQ in zones of endemicity with the possibility of the emergence of drug-resistant *Schistosoma*, combined with the lack of any other effective antischistosomal drug, requires new effective schistosomicidal compounds to be developed and further studies to be carried out with a view to developing alternative therapies that could either replace or complement the use of PZQ for treatment of *S. mansoni* infection (4, 13).

It is well known that hydrazones, thiosemicarbazones, and phthalimides as well as thiazoles are considered privileged structures as leads in medicinal chemistry (19–21). These core structures have figured prominently in a vast number of structural subunits used for a broad spectrum of activities, and the mode of action of the nuclei of these pharmacophores is usually attributed to the inhibition of multiple targets. For example, hydrazones and thiosemicarbazones have been shown to possess antimicrobial,

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anticonvulsant, analgesic, anti-inflammatory, antiplatelet, antitubercular, and antitumor properties, among others (22).

Likewise, hydrazones, thiazolidinone, and their bioisoster thiazole derivatives are known for their potential pharmaceutical applications and possess antimicrobial (23), antischistosomal (24), antifungal (25), antimalarial (26), herbicidal (27), antiviral (28), antidiabetic (29), and antioxidant (30) properties. Thiazole derivatives have been used to prepare various drugs that are important for antimicrobial (31), antibacterial (32, 33), antifungal (32), anti-inflammatory (34), and antitubercular (35) treatment, and some of the thiazole derivatives are used as antiprotozoals (36).

On the other hand, phthalimide derivatives, such as thalidomide, are known for their immunomodulatory activities, inhibiting the cytokine tumor necrosis factor alpha (TNF-α), interleukins-1β (IL-1β), IL-6, and IL-12, and granulocyte-macrophage colony-stimulating factor. They also activate the Th1 response, by increasing gamma interferon (IFN-γ) and IL-2, have antiangiogenic and antiproliferative properties, activate apoptosis, T cells, and NK cells, and inhibit cell adhesion (37, 38).

With this in mind, we performed a synthesis of a set of molecules whose structures have a hydrazine and/or thiazole nucleus as a common group. With a view to ascertaining whether the new thiosemicarbazone, phthalyl thiosemicarbazone, phthalyl thiazole, and phthalyl thiazolidinone pharmacophores are an essential requirement for antischistosomal activity, 10 compounds were synthesized, and their antischistosomal potentials were determined.

The efficacy of the compounds was examined in terms of (i) schistosome survival, (ii) egg output (oviposition), (iii) motor activity, (iv) ultrastructural alterations in the tegument of *S. mansoni* as determined by scanning electron microscopy (SEM), and (v) cytotoxicity and immunomodulatory activity induced by these new compounds on splenocytes and macrophages, respectively.

MATERIALS AND METHODS

Compounds. The compounds 2-(1-phenoxypropan-2-ylideno)thiosemicarbazide (LpQM-01), 2-(1-phenoxypropan-2-ylideno)-4-phenylthiosemicarbazide (LpQM-02), and 2-(1-phenoxypropan-2-ylideno)-4-methylthiosemicarbazide (LpQM-03) were prepared as described by Moreira et al. (39). The compounds 2-(2-(1,3-dioxoisoindolin-2-yl)ethylidene)-1-methylthiosemicarbazide (LpQM-38), (3-methyl-4-oxothiazolidin-2-ylidene)hydrazono)ethyl) isoindoline-1,3-dione (LpQM-40), 2-(2-(2-(4-(4-fluorophenyl)thiazol-2-yl) hydrazono)ethyl)isoindoline-1,3-dione(LpQM-43), 2-(2-(2-(4-(4-methoxyphenyl)thiazol-2-yl)hydrazono)ethyl)isoindoline-1,3-dione (LpQM-45), and 2-(2-(2-(4-(4-chlorophenyl)thiazol-2-yl)hydrazono)ethyl)isoindoline-1,3-dione (LpQM-47) were prepared as described by Pessoa et al. (40); compounds 2-(2-(1-(3-bromophenyl)propylidene)hydrazinyl)-4-methoxyphenyl)thiazole (LpQM-14) and 2-(2-(1-(3-bromophenyl)propylidene)hydrazinyl)-4-(4-nitrophenyl)thiazole (LpQM-17) were also used (C. L. Leite and P. A. T. Gomes, unpublished data). All compounds were chemically characterized by nuclear magnetic resonance (NMR), infrared, and mass spectra and by elemental analysis and presented purity of >95%. PZQ (catalog no., 4668; bench, BCBD3257V) was purchased from Sigma-Aldrich (St. Louis, MO, USA).

Parasites and intermediary and definitive hosts. The LE strains (Belo Horizonte, Minas Gerais, Brazil) of *S. mansoni* were used throughout this study. These strains were maintained in *Biomphalaria glabrata* snails and Swiss mice, in the laboratory at the Aggeu Magalhães Research Center (CPqAM) of the Oswaldo Cruz Foundation (FIOCRUZ, PE, Brazil).

Female Swiss mice weighing 20 ± 2 g were used as the definitive host and were infected transcutaneously with about 120 cercariae (LE strain). The animals were kept in a controlled temperature and light environment and had access to food and water *ad libitum*. After 55 days of infection, adult *S. mansoni* specimens were recovered from the mice by perfusion, using the technique developed by Duvall and De Witt (41).The experiments were approved by the Ethics Committee on Animal Use (CEUA), FIOCRUZ (process number 22/2011).

***In vitro* assay.** *S. mansoni* worms harvested from Swiss mice were kept in RPMI 1640 medium (Sigma-Aldrich, St. Louis, MO, USA) buffered to pH 7.5, supplemented with HEPES (20 mM), 10% fetal bovine serum, penicillin (100 U/ml), and streptomycin (100 $\mu g \cdot ml^{-1}$). Incubation was carried out at 37°C in a humid atmosphere containing 5% CO_2 gas. LpQM-43, LpQM-45, LpQM-47, and LpQM-14 compounds were dissolved in 1.6% dimethyl sulfoxide (DMSO) and used in concentrations varying from 40 to 100 $\mu g \cdot ml^{-1}$; compounds were added to the medium containing the worms after a 2-h period of adaptation to the culture medium. One pair of adult worms/well was used in this study. The control worms were assayed in RPMI 1640 medium with 1.6% DMSO as a negative-control group. All experiments were carried out in five replicates and were repeated at least three times. The motor activity, egg output (oviposition), tegumental alterations, and survival of the parasites were monitored every 24 h for 192 h using an inverted microscope (SMZ 1000; Nikon).

SEM. Male and female worms after *in vitro* exposure to LpQM-45 compound over a period of 24 and 48 h were fixed overnight at room temperature with 2.5% glutaraldehyde, 4% formaldehyde, and 0.1 M cacodylate buffer at pH 6.8. They were then postfixed in 2% osmium tetroxide (OsO_4) in a 0.1 M cacodylate buffer at pH 6.8 for 60 min in the absence of light at room temperature. The next steps included washing and dehydration in a graded ethanol series for 15 min each. The worms were critical-point dried using liquid CO_2, directly sputter coated with colloidal gold for 1 min, and examined under a JEOL-5600LV microscope.

Animals used for cytotoxicity and immunological assays. Male BALB/c mice (6 to 8 weeks old) were raised at the animal facility of the Oswaldo Cruz Foundation (Rio de Janeiro, Brazil) and maintained at the animal facility of the Aggeu Magalhães Research Center, Oswaldo Cruz Foundation, in Recife, Brazil. All mice were euthanized, and their spleens were removed in accordance with the guidelines of the Oswaldo Cruz Foundation Commission for Experiments with Laboratory Animals (Ministry of Health, Brazil, 0266/05).

Spleen cell harvesting. Spleen cells were harvested according to a previous protocol (42). After the BALB/c mice were euthanized with CO_2 gas, the spleen of each mouse was removed aseptically and placed in a Falcon tube containing incomplete RPMI 1640 medium with fetal calf serum (complete medium). In a vertical flow, each spleen was transferred to a petri dish where it was soaked. The cell suspensions obtained were transferred to Falcon tubes containing approximately 10 ml of incomplete medium per spleen and centrifuged at 4°C and 200 × *g* for 5 min. After the supernatant was discarded, distilled water was added to the sediment to trigger red blood cell lysis. The supernatant (containing no cell debris) was collected and centrifuged at 4°C and 200 × *g* for 5 min. The resulting sediment (containing cells) was resuspended in complete RPMI 1640 medium. An aliquot of each cell suspension was separated and diluted in trypan blue for quantification in a Neubauer chamber, and the viability of cells was determined.

***In vitro* cytotoxicity assay.** Spleen cells (6×10^5 cells/well), obtained as described in the previous paragraph, were cultured in 96-well plates containing RPMI 1640 medium. These cells were incubated with the compounds at six concentrations (1, 5, 10, 25, 50, and 100 $\mu g \cdot ml^{-1}$) in the presence of [^{3}H]thymidine (Amersham Biosciences, USA) (1 $\mu Ci \cdot well^{-1}$) for 24 h at 37°C and 5% CO_2. Cells treated with saponin (0.05%) were used as a positive control, and cells treated with DMSO (1%) were used as a negative control. Each drug was tested in triplicate.

The contents of the plate were then harvested to determine [^{3}H]thymidine incorporation using a beta-radiation counter (Wallac 1209; Rackbeta Pharmacia, Stockholm, Sweden). Compound toxicity was determined by comparing the percentage of [^{3}H]thymidine incorporation (as an indicator of cell viability) in treated cells with that in untreated cells. Noncytotoxic concentrations were defined as those where [^{3}H]thymidine incorporation was

30% lower than the level in untreated controls. Six concentrations were also used for PZQ (1, 5, 10, 25, 50, and 100 $\mu g \cdot ml^{-1}$).

Measurement of cytokine levels in macrophage supernatants. Cytokines were quantified in supernatants of macrophage cultures treated *in vitro* after 48 h and 72 h with LpQM-43, LpQM-45, LpQM-47, LpQM-14, and PZQ at 56, 58, 55, 50, and 51 $\mu g \cdot ml^{-1}$, respectively (50% cytotoxic concentration [CC_{50}] in macrophages). As a positive control, cells were stimulated with the mitogens lipopolysaccharide (LPS at 50 $\mu g \cdot ml^{-1}$) and concanavalin A (ConA at 2.5 $\mu g \cdot ml^{-1}$), while for the negative controls, cells did not receive either mitogen or drugs. The levels of the IL-6, IL-10, IL-12, and TNF-α cytokines were measured using sandwich enzyme-linked immunosorbent assays (ELISAs), according to the manufacturer's suggested protocols. The monoclonal antibodies used were the OptEIA (BD Biosciences) kit, and these were used after titration. Plates with 96 wells (NalgeNunc International Corp.) were sensitized with specific anticytokine antibodies (according to the manufacturer's instructions) and incubated overnight at 4°C. Cytokine standards were added after serial dilutions from their initial concentrations (16,000 $pg \cdot ml^{-1}$). After a washing step, 50 μl of all samples and standards was added in duplicate, and the plate was incubated for 2 h at room temperature. The specific antibodies were then combined with biotin (according to the manufacturer's instructions) and incubated for 1 h 30 min at room temperature. Revealer solution containing 2,2-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt (ABTS) was added. The reaction was blocked with 1 M sulfuric acid, and the reading was carried out on a spectrophotometer (3550; Bio-Rad, Hercules, CA) at 415 nm. Sample concentrations were calculated in the linear region of the titration curve of cytokine standards, and final concentrations were expressed in $pg \cdot ml^{-1}$, using Microplate Manager, version 4.0, software (Bio-Rad Laboratories).

***In vitro* nitrite analysis.** Nitric oxide (NO) production was measured as nitrite (a stable breakdown product of NO) accumulated in the supernatant of the macrophage culture stimulated with LpQM-43, LpQM-45, LpQM-47, LpQM-14, and PZQ at 56, 58, 55, 50, and 51 $\mu g \cdot ml^{-1}$, respectively (the 50% cytotoxic concentration [CC_{50}] in the macrophages) Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% heat-inactivated fetal bovine serum and LPS (50 $ng \cdot ml^{-1}$) were used as positive and negative controls, respectively.

The Griess indirect method (43) was used, and nitrite levels were quantified using 50 μl of each supernatant and an equal volume of Griess reagent [1% sulfanilamide, 0.1% dihydrochloride of *N*-(1-naphthyl)-ethylenediamine, 2.5% H_3PO_4], and samples were incubated at room temperature for 10 min. Absorbance was measured on a 540-nm reader (Multiskan FC; Thermo Scientific) at 540 nm, and the nitrite levels in each sample after 24 h, 48 h, and 72 h were determined by extrapolation from a previously determined standard curve.

Statistical analysis. The differences between groups were analyzed using Mann-Whitney *U* and Dunnett's nonparametric tests. All results are expressed as mean values of groups ± standard deviations, and a *P* value of <0.05 was taken to be statistically significant.

RESULTS

Effect of new compounds on adult *S. mansoni* survival. Ten derivatives containing the pharmacophore phthalimide, thiazole, thiazolidinone, and thiosemicarbazone nuclei were tested for schistosomicidal properties (Table 1). Compounds were evaluated at a concentration of 40 to 100 $\mu g \cdot ml^{-1}$ every 24 h for a period of 192 h, and mortality, motility, and alterations in the integument of the worms were observed. The mortality after 144 h of exposure at a concentration of 100 $\mu g \cdot ml^{-1}$ was initially used to perform a screening of compounds. Of the tested compounds, those formed of thiazole and phthalimide led to higher mortality among worms (Fig. 1). One exception was LpQM-17, a thiazole derivative that did not kill worms under the conditions cited. We investigated the action of four compounds, LpQM-43, LpQM-45, LpQM-47, and LpQM-14, in greater detail, examining the effect of these compounds on the mortality rate with respect to concentration and incubation time. Other factors evaluated included changes in motility and integument and the 50% inhibitory concentration (IC_{50}; 50% mortality).

All compounds were tested in concentrations of 5, 10, and 20 $\mu g \cdot ml^{-1}$, but they did not cause worm mortality in these concentrations (data not shown). Because of this, only concentrations above 40 $\mu g \cdot ml^{-1}$ are described in Table 2. The phthalyl thiazoles LpQM-45 and LpQM-14 caused 100% worm mortality at concentrations of 100 and 80 $\mu g \cdot ml^{-1}$ within 144 and 168 h, respectively. Similarly, the phthalyl thiazoles LpQM-43 and LpQM-47 caused 67% and 95% worm mortality, respectively, in 192 h at a concentration of 100 $\mu g \cdot ml^{-1}$ (Table 2). Interestingly, oviposition by adult worms was not seen with any of the four compounds examined. All of the worms in the control group remained viable until the end of the experiment.

As can be seen in Table 3, there was a significant reduction in motility under the treatment with the LpQM-45 compound at all concentrations. The LpQM-47 compound brought about a reduction in motility at all concentrations, and LpQM-14 reduced motility at 60 to 100 $\mu g \cdot ml^{-1}$. LpQM-43 caused only a partial reduction in worm motility. Many physiological alterations were observed in adult worms exposed to the new compounds (Table 2). The IC_{50}, the concentration of compound required to cause 50% mortality of worms, was another parameter used to evaluate schistosomicidal activity, and the results are shown in Table 2.

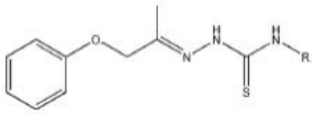
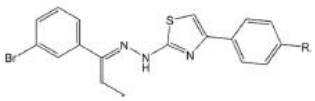
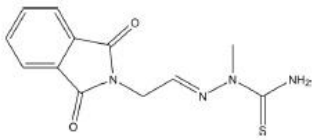
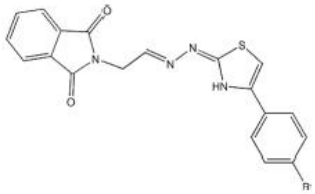
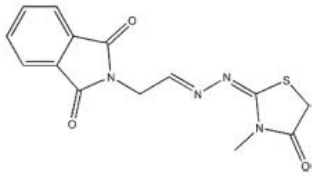
The activity of compounds was evaluated after 120 h of exposure in terms of changes in motility and the tegument of the worms and according to the criteria established by Ramirez et al. (44). According to these criteria, LpQM-43 was considered partially active at a concentration range of 40 to 100 $\mu g \cdot ml^{-1}$, while LpQM-45 and LpQM-47 were considered active at a concentration of 40 to 100 $\mu g \cdot ml^{-1}$, and LpQM-14 was active at a concentration of 60 to 100 $\mu g \cdot ml^{-1}$ and partially active at a concentration of 40 $\mu g \cdot ml^{-1}$.

Scanning electron microscope examination. The compound LpQM-45 caused 100% worm mortality, and it was the most potent of the compounds; therefore, we studied its effects on the worm morphology.

The tegument surface of male and female *S. mansoni* worms after *in vitro* exposure to LpQM-45 for periods of 24 and 48 h was examined using scanning electron microscopy (SEM). The worms exposed to DMSO and to medium alone (controls) were also examined using SEM. The control male and female worms that were not exposed to any drugs (negative controls) presented normal surface membrane topography. The male worms exhibited a large number of tubercles with typical spines, sensory papillae, oral and ventral suckers, and no abnormality, and, in the anterior part of the body, the gynecophoral canal showed no abnormality after 24 and 48 h (Fig. 2A to D). In the female worms, parallel fissures, tegument spines, and sensory papillae with no abnormality were observed at 24 and 48 h (Fig. 2E to H).

Exposure of the worms to compound LpQM-45 resulted in ultrastructural alterations, which were already apparent during the first period of exposure (24 h), revealing a variety of changes in the tegument surface. In the male worm, complete destruction of some tubercles was found, with extensive sloughing and exposure of the subtegument layer of muscle tissue (Fig. 3A to D). The

TABLE 1 Structure of new thiosemicarbazone analogs used in this study

Analog	Structure	Compound	Identification of R
Phenoxy-thiosemicarbazones		LpQM-01	—H
		LpQM-02	
		LpQM-03	—CH_3
Phenyl-thiazoles		LpQM-14	—O—CH_3
		LpQM-17	—NO_2
Phthalyl-thiosemicarbazone		LpQM-38	
Phthalyl-thiazoles		LpQM-47	—Cl
		LpQM-43	—F
		LpQM-45	—O—CH^3
Phthalyl-thiazolidinone		LpQM-40	

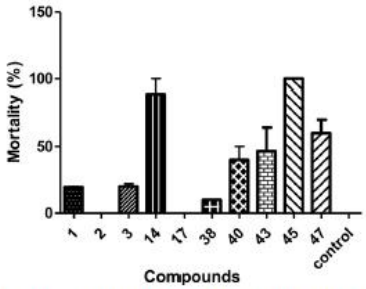


FIG 1 Effects of LpQM compounds on the mortality rate of *S. mansoni* at a concentration of 100 μg · ml^{-1} after 144 h. Compounds are indicated by the numerical suffix following LpQM.

severity of tegument damage was higher in the males after 48 h of exposure, with an increasing number of tubercles completely destructed or eroded, a roughened surface in the same areas, and disintegration of the tegument in this area, as revealed by higher magnification (Fig. 3E to H).

The female worms exposed to LpQM-45 showed serious damage, with extensive sloughing and disintegration of the tegument and exposure and injury of the layer of muscle tissue after 24 and 48 h (Fig. 4).

Cytotoxic activity in splenocytes. After determining the antiparasitic activity against *S. mansoni* worms, we determined the cytotoxicity in splenocytes of BALB/c mice for the most potent antiparasitic compounds. The evaluation of cytotoxic compounds showed that LpQM-43, LpQM-45, and LpQM-47 presented nontoxic effects at concentrations up to 100 μg · ml^{-1}, while LpQM-14 presented nontoxic effects at concentrations up to 25 μg · ml^{-1} (Table 2). On the other hand, PZQ showed higher toxicity in splenocytes (<1 μg · ml^{-1}) at all concentrations tested.

TABLE 2 *In vitro* effects of LpQM-43, LpQM-45, LpQM-47, and LpQM-14 against adult worms of *S. mansoni*

Drug	Time (h)	Mortality (%) at the indicated concn (μg/ml)				IC_{50} (μg/ml)	Cytotoxicity (μg/ml)[a]	Worm characteristic(s) observed
		100	80	60	40			
LpQM-43	24	13	0	7	0	82.24	>100	Not paired
	48	23	3	10	0	84.13		No sucker adherence
	72	30	7	10	0	83.53		Absence of eggs
	96	37	13	16	0	73.65		Nontransparent blackish tegument
	120	40	20	24	0	64.13		
	144	47	37	29	0	58.01		
	168	60	40	45	0	55.83		
	192	67	53	56	53	32.93		
LpQM-45	24	5	36	9	32	46.20	>100	Not paired
	48	40	41	28	41	19.97		No sucker adherence
	72	60	60	57	51	26.36		Absence of eggs
	96	70	80	62	56	31.53		Integument morphology altered (nontransparent blackish tegument, appearance of bubbles)
	120	90	95	76	61	33.78		
	144	100	100	90	70	32.09		
	168	100	100	90	85	25.88		
	192	100	100	90	95	24.69		
LpQM-47	24	5	9	10	5	40.08	>100	Not paired
	48	5	9	19	15	51.38		No sucker adherence
	72	15	25	39	21	41.90		Absence of eggs
	96	20	29	53	44	37.48		Integument morphology altered (nontransparent, blackish tegument, appearance of bubbles)
	120	40	46	66	66	23.78		
	144	60	54	74	73	24.92		
	168	80	58	88	83	26.97		
	192	95	58	91	88	30.95		
LpQM-14	24	5	0	5	0	65.70	25	Not paired
	48	25	0	20	0	72.77		No sucker adherence
	72	50	10	25	0	76.17		Absence of eggs
	96	50	40	25	5	60.15		Integument morphology altered (nontransparent, blackish tegument, appearance of bubbles)
	120	78	75	39	22	53.95		
	144	89	95	52	37	48.06		
	168	100	100	57	47	43.41		
	192	100	100	67	61	33.52		

[a] The highest nontoxic concentration on spleen cells of BALB/c mice. Saponin (<1.0 μg/ml) was used as a positive control.

Immunomodulatory activity in macrophages treated with the compounds. The ability of the compounds to stimulate the secretion of IL-6, IL-10, IL-12, and TNF-α was investigated through the measurement of these cytokines in the supernatants of macrophage cultures. Results are shown in Fig. 5. In comparison to the untreated cells (negative control), a significant ($P < 0.001$) production of TNF-α was observed after 48 h of treatment with the compound LpQM-47. A similar finding was observed for PZQ-treated cells. After 72 h of treatment, compounds LpQM-43, LpQM-45, and LpQM-47 caused significant TNF-α secretion. In contrast, compound LpQM-14 caused significant TNF-α secretion only after 72 h of treat-

TABLE 3 Motility score of control and worms treated with PZQ and LpQM-43, LpQM-45, LpQM-47, and LpQM-14 for 120 h

Group	Percentage of worms by motility score after drug treatment at:[a]															
	100 μg/ml				80 μg/ml				60 μg/ml				40 μg/ml			
	3	2	1	0	3	2	1	0	3	2	1	0	3	2	1	0
Control	100	0	0	0	100	0	0	0	100	0	0	0	100	0	0	0
PZQ	0	0	0	100	0	0	0	100	0	0	0	100	0	0	0	100
SC-43	17	29	17	38	3	50	27	20	13	46	18	23	4	48	10	38
SC-45	0	0	10	90	0	5	0	95	0	5	19	76	0	20	19	61
SC-47	5	10	45	40	8	13	34	45	0	4	30	66	0	13	21	66
PT-1.4	5	11	6	78	0	20	5	75	0	23	39	39	16	42	20	22

[a] The measurement of mean worm motility is scored on a scale of 0 to 3 as follows: 3, normally active; 2, slowed activity; 1, minimal activity with occasional movement of head and tail and absence of motility apart from gut movements; 0, total absence of mobility.

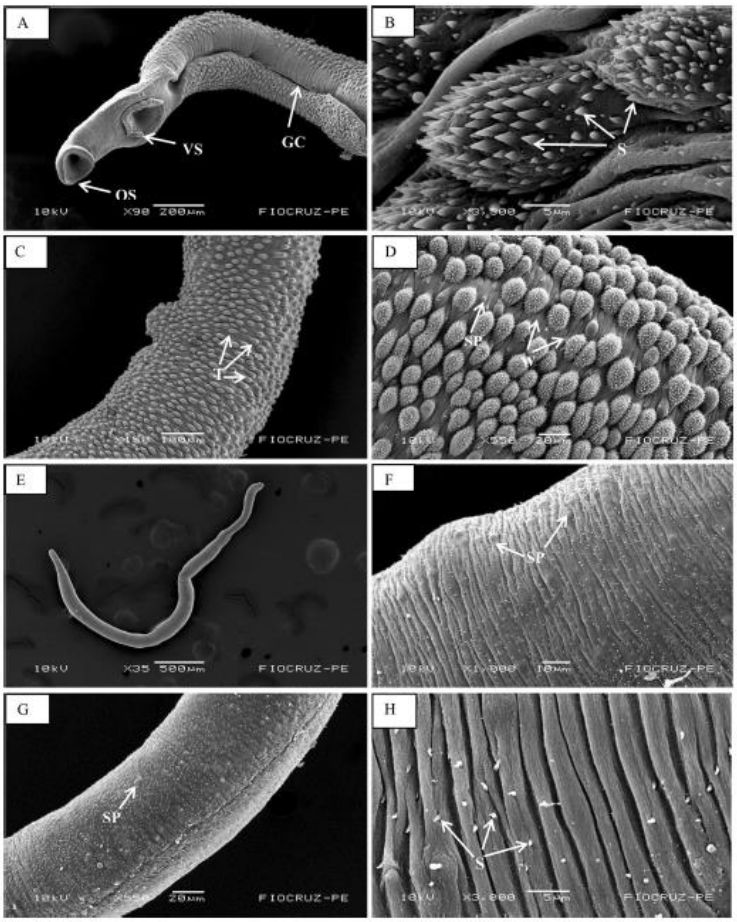


FIG 2 Scanning electron micrographs of adult male and female *S. mansoni* worms from the control group. (A) Male worms kept in medium and DMSO after 24 h, showing the anterior portion of the body with oral (OS) and ventral suckers (VS) and the gynecophoral canal (GC) with no abnormalities. (B) Male worms kept in medium and DMSO after 48 h, showing in detail numerous spines (S) covering the tubercules (T). (C) The dorsal region of male worms kept in medium alone after 48 h, showing numerous tubercles distributed along the body. (D) Male worms kept in medium only after 24 h, showing in detail the dorsal region with sensory papillae (SP) and parallel wrinkles (W) visible. (E) Female worms kept in medium and DMSO after 24 h, showing the whole extent of the body. (F) Female worms kept in medium and DMSO after 48 h, showing the sensory papillae (SP). (G) Female worms kept in medium alone showing the integrity of the tegument. (H) Female worms kept in medium alone, showing spines (S) in detail.

ment. In addition, we observed that the TNF-α content under treatment with compound LpQM-14 was quite similar to that observed for LPS-stimulated cells (positive control), indicating that this compound substantially modulates the immune response. After the TNF-α content was measured, the secretion of IL-6, IL-10, and IL-12 was evaluated under compound treatment. However, in comparison to the LPS-stimulated cells (positive control), none the compounds induced the secretion of IL-6, IL-10, and IL-12 in macrophages until 72 h after drug incubation (data not shown).

NO content in macrophages. In another set of experiments, the nitrite content was determined in the supernatant of macrophages treated with the drugs LpQM-43, LpQM-45, LpQM-47, and LpQM-14. LPS-treated cells (positive control) and untreated cells (negative control) were included in the experiment (Fig. 6). In comparison to untreated cells, the production of nitrite was significantly higher under compound LpQM-14 treatment, and this was observed during the three data points (24, 48, and 72 h after incubation). In contrast, compound LpQM-45 produced a statistically significant amount of nitrite only at a time point under 48 h after incubation, while PZQ had no effect on the nitrite production.

DISCUSSION

The 10 tested compounds all had a phthalimide nucleus, a hydrazone moiety, and/or a thiazole ring system. In general, compounds that possessed a phthalimide and thiazole ring system showed significant

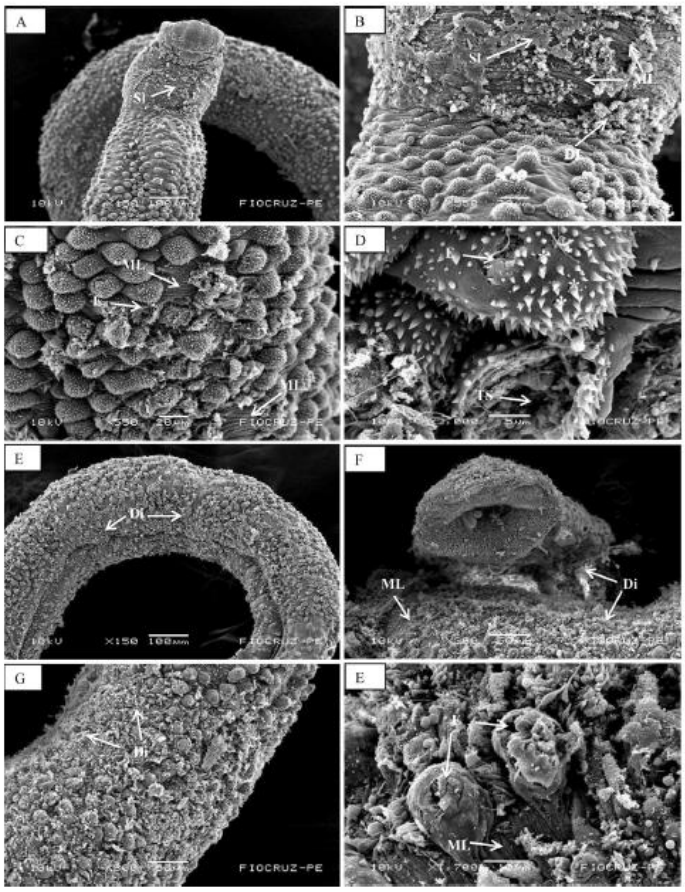


FIG 3 Scanning electron micrographs of adult male *S. mansoni* worms exposed to 80 $\mu g \cdot ml^{-1}$ LpQM-45. (A to D) Worms after 24 h of incubation showing extensive sloughing (Sl), erosion (E), loss of spines (*) and disintegration (Di) of tegument with exposure of subtegumental tissue (TS) and muscle layer (ML). (E to H) Worms after 48 h of incubation showing a greater area of erosion (E) and disintegration (Di) of the tegument and exposure of muscle layer (ML).

antiparasitic activity against *S. mansoni* worms. No antiparasitic activity was observed in the case of LpQM-17, a thiazole derivative that also has a nitro group in the 4-position of the phenyl ring. The cytotoxicity in mouse cells of PZQ was greater than that of any of the tested compounds that showed anti-*S. mansoni* activity; therefore, the compounds described here were more selective. In terms of the oviposition profile of adult worms (absence), the tested compounds exhibited activity similar to that observed for PZQ.

The thiazole nucleus, which is present in an important class of heterocyclic compounds present in many biologically potent active molecules (45), is also present in the most active compounds of the whole series. Some thiazoles have, in fact, been shown in the literature as schistosomicidal agents (19, 46). The phthalyl thiazoles LpQM-43, LpQM-45, and LpQM-47 and the thiazole LpQM-14 have been shown to have antischistosomal properties, with the IC_{50}s ranging from 82.24 to 32.93 $\mu g \cdot ml^{-1}$, 46.20 to 24.69 $\mu g \cdot ml^{-1}$, 40.08 to 30.95 $\mu g \cdot ml^{-1}$, and 65.70 to 33.52 $\mu g \cdot ml^{-1}$, respectively. These results suggest that the efficacy varies according to the substituent in the 4-position of the phenyl group. LpQM-45 exhibited the best schistosomicidal properties in relation to other compounds, with 100% mortality within 144 h at concentrations of 100 and 80 $\mu g \cdot ml^{-1}$. LpQM-14 also showed significant schistosomicidal activity, with 100% mortality within 168 h at concentrations of 100 and 80 $\mu g \cdot ml^{-1}$.

The compounds LpQM-14 and LpQM-45 have in common a methoxyl group attached in the 4-position of the phenyl ring.

In comparison to the literature findings, it was also observed that heterocyclic compounds containing a methoxyl group exhibited higher schistosomicidal activity than compounds without this group (47). Another interesting structure-activity relationship is observed for the compound LpQM-47, which produced a mortality rate of 95% at 100 $\mu g \cdot ml^{-1}$ and 91% at 60 $\mu g \cdot ml^{-1}$ after 192 h of incubation. This compound has a chloro atom attached to the 4-position of the phenyl ring. In agreement with this, it is described in the literature that the attachment of a chloro atom im-

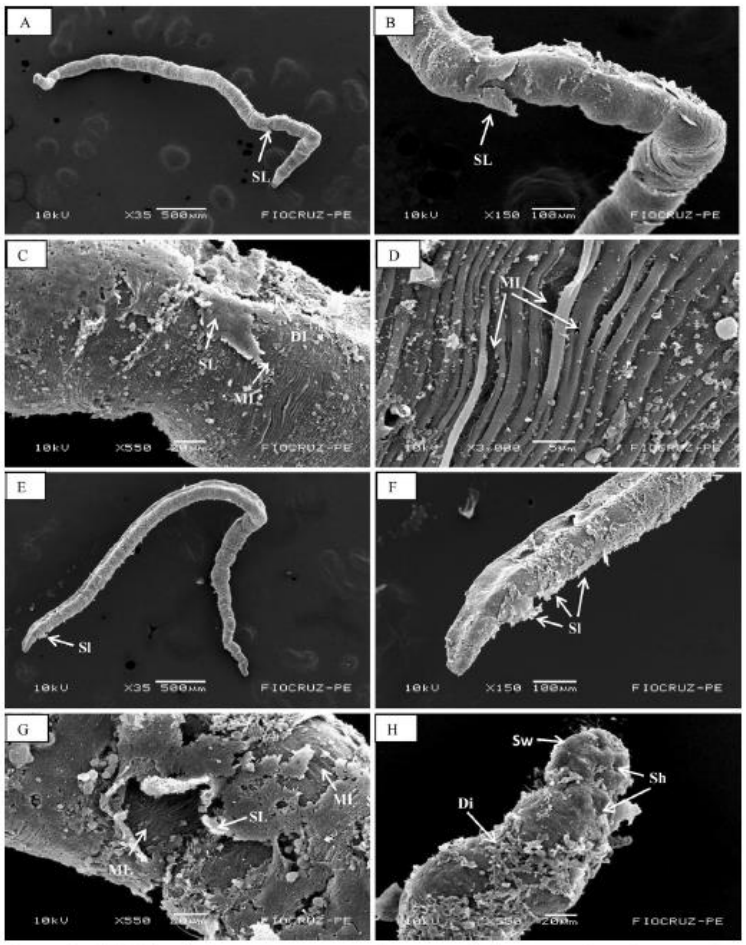


FIG 4 Scanning electron micrograph of adult female *S. mansoni* worms exposed to 80 $\mu g \cdot ml^{-1}$ LpQM-45. (A to D) Worms after 24 h of incubation showing sloughing (Sl), disintegration (Di) of tegument, and exposure of subtegumental muscle layer (ML) with muscle damage (MI). (E to H) Worms after 48 h of incubation showing sloughing (Sl), disintegration (Di) of the tegument with exposure of muscle layer (ML), swelling (Sw), and shrinking (Sh).

proves the schistosomicidal activity for 2-thioxoimidazolidin-4-one compounds (48).

Another common feature of all active compounds described here is the presence of a hydrazone moiety. Some 9-acridanone hydrazones were found to be effective against *S. mansoni* in mice, killing almost all of the skin schistosomules, when administered at a dose of 100 mg/kg (49). In another study, 9-acridanones derived from thiazoles were effective against *S. mansoni* in the skin phase, killing almost all of the parasites in mice at a dose of 100 mg/kg, 24 h after penetration by cercariae. This same study showed that when the compound is administered to monkeys at a dose of 25 mg/kg, worms and eggs are absent from liver tissue and rectal mucosa 7 days after infection, which constitutes cure (20).

Detailed microscopic observation showed that LpQM-43, LpQM-45, LpQM-47, and LpQM-14 molecules caused alterations in the teguments of the worms compared with the untreated control group. These compounds also appear to influence the oviposition of parasites since no eggs were found in the culture medium.

The thick tegument that covers the entire surface of schistosomes is an important target for drugs because the functioning of the surface membrane and the integrity of the tegument are critical for the survival and proliferation of the *Schistosoma* parasite (13). These structures play vital roles in the immune evasion, nutrient absorption, and cholesterol metabolism of the host (13, 50). Alterations in the surface ultrastructure of schistosome worms have been investigated by a number of authors in order to evaluate antischistosomal drugs (13, 24, 50, 51, 52, 53). The present study thus examined the surface topography of male and female worms to determine the schistosomicidal effect of LpQM-45. We found extensive damage to the tegument in both male and female worms after 24 and 48 h of exposure.

SEM analysis revealed progressive damage to the tegument

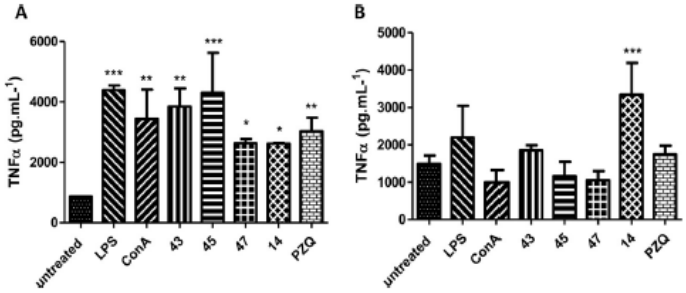


FIG 5 TNF-α production in supernatants of the macrophage culture in the presence of LpQM-43,-45,-47, and -14 and PZQ at 56, 58, 55, 50, and 51 μg · ml^{-1}, respectively. Assays were performed at 48 h (A) and 72 h (B). The horizontal bars represent median values. *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$.

surface, causing destruction of tubercles in the male, extensive sloughing with disintegration of the tegument in the same areas, and exposure of subtegumental tissue and the layer of muscle tissue in both male and female worms. Similar changes occurred in response to different drugs (13, 50). For instance, Manneck et al. (50) have presented a detailed study of tegument surface alterations caused by 100 μg · ml^{-1} of mefloquine in *S. mansoni* worms, finding in females extensive sloughing, with the base membrane exposed along with roughened teguments which have already started to disintegrate. The males showed a roughened surface with disintegration of the tegument, resulting in a fibrous appearance, loss of tubercles, spines, and parallel wrinkles. Bertão et al. (13) also found severe damage to the surface of adult male schistosomes caused by exposure to miltefosine, which was characterized by peeling of the tegument, reduction in the size of the spine, erosion, the formation and rupturing of blisters, and the emergence of holes with exposure of layers of muscle tissue.

After 3 h of exposure, PZQ cause severe muscle contraction; the worms became curved, resulting in a decrease in body size (13, 54). In contrast, LpQM-45 caused severe damage to the surface of the worm but no muscle contraction. The lesions were more numerous in parasites exposed to LpQM-45 than in those treated with PZQ. Similarly, miltefosine (13) and thioxoimidazolidine (54) have also been shown to be more effective than PZQ in causing tegument damage in *S. mansoni*. In terms of differences between the changes in males and females, it was noted that the tegument of female worms was slightly more affected and that mefloquine (13) and artemether (55) likewise tend to have a greater effect on females.

The outer membrane and lipid bilayers of *S. mansoni* worms proved to be extremely sensitive to LpQM-45. The morphological

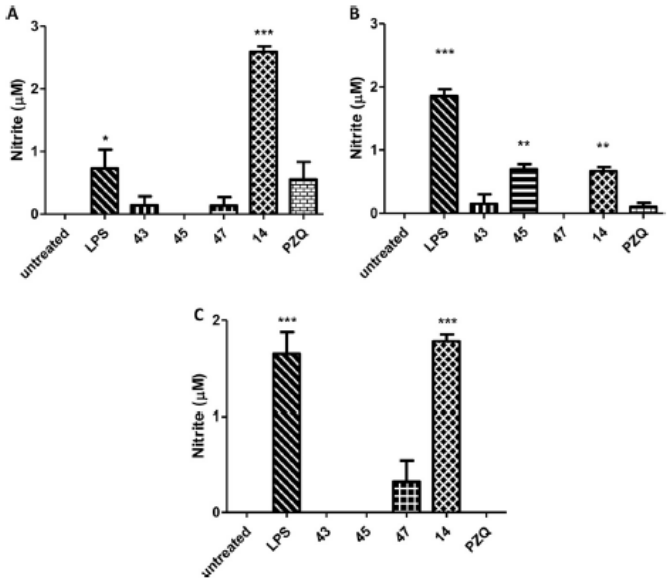


FIG 6 Nitrite production in supernatants of the macrophage culture in the presence of LpQM-43, -45, -47, and -14 and PZQ at 56, 58, 55, 50, and 51 μg · ml^{-1}, respectively. Assays were performed at 24 h (A), 48 h (B), and 72 h (C). The horizontal bars represent median values. *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$.

changes brought on by LpQM-45 may therefore have exerted a profound effect upon the metabolic activity of the parasite and may be the mechanism that causes these compounds to kill the worms. The damage to the tegument along the worm's body may impair the functioning of the tegument and destroy the worm's defense system so that it is easy prey to the host's immune system (24).

The morbidity caused by human schistosomiasis is attributed to the granulomatous inflammation caused by an immune response to the egg antigen. Many studies have shown that in schistosomotic patients, there is a balance between the Th1 and Th2 responses (12). Furthermore, these same studies strongly suggest that resistance to infection is multifactorial and that it cannot be clearly correlated with a single immune mechanism. The Th1 immune response, generated by tumor necrosis factor alpha (TNF-α), IL-1, and IL-6, seems to predominate in the acute phase, but it is replaced by a Th2 immune response upon egg antigen production. The main Th2 cytokine responsible for fibrosis is IL-13 (56). Some mediators such as IL-12, TNF-α, NO, and gamma interferon (IFN-γ) prevent production of excess IL-13 during *S. mansoni* infection (9). For immunological assays, the present study investigated IL-6, IL-10, IL-12, and TNF-α cytokines and NO production in the supernatants of macrophage cultures stimulated *in vitro* with LpQM-43, LpQM-45, LpQM-47, and LpQM-14 compounds.

In terms of cytokine production, statistically significant levels of TNF-α were observed, compared to those of the negative control, for all four compounds analyzed. In the case of LpQM-47, peak production of TNF-α was observed after 24 h, decreased production was noted after 48 h, and there was no significant production after 72 h. LpQM-43 and LpQM-45 affected peak production after 48 h, and LpQM-14 had an effect after 72 h. These results demonstrate that the compounds analyzed stimulate a response with a Th1 cytokine profile. Some compounds that stimulate production of TNF-α have shown antiparasitic activity, including meglumine antimoniate, which has been shown to have antileishmania properties involving increased production of TNF-α (57).

NO has been shown to be an important cytotoxic and cytostatic effector for a number of pathogens, including viruses, bacteria, fungi, and parasites (58). It is implicated as an integral component of the host armament against invading parasites, and evidence has been put forward for the beneficial role of NO during helminthic infections. In the case of *Schistosoma mansoni*, for example, NO plays a role in regulation of egg-induced inflammation, acting as an antifibrogenic substance and preventing hepatocyte death and widespread tissue damage, in addition to being toxic to the schistosomula (9, 59, 60). It is significant that schistosomes are known to be more susceptible to oxidative stress than the hosts (61). The production of NO may also contribute to worm mortality by way of *S*-nitrosylation of cysteine proteases, given that schistosomes express cysteine proteases that play a role in digestion, reproduction, and protein turnover and that this appears to be a common and widespread mechanism (58, 62). Studies have also shown that compounds that induce the NO release possess potential immunomodulatory properties (63–65). The present study found that LpQM-14 stimulated NO production for the three incubation times analyzed, while LpQM-45 stimulated production only after 48 h of exposure. This behavior may indicate the immuno-stimulant properties of these compounds, especially when it is noted that the same compounds caused production of TNF-α.

Conclusions. To sum up, our results indicate that the thiazoles, especially those containing a methoxyl or chloro group, are antiparasitic agents and that they were more potent than their analogs, the thiosemicarbazones. These compounds showed substantial schistosomicidal properties against adult *S. mansoni* worms, with a significant reduction in motility, severe alterations in the integument and mortality of worms, lower toxicity than the reference drug (PZQ), and production of nitric oxide, inhibiting oviposition. The present study revealed LpQM-45 to possess the most effective schistosomicidal properties, suggesting its use as a prototype for the development of new schistosomicidal compounds. The present findings provide a sound basis for further in-depth studies of the antischistosomal properties of phthalyl thiazoles, particularly LpQM-45. It is also important that, in view of the results obtained, further biological studies are needed to shed light on the mechanism(s) of schistosomicidal action.

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