Scientific Research Publishing

Advances in Biological Chemistry, 2025, 15(6), 137-151
https://www.scirp.org/journal/abc
ISSN Online: 2162-2191
ISSN Print: 2162-2183

In Vitro Macrofilaricidal and Microfilaricidal Activities against *Onchocerca ochengi*, Cytotoxicity and Acute Toxicity of Seventeen Thiazolidinone Derivatives

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How to cite this paper: Kang, T.R., Beteck, R.M., Legoabe, L.J., Lahngong, M.S. and Ngemenya, M.N. (2025) *In Vitro* Macrofilaricidal and Microfilaricidal Activities against *Onchocerca ochengi*, Cytotoxicity and Acute Toxicity of Seventeen Thiazolidinone Derivatives. *Advances in Biological Chemistry*, **15**, 137-151.
https://doi.org/10.4236/abc.2025.156011

Received: November 17, 2025
Accepted: December 23, 2025
Published: December 26, 2025

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Abstract

In the search for suitable drugs for onchocerciasis, 17 synthetic thiazolidinone derivatives (TZDs) were screened for activity against the worm stages of *Onchocerca ochengi*, which cause the disease and the safety of the most active compounds was also assessed. Anti-onchocerca activity was assessed against the adult worms and microfilariae isolated from cattle skin, using worm motility and MTT formazan reduction assays. The cytotoxicity of the most active compounds was assessed on LLCMK2 (monkey kidney epithelial) cells and acute toxicity was assessed in BALB/c mice. Of the 17 TZDs, 8 and 4 compounds were highly active against adult male (AM) and adult female (AF) worms, respectively (≥90% inhibition, IC_{50} < 10 μM); they were not cytotoxic and their selectivity index (SI) values were ≥ 10. Compound **3** was the most active (IC_{50} = 0.098 μM) against AM; compound **5** was the most active (IC_{50} = 0.302 μM) against AF and **16** was the most selective (SI = 607.5). Sixteen compounds were active against microfilariae and 9 showed high activity against all three worm stages. No mortality, no adverse effects and no significant changes in liver and kidney functions were observed in acute toxicity test of the most active compounds in mice ($p < 0.05$). Some thiazolidinone derivatives demonstrated high macrofilaricidal and microfilaricidal activities at micromolar potency, high selectivity, no cytotoxicity and no adverse effects in mice. These broad-acting compounds have the potential for use in the eradication of onchocerciasis. Further studies should be pursued towards lead characterization.

Keywords

Onchocerciasis, *Onchocerca ochengi*, Thiazolidinones, Toxicity

1. Introduction

Human onchocerciasis is a neglected tropical disease of public health concern which is endemic in sub-Saharan Africa and Latin America. It is caused by the filarial nematode *Onchocerca volvulus* transmitted through the bite of an infected blackfly of *Simulium species* [1]. It is also referred to as river blindness because the blackflies breed in rapidly flowing freshwater streams and rivers. Onchocerciasis affected about 40 million people in 2022 in 37 countries in tropical Africa and South America where the population is at risk [1]. The main clinical manifestations are onchodermatitis, onchocercoma, visual impairment or blindness and onchocerca-associated epilepsy [1]. These pathological effects compromise quality of life with resultant significant negative social and economic impact [2]. Presently, management of onchocerciasis is solely by mass administration in community-directed treatment with ivermectin. However, ivermectin has several limitations; it has only microfilaricidal activity, but lacks efficacy against the adult worms (macrofilariae), which are responsible for sustaining long-term infection. There is emerging resistance with decreasing susceptibility of the parasite to ivermectin in some sub-Saharan African countries [3] [4]. Additionally, ivermectin exhibits severe and sometimes fatal, adverse neurological reactions in cases of co-infection with *Loa loa* [5]. Therefore, the quest for novel, safer and potent filaricides that will target both the macro- and micro-filariae worms is highly imperative. Several approaches are presently being explored to discover new, more efficacious and safer filaricides which are active against the adult stages. These include screening of synthetic compounds derived from medicinal chemistry, molecular modelling, modification and repurposing of existing molecules, drug combination and exploration of natural products among others [5] [6].

A few studies have demonstrated the potential of synthetic compounds to combat parasitic nematodes. Bulman *et al.* [7] demonstrated that Auranofin possesses significant nematocidal activity against multiple filarial species. Another study reported significant activity for synthetic thienylazoryl dyes against *O. ochengi* adult male and microfilariae respectively [8].

The thiazolidinones (TZDs) are heterocyclic compounds with a five-membered ring containing sulfur atom at position 1, nitrogen at position 3, and carbonyl at positions 2, 4, or 5 [9]. It is an important pharmacophore in several drugs which confers a wide range of pharmacological properties including antimicrobial, anticancer, antidiabetic, anticonvulsant, anti-inflammatory, antihypertensive among others. Examples of drugs containing this moiety in their core structure are Proglitazone and Rosiglitazone, used as antidiabetics, Darbufelone (anti-inflammatory and anticancer) and Actithiazic acid, an antibiotic [9]. A wide variety of molecules

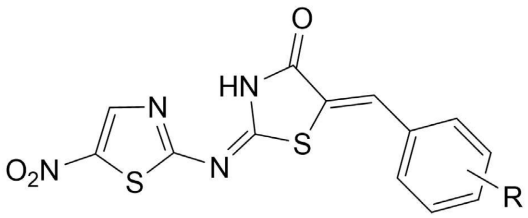
with various biological activities have been derived from the thiazolidinone scaffold by substitution at positions 2, 3 or 5. They have been explored in molecular hybridization with other heterocycles such as pyrazole, triazole, pyridine/pyrimidine among others to obtain several new drugs and compounds with promising pharmacological properties [10].

Studies on the anthelmintic activity of thiazolidinones are rare. A study of the nine substituted aryloxy-4-thiazolidinones revealed good anthelmintic activity against earthworms (*Portooscoplex corethrusus*) [11]. In a repurposing study of 106 FDA-approved drugs, several showed high activity against *O. gutturosa* [12]. The rationale of this study was to target the adult stages, hence blocking production of microfilariae and resulting in the eradication of the disease. This study therefore aimed to assess the macro- and also the microfilaricidal activity of 17 thiazolidinone derivatives using the *O. ochengi in vitro* model. The safety of highly active compounds was also assessed.

2. Materials and Methods

2.1. Source of Pure Compounds

The synthesis and structural characterization of the seventeen (17), thiazolidinones derivatives (TZDs) has been reported in detail elsewhere [13]. Briefly, the synthesis employed molecular hybridization between a 2-amino-5-nitrothiazole moiety and thiazolidin-4-one. This was followed by exploration of structure-activity relationships (SARs) by addition of a substituted benzylidene moiety on the thiazolidin-4-one, to obtain a library of seventeen (17) thiazolidinone derivatives using Knoevenagel condensation. The structures of target compounds were determined using Fourier-transform infrared spectroscopy (FTIR), ^{1}H and ^{13}C-NMR, and high-resolution mass spectra. The structures of the compounds are shown in **Figure 1**.



R= **1:** H **2**: 4-F **3**: 4-Cl **4**: 4-N0$_2$ **5**: 2-Me **6**: 2,4-MeO **7**: 4-OH **8**: 3-MeO **9**: 3-F
10: 3-Cl **11**: 3-Br **12**: 2-F **13**: 2-Cl **14**: 2-NO$_2$ **15**: 2,4,6-MeO **16**: 3,4-Cl **17**: 4-Me

Figure 1. Chemical structures of the thiazolodinone derivatives [13].

2.2. Preparation of Stock Solutions and Medium

Stock solutions of compounds (30 mM) were prepared by dissolving in Dimethyl sulfoxide (DMSO), (Sigma Aldrich, Germany), vortexed (Labnet VX 100, USA) for homogeneity and kept at −20˚C for subsequent use. Complete culture medium (CCM) was prepared by supplementing RPMI-1640 with L-glutamine (BioCon-

cept, Switzerland), 5% newborn calf serum (SIGMA, USA), 200 units/mL penicillin, 200 µg/mL streptomycin, and 0.25 µg/mL amphotericin B (SIGMA, USA), pH 7.4.

2.3. Extraction of *Onchocerca ochengi* Adult Worms

Adult worms were isolated as described [14] [15]. Briefly, fresh pieces of umbilical cattle skin rich in palpable nodules were obtained from slaughterhouses in Douala, thoroughly washed, cleaned and processed. The dampness of the skin was reduced by dabbing with a dry towel and then spread on a sterile wooden board in a laminar flow hood and disinfected with 95% and 70% ethanol consecutively. Worm masses were dissected out of the nodules and each was immersed in 2 mL CCM per well in a 12-well plate (BioConcept, Switzerland; NUNC, USA). The plates were incubated overnight at 37˚C, 5% CO_2 (HERACELL-150i, USA), and worm viability and sterility of cultures were evaluated using an inverted microscope (Nikon Eclipse TS100, China). Before use in primary and secondary screens, 1 mL of CCM was added to each well, giving a volume of 3 mL.

2.4. Isolation of *O. ochengi* Microfilariae

O. ochengi microfilariae (MFs) were isolated from infected umbilical cattle skin with palpable nodules as described [14] [15]. The skin was carefully cleaned, shaved, and sterilized as previously described. Skin slivers were cut out, placed in CCM for two hours at room temperature for MFs to emerge. The emerged highly motile MFs were concentrated by centrifugation (2000 rpm, 10 min), re-suspended in CCM and distributed into wells (about 15 MFs/100 µL of CCM/well) of the 96-well microtitre plate that contained a layer of monkey kidney epithelial (LLCMK2) cells. Their viability and sterility were assessed for 24 hours before compounds were added [14] [15].

2.5. Culture of Mammalian Cells

Mammalian (monkey kidney epithelial) cells (LLCMK2; ATCC, Virginia, USA), were cultured to confluence in CCM at 37˚C and 5% CO_2 as described [14] [15]. The medium was decanted and cells dislodged using 0.5 mM EDTA and 0.125% trypsin in incomplete culture medium (ICM), re-suspended in 10 mL of CCM and centrifuged (560 g, 10 mins) to eliminate the trypsin. The cells were then transferred into 96-well plates (100 µL/well) and incubated as above. LLCMK2 cells served as a feeder layer for microfilariae cultures and were also used for cytotoxicity studies [14].

2.6. Anti-Onchocerca Bioassays

2.6.1. Primary Screen on Adult Worms

This was done as reported [14] to identify active compounds. The stock solution (30 mM) of each compound was diluted to four times the final concentration with CCM; then 1 mL was added per well into 12-well plates containing worm masses

submerged in 3 mL making a final volume of 4 mL (DMSO concentration < 2%), a final concentration of 30 μM. Positive control wells contained auranofin at (30 μM) while negative controls contained the diluent (≤2% DMSO in CCM). All wells were set up in triplicate and the plates were incubated as above for five and seven days, for adult male (AM) and female (AF) worm assays, respectively, then worm viability was evaluated. Adult male worm viability was assessed based on motility using an inverted microscope on the following scale: 100% (complete inhibition of motility), 75% (only head or tail of worm shaking occasionally), 50% (whole worm motile, but sluggishly), 25% (only little change in motility), to 0% (no observable change in motility).

Thereafter, the worm masses were incubated in 500 μL of 0.5 mg/mL MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) for 30 minutes and female worm viability was evaluated by visual estimation of the percentage inhibition of formazan formation (blue coloration); from 100% parasite killing (no blue formazan coloration seen), 90%, 75%, 50%, 25%, to 0% (the entire worm appears blue as in the negative control). A compound was deemed active if there was ≥90% inhibition of male worm motility or of formazan formation; moderately active with 50% - 89% inhibition of male worm motility or of formazan formation; and inactive with < 50% inhibition of worm motility or formazan formation.

2.6.2. Secondary Screen on Adult Worms

The assay was repeated to confirm activity established in the primary screen, ascertain the compounds' dose-dependent response and determine their 50% inhibitory concentrations (IC_{50} value). Compounds with 90% - 100% inhibitory activities on both male and female adult worms at the primary screen were selected for secondary screens. The assay was set up at concentrations of 0.1 - 30 μM in triplicate and plates incubated as above. Activities of the compounds were determined using the same methods as above.

2.6.3. Primary Screen on Microfilariae

A primary screen was performed similarly as above to identify active compounds [8] [14] with minor adjustments. The monkey kidney epithelial cells were cultured to confluence as mentioned above, the medium was decanted quickly and the adhered cells were used as feeder layer. Microfilariae (MFs) in 100 μL of fresh CCM were added to each well of the 96-well plate (Greiner Bio-One, Austria) and incubated overnight. Before compound addition, sterility of the culture was verified and 50 μL of fresh CCM was added to each well. Each compound was added (50 μL per test well) at a single final concentration of 100 μM. Wells were set up in duplicate. Amocarzine (30 μM) and ≤2% DMSO in CCM were included as positive and negative controls, respectively and incubated as above with daily evaluation of worm viability by microscopy. Culturing was terminated after 120 hours. The percentage motility inhibition was determined daily as 100% (all MF immotile), 75% (only head or tail of MF shaking, occasionally), 50% (whole body of MF motile but sluggishly or with difficulties), 25% (almost vigorous motility), 0%

(vigorous motility). A compound was considered active on the MFs if there was 90% to 100% mean reduction in MFs motility; moderately active between 50% - 89% mean reduction in motility and inactive if less than 50% compared to untreated controls.

2.6.4. Secondary Screen on Microfilariae

This was carried out to confirm the activity in the primary screen and determine the IC_{50} values. Compounds with ≥90% inhibitory activity in the primary screen were tested at 0.03 to 100 µM using a feeder layer as above. Each test solution (50 µL) was added into a 96-well plate that contained MFs in 150 µL of CCM and the plate was incubated. The MFs motility was scored daily, cultures were stopped 120 hours later, and compound activities were recorded as above.

2.7. Cytotoxicity Test

Cytotoxicity of 12 compounds with ≥90% inhibition in the primary screen was evaluated using monkey kidney epithelial cells [8] [14]. Briefly, the cells were cultured in CCM to confluence as described above, the medium was decanted and the cells were rinsed twice with ICM. About 3000 cells/100µL in CCM were seeded in duplicate in a 96-well flat-bottom microtitre plate (Greiner Bio-one, Austria) and incubated for three days until fully confluent. Cells were examined by microscopy for confluence, the medium was discarded by flipping the plate and drying on a tissue, and 150 µL of fresh medium (CCM) was added per well. Then 50 µL of test compound (8 final concentrations: 0.03 µM to 100 µM), and controls were added to corresponding wells in duplicate and incubated for 5 days. Thereafter, the medium was discarded, the cells were washed by shaking twice in ICM for five minutes at 600 rpm (IKA Labortechnik KS125 basic shaker), and 100 µL of MTT (1 mg/mL ICM) was added and then incubated for 2 hours as above. Post incubation, the MTT solution was discarded and 100 µL of DMSO was added to dissolve the formazan precipitate, then homogenized and optical densities were read at 630 nm using an ELISA plate reader (Sinothinker SK201, China). Percentage inhibition was calculated using the formula below:

$$\%\ \text{inhibition} = \frac{\Delta\text{OD Negative control} - \Delta\text{OD of compound}}{\Delta\text{OD Negative control}} \times 100 \quad (1)$$

The cytotoxic concentration (CC_{50}) was determined as described above, while selectivity index was determined using the formula below:

$$\text{Selectivity Index } (\text{SI}) = \frac{\text{CC50 on LLCMK2 cells}}{\text{IC50 on parasite}} \quad (2)$$

2.8. Acute Toxicity Test

This was done for three compounds (**5**, **7** and **16**), selected based on the lowest IC_{50} values and highest SI values on adult worms; **7** was the most active (lowest IC_{50}) and most selective (highest SI) among the nine compounds active against all three worm stages in the primary screen. The test was conducted as previously

described [16] [17] and in accordance with guidelines of the Organization for Economic Cooperation and Development, version 423 [18] and ARRIVE, on animal research. Ethical clearance was obtained from the university's Institutional Animal Care and Use Committee (No. UB-IACUC Nº 18/2025 issued on 20 June 2025). BALB/c mice (20) were handled and processed as described in previous works [16] [19], then separated into four groups (3 test groups and a control group) with five mice per group. One mouse was fasted overnight (with water), weighed, administered 2000 mg/kg of compound, fasted for a further 2 hours and observed till 24 hours with food provided. Following the survival of the treated mouse, the others were treated the same and observed for 14 days (with food and water), then weighed. The mice were again fasted overnight, anesthetized with ketamine/xylazine (90/10 mg/kg); blood was collected by retro-orbital bleeding, allowed to coagulate then centrifuged (2000 rpm × 5 mins). The serum was used for biochemical analysis of two markers of hepatocellular damage, alanine aminotransferase (ALT) and aspartate aminotransferase (AST), and renal damage (urea and creatinine), using test kits (Chronolab, Spain) according to the manufacturer's procedure.

2.9. Data Analysis

The average percentage inhibition from secondary screens was used to determine the IC_{50} values of active compounds by nonlinear regression in Graphpad Prism 5.0 software and Excel 2019. Dose-response curves were also plotted using Graphpad Prism. CC_{50} values were determined from average percentage inhibition of compounds on cells using Graphpad Prism. Biochemical data of control and test mice were compared using an unpaired t-test. Statistical significance was set at $P < 0.05$.

3. Results and Discussion

3.1. Anti-Onchocerca Activity of Thiazolidinone Derivatives against Adult Worms

Out of the 17 thiazolidinone derivatives (TZDs) tested in the primary screen, 12 and 9 compounds showed high anti-onchocerca activity (≥90% inhibition), against adult male (AM) and adult female (AF) worms respectively (**Table 1**). All compounds showed activity against at least one worm stage. Meanwhile, 1 and 5 compounds were highly active only against AM and MFs respectively. Two were active against both AM and MF only, and 9 were active against all three stages (**Table 1**). No compound was active on adults only.

In the secondary screen, the IC_{50} values of compounds ranged from 0.098 to 8.5 μM against AM and 0.30 to 15.38 μM against AF. Among the 12 compounds tested against AM, compound **3** was the most active with an IC_{50} of 0.0988 μM. Of the 9 compounds tested against AF, compound **5** was the most active with an IC_{50} of 0.3029 μM. The macrofilaricidal activity of each compound showed a dose-dependent response for adult worms (**Figure 2**). Seven compounds had lower IC_{50}s

against AM compared to AF. Only 2 compounds had lower IC_{50}s against AF than AM (Table 2).

Table 1. Inhibition of *Onchocerca ochengi* motility by thiazolodinone derivatives at 100 µM.

Compound code	Average inhibition (%)			Worm Stages
	Male	Female	Microfilariae	
1	100	100	100	A
2	100	100	100	A
3	100	100	100	A
4	75	0	100	MFs
5	100	95	100	A
6	66.666	16.666	100	MFs
7	100	100	100	A
8	75	75	100	MFs
9	100	0	100	AM, MFs
10	100	58.333	100	AM, MFs
11	100	100	100	A
12	75	33.33	100	MFs
13	91.66	100	100	A
14	87.5	33.33	100	MFs
15	100	0	50	AM
16	100	100	100	A
17	100	100	100	A
Auranofin (30 µM)	100	100	-	AM, AF
	100	100	-	AM, AF
Amocarzine (30 µM)	-	-	100	MFs
	-	-	100	MFs

A: all stages; AM: Adult male; AF: Adult female; MFs: Microfilariae. Positive controls Auranofin, Amorcazine.

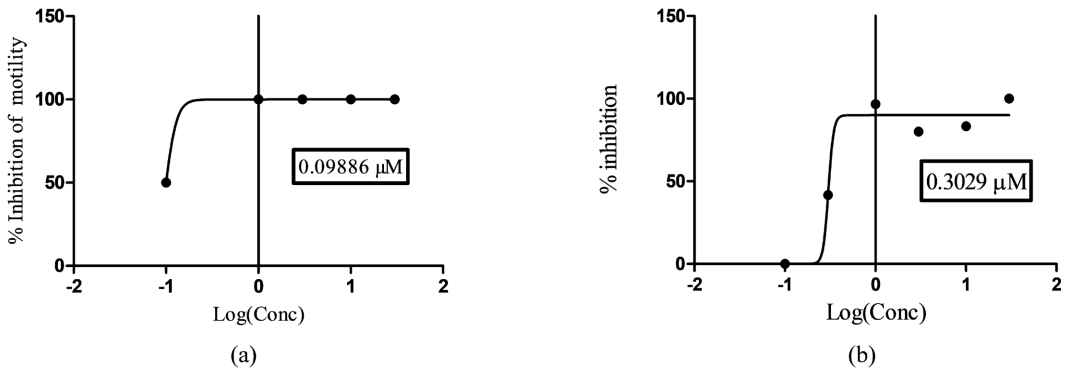


Figure 2. Dose-response curves with IC_{50}s of the most active thiazolidinones: (a) compound 3 and (b) compound 5 on *O. ochengi* adult male and female worms, respectively.

Table 2. Cytotoxicity, filaricidal activity and selectivity index (SI) values of active thiazolodinone derivatives on *O. ochengi* worm stages.

Compound code	CC_{50} on Cells (μM)	Filaridical activity IC_{50} (μM)			Selectivity Index (SI)		
		AM	AF	MFs	AM	AF	MFs
1	22.49	0.3294	1.172	.	68.2757	19.1894	.
2	22.74	0.9945	15.3836	.	22.8658	1.4782	.
3	22.85	0.0988	7.170	.	231.0415	3.1869	.
5	23.04	0.3156	0.3029	.	73.0038	76.0647	.
7	16.29	2.560	0.5669	.	6.3633	28.7352	.
9	>100	0.3154	NA	.	>317.0577	NA	.
10	30.51	2.851	NA	.	10.7015	NA	.
11	9.706	1.000	2.555	.	9.706	3.7988	.
13	6.68	7.517	5.181	28.9992	0.8886	1.2893	0.2304
15	166.1774	8.519	NA	NA	19.5067	NA	NA
16	75.21	0.1238	1.115	.	607.5121	67.4529	.
17	4.876	3.300	6.4535	5.6465	1.4776	0.7556	0.8635

Cutoff for cytotoxicity, CC_{50}: < 10 μM. NA: Not highly active, -: Not determined. Some key values are shown in bold.

Out of the 17 TZDs, 8 hits against AM and 4 hits against AF were found based on the following criteria: ≥90% inhibition, IC_{50} < 10 μM, cytotoxicity (CC_{50}) > 10 μM and a selectivity index (SI) ≥ 10 (**Table 1** and **Table 2**) [20]-[22]. The classification of these compounds as hits is supported by data from a study of a large library of over 2000 drugs in clinical use where ≥50% inhibition at 10 μM was considered a hit [20]. The AM shares three of the four hits on AF (compounds **1**, **5** and **16**). To the best of our knowledge, this is likely the first report on the activity of thiazolidinone derivatives against a causative agent of onchocerciasis, *O. ochengi*, albeit in cattle. Compound **3**, the most active (IC_{50} = 0.098), was also highly selective (SI = 231) against AM; but was only moderately active (IC_{50} = 7.17 μM), with low selectivity (SI = 3.18) against AF. Compound **3** also demonstrated 100% inhibition against microfilariae (MFs) at 100 μM. Compound **5**, the most active against AF (IC_{50} = 0.302 μM) showed similar activity against AM. TZDs which are macrofilaricidal against both adult stages have great potential as killing the adult stages will consequently lead to the absence of MFs which contribute significantly to the devastating pathology of human onchocerciasis. When compared to data from a previous study, 12 compounds had either a lower or comparable IC_{50} to Albendazole (15.7 μM), Levamisole (5.1 μM) and Ivermectin (1.3 μM) against AM *O. ochengi*, while 8 compounds had a similar trend against AF [23], As earlier mentioned, Ivermectin is only microfilaricidal alongside other limitations and acts mainly by inhibiting glutamate-gated chloride channels in invertebrates leading to paralysis [24]. The TZD hits which are highly active against adult stages are likely acting on different targets to that of ivermectin hence producing their effects

by different mechanisms. These hits therefore have the potential to overcome the limitations of ivermectin.

3.2. Activity of Compounds against Microfilariae

Out of the 17 TZDs, 16 (except compound **15**), showed high microfilaricidal activity (≥90% inhibition at 100 μM) against MFs in the primary screen. The IC_{50} was determined for only two compounds, **13** and **17**, with the values 28.9992 μM and 5.6465 μM respectively. Though almost all the TZDs were highly active against MFs (Table 1), IC_{50}s were not determined for most of them due to scarcity of MFs following treatment of bovine onchocerciasis with ivermectin in the study area. Ivermectin is used in veterinary medicine to kill MFs of *O. ochengi*, the bovine parasite, in order to improve animal health and beef productivity [24]. This was a major setback to identifying broad-acting TZDs which will be pursued as a priority in future work.

Further studies of the 9 compounds with high activity against all three worm stages may afford broad-acting anti-onchocerca agents which could be used to interrupt transmission and hence eradication of onchocerciasis.

A previous study had reported macrofilaridical activity against AM and microfilaricidal activity for 7 thienylazoryl dyes [8]. Studies on the anthelmintic activity of synthetic thiazolidinones are uncommon. A study [25] reported high activity of some novel thiozolidine-2,4-dione derivatives against adult earth-worm *Pheretima posthuma*.

3.3. Structure-Activity Relationship of Compounds

In terms of SARs, the unsubstituted benzylidene moiety (parent compound), (Figure 1), possesses high activity (compound **1**, R = H), against all three worm stages with very low IC_{50}s (0.32 to 1.17 μM) and high SI values (19.1 to 68.2). This activity is modulated following substitution on this moiety. Considering compounds active against AM, substitution with electron-withdrawing groups in the *para*-position of the benzylidene moiety [**3** (R = 4-Cl) and **16** (R = 3,4-Cl)], greatly enhances activity. Hence, compound **3** was the most active of all the TZDs. Compounds **2** (R = 4-F) and **11** (R = 3-Br), which are also electron-withdrawing had relatively high activities while **7** (R = 4-OH) and **10** (R = 3-Cl), had relatively lower activities. The substitutions in the *ortho*- and *meta*-positions in compounds **5** (R = 2-Me), and **9** (R = 3-F), respectively, did not alter activity compared to the parent compound **1**. Substituents in the *ortho*-position 2 in **13** (R = 2-Cl) and electron-donating groups [compounds **15** (R = 2,4,6-MeO) and **17** (R = 4-Me)], significantly decreased activity.

Considering the SARs for AF, the contrary to AM was observed whereby electron-donating substituents in the *para*-position (compound **7**, R = 4-OH) and *ortho*-position-2 (**5**, R = 2-Me), greatly enhanced activity compared to compound **1**. The electron-withdrawing substituents [Compounds **2** (R = 4-F), **3** (R = 4-Cl), **11** (R = 3-Br), **13** (R = 2-Cl), **15** (R = 2,4,6-MeO), and **16** (R = 3,4-Cl)] decreased

activity. Compound **19** (R = Me) did not enhance activity because it is weakly electron-donating. The plausible basis for the difference in the pattern of activity between AM and AF is likely that the adult stages have different target sites for the active compounds. Further evidence of acting at a target site is the sigmoid dose-dependent profiles in Figure 2. These targets need to be identified in future work.

3.4. Cytotoxicity of Thiazolidinone Derivatives

Of the 12 active compounds tested, the CC_{50} values of 9 compounds on monkey kidney epithelial cells were above the cut-off value for cytotoxicity of pure compounds (CC_{50} < 10 μM; according to other works [21] [22]. This indicates they were non-toxic against this cell line. Three compounds, **11**, **13** and **17** had CC_{50} values <10 μM indicating cytotoxicity (Table 2). In terms of selectivity, compound **16** had the highest selectivity index (SI) of 607.5 while the most active compounds with the lowest IC_{50}s, against AM (**3**) and against AF, (**5**) had relatively high SI values of 231 and 76 respectively (Table 2). The high SI values indicate a very low risk of harmful effects if used in the treatment of human onchocerciasis.

3.5. Acute Toxicity of Compounds

There was an increase in body weight of animals but the difference between control and test mice was not significant (compounds **5**, $p = 0.637$; **7**, $p = 0.604$ and **16**, $p = 0.487$). No adverse effects and no mortality were observed. There was also no significant difference between control and test mice for all biochemical parameters (ALT, AST, urea and creatinine), as all p-values were > 0.05, *i.e.*, compound **5** ($p = 0.594$ - 0.867), **7** ($p = 0.141$ - 0.999), and **16** ($p = 0.053$ - 0.564), Table 3.

Table 3. Effects of highly active thiazolodinone derivatives on liver and kidney function in acute toxicity test BALB/c mice.

Treatment Group (n= 5)	Liver		Kidney	
	ALT (U/L)	AST (U/L)	Creatinine (mg/dL)	Urea (mg/dL)
Control	16.80 ± 4.215	38.50 ± 11.54	1.757 ± 0.6791	4.600 ± 2.821
5	16.10 ± 8.058	41.65 ± 5.308	1.888 ± 0.3422	4.200 ± 1.217
7	21.35 ± 10.31	28.00 ± 8.573	1.757 ± 0.3428	5.700 ± 3.452
16	23.63 ± 4.630	47.69 ± 19.97	2.394 ± 1.058	3.625 ± 1.702

Dose: 2000 mk/kg observed for 14 days. P values for mean ± SD ALT, AST, urea and creatinine: **5**, $P = 0.867, 0.594, 0.709, 0.778$; **7**, $P = 0.387, 0.141, 0.999, 0.596$ and **16**, $P = 0.053, 0.412, 0.307, 0.564$, respectively.

The overall absence of harmful effects in the acute toxicity is consistent with the high selectivity of the tested compounds. Abnormally high levels of biochemical markers in serum are an indication of tissue damage in the corresponding organ. Toxicity studies of TZDs are few and mostly focused on anticancer screening. A

study [26] reported moderate anticancer activity for some TZDs against three cancer cell lines, but they were not toxic to Vero cells. Another study [27] reported high inhibitory activity (IC_{50} = 0.27310 - 0.533 μM) on aldose reductase for a series of 2,4-thiazolidinediones with CC_{50} values < 10 μM on L929 fibroblasts; despite the CC_{50}s being low and below the cut-off for cytotoxicity (≤10 μM) [21] [22], the compounds were considered safe because their SI values were relatively high.

3.6. Strength and Limitations of the Study

As a main strength, this is the first study, to the best of our knowledge, to demonstrate anti-onchocerca activity for TZDs with hits found in this class of compounds. However, there are some limitations to the study. Though *O. ochengi* is genetically a close relative of the human parasite, *O. volvulus*, the *O. ochengi in vitro* model does not exactly replicate the activity of the TZDs on *O. volvulus*. The mechanisms of action of the potent compounds were not determined in this study and secondary screen was not done for most compounds active against microfilariae to permit a better appraisal of their activity on the three worm stages. Acute toxicity test needs to be done for the other highly active compounds in order to identify the safest ones, and extended to repeated-dosing (sub-acute) with histopathological analysis for a better assessment of the toxicity of the active compound on long-term use.

4. Conclusion

Herein, we have identified thiazolidinones derivatives having broad-spectrum anti-onchocerca activity targeting all stages of the parasite. Most of these compounds showed sub-micromolar potency against the adult forms of the worm, superior to most clinically approved antifilarial agents. Selected hit compounds showed no cytotoxicity, suggesting they exhibit intrinsic antifilarial activity. *In vivo* acute toxicity evaluation of selected hit compounds at a high dose of 2000mg/kg revealed that the compounds are nontoxic as they did not lead to sudden animal death nor alter essential metabolic processes of the liver and kidney. These compounds therefore have the potential for use in the eradication of onchocerciasis following further drug development. The compounds should also be screened against *O. volvulus*, the species that infects humans. Further work should be done to elucidate the *in vivo* efficacy of the hits. This will be followed by lead identification by optimizing potency through structure-based design and high-throughput screening. Thereafter, the optimized lead will be screened for potential interaction with specific target proteins to determine the possible mechanism of action, followed by preliminary *in vitro* pharmacokinetic studies.

Acknowledgements

The Biotechnology Unit, Faculty of Science, University of Buea, provided facilities for the conduct of this work.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

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