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RESEARCH ARTICLE

Schistosomicidal and molluscicidal activities of aminoalkylamino substituted neo- and norneocryptolepine derivatives

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Abstract

Context: The cryptolepines originate from the roots of the climbing shrub *Cryptolepis sanguinolenta* (Lindi) Schitr (Periplocaeae) which is used in Central and West Africa in traditional medicine for the treatment of malaria.

Objectives: Evaluation for the first time of a series of chloro- and aminoalkylamino derivatives of neo- and norneocryptolepines for potential schistosomicidal and molluscicidal activities.

Materials and methods: A series of chloro- and aminoalkylamino substituted neo- and norneocryptolepine derivatives were synthesized. They were tested *in vitro* against viable *Schistosoma mansoni* Sambon mature worms in culture medium with fetal serum and antibiotics and in dechlorinated water against the snail vector *Biomphalaria alexandrina* Ehrenberg. Active compounds were further subjected to determination of their IC_{50} values.

Results: Results showed that six neocryptolepine and two norneocryptolepine derivatives had *in vitro* schistosomicidal activity on Egyptian and Puerto Rican strains of *S. mansoni*. The most effective derivative (2-chloro-5-methyl-*N*-(2-morpholin-4-ethyl)-*5H*-indolo[2,3*b*]quinoline-11-amine) has IC_{50} and IC_{90} 1.26 and 4.05 μM and 3.54 and 6.83 μM with the Egyptian and Puerto Rican strains of *Schistosoma,* respectively. All eight derivatives showed molluscicidal activity against the vector snail *B. alexandrina*. The most active compound (2-chloro-11-(4-methylpiperazin-1-yl)-*6H*-indolo [2,3-*b*] quinoline) has LC_{50} 0.6 and LC_{90} 3.9 ppm after 24 h.

Discussion and conclusions: The findings demonstrate that introducing chloro- and aminoalkylamino side chain initiated both schistosomicidal and molluscicidal activities in these derivatives. The structure–activity relationship of this series of compounds is discussed.

Keywords: Schistosomiasis; *Schistosoma mansoni*; aminoalkylamino neocryptolepine; norneocryptolepine; *in vitro*

Introduction

Schistosomiasis is the second most prevalent disease in the world after malaria, with about 200 million human beings infected in 74 countries. It is estimated that 20 million of them have serious forms of the disease or related disability, and that 200,000 people die from the disease every year (World Health Organization, 2002). Chemotherapeutic measures have been the mainstay in the control of this disease (Fenwick & Webster, 2006). Since 1970, praziquantel has become the drug of choice against the three major human species of schistosomes (Schistosomatidae), *Schistosoma mansoni* Sambon, *S. hematobium* (Bilharz) and *S. japonicum* (Katsurada) (Gönnert & Andrews, 1977; pica-Mattoccia 2004; Doenhoff & Pica-Mattoccia, 2006). It is a relatively safe, orally administered drug that leads to reducing the prevalence of schistosomiasis (Southgate et al., 2005). Consequently, a targeted as well as mass drug administration program presently relies heavily on this drug for the control of schistosome-induced morbidity. However, with only one drug of choice for treatment

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and the possibility of development of parasite resistance (Ismail et al., 1999; Doenhoff et al., 2002; Botros & Bennett, 2007), the present situation is dangerous. Therefore, there is a real need for discovery of newer drugs. To reach this objective, the first step could be by testing compounds for antischistosomal activity on mature worms in culture (*in vitro*) to isolate potentially effective ones. This should be followed by testing top active compounds *in vivo*. Moreover, it should be useful to test these compounds for other biological activities, especially molluscicidal activity against snail vector of the parasite.

The present work has been carried out as part of our ongoing program for developing novel antimalarial drugs which are based on a natural product. This is isolated from the roots of the climbing shrub *Cryptolepis sanguinolenta* (Lindi) Schitr (Alajarin et al., 1997) that is used in Central and West Africa in traditional medicine for the treatment of malaria (Wright, 2005).

As far as can be ascertained, cryptolepine **2** (Wright, 2007) and neocryptolepine **3** (Jonckers et al., 2002; El Sayed et al., 2009) have never been reported to exhibit antischistosomal activity (Figure 1).This initiated searching for analogues of both compounds as potential antischistosomal agents. Side chains were incorporated at different core positions which proved to be an important feature for the antimalarial activity.

Materials and methods

Chemical

The source of the neocryptolepine core used in this study is the roots of the climbing shrub *C. sanguinolenta* (Lindi) Schitr (Periplocaeae). A series of neo- and norneocryptolepines, (**4** and **5** respectively, Figure 2) with various basic aminoalkyamino side chains at different core position were synthesized (Tables 1 and 2). This was performed according to methods previously described by Shi et al. (1999) and El Sayed et al. (2009). The aminoalkylamino- or aminoalkyl group was chosen because of their indispensable importance for the antimalarial drug chloroquine. The active compounds were further subjected to determination of their IC_{50} and IC_{90} values. The starting materials used here were either commercially available or prepared. Anhydrous THF (tetrahydrofuran), toluene and dioxane were obtained from Sigma-Aldrich (St. Louis, MO) or Acros. Moisture-sensitive reactions were carried out under nitrogen or an argon atmosphere. Analytical thin-layer chromatography was performed on silica gel 60 F_{254} (Merck, Whitehouse Station, NJ). Column chromatography was carried out on silica gel 60 (230–400 mesh, Merck). Characterization of all compounds was done with ^{1}H-NMR and mass spectrometry. ^{1}H-NMR spectra were recorded on a 400 MHz.Bruker Avance DRX-400 spectrometer with NMR shifts being expressed in ppm downfield from internal TMS. ES Mass spectra were obtained from an Esquire 3000 plus iontrap mass spectrometer from Bruker Daltonics. Purity was verified using two diverse HPLC systems using respectively a mass and UV-detector. Water (A) and ACN (B), were used as eluents. LC-MS spectra were recorded on an Agilent 1100 Series HPLC system using a Alltech Prevail C18 column (2.1, 50 mm, 3 µm) coupled with an Esquire 3000plus as MS detector and a 5–100% B, 20-min gradient was used with a flow rate of 0.2 mL/min. 0.1% formic acid was added to solvent A and B. Reversed phase

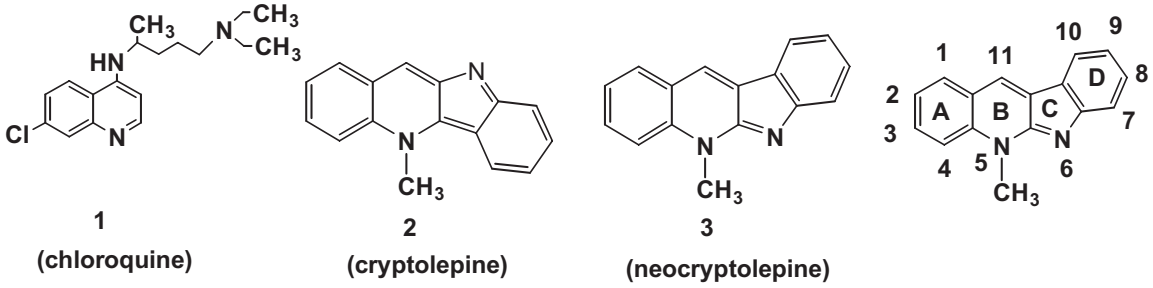


Figure 1. Structures of chloroquine (**1**), cryptolepine (**2**), neocryptolepine (**3**).

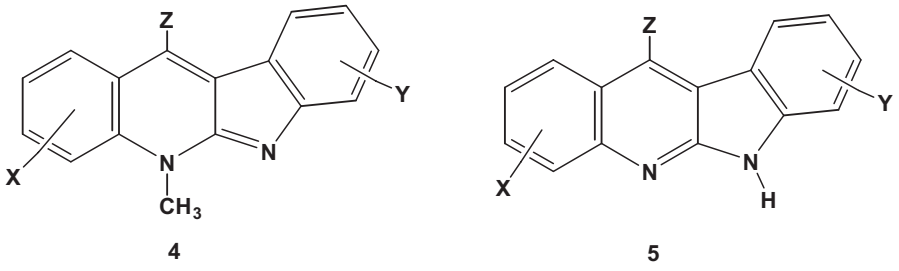


Figure 2. General structures of neo- (**4**) and norneocryptolepine analogs (**5**).

HPLC was run on a Gilson instrument equipped with an Ultrasphere ODS column (4.6, 250 mm, 5 μm). A 10–100% B, 35-min gradient was used with a flow rate of 1 mL/min. 0.1% of trifluoroacetic acid was added to solvent A and B. 214 nm was used as wavelength. Flash chromatography was carried out using Flash Master II (Jones Chromatography, Lakewood, CO) using Merck silica gel 60 (230–400 mesh).

General procedure for synthesis of the new neocryptolepine analogues

A round-bottom flask was charged with chloroindoloquinoline (0.5 mmol), the appropriate amine (0.75 mmol), and NaO*t*Bu (67.30 mg, 0.7 mmol) followed by dry toluene (1 mL) in air. Subsequently, the flask was flushed with Ar for a few minutes under magnetic stirring. A stock solution (1 mL) of Pd-catalyst (4 mol%) was added via a syringe and the flask was flushed with Ar for an additional 3 min. The resulting mixture was heated at reflux (oil bath temperature: 105–110°C) for 2–24 h under magnetic stirring and an argon atmosphere. After cooling to room temperature dichloromethane (DCM, 25 mL) was added and the suspension filtered over a path of celite and rinsed with DCM (30 mL). The solvent was removed under reduced pressure and the residue purified by flash chromatography using DCM-2.0 *N*-ammonia in MeOH (90:10) as the eluent to yield title compounds **g** [*N*-(5-*methyl*-*5H*-indolo[2,3-*b*]quinolin-2-yl)-*N*-(*1*-phenylethyl)amine] and **h** {5-*methyl*-2-(4-morpholinyl)-*5H*-indolo[2,3-*b*]quinoline}.

Analytical and spectra data

Preparation of 4 mol% stock solution of the catalyst: A 250 mL bottle was charged with Pd $(OAc)_2$ as Pd(0) source (89.8 mg, 0.4 mmol), and DCPB [2-(dicyclohexylphosphanyl)biphenyl] or DTPB [2-(di-*t*-butylphosphanyl)biphenyl] as ligand (0.8 mmol) and toluene (10 mL) in air. Subsequently, the bottle was flushed with Ar for 10 min under magnetic stirring. The stock solutions were stored under an Ar atmosphere. When DTPB was used as ligand for the catalyst the stock solution was stirred for 16 h prior to its use.

N-(5-methyl-5H-indolo[2,3-b]quinolin-2-yl)-N-(1-phenylethyl) amine 4 g

Yield: 74 mg (42%), ^{1}H NMR ($CDCl_3$) δ 1.61 (d, 3H, J = 6.8 *Hz*), 4.3 (s, 3H), 4.6 (m, 1H), 7.17 (m, 2H), 7.26 (m, 1H), 7.35 (m, 2H), 7.43 (m, 2H), 7.51 (m, 1H), 7.51 (m, 1H), 7.54 (d, 1H, J = 9.8 *Hz*), 7.7 (s, 1H, J = 8 *Hz*), 7.95 (d, 1H, J = 7.6 *Hz*), 8.29 (s, 1H). HPLC: 214 nm t_r 21.78 min. 100%. LC/MS: t_r 14.5 min. 100%. MS (ESI): m/z = 352 (M^{+1}).

5-methyl-2-(4-morpholinyl)-5H-indolo[2,3-b]quinoline 4 h

Yield: 124 mg (78%), ^{1}H NMR ($CDCl_3$) δ 3.21 (m, 4H), 3.92 (m, 4H), 4.27 (s, 3H), 7.18 (m, 1H), 7.28 (m, 1H), 7.39 (m, 1H), 7.51 (m, 1H), 7.59 (d, 1H, J = 9.2 *Hz*), 7.7 (d, 1H, J = 7.6 *Hz*), 7.98 (d, 1H, J = 7.6 *Hz*), 8.37 (s, 1H). HPLC: 214 nm: t_r 16.39 min. 100%. LC/MS t_r 12 min. 95%. MS (ESI): m/z = 318 (M^{+1}).

In vitro schistosomicidal bioassay

The schistosomicidal bioassay used here followed the main procedure previously described by Yousif et al. (2007) and Ramirez et al. (2007).Thus, the parasite material was *S. mansoni* mature worms of two strains (Egyptian and Puerto Rican strains) maintained at the Schistosome Biological Supply Centre (SBSC), Theodor Bilharz Research Institute (TBRI), Cairo, Egypt. The mature worms were obtained from hamsters (*Mesocricetus auratus*) (Waterhouse, 1839) percutaneously infected with cercariae 7 weeks earlier. They were obtained by perfusion using citrated saline and the recovered worms were washed from blood in small sieves (20 μm mesh) by phosphate buffer. Worms were washed three times with the culture medium, which is used for the assay under a sterilized laminar flow chamber. The culture medium is RPMI 1640 + L-glutamine + 20% fetal calf serum + antibiotics (300 μg streptomycin + 300 IU penicillin + 160 μg gentamycin per ml). The bioassay was carried out using 24-well tissue culture plates. Stock solutions 5 mg/ml of compounds were prepared in 100% dimethyl sulfoxide (DMSO) immediately before being used or stored at −20°C. Successive dilutions were made using DMSO and water (1:1). Three pairs of worms, males and females equally, were used for each test (well), and two replicates were set up. Exposure of worms to a standard concentration of 5 μg/ml of each compound was made for 5 days at 37°C ± 0.5°C in 5% CO_2 incubator. A pure medium and medium with 0.5% DMSO were used as negative control while praziquantel was used as a reference drug. Worms were examined for their viability using a stereomicroscope and those not showing motility for 1 min were considered dead. The mortality rate of worms was calculated after 5 days exposure. Compounds showing activity in the primary screen were retested (secondary screen) using the same technique by successive descending dilutions (five dilutions) of the solution. Two replicates were used for six worms in each and the mortality of worms was determined in each case. IC_{50} and IC_{90} values were calculated using the statistical program SPSS version 7.5.

Molluscicidal tests

Adult *Biomphalaria alexandrina* (Ehrenberg) (Planorbidae) snails, the intermediate host of *S. mansoni* in Egypt, were collected from the irrigation system in the Nile Delta and maintained in the laboratory for 3 weeks before being used. The efficacy of the compounds was primarily determined against the snails using the standard method. Thus, 1 L of dechlorinated water with a concentration 5 ppm of each compound was prepared and ten snails were added. They were maintained in the solution for 24 h at 25°C ± 1°C. After the exposure period, the snails were washed thoroughly with dechlorinated water and maintained in fresh water for another 24 h for recovery. In each case, two replicates were carried

out and two groups of snails were used as negative control. The conventional molluscicide (niclosamide) at the same concentration was used as positive control. The compounds showing molluscicidal activity were retested by the same method using descending concentrations for LC_{50} and LC_{90} determination by SPSS statistical program.

Results and discussion

The synthetic strategy of neocryptolepine and norneocryptolepine compounds was based on the amination of chlorosubstituted compounds obtained through Graebe-Ullman condensation (Peczynska-Czoch et al., 1994; Kaczmarek et al., 1988). This method was used for

Table 1. *In vitro* schistosomicidal activity of neocryptolepine (5-methyl-5*H*-indolo[2,3- *b*]quinoline) derivatives on adult *Schistosoma mansoni* worms (results after 5 days exposure).

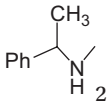
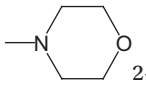
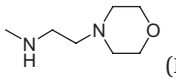
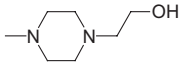
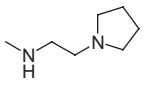
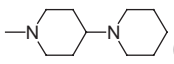
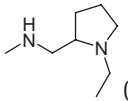
		Primary bioassay (5 μg/ml)	Secondary bioassay			
			IC_{50} (μM)		IC_{90} (μM)	
Neocryptolepines **4**	Core substitution	Worm mortality %	Egyptian strain	Peurto Rican	Egyptian strain	Peurto Rican
a	5-Me, 9-Cl	0	—	—	—	—
b	3-Cl, 5-Me, 11-Cl	0	—	—	—	—
c	2-NHCH(CH_3)(CH_2)$_3$NEt_2, 5-Me (MW:388.55)	66.70	—	—	—	—
d	3-NHCH(CH_3)(CH_2)$_3$NEt_2, 5-Me	0	—	—	—	—
e	9-NHCH(CH_3)(CH_2)$_3$NEt_2, 5-Me	0	—	—	—	—
f	5-Me, 11-NHCH(CH_3)(CH_2)$_3$NEt_2 (MW:388.55)	25	—	—	—	—
g	 2 , 5-Me	0	—	—	—	—
h	 2- 5-Me	0	—	—	—	—
i	2-Cl, 5-Me, 11-NHCH(CH_3)(CH_2)$_3$NEt_2 (MW:422.99)	100	11.74	14.98	16.78	21.65
j	3-Cl, 5-Me, 11--NHCH(CH_3)(CH_2)$_3$NEt_2 (MW:422.99)	100	19.26	29.33	24.32	32.83
k	2-Cl, 5-Me, 11-  (MW:394.90)	100	1.26	3.54	4.05	6.83
l	2-Cl, 5-Me, 11-  (MW:394.90)	100	1.77	3.29	4.55	5.57
m	2-Cl, 5-Me, 11-NH(CH_2)$_2$NMe_2 (MW:352.86)	100	3.68	5.95	7.65	13.03
n	2-Cl, 5-Me, 11-  (MW:378.90)	91.7	—	—	—	—
o	2-Cl, 5-Me,11-  (MW:432.99)	100	3.46	7.85	8.31	13.39
p	2-Cl, 5-Me, 11-  (MW:392.92)	58.7	—	—	—	—
Reference drug	Praziquantel (MW: 312.4)	100	0.6	0.89	1.08	1.5

Table 2. *In vitro* schistosomicidal activity of norneocryptolepine (quinindoline, 6H-indolo [2,3-b]quinoline) derivatives for *Schistosoma mansoni* worms (results after 5 days exposure).

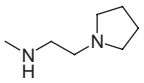
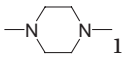
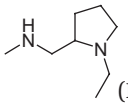
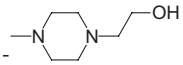
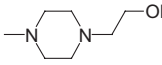
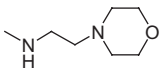
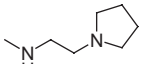
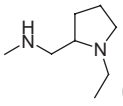
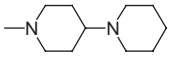
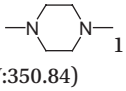
		Primary bioassay (5 μg/ml)	Secondary bioassay			
			IC_{50} (μM)		IC_{90} (μM)	
Norneocryptolepines **5**	Core substitution	Worm mortality %	Egyptian strain	Peurto Rican strain	Egyptian strain	Peurto Rican strain
a	2-Cl, 11-Cl	0	—	—	—	—
b	3-Cl, 11-Cl	0	—	—	—	—
c	2-Cl, 11-$NHCH(CH_3)(CH_2)_3NEt_2$ (MW:408.97)	50	—	—	—	—
d	3-Cl, 11-$NHCH(CH_3)(CH_2)_3NEt_2$	0	—	—	—	—
e	2-Cl, 11-$NH(CH_2)_2NMe_2$ (MW:338.83)	83.3	—	—	—	—
f	2-Cl, 11-  (MW:364.87)	66.7	—	—	—	—
g	2-Cl  11- (MW:350.84)	100	5.41	6.27	13.68	17.95
h	2-Cl, 11-  (MW:378.16)	83.3	—	—	—	—
i	2-Cl, 11-  (MW:380.87)	100	3.41	7.61	6.30	11.02
Reference drug	Praziquantel (MW: 312.4)	100	0.19	0.28	0.34	0.47

Table 3. Molluscicidal activity of substituted neo- and norneocryptolepines on *Biomphalaria alexandrina* snails (results after 24 h).

			Primary test (5 ppm)	Secondary test	
Derivatives	Code	Indoloquinoline side chains	Snail mortality %	LC_{50} (ppm)	LC_{90} (ppm)
Neocryptolepine	l	2-Cl, 5-Me, 11-  (MW:394.90)	100	3.9	4.8
	k	2-Cl, 5-Me, 11-  (MW:394.90)	100	1.3	2.2
	m	2-Cl, 5-Me, 11-$NH(CH_2)_2NMe_2$ (MW:352.86)	100	1.47	2.12
	n	2-Cl, 5-Me, 11-  (MW:378.90)	100	1.74	2.64
	p	2-Cl, 5-Me, 11-  (MW:392.92)	100	2.95	4.1
	o	2-Cl, 5-Me,11-  (MW:432.99)	100	2.6	3.5
Norneocryptolepines	g	2-Cl,  11 (MW:350.84)	100	0.63	0.91
Reference molluscicide		Niclosamide	100	0.2	0.6

synthesis of neocryptolepines with substitutions on the A or D ring (2-, 3-, 8- and 9 substitution).

The chloroquine-derived N^1, N^1-diethyl-1,4-pentanediamine was firstly used as basic side chain. This chain was introduced on 2-, 3- and 9-chloroneocryptolepines with a palladium-catalyzed amination reaction using a Buchwald-Hartwig amination (Sheng & Hartwig, 2008; Zim & Buchwald, 2003). A series of neo- and norneocryptolepines were also prepared with various aminoalkylamino groups in position 11 and a chlorine atom at the 2-position by following the procedure previously reported by El Sayed et al. (2009) starting from 1*H*-methyl indole-3-carboxylate and aniline derivatives. The analytical and spectral data of the newly synthesized neocryptolepine compounds **g** and **h** agreed very well with the proposed structures (*cf.* material and methods section). All other derivatives of neo-and norneocryptolepines revealed analytical and spectral data consistent with those reported by El Sayed et al. (2009). The synthesized compounds used for biological screening are listed in Tables 1 and 2.

Schistosomicidal activity

The schistosomicidal (primary bioassay using Egyptian strain) showed that 6 neocryptolepine compounds out of 16 and 2 norneocryptolepines out of 9 exhibited 100% worm mortality at the concentration used (5 μg/ml) after 5 days. IC_{50} and IC_{90} of the schistosomicidally effective neocryptolepine were lowest in compounds **k** and I being 1.26 and 4.05 μM and 1.77 and 4.55 μM for *S. mansoni* Egyptian strain respectively. IC_{50} and IC_{90} were 3.54 μM and 6.83 μM and 3.29 and 5.57 μM for the Puerto Rican strain, respectively, thus showing more sensitivity of Egyptain than Puerto Rican strain. However, the efficacy of these compounds is still less than that of the reference drug Praziquantel which has IC_{50} and IC_{90} 0.6 and 1.08 μM for the Egyptian strain and 0.89 and 1.5 μM for the Puerto Rican strain respectively (Table 1). The structure–activity relationship studies revealed that all mono- and dichlorosubstituted neocryptolepines **4a** and **4b** and norneocryptolepines **5a** and **5b** exhibited no antischistosomal activity (Tables 1 and 2). In general, introduction of the aminoalkylamino side chain into the indoloquinoline core in combination with the chlorine atom at the A ring resulted in significant increases in the antischistosomal activity as shown in compounds **4i-p** for neocryptolepines (Table 1) and **5c** and **5e-i** for norneocryptolepines (Table 2). However, the activity is completely lost by switching the position of the aminoalkylamino side chain from 11 to 3 or 9 position with the absence of chlorine at the A ring, as in compounds **4d** and **4e**, respectively (Table 1). In case of norneocryptolepine series compounds **5g** and **5i** with piperazinyl moiety as side chain were the most active compounds (Table 2). The absence of activity of chloroneocryptolepines **4a-b** and chloronorneocryptolepine **5a-b**, which have no basic side chain, gives credence to the view that an aminoalkylamino side chain is important for antischistosomal activity. The possibility to obtain potent derivatives with dibasic side chains is a potential lead worthy of exploration. Further work will be directed toward the synthesis and evaluation of more neocryptolepines with this structural form.

Molluscicidal activity

Concerning the molluscicidal activity seven compounds (six neocryptolepines and one norneocryptolepine) provided 100% snail mortality at the tested concentration of 5 ppm (Table 3). The LC_{50} and LC_{90} for these compounds ranged between 0.63–3.9 ppm and 0.91–4.8 ppm, respectively. The norneocryptolepine compound (g) gave the highest effect since LC_{50} and LC_{90} were 0.63 ppm and 0.91 ppm, respectively. However, comparing these results with the activity of the conventional molluscicides, niclosamide, and the latter compound shows a better activity. The observed LC_{50} and LC_{90} values for niclosamide were 0.2 ppm and 0.6 ppm, respectively.

Declaration of interest

The authors report no conflicts of interest.

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