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SYNTHESIS AND BIOASSAY OF SOME BENZOPYRANO [2, 3-B] PYRIDINE DERIVATIVES

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Abstract

One 2-aminopyridine derivative **7** and three nitrogen analogues of xanthone (1-azaxanthones) **8-10** have been synthesized from 3-formylchromone **(2)** and 3-cyanochromone **(5),** respectively. The prepared compounds have been screened for activity against *Biomphalaria glabrata*, miracidia of *Schsitosoma haematobium* and cercariae of *Shistosoma mansoni*.

1. Introduction

The chemistry of 4-oxo-4H-benzopyrone, commonly called chromone, continues to be of interest to a number of investigators. Some of the attractions to this class of heterocyclic compounds include its natural occurrence (Ellis, 1977), wide range of biological activities (Hutter and Dale, 1951; Devi *et al.*, 1988; Achaiah and Reddy, 1991; Lacova *et al.*, 1995; El-Shaaer *et al.*, 1998) and interesting synthetic possibilities (Nohara *et al.*, 1977; Fitton *et al.*, 1979; Kubicova *et al.*, 2002; Lacova *et al.*, 1998). Khellin **1** (a furanochromone), an active ingredient from the extract of the fruit of a Middle Eastern plant of the *Umbelliferae* family, *Amni visnaga*, has been in use for centuries in folk medicine and has been attributed with many therapeutic properties, including its use as an antispasmodic in asthma and a coronary vasodilator in *Angina pectoris* (Hutter and Dale, 1951). The discovery by Nohara and co-workers that a carbonyl group (C=O) at C-3 position enhanced the anti-allergic activities of chromones led to intensive investigations of 3-substituted chromones (Nohara *et al.*, 1977). 3-Formylchromones **2** and/or some of their condensation products with primary amines have been reported to exhibit antibacterial and antifungal activities (Achaiah and Reddy, 1991; El-Shaaer *et al.*, 1998), antimycobacterial activity (Lacova *et al.*, 1995; El-Shaaer *et al.*, 1998), anti-inflammatory and analgesic properties (Devi *et al.*, 1988, 1988), antiallergic (Nohara *et al.*, 1974, 1977 Achaiah *et al.*, 1991).

In general, 3-acylchromones **2** are versatile synthons in heterocyclic chemistry (Fitton *et al.*, 1979; Lacova *et al.*, 1998; Kubicova *et al.*, 2002). They react easily with nucleophiles, with C-2 being the preferred site of attack. The C-2, C-4 and HC=O positions are electron-deficient, hence 3-formylchromones react smoothly with nucleophiles under mild conditions (Fitton *et al.*, 1979; Lacova *et al.*, 1998)

The initial product **3** may form 'ring-opened' products **4** which may react further to form new heterocyclic compounds. With diamines, **2** gives pyrimidines. On the other hand, **2** and its derivatives react with active methylene compunds, like malonic acid derivatives and enamino-ketones, to give condensation products and benzopyrano-pyridines (Elden, 1981), commonly known as 1-azaxanthones (e.g. **8**). Such compounds have not been studied as potential drugs against infestations with schistosomes. Only lucanthone, a thioxanthone has been studied as an agent against *S. mansoni* infestations (Davis *et al.*, 1965).

This paper describes the reaction of 3-formylchromone **2** and 3-cyanochromone **5** with some active methylene compounds and the investigation of the activity of the reaction products as molluscicidal and antischistosomal agents.

2. Materials and Methods

(a) Bioassay Procedures

Biomphalaria glabrata were reared as described by Madsen (Madsen, 1984). Molluscicidal tests were carried out, according to the WHO provisional plan of 1961 (WHO 1961; Adewunmi *et al.*, 1987). Eggs of *schitosoma haematobium*, recovered from urine of infected school children by standard parasitological techniques, were hatched by exposing them to bright light for 60 min. Five miracidia recovered from hatched eggs were used for each concentration of the synthetic chemicals. The activity

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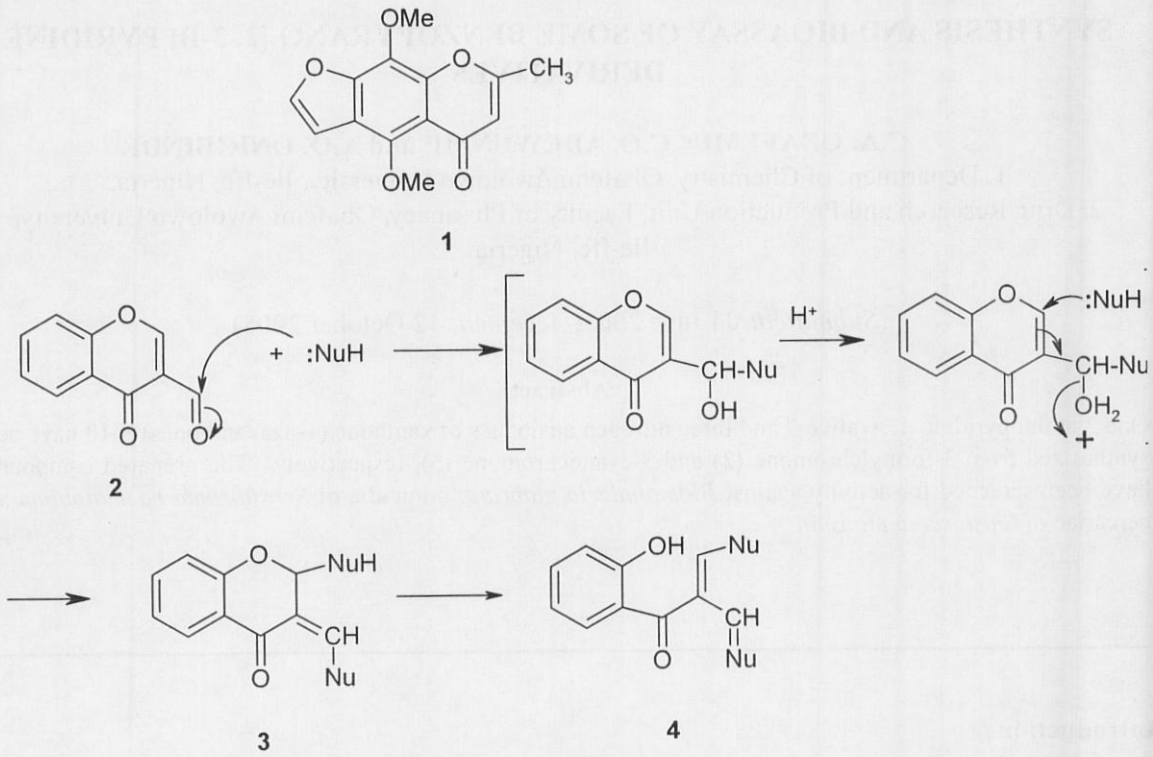


Scheme 1

(e.g Nu = primary amines)

of the miracidia was monitored at 1 h and 2 h exposure to the chemicals.

Schistosoma mansoni cercariae were harvested from *B. pfeifferi* by standard techniques. Groups of 5 cercariae were exposed to 100 ppm of the compounds for 2 h (Adewunmi and Furu, 1989).

(b) Synthesis

All melting points were determined with open capillary tubes, on a Gallenkamp (variable heater) melting point apparatus and are uncorrected. UV spectra were run in methanol solution on an SP8-400 uv/vis spectrometer (l max in nm (e)). IR spectra were recorded on a Perkin-Elmer 727B machine (in KBr or nujol, n_{max} in cm^{-1}). ^1H- and ^{13}C-NMR were recorded in CF_3COOD or DMSO-d_6 solutions on a Varian FT 80 NMR spectrometer (d in ppm relative to Me_4Si) (at the Polytechnic of North London, UK).

4-Oxo-4H-chromene-3-carbonitrile: (3-cyanochromone), 5.

Hydroxylaminehydrochloride (3.4 g, 48.9 mmol) and hydrochloric acid (8.0 mL) were added to a solution of 3-formylchromone (8.5 g, 48.9 mmol) in 90 % ethanol (40 mL) and then refluxed for 12 h. The reaction mixture was cooled and the resulting yellow precipitate filtered and recrystallised from ethanol to give **5**, (8.1 g, 97 %), mp 158-60 °C. (Found: C, 70.10; H, 2.90; N, 8.28; $C_{10}H_5NO_2$ calculated: C, 70.18; H, 2.94; N, 8.18). IR (cm^{-1}): 3030, 1650 (C=O), 2210 (CN), 1140. lmax (nm), (e): 286 (20,000), 240 (48,800). ^1H nmr (d): 9.10 (s, IH, H-2), 8.25-7.50 (m, 4H, Ar).

Reaction of 3-Formylchromone with Malononitrile to give [(4-Oxo-4H-chromen-3-yl) methylene] malononitrile, 6

Malononitrile (0.19 g, 2.9 mmol), containing 2 drops of pyridine, was added to 3-formylchromone **2** (0.5 g, 2.9 mmol) in ethanol (20 mL) and the mixture refluxed for 4 h. Cooling of the reaction mixture gave a red precipitate which was filtered and recrystallised from ethanol to give **6** (0.3 g, 47 %), mp (dec) >290 °C. (Found: C, 70.27; H, 2.60; N, 12.68; $C_{13}H_6N_2O_2$ calculated: C, 70.27; H, 2.27; N, 12.61). IR (cm^{-1}): 3030, 2220 (CN), 1700 (C=O), 1602, 1070: lmax (nm) (e): 340 (5000), 286 (5500), 240 (7000).

2-Amino-5-(2-hydroxybenzoyl)nicotinonitrile, 7

A mixture of malononitrile (0.38 g, 5.8 mmol) and ammonium acetate (0.44 g, 5.8 mmol) was added to a solution of 3-formylchromone (1.0 g, 5.8 mmol) in ethanol (20 mL), followed by refluxing for 4 h. The reaction mixture was cooled and the resulting solid filtered and then recrystallised from ethanol to give light brown crystals of **7** (0.75 g, 55 %), mp (dec.) 207-210 °C. Found: C, 65.30; H, 3.84; N, 17.50; $C_{13}H_9N_3O_2$ calculated: C, 65.27; H, 3.79; N,

17.56). IR (cm^{-1}): 3380, 3290, 3200 (OH, NH_2), 3030, 2220 (CN), 1660 (C=0), 1110. lmax (nm) (e): 328 (12200), 232 (13200). ^1H nmr (d, DMSO-d6)): 10.10 (br s, 1H, OH, D_2O exchangeable), 8.45 (d, 1H, H-4); 8.10 (d, 1H, H-6,); 7.50 (br s, 2H, NH_2, D_2O exchangeable); 7.41-6.50 (m, 4H, Ar).

2-Amino-5-oxo-5H-chromeno[2, 3-b]pyridine-3-carbonitrile, 8

Compound **8** was prepared as described for compound **6**, from the reaction of 3-cyanochromone with malononitrile (5.9 mmol each) containing 2 drops of pyridine. It was obtained as red crystals, yield = 0.47 g, 34%, mp (dec.) 225-227 °C (Found: C, 65.60; H, 2.90: N, 17.95; $C_{13}H_7N_3O_2$ calculated: C, 65.82; H, 2.97; N, 17.71). IR (cm^{-1}): 3380, 3140 (NH_2), 3030, 2220 (CN), 1650 (C=O), 1120. l max (nm) (e): 344 (4800), 310 (3000), 280 (3500), 240 (9400). 1H nmr (d, DMSO-d_6): 7.71-7.20 (m, 5H, Ar), 6.90 (br, s, 2H, NH_2, D_2O exchangeable).

3-Acetyl-2-methyl-5H-chromeno[2, 3-b]pyridin-5-one, 9

Compound **9** was obtained as brown crystals, from the reaction of **5** with acetylacetone using piperidine as base, as described above. It was recrystallized from chloroform-toluene (1:9) to give the pure crystals of **9,** mp 210-212 °C. (Found: C, 71.10; H, 4.11; N, 5.72; $C_{15}H_{11}NO_3$ calculated: C, 71.14; H, 4.38; N, 5.53). IR (cm^{-1}): 3030, 1670 (ArCO), 1650 (pyrone C=O), 1570, 1130. lmax (nm) (e): 322 (20,000), 239 (70,400). ^1H nmr (d, DMSO-d_6): 8.90 (s, IH, H-4); 8.20-7.25 (m, 4H, Ar): 2.65 (s, 3H, CH_3); 2.64 (s, 3H, CH_3).

6H-Chromeno[3',2':5,6]pyrido[2,3-d]pyrimidine-2,4,6(1H,3H)-trione, 10.

3-Cyanochromone **5** (10.0 g, 58 mmole) was dissolved in ethanol (20 mL). Barbituric acid (5.90 g, 46 mmole) and pyridine (3 drops) were added and the resulting mixture refluxed for 6 h. Cooling gave orange precipitate of **10**. This was recrystallised from ethanol to give 8.5 g (66%) of **10**. Mp > 320 °C. (Found: C, 59.58; H, 2.69; N, 14.75. $C_{14}H_7N_3O_4$ calculated: C, 59.80; H, 2.51; N, 14.94). IR (cm^{-1}); 3400, 3030, 1680, 1650, 1590, 1110. lmax (nm) (e): 342 (16800), 241 (44000). ^1H nmr (CF_3COOD/ DMSO-d_6): 11.40, 11.31 (br, s, 2 NH, D_2O exchangeable), 8.68(s, 1H), 7.90-7.28 (m, 4H, Ar).

3. Results and Discussion

(a) Chemistry

3-Formylchromone **(2)** was prepared according to a literature procedure, via Vielsmeier-Haack double acylation (Nohara et al., 1974). The reactions of **2** with malononitrile in the presence of few drops of pyridine in ethanol gave the dicyanomethylidene derivative **6** as red crystals (scheme 2). When the same reaction was carried out using ammonium acetate (one mole equivalent) as base instead of pyridine, a 'ring-opened' pyridine derivative **7** was formed, similar to the observation of Nohara et al (Nohara et al., 1974). The reaction, most probably, initially takes place by condensation of malononitrile with the formyl group, followed by attack of NH_3 (from NH_4OCOCH_3) on the C-2 atom of the pyrone ring with opening of the ring (Scheme 3).

The 'H nmr spectrum of **7** showed the OH proton as broad singlet at d 10.10 ppm and the NH_2 protons at d 7.50 ppm, both exchangeable with D_2O. The ^{13}C nmr spectrum is readily assigned and agree well with the assigned structure.

Compound **2** was converted to the corresponding 3-cyanochromone **5** following a literature procedure, by refluxing **2** with hydroxylamine-hydrochloride in ethanol containing HCl (Nohara et al., 1977).

5 condenses with the appropriate active methylene compounds (malononitrile, acetylacetone and barbituric acid) in ethanol, in the presence of pyridine or piperidine, to give 5-oxo-5H[1]benzopyrano[2,3-b]pyridine derivatives (5-oxo-5H-chromeno[2,3-b]pyridines) **8**, **9** and **10**, respectively. The products are presumably formed via a derivative of 2-amino-3-formylchromone **11**. Compound **5** is known to react in an aqueous or slightly basic medium in the form of **11** (Ishiguro et al., 1981). The reactive methylene compounds (e.g. acetylacetone) undergo Michael addition to the a,b-unsaturated ketone with concomitant ring-opening of the pyrone heterocycle to give the intermediate 2-hydroxybenzoyl alkene derivative **12,** followed by a double cyclization to give the benzopyranopyridine derivatives, as shown in scheme 4.

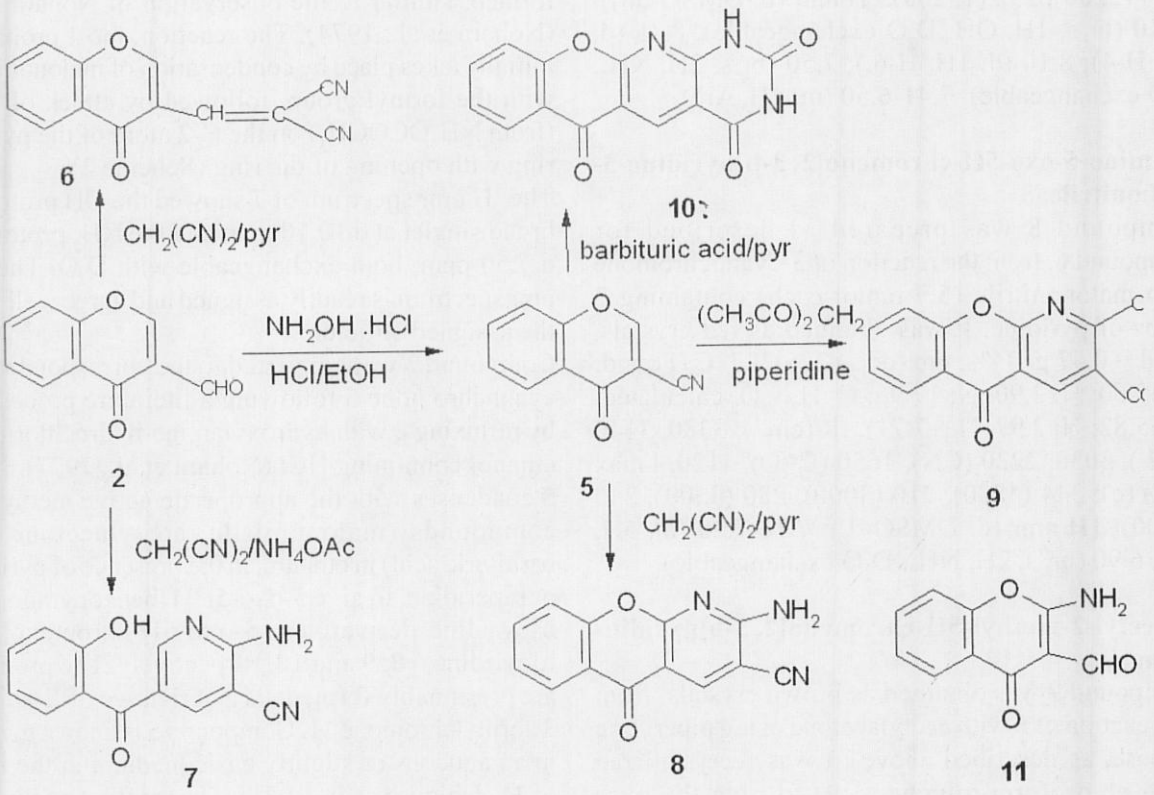
The structures of the synthesized compounds were assigned by their spectral and elemental analyses. The infrared spectra of the compounds showed absorption bands for the CN, NH_2, CONH and CO groups at the expected regions.

Table 1: Molluscicidal action of 7, 8, 9, & 10 on *Biomphalaria glabrata* at a concentration of 100 ppm

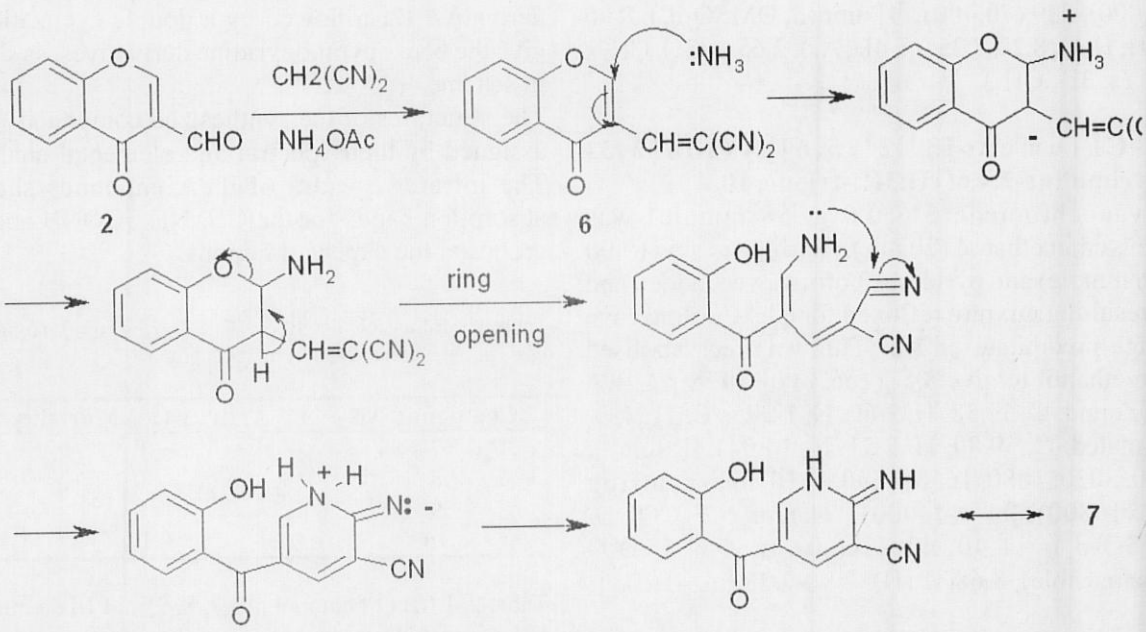
Compound No	Activity (%) Mortality
7	0
8	0
9	0
10	10

Table 2: Effect of compounds 7, 8, 9, and 10 on miracidia of *S. haematobium* and cercariae of S. mansoni at a concentraton of 100 ppm

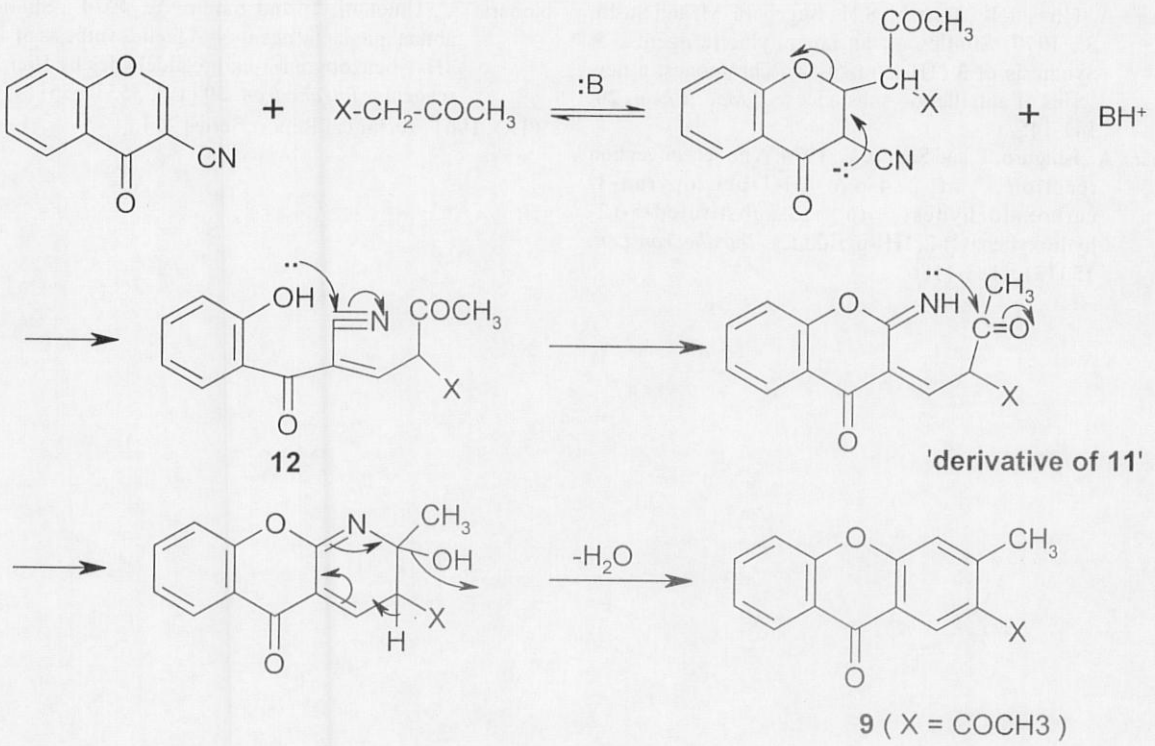
Compound No	*S. haematobium miracidia (% Mortality)*		*S. mansone cercariae (% mortality)*	
	1 h	2 h	1 h	2 h
7	60	80	100	100
8	0	20	60	100
9	0	40	100	100
10	0	80	100	100



Scheme 2



Scheme 3



Scheme 4

(b) Biological activity

The results of the molluscicidal assays are shown in Table 1 while the schistosomicidal results on the miracidia of *S. haematobium* and cercariae of *S. mansoni* are shown in Table 2. Compound **10** showed weak activity while the others, **7**, **8**, and **9**, have no activity.

In contrast to their weak or non-molluscicidal potential, compounds **7** and **10** exhibited strong miracidal activity, while the cercaricidal activity of the compounds are very impressive.

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