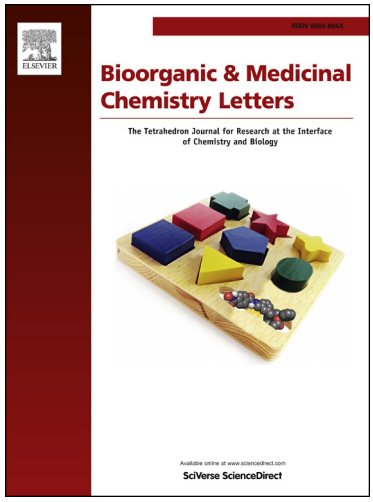

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PII: S0960-894X(13)00575-1
DOI: http://dx.doi.org/10.1016/j.bmcl.2013.04.085
Reference: BMCL 20450

To appear in: *Bioorganic & Medicinal Chemistry Letters*

Received Date: 16 January 2013
Revised Date: 24 April 2013
Accepted Date: 30 April 2013

Please cite this article as: Chen, J., Sun, W., Yang, J., Sun, H., Wang, Z., Dong, L., Qiao, C., Xia, C-m., Development of a Novel Class of Pyrrolo-[1,2,5]benzothiadiazepine Derivatives as Potential anti-Schistosomal Agents, *Bioorganic & Medicinal Chemistry Letters* (2013), doi: http://dx.doi.org/10.1016/j.bmcl.2013.04.085

Development of a Novel Class of Pyrrolo-[1,2,5]benzothiadiazepine Derivatives as Potential anti-Schistosomal Agents

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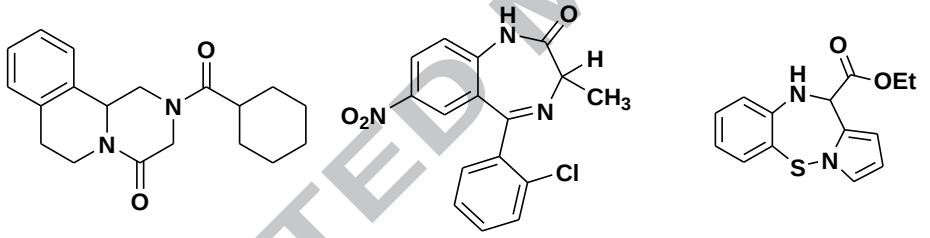
Abstract: Analogues of pyrrolo-[1,2,5]benzothiadiazepine were prepared and evaluated against *Schistosoma japonica*. The biological data revealed that most benzothiazepine derivatives show anti-schistosomal activity to some extent, while α-chloronation of the title compound and another bioisosteric derivative pyrrolo-[1,2,5]benzodiazepine displayed the most distinct worm killing activity. This study proved that benzodiazepine may serve as a novel structural skeleton for the development of anti-schistosomal agents.

Key word: benzothiadiazepine derivatives, anti-schistosomal

Schistosomiasis is one of the most burdensome nevertheless neglected tropical diseases. The World Health Organization (WHO) estimates that the number of people that treated for schistosomiasis has risen from 12.4 million in 2006 to 33.5 million in 2010.[1] Over the past 40 years, praziquantel (PZQ, Figure **1**) has been used as the only effective drug for schistosomal disease.[2,3]There are no back-up drugs for the treatment of schistosomiasis should praziquantel become less effective. Thus, there is an urgent need to develop newer anti-schistosomal agents.

A benzodiazepine derivative 3-methylclonazepam (Figure **1**, compound **2**) was

first reported by Kelly Chibale to show a marked degree of therapeutic and prophylactic activity against all stages of schistosomiasis caused by *Schistosoma mansoni* and *S. haematobium*.[4] Mechanism of action study indicated that this compound caused an increase in the influx of extracellular calcium and subsequent spastic paralysis of the parasite's musculature by binding to a low affinity benzodiazepine receptor on the epidermis of the schistosome.[5a,5b] Later on, another pyrrolo[1,2,5]benzothiadiazepine derivative (Figure **1,** compound **3**) was revealed to demonstrate good anti-schistosomal efficacy.[6] It is concealed that cytochalasin D (CyD) and calcium channel blockers counteracted the activity of compound **3**, both cytochalasin D (CyD) and calcium channel blockers may be involved in the calcium channels of schistosome.[7a,7b] These results prompted us to deepen our insight into a novel skeleton of pyrrolo[1,2,5]benzothiadiazepine as therapeutic agents against schistosomiasis.

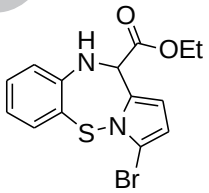


1. Praziquantel (PZQ) **2**. 3-Methylclonazepam **3**. Pyrrolo-[1,2,5]benzothiadiazepine

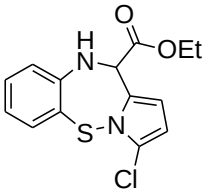
Figure 1. Structures of reported compounds with anti-schistosomal activities

Pyrrolo[1,2,5]benzothiadiazepine **3** was initially synthesized as non-nucleoside reverse transcriptase inhibitors,[8] but didn't show appreciable activity against HIV-1. Following the reported preparation method, compound **3** was synthesized. Its derivatives **SW-1** and **SW-2** (Figure 2) were then prepared efficiently using N-bromosuccinimide (NBS) and N-chlorosuccinimide (NCS) as halogenation reagents, respectively. Briefly, a mixture of compound **3** and halogenation reagents in acetonitrile was stirred at -20 ℃ for 8 hours, the reaction mixture was partitioned between aqueous and organic phase, purification by flash silica gel column afforded the desired compounds. In both cases, only α-halogenation products were obtained and nuclear magnetic resonance spectra were analyzed.[9] Introduction of the electron-withdrawing halogen atom to the electron-rich pyrrole ring was supposed to

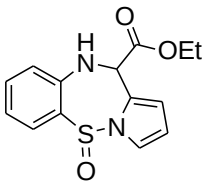
increase the chemical structure stability. **SW-11**[10] was prepared and evaluated because it possessed the same structural skeleton as compound **3**. Given the high structural similarity between pyrrolo[1,2,5]benzothiadiazepine and pyrrolo[1,2-*b*][1,2,5]benzothiadiazepine, 5-monooxide **SW-3**,[10] and pyrrolo[1,2-*b*][1,2,5]benzothiadiazepine 5,5-dioxides,[11] **SW-4**[12] as well as **SW-5**[12] were prepared (Figure **2**). Employing isosteric replacement principle, the sulfur atom in **SW-1** was replaced by nitrogen in **SW-6**, [11] **SW-7**, [13] **SW-8** and **SW-9**[14] (Figure **2**). The synthesis of these compounds were previously reported by us or other groups, and their biological activity against schistosomiasis was not fully described.[15] Analogously, one carbon takes the sulfur position provided compound **SW-10**, the synthesis of **SW-10** was shown in Scheme 1. Briefly, bromination of 1-methyl-2-nitro benzene by NBS provided 1-(bromomethyl)-2-nitrobenzene, following the reaction with hexamethylenetetramine in the same reaction pot, a white precipitation was formed and filtered, the benzylbromide was converted to amine after treating this reaction intermediate with conc. HCl in aqueous solution. 1-(2-Nitrobenzyl)-1*H*-pyrrole was prepared in 80% yield by treating the previous amine with 2,5-dimethoxy tetrahydrofuran. Next, the nitro group was reduced to amine using sodium hypophosphite, following the reaction with ethyl 2-ethoxy-2-hydroxyacetate, **SW-10** was prepared and its structure was fully characterized.[16] All the prepared compounds were characterized by ^{1}H, ^{13}C NMR and HRMS analysis.



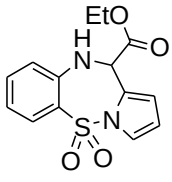
SW-1



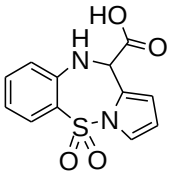
SW-2



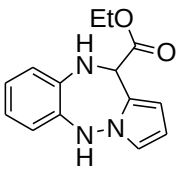
SW-3



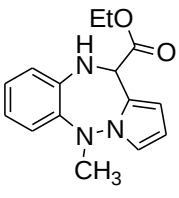
SW-4



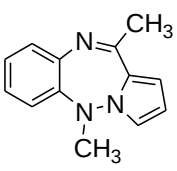
SW-5



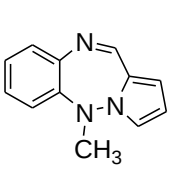
SW-6



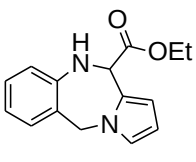
SW-7



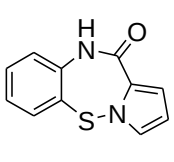
SW-8



SW-9

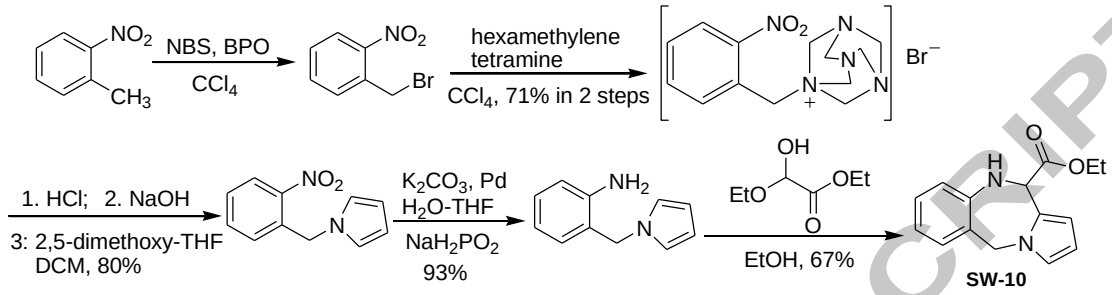


SW-10



SW-11

Figure 2. Design and prepared pyrrolo-[1,2,5]benzothiadiazepine derivatives



Scheme 1. Synthesis of compound **SW-10**.

The newly prepared analogues were evaluated for their ability against adult *Schistosoma japonica in vitro*. According to previously described method,[17] 6 male worms obtained from rat infections were distributed in duplicate tissue culture dishes (3.5 cm) in Dulbecco's modified minimum Eagle's medium (bicarbonate buffered) supplemented with 20% newborn calf serum, 100 U mL^{-1} penicillin, 100 μg mL^{-1} streptomycin and 0.5 μg mL^{-1} amphotericin B. Cultures were kept at 37℃in an atmosphere of 5% CO_2 in air and were observed under a Leica MZ 12.5 stereomicroscope. Worms of *Schistosoma japonica* were exposed overnight (16 h) to various compounds, washed and subsequently cultured in compound-free medium. Worms were obtained at day 42 infection. Compounds were then added from 5.0 mM DMSO stock solutions to achieve a concentration of 50 μM. Compound activity was assessed by percentage of survival and vitality score within 72 hours. The total score of 6 worms was counted for *in vitro* test. Data representative of repeated experiments was shown in Table **1**.

Table **1**. *In vitro* activity of pyrrolo-[1,2,5]benzothiadiazepine analogues against adult *Schistosoma japonica.*

	24 h		48 h		72 h	
Compd.	% worm survival	Vitality score	% worm survival	Vitality score	% worm survival	Vitality score

SW-1	100	14	100	13	66.7	9
SW-2	100	13	83.3	7	33.3	2
SW-3	100	14	100	16	100	14
SW-4	100	13	100	16	100	16
SW-5	100	14	100	16	100	14
SW-6	100	12	100	15	100	15
SW-7	100	14	100	11	83.3	10
SW-8	100	12	100	12	83.3	10
SW-9	100	14	100	14	83.3	10
SW-10	100	12	100	9	83.3	8
SW-11	100	11	100	13	100	13
PZQ	66.7	3	33.3	2.0	33.3	2.0
Comp 3	100	13	66.7	3.0	16.7	2
Control	100	18	100	18	100	18

The vitality score for each worm is illustrated as: 3 scores: the highest score, as observed in the control group during the observation period. Worms moved actively and softly, and the body was transparent. The highest score for 6 tested worms is 3x6=18; 2 scores: worms acted all over the body, but stiffly and slowly, with the body translucent and shrunken; 1 score: parasites moved partially with opaque and vacuole appearance. 0 score: the worm remained contracted and show severe tegument damage, did not resume movements, we could deem it 'dead'; Compound stock solutions (50.0 mM) were prepared in 100% DMSO immediately before use.

For the compounds tested against adult *Schistosoma japonica* in vitro, **SW-1, SW**-2, **SW-7, SW-8, SW-9** and **SW-10** show obvious effect within 72 hours. **SW-2** reduced worm motility significantly, the worm survival rate is only 33.3%. For comparison, PZQ demonstrated close worm killing capability as **SW-2** in this experiment. The activity of compound **SW-1** was weaker compared to **SW-2**, the worm survival percentage is 66.7% in 72 hours. Compounds **SW-7, SW-8, SW-9,** and **SW-10** exhibits almost the same moderate worm killing capability. Other derivatives **SW-3, SW-4, SW-5** and **SW-6** showed only very weak ability to reduce worm

survival rate within 24 hours. It is also interesting to notice that compound **3** killed most worms within 72 hours, the bromo- and chloro- substituted anologues **SW-1** and **SW-2** displayed reduced activity compared to compound **3**, but the in vitro worm killing activity is still the most potent among all tested compounds.

Based on the *in vitro* biological activity, compounds displayed moderate to good potency at 50 μM were further evaluated for their *in vivo* worm killing ability (Table **2**). Female ICR mice were infected with ca. 100 *Schistosoma japonicum* cercariae, on day 42 post-infection (adult stage), the infected mice were administrated with 3 consecutive days of 200 mg/kg oral doses of the test compounds, which was suspended in distilled water. Each experimental group used ten mice. At 21 days post-treatment, animals were sacrificed and dissected to assess total worm reduction as described in detail.[18]

Table **2** Effect of compounds **3**, **SW-1, SW-2**, **SW-7**, **SW-8 SW-9,** and **SW-10** to mice harboring 42-day-old adult *Schistosoma japonica*.

Compounds	Number of detected worms/mice (x±s)	Total worm reduction (%)
control	66±7	0
Compd 3	63±7	4.5
SW-1	58±5	12.0
SW-2	32±5	51.5
SW-7	43±4	34.8
SW-8	50±6	24.2
SW-9	62±8	6.1
SW-10	17±5	74.2
PZQ	2±2	96.9

The in vivo activity data revealed that among these compounds, **SW-2** and **SW-10** are the two compounds with significant activity, reduced the worm load more than 50%. Especially compound **SW-10**, which displayed only week in vitro efficacy,

demonstrated high in vivo capability and note worthily reduced worm burden in mice, whether or not this remarked potency is derived from the possible metabolite should be further investigated. Other evaluated compounds show marginal activity (**SW-1**, **SW-7** and **SW-8**), and **SW-9** is almost inactive. Although compound **3** almost lost its in vivo worm killing capability, its chlorination derivative **SW-2** exhibited fairly potent efficacy to reduce mice worm burden.

The structural activity relationship of pyrrolo-[1,2,5]benzothiazepine anologues revealed that bromination (**SW-1**) or chlorination (**SW-2**) of the pyrole ring would attenuate the parent compound antischistosomal activity both in vitro and in vivo. The oxidized sulfinyl and sulfonyl analogues, **SW-3**, **SW-4** and **SW-5** displayed reduced efficacy, suggesting the oxygen atom exerted an unfavorable effect for the compound worm killing ability. Replacement of sulfur atom by nitrogen (**SW-6**, **SW-7**, **SW-8** and **SW-9**) would retain the compounds capability in vitro; Meanwhile, this displacement did not endowed the compounds high worm killing capability in vivo. Finally, the pyrrolo-[1,2,5]benzodiazepine analogue **SW-10** exhibited the highest worm reducing capability in vivo. Considering its relative low in vitro efficacy, it is reasonable to believe that its good in vivo worm reducing ability may come from the metabolite form of **SW-10**. Therefore, further study of compound **SW-10** will be investigated.

In summary, we have identified pyrrolo-[1,2,5]benzothiadiazepine derivatives with activities against *Schistosoma japonica*. Although none of these analogues displayed activity equal to PZQ, the structural activity relationship revealed that chlorination of the pyrrol ring in the parent compound displayed elevated worm killing capability; Another pyrrolo-[1,2,5]benzodiazepine skeleton also displayed marked in vivo worm reducing efficacy. Therefore, more analogues with this skeleton is worthy of further exploration in the near future.

Acknowledgments

This project is funded by the Priority Academic Program Development of Jiangsu Higher Education Institutions.

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8. **SW-1**: ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.25–7.20 (m, 1H), 7.05 (t, *J* = 8.4 Hz, 1H), 6.58– 6.52 (m, 2H), 6.21 (d, *J* = 4.4 Hz, 1H), 6.17 (d, *J* = 3.9 Hz, 1H), 5.96 (d, *J* = 3.8 Hz, 1H), 5.04 (d, *J* = 4.2 Hz, 1H), 4.43–4.30 (m, 2H), 1.33 (t, *J* = 7.1 Hz, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 170.62, 147.20, 136.95, 132.57, 131.70, 118.17, 118.00, 115.84, 111.58, 108.69, 108.14, 62.62, 54.83, 14.26. HRMS $[ESI]^+$ cacul. for $[M+H]^+$: 352.9954; found: 352.9966, error 4.53 ppm. **SW-2**: ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.22 (d, *J* = 7.6 Hz, 1H), 7.06 (t, *J* = 7.7 Hz, 1H), 6.56 (t, *J* = 7.9 Hz, 2H), 6.18 (d, *J* = 5.1 Hz, 1H), 6.06 (d, *J* = 3.9 Hz, 1H), 5.93 (d, *J* = 3.8 Hz, 1H), 5.04 (d, *J* = 4.9 Hz, 1H), 4.49–4.22 (m, 2H), 1.34 (t, *J* = 7.1 Hz, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 170.60, 147.28, 135.43, 132.58, 131.70, 121.96, 118.21, 118.00, 115.83, 107.44, 107.05, 62.63, 54.62, 14.27. HRMS $[ESI]^+$ cacul. for $[M+H]^+$: 309.0459; found: 309.0461, error 0.64 ppm.
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16. **SW-10**: ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.05 (t, *J* = 7.7 Hz, 1H), 6.99 (d, *J* = 7.6 Hz, 1H), 6.68 (s, 1H), 6.66 – 6.59 (m, 2H), 6.04 (d, *J* = 3.0 Hz, 1H), 5.96 (s, 1H), 5.54 (s, 1H), 5.42 (d, *J* = 15.5 Hz, 1H), 4.87 (d, *J* = 15.5 Hz, 1H), 4.77 (s, 1H), 4.35 (p, *J* = 7.0 Hz, 2H), 1.34 (t, *J* = 7.1 Hz, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 171.11, 144.78, 129.52, 129.03, 128.47, 121.34, 120.57, 118.42, 118.39, 106.58, 106.40, 62.12, 54.30, 51.44, 14.25. HRMS:$[ESI]^+$ cacul. for $[M+H]^+$: 255.1128; found: 255.1127, error 0.39 ppm.
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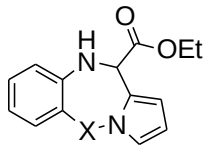
Graphical Abstract



X = S, C , NH, $NHCH_3$,  or 

with anti-schistosomal activity