

Article

Synthesis and Anti-Intestinal Nematode Activity of Variously Substituted Benzonaphthyridine Derivatives

Li-Ping Duan, Ai-Dan Wen, Ning-Bo Wu, Yi Tao and Hao-Bing Zhang *

Key laboratory of Parasitology and Vector Biology at National Institute of Parasitic Diseases, Chinese Center for Disease Control and Prevention, WHO Collaborating Centre for Malaria, Schistosomiasis, and Filariasis, National Institute of Parasitic Diseases, Shanghai 200025, China

* Author to whom correspondence should be addressed; E-Mail: zhanghaobing@hotmail.com.

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Abstract: A series of benzonaphthyridine derivatives bearing the C=N linkage moiety were designed and synthesized. The structures of all the newly synthesized compounds were identified by elemental analysis, ¹H-NMR, ¹³C-NMR and MS. Their anti-intestinal nematode activities against *Nippostrongylus brazilliensis* were evaluated *in vivo* by an oral route in male rats. Among these compounds, at concentrations of 10 mg/kg of rat, the compound 7-chloro-2-methoxy-10-(4-(4'-(1*H*-indol-5'-yl)methylene)aminophenyl)-amino-benzo[b][1,5] naphthyridine (**4n**) produced the highest activity, with 80.2% deparasitization. These compounds may find usefulness in the discovery and development of new anti-intestinal drugs.

Keywords: benzonaphthyridine; C=N linkage; *Nippostrongylus brazilliensis*

1. Introduction

Intestinal parasitic nematodes or roundworms infect over 1 billion people in tropical countries. According to a 2005 report by the World Health Organization (WHO), approximately 0.807–1.221 billion humans have *ascariasis*, 604–795 million have *trichuriasis*, and 576–740 million have hookworm infections worldwide [1]. Despite their harmfulness, few drugs for dealing with them exist [2-4]. Tribendimidine has good single-dose efficacy against some roundworm parasites [5], and in studies [6-8] tribendimidine showed high efficacy against *Nippostrongylus brazilliensis*, *Necator*

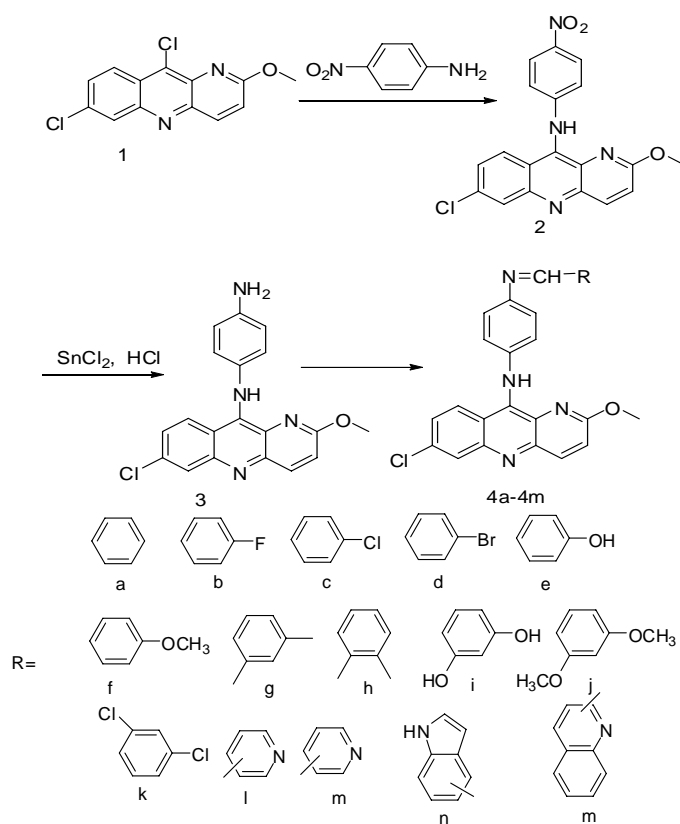
americanus, *Ancylostoma caninum*, and *Toxocara canis*. Furthermore, tribendimidine can continue to be effective in areas where albendazole has become less so due to resistance. This has been attributed to the presence of a toxophoric C=N linkage [5]. In our research group, we have been interested in studying the design, synthesis, and biological activity of compounds, especially heterocyclic compounds [9-14]. During the last 30 years a large number of derivatives belonging to the general benzonaphthyridine class have been prepared and evaluated extensively as antimalarial, antileishmanial and antitrypanosomal compounds [15-19]. This inspired us to assume that benzo[b][1,5]-naphthyridine derivatives incorporating a Schiff base might have some improved or different biological activities. In the present work, we describe the synthesis and results of some preliminary *in vivo* biological tests of 15 different novel benzonaphthyridine derivatives bearing a C=N linkage moiety.

2. Results and Discussion

2.1. Synthesis and Characterization of substituted benzo-naphthyridine derivatives (4a-4o)

The synthetic route to the target compounds **4a-4o** is shown in Scheme 1. Benzo[b][1,5]-naphthyridine derivative **1** had already been described [20].

Scheme 1. Synthesis of compounds **4a-4o**.



Direct nucleophilic substitution of chlorine at the 10 position of the benzo[b][1,5]-naphthyridine ring by *p*-nitroaniline afforded desired chloride **2** in good yield. The subsequent reduction of **2** using SnCl₂ as reducing agent led to the key intermediate **3**. Then **3** and different aromatic aldehydes were

mixed in boiling ethanol affording the target compounds **4a–4o**, which were recrystallized twice from ethanol to give satisfactory yields of 35–50%, respectively. Compounds **4a–4o** were characterized by elemental analysis, $^1\text{H-NMR}$, $^{13}\text{C-NMR}$ and MS. All results were in full agreement with the proposed structures. For example, the $^1\text{H-NMR}$ spectrum of compound **4b** showed a singlet at 4.18 ppm (OCH_3), singlet at 8.45 ppm (NH) and a singlet at around $\delta = 8.40$ due to one proton of the Schiff base. This was also confirmed by $^{13}\text{C-NMR}$ spectrum, which showed C=N signals at 160.71 ppm, moreover, the $^{13}\text{C-NMR}$ spectrum showed δ 53.90(OCH_3), 159.73, 154.89, 148.65, 148.02, 146.56, 143.71, 142.92, 142.50, 142.20, 140.49, 136.62, 135.11, 129.95, 129.09, 128.87, 127.76, 127.71, 126.60, 124.51, 122.58, 122.18, 119.42, 118.59, 115.76 (Ar-C), all consistent with its proposed structure. The elemental analyses results were in good agreement with those calculated for the suggested formula. Further confirmation for the structure of compound **4b** was obtained by MS, which showed the expected $[\text{M}+1]^+$ ion at 457.27. Additionally, the melting points are sharp, suggesting the purity of these compounds.

2.2. Anti-intestinal nematode activity

From Table 1, we can see that some of compounds showed significant anti-intestinal nematode activity in a two-day *in vivo* test in rats. At concentrations of 10 mg/kg of rat, compound **4n** produced the highest activity against *Nippostrongylus brazilliensis*, with 80.3% deparasitization.

Table 1. Effect of benzo[b][1,5] naphthyridine derivatives in treatment of rats infected with NbL3 for 11 days (**4a–4o**).

| Compound | Dose(mg/kg) | Number of rats | Worm recovery | | |
|-------------|-------------|----------------|---------------|-------|---------------|
| | | | Expelled | Total | Reduction (%) |
| Albendazole | 10 | 5 | 245 | 248 | 98.8 |
| 4a | 10 | 5 | 114 | 543 | 21.0 |
| 4b | 10 | 5 | 75 | 297 | 25.2 |
| 4c | 10 | 5 | 74 | 322 | 23.0 |
| 4d | 10 | 5 | 48 | 237 | 20.2 |
| 4e | 10 | 5 | 100 | 426 | 23.4 |
| 4f | 10 | 5 | 75 | 269 | 27.9 |
| 4g | 10 | 5 | 68 | 326 | 20.8 |
| 4h | 10 | 5 | 69 | 287 | 24.0 |
| 4i | 10 | 5 | 82 | 306 | 26.8 |
| 4j | 10 | 5 | 89 | 370 | 24.0 |
| 4k | 10 | 5 | 79 | 310 | 25.4 |
| 4l | 10 | 5 | 95 | 189 | 50.2 |
| 4m | 10 | 5 | 101 | 197 | 51.2 |
| 4n | 10 | 5 | 215 | 268 | 80.2 |
| 4o | 10 | 5 | 176 | 248 | 70.9 |

Expelled: number of expelled worms; Total: total worm number; Reduction: worm burden reduction.

To investigate the role of substituent effects on activity, a variety of substituents could be introduced on the phenyl ring. The F, OCH_3 , OH, Cl and Br-substituted derivatives all have almost the

same activity as **4a**. Furthermore, disubstituted derivatives also appeared to have similar potency as the monosubstituted phenyl analogues. For instance, the replacement of the 4'-F in **4'** by a hydroxyl group led to only slightly less potency, and compound **4f** inhibited *Nippostrongylus brazilliensis* with a value similar to that of the 2,4-disubstituted analogue **4j**. In contrast, derivatives **4g** and **4l** were slightly less active than the corresponding isomers **4h** and **4m**. On the other hand, replacement of the phenyl ring by a pyridyl ring, indole group, or quinoline group resulted in increased effects, suggesting a preference for hetero-aromatic rings in maximizing activity. This indicated that indole group was the most important group involved in this activity. The quinoline group also seems to influence this activity, while the pyridyl group appeared to give only a small contribution. Because compound **4n** displayed anti-intestinal nematode potency that was comparable to albendazole (10 mg/kg), further anti-intestinal nematode activity assays were carried out for compound **4n**. It was found that at concentrations of 30 mg/kg of rat, **4n** produced the highest activity against *Nippostrongylus brazilliensis* with 98.8% effectiveness, which implies further possibilities for the development of new anti-intestinal drugs.

3. Experimental

3.1. Materials and reagents

All solvents and other reagents were of high purity (Aldrich and Sigma) and were used without further purification. Elemental analysis was performed on a PE-2400 Elemental Analyzer, the C, H and N analysis were repeated twice. ¹H-NMR and ¹³C-NMR spectra were obtained in CDCl₃ with TMS as internal standard on a Bruker AM-400 spectrometer. Chemical shifts were reported as ppm. Mass spectra were measured on a HP 1100 LC-MS spectrometer. Melting points were determined by an BÜCHI melting point B-540 apparatus and are uncorrected.

3.2. Synthesis of 7-chloro-2-methoxy-10-aminobenzo[b][1,5]naphthyridine (**2**)

A solution obtained dissolving equimolar amounts of the starting compound 7-chloro-2-methoxy-10-chloro-benzo[b][1,5]naphthyridine (**1**) and *p*-nitroaniline in absolute ethanol (150 mL) was refluxed for 2.5–3 h until the starting materials disappeared (TLC, ethyl acetate/*n*-hexane 1:4). On standing and cooling a red-orange precipitate formed which was filtered off and dried (70–80% yields). Purification was accomplished by repeated crystallization from ethanol. LC-MS: [M+1]⁺ 381.17.

3.3. Synthesis of 7-chloro-2-methoxy-10-(4-aminophenyl)-aminobenzo[b][1,5] naphthyridine (**3**)

Compound **2** (3.80 g, 0.01 mol) was added to a previously prepared solution of glacial acetic acid (40 mL) containing SnCl₂ (15.3 g, 0.08 mmol) and aerated with hydrogen chloride. The reaction mixture was stirred for 1 h. The precipitated solid was collected by filtration and washed with water. Crystallization from acetic acid gave **3** as red–orange needles: 3.46 g (98.8%); LC-MS: [M+1]⁺ 351.29.

3.4. General procedure for the synthesis of Schiff bases 4a-4o

To a mixture of **3** (0.005 mol) and substituted aromatic aldehyde (0.005 mol) in absolute ethanol (40 mL) was added few drops of glacial acetic acid. Then the mixture was refluxed on water bath for 5–6 h. The excess of solvent was distilled off, poured onto ice cold water. The separated solid was filtered, washed and recrystallized from ethanol.

7-Chloro-2-methoxy-10-(4-benzylideneaminophenyl)-aminobenzo[b][1,5]naphthyridine (4a). Yield 35%, m.p. 180.2–181.3 °C. $^1\text{H-NMR}$ δ : 8.41 (s, 1H, Ar-NH), 8.37(s, 1H, CH=N), 8.24–6.82 (m, 14H, Ar-H), 4.16 (s, 3H, OCH₃); $^{13}\text{C-NMR}$ δ : 160.20, 159.62, 154.70, 149.00, 148.12, 146.45, 143.71, 142.00, 140.50, 137.63, 131.65, 130.86, 130.78, 129.24, 124.78, 123.16, 123.10, 122.17, 122.16, 121.76, 119.28, 119.16, 115.78, 114.44, 113.15, 53.69; Anal. Calcd. for C₂₆H₁₉ClN₄O (439.82): C 71.15, H 4.36, N 12.77; found C 71.16, H 4.38, N 12.77.

7-Chloro-2-methoxy-10-(4-(4'-fluorobenzylidene)aminophenyl)-aminobenzo[b][1,5]naphthyridine (4b). Yield 40%, m.p. 191.6–191.8 °C. $^1\text{H-NMR}$ δ : 8.45 (s, 1H, Ar-NH), 8.40 (s, 1H, CH=N), 8.29–7.02 (m, 13H, Ar-H), 4.18 (s, 3H, OCH₃); $^{13}\text{C-NMR}$ δ : 160.71, 159.73, 154.89, 148.65, 148.02, 146.56, 143.71, 142.92, 142.50, 142.20, 140.49, 136.62, 135.11, 129.95, 129.09, 128.87, 127.76, 127.71, 126.60, 124.51, 122.58, 122.18, 119.42, 118.59, 115.76, 53.90; Anal. Calcd. for C₂₆H₁₈ClFNaO (457.27): C 68.39, H 4.00, N 12.07; found C 68.39, H 4.00, N 12.07.

7-Chloro-2-methoxy-10-(4-(4'-chlorobenzylidene)aminophenyl)-aminobenzo[b][1,5]naphthyridine (4c). Yield 42%, m.p. 193.4–194.1 °C. $^1\text{H-NMR}$ δ : 8.45 (s, 1H, Ar-NH), 8.40 (s, 1H, CH=N), 8.25–6.98 (m, 13H, Ar-H), 4.16 (s, 3H, OCH₃); $^{13}\text{C-NMR}$ δ : 160.83, 159.80, 154.70, 149.79, 148.32, 144.40, 143.87, 142.21, 140.36, 138.06, 132.56, 131.45, 129.65, 126.09, 124.87, 123.76, 122.67, 122.48, 121.69, 119.20, 119.17, 117.09, 116.47, 115.20, 53.80 ; Anal. Calcd. for C₂₆H₁₈Cl₂N₄O (474.25): C 65.97, H 3.83, N 14.98; found C 65.99, H 3.83, N 14.98.

7-Chloro-2-methoxy-10-(4-(4'-bromobenzylidene)aminophenyl)-aminobenzo[b][1,5]naphthyridine (4d). Yield 37%, m.p. 188.6–191.0 °C. $^1\text{H-NMR}$ δ : 8.47 (s, 1H, Ar-NH), 8.40(s, 1H, CH=N), 8.25–7.00 (m, 13H, Ar-H), 4.17 (s, 3H, OCH₃); $^{13}\text{C-NMR}$ δ : 160.83, 159.60, 149.71, 148.38, 144.84, 143.89, 142.41, 140.40, 138.96, 132.66, 131.95, 129.75, 126.49, 125.67, 124.86, 123.69, 123.68, 120.69, 119.29, 119.20, 118.89, 117.57, 116.27, 115.48, 53.90 ; Anal. Calcd. for C₂₆H₁₈BrClN₄O (519.21): C 60.31, H 3.50, N 10.82; found C 60.31, H 3.51, N 10.85.

7-Chloro-2-methoxy-10-(4-(4'-hydroxybenzylidene)aminophenyl)-amino-benzo[b][1,5] naphthyridine (4e). Yield 39%, m.p. 201.3–201.5 °C. $^1\text{H-NMR}$ δ : 8.44 (s, 1H, Ar-NH), 8.20 (s, 1H, CH=N), 8.28–6.84 (m, 13H, Ar-H), 5.50 (s, 1H, OH), 4.18 (s, 3H, OCH₃); $^{13}\text{C-NMR}$ δ : 160.83, 160.21, 159.74, 149.68, 148.45, 144.91, 143.56, 142.35, 138.95, 132.45, 130.68, 129.87, 127.49, 125.86, 123.89, 123.70, 123.45, 120.69, 119.34, 119.08, 118.54, 116.60, 116.27, 115.50, 114.56, 59.62 ; Anal. Calcd. for C₂₆H₁₉ClN₄O₂ (455.28): C 64.43, H 4.05, N 11.58; found C 64.43, H 4.05, N 11.58.

7-Chloro-2-methoxy-10-(4-(4'-methoxybenzylidene)aminophenyl)-aminobenzo[b][1,5] naphthyridine (4f). Yield 45%, m.p. 170.8–171.1 °C. ¹H-NMR δ: 8.87 (s, 1H, Ar-NH), 8.40(s, 1H, CH=N), 8.20–6.68 (m, 13H, Ar-H), 4.18 (s, 3H,OCH₃), 3.82 (s, 3H,OCH₃); ¹³C-NMR δ: 160.73, 159.23, 149.91, 148.98, 145.82, 143.90, 142.53, 138.96, 132.70, 131.90, 129.86, 127.31, 126.77, 125.77, 123.54, 123.25, 121.99, 120.29, 119.78, 118.96, 116.78, 116.20, 106.48, 103.76, 54.60, 53.82; Anal. Calcd. for C₂₇H₂₁ClN₄O₂ (419.18): C 69.15, H 4.51, N 11.95; found C 69.16, H 4.51, N 11.96.

7-Chloro-2-methoxy-10-(4-(2',4'-dimethylbenzylidene)aminophenyl)aminobenzo[b][1,5] naphthyridine (4g). Yield 46%, m.p. 161.1–161.9 °C. ¹H-NMR δ: 8.80 (s, 1H, Ar-NH), 8.40 (s, 1H, CH=N), 8.39–6.78 (m, 12H, Ar-H), 4.18 (s, 3H,OCH₃), 2.55(s, 3H, CH₃), 2.35 (s, 3H, CH₃); ¹³C-NMR δ: 160.61, 158.34, 149.11, 148.74, 143.12, 142.87, 141.49, 140.81, 140.48, 138.58, 135.18, 131.88, 131.48, 125.77, 128.86, 128.41, 127.91, 127.26, 126.75, 124.28, 122.79, 122.15, 119.37, 115.46, 53.79, 21.57,19.47; Anal. Calcd. for C₂₈H₂₃ClN₄O (467.29): C 69.10, H 5.34, N 11.49; found C 69.18, H 5.34, N 11.43.

7-Chloro-2-methoxy-10-(4-(2',3'-dimethylbenzylidene)aminophenyl)-aminobenzo[b][1,5] naphthyridine (4h). Yield 48%, m.p. 125.1–125.7 °C. ¹H-NMR δ: 8.45 (s, 1H, Ar-NH), 8.40(s, 1H, CH=N), 8.20–7.01 (m, 12H, Ar-H), 4.18 (s, 3H,OCH₃), 2.34(s, 6H, CH₃); ¹³C-NMR δ: 160.62, 158.34, 149.11, 148.74, 143.10, 142.80, 141.48, 140.80, 140.47, 138.56, 135.08, 131.78, 131.48, 125.77, 128.86, 128.41, 127.90, 127.26, 126.70, 124.28, 122.78, 122.05, 119.37, 115.46, 53.89, 21.47,19.37; Anal. Calcd. for C₂₈H₂₃ClN₄O (467.28): C 71.87, H 4.62, N 11.68; found C 71.87, H 4.62, N 11.68.

7-Chloro-2-methoxy-10-(4-(2',4'-dihydroxybenzylidene)aminophenyl)-aminobenzo[b][1,5]naphthyridine (4i). Yield 44%, m.p. 232.1–232.9 °C. ¹H-NMR δ: 8.45 (s, 1H, Ar-NH), 8.40(s, 1H, CH=N), 8.20–7.01 (m, 12H, Ar-H), 4.18 (s, 3H,OCH₃), 1.44(s, 2H, OH); ¹³C-NMR δ: 160.63, 158.38, 149.16, 148.77, 143.13, 142.80, 141.48, 140.83, 140.49, 138.58, 135.68, 131.80, 131.56, 125.79, 128.89, 128.43, 127.92, 127.36, 126.78, 124.59, 122.78, 122.16, 119.39, 115.48, 53.91; Anal. Calcd. for C₂₆H₁₉ClN₄O₃ (471.27): C 66.07, H 3.76, N 11.50; found C 66.07, H 3.76, N 11.50.

7-Chloro-2-methoxy-10-(4-(2',4'-methoxybenzylidene)aminophenyl)-aminobenzo[b][1,5] naphthyridine (4j).Yield 45%, m.p. 160.2–161.8 °C. ¹H-NMR δ: 8.89 (s, 1H, Ar-NH), 8.40 (s, 1H, CH=N), 8.20–6.55 (m, 12H, Ar-H), 4.18 (s, 3H,OCH₃), 3.83 (s, 3H,OCH₃), 3.82 (s, 3H,OCH₃); ¹³C-NMR δ: 160.73, 159.23, 149.91, 148.98, 145.82, 143.90, 142.53, 138.96, 132.70, 131.90, 129.86, 127.31, 126.77, 125.77, 123.54, 123.25, 121.99, 120.29, 119.78, 118.96, 116.78, 116.20, 115.48, 114.76, 54.60, 53.82, 53.80; Anal. Calcd. for C₂₈H₂₃ClN₄O₃ (499.29): C 66.21, H 5.19, N 10.52; found C 66.21, H 5.19, N 10.52.

7-Chloro-2-methoxy-10-(4-(2',4'-dichlorobenzylidene)aminophenyl)-aminobenzo[b][1,5] naphthyridine (4k). Yield 39%, m.p. 220.1–221.2 °C. ¹H-NMR δ: 8.59 (s, 1H, Ar-NH), 8.40 (s, 1H, CH=N), 8.20–6.99 (m, 12H, Ar-H), 4.18 (s, 3H,OCH₃); ¹³C-NMR δ: 160.63, 159.13, 149.00, 148.88, 145.83, 143.91, 142.59, 138.99, 132.72, 131.91, 129.88, 127.32, 126.79, 125.79, 123.56, 123.35, 121.99,

120.39, 119.79, 118.98, 116.79, 116.21, 115.49, 114.78, 53.90; Anal. Calcd. for C₂₆H₁₇Cl₃ N₄O (507.21): C 60.65, H 3.70, N 10.69; found C 60.90, H 3.53, N 10.87.

7-Chloro-2-methoxy-10-(4-(2'-pyridylidene)aminophenyl)-amino-benzo[b][1,5] naphthyridine (4l). Yield 48%, m.p. 227.2–228.7 °C. ¹H-NMR δ: 8.75 (s, 1H, Ar-NH), 8.41(s, 1H, CH=N), 8.80–7.05 (m, 13H, Ar-H), 4.18 (s, 3H, OCH₃); ¹³C-NMR δ: 160.69, 159.23, 149.09, 148.78, 145.85, 143.92, 142.59, 138.99, 132.76, 131.93, 129.88, 127.35, 126.69, 125.81, 123.66, 123.36, 121.98, 120.49, 119.89, 118.45, 116.80, 116.20, 115.38, 114.78, 53.91; Anal. Calcd. for C₂₅H₁₈ClN₅O (440.26): C 62.76, H 3.79, N 14.40; found C 62.70, H 3.94, N 14.42.

7-Chloro-2-methoxy-10-(4-(3'-pyridylidene)aminophenyl)-aminobenzo[b][1,5]-naphthyridine (4m). Yield 41%, m.p. 190.1–190.2 °C. ¹H-NMR δ: 8.76 (s, 1H, Ar-NH), 8.40 (s, 1H, CH=N), 8.91–7.09 (m, 13H, Ar-H), 4.18 (s, 3H, OCH₃); ¹³C-NMR δ: 160.67, 157.97, 148.73, 147.91, 142.91, 141.22, 140.55, 135.07, 130.75, 130.68, 129.88, 127.32, 126.79, 125.79, 123.56, 123.35, 121.99, 120.39, 119.79, 118.98, 116.79, 116.21, 115.49, 114.78, 53.90; Anal. Calcd. for C₂₅H₁₈ClN₅O (440.26): C 68.26, H 4.12, N 15.92; found C 68.28, H 4.14, N 15.94.

7-Chloro-2-methoxy-10-(4-(4'-(1H-indol-5'-yl)methylene)aminophenyl)-aminobenzo[b][1,5]-naphthyridine (4n). Yield 45%, m.p. 226.8–227.7 °C. ¹H-NMR δ: 8.68 (s, 1H, Ar-NH), 8.40 (s, 1H, CH=N), 8.45–7.10 (m, 14H, Ar-H), 6.61 (s, 1H, Pyrrol-NH), 4.18 (s, 3H, OCH₃); ¹³C-NMR δ: 160.60, 157.98, 148.76, 147.87, 142.96, 141.24, 140.55, 140.33, 139.80, 136.31, 135.76, 130.69, 130.65, 129.89, 127.36, 126.80, 125.78, 123.58, 123.38, 122.01, 120.39, 119.82, 118.98, 116.79, 116.21, 115.47, 114.96, 53.78; Anal. Calcd. for C₂₈H₂₀ClN₅O (478.30): C 69.33, H 4.31, N 14.23; found C 69.33, H 4.31, N 14.23.

7-Chloro-2-methoxy-10-(4-(4'-(quinoline-2'-yl)methylene)aminophenyl)-aminobenzo[b][1,5]naphthyridine (4o). Yield 44%, m.p. 219.7–220.9 °C. ¹H-NMR δ: 9.24 (s, 1H, Ar-NH), 8.40 (s, 1H, CH=N), 9.05–7.15 (m, 15H, Ar-H), 4.18 (s, 3H, OCH₃); ¹³C-NMR δ: 160.67, 159.21, 158.45, 158.21, 157.99, 148.84, 147.68, 142.91, 141.20, 140.59, 135.14, 130.36, 130.23, 129.89, 127.33, 126.76, 125.78, 123.56, 123.35, 121.99, 120.39, 119.87, 118.98, 116.82, 116.71, 115.49, 115.41, 53.92; Anal. Calcd. for C₂₉H₂₀ClN₅O (490.32): C 70.98, H 4.17, N 14.04; found C 71.01, H 4.18, N 14.12.

3.5. Biological assays

All analogues were tested against *N. brazilliensis* to evaluate their anti-intestinal nematode activities. These compounds were tested on 15 groups of rats, each containing five rats. Eighty male rats, weighing 70–80 g, were provided by the Shanghai Animal Center (Chinese Academy of Sciences). Rats were kept at the animal facility of our institute. Animals were acclimatized for 1 week before infection, and they had free access to water and food. The life cycle of *N. brazilliensis* were maintained in our laboratory. Egg-positive feces were obtained from *N. brazilliensis*-infected rats at 12–20 days post-infection, and then used for the cultivation of *N. brazilliensis* third stage infective larvae (NbL3) as described previously [21]. For the *in vivo* test, rats were each infected subcutaneously

with 300 NaL3, all infected rats were treated with compounds 11 days post-infection. For oral administration, compounds were suspended or dissolved in 7% Tween 80 and 3% alcohol, and the concentrations of each drug were 10 mg/kg. The reference compound was albendazole, and water containing 3% alcohol and 7% Triton X-80 was used as a negative control. The percentage deparasitization was calculated using the following formula:

$$n/N \times 100$$

where N = average number of total worms found from small intestine, large intestine and feces in the control animals and n = average number of worms found from large intestine and feces in the control animals. Evaluations were based on a percentage scale of 0–100, in which 100 was total kill and 0 was no activity.

4. Conclusions

In summary, various types of substituted benzonaphthyridine derivatives were synthesized and their biological activities towards the *N. brazilliensis* were demonstrated. Among these compounds, **4n** produced the highest activity against *N. brazilliensis*, with 80.2% deparasitization. The present work suggested that **4n** may be a useful lead compound for anti-intestinal nematode medicine development. Further studies of the structure-activity relationships around the designed compounds are underway.

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References and Notes

1. WHO. Deworming for health and development. In Proceedings of the third global meeting of the partners for parasite control, Geneva, Switzerland, 29-30 November, 2004; World Health Organization: Geneva, Switzerland, 2005.
2. Bennett, A.; Guyatt, H. Reducing intestinal nematode infection: Efficacy of albendazole and mebendazole. *Parasitol. Today* **2000**, *16*, 71-74.
3. Kaplan, R.M. Drug resistance in nematodes of veterinary importance: A status report. *Trends Parasitol.* **2004**, *20*, 477-481.
4. Flohr, C.; Tuyen, L.N.; Lewis, S.; Minh, T.T.; Campbell, J. Low efficacy of mebendazole against hookworm in Vietnam: Two randomized controlled trials. *Am. J. Trop. Med. Hyg.* **2007**, *76*, 732-736.
5. Xiao, S.H.; Hui, M.W.; Tanner, M.; Utzinger, J.; Chong, W. Tribendimidine: A promising, safe and broad-spectrum anthelmintic agent from China. *Acta Trop.* **2005**, *94*, 1-14.
6. Zhang, J.H.; Xiao, S.H.; Wu, Z.X.; Qiu, D.C.; Wang, S.H.; Wang, S.Q.; Wang, C. Tribendimidine enteric coated tablet in treatment of 1,292 cases with intestinal nematode infection—a phase IV clinical trial (in Chinese). *Chin. J. Parasitol. Parasit. Dis.* **2008**, *26*, 6-9.

7. Xiao, S.H.; Jian, X.; Tanner, M.; Yong, N.Z.; Keiser, J.; Utzinger, J.; Qiang, H.Q. Artemether, artesunate, praziquantel and tribendimidine administered singly at different dosages against *Clonorchis sinensis*: a comparative *in vivo* study. *Acta Trop.* **2008**, *106*, 54-59.
8. Keiser, J.; Xiao, S.H.; Chollet, J.; Tanner, M.; Utzinger, J. Evaluation of the *in vivo* activity of tribendimidine against *Schistosoma mansoni*, *Fasciola hepatica*, *Clonorchis sinensis*, and *Opisthorchis viverrini*. *Antimicrob. Agents Chemother.* **2007**, *51*, 1096-1098.
9. Duan, L.P.; Xu, Y.F.; Qian, X.H.; Zhang, Y.X.; Liu, Y. Novel naphthalimide derivatives with near-infrared emission: Synthesis via photochemical cycloaromatization, fluorescence in solvents and living cell. *Tetrahedron Lett.* **2009**, *50*, 22-25.
10. Duan, L.P.; Xu, Y.F.; Qian, X.H. Highly Sensitive and Selective Pd²⁺ Sensor of Naphthalimide Derivative Based on Complexation with Alkynes and Thio-heterocycle. *Chem. Commun.* **2008**, 6339-6341.
11. Cheng, T.Y.; Xu, Y.F.; Zhang, S.Y.; Qian, X.H.; Duan, L.P. A highly sensitive and selective OFF-ON fluorescent sensor for cadmium in aqueous solution and living cell. *J. Am. Chem. Soc.* **2008**, *130*, 16160-16161.
12. Xue, S.J.; Duan, L.P.; Ke, S.Y. Crystal structure and herbicidal activity 5,7-dimethoxy-(2,4-dichlorophenoxyacetylmino)-2H-1,2,4-thiadiazolo[2,3-a]pyrimidine. *J. Chin. Struct. Chem.* **2005**, *6*, 730-734.
13. Xue, S.J.; Ke, S.Y.; Duan, L.P. Ultrasonic irradiated synthesis and herbicidal activities of 5,7-disubstituted-2-(substitutedpyridine-3-formylimino)2H-1,2,4-thiadiazolo[2,3-a]pyrimine derivatives. *J. Indian Chem. Soc.* **2005**, *82*, 79-82.
14. Duan, L.P.; Zhao, Q.F.; Zhang, H.B. Novel 2H-[1,2,4]thiadiazolo[2,3-a]pyrimidine derivatives bearing chiral S-2-(4-chlorophenyl)-3-methylbutyric acid moiety: Design, synthesis and biology activity. *Arab. J. Chem.* **2010**, *3*, 225-228.
15. Chavalitsheewinkoo, P.; Wilairat, P.; Gamage, S.A.; Denny, W.A.; Figgitt, D.P.; Ralph, R. Structure-activity relationships and modes of action of 9-anilinoacridines against chloroquine-resistant *Plasmodium falciparum* in vitro. *Antimicrob. Agents Chemother.* **1993**, *37*, 403-406.
16. Gamage, S.A.; Tepsiri, N.; Wilairat, P.; Wojcik, S.J.; Denny, W.A. Synthesis and *in vitro* evaluation of 9-Anilino-3,6-diaminoacridines Active Against a Multidrug-Resistant Strain of the Malaria Parasite *Plasmodium falciparum*. *J. Med. Chem.* **1994**, *37*, 1486-1494.
17. Mael, J.; Denny, W.A.; Gamage, S.A.; Ralph, R.; Wojcik, S.J.; Figgitt, D.P.; Ralph, R. 9-Anilinoacridines as potential antileishmanial agents. *Antimicrob. Agents Chemother.* **1993**, *37*, 991-996.
18. Gamage, S.A.; Figgitt, D.P.; Wojcik, S.J.; Ralph, R.; Ransijn, A.; Mael, J.; Yardley, V.; Snowdon, D.; Croft, S. L.; Denny, W.A. Structure-activity relationships for the Antileishmanial and Antitrypanosomal Activities of 1-Substituted 9-Anilinoacridines. *J. Med. Chem.* **1997**, *40*, 2634-2642.
19. Figgitt, D.P.; Denny, W.A.; Chavalitsheewinkoo, P.; Wilairat, P.; Ralph, R. *In vitro* study of anticancer acridines as potential antitrypanosomal and antimalarial. *Antimicrob. Agents Chemother.* **1992**, *36*, 1644-1647.
20. Denny, W.A.; Cain, B.F.; Atwell, G.L.; Hansch, C.; Panthanickal, A.; Leo, A. Potential antitumor agents: Quantitative relationships between experimental antitumor activity, toxicity,

and structure for the general class of 9-anilinoacridine antitumor agents. *J. Med. Chem.* **1982**, *25*, 276-315.

21. Xue, J.; Liu, S.; Qiang, H.Q.; Ren, H.N.; Li, T.H.; Xue, H.C.; Hotez, P.J.; Xiao, S.H. Necator americanus: maintenance through one hundred generations in golden hamsters (*Mesocricetus auratus*).I. Host sex-associated differences in hookworm burden and fecundity. *Exp. Parasitol.* **2003**, *104*, 62-66.

Sample Availability: Samples of the compounds **4a-4o** are available from the authors.

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