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Development of chiral praziquantel analogues as potential drug candidates with activity to juvenile *Schistosoma japonicum*

Yang Zheng [a,†], LanLan Dong [b,†], Changyan Hu [a], Bo Zhao [b], Chunhua Yang [a], Chaoming Xia [b,*], Dequn Sun [a,*]

[a] Marine College, Shandong University at Weihai, No. 180, Wenhua West Road, Weihai 264209, PR China
[b] Department of Parasitology, Medical College of Soochow University, Jiangsu 215123, PR China

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ABSTRACT

A series of chiral praziquantel analogues were synthesized and evaluated against *Schistosoma japonicum* both in vitro and in vivo. All compounds exhibited low to considerable good activity in vivo. Remarkably, worm reduction rate of R-**3** was 60.0% at a single oral dose of 200 mg/kg against juvenile stage of *Schistosoma japonicum*. The target compounds displayed in vivo antischistosomal activity against both *Schistosoma japonicum* and *Schistosoma mansoni*. Furthermore, all R-isomers displayed stronger antischistosomal activity than S-isomers in vivo, indicating R-isomers were the active enantiomers, while S-isomers were less active ones. This structure activity relationship (SAR) could have important implications in further drug development for schistosomiasis.

Nowadays, there is growing awareness of the huge public health significance of the so-called neglected tropical diseases (NTDs). The huge global burden due to NTDs is estimated to be similar to human immunodeficiency virus/AIDS, malaria, and tuberculosis.[1,2] As an important member of NTDs,[1,3] schistosomiasis is an ordinary chronic parasitic disease in the world, especially in tropical and subtropical regions.[4,5] About 200 million people are infected with schistosomiasis, with 650 million people at a risk of infection all over the world.[6,7] In spite of this severe situation, the most important drug in clinical use to control all forms of schistosomiasis is only praziquantel (PZQ). After the introduction since 1970s, PZQ is risking drug resistance or tolerance. Recently, schistosome isolated with diminished sensitivity to PZQ continues to be identified.[8] It is very dangerous to lack of back-up drugs for the treatment of schistosomiasis. PZQ is well known to have excellent activity against adult schistosome. However, its annoying, even fatal weakness is its low efficacy against juvenile stages of schistosomes,[9] this is absolutely the exclusive reason for the endless schistosomiasis and cause the unconditional necessity to find molecules with good activity against juvenile stages of schistosomes in order to cease schistosomiasis completely. However, due to the unclear action mechanism of PZQ, it is blind and difficult to design the rational molecule with potential anti-schistosomal activity. So far, several attempts have been made to find bioactive PZQ derivatives,[10–13] as well as other candidates for the treatment of schistosomiasis.[14,15]

It has been reported that[16] the PZQ metabolites (Fig. 1), including the major trans-cyclohexanol metabolite, are less active than the parent compound itself.

In order to impede the metabolism and increase metabolic stability, racemate **3** (Fig. 2, Rac-**3**) with carbonyl group at metabolic liable position was designed, its worm killing activity against both juvenile and adult stages of *S. mansoni* in infected mice was evaluated.[10]

It was interestingly found this compound showed significant in vivo activity to *S. mansoni* even with no in vitro activity.

This interesting phenomenon impelled us to conjecture that such kind of compounds might have similar bioactivity against *S. japonicum*. Furthermore, PZQ used in clinic is a mixture of R-PZQ

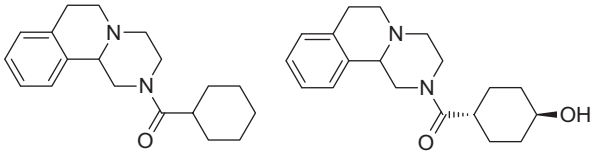

Figure 1. Praziquantel (PZQ) and its major metabolite.

* Corresponding authors.
E-mail addresses: xiachaoming@suda.edu.cn (C. Xia), dequn.sun@sdu.edu.cn (D. Sun).
† These authors contribute equally.

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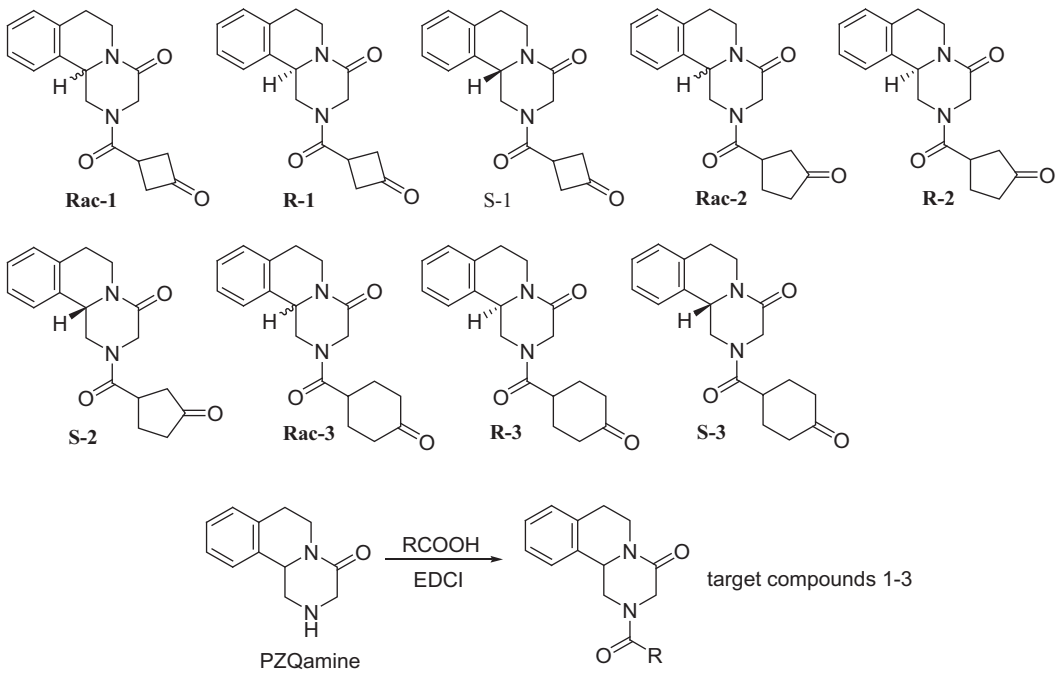

Figure 2. PZQ analogues, their chiral isomers and preparation.

and S-PZQ, and the R-isomer is the eutomer, while the S-isomer is the inactive enantiomer. The isomers have very different antischistosomal activity.[17] Chiral isomers of compound **3** and its analogues have not been synthesized and evaluated for their activity against *S. japonicum*. We further hypothesised that chiral isomers of carbonyl compounds (**1**, **2**, **3**) would reveal similar activity behaviour like PZQ and its chiral isomers regarding to *S. japonicum*. Therefore, in order to study their SAR and to verify our hypothesis, three carbonyl compounds and their corresponding chiral isomers (Fig. 2) were prepared to estimate both in vitro and in vivo activity against *S. japonicum*.

All nine test compounds were prepared by standard condensation reaction between PZQ amines and corresponding acids using EDCI as coupling reagent. The nine target compounds were obtained in 73–80% yield.[18] The chiral PZQ amines used for preparation of chiral target compounds were obtained through the reported resolution method reported.[19] The ee value of chiral PZQ amines was measured as over 99% by HPLC (DAICEL AD-H, 4.6 mm*250 mm, with hexane/ethanol/triethylamine = 80:20:0.1 as eluent, flow rate 1.0 ml/min).

All prepared compounds were evaluated for their activity against adult and juvenile *S. japonicum*. Female ICR Kunming strain mice (4–6 weeks, 20–22 g) were provided by the Experimental Animal Center of Soochow University, and *S. japonicum* cercariae released from infected intermediate host snail *Oncomelania hupensis* were purchased from the Institute of Schistosomiasis Control in Jiangsu Province (Wuxi, China). In vitro and in vivo activity test was conducted according to described method.[20,21] Five adult male worms (6 weeks post-infection) and nine juvenile worms (17 days post-infection) were distributed in duplicate tissue culture dishes (3.0 cm) in Dulbecco-modified Minimum Eagle's Medium (bicarbonate buffered) supplemented with 20% newborn calf serum, 100 U/ml penicillin, 100 μg/ml streptomycin and 0.5 μg/ml amphotericin B. Cultures were kept at 37 °C in an atmosphere of 5% CO_2 in air and were observed daily under a Leica MZ12.5 stereomicroscope. Cultures were exposed over-night to different PZQ analogues and the next morning worms were washed thrice and transferred to new dishes containing drug-free medium. PZQ and its analogues were dispensed from a stock solution in DMSO and diluted to the concentration of 50–100 μM. Compound activity was assessed by worm survival rate and vitality reduction rate within 72 h. The score of worm vitality is illustrated as: 3 scores: the highest score, as observed in the control group during the observation period. Worms moved more actively and softly, and the body was transparent. 2 scores: worms acted all over the body, but stiffly and slowly, with the body translucent. 1 score: parasites moved partially with opaque appearance. 0 score: the worm remained contracted, did not resume movements, we could deem it 'dead'. The total vitality score and worm survival rate of adult and juvenile worms was separately counted for in vitro test and the data representative of repeated experiments (5 adult worms or 9 juvenile worms each group) was summarised in Tables 1 and 2.

For the in vivo test, female ICR Kunming strain mice infected with 60 ± 2 bisexual *S. japonicum* cercariae were treated with 200 mg/kg of nine analogues suspended in corn oil once a day for 5 days beginning on day 14 after infection (juvenile stage). 21 days post-treatment, mice were sacrificed and dissected to assess worm reduction and the result of repeated experiments (5 mice each group) was given in Table 3.

For the nine target compounds tested against adult *S. japonicum* in vitro (Table 1), all of them displayed nearly no activity with the exception of Rac-**3** and R-**3**. Rac-**3** reduced worm vitality by 66.7% in 72 h at the concentration of 50 μM, which was totally different from its activity against adult *S. mansoni*.[10] And worm mortality rate induced by R-**3** was 40.0%in 72 h at the concentration of 100 μM.

Regarding activity to juvenile *S. japonicum* in vitro (Table 2), more compounds exhibited activity at the concentration of 50 μM. Rac-**2**, Rac-**3** and R-**3** showed modest antischistosomal activity and other compounds exhibited very low mortality rate. 45.8% of juvenile worms were killed by Rac-**2**within 72 h. Worm mortality rate induced by Rac-**3** was 30.8% within 72 h. Compound with best antischistosomal activity was R-**3**, which reduced worm survival rate to 44.1% after 72 h.

However, all compounds displayed low to considerable good antischistomal activity against juvenile *S. japonicum* in vivo

Table 1
Nine PZQ analogues activity against adult *S. japonicum* in vitro

Compound	Conc (μM)	Worm number	24 h		48 h		72 h	
			[b]Worm survival%	[c]Vitality total score/ Vitality reduction%	Worm survival%	Vitality total score/ Vitality reduction%	Worm survival%	Vitality total score/ Vitality reduction%
Control		5	100	15 ± 0/0	100	15 ± 0/0	100	15 ± 0/0
[a]DMSO		5	100	14.7 ± 0.6/2.2	100	15 ± 0/0	100	15 ± 0/0
Rac-**1**	50	5	100	13 ± 0/13.3	100	15 ± 0/0	100	15 ± 0/0
	100	5	100	14.5 ± 0.6/6.7	100	13.5 ± 0.6/10	100	13.5 ± 0.6/10
R-**1**	50	5	100	12.3 ± 0.6/17.8	100	12.7 ± 0.6/15.5	100	12.7 ± 1.2/15.3
	100	5	100	11.3 ± 0.6/24.5	100	11.7 ± 0.6/22	100	11.6 ± 0.6/22.7
S-**1**	50	5	100	14.3 ± 0.6/4.5	100	15 ± 0/0	100	15 ± 0/0
	100	5	100	14 ± 0/6.7	100	13.7 ± 0.6/8.7	100	13.7 ± 0.6/8.9
Rac-**2**	50	5	100	15 ± 0/0	100	15 ± 0/0	100	15 ± 0/0
	80	5	100	11.5 ± 0.6/23.3	100	13 ± 0.8/13.3	100	15 ± 0/0
	100	5	100	12.3 ± 0.6/17.8	100	13 ± 1/13.3	100	13.7 ± 0.6/8.7
R-**2**	50	5	100	12.7 ± 0.6/15.5	100	15 ± 0/0	100	14 ± 0/6.7
	100	5	100	13 ± 1/13.3	100	13.3 ± 1.1/11.1	100	13.3 ± 1.1/11.1
S-**2**	50	5	100	14.3 ± 0.6/4.5	100	15 ± 0/0	100	15 ± 0/0
	100	5	100	12.6 ± 0.5/16	100	12.7 ± 0.6/15.5	100	12.7 ± 0.6/15.3
Rac-**3**	50	5	100	9 ± 0/40	100	5.3 ± 0.6/64.5	100	5 ± 0/66.7
	80	5	100	7.7 ± 0.7/48.9	100	5.3 ± 0.6/64.5	100	5.33 ± 0.5/64.5
	100	5	100	8 ± 0/46.7	100	6 ± 0/60	100	6.7 ± 0.6/55.3
R-3	50	5	**60**	4 ± 0/73.3	80	6 ± 0/60	100	6.7 ± 0.6/55.5
	80	5	**60**	2.7 ± 0.5/82	100	4.6 ± 0.6/69.3	100	6 ± 0/60
	100	5	**40**	2 ± 0/86.7	**60**	2.7 ± 0.6/82.2	**60**	3.3 ± 0.6/77.8
S-**3**	50	5	100	15 ± 0/0	100	15 ± 0/0	100	15 ± 0/0
	100	5	100	13.7 ± 0.6/8.7	100	12.7 ± 0.6/15.3	100	12.7 ± 0.6/15.5

[a] 3 μL DMSO was added with no compound.
[b] Worm survival% $= \frac{\text{number of survival worm}}{\text{number of total worm}} \times 100\%$.
[c] Vitality reduction% $= \frac{\text{total score of worm vitality for control group} - \text{total score of worm vitality for test group}}{\text{total score of worm vitality for control group}} \times 100\%$.

Table 2
Nine PZQ analogues activity against juvenile *S. japonicum* in vitro

Compound (50 μM)	Worm number	24 h		48 h		72 h	
		[b]Worm survival%	[c]Vitality total score/vitality reduction%	Worm survival%	Vitality total score/vitality reduction%	Worm survival%	Vitality total score/vitality reduction%
[a]Control	9	100	27 ± 0/0	100	27 ± 0/0	100	27 ± 0/0
Rac-**1**	9	82.5	17.3 ± 1.2/35.8	82.5	17.3 ± 1.2/35.8	82.5	17 ± 1.7/37
R-**1**	9	100	18.3 ± 3.8/32.1	100	17 ± 4.6/37	100	16.7 ± 4/38.3
S-**1**	9	88.3	18.7 ± 0.6/30.9	88.3	18.7 ± 2.1/30.9	88.3	17.7 ± 1.2/34.6
Rac-**2**	9	**54.2**	12 ± 0/55.6	**54.2**	12 ± 0/55.6	**54.2**	12.3 ± 0.6/54.3
R-**2**	9	100	16.7 ± 2.9/38.3	100	16.7 ± 2.9/38.3	88.3	13.3 ± 1.3/50.6
S-**2**	9	100	14.7 ± 3.1/45.7	100	14.7 ± 3.1/45.7	85	12.7 ± 1.2/53.1
Rac-**3**	9	**69.2**	17.3 ± 5.7/35.8	**69.2**	17.7 ± 5.1/34.6	**69.2**	17.7 ± 5.1/34.6
R-3	9	**51.6**	10.7 ± 5.5/60.5	**48.3**	9.7 ± 5.5/64.2	**44.1**	9 ± 4.4/66.7
S-**3**	9	88.3	19 ± 1.7/29.6	88.3	19 ± 1.7/29.6	88.3	19 ± 1.7/29.6

[a] 3 μL DMSO was added with no compound.
[b] Worm survival% $= \frac{\text{number of survival worm}}{\text{number of total worm}} \times 100\%$.
[c] Vitality reduction% $= \frac{\text{total score of worm vitality for control group} - \text{total score of worm vitality for test group}}{\text{total score of worm vitality for control group}} \times 100\%$.

Table 3
Nine PZQ analogues activity against juvenile *S. japonicum* in vivo. (oral dose 200 mg/kg)

Compound	Worm number ($\bar{x} + s$)	[b]Worm reduction%
[a]Control	48.8 ± 10.1	0
PZQ	40.2 ± 2.2	19.9
Rac-**1**	45 ± 7.4	7.7
R-**1**	28.8 ± 4.1	41.0
S-**1**	39.5 ± 7.6	19.0
Rac-**2**	30.5 ± 2.1	37.4
R-**2**	27.3 ± 7.3	44.1
S-**2**	36.8 ± 4.1	24.6
Rac-**3**	35.3 ± 7.7	27.7
R-**3**	19.5 ± 5	60.0
S-**3**	40 ± 3.4	18.0

[a] Mice were given equal volume of corn oil.
[b] Worm reduction % $= \frac{\text{total worm number for control group} - \text{total worm number for test group}}{\text{total worm number for control group}} \times 100\%$.

(Table 3) in spite of low activity in vitro, this result was in coincidence with the report[10] that Rac-**3** exhibited modest activity against *S. mansoni* in vivo even with no activity in vitro, which confirmed our hypothesis.

Remarkably, six compounds (R-**1**, Rac-**2**, R-**2**, S-**2**, Rac-**3**, R-**3**) displayed stronger activity (24.6–60.0%) against juvenile *S. japonicum* than PZQ (19.9%). Three compounds (R-**1**, R-**2**, R-**3**) showed significant ($P < 0.01$) activity compared with control group. The result of particular significance was for R-**3**, its worm reduction rate was 60.0%, which might be a better drug candidate.

All three R-isomers exhibited better antischistosomal activity than their corresponding S-isomers, which revealed consistency with the activity of R-PZQ and S-PZQ. The activity of R-**1** (41.0%) was about 2.1 times of S-**1** (19.0%); R-**2** (44.1%) showed nearly 20% stronger activity than S-**2** (24.6%); Of all the activity difference between isomers, the greatest one was isomers of Rac-**3**,

antischistosomal activity of R-**3** (60.0%) was over three times as much as that of S-**3** (18.0%).

The above results indicated that all R-isomers were the active enantiomers in accordance with the report[17] that R-PZQ was the active enantiomer for PZQ. Furthermore, as the size of ring with carbonyl group enlarged, antischistosomal activity of R-isomers increased from 41.0% to 60.0%, while worm reduction induced by S-isomers did not change obviously (from 18.0% to 24.6%). This implied that the size of the ring with carbonyl group in PZQ analogues had appreciable impact on the activity of R-isomers (active enantiomers), but less impact on S-isomers (inactive enantiomers).

We have summarised the SAR of three racemic PZQ analogues and their chiral isomers with activity against adult and juvenile *S. japonicum* in vitro, and the in vivo activity to juvenile *S. japonicum*. Even with no obvious activity in vitro, most compounds were more effective against juvenile *S. japonicum* than PZQ in vivo. Especially, three R-isomers displayed considerably stronger activity against juvenile *S. japonicum* in vivo than their corresponding S-isomers, which suggested that R-isomer of PZQ analogues might be a more valuable tool to probe the action mechanism of PZQ. Notably, six compounds (R-**1**, Rac-**2**, R-**2**, S-**2**, Rac-**3**, R-**3**) showed better antischistosomal activity than PZQ against juvenile *S. japonicum*, meanwhile, Rac-3 exhibited moderate activity to juvenile *S. mansoni*,[10] biological assay of these compounds against juvenile *Schistosoma haematobium* and the cross drug resistance should be further investigated in order to explore drug candidates with broad-spectrum activity to cease schistosomiasis completely.

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bmcl.2014.07.039.

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18. Melting points (mp) of the products were determined in open capillary tubes and are uncorrected. ^{1}H and ^{13}C NMR spectra were recorded on a 400 MHz spectrometer in $CDCl_3$ solvent. All chemical shifts are reported in parts per million (ppm) and are relative to internal $(CH_3)_4Si$ (0 ppm) for ^{1}H NMR, and $CDCl_3$ (77.0 ppm) for ^{13}C NMR. Mass spectra were recorded with a JEOL MS-D 300 mass spectrometer. Optical rotation values for each PZQ amine enantiomer and PZQ analogue were determined in Jasco p-1030 Automatic Polarimeter with $CHCl_3$ as the solvent.
PZQ-amine: yield: 91.2%; mp: 115–117 °C (116–118°C[12]); ^{1}H NMR: δ: 7.12–7.29 (m, 4H), 4.40–4.42 (t, 1H), 4.04–4.11 (m, 1H), 3.39(s, 4H), 3.26(s, 2H), 2.72–2.73(d, 1H), 2.63–2.67 (t, 2H). *R-PZQ-amine:* $[\alpha]_D^{26.5}$ −268.36 (c = 0.5, $CHCl_3$), *S-PZQ-amine:* $[\alpha]_D^{26.5}$ +264.69 (c = 0.5, $CHCl_3$).
*2-(3-Oxocyclobutanecarbonyl)-2,3,6,7-tetrahydro-1H-pyrazino[2,1-a]isoquinolin-4(11bH)-one (Rac-**1**):* yield: 73.4%; mp 150–152 °C; ^{1}H NMR δ 7.25–7.30 (m, 4H), 5.13–5.17 (m, 1H), 4.81–4.88 (m, 2H), 4.10–4.42 (dd, 2H, J = 17.2), 3.50–3.51 (t, 3H), 3.29–3.35 (m, 4H), 2.91–2.97 (m, 2H), 2.79–2.83 (d, 1H, J = 16.0); ^{13}C NMR δ 203.05, 171.20, 63.61, 134.73, 132.29, 129.36, 127.61, 127.04, 125.41, 54.83, 50.99, 48.81, 45.73, 39.13, 28.65, 25.82. HR-MS (ESI) calcd. for $C_{17}H_{18}N_2O_3$ (M+1), 299.1396; found, 299.1397. ^{1}H NMR of R-**1** and S-**1** were the same as those of Rac-**1**. R-**1**: $[\alpha]_D^{26.6}$ −154.46 (c = 0.5, $CHCl_3$), S-**1**: $[\alpha]_D^{26.6}$ +126.07 (c = 0.5, $CHCl_3$).
*2-(3-Oxocyclopentanecarbonyl)-2,3,6,7-tetrahydro-1H-pyrazino[2,1-a]isoquinolin-4(11bH)-one (Rac-**2**):* yield: 83.7%, mp 154–156 °C, ^{1}H NMR δ 7.19–7.28(m, 4H), 5.15(t, 1H), 4.83(m, 3H), 4.46(m, 1H), 4.153(m, 2H), 3.357(m, 1H), 2.895(m, 4H), 2.684(m, 2H), 2.437(m, 2H), 2.270(m, 4H); ^{13}C NMR δ 216.26, 172.26, 163.72, 135.44, 134.67, 132.33, 131.62, 129.43, 127.33, 54.77, 48.83, 45.48, 41.38, 39.11, 38.25, 37.09, 28.63. HR-MS (ESI) calcd. for $C_{18}H_{20}N_2O_3$ (M+1), 313.1552; found, 313.1553. ^{1}H NMR of R-**2** and S-**2** were the same as those of Rac-**2**. R-**2**: $[\alpha]_D^{26.7}$ −151.35 (c = 0.5, $CHCl_3$), S-**2**: $[\alpha]_D^{26.7}$ +148.25 (c = 0.5, $CHCl_3$).
*2-(4-Oxocyclohexanecarbonyl)-2,3,6,7-tetrahydro-1H-pyrazino[2,1-a]isoquinolin-4(11bH)-one (Rac-**3**):* yield: 76.8%, mp 176–179 °C, ^{1}H NMR δ 7.29(m, 4H), 5.14(m, 1H), 4.81(m, 2H), 4.53(d, 1H),4.19(d, 1H), 2.94(m, 4H), 2.81(m, 1H), 2.54(m,2H), 2.39(m,2H), 2.08(m,4H); ^{13}C NMR δ 209.36, 172.73, 163.85, 135.05, 131.98, 129.43, 127.33, 125.47, 54.81, 49.10, 45.33, 39.77, 39.12, 38.21, 28.73, 28.64. HR-MS (ESI) calcd. for $C_{19}H_{22}N_2O_3$ (M+1), 327.1709; found, 327.1715. ^{1}H NMR of R-**3** and S-**3** were the same as those of Rac-**3**. R-**3**: $[\alpha]_D^{26.8}$ −138.18 (c = 0.5, $CHCl_3$), S-**3**: $[\alpha]_D^{26.8}$ +130.33 (c = 0.5, $CHCl_3$).
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