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A straightforward and efficient synthesis of praziquantel enantiomers and their 4′-hydroxy derivatives

Alberto Cedillo-Cruz [a,*], María Isabel Aguilar [a], Marcos Flores-Alamo [a], Francisca Palomares-Alonso [b], Helgi Jung-Cook [a,b]

[a] *Departamento de Farmacia, Facultad de Química, Universidad Nacional Autónoma de México, Cd. Universitaria, Coyoacán, México 04510, Mexico*
[b] *Laboratorio de Neuropsicofarmacología, Instituto Nacional de Neurología y Neurocirugía, México, DF 14269, Mexico*

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ABSTRACT

A new method for the synthesis of praziquantel enantiomers via resolution of praziquanamine with (*S*)-(+)-naproxen was developed. The four 4′-hydroxy derivatives were obtained through each single praziquanamine enantiomer, coupling with *cis*- and *trans*-4-(benzyloxy)cyclohexanecarboxylic acids and subsequent hydrogenolysis for the deprotection of the 4′-OH cyclohexane residue. Additionally, the in vitro cysticidal activity of the compounds was tested, finding that (*R*)-(−)-praziquantel is the eutomer.

1. Introduction

Praziquantel (PZQ) is a racemic compound containing equal parts of its isomers, (*R*)-(−)-**4a** and (*S*)-(+)-praziquantel **4b**. PZQ is a pyrazinoisoquinolone derivative, which has been shown to be highly effective against a broad range of cestodes and trematodes in humans and animals;[1] the mechanism of the action of PZQ is not known exactly. PZQ undergoes rapid first-pass drug metabolism and its principal metabolites are the mono- and di-hydroxy derivatives, highlighting the *trans*-4′-hydroxypraziquantel by high plasma concentrations.[2]

Experimental and clinical studies with the trematodes *Schistosoma japonicum* and *Schistosoma mansoni* have demonstrated that **4a** is the active component against these species.[3] Moreover, the use of the pure eutomer resulted in fewer side effects with respect to the racemate.[4] (*R*)-(−)-*trans*-4′-Hydroxypraziquantel **9b** has also been shown to be an effective schistosomicidal agent, but not at the same level of potency as **4a**.[5]

Praziquantel is also effective in the treatment of taeniasis; the cysticercosis is more complex to treat, nevertheless a combination of drugs such as praziquantel or albendazole with corticosteroids may be used.[6] To date, studies have not been carried out in order to determine if **4a** and its 4′-hydroxy derivatives are the only active stereoisomers against *Taenia* cysts.

For the production of **4a**, the following methods have been used: (1) enantioselective chromatography;[7] (2) enantioselective synthesis;[8] (3) enrichment of partially resolved mixtures;[9] and (4) resolution by the formation of diastereomers via: (a) diastereoisomeric salt formation[10] and (b) diastereoisomeric covalent bond formation.[11] Hence, based on this last point, (b) is a suitable method for the production of large quantities of enantiomerically pure **4a** and **4b**, allowing the determination of the diastereoisomer ratio by TLC, ^{1}H NMR, or HPLC and thus providing direct information of the enantiomeric purity of the final product.

Herein we report a new method for the resolution of praziquanamine as a key intermediate for the formation of praziquantel enantiomers, the synthesis of its 4′-hydroxy derivatives, single-crystal X-ray diffraction analysis as well as the in vitro cysticidal activity of PZQ and compounds **4a**, **4b**, (*R*)-(−)-*cis*-4′-hydroxypraziquantel **9a**, **9b,** and (*S*)-(+)-*trans*-4′-hydroxypraziquantel monohydrate **9d**.

2. Results and discussion

2.1. Chemistry

The synthesis of the praziquantel enantiomers **4a** and **4b** in enantiomerically pure form is outlined in Scheme 1. The racemic praziquanamine **1** was synthesized following the procedures reported by Kim et al.[12] Next, **1** was acylated with (*S*)-(+)-naproxen acid chloride followed by separation of the resulting diastereoisomeric amides **2a** and **2b** using flash column chromatography (SiO_2, hexane–ethyl acetate). In order to define the absolute configuration of the C11*b* stereogenic center of compounds **2a** and **2b**, diverse crystallization conditions were explored. Only compound **2a** formed crystals suitable for further X-ray investigation. The absolute C11*b* configuration of compound **2a** was determined as (*R*), assigned by reference to the unchanged stereogenic center of the naproxen acid moiety with an (*S*)-configuration (Fig. 1). On

* Corresponding author. Tel.: +52 55 5622 5281; fax: +52 55 5622 5329.
E-mail address: albertocedillo_cruz@comunidad.unam.mx (A. Cedillo-Cruz).

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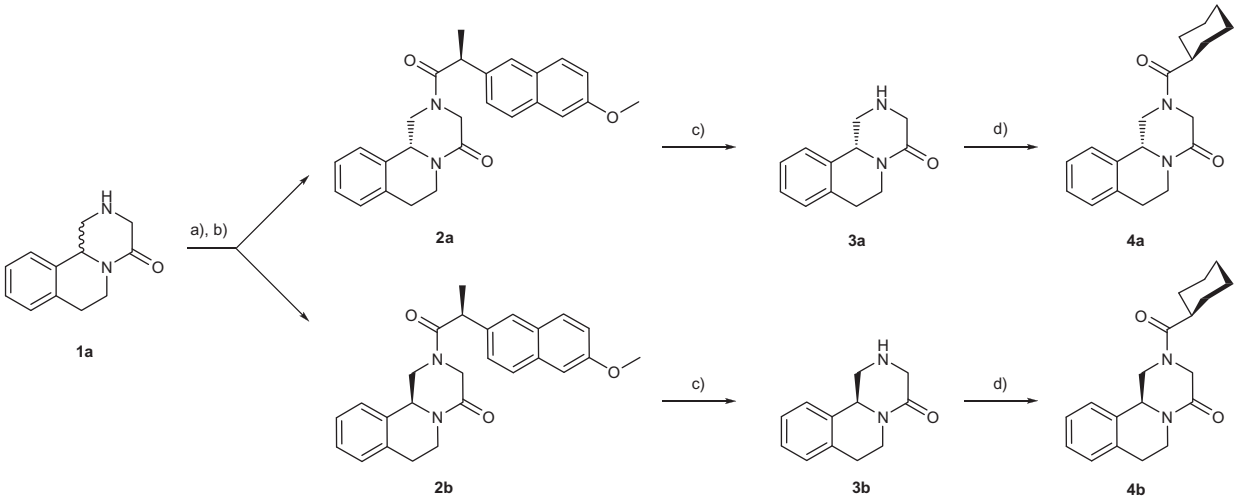


Scheme 1. Synthesis of enantiomerically pure **4a** and **4b**. Reagents and conditions: (a) (*S*)-(+)-naproxen acid chloride, NMM, CH_2Cl_2, room temp, 4 h; (b) separation by flash chromatography; (c) 85% H_3PO_4, MW, 150 °C, 20 min then 5 M NaOH; (d) Na_2CO_3, cyclohexanecarbonyl chloride, CH_2Cl_2, 0 °C to room temp, 2 h.

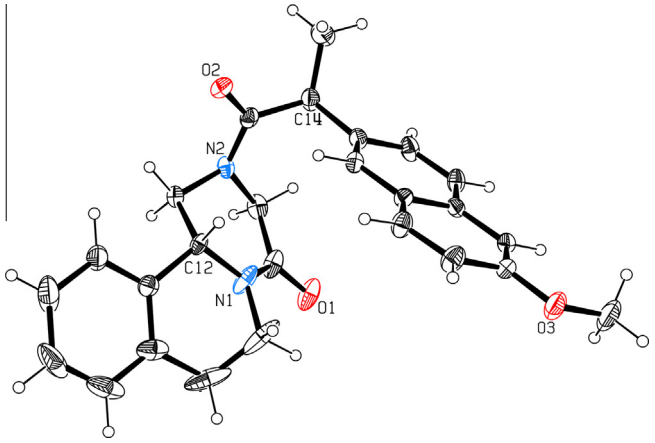


Figure 1. Molecular structure of **2a** with thermal ellipsoids at the 50% probability level.

the basis of this analysis we assigned the (11*bS*,2′*S*)-configuration to diastereoisomer **2b**.

From the NMR spectra of **2a** and **2b** at room temperature, a pair of rotamers derived from slow rotation of the amide bond was identified. Dynamic ^{1}H NMR measurements were performed for these two compounds. Coalescence for **2a** was observed at 60 °C. The presence of rotamers in these types of structures derived from PZQ was inferred by Blaschke[13] through molecular mechanics calculations; it was pointed out that their concentration in solutions should depend on the solvent polarity and the value of the dipole moment for the respective form of the molecule.

The hydrolysis of **2a** by refluxing with 1 M HCl for 7 days gave **3a** in 52% yield, whereas the hydrolysis in concentrated H_3PO_4 at 100 °C for 3 days gave **3a** in 72% yield. As can be seen, both conditions provided the desired product in low to moderate yields. In order to reduce the reaction time, we carried out the hydrolysis under microwave irradiation. The optimum conditions were found with irradiation at 150 °C and 85 W maximum power for 20 min in 85% w/w H_3PO_4 with 88% yield being obtained after chromatographic purification. Using this methodology it was possible to quickly prepare. In contrast to conventional heating, the use of microwave irradiation drastically reduced the reaction time of at least 3 days to only 20 min with excellent yield. Finally, **4a** or **4b** was synthesized from the single praziquanamine enantiomer via acylation with cyclohexanecarbonyl chloride. The structure of **4a** was confirmed by X-ray diffraction (Fig. 2).

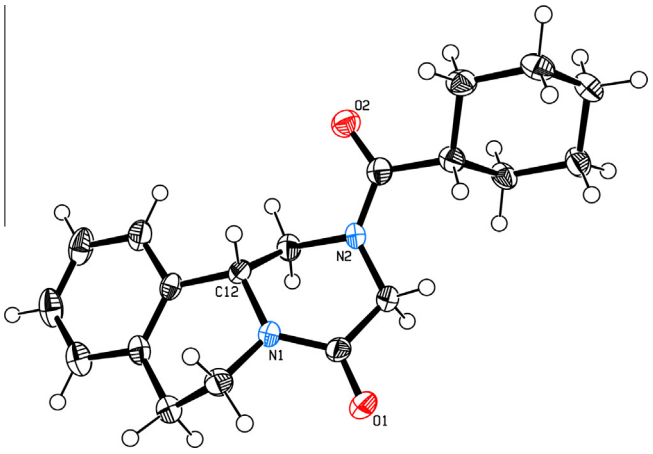
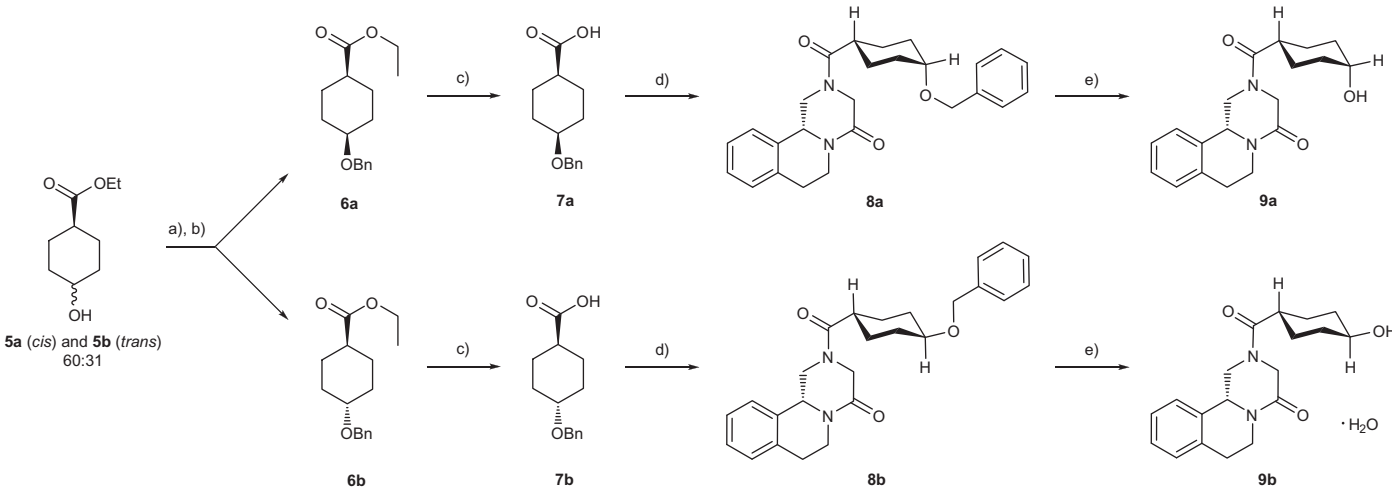


Figure 2. Molecular structure of **4a** with thermal ellipsoids at the 50% probability level.

In order to prepare the 4′-hydroxy derivatives of (*R*)-(−)-praziquantel **9a** and **9b**, a mixture of *cis*- and *trans*-ethyl 4-hydroxycyclohexanecarboxylates (**5a** and **5b**) with benzyl bromide, sodium hydride in THF or DMF gave **6a** and **6b** in poor yields as complex mixtures of several compounds (visualized by GC/MS). When the mixture of **5a** and **5b** was subjected to reductive etherification,[14] the diastereoisomers **6a** and **6b** were obtained and separated in good yields using flash column chromatography (SiO_2, hexane–ethyl acetate). A detailed analysis of the ^{1}H NMR spectrum of **6a** showed the 1′-H atom to be in the axial position ($J_{1\text{-Ha/2-Ha}}$ = 9.6 Hz, $J_{1\text{-Ha/2-He}}$ = 4.0 Hz) and the 4′-H atom to be in the equatorial position ($J_{4\text{–He/3–Ha}}$ = 5.1 Hz, $J_{4\text{–He/3–He}}$ = 2.8 Hz), while the ^{1}H NMR spectrum of isomer **6b** indicated that the 1′-H atom exists in the axial position ($J_{1\text{-Ha/2-Ha}}$ = 11.6 Hz, $J_{1\text{-Ha/2-He}}$ = 3.6 Hz) as well as the 4′-H atom ($J_{4\text{–Ha/3–Ha}}$ = 10.3 Hz, $J_{4\text{–Ha/3–He}}$ = 4.1 Hz), thus confirming the *cis*- and *trans*-conformations for **6a** and **6b**, respectively. Basic hydrolysis of each diastereoisomer gave the corresponding carboxylic acids, **7a** or **7b** and both acids were



Scheme 2. Synthesis of enantiomerically pure **9a** and **9b**. Reagents and conditions: (a) PhCHO, Et_3SiH, 5% mol $FeCl_3$, $MeNO_2$, room temp, 1 h; (b) separation by flash chromatography; (c) 12.5 M NaOH, MeOH/THF, room temp, 6 h; (d) **3a**, PyBOP, NMM, DMSO, room temp, 30 min; (e) ethanol, Pd/C (10%), H_2, room temp to 45 °C, 3 h.

coupled with amine **3a**. The hydrogenolysis of (*R*)-(−)-*cis*-4′-benzyloxypraziquantel **8a** with palladium on carbon at room temperature for 6 h gave (*R*)-(−)-*cis*-4′-hydroxypraziquantel **9a** in very low yield, however the reaction time for the complete conversion could be reduced to 3 h at 45 °C. Thus, the synthesis of (*R*)-(−)-*trans*-4′-hydroxypraziquantel **9b** was also carried out under those conditions (Scheme 2). With respect to the spectroscopic characterization of **9a** and **9b** by ^{1}H NMR, we found a discrepancy in some chemical shift values for this compound with those reported in the literature.[15] The molecular structures of compounds **8a**, **8b**, and **9b** were confirmed by single-crystal X-ray analysis (Figs. 3–5).

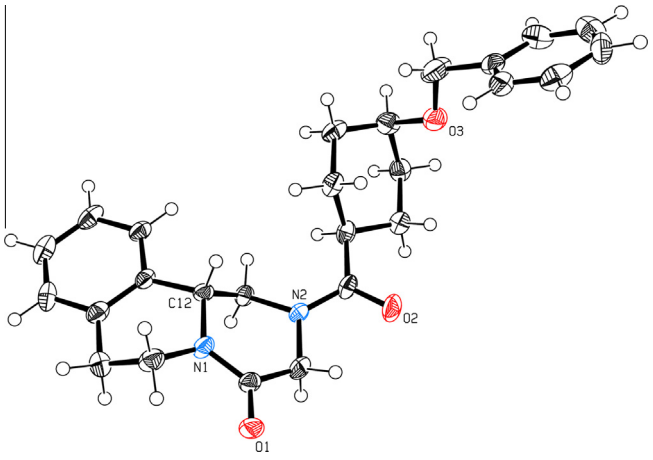


Figure 3. Molecular structure of **8a** with thermal ellipsoids at the 50% probability level.

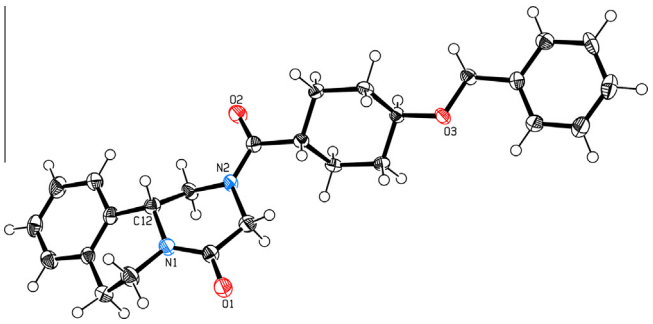


Figure 4. Molecular structure of **8b** with thermal ellipsoids at the 50% probability level.

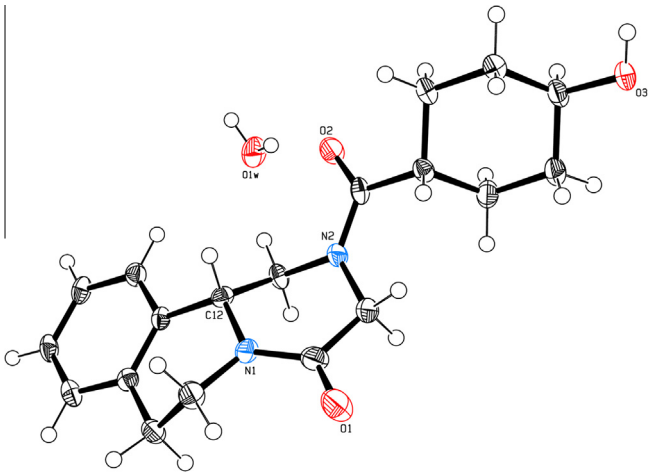


Figure 5. Molecular structure of **9b** with thermal ellipsoids at the 50% probability level.

It is noteworthy that *trans*-isomers **9b** and **9d** crystallized with one water molecule, in contrast with the *cis*-isomers **9a** and **9c**, which crystallized as anhydrous forms. This was observed by the elemental analysis and was confirmed by single-crystal X-ray diffraction of **9b** (Fig. 5). In this compound, the crystal structure is stabilized by a two-dimensional network of O–H···O hydrogen bonds. Intermolecular hydrogen bonds were observed between the O1W atom of the water molecule and the O2 atom of the amide carbonyl group and also with the O3 atom of the hydroxyl group, which seemed to be very effective in the stabilization of the crystal structure.

For derivatives **2a**, **4a**, **8b,** and **9b** with a praziquanamino moiety, the most stable conformation was observed in which the O=C_{amide} group was *anti* to the N–C(C=O_{lactam}) moiety; the

syn-conformation occurs at 0°, while the *anti* conformation at 180°. Compound **8a** showed a *syn* conformation with a dihedral angle of −1.4 and −4.0° for the two independent molecules per asymmetric unit. Nevertheless, it is possible to find another conformation due to the rotation of the amide bond, such as that observed in the complex between glutathione *S*-transferase (*Schistosoma japonica*) and PZQ[16] with a *gauche* conformation and a dihedral angle of −71.05°.

2.2. Cysticidal activity

This is the first study in which the in vitro cysticidal activity of PZQ and compounds **4a**, **4b**, **9a**, **9b,** and (*S*)-(+)-*trans*-4′-hydroxypraziquantel monohydrate **9d** was evaluated on *Taenia crassiceps* cysts (ORF strain). The results showed that the EC_{50} values for PZQ and **4a** were 61.798 (56.567–66.942) and 29.654 (27.237–32.461) nM, respectively, showing that the potency of **4a** was 2 times greater than that for PZQ. We found that, as in *Schistosoma japonicum* and *S. mansoni*,[3] for cysticidal activity, **4a** is the eutomer of PZQ. Moreover, **4b**, **9a,** and **9d** did not display cysticidal activity; only **9b** showed 20% of activity at a concentration of 2886.66 nM.

3. Conclusion

A new method for the resolution of praziquanamine as a key intermediate for the formation of the praziquantel enantiomers (*R*)-(−)-**4a** and (*S*)-(+)-praziquantel **4b** and the synthesis of its 4′-hydroxy derivatives ((*R*)-(−)-*cis*-4′-hydroxypraziquantel **9a**, (*R*)-(−)-*trans*-4′-hydroxypraziquantel **9b** and the optical antipodes) was developed. The method involved the formation of a pair of diastereoisomers derived from (*S*)-(+)-naproxen **2a** and **2b**, which were easily separated by flash column chromatography, with a subsequent acid hydrolysis and acylation of each resultant single praziquanamine enantiomer with cyclohexanecarbonyl chloride to provide **4a** and **4b**, respectively with overall yields of 70%. The 4′-hydroxy *cis*- and *trans*-derivatives of **4a** and **4b, 9a**, **9b**, **9c,** and **9d** were obtained by a 4-step sequence. In each case, they were obtained with overall yields of 72% and their structures were confirmed by spectroscopic techniques and single-crystal X-ray diffraction. The in vitro cysticidal activity of praziquantel toward *Taenia crassiceps* (ORF strain) was attributed to enantiomer **4a**, while the contribution of **4b** to the cysticidal activity of the racemic mixture was negligible.

4. Experimental

4.1. General

All commercial reagents used for the synthesis were purchased from Sigma–Aldrich (Toluca, Mexico) unless otherwise specified. Flash column chromatography was carried out on Merck Silicagel 60 (0.015–0.040 mm). Melting points were determined on a Fisher–Johns apparatus and are uncorrected. Optical rotations were measured with a Perkin–Elmer model 343 polarimeter. The IR spectra were registered with a Perkin–Elmer Spectrum 400 FT-IR/FT-FIR spectrometer. Elemental analyses were determined with a Perkin–Elmer 2400 Series II CHNS/O Analyzer. Mass spectra were registered in a Thermo-Electron spectrometer model DFS (Double Focus Sector). NMR spectra were recorded in $CDCl_3$ solution in a Varian INOVA 400 spectrometer using the solvent signal 7.26 ppm for ^1H and 77.16 ppm for ^{13}C as references. NMR signals assignments were made with a combination of 2D homonuclear (^1H–^1H) and heteronuclear (^1H–^{13}C) correlation techniques, which included ^1H–^1H COSY, ^1H–^1H Nuclear Overhauser Effect Spectroscopy (NOESY), Heteronuclear Single Quantum Correlation (HSQC) and Heteronuclear Multiple Bond Correlation (HMBC). All 2D NMR spectra were recorded using the standard pulse sequences and parameters recommended by the manufacturer. Dulbecco's Modified Eagle's Minimal Essential Medium (Sigma-Aldrich Co., U.S.A.) culture medium was used for the cysticidal activity, supplemented with 10% fetal calf serum, 2 mM L-glutamine, 8 mg/dL of gentamicin sulfate and 200,000 IU/dL of penicillin G sodium (Gibco, U.S.A.). The *Taenia crassiceps* cysts were observed with an inverted light microscope (Reichert, 569).

4.1.1. Racemic praziquanamine 1

Racemic praziquanamine **1** was prepared according to published methods.[12]

4.1.2. (*R*)-(−)-2-((*S*)-2-(6-Methoxynaphthalen-2-yl)propanoyl)-1,2,3,6,7,11*b*-hexahydro-4*H*-pyrazino[2,1-*a*]isoquinolin-4-one 2a and (*S*)-(+)-2-((*S*)-2-(6-methoxynaphthalen-2-yl)propanoyl)- 1,2,3,6,7,11*b*-hexahydro-4*H*-pyrazino[2,1-*a*]isoquinolin-4-one 2b

A solution of (*S*)-(+)-naproxen acid chloride (2.705 g, 10.88 mmol) in dichloromethane (20 mL) was slowly added to a stirred mixture of **1** (2 g, 9.89 mmol) and 4-methylmorpholine (3.25 mL, 29.56 mmol) in dichloromethane (20 mL). The mixture was stirred at room temperature for 4 h, then washed consecutively with 1 M HCl (3 × 50 mL), a saturated solution of $NaHCO_3$ (3 × 50 mL), water (3 × 50 mL), and dried over Na_2SO_4, filtered, and concentrated. The residue was purified by column chromatography (SiO_2, hexane–ethyl acetate from 100:0, v/v to 30:70, v/v) and gave **2a** (faster eluting diastereoisomer, 1.927 g, 47%), and **2b** (slower eluting diastereoisomer, 1.763 g, 43%). Overall yield: 90%. Compound **2a**: mp 154–156 °C. $[\alpha]_D = -2.32$ (*c* 1, $CHCl_3$). IR ν_{max} 1641, 1603, 1481, 1441, 1202, 1031 cm^{-1}. MS (EI) *m*/*z* (relative intensity, %) 414 (M^+, 9), 312 (17), 256 (10), 228 (8), 201 (72), 185 (100), 173 (18), 145 (28), 132 (82). HRMS (EI) Calcd for $C_{26}H_{26}N_2O_3$ (M^+): *m*/*z* 414.1938, Found: 414.1948. Anal. Calcd for $C_{26}H_{26}N_2O_3$: C, 75.34; H, 6.32; N, 6.76. Found: C, 75.26; H, 6.11; N, 6.78. For clarity, the NMR spectra of the two rotamers are separately described.

Major rotamer (73.40%): ^1H NMR (400 MHz, $CDCl_3$) δ 7.77–7.66 (m, 3H, $HC_{4'',8'',1''}$), 7.43 (d, J = 8.5 Hz, 1H, $HC_{3''}$), 7.34–7.21 (m, 3H, $HC_{11,10,9}$), 7.19–7.11 (m, 3H, $HC_{7'',8,5''}$), 5.15 (dd, J = 13.4, 3.5 Hz, 1H, HC_1), 4.67 (dd, J = 10.3, 3.4 Hz, 1H, HC_{11b}), 4.64–4.55 (m, 1H, HC_6), 4.35 (AB, J = 17.2 Hz, 1H, HC_3), 4.16 (AB, J = 17.2 Hz, 1H, HC_3), 3.97 (q, J = 6.8 Hz, 1H, $HC_{2'}$), 3.93 (s, 3H, $HC_{4'}$), 2.96–2.81 (m, 2H, $HC_{1,7}$), 2.78–2.67 (m, 2H, $HC_{6,7}$), 1.56 (d, J = 6.8 Hz, 3H, $HC_{3'}$) ppm. $^{13}C\{^1H\}$ NMR (101 MHz, $CDCl_3$) δ 172.9 ($C_{1'}$), 164.1 (C_4), 157.9 ($C_{6''}$), 136.0 ($C_{2''}$), 135.0 (C_{7a}), 133.8 ($C_{4a''}$), 132.6 (C_{11a}), 129.4 (C_8), 129.3 ($C_{8''}$), 129.2 ($C_{8a''}$), 128.0 ($C_{4''}$), 127.6 (C_9), 127.1 (C_{10}), 126.0 ($C_{3''}$), 125.7 (2C, $C_{1'',11}$), 119.3 ($C_{7''}$), 105.8 ($C_{5''}$), 55.4 ($C_{4'}$), 54.8 (C_{11b}), 48.8 (C_3), 45.8 (C_1), 43.6 ($C_{2'}$), 39.0 (C_6), 28.8 (C_7), 20.8 ($C_{3'}$).

Minor rotamer (26.60%): ^1H NMR (400 MHz, $CDCl_3$) δ 7.78–7.67 (m, 2H, $HC_{4'',8''}$), 7.64 (s, 1H, $HC_{1''}$), 7.39 (d, J = 8.6 Hz, 1H, $HC_{3''}$), 7.36–7.20 (m, 2H, $HC_{8,10}$), 7.19–7.09 (m, 3H, $HC_{9,7'',5''}$), 7.00 (m, 1H, HC_{11}), 4.93 (AB, J = 18.6 Hz, 1H, HC_3), 4.89–4.79 (m, 1H, $HC_{11b,6}$), 4.43 (d, J = 12.3 Hz, 1H, HC_1), 4.18–4.13 (m, 1H, $HC_{2'}$), 3.93 (s, 3H, $HC_{4'}$), 3.85 (AB, J = 18.7 Hz, 1H, HC_3), 2.97–2.78 (m, 3H, $HC_{7,6,1}$), 2.78–2.67 (m, 1H, HC_7), 1.62 (d, J = 6.6 Hz, 3H, $HC_{3'}$). $^{13}C\{^1H\}$ NMR (101 MHz, $CDCl_3$) δ 172.1 ($C_{1'}$), 165.3 (C_4), 157.9 ($C_{6''}$), 136.2 ($C_{2''}$), 135.5 (C_{7a}), 133.7 ($C_{4a''}$), 132.1 (C_{11a}), 129.7 (C_8), 129.6 ($C_{8a''}$), 129.3 ($C_{8''}$), 128.0 ($C_{4''}$), 127.7 (C_9), 127.1 (C_{10}), 125.9 ($C_{3''}$), 125.4 ($C_{1''}$), 125.1 (C_{11}), 119.4 ($C_{7''}$), 105.8 ($C_{5''}$), 55.6 (C_{11b}), 55.4 ($C_{4'}$), 49.5 (C_1), 46.9 (C_3), 44.1 ($C_{2'}$), 38.7 (C_6), 28.8 (C_7), 20.5 ($C_{3'}$).

Compound **2b**: mp 92–94 °C. $[\alpha]_D = +128.4$ (*c* 0.5, $CHCl_3$). IR ν_{max} 1644, 1604, 1443, 1417, 1207, 1031 cm^{-1}. MS (EI) *m*/*z* (relative intensity, %) 312 (32), 201 (100), 185 (24), 173 (18), 145

(32), 132 (98). HRMS (EI) Calcd for $C_{26}H_{26}N_2O_3$ (M^+): m/z 414.1938, Found: 414.1943. Anal. Calcd for $C_{26}H_{26}N_2O_3$: C, 75.34; H, 6.32; N, 6.76. Found: C, 75.32; H, 6.74; N, 6.84.

Major rotamer (52.21%): ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.83–7.72 (m, 3H, $HC_{4'',8'',1''}$), 7.43 (dd, J = 8.4, 1.3 Hz, 1H, $HC_{3''}$), 7.19–7.07 (m, 3H, $HC_{7'',9,5''}$), 7.07–6.97 (m, 2H, $HC_{8,10}$), 6.32 (d, J = 7.9 Hz, 1H, HC_{11}), 4.98 (AB, J = 18.8 Hz, 1H, HC_3), 4.82–4.78 (m, 1H, HC_6), 4.39 (dd, J = 13.3, 2.7 Hz, 1H, HC_1), 4.03 (qd, J = 6.8, 3.2 Hz, 1H, $HC_{2'}$), 3.91–3.86 (m, 3H, $HC_{4'}$), 3.84 (AB, J = 18.9 Hz, 1H, HC_3), 3.77 (dd, J = 10.6, 4.0 Hz, 1H, HC_{11b}), 3.05 (dd, J = 13.4, 10.6 Hz, 1H, HC_1), 2.84–2.73 (m, 1H, HC_7), 2.57 (dt, J = 16.2, 2.8 Hz, 1H, HC_7), 2.48 (td, J = 12.7, 3.4 Hz, 1H, HC_6), 1.55 (d, J = 6.8 Hz, 3H, $HC_{3'}$) ppm. ^{13}C{^{1}H} NMR (101 MHz, $CDCl_3$) δ 171.6 ($C_{1'}$), 165.2 (C_4), 157.8 ($C_{6''}$), 136.8 ($C_{2''}$), 135.0 (C_{7a}), 133.6 ($C_{4a''}$), 131.7 (C_{11a}), 129.3 (C_8), 129.1 ($C_{8a''}$), 128.8 ($C_{8''}$), 128.2 ($C_{4''}$), 127.1 (C_9), 126.5 (C_{10}), 125.6 ($C_{3''}$), 125.6 ($C_{1''}$), 125.2 (C_{11}), 119.6 ($C_{7''}$), 105.7 ($C_{5''}$), 55.2 ($C_{4'}$), 55.1 (C_{11b}), 49.5 (C_1), 46.4 (C_3), 44.2 ($C_{2'}$), 38.2 (C_6), 28.7 (C_7), 20.8 ($C_{3'}$) ppm.

Minor rotamer (47.79%): ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.72–7.63 (m, 2H, $HC_{4'',8''}$), 7.58 (d, J = 1.7 Hz, 1H, $HC_{1''}$), 7.36–7.06 (m, 6H, $HC_{3'',11,10,9,8,7'',5''}$), 5.22 (dd, J = 13.3, 2.8 Hz, 1H, HC_1), 4.83 (dd, J = 10.3, 4.3 Hz, 1H, HC_{11b}), 4.78–4.73 (m, 1H, HC_6), 4.49 (AB, J = 17.7 Hz, 1H, HC_3), 4.03 (qd, J = 6.8, 3.2 Hz, 1H, $HC_{2'}$), 3.91–3.85 (m, 3H, $HC_{4'}$), 3.63 (AB, J = 17.7 Hz, 1H, HC_3), 2.94–2.66 (m, 4H, $HC_{7,6,1,7}$), 1.55 (d, J = 6.8 Hz, 3H, $HC_{3'}$) ppm. ^{13}C{^{1}H} NMR (101 MHz, $CDCl_3$) δ 172.4 ($C_{1'}$), 164.1 (C_4), 157.6 ($C_{6''}$), 135.5 ($C_{2''}$), 134.7 (C_{7a}), 133.5 ($C_{4a''}$), 132.6 (C_{11a}), 129.2 (C_8), 129.1 ($C_{8''}$), 129.0 ($C_{8a''}$), 127.7 ($C_{4''}$), 127.4 (C_9), 126.9 (C_{10}), 125.8 ($C_{3''}$), 125.7 ($C_{1''}$), 125.4 (C_{11}), 119.1 ($C_{7''}$), 105.5 ($C_{5''}$), 55.2 ($C_{4'}$), 54.9 (C_{11b}), 49.1 (C_3), 45.2 (C_1), 43.3 ($C_{2'}$), 39.0 (C_6), 28.6 (C_7), 20.4 ($C_{3'}$) ppm.

4.1.3. (*R*)-(−)-1,2,3,6,7,11*b*-Hexahydro-4*H*-pyrazino[2,1-*a*]isoquinolin-4-one 3a

Compound **2a** (100 mg, 0.241 mmol) was suspended in 85 w/w H_3PO_4 (2 mL) in a proper vial which was heated to an external temperature of 150 °C over a 3 min period ramping up to a microwave power of 300 W (Synthos 3000). The vial was held at this temperature for 20 min to a microwave power of 85 W. The mixture was cooled to 4 °C, poured into crushed ice (15 mL) and an aqueous solution of 5 M NaOH was added to pH 12. The aqueous layer was extracted with dichloromethane (3 × 15 mL). The organic layers were combined, dried over Na_2SO_4, filtered, and concentrated. The residue was purified by column chromatography (SiO_2, dichloromethane–methanol gradient from 100:0, v/v to 90:10, v/v). A white crystalline product was obtained (43.1 mg, 88% yield). mp 124–126 °C. $[\alpha]_D = -306.1$ (c 1, $CHCl_3$). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.25–7.10 (m, 4H, $HC_{10,9,8,11}$), 4.86 (ddd, J = 12.5, 5.1, 2.6 Hz, 1H, HC_6), 4.79 (dd, J = 10.0, 4.6 Hz, 1H, HC_{11b}), 3.73 (ddd, J = 13.0, 4.7, 1.4 Hz, 1H, HC_1), 3.72 (AB, J = 17.6 Hz, 1H, HC_3), 3.52 (AB, J = 17.3 Hz, 1H, HC_3), 3.03–2.93 (m, 1H, HC_7), 2.92–2.85 (m, 1H, HC_1), 2.87–2.79 (m, 1H, HC_6), 2.78–2.70 (m, 1H, HC_7), 1.98 (s, 1H, HN_2) ppm. ^{13}C{^{1}H} NMR (101 MHz, $CDCl_3$) δ 167.4 (C_4), 135.1 (C_{7a}), 134.3 (C_{11a}), 129.5 (C_8), 127.1 (C_9), 126.7 (C_{10}), 124.8 (C_{11}), 57.0 (C_{11b}), 50.1 (C_3), 49.9 (C_1), 38.9 (C_6), 28.9 (C_7) ppm. IR ν_{max} 3319, 2878, 1618, 1432 cm^{-1}. MS (EI) m/z (relative intensity, %) 202 (M^+, 20), 173 (76), 145 (100), 131 (71), 117 (33), 103 (16), 77 (17). HRMS (EI) Calcd for $C_{12}H_{14}N_2O$ (M^+): m/z 202.1101, Found: 202.1102. Anal. Calcd for $C_{12}H_{14}N_2O$: C, 71.26; H, 6.98; N, 13.85. Found: C, 71.30; H, 6.92; N, 14.02.

4.1.4. (*S*)-(+)-1,2,3,6,7,11*b*-Hexahydro-4*H*-pyrazino[2,1-*a*]isoquinolin-4-one 3b

Following the procedure reported above for the preparation of **3a** and starting with amide **2b** (100 mg, 0.241 mmol) compound **3b** was obtained as a white crystalline solid (42.8 mg, 88% yield). mp 124–126 °C. $[\alpha]_D = +305.6$ (c 1, $CHCl_3$). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.35–7.04 (m, 4H, $HC_{10,9,8,11}$), 4.86 (ddd, J = 12.5, 5.1, 2.6 Hz, 1H, HC_6), 4.79 (dd, J = 10.0, 4.5 Hz, 1H, HC_{11b}), 3.73 (ddd, J = 13.0, 4.7, 1.5 Hz, 1H, HC_1), 3.66 (AB, J = 17.4 Hz, 1H, HC_3), 3.52 (AB, J = 17.3 Hz, 1H, HC_3), 3.04–2.93 (m, 1H, HC_7), 2.92–2.85 (m, 1H, HC_1), 2.87–2.79 (m, 1H, HC_6), 2.78–2.70 (m, 1H, HC_7), 1.85 (s, 1H, HN_2) ppm. ^{13}C{^{1}H} NMR (101 MHz, $CDCl_3$) δ 167.4 (C_4), 135.1 (C_{7a}), 134.4 (C_{11a}), 129.5 (C_8), 127.1 (C_9), 126.7 (C_{10}), 124.8 (C_{11}), 57.0 (C_{11b}), 50.2 (C_3), 50.0 (C_1), 38.9 (C_6), 29.0 (C_7) ppm. IR ν_{max} 3318, 2878, 1618, 1432 cm^{-1}. MS (EI) m/z (relative intensity, %) 202 (M^+, 30), 173 (92), 145 (100), 131 (70), 117 (23), 103 (10), 77 (8). HRMS (EI) Calcd for $C_{12}H_{14}N_2O$ (M^+): m/z 202.1101, Found: 202.1100. Anal. Calcd for $C_{12}H_{14}N_2O$: C, 71.26; H, 6.98; N, 13.85. Found: C, 70.79; H, 7.14; N, 13.73.

4.1.5. (*R*)-(−)-2-(Cyclohexanecarbonyl)-1,2,3,6,7,11*b*-hexahydro-4*H*-pyrazino[2,1-*a*]isoquinolin-4-one 4a

To a solution of **3a** (200 mg, 988.9 μmol) and Na_2CO_3 (184.7 mg, 1.74 mmol) in dichloromethane (5 mL) was added dropwise a solution of cyclohexanecarbonyl chloride (135 μL, 1.01 mmol) in dichloromethane (5 mL) at 0 °C. After stirring at room temperature for 2 h, dichloromethane (20 mL) was added, and the mixture was then washed successively with 1 M HCl (3 × 30 mL), saturated solution of $NaHCO_3$ (3 × 30 mL), water (3 × 30 mL), and dried over Na_2SO_4, filtered, and concentrated. The residue was purified by column chromatography (SiO_2, hexane–ethyl acetate 70:30, v/v). A white powder was obtained (271.7 mg, 88% yield). Mp 108–110 °C. $[\alpha]_D = -147.5$ (c 1, $CHCl_3$). IR ν_{max} 2933, 2923, 1637, 1495, 1439, 1418, 1296, 1214, 757 cm^{-1}. MS (EI) m/z (relative intensity, %) 312 (M^+, 44), 201 (100), 185 (17), 173 (15), 145 (25), 132 (70). HRMS (EI) Calcd for $C_{19}H_{24}N_2O_2$ (M^+): m/z 312.1832, Found: 312.1840. Anal. Calcd for $C_{19}H_{24}N_2O_2$: C, 73.05; H, 7.74; N, 8.97. Found: C, 72.76; H, 7.67; N, 8.92. For clarity, the NMR spectra of the two rotamers are described separately.

Major rotamer (77.52%): ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.34–7.19 (m, 4H, $HC_{11,10,9,8}$), 5.18 (dd, J = 13.3, 2.6 Hz, 1H, HC_1), 4.87–4.79 (m, 2H, $HC_{6,11b}$), 4.49 (AB, J = 17.5 Hz, 1H, HC_3), 4.10 (AB, J = 17.4 Hz, 1H, HC_3), 3.06–2.78 (m, 4H, $HC_{7,6,1,7}$), 2.49 (t, J = 11.4 Hz, 1H, $HC_{1'}$), 1.94–1.69 (m, 5H, $HC_{3',2',4'}$), 1.69–1.49 (m, 2H, $HC_{2'}$), 1.41–1.21 (m, 3H, $HC_{4',3'}$) ppm. ^{13}C{^{1}H} NMR (101 MHz, $CDCl_3$) δ 174.9 (C_{12}), 164.5 (C_4), 134.9 (C_{7a}), 132.92 (C_{11a}), 129.4 (C_8), 127.6 (C_9), 127.1 (C_{10}), 125.6 (C_{11}), 55.1 (C_{11b}), 49.2 (C_3), 45.3 (C_1), 40.9 ($C_{1'}$), 39.2 (C_6), 29.4 ($C_{2'}$), 29.2 ($C_{2'}$), 28.9 (C_7), 25.9 ($C_{3',4'}$) ppm.

Minor rotamer (22.48%): ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.34–7.19 (m, 4H, $HC_{9,10,8,11}$), 4.94–4.79 (m, 3H, $HC_{6,11b,3}$), 4.39 (d, J = 12.0 Hz, 1H, HC_1), 3.89 (AB, J = 18.4 Hz, 1H, HC_3), 3.28 (t, J = 11.6 Hz, 1H, HC_1), 3.06–2.78 (m, 3H, $HC_{7,6,7}$), 2.64–2.54 (m, 1H, $HC_{1'}$), 1.94–1.69 (m, 5H, $HC_{3',2',4'}$), 1.69–1.49 (m, 2H, $HC_{2'}$), 1.41–1.21 (m, 3H, $HC_{4',3'}$) ppm. ^{13}C{^{1}H} NMR (101 MHz, $CDCl_3$) δ 174.5 (C_{12}), 165.7 (C_4), 135.7 (C_{7a}), 132.2 (C_{11a}), 129.8 (C_8), 127.8 (C_9), 127.1 (C_{10}), 125.3 (C_{11}), 55.9 (C_{11b}), 49.7 (C_1), 46.5 (C_3), 40.9 ($C_{1'}$), 38.8 (C_6), 29.3 ($C_{2'}$), 29.2 ($C_{2'}$), 28.9 (C_7), 25.9 ($C_{3',4'}$) ppm.

4.1.6. (*S*)-(+)-2-(Cyclohexanecarbonyl)-1,2,3,6,7,11*b*-hexahydro-4*H*-pyrazino[2,1-*a*]isoquinolin-4-one 4b

Following the procedure reported above for the preparation of **4a** and starting with amine **3b** (138 mg, 0.36 mmol), compound **4b** was obtained as a white powder (268.6 mg, 87% yield). Mp 107–109 °C. $[\alpha]_D = +147.2$ (c 1, $CHCl_3$). IR ν_{max} 2933, 2923, 1637, 1495, 1439, 1418, 1296, 1214, 757 cm^{-1}. MS (EI) m/z (relative intensity, %) 312 (M^+, 29), 201 (92), 185 (28), 173 (18), 145 (32), 132 (100). HRMS (EI) Calcd for $C_{19}H_{24}N_2O_2$ (M^+): m/z 312.1832, Found: 312.1826. Anal. Calcd for $C_{19}H_{24}N_2O_2$: C, 73.05; H, 7.74; N, 8.97. Found: C, 72.67; H, 7.58; N, 8.89.

4.1.7. Ethyl *cis*-4-(benzyloxy)cyclohexanecarboxylate 6a and ethyl *trans*-4-(benzyloxy)cyclohexanecarboxylate 6b

This compound was obtained by the procedure described by Oriyama et al.[14] To a suspension of anhydrous iron(III) chloride

(21.0 mg, 0.13 mmol) and benzaldehyde (264 μL, 2.60 mmol) in nitromethane (13 mL) were added successively ethyl 4-hydroxycyclohexanecarboxylate, a mixture of *cis* and *trans* (dr value 69:31, determined by NMR) (500 μL, 3.10 mmol) and triethylsilane (495 μL, 3.10 mmol) at room temperature under a nitrogen atmosphere. After stirring for 1 h, water (100 mL) was added, and the aqueous layer was extracted with dichloromethane (3 × 30 mL). The organic layers were combined, dried over Na_2SO_4, filtered, and concentrated. The residue was purified by column chromatography (SiO_2, hexane–ethyl acetate from 100:0, v/v to 95:5, v/v) to give **6a** (faster eluting diastereoisomer, 438.5 mg, 64%), and **6b** (slower eluting diastereoisomer, 180.1 mg, 26%). Overall yield: 90%. Compound **6a**: ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.39–7.30 (m, 4H, $HC_{7,8}$), 7.30–7.23 (m, 1H, HC_9), 4.51 (s, 2H, HC_5), 4.13 (q, J = 7.1 Hz, 2H, HC_{11}), 3.58 (tt, J = 5.1, 2.8 Hz, 1H, HC_4), 2.36 (tt, J = 9.6, 4.0 Hz, 1H, HC_1), 1.97 (ddd, J = 10.7, 8.7, 3.4 Hz, 2H, HC_2), 1.93–1.84 (m, 2H, HC_3), 1.68 (ddd, J = 12.9, 8.8, 4.2 Hz, 2H, HC_2), 1.61–1.50 (m, 2H, HC_3), 1.25 (t, J = 7.1 Hz, 3H, HC_{12}) ppm. ^{13}C {^{1}H} NMR (101 MHz, $CDCl_3$) δ 175.6 (C_{10}), 139.2 (C_6), 128.4 (C_8), 127.5 (C_7), 127.4 (C_9), 73.2 (C_4), 69.8 (C_5), 60.3 (C_{11}), 42.0 (C_1), 29.2 (C_3), 24.0 (C_2), 14.4 (C_{12}) ppm. IR ν_{max} 2937, 2866, 1727, 1453 cm^{-1}. MS (FAB) m/z (relative intensity, %) 263 (M^++H^+, 100), 217 (8), 181 (9), 155 (43), 91 (75). HRMS (FAB) Calcd for $C_{16}H_{23}O_3$ [M+H]$^+$: m/z 263.1642, Found: 263.1629. Anal. Calcd for $C_{16}H_{22}O_3$: C, 73.25; H, 8.45. Found: C, 72.71; H, 8.33. Compound **6b**: ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.37–7.34 (m, 4H, $HC_{7,8}$), 7.33–7.26 (m, 1H, HC_9), 4.58 (s, 2H, HC_5), 4.14 (q, J = 7.1 Hz, 2H, HC_{11}), 3.37 (tt, J = 10.3, 4.1 Hz, 1H, HC_4), 2.30 (tt, J = 11.6, 3.6 Hz, 1H, HC_1), 2.16 (dd, J = 12.4, 2.5 Hz, 2H, HC_3), 2.05 (d, J = 12.0 Hz, 2H, HC_2), 1.57–1.44 (m, 2H, HC_2), 1.43–1.30 (m, 2H, HC_3), 1.27 (t, J = 7.1 Hz, 3H, HC_{12}) ppm. ^{13}C {^{1}H} NMR (101 MHz, $CDCl_3$) δ 175.7 (C_{10}), 139.0 (C_6), 128.5 (C_8), 127.7 (C_7), 127.6 (C_9), 76.6 (C_4), 70.1 (C_5), 60.4 (C_{11}), 42.6 (C_1), 31.4 (C_3), 27.3 (C_2), 14.3 (C_{12}) ppm. IR ν_{max} 2937, 2863, 1728, 1454 cm^{-1}. MS (FAB) m/z (relative intensity, %) 263 (M^++H^+, 17), 217 (3), 181 (4), 155 (29), 91 (100). HRMS (FAB) Calcd for $C_{16}H_{23}O_3$ [M+H]$^+$: m/z 263.1642, Found: 263.1632. Anal. Calcd for $C_{16}H_{22}O_3$: C, 73.25; H, 8.45. Found: C, 73.30; H, 8.42.

4.1.8. *cis*-4-(Benzyloxy)cyclohexanecarboxylic acid 7a

To a solution of **6a** (262.3 mg, 1.00 mmol) in 1:1 methanol/tetrahydrofuran (4 mL) was added dropwise 12.5 M sodium hydroxide (400 μL, 5.00 mmol) at room temperature. After stirring for 6 h, it was concentrated and water (20 mL) was added. The aqueous phase was washed with diethyl ether (3 × 20 mL) and an aqueous solution of 4 M HCl was added to pH 1. The aqueous layer was extracted with dichloromethane (3 × 30 mL). The organic layers were combined, dried over Na_2SO_4, filtered, and concentrated. A white powder was obtained (230.5 mg, 98% yield). Mp 87–88 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.40–7.25 (m, 5H, $HC_{7,8,9}$), 4.54 (s, 2H, HC_5), 3.61 (tt, J = 5.2, 2.9 Hz, 1H, HC_4), 2.45 (tt, J = 9.6, 4.0 Hz, 1H, HC_1), 2.06–1.98 (m, 2H, HC_2), 1.96–1.90 (m, 2H, HC_3), 1.77–1.71 (m, 2H, HC_2), 1.63–1.56 (m, 2H, HC_3) ppm. ^{13}C{^{1}H} NMR (101 MHz, $CDCl_3$) δ 181.8 (C_{10}), 139.2 (C_6), 128.5 (C_8), 127.5 (C_7), 127.5 (C_9), 73.1 (C_4), 69.8 (C_5), 41.7 (C_1), 29.2 (C_3), 23.8 (C_2) ppm. IR ν_{max} 2940, 2864, 1691, 1242 cm^{-1}. MS (EI) m/z (relative intensity, %) 234 (M^+, 2), 216 (3), 188 (2), 172 (2), 107 (50), 91 (100). HRMS (FAB) Calcd for $C_{14}H_{19}O_3$ [M+H]$^+$: m/z 235.1329, Found: 235.1315. Anal. Calcd for $C_{14}H_{18}O_3$: C, 71.77; H, 7.74. Found: C, 72.05; H, 7.74.

4.1.9. *trans*-4-(Benzyloxy)cyclohexanecarboxylic acid 7b

Following the procedure reported above for the preparation of **7a** and starting with ester **6b** (180.1 mg, 0.69 mmol), compound **7b** was obtained as a white powder (158.1 mg, 98% yield). Mp 105–107 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.38–7.25 (m, 5H, $HC_{7,8,9}$), 4.58 (s, 2H, HC_5), 3.37 (tt, J = 10.1, 4.0 Hz, 1H, HC_4), 2.35 (tt, J = 11.4, 3.6 Hz, 1H, HC_1), 2.20–2.05 (m, 4H, $HC_{3,2}$), 1.59–1.44 (m, 2H, HC_2), 1.44–1.31 (m, 2H, HC_3) ppm. ^{13}C{^{1}H} NMR (101 MHz, $CDCl_3$) δ 180.3 (C_{10}), 139.0 (C_6), 128.5 (C_8), 127.7 (C_7), 127.6 (C_9), 76.5 (C_4), 70.2 (C_5), 42.1 (C_1), 31.3 (C_3), 27.0 (C_2) ppm. IR ν_{max} 2940, 2863, 1687, 1219, 1112 cm^{-1}. MS (FAB) m/z (relative intensity, %) 235 (M^++H^+, 7), 217 (5), 143 (3), 107 (9), 91 (100). HRMS (FAB) Calcd for $C_{14}H_{19}O_3$ [M+H]$^+$: m/z 235.1329, Found: 235.1312. Anal. Calcd for $C_{14}H_{18}O_3$: C, 71.77; H, 7.74. Found: C, 71.91; H, 7.50.

4.1.10. (*R*)-(−)-2-(*cis*-4-(Benzyloxy)cyclohexanecarbonyl)-1,2,3,6,7,11*b*-hexahydro-4*H*-pyrazino[2,1-*a*]isoquinolin-4-one 8a

Compound **3a** (101.1 mg, 0.50 mmol), **7a** (117.2 mg, 0.50 mmol), PyBOP (284.3 mg, 0.55 mmol), and 4-methylmorpholine (272 μL, 2.47 mmol) were dissolved under a nitrogen atmosphere in DMSO (2 mL), and the mixture was stirred at room temperature for 30 min. It was then poured into a funnel and dichloromethane (20 mL) was added. The organic layer was successively washed with 1 M HCl (3 × 10 mL), a saturated solution of $NaHCO_3$ (3 × 30 mL), water (3 × 30 mL) and dried over Na_2SO_4, filtered, and concentrated. The residue was purified by column chromatography (SiO_2, ethyl acetate 100%). A white powder was obtained (185.7 mg, 89% yield). Mp 132–135 °C. $[\alpha]_D = -99.2$ (c 1, $CHCl_3$). IR ν_{max} 2927, 2862, 1645, 1625, 1439, 1420, 1206, 1066, 735 cm^{-1}. MS (EI) m/z (relative intensity, %) 418 (M^+, 14), 327 (36), 201 (100), 185 (4), 173 (10), 145 (16), 132 (50). HRMS (EI) Calcd for $C_{26}H_{30}N_2O_3$ (M^+): m/z 418.2251, Found: 418.2236. Anal. Calcd for $C_{26}H_{30}N_2O_3$: C, 74.61; H, 7.22; N, 6.69. Found: C, 74.37; H, 6.62; N, 6.74. For clarity, the NMR spectra of the two rotamers are separately described.

Major rotamer (81.28%): ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.39–7.14 (m, 9H, $HC_{7',8',11,10,9,9',8}$), 5.16 (d, J = 12.6 Hz, 1H, HC_1), 4.88–4.77 (m, 2H, $HC_{6,11b}$), 4.50 (s, 2H, $HC_{5'}$), 4.46 (AB, J = 17.6 Hz, 1H, HC_3), 4.08 (AB, J = 17.4 Hz, 1H, HC_3), 3.68 (s, 1H, $HC_{4'}$), 3.03–2.74 (m, 4H, $HC_{7,6,1,7}$), 2.51 (t, J = 10.6 Hz, 1H, $HC_{1'}$), 2.13–1.91 (m, 4H, $HC_{3',2'}$), 1.60–1.41 (m, 4H, $HC_{2',3'}$) ppm. ^{13}C{^{1}H} NMR (101 MHz, $CDCl_3$) δ 174.4 (C_{12}), 164.5 (C_4), 139.2 ($C_{6'}$), 134.8 (C_{7a}), 132.9 (C_{11a}), 129.4 (C_8), 128.4 ($C_{8'}$), 127.5 (C_9), 127.4 ($C_{7'}$), 127.4 ($C_{9'}$), 127.1 (C_{10}), 125.6 (C_{11}), 72.0 ($C_{4'}$), 69.8 ($C_{5'}$), 55.1 (C_{11b}), 49.2 (C_3), 45.3 (C_1), 40.3 ($C_{1'}$), 39.2 (C_6), 29.2 ($C_{3'}$), 28.8 (C_7), 23.7 ($C_{2'}$), 23.5 ($C_{2'}$) ppm.

Minor rotamer (18.72%): ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.39–7.14 ($_{7',8',9,10,9',8,11}$), 4.90–4.73 (m, 3H, $HC_{6,11b,3}$), 4.50 (s, 2H, $HC_{5'}$), 4.34 (d, J = 12.1 Hz, 1H, HC_1), 3.86 (AB, J = 17.5 Hz, 1H, HC_3), 3.68 (s, 1H, $HC_{4'}$), 3.25 (t, J = 12.7 Hz, 1H, HC_1), 3.03–2.72 (m, 3H, $HC_{7,6,7}$), 2.51 (t, J = 11.3 Hz, 1H, $HC_{1'}$), 2.13–1.91 (m, 4H, $HC_{3',2'}$), 1.60–1.41 (m, 4H, $HC_{2',3'}$) ppm. ^{13}C{^{1}H} NMR (101 MHz, $CDCl_3$) δ 173.7 (C_{12}), 165.6 (C_4), 139.2 ($C_{6'}$), 135.7 (C_{7a}), 132.2 (C_{11a}), 129.8 (C_8), 127.8 (C_9), 127.4 ($C_{7'}$), 127.4 ($C_{9'}$), 127.1 (C_{10}), 125.2 (C_{11}), 72.1 ($C_{4'}$), 69.6 ($C_{5'}$), 55.9 (C_{11b}), 49.7 (C_1), 46.4 (C_3), 40.3 ($C_{1'}$), 38.7 (C_6), 29.4 ($C_{3'}$), 29.2 (C_7), 24.0 ($C_{2'}$), 23.7 ($C_{2'}$) ppm.

4.1.11. (*R*)-(−)-2-(*trans*-4-(Benzyloxy)cyclohexanecarbonyl)-1,2,3,6,7,11*b*-hexahydro-4*H*-pyrazino[2,1-*a*]isoquinolin-4-one 8b

Following the procedure reported above for the preparation of **8a** and starting with amine **3a** (101.1 mg, 0.50 mmol) and acid **7b** (117.2 mg, 0.50 mmol), compound **8b** was obtained as a white powder (183.9 mg, 88% yield). Mp 135–137 °C. $[\alpha]_D = -97.0$ (c 1, $CHCl_3$). IR ν_{max} 2935, 2863, 1644, 1451, 1418, 1202, 1076, 734 cm^{-1}. MS (EI) m/z (relative intensity, %) 418 (M^+, 16), 327 (10), 201 (75), 185 (9), 173 (17), 145 (25), 132 (70), 91 (100). HRMS (EI) Calcd for $C_{26}H_{30}N_2O_3$ (M^+): m/z 418.2251, Found: 418.2259. Anal. Calcd for $C_{26}H_{30}N_2O_3$: C, 74.61; H, 7.22; N, 6.69. Found: C, 74.09; H, 7.08; N, 6.80. For clarity, the NMR spectra of the two rotamers are described separately.

Major rotamer (76.54%): ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.41–7.08 (m, 9H, $HC_{7',8',11,10,9,9',8}$), 5.13 (dd, J = 13.5, 2.7 Hz, 1H, HC_1), 4.88–4.73 (m, 2H, $HC_{6,11b}$), 4.57 (s, 2H, $HC_{5'}$), 4.46 (AB, J = 17.5 Hz, 1H, HC_3), 4.09 (AB, J = 17.5 Hz, 1H, HC_3), 3.44–3.33 (m, 1H, $HC_{4'}$),

3.03–2.75 (m, 4H, $HC_{7,6,1,7}$), 2.46 (t, J = 11.6 Hz, 1H, $HC_{1'}$), 2.20 (d, J = 12.0 Hz, 2H, $HC_{3'}$), 1.90–1.79 (m, 2H, $HC_{2'}$), 1.70–1.51 (m, 2H, $HC_{2'}$), 1.40–1.31 (m, 2H, HC_3) ppm. $^{13}C\{^1H\}$ NMR (101 MHz, $CDCl_3$) δ 174.2 (C_{12}), 164.3 (C_4), 138.9 ($C_{6'}$), 134.9 (C_{7a}), 132.8 (C_{11a}), 129.4 (C_8), 128.5 ($C_{8'}$), 127.7 ($C_{7'}$), 127.6 (C_9), 127.6 ($C_{9'}$), 127.1 (C_{10}), 125.6 (C_{11}), 76.7 ($C_{4'}$), 70.2 ($C_{5'}$), 55.0 (C_{11b}), 49.1 (C_3), 45.3 (C_1), 40.1 ($C_{1'}$), 39.2 (C_6), 31.7 ($C_{3'}$), 31.6 ($C_{3'}$), 28.8 (C_7), 27.6 ($C_{2'}$), 27.4 ($C_{2'}$) ppm.

Minor rotamer (23.46%): ^1H NMR (400 MHz, $CDCl_3$): δ 7.38–7.12 (m, 9H, $HC_{7',8',9,10,9'_{,,}}$), 4.91–4.70 (m, 3H, $HC_{6,11b,3}$), 4.57 (s, 2H, $HC_{5'}$), 4.35 (d, J = 13.4 Hz, 1H, HC_1), 3.88 (AB, J = 18.6 Hz, 1H, HC_3), 3.44–3.33 (m, 1H, $HC_{4'}$), 3.28 (t, J = 11.9 Hz, 1H, HC_1), 3.03–2.75 (m, 3H, $HC_{7,6,7}$), 2.46 (t, J = 11.6 Hz, 1H, $HC_{1'}$), 2.20 (d, J = 12.0 Hz, 2H, $HC_{3'}$), 1.90–1.79 (m, 2H, $HC_{2'}$), 1.70–1.51 (m, 2H, $HC_{2'}$), 1.40–1.31 (m, 2H, $HC_{3'}$) ppm. $^{13}C\{^1H\}$ NMR (101 MHz, $CDCl_3$) δ 173.7 (C_{12}), 165.5 (C_4), 138.9 ($C_{6'}$), 135.6 (C_{7a}), 132.0 (C_{11a}), 129.8 (C_8), 128.5 ($C_{8'}$), 127.9 (C_9), 127.7 ($C_{7'}$), 127.6 ($C_{9'}$), 127.1 (C_{10}), 125.3 (C_{11}), 76.7 ($C_{4'}$), 70.2 ($C_{5'}$), 55.9 (C_{11b}), 49.8 (C_1), 46.5 (C_3), 40.1 ($C_{1'}$), 38.8 (C_6), 31.7 ($C_{3'}$), 31.6 ($C_{3'}$), 29.2 (C_7), 27.6 ($C_{2'}$), 27.4 ($C_{2'}$) ppm.

4.1.12. (*R*)-(−)-2-(*cis*-4-Hydroxycyclohexanecarbonyl)-1,2,3,6,7,11*b*-hexahydro-4*H*-pyrazino[2,1-*a*]isoquinolin-4-one ((*R*)-(−)-*cis*-4′-hydroxypraziquantel) 9a

Compound **8a** (150 mg, 0.36 mmol) was dissolved in ethanol (30 mL), 10% w/w Pd/C (38. 2 mg, 35.90 μmol) was added and an atmosphere of hydrogen was introduced at room temperature. The reaction mixture was stirred under hydrogen at 45 °C for 3 h (Parr shaker hydrogenation apparatus); it was then cooled and filtered through Celite, washed with ethanol (3 × 10 mL), and the solvent was removed under reduced pressure. A white powder was obtained (114.2 mg, 92% yield). Mp 161.09 °C $[\alpha]_D = -148.0$ (c 1, $CHCl_3$). IR ν_{max} 3423, 2926, 2866, 1631, 1419, 1204 cm^{-1}. MS (EI) m/z (relative intensity, %) 328 (M^+, 20), 201 (62), 185 (15), 173 (48), 145 (60), 132 (100). HRMS (EI) Calcd for $C_{19}H_{24}N_2O_3$ (M^+): m/z 328.1781, Found: 328.1793. Anal. Calcd for $C_{19}H_{24}N_2O_3$: C, 69.49; H, 7.37; N, 8.53. Found: C, 69.39; H, 7.37; N, 8.68. For clarity, the NMR spectra of the two rotamers are described separately.

Major rotamer (73.49%): ^1H NMR (400 MHz, $CDCl_3$) δ 7.32–7.13 (m, 4H, $HC_{11,10,9,8}$), 5.15 (d, J = 12.8 Hz, 1H, HC_1), 4.87–4.71 (m, 2H, $HC_{6,11b}$), 4.44 (AB, J = 17.3 Hz, 1H, HC_3), 4.07 (AB, J = 17.3 Hz, 1H, HC_3), 4.03 (s, 1H, $HC_{4'}$), 3.03–2.73 (m, 4H, $HC_{7,6,1,7}$), 2.50 (t, J = 11.2 Hz, 1H, $HC_{1'}$), 2.07–1.75 (m, 4H, $HC_{2',3'}$), 1.68–1.50 (m, 4H, $HC_{3',2'}$) ppm. $^{13}C\{^1H\}$ NMR (101 MHz, $CDCl_3$) δ 174.3 (C_{12}), 164.4 (C_4), 134.8 (C_{7a}), 132.8 (C_{11a}), 129.4 (C_8), 127.6 (C_9), 127.1 (C_{10}), 125.6 (C_{11}), 65.6 ($C_{4'}$), 55.1 (C_{11b}), 49.2 (C_3), 45.3 (C_1), 39.8 ($C_{1'}$), 39.3 (C_6), 32.1 ($C_{3'}$), 28.8 (C_7), 23.3 ($C_{2'}$), 23.1 ($C_{2'}$) ppm.

Minor rotamer (26.51%): ^1H NMR (400 MHz, $CDCl_3$): δ 7.32–7.13 (m, 4H, $HC_{9,10,8,11}$) 4.86–4.74 (m, 3H, $HC_{6,11b,3}$), 4.34 (d, J = 12.1 Hz, 1H, HC_1), 4.03 (s, 1H, $HC_{4'}$), 3.86 (AB, J = 19.2 Hz, 1H, HC_3), 3.25 (t, J = 11.8 Hz, 1H, HC_1), 3.02–2.73 (m, 3H, $HC_{7,6,7}$), 2.60 (t, J = 10.5 Hz, 1H, $HC_{1'}$), 2.07–1.75 (m, 4H, $HC_{2',3'}$), 1.68–1.50 (m, 4H, $HC_{3',2'}$). $^{13}C\{^1H\}$ NMR (101 MHz, $CDCl_3$) δ 173.7 (C_{12}), 165.7 (C_4), 135.7 (C_{7a}), 132.2 (C_{11a}), 129.8 (C_8), 127.8 (C_9), 127.1 (C_{10}), 125.3 (C_{11}), 65.6 ($C_{4'}$), 55.9 (C_{11b}), 49.8 (C_1), 46.5 (C_3), 39.8 ($C_{1'}$), 38.8 (C_6), 32.1 ($C_{3'}$), 28.8 (C_7), 23.7 ($C_{2'}$), 23.3 ($C_{2'}$).

4.1.13. (*R*)-(−)-2-(*trans*-4′-Hydroxycyclohexanecarbonyl)-1,2,3,6,7,11*b*-hexahydro-4*H*-pyrazino[2,1-*a*]isoquinolin-4-one monohydrate, ((*R*)-(−)-*trans*-4′-hydroxypraziquantel monohydrate) 9b

Following the procedure reported for the preparation of **9a** and starting with **8b** (150 mg, 0.36 mmol) compound **9b** was obtained as a white powder (115.7 mg, 93% yield). Mp 98.66 °C $[\alpha]_D = -135.4$ (c 1, $CHCl_3$). IR ν_{max} 3452, 3368, 3252, 2934, 2854, 1648, 1631, 1495, 1457, 1437, 1423, 1070, 767 cm^{-1}. MS (EI) m/z (relative intensity, %) 328 (M^+, 41), 201 (100), 185 (19), 173 (20), 145 (33), 132 (85). HRMS (EI) Calcd for $C_{19}H_{24}N_2O_3$ (M^+): m/z 328.1781, Found: 328.1795. Anal. Calcd for $C_{19}H_{24}N_2O_3{\cdot}H_2O$: C, 65.87; H, 7.56; N, 8.09. Found: C, 65.73; H, 7.62; N, 8.24. For clarity, the NMR spectra of the two rotamers are described separately.

Major rotamer (67.11%): ^1H NMR (400 MHz, $CDCl_3$) δ 7.37–7.16 (m, 4H, $HC_{11,10,9,8}$), 5.16 (dd, J = 13.3, 2.8 Hz, 1H, HC_1), 4.94–4.74 (m, 2H, $HC_{6,11b}$), 4.48 (AB, J = 17.4 Hz, 1H, HC_3), 4.12 (AB, J = 17.4 Hz, 1H, HC_3), 3.68 (tt, J = 10.8, 3.8 Hz, 1H, $HC_{4'}$), 3.06–2.78 (m, 4H, $HC_{7,6,1,7}$), 2.45 (tt, J = 11.7, 3.3 Hz, 1H, $HC_{1'}$), 2.11 (d, J = 12.5 Hz, 2H, $HC_{3'}$), 1.95–1.75 (m, 2H, $HC_{2'}$), 1.76–1.56 (m, 2H, $HC_{2'}$)., 1.43–1.27 (m, 2H, $HC_{3'}$) ppm. $^{13}C\{^1H\}$ NMR (101 MHz, $CDCl_3$) δ 174.2 (C_{12}), 164.4 (C_4), 134.9 (C_{7a}), 132.8 (C_{11a}), 129.4 (C_8), 127.6 (C_9), 127.1 (C_{10}), 125.6 (C_{11}), 69.9 ($C_{4'}$), 55.1 (C_{11b}), 49.2 (C_3), 45.4 (C_1), 39.8 ($C_{1'}$), 39.3 (C_6), 34.8 ($C_{3'}$), 28.8 (C_7), 27.6 ($C_{2'}$), 27.4 ($C_{2'}$) ppm.

Minor rotamer (32.89%): ^1H NMR (400 MHz, $CDCl_3$): δ 7.37–7.16 (m, 4H, $HC_{9,10,8,11}$), 4.94–4.74 (m, 3H, $HC_{6,11b,3}$), 4.37 (d, J = 12.6 Hz, 1H, HC_1), 3.91 (AB, J = 18.9 Hz, 1H, HC_3), 3.68 (tt, J = 10.8, 3.8 Hz, 1H, $HC_{4'}$), 3.31 (t, J = 11.9 Hz, 1H, HC_1), 3.06–2.77 (m, 3H, $HC_{7,\ 6,\ 7}$), 2.59–2.51 (m, 1H, $HC_{1'}$), 2.11 (d, J = 12.5 Hz, 2H, $HC_{3'}$), 1.95–1.75 (m, 2H, $HC_{2'}$), 1.76–1.56 (m, 2H, $HC_{2'}$), 1.43–1.27 (m, 2H, $HC_{3'}$) ppm. $^{13}C\{^1H\}$ NMR (101 MHz, $CDCl_3$) δ 173.8 (C_{12}), 165.6 (C_4), 135.7 (C_{7a}), 132.1 (C_{11a}), 129.8 (C_8), 128.0 (C_9), 127.1 (C_{10}), 125.3 (C_{11}), 69.9 ($C_{4'}$), 55.9 (C_{11b}), 49.8 (C_1), 46.5 (C_3), 39.8 ($C_{1'}$), 38.8 (C_6), 34.8 ($C_{3'}$), 29.0 (C_7), 27.8 ($C_{2'}$), 27.6 ($C_{2'}$) ppm.

(*S*)-(+)-*cis*-4′-Hydroxypraziquantel **9c** and (*S*)-(+)-*trans*-4′-hydroxypraziquantel monohydrate **9d** were obtained in a similar manner, starting from (*S*)-(+)-praziquanamine **3b**.

4.2. X-ray crystal structure determinations

The crystals of **2a**, **4a, 8a, 8b,** and **9b** mounted on glass fiber were studied with an Oxford Diffraction Gemini 'A' diffractometer with a CCD area detector; with radiation source of $\lambda_{MoK\alpha}$ = 0.71073 Å for **4a, 8b,** and **9b,** and $\lambda_{CuK\alpha}$ = 1.5418 Å for **2a** and **8a** using graphite-monochromatized radiation. CrysAlisPro and CrysAlis RED software packages[17] were used for data collection and data integration. Data sets consisted of frames of intensity data collected with a frame width of 1° in ω and α crystal-to-detector distance of 55.00 mm. The double pass method of scanning was used to exclude any noise. The collected frames were integrated by using an orientation matrix determined from the narrow frame scans. Final cell constants were determined by a global refinement; collected data were corrected for absorbance by using analytical numeric absorption correction[18] using a multifaceted crystal model based on expressions upon the Laue symmetry using equivalent reflections.

Structure solution and refinement were carried out with the program(s): SHELXS97[19] for molecular graphics: ORTEP-3 for Windows;[20] and the software used to prepare material for publication: WinGX 1.80.05.[21]

Full-matrix least-squares refinement was carried out by minimizing $(Fo^2 - Fc^2)^2$. All non-hydrogen atoms were refined anisotropically. The H atoms of the hydroxyl group and water molecule were located in a difference Fourier map and refined isotropically with $U_{iso}(H) = 1.5\ U_{eq}$. The H atoms attached to C atoms were placed in geometrically idealized positions and refined as riding on their parent atoms, with C═H = 0.93–1.00 Å with $U_{iso}(H) = 1.2\ U_{eq}(C)$ for methylene, methyne and aromatic groups, and $U_{iso}(H) = 1.5\ U_{eq}(C)$ for methyl groups. CCDC 945871 for **2a**, 945872 for **8b**, 945874 for **8a**, 945875 for **4a** and 945876 for **9b** contain the supplementary crystallographic data for this paper. These data can be obtained free of charge from The Cambridge Crystallographic Data Centre via http://www.ccdc.cam.ac.uk/Community/Requestastructure/Pages/DataRequest.aspx?.

4.3. Cysticidal activity

Stock solutions of PZQ, **4a**, **4b**, **9a**, **9b,** and **9d** were prepared in DMSO. In order to determine the concentration that produced 50% effect (EC_{50}), working solutions of compounds were prepared in Dulbecco's culture medium to obtain concentrations between 16.01 and 86.07 nM for PZQ, 11.43 and 43.92 nM for **4a**, 46.09 and 870.98 nM for **4b**, 97.06 and 2888.59 nM for **9a** and **9b** and 97.06 nM for **9d**. A 0.07% solution of DMSO in water was prepared as a control.

24-Well cell culture flat-bottom microplates were carefully filled with 2 mL of culture medium containing each compound or control solution. Ten cysts were deposited into each well and were incubated at 37 °C with 5% CO_2 atmosphere and 98% relative humidity for 11 days. Each experiment was run in triplicate. In order to observe the effect of the compounds over the cysts, the parasites were observed every day and were monitored for integrity, motility, and morphological aspect using an inverted light microscope. The criteria to assess parasite mortality were loss of vesicular fluid, paralysis of membrane, and collapse of parasites. The mortality was confirmed on day 11 using the Trypan blue exclusion test.

The mortality results were analyzed using nonlinear regression (Probit model) to obtain the corresponding EC_{50} and the confidence limits of each compound. The analysis was performed using IBM® SPSS® Statistics version 21.

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