



Study on the absolute configuration of levetiracetam via density functional theory calculations of electronic circular dichroism and optical rotatory dispersion

Li Li*, Yi-Kang Si

Institute of Materia Medica, Chinese Academy of Medical Sciences & Peking Union of Medical College, 1 Xian Nong Tan Street, Beijing 100050, PR China

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ABSTRACT

Theoretical calculation of chiroptical properties has been a powerful tool for the absolute configuration assignment of chiral compounds including synthetic drugs and natural products. In the present work, time-dependent density functional theory (TDDFT) calculations of electronic circular dichroism (ECD) and optical rotatory dispersion (ORD) were employed to investigate the absolute configuration of levetiracetam, which is a widely used anticonvulsant drug. Nine conformers were generated by conformation search using the MMFF94 molecular mechanics force field, and the geometries were then optimized using the Becke 3–Lee–Yang–Parr (B3LYP) exchange–correlation functional. The population-averaged ECD spectrum was obtained by adding ECD spectrum of each conformer using Boltzmann statistics. The predicted ECD spectrum is in excellent agreement with the measured ECD spectrum of levetiracetam. Theoretical ORD spectra show the same tendency as the experimental data of levetiracetam, further confirming the absolute configuration derived from the ECD spectra. Our results demonstrated that the only chiral carbon atom of levetiracetam is unambiguously to be S configuration.

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1. Introduction

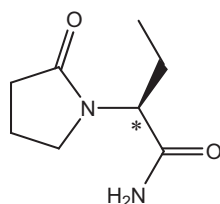
Different enantiomers of chiral compounds may exert different or even totally opposite functions. Enantiopure drugs have been the focus of pharmaceutical industry since they often display higher efficiency and less toxicity than racemates [1]. Therefore, determination of the absolute configuration of bioactive compounds is crucial for drug discovery and development. Many countries have introduced requirements on the identification and characterization of the stereochemistry of novel drugs. Single-crystal X-ray diffraction is conventionally regarded as the only direct way to assign the absolute configuration. However, this method requires a sizable single crystal of a pure enantiomer and the lack of heavy atoms would prevent use of Flack's methodology. Electronic circular dichroism (ECD) is a convenient and sensitive technique to detect the stereochemistry of organic and inorganic molecules [2]. A good ECD spectrum can be obtained using 0.1–1.0 mg of the sample, which can be solid or liquid. ECD is associated to the electronic transitions of molecules and often gives complex curve, which contains important stereochemical information. Thus, the interpretation of ECD spectra is pivotal to assign the absolute configuration. Several empirical rules and semi-empirical models

have been established including octant rule and exciton chirality method. However, specific requirements of molecular structures limit the application of these approaches. In the past 10 years, a novel alternative methodology based on the chiroptical properties has been developed and implemented via comparison of *ab initio* quantum chemical calculations with experimental results [3–6]. Amongst all the theoretical prediction methodologies, time-dependent density functional theory (TDDFT) has been proved to be a reliable and feasible approach. Many studies on the application of TDDFT to the ECD calculation have been reported over the past few years [7]. Combined with other chiroptical properties, ECD prediction has been demonstrated to be a most valuable tool for the absolute configuration assignment of chiral compounds [8].

Levetiracetam ((S)-2-(2-oxopyrrolidin-1-yl)butanamide) obtained marketing authorization from the Food and Drug Administration (FDA) as an anticonvulsant medication used to treat epilepsy and neuropathic pain (Scheme 1) [9]. Levetiracetam has also potential benefits for other psychiatric and neurologic conditions [10]. It is the S-enantiomer of etiracetam, structurally similar to the prototypical nootropic drug piracetam. Levetiracetam can be prepared from chiral source (S)-2-aminobutanoic acid or (S)-2-aminobutanamide, which is synthesized and optically resolved with tartaric acid. Neither single-crystal X-ray nor circular dichroism data of levetiracetam has been reported to identify its absolute configuration. In this paper, the predicted ECD and ORD

* Corresponding author. Tel.: +86 10 63165247; fax: +86 10 63165247.

E-mail address: annaleelin@imm.ac.cn (L. Li).



Scheme 1. Structure of levetiracetam ((S)-1).

spectra by quantum chemical calculation were compared with the experimental data of levetiracetam to determine its absolute configuration.

2. Materials and methods

Levetiracetam was synthesized and characterized according to literature methods. ECD spectra of levetiracetam, at a concentration of 0.2–1.0 mg/mL in distilled water, were recorded in a quartz cuvette of 1 mm optical path length using a Jasco J-815 CD spectrometer (Jasco Inc., Japan). The conditions of measurement were as follows: scanning speed, 50 nm/min; bandwidth, 1 nm; and 3 accumulations. ECD spectrum of the solvent was used as the baseline and subtracted from the experimental spectra. Optical rotations of levetiracetam were measured on a Perkin-Elmer Model 341 LC polarimeter (Perkin-Elmer Inc., Norwalk, CT) in H₂O or CH₂Cl₂ at different wavelengths (589, 546, 436 and 365 nm) and room temperature.

3. Computational details

All calculations have been carried out on S configuration of compound 2-(2-oxopyrrolidin-1-yl)butanamide ((S)-1, Scheme 1). Initial conformational analysis was performed using the MMFF94 molecular mechanics force field via the MOE software package [11]. Full geometry optimization of the MMFF94 conformations obtained was then completed in the framework of density functional theory (DFT) using the Becke 3-Lee-Yang-Parr (B3LYP) exchange-correlation functional at the 6-31+G(d,p) basis set level. It has been checked that stationary points were true minima of the potential energy surface by verifying they do not exhibit vibrational imaginary frequencies. The polarizable continuum model (PCM) was adopted to consider solvent effects using the dielectric constant of water (78.36) and dichloromethane (8.93). The 20 lowest electronic transitions were calculated for each conformer. Rotational strengths of electronic excitations were showed using both

dipole length (R_{len}) and dipole velocity (R_{vel}) representations. ECD spectra were simulated by using a Gaussian function

$$\Delta \epsilon(E) = \frac{1}{2.297 \times 10^{-39}} \frac{1}{\sqrt{2\pi}\sigma} \sum_i^A \Delta E_i R_i e^{-((E-\Delta E_i)/2\sigma)^2}$$

where σ is the width of the band at 1/e peak height, and ΔE_i and R_i are excitation energies and rotatory strength for transition i , respectively. Equilibrium populations of conformers at 298.15 K were calculated from their relative free energies (ΔG) using Boltzmann statistics. The overall ECD spectra were then generated according to the Boltzmann weighting of each conformer. All quantum computations were performed by using Gaussian03 program package [12], on an IBM cluster machine located at the High Performance Computing Center of Peking Union Medical College.

4. Results and discussion

4.1. Conformation analysis

Since compound (S)-1 is a relatively flexible molecule, a complete conformational analysis was carried out to obtain the multiple possible conformations. A systematic conformation search using the MMFF94 molecular mechanics force field identified nine conformations of (S)-1 within a 10 kJ/mol window. Conformers **1a–1d** and **1la–1le** differ principally with regard to the position of β -carbon atom C4, below and above the C1C2N10C7 plane of cyclic amide, respectively (Fig. 1).

The geometries of the MMFF94 conformers were then re-optimized using DFT at the B3LYP/6-31+G(d,p) level and nine conformers were obtained (Fig. 2). Harmonic vibrational frequencies were then calculated to check the stability and also to give the free energies of each conformer, with the results shown in Table 1. Solvents may affect the population of conformers and the calculation of chiroptical properties through the solute-solvent interaction. Regarding (S)-1 in aqueous solution, it may form hydrogen bond with the solvent. Therefore, the influence of H₂O has been taken into account and compared with CH₂Cl₂ using PCM, which is a commonly used method to model solvation effects. It is interesting to find that (S)-1 possesses a similar conformation distribution in H₂O and CH₂Cl₂ regardless the possible formation of hydrogen bond with the solvent. Conformers **1a**, **1b**, **1lb**, **1lc** and **1le** are dominant, constituting >98% in the solution phase and >80% in the gas phase, respectively.

The difference between **1a** and **1b** lies in that the torsional angle of C11–C13 bond and C14–C17 bond changed from anti-periplanar to 65°. Conformers **1c** and **1d** differ with **1a** and **1b** in the N10C11C13N24 dihedral angle, respectively. The relative free

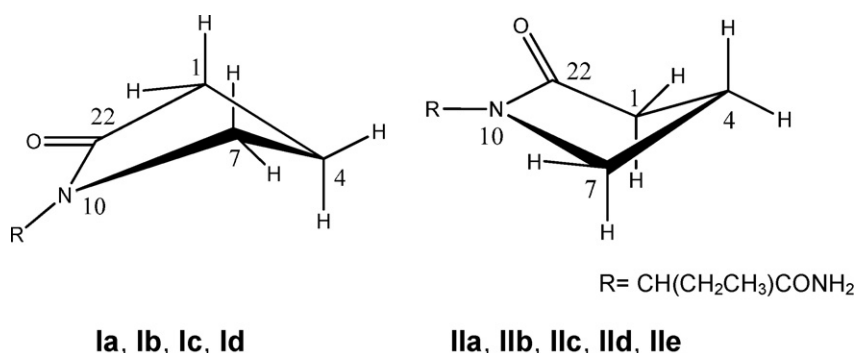


Fig. 1. Schematic representation of the envelope conformations of (S)-1, with the β -carbon atom below (conformers **1a**, **1b**, **1c** and **1d**) and above the C1C2N10C7 plane (conformers **1la**, **1lb**, **1lc**, **1ld** and **1le**).

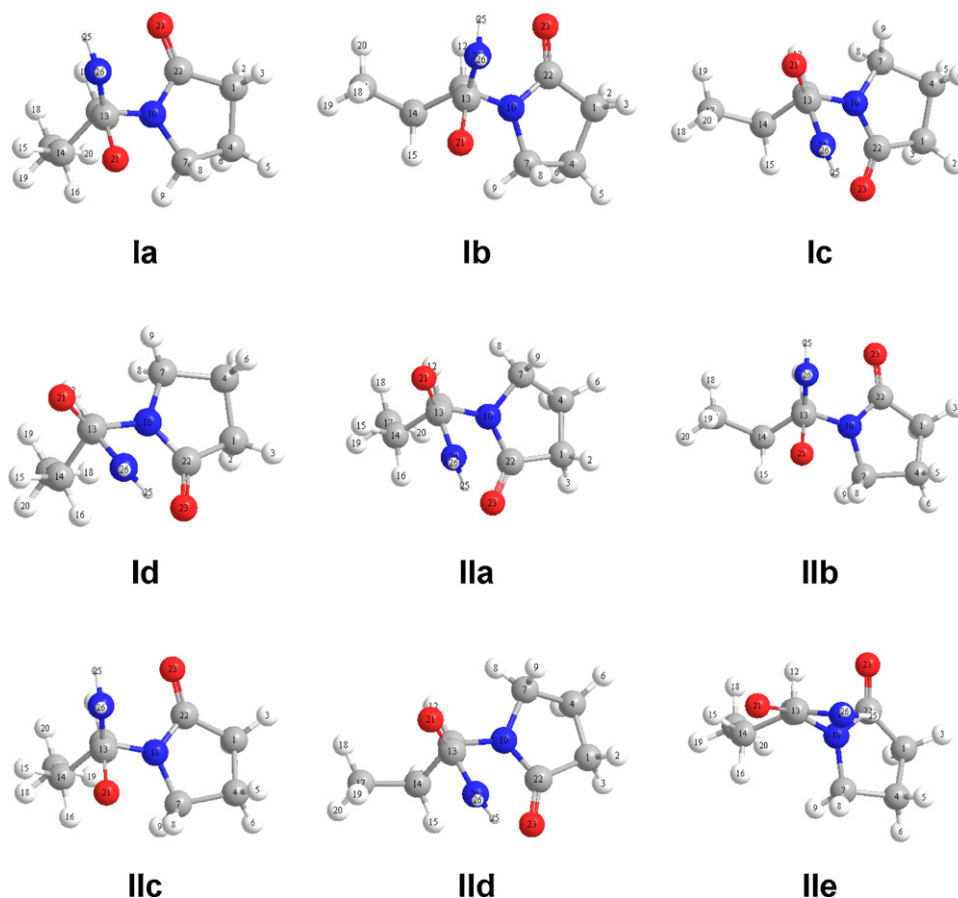


Fig. 2. B3LYP/6-31+G(d,p) structures of the (S)-1 conformers.

energies of **Ic** and **Id** are computed to be more than 10 kJ/mol higher than the counterpart in H₂O and CH₂Cl₂, therefore should not have a significant thermal population in both solvents at ambient temperature. Conformers **Ia** and **Ib** are a little more stable than the counterpart **IIc** and **IIb** because of the lower steric force between the β -methylene group and bulky N-substituent group. The relative free energies and Boltzmann statistics were used to predict the room temperature equilibrium population of conformations. On this basis, none of the conformers predominates the population, with **Ia** and **Ib** showing slightly more contribution than **IIc** and **IIb**.

4.2. ECD spectra

The oscillator strengths and rotational strengths of the nine conformations of (S)-1 have been calculated at the B3LYP/6-31+G(d,p) equilibrium geometries in gas phase and two experimental solvents. Rotational strength is the quantum mechanical quantity describing ECD and is calculated using length and velocity representations, both of which could be used to simulate the ECD spectra. The origin-independent velocity rotational strengths and ECD spectra obtained using Gaussian band shapes are plotted in Fig. 3. It is obvious that the ECD spectra strongly depend on the molecular con-

Table 1

The relative energies (ΔE)^a and relative free energies (ΔG)^a of the conformers of (S)-1.

Conformer	MMFF94 ΔE	B3LYP/6-31+G(d,p)					
		In gas phase		In CH ₂ Cl ₂		In H ₂ O	
		ΔG	<i>P</i> (%) ^b	ΔG	<i>P</i> (%) ^b	ΔG	<i>P</i> (%) ^b
Ia	0.00	0.00	21.03	0.00	40.45	0.00	31.13
Ib	0.64	-0.64	27.17	0.86	28.58	0.93	21.35
Ic	2.54	4.56	3.34	12.12	0.30	12.57	0.20
Id	2.85	3.86	4.43	10.90	0.50	11.49	0.30
IIa	3.89	2.86	6.64	9.97	0.72	10.61	0.43
IIb	4.77	0.62	16.39	3.66	9.25	1.21	19.08
IIc	4.78	1.84	10.01	2.40	15.36	1.38	17.81
IIId	5.06	4.77	3.07	12.56	0.25	12.79	0.18
IIe	6.29	2.42	7.93	5.40	4.58	2.94	9.52

^a In kJ/mol.

^b Population percentages based on ΔG , assuming Boltzmann statistics at $T=298.15$ K.

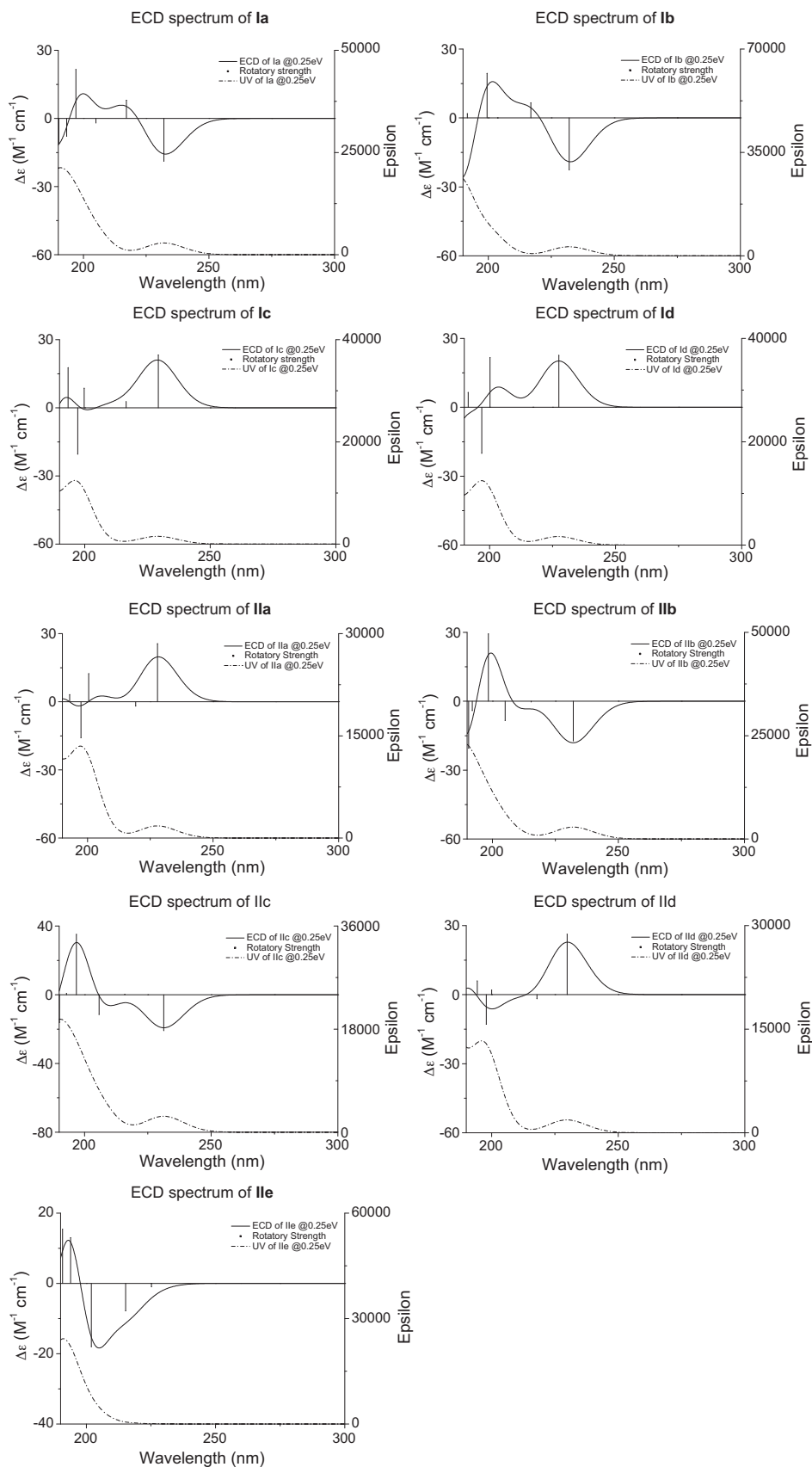


Fig. 3. Calculated ECD and UV spectra of (S)-1 conformers in water at the B3LYP/6-31+G(d,p) level. The calculated ECD spectra are simulated using rotatory strengths and Gaussian functions with $\sigma = 0.25$ eV. Upper part: the calculated ECD spectra (solid line) and rotatory strength (bar); lower part: the calculated UV spectra (dash dot line).

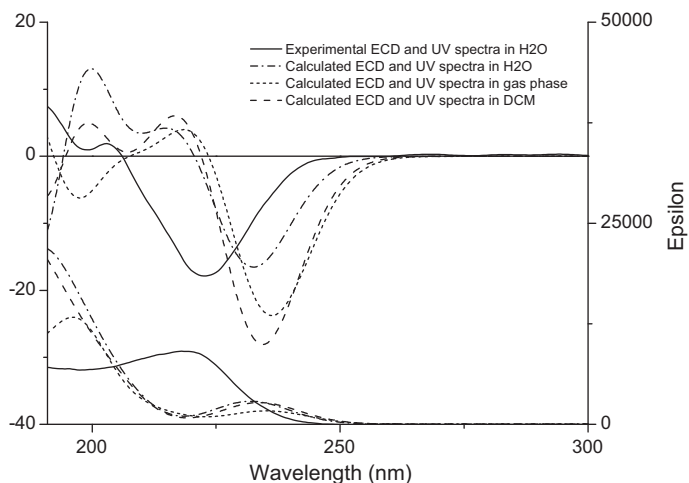


Fig. 4. Comparison of the calculated spectra of (S)-1 and the experimental spectra of levetiracetam. Calculated ECD and UV spectra of (S)-1 in gas phase (short dash line) and different solvents (H₂O: dash dot line, CH₂Cl₂: dash line) at the B3LYP/6-31+G(d,p) level, and the experimental ECD and UV spectra of levetiracetam in water (solid line).

formations. The predicted ECD spectra of conformers **Ic**, **Id**, **Ila** and **Ild** differ significantly from those of **Ia**, **Ib**, **Ilb**, **Ilc** and **Ile**.

The experimental ECD spectrum of levetiracetam was measured at room temperature and thus represents a thermal average over the ECD spectra of many different conformers. The population-averaged ECD spectra of (S)-1 were compared to the experimental spectrum of levetiracetam in water over the range 190–300 nm (Fig. 4). As shown in the figure, the agreement between the simulated ECD spectrum and experimental ECD spectrum of levetiracetam is very satisfactory, and identifies the chiral carbon atom of levetiracetam as being S configuration. One diagnostic negative Cotton effect of the measured ECD spectrum was observed at 223 nm. As predicted by TDDFT calculation, this band can be reasonably attributed to the transition from HOMO (MO46) to LUMO (MO47) of conformers **Ia**, **Ib**, **Ilb** and **Ilc**, being the character of an amide $n(O) \rightarrow \pi^*$ transition (Fig. 5). The weak positive band at 203 nm of experimental ECD spectrum involved mainly the transition from HOMO-1 (MO45) to LUMO. The calculated band is slightly red-shifted, which is not untypical of B3LYP functional used here. The intense positive amide $n(N) \rightarrow \pi^*$ transition is located at the blue end of the experimental ECD spectrum.

4.3. ORD spectra

The specific optical rotations of (S)-1 have been calculated in the gas phase, H₂O and CH₂Cl₂ at the B3LYP/6-31+G(d,p) level and B3LYP/aug-cc-pVDZ level using B3LYP/6-31+G(d,p) geometries, with the results given in Table 2. The predicted specific

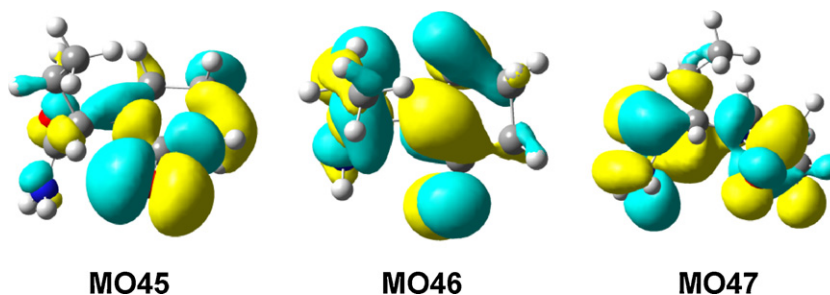


Fig. 5. Molecular orbitals involved in key transitions in the calculated ECD spectra of (S)-1 at B3LYP/6-31+G(d,p) in water.

Table 2
Comparison of the calculated^a specific optical rotations of (S)-1 and the experimental data of levetiracetam.^b

	$[\alpha]_D$	$[\alpha]_{546}$	$[\alpha]_{436}$	$[\alpha]_{365}$
At B3LYP/6-31+G(d,p) level				
(S)-1 in gas phase	-258.24	-311.83	-579.18	-1028.39
(S)-1 in CH ₂ Cl ₂	-319.65	-386.14	-718.18	-1276.09
(S)-1 in H ₂ O	-185.27	-223.93	-417.25	-742.23
At B3LYP/aug-cc-pVDZ//B3LYP/6-31+G(d,p) level				
(S)-1 in gas phase	-265.34	-320.85	-599.80	-1075.36
(S)-1 in CH ₂ Cl ₂	-314.27	-380.52	-714.87	-1287.38
(S)-1 in H ₂ O	-176.94	-214.49	-404.85	-732.53
Experimental data				
Levetiracetam in CH ₂ Cl ₂	-201.11	-232.22	-436.02	-793.33
Levetiracetam in H ₂ O	-81.36	-98.25	-185.05	-326.31

^a Population-weighted average.

^b Specific optical rotations in degrees $[\text{dm g/cm}^3]^{-1}$, c1 in H₂O or CH₂Cl₂.

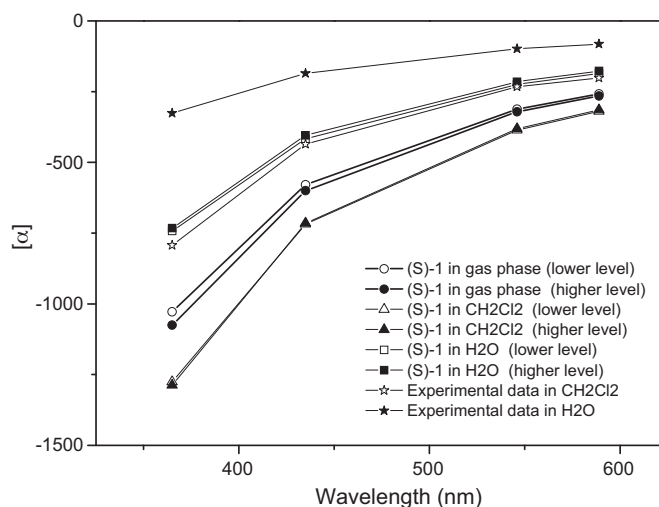


Fig. 6. Comparison of the calculated ORD of (S)-1 and the experimental data of levetiracetam. ORD of (S)-1 are calculated in gas phase (hollow or solid circle), CH₂Cl₂ (hollow or solid triangle) and H₂O (hollow or solid square) at the B3LYP/6-31+G(d,p) (lower level) and B3LYP/aug-cc-pVDZ//B3LYP/6-31+G(d,p) level (higher level), respectively. Experimental specific optical rotations of levetiracetam are collected in CH₂Cl₂ (hollow star) and H₂O (solid star).

optical rotations are negative in the sign and decrease with decreasing wavelength from 589 to 365 nm. The experimental ORD data of levetiracetam in water and dichloromethane showed the same sign and tendency as the calculated data (Fig. 6), further supporting the absolute configuration assignment derived from the ECD spectrum. The basis set aug-cc-pVDZ performs a little better than 6-31+G(d,p), but requires longer computational time.

5. Conclusion

The ECD and ORD spectra of levetiracetam have been presented in this paper and the chiral carbon atom is confirmed as being S configuration using TDDFT calculations. As to this quantum chemical calculation methodology, no empirical data or reference compound is needed for the absolute configuration identification. It may be convenient and feasible to use ECD spectroscopy and other chiroptical detections combined with the high-quality quantum chemical computation as a routine method for reliable absolute configuration assignments of chiral drugs.

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