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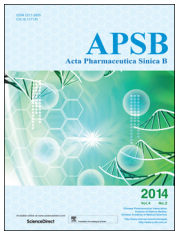

SHORT COMMUNICATION

Electronic circular dichroism behavior of chiral Phthiobuzone

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KEY WORDS

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Abstract Phthiobuzone is a bis(thiosemicarbazone) derivative with a single chiral center which has been used as a racemate in the clinical treatment of herpes and trachoma diseases. In this study, its two enantiomers were prepared from chiral amino acids and their absolute configurations were investigated by electronic circular dichroism (ECD) combined with modern quantum-chemical calculations using time-dependent density functional theory. It was found that solvation changed both the conformational distribution and the ECD spectrum of each conformer. The theoretical ECD spectra of the two enantiomers were in good agreement with the experimentally determined spectra of the corresponding isomers in dimethyl sulfoxide. The ECD behavior of the bis(thiosemicarbazone) chromophore in a chiral environment is also discussed. Our results indicate that ECD spectroscopy may be a useful tool for the stereochemical evaluation of chiral drugs.

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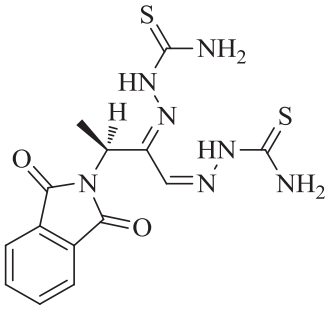
1. Introduction

Despite the development of versatile technologies to discover novel drugs and despite increasing expenditure, the number of first-in-class drug approvals continues to disappoint[1]. Perhaps because of this, old drugs continue to attract attention as potential sources of new drugs if only because their metabolism and clinical side-effects are generally well-understood[2]. Thus much effort continues to be devoted to the investigation of novel pharmacological effects of old drugs and their mechanisms of action. In particular, drugs with chiral centers have been examined either with a view to the potential benefits of switching from racemates to single enantiomers or for their use in new indications. An often-quoted example is that of thalidomide, the (*S*)-isomer of which caused the enormous tragedy of congenital abnormalities. During the last three decades, thalidomide was subjected to a full re-evaluation by pharmacologists and eventually approved by the Food and Drug Administration of the USA for the treatment of leprosy and multiple myeloma[3].

Phthiobuzone (**1**, Fig. 1) has been used for more than 30 years to treat herpes and trachoma diseases in China. It has a unique antiviral mechanism against herpes simplex virus 1 and 2 (HSV-1 and HSV-2), which is different from nucleotide derivatives[4]. As shown in Fig. 1, the chemical structure of **1** consists of a core phthalimide and a side chain bis(thiosemicarbazone). The latter is present in many biologically active compounds and continues to attract the attention of medicinal chemists[5,6]. In fact, some chiral analogs of Phthiobuzone have been synthesized in the search for novel antiviral compounds[7,8].

Electronic circular dichroism (ECD) is a powerful spectroscopic method for solving stereochemical problems of chiral molecules including natural products and synthetic compounds[9,10]. Together with quantum chemical calculations using time-dependent density functional theory (TDDFT), ECD has become a rapid and reliable way to establish the absolute configuration of chiral compounds[11,12]. Phthalimide is an inherently symmetric chromophore with a strong charge-transfer $\pi \rightarrow \pi^*$ transition at 220 nm which has often been used in stereochemical studies of chiral amino groups using ECD[13,14]. However, to date ECD studies of derivatives containing the bis(thiosemicarbazone) group have not been reported. Thus, prompted by our continuous efforts to apply chiroptical methods to the study of chiral compounds, we employed ECD and TDDFT to assign the absolute configuration of the enantiomers of **1**. The present study also provides preliminary information regarding the ECD behavior of the bis (thiosemicarbazone) group.



(*S*)-**1**

Figure 1 Chemical structure of (*S*)-**1**.

2. Experimental and computational methods

2.1. Material and methods

Enantiomers of **1** were synthesized from (*R*)- and (*S*)-alanine according to the literature method[15]. The ECD spectra of (*R*)-**1** and (*S*)-**1** in DMSO at a concentration of 0.1–0.3 mg/mL were recorded in a 1 mm path length quartz cuvette using a Jasco J-815 CD spectrometer (Jasco Inc., Japan).

2.2. Quantum chemical calculations

All quantum chemical calculations were carried out on the (*S*) enantiomer of **1**. Primary conformers were identified by a standard conformational search using the MMFF94 molecular mechanics force field in the MOE software package[16]. These conformers were further optimized and verified as true minima of the potential energy surface using Gaussian 09 software in the framework of TDDFT at the B3LYP/6–31+G(d,p) level[17]. The polarizable continuum model (PCM) was used to take into account solvent effects using a value of 46.8 for the dielectric constant of DMSO. Oscillator strengths and rotational strengths in both dipole length and dipole velocity representations of the 45 lowest electronic transitions were calculated for each conformer. Because rotatory strengths in length and velocity representations showed only small differences, only velocity representations were used to simulate the ECD spectra with a Gaussian function. The overall ECD spectra were generated by Boltzmann statistics.

3. Results and discussion

3.1. Conformational analysis

Since different conformers of a specific stereochemical configuration can give different or even opposite ECD spectra, it is crucial to identify all stable conformers in order to predict the ECD spectrum. Compound **1** has several freely rotatable single bonds giving rise to multiple possible conformers. Therefore, a standard conformational analysis was performed in the MMFF94 force field and 11 conformations were identified within an energy window of 6 kcal/mol. These conformers were then re-optimized and verified as true minima of the potential energy surface using the B3LYP hybrid functional. This has been frequently used in TDDFT calculations and can provide acceptable results for many molecular properties[9]. In addition, the polarizable continuum model (PCM) was utilized to mimic environmental effects.

Relative free energies, equilibrium populations and key dihedral angles of all stable conformations of (*S*)-**1** in dimethyl sulfoxide (DMSO) and in the gas phase are listed in Table 1. It was found that solvation markedly affected the number and relative amount of each conformer. Thus, in DMSO, the number of stable conformers was eight both at the B3LYP/6-31+G(d,p) level and the lower B3LYP/6-31G(d) level (data not shown). Whereas, in the gas phase, the number reduced to seven because conformer **1c** was able to readily transform into **1a**. Even when conformer **1c** in DMSO was used as input geometry, the transformation still took place. For conformers **1a**, **1b** and **1c** in DMSO, two C=N bonds adopted the s-*cis* configuration and formed six-membered rings through intramolecular hydrogen bonds which greatly decreased the free energies (Fig. 2). In the gas phase, the lowest-energy conformer was **1b** which has similar structural characteristics with

Table 1 Equilibrium distribution and key dihedral angles of conformers of (*S*)-**1** at the B3LYP/6–31+G(d,p) level.

Conformer	In DMSO		In the gas phase	
	P (%)	D(N8-C12-C13-N14)	*P* (%)	D(N8-C12-C13-N14)
1a	63.00	−127.5	29.73	−114.0
1b	15.60	113.3	68.35	109.2
1c	10.60	−15.6	–	
1d	5.53	−118.1	0.75	−112.9
1e	4.95	−91.7	1.10	−95.5
1f	0.22	103.1	0.07	106.2
1g	0.09	87.0	0.00	80.6
1h	0.02	−90.0	0.00	−95.1

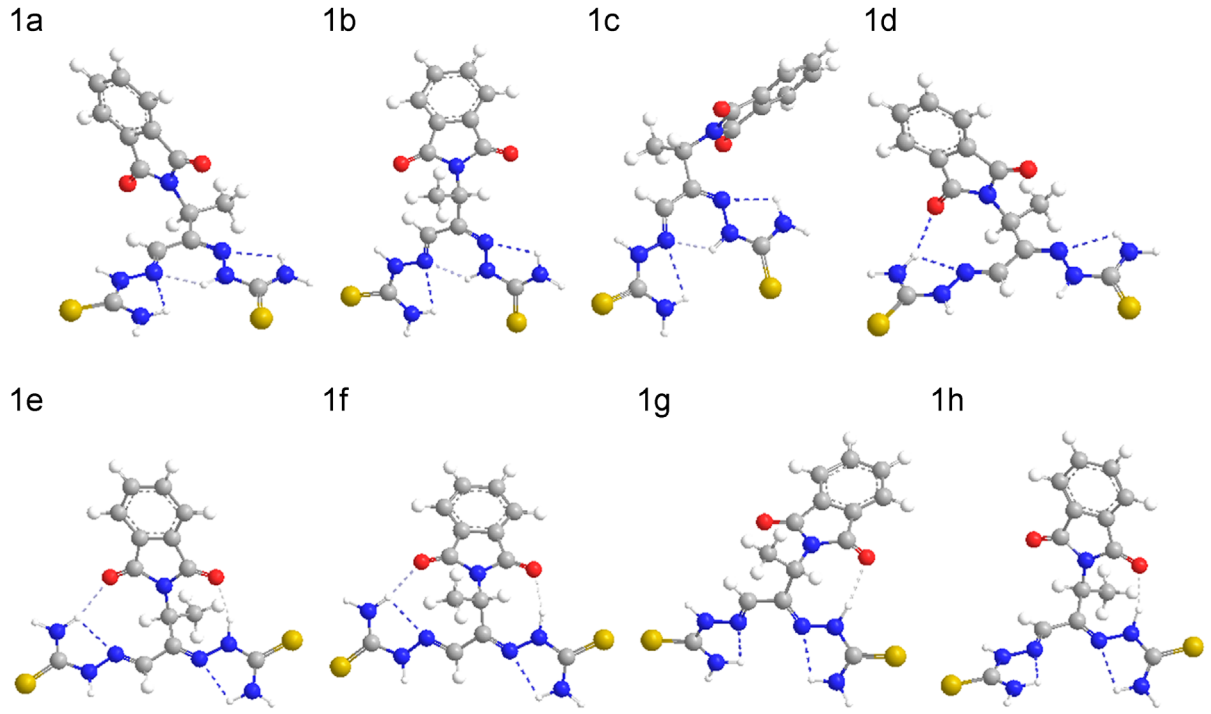


Figure 2 Main conformers of (*S*)-**1** in DMSO at the B3LYP/6–31+G(d,p) level.

its single crystal[18]. The dihedral angles D(N8-C12-C13-N14) of conformers in DMSO were slightly different from the values of the corresponding conformers in the gas phase.

3.2. *ECD spectra*

Synthesis of the enantiomers of **1** began with the condensation of chiral amino acids and phthalic anhydride. The resulting acids were transformed to diazoketones and then reacted with hydrogen bromide. The bromoketone products were oxidized to ketoaldehydes and subsequently condensed with thiosemicarbazone, at which step partial racemization took place because of the release of hydrogen bromide. The specific optical rotations of (*S*)- and (*R*)-**1** were −221° (*c* 0.11, acetone) and +289° (*c* 0.11, acetone) respectively indicating that the optical purity of (*S*)-**1** was lower than that of (*R*)-**1**. Because compound **1** is almost insoluble in the solvents normally used for ECD determination (methanol, acetonitrile), DMSO was used with a terminal wavelength of 250 nm because of its intrinsic absorption. In the experimental ECD curve of (*R*)-**1**, the diagnostic Cotton effects (CEs) were two strong positive signals near 290 nm and 355 nm; for (*S*)-**1**, the ECD spectrum was opposite to that of (*R*)-**1** with slightly weaker intensities consistent with the optical rotation results.

CEs in the ECD spectrum arise from chiral perturbations of the UV absorption chromophore during excitation. The chromophores in compound **1** are the phthalimide and bis(thiosemicarbazone) groups. The phthalimide chromophore has been used as a chromophoric tag in determining the absolute configuration of chiral amines or amino acids using the ECD method [13]. Its intramolecular charge-transfer $\pi \rightarrow \pi^*$ band at 220 nm is suitable for exciton coupling with $\pi \rightarrow \pi^*$ transitions of other chromophores. However, the ECD behavior of the bis(thiosemicarbazone) group remains unknown. Thus, the ECD and UV spectra of **1** were predicted by quantum-chemical calculation using TDDFT not only to verify the assignment of absolute configuration but also to investigate the ECD behavior of the bis(thiosemicarbazone) group.

Calculation of the ECD spectra of the principal conformers of **1** at the B3LYP/6–31+G(d,p) level in both DMSO and the gas phase revealed that different conformers show different ECD

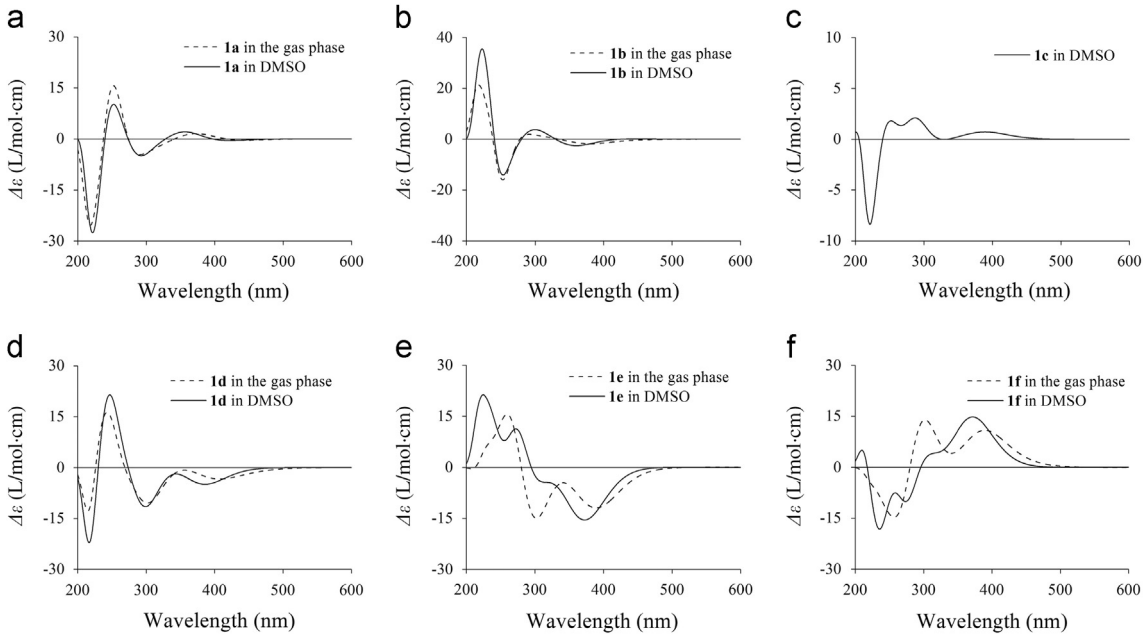


Figure 3 Calculated ECD spectra of conformers **1a**–**1f** at the B3LYP/6–31+G(d,p) level. $\sigma = 0.35$ eV.

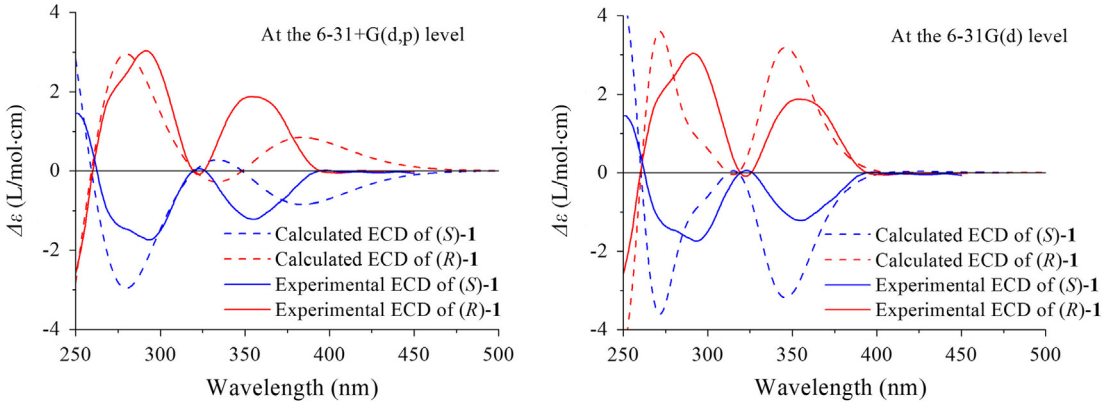


Figure 4 Comparison of experimental ECD spectrum of chiral **1** in DMSO (After blue shift 20 nm).

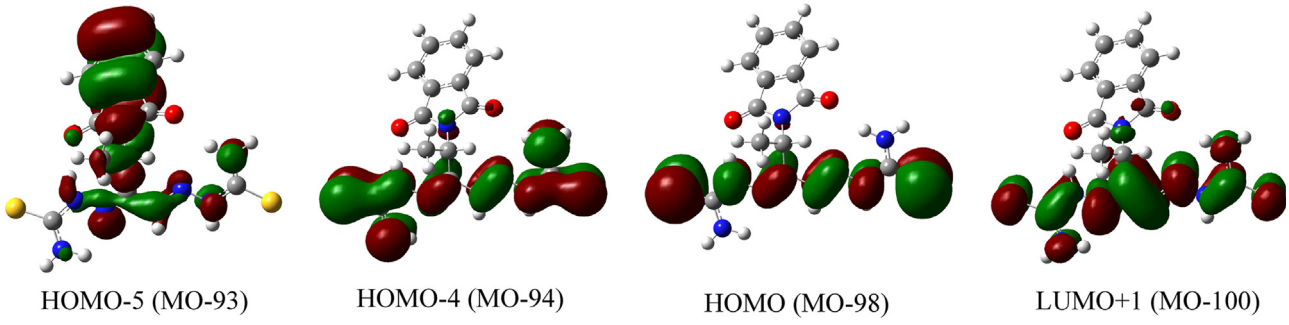


Figure 5 Molecular orbitals involved in the key electron transitions of (*S*)-**1** at the 6–31+G(d,p) level in DMSO.

spectra in the same medium with conformers **1a** and **1b** giving nearly opposite ECD spectra (Fig. 3). This may be ascribed to different or opposite exciton coupling between the phthalimide and bis(thiosemicarbazone) chromophores which is consistent with the D(N8-C12-C13-N14) dihedral angles. Conformers gave similar ECD curves in DMSO and the gas phase with the exception of **1e** and **1f** which showed greater differences due to subtle solvation effects.

The overall UV and ECD spectra of **1** shown in Fig. 4 were obtained by averaging the ECD spectra of each conformer using Boltzmann statistics. Based on our experience and on the literature, a lower level basis set can sometimes give better

concordance with the experimental data due to the error compensation in the calculation[19,20]. Thus, the ECD spectra of the enantiomers of **1** were also calculated at the B3LYP/6–31G(d) level using the optimized geometries at the B3LYP/6–31+G(d,p) level in DMSO. Since the TDDFT method has a tendency to underestimate the excitation energy, it is necessary to perform a correction to compensate for systematic computational errors. In this case, the long wavelength absorption band at 349 nm was used for wavelength correction. After a blue shift of 20 nm, the calculated ECD spectra of the enantiomers of **1** were in good agreement with their corresponding experimental ECD spectra. In the gas phase, the theoretical and experimental ECD spectra differed greatly from those in DMSO indicating that solvation has a major effect on both the conformational distribution and excitation properties.

For **1**, the lowest energy CE was observed at 355 nm corresponding to the absorption peak at 349 nm due to the HOMO (MO-98)→LUMO+1 (MO-100) transition (Fig. 5). Another diagnostic CE at 290 nm originated from the interaction between the phthalimide and bis(thiosemicarbazone) groups, the corresponding transitions being from HOMO-4 (MO-94)→ LUMO+1 (MO-100) and HOMO-5 (MO-93)→LUMO+1 (MO-100).

As recommended by the Chinese Pharmacopoeia, the content of Phthiobuzone in crude drug and pharmaceutical dosage forms can be determined using its absorption band at 349 nm[21]. Since **1** has only one chiral center, the CE at around 349 nm could then provide a convenient means to assign the absolute configuration of **1** and its chiral derivatives, the positive sign corresponding to the *R* configuration. It could also be useful to assign the absolute configuration of novel bis(thiosemicarbazone) derivatives.

4. Conclusions

The absolute configurations of the enantiomers of Phthiobuzone have been verified by a comparison of their experimental and theoretical ECD spectra using quantum chemical calculations. To assign the absolute configuration of Phthiobuzone and its chiral analogs, the Cotton effect at 349 nm can be used being negative for the *S* isomer and positive for the *R* isomer. Our results demonstrate that the ECD method is a powerful and reliable tool for the stereochemical evaluation of chiral drugs.

Acknowledgments

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