A new dehydroabietic acid-based arylamine fluorescent probe: Synthesis, structure analysis and in vitro biodiagnose function

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A B S T R A C T
A new fluorescent probe (methyl 13-(α-naphthalene)aminodeisopropyldehydroabietate) has been synthesized, and its structure was optimized by theoretical DFT calculation and determined by single-crystal X-ray diffraction analysis. The optimized data are in agreement with the experimental values. The fluorescence properties, photostability, cell toxicity and in vitro fluorescence imaging of the compound have been investigated. The results indicated that it can be effectively taken up by HeLa, 7721, 7901 and A549 cells and strong blue fluorescence signals were detected in these cells.

Development of useful dyes for testing cells is an important task. There are many dyes, fluorescent included, that have already been developed. The dyes serve specific purposes: ion- and pH-sensitive dyes to test ion metabolism in the cells,1–3 intracellular enzyme substrates with fluorogenic characteristics to test activity of intracellular enzymes, viability dyes etc.4,5 A variety of fluorescence probes have been developed for their biological and biomedical applications in vitro and in vivo imaging, which is a most popular modality due to its high sensitivity with a high temporal resolution.6–11

Dehydroabietic acid is one of important renewable resource, it is a readily available hydrophenanthrene derivative which offers promise of becoming a useful starting material for the synthesis of industrially or physiologically important products by the introduction of suitable substituents into the aromatic ring.12 In recent years, many fluorescence derivatizing reagents were synthesized based on the aromatic ring, such as dehydroabietic acid-based diarylamines, triarylamines quinoxaline, triarylamines styylene substituent, nitrogen-containing heterocycles, and C-12 substituted compounds, which are a family of excellent fluorescent emitters widely used as doped emitters in organic light-emitting diodes (OLEDs).13 However, the reports concerning their fluorescent probes and the bioimaging applications in living cells were still very scarce. In order to discover the novel optical materials with fluorescent probe function, we plan to synthesize title compound, in which naphthalene moiety with a big conjugated system was instituted in place of the N atom connecting to benzene ring of dehydroabietic acid. We expect that this structural modification may lead to the discovery of an improved fluorescence property compound.

The synthesis of title compound was accomplished by synthetic route shown in Scheme 1. The intermediate product (methyl 13-aminodeisopropyldehydroabietate) was prepared via esterification, bromation, nitration, and reduction with the starting material dehydroabietic acid according to literature procedure.14–17 Title compound (methyl 13-(α-naphthalene)aminodeisopropyldehydroabietate) was synthesized from methyl

Scheme 1. Synthetic route for title compound.
Figure 1. Molecular structure of title compound by single-crystal X-ray diffraction (a) and by DFT studies (b); molecular structures of Model 1, 2, 3 by DFT.
Title compound contains four crystallographically unique rings: two cyclohexane rings A and B, a aromatic ring C, and a naphthalene ring D (labeled in Scheme 1). Most of the optimized bond lengths are slightly longer than the experimental values, because the theoretical calculations are based on the isolated molecules in the gaseous phase, while the experimental results are based on the molecules in the solid state. In solid state, the existence of the crystal field along with the intermolecular interactions have connected the molecules together, which result in the differences in bond parameters between the calculated and experimental values. In order to investigate the spatial structure with the aim of forecasting fluorescent properties of title compound, we optimized its geometry with the DFT/B3LYP method at the 6-31G level of GAUSSIAN 03, and determined crystal structure by X-ray diffraction as verification (CCDC ID: 882322). The experimental methods and details of crystal data collection and structure refinement for compound can be seen in Supplementary data. The molecular structures are shown in Figure 1. Some selected geometric parameters are listed in Table 1.

Table 1

<table>
<thead>
<tr>
<th>Bond lengths</th>
<th>Experimental</th>
<th>Optimized</th>
<th>Model 1</th>
<th>Model 2</th>
<th>Model 3</th>
</tr>
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<tbody>
<tr>
<td>N–C13</td>
<td>1.398 (5)</td>
<td>1.401</td>
<td>1.373</td>
<td>1.457</td>
<td>1.399</td>
</tr>
<tr>
<td>N–C19</td>
<td>1.403 (5)</td>
<td>1.404</td>
<td></td>
<td></td>
<td>1.406</td>
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<tr>
<td>C13–C14</td>
<td>1.371 (5)</td>
<td>1.399</td>
<td></td>
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</tr>
<tr>
<td>C1–C2</td>
<td>1.524 (5)</td>
<td>1.535</td>
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</tbody>
</table>

<table>
<thead>
<tr>
<th>Bond angles</th>
<th>Experimental</th>
<th>Optimized</th>
<th>Model 1</th>
<th>Model 2</th>
<th>Model 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>C3–C2–C1</td>
<td>111.0 (3)</td>
<td>111.81</td>
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<tr>
<td>C26–C25–C24</td>
<td>129.3 (4)</td>
<td>121.2</td>
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<tr>
<td>C13–N–C19</td>
<td>129.3 (3)</td>
<td>127.8</td>
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<tr>
<td>C13–N–H0A</td>
<td>115.4</td>
<td>114.3</td>
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<tr>
<th>Torsion angles</th>
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<th>Model 1</th>
<th>Model 2</th>
<th>Model 3</th>
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<tbody>
<tr>
<td>C19–N–C13–C12</td>
<td>20.9 (6)</td>
<td>36.8</td>
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<tr>
<td>C2–C3–C4–C5</td>
<td>52.7 (4)</td>
<td>51.1</td>
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<tr>
<td>C6–C5–C10–C9</td>
<td>–55.8 (3)</td>
<td>–55.5</td>
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<tr>
<td>C8–C9–C11–C12</td>
<td>–6.7 (5)</td>
<td>–0.6</td>
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<tr>
<td>C19–C20–C21–C22</td>
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<td>–1.5</td>
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<tr>
<td>C24–C25–C26–C27</td>
<td>1.6 (6)</td>
<td>–0.3</td>
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Figure 2. Absorption (left, \( \lambda_{\text{max}} = 344 \text{ nm} \)) and fluorescence (right, \( \lambda_{\text{em, max}} = 442 \text{ nm} \)) spectra of compound in methanol, abs: absorption, em: emission, ex: excitation.
The high photostability is important property for each photo device such as fluorescent probe in regard with their practical usage. Materials exposed to light undergo degradation which shortens their service life, mainly by a sequence of photooxidation steps. Fluorescence spectra of sample solution were recorded before and after UV exposure to an ultraviolet lamp light (9 W) continuously for a span of time (3 min). Before measurement, title compound was dissolved in cosolvent DMSO at concentration 25 mM, and the resulting solution was diluted by deionized water to several concentrations: 1, 2, 5, 10, 12.5, 15, and 20 $\text{mM}$. It can be found the 15 $\text{mM}$ sample solution is still transparent. It is that, the solubility of title compound in water is above 15 $\text{mM}$. We carried out the photostability experiment using 10 $\text{mM}$ sample solution, the distance between the sample solution and the ultraviolet lamp was fixed at 20 cm. Subtraction of emission spectra after irradiation at different time points from the spectrum before irradiation at emission wavelength 391 and 420 nm are shown in Figure 4. The fluorescence intensity before irradiation (time 0 min) is 450 (a.u.) at 391 nm and 370 (a.u.) at 420 nm, and which after irradiation (time 30 min) is 400 (a.u.) and 284 (a.u.) at corresponding emission wavelength. It seems that the first shoulder (420 nm) is

Figure 3. Fluorescence decay lifetime of title compound.

Figure 5. Fluorescence emission spectra of title compound ($1 \times 10^{-6}$ M) in various solvents. (1) Water ($\lambda_{ex} = 280$ nm, $\lambda_{em} = 391/420$ nm), (2) DMSO ($\lambda_{ex} = 366$ nm, $\lambda_{em} = 454$ nm), (3) methanol ($\lambda_{ex} = 363$ nm, $\lambda_{em} = 442$ nm), (4) dichloromethane ($\lambda_{ex} = 348$ nm, $\lambda_{em} = 436$ nm), (5) cyclohexane ($\lambda_{ex} = 348$ nm, $\lambda_{em} = 427$ nm).

Figure 6. Survival percent of A549 with different drug concentration for 24 h.

Figure 7. Cytotoxicity of cells incubated with title compound for 24 h.
slightly more light sensitive than the main peak at 391 nm. However, in general, fluorescence intensity of two emission wavelength has not obviously decrease as increase in irradiation duration, showing its good photostability.

It can be seen from Figures 2 and 4, the shift of the emission maximum from 442 nm in a methanol solution (Fig. 2) to 391 nm in water solution (Fig. 4), this could be an indication of the sensitivity of the fluorescent parameters of title compound to polarity of its microenvironment. To further explore the phenomenon, the fluorescence spectra of title compound were studied in five solvents of different polarity: water, DMSO, methanol, dichloromethane, and cyclohexane. As shown in Figure 5, compared to nonpolar solvent cyclohexane, the emission spectra exhibits a blueshift accompanied by the appearance of vibrational fine structure (the typical two sub-peaks) in water but a redshift in DMSO, methanol and dichloromethane. The blueshift value is 36 nm in water and maximum redshift value is 27 nm in DMSO. Such sensitivity to microenvironment may be an advantage toward achieving a fluorescent sensor with good photophysical properties. Judging from the shift in fluorescence spectra, it may be concluded that solvent polarity is one of important influence factors, however, there may be other influence factors, such as hydrogen-bonding between...
solvents and the compound, etc. The actual measured shift of fluorescence spectral is the net result of the combination of solvent effect and hydrogen bonding interactions on solute molecules, and so on, which needs further investigations.

The cell toxicity is very important index for fluorescent probe. Figure 6 shows the percentage of cell survival after treatment of different concentration title compound to A549 cell, the cell survival percent was not markedly affected when the dosage increase from 0 to 25 μM. Figure 7 shows the cell survival percentage after treatment of title compound (1 μM) to different cells, including Hela, SMMC-7721, SGC-7901 and A549 cells, experimental procedures can be seen in the Supplementary data. The results indicate that cells are viable after treated with title compound for same amount of time; and their cell survival percentages were 97.35%, 96.05%, 94.49% and 93.07% using the untreated cells as control. Above results show title compound has very low cytotoxicity at this concentration, suggesting it can be used as an effective fluorescent probe in vitro.

For fluorescence imaging, after 4 h incubation in incubator, cells were washed with PBS (pH 7.4) three times and the cellular uptake of title compound was determined using fluorescence microscopy (Nikon Ti fluorescence microscope). As seen in Figure 8, title compound is all successfully taken up by all of the cells, including HeLa, SMMC-7721, SGC-7901 and A549 cells. So, the product could be applied as fluorescent probe in biological imaging and improved the detection sensitivity.

Besides, similar distribution patterns are obtained for title compound treated cells, including Hela, SMMC-7721, 7901, and A549 cells. After entrapping inside the cells, the fluorescence intensity of title compound are not uniform. As showed in Figure 9, the fluorescence intensity from cytoplasm is much stronger than that from nuclear, which indicates that title compound accumulated in cytoplasm.

In summary, a fluorescent probe was synthesized from dehydroabietic acid. The structure analysis results show the data obtained from DFT calculation are in good agreement with those from X-ray crystallography analysis. The compound owns high photostability and low cytotoxicity. And in vitro experiments indicate that can be effectively taken up by Hela, SMMC-7721, 7901, and A549 cells, and strong blue fluorescence signals are detected in these cells. Above properties indicate that such compound has potential to be used as fluorescent probes in biological diagnostic.

Acknowledgment

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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.2013.01.035.

References and notes