Synthesis, Spectroscopic Characterization, X-Ray Structure, and DFT Calculations of Some New 1,4-Dihydro-2,6-Dimethyl-3,5-Pyridinedicarboxamides

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Abstract
A series of novel 1,4-dihydro-2,6-dimethyl-3,5-pyridinedicarboxamides were synthesized and characterized by infrared absorption spectrum (IR), proton nuclear magnetic resonance (1H NMR), elemental analysis, ultraviolet spectrum (UV), and fluorescence techniques, together with X-ray single crystal diffraction. The results of density functional theory (DFT) and time-dependent density functional theory (TDDFT) calculations provided a reasonable explanation on the molecular structures, the molecular frontier orbital, and the spectra of electronic absorption and emission. The present work will be helpful to systematically understanding of the structures and the optical properties of 1,4-dihydropyridines for studying the structure-activity relationship and to develop new drugs and their analytical methods.

Introduction
1,4-Dihydropyridines (1,4-DHPs) are very important bioactive molecules in the field of drug and pharmaceuticals. These compounds are well known as calcium channel modulators and have emerged as one of the most important classes of drugs for the treatment of hypertension and so on [1–3]. Owing to the potential importance of 1,4-DHPs from pharmaceutical, industrial and synthetic points of view, the synthesis of 1,4-DHPs has attracted much attention and various methods have been developed [4]. Hantzsch reaction is the most classical method for the synthesis of 1,4-DHPs, by which a mixture of β-keto ester, an ammonium salt and an aldehyde in organic solvents is heated together [5–7]. It has been demonstrated that substitution of aryl-amide group for rings is the axis of symmetry of molecules. The amide bond lengths through the N atom and the C atom at the 4-position of pyridine through the N atom and the C atom at the 4-position of pyridine are within normal ranges (1.325–1.352 Å) [11]. The dihedral angle between phenyl ring and pyridine ring is 77.88° in α and 6.04° in β. It indicates that the phenyl rings are orthogonal to the pyridine ring in α, and nearly in co-plane to the pyridine ring in β. The dihedral angle of two phenyl rings is 85.19° in α and 122° in β. The intramolecular C-H···O H-bonds in the two molecules result in the formation of four non-planar pseudo six-member rings with envelope conformations. The dihedral angle of the carbonyl and the pyridine ring is 45.02° in α and 32.37° in β.

Results and Discussion
Description of the crystal structures
The crystals of α and β were prepared and determined by single crystal X-ray diffraction. Their crystal data and structure refinement are shown in Table 1. The selected bond lengths and angles are tabulated in Table 2. The observed hydrogen bonds are listed in Table 3. The molecular ellipsoid and the unit cell accumulation are shown in Figs 2 and 3, respectively.

The molecules of α and β are axial symmetry. The line passing through the N atom and the C atom at the 4-position of pyridine ring is the axis of symmetry of molecules. The amide bond lengths of N1-C7 (1.348(3) Å) in α and N2-C8 (1.347(4) Å) in β are within normal ranges (1.325–1.352 Å) [11]. The dihedral angle between phenyl ring and pyridine ring is 77.88° in α and 6.04° in β. It indicates that the phenyl rings are orthogonal to the pyridine ring in α, and nearly in co-plane to the pyridine ring in β. The dihedral angle of two phenyl rings is 85.19° in α and 122° in β. The 

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The molecules of \textit{a} are stabilized by intermolecular N-H\cdots O H-bonds and C-H\cdots \pi stacking interactions, while \textit{b} is organized only by C-H\cdots \pi stacking interactions. The molecules are interlinked by the intermolecular hydrogen bonds to form an infinite chain.

Geometric optimization and conformational study

The structures of \textit{a-e} have been optimized with DFT/B3LYP/6-31G (d). The five molecular conformations look like five different butterflies with beautiful symmetry (Fig. 4).

In order to confirm the molecular structures of the compounds, the calculated results of \textit{a} and \textit{b} are presented in Table 2, together with the X-ray diffraction data. Because the results of the two calculation methods (6-31G (d) and 6-31G (d, p)) were similar, we used the results calculated by 6-31G (d) here. The biggest difference between the calculated and the X-ray values of the bond length and bond angle of both \textit{a} and \textit{b} are at the 4-position of the pyridine ring. The calculated bond lengths are longer (0.1331 Å in \textit{a}, C8-C9, and 0.127 Å in \textit{b}, C1-C5) than the experimental values. The calculated bond angles are smaller (6.0508 \degree in \textit{a}, C8-C9-C8A, and 6.6361 \degree in \textit{b}, C5–C1–C2) than the X-ray values. The reason may be the inference by the intramolecular C-H\cdots OH - bonding in both molecules.

Vibration assignments

The FT-IR spectrum of the five compounds were recorded in the frequency region of 4000–400 cm\textsuperscript{-1}, and the harmonic vibrational frequencies calculated by using B3LYP with 6-31G (d) basis set are given along with the experimental ones in Table 4. The FT-IR and predicted spectra for the compounds are given in Fig. 5. None of the predicted vibrational spectra have any imaginary frequency prove that optimized geometry is located at the lowest point on the potential energy surface. It is well known that DFT levels systematically overestimate the vibrational wave-numbers. So, the scaling factor values of 0.96 were used in order to correct anharmonicity and neglected part of electron correlation [12,13]. The assignments of various bands in different compounds, in general, have been reported in detail [14,15]. The B3LYP method with 6-31G (d) basis set has good ability to predict the IR spectra of the five compounds.

| Table 1. Crystal data and structure refinement for \textit{a} and \textit{b}. |
|-----------------|-----------------|
| \textbf{a}     | \textbf{b}     |
| CCDC No.       | 851885          | 851886          |
| empirical formula | C\textsubscript{21}H\textsubscript{19}N\textsubscript{3}O | C\textsubscript{23}H\textsubscript{25}N\textsubscript{3}O\textsubscript{2} |
| formula weight | 345.40          | 375.46          |
| temperature [K] | 293(2)          | 293(2)          |
| wavelength [Å] | 0.71073         | 0.71073         |
| crystal system  | Orthorhombic     | Orthorhombic     |
| space group     | Pbcn            | Pbca            |
| unit cell dimensions |
| \textit{a} [Å]  | 23.278(5)       | 23.278(5)       |
| \textit{b} [Å]  | 8.3400(17)      | 8.3400(17)      |
| \textit{c} [Å]  | 9.4260(19)      | 9.4260(19)      |
| \textit{a} [\degree] | 90.00         | 90.00           |
| \textit{b} [\degree] | 90.00          | 90.00           |
| \textit{c} [\degree] | 90.00          | 90.00           |
| volume [Å\textsuperscript{3}] | 1829.9(6)     | 4230.7(15)      |
| \textbf{Z}     | 4               | 8               |
| \textit{μ} [g cm\textsuperscript{-1}] | 1.254          | 1.179           |
| \textit{μ} [mm\textsuperscript{-1}] | 0.075          | 0.076           |
| \textit{f}(000) | 728             | 1600            |
| crystal size [mm\textsuperscript{3}] | 0.10 \times 0.20 \times 0.30 | 0.10 \times 0.10 \times 0.20 |
| \beta range [\degree] for data collection | 1.75 to 25.37 | 1.80 to 25.37 |
| index ranges   |
| \textit{h} = 0 to \textit{k} = 28 | 0 to \textit{h} = 10 |
| \textit{i} = 0 to \textit{k} = 26 |
| \textit{i} = 0 to \textit{h} = 11 |
| \textit{h} = 0 to \textit{k} = 27 |
| reflections collected | 3304          | 4308           |
| independent reflections | 1676 [\textit{R} = 0.040] | 3879 [\textit{R} = 0.075] |
| max. and min. transmission | 0.9918/0.9757 | 0.9924/0.9849 |
| data/restraints/parameters | 1676/0/120 | 3879/8/253 |
| goodness-of-fit on \textit{R}² | 1.000 | 1.000 |
| final \textit{R} indices [\textit{I} > 2\textit{σ(\textit{I})}]: \textit{R}₁, \textit{wR}₂ |
| \textit{R}₁, \textit{wR}₂ (all data) | 0.0506, 0.1382 | 0.0772, 0.1140 |
| largest diff. peak and hole [eÅ\textsuperscript{-1}] | 0.241 and -0.150 | 0.149 and -0.180 |

The molecules of \textit{a} are stabilized by intermolecular N-H\cdots O H-bonds and C-H\cdots \pi stacking interactions, while \textit{b} is organized only by C-H\cdots \pi stacking interactions. The molecules are interlinked by the intermolecular hydrogen bonds to form an infinite chain.
Frontier molecular orbital and energy

HOMO and LUMO energies are very important parameters for quantum chemistry. LUMO as an electron acceptor represents the ability to obtain an electron, whereas HOMO represents the ability to donate an electron [16]. Energy gap (Eg) between HOMO and LUMO characterizes the molecular chemical stability and it is a critical parameter in determining molecular electrical transport properties because it is a measure of electron conductivity [17].

Fig. 4 shows the patterns of the HOMO and LUMO of the five compounds calculated with the B3LYP level. The positive phase is symbolized with blue and the negative phase green. HOMO and LUMO energies of the compounds are listed in Table 5.

It can be seen that the EgS of all the five compounds are small (about 0.15 eV). They have delocalized π systems. It is easier for the vertical transitions of the delocalized π electrons from HOMO to LUMO.

Molecular electrostatic potential map

The molecular electrostatic potential (MEP) map is useful to study the electrophile attracted negative regions (where the electron distribution effect is dominant) [18]. The importance of MEP lies in the fact that it simultaneously displays molecular size, shape as well as positive, negative and neutral electrostatic potential regions in terms of color grading. Regions of negative are usually associated with the lone pair of electronegative atoms. The regions having the negative potential are electron deficiency with hydrogen atoms.

The regions having the positive potential are electron excess with the electronegative atoms (C = C group, oxygen and nitrogen atoms), while the regions having the positive potential are electron deficiency with hydrogen atoms.

Electronic spectra

The electronic absorption spectra of a–e determined in ethanol are shown in Fig. 7 and listed in Table 6. Since the presence of an aromatic ring and a heterocyclic, compounds of a–e have 2 to 5 electron donors.

Table 2. Selected crystal structure parameters of a and b.

| Parameters | a | B3LYP/6-31G(d) | B3LYP/6-31G(d, p) |
|------------|---|----------------|-------------------|
| Bond lengths (Å) | Experimental | | |
| O-C7 | 1.227(2) | 1.2335 | 1.2337 |
| N1-C7 | 1.348(3) | 1.387 | 1.3866 |
| N1-C6 | 1.405(3) | 1.4098 | 1.4093 |
| N2-C10 | 1.343(3) | 1.384 | 1.3837 |
| Bond angles (°) | | | |
| C7-N1-C6 | 127.10(18) | 129.1118 | 129.0683 |
| C10-N2-C10A | 120.9(3) | 125.4339 | 125.3796 |
| N1-C7-C8 | 114.13(17) | 114.7855 | 114.7569 |
| C1-C6-N1 | 122.7(2) | 123.9904 | 123.9536 |
| C5-C6-N1 | 118.3(2) | 116.8468 | 116.8731 |
| | | | |
| Bond lengths (Å) | Experimental | | |
| N2-C8-C5 | 123.4(2) | 125.4196 | 125.3645 |
| N1-C4-C7 | 116.7(3) | 114.7855 | 114.7569 |
| N1-C4 | 1.331(4) | 1.3841 | 1.3838 |
| Bond angles (°) | | | |
| C8-N2-C9 | 122.5(2) | 123.5064 | 123.481 |
| C1-C6 | 118.3(2) | 116.8468 | 116.8731 |

Table 3. Parameters (Å, °) for the intra- and intermolecular interactions in a and b.

| Comp. | D-H...A | D-H | H...A | D...A | D-H...A |
|-------|---------|-----|-------|-------|---------|
| (a) Intermolecular and intramolecular hydrogen bond | | | | |
| a | C1-H1B...O | 0.9300 | 2.4909 | 2.940(3) | 110.00 |
| b | C1-H1B...O | 0.8600 | 2.0700 | 2.907(2) | 166.00 |
| (b) C-H...π interactions | | | | |
| a | C1-H1B...Cg1b | 0.9300 | 3.1078 | 3.942(3) | 150.26 |
| b | C1-H1B...Cg1b | 0.9300 | 3.3216 | 4.212(4) | 108.05 |

Symmetry codes: (1) x, 1−y, 1/2+z; (2) −x,1−y,−z; (3) 1/2−x, 1/2+y, z; (4) x−1+y, z; (5) 1/2−x,−1/2+y, z.
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3 absorption bands with the $\lambda_{\text{max}}$ between 250 to 380 nm. Comparing to $a$, the absorption bands of $b$–$d$ with alkyl substituent on the benzene rings are blue-shift. The absorption band of $e$ with auxochrome groups of -OCH$_3$ and -Cl is a certain degree of red-shift and the $\lambda_{\text{max}}$ is also increased.

To further understand the electronic transitions of $a$–$e$, TDDFT/B3LYP/6-31G (d) was used to study the nature and the energy of absorption spectra on the basis of the optimized geometries. The electronic absorption spectra were simulated by the Gaussian functions based on the 20 lowest singlet energies from the calculations and illustrated in Fig. 7. Table 7 lists the main transition configurations and oscillator strengths for the most relevant singlet excited states of $a$–$e$ both in vacuum and in ethanol.

The data of calculation and experiment are basically identical. The relative errors calculated in ethanol and in gas phase are 0.3–2.9% and 0.8–5.1%, respectively. Compared with the experimental data, calculated values in solution are superior to that in gas phase. The model considering the effect of the solvent is closer to the actual situation than the model of gas phase.

**Fluorescence spectra**

The fluorescence mechanism can be simply expressed as: $S_1 \rightarrow S_0 + hv$. The electrons from the first excited state drop back to the ground state and emit the radiation of degradation. Strong fluorescent substances have such structure characteristics that the molecules have rigid plane, greater delocalization of $\pi$ bond, and lower singlet electronic excited states. Geometry optimization results show that the five compounds have the above characteristics of configuration.

The fluorescence spectra of $a$–$e$ determined in ethanol are shown in Fig. 8 and listed in Table 8. The excitation and emission spectra of $a$–$e$ are similar. The maximum excitation and emission wavelengths are near 375 nm and 450 nm, respectively. The Stokes shift is about 71–80 nm. The results indicate that the alkyl substitution has the effect of blue-shift on the fluorescence spectra of $b$–$d$. The maximum excitation and emission wavelengths of $e$ with auxochrome groups of -OCH$_3$ and -Cl are a certain degree of red-shift and the Stokes shift is bigger because the planarity of the molecule structure is increased by the lone pair electrons of -OCH$_3$ and -Cl conjugating with the benzene rings.
Figure 4. The optimized geometries and the surfaces of the frontier molecular orbital of a-e obtained at the B3LYP/6-31G (d) level.
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Figure 5. Experimental (Red) and simulated (Blue) Infrared spectra of a-e.
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Conclusions

Five new 1,4-dihydro-2,6-dimethyl-3,5-pyridinedicarboxamides a–e have been synthesized and characterized by spectrometry and X-ray diffraction.

The experimental electronic absorption spectra in ethanol solution show 2 to 3 absorption bands with the $\lambda_{\text{max}}$ between 250 to 380 nm. The predicted electronic absorption spectra were achieved by TDDFT in gas phase and in ethanol solution. The model considering the effect of the solvent is closer to the actual situation than the model of gas phase.

The results of the single crystal X-ray show that the compounds look like beautiful butterflies. The same intramolecular C-H??O H-bonds in the molecules result in the formation of four non-planar pseudo rings with envelope conformations. The geometric parameters calculated by DFT/B3LYP/6-31G (d) represent a good approximation to the experimental data.

Table 4. Experimental and calculated vibrational frequencies (cm$^{-1}$) with DFT method.

| Assignments | a | Exp. (IR) | B3LYP/6-31G (d) | b | Exp. (IR) | B3LYP/6-31G (d) | c | Exp. (IR) | B3LYP/6-31G (d) | d | Exp. (IR) | B3LYP/6-31G (d) | e | Exp. (IR) | B3LYP/6-31G (d) |
|-------------|---|-----------|-----------------|---|-----------|-----------------|---|-----------|-----------------|---|-----------|-----------------|---|-----------|-----------------|
| $\nu_{\text{N-H}}$ | 3405 | 3395 | 3407 | 3401 | 3373 | 3294 | 3171 | 3161 |
| $\nu_{\text{C-H}}$ | 3070 | 3056 | 3093 | 3050 | 3055 | 3012 | 3038 | 3017 | 3024 | 3037 | 3020 |
| $\nu_{\text{C=O}}$ | 2910 | 2921 | 2920 | 2970 | 2916 | 2928 | 2942 | 2931 |
| $\nu_{\text{C-C}}$ | 1678 | 1684 | 1673 | 1675 | 1681 | 1689 | 1675 | 1670 | 1675 | 1666 | 1651 | 1653 |
| $\nu_{\text{C-N}}$ | 1593 | 1587 | 1596 | 1579 | 1605 | 1598 | 1640 | 1601 | 1622 |
| $\nu_{\text{C-Cl}}$ | 1525 | 1536 | 1509 | 1513 | 1505 | 1502 | 1517 | 1517 | 1515 | 1517 |
| $\nu_{\text{C-H}}$ | 1498 | 1492 | 1450 | 1448 | 1482 | 1491 |
| $\nu_{\text{C-H}}$ | 1432 | 1446 |
| $\nu_{\text{C-O}}$ | 1369 | 1369 | 1400 | 1355 | 1352 | 1397 | 1408 |
| $\nu_{\text{C-C}}$ | 1351 | 1352 |
| $\nu_{\text{C-N}}$ | 1310 | 1325 | 1318 | 1325 | 1313 | 1312 | 1311 | 1320 | 1305 | 1288 |
| $\nu_{\text{C-Cl}}$ | 1290 | 1284 | 1285 | 1285 | 1283 | 1281 | 1268 | 1269 | 1214 | 1219 |
| $\nu_{\text{C-H}}$ | 1238 | 1231 | 1216 | 1217 | 1230 | 1267 | 1212 | 1236 | 1165 | 1183 |
| $\nu_{\text{C-H}}$ | 1210 | 1212 | 1121 | 1114 | 1205 | 1134 | 1142 | 1126 | 1139 |
| $\nu_{\text{C-H}}$ | 1118 | 1119 | 1014 | 1018 | 1132 | 1141 | 1012 | 1017 | 1036 | 1038 |
| $\nu_{\text{C-Cl}}$ | 1035 | 1030 | 1014 | 1021 |
| $\nu_{\text{C-S}}$ | 751 | 756 | 813 | 820 | 841 | 852 | 861 | 857 | 846 |
| $\nu_{\text{C-H}}$ | 692 | 694 | 751 | 755 | 751 | 753 | 808 | 807 | 822 | 830 |
| $\nu_{\text{C-Cl}}$ | 732 | 718 |

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Table 5. Frontier orbitals and energy gaps (Eg).

| Comp. | HOMO-3 | HOMO-2 | HOMO-1 | HOMO | LUMO | LUMO+1 | LUMO+2 | LUMO+3 | Eg (eV) |
|-------|--------|--------|--------|------|------|--------|--------|--------|--------|
| a     | -0.247 | -0.220 | -0.216 | -0.196 | -0.050 | -0.011 | 0.000  | 0.000  | 0.146  |
| b     | -0.246 | -0.215 | -0.211 | -0.195 | -0.049 | -0.010 | 0.000  | 0.000  | 0.146  |
| c     | -0.240 | -0.225 | -0.223 | -0.194 | -0.044 | -0.005 | 0.003  | 0.004  | 0.15   |
| d     | -0.239 | -0.220 | -0.218 | -0.193 | -0.043 | -0.003 | 0.003  | 0.004  | 0.15   |
| e     | -0.239 | -0.215 | -0.214 | -0.192 | -0.044 | -0.018 | -0.011 | 0.002  | 0.148  |

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The present work will be helpful to systematically understanding of the structures and the optical properties of 1,4-dihydropyridines for studying the structure-activity relationship and to develop new drugs and their analytical methods.

**Experimental**

*Materials and instruments*

Acetoacetanilides (Sinopharm Chemical Reagent Ltd.). Other chemicals and solvents were reagent grade and were used without further purification.

Melting points were measured on an X-4 microscope electrothermal apparatus (Taike, China) and were uncorrected. IR spectra were obtained on a Nicolet 380 FT-IR spectrophotometer (KBr Pellets). $^1$H NMR spectra were recorded on a Bruker AV-300 spectrometer at 300 MHz using CDCl$_3$ or DMSO-d$_6$ as the solvent, with tetramethylsilane (TMS) as internal standard. The chemical shifts were reported in $\delta$ ppm and the coupling constants in $\text{J}$ Hz. The elemental analyses were performed with a Flash EA 1112 elemental analyzer. Electronic absorption spectra were obtained using a Cary5000 UV/vis/near-IR Spectrophotometer (Varian). The X-ray crystallographic analysis was performed on a Nonius CAD4 single-crystal diffractometer using graphite-monochromated Mo K$_\alpha$ radiation ($\lambda=0.71073$ A). Purity of the compounds was checked on thin layer chromatography (TLC) plates (silica gel G), the spots were located under UV light. Fluorimetric measurements were carried out using a spectrofluorometer (FP-6200, Jasco) equipped with a xenon lamp, dual monochrometers, and a controlling computer with operating software (Microsoft Windows). The slit widths for both excitation and emission were set at 5 nm. The sample solution was transferred to a conventional 1×1-cm quartz cell and then mounted on a cell holder. Subsequently, fluorescence spectra and their associated intensities were observed using the standard method.

Yellow color crystals of a and b suitable for X-ray analysis were grown from ethanol. A crystal was put on a glass fiber. The diffraction data were collected by using a $\omega/2\theta$ scan mode at 293 K. The crystal structure was solved by the direct method and refined by the full-matrix least-squares procedure on $F^2$ using SHELXL-97 program [19]. Positions of hydrogen atoms were located by geometrical calculation ($x$, $y$, $z$ and $U_{\text{iso}}$ fixed to 1.2 times $U_{\text{iso}}$ of the atom they are bound to).

**Computation details**

DFT methods of hybrid B3LYP were used to optimize the molecular structures and to study the properties of a–e. The 6-31G (d) and 6-31G (d, p) basis sets were used. The electronic 300 spectrometer at 300 MHz using CDCl$_3$ or DMSO-d$_6$ as the solvent, with tetramethylsilane (TMS) as internal standard. The chemical shifts were reported in $\delta$ ppm and the coupling constants in $\text{J}$ Hz. The elemental analyses were performed with a Flash EA 1112 elemental analyzer. Electronic absorption spectra were obtained using a Cary5000 UV/vis/near-IR Spectrophotometer (Varian). The X-ray crystallographic analysis was performed on a Nonius CAD4 single-crystal diffractometer using graphite-monochromated Mo K$_\alpha$ radiation ($\lambda=0.71073$ A). Purity of the compounds was checked on thin layer chromatography (TLC) plates (silica gel G), the spots were located under UV light. Fluorimetric measurements were carried out using a spectrofluorometer (FP-6200, Jasco) equipped with a xenon lamp, dual monochrometers, and a controlling computer with operating software (Microsoft Windows). The slit widths for both excitation and emission were set at 5 nm. The sample solution was transferred to a conventional 1×1-cm quartz cell and then mounted on a cell holder. Subsequently, fluorescence spectra and their associated intensities were observed using the standard method.

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**Computational details**

DFT methods of hybrid B3LYP were used to optimize the molecular structures and to study the properties of a–e. The 6-31G (d) and 6-31G (d, p) basis sets were used. The electronic

| Comp. | $\lambda_{\text{max}}$/nm | $\varepsilon$ $\lambda_{\text{max}}$/(L/mol·cm) |
|-------|--------------------------|---------------------------------------------|
| a     | 266/368                  | $2.39\times10^4/1.01\times10^4$           |
| b     | 268/366                  | $2.66\times10^4/1.10\times10^4$           |
| c     | 256/364                  | $2.04\times10^4/1.06\times10^4$           |
| d     | 254/364                  | $1.81\times10^4/0.88\times10^4$           |
| e     | 272/310/373              | $2.61\times10^4/2.74\times10^4/1.72\times10^4$ |
absorption spectra were calculated and simulated with the time dependent density functional theory (TDDFT) method. All calculations were carried out using the Gaussian 09 program [20].

### General procedure for synthesis of a–e

A mixture of acetoacetanilide derivative (0.01 mol), hexamethylene tetramine (0.01 mol), ammonium acetate (0.005 mol), and water (5 mL) were transferred to a round bottom flask containing 15 mL of ethanol. The reaction mixture was refluxed for 10–16 h. The reaction was monitored by TLC using the solvent system (ethyl acetate: petroleum ether = 2:1). Soon after the reaction was completed, the reaction mixture was allowed to cool. The solid product formed was filtered and washed with cold ethanol to get the 1,4-DHPs. The physical properties and \(^1\)H NMR data of a–e are listed in Table 9 and Table 10, respectively.

#### Table 7. Calculated absorption spectra of a–e in vacuum and in ethanol.

| Molecular States | Transition | Coefficient | Strength / \(f\) (cal.) | \(\lambda_{(nm)}\) (cal.) | \(\lambda_{(nm)}\) (exp.) | Relative error (%) |
|------------------|-------------|-------------|--------------------------|--------------------------|--------------------------|-------------------|
| a                | gas-phase \(S_0\rightarrow S_1\) | 0.66371      | 0.3068                   | 361                      | 368                      | 1.9               |
|                  | ethanol     | 0.66600      | 0.3530                   | 376                      | 368                      | 2.1               |
| b                | gas-phase \(S_0\rightarrow S_1\) | 0.66471      | 0.3433                   | 363                      | 368                      | 0.8               |
|                  | ethanol     | 0.66743      | 0.3852                   | 377                      | 366                      | 2.9               |
| c                | gas-phase \(S_0\rightarrow S_1\) | 0.65822      | 0.2732                   | 352                      | 364                      | 3.4               |
|                  | ethanol     | 0.66113      | 0.3138                   | 365                      | 364                      | 0.3               |
| d                | gas-phase \(S_0\rightarrow S_1\) | 0.65954      | 0.2948                   | 353                      | 364                      | 3.1               |
|                  | ethanol     | 0.66192      | 0.3315                   | 365                      | 364                      | 0.3               |
| e                | gas-phase \(S_0\rightarrow S_1\) | 0.66073      | 0.3270                   | 355                      | 364                      | 5.1               |
|                  | ethanol     | 0.66218      | 0.3605                   | 368                      | 373                      | 1.4               |

#### Supplementary material

Crystallographic data for the structural analysis of the synthesized compounds have been deposited at the Cambridge Crystallographic Data Center, 12 Union Road, Cambridge, CB2 1EZ, UK, and are available free of charge from the Director.

#### Table 8. Florescence spectra of a–e in ethanol.

| Comp. | \(\lambda_{ex}\) (nm) | \(\lambda_{em}\) (nm) | Stokes shift (nm) |
|-------|------------------------|------------------------|-------------------|
| a     | 374                    | 452                    | 78                |
| b     | 375                    | 450                    | 75                |
| c     | 370                    | 444                    | 74                |
| d     | 370                    | 441                    | 71                |
| e     | 380                    | 460                    | 80                |

Figure 8. Experimental fluorescence spectra of a–e.

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Table 9. Yield, melt point, and EA data of compounds a–e.

| Compd. | Yield (%) | Physical state | m.p./°C | Elemental anal. (% Calcd.) |
|--------|-----------|----------------|---------|---------------------------|
| a      | 78.3      | Light yellow cryst. | 224–226 | C 72.36(72.60), H 6.11(6.09), N 12.15(12.10) |
| b      | 76.1      | Yellow cryst. | 232–234 | C 73.32(73.57), H 6.74(6.71), N 11.15(11.19) |
| c      | 70.3      | Light yellow cryst. | 244–247 | C 73.31(73.57), H 6.68(6.71), N 11.23(11.19) |
| d      | 73.8      | Light yellow cryst. | 307–309 | C 74.69(74.41), H 7.27(7.24), N 10.36(10.41) |
| e      | 62.6      | Yellow cryst. | 238–241 | C 56.23(55.98), H 5.10(5.07), N 7.79(7.83) |

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Table 10. 1H NMR data of compounds a–e.

| Compd. | Value (s) | Assign. | J (Hz) | Compd. | Value (s) | Assign. | J (Hz) |
|--------|-----------|---------|--------|--------|-----------|---------|--------|
| a      | 8.26      | s, 2 H, NH |        | b      | 8.28      | s, 2 H, NH |        |
| a      | 7.60      | s, 2 H, NH | 8.53   | c      | 8.29      | s, 2 H, NH | 8.52   |
| a      | 6.90      | s, 2 H, NH | 8.57   | d      | 8.29      | s, 2 H, NH | 8.56   |
| a      | 6.40      | s, 2 H, NH | 8.60   | e      | 8.29      | s, 2 H, NH | 8.62   |

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Author Contributions
Conceived and designed the experiments: FSI, YL, YYL. Performed the experiments: YL, YYL, XJC. Analyzed the data: YL, XHX. Contributed reagents/materials/analysis tools: YL, XHX. Wrote the paper: YL, YYL, FSI.

References
1. Janis RA, Triggle DJ (1983) New developments in Ca2+ channel antagonists. J Med Chem 26: 775–785.
2. Shah R, Velazquez C, Knaus EE (2004) Syntheses, Calcium Channel Agonist- Antagonist Modulation Activities, and Nitric Oxide Release Studies of Nitrooxyalkyl 1,4-Dihydropyridine-2,6-Dimethyl-3-nitro-1-(2,1,3-benzoxadiazol-4-yl)-pyridine-5-carboxylate Racemates, Enantiomers, and Diastereomers. J Med Chem 47: 254–261.
3. Mojarrad JS, Miria R, Knaus EE (2004) Design and synthesis of methyl 2-methyl-7,7-dihalo-5-phenyl-2-azabicyclo[4.1.0]hept-3-ene-4-carboxylates with calcium channel antagonist activity. Bioorg Med Chem 12: 3215–3220.
4. Stout DM, Meyers AI (1982) Recent advances in the chemistry of dihydropyridines. Chem Rev 82: 223–243.
5. Suarez M, Verdecia Y, Illescas B, Martinez-Alvarez R, Avarez A, et al. (2003) Synthesis and study of novel fulleropyrrolidines bearing biologically active 1,4-dihydropyridines. Tetrahedron 59: 9179–9186.
6. Fukui K (1982) Role of frontier orbitals in chemical reactions. Science 218: 747–748.
7. Goodman M, Ganis P, Avitabile G, Migdal S (1971) Crystal structures of N-ethyl-N-p-nitrophenylcarbamoyl chloride and of N-phenylurethane. J Am Chem Soc 93: 3320–3331.
8. Merrick JP, Moran D, Radom L (2007) An Evaluation of Harmonic Vibrational Frequency Scale Factors. J Phys Chem A111: 11683–11700.
9. Li Y, Liu YY, Wang HW, Xiong XH, Wei P, et al. (2013) Synthesis, crystal structure, vibration spectral and DFT studies of 4-aminoantipyrine and its derivatives. Molecules 18: 877–893.
10. Fassihi A, Azadpour Z, Delbari N (2009) Synthesis and antitubercular activity of novel 4-substituted imidazo[2,1-b]imidazol-2-yl-3,5-bis-N-(substituted phenyl)carbamoyl-1,4-dihydropyridine-3,5-dicarboxamides. Eur J Med Chem 44: 3255–3260.
11. Goodman M, Garin P, Avitabile G, Migdal S (1971) Crystal structures of N-ethyl-N-p-nitrophenylcarbamoyl chloride and of N-phenylurethane. J Am Chem Soc 93: 3320–3331.
12. Silverstein RM, Badu GC, Morrill TC (1991) Spectrometric Identification of Organic Compounds, John Wiley and Sons, New York.
13. Kemp W (1996) Organic Spectroscopy, MacMillan, London.
14. Fukui K (1982) Role of frontier orbitals in chemical reactions. Science 218: 747–748.
15. Li Y, Yang MM, Liu YY, Wei RQ, Liu XN, et al. (2011) Synthesis, characterization and structural aspects of new haptenes for Pahs. J Mol Struct 987: 206–213.
17. Li Y, Zhang H, Liu YY, Li FS, Liu XN (2011) Synthesis, characterization, and chemical quantum calculation studies on 5-(3-nitrophenylsulfonyl)aniline. J Mol Struct 997: 110–116.
18. Sheldrick GM (2008) A short history of SHELX. Acta Crystallogr A 64: 3215–3220.
19. Frisch MJ, Trucks GW, Schlegel HB (2009) Gaussian 09, Revision C.01, Wallingford CT, Gaussian, Inc.