Tetrahedron 67 (2011) 4869–4873

Contents lists available at ScienceDirect

Tetrahedron

journal homepage: www.elsevier.com/locate/tet

A ratiometric and exclusively selective Cu^{II} fluorescent probe based on internal charge transfer (ICT)

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ARTICLE INFO

Article history:
Received 11 February 2011
Received in revised form 21 April 2011
Accepted 3 May 2011
Available online 7 May 2011

Keywords:
Ratiometric fluorescent probe
Exclusively selective Cu^{II}
Imaging agent
Blue shift

ABSTRACT

A new ratiometric and exclusively selective fluorescent probe *N*-butyl-4,5-di[*N*-(phenyl)-2-(amino)-acetamino]-1,8-naphthalimide (**1**) was designed and synthesized on the basis of the mechanism of internal charge transfer (ICT). The probe **1** showed exclusively selectivity for Cu^{II} in the presence of a variety of other metal ions in aqueous ethanol solutions and the binding mode of probe **1** with Cu^{II} was 1:1 metal–ligand complex. Fluorescent emission spectra of probe **1** in the presence of Cu^{II} showed a 50 nm blue shift, which is from 521 nm to 471 nm. Furthermore, probe **1** shows the same fluorescent change with the Cu^{II} in living cells.

1. Introduction

The development of highly selective and ratiometric fluorescent probes capable of reporting transition–metal ions has attracted considerable attention.[1] In particular, the design of ratiometric fluorescent sensors for copper ions in the presence of a variety of other metal ions is actively investigated.[2–5] As is well-known, copper is a vital trace element, the third most abundant in humans, and is present at low levels in a variety of cells and tissues with the highest concentrations in the liver.[6] Cu^{II} plays an important role in living systems, such as those occurring in the human nervous system, gene expression, and the functional and structural enhancement of proteins.[7] Therefore fast detection of Cu^{II} in aqueous solution or biosystems is significant for life sciences and environmental sciences. However, design and synthesis of excellent fluorescent probes for selective detection of transition metal ions in biosystems remain a great challenge, because they often coexist and most of them have a similar reactivity with common fluorescent probes. To achieve Cu^{II}-only ratiometric sensing in aqueous solution or biosystems, fluorescent probes require deliberate design.

1,8-Naphthalimide with an electron donor and an acceptor (EDA) group is characteristic of an internal charge transfer (ICT) chromophore.[4] In our previous study, two 2-(aminomethyl)pyridine ligands had been introduced to 4 and 5 positions of 1,8-naphthalimide (Fig. 1, **A**),[4a] forming a tetradentate receptor, which displayed a special cavity and had a strong binding with Cu^{II}. As a result ratiometric fluorescence responses based on ICT mechanism were obtained. Soon afterward, it was observed that the amines conjugated to 1,8-naphthalimide can be deprotonated in the presence of Cu^{II}, which resulted in the color change from primrose yellow to pink (probe **B**).[4b] But some disadvantages exist in these two probes, probe **A** is not a highly selective fluorescent sensor, it is interfered by other metal ions, such as Co^{2+}, Fe^{2+}, Ni^{2+}, Ag^{+}; probe **B** has a lower sensitivity for Cu^{II} and poor fluorescent quantum yield because of the PET from aniline to fluorophore. Recently, Xu et al. have shown that the addition of a carbonyl group between 1,8-naphthalimide and di-2-picolylamine (probe **C**)[8] demonstrated the ability to not only block heavy and transition metal (HTM) ions from interacting with the naphthalimide but also increase the oxidation potential of naphthalimide and to act as a sacrificial donor in order to maintain fluorescence.[9] For probe **1** in this work, two 2-amino-*N*-phenyl-acetamide ligands were introduced to 4 and 5 positions of 1,8-naphthalimide bases on probe **B** for the high selectivity with the similar tetradentate receptor, the addition of a carbonyl group at the side of aniline not only provides more Lewis basic binding site but also reduces the steric hindrance by avoiding the interaction of two aniline groups. Besides, it repressed PET from aniline to fluorophore, thus increasing the fluorescent quantum yield.

2. Results and discussion

2.1. Synthesis

Probe **1** synthesized by conjugating compound **2** and **3** was shown in Scheme 1. The intermediate compound **2** was synthesized

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doi:10.1016/j.tet.2011.05.001

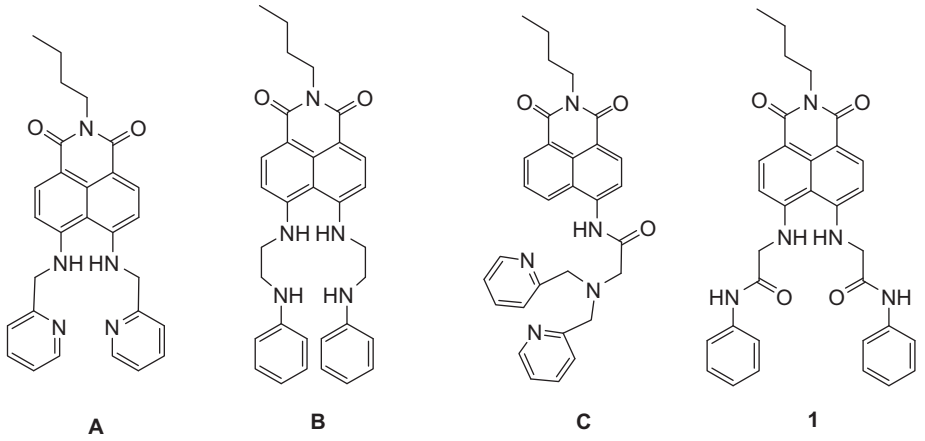
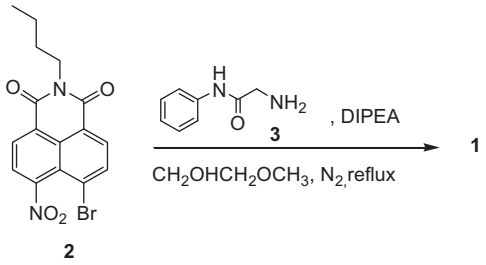


Fig. 1. Structures of probe **A**, **B**, **C**, and **1**.

from acenaphthene following a literature procedure.[10] 2-Azido-*N*-phenylacetamide was prepared in 90% yield by the condensation of 2-bromine-*N*-phenylacetamide with sodium azide and was subsequently reduced into compound **3** in 100% yield, probe **1** was easily synthesized by conjugating compound **2** and compound **3** in 83.5% yield.



Scheme 1. Synthesis of probe **1**.

2.2. The effect of pH

Fluoroionophores are usually disturbed by a proton in the detection of metal ions. Thus, the influence of pH on the fluorescence of **1** was first determined by fluorescence titration (Fig. 2). The fluorescence of **1** at 521 nm remains unaffected between pH 12.56–1.99 and rapidly decreases from pH 1.99 to 0.96 and pH 12.56 to 13.04, leading to a vaulted curve. The fluorescence quenching was most likely caused by the photoinduced electron transfer (PET) from the fluorophore to protonated aniline in strong acids condition,[11] which was similar to the findings of de Silva in the design of an 'off-on-off' fluorescent PET sensor.[12] And PET from deprotonated aniline to the fluorophore in strong alkali condition. Therefore, further fluorescence studies were carried out at pH 7.4 maintained with HEPES buffer (30 mM).

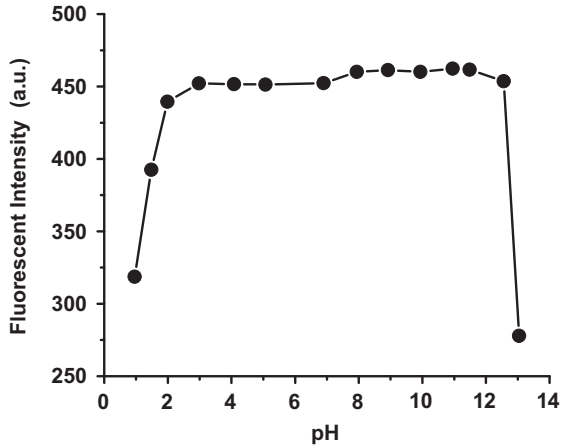


Fig. 2. Influence of pH on the fluorescence of **1** in the ethanol–water solution (90:10, v/v). Excitation wavelength is 447 nm, [**1**]=10 μM. The pH was modified by adding 75% $HClO_4$ or 25% $N^+(CH_3)_4OH^-$.

2.3. Optical behavior of 1 with Cu^{II}

The emission spectra of **1** and its fluorescence titration with Cu^{II} were recorded in an ethanol–water solution (90:10, v/v) (Fig. 3), and the emission spectrum of free **1** displays a broad band with a maximum at 521 nm. When Cu^{II} was added to the solution of **1**, the fluorescence emission intensity at 521 nm decreased significantly and a blue-shifted emission band centered at 471 nm showed up, which was attributed to the formation of a **1**/Cu^{II} complex. The inset in Fig. 3 exhibits the dependence of the intensity ratios of emission at 471 nm to that at 521 nm (I_{471}/I_{521}) on the concentrations of Cu^{II}, which indicates the formation of a **1**/Cu^{II} adduct of 1:1 stoichiometry. The Φ_F values of free **1** and **1**/Cu^{II} adduct (1:1) are 0.5378 and 0.1138, respectively.[13] In the UV–vis absorption spectra of **1**, no significant changes was found with the addition of Cu^{2+}. This would indicate that the blue shift of fluorescence spectra was caused by a change of the charge-transfer character of the emissive species.

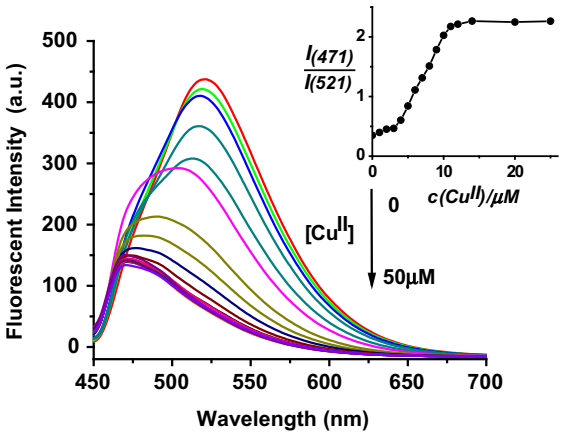


Fig. 3. Fluorescent emission spectra of **1** in the presence of different concentrations of Cu^{II}(0, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25, 30, 50 μM) in ethanol–water solution (90:10, v/v, 30 mM HEPES buffer, pH 7.4). Excitation wavelength was 447 nm, and emission was at 471 and 521 nm. The concentration of **1** was 10 μM. Inset: ratiometric calibration curve I_{471}/I_{521} as a function of Cu^{II} concentration.

The fluorescence titration of **1** with various metal ions in ethanol–water solution was conducted to examine the selectivity. As shown in Fig. 4b, the addition of Cu^{2+} induced a selective increase in emission band at 471 nm. The addition of other metal ions, such as Li^{+}, Na^{+}, K^{+}, Mg^{2+}, Ca^{2+}, Mn^{2+}, Al^{3+}, Co^{2+}, Ni^{2+}, Zn^{2+}, Cd^{2+}, Fe^{2+}, Fe^{3+}, Cr^{3+}, Ag^{+}, Hg^{2+}, Pb^{2+}, Ce^{2+}, and Ba^{2+}, produced a negligible change in the fluorescence spectra of **1**. Fig. 4a shows the dependence of the intensity ratios (I_{475}/I_{525}) on the metal ions. The competition experiments were conducted in the presence of Cu^{2+} at 10 μM mixed with other metal ions at 30 μM, as well as in a mixture of the metal ions, respectively, no significant variation in the intensity ratios (I_{471}/I_{521}) was found by comparison with that without the other metal ions besides Cu^{2+} (Fig. 4c). Therefore, in aqueous solutions, probe **1** is an outstandingly high selectivity fluorescent sensor for Cu^{II}.

Proposed mechanism of binding mode of probe **1** with Cu^{2+} is shown in Fig. 5, which is similar to probe **A**'s binding way.[4a] Probe **1** bases on probe **B** for the high selectivity with the similar tetradentate receptor, but the addition of a carbonyl group at the side of aniline lead to forming a tetradentate receptor site with the carbonyl oxygen donors, which provides the more Lewis basic binding site to bind Cu^{2+} more strongly within the cavity, making it unavailable for fluorescence quenching, and as a result fluorescence is maintained. In other words, the M (Cu^{II})–R (receptor) interaction has been increased to reduce indirectly the communication between M and F (fluorescence).[14] Also, the amide linkage can increase the inflexibility of the receptor to reduce the steric hindrance by avoiding the interaction of two aniline groups. The capture of Cu^{2+} by the tetradentate receptor resulted in the reduction of the electron-donating ability of the two amino groups conjugated to the naphthalene ring, thus, the receptor showed a 50 nm blue shift of fluorescence emission base on the internal charge transfer (ICT).

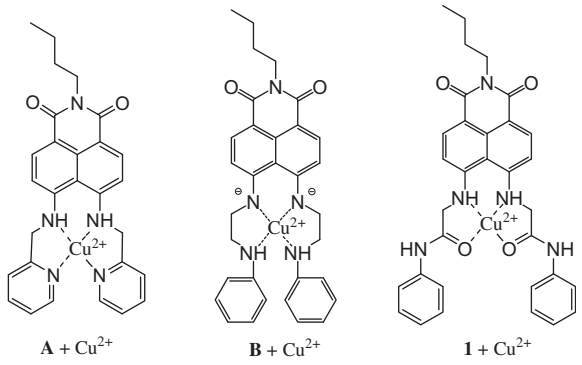


Fig. 5. Proposed mechanism of binding mode of **A**, **B**, and **1** with Cu^{2+}.

2.4. Cell imaging of 1 with Cu^{II}

The utility of probe **1** for fluorescence imaging of Cu^{2+} in living cells was investigated. To determine the cell permeability of **1**, MCF7 cells were incubated with 10 μM **1** for 30 min at 37 °C and washed with PBS to remove the remaining **1**. A clear yellow fluorescent image could be observed obviously from fluorescence microscopy as shown in Fig. 6c. In Fig. 6d, the cells were pretreated with Cu^{2+} in the growth medium for 30 min. The cells were then washed with PBS to remove the remaining Cu^{2+} and further incubated with probe **1** for 30 min. The resulting bright green fluorescence image demonstrates that probe **1** is cell membrane permeable and able to display a fluorescence blue-shift response to Cu^{2+} in the living cells. It should be potentially useful for the study of the toxicity or bioactivity of Cu^{2+} in living cells.

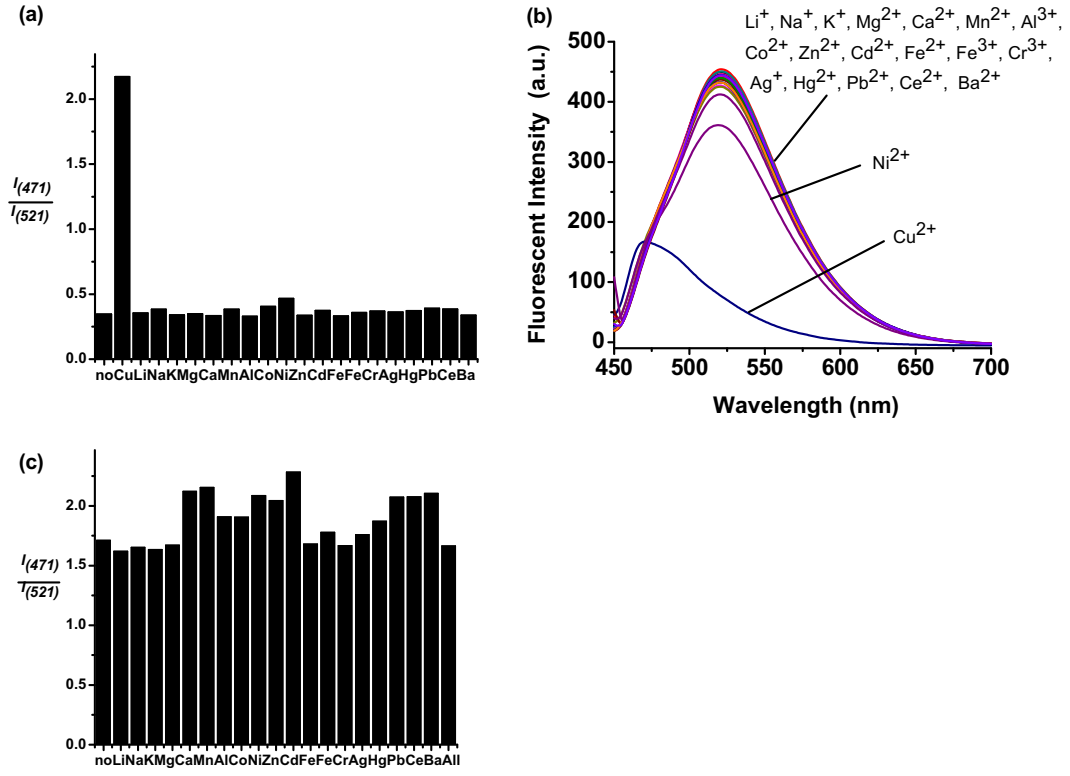


Fig. 4. (a) Fluorescence response of **1** to various metal ions (30 μM) in ethanol–water solution (90:10, v/v, 30 mM HEPES buffer, pH 7.4). (b) Fluorescence spectra of **1** in the presence of different HTM ions (30 μM). (c) The fluorescent response of **1** containing 10 μM Cu^{2+} to the selected metal ions (30 μM). Excitation was at 447 nm, and emission was at 471 and 521 nm. The concentration of **1** was 10 μM.

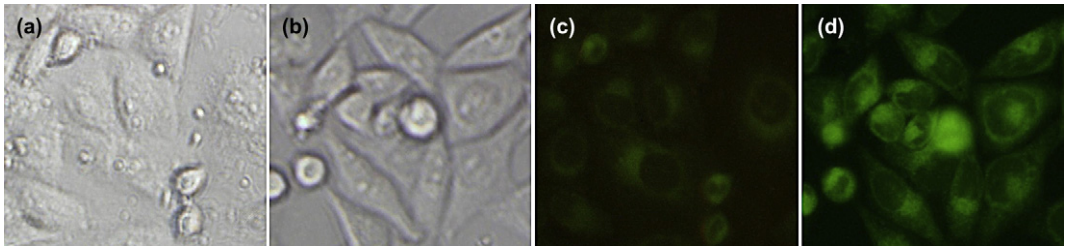


Fig. 6. Bright field and fluorescence images of MCF7 cells. (a) bright field images of MCF7 cells were incubated with probe **1** (10 μM) for 30 min at 37 °C; (b) bright field images of MCF7 cells pre-treated with Cu^{II} (4 equiv) for 30 min at 37 °C and then further incubated with probe **1** (10 μM) for 30 min at 37 °C; (c) fluorescence images of MCF7 cells shown in (a); (d) fluorescence images of MCF7 cells shown in (b).

3. Conclusions

Probe **1** was designed with the extra carbonyl group based on strategies that not only provides more Lewis basic binding site but also reduces the steric hindrance by avoiding the interaction of two aniline groups, and we have demonstrated that probe **1** displayed colorimetric response with fluorescence spectra from yellow to green, which was useful for easy detection of Cu^{II} with a ratiometrically and exclusively selectivity in the presence of a variety of other metal ions in aqueous solutions. And especially, probe **1** can be used to successfully detect Cu^{II} in cultured cells with the same fluorescent change. Blue shift emission (50 nm) was attributed to the reduction of the electron-donating ability of the two amino groups conjugated to the naphthalene ring. The design strategy of the sensor with the addition of a carbonyl group will help to improve the development of fluorescent sensors for detect other metal ions.

4. Experimental

4.1. Materials and methods

Unless otherwise noted, materials were obtained from commercial suppliers and were used without further purification. ^{1}H NMR were measured on a Bruker AV-400 spectrometer with chemical shifts reported as parts per million (in $CDCl_3$/DMSO-d_6, TMS as internal standard). Mass spectra were measured on an HP 1100 LC–MS spectrometer. Melting points were determined by an X-6 micro-melting point apparatus and are uncorrected. IR spectra were recorded on a Nicolet Nexus 770 spectrometer. All pH measurements were made with a Sartorius basic pH-Meter PB-20. Fluorescence spectra were determined on a Hitachi F-4500. Absorption spectra were determined on a PGENERAL TU-1901 UV–vis Spectrophotometer.

4.2. Preparation of fluorometric metal ion titration solutions

All the solvents were of analytic grade and used as received. The solutions of metal ions were prepared from $LiClO_4\cdot 3H_2O$, $NaClO_4$, $KClO_4$, $BaCl_2\cdot 2H_2O$, $CaCl_2$, $FeCl_2\cdot 4H_2O$, $MnCl_2\cdot 4H_2O$, $CoCl_2\cdot 6H_2O$, $NiCl_2\cdot 6H_2O$, $ZnCl_2$, $CdCl_2\cdot 1/2H_2O$, $HgCl_2$, $AlCl_3$, $FeCl_3\cdot 6H_2O$, $AgNO_3$, $Pb(NO_3)_2$, $Mg(NO_3)_2\cdot 2H_2O$, $Cu(NO_3)_2\cdot 3H_2O$, $Ce(NO_3)_3\cdot 6H_2O$, $Cr(NO_3)_3\cdot 9H_2O$, respectively, and were dissolved in distilled water. Stock solutions of host (0.01 mM) in DMSO were also prepared. Test solutions were prepared by placing 4–40 mL of the probe stock solution into a test tube, adding an appropriate aliquot of each metal stock, and diluting the solution to 4 mL with 0.3 M HEPES (pH 7.4). For all measurements, excitation was at 447 nm. Both excitation and emission slit widths were 3 nm or 5 nm.

4.3. Synthesis of 2-amino-*N*-phenylacetamide 3

2-Bromine-*N*-phenylacetamide (1.07 g, 1.0 equiv) was added to a reaction vessel containing a solution of NaN_3 in DMSO (1.1 equiv, 0.5 M). The reaction was monitored by NMR analysis. Upon completion of the reaction, water (50 mL) was added and the product was extracted with ether (3×50 mL). The combined organic layers were washed with water (2×50 mL) and brine (50 mL) and dried with natrium sulfate. The organic solvent was removed to provide 2-azido-*N*-phenylacetamide as a colorless liquid in 90% yield. ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.00 (bd s, 1H), 7.54 (d, J=8.6 Hz, 2H), 7.35 (dd, J_1=8.4 Hz, J_2=7.5 Hz, 2H), 7.16 (d, J=8.6 Hz, 1H), 4.15 (s, 2H).[15]

A solution of 2-azido-*N*-phenylacetamide (5 mmol) in 40 mL of methanol was hydrogenated over 3.5% Pd/C (300 mg) in autoclave (1 Mp) for overnight, and starting material disappeared, then catalyst was removed by filtration through Celite and washed with 5 mL of methanol. The filtered solution was concentrated by rotary evaporation to give 2-amino-*N*-phenylacetamide **3** as colorless liquid (100% yield). ^{1}H NMR ($CDCl_3$, 400 MHz) δ 1.69 (s, N–H), 3.32 (s, 2H), 7.03 (t, J=7.2 Hz, 1H), 7.27 (t, J=7.6 Hz, 2H), 7.55 (d, J=8.0 Hz, 2H), 9.45 (s, N–H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 45.12, 119.58, 124.05, 128.90, 137.80, 171.46; IR (KBr, cm^{-1}): 3320, 3275, 3058, 2915, 1655, 1600, 1498, 1447, 1313, 1076, 755, 692; HRMS (ES) calcd for $C_8H_{11}N_2O$ [MH^+] 151.0871, found 151.0873.

4.4. Synthesis of probe 1

2-Amino-*N*-phenylacetamide **3** (7.72 mmol) was added dropwise to a solution of 145 mg (0.38 mmol) *N*-butyl-4-bromo-5-nitro-1,8-naphthalimide **2** and 128 μl (2.0 equiv) *N*,*N*-diisopropylethylamine (DIPEA) in 3 mL 2-methoxyethanol, and then the mixture was heated to reflux for 3 h and monitored by TLC. After the reaction was completed, the solution was cooled at room temperature to give yellow needle crystals. The product was filtered off, washed with 2-methoxyethanol, and then dried in the air in 83.5% yield (177 mg). Mp: 276.2–278.4 °C; ^{1}H NMR (DMSO-d_6, 400 MHz) δ 0.90 (t, J=7.2 Hz, 3H), 1.28–1.34 (m, J=7.2 Hz, 2H), 1.54–1.57 (m, J=7.6 Hz, 2H), 3.98 (t, J=7.2 Hz, 2H), 4.21 (s, 4H), 6.75 (d, J=8.8 Hz, 2H), 7.07 (t, J=7.2 Hz, 2H), 7.32 (t, J=8.0 Hz, 4H), 7.59 (d, J=7.6 Hz, 4H), 7.71 (s, N–H), 8.25 (d, J=8.4 Hz 2H), 10.22 (s N–H); ^{13}C NMR (DMSO-d_6, 100 MHz) δ 14.23, 20.29, 30.35, 40.03, 48.20, 107.20, 110.73, 110.86, 119.99, 124.06, 129.26, 132.19, 133.66, 139.03, 152.67, 163.75, 168.15; IR (KBr, cm^{-1}): 3342, 2956, 2872, 1666, 1626, 1600, 1557, 1499, 1446, 1314, 1107, 748, 691; HRMS (EI) calcd for $C_{32}H_{31}N_5O_4$ [M^+] 549.2376, found 549.2368.

Acknowledgements

We are grateful to the National Natural Science Foundation of China (90713030; 20876025).

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