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Graphical Abstract

Synthesis and optical properties of novel Tröger's base derivatives

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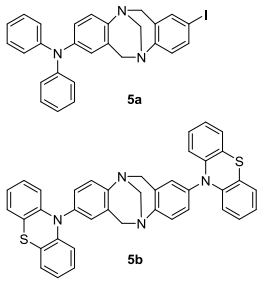
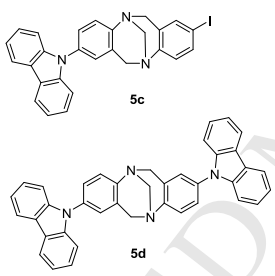
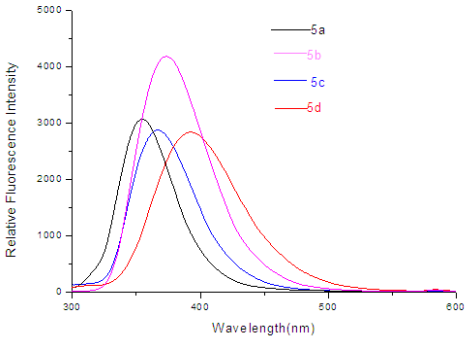
Diphenylamine, phenothiazine or carbazole were introduced into Tröger's base skeleton *via* Ullmann or Suzuki coupling to afford new TB derivatives. Their optical properties tests, density functional theory (B3LYP; 6-31G*) calculations were investigated. Incidentally anti-cancer activity against human hepatoma HepG2 cells in vitro (MTT assay) were investigated. All the results indicated that these novel compounds have the potential as fluorescence materials.







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ABSTRACT

Diphenylamine, phenothiazine or carbazole were introduced into Tröger's base skeleton *via* Ullmann or Suzuki coupling to afford new TB derivatives. Their optical properties tests, density functional theory (B3LYP; 6-31G*) calculations were investigated. Incidentally anti-cancer activity against human hepatoma HepG2 cells in vitro (MTT assay) were investigated. All the results indicated that these novel compounds have the potential as fluorescence materials.

1. Introduction

The unique structure of Tröger's base (TB, Figure **1**) and its derivatives has received more attention since found in 1887[1]. Structurally, TB is an excellent natural candidate for luminescence material with high controllability and obvious advantages: (1) because TB skeleton contains non-coplanar V-type twisted structure which cannot be rotated, so it can prevent the intermolecular π-π close stacking and dipole-dipole interaction. (2) its large dihedral angle (80-104°)[2] allows wider Stokes shift and less self-absorption. (3) the highly rigidity and steric hindrance can restrict the internal rotation (RIR) and reduce non-radioactive transition. (4) the two bridge nitrogen atoms on the skeleton can form N*---H-C bond which make TB acting as a hydrogen bond acceptor. Therefore, it is convenient to adjust the wavelength and intensity of luminescence *via* binding with large cavity and combining with various kinds and different numbers of objects. (5) the intermolecular distance between two terminals is approximate to 1 nm and the minimum distance between the methylene adjacent to bridge nitrogen atom and aromatic ring is 2.91Å.[2-3] (6) there are eleven sites in the framework that can be modified, without considering the side chain. So, the TB structure can be designed, assembled and tailored to meet the needs easily. All of these advantages can greatly reduce the fluorescence quench and ~~enhance t~~he fluorescence quantum efficiently.

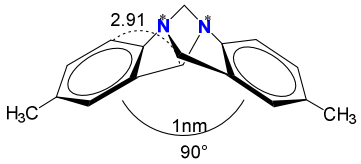

Figure1. Structure of Tröger's Base

Moreover, the unique V-type twisted structure of Tröger's base (can be classified as a family of molecular tweezers[4]) enable it selectively embed specific DNA double helix and cut them off. Recent studies[5-11] indicated that Tröger's base derivatives cut off the A-T-T base pairs on DNA selectively during the process of unwinding and rewinding of the DNA helix. To the best of our knowledge, the hindrance of the rewinding of the DNA helix will lead to the depression of multiplication of cancer cell. Because there may be more A-T-T base pairs in tumor cells than that in normal ones[12], Tröger's bases can be used as tumor inhibitor.

Therefore, introducing suitable groups to TB skeleton is an efficient way to take advantage of TB and get novel fluorescence materials and tumor inhibitor.

Although there are many sites on TB framework can be modified, the species of new TB derivatives are relatively few in

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number. In most cases aromatic nucleus are modified to afford unilateral or bilateral TB derivatives. Halo-substituted TB was chosen as starting material, *via* Buchwald-Hartwig amination and various coupling reactions such as Corrius-Kumada, Sonogashira and Suzuki coupling to obtain novel TB derivatives[13-16]. Unfortunately, most of them focused on improving yields by optimizing the reaction conditions.

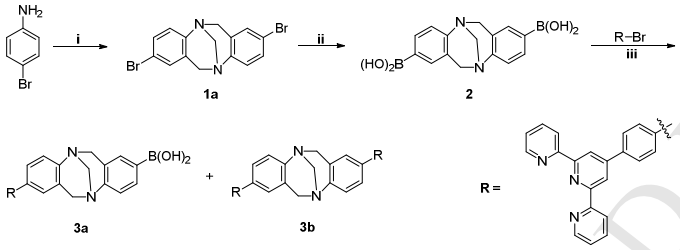
Recently, the luminescence properties, molecular recognition abilities and host-guest chemical properties applications of TB and its derivatives have been reported[17-19], but taken altogether, there are few researches on the luminescence properties of new TB derivatives, also, no researches about the bioactivity have been mentioned.

Diphenylamine (DPA), phenothiazine and carbazole are widely used in the field of dyes[20-21] and pharmaceuticals aspects[22-23]. In this work, they were introduced into TB skeleton to obtain series of new TB derivatives and the optical properties and anticancer activity of products were studied subsequently.

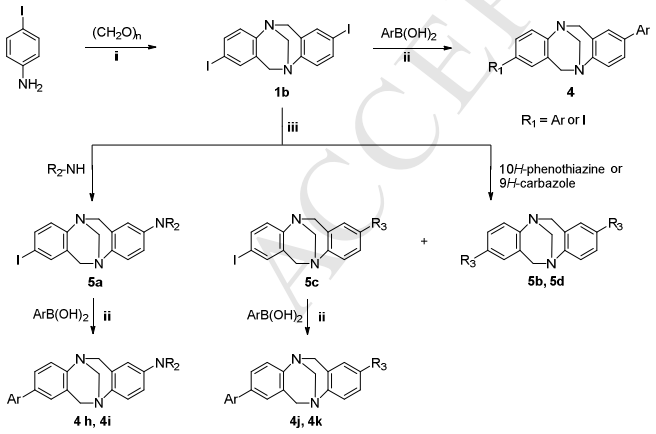
2. Results and discussion

*2.1. Synthesis of new compounds **3a-b**, **4a-k** and **5a-d***

Seventeen novel TB derivatives were prepared according to the synthetic routes showed in Scheme **1** and **2**. Thirteen of them (**3a-b**, **4a-k**) were obtained *via* Suzuki C-C coupling, and four of them (**5a-d**) were obtained *via* Ullmann C-N coupling. (Scheme **1** and Figure **2**).



Scheme 1. Synthetic route of **3a** and **3b**: (i) paraformaldehyde, TFA, -15°C to 0°C, 6 d; (ii) *n*-BuLi, trimethylborate,-78°C to r.t.; (iii) $Pd(PPh_3)_4$, K_2CO_3, toluene, 110 °C (or THF, 70°C).



Scheme 2. Synthetic route of **4a-k** and **5a-d**: (i) TFA, -15°C, 6 d; (ii) Pd $(PPh_3)_4$, K_2CO_3, toluene, 110°C; (iii) CuI, 1,10-Phenanthroline, Cs_2CO_3, toluene, 110°C.

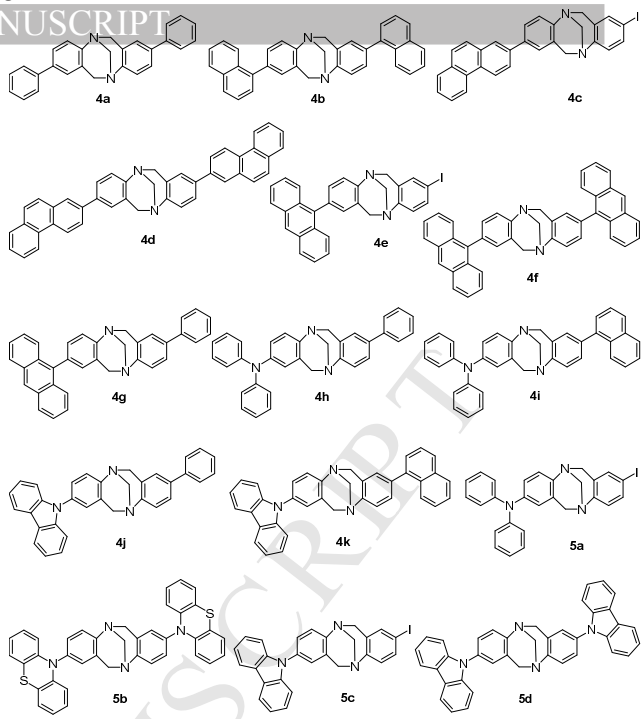


Figure 2. Compound **4a-k** and **5a-d**

In order to enhance the total yield, reaction conditions were optimized. Table **1** showed that tetrahydrofuran was the best solvent for **3a** and **3b** because of the good solubility. Toluene was the ideal solvent for **4a-b**, **4e**, **4h-i** and **5a-d** and benzene was the best solvent for **4c**, **4g**, **4j** and **4k**. The optimum reaction time for **5a-b** was 48 h, while **5c** was 36 h and **5d** was 24 h. (Table **2**)

Table 1 The effect of different solvents on total yield.[a]

Compound	Toluene[b] / %	Tetrahydrofuran[c] / %	Benzene[d] / %
3a	35	54	28
3b	30	62	31
4a	68	58	40
4b	52	43	38
4c	25	32	17
4d	15	27	32
4e	30	25	27
4f	18	16	20
4g	26	30	28
4h	46	35	37
4i	25	19	18
4j	38	42	36
4k	30	32	25
5a	37	29	21
5b	22	17	17
5c	42	31	29
5d	27	23	24

[a]The loading of **2** and **1b** was 0.5 mmol. [b]At 110°C. [c]At 65°C. [d]At 80°C.

Table **2** The effect of different reaction time on total yield.[a]

Compound	24 h / %	36 h / %	48 h / %
5a	22	32	37
5b	10	10	14
5c	42	45	38
5d	27	25	25

[a]The loading of **2** and **1b** was 0.5 mmol, in toluene at 110°C.

2.2. Optical Properties

UV-vis absorption spectra of the new compounds (**4a-k** and **5a-d**) in DCM were shown in Figure **3** and Figure **4**. The maximum UV-vis absorption wavelength of **4a-k** was in the range of 250-340 nm and **5a-d** was in the range of 285-310 nm. Due to the more delocalized and extended π-conjugated system, there was a strong maximum absorption peak at 275 nm for compound **4c**. Compound **4d** red-shifted because more conjugated groups were introduced.

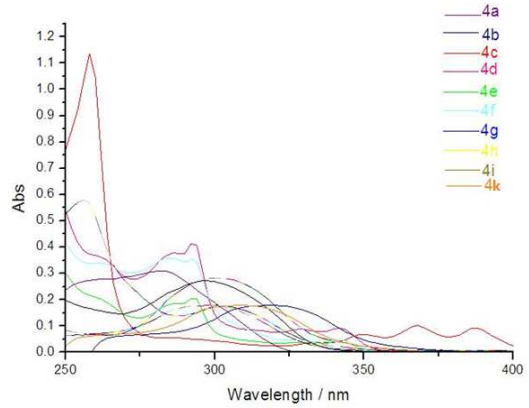


Figure 3. The absorption spectra of **4a-k** (1×10^{-6} mol·L^{-1} in CH_2Cl_2)

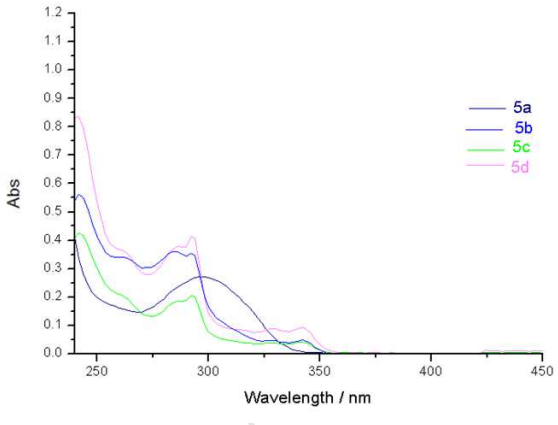


Figure 4. The absorption spectra of **5a-d** (1×10^{-6} mol·L^{-1} in CH_2Cl_2)

The fluorescence emission spectra of the new compounds (**3a-b**, **4a-k** and **5a-d**) in dichloromethane were shown in Figure **5**, **6** and **7**. Compound **3a** had a strong emission peak at 435 nm which caused by the π-π* transitions. Compound **3b** red-shifted and the emission intensity decreased which may due to the longer conjugated chain. (Figure **5**)

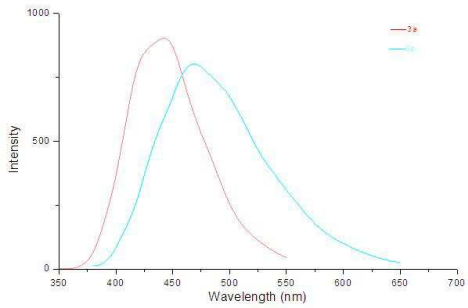


Figure 5. The fluorescence emission spectra of **3a** and **3b** (1×10^{-6} mol·L^{-1} in CH_2Cl_2)

The fluorescence emission spectra of **4a-k** were in the range of blue or green light 382-447 nm excitated at 300 nm (Figure **6**). When more conjugate groups were introduced, the new compounds red-shifted except **4c**. The fluorescence emission spectra of **4c** exhibited strong fluorescence emission at 445 nm, and it may be caused by the unilateral introduction of phenanthrene.

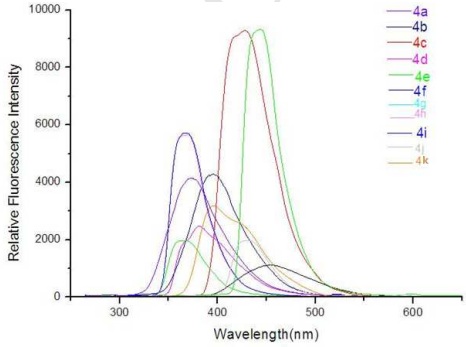


Figure 6. The fluorescence emission spectra of **4a-k** (1×10^{-6} mol·L^{-1} in CH_2Cl_2)

The fluorescence emission spectra of **5a-d** were in the range of 350-430 nm. Figure **7** showed that compound **5d** red shifted, partly because the introduction of larger conjugated system made the flow of electrons smoother.

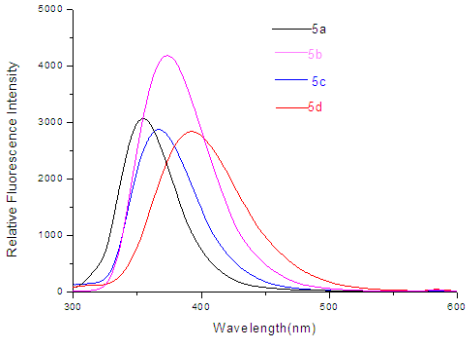


Figure 7. The fluorescence emission spectra of **5a-d** (1×10^{-6} mol·L^{-1} in CH_2Cl_2)

2.3. DFT calculations

The electronic configurations were performed on compounds **3a-b**, **4a-k** and **5a-d** using Gaussian 09 program[24] and the B3LYP/6-31G(d) basis sets were used for the calculations. The HOMO and LUMO distributions of all the compounds were shown in Table **3**. Because of the introduction of electron donating or electron-withdrawing groups and the extended

conjugated system, the band gaps of HOMOs and LUMOs were reduced which conducive to efficient electronic transmission. The result indicated that these new TB derivatives possess significant charge-transfer property.

Table 3 Electrochemical property of **3a**-**5d**

Compound	HOMO (eV)	LUMO (eV)	Band (eV)
TB	-5.33	-0.13	5.2
3a	-6.13	-2.32	3.81
3b	-5.78	-1.67	3.51
4a	-4.69	-0.92	3.77
4b	-3.78	-1.13	2.65
4c	-4.75	-0.87	3.88
4d	-4.87	-1.13	3.74
4e	-3.98	-0.67	3.31
4f	-5.37	-1.32	4.05
4g	-4.86	-0.97	3.89
4h	-4.33	-1.04	3.29
4i	-4.58	-1.23	3.35
4j	4.91	-1.57	3.34
4k	-5.13	-0.89	4.24
5a	-4.75	-0.54	4.21
5b	-5.39	-2.81	2.58
5c	-4.35	-1.86	2.49
5d	-5.21	-2.04	3.17

2.4. Anti-cancer activity against human hepatoma HepG2 cells

Table 4 Inhibition rate (%) of TB derivatives (5 μg/mL) on HepG2 cell[a]

Entry	Inhibition rate (%)	Entry	Inhibition rate (%)
1a	0.0±3.1	**4f**	3.8±2.4
1b	5.3±1.8	**4g**	1.4±2.8
2	-5.0±1.4	**4h**	0.5±3.1
3a	18.1±1.1	**4i**	2.3±1.7
3b	30.0±1.4	**4j**	2.1±1.4
4a	-5.6±3.1	**4k**	-1.3±0.7
4b	1.3±2.7	**5a**	6.4±3.8
4c	1.7±2.5	**5b**	-3.±1.8
4d	11.6±1.8	**5c**	-2.5±2.7
4e	-0.6±2.1	**5d**	-0.9±1.9

[a]TB derivatives were dissolved in DMSO, and the absorbance value was measured at 490 nm in a Enzyme Linked Immunosorbent Assay (ELISA). Experiments were performed in triplicate, and the results were expressed as mean of percentage inhibition.

Then compounds **3a**, **3b** and **4b** were determined further in different concentrations. (Table **5**) The result showed that the inhibition rate was increased with the increase of their concentration. Compound **4b** showed a significantly increased of inhibition rate when the concentration is 50 μg/mL compare to 25 μg/mL. 25 μg/mL of **3a** and **3b** showed very good inhibitory effects against human hepatoma HepG2 cells in vitro and the inhibition rate is 73.5% and 76.8% respectively. The result indicated these compounds have the profound value for further study.

Because some of the new compounds may have the potential anti-tumor activity, so incidentally compounds **3a**-**b**, **4a**-**k** and **5a**-**d** were subjected to biological assessment against human hepatoma HepG2 cell lines in vitro. Cell proliferation was determined by MTT assay and HepG2 cells were firstly treated with 5 μg/mL of the compounds. The results indicated the inhibition rate of compounds **3a**, **3b** and **4b** on the HepG2 cells was over 10% which showed their effectiveness on this cancer cell. (Table **4**)

Table 5 Inhibition rate (%) and IC50 of TB derivatives on HepG2 cell in different concentration[a]

Entry	Inhibition rate in difference concentration (μg/mL)			IC50 (μM)
	5	25	50	
3a	18.1±1.1	73.5±2.6	81.0±1.5	24.68±1.39
3b	30.0±1.4	76.8±1.8	78.8±1.6	12.61±1.10
4d	11.6±1.8	28.2±1.0	80.9±1.5	50.34±1.70

[a]The TB derivatives were dissolved in DMSO, and the absorbance value was measured at 490 nm in a Enzyme Linked Immunosorbent Assay (ELISA). Experiments were performed in triplicate, and the results were expressed as mean of percentage inhibition.

3. Conclusions

In this work, diphenylamine (DPA), phenothiazine or carbazole were introduced into TB skeleton *via* Ullmann or Suzuki coupling and series of new TB derivatives were synthesized. The results of their optical properties indicated that some product exhibited strong fluorescence emission. The electronic configurations calculated by DFT indicated the extended conjugated system conducive to efficient electronic transmission and the new TB derivatives possess significant charge-transfer property. The results showed they have the potential as fluorescence materials. Finally, the results of their anticancer activity on the HepG2 cells indicated that three compounds could inhibit the growth of cancer cells effectively. So, they are worth further study and can be developed as high-performance anticancer drugs in the future.

Acknowledgments

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Supplementary Material

Supplementary data (experimental details including synthetic procedures, NMR spectra, mass spectrometry, IR) associated with this article can be found, in the online version, at http://dx.doi.org/

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