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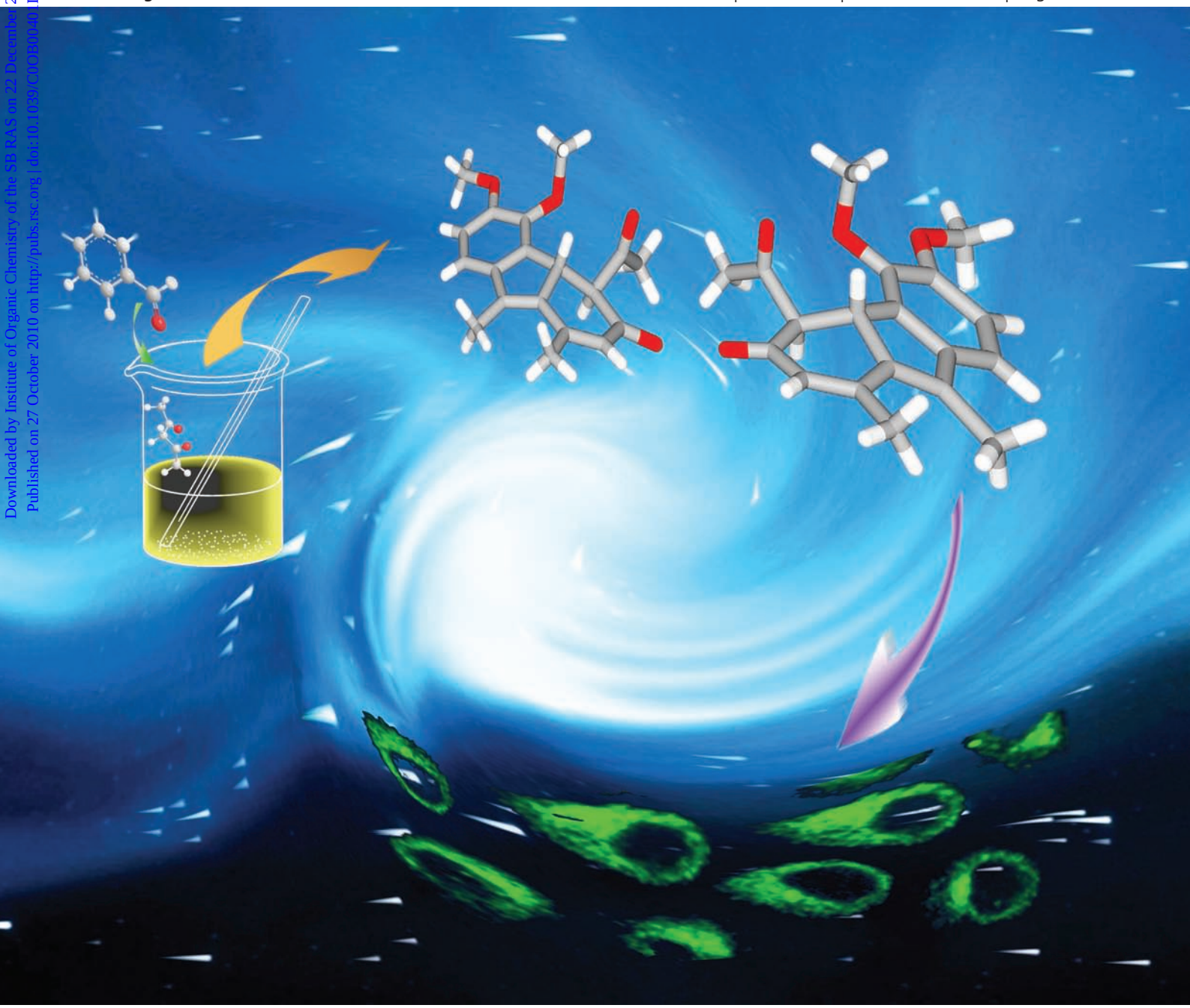

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New fluorescent *trans*-dihydrofluoren-3-ones from aldol–Robinson annulation–regioselective addition involved one-pot reaction†

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An unexpected discovery of new *trans*-4-acetyl-1,9-dimethyl-4,4a-dihydro-3*H*-fluoren-3-ones from one pot reactions of benzaldehydes and acetylacetone is described. The synthetic mechanism and stereochemistry were discussed. These new derivatives exhibit good fluorescent properties in solutions.

Fluorescent probes have been of great interest to chemists because of their wide usage as biological imaging and chemical sensing reagents[1] for various purposes such as chemical species analysis,[2] cellular process monitoring[3] and tissue visualization.[4] Fluorene derivatives such as 9-fluorenones, which exhibit an extensive aromatic π system, have attracted much attention owning to their antiviral and interferon inducing ability[5] as well as antitumor potency;[6] besides, 9-fluorenones exhibited excellent fluorescence properties[7] and could be used as fluorescent probes for detecting amino acids,[8] hydrogen binding interactions in cyclodextrin[9] and Ca^{2+} ions,[10] *etc*. The skeleton of 9-fluorenones could be formed by intramolecular arylation of biphenyl-2-carbaldehyde[11] or 2-biphenyl carboxylic acid,[12] and other cyclization processes.[13] Despite all the investigations on synthetic mothedologies and various properties of 9-fluorenones, the non-extensive aromatic π system derivatives such as dihydrofluorenones, had rarely been investigated especially on their fluorescence. We herein describe an unexpected discovery of a new series of substituted *trans*-4,4a-dihydro-3*H*-fluoren-3-ones as novel fluorophores from one pot reactions of readily available starting materials.

In an exploration to the synthesis of 3-benzylidenepentane-2,4-dione derivatives, which acted as intermediates in our ongoing project on novel curcumin analogues for tumor chemopreventive agents screening, 2 eq concentrated sulfuric acid was adopted as the catalyst in the reaction of 3-ethoxy-4-hydroxybenzaldehyde (**1a**) and excess actylacetone in cold glacial acetic acid. After 3 h stirring, thin-layer chromatography (TLC) revealed a product with apparent green-to-yellow fluorescence. This product was isolated and purified for structural characterization. The MS and elemental analysis of this product indicated a formula of $C_{19}H_{20}O_4$, which differed from the targeted 3-(3-ethoxy-4-hydroxybenzylidene) pentane-2,4-dione ($C_{14}H_{16}O_4$). In the ^1H NMR spectrum, two saturated hydrogen atoms were found at δ_H 4.32 ppm (dd, J = 12.79, 1.38 Hz, 1H) and 3.32 ppm (d, J = 12.80 Hz, 1H) respectively, both of which were further proven to be adjacent by COSY analysis. In the HMBC spectrum, the signal at δ_H 3.32 ppm was found to have correlation to two carbonyl carbons signals at δ_C 207.2 and 196.3 ppm respectively. The signal at δ_C 196.3 ppm showed close correlation with a double bond hydrogen at δ_H 5.73 ppm, 1H while the signal at δ_C 207.2 showed correlation with a methyl group at δ_H 2.42 ppm (s, 3H) and suggesting an acetyl group nearby (Fig. 1).

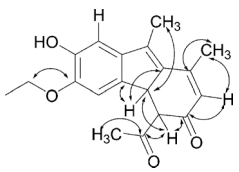
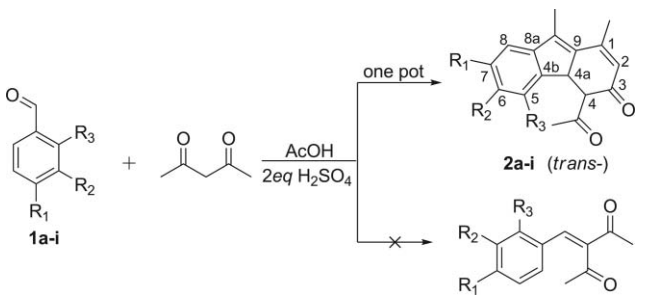


Fig. 1 H–C correlations of the product in HMBC.

Meanwhile, we found the signal at δ_H 5.77 ppm was correlated with another methyl group at δ_H 2.37 (d, J = 1.17 Hz, 3H) in the H–H COSY spectrum, which meant that a structural fragment of –CH═C(CH_3)– existed.[14] It is obvious that a methyl and acetyl substituted cyclohex-2-enone moiety presented based on above analysis. On the other hand, only two independent Ar–H were found at δ_H 7.00 (s, 1H) and 6.74 (s, 1H) ppm, implying that the third Ar–H of **1a** had been substituted. Furthermore, signal at δ_H 4.32 ppm was found to exhibit an additional correlation with an aromatic carbon at 140.2 ppm. Combining all of the informations, the product was finally identified as 4-acetyl-6-ethoxy-7-hydroxy-1,9-dimethyl-4,4a-dihydro-3*H*-fluoren-3-one (**2a**) (Table 1, Scheme 1).



Scheme 1

The revealed structure has two chiral carbons present in 4a and 4 position respectively; according to the *Karplus equation* (J = 7cosθ + 5cos2θ,[15] see the ESI†), the dihedral angel $\theta_{(H\text{-}C4a\text{-}C4\text{-}H)}$ can be calculated from coupling constant between the H_4 and H_{4a} signals. We therefore caculated the $\theta_{(H\text{–}C4a\text{–}C4\text{–}H)}$ of **2a**, the

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† Electronic supplementary information (ESI) available: Synthesis, characterization data of new compounds, stereochemistry discussion and the crystallographic data for **2b**. CCDC reference number 762211. For ESI and crystallographic data in CIF or other electronic format see DOI: 10.1039/c0ob00401d

Table 1 Structure information, yields, UV/vis and fluorescence data for compounds **2a–i**[a]

Entry	Benzaldehyde	R_1	R_2	R_3	Compd	Yield (%)	M.P./°C	H–C_{4a}–C_4–H		UV/vis	Fluorescence		Stoke's shift/nm
								J/Hz	Dihedral angle θ^i	λ_{Ex}/nm	λ_{Em}/nm	ϕ^e	
1	**1a**	OH	OC_2H_5	H	**2a**	15	177.5–180.0	12.80	≈180°	404	542	0.036	138
2	**1b**	H	OCH_3	OCH_3	**2b**	20	165.0–166.2	11.79	162°	360	481	0.20[f]	121
										360	484	0.26[g]	124
										381	516	0.34	135
										382	497	0.77[h]	115
3	**1c**	OC_2H_5	OCH_3	H	**2c**	16	157.2–160.4	12.79	≈180°	398	534	0.18	136
4	**1d**	OH	OCH_3	H	**2d**	18	163.9–165.5	12.87	≈180°	401	539	0.033	138
5	**1e**	OCH_3	OCH_3	H	**2e**	15	159.2–161.4	12.79	≈180°	398	533	0.12	135
6	**1f**[a]	$OCH_2CH{=}CH_2$	OCH_3	H	**2f**	18	131.8–133.2	12.79	≈180°	396	531	0.22	135
7	**1g**[b]	$OCH(CH_3)_2$	OCH_3	H	**2g**	15	138.9–140.8	12.81	≈180°	392	536	0.065	142
8	**1h**[c]	$OCH_2CH{=}CH_2$	OC_2H_5	H	**2h**	11	126.9–130.4	12.80	≈180°	396	532	0.15	136
9	**1i**[d]	$OCH(CH_3)_2$	OC_2H_5	H	**2i**	14	101.0–103.7	12.82	≈180°	392	537	0.16	145

[a] For synthesis details and structure characterization data, please see the ESI;† *a*,*c*: aldehydes **1f** and **1h** were prepared from allylation of **1d** and **1a** respectively; *b*,*d*: aldehydes **1f** and **1h** were prepared by isopropylation of **1d** and **1a** respectively; *e*: determined using quinine sulfate in 1 N H_2SO_4 as a standard (1 μmol L^{-1}), and compounds **2a–i** were dissolved in EtOH as 1 μmol L^{-1} solution except when mentioned alternatively; *f*: in acetone; *g*: in CH_2Cl_2; *h*; in DMSO; *i*: calculated based on *Karplus equation*.

determined dihedral angel is about 180° (Table 1), indicating a *trans*-conformation of **2a**.

The above finding indicated that the skeleton of *trans*-4,4a-dihydro-3*H*-fluoren-3-one formed in an acceptable isolated yield from readily available starting materials. Though in low yields, the product **2a** can be easily distinguished and purified by column chromatography from the reaction mixture, which is mainly constituted of unreacted starting materials and polymer by-products; moreover, we found **2a** showed strong fluorescence in solution. We therefore adopted this procedure for more derivatives synthesis. Using various benzaldehydes instead of **1a**, another 8 new compounds were obtained successfully in 11–20% isolated yields, structure characterization indicated all of them have the same skeleton as **2a** (Table 1, Scheme 1 **2b–i**), the dihedral angel $\theta_{(H\text{-}C4a\text{-}C4\text{-}H)}$ calculation also revealed a *trans*-conformation of **2b–i** (Table 1); besides, the skeleton of *trans*-4,4a-dihydro-3*H*-fluoren-3-one was further supported by the X-ray data of **2b** (Fig. 2).[16]

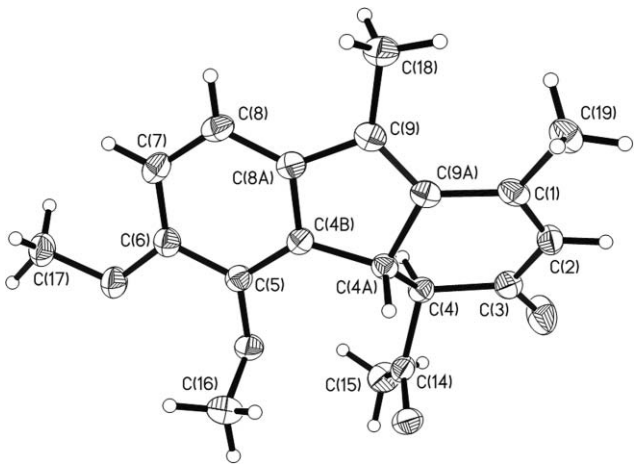
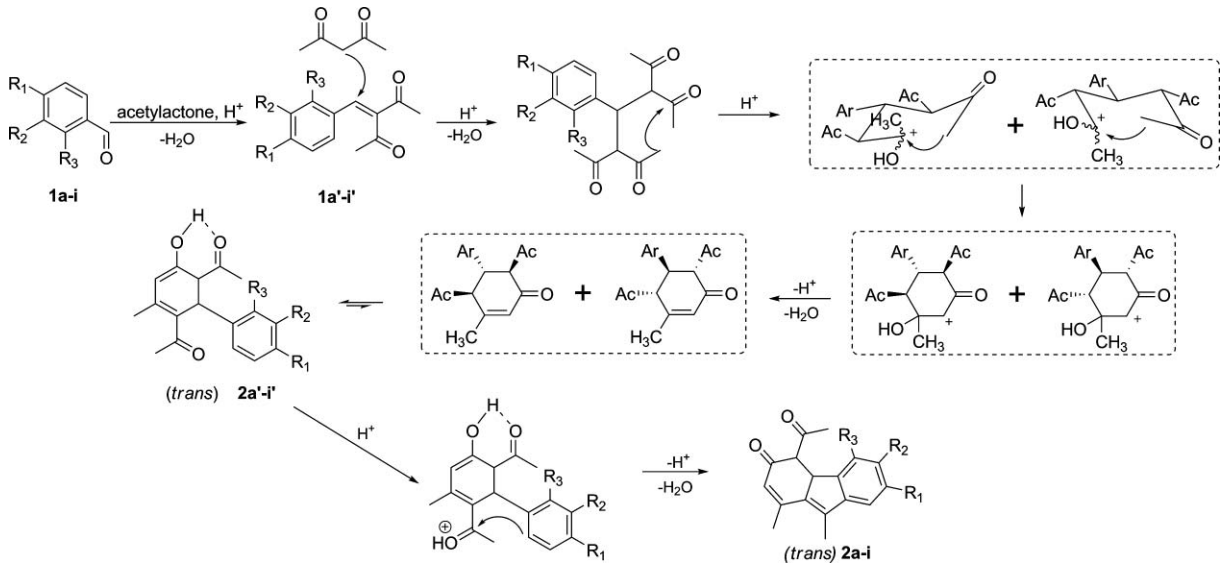


Fig. 2 X-Ray structure of **2b** (CCDC no. 762211).

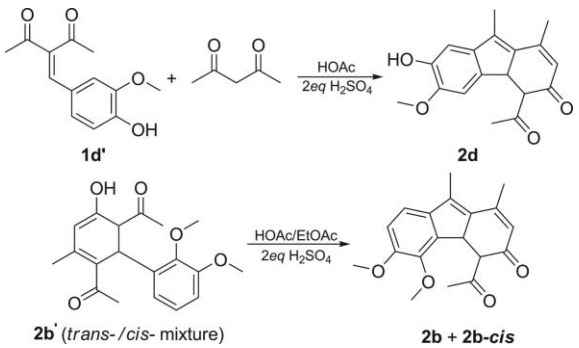
Noticeably, we found only benzaldehydes containing electron donating R_2 could transformed into the final products **2** while other benzaldehydes failed during the synthesis (data not shown), indicating the electron density of the phenyl ring played a critical role during the final production formation. Based on this information and the structure of products, the synthetic mechanism was proposed as Scheme 2. In this procedure, an aldol reaction followed by Robinson annulation occurred, and the final products were formed through a carbonyl-selective intramolecular aromatic electrophilic addition–elimination process, the regioselectivity was achieved probably owning to the possible hydrogen bond between the enol form of the β-diketone moiety during the aromatic electrophilic addition process.

It should be pointed out that though the reaction takes place in an achiral environment, only the *trans* products were found in current study. We reasoned that this phenomenon would result from the low-level energy state of reaction intermediate during the Robinson annulation step, the cyclohexanone-like intermediate presented a chair form during the attacking; the energy and bulk effect would be lower when large groups were at *e*-position, resulting in the *trans* conformation between H_4 and H_{4a} (Scheme 2).

To further verify the mechanism, we prepared the intermediate **1d′** (R_1 = OH, R_2 = OCH_3 R_3 = H) and the Robinson annulation product **2b′** (R_1 = H, R_2 = R_3 = OCH_3) (Scheme 2) respectively. Both **1d′** and **2b′** cannot be obtained in the current acid catalysis condition and therefore were synthesized under piperidine catalysis. Noticeably, the intermediate **2b′** would be *trans*- and *cis*-isomer mixture in the base catalysis condition.[17] After that, the acetylacetone was mixed with **1d′** under the same condition as in the synthesis of **2d** (Scheme 3). The second reaction started from the intermediate **2b′**, which was treated directly with acetic acid and 2 eq concentrated sulfuric acid. Expectedly, intermediates **1d′** resulted in the target product **2d** with higher yield (45%); in the case of intermediate **2b′**, the *cis*- and *trans*-forms of 4-acetyl-5,6-dimethoxy-1,9-dimethyl-4,4a-dihydro-3*H*-fluoren-3-one (**2b**-*cis* and **2b**) were obtained in 28% and 16% yield respectively (total yield, 44%). NMR revealed 6.11 Hz of $J_{H4a\text{-}H4}$ in **2b**-***cis***, the θ(H–C_{4a}-C_4–H) of **2b**-***cis*** was therefore calculated to be 46°, which match the *cis* H-C4a-C4-H form in **2b**-***cis***. Regardless



Scheme 2 Proposed mechanism of **2a–i** synthesis.



Scheme 3 Synthesis of **2b**, **2b**-*cis* and **2d** from intermediates.

of the **2b**-***cis*** obtained from a racemic mixture **2b′**, the obtained results provided evidences to the mechanism shown in Scheme 2.

We next investigated the fluorescence properties of **2a–i**. The results are summarized in Table 1. In ethanol, λ_{Ex} and λ_{Em} of **2a**, **2c–i** were all round 395 nm and 530 nm respectively, while those of **2b** were slightly different, with λ_{Ex} = 381 nm and λ_{Em} = 516 nm (Fig. 3). The Stoke's shifts of all compounds were as large as 130 nm approximately, showing a broad emission peak (see Fig. 3). Among all compounds, the fluorescence intensity and quantum yield[18] of **2a** and **2d** were comparatively lower than those of the other ones, indicating that the hydroxyl group at the 7 position would decrease fluorescence.

Remarkably, the 5,6-dimethyoxyl derivative **2b** showed the strongest fluorescence intensity and highest quantum yield in ethanol. The quantum yield of **2b** in various solutions (Fig. 4) were therefore measured and the result showed that Φ of **2b** depended on the polarity of solvent (Φ = 0.77 in DMSO, and 0.20, 0.26, 0.34 in acetone, CH_2Cl_2 and ethanol respectively, Table 1).

Furthermore, we investigated preliminarily the distribution of **2b** in lung cancer cell line A549 by laser confocal microscopy imaging. The result indicates that **2b** distributed in the cytoplasm equably but kept out of the nucleus (Fig. 5). These results suggested the structure of **2b** might be an useful model for novel fluorescent probes discovery based on dihydrofluoren-3-one.

In summary, we found 4,4a-dihydro-3*H*-fluoren-3-one derivatives can be synthesized under simple conditions with readily available starting materials and 9 new analogues were obtained successfully in acceptable isolated yield. The mechanism was proposed; to the best of our knowledge, only Anastassiou *et al.* reported the preparation of single compound 4,4a-dihydro-3*H*-fluoren-3-one by acid catalyzed pericyclization of benzo[9]annulenone in 1981[19] and no further investigation on this skeleton was found ever since. Compared with the reported method which needed expensive starting material, long synthetic steps and low diversity, our finding afforded a simple, economical procedure to

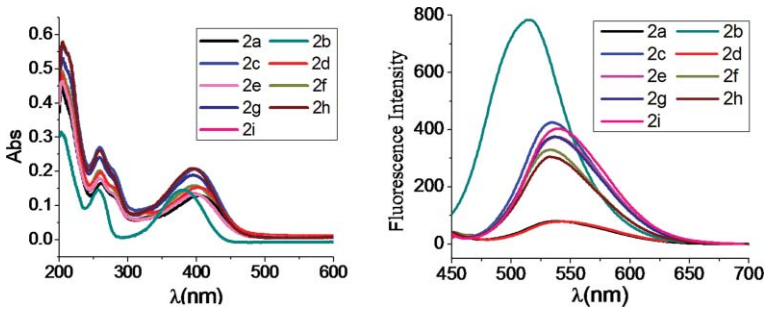


Fig. 3 UV-vis (left) and emission (right) spectrum of 10 μmol L^{-1} **2a–i** in ethanol.

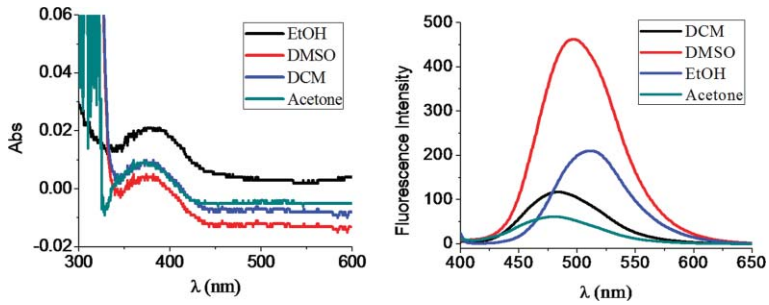


Fig. 4 UV-vis (left) and emission (right) spectrum of 1 μmol L^{-1} **2b** in different solvents.

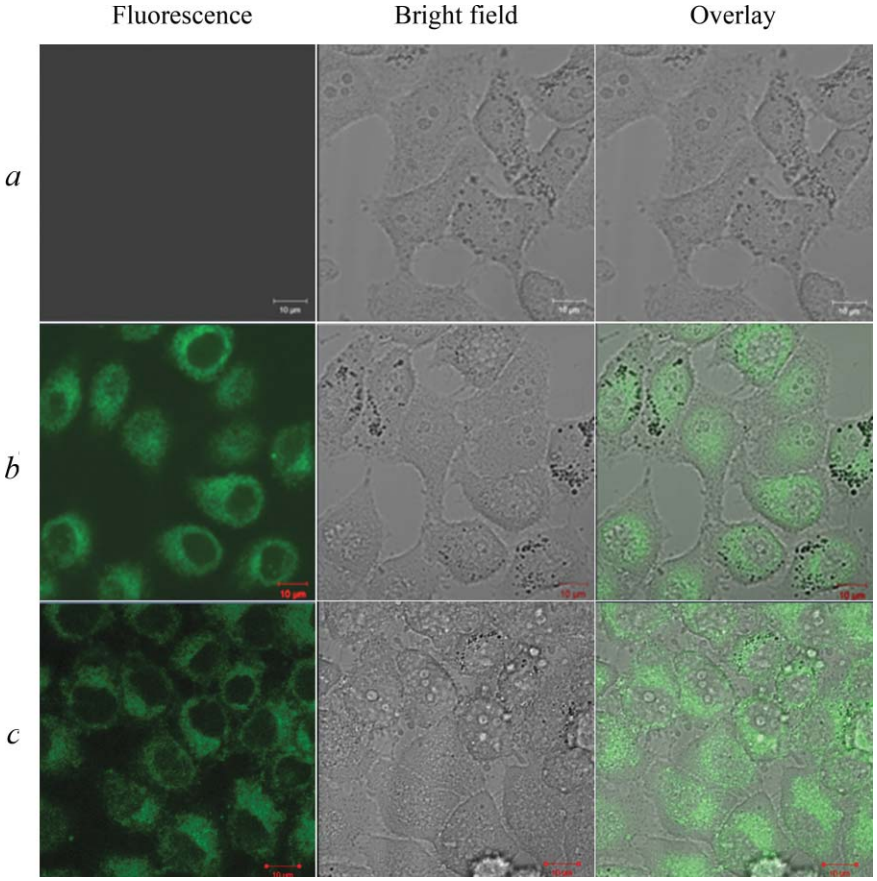


Fig. 5 Confocal imaging of **2b** treated A549 cells. *a*, without **2b**; *b*, with 0.5 μM **2b**, 2 h; *c*, with 0.5 μM **2b**, 6 h.

generate more 4-acetyl-1,9-dimethyl-4,4a-dihydro-3*H*-fluoren-3-one diversities though the final yields are relative low. Remarkably, we found the obtained compounds **2a–i**, especially **2b**, exhibit good fluorescence properties. The high fluorescence quantum yield, large Stoke's shift and lack of ionic charge for fluorescence emission of them predicated the significance of our finding in searching for novel fluorophores based on dihydrofluoren-3-one, distinguishing from the well studied 9-fluorenone with extensive π-aromatic system, and such investigations are now in progress in our group.

Acknowledgements

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Notes and references

1 (*a*) M. S. T. Gonçalves, *Chem. Rev.*, 2009, **109**, 190–212; (*b*) M. Leopoldo, E. Lacivita, F. Berardi and R. Perrone, *Drug Discovery Today*, 2009, **14**(13–14), 706–712; (*c*) M. Fernández-Suárez and A. Y. Ting, *Nat. Rev. Mol. Cell Biol.*, 2008, **9**(12), 929–943.
2 (*a*) N. Inoue, Y. Suzuki, K. Yokoyama and I. Karube, *Biosci., Biotechnol., Biochem.*, 2009, **73**(5), 1215–1217; (*b*) Z. Cao, P. Nandhikonda and M. D. Heagy, *J. Org. Chem.*, 2009, **74**, 3544–3546.
3 (*a*) T. Kihara, C. Nakamura, M. Suzuki, S.-W. Han, K. Fukazawa, K. Ishihara and Miyake, *J. Biosens. Bioelectron.*, 2009, **25**, 22–27; (*b*) S. Fakih, M. Podinovskaia, X.-L. Kong, U. E. Schaible, H. L. Collins and R. C. Hider, *J. Pharm. Sci.*, 2009, **98**(6), 2212–2226.

4 (*a*) Y. Urano, *Anal. Sci.*, 2008, **24**, 51–53; (*b*) M. Sawa, T.-L. Hsu, T. Itoh, M. Sugiyama, S. R. Hanson, P. K. Vogt and C.-H. Wong, *Proc. Natl. Acad. Sci. U. S. A.*, 2006, **103**(33), 12371–12376.

5 (*a*) S. A. Lyakhov, E. A. Lyakhova, A. S. Karpenko, G. V. Mal'tsev, I. V. Vel'cheva, L. A. Litvinova, M. N. Lebedyuk, G. A. Khorokhorina and V. P. Fedchuk, *Pharm. Chem. J.*, 2004, **38**(3), 128–131; (*b*) S. Alcaro, A. Artese, J. N. Iley, R. Maccari, S. Missailidis, F. Ortuso, R. Ottanà, P. Ragazzon and M. G. Vigorita, *Bioorg. Med. Chem. Lett.*, 2007, **17**, 2509–2514.

6 N. S. Youssef, E. A. El Zahany, M. M. Anwar and S. A. Hassan, *Phosphorus, Sulfur Silicon Relat. Elem.*, 2009, **184**, 103–125.

7 (*a*) J. R. Heldt, J. Heldt, M. Józefowicz and J. Kamiǹski1, *J. Fluoresc.*, 2001, **11**(1), 65–73; (*b*) H.-X. Shao, X.-P Chen, Z.-X Wang and P. Lu, *J. Lumin.*, 2007, **127**, 349–354.

8 T.-J. Hsieh and S. Chen, *Amino Acids*, 2007, **33**, 97–104.

9 L. J. Biczok, *J. Inclusion Phenom. Mol. Recognit. Chem.*, 1994, **18**, 237–245.

10 N. T. Player, S. Shinoda and H. Tsukube, *Org. Biomol. Chem.*, 2005, **3**, 1615–1616.

11 J. Barluenga, M. Trincado, E. Rubio and Jose M. Gonzalez, *Angew. Chem., Int. Ed.*, 2006, **45**, 3140–3143.

12 (*a*) D. Tilly, S. S. Samanta, D. Asish, A. Castanet and J. Mortier, *Org. Lett.*, 2005, **7**, 827–830; (*b*) D. Tilly, S. S. Samanta, F. Faigl and J. Mortier, *Tetrahedron Lett.*, 2002, **43**, 8347–8350.

13 (*a*) X.-X. Zhang and R. C. Larock, *Org. Lett.*, 2005, **7**, 3973–3976; (*b*) J. Zhao, D.-W. Yue, M. A. Campo and R. C. Larock, *J. Am. Chem. Soc.*, 2007, **129**, 5288–5295; (*c*) V. S. Thirunavukkarasu, K. Parthasarathy and C.-H. Cheng, *Angew. Chem., Int. Ed.*, 2008, **47**, 9462–9465.

14 N. E. Jacobsen, University of Arizona, *NMR Spectroscopy Explained-Simplified Theory, Applications and Examples for Organic Chemistry and Structural Biology*, John Wiley & Sons, Inc., Hoboken, New Jersey, chapter 9.

15 N. E. Jacobsen, University of Arizona, *NMR Spectroscopy Explained-Simplified Theory, Applications and Examples for Organic Chemistry and Structural Biology*, John Wiley & Sons, Inc., Hoboken, New Jersey, chapter 2.

16 CCDC reference number 762211.

17 A. Sharma, J. Pandey and R. P. Tripathi, *Tetrahedron Lett.*, 2009, **50**(16), 1812–1816.

18 Franklyn G. Prendergast, Michael Meyer, Gerald L. Carlson, Shozo Iida and Jame D. Potter, *J. Biol. Chem.*, 1983, **258**(12), 7541–7544.

19 A. G. Anastassiou, H. S. Kasmai and M. R. Saadein, *Helv. Chim. Acta*, 1981, **64**(2), 617–619.

