Chinese Chemical Letters 33 (2022) 4203–4207

Contents lists available at ScienceDirect

Chinese Chemical Letters

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

Synthesis and characterization of fluorescence active G_4-quartet and direct evaluation of self-assembly impact on emission

Ying He[a], Mengjia Liu[a], Shun Teng[a], Lukasz Wojtas[a], Guangxin Gu[b,*], Xiaodong Shi[a,*]

[a] Department of Chemistry, University of South Florida, Tampa, FL 33620, United States
[b] Department of Materials Science, Fudan University, Shanghai 200438, China

ARTICLE INFO

Article history:
Received 22 October 2021
Revised 27 February 2022
Accepted 2 March 2022
Available online 5 March 2022

Keywords:
Self-assembly
H-bonding
G_4-quartet
Fluorescence
Aggregation induced emission

ABSTRACT

Fluorescence (FL) active 8-aryl guanosine derivatives were prepared and applied for cation mediated self-assembly to form the H-bonded G_8-quadruplexes. The *p*-cyano (*p*-CN) and 8-anthracene (8-An) substituted guanosines were identified to give the strongest fluorescence with the formation of G_8-octamers (G_8) both in solution (NMR) and solid state (X-ray). This well-defined G_8-octamer system has provided the first direct evidence on the self-assembled G-quadruplex fluorescence emission with aggregation-induced emission (AIE), which could be applied as the foundation for FL molecular probe design toward G-quadruplex recognition.

The G-quadruplex refers to the cylindrical structure based on the stacking of planar G_4-quartets (G_4), which are the H-bond assemblies from four guanine units [1–3]. As a well-known nucleic acid secondary structure, G-quadruplex has received tremendous attention over the past two decades [4–9]. Considering that G_4-quartet is a planar structure containing four aromatic purines, efforts have been made to the design of planar aromatic compounds as potential receptors to interact with G_4-quartet through π-π interactions [10,11]. As shown in Scheme 1, one example of G-quadruplex binding/recognition was the application of external chromophore (Thioflavin T, ThT) to interact with the G_4-quartet plane [12]. This strategy has been successfully applied to the development of G-quadruplex receptor and fluorescence sensors [12–16]. Although both terminal and internal binding have been proposed in the past, the influence of photoemission upon the formation of H-bonded self-assembly remains unclear, mainly due to the lack of well-defined and stable G-quadruplex systems as the direct fluorophore to evaluate photoluminescent (PL) activities [17–23].

With the interest in understanding how H-bond aggregation influences purine conjugation and G_4-quartet fluorescence, in this work, we prepared a series of fluorescence active 8-aryl guanosine and evaluated their FL emission upon self-assembly. Fluorescence active G-quadruplexes were successfully prepared and characterized both in solution and solid state. *para*-Cyano (*p*-CN) and 8-anthracene (8-An) substituted guanosine analogs were identified to maintain the FL emission while holding the G_4-quartet assembly. Interesting photoluminescent activity was observed between monomeric guanosine and G_4-assembly, confirming the ligand-controlled G_4 emission for the potential π-π stacking system, which provides an important foundation for future FL molecular probe design.

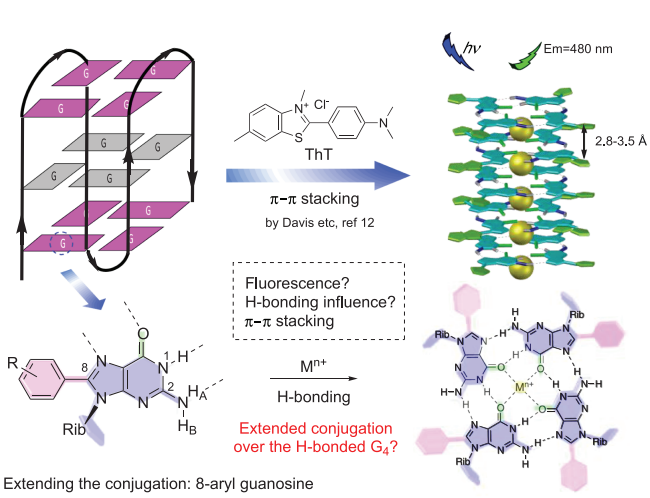
Recently, our group developed a well-defined guanosine G_8-octamer system through the application of conformationally defined guanosine building blocks [24,25]. The key to conformational control is the introduction of steric hindrance both on guanine (C8-aryl) and ribose (2′,3′-isopropylidene), which allows the G_4-quartet to adopt a tight "bowl-shape" conformation and leads to the "bottom-to-bottom" stacked G_8 as the discrete species in solution and solid state. This dual modification is critical for the formation of well-defined G-quadruplex as modification on either C-8 or ribose alone resulted in the formation of mixtures of various stacking isomers (Fig. 1A, see details in Supporting information).

To systematically study the fluorescence difference between G-monomer and G_4-quartet, a library of guanosine derivatives was prepared (Fig. 1B) with variations on both 8-aryl and ribose. It is known that the introduction of 8-aryl group on purine could introduce fluorescence emission [11,21,26,27]. To explore the FL activity of these guanosine monomers, we first evaluated the photoactivity of these compounds in dimethyl sulfoxide (DMSO). The results are summarized in Table 1.

As expected, a wide range of FL emission was achieved with these modified guanosine derivatives (Fig. 2A). With purine as the

* Corresponding auhtors.
E-mail addresses: Guangxingu@fudan.edu.cn (G. Gu), xmshi@usf.edu (X. Shi).

https://doi.org/10.1016/j.cclet.2022.03.007
1001-8417/



Scheme 1. G-quadruplex as fluorescence sensors.

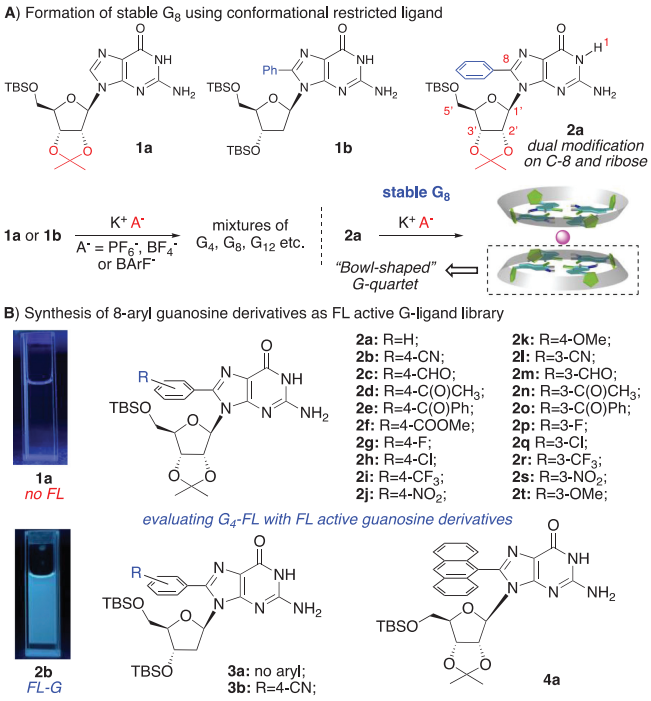


Fig. 1. Exploring π-conjugation with FL active guanosines.

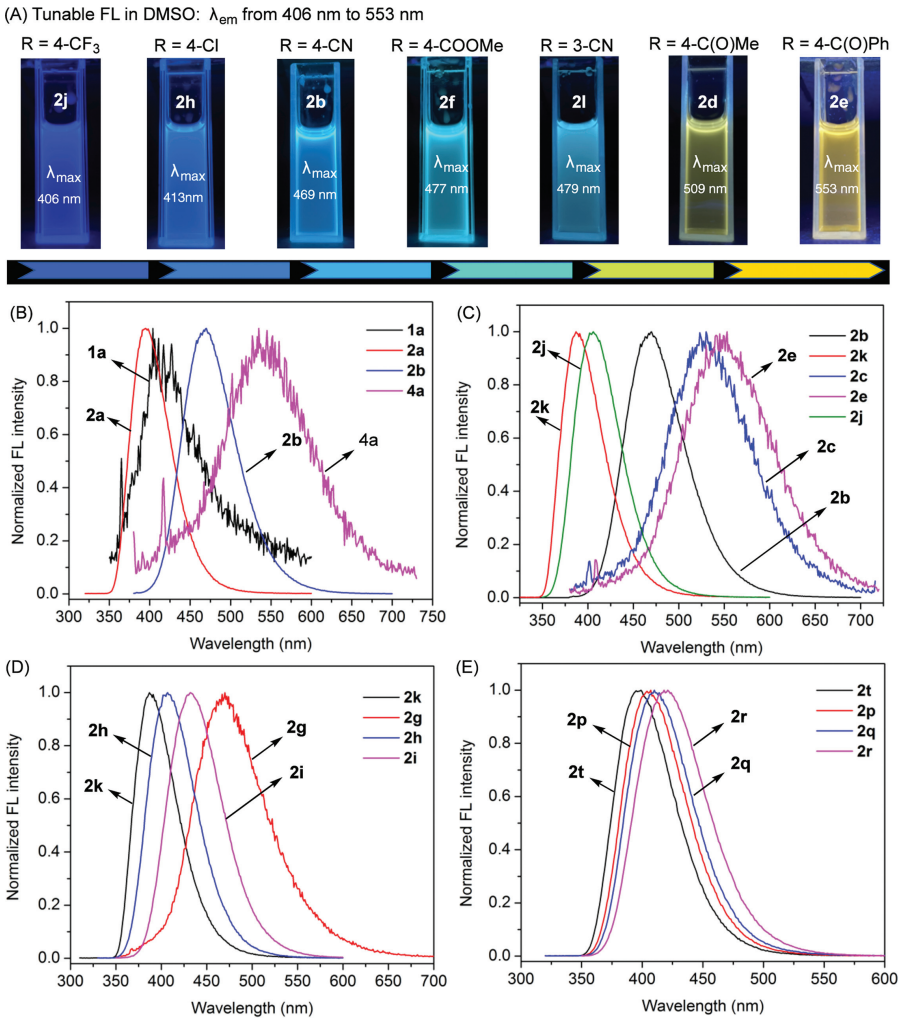


Fig. 2. (A) Tuning the fluorescence with 8-aryl substituents in DMSO solution under irradiation of 365 nm UV light (1.0 mmol/L). (B) FL emission of guanosine **1a**, **2a**, **2b**, and **4a** in DMSO. (C) Normalized FL emission of *para*-substituted 8Ar-G, including compound **2b**, **2k**, **2c**, **2e**, or **2j** in DMSO. (D) Normalized FL emission of *para*-substituted 8Ar-G, including compound **2g, 2h, 2i,** or **2k** in DMSO. (E) Normalized FL emission of *meta*-substituted 8Ar-G, including compound **2t**, **2p**, **2q**, or **2r** in DMSO.

Table 1
Optical properties of monomeric guanosine in DMSO.[a]

Entry	G	λ_{em}/nm	Φ (%)	Entry	G	λ_{em}/nm	Φ (%)
1	**1a**	404	<1	14	**2l**	479	18
2	**1b**	398	>90	15	**2m**	395, 496	16
3	**2a**	395	85	16	**2n**	407	<1
4	**2b**	469	>90	17	**2o**	448	4
5	**2c**	530	17	18	**2p**	404	>
6	**2d**	509	20	19	**2q**	410	>90
7	**2e**	553	13	20	**2r**	421	>90
8	**2f**	477	87	21	**2s**	391	2
9	**2g**	470	52	22	**2t**	397	66
10	**2h**	413	87	23	**3a**	399	10
11	**2i**	432	74	24	**3b**	472	>90
12	**2j**	406	>90	25	**4a**	539	3
13	**2k**	387	64				

[a] $\varepsilon = A/(c \times l) = 6 \times 10^4$, concentration: 10 μmol/L in DMSO.

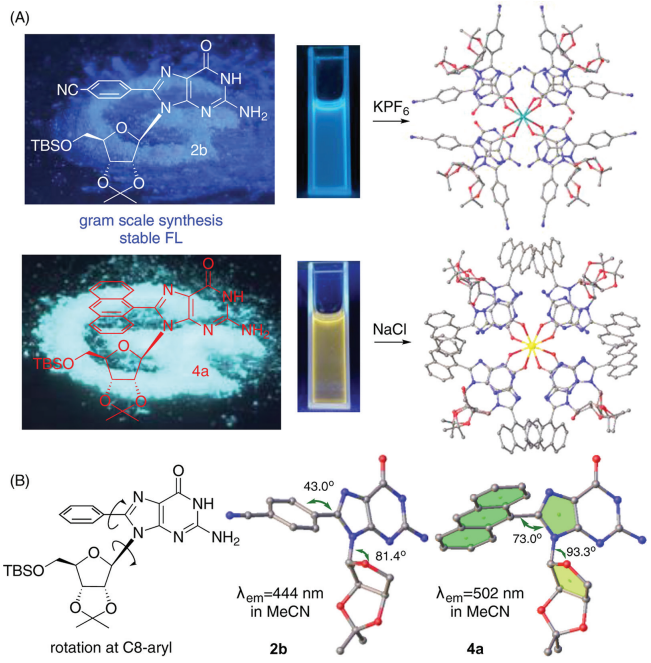


Fig. 3. Crystal structures of **2b** and **4a** G_8-octamer. (A) FL of monomeric **2b** and **4a** in the solid state under irradiation of 365 nm UV light (left); top view of crystal structures of G_8-octamer formed by **2b** and **4a** through self-assembly with KPF_6 and NaCl respectively (right); (B) Crystal structures showing large dihedral angles of **2b** and **4a.**

electron rich aromatic ring, incorporation of electron-withdrawing groups (EWG) on 8-aryl should facilitate a red-shift of the emission. To our delight, compared to guanosine **1a** and **2a**, 8-(4-cyano)phenyl guanosine **2b** exhibited a significant red-shift with strong fluorescence emission maxima at 469 nm with a bright blue-green light in DMSO (Fig. 2B). Other 8-aryl guanosines with *para*-substituted EWGs were also examined and compared. Overall, guanosine **2c**, **2d**, **2e**, and **2f** exhibited obvious redshifted emission (λ_{max} > 470 nm) compared to other guanosines though with comparatively lower quantum yields. It is noteworthy that the emission λ_{max} of 8-aryl guanosines with carbonyl substituents is longer compared to **2g**, **2i**, and **2j** with stronger electron withdrawing abilities. The red-shift emission achieved by *p*-CN, and *p*-carbonyl substitution can be attributed to an extended conjugated structure with smaller energy difference between the highest occupied molecular orbital (HOMO) and the lowest unoccupied molecular orbital (LUMO) [28]. As shown in Fig. 2C, compound **2e** demonstrated the longest FL emission maxima (λ_{max} = 553 nm) likely due to the further extended conjugation by benzyl group, though with a compromise of lower emission efficiency (Φ = 13%). Installing the electron donating group (EDG) at the *para* position of 8-aryl (**2k**, λ_{max} = 387 nm) led to a notably blue-shifted emission (up to 160 nm). For compounds with no substitution's conjugations, including **2k**, **2g**, **2h**, and **2i**, the λ_{max} increases with the increase of electron withdrawing ability. This observation highlights the effective emission control by tuning the electronic property on the 8-aryl group. These results also suggest that electronic effect and conjugation are the synergistic factors to influence the fluorescence performance on guanine.

Tuning electronic density of the 8-aryl group with various *meta*-substituted analogues gave a similar trend but with reduced impact (<24 nm difference) on the emission (Fig. 2E). Most EWG at *meta*-substitution resulted in a blue-shift and lower quantum yields, compared to *para*-substituted derivatives (**2m-2o**, λ_{max} = 395–448 nm, Fig. S14 in Supporting information).

To better examine the influence of electronic effect on the FL property, compounds with substituents possessing simple electronic effect (**2k**, **2g**, **2h**, **2i** and **2t**, **2p**, **2q**, **2r**) were examined (Figs. 2D and E). The Hammett correlation was applied to evaluate the substituent effects on 8-aryl guanosine absorption and fluorescence spectra (see Supporting information for detail). These results confirmed that electron density difference on the 8-phenyl ring and guanine can influence the luminesce as we proposed.

The influence of the sugar ribose on the fluorescence was also evaluated. Guanosine **1b**, **3a**, and **3b** were synthesized and the absorption and emission spectra were collected (Fig. S13 in Supporting information). As expected, the FL emission data of 2′-deoxyguanosine demonstrated a very similar emission trend as 2′-oxy-guanosine, suggesting the strong influence of purine over ribose for the resulting FL emission. The 8-anthracene substituted guanosine **4a** was synthesized with the expectation to extend purine conjugation. Interestingly FL red shift (λ_{max} = 539 nm in DMSO) was received as shown in Fig. 2B. Switching the solvent to less polar dichloroethane (DCE) led to a blue shift (λ_{max} = 476 nm) with significantly improved intensity (see Supporting information for the optical activity of all other compounds in DCE).

With the FL emission of guanosine monomers tested, we continue to explore the fluorescence upon self-assembly in non-polar organic solvents. Treating **2b** and **4a** with K^+ or Na^+ in either $CDCl_3$ or CD_3CN gave a complex with one set of ^1H NMR signals (Figs. S2 and S3 in Supporting information). The guanosine to cation ratio of these complexes is 8:1, indicating the formation of octamers. Fortunately, X-ray single crystal structures of $[(\mathbf{2b})_8\,K]^+PF_6^-$ and $[(\mathbf{4a})_8Na]^+Cl^-$ were successfully determined, which confirmed the formation of H-bonded G_8-octamers (Fig. 3).

The X-ray crystal structures of $[(\mathbf{2a})_8\,K]^+PF_6^-$ revealed the twisted conformation between 8-aryl and purine with a dihedral angle of 43° (Fig. 3B). A larger dihedral angle (73°) was observed with 8-An substituted guanosine complex $[(\mathbf{4a})_8Na]^+Cl^-$, resulting in the stronger steric hindrance between the modified purine and ribose to adopt a *syn* conformation. These crystal data suggest the twisted intermolecular charge transfer (TICT) mechanism for the observed FL emission.

With the conditions for both G-monomer (in DMSO) and G_8 complexes confirmed, their FL emissions were compared. As shown in Fig. 4, a blue shift emission (around 30 nm) was observed with G_8 complexes relative to monomer for both **2b** and **4a**. This result strongly suggested that no extended conjugation was formed in the H-bond linked poly-purine G_4-quartet aggregation (would lead to the red shift) due to the interrupted p-conjugation. To the best of knowledge, this is the first direct evidence for FL emission evaluation upon G_4-quartet formation over monomer, though in different solvents (CH_3CN *vs.* DMSO). The ^1H NMR titration (mixture of

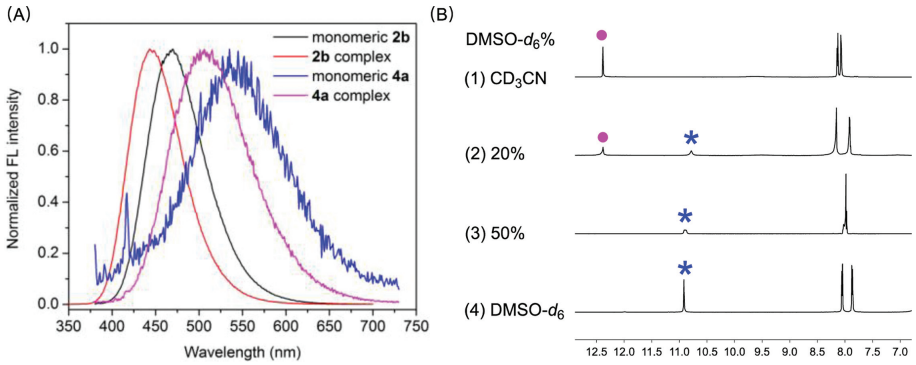


Fig. 4. (A) FL emission of monomeric **2b** and **4a**, $[(\mathbf{2b})_8\,K]^+PF_6^-$ and $[(\mathbf{4a})_8Na]^+Cl^-$. (B) ^1H NMR titration experiment of $[(\mathbf{2b})_8\,K]^+PF_6^-$ complex in (1) CD_3CN, (2) 20% of DMSO–d_6 and CD_3CN solvent mixture, (3) 50% of DMSO–d_6 and CD_3CN solvent mixture, (4) DMSO–d_6. The characteristic proton NH(1) on guanosine was marked by dots and stars. The dot represents the NH(1) proton of complexes while the star represents NH(1) proton of monomers.

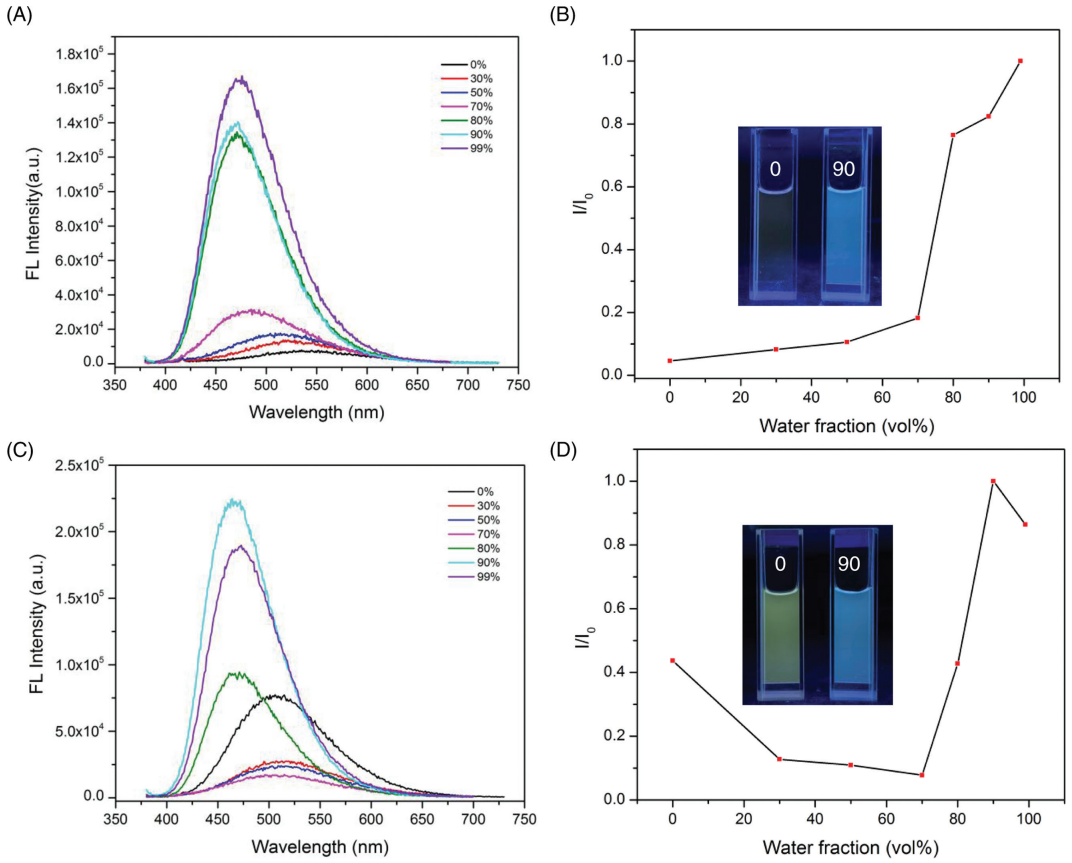


Fig. 5. The titration PL spectra of (A) **4a** (10 μmol/L) in DMSO/water mixtures and (C) $[(\mathbf{4a})_8Na]^+Cl^-$ complex in CH_3CN/water mixtures with increasing water fractions (f_w). Plots of emission intensity of (B) **4a** and (D) $[(\mathbf{4a})_8Na]^+Cl^-$ complex *versus* the water mixtures.

CD_3CN and DMSO–d_6) experiments were also conducted to ensure that the supramolecular equilibrium was between G-monomer and G_8-octomer without observation of intermediate aggregations as shown in Fig. 4B.

The twisted conformations of **2b** and **4a** from the crystal structures raised our interest to know the fluorescence of these guanosine ligands in the solid state. With the 8-aryl guanosines synthesized in gram scale of these guanosine derivatives, their FL emissions were explored under solid state. Interestingly, strong fluorescence was observed for most of these compounds in the solid state (Fig. 3A, see Supporting information for detail). This strong solid-state emission initiated our interest in exploring their potential aggregation-induced emission (AIE) in solution [29–31]. The pioneering work of applying the AIE property for the "turn-on" G-quadruplex probe was reported by Benzhong Tang and co-workers [32]. In their studies, an AIE active salts tetraphenylethenes (TPE), which is a non-emissive dye, was applied and showed surprisingly high luminescent emission after binding to the G_4-quartet by electrostatic interaction. However, no FL-active G_4-quartet has been reported with potential AIE property. To explore the potential AIE property with these FL-active guanosine analogs, compounds **1a**, **2b**, **3b**, and **4a** were applied into the solvent-titration experiments. As shown in Figs. 5A and B, addition of water into the **4a** in DMSO solution gave the drastic increase of FL emission (as of 70%), reflecting a typical AIE phenomenon in solution. Similar AIE effective was observed with the $[(\mathbf{4a})_8Na]^+Cl^-$ complex in CH_3CN

(Figs. 5C and D), which likely attributed to the complex dissociation in protic solvent. Notably, all other tested guanosine derivative did not show this AIE property (Figs. S38-S40 in Supporting information), suggested the unique photoactivity of guanosine **4a**. Detailed mechanistic studies and application of these special AIE guanosine ligands are currently under investigation in our lab.

In this study, we provided the first direct evaluation of FL activity of guanosine upon formation of G_4-quartet. The introduction of various aryl groups on purine C-8 position results in tunable FL emission with strong blue to yellow light emission and large Stokes shift (up to 190 nm). The direct confirmation of guanosine monomer and G-quadruplex FL emission revealed no extended conjugation within G_4-quartet. Interesting AIE property was observed with the *p*-anthracene guanosine, suggesting the promising applications of this new FL system in chemical and biological research.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgments

We are grateful to the National Natural Science Foundation (No. CHE-1665122), the National Institutes of Health (No. 1R01GM120240–01) for financial support.

Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.cclet.2022.03.007.

References

[1] J.T. Davis, G.P. Spada, Chem. Soc. Rev. 36 (2007) 296–313.
[2] J.T. Davis, Angew. Chem. Int. Ed. 43 (2004) 668–698.
[3] M. Martín-Arroyo, A. Del Prado, R. Chamorro, N. Bilbao, D. González-Rodríguez, Angew. Chem. Int. Ed. 59 (2020) 9041–9046.
[4] A.N. Lane, J.B. Chaires, R.D. Gray, J.O. Trent, Nucleic Acids Res. 36 (2008) 5482–5515.
[5] G. Biffi, D. Tannahill, J. McCafferty, S. Balasubramanian, Nat. Chem. 5 (2013) 182–186.
[6] M.L. Bochman, K. Paeschke, V.A. Zakian, Nat. Rev. Genet. 13 (2012) 770–780.
[7] S. Xiao, W. Lee, F. Chen, et al., Chem. Commun. 56 (2020) 6981–6984.
[8] S. Xiao, P.J. Paukstelis, R.D. Ash, P.Y. Zavalij, J.T. Davis, Angew. Chem. Int. Ed. 58 (2019) 18434–18437.
[9] K.B. Sutyak, W. Lee, P.V. Zavalij, O. Gutierrez, J.T. Davis, Angew. Chem. Int. Ed. 57 (2018) 17146–17150.
[10] D. Luo, Y. Mu, J. Phys. Chem. 119 (2015) 4955–4967.
[11] M. Sproviero, R.A. Manderville, Chem. Commun. 50 (2014) 3097–3099.
[12] G.M. Peters, L.P. Skala, J.T. Davis, J. Am. Chem. Soc. 138 (2016) 134–139.
[13] M.I. Umar, D. Ji, C.Y. Chan, C.K. Kwok, Molecules 24 (2019) 2416.
[14] P. Zhu, Y. Zhang, S. Xu, X. Zhang, Chin. Chem. Lett. 30 (2019) 58–62.
[15] H. Cai, C. Zhou, Q. Yang, et al., Chin. Chem. Lett. 29 (2018) 531–534.
[16] L. Yu, Q. Yang, Y. Tang, Chin. Chem. Lett. 30 (2019) 694–697.
[17] Y. Saito, R.H.E. Hudson, J. Photochem. Photobiol. C 36 (2018) 48–73.
[18] D.J.M. Blanchard, T.Z. Cservenyi, R.A. Manderville, Chem. Commun. 51 (2015) 16829–16831.
[19] D.J.M. Blanchard, K.L. Fadock, M. Sproviero, et al., Chem. Comm. 4 (2016) 2915–2924.
[20] K.L. Fadock, R.A. Manderville, P. Sharma, S.D. Wetmore, Org. Biomol. Chem. 14 (2016) 4409–4419.
[21] A. Dumas, N.W. Luedtke, Nucleic Acids Res. 39 (2011) 6825–6834.
[22] A.A. Tanpure, S.G. Srivatsan, Nucleic Acids Res. 43 (2015) 149-149.
[23] T.X. Chen, F. Ning, H.S. Liu, et al., Chin. Chem. Lett. 28 (2017) 1380–1384.
[24] Y. He, Y. Zhang, L. Wojtas, et al., Chem. Sci. 10 (2019) 4192–4199.
[25] Y. He, Y. Zhang, L. Wojtas, Chem. Asian J. 15 (2020) 1030–1034.
[26] Y. Zhang, Y. He, L. Wojtas, X. Shi, H. Guo, Cell Rep. Phys. Sci. 1 (2020) 100211.
[27] Y. He, Y. Zhang, M. Liu, et al., Cell Rep. Phys. Sci. 2 (2021) 100519.
[28] A. Jablonski, Nature 131 (1933) 839–840.
[29] Y. Hong, J.W.Y. Lam, B.Z. Tang, Chem. Soc. Rev. 40 (2011) 5361–5388.
[30] M.H. Lee, A. Sharma, M.J. Chang, et al., Chem. Soc. Rev. 47 (2018) 28–52.
[31] R.T.K. Kwok, C.W.T. Leung, J.W.Y. Lam, B.Z. Tang, Chem. Soc. Rev. 44 (2015) 4228–4238.
[32] Y. Hong, M. Haubler, J.W.e.Y. Lam, et al., Chem. Eur. J. 14 (2008) 6428–6437.