J Fluoresc (2013) 23:853–857
DOI 10.1007/s10895-013-1216-8

SHORT COMMUNICATION

Selective FL Quenching or Enhancing of Diimine Ligands by Guanine

Srung Smanmoo · Shinya Kawasaki · Pramuan Tangboriboonrat · Takayuki Shibata · Tsutomu Kabashima · Masaaki Kai

Received: 3 September 2012 / Accepted: 1 April 2013 / Published online: 25 April 2013
© Springer Science+Business Media New York 2013

Abstract Diimine ligand (DL) **1** significantly exhibited the fluorescence quenching upon binding to guanine. Changing at the *para*-substituent of the phenyl ring from the hydroxyl to bromo groups reversely enhanced the fluorescence in the presence of guanine. The reverse in the fluorescence selectivity indicated the profound effect of the substituent at the *para*-position of the phenyl ring. The simple synthesis of DL **1** and DL **2** with good selectivity for guanine offers these DLs as promising compounds for chemosensors of other guanine derivatives.

Keywords Fluorescence (FL) sensor · Guanine chemical sensor · Diimine ligand (DLs) · Nitrogenous base sensor

Introduction

Molecular recognition is the term defined as the recognition between host and guest molecules by noncovalent bonding, e.g., hydrogen bonding, metal coordination and hydrophobic forces [1–4]. In biological systems, molecular recognition plays a number of important roles [5–7]. For example, the recognition between antibiotic vancomycin and a bacterial peptide, D-alanyl-D-alanine [8]. Recently, Zou et al. has demonstrated the molecular recognition between Puerarin to human serum albumin (HSA) using fluorescence spectroscopy [9]. The recognition of Puerarin by HSA is the result of the combination between a hydrogen bonding and an electrostatic interaction.

Supramolecular system is the artificial molecular recognition. The combination between the molecular recognition part (ionophore) and the fluorophore is highly desired for the development of highly sensitive and selective chemosensor. Changing the physical properties of host molecules upon binding of guests utilizes chemosensor as one of the powerful tools in analytical researches [10–14]. The most renowned host in supramolecular system is crown ether. Crown ether and its derivatives have a high selectivity and specificity to cations depending on the size, type and number of heteroatom present in their structure [15–18]. 15-Crown-5-anthracene has recently been developed for selective recognition of cesium ion [19]. Upon the binding to different concentrations of cesium ions, the fluorescence enhancement of 15-crown-5-anthracene is observed. When one of the oxygen atom in this crown ether is replaced by a nitrogen atom, the selectivity favors barium ion [20].

The detection of the nitrogenous bases is highly desirable because of their significance in medical and biological implications [21–24]. Recently, Jang et al. has demonstrated the first example of adenine recognition by diimine ligand (DL) [25]. Among other nitrogenous bases, the corresponding DL exhibited a good degree of selectivity to adenine. Upon the binding of adenine to the corresponding DL, the fluorescence (FL) was enhanced. The molecular recognition of adenine by DL via the hydrogen bonding inside the ligand's pocket is suggested.

S. Smanmoo (✉)
Bioresources Research Unit, National Center for Genetic Engineering and Biotechnology (BIOTEC), 113 Thailand Science Park, Phaholyothin Road,
Klong Luang, Pathumthani 12120, Thailand
e-mail: srung.sma@biotec.or.th

S. Kawasaki · T. Shibata · T. Kabashima · M. Kai (✉)
Graduate School of Pharmaceutical Sciences, Faculty of Biomedical Sciences, Nagasaki University, 1-14 Bunkyo-Machi, Nagasaki 852-8521, Japan
e-mail: ms-kai@nagasaki-u.ac.jp

P. Tangboriboonrat
Department of Chemistry, Faculty of Science, Mahidol University, Rama 6 Road, Phyathai,
Bangkok 10400, Thailand

We demonstrated the simple method for the FL detection of guanine with DLs. The guanine detection is based on the fluorescence quenching or enhancing of DLs. by guanine. The changing at the *para*-substituent of phenyl rings had a profound effect in FL selectivity. DL **1** exhibited a significant degree of fluorescence quenching upon binding to guanine while the fluorescence intensity of DL **2** is enhanced in the presence of guanine. Guanine titration experiments indicated the binding ratio of guanine to DLs **1** and **2** are to be 1:1 with a high binding constant. The recognition of guanine by DLs **1** and **2** is based on hydrogen bonding between diimine nitrogens and NH groups of guanine.

Experimental Section

Apparatus

Fluorescence measurements were carried out using a FP-6300 spectrofluorometer (JASCO) equipped with a xenon lamp source and a 1.0-cm quartz cell, and the scan speed was 600 nm min^{-1}. ^{1}H spectrum was recorded on Bruker DPX 400 MHz spectrometer in $CDCl_3$ using TMS as the internal standard. Mass spectra were recorded on Bruker Esquire and Finnigan MAT INCOS 50 mass spectrometers.

Reagents

All reagents for the synthesis of diimine ligand phosphates obtained commercially were used without further purification. The corresponding nitrogenous bases (adenine, guanine, thymine, cytosine and uracil) used in this study were purchased from Sigma Aldrich (USA) and used without further purification. Methanol was used as a HPLC grade. All other chemicals used were supplied from Sigma Aldrich (USA) as analytical grade and used without further purification. MilliQ water was used throughout this study. The concentration of stock solution of metal ions was 1 mM. DL **2** was prepared according to the literature [26].

Synthesis of 4,4′-[1,2-Phenylenebis(nitrilomethylidene)]bisphenol (DL 1)

DL **1** was synthesized from the condensation between a corresponding 4-hydroxybenzaldehyde (1 mmol) and *o*-phenylenediamine (1 mmol) in the presence of distilled water (10 mL). After the reaction mixture was left stirring for 6 h, the crude solid was collected by filtration and crystallized from methanol to obtain the final product in good yields. DL2 was obtained as white crystals (2.40 g, 82 %). ^{1}H NMR (DMSO-*d6*) δ_H 8.94 (s, 2H), 7.67 (d, 2H), 7.46 (m, 4H), 7.41 (m, 4H), 6.97 (m, 4H). ^{13}C NMR δ 117.6, 119.1, 119.4, 119.8, 127.9, 132.5, 133.5, 142.6, 161.5, 163.8. MS (ESI) $[M+H]^+$ 317.35.

Results and Discussion

DLs **1** and **2** were prepared according to the literature procedure and obtained in good yields (89 % and 92 %) (Fig. 1) [12, 26]. Although, DL **1** and DL **2** dissolved in various organic solvents, methanol was chosen as a solvent system. When excited at 310 nm, DL **1** was shown with the maximum fluorescence emission at 355 nm while DL **2** emitted the fluorescence at 350 nm. The fluorescence spectra of DLs **1** and **2** were recorded at 2.5×10^{-6} M concentration in MeOH:H_2O (10:90, v/v).

The recognition of other nitrogenous bases was then investigated. Five nitrogenous bases (adenine, guanine, cytosine, thymine and uracil) were screened in the presence and absence of DLs **1** and **2**. As expected, no FL emission was observed for all nitrogenous bases. In the presence of DL **1**, adenine, cytosine, thymine and uracil slightly quenched the fluorescence emission intensity of DL **1**. Interesting, there was a significant FL quenching of DL **1** when guanine was added. It was clear that guanine exhibited a good selectivity for the FL quenching of DL **1** (Fig. 2a). Next, same set of nitrogenous bases were investigated. This time, adenine, cytosine, thymine and uracil slightly quenched the FL intensity of DL **2**. However, in the presence of guanine, the fluorescence emission intensity of DL **2** was significantly enhanced. Therefore, DL **2** confers a good selectivity for the FL enhancement in the presence of guanine. These results indicated the determining roles of *para*-substituents of DLs' phenyl rings for the fluorescence selectivity.

To confirm the FL quenching and enhancing of DLs **1** and **2** by guanine, the fluorescence ratio $(I\text{-}I_0)/I_0$ was determined (Fig. 3). From Fig. 3, the high selectivity for FL quenching by guanine was clearly observed for DL **1**. In the presence of other nitrogenous bases, no significant degree of FL quenching for DL **1** was observed (Fig. 3a). DL **2** exhibited the significant FL enhancing effect upon binding to guanine and showed less response to other nitrogenous bases (Fig. 3b).

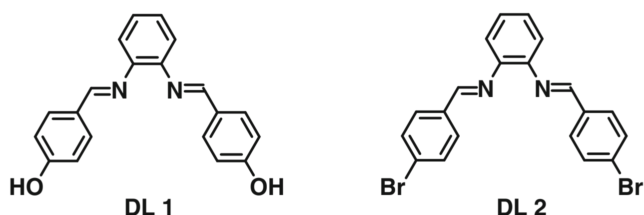

Fig. 1 DLs **1** and **2**

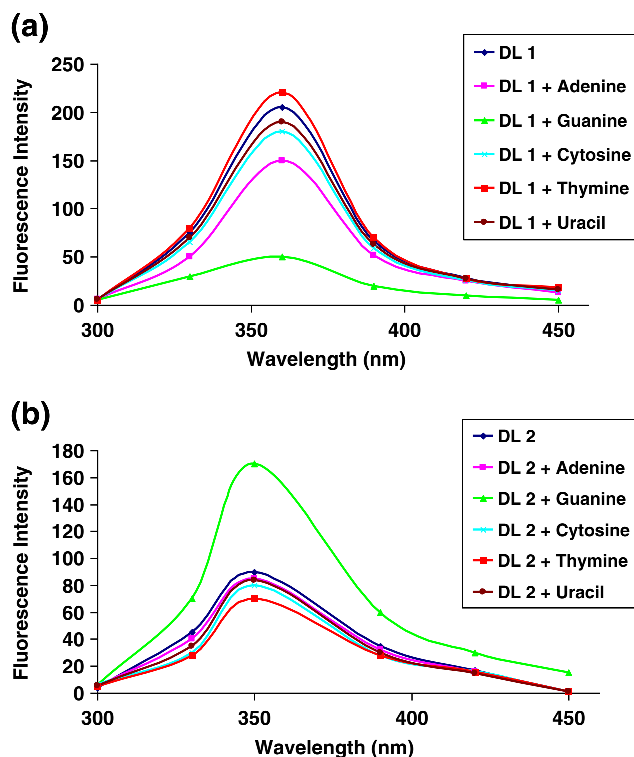

Fig. 2 **a** Changes in fluorescence emission intensity of DL **1** (2.5×10^{-6} M) and **b** changes in fluorescence emission intensity of DL **2** (2.5×10^{-6} M) upon the addition of 1 equiv of different nitrogenous bases (Adenine, Guanine, Cytosine, Thymine and Uracil) in MeOH: H_2O (10:90, v/v) with the excitation at 310 nm

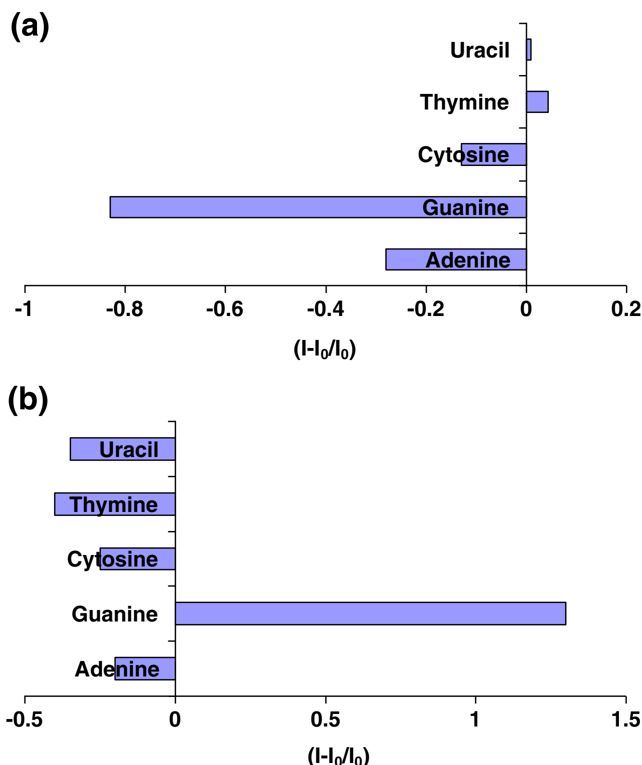

Fig. 3 Fluorescence ratio (I-I_0/I_0) of **a** DL **1** (2.5×10^{-6} M) and **b** DL **2** (2.5×10^{-6} M) upon the addition of 1 equiv of different nitrogenous bases in MeOH:H_2O (10:90; v/v)

The recognition of guanine by DLs **1** and **2** is suggested to occur via the hydrogen bonding between the diimine nitrogens and the NH groups of guanine (Fig. 4). Unlike the Jang's binding model, the hydrogen bonding for DLs **1** and **2** is only from the diimine nitrogens of DLs and the NH groups of guanine [12, 25]. The participation of the hydrogen bonding between the NH groups of guanine and the hydroxyl groups at the *para*-position of the phenyl rings is unlikely to occur as they are to far for the interaction.

Next the fluorescence titrations of DLs **1** and **2** by guanine were carried out. When 1 equiv of guanine was added to the DL **1** solution, a significant FL quenching was observed as shown in Fig. 5a. Increasing the guanine concentration did not induce a significant quenching of DL **1**. The change of FL quenching after the addition of 1 equiv of guanine to DL **1** indicated the critical molar ratio of 1:1 between DL **1** and guanine. As expected, in the presence of guanine, the fluorescence of DL **2** was significantly enhanced. The FL enhancement of DL **2** was noticed when 1 equiv of guanine was added. Further addition of guanine slightly improved the FL enhancement of DL **2**. The saturation for the FL enhancement of DL **2** was observed after 1 equiv guanine was added (Fig. 5b). Therefore, the molar ratio (1:1) between DLs **1** or **2** and guanine was the same. The molar ratio was confirmed by plotting the change of fluorescence intensity against the molar ratio between guanine and DLs. It was obvious that a significant change in the difference of fluorescence intensity was detected at a 1:1 ratio between guanine and DLs (Fig. 6). Beyond this ratio, there was no further change of fluorescence intensity. This **2** indicated the same

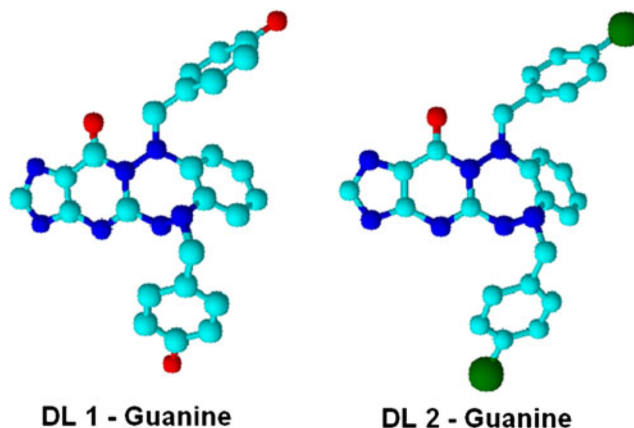

Fig. 4 MM2 energy-optimized complexes (MacroModel 7.1, MM2* force field) [27] of DL **1**·Guanine and DL **2**·Guanine

mode of binding between DLs **1** and **2** to guanine [25]. The FL enhancement of DL **2** by guanine was also suggested by Jang et al. [25]. The increasing of FL intensity upon the increasing concentration of guanine indicates the rigidity of the conformational complexes formed between guanine and DL **2**. The rigidity of the complex prohibits the non-radiative decay from the excited state which results in increasing of the FL intensity.

It is interesting that whether the presence of other nitrogenous bases could disturb the effects of FL quenching or enhancing of DLs **1** and **2** by guanine, the interference was subsequently investigated. As shown in Fig. 7, no interference of the FL quenching of DL **1** by guanine was observed in the presence of other nitrogenous bases. The FL quenching of DL **1** by guanine was the same either in the absence or presence of other nitrogenous bases.

The similar result was observed for DL **2**, i.e., the presence or absence of other nitrogenous bases had no effect for the FL enhancement of DL **2** by guanine. Therefore, it was clear that DLs **1** and **2** exhibited a very good selectivity for guanine. The presence of other nitrogenous bases did not affect the FL quenching or enhancing of DLs by guanine.

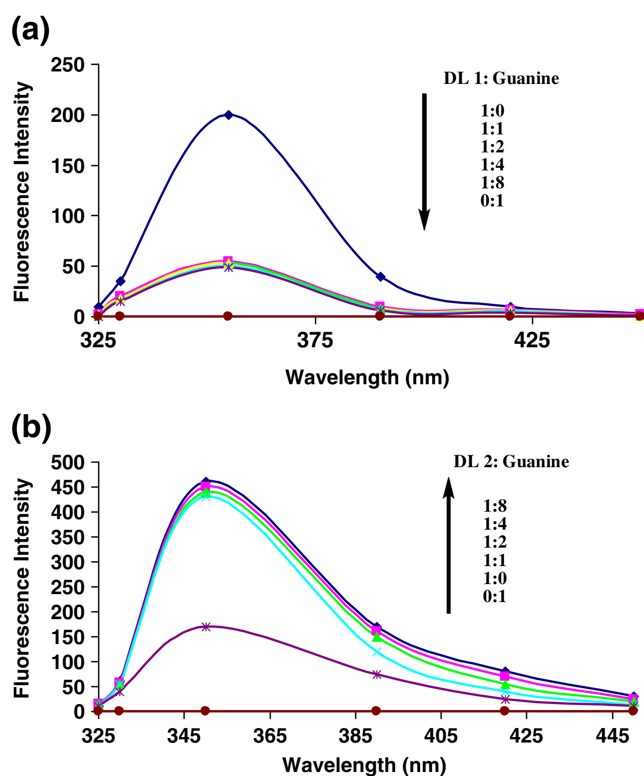

Fig. 5 Fluorescence spectra changes of **a** DL **1** and **b** DL **2** upon the addition of guanine excited at 310 nm in MeOH:H_2O (10:90; v/v). [DL]=2.5×10^{-6} M

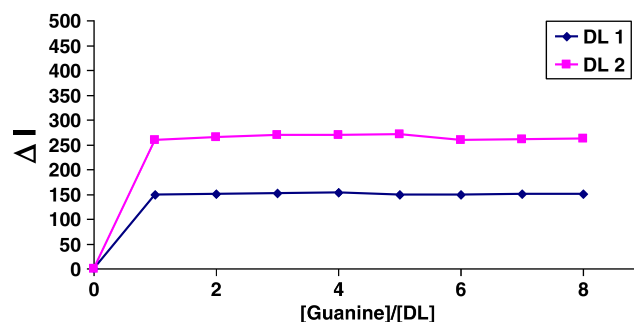

Fig. 6 Fluorescence titration curves ([Guanine]/[DL]) vs change in FL emission (λex=310 nm) in MeOH:H_2O (10:90; v/v). [DL]=2.5×10^{-6} M

Conclusion

In conclusion, DLs **1** and **2** were the first-time evaluated as promising selective chemosensors for the FL sensing of nitrogenous base, guanine. The complex formation constants (log *K*) of DLs **1** and **2** showed with the high affinity to guanine (5.68 and 5.54 for DL **1** and **2**, respectively) [27]. The detection limit by these DLs is as low as 1×10^{-6} M. The mode of recognition for guanine by DLs **1** and **2** was suggested to be similar to what is observed by Jang et al. [25]. which DLs's diimine nitrogens and NH groups of guanine play a determining role for this recognition. The selectivity of FL is dependent on *para*-substituents of the DLs' phenyl rings. Changing the *para*-subtituents from hydroxyl to bromo groups reverses the FL selectivity from quenching to the enhancement. The role of the substituents at this position is currently being investigated. The binding ratio between DLs **1** and **2** with guanine was determined to be 1:1 which is similarly observed by Jang et al. The simple synthesis of DLs and a high selectivity for guanine offer these DLs as promising chemosensors for further development for FL sensing other guanine derivatives.

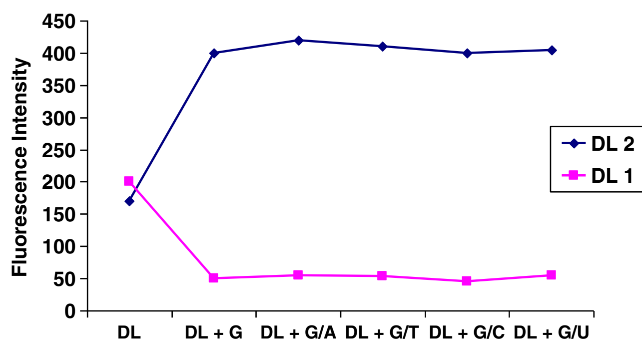

Fig. 7 The effect of other nitrogenous bases (1 equiv) as the interferences in MeOH:H_2O (10:90; v/v)

Acknowledgments We thank the Japan Society for the Promotion of Science (JSPS) and The Thailand Research Fund (TRF)/the Commission on Higher Education (CHE)'s research grant to P.T. and S.S. for the financial support.

References

1. Ariga K, Kunitake T (1998) Molecular recognition at air-water and related interfaces: complementary hydrogen bonding and multisite interaction. Acc Chem Res 31:371
2. Kienberger F, Ebner A, Gruber HJ, Hinterdorfer P (2006) Molecular recognition imaging and force spectroscopy of single biomolecules. Acc Chem Res 39:29
3. Pietraszkiewicz M, Prus P, Pietraszkiewicz O (2004) Synthesis of novel, boron-containing cavitands derived from calix[4] resorcinarenes and their molecular recognition of biologically important polyols in Langmuir films. Tetrahedron 60:10747
4. Idzik KR, Cywinski PJ, Cranfield CG, Mohr GJ, Beckert R (2011) Molecular recognition of the antiretroviral drug abacavir: towards the development of a novel carbazole-based fluorosensor. J Fluoresc 21:1195
5. Wang J (2010) Cellular and biomolecular recognition: synthetic and non-biological molecules. J Am Chem Soc 132:6611
6. Gellman SH (1997) Introduction: molecular recognition. Chem Rev 97:1231
7. Wang H, Yang R, Yang L, Tan W (2009) Nucleic acid conjugated nanomaterials for enhanced molecular recognition. ACS Nano 3:2451
8. Knox JR, Pratt RF (1990) Different modes of vancomycin and D-alanyl-D-alanine peptidase binding to cell wall peptide and a possible role for the vancomycin resistance protein. Antimicrob Agents Chemother 34:1342
9. He Y, Wang YW, Tang LF, Liu H, Chen W, Zheng ZL, Zou GL (2007) Binding of puerarin to human serum albumin: a spectroscopic analysis and molecular docking. J Fluoresc 18:433
10. Goswami P, Das K (2012) N, N, N, N-tetradentate macrocyclic ligand based selective fluorescent sensor for zinc (II). J Fluoresc 22:1081
11. Martínez-Máñez R, Sancenón F (2005) New advances in fluorogenic anion chemosensors. J Fluoresc 15:267
12. Nasomphan W, Tangboriboonrat P, Smanmoo S (2011) Selective fluorescence sensing of deoxycytidine 5′-monophosphate (dCMP) employing a bis(diphenylphosphate)diimine ligand. J Fluoresc 21:187
13. Kuhn KJ, Dyke JT (1996) A renewable-reagent fiber-optic sensor for measurement of high acidities. Anal Chem 68:2890
14. Xu S, Mutharasan R (2011) Cell viability measurement using 2′,7′-bis-(2-carboxyethyl)-5-(and-6)-carboxyfluorescein acetoxymethyl ester and a cantilever sensor. Anal Chem 83:1480
15. Shirai M, Matoba Y, Tsunooka M (1997) Cation-responsive fluorescence of dansyl-labeled polyanions bearing crown ether units. J Fluoresc 4:245
16. Shirai M, Murakami H, Tsunooka M (1996) Cation-sensitive fluorescence of anionic polymers bearing naphtho-18-crown-6 units. J Fluoresc 6:237
17. Seo HS, Lee SH (2011) A novel fluorescent cesium ion-selective optode membrane based on 15-crown-5-anthracene. J Fluoresc 21:747
18. Batista RMF, Oliveira E, Costa SPG, Lodeiro C, Raposo MMM (2011) Imidazo-benzo-15-crown-5 ether bearing arylthienyl and bithienyl moieties as novel fluorescent chemosensors for Pd^{2+} and Cu^{2+}. Tetrahedron 67:7106
19. Seo HS, Karim MM, Lee SH (2008) Selective fluorimetric recognition of cesium ion by 15-crown-5-anthracene. J Fluoresc 18:853
20. Kondo S, Takahashi T, Takiguchi Y, Unno M (2011) Synthesis and photophysical properties of a 2,2′-bianthracene-based receptor bearing two aza-15-crown-5 ether for naked-eye detection of barium ion. Tetrahedron Lett 52:453
21. Huang W, Morita K, Sankaran NB, Nishizawa S, Teramae N (2006) Electrochemical detection at low temperature for a specific nucleobase of target nucleic acids by an abasic site-containing DNA binding ligand. Electrochem Commun 8:395
22. Cui Q, Ma K, Shao Y, Xu S, Wu F, Liu G, Teramae N, Bao H (2012) Gap site-specific rapid formation of fluorescent silver nanoclusters for label-free DNA nucleobase recognition. Anal Chim Acta 724:86
23. Goyal RN, Chatterjee S, Rana ARS, Chasta H (2011) Application of modified pyrolytic graphite electrode as a sensor in the simultaneous assay of adenine and adenosine monophosphate. Sensors Actuators B:Chem 156:198
24. Ghosh K, Sen T, Fröhlich R (2007) Adenine-based receptor for dicarboxylic acids. Tetrahedron Lett 48:7022
25. Singh N, Lee GW, Jang DO (2008) Highly selective imine-linked fluorescent chemosensor for adenine employing multiple hydrogen bonding. Tetrahedron Lett 49:44
26. Issa RM, Marghalani YA, El-Masry AM (1979) Infrared spectroscopic investigation of some ortho-, meta-, and para-dianils. Proc Pak Acad Sci 15:1
27. The modeling study was carried out using MM2* force field implemented in MacroModel 7.1 program on a Silicon Graphics Indigo IMPACT workstation. To find an energy-minimized structure, 1000 separate search steps were conducted with a Monte Carlo conformational search