Bioorganic & Medicinal Chemistry Letters 22 (2012) 4668–4671

Contents lists available at SciVerse ScienceDirect

Bioorganic & Medicinal Chemistry Letters

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

Photoinduced DNA cleavage by anthracene based hydroxamic acids

Nilanjana Chowdhury, Swagata Dasgupta, N. D. Pradeep Singh *

Department of Chemistry, Indian Institute of Technology Kharagpur, Kharagpur 721302, India

ARTICLE INFO

Article history:
Received 31 January 2012
Revised 23 April 2012
Accepted 23 May 2012
Available online 30 May 2012

Keywords:
Hydroxamic acid
DNA cleavage
UV light

ABSTRACT

Two different series of naphthalene and anthracene based hydroxamic acids having amino acid derivatives were synthesized. Single strand DNA cleavage was achieved on irradiation of newly synthesized hydroxamic acids by UV light ($\geqslant$350 nm). Both reactive oxygen species (ROS) and generated radicals from hydroxamic acids were shown to be responsible for the DNA cleavage. Further, DNA cleaving ability of hydroxamic acids was found to be dependent on its concentration and on its structure.

Hydroxamic acids are a family of weak organic acids having general formula RCONR′OH. They are generally considered to be derivatives of hydroxylamine and carboxylic acids. Hydroxamic acids and its derivatives have attracted considerable attention due to their wide spectrum of biological activities such as anti-inflammatory, antimetastatic, anti-asthmatic, anti-biotic, psychotropic, insecticidal and nematocidal activity.[1]

During the last few decades, there has been continuous upsurge of interest in the development of hydroxamic acid derivatives as potential anticancer agents mainly due to their ability to inhibit Histone deacetylase (HDAC).[2] In 2001, Richon and its co-worker[3] reported that suberoylanilide hydroxamic acid (SAHA) as HDAC inhibitor that induces cell cycle arrest, differentiation and/or apoptosis in transformed cultured cell lines. Recently, SAHA has been approved by the FDA for the treatment of cutaneous T-cell lymphoma.[4] Further, several groups have also reported potent HDAC inhibitors based on hydroxamic acid analogous as promising chemotherapeutic agents such as trichostatin A,[5] scriptaid[6] and oxamflatin.[7] Apart from HDAC inhibitors other types of hydroxamic acid derivatives are also known which causes DNA damage using their hydrolytic and oxidative capabilities in the presence of metal complexes.[8]

There have been an increasing number of reports concerning light-induced DNA scission by a variety of metal complexes having hydroxamic acid groups attached to the ligand system.[8] But, till date there has been no report on the photoinduced DNA cleaving ability of hydroxamic acids in the absence of metal complexes.

After 1980s, significant progress has been made to understand the photochemical behavior of hydroxamic acids.[9] So far two types of photodisassociation mechanism has been reported on direct irradiation of hydroxamic acid (i) homolytic N–O bond cleavage from the singlet excited state resulting in the formation of anilides and amides,[10] (ii) generation of primary intermediate acylaminyl oxyl radicals by loss of hydrogen radical from hydroxyl group.[11] The promising anticarcinogenic ability and facile photocleavage property of hydroxamic acids tempted us to investigate their DNA cleaving ability under light.

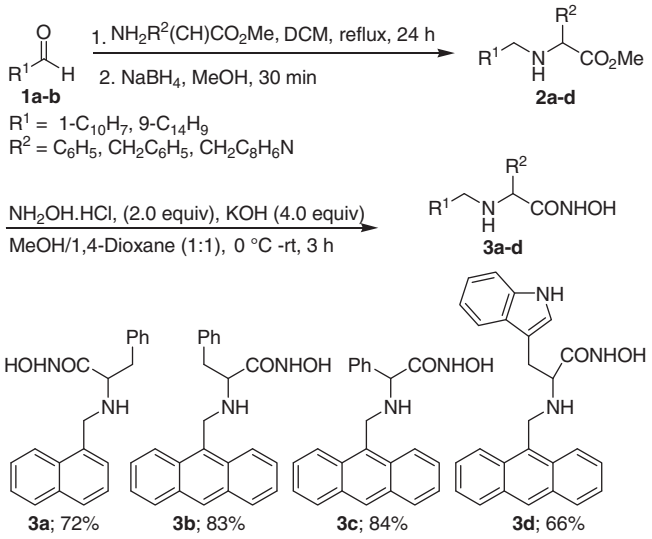
Synthesis of hydroxamic acids, **3a**–**d** of *series-I* were carried out as depicted in Scheme 1. First, reductive amination of aldehydes **1a–b** with amino acid esters furnished desired esters **2a–d**. Next, treatment of esters **2a–d** with $NH_2OH{\cdot}HCl$ in methanol/1,4 -dioxane mixture provided hydroxamic acids **3a–d** in moderate to good yield.

Second series of hydroxamic acid derivatives, **6a**–**d**, were synthesized using the procedure as shown in Scheme 2. Cinnamic acids **4a–b** were initially converted to corresponding acid chlorides by using oxalyl chloride in DCM in presence of catalytic amount of DMF. The freshly prepared acid chlorides were then treated with various amino acid esters in presence of triethyl amine to produce corresponding condensation products **5a–d**. Treatment of **5a–d** with $NH_2OH{\cdot}HCl$ in methanol/1,4-dioxane mixed solvent resulted hydroxamic acids **6a–d** in moderate to good yields. Hydroxamic acids **3a–d** and **6a–d** were characterized by NMR, mass spectrum and elemental analysis.

The UV/vis absorption and emission spectra of degassed 4×10^{-6} M solution of hydroxamic acids (**3a–d** and **6a–d)** in absolute ethanol were recorded. The absorption and emission maxima, molar absorptivities (ε) and fluorescence quantum yield (Φ_F) of above hydroxamic acids were summarized in Table 1. Fluorescence quantum yields were calculated using anthracene as standard ($\Phi_F = 0.27$ in ethanol).[12]

* Corresponding author. Tel.: +91 3222 282324; fax: +91 3222 282252.
E-mail address: ndpradeep@chem.iitkgp.ernet.in (N.D. Pradeep Singh).

0960-894X/$ - see front matter © 2012 Elsevier Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.bmcl.2012.05.084



Scheme 1. Synthesis of naphthalene and anthracene based hydroxamic acids **3a–d.**

To find out the optimum concentration for DNA cleavage by the above mentioned hydroxamic acids, initially we irradiated compound **3b** at different concentration (10–100 μM) dissolved in sodium phosphate buffer (pH 7.0, 10 mM) with 50% DMF containing the pBR322 plasmid DNA (form I; 62.5 μg/ml) for 1 h under aerobic condition. By analyzing the results from gel electrophoresis on 1.0% agarose gel with ethidium bromide staining, we found that compound **3b** caused the single strand cleavage of DNA (form I) to give the relaxed circular DNA (form II) and the DNA cleaving ability of **3b** enhanced with the increase of its concentration (Fig. 1). Based on the above concentration study, the DNA cleaving ability of all hydroxamic acids were carried out using 100 μM concentration for a period of 1 h and the results are

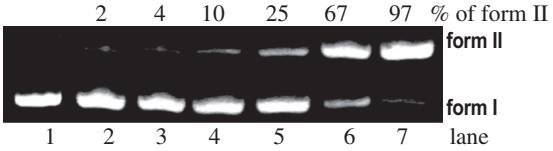
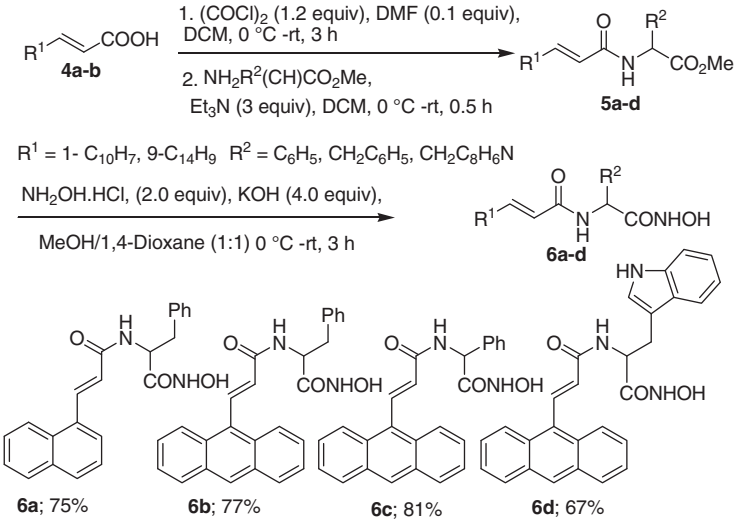


Figure 1. Single strand cleavage of supercoiled circular pBR322 DNA (form I) to relaxed circular DNA (form II) was carried out by compound **3b** at different concentration (10–100 μM) in a sodium phosphate buffer (pH 7.0, 10 mM) upon irradiation of UV light (⩾350 nm) under aerobic condition at room temperature for 1 h. The resultant products were subjected to electrophoresis on 1% agarose gel followed by ethidium bromide staining under UV light. Lane 1, DNA alone; lane 2, DNA + **3b** (10 μM); lane 3, DNA + **3b** (25 μM); lane 4, DNA + **3b** (40 μM); lane 5, DNA + **3b** (50 μM); lane 6, DNA + **3b** (75 μM); lane 7, DNA + **3b** (100 μM).



Scheme 2. Synthesis of naphthalene and anthracene based hydroxamic acids **6a–d.**

Table 1
UV/vis and fluorescence data of hydroxamic acids (**3a–d** and **6a–d**) in absolute ethanol

Entry	Compound	UV/vis		Fluorescence		
		λ_{max}[a] (nm)	log ε[b]	λ_{max}[c] (nm)	Stokes' shift[d] (nm)	Φ_F[e]
1	**3a**	315	4.00	–	–	–
2	**3b**	384	3.84	412	28	0.012
3	**3c**	385	3.98	413	28	0.010
4	**3d**	387	3.75	412	25	0.008
5	**6a**	317	4.06	–	–	–
6	**6b**	390	3.93	503	113	0.015
7	**6c**	390	3.84	502	112	0.018
8	**6d**	391	3.72	504	113	0.010

[a] Maximum absorption wavelength.
[b] Molar absorption coefficient (in mol^{-1} L cm^{-1}) at the maximum absorption wavelength.
[c] Maximum emission wavelength.
[d] Difference between maximum absorption wavelength and maximum emission wavelength.
[e] Fluorescence quantum yield (error limit within ± 5%).

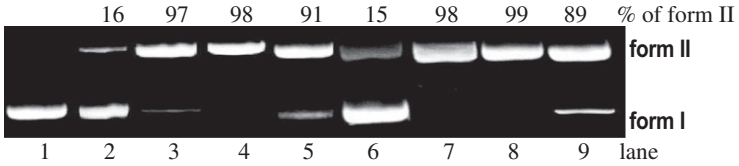


Figure 2. Single strand cleavage of supercoiled circular pBR322 DNA (form I) to relaxed circular DNA (form II) was carried out by compounds **3a–d** and **6a–d** (100 μM) upon irradiation of UV light (⩾350 nm) in a sodium phosphate buffer (pH 7.0, 10 mM) under aerobic condition at room temperature for 1 h. The resultant products were subjected to electrophoresis on 1% agarose gel followed by ethidium bromide staining under UV light. Lane 1, DNA alone; lane 2, DNA + **3a;** lane 3, DNA + **3b**; lane 4, DNA + **3c**; lane 5, DNA + **3d**; lane 6, DNA + **6a**; lane 7, DNA + **6b**; lane 8, DNA + **6c;** lane 9, DNA + **6d.**

presented in Figure 2. All hydroxamic acids showed remarkable DNA cleaving ability except naphthalene based hydroxamic acids **3a** and **6a**. Anthracene based hydroxamic acids containing phenylalanine and phenylglycine amino acid residue showed better DNA cleaving ability. Both *series I* and *series II* compounds exhibited almost similar DNA cleaving ability.

To understand the mechanistic aspects of the photoinduced DNA cleavage by hydroxamic acids, compound **3b** was studied under different conditions. The DNA photocleavage reactions involving molecular oxygen could proceed *via* two major pathways, viz the type-II process forming singlet oxygen ($^1O_2$) species or a photo-redox pathway forming reactive hydroxyl radicals. To understand the involvement of the singlet oxygen during the course of DNA cleavage, control experiments were carried out using NaN_3, a known singlet oxygen quencher.[13] The experiments were carried out by adding increasing amounts of NaN_3 in the phosphate buffer solution containing compound **3b** and supercoiled circular pBR322 DNA. The percentage of form II DNA decreased from 97% to 64% on increasing the amount of NaN_3 from 0 to150 mM (Fig. 3). Further increase in the amount of NaN_3 from 150 to 600 mM has no effect on the percentage of form II DNA. Thus the contribution resulting from singlet oxygen to DNA cleavage is ≈33%.

Further, hydroxyl radical scavengers, viz DMSO also showed partial inhibition in the DNA cleavage activity[14] (Fig. 4). The above results suggested that formation of both singlet oxygen and hydroxyl radicals as reactive oxygen species (ROS) are responsible for the photocleavage of DNA. The singlet oxygen formation was also confirmed from the enhancement of the DNA cleavage activity in D_2O due to longer lifetime of $^1O_2$ in D_2O medium[15] (Fig. 4).

To investigate the role of radicals generated from hydroxamic acid in the DNA photocleavage process, we carried out control experiment in presence of ascorbic acid, a known radical scavenger. Figure 4 showed that addition of ascorbic acid to supercoiled circular DNA partially inhibited the photoinduced DNA cleavage ability of **3b**.

Finally, To understand the essential role of hydroxamic acid moiety in the photoinduced DNA cleavage process, we have synthesized compound **7** and **8** [which are analogous to compound **3b** and **6b** except hydroxamic acid (CONHOH) moiety was replaced by carboxylic acid (COOH)] as shown in Scheme 3 (for synthetic procedure see Supplementary data).

To compare the DNA cleaving ability of carboxylic acids **7** and **8** with corresponding hydroxamic acids **3b** and **6b**, we irradiated supercoiled circular pBR322 DNA for 1 h in presence of 100 μM of above said acids individually and the results were presented in Figure 5. The results revealed that hydroxamic acids **3b** and **6b** showed better DNA cleaving ability compared to their corresponding acid analogous **7** and **8** indicating the importance of hydroxamic acid moiety in DNA cleavage process.

In conclusion, we have reported the simple, convenient and high yielding synthesis of naphthalene and anthracene based hydroxamic acids of amino acid derivatives. The gel electrophoresis data showed concentration and substrate dependent DNA

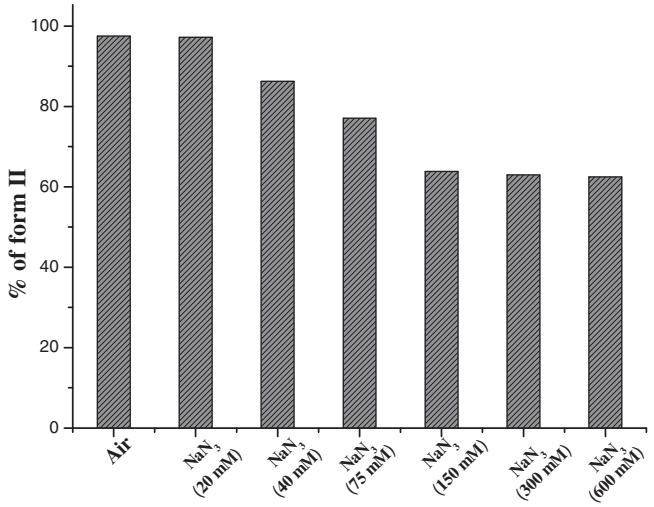


Figure 3. Bar diagram displaying the single strand cleavage of supercoiled circular pBR322 DNA (form I) to relaxed circular DNA (form II) by compounds **3b** (100 μM) in presence of increasing amount of NaN_3 (20–600 mM) in a sodium phosphate buffer (pH 7.0, 10 mM) upon irradiation of UV light (⩾350 nm) under aerobic condition at room temperature for 1 h. The resultant products were subjected to electrophoresis on 1% agarose gel followed by ethidium bromide staining under UV light.

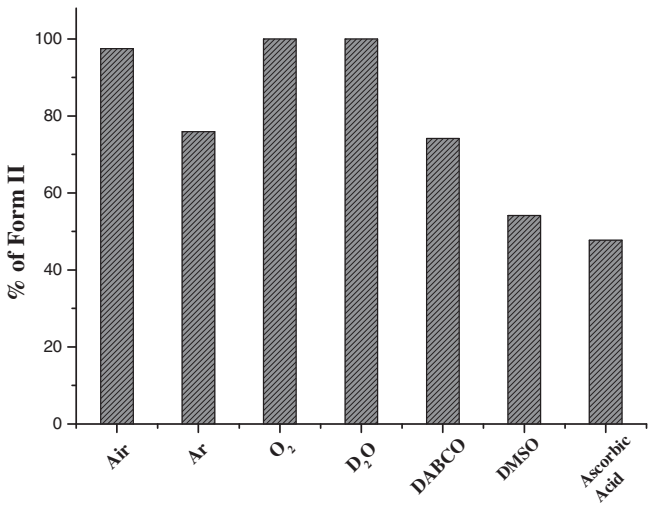
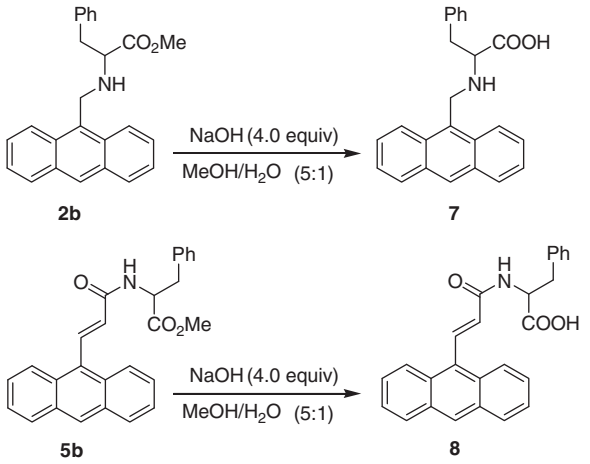


Figure 4. Bar diagram displaying the single strand cleavage of supercoiled circular pBR322 DNA (form I) to relaxed circular DNA (form II) by compounds **3b** (100 μM) in presence of various additives in a sodium phosphate buffer (pH 7.0, 10 mM) upon irradiation of UV light (⩾350 nm) under aerobic condition at room temperature for 1 h. The resultant products were subjected to electrophoresis on 1% agarose gel followed by ethidium bromide staining under UV light. The additive concentrations/quantities are: D_2O, 4 μL; DABCO, 50 mM; DMSO, 4 μL, Ascorbic acid, 50 mM.



Scheme 3. Synthesis of naphthalene and anthracene based carboxylic acids **7** and **8.**

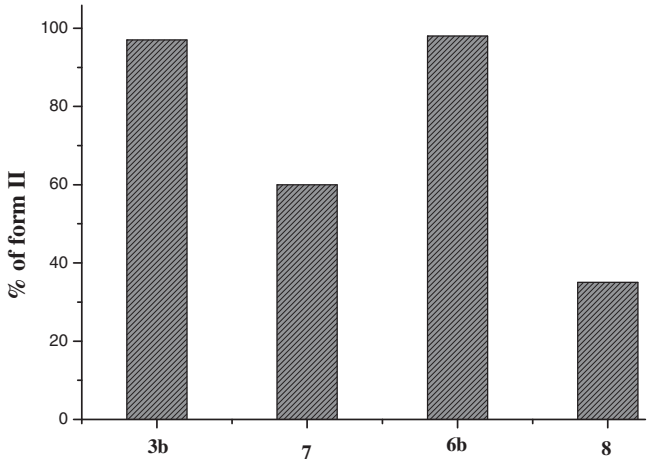


Figure 5. Bar diagram displaying the single strand cleavage of supercoiled circular pBR322 DNA (form I) to relaxed circular DNA (form II) by compounds **3b, 7, 6b** and **8** (100 μM) in a sodium phosphate buffer (pH 7.0, 10 mM) upon irradiation of UV light (⩾350 nm) under aerobic condition at room temperature for 1 h. The resultant products were subjected to electrophoresis on 1% agarose gel followed by ethidium bromide staining under UV light**.**

cleaving ability of hydroxamic acids in the presence of UV light (⩾350 nm).

Acknowledgments

We thank DST (SERC Fast Track Scheme) for financial support, DST-FIST for 400 MHz NMR. Nilanjana is thankful to UGC for research fellowship.

Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bmcl.2012.05.084.

References and notes

1. (a) Neilands, J. B. *Science* **1967**, *156*, 1443; (b) Weber, G. *Cancer Res.* **1983**, *43*, 3466; (c) Miller, M. *J. Chem. Rev.* **1989**, *89*, 1563.
2. (a) Hockly, E.; Richon, V. M.; Woodman, B.; Smith, D. L.; Zhou, X.; Rosa, E.; Sathasivam, K.; Ghazi-Noori, S.; Mahal, A.; Lowden, P. A. S.; Steffan, J. S.; Marsh, J. L.; Thompson, L. M.; Lewis, C. M.; Marks, P. A.; Bates, G. P. *Proc. Natl. Acad. Sci. U.S.A.* **2003**, *100*, 2041; (b) Kelly, W. K.; Marks, P. A. *Nat. Rev. Clin. Oncol.* **2005**, *2*, 150; (c) Bieliauskas, A. V.; Weerasinghe, S. V. W.; Pflum, M. K. H. *Bioorg. Med. Chem. Lett.* **2007**, *17*, 2216; (d) Yin, D.; Ong, J. M.; Hu, J.; Desmond, J. C.; Kawamata, N.; Konda, B. M.; Black, K. L.; Koeffler, H. P. *Clin. Cancer Res.* **2007**, *13*, 1045; (e) Fiskus, W.; Ren, Y.; Mohapatra, A.; Bali, P.; Mandawat, A.; Rao, R.; Herger, B.; Yang, Y.; Atadja, P.; Wu, J.; Bhalla, K. *Clin. Cancer Res.* **2007**, *13*, 4882; (f) Marks, P. A.; Rifkind, R. A.; Richon, V. M.; Breslow, R. *Clin. Cancer Res.* **2001**, *7*, 759; (g) Hebbel, R. P.; Vercellotti, G. M.; Pace, B. S.; Solovey, A. N.; Kollander, R.; Abanonu, C. F.; Nguyen, J.; Vineyard, J. V.; Belcher, J. D.; Abdulla, F.; Osifuye, S.; Eaton, J. W.; Kelm, R. J., Jr.; Slungaard, A. *Blood* **2010**, *115*, 2483; (h) Acharya, M. R.; Sparreboom, A.; Venitz, J.; Figg, W. D. *Mol. Pharmacol.* **2005**, *68*, 917.
3. Butler, L. M.; Zhou, X.; Xu, W. –S.; Scher, H. I.; Rifkind, R. A.; Marks, P. A.; Richon, V. M. *Proc. Natl. Acad. Sci. U.S.A.* **2002**, *99*, 11700.
4. (a) Mann, B. S.; Johnson, J. R.; Cohen, M. H.; Justiceand, R.; Pazdur, R. *Oncologist* **2007**, *12*, 1247; (b) Saharan, R.; Singh, R.; Nagar, N.; Verma, S.; Sharma, V.; Yadav, S.; Nagori, B. P. *Pharmacophore* **2011**, *2*, 104.
5. (a) Vigushin, D. M.; Ali, S.; Pace, P. E.; Mirsaidi, N.; Ito, K.; Adcock, I.; Coombes, R. C. *Clin. Cancer Res.* **2001**, *7*, 971; (b) Platta, C. S.; Greenblatt, D. Y.; Kunnimalaiyaan, M.; Chen, H. *J. Surg. Res.* **2007**, *168*, 197; (c) Zhang, X.; Yashiro, M.; Ren, J.; Hirakawa, K. *Oncol. Rep.* **2006**, *16*, 563; (d) Ailenberg, M.; Silverman, M. *Biochem. Biophys. Res. Commun.* **2002**, *298*, 110; (e) Yoshida, M.; Horinouchi, S.; Beppu, T. *BioEssays* **1995**, *17*, 423.
6. (a) Kuribyashi, T.; Maki ohara, M.; Sora, S.; Kubota, N. *Int. J. Mol. Med.* **2010**, *25*, 25; (b) Thuan, N. V.; Bui, H. T.; Kim, J. H.; Hikichi, T.; Wakayama, S.; Kishigami, S.; Mizutani, E.; Wakayama, T. *Reproduction* **2009**, *138*, 309; (c) Lee, E. J.; Lee, B. B.; Kim, S. J.; Park, Y. D.; Park, J.; Kim, D. H. *Int. J. Oncol.* **2008**, *33*, 767; (d) Ying, H.; Zhang, Y.; Lin, S.; Han, Y.; Zhu, Z. H. *Int. J. Mol. Med.* **2010**, *26*, 265; (e) Strobl, J. S.; Cassell, M.; Mitchell, S. M.; Reilly, C. M.; Lindsay, D. S. *J. Parasitol.* **2007**, *93*, 694.
7. (a) Kim, Y. B.; Lee, K. H.; Sugita, K.; Yoshida, M.; Horinouchi, S. *Oncogene* **1999**, *18*, 2461; (b) Dear, A. E.; Medcalf, R. L. *Biochim. Biophys. Acta* **2000**, *1492*, 15; (c) Sonoda, H.; Nishida, K.; Yoshioka, T.; Ohtani, M.; Sugita, K. *Oncogene* **1996**, *13*, 143; (d) Dear, A. E.; Liu, H. B.; Mayes, P. A.; Perlmutter, P. *Org. Biomol. Chem.* **2006**, *4*, 3778.
8. (a) Hashimoto, S.; Nakamura, Y. *J. Chem. Soc., Chem. Commun.* **1995**, 1413; (b) Hashimoto, S.; Nakamura, Y. *J. Chem. Soc., Perkin Trans. 1* **1996**, 2623; (c) Hashimoto, S.; Itai, K.; Takeuchi, Y.; Nakamura, Y. *Nucleic Acids Symp. Ser.* **1997**, *37*, 7; (d) Hashimoto, S.; Nakamura, Y. *Chem. Pharm. Bull.* **1998**, *46*, 1941; (e) Hashimoto, S.; Inui, T.; Nakamura, Y. *Chem. Pharm. Bull.* **2000**, *48*, 603.
9. (a) Kochany, E. L. *Chem. Rev.* **1991**, *91*, 477; (b) Sałdyka, M.; Mielke, Z. *Phys. Chem. Chem. Phys.* **2003**, *5*, 4790; (c) Kaczor, A.; Szczepanski, J.; Vala, M.; Proniewicz, L. M. *Phys. Chem. Chem. Phys.* **2005**, *7*, 1960.
10. Walling, C.; Naglieri, A. N. *J. Am. Chem. Soc.* **1960**, *82*, 1820.
11. (a) Lipczynska-Kochany, E.; Kochany, J. *J. Photochem.* **1987**, *38*, 331; (b) Lipczynska-Kochany, E.; Kochany, J. *J. Photochem. Photobiol.* **1988**, *44*, 317.
12. Endo, A.; Sujki, K.; Yoshihara, T.; Tobita, S.; Yahiro, M.; Adachi, C. *Chem. Phys. Lett.* **2008**, *460*, 155.
13. (a) Deby-Dupont, G.; Deby, C.; Mouithys-Mickalad, A.; Hoebeke, M.; Jadoul, L.; Vandenberghe, A.; Lamy, M. *Biochim. Biophys. Acta* **1998**, *1379*, 61; (b) Hwu, J. R.; Lin, C. C.; Chuang, C. H.; King, K. Y.; Su, T. R.; Tsay, S. C. *Bioorg. Med. Chem.* **2004**, *12*, 2509.
14. Hussain, A.; Gadadhar, S.; Goswami, T. K.; Karande, A. A.; Chakravarty, A. R. *Dalton Trans.* **2012**, *41*, 885.
15. (a) Khan, A. U. *J. Phys. Chem.* **1976**, *80*, 2219; (b) Merkel, P. B.; Kearns, D. R. *J. Am. Chem. Soc.* **1972**, *94*, 1029.