Organic & Biomolecular Chemistry

Dynamic Article Links

Cite this: *Org. Biomol. Chem.*, 2012, **10**, 1040

www.rsc.org/obc

PAPER

Synthesis of directly linked diazine isosteres of pyrrole-polyamide that photochemically cleave DNA†

Chi Wi Ong,*[a] Ya-Ting Yang,[a] Meng-Chi Liu,[a] Keith R. Fox,[b] Ping Hao Liu[a] and Hung-Wei Tung[a]

Received 27th October 2011, Accepted 2nd November 2011
DOI: 10.1039/c1ob06803b

A distamycin model containing an isosteric diazine linked pyrrole has been designed and synthesized. The key steps of the synthesis involved the successful diazotization of the 4-amino-pyrrole derivatives to give the diazomium salts, which undergo coupling reactions with *N*-methylpyrrole to yield the directly linked diazine compounds. The amide isosteric-diazine pyrrole **I** demonstrated photo-induced DNA damage upon iradiation with UV light (365 nm). Spectrophotometric and mass spectrometric identification suggest that the azo-linkage in **I** did not dissociate during irradiation. Moreover, compound **I** produced DNase I footprints with the HexB DNA fragment at AT sites, as well as some other mixed sequences (5′-ATGTCG-3′), indicative of the additional role of the diazine-linkage for interaction at the duplex DNA.

Introduction

The natural products netropsin and distamycin are well-known small molecules that bind to the minor groove of DNA and there has been considerable interest in modifying their structures in order to target specific DNA sites in the genome for applications in molecular biology and human medicine.[1,2] Analogues of polyamides, constructed from a variety of combinations of *N*-methylpyrrole, *N*-methylimidazole, thiazole, furan, thiophene and *N*-methylhydroxypyrrole, have been synthesized to investigate their binding to the DNA minor groove.[3,4] The introduction of an H-bond acceptor heteroatom, such as imidazole[5] or pyridine,[6] to the heterocyclic ring that faces towards the DNA minor-groove, has been reported to render binding to G–C base pairs. Altering the curvature of DNA minor-groove binding agents through the choice of linking heterocycles also offers a further means for tuning their binding ability, enabling them to better fit the natural curvature of the DNA minor groove.[7,4] Through computational studies[7] it was proposed that vinylexins containing –C=C– linkages might be an optimum fit for the DNA minor groove. Recently, alkene-containing minor-groove binders have been successfully synthesized.[8]

We have been working on modifying the carboxamide bond in distamycin with urea and carbonyl-urea.[9] Photoactive agents are potentially useful as DNA cleaving agents as they are inactive until triggered by irradiation.[10,11] Recent research has focused on the development of photonucleases that damage DNA directly upon irradiation, without requiring the activation of oxygen to form reactive oxygen species. Thus, the photoinduced extrusion of nitrogen from diazine-containing molecules to generate radical species has been reported as a new class of photonuclease.[10,12] Following this new concept, we report a new approach of introducing the diazine moiety as an amide-isostere, linking two pyrrole rings to obtain the Dystamycin mimic presented in Fig. 1.

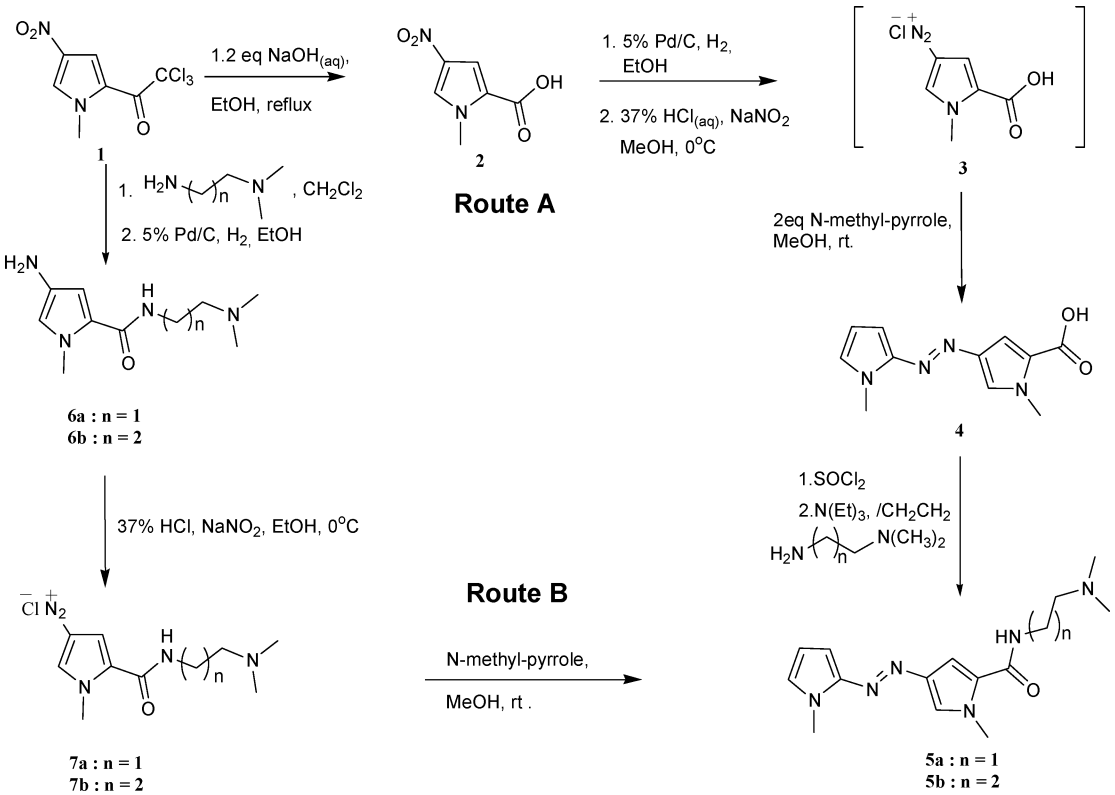
Although groove binding molecules containing diazine have been reported,[12–16] the direct coupling of photosensitive moiety into groove binding agent has not been studied. Furthermore, the diazine (–N=N–) moiety in the newly designed distamycin model, having a nitrogen lone pair, may also promote binding at the minor groove, a criterion that is absent in the alkene-distamycin model.

In this study, we describe the efficient synthesis of a directly linked diazine-containing pyrrole polyamide (Scheme 1), replacing the carboxamide with a diazine bond. We have conducted a detailed study on the photochemical *E-Z* isomerization of the amide-isosteric diazine model **I** (Fig. 1) along with its propensity to cleave DNA upon irradiation with non-photodamaging UV light (365 nm). The binding sites of diazine model **I** to DNA were followed by a footprinting experiment using HexB DNA as the substrate. DNA fragmentation indicated the binding of the diazine model **I** to A–T sites as well as to a mixed site (5′-ATGTCG-3′), consistent with the involvement of the diazine

[a]*Department of Chemistry, National Sun Yat Sen University, No. 70, Lienhai Rd., Kaohsiung, 804, Taiwan, R.O.C. E-mail: cong@mail.nsysu.edu.tw; Fax: +886-7-5253908; Tel: +886-7-5252000-3923*

[b]*School of Biological Sciences, Life Sciences Building, University of Southampton, Southampton, SO17 1BJ, United Kingdom. E-mail: krf1@soton.ac.uk; Fax: +44 23 8059 4459; Tel: +44 23 8059 4374*

† Electronic supplementary information (ESI) available: Supplementary data for **4** and **Ib** for melting curves of UV-Vis spectrophotometer and EtBr displacement assay and NMR spectra of **4**, **5a**, **5b**, **Ia**, and **Ib** can be found online. See DOI: 10.1039/c1ob06803b



Scheme 1 Feasible synthetic routes to diazine-pyrrole polyamides.

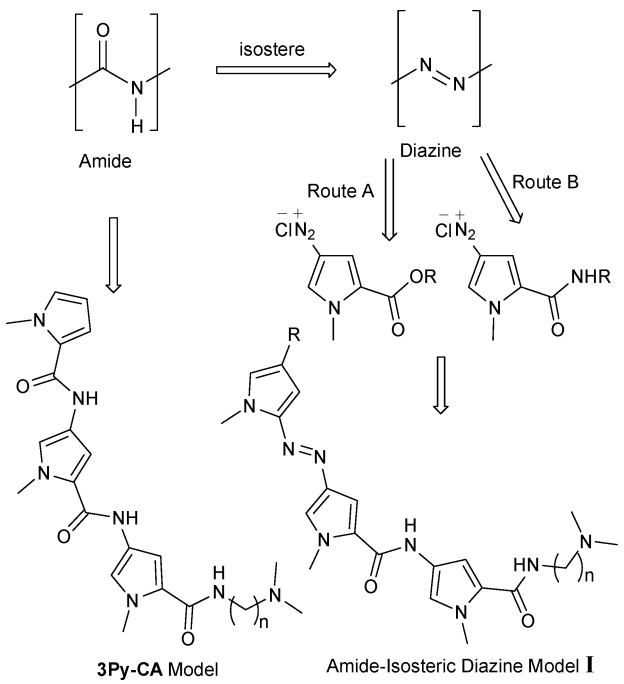


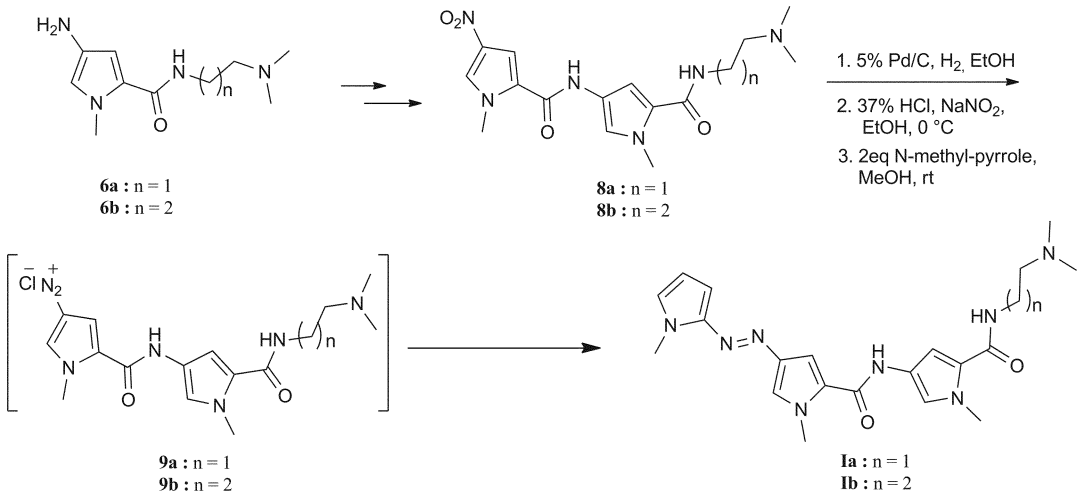
Fig. 1 Two possible routes towards a directly linked diazine model for distamycin.

moiety in modulating the binding of this compound to the minor groove.

Results and discussion

Synthesis

There are two possible synthetic routes for the proposed pyrrole-polyamide analogue containing a diazine linkage in place of carboxylamide, as outlined in Fig. 1. At the onset, it was thought that Route A would provide a greater versatility for the synthesis of a vast number of anologues. The starting point was the 2-trichloroacetyl-4-nitropyrrole, **1**, which has been previously prepared from the trichloroacylation of *N*-methylpyrrole, followed by nitration.[9] The diazonium salt of pyrrole-2-carboxylic acid **3** was prepared by reduction of the nitro-group of **2**, followed by diazotation according to reported procedure.[17] Compound **3** was reacted immediately with pyrrole to give the diazine-pyrrole carboxylic acid product **4**. Treatment of **4** with thionyl chloride to generate the acid chloride, followed by treatment with the appropriate amine gave the requisite product, **5**, in low yield. In view of the low yield, we decided carry out the synthesis of **5** using the alternative route, B. The nitro group of compound **1** was reduced to the amine by a known method,[18] and directly diazotized to give the diazonium salt, **7**. The coupling of the diazonium salt, **7**, with pyrrole furnished the diazine-pyrrole polyamide **5** in approx. 40% purified yield after three steps. It has been indicated that the presence of a minimum of three pyrrole rings without the leading amide at the terminus is necessary to provide at least a minimal binding with DNA.[19b] With the success of using Route B in the previous synthesis, compound **6** was first reacted with 2-trichloroacetyl-4-nitropyrrole (**1**) to give **8** using reported



Scheme 2 Synthesis of the tri-pyrrole diazine polyamide.

Table 1 Inhibition effect of azo-derivatives on the growth of tumor cells *in vitro*

Cell lines (ED_{50} in μg mL^{-1})			
Compound	Hepa59T/VGH	KB	Hela
Ia	>20	>20	>20
Ib	12.92	14.8	15.6
Doxorubicin	0.09	0.11	0.09

procedure (Scheme 2).[18] To our delight, the amino group from the reduction of the nitro group in **8** smoothly underwent the diazotization reaction to give the diazonium salt intermediate, **9**. The coupling reaction of **9** with *N*-methylpyrrole took place readily to provide the requisite diazine-distamycin model **Ia** in 34.2% and **Ib** in 30% purified yield.

The growth inhibitions (ED_{50}) of compounds **Ia** and **Ib** were evaluated *in vitro* on three human cancer cell lines: Hepa-59T/VGH (human liver carcinoma), KB (human oral epidermoid carcinoma) and HeLa (human cervical epitheloid carcinoma). The results obtained in this study are summarized in Table 1. A moderate antiproliferation effect was observed for **Ib**.

Photoinduced DNA cleavage and mechanistic characterization

The DNA cleavage ability of diazine model **Ib** was first studied using ΦX174 RFI plasmid DNA irradiated at 365 nm and agarose gel electrophoresis was used to monitor the conversion of the covalently closed circular DNA (ccc-DNA) to the nicked open form. Results are shown in Fig. 2. The amount of nicked form DNA increased with increasing concentration of **Ib**, with almost all of the ccc-DNA being converted to the nick form at 20 μM after 1 h irradiation (Fig. 2a, lane 7). On the other hand, distasmycin at the same concentration and after irradiation at 365 nm for 1 h, did not show any significant cleavage of ccc-DNA. We further studied the reactive species that might be involved in the photocleavage by **Ib** using various reported radical scavengers[20] (Fig. 2b). Thiourea and potassium iodide reduce hydroxyl radicals, sodium azide serves as a singlet oxygen scavenger and mannitol quenches free radical species.[21] It can be seen from the scavenging experiment (Fig. 2b) that sodium azide and mannitol did not inhibit the photoinduced DNA cleavage of **Ib**, while thiourea and KI showed some inhibition (less than 50%) from which we can conclude that singlet oxygen and hydroxyl radicals are not the main species involved in this DNA cleavage. The photoexcitation of **Ib** must therefore generate reactive species that can directly induce DNA cleavage. We studied the requirement for bringing the reactive species into close proximity to the DNA to induce photocleavage by synthesizing compound **4** that possesses only two pyrrole rings, linked by the diazine-group and also lacking the dimethylamino side chain for interacting with the DNA phosphate back-bone. As such, compound **4** is postulated to have only very weak interaction with the minor groove of duplex DNA. Irradiation of **4** in the presence of ccc-DNA did not show any significant cleavage (Fig. 2c). From these results, it is thought that the photonuclease activity of the diazine model **Ib** is associated with the generation of the radical species in close proximity to the DNA.

It has been reported that photoirradiation of an azo-compound can lead to photoisomerization and the photoextrusion of nitrogen to produce reactive radicals.[10,16] We therefore checked whether compound **Ib** could follow these reported pathways. Irradiating **Ib** at 365 nm for 1 h led to a large decrease in absorbance at the longer wavelength peak (around 400 nm) and an enhancement at the shorter wavelengths (around 350 nm). This is consistent with the suggestion that photo-irradiation at 365 nm transforms the *E*- to *Z*-isomer. Importantly, this isomerization process did not cause any photo-decomposition after 1 h photo-irradiation at 365 nm (Fig. 3a). However, when **Ib** was photo-irradiated at a shorter wavelength (312 nm), the absorption band at 400 nm diminished without the appearance of any other peaks. This process was irreversible (Fig. 3b), suggesting the decomposition of **Ib**.

MALDI mass spectrometry (Fig. 4) is a useful technique for determining the molecular weight of compounds, which can then be used to correlate the structure(s) produced after photo-irradiation of **Ib**. The mass spectra show that photo-irradiation of **Ib** at 365 nm for 1 h did not give any new fragment(s), except the original mass number of 439 (M + 1) (Fig. 4c), *i.e.*

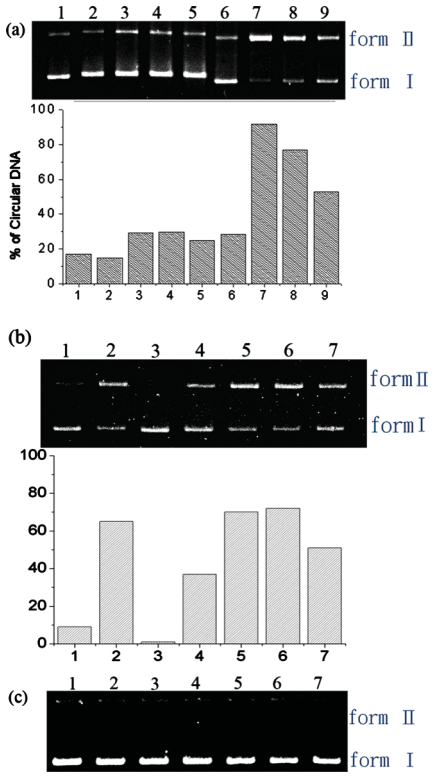


Fig. 2 Agarose gel electrophoresis patterns for DNA photocleavage. Each experiment contained 100 ng ΦX174 RFI plasmid DNA and were performed in a PBS buffer, pH 6.8, and exposed to 365 nm light (8 W) for 1 h. (a) Compound **Ib** and distamycin: lane 1, DNA control; lane 2, DNA and 20 μM distamycin without irradiation; lanes 3–5, DNA and 20, 10, and 5 μM distamycin, respectively; lane 6, DNA and 20 μM **Ib** without irradiation; lanes 7–9, DNA and 20, 10 and 5 μM **Ib**, respectively. (b) Inhibition of photocleavage by free radical scavengers: lane 1, DNA control; lanes 2–7 all contain DNA and 20 μM **Ib**, lane 3 was left in the dark for 1 h without irradiation, lanes 2 and 4–7 were irradiated at 365 nm for 1 h; the following scavengers were added, each at 2 mM: lane 4, thiourea; lane 5, sodium azide; lane 6, D-mannitol; lane 7, potassium iodide. (c) Compound **4**: lane 1, DNA control; lanes 2–6, DNA and 25, 50, 75, 100, 125 μM **4**, respectively, after irradiation at 365 nm for 1 h; lane 7, DNA and 125 μM **4** without irradiation.

no photoextrusion of nitrogen was observed. This observation highlights a new photo-nuclease activity of **Ib**, whereby the radical species is derived directly from the excitation of the azo π-bond. The reported photoextrusion of nitrogen was only observed when **Ib** was photo-irradiated at 312 nm, as indicated by the disappearance of mass number 439 and the appearance of a new fragment with mass number of 332 (M + 1) (Fig. 4d). UV irradiation at 312 nm is not suitable for photochemotherapy as this also kill cells. From these results it can be postulated that the energy from irradiation at 365 nm can only lead to the homolytic cleavage of the diazine N═N π-bond which can lead to isomerization in solution and in close proximity to DNA, cause DNA cleavage. The importance of the proximity effect has been further illustrated with **4**, which has the diazine group but does not bind and cleave DNA. On the other hand, irradiation at higher energy (315 nm) results in homolytic cleavage of the C–N bond to generate the pyrrole radicals with extrusion of nitrogen.

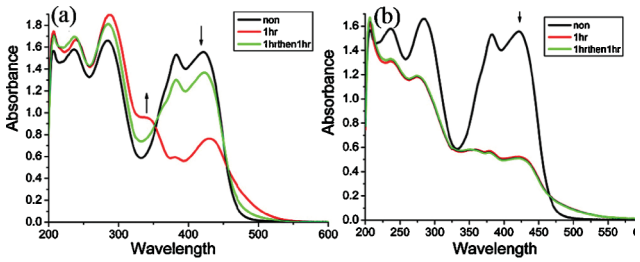


Fig. 3 (a) Black line: absorbance of compound **Ib** before exposure to light. Red line: absorbance of compound **Ib** after exposure to 365 nm UV-light for 1 h. Green line: absorbance of compound **Ib** after exposure of 365 nm UV-light for 1 h followed by 1 h recovery (b) Black line: absorbance of compound **Ib** before exposure to UV-light. Green line: absorbance of compound **Ib** after exposure to 312 nm UV-light for 1 h. Red line: absorbance of compound **Ib** after exposure to 312 nm UV-light for 1 h followed by recovery for 1 h.

Binding studies and DNase I footprinting for Ib

It has been reported that a minimum of three pyrrole carboxamide units (**3Py-CA**, Fig. 1) are necessary for the onset of DNA binding, whereas two pyrrole caboxamide units failed to exhibit any detectable binding.[19] The binding affinity of the diazine-model **Ib** was first investigated by UV melting experiments with calf thymus DNA. The diazine **Ib** showed a detectable increase in the melting temperature of ΔT_m = 0.8 °C, indicative of interaction with DNA, whereas **3Py-CA** showed negligible ΔT_m. The ethidium bromide (EtBr) displacement assay for **3Py-CA** has been reported, conferring a binding affinity of 6×10^4 M^{-1} [19b] The diazine model **Ib** was found to have a binding affinity of 4.26×10^5 M^{-1} using an EtBr displacement assay,[19a] about 7 fold higher as compared to **3Py-CA** having three pyrrole carboxamide (ESI, Fig. S1†). This observation is important as this suggests that the diazine-isoster did improve binding of **Ib** to DNA. The overall results substantiate the requirement of a cooperative effect, that is, binding for localizing the photoactive diazine moiety in close proximity of DNA for the observed photoinduced cleavage of DNA.

DNase I footprinting experiments were performed using the HexB DNA fragment[22] to determine sequence selective binding of diazene compound **Ib** (Fig. 5). The gel showed some protected regions (footprints) with increasing concentrations of compound **Ib** and, to a much lesser extent, footprinting for distamycin. Compound **4** showed no footprints, consistent with the EtBr assay which showed no DNA binding. Differential cleavage analyses of the gels are presented in Fig. 5c. Diazine **Ib** showed numerous protected regions at (A/T)AATT(A/T) and (A/T)TTAA(A/T) sites, similar to the distamycin counterpart.[22] Although the binding was weak, there are also several new regions of protection with **Ib**, namely 5′-GTATAC-3′, and an interesting mixed-sequence, 5′-ATGTCG-3′. This is the first observation in which the replacement of the carboxamide with an azo linkage has induced binding at a mix sequence, similar to that reported for replacing the pyrrole ring with imidazole[5] and pyridine[6] rings having a nitrogen lone pair which also binds at mixed sequences of DNA. Despite this, a high drug concentration did not give complete protection of the minor groove from DNase I, indicating that **Ib** has both poor and to some extent a lack of specificity for binding to the Hex-B DNA fragment. These experimental results from the two

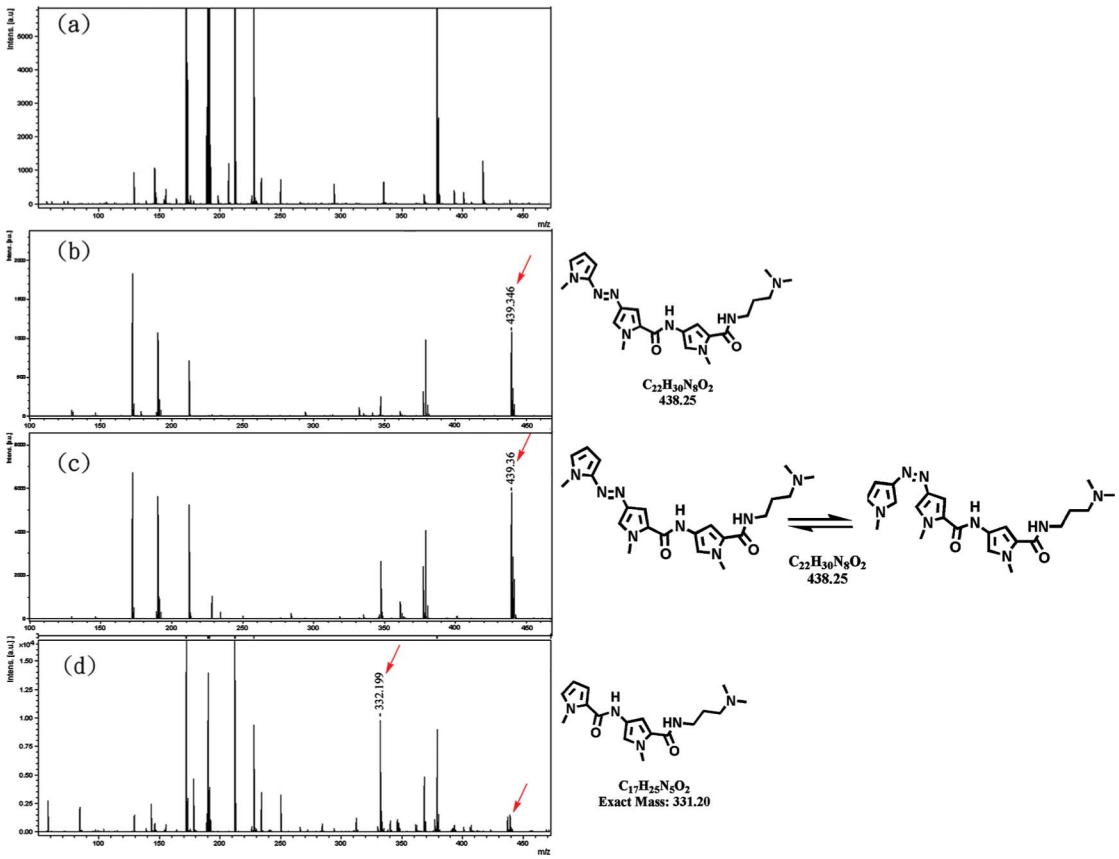


Fig. 4 Mass spectra of compound **Ib** after exposure to UV light at 365 nm and 312 nm. (a) Matrix (b) MALDI mass of compound **Ib** (c) MALDI mass of compound **Ib** after exposure to UV light at 365 nm for 1 h (d) MALDI mass of compound Ib after exposure to UV light at 312 nm for 1 h.

techniques, namely ethidium bromide displacement assay and gel electrophoresis footprinting, might be indicative that **Ib** can also bind to DNA by intercalation.

Conclusions

This paper described the synthesis of a new photoactive diazine moiety directly linked to a minor groove binding molecule. The model **Ib** shows good DNA cleaving efficiency upon photo-irradiation. Preliminary DNA binding studies showed an improved interaction of the directly link diazine containing polypyrrole amide. The participation of the diazine moiety in binding is further illustrated in the DNase I footprinting, which shows binding at mixed sequence sites. This study shows that replacement of the carboxamide linkage of distamycin by the diazine isotere presents new interesting biological functions. This new class of molecules may have a potential for use in novel photochemotherapuetic agents. Further studies in these areas are under way in our laboratory.

Experimental

General

All reactions were conducted under an atmosphere of nitrogen in oven-dried glassware. CH_2Cl_2 was distilled over calcium hydride. DMSO, MeOH and EtOH were purchased from Merck and used as received. 1-Methyl-4-nitro-1*H*-pyrrole-2-carboxylic acid(3-dimethylaminopropyl)-amide, the nitro precursor of **6b**, and 3-[1-methyl-4-(1-methyl-4-nitropyrrole-2-carbonylamino)pyrrole-2-carbonylamino]propyl-dimethylamine, **8b**, were prepared according to published procedures[18] Nuclear magnetic resonance spectra were recorded on a Varian Gemini-200 MHz and Varian Unity-INOVA-500 MHz spectrometer. ($CDCl_3$). Chemical shifts are reported in ppm relative to residual $CHCl_3$ (δ = 7.26, ^{1}H; 77.0, ^{13}C). Mass spectra were obtained on JEOL JMS-HX110, ESI on Bruker APE (II) FT-MS or Bruker Autoflex MALDI-TOFMS.

DNA melting temperature (ΔT_m) measurement

DNA melting experiments were conducted with a Pharmacia Biotect Ultrospec 4000 UV/Visible spectrophotometer with a temperature controller in 3 mL quartz cuvettes with a Teflon cap. The absorbance of the DNA-ligand complex was monitored at 260 nm as a function of temperature and DNA without compound was used as a control. Cuvettes were mounted in a thermal block and the solution temperatures were monitored with a heating rate of 0.5 °C min^{-1}. The concentration of DNA was determined by measuring the absorbance at 260 nm. A ratio of [compound] : [DNA] = 0.18 (5 μM : 27.7 μM) were used in the studies where the DNA concentration is expressed in nucleotide phosphates. The experiments were carried out in a PBS buffer (pH

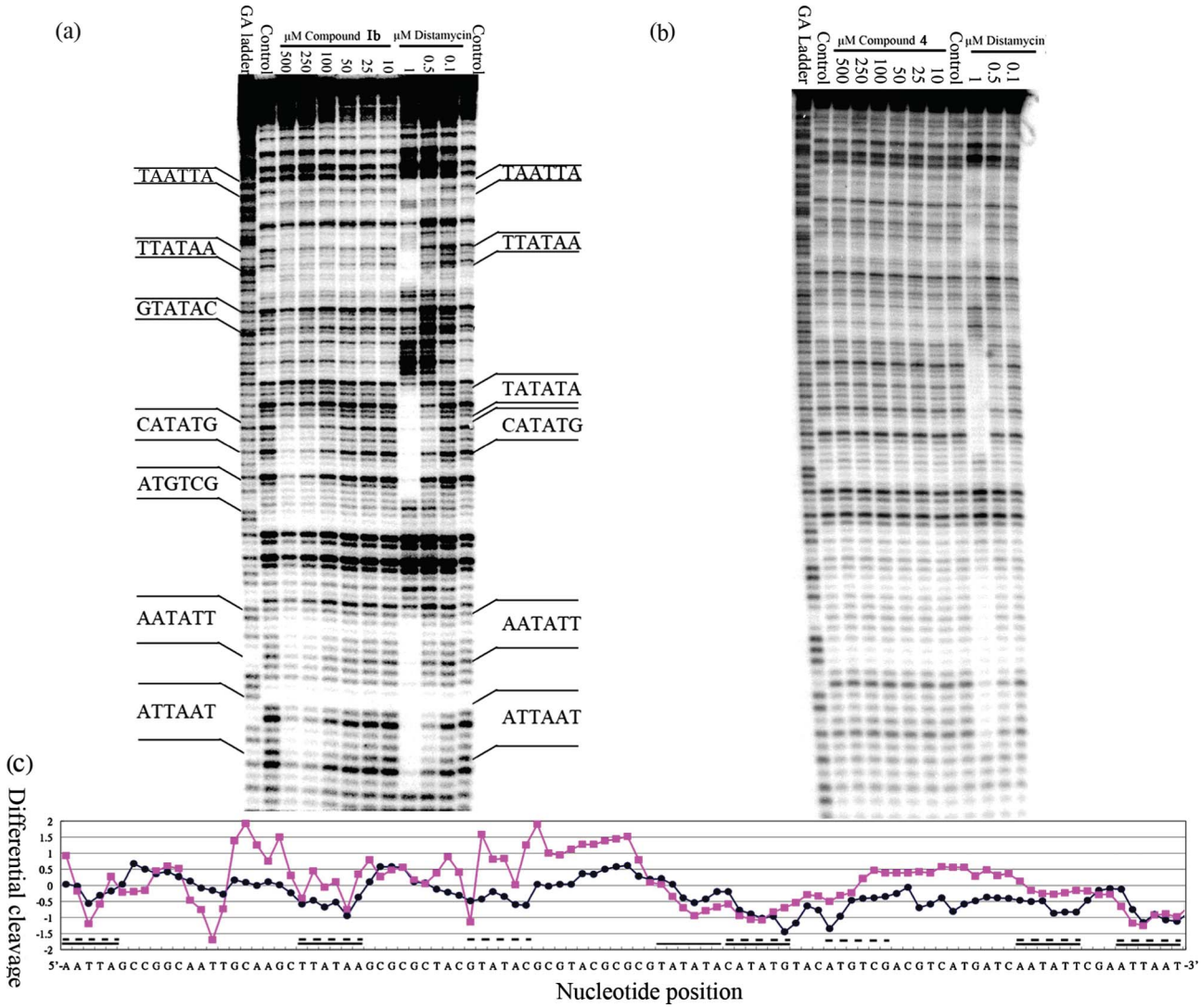


Fig. 5 (a) DNase I footprinting of azo-modified compound **Ib** and distamycin on the HexB DNA fragment. The ligand concentration (μM) is shown at the top of each lane. The binding sites for **Ib** are shown on left hand side, while those for distamycin are shown on at right hand side. Digestion of the DNA in the absence of added ligand is indicated by 'control'. Tracks labelled 'GA ladder' are Maxam–Gilbert sequence markers specific for purines. (b) DNase I footprinting of azo-modified compound **4** and distamycin on the HexB DNA fragment. The ligand concentration (μM) is shown at the top of each lane. (c) Differential cleavage plots showing differences in susceptibility of the HexB fragment to DNase I cleavage in the presence of (●) azo-modified compound **Ib** (500 μM) and (■) distamycin (0.5 μM). Vertical scales are in units of ln(fa) – ln(fc), where fa is the fractional cleavage at any bond in the presence of the drug and fc is the fractional cleavage of the same bond in the control, given closely similar extents of overall digestion. Positive values indicate enhancement and negative values indicate protection. The binding sites are also indicated by a dashed black line for Azo-modified compound **Ib** (500 μM) and a solid black line for distamycin (0.5 μM).

6.8). Compounds were dissolved in DMSO and diluted with buffer so that the final DMSO concentration was always less than 1%.

Ethidium bromide displacement assay

A 3 mL quartz cuvette was loaded with Tris buffer (0.1 M Tris-HCl, pH 8.0, containing 0.1 M NaCl) and ethidium bromide (13.9 μM final concentration). The fluorescence was measured (excitation 545 nm, emission 595 nm) and normalized to 0% relative fluorescence. Calf thymus DNA was added (27.7 μM base pairs final concentration) and the fluorescence was measured again and normalized to 100% relative fluorescence. Aliquots of the compounds (stock concentration 200 mM in DMSO) were added and the fluoresence was measured after 5 min. Additions were continued until the fluorescence decreased to lower than 50%.

Synthesis

3-{1-Methyl-4-[1-methyl-4-(1-methyl-2-diazopyrrole)pyrrole-2-carbonylamino]pyrrole-2-carbonylamino}ethyldimethylamine (Ia). Compound **8a** (100 mg, 0.33 mmol) was reduced with 5% Pd/C (0.05 mg) under 30 psi H_2 gas in EtOH (20 mL) containing $Na_2(CO_3)$ (30 mg). After tracking the reaction by TLC, the filtrate was collected and added to 10% aqueous HCl (0.3 ml, 3.6 mmol) at 0 °C for 10 min. Excess $NaNO_2$ (0.1 mg, 1.45 mmol) was added in portion and reacted for 1 h from 0 °C to rt. The solvent and excess HCl were removed under vacuum without heating, in order to keep

the temperature below room temperature. The residue was reacted with 2 eq N-methypyrrole (0.041 ml, 0.46 mmol) in MeOH (10 mL) overnight. After removing solvent under vacuum the diazine-modified compound was subjected to column chromatography on basic aluminum oxide with CH_2Cl_2 : MeOH = 20 : 1 as eluent. Yield **Ia**, 35.0 mg, 34.2%. Mp Mp 98–100 °C; IR (solid) 3163–3440, 2926, 1640, 1438 cm^{-1}; UV 330–500 nm ^{1}H-NMR (500 MHz, $CDCl_3$) δ 7.56 (s, 1H), 7.33 (d, J = 1.8 Hz, 1H), 7.16 (d, J = 2.0 Hz, 1H), 6.94 (d, J = 1.8 Hz, 1H), 6.81 (t, J = 4.0 Hz, 1H), 6.55–6.52 (m, 2H), 6.49 (s, 1H), 6.23 (dd, J = 6.8 Hz, 1H), 4.00 (s, 3H), 3.90 (s, 3H), 3.89 (s, 3H), 3.42(q, J = 17.0 Hz, 2H), 2.45 (t, J = 12.6 Hz, 2H), 2.23 (s, 6H); ^{13}C-NMR (125 MHz, $CDCl_3$) δ 161.66, 159.04, 146.26, 142.52, 127.57, 126.21, 125.18, 123.71, 120.10, 118.74, 109.63, 103.31, 100.90), 98.38, 57.90, 45.15, 37.27, 36.64, 36.49, 33.23; HRMS (ESI†): calcd for $C_{21}H_{28}N_8O_2$ [M^+ + H] 425.2410, found 425.2413.

3-{1-Methyl-4-[1-methyl-4-(1-methyl-2-diazopyrrole)pyrrole-2-carbonylamino]pyrrole-2-carbonylamino}propyldimethylamine (Ib). Compound **Ib** was similarly prepared as above with the use of **8b** (100 mg). Yield **Ib**, 35.0 mg, 30%. Mp 98–100 °C; IR (solid) 3164–3440, 2932 1640, 1439 cm^{-1}; UV 330–500 nm; ^{1}H-NMR (500 MHz, $CDCl_3$) δ 7.94 (s, 1H), 7.56 (s, 1H), 7.34(d, J = 1.0 Hz, 1H), 7.28 (d, J = 1.5 Hz, 1H), 7.06 (d, J = 1.0 Hz, 1H), 6.83 (dd, J = 2.5, 1.5 Hz, 1H), 6.61 (d, J = 1.5 Hz, 1H), 6.55 (d, J = 4.0, 1.5 Hz, 1H), 6.25 (dd, J = 4.0, 2.5 Hz, 1H), 4.02 (s, 3H), 3.92 (s, 3H), 3.91 (s, 3H), 3.47 (q, J = 17.5 Hz, 2H), 2.70 (t, J = 13.0 Hz, 2H), 2.49 (s, 6H), 1.87(t, J = 12.5 Hz, 2H); ^{13}C-NMR (125 MHz, $CDCl_3$) δ 161.91, 159.01, 146.25, 142.50, 127.51, 126.32, 125.16, 123.36, 121.16, 118.91, 109.60, 103.29, 101.10, 98.32, 57.50, 44.50, 37.84, 37.28, 36.69, 33.27, 25.29; HRMS (ESI†): calcd for $C_{22}H_{30}N_8O_2$ [M^+ + H] 439.2567, found 439.2570.

11-Methyl-4-(1-methyl-1*H*-pyrrol-2-ylazo)-1*H*-pyrrole-2-carboxylic acid (4). Compound **4** was similarly prepared as above with the use of **2** (100 mg). Yield **4**, 58.7 mg, 43%. Mp > 300 °C; IR (solid) 3185–3372, 2926, 1705, 1435 cm^{-1}; UV 350–480 nm; ^{1}H-NMR (500 MHz, MeOD) δ 7.27 (d, J = 2.0 Hz, 1H), 7.05 (d, J = 2.0 Hz, 1H), 6.90 (bs, 1H), 6.42 (dd, J = 4.5, 1.5 Hz, 1H), 6.17 (dd, J = 4.3, 1.5 Hz, 1H), 3.96 (s, 3H), 3.87 (s, 3H); ^{13}C-NMR (125 MHz, MeOD) δ 169.81, 147.54, 143.56, 132.80, 127.34, 126.20, 110.44, 105.06, 98.26, 37.57, 33.28; HRMS (ESI†): calcd for $C_{16}H_{24}N_6O$ [M^+ + H] 233.1038, found 233.1037.

3-[1-Methyl-4-(1-methyl-2-diazopyrrole)pyrrole-2-carbonylamino]ethyldimethylamine (5a). Compound **5a** was similarly prepared as above for **Ia** starting from **6**. Yield yellow oil **5a**, 58.7 mg, 40%. IR (solid) 3158–3410, 2927, 1643, 1465 cm^{-1}; UV 314–500 nm; ^{1}H-NMR (500 MHz, $CDCl_3$) δ 7.28 (d, J = 1.5 Hz, 1H), 6.91 (d, J = 1.5 Hz, 1H), 6.80 (dd, J = 2.0, 1.5 Hz, 1H), 6.62 (s, 1H), 6.53 (dd, J = 4.0, 1.5 Hz, 1H), 6.23 (dd, J = 4.0, 2.0 Hz, 1H), 3.79 (s, 3H), 3.89 (s, 3H), 3.45 (q, J = 19.0 Hz, 2H), 2.48 (t, J = 12.0 Hz, 2H), 2.25 (s, 6H); ^{13}C-NMR (125 MHz, $CDCl_3$) δ 161.79, 146.21, 142.26, 126.61, 126.57, 124.89, 109.41, 101.22, 98.21, 57.51, 37.10, 36.60, 33.17; HRMS (ESI†): calcd for $C_{15}H_{22}N_6O$ [M^+ + H] 302.1885, found 302.1884.

3-[1-Methyl-4-(1-methyl-2-diazopyrrole)pyrrole-2-carbonylamino]propyldimethylamine (5b). Yield yellow oil **5b**, 58.7 mg, 30%. IR (neat liquid) 3158–3537, 2946, 1638, 1488 cm^{-1}; UV 315–510 nm ^{1}H-NMR (500 MHz, $CDCl_3$) δ 8.16 (s, 1H), 7.27 (d, J = 1.5 Hz,1H), 6.91 (m, 1H), 6.53 (dd, J = 4.0, 1.5 Hz, 1H), 6.23 (dd, J = 4.0, 2.5 Hz, 1H), 3.799 (s, 3H), 3.87 (s, 3H), 3.47 (q, J = 17.5 Hz, 2H), 2.46 (t, J = 12.0 Hz, 2H), 2.29 (s, 6H), 1.72 (m, 2H); ^{13}C-NMR (125 MHz, $CDCl_3$) δ 161.67, 146.21, 142.42, 126.94, 126.73, 124.72, 109.42, 100.55, 97.64, 59.18, 45.39, 39.74, 37.12, 32.90, 25.37. HRMS (ESI†): calcd for $C_{16}H_{24}N_6O$ [M^++H] 316.2012, found 316.2009.

Acknowledgements

We thank the National Sciences Council (NSC) of Taiwan for the financial support to Mr. Meng-Chi Liu for research funding at the University of Southampton with Prof. K. R. Fox and Postdoctoral funding to Ya-Ting Yang (Tina)

Notes and references

1 A. Z. Ansari and A. K. Mapp, *Curr. Opin. Chem. Biol.*, 2002, **6**, 765.
2 P. B. Dervan and B. S. Edelson, *Curr. Opin. Struct. Biol.*, 2003, **13**, 284.
3 P. B. Dervan, *Bioorg. Med. Chem.*, 2001, **9**, 2215.
4 M. A. Marques, R. M. Doss, A. R. Urbach and P. D. Dervan, *Helv. Chim. Acta*, 2002, **85**, 4485.
5 (*a*) J. W. Lown, K. Krowicki, U. G. Bhat, A. Skorobogaty, B. Ward and J. C. Dabrowiak, *Biochemistry*, 1986, **25**, 7408; (*b*) J. W. Lown, *J. Mol. Recognit.*, 1994, **7**, 79.
6 W. S. Wade, M. Mrksich and P. B. Dervan, *J. Am. Chem. Soc.*, 1992, **114**, 8783.
7 C. Bailly and J. B. Chaires, *Bioconjugate Chem.*, 1998, **9**, 513.
8 N. G. Anthony, D. Breen, G. Donoghue, A. I. Khalaf, S. P. Mackay, J. A. Parkinson and J. C. Suckling, *Org. Biomol. Chem.*, 2009, **7**, 1843.
9 M.-C. Liu and C. W. Ong, *Tetrahedron*, 2009, **65**, 8389.
10 B. Armitage, *Chem. Rev.*, 1998, **98**, 1171.
11 T. D. Ros, G. Spalluto, A. S. Boutorine, R. V. Bensasson and M. Prato, *Curr. Pharm. Des.*, 2001, **7**, 1781.
12 T. M. Bregant, J. Groppe and R. D. Little, *J. Am. Chem. Soc.*, 1994, **116**, 3635.
13 P. J. Ceolho, L. M. Carvalho, A. M. C. Fonseca and M. M. M. Raposo, *Tetrahedron Lett.*, 2006, **47**, 3711.
14 D. G. Flint, J. R. Kumita, O. S. Smart and G. A. Woolley, *Chem. Biol.*, 2002, **9**, 391.
15 (*a*) D. Liu, J. Karanicolas, C. Yu, Z. Zhang and G. A. Woolley, *Bioorg. Med. Chem. Lett.*, 1997, **7**, 2677; (*b*) I. Willner and S. Rubin, *Angew. Chem., Int. Ed. Engl.*, 1996, **35**, 367; (*c*) D. G. Flint, J. R. Kumita, O. S. Smart and G. A. Woolley, *Chem. Biol.*, 2002, **9**, 391; (*d*) H. Asanuma, X. Liang, T. Yoshida and M. Komiyama, *ChemBioChem*, 2001, **2**, 39; (*e*) X. Liang, H. Asanuma and M. Komiyama, *J. Am. Chem. Soc.*, 2002, **124**, 1877; (*f*) M. Kar and A. Basak, *Chem. Commun.*, 2006, (36), 3818; (*g*) S. Ghosh, D. Usharani, S. De, E. D. Jemmis and S. Bhattacharya, *Chem.–Asian J.*, 2008, **3**, 1949; (*h*) A. M. Caamaño, M. E. Vázquez, J. Martínez-Costas, L. Castedo and J. L. Mascareñas, *Angew. Chem.*, 2000, **112**, 3234; (*i*) L. Guerrero, O. S. Smart, C. J. Weston, D. Burns, G. A. Woolley and R. K. Allemann, *Angew. Chem., Int. Ed.*, 2005, **44**, 7778.
16 S. Ghosh, D. Usharani, Paul, A. S. De, E. D. Jemmis and S. Bhattacharya, *Bioconjugate Chem.*, 2008, **19**, 2332.
17 H. Li and Z. Liu, *Synth. Commun.*, 1998, **28**, 3779.
18 J. H. Xiao, G. Yuan, W. Q. Huang, A. S. C. Chan and K. L. D. Lee, *J. Org. Chem.*, 2000, **65**, 5506.
19 (*a*) D. L. Boger, B. E. Fink, S. R. Brunette, W. C. Tse and M. P. Hedrick, *J. Am. Chem. Soc.*, 2001, **123**, 5878; (*b*) M. Thomas, U. Varshney and S. Bhattacharya, *Eur. J. Org. Chem.*, 2002, **21**, 3604.
20 N. Saglam, A. Colak, K. Serbest, S. Karaböcek and S. Güner, *Monatshefte für Chemie*, 2004, **135**, 1023.
21 G. J. Quinlan and J. M. C. Gutteridge, *Biochem. Pharmacol.*, 1987, **36**, 3629.
22 A. J. Hampshire and K. R. Fox, *Biochimie*, 2008, **90**, 988.

