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Recent advances in the development of 1,8-naphthalimide based DNA targeting binders, anticancer and fluorescent cellular imaging agents†

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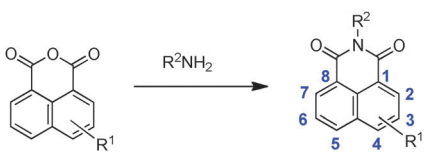
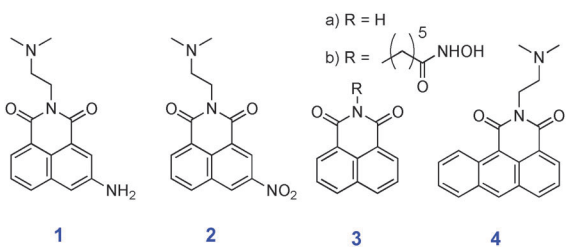
The development of functional 1,8-naphthalimide derivatives as DNA targeting, anticancer and cellular imaging agents is a fast growing area and has resulted in several such derivatives entering into clinical trials. This review gives an overview of the many discoveries and the progression of the use of 1,8-naphthalimides as such agents and their applications to date; focusing mainly on mono-, bis-naphthalimide based structures, and their various derivatives (*e.g.* amines, polyamine conjugates, heterocyclic, oligonucleotide and peptide based, and those based on metal complexes). Their cytotoxicity, mode of action and cell-selectivity are discussed and compared. The rich photophysical properties of the naphthalimides (which are highly dependent on the nature and the substitution pattern of the aryl ring) make them prime candidates as probes as the changes in spectroscopic properties such as absorption, dichroism, and fluorescence can all be used to monitor their binding to biomolecules. This also makes them useful species for monitoring their uptake and location within cells without the use of co-staining. The photochemical properties of the compounds have also been exploited, for example, for photocleavage of nucleic acids and for the destruction of tumour cells.

1. Introduction

In the area of anticancer research, the development of small molecules capable of binding to deoxyribonucleic acid (DNA) and exhibiting anticancer activities has received enormous attention in recent times.[1] Amongst these it has been shown that 1,8-naphthalimides (benz[*de*]isoquinolin-1,3-diones) possess high antitumour activity towards various human and murine cells[2] and the aim of this review is to highlight their use as potential anticancer agents. Naphthalimides are conveniently synthesised from the corresponding 1,8-naphthalic anhydrides by reaction with an amine (Scheme 1). This allows the production of a large family of derivatives (as will be well illustrated by the range of molecules presented below – including Bis-naphthalimides, polyamine and amino-acid derivatives). Additionally the naphthalimide ring can be substituted, for example, at the 3- or 4-position by amino or nitro groups, *e.g.* **1** and **2**. This not only allows the introduction of other functional groups, which can be used for targeting biomolecules, but can have a major effect on the electronic properties with a consequent influence on the chemical, photochemical and spectroscopic properties. Another fruitful approach for controlling the



Scheme 1 General synthetic route and numbering of 1,8-naphthlimide.



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properties of naphthalimides is to extend the aromatic ring system to create aromatic- or heteroaromatic-fused derivatives, as will be outlined in Sections 2.4 and 2.5.

As mentioned above the optical and photophysical properties of 1,8-naphthalimides are very sensitive to substitution in the aromatic ring. For example functionalisation with an amino function at the 3, 4, 5 or 6 position of the ring (see numbering in Scheme 1) produces compounds which possess internal charge transfer (ICT) transitions. The resultant band in the absorption spectrum is shifted to the visible and shows a marked solvatochromic effect.[3a] Many of the compounds are also strongly fluorescent, with a marked Stokes shift, Fig. 1. This emission is often in the green part of the electromagnetic spectrum and can be directed further towards the red by altering the nature of the ring substituent or that of the imide. This yields particularly attractive derivatives since they can partially overcome auto-fluorescence and light scattering from any biological environments. These tuneable photophysical properties thus make them excellent compounds to probe the microenvironment of biological systems as well as finding applications in the field of supramolecular chemistry.[3] As will be illustrated below, these properties are the basis for their use as dual therapeutic and fluorescent imaging agents.

(top row) Swagata Banerjee, Emma Veale, Caroline Phelan, Samantha Murphy; (bottom row) Gillian Tocci, Lisa Gillespie, Daniel Frimannsson, John Kelly, Thorfinnur Gunnlaugsson

Swagata Banerjee completed her BSc (Chemistry Hons) from Presidency College (University of Calcutta) and MSc in Biophysics and Molecular Biology from the University of Calcutta. She completed her PhD degree in 2012 *under the joint supervision of Prof. T Gunnlaugsson and Prof. J. M. Kelly from the School of Chemistry, Trinity College Dublin, where her project focuses on the development and photophysical studies of* 1,8-*naphthalimide derivatives and their interactions with DNA.*

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Dr Caroline M. Phelan completed her BSc Hon. degree in Chemistry from University College Dublin before undertaking a PhD in Chemistry from Trinity College Dublin in the area of naphthalimide anticancer agents. She has since then worked in various pharmaceutical companies in Ireland; currently working as a Projects Operations Engineer at MSD Ireland, a subsidiary of Merck Ltd.

Dr Samantha A. Murphy completed her Moderatorship (BA Hon) in Medicinal Chemistry from Trinity College Dublin before undertaking her PhD studies in the area of naphthalimide anticancer drugs at Trinity College Dublin, working on the development of bis-naphthalimides functionalised Tröger's bases. She is currently employed by Intel Ireland Ltd. as process engineer.

Dr Gillian M. Tocci completed her Moderatorship (BA Hon) in Chemistry from Trinity College Dublin. She remained on for a short postdoctoral fellowship within the group of Prof. Gunnlaugsson before undertaking a postdoctoral fellowship in the group of Dr Chris J. Michejda at National Cancer Institute at Frederick, Maryland, USA. In the Molecular Aspects of Drug Design group at NCI, she carried out research on glycopeptide derivatives of the anti-proliferative factor of interstitial cystitis. In 2006, *Dr Tocci joined the Analytical Services group at Particle Sciences, Bethlehem, Pennsylvania, USA.*

Dr Lisa J. Gillespie completed her Moderatorship (BA Hon) in Chemistry from Trinity College Dublin. She undertook a PhD in the area of naphthalimide conjugates as DNA targeting drugs and supramolecular chemistry with Prof. Gunnlaugsson. Since completing her postgraduate training she has worked in publishing in London and as a writer; focusing on the writing of scientific literature and history.

Dr Daniel O. Frimannsson graduated from the University of Iceland with a BSc in Biochemistry before undertaking a PhD in chemistry under the guidance of Prof. Gunnlaugsson and Prof. Mark Lawler at the Department of Haematology, focusing on the biological chemistry of naphthalimides and anticancer agents. After completing his postgraduate studies he undertook a postdoctoral fellowship in the group of Prof. Donal O'Shea at University College Dublin. He has since 2010 *been a postdoctoral fellow in the Stanford Medical School.*

Prof. John M. Kelly obtained his BSc from the University of Manchester in 1965, *his MSc in organic photochemistry from McMaster University (supervisor, John J. McCullough) and his PhD from the University of London (supervisor, George Porter) in* 1970. *After a Leverhulme Teaching Fellowship at the University of the West Indies, Jamaica, he carried out postdoctoral work in Ernst Koerner von Gustorf's group at the Max Planck Institut für* 1420 *Strahlenchemie, Mülheim. He joined Trinity College Dublin in* 1973 *and was the Head of Department from* 1994–2000. *He is a Fellow of the Royal Society of Chemistry and a Member of the Royal Irish Academy (MRIA). His research interests include the transient spectroscopy and photochemistry of DNA and metal containing compounds and the synthesis of micro- and nanoparticles of metals and metal oxides for applications in electronic devices and biosensors.*

Prof. Thorri Gunnlaugsson MRIA holds a Person Chair, Professor of Chemistry, in the School of Chemistry, Trinity College Dublin, and is a PI at the Trinity Biomedical Science Institute. He undertook his PhD in Queen's University Belfast under the supervision of Prof. A. P. de Silva MRIA and postdoctoral work with Prof. David Parker FRS at Durham University before moving to the University of Dublin in September 1998. *He was elected as a Member of the Royal Irish Academy in* 2011 *and he is a recipient of the Bob Hay Lectureship of the RSC Macrocyclic and Supramolecular Group. He has held several Honorary Professorships in Europe, Australia and New Zealand. He is the author of over* 150 *publications in the areas of supramolecular and medicinal chemistry.*

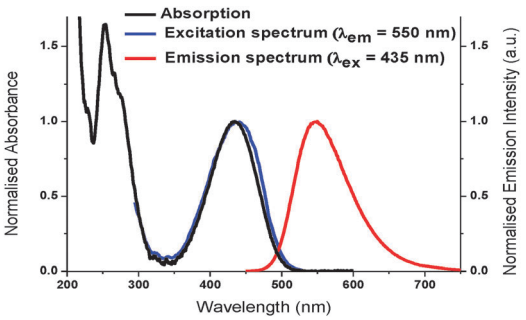


Fig. 1 The absorption, excitation and emission spectra of a 4-aminonaphthalimide derivative in 10 mM phosphate buffer at pH 7.0 (for structure **68** discussed in Section 2.9).

Another feature of the naphthalimides is their ability to target biomolecules and in particular nucleic acids. Indeed many naphthalimides already form strong intermolecular complexes with mononucleotides. The planar nature of the aromatic core suggests that the molecule should intercalate itself between the base-pairs of DNA and this behaviour has been assumed in many cases. However, it should always be borne in mind that small molecules can bind to DNA in a number of ways – for example by binding to the grooves (most often the minor one) or externally (especially if the molecules show a propensity for stacking). Also it is possible that the binding mode may depend on the DNA sequence. UV/visible absorption and fluorescence spectroscopy are excellent techniques for monitoring the binding to nucleic acids, *e.g.* Fig. 2. Binding constants can then be determined by fitting the changes, as illustrated in the inset of Fig. 2.[4a,b] However, other methods are required to define the precise nature of the binding sites. Ideally one would determine this through X-ray crystallography, but to the best of our knowledge there are currently no such reports for naphthalimides bound to DNA. There are also few detailed NMR studies, probably because of the problems of exchange processes. Excellent methods for distinguishing intercalation from other DNA binding modes include dichroism spectroscopies (especially linear dichroism), hydrodynamic studies, such as viscometry, or biophysical measurements such as topoisomerisation[4c] and such studies have been carried out in a number of cases.

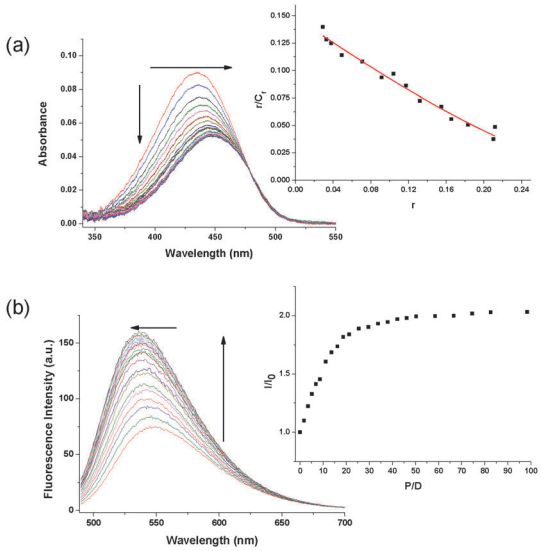


Fig. 2 (a) Changes in the UV-vis spectra of a 4-amino-1,8-naphthalimide derivative in the presence of increasing concentration of salmon testes (st)-DNA in 10 mM phosphate buffer (pH 7.0); inset: plot of r/C_f *vs.* r (■) and the best fit of the data (—) using the McGhee–von Hippel model. (b) Changes in the steady state emission of a 4-amino-1,8-naphthalimide derivative in the presence of increasing concentration of st-DNA (λ_{ex} = 480 nm) in 10 mM phosphate buffer (pH 7.0); inset: plot of I/I_0 *vs.* DNA nucleotide phosphate/ligand (P/D) (for structure **68** discussed in Section 2.9).

The uses of the 1,8-naphthalimide core extend beyond their application as DNA-binding motifs and anticancer agents. They have been extensively used within the field of supramolecular chemistry (such as in anion sensing), and they have found their applications as fluorescent brighteners, fluorescent bio-probes,[5a] as solar energy collectors,[5b,c] and in laser dyes.[5d,e] Recently attention has focused on sulfonated derivatives of 1,8-naphthalimides and it has been reported that these compounds can act as antiviral agents with selective *in vitro* activity against the human immunodeficiency virus, HIV-1.[5f] 1,8-Naphthalimides brominated at the 3 and 4 positions of the ring have been proposed as good candidates for the photochemotherapeutic inhibition of enveloped viruses in blood and in blood products.[5g–i] Moreover, the 1,8-naphthalimides are powerful photo-reagents, which can induce lesions in DNA molecules and, as such, possess the ability to kill cells when photoactivated.[5j] This opens up possible applications in photo-therapy. Finally it should again be emphasised that an important feature of 1,8-naphthalimides is that they are relatively easy to synthesise in high purity on a large scale.

2. 1,8-Naphthalimides as anticancer agents

The 1,8-Naphthalimides constitute a class of DNA-binding agents developed initially by Braña and co-workers.[2] Fabrication of these early naphthalimide derivatives was achieved by the incorporation of the structural elements from various known anticancer agents into a single structure, for example the β-nitronaphthalene of aristolochic acid, basic side chain from tilorone and morpholine β-thalidomide and the glutarimide unit of cycloheximide.

Two leading members of this family *amonafide*, **1** (a 3-amino-1,8-naphthlimide), and *mitonafide*, **2** (a 3-nitro-1,8-naphthalimide), have entered into phase II clinical trials. Clinical studies showed both exhibit high antitumour activity with IC_{50} values (the concentration of a drug required to inhibit a given biological/biochemical process by 50%) of 0.47 μM and 8.80 μM, respectively, against HeLa cell lines.[2,6a] The dihydrochloride salt of **1** developed by *ChemGenex Pharmaceuticals* has successfully entered into phase II clinical trials for prostate cancer under the generic name of Quinamed®, also known as amonafide.[6b] These compounds have also been found to stabilise double stranded DNA against heat denaturation.[6] Furthermore, **1** has been found to induce DNA strand breaks and

protein–DNA crosslinking in cultured mammalian cells[7] and can inhibit nucleic acid synthesis at a concentration where protein synthesis is generally unaffected.[6a] The 3-nitro derivative **2** can cause unwinding of closed circular DNA and increases the viscosity of sonicated DNA.[8] Both **1** and **2** can bind to DNA *via* intercalation and inhibit topoisomerase II activity by interfering with the breakage–rejoining step of the enzymatic cycle and stabilise the enzyme–DNA cleavage complex.[9]

Earlier structure–activity relationship (SAR) studies have pointed out some crucial parameters, which influence the anticancer property of this kind of naphthalimide (*i.e.* related to structures **1** and **2**). The presence of a basic terminal group in the side chain and of two or three methylene units separating the terminal nitrogen of the side chain from the naphthalene ring was shown to play a key role in their anticancer activity.[6a] Consequently, a large number of examples of such naphthalimides have been developed to date, but it is important to stress that such SAR has been found to be highly dependent on the nature of the group at the imide side of the naphthalimide structure (see later discussion) but in the case of structures related to **1** and **2**, the nature of the terminal amino nitrogen has been shown to play an important role in determining the anticancer activity of structures possessing two carbon spacers. Similarly, for such structures, the 3-nitro substituted 1,8-naphthalimides have been found to exhibit better antitumour activity compared to the 4-nitro analogues;[10] a phenomenon that is also dependent on the nature of the imide substituent. In the case of derivatives based on **1** and **2** (and in fact many other systems) this is presumably because of better stacking interactions between the 3-nitro-1,8-naphthalimides and DNA, where the nitro group can assume a coplanar orientation with the imide ring. However, for the 4-nitro derivative, the angular orientation of the nitro group with respect to the imide plane destabilises the stacking interaction.

Zee-Cheng and Cheng reported the development of *N*-(dialkylaminoethyl)-derivatives of 3,6-dinitro and 3,6-diamino-1,8-naphthalimides as potential DNA binders.[11] These derivatives showed high anticancer activity against leukemia with IC_{50} values of 0.036 and 0.33 μM, respectively, and colon adenocarcinoma cell lines (IC_{50} values of 0.041 and 0.68 μM respectively). These compounds also exhibited high anticancer activity *in vitro* against the P388 leukemia model. Subsequently, Braña and co-workers reported the development of a series of 3-amino-6-nitro-1,8-naphthalimide derivatives.[12] These compounds exhibited very high cytotoxicity compared to **1** and **2** against human CX-1 colon carcinoma and LX-1 lung carcinoma cell lines. However, the presence of alkyne substitution at the 3- and 4-positions of the naphthalene ring was found to decrease the cytotoxic activity of these compounds.[13]

The parent compound (ring unsubstituted) **3** has also been used successfully developed for anticancer treatment and derivatives of this structure have entered clinical trials. Compound **3a**, benzisoquinolinedione, which is also known as *Nafidimide*, was also synthesised by Braña *et al.* and was found to show considerable activity in a series of animal tumours both *in vitro* and *in vivo*.[6a] Nafidimide's pronounced activity *in vivo* against tumours in animal models facilitated its entry into clinical phase I trials. An investigation was undertaken by Andersson *et al.* to study its antileukemic activity *in vitro*, cellular drug transport, and molecular mechanism of action with DNA.[7a] Compound **3a** was found to be cytotoxic against human myeloid leukemia cells (KBM-3, HL-60). However the drug also reduced the survival of normal human bone marrow cells. Agarose gel electrophoresis of various topoisomers produced by the relaxing action of topoisomerase I on supercoiled DNA in the presence of **3a** confirmed that **3a** intercalates into DNA. It was also found to behave as a topoisomerase II inhibitor.[7a] Related to this structure is *Scriptaid*, **3b**,[7b] another unsubstituted ring naphthalimide structure, which possesses a hexanoic acid hydroxamide unit at the imide site. This structure was found to inhibit histone deacetylase and in combination with 5-aza 2′-deoxycytidine, **3b** was shown to enhance the expression of estrogen receptor α in estrogen receptor negative human breast cancer cells.[7c] **3b** has also been approved by FDA, clearly demonstrating that even the most simple naphthalimide structures have great potential for clinical use.

Azonafide, **4**, represents another important compound, where an anthracene moiety is introduced in the place of the naphthalene ring.[14] The derivative **4** showed significantly enhanced antitumour activity *in vitro* compared to **1**. Among various derivatives of **4**, basicity of the side chain nitrogen, length of the side chain and size of the substituent on the anthracene moiety were found to be important in determining the antitumour activity.[15,16] It has also been shown that the 4-, 5-, 7- and 9-amino derivatives exhibited significantly higher potency than the unsubstituted compound **4** against leukemia cell lines.[16]

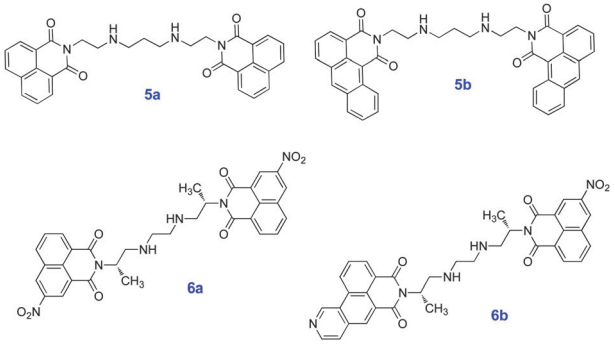
In order to achieve improved affinity for DNA and increase the cytotoxic potential several bis-1,8-naphthalimide derivatives have been developed, and these will be discussed in the following section.

2.1 Bis-naphthalimide based anticancer agents

Bis-naphthalimides, where two 1,8-naphthalimide moieties are connected by a polyamine spacer to enhance the DNA binding and antitumour activity, were initially developed by Braña and co-workers.[17] Generally the nitro/amino substituted derivatives exhibited better antitumour activity.[18] However, the bis-naphthalimide, elinafide (LU79553) **5a**, developed by Braña *et al.* lacks any such substitution and was found to exhibit high activity against a variety of human xenograft models such as LX-1 (lung), CX-1 (colon), and LOX (melanoma).[19] The bis-naphthalimide, **5a**, has been shown to be a bis-intercalator, as demonstrated by its ability to unwind and consequently alter the viscosity of closed circular plasmid DNA, binding to DNA along the major groove; interacting with DNA in a sequence specific manner where it was found to exhibit preference for alternating purine–pyrimidine dinucleotide steps.[5j]

Using a combination of NMR spectroscopy and molecular dynamics, Gallego and Reid showed that two naphthalimide chromophores of **5a** bisintercalate at TpG and CpA steps in the

hexameric d(ATGCAT)$_2$ sequence.[20] NMR spectroscopy showed that the binding of **5a** with DNA involves a two-step interaction with dissociation rates of 10^{-2} s^{-1} and 1–4 s^{-1}.[21] The sequences flanking the tetra nucleotide binding site have been found to influence the overall binding, particularly the intercalation step. The interaction is strongly disfavoured in the presence of A-rich tract at the 3′-end of the tetranucleotide motif apparently due to poor stacking interaction between the naphthalimide–DNA and among the DNA basepairs.

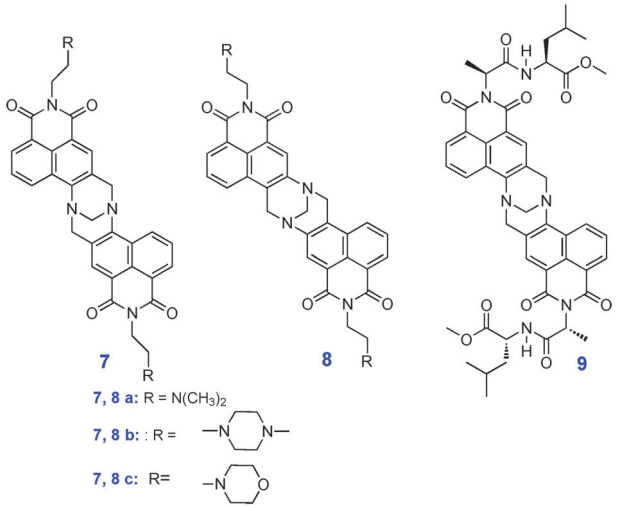


An anthracene derivative, **5b** (also known as *Bibenoline*), has also been developed.[22] This compound had similar activity to **5a**; the IC_{50} values for HT-29 human colon cell lines being reported as 0.004 and 0.014 μM for **5a** and **b**, respectively.

Chen and co-workers reported the development of another bis-naphthalimide DMP 840, **6a**, that exhibited potent proliferative activity against leukemia and various solid tumours *in vitro*.[18a] Mechanistic studies showed that **6a** inhibits DNA and RNA biosynthesis by interfering with the incorporation of thymidine and uridine respectively and by inducing DNA single strand breaks.[18b] Moreover, **6a** can also act as a eukaryotic topoisomerase II poison and stabilises the cleavage complex of topoisomerase II with DNA, hence causing cell death.[18c]

A series of compounds related to **6a** have been synthesised, differing in the type of chromophores at one end of the molecule, but leaving one of the naphthalimides unchanged from that of **6a**.[23] The compounds were evaluated *in vitro* for DNA binding (ethidium bromide displacement), and growth inhibition (L1210 murine leukaemia). A particular example from this family of structures is **6b**; the incorporation of a phenanthrene chromophore was found to be an efficient DNA binder. However, it was established that electron donating and withdrawing groups at position 6 of the phenanthrene neither helped DNA binding nor inhibition. Compound **6b** was found to display excellent L1210 activity with an IC_{50} of 0.035 μM, which was similar to that reported for **5a** (IC_{50} = 0.034 μM).

In an interesting extension to the aforementioned bis-naphthalimide derivatives, Gunnlaugsson *et al.* developed a series of bis-naphthalimides **7–9** linked by the Tröger's base (TB) moiety for DNA targeting.[24,25] The molecules **7** and **8** were specifically designed such that the terminal nitrogen atom in side chain of all three bis-naphthalimides is protonated at physiological pH, thereby increasing their water solubility and favouring electrostatic interactions with the negatively charged phosphate backbone of DNA. These bis-naphthalimides were found to bind to calf thymus (*ct*)-DNA with significantly high affinity ($\sim 10^6$ M^{-1}) and stabilise *ct*-DNA against thermal denaturation to a great extent (ΔT_m > 15 °C).[24] The molecules are taken up readily by HL-60 cells (leukaemia cell line) and localised within the nucleus. This was shown by using confocal florescence microscopy, exploiting the green emission from their ICT excited states. It was also demonstrated that these compounds are taken up into cells rapidly. Their localisation was further confirmed by co-staining using commercially available agents. Compounds **7a** and **7b** were found to have significantly high cytotoxicity (LD_{50} 5.21 and 5.5 μM, respectively) compared to their 4-amino substituted mononaphthalimide precursors (27.7 and 80.9 μM, respectively).[24]



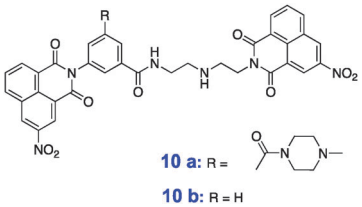
As an extension to this work, the TB-derivatives **8a–c** derived from 3-amino-1,8-naphthalimide were also developed.[25a] These derivatives were found to have lower fluorescence quantum yields compared to their 4-substituted analogues **7a–c**. Photophysical measurements showed that they bind to DNA with similar affinity and display dual mode of binding with one naphthalimide ring intercalated between DNA basepairs while the second one is groove-bound. Several additional TB-derivatives incorporating various amino acids and peptide side chains *e.g.* **9** have been subsequently developed.[25a] Compound **9** is formed from synthons that possess amino acid or peptide residues and have been shown by Gunnlaugsson *et al.* to be highly active anticancer agents in resilient cancer cell lines such as the CML based K562 cell line.[25b] Furthermore, the development of alternative bis-intercalators where the two naphthalimide units were spaced by an alkyl or (poly)-amino-alkyl based spacer, conjugated *via* a peptide linkage to such amino acid derived structures was also undertaken and shown to give rise to highly active agents that could bind to DNA with high affinity.[25c]

To achieve sequence selective DNA binding Suzuki *et al.* developed novel naphthalimidobenzamide derivatives **10a** and **b** and analysed their interaction with various DNA sequences by ethidium bromide displacement assay.[26] The strength of binding of **10b** was approximately 350 times stronger to G·C-repeats than to A·T and A·A-repeating oligomers.

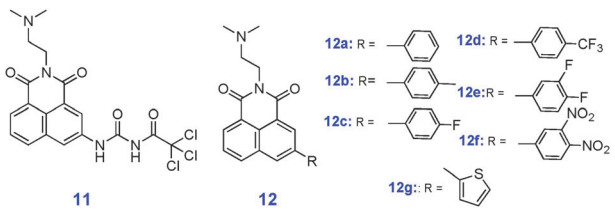
The interaction of **10b** with a specific DNA sequence induced p300 gene expression and exhibited significant anticancer activity in human solid tumour xenografts, thereby showing that DNA binding agents can be sequence specific and have the potential to control transcription of tumour related genes and inhibit tumour growth.

2.2 Naphthalimide-amine and related conjugates

In spite of their potent cytotoxic activities, clinical evaluation of most of the naphthalimides is limited because of the associated adverse side effects such as central neurotoxicity. Clinical studies suggested that **1** is converted to *N*-acetyl amonafide by the *N*-acetyltransferase enzyme during metabolism in human.[27] This metabolite can result in various unpredictable toxicities. Moreover, differential extent of *N*-acetylation between individuals also causes obstruction in clinical development. Several strategies have been developed to modify the naphthalimide chromophore to improve its potency and lower the side effects.

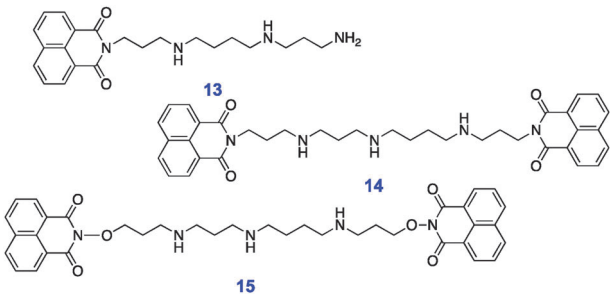


To improve the antitumour effects of **1**, as well as to minimise its toxic side effects, Quaquebeke *et al.* developed several analogues of **1**, by incorporating amide, urea, imine, amine and thiourea functional groups at the 3-position of the naphthalene ring.[28] SAR studies showed that the urea derivatives exhibited improved anticancer activity both *in vitro* and *in vivo* compared to the amide, amine, imine and thiourea derivatives. Especially compound **11** was found to have a *ca.* 4-fold higher maximum tolerated dose compared to **1** and did not induce hematotoxicity in a mice model at a dose that caused significant antitumour effects. However, the intercalation efficiency of compound **11** was found to be much weaker than **1**. The antiproliferative effect exerted by **11** has been attributed to its ability to induce autophagy and senescence in cancer cells.



As a strategy to modify the 3-amino substituent of **1**, a series of naphthalimides, **12a–g**, containing a phenyl moiety at the 3-position have been synthesised.[29] Ethidium bromide displacement and viscosity measurements suggested that these compounds bind to *ct*-DNA *via* intercalation and displayed high cytotoxicity against HeLa and P388D1 (murine lymphoid neoplasm) cell lines compared to **1**, showing the importance of the phenyl ring in improving the activity of these derivatives.

In order to achieve tumour cell specific entry of naphthalimide derivatives, several research groups have developed naphthalimide–polyamine conjugates. Thus recently, Tian *et al.* reported the synthesis and cytotoxic activity of a series of 1,8-naphthalimide–polyamine conjugates.[30] The presence of the triamine moiety and the spermine/homospermine skeleton was found to be crucial for their anticancer activity. Compound **13**, bearing a spermine side chain, has been shown to cause caspase activation and to induce apoptosis by lowering the expression of the Bcl2 protein and releasing cytochrome c from mitochondria. Derivative **13** was also found to up-regulate the expression of polyamine oxidase and lead to accumulation of reactive oxygen species through the depletion of reduced glutathione (GSH) pool.[30b]

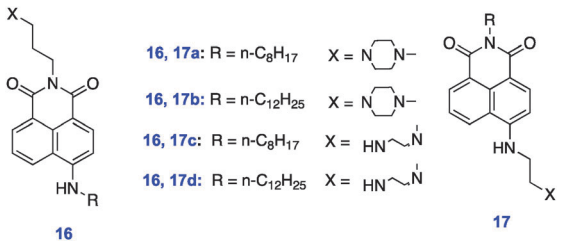


In a related study, Xie *et al.* demonstrated that the 3-nitro-naphthalimide–norspermine conjugate (NPC-16) can be taken up by tumour cells *via* the active polyamine transporter (PAT). NPC-16 displays high cytotoxicity against Bel-7402 and HepG3 cells and induces apoptosis.[31] However, the mechanism of apoptosis was different in the two cell lines. Thus in the Bel7402 cell lines, NPC-16 induced caspase activation and apoptosis *via* a mitochondrial pathway, whereas in HepG2 cell lines, it induced formation of autophagosomes and increased lysosomal activity followed by cell death.

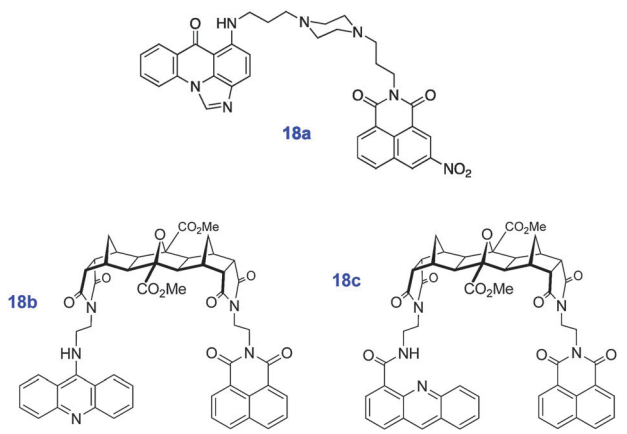
Lin and Pavlov have designed a series of bis-naphthalimide conjugates using spermine and spermidine as the linkers.[32] The spermidine conjugate **14** exhibits high cytotoxicity against the colon adenocarcinoma cell lines Caco-2 and HT29. The polyamine conjugate was found to induce apoptosis through DNA fragmentation, chromatin condensation and caspase activation.[33] This study was further extended to develop bis-oxy-naphthalimidopolyamine by incorporating oxygen atoms at the α position with respect to the naphthalimide ring (for example **15**).[34] Thermal melting and agarose gel electrophoretic mobility assays suggested that **15** behaves as a DNA intercalator. However, this compound was found to be less cytotoxic than **14**. The reduced cytotoxicity of **15** is thought to be due to poor cellular uptake by the polyamine transporter due to the presence of heteroatoms in the conjugates.

To avoid the *in vivo* acetylation of amonafide, Chen *et al.* developed a novel class of naphthalimide derivatives functionalised at the imide *N*- and the 4-position of the naphthalene ring with polyamines and long alkyl chains (**16a–d** and **17a–d**).[35] These derivatives show moderately high affinity for *ct*-DNA ($\sim 10^5$ M^{-1} in 20 mM TRIS buffer) and inhibit

topoisomerase II activity. Linear and flexible polyamine conjugates (**16c,d** and **17c,d**) displayed higher inhibitory activity. The majority of these derivatives show high antiproliferative activity compared to **1** against a variety of human cancer cell lines. These derivatives were found to induce lysosomal membrane permeabilisation, which releases several proteases (such as cathepsin) in the cytosol.



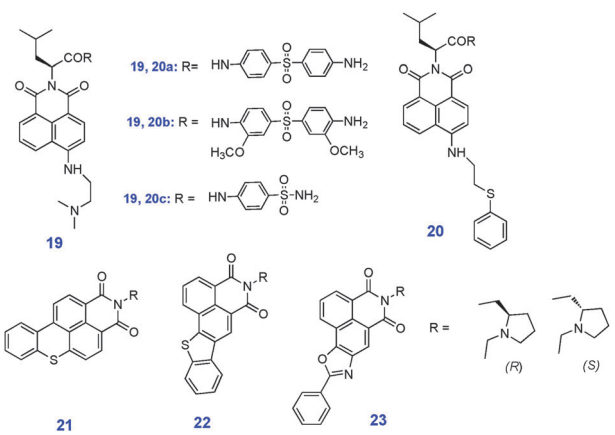
Other interesting examples of structures possessing naphthalimide units with additional intercalating units have been developed. An example of such design is that of Cholody *et al.*[36,37] who have investigated a series of asymmetrical bi-functional antitumour agents, accomplished by linking an imidazoacridone moiety to the naphthalimide core to give **18a**. This compound was thought to interact with DNA and has been shown to induce apoptosis in sensitive cells *in vitro* at low nanomolar concentrations. Furthermore, substitution on the naphthalimide ring proved significant in terms of cytotoxicity. The presence of a nitro group at position 3 of the naphthalimide ring was found to be essential for the cytotoxic activity of **18a**. Reduction of the nitro group to the corresponding amine was found to reduce the activity. The 4-nitro derivative also showed significantly lower cytotoxicity compared to **18a**, which is in accordance with the SAR studies carried out on structures such as **1** and **2**.[37b] A second family of such bifunctional intercalators is that of Waring and co-workers, who developed the acridine–naphthalimide conjugates **18b** and **18c** based on polynorbornane scaffold.[38] These compounds were shown to possess DNA unwinding properties and were also reported to have some Topoisomerase I mediated relaxation effect on closed circular DNA.



2.3 Naphthalimide–amino acid and other chiral conjugates

As discussed above, Gunnlaugsson *et al.* have developed TB derivatives based on the use of short peptide based 1,8-naphthalimide derivatives, where the peptide is conjugated to the ring through the ε-amino moiety. The precursors to these structures have also been found to be highly luminescent and to be active anticancer reagents with low μM activity in CML cell lines.[25c] In order to overcome the poor aqueous solubility and to achieve enhanced cellular uptake, Qian and co-workers have recently also developed related 1,8-naphthalimide based derivatives conjugated to a leucine amino acid.[39] These structures, **19–20**, were shown to possess moderately high affinity ($\sim 10^4$ M^{-1}) towards *ct*-DNA, to bind to DNA *via* intercalation as shown by their increase in the viscosity of *ct*-DNA. These naphthalimide–leucine conjugates were shown to have significant cytotoxicity against a wide range of tumour cells. Moreover, they exhibited strong interaction with bovine serum albumin, which is the bovine analogue of the most abundant transport protein of blood plasma. Compound **19** is also a PET sensor for protons and would be expected to show changes in the emission spectra depending on the local pH environment. The pH is expected to be different in cancer cells compared to healthy cell lines. Hence, these systems can function as cellular imaging agents for pH as well as being a therapeutic moiety. Saito and co-workers have also developed 1,8-naphthalimides conjugated to the side-amino functional group of L-lysine. These compounds are photocleavage agents and are discussed further in Section 2.9.

Qian and co-workers have developed several excellent examples of α-amino acid derived naphthalimide derivatives based on structures such as **21–23**; having chiral amino side chains at the imide position.[40] Among these chiral intercalators, the *S*-enantiomers showed higher affinity for DNA and greater photocleaving ability than the corresponding *R*-enantiomers.

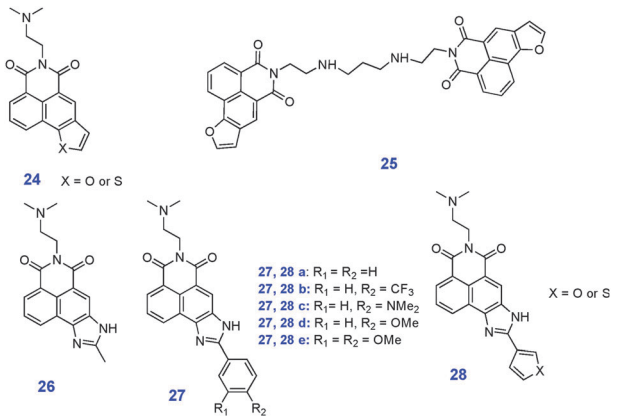


2.4 Heterocyclic fused naphthalimide derivatives

Braña *et al.* reported the synthesis of a series of mono 1,8-naphthalimides, where the naphthalene ring was fused to a furan or thiophene ring, **24**.[41] The derivatives containing the furan ring, which was shown to be oriented towards the outside of the naphthalimide moiety, were found to be the most active.

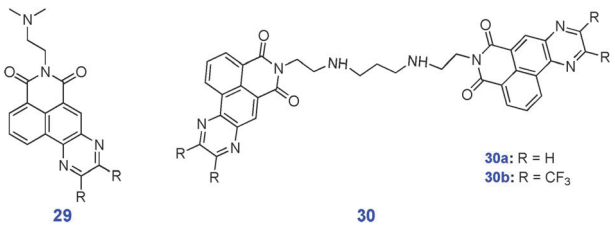
The dimerisation of this moiety using a polyamine linker **25** was also developed, and in line with what has been discussed above, this was found to increase the binding affinity of this structure for DNA. Dimerisation of these furano-naphthalimides also enhanced the cytotoxicity against CEM leukemia cell lines by more than 100 times compared to the corresponding mono-naphthalimides.[42]

In related work, Bailly *et al.* showed that the bis-naphthalimide **25** exhibits different sequence selectivity with a marked preference for GC steps compared to that seen for compound **5a**; which suggests that a furan ring plays a crucial role in determining the sequence selectivity.[42] It was suggested that the drug–DNA complex is stabilised by stacking and a hydrogen bonding interaction between the furan ring and the amino group of guanine. Moreover, the hydrogen bonding interactions between the protonated side chain of the ligand and O6 and N7 atoms of guanine base in the major groove act as an anchor and maintain the stability of the drug–DNA complex.



Molecular modelling studies by Braña and co-workers on monomer **24** and the corresponding bis-compound binding to short sequences of DNA were performed subsequently. For the bis-1,8-naphthalimide in the presence of $d(ATGCAT)_2$ it was revealed that in the most stable conformation the naphthalimide rings adopt a relative antiparallel orientation and are located in the major groove.[41] However, this group did not report any hydrogen bonding interaction between the furan–oxygen and the amino group of guanine in the minor groove, which was postulated to be crucial for the GC selectivity by Bailly and co-workers.[42] In contrast to these results, dimerisation of related imidazo-naphthalimides using polyamine linkers did not improve their cytotoxic activity.[43] Molecular modelling studies suggested that the linker length is not sufficiently long in these conjugates to form a stable complex with DNA. However, the corresponding mono-imidazo-naphthalimide **26** showed enhanced cytotoxic activity compared to **1** against human colon carcinoma cell lines. This increased affinity towards DNA may be attributed to the presence of an additional heterocyclic ring that increases the stacking interaction. Subsequently Qian and co-workers developed a series of "4–1" pentacyclic naphthalimides, where the naphthalimide ring is fused onto an imidazole ring bearing an un-fused aryl (**27**) or heteroaryl ring (**28**).[44] Viscosity measurements suggested that these derivatives bind to DNA *via* intercalation and stabilised DNA against heat denaturation to a great extent (ΔT_m 4–13.3 °C).[44]

Replacement of the imidazole moiety with a π-deficient pyrazine ring was found to increase the DNA binding and cytotoxic activity of the naphthalimide derivatives, **29** and **30**, presumably due to enhanced stacking interaction with the DNA bases and favoured intercalation.[45] The monomer **29a** and the bis-naphthalimide **30a** were also shown to have strong preference for GC rich DNA sequences.[46] Additionally, derivative **30a** was also found to inhibit DNA relaxation by topoisomerase I. In contrast, the bis-naphthalimide derivative **30b**, bearing two trifluoromethyl groups, did not exhibit any improved activity. This has been rationalised in terms of steric hindrance resulting from the presence of two trifluoromethyl groups interfering with the bis-intercalation.[45]

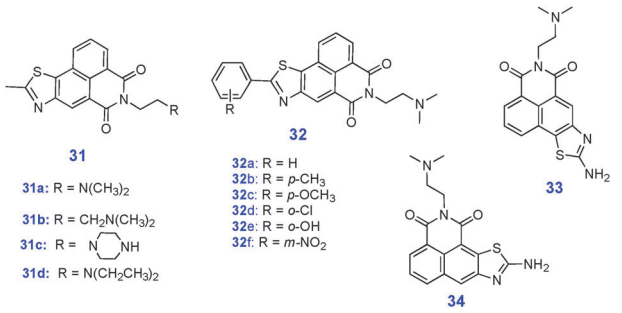


Methylation of the two *N*-atoms of the linker in **30a** was found to reduce the binding affinity of the dimer.[46] Binding to major/minor groove of DNA has been probed using oligonucleotides containing modified nucleotides 7-deazaguanine (C^7-G), 5-methyl cytosine (M) and inosine (I). Nucleotide modifications in the major groove such as G → C^7-G and C → M substitutions did not interfere with the binding of **30a** to DNA, whereas G → I substitution, which causes removal of the exocyclic 2-amino group of guanine in the minor groove nearly abolishes the binding. This suggests the possibility of binding along the minor groove of DNA. The authors have also suggested an alternative possibility of reduced stacking interaction between the pyrazino-naphthalimide and I.C basepairs. The faster breathing motion (basepair opening rate) in the I.C base pairs may also be reflected in the binding affinity determined by the Surface Plasmon Resonance (SPR) technique.[46] It should be mentioned here that neither the heterocyclic naphthalimide nor the bis-naphthalimide derivatives were found to inhibit topoisomerase II activity as observed for **1** and **4**. The mechanisms for the high cytotoxicity of most of these compounds have not been reported.

2.5 Thio-heterocyclic fused 1,8-naphthalimide derivatives

Thiazole and polythiazole moieties have been exploited in the design of several photonucleases and anticancer bleomycin-type antibiotics.[47,48] Qian and co-workers reported the synthesis of **31a–d** as photonucleases, whereby they combined the thiazole moiety with the intercalating 1,8-naphthalimide units.[49] These derivatives bind to *ct*-DNA with a significantly high affinity ($\sim 10^5$ M^{-1}) presumably by intercalation. All of these methyl-thiazonaphthalimide derivatives have been evaluated against A549 (human lung cancer cell) and P388 (murine leukemia cell),

for their antitumor activities. The cytotoxic potency of the derivatives was found to be highly dependent on the structure of the aminoalkyl side chain as discovered by SAR studies.



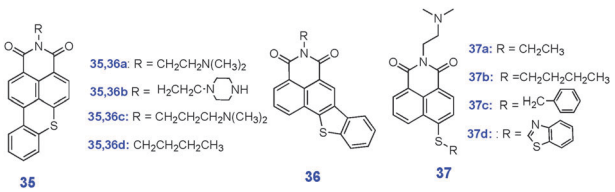
Compound **31a** gave IC_{50} values of 82.8 and 31 nM against A549 and P388 cell lines, respectively, while **31c** gave a IC_{50} value of 20.8 nM against A549 cell line; showing about 6 and 50 fold higher antitumour activity, respectively, when compared to **1** (IC_{50} 1100 and 200 nM against A549 and P388 cell lines, respectively). The high cytotoxicity of these derivatives compared to **1** has been attributed to the presence of the fused methylthiazole rings. Moreover, photoirradiation (λ = 360 nm) of these derivatives was found to induce strand cleavage of closed circular pBR322 plasmid DNA. This was proposed to proceed *via* the formation of superoxide anion generated through electron transfer from the naphthalimide chromophore to O_2. The order of photocleavage activity was found to parallel their DNA binding ability **31b** > **31a** > **31c** > **31d**. However, **31b** exhibited the weakest cytotoxicity among all the derivatives.

Cytotoxicity of a molecule is determined by both its DNA binding ability as well as its ability to penetrate the cell membrane. It also depends on the basicity of the side chain *N* atoms as discussed above. Derivatives **31a–d** would be expected to be protonated to different extents within the physiological pH window. A high degree of protonation of the side chain favors DNA binding, and similar results have also been seen by other researchers for structures possessing protonated sites.[25] However, cellular uptake of highly charged molecules can in some cases be less efficient (and beneficial in others), and therefore, a balance between these two factors determines the cytotoxicity. It is possible therefore that poor cellular uptake, which is probably lowest for **31b** within this set of compounds, results in weaker cytotoxicity.

Qian and co-workers have also reported the synthesis of the 1,8-naphthalimide phenyl substituted thiazole moiety derivatives **32a–f**.[50] All of these derivatives were found to induce strand cleavage under photo-irradiated conditions, with the order of photocleavage being **32a** (H) > **32d** (*o*-Cl) > **32b** (*p*-Me) > **32f** (*m*-NO_2) > **32c** (*p*-OMe) >**32e** (*o*-OH). It was suggested by these authors that the photoirradiation produces a naphthalimide-thiazole radical through its excited triplet state, which in turn caused DNA damage due to hydrogen abstraction. Semiempirical calculations indicated that in the triplet state the electron clouds are mainly localized on the thiazole ring and the electron density on the C–N is highest in compound **32a**, which probably accounts for its highest activity.

Li *et al.* reported the development of a new 1,8-naphthalimide series **33–34** containing the 2-aminothiazole moiety,[51] which has been extensively exploited in the development of antiparkinsonian agents. These derivatives only show moderate affinity towards *ct*-DNA (10^4 M^{-1}). The intercalating ability of **34** with a linear heterocyclic fused chromophore was found to be higher than **33** with its angular chromophore. These compounds also exhibit photocleavage activity. Liang *et al.* demonstrated that **34** can induce expression of tumour suppressor gene p53 in HeLa cells and MCF7 (human breast cancer) cell lines.[52] Compound **34** was found to increase the activity of p53, which can interact with the promoter region of Bcl2, an important regulator of apoptosis and can also down regulate the expression of Bcl2 and induce apoptosis in a caspase independent manner.[53]

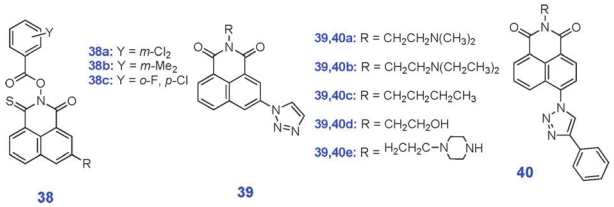
Qian *et al.* reported that an *N*-[β-(*N*′,*N*′-dimethyl amino) ethyl] dithiono-1,8-naphthalimide (where the oxygen of the imides are replaced by sulphur) can cause DNA photocleavage more efficiently than its oxo counterpart.[54] This led to the further development of a series of 1,8-naphthalimides fused with a thio-heterocyclic ring **35a–d**.[55] These molecules were found to absorb in the visible region and demonstrated efficient photocleavage of closed supercoiled pBR322 DNA after photo-irradiation with visible light (λ = 450 nm). The presence of a *N*,*N*-dimethyl aminoethyl or analogous group on the imide-*N* was found to be important for the photocleaving ability of **35a–c**, while **35d** did not exhibit significant photocleavage. The order of photocleavage activity of **35a–d** parallels the order of their fluorescence quantum yield under physiological pH. Under such conditions, the *N*,*N*-dimethyl aminoethyl group or analogue exists in protonated form, thereby inhibiting the intramolecular photoinduced electron transfer (PET) process. This results in an enhanced emission of these derivatives under physiological pH. The photocleavage reaction has been speculated to involve superoxide ions. These derivatives were also found to degrade maize genomic DNA under photoirradiated conditions without impairing the activity of biologically significant enzymes such as trypsin suggesting their potential use in the removal of transgenic materials during biochemical preparation of proteins and enzymes.[56]



These authors also reported the development of structural isomers **36a–d** bearing a five membered thio-heterocyclic ring.[57a] These compounds exhibit very high affinity for *ct*-DNA ($\sim 10^5$ M^{-1}). However, the six membered heterocyclic isomers were found to have higher photocleavage activity than the five membered heterocyclic compounds. Derivative **36a** displayed very high cytotoxicity against a number of human

cancer lines compared to **1**. Compound **36a** can also bind to the ATPase domain of topoisomerase II and as such function as a topoisomerase poison. It can also trigger cell cycle arrest and apoptosis by inducing double strand DNA break by stabilising the topoisomerase II–DNA cleavage complex.[57b] Based on the structure of **36a**, several non-ring fused naphthalimide derivatives, **37a–d**, have been developed containing sulphur substitution at the 4-position of the naphthalene ring.[58] These compounds showed efficient photocleavage of pBR322 closed supercoiled DNA and high photocytotoxicity against human mammary cancer and colon cancer cell lines. The antiproliferative activity of these compounds has been assigned to their ability to inhibit the expression of topoisomerase II.

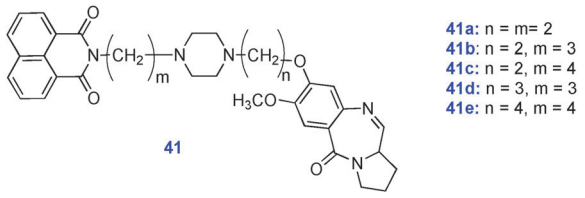
Xu *et al.* reported the development of novel *N*-aroyloxylthioxo-1,8-naphthalimide based photonucleases **38a–c**.[59] Semi-empirical calculations suggested that the photocleaving activities of these derivatives are correlated with the electron density on the N–O bond in the triplet state. For the *m*-dichloro analogue **38b**, the electron cloud density on the N–O bond is very low resulting in the easy breakage of the N–O bond and generation of an aryoyloxyl radical upon photoirradiation, which in turn induces DNA cleavage.



2.6 Other modified naphthalimide derivatives

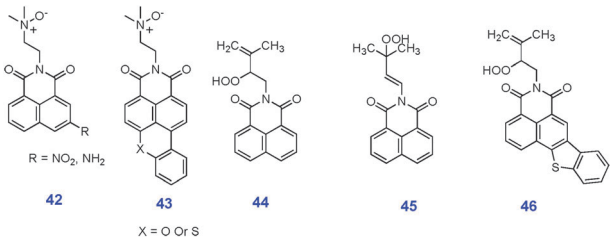
Qian and co-workers have developed a series of 1,8-naphthalimide derivatives incorporating a triazole moiety at the 3- and 4-positions of the naphthalene ring (**39a–e** and **40a–e**).[60] These derivatives possess high affinity towards *ct*-DNA ($\sim 10^5$ M^{-1}) and exhibit good cytotoxicity against a variety of human cancer cell lines compared to amonafide **1**. The enhanced cytotoxicity has been attributed to the presence of the triazole ring. Moreover, the presence of a basic side chain was found to be crucial for the cytotoxic activity. All of the triazole-substituted derivatives were found to induce DNA cleavage under photoirradiated conditions.

Recently, Kamal and co-workers have reported the synthesis and cytotoxicity study of a series of hybrid molecules, where the naphthalimide unit was conjugated with pyrrolo[2,1-c][1,4]benzodiazepines **41a–e** through a pyrazine moiety with an alkane spacer.[61] Among this series, the hybrid molecules, **41b–c**, having



three or four ethylene units between the pyrrolobenzodiazepine and piperazine rings showed the highest binding affinity and substantial increase in cytotoxicity.[61]

To reduce any undesirable toxicity of 1,8-naphthalimide derivatives and improve the therapeutic index, several 1,8-naphthalimide based prodrugs, **42–46**, have been synthesised by incorporating the tertiary amine *N*-oxide moiety in the side chain.[62] Of these, derivatives **42** and **43** showed much lower affinity towards double stranded DNA compared to the corresponding amine. This may be due to lack of the protonated side chain in the ligand, which favours electrostatic binding with DNA. Importantly, the *N*-oxide derivatives exhibit less cytotoxicity in oxic A375 cell lines compared to the corresponding amine, while they are found to be highly cytotoxic in hypoxic tumour cell lines. This selectivity arises from the bioreduction of the *N*-oxides to the corresponding amines by the CYP3A isozyme of NADPH:Cytochrome C (P-450). The resulting amines can therefore bind to DNA and inhibit topoisomerase function.

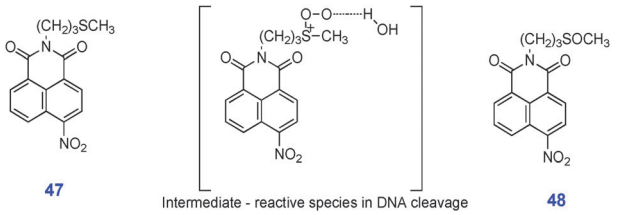


Recently, Yin *et al.* demonstrated that these *N*-oxides of naphthalimides could also be used as fluorescent markers for hypoxic cells.[63] Under physiological conditions the fluorescence intensity of *N*-oxide derivatives is quite low due to aggregation and hydrogen bonding interaction with polar water molecules. In contrast the corresponding amines show higher fluorescence quantum yield. Therefore, obvious differential fluorescence response can be observed from hypoxic and oxic cells due to preferential reduction of the *N*-oxides under hypoxic conditions. For compound **42** this fluorescence difference was found to be $\sim$17 times higher between hypoxic and oxic cells probably due to a bis-bioreduction mechanism.

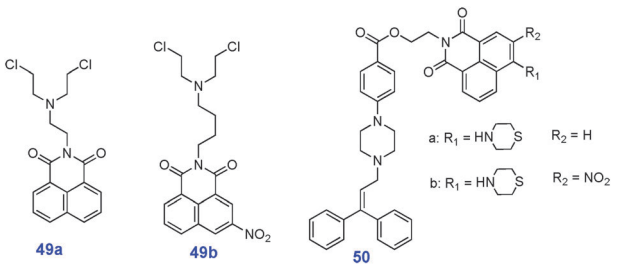
Photo-Fenton reagents based on the 1,8-naphthalimide-hydroperoxide derivatives **44–45** have been designed by Tao *et al.*[64] These molecules were demonstrated to intercalate into DNA and generate $OH^{\bullet}$ radical on photoactivation, which in turn causes DNA cleavage. Further studies were carried out on reagents with a larger conjugated planar heterocyclic fused naphthalene ring such as **46**.[65] These compounds showed higher cleaving abilities, compared to **44**, of supercoiled circular pBR322 DNA, on photoirradiation. The enhanced DNA intercalating capability of **46** was thought to contribute to this.

Matsugo *et al.* synthesised and studied a series of thio ether based 1,8-naphthalimides, such as **47**, as photoinduced DNA cleavers.[66] It was found that **47** only caused cleavage of supercoiled circular ϕx 174 DNA after photoirradiation under aerobic conditions. It was proposed that **47**, in its excited state, reacts with molecular oxygen yielding singlet oxygen,

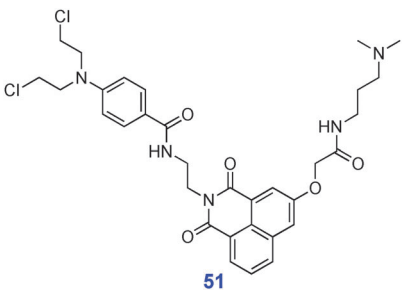
which then forms a persulfoxirane-type intermediate. This intermediate then reacts with another equivalent of **47** to produce two moles of **48**, but also acts as the reactive species in DNA cleavage.



A nitrogen mustard derivative **49a** has been developed by combining the naphthalimide unit with the *N*,*N*′-bis(2-chloroethyl)amino group. This showed high cytotoxicity against murine carcinoma cell lines.[67] Recent studies on 3-nitro-1,8-naphthalimide conjugated with nitrogen mustard **49b** showed significantly high antitumour activity and low systemic toxicity against hepatocellular carcinoma compared to **1**.[68] Recent studies have also demonstrated that the naphthalimide–benzoic acid conjugates **50a–b** exhibit cytotoxic activity comparable to that of **1** in a variety of cancer cell lines.[69] These compounds were found to induce cell cycle arrest at G2/M boundary and trigger apoptosis.



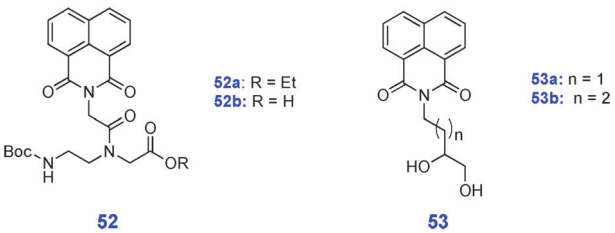
Gupta *et al.* have also developed a range of 1,8-naphthalimide derivatives with nitrogen mustard functionalities at the imine position for example **51** in an effort to combine their individual antitumour activities.[70] These compounds were found to achieve sequence specific alkylation at the guanine N7 position and show considerable anticancer activities. This is a prime example of how it is possible to manipulate 1,8-naphthalimide moieties by incorporating other structural motifs to enhance their activities and specificities.



2.7 1,8-Naphthalimide–oligonucleotide/peptide nucleotide conjugates

In recent times, several research groups have coupled the intercalating 1,8-naphthalimide moiety with peptide or oligonucleotide sequences to construct potential candidates for photodynamic therapy.

Peptide nucleic acids (PNAs) have received much attention due to their high affinity and selective recognition of nucleic acid sequences, stability towards cellular nucleases and proteases, easy synthetic pathway using solid state peptide chemistry.[71] Ikeda *et al.* reported the synthesis of PNA incorporating the 1,8-naphthalimide unit **52**.[72] PNA oligomers containing naphthalimide at the amino terminus were found to be more stable against heat denaturation presumably due to an extended π–π interaction by the naphthalimide moiety. This study illustrates that the naphthalimide photosensitiser can be selectively incorporated at a predetermined site into PNA oligomers and represents potential candidates for photodynamic therapy.

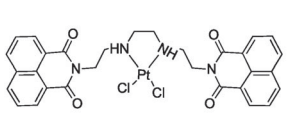


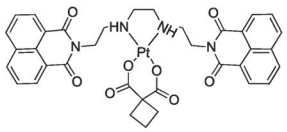
Wamberg *et al.* reported the synthesis of a series of intercalating nucleic acids incorporating 1,8-naphthalimide (that were then conjugated *via* a phosphoramidite) as the intercalating unit **53a–b**.[73] Thermal melting studies demonstrated that the intercalators with a short linker length (<five carbon atoms) were unable to stabilise the DNA–DNA duplex because the chain length is probably not optimal to position the intercalator for base stacking without disturbing the DNA backbone. However, compound **53b** with a five carbon linker length has been shown to discriminate between DNA and RNA. It can stabilise the DNA–DNA duplex while destabilising the DNA–RNA duplex. Intercalator **53b** was also found to be a sensitive reporter of nucleotide mismatch in an oligonucleotide sequence showing a decrease in melting temperature of the oligonucleotide up to 29 °C.

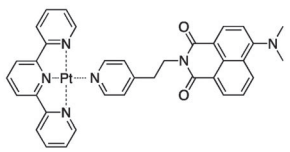
Majima and co-workers have demonstrated the incorporation of 4-amino-1,8-naphthalimide and pyrene chromophores into oligonucleotide sequences to construct a FRET donor–acceptor system, which can be useful to study structure and conformational dynamics of nucleic acids.[74] Incorporation of dye molecules did not interfere with duplex stability and the quantum yield of emission of the fluorophore. This group has also illustrated the use of oligonucleotides modified with the naphthalimide photosensitiser to study charge transport in DNA (see below).[75]

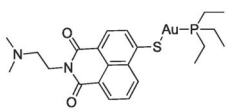
2.8 Naphthalimide–metal complex conjugates

Over the past couple of decades, transition metal complexes have received great deal of interest for developing cancer chemotherapeutic agents.[76] In order to achieve enhanced DNA binding and cytotoxicity Pérez *et al.* developed novel Pt-bis-naphthalimide complexes such as **54a–b**, which overcome the cellular resistance to *cis*-platin, a common problem encountered in *cis*-platin based chemotherapeutics.[77] The high cytotoxicity exerted by these derivatives results from the intercalation of the bis-naphthalimide unit combined with the platination of DNA bases. Banerjee *et al.* developed a Pt(II) based bifunctional DNA binder **55** which incorporates the terpyridyl and the 4-*N,N′*-dimethylamino-1,8-naphthalimide. This shows enhanced DNA binding affinity and cytotoxicity (IC_{50} 18 μM) against MCF-7 cell lines.[78]

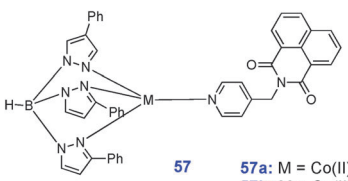


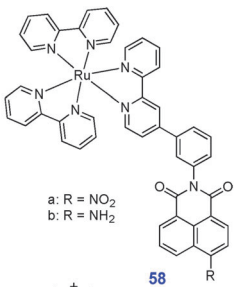


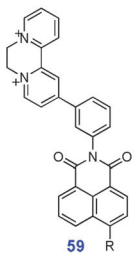


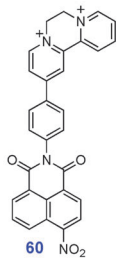


Development of gold(I) phosphine complexes bearing a thionaphthalimide ligand such as **56** was found to be an important design strategy to enhance the antiproliferative activity of gold(I)-complexes.[79] Compound **56** was shown to display significant growth inhibitory effects in MCF-7 breast cancer and HT-29 colon carcinoma cells. The presence of a thionaphthalimide ligand also increased the cellular uptake and accumulation of gold in the nuclei of tumour cells compared to the naphthalimide free analogue.







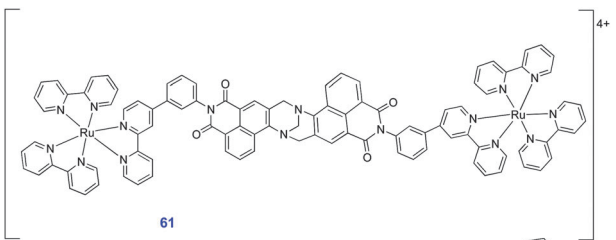


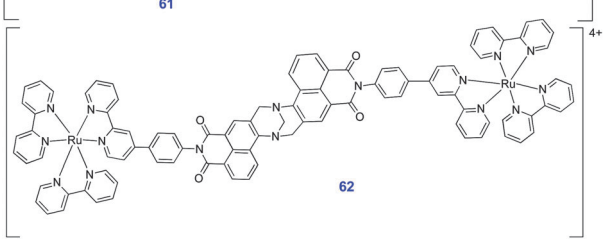
In a recent study, Chakravarty and co-workers reported the development of the "3d-metal Scorpionates"-bearing 1,8-naphthalimide chromophores, **57a–c**, which exhibit moderately high affinity towards *ct*-DNA ($\sim 10^5$ M^{-1}).[80] Additionally, the Co(II) and Cu(II) complexes **57a** and **57b** were found to induce DNA cleavage and to exhibit significantly high cytotoxicity against HeLa cervical cancer cells upon UV irradiation.

Several Ru(II)–naphthalimide conjugates have been developed by Gunnlaugsson and co-workers as potential DNA binders and photocleaving agents. This resulted in the development of ruthenium [Ru(II)] polypyridyl complexes conjugated with 1,8-naphthalimide derivatives using flexible and rigid linkers. The use of a rigid aromatic linker as shown by Quinn *et al.* in **58** was found to provide control over the relative orientation of the two chromophores, placing the naphthalimide in close proximity to the metal complex.[81a] These complexes show significant hypochromism and a red shift in λ_{max} of the MLCT band in the presence of DNA with binding constants $\sim 10^6$ M^{-1} in 10 mM phosphate buffer (pH 7.0). Moreover, these systems cause DNA cleavage under irradiation in aerated solution (λ_{ex} > 390 nm). Of these systems, the 4-aminonaphthalimide conjugate was found to be a more active photocleaving agent than the 4-nitro analogue correlating with the higher quantum yield of emission of the former.

Gunnlaugsson, Quinn *et al.* also reported the synthesis and photophysical evaluation of a new family of fluorescent quaternary bipyridyl–1,8-naphthalimide conjugates **59** and **60** as DNA targeting agents.[81b] Even though these systems lack the presence of metal ions, it was found that they exhibited binding affinities, which were greater than those previously reported for the 1,8-naphthalimide systems and comparable to those exhibited by the metal complex **58**.

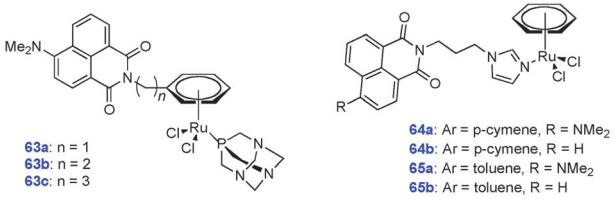
As an extension to the above work, Gunnlaugsson, Williams and coworkers have developed several 1,8-naphthalimide based Tröger base derivatives conjugated with Ru(II)-polypyridyl complexes **61–62**.[82] The luminescence of these complexes was found to be much less affected upon binding to DNA than the corresponding Ru(II)–naphthalimide conjugates. However, thermal denaturation studies demonstrated that they gave rise to enhanced affinity compared to the precursors. Furthermore, both of these conjugates were readily taken up by cervical cancer cell lines and were found to





cause membrane blebbing, which opens up the use of these complexes for imaging and therapeutic purposes.

Recently, two series of naphthalimide based ruthenium(II) arene complexes **63–65** were prepared by Kilpin, Dyson and co-workers and it was found that for both the presence of the intercalating moiety of the 1,8-naphthalimide increased the cytotoxicity (2–49 μM) of the ruthenium arene unit with cancer cells.[83] In their findings, it was reported that the intercalation mechanism of the naphthalimide unit within DNA along with binding of the ruthenium(II) arene unit with proteins led to the observed increase in anticancer selectivity for **64–65** over model healthy cells and for overcoming *cis*-platin acquired drug resistance.

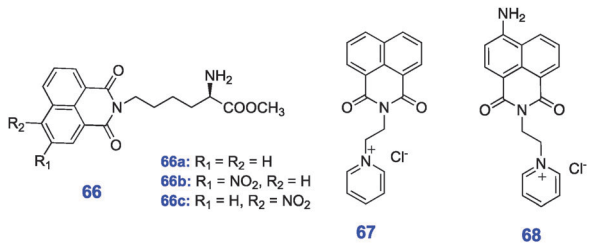


2.9 Mechanistic studies on nucleic acid photooxidation by 1,8-naphthalimide based photosensitisers

As demonstrated by many examples above, photoexcitation of various 1,8-naphthalimide derivatives has been shown to induce strand cleavage and other chemical reactions on DNA, often with sequence selectivity. In many cases this process is initiated by electron transfer from the nucleobase (most commonly guanine, which is the most easily oxidised of the four common bases) to the naphthalimide (eqn (1)).

$$NI^* + G \rightarrow NI^{\bullet -} + G^{\bullet +} \quad (1)$$

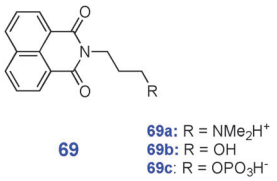
This was demonstrated by Saito and co-workers with the L-lysine-conjugated 1,8-naphthalimide **66a**, which caused photoinduced cleavage after alkaline treatment. The oxidation was shown to proceed preferentially (84%) at the 5′-G residue of the GG sequence in the target duplex 5′TTGGTA/5′TACCAA.[84a] This site preference was not affected by deaerating the solution. Studies with other oligodeoxynucleotides showed that the preference for cleavage was in the order GGG > GG > GA >> GC, GT, which correlated well with the lowest ionisation potential calculated *ab initio*.[84b] Nanosecond laser flash photolysis of **66a** in the presence of the duplex confirmed the formation of the reduced radical anion of **66a** from the initially formed triplet excited state. In contrast to **66a**, the 3-nitro derivative



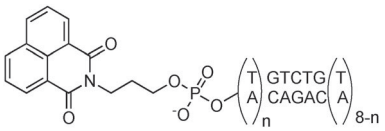
66c was shown to photocleave DNA preferentially at the T residues.[84c] The thymine specific cleavage is thought to be initiated by H-abstraction from the methyl group of thymine by the photoexcited **66c**. However, with the 4-nitro derivative **66b**, both 5′-GG and T specific cleavages were observed.

Kelly and co-workers have reported the interaction of mononucleotides and DNA with cationic naphthalimide, **67** or its corresponding naphthalene diimide.[85] **67** induced photocleavage of supercoiled DNA in the absence of oxygen.[85c] Laser flash photolysis showed that in the presence of mononucleotides and DNA the triplet state of **67** is quenched with concomitant growth of imide radical anion ($NI^{\bullet -}$).[85a,b] Raising the DNA concentration increases the fraction of DNA bound chromophore and leads to a decrease in the yield of triplet excited state ($^3NI^*$). This was explained in terms of increased singlet state quenching within the DNA-bound imide complex, which produces a drop in the efficiency of intersystem crossing. (ISC quantum yield decreased from 0.71 for free **67** to 0.08 when **67** was fully bound to DNA). Banerjee *et al.* carried out a detailed study of the interactions with nucleotides and various DNAs of **68**, a 4-amino analogue of **67**.[86a] Interestingly the compound intercalates but shows a strong preference for AT-rich sites.[86a] **68** shows an enhanced fluorescence emission when bound to AT rich DNA or to AMP, but is quenched by guanine, indicating that its singlet excited state can oxidise guanine but not adenine. Ultrafast laser excitation of **68** complexed to GMP, however, did not show the formation of oxidised guanine or the naphthalimide radical anion, suggesting that the back reaction of the naphthalimide radical anion and the guanine radical cation proceeds very rapidly (<700 ps).[86b]

Majima and co-workers investigated the interaction of cationic, anionic and neutral naphthalimides **69a–c** with oligonucleotide sequences.[87] The cationic naphthalimide **69a** exhibited strong association with oligonucleotides; however, the naphthalimide radical anion ($NI^{\bullet -}$) was not observed in nanosecond transient absorption measurements. This again is consistent with very rapid back electron transfer following the guanine oxidation by the singlet excited naphthalimide. In contrast $NI^{\bullet -}$ is observed for the anionic naphthalimide **69c**, which does not bind with negatively charged oligonucleotides. Additionally, the charge separation efficiency was found to increase when the oligonucleotide contained sequential G′s as the oxidation potential of G has been shown to decrease by stacking interactions.[84b]



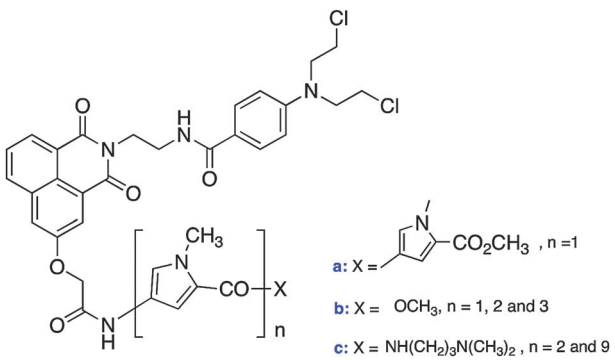
Majima and co-workers have also investigated the photosensitised damage in DNA using various oligonucleotide sequences covalently attached to the naphthalimide chromophore as shown in structure **70** and correlated the results with laser flash photolysis measurements.[75] The oxidative damage to guanine was found to be very sensitive to the number of intervening AT base-pairs, being very small for $n < 4$. This chemical yield was shown to depend on the lifetime of the charge-separated state of $NI^{\bullet -}$ and $G^{\bullet +}$ which is determined by the back electron transfer. This study nicely demonstrates how several factors are important in determining the photooxidative damage to DNA.



70

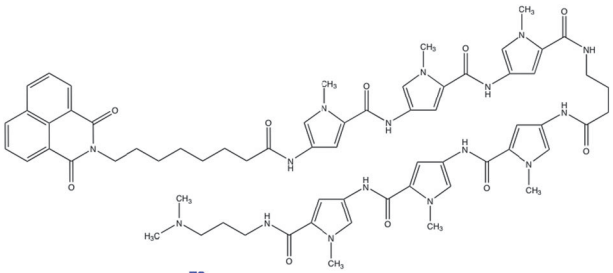
2.10 Naphthalimide derivatives with specific DNA sequence selectivity

The potential of small molecules that target DNA in a sequence specific manner has long been realised and has had major implications in medicine, as well as for basic research. One way of achieving this is by combining the effects of different DNA binding modes, such as intercalation, electrostatic and hydrogen bonding interactions in a single molecular target. To date several excellent examples have been published that attempted to achieve this, by linking the naphthalimide structures to known minor groove-binding agents. An example of such design is that of Gupta *et al.* who reported on the synthesis, DNA sequence specificity and biological evaluation of DNA-directed alkylating agents **71a–c** comprising of naphthalimide, nitrogen mustard and lexitropsin moieties.[70] The biological properties of these compounds were tested against human nasopharyngeal tumour cells and, interestingly, the compound lacking the lexitropsin moiety was the most active.



71

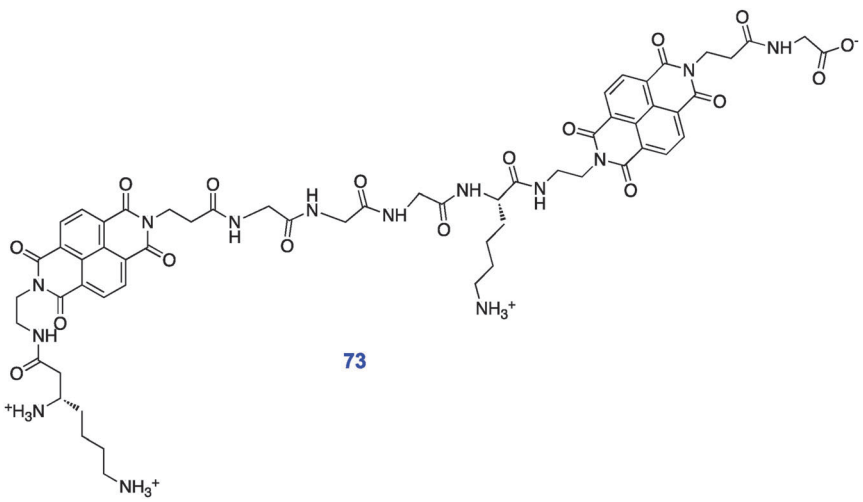
Another example is that of Lee *et al.*[88] who combined the intercalation effect of the 1,8-naphthalimide chromophore with the groove binding effect of pyrrole-imidazole polyamides, giving conjugate **72**. Ethidium bromide displacement studies showed that the 1,8-naphthalimide moiety of **72** intercalated into DNA upon binding. In addition, DNAse I footprinting, thermal denaturation, circular dichroism and Surface Plasmon Resonance (SPR) studies established that sequence selective binding to a topoisomerase II gene promoter regulatory sequence was driven by the pyrrole-imidazole portion of **72**.



72

Another example of the use of chromophores conjugated to peptidyl chains with the view of achieving sequence selective recognition of DNA is that of by Iverson *et al.*[89] who developed the peptide **73** (and many other related structures) as a DNA bis-intercalator. While the scope of this review is not to explore the field of these related naphthalimide structures, we end this review on this particular *naphthalene diimide* example, giving its close relation to the topic herein.

This compound was shown to bind to DNA by intercalation, or more specifically, by the threading of the naphthalene diimide *via* the major groove, in a similar manner to that of many bis-1,8-naphthalimide derivatives discussed above. In **73**, the naphthalene diimide parts showed a preference for the binding to G:G steps, which was possibly enhanced through the formation of specific hydrogen bonds between the amide N–H groups and the oxygen of guanine. Furthermore, the NH_3^+ from the lysine residue was folded towards the negatively charged



73

phosphate backbone of DNA, thus improving the DNA recognition and the overall strength of the binding. This work has been continued by the Iverson group, who recently demonstrated the sequence specific threading intercalation of a naphthalene diimide derivative containing four intercalating moieties.[90]

3. Conclusion

This review has given an overview of some of the many 1,8-naphthalimide derivatives that have been developed to date as DNA binders, as anticancer agents and as potential imaging agents for cellular environments, and potential markers for cancers. As can be seen from our review, which builds on the earlier work of Braña *et al.*, the field is thriving and more and more examples are being published every year; with many more being developed by both academic institutions and companies. The 1,8-naphthalimide structure has a bright future in the field of small molecules targeting therapeutics; its ease of synthesis and the ability to adapt and modify the basic structure is the key to its success.

We have tried to give as a broad overview as possibly of the field, pointing out some of the most promising structures to date that have entered into clinical trials and shown to possess high anticancer activity in various types of cell lines. Necessarily we have had to restrict our discussion at the same time as we have tried to be as detailed as possible, comparing and contrasting the results from both physiochemical as well as biological studies.

Acknowledgements

We grateful to the many undergraduate project and postgraduate students who have helped us out with developing the naphthalimide project at Trinity College Dublin. Some of their names are listed in the references and have been discussed above; but many more have contributed to a large body of work that is still to be published. We, in particularly, grateful to Dr Celine Blais and Dr Anthony McElligot for their major contributions early in this programme, and Prof. Mark Lawler and Prof. Clive Williams (Trinity College Dublin) who have spearheaded the investigation into the biological properties of so many of our compounds. Their support and collaboration throughout the last ten years have been vital to our success. We also grateful to Dr Susan Quinn (University College Dublin) and Dr Jonathan Kitchen (University of Southampton) for their collaborations and contributions and our former PhD students Dr Robert Elmes (now at Sydney University) and Gary Ryan for their contributions to the development of naphthalimide based metal ion complexes. We grateful to Prof. Paul E. Kruger (University of Canterbury, NZ) and Prof. Fred Pfeffer (Deakin University, Australia) for their collaborations on the use of naphthalimides as anion sensors.[3a] We particularly like to thank Science Foundation Ireland (SFI: RFP 2006 and RFP 2009), CSCB, PRTLI for Cycle 4, and Enterprise Ireland (EI) as well as Kinerton Ltd. (Now Ibsen Ltd.) for financial support of this project, spanning over 14 years. This review is dedicated to the memory of Dr Chris J. Michejda (formerly at National Cancer Institute at Frederick, Maryland, USA) for his inspiration and friendship.

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