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Multi-nuclear platinum complexes as anti-cancer drugs

Nial J. Wheate [a,b], J. Grant Collins [a,*]

[a] School of Chemistry, University College, University of New South Wales, Australian Defence Force Academy, Canberra, ACT, 2600, Australia
[b] Joint Health Support Agency, Department of Defence, Campbell Park Offices, Canberra, ACT, 2600, Australia

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Contents

Abstract

This article reviews investigations over the last 15 years into the development of multi-nuclear platinum complexes as anti-cancer agents, with the purpose of providing an insight into the benefits of, and reasons for, their success. The cytotoxicity of multi-nuclear platinum complexes is compared, as is their ability to overcome both natural and acquired drug resistance. Possible structure-activity relationships are outlined. While the multi-nuclear platinum complexes exhibit excellent anti-cancer activity, the associated toxicity could limit their clinical use. Given that these complexes derive their activity from the novel adducts they form with DNA, three important aspects of their binding are discussed; their DNA pre-association, the DNA adducts formed and the DNA conformational changes induced.

Keywords: Anti-cancer; Multi-nuclear platinum complexes

1. Introduction

In the mid 1970s initial anti-tumour studies with cisplatin (Fig. 1) indicated considerable activity against sarcoma 180 and leukemia L1210 tumours in mice [1]. Soon after, the US Food and Drug Administration

* Corresponding author. Tel.: +61-2-6268-8316; fax: +61-2-6268-8002.
E-mail address: g-collins@adfa.edu.au (J.G. Collins).

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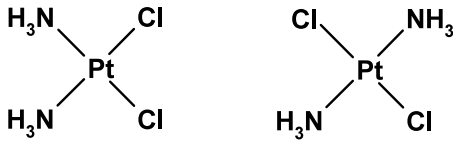

Fig. 1. The anti-cancer platinum complex (A) cisplatin and its inactive isomer (B) transplatin.

(FDA) approved cisplatin for clinical use and it has remained in use ever since [1]. Interestingly, the *trans*-isomer (see Fig. 1) of this complex shows almost no activity against tumours, either because it is stereochemically incapable of forming particular intrastrand adducts or possibly because the biological processing of adducts from this complex is different from those for the *cis*-isomer [1].

Cisplatin is currently used in the treatment of testicular, ovarian, bladder, head and neck, lung, and cervical cancers [1], but does however, have several major drawbacks. A large percentage of human cancers have a natural resistance to treatment with cisplatin [2]. While, of the cancers that do initially respond to cisplatin treatment, many later acquire resistance to the drug [2]. Acquired resistance can occur for at least four reasons: reduced cellular uptake; increased repair of cisplatin/DNA lesions; increased tolerance of cisplatin/DNA lesions; and deactivation of cisplatin by thiol-containing proteins [3]. Acquired resistance to cisplatin is generally the reason for treatment failure [2]. Cisplatin also has large dose-limiting side effects. Patients treated with cisplatin have shown severe signs of nephrotoxicity (kidney damage), neurotoxicity (nervous system damage) and ototoxicity (hearing loss) [1,2]. Other side effects recorded include elevated blood pressure, diarrhea, severe nausea and vomiting [1].

While it has been found that intravenous hydration has been able to reduce the level of nephrotoxicity [4], the side effects of cisplatin still pose a major drawback to the use of this drug. Over the past 25 years much interest has been centred on developing drugs similar to cisplatin that have fewer side effects. These cisplatin derivatives have demonstrated improvements in reducing side effects, in both the laboratory and in clinical studies, and in overcoming acquired cisplatin resistance in some cancer cell lines [2].

Carboplatin (Fig. 2) developed by Johnson Matthey Technology, displays the same spectrum of activity

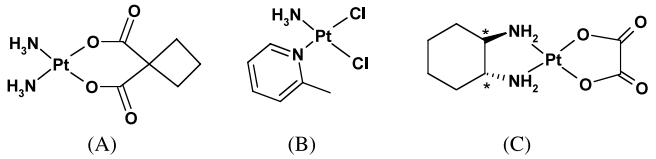

Fig. 2. Second generation anti-cancer platinum complexes (A) carboplatin (B) AMD-473 and (C) oxaliplatin (*, indicates a chiral centre).

against cancer cell lines as cisplatin, with bone marrow toxicity being the dose-limiting factor [5], with little or no nephrotoxicity or neurotoxicity. The reduced toxicity is attributed to the slow nucleophilic substitution (by water) of the chelated dicarboxylato leaving group, with the low reactivity of the platinum complex being attributed to its slow ring opening [6]. As a result, carboplatin can be administered at much higher doses (900 mg m^{-2}) than cisplatin (60–120 mg m^{-2}) [3]. Carboplatin is the only drug, other than cisplatin, to obtain worldwide approval for use in the clinic to date [3].

AMD-473 (*cis*-dichloroamine-2-methylpyridineplatinum(II), see Fig. 2) co-developed by Johnson Matthey and the Cancer Research Campaign (UK), is a sterically hindered platinum complex which is under development as a potential treatment for cisplatin resistant cancers [7]. Crystal structures of the complex have shown that the pyridine ring is tilted 102.7° with respect to the PtN_2Cl_2 square plane [8]. The sterically hindered non-leaving group slows associative substitution reactions on the square planar platinum. This is important for biological nucleophiles that are able to bind Pt without prior hydrolysis of the chloro ligand (e.g. thiol groups of proteins or peptides) [7]. The strategy was successful, with a lower reactivity of the drug to soft nucleophiles (such as sulphur) being observed while maintaining reactivity towards DNA [9]. AMD-473 hydrolyses more slowly (twofold) than cisplatin and also shows reduced rates of reaction with thiourea and methionine [9]. AMD-473 also displays (in vitro) cytotoxicity intermediate between cisplatin and carboplatin [10]. In a growth inhibition assay using 11 human ovarian cancers the mean IC_{50} of AMD-473 was 8.1 μM, higher than the mean for cisplatin (2.6 μM) but lower than the mean for carboplatin (20.3 μM) [10].

As well as overcoming cisplatin resistance from deactivation by thiol-containing proteins, AMD-473 has also been shown to overcome other modes of resistance such as reduced cellular uptake and enhanced DNA repair [10]. Currently Anormed (the development company) is looking for a partner to help complete further clinical trials [11].

Oxaliplatin (see Fig. 2) was approved by the FDA in August 2002 under the name Eloxatin [12]. Eloxatin is given in combination with 5-fluorouracil plus leucovorin (5FU/LV). It is used to treat patients with colorectal cancer whose disease has recurred or become worse following initial therapy with a combination of other drugs [12]. The combination, including Eloxatin, was shown to shrink tumours in some patients and delay resumed tumour growth [12].

While there has been some success in lowering the toxicity of platinum drugs (carboplatin) and limited success in overcoming acquired cisplatin resistance (AMD-473, oxaliplatin) there has been little success in

developing drugs that show activity in cancer cell lines that have a natural resistance to cisplatin and carboplatin. In an effort to overcome both natural and acquired resistance in human cancer cell lines many researchers have turned to multi-nuclear platinum complexes.

The purpose of this review is to summarise the reported studies into multi-nuclear platinum complexes with regard to their history and development, structure-activity relationships, toxicity, pre-association with DNA and the DNA covalent adducts formed. In the last 3 years there has been little review of multi-nuclear platinum complexes as anti-cancer agents and the reviews that have been published have tended to focus primarily on specific examples rather than a more general review [13–15]. Here we present a more comprehensive review of the multi-nuclear platinum complexes synthesised and tested in the effort to improve platinum chemotherapy.

2. Development of multi-nuclear complexes

Although some progress had been made in reducing the toxic side effects and overcoming resistance, little improvement in cytotoxicity or spectrum of activity had been observed for the many analogues of cisplatin [3]. Evidence suggests that this is due to the cisplatin analogues forming a similar array of DNA adducts as cisplatin [3]. Consequently, attention turned to the synthesis of 'non-classical' platinum complexes which were capable of forming a different range of DNA adducts—which could therefore display a different spectrum of anti-cancer activity compared to cisplatin. Non-classical platinum complexes include *trans* complexes, of the general formula *trans*-[$PtCl_2(NH_3)L$] where L is a planar N donor [16], mono-functional complexes, like *cis*-$[Pt(NH_3)_2(Am)Cl]^+$ where Am = pyridine, pyrimidine, or purine [17–19] and multi-nuclear complexes. Multi-nuclear platinum complexes contain two or more linked platinum centres that can each covalently bind to DNA, and hence are capable of forming a completely different range of DNA adducts compared to cisplatin and its analogues [14]. These multi-nuclear complexes represent a completely new paradigm for platinum based anti-cancer complexes [14], and appear to offer great potential as new anti-cancer agents.

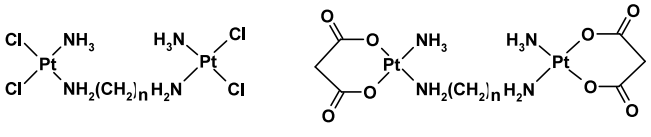

Fig. 3. Dinuclear platinum complexes containing two linked cisplatin centres, where $n = 2–6$.

2.1. Bi-functional platinum centres

Initially, the synthesis of multi-nuclear platinum complexes (Fig. 3) was based upon the linking of two cisplatin-like centres [20–29]. The first complex developed (2,2/c,c) (**1**) was a simple cisplatin derivative with two [$PtCl_2(NH_3)$] centres linked by a flexible diamine chain [25,27]. This kept with the traditional structure-activity rules of a neutral complex consisting of a square planar platinum with two *cis*-chloro ligands and with at least one am(m)ine group. The replacement of the chloro ligands with malonate (**2**), to improve water solubility, resulted in a series of 2,2/c,c complexes with good activity in cisplatin resistant cancer cell lines [24]. While these complexes were capable of binding DNA in a manner similar to cisplatin, GpG intrastrand cross-links, they were also capable of forming longer range intra- and interstrand adducts [24]. Indeed, mechanistic studies demonstrated that the 2,2/c,c complexes produced a high percentage of interstrand adducts [24].

A similar approach was also adopted by Broomhead and co-workers (Fig. 4), who linked two cisplatin-like centres together with the 4,4′-dipyrazolylmethane (dpzm) ligand [20–23]. A series of double bridged dinuclear platinum complexes with both square planar and octahedral geometries were synthesised and tested for anticancer activity, both in vitro and in vivo (**3**–**7**) [20–22]. The series was then extended to single bridged dinuclear complexes, with either two chloro or two DMSO ligands on each platinum centre (Fig. 5) (**8**–**11**) [23]. While these complexes exhibited better cytotoxicity than the double bridged complexes in three cancer cell lines, they were insoluble in water and did not show any significant advantage compared to cisplatin [23].

2.2. Mono-functional platinum centres

While the synthesis of dinuclear complexes with two bi-functional platinum centres resulted in complexes that could bind to DNA in a different manner than cisplatin, the more important development was the subsequent synthesis of dinuclear complexes that contained mono-functional platinum centres (Fig. 6) (**12**–**15**) [24,30–42]. The first complexes, called 1,1/t,t (**12**) and 1,1/c,c, (**13**) represented a truly new class of metal-based anti-cancer compound, as they were cationic and contained only one chloro leaving group on each platinum centre. These complexes show good water solubility and excellent activity in both cisplatin sensitive and resistant cell lines.

To a smaller extent other groups have also studied multi-nuclear platinum complexes with one leaving group on each platinum with mixed results (Fig. 7). A small series of dipyridyl linked complexes (**16**) have been synthesised by Zhao et al. [37]. Wheate et al. have synthesised dinuclear and trinuclear platinum complexes

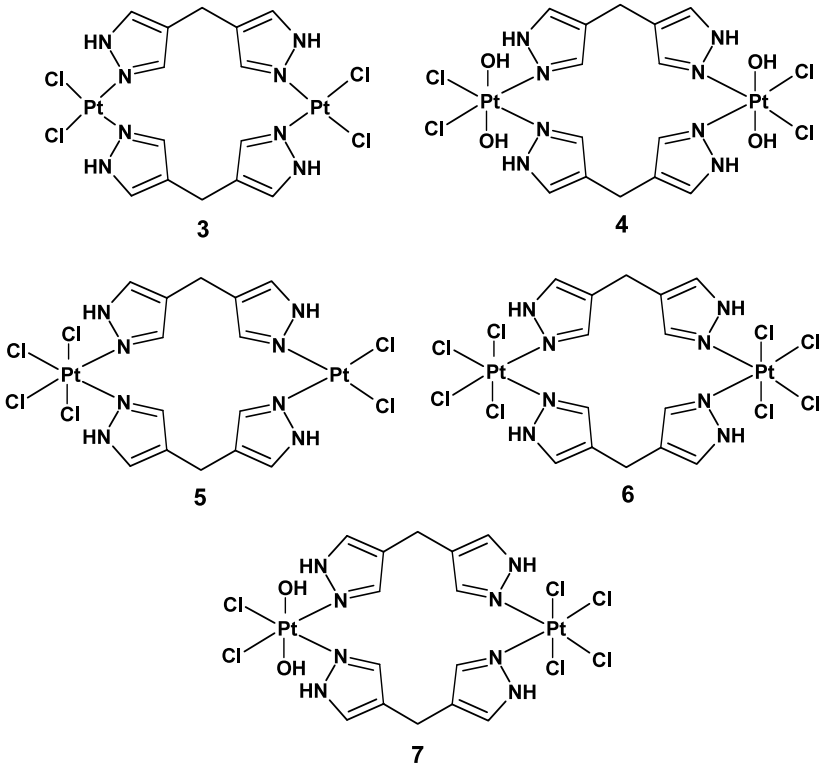

Fig. 4. Double bridged, multi-nuclear platinum complexes linked by the 4,4′-dipyrazolylmethane (dpzm) ligand synthesised by Broomhead and co-workers.

that are linked by the dipyrazolylmethane (dpzm) ligand (**17** and **18**) [38]. These two complexes are the dpzm analogues of 1,1/t,t ($n = 6$, **12**) and BBR3464 (see below), and hence, provide a direct comparison of the effect of replacing the aminoalkane linking ligand for a less flexible aromatic linking ligand. Also of interest is the tetranuclear complex (**19**) of Jansen et al. [39], using a branched aminoalkane linking chain and the bis(thiourea) linked complex (**20**) of Bierbach et al. (Fig. 8) [40].

In a different approach, Komeda et al. produced a series of pyrazole linked complexes (Fig. 9) [41,42]. These pyrazole linked complexes (**21**–**23**) were synthesised to mimic cisplatin binding (e.g. formation of a GpG intra-strand adduct) without the major DNA kinking [41,42]. The distance between the two platinum centres is similar to the distance between two sequential G bases in B-type DNA. The complexes were therefore hoped to bind DNA in such a way that the DNA helix structure should remain relatively undisturbed [41,42].

2.3. Complexes incorporating cationic and hydrogen-bonding linking ligands

The development of trinuclear complexes, and di-nuclear complexes linked with polyamine ligands, that incorporate charge and hydrogen-bonding functionality into the bridging ligand further increased the multi-

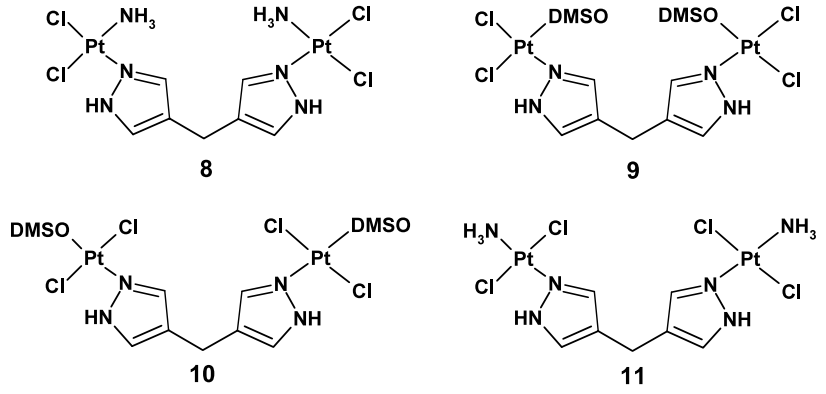

Fig. 5. Singularly bridged, multi-nuclear platinum complexes linked by the 4,4′-dipyrazolylmethane (dpzm) ligand.

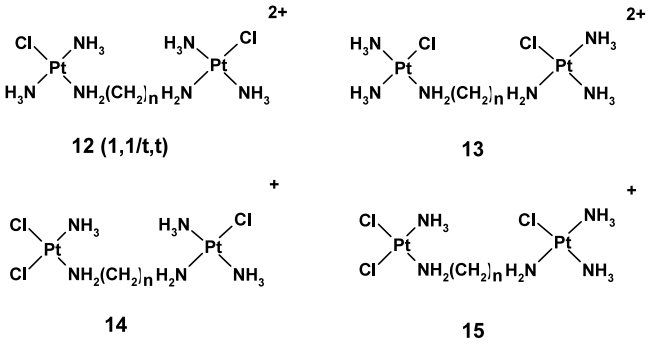

Fig. 6. The range of dinuclear platinum complexes synthesised by Farrell and co-workers, that contain charged mono-functional platinum centres.

nuclear series (Fig. 10) (**24**–**27**) [14,43–53]. The am(m)ine groups of the polyamine linkers (**25**–**27**), or the central platinum centre in **24,** provide groups capable of hydrogen bond formation with DNA atoms such as the O6 of guanine or the O3 of thymine. These complexes show excellent solubility in water, which is good for administration of the drugs. More importantly, whereas the dinuclear platinum complexes like **12** and **13** exhibit cytotoxicity at micromolar concentrations, some of these complexes are active at nanomolar concentrations. These complexes (**24**–**27**) also show activity in vivo at concentrations lower than their maximum tolerated dose (MTD) in mice [14]. Although the MTD of these complexes ($\sim$0.25 mg kg^{-1}) are much

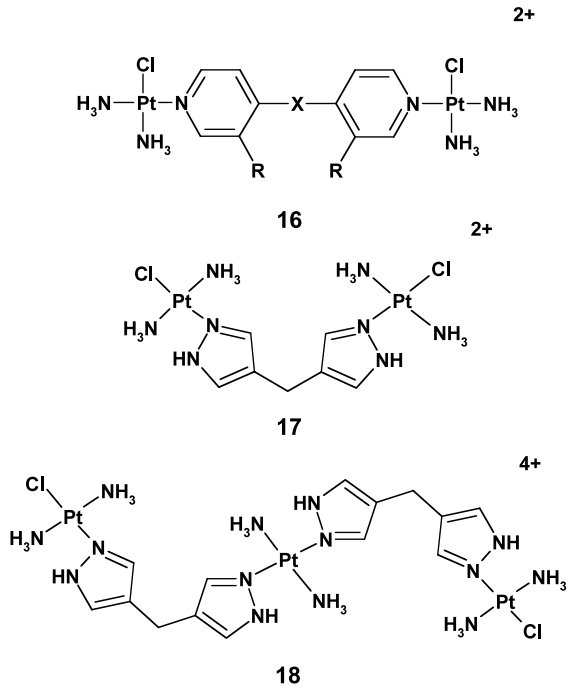

Fig. 7. The dinuclear platinum complexes synthesised by Lin et al. (**16**) where X is S or Se, R is H or CH_3, and the multi-nuclear platinum complexes containing mono-functional platinum groups of Wheate et al. (**17** and **18**).

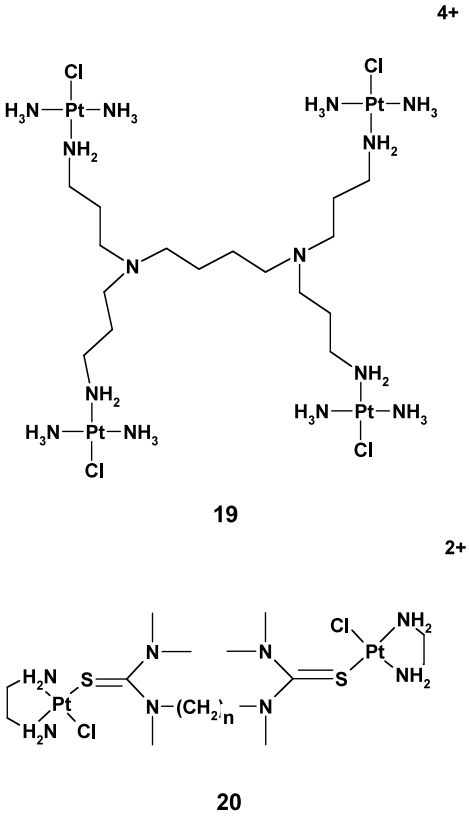

Fig. 8. A tetranuclear platinum complex (**19**) and the bis(thiourea) linked platinum complex (**20**).

lower than the available doses for either cisplatin or carboplatin (see Section 1), **24** entered Phase II clinical trials 3 years ago, under the auspices of Novuspharma SpA, and **25**–**27** are about to enter Phase I trials [54].

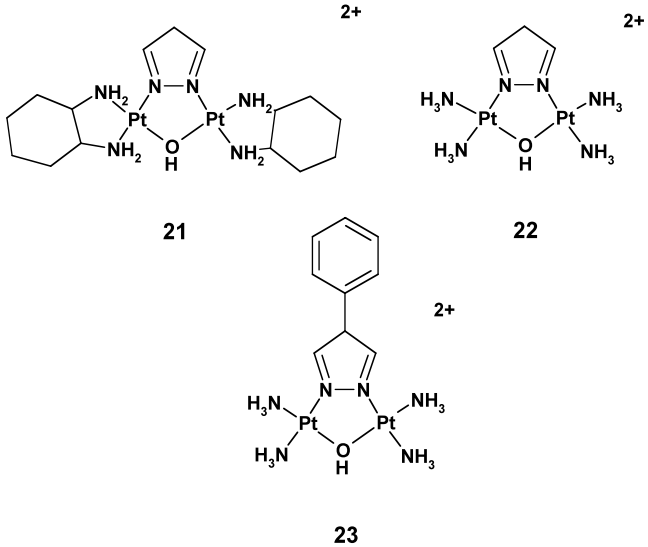

Fig. 9. The dinuclear pyrazole linked complexes of Komeda et al. designed to mimic the binding of cisplatin to sequential G bases of DNA.

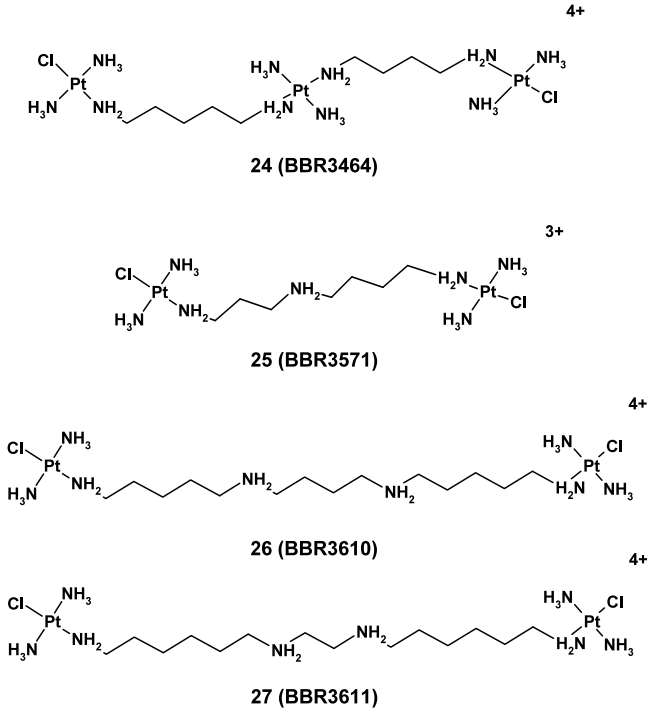

Fig. 10. A selection of multi-nuclear platinum complexes, synthesised by Farrell and co-workers, that incorporate hydrogen-bonding capacity and charge into the linker.

2.4. Miscellaneous complexes

Following a different line, Woodhouse and Rendina have synthesised two dinuclear platinum complexes (Fig. 11), **28** and **29**, linked by a dicarba-*closo*-dodecaborane ligand [55]. These complexes, rich in boron atoms, are not designed as conventional platinum drugs. Although they contain cisplatin-like centres, and monofunctional centres, they are vehicles to get the boron cage as close to the cellular DNA as possible for increased effectiveness in Boron Neutron Capture Therapy (BNCT).

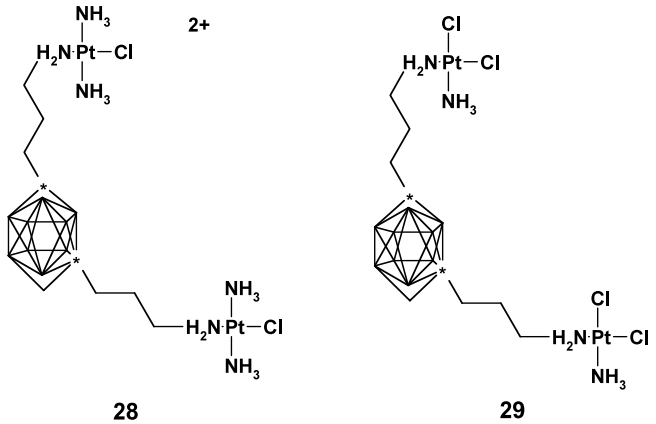

Fig. 11. Carborane containing complexes synthesised by Woodhouse and Rendina for use in BNCT. Each point in the cage represents a boron atom and each *, represents a carbon atom.

3. Cytotoxicity and structure-activity relationships

3.1. Cytotoxicity

Over 50 multi-nuclear platinum complexes have been synthesised and tested for cytotoxicity in a variety of cancer cell lines in various studies. Table 1 shows the cytotoxicities and resistance factors (R_f) of a selection of multi-nuclear platinum complexes in the matched cancer cell lines L1210 and L1210/DDP. Most of these complexes show lower R_f values than cisplatin in matched cell lines, but the level of cytotoxicity is affected by the nature of the ligands used (see Section 3.2). Previously it has been shown that the cisplatin resistance in L1210/DDP is due to several factors including reduced uptake, increased thiol concentrations and increased cellular repair [56], making this a good cell line model for determining the potential of the complexes to overcome resistance.

From all the complexes tested, the first to enter clinical trials based on in vitro and in vivo trials was the tri-nuclear complex BBR3464 (**24**). BBR3464 is much more active than cisplatin in all cancer cell lines tested, showing comparable activity at up to 1/500th the dose in vitro [48,57]. In an assay of 7 human cancer cell lines the mean IC_{50} of BBR3464 (0.6 μM) was much lower than that observed for cisplatin (27.8 μM) [58]. BBR3464 is also capable of overcoming cisplatin resistance [47]. Most importantly, however, BBR3464 shows activity in several cancer cell lines that have a natural resistance to cisplatin [48]. A list of the cytotoxicities of BBR3464 in a range of cancer cell lines is given in Table 2.

From clinical trials it appears that BBR3464 will be most useful in the treatment of non-small cell lung cancer, gastric cancer, ovarian cancer, small-cell lung cancer and possibly other solid tumours including pancreatic cancer [54].

3.2. Structure-activity relationships

From the studies of multi-nuclear platinum complexes in differing cancer cell lines and DNA binding studies, some important structure-activity rules are becoming apparent, and have previously been summarised in some detail by Farrell [14]. The three overriding factors in designing multi-nuclear platinum drugs appear to be chain length and flexibility, hydrogen-bonding capacity and charge of the linking chain, and the geometry of the chloro ligand to the linking chain. For the aliphatic chains used by Farrell and co-workers the ideal length of the linker appears to be 8 atoms (two ammine and six methylene groups). This is evident in the dinuclear and trinuclear complexes 1,1/t,t ($n = 6$, **12**) and BBR3464 (**24**). Both are much more active than the other analogues where the linking chain is either shorter

Table 1
Cytotoxicity levels (IC_{50}) of selected multi-nuclear platinum complexes showing their ability to overcome cisplatin resistance in the matched leukaemia cancer cell lines L1210 and L1210/DDP or L1210/2

Complex	Variables	IC_{50} (μM)			IC_{50} (μM)			R_f		
		Cell line	Complex	Cisplatin	Cell line	Complex	Cisplatin	Complex	Cisplatin	Ref.
1	$n=5$	L1210/0	1.16	0.23	L1210/DDP	2.33	5.7	2.0	25	[29]
12	$n=4$	L1210/0	3.5	0.22	L1210/DDP	0.87	9.89	0.25	45	[24]
13	$n=6$	L1210	0.24	0.43	L1210/DDP	2.6	11	11	26	[60]
16	X = S, R = H	L1210	0.83	0.47	–	–	–	–	–	[37]
16	X = Se, R = CH_3	L1210	5.91	0.47	–	–	–	–	–	[37]
17	–	L1210	3.8	0.8	L1210/DDP	8.8	6.9	2.3	8.6	[38]
18	–	L1210	2.5	0.8	L1210/DDP	3.6	6.9	1.4	8.6	[38]
19	–	L1210/0	12.4	1.5	L1210/2	9.3	4.6	0.8	3.1	[39]
20	$n=6$	L1210/0	6.93	0.19	L1210/DDP	56.63	11.63	8.2	61	[40]
22	–	L1210	0.5	4.8	L1210(cisPt)	1.1	19.3	2.2	4.0	[42]
23	–	L1210/0	2.0	4.8	L12110(cisPt)	11.6	19.3	5.8	4.0	[42]
24	–	L1210	0.094	3	L1210/CDDP	0.075	28	0.8	9.3	[46]
25	–	L1210/0	0.41	0.31	L1210/DDP	0.02	8.9	0.05	29	[44]
26	–	L1210	1.15×10^{-3}	3.0	L1210/CDDP	1.06×10^{-3}	27.7	0.92	9.2	[14]
27	–	L1210	4.82×10^{-4}	3.0	L1210/CDDP	3.95×10^{-4}	27.7	0.82	9.2	[14]
34	$n=4$	L1210/0	>10	–	L1210/DDP	>10	–	–	–	[91]
35	$n=6$	L1210	19.2	1.5	L1210/DDP	23.8	4.6	1.2	3.1	[34]

These complexes, with the exception of **23**, all show lower R_f values compared to cisplatin, with the highest cytotoxicities displayed by the clinical agents BBR3610 (**26**) and BBR3611 (**27**). Complexes **34** and **35** (structures not shown) are derivatives of the 1,1/t,t complex (**12**), synthesised by Farrell and co-workers, that contain pyridine ligands.

($n=2$–5) or longer ($n=7$) [59]. For the polyamine family of multi-nuclear platinum complexes, it is evident that increasing the charge and the chain length, e.g. in BBR3610 (**26**) and BBR3611 (**27**), leads to complexes which are more active [14].

However flexibility also appears to be a major factor. In studies using the dpzm ligand, both the dinuclear (**17**) and trinuclear (**18**) complexes were much less active than their aliphatic equivalents 1,1/t,t ($n=6$, **12**) and BBR3464 (**24**) [38]. As the linkers were ca. the same length as the those in the BBR complexes, but were much more rigid, the decrease in activity was attributed to this rigidity [38]. It was proposed that due to their rigid nature, these two dpzm complexes probably could not form a sub-set of the DNA adducts formed by both 1,1/t,t ($n=6$, **12**) and BBR3464 (**24**) and indeed this may also explain why the shorter 1,1/,t,t ($n=2$–5) and 1,0,1/t,t,t ($n=2$–5) complexes are less active [38]. The length of these complexes would also be prohibitive in the formation of some types of DNA adducts.

From analysis of the data in Table 1, a trend can be seen where almost all the complexes containing aromatic ligands show poorer cytotoxicities, with values greater than 1 μM being generally observed. This would indicate that straight chain aliphatic, or NH_3, ligands are preferable, even when not incorporated as linking ligands, over aromatic ones. The best linking ligands appear to contain both positive charges and hydrogen-bonding capacity in the form of either a charged platinum centre and/or charged am(m)ine groups.

Geometric isomerisation also plays a part. While all complexes synthesised with the chloro ligand *cis* or

Table 2
Cytotoxicity levels (IC_{50} expressed as either μg ml^{-1} [*] or μM) of BBR3464 in a variety of cancer cell lines

IC_{50}				IC_{50}				IC_{50}			
Cell line	BBR3464	Cisplatin	Ref.	Cell line	BBR3464	Cisplatin	Ref.	Cell line	BBR3464	Cisplatin	Ref.
U2-OS*	1.1	4.1	[47]	SK-N-DZ	0.12	6.37	[95]	IGROV-1*	8.0	4.3	[48]
U2-OS/Pt	1.2	21.3	[47]	BE(12)M17	0.017	5.50	[95]	SW626*	0.08	6.3	[48]
L1210/0	0.35	1.3	[57]	LAN-1	0.0017	7.40	[95]	SAOS*	0.1	3.0	[48]
L1210/DDP	0.12	59	[57]	A2780*	0.012	0.06	[48]	POGB*	0.009	0.25	[48]
L1210/MGBG	0.005	2.6	[57]	A2780/CP*	0.29	0.73	[48]	A431*	3.1	10.4	[48]
ROSS*	5.4	8.8	[94]	M14*	7.0	10.7	[94]	CL2/21*	0.16	2.7	[94]
JR8*	1.6	7.8	[94]	CL2/60*	0.05	10.6	[94]	SK-MEL-5*	7.5	8.8	[94]
OLI*	15	24.5	[94]	GRIG*	13	13	[94]	OAW42*	6.5	2.6	[94]
OVCAR5*	0.58	6.8	[94]	OVCA-432*	28	1.94	[94]	A2780Cp8*	0.25	18	[94]

trans to the bridging ligand show good activity, it is the complexes in the *trans* configuration that are generally more active, particularly in cisplatin resistant cell lines [14,60]. This is seen in several comparisons including the dinuclear complexes (**12** and **13**) and the various geometric variations of the trinuclear complex BBR3464 (**24**) [14,60]. Again, the reason for this difference in activity is probably due to the *cis* complexes being able to only form a sub-set of the DNA adducts formed by the *trans* complexes, although other factors such as the rate of hydrolysis can not be excluded.

4. Toxicity

Although only a small amount of data has been published to date on multi-nuclear platinum complexes all evidence points to them being much more toxic, and with different toxicity profiles, compared to both cisplatin and carboplatin. Whereas carboplatin and cisplatin can be administered in much higher doses (900 and 60–120 mg m^{-2}, respectively) [3], from Phase I trials it was found that the MTD of BBR3464 is only 1.1 mg m^{-2} [61,62]. BBR3464 also displays a different toxicity profile from cisplatin and carboplatin, with the dose-limiting toxicity being neutropenia, often leading to diarrhea and nausea. The side-effects were found to be dose-related, with diarrhea and neutropenia reaching grade IV by the 1.1 mg m^{-2} stage. At no stage was there evidence of nephrotoxicity (kidney damage), neurotoxicity (nervous system damage), pulmonary toxicity or severe emesis [61]. From a recent Phase II trial a MTD of only 0.9 mg m^{-2} was reported for 5 patients and from the 45 patients participating in the trial the main toxicity observed, was again, neutropenia (G3: 40%, G4: 40%) [63].

Recently it has been found that the MTD of BBR3571 in mice was ca. 0.25 mg kg^{-1} [14], with a tolerability similar to that of BBR3464. Similar levels of toxicity for BBR3571 means it also can only can be administered at very low levels, but BBR3610 and BBR3611 are even more toxic and would have to be administered at doses ten times lower [14].

5. Pre-association with DNA

It is generally accepted that *cis*-$[Pt(NH_3)_2(Cl)(H_2O]^+$, formed by the hydrolysis of one Pt–Cl bond in cisplatin, pre-associates with DNA before covalently binding to specific purine residues [64]. Lippard and co-workers have demonstrated that the rate of cisplatin covalent binding to oligonucleotides was facilitated by the pre-association of the metal complex with the DNA [65], while Wang et al. have recently provided direct evidence of this pre-association [64]. Given that the pre-association is largely stabilised by electrostatic forces, it is reasonable to assume that the pre-association of cationic multi-nuclear platinum complexes with DNA would be even stronger, and hence, more important.

The pre-covalent association of cationic multi-nuclear platinum complexes with polyanionic DNA will significantly affect the rate and site of platination, as an increased local concentration will increase the probability of a covalent reaction at these sites. In addition, the pre-association may induce a local conformational change in the DNA that may aid binding at particular sites. Using inert analogues of dinuclear platinum anti-cancer complexes, Farrell and co-workers have demonstrated that these complexes will, relatively, strongly associate with DNA [66]. Furthermore, depending upon the base sequence of the DNA, the inert platinum complexes can induce a B- to Z-type DNA conformational change [66]. With poly(dG-dC)·poly(dG-dC), the complex $[\{Pt(NH_3)_3\}_2\mu\text{-}H_2N\text{–}(CH_2)_6\text{–}NH_2]^{4+}$ was shown to induce a B- to Z-type conformational change, and at a lower concentration than $[Co(NH_3)_6]^{3+}$, a well known agent for converting B-type DNA to Z-type DNA [66]. Johnson et al. proposed that the initial pre-association might induce a reversible DNA conformational change, which could become 'locked' upon covalent binding (Fig. 12) [66].

In order to determine the preferred site for the pre-covalent binding association of cationic multi-nuclear platinum complexes with DNA (Fig. 13), Wheate et al. were the first to show minor groove pre-association when they studied the binding of inert analogues of dinuclear platinum anti-cancer complexes with sequence specific oligonucleotides by NMR spectroscopy [67–69]. For the dodecanucleotide $d(CGCGAATTCGCG)_2$, the complexes **30**–**33** (Fig. 13) were shown to preferentially bind at the central AATT sequence in the DNA minor groove [67,68]. In order to confirm that the observed preference was for A/T rich regions, rather than simply at the centre of the short segment of DNA, the binding of **30** was further examined with the dodecanucleotide $d(CAATCCGGATTG)_2$ [67]. Again, the metal complex

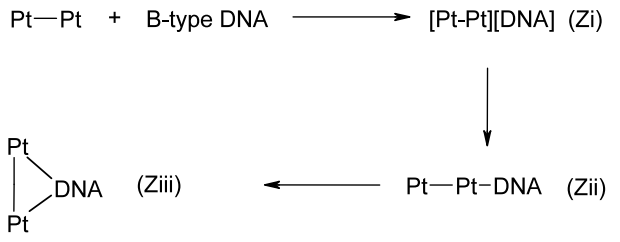

Fig. 12. The three step induction of DNA conformation change by multi-nuclear platinum complexes proposed by Johnson et al. First, Zi, initial association with the DNA and conformation change from B-type to either Z-type or A-type. Second, Zii, mono-adduct formation and third, Ziii, bis-adduct formation locking the DNA into either Z-type of A-type DNA.

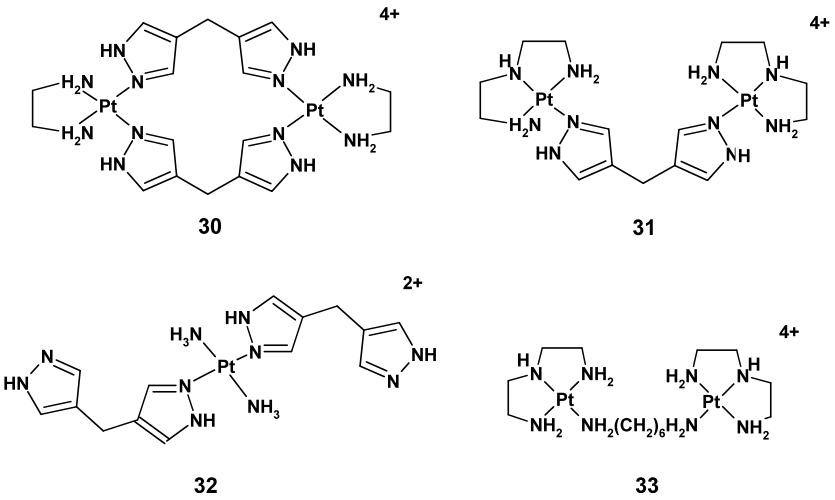

Fig. 13. Mononuclear and dinuclear platinum complexes used to study the pre-covalent association of multi-nuclear platinum complexes with DNA by Wheate et al.

was shown to bind at the A/T rich regions in the minor groove, with an affinity $>10^5$ M^{-1}, rather than at the central CCGG sequence. These results demonstrated that square planar multi-nuclear platinum complexes will preferentially associate in the DNA minor groove regardless of the charge, shape, steric bulk, or the linking ligand used.

It is generally considered that preferential minor groove binding at A/T rich sequences is due to both favourable electrostatic interactions and van der Waals forces, and unfavourable steric interactions of the guanine amino group at G/C rich sequences. In support of this Sehlstedt et al. found that the well characterised minor groove binder 4′,6-diamino-2-phenylindole binds in the major groove of poly(dG-dC)·poly(dG-dC), but in the minor groove of poly(dI-dC)·poly(dI-dC) [70]. They proposed that as inosine is similar to guanine, but lacks the amino group that projects into the minor groove, the major groove selectivity was due to the guanine amino group. However, in a study of the binding of **30** with a decanucleotide that only contained G/C residues, $d(GC)_5$, Wheate et al. showed that the metal complex still preferentially associated in the minor groove, although with a significantly reduced binding constant (10^3 M^{-1}) [69]. This result was somewhat surprising, given the steric hindrance and decreased electrostatic interactions, caused by the guanine amino group, in the DNA minor groove. Taken together, these results indicate that cationic square planar multi-nuclear platinum complexes will preferentially associate in the DNA minor groove, rather than in the major groove where the covalent binding occurs. Recently, further evidence of pre-association in the minor groove has been demonstrated by Berners-Price and co-workers in their studies of the DNA binding of the 1,1/t,t ($n=6$) complex [71,72].

The relatively high affinity of the cationic multi-nuclear platinum complexes for the DNA minor groove may provide an explanation for the observed lower reactivity of BBR3464 with duplex DNA than with single strand DNA as has been reported (see Section 6.1). In addition, the preferred minor groove pre-covalent association may allow the formation of covalent adducts in the minor groove. Indeed, Farrell and co-workers have reported the observation of covalent binding at an A of an ATAĀTTAAA sequence for 1,1/t,t ($n=6$, **12**), which may represent a minor groove covalent adduct [45]. However, as the reported A-binding was based on a polymerase stop assay and the DNA was not dialyzed after incubation with the complex, the stop site could have been produced by a non-covalent adduct.

6. DNA covalent adducts

Cisplatin binds at the N7 position of both guanine (G) and adenine (A) bases, with the major adducts formed being the (GpG) (47–50%) and (ApG) (23–28%) intrastrand cross-links, with interstrand cross-links (1–7%) also having been reported [1]. Cisplatin binding produces a rigid bend in DNA of 30–35° directed toward the major groove, and a localised unwinding of the DNA double helix of 13° [1].

Conversely, the DNA binding of multi-nuclear platinum complexes is generally characterised by flexible, non-directional DNA adducts, a greater percentage of interstrand to intrastrand adducts and the ability to induce DNA conformational changes to both A- and Z-type DNA [45,66,73–75]. Since global conformational changes such as winding and bending are important features in protein recognition of platinum damaged DNA, and the adducts formed by multi-nuclear plati-

num complexes are vastly different from the adducts formed by cisplatin, it has been suggested that the distortions induced by these complexes are only weakly recognised by DNA repair proteins [76,77]. This results in a systematic bypass allowing the platinum complexes to inhibit DNA transcription and replication [77,78].

6.1. Rate of reaction with DNA

Using [^{1}H,^{15}N] HSQC 2D NMR spectroscopy, Cox et al. have shown that like cisplatin, the monoaqua monochloro complex is formed prior to the covalent binding of 1,1/t,t ($n=6$, **12**) to DNA and aquation is the rate limiting step in the formation of the monofunctional adduct [71]. These studies also suggested that aquation is the rate limiting step for the formation of the bifunctional adduct, and the rate of the ring closure is significantly faster than closure to the 1,2-GpG intrastrand adduct formed by cisplatin [71]. It has been shown that the rate of adduct closure is faster for longer chains than for shorter chains, with $n=6>n=4>>n=2$ [79].

The rate of reaction of multi-nuclear platinum complexes can vary considerably, depending upon the nature of the linking ligand, the geometry of the platinum centre and the charge of the complex. BBR3464 reacts more rapidly with DNA than does cisplatin, with $t_{1/2}$ being 0.7 and 4 h, respectively [14,45]. It is thought that the 4+ charge of BBR3464 facilitates the rapid binding to polyanionic DNA [14]. Interestingly, in a study examining the rate of DNA binding by BBR3464 to different types of DNA it was found that BBR3464 reacted faster with single stranded DNA and RNA than it did with double-stranded DNA [80]. It was postulated that this may have been due to the conformation of the double-stranded DNA compared to the single strand DNA/RNA [80]. More recently, Davies et al. established that the rate of aquation of the dinuclear complex 1,1/t,t (**12**), the rate limiting step, is significantly faster in the presence of single stranded DNA than double-stranded DNA [81]. However, it is also possible that the slower rate of reaction with duplex DNA may be due to the initial association of the complex in the double-stranded DNA minor groove, as outlined in Section 5.

The dinuclear complex 1,1/t,t ($n=6$, **12**) (2+) reacts with DNA at about the same rate as cisplatin, while the corresponding *cis*-isomer 1,1/c,c (**13**) reacts more slowly with DNA [79]. In the latter complex the chloro ligands are sterically inaccessible and this is reflected in slower reaction rates [79]. In a separate study, the dpzm-based di- and trinuclear complexes (**17** and **18**) were found to react with guanosine considerably more slowly than does cisplatin [82].

6.2. Interstrand vs. intrastrand adducts

Multi-nuclear platinum complexes form a greater percentage of interstrand DNA cross-links in comparison to cisplatin [14,38,83–86]. The geometry of the 1,1/c,c complex (**13**, $n=4$ or 6) causes it to form only interstrand DNA cross-links, as steric hindrance prevents the formation of intrastrand adducts [77,79]. Additionally, some conformationally flexible complexes also form a high percentage of interstrand DNA cross-links. For example, the simple dinuclear complex 1,1/t,t ($n=6$, **12**) forms 70–90% interstrand cross-links, compared to the 1–7% interstrand adducts formed by cisplatin [45]. However, the trinuclear analogue of 1,1/t,t ($n=6$, **12**), BBR3464 (**24**), only forms 20% interstrand DNA cross-links [45]. The relatively rigid dpzm-based di- and trinuclear complexes (**17** and **18**, respectively) also predominantly form interstrand DNA cross-links [38]. Conversely, the pyridyl-linked complex **16**, which is structurally similar to complex **17**, shows much reduced interstrand cross-link formation [87]. However, in general, these results suggest that dinuclear platinum complexes will form more interstrand DNA cross-links than trinuclear platinum complexes.

DNA interstrand cross-linking is thought to be implicated in cytotoxicity [14]. However, there does not appear to be a strong correlation between the degree of interstrand cross-linking and the cytotoxicity. BBR3464 is far more cytotoxic than either 1,1/t,t (**12**, $n=6$) or 1,1/c,c (**13**, $n=6$) even though it forms a considerably lower percentage of interstrand cross-links. Furthermore, there does not seem to be a strong correlation between the ability of the platinum complex to overcome platinum resistance and the degree of interstrand cross-linking. For example, even though both 1,1/t,t ($n=6$, **12**) and 1,1/c,c ($n=6$, **13**) form predominantly interstrand cross-links, the 1,1/c,c complex becomes markedly less active in the L1210/DDP sub-line compared to the parent L1210 cells, whereas, 1,1/t,t ($n=6$, **12**) is considerably more active in the L1210/DDP sub-line compared to L1210 cells [58].

Although multi-nuclear complexes form a greater percentage of interstrand DNA cross-links than mononuclear complexes, it is the type of interstrand and intrastrand adducts formed and their subsequent affect on DNA conformation that lead to their great potential as anti-cancer agents.

6.3. Long-range intrastrand and interstrand adducts

Perhaps the feature that most distinguishes multinuclear platinum complexes from cisplatin and other similar mononuclear platinum complexes is their ability to form long-range adducts with DNA. Although dinuclear complexes such as 1,1/t,t ($n=2$, 4 or 6, **12**) can form short range 1,2-intrastrand cross-links at GpG

and ApG sequences like cisplatin, they predominantly form interstrand cross-links [88]. The interstrand adducts can be both short range 1,2-cross-links at GC and CG sequences, or because the complexes can span 4 base-pairs, long-range 1,3- and 1,4-cross-links [88]. Interestingly, the length of alkane chain does not appear to affect the distribution of these interstrand cross-links [88].

For the trinuclear complex BBR3464, where the distance between the two reactive Pt–Cl centres is even greater, DNA interactions could extend from 1,2- to 1,6-interstrand and intrastrand cross-links [45,80]. Simple molecular modelling, using HyperChem, suggested that long-range intrastrand and interstrand cross-links are almost equally favoured [45]. This could be due to either the length or flexibility of the chain linking between the two reactive Pt–Cl groups. More recently, Kasparkova et al. have established that interstrand cross-links are preferentially formed between guanine residues separated by two base-pairs [89]. These 1,4-interstrand cross-links are formed in both the 3′-3′ and 5′-5′ directions and induce a directional bending of the helix and local unwinding of the duplex [89]. Interestingly, these structural distortions are not recognised by high mobility group proteins. Furthermore, Kasparkova et al. established that unlike intrastrand adducts formed by BBR3464 (**24**) 1,4-interstrand cross-links are not removed by nucleotide repair excision [89]. This suggests that the 1,4-interstrand cross-links might persist longer than intrastrand adducts and hence, increase the cytotoxicity of the interstrand cross-links [89].

6.4. DNA conformational changes

Unlike cisplatin, studies with multi-nuclear platinum complexes (particularly the 1,1/t,t complex, **12**) bound to DNA indicate that the adducts formed are flexible and non-directional [78,90]. The major differences between the binding of **12** and cisplatin is the absence in the dinuclear adduct of the 100% N-type sugar conformation of the 5′-guanine base when compared to cisplatin [90]. An equilibrium between *syn*/*anti*-*syn* conformation around the 3′-guanine, which had previously never been observed for cisplatin and the ability of the 5′-base to remain stacked with the bound neighbouring guanine for the dinuclear adduct, was observed [90].

Both multi-nuclear complexes and cisplatin unwind DNA by ca. the same amount. The 1,1/t,t ($n=4$, **12**) and the 1,1/c,c ($n=4$, **13**) complexes have been shown to unwind supercoiled DNA by 10–12°, which is comparable to that caused by cisplatin, although the exact degree of topological unwinding is dependent on the geometry and structure of the specific DNA adduct [91].

However, the more important DNA binding feature of multi-nuclear platinum complexes, as proposed by Farrell and co-workers, is their ability to induce DNA conformational changes to A- and Z-type DNA [14,65,92]. Unlike some agents where the transition is reversible, it has been found that the transitions induced by the covalent binding complexes are irreversible [92]. Interestingly, the 2,2/c,c (**1**) complex does not induce any DNA conformational changes, as do complexes **12** and **13** [24]. It was therefore proposed that mono-functional platinum centres are required to induce DNA conformational changes.

The typical DNA conformational changes induced are from B to Z-type in poly(dG-dC)·poly(dG-dC) DNA and from B to A-type in poly(dG)·poly(dC) DNA [66,73,92]. These conformational changes can be induced at concentrations as low as 12.5 μM or at a complex to DNA ratio (r_b) of 0.013 [66]. However, not all complexes with mono-functional platinum centres can induce DNA conformation changes. In studies with complex **16** (X = S, R = H) it was found that it did not induce a B- to Z-type DNA conformation change when reacted with poly(dG-dC)·poly(dG-dC) DNA in concentrations of up to 50 μM of the complex [87].

Qu et al. used ^{1}H-NMR spectroscopy to determine a partial structure of a 1,4-interstrand cross-link formed between BBR3464 (**24**) and d(ATGTACAT)$_2$ [72]. The structure was characterised by a lack of severe DNA distortions that are generally observed for mononuclear complexes [72]. However, NMR analysis did show that the A1 and A7 residues adopted a *syn* conformation, a conformation not usually observed for adenine residues and bases not directly involved in the cross-link [72]. In addition, NOE evidence indicated that the central, inert, linker was located in the DNA minor groove [72].

A unique conformational change has also been observed in the adduct formed by the reaction of the 1,1/t,t ($n=4$, **12**) complex and the oligonucleotide d(CATGCATG)$_2$ [93]. The complex bound via a 1,2-(GG) interstrand adduct and induced a conformational change that resulted in the DNA adopting a dumbbell type structure. While the complex bound at the N7 position of both guanines, the DNA conformational change induced resulted in the complex moving to the minor groove [93].

7. Conclusions

The investigation of multi-nuclear platinum complexes remains a new and exciting area in the treatment of human cancers. New complexes undergoing clinical evaluation like BBR3464, BBR3571, BBR3610 and BBR3611 may be the key to overcoming both intrinsic and acquired resistance to conventional platinum treatment. These complexes are active at doses much lower than cisplatin and carboplatin but are also much more toxic and for these complexes to be widely used in the clinic, this may need to be addressed.

However, despite the success of these complexes in overcoming resistance, relatively little is known about how this is accomplished. Currently, the general consensus is that they derive their activity through the different adducts that they form with DNA compared to cisplatin. These complexes form a large percentage of interstrand DNA cross-links ranging from short range 1,2-adducts to a possible 1,6-adduct.

It is now important to determine which adducts are responsible for the excellent cytotoxicity observed, the flexible, non-directional DNA intrastrand adducts, the long-range DNA interstrand adducts or even DNA minor groove adducts.

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