Electrophilic Attack of $[I(py)_2]^+(NO_3^-)$ on Three-Coordinate Pt^0 Precursors: Synthesis and In Vitro Antitumor Activity of Water-Soluble, Five-Coordinate Pt^{II} Complexes

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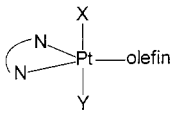
$[I(py)_2]^+(NO_3^-)$ oxidatively adds to the three-coordinate platinum(0) precursors [Pt(*N*,*N*-chelate)(olefin)] affording the cationic five-coordinate products [Pt(*N*,*N*-chelate)I(py)(olefin)]$^+(NO_3^-)$ (**1**) {*N*,*N*-chelate = 2,9-Me_2-1,10-phenanthroline (dmphen), 2-methyl-6-[(phenylimino)methyl]pyridine (pimpy) and 2-methyl-6-[(4-methoxyphenylimino)methyl]pyridine (mpimpy)}. The crystal structure of [Pt(pimpy)I(py)(dimethyl fumarate)]$^+(NO_3^-)$ is reported. The crystals are triclinic, space group *P*1(bar), with *a* = 13.511(2), *b* = 13.754(2), *c* = 8.678(1) Å, α = 95.2(1)°, β = 92.5(2)°, γ = 64.7(1)°, *Z* = 2. Type **I** complexes, which are soluble both in chlorinated solvents and in water, were tested for cytotoxicity against a panel of tumour cell lines of various histotypes including NSCLC H460, leukaemic HL-60, ovarian A 2780, IGROV-1, SKOV-3, and an ovarian carcinoma A 2780/CP selected for resistance to *cis*-platin [DDP = *cis*-diamminedichloroplatinum(II)]. [Pt(dmphen)I(py)(methyl acrylate)]$^+(NO_3^-)$ was the most active in all cell lines tested, its antitumoural activity in vitro being comparable to that of DDP.

Introduction

Cisplatin [DDP = *cis*-diamminedichloroplatinum(II)] is an extensively investigated anticancer drug used in the treatment of some solid tumours such as ovarian, testicular and bladder cancers.[1] Its therapeutic efficacy is often limited by its dose-related nephrotoxicity and by the development of drug resistance, depending on the increase of intracellular glutathione,[2] increased tolerance to DNA damage or increased DNA repair.

Many analogues have been synthesised in an attempt to either decrease the toxic side effects of the parent drug or to overcome the intrinsic and induced resistant phenotype and so broaden the clinical spectrum of antitumour activity.



I

Figure 1. General formula for type **I** complexes

In the last few decades interest in five-coordinate platinum(II) complexes (**I**) (Figure 1) has increased.[3]

The stability of these species is prompted by the presence of sterically hindered nitrogen chelates, e.g. 2,9-Me_2-1,10-phenanthroline (dmphen). Such ligands inhibit olefin loss because the resulting square-planar product would suffer severe constraints in the coordination plane.[3] Some type **I** complexes of formula $[PtCl(Me)(dmphen)(olefin)]BF_4$[4] and $[PtCl(SnMe_nCl_{4-n})(dmphen)(dimethyl\ fumarate)]$[5] have been tested in vitro against some tumour cell lines with promising results. As far as we know, this was the first investigation on coordinatively saturated platinum(II) derivatives, since a literature search shows that other studies have been concerned with square-planar platinum(II)[6] or octahedral platinum(IV) species.[7] The insolubility in water of the above-mentioned species of type **I** limits a further development of these studies. Thus, we were prompted to investigate the feasibility of related compounds with properties more suitable to pharmacological uses. Keeping in mind that a charged complex may display a good solubility in water without loss of pharmacological activity,[8] we aimed to prepare cationic water-soluble complexes of type **I**. A straightforward procedure was found to be the oxidative addition of the readily available iodonium salt $[I(py)_2]^+(NO_3^-)$[9] to three-coordinate platinum(0) precursors[10] (Scheme 1).

Herein we describe the synthesis of novel type **I** species, their NMR characterisation and the crystal structure of a

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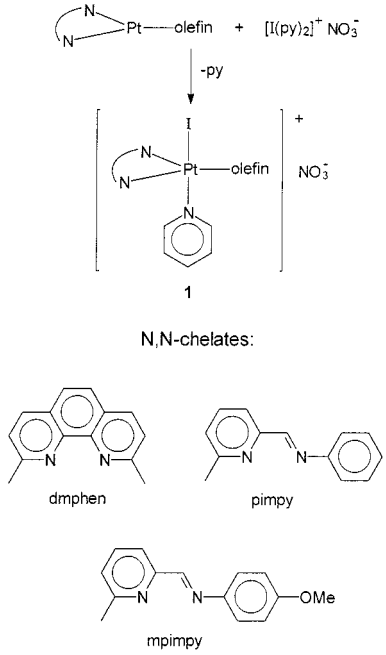

Scheme 1. Synthesis of type-**1** complexes and used *N,N* ligands

representative complex. This paper reports the pharmacological advantages of new modifications introduced in the type **I** species on the inhibitory effect of the growth of selected tumoural DDP resistant and sensitive cell lines.

Results and Discussion

Synthesis, NMR Characterisation and Reactivity of Type I Complexes

Cationic five-coordinate Pt^{II} complexes are known.[11,12] They were obtained by chloride abstraction starting from a suitable precursor in the presence of a silver salt [Equation (1) and Equation (2)]:

$$[PtCl(R)(N,N\text{-chelate})(olefin)] + AgBF_4 + MeCN \rightarrow [Pt(R)(MeCN)(N,N\text{-chelate})(olefin)](BF_4) + AgCl \quad (1)$$

$$cis\text{-}[PtCl_2(MeCN)(olefin)] + AgBF_4 + N,N\text{-chelate} \rightarrow [PtCl(MeCN)(N,N\text{-chelate})(olefin)](BF_4) + AgCl \quad (2)$$

Route 1 allowed the synthesis of type **I** derivatives containing a hydrocarbyl group R and a neutral ligand (e.g. MeCN) in the axial positions. The second procedure afforded the related chloro derivatives. In the latter case, only products with electron-rich olefins could be obtained, since the required precursors *cis*-$[PtCl_2(L)(olefin)]$ are not available when the olefin bears electron-withdrawing substituents. The synthetic procedure herein described bypasses this obstacle and allows the attainment of several cationic halo complexes containing electron-acceptor olefins.

The method is based on the well-known ability of three-coordinate platinum(0) complexes of general formula [Pt(*N,N*-chelate)(olefin)] to undergo electrophilic attack.[10] Thus, the iodonium(I) salt $[I(py)_2]^+(NO_3^-)$[9] oxidatively adds to the suitable Pt^0 complexes with formation of pyridine and of the corresponding five-coordinate complexes, which contain a pyridine and an iodide in the axial positions (Scheme 1).

The N,N ligands used in this work are shown in Scheme 1. The products **1** are listed in Table 1 with their labels. The reactions were performed in chloroform, which is a good solvent for both reagents and products. The only exception was the addition to [Pt(dmphen)(maleic anhydride)], which required the use of tetrachloroethane due to the poor solubility of the Pt^0 precursor.

Type **I** complexes could be crystallised by slow addition of diethyl ether to the reaction mixture. They are soluble both in water and chlorinated solvents, except for the dibenzoylethylene derivative, which is only soluble in chlorinated solvents. The products are fairly stable in solution, and no appreciable decomposition could be seen even after several hours.

The possibility that water may substitute iodide in one of the apical positions has been ruled out by conductivity measurements. Actually, if hydrolysis occurred, the dipositive cation $[Pt(N,N\text{-chelate})(H_2O)(py)(olefin)]^{2+}(I^-)(NO_3^-)$ should form. However, the molar conductivities of type **I** complexes in water are in the range $1.0-1.3 \times 10^2$ $ohm^{-1}\cdot cm^2\cdot mol^{-1}$. These values are close to that measured (1.4×10^2 $ohm^{-1}\cdot cm^2\cdot mol^{-1}$) for an authentic 1:1 salt, i.e. $K[PtCl_3(C_2H_4)]$. On the other hand, the conductivity of a 2:1 salt such as K_2PtCl_4 in water is significantly higher (2.3×10^2 $ohm^{-1}\cdot cm^2\cdot mol^{-1}$).

The NMR spectra were recorded in D_2O in most cases (Table 1). It is possible to assign unequivocally a trigonal bipyramidal geometry to the products on the basis of arguments already thoroughly discussed for related species.[3] As for the dmphen derivatives **1a-e**, the number of isomers theoretically predicted depends on the symmetry of the alkene. Dimethyl maleate and maleic anhydride can afford two rotamers, while methyl acrylate could afford two enantiomeric pairs. In all cases, only one spectral pattern was observed, indicating that the olefins coordinate with a high degree of stereoselectivity. Dimethyl fumarate gives rise to a pair of enantiomers, obviously not distinguishable by NMR spectroscopy in the absence of chiral interference.

In the case of pimpy (**1h**), we observed the expected two diastereomers (each one a pair of enantiomers) for the complex with dimethyl fumarate. Slow recrystallization of the complex affords only one pair whose X-ray crystal structure is described below. Finally, complex **1i** with mpimpy and dimethyl maleate should also exist as two diastereomers (pairs of enantiomers). The high degree of stereoselectivity in olefin coordination allows us to detect only one pair in solution.

Description of the Molecular Structure of 1h

A view of the structure of the cation giving the atom numbering scheme is shown in Figure 2. Selected bond lengths and angles and torsion angles are listed in Table 2.

The bipyramidal trigonal coordination about the metal is as expected and does not differ substantially from that found for similar five-coordinate Pt^{II} complexes.[3] The li-

Table 1. Selected spectroscopic and analytical data for type **I** complexes

Compound	^{1}H NMR[a]			Analysis (%) found (calcd)		
	H-olefin[b]	Me-chelate	Other selected signals	C	H	N
1a [PtI(py)(dmphen) (dimethyl maleate)](NO_3)	5.19 (s, 2 H, 80)	3.53 (6 H)	7.92 (d, 2 H, 2,6-H-py) 3.64 (s, 6 H, OMe)	36.61 (36.82)	3.15 (3.09)	6.69 (6.87)
1b[PtI(py)(dmphen) (dimethyl fumarate)](NO_3)	5.52 (d, 1 H) 4.95 (d, 1 H)	3.55 (3 H) 3.38 (3 H)	7.90 (d, 2 H, 2,6-H-py) 3.86 (s, 3 H, OMe) 3.55 (s, 3 H, OMe)	36.88 (36.82)	3.18 (3.09)	7.02 (6.87)
1c [PtI(py)(dmphen) (maleic anhydride)](NO_3)	5.16 (s, 2 H, 76)	3.56 (6 H)		35.75 (35.90)	2.60 (2.49)	7.41 (7.28)
1d [PtI(py)(dmphen) (methyl acrylate)](NO_3)	5.12 (dd, 1 H, 65) 4.17 (d, 1 H, 59) 3.82 (d, 1 H, 55)	3.51(3 H) 3.36 (3 H)	7.98 (d, 2 H, 2,6-H-py) 3.54 (s, 3 H, OMe)	36.28 (36.47)	2.98 (3.06)	7.24 (7.40)
1e[c] [PtI(py)(dmphen) (dibenzoylethylene)](NO_3)	5.15 (s, 2 H, 78)	2.71 (6 H)	8.20 (d, 2 H, 2,6-H-py)	46.36 (46.32)	3.27 (3.22)	6.29 (6.17)
1f [PtI(py)(dmphen) (diethylmaleate)](NO_3)	5.06 (s, 2 H, 76)	3.48 (6 H)	7.92 (d, 2 H, 2,6-H-py) 4.05 (m, 4 H, OCH_2) 1.14 (t, 6 H, CH_2*Me*)	38.57 (38.44)	3.38 (3.47)	6.78 (6.64)
1g [PtI(py)(dmphen) (diethyl fumarate)](NO_3)	5.51 (d, 1 H, 70) 4.95 (d, 1 H, 68)	3.56 (6 H)	8.03 (d, 2 H, 2,6-H-py) 4.30 (q, 2 H,OCH_2) 3.91 (m, 1 H, OCH_2) 3.65 (m, 1 H,OCH_2) 1.38 (t, 3 H, CH_2*Me*) 1.14 (t, 3 H, CH_2*Me*)	38.22 (38.44)	3.33 (3.47)	6.50 (6.64)
1h [PtI(py)(pimpy) (dimethyl fumarate)](NO_3) (more abundant isomer)	5.50 (d, 1 H) 4.48 (d, 1 H)	3.41 (3 H)	9.31 (s, 1 H, CH=N) 3.87 (s, 3 H, OMe) 3.39 (s, 3 H, OMe)	36.04 (35.88)	3.11 (3.14)	7.12 (6.97)
1i [PtI(py)(mpimpy) (dimethylmaleate)](NO_3)	5.25 (ABq, 2 H)	3.37 (3 H)	9.19 (s, 1 H, CH=N) 3.94 (s, 3 H, OMe) 3.55 (s, 3 H, OMe) 3.46 (s, 3 H, OMe)	36.28 (36.03)	3.19 (3.26)	6.57 (6.72)

[a] At 250 or 200 MHz and 298 K; in D_2O, HDO (δ = 4.8) as internal standard; abbreviations: ABq: AB quadruplet; d: doublet; dd: doublet of doublets; m: multiplet; s: singlet; q: quadruplet; t: triplet. – [b] $^2J_{Pt-H}$ (Hz) in parentheses (when measurable). – [c] In $CDCl_3$, $CHCl_3$ (δ = 7.26) as internal standard.

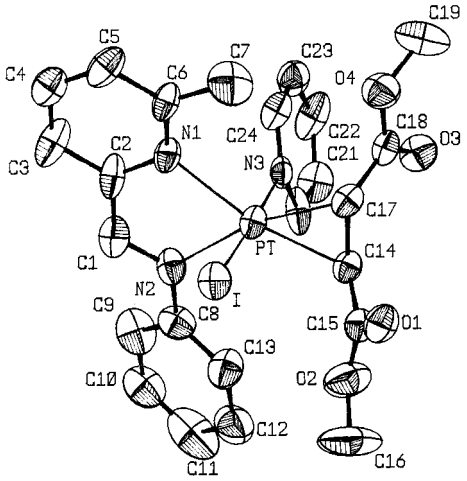

Figure 2. The molecular structure of the cation [PtI(py)(pimpy)(dimethyl fumarate)]$^+$ with the atom numbering scheme; the trigonal bipyramidal coordination sphere is clearly shown

gands iodide and pyridine occupy the axial positions, the pimpy ligand and the fumarate double bond are in the equatorial coordination sites. All the atoms of pimpy, except those of the phenyl group bonded to N2, lie in a plane almost coincidental with the equatorial coordination plane of Pt (the corresponding dihedral angle is ca. 3°). Also C14 and C17 of the olefin bond of dimethyl fumarate are contained in this plane with deviations of less than 0.01–0.02 Å. The phenyl group bonded to N2 is rotated about the

Table 2. Selected geometrical parameters for **1h**

Bond lengths (Å)	
Pt–I	2.616(2)
Pt–N(1)	2.209(8)
Pt–N(2)	2.21(1)
Pt–N(3)	2.08(1)
Pt–C(17)	2.10(1)
Pt–C(14)	2.116(9)
C(14)–C(17)	1.44(1)
Bond angles (°)	
C(14)–Pt–I	92.1(3)
N(1)–Pt–N(3)	91.9(4)
C(17)–Pt–I	93.2(3)
N(2)–Pt–N(3)	88.4(4)
N(1)–Pt–I	85.5(3)
C(17)–Pt–N(1)	122.2(4)
N(2)–Pt–I	90.5(3)
C(14)–Pt–N(2)	123.1(4)
C(14)–Pt–N(3)	90.5(4)
N(1)–Pt–N(2)	75.1(4)
C(17)–Pt–N(3)	88.5(5)
I–Pt–N(3)	177.4(4)
Torsion angles(°)	
C(18)–C(17)–C(14)–C(15)	133.6
C(1)–N(2)–C(8)–C(9)	26.9
C(14)–C(15)–O(2)–C(16)	–175.1
C(17)–C(18)–O(4)–C(19)	177.8

bond C8−N2 in order to relieve short contact interactions between HC9 and HC1 (here at 2.1 Å). The rotation is imposed by the pyridine group in the apical position on Pt such as to produce the most favourable contact distances between these two groups. The atoms C17 and C14, at distances of 1.44 Å, reveal, as found in several similar complexes,[13,14] a sharp dehybridization $sp^2 \rightarrow sp^3$ due to a consistent platinum-to-olefin back-donation. As a consequence, a rotation about the bond C14−C17 is allowed (here it is ca. 132°, i.e. 48° from a *trans* conformation). This rotation is imposed by stereochemical requirements in order to relieve intramolecular interactions, with I on one side (I···O1 = 3.4 Å; I···C15 = 3.3 Å) and the pyridine group on the other side (N3···C18 = 2.88 Å; N3···O3 = 2.95 Å). Repulsions between pimpy and dimethyl fumarate also cooperate to this effect, in particular C7 with O4, and C18 and C13 with O2. These interactions cannot be avoided through a rotation about the coordination axis of the olefin owing to demands imposed by the coordination geometry about the metal.[14] Dimethyl fumarate in its observed configuration seems to have no influence on the conformation or on the choice of coordination isomerism by the Schiff base at Pt (or vice versa, the Schiff base does not appear to induce any preference for the coordination of dimethyl fumarate in either of its prochiral configurations). Thus, we believe that the diastereoisomer examined here is the one favoured in the crystal only because of crystal field forces. All the other geometrical parameters are normal and have been exhaustively discussed in our previous structural reports.[12−14]

Cytotoxic Activity

The cytotoxic activity of six cisplatin (DDP) analogues were studied on a panel of three human carcinoma cell lines in comparison with the parent compound. The drugs were dissolved in DMSO and diluted with 0.9% NaCl solution. After further dilution in culture, DMSO is present at a concentration ranging from 0.01 to 1%. The pattern of cellular response is shown in Table 3.

Table 3. Cytotoxicity (IC_{50} μM) of type **I** compounds in comparison to DDP

Compound	A-2780	GLC-4	H-460
DDP	0.33 ± 0.06	0.53 ± 0.10	0.67 ± 0.10
1a	0.49 ± 0.18	1.59 ± 0.12	1.28 ± 0.49
1b	4.41 ± 0.02	1.96 ± 0.25	2.69 ± 0.60
1d	0.40 ± 0.07	0.40 ± 0.13	0.092 ± 0.003
1f	0.25 ± 0.16	0.58 ± 0.21	3.08 ± 0.95
1g	1.30 ± 0.12	1.85 ± 0.27	2.96 ± 0.59
1i	4.2 ± 0.6	2.39 ± 0.48	4.43 ± 1.44

Compound **1d** showed an activity similar to DDP on A2780 and GLC-4 cell lines, while on the H460 cell line the IC_{50} value was at least three orders of magnitude greater than that of the parent compound. The other analogues exhibited a cytotoxic potency lower or, at best, similar to that of DDP. The cytotoxic activity of **1d** was further evaluated on two additional ovarian cell lines (IGROV and SKOV-3) and on two leukaemia cell lines (human HL-60 and murine L1210). It exhibited a cytotoxic activity similar to (SKOV-3 cells) or three times higher (IGROV cells) than DDP, whereas on HL-60 and L1210 leukaemia its potency was lower (Table 4).

Table 4. Cytotoxicity (IC_{50} μM) of **1d** in comparison to DDP

Cell lines	DDP	**1d**
A 2780	0.33 ± 0.07	0.40 ± 0.07
A 2780/CP	3.3 ± 0.1	1.32 ± 0.03
H460	0.67 ± 0.10	0.09 ± 0.003
SKOV-3	4.2 ± 2.5	1.45 ± 0.33
IGROV	1.0 ± 0.7	0.13 ± 0.03
HL-60	0.40 ± 0.10	1.08 ± 0.35
L1210	1.0 ± 0.13	1.65 ± 0.07
GLC-4	0.53 ± 0.10	0.40 ± 0.13

In the A2780 sub-line selected for resistance to DDP (A2780/CP), characterised by an increased ability to repair drug-induced DNA damage, compound **1d** was cross-resistant to DDP with a Resistance Index (RI) value of 3 (vs. 10).

Conclusion

New cationic, water-soluble, trigonal bipyramidal complexes of platinum(II) were prepared by oxidative addition of $[I(py)_2]^+(NO_3^-)$ to suitable platinum(0) precursors. The products were tested for cytotoxicity against a panel of tumour cell lines of various histotypes. The data show that the new class of Pt^{II} five-coordinate complexes is endowed with promising antitumor properties. The next step will therefore be the characterisation of the antitumour efficacy of the compounds in vivo. In the meantime, studies evaluating the potential nephrotoxic effects of the molecule will be performed.

Experimental Section

General: NMR spectra were recorded at 250 or 200 MHz on a Bruker AC-250 or a Varian XL-200 spectrometer. The three-coordinate precursors [Pt(*N*,*N*-chelate)(olefin)][10] and $[I(py)_2]^+(NO_3^-)$[9] were prepared according to literature procedures. Solvents and reagents were of analytical grade and were used without further purification.

Type-1 Complexes: To a solution of the appropriate [Pt(*N*,*N*-chelate)(olefin)] precursor (0.20 mmol) in chloroform (1 mL) was added $[I(py)_2]^+(NO_3^-)$ (0.20 mmol) previously dissolved in the minimum amount of chloroform. Careful addition of diethyl ether prompted the crystallisation of the product, which was washed with diethyl ether and dried under vacuum (yield: 80−90%). In the case of the precursor [Pt(dmphen)(maleic anhydride)] the reaction was performed in tetrachloroethane.

X-ray Data Collection and Refinement of the Structure of 1h: A crystal of **1h** suitable for X-ray analysis was obtained from dichloro-

Table 5. Crystal data and experimental details for **1h**

Formula	$C_{24}H_{25}IN_4O_7Pt$[a]
M (g/mol)	803.5
Cryst. Syst.	Triclinic
Space group	*P*1(bar)
a, Å	13.511(2)
b, Å	13.754(2)
c, Å	8.678(1)
α (°)	95.2(1)
β (°)	92.5(1)
γ (°)	64.7(1)
Z	2
D (g/cm^3)	1.84
μ (Mo-*K*α) (cm^{-1})	56.67
F(000)	768
Cryst. Size (mm)	0.2×0.2×0.3
Scan speed (°/min)	2.0 in 2θ scan mode
2θ range (°)	$3.0 \leq 2\theta \leq 45$
Measd. reflns.	6927
Scan width (°)	1.2
No. of data used [$I > 2\sigma(I)$]	5721
No. of params. refined	383
R[b], R_w[c]	0.060, 0.064
GOF[d]	1.11
Highest map residues (e/Å^3)	1.2[e]

[a] The probable presence of water of crystallization has not been considered. See text. – [b] $R = \Sigma||F_o| - |F_c||/\Sigma|F_o|$. – [c] $R_w = [\Sigma w(|F_o| - |F_c|)^2/\Sigma w|F_o|^2]^{1/2}$. – [d] GOF = $[\Sigma w(|F_o| - |F_c|)^2/(ND - NU)^2]^{1/2}$. – [e] This value is explained in the Experimental Section.

methane/diethyl ether. Crystal data, intensity data and processing details for **1h** are shown in Table 5.

The data were obtained with a Philips PW-100 four-circle automated diffractometer with graphite monochromator. Intensity data were collected at 25 °C using the θ−2θ scan method. Two reference reflections, monitored periodically, showed no significant variations in intensity. Data were corrected for Lorentz and polarization effects and an empirical absorption correction was applied (Ψ scan). The position of Pt was determined from a three-dimensional Patterson function. All the remaining atoms, except for the hydrogens, were located from successive Fourier maps using SHELX-76. The hydrogen atoms were located at calculated positions and refined with a fixed geometry with respect to their carrier atoms (C−H = 1.08 Å). Anisotropic thermal parameters were used for all the atoms except for the anion NO_3^- and the hydrogens. Blocked-cascade least-square refinements converged to $R = 0.060$.

Actually, the structure shows an appreciable degree of disorder due to the high libration of the anionic NO_3^- group which seems also to be solvated with one (or more likely less) water molecule through hydrogen bonds. This question could not be resolved through density measurements. The corresponding disorder could not be established with certainty from structure refinements and difference Fourier syntheses. These facts explain the relatively high value of the *R* index and the highest map residuals (see Table 5). Nonetheless, they do not invalidate the most relevant structural features here described.

Crystallographic data (excluding structure factors) for the structure reported in this paper have been deposited with the Cambridge Crystallographic Data Centre as supplementary publication no CCDC-143825. Copies of the data can be obtained free of charge on application to CCDC, 12 Union Road, Cambridge CB2 1EZ, UK [Fax: (internat.) + 44-1223/336-003; E-mail: deposit@ccdc.cam.ac.uk].

Evaluation of Cytotoxic Activity: The cytotoxicity of all compounds was studied on a variety of human tumour cell lines of different types, using the SRB assay.[15] Briefly, the A 2780 (1.0×10^4 cells/mL), A 2780/CP (7.0×10^3 cells/mL), SKOV-3 (4.0×10^4 cells/mL), IGROV-1 (2.5×10^4 cells/mL), L1210 (5.0×10^3 cells/mL), HL-60 (5×10^3 cells/mL), H460 (7×10^3 cells/mL) or GLC-4 (7×10^3 cells/mL) cell line was seeded in 96-well microtiter plates and incubated for 24 h at 37 °C in a 5% CO_2 incubator. Drugs were then added to the wells to achieve final drug concentrations ranging from 0.1 to 100 μM. After 24 h of drug exposure, the cells were washed twice with PBS and incubated in a drug-free medium for about three doubling times (72 h). The cells were then fixed with 16% TCA, incubated at 4 °C for 1 h and finally rinsed five times with tap water and dried thoroughly. The cells were stained with 100μL/well of SRB (0.4% in 1% acetic acid) for 15 min, rinsed five times in 1% glacial acetic acid and suspended in 10 mM TRIS-HCl (pH = 10.5) buffer solution. The absorbance was measured with Microplate Reader (BioRad MR 3550) at 570 nm. The results were expressed as IC_{50}, calculated from dose-response curves, and defined as the drug concentration required for a 50% reduction of absorbance compared to control cells. Each experiment was repeated at least three times.

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