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Synthesis, cytotoxicity and structure-activity relationships between ester and amide functionalities in novel acridine-based platinum(II) complexes

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ABSTRACT

In order to improve the pharmacological profile of the anticancer drug cisplatin, several new acridine-based tethered (ethane-1,2-diamine)platinum(II) complexes connected by a polymethylene chain were synthetized. Activity-structure relationship between amide or ester functionalities was explored by changing acridine-9-carboxamide into acridine-9-carboxylate chromophore. The *in vitro* cytotoxicity of these new complexes was assessed in human colic HCT 116, SW480 and HT-29 cancer cell lines. Series of complexes bearing the acridine-9-carboxylate chromophore displayed higher cytotoxic effect than acridine-9-carboxamide complexes, with gradual effect according to the size of the polymethylene linker.

1. Introduction

Platinum (Pt) compounds, such as cisplatin and oxaliplatin (Fig. 1) exhibit antitumor activity by formation of DNA-Pt adducts, which led to DNA structural alterations and activation of various cell death pathways such as apoptosis [1,2]. However, the development of cellular resistance to platinum complexes is a common feature in mammalian cells [3,4]. Mechanisms of resistance are multifactorial and may involve low cellular uptake of the drug, or inactivation by high levels of cellular low molecular weight thiol containing molecules, such as glutathione or metallothioneine, and/or enhancement of DNA repair systems [4]. In clinic, Pt complexes are involved in chemotherapeutic protocols against various types of solid tumors including colorectal cancers (CRC) [1,5]. CRC is one of the most common cancer in western industrialized countries and a major cause of cancer death [6]. Most CRC develop through ordered multi-step carcinogenic pathways which combine specific histological lesions with genetic alterations [5–8]. Oxaliplatin, in association with fluoropyrimidines, represent a mainstay of colorectal cancer chemotherapy in both adjuvant and metastatic settings, whereas cisplatin is poorly active in clinic [1,4,5,9].

Resistance to and high toxicity of oxaliplatin and cisplatin could be circumvent by better understanding of the pathogenesis of colorectal cancer as well as the development of novel platinum-based agents [10,11]. Among the thousands of compounds already synthesized, very few of them were successfully transferred to clinic. Such disappointments focused basic scientists towards new concepts of drug design, for example, on increasing DNA targeting by incorporation of platinum-binding functionalities into suitable "carrier" ligands such as 9-anilinoacridine [12], 9-aminoacridine [13] or 1,10-phenanthroline [14]. Several families of bifunctional molecules containing both entities were developed such as bis(carboxylate)platinum(IV) [15] complexes or [PtCl(ethylenediamine)-(1-[2-(acridin-9-ylamino)ethyl]-1,3-dimethylthiourea)] (PT-ACRAMTU) [16] (Fig. 1), in which acridine core drives platination into DNA minor groove [17]. Acridine orange-ring has been rapidly recognized as a potential "carrier" [18–20], as it could easily go across cell membrane, and intercalate into DNA structure [18]. Bifunctional molecules containing acridine orange moiety tethered to cisplatin-like group by a polymethylene linker were shown to have a dual mode of action in which the platinum moiety binds covalently to DNA while the acridine orange moiety is intercalated one or two base pairs away [20]. Structure-activity relationship studies highlighted the influence of the chemical structure and substituted state of the intercalator pharmacophore, as well as the size of the linker chain connecting the two functionalities on the cytotoxicity of Pt complexes.

In our study, the synthesis, characterization and cytotoxic activity of novel acridine-based tethered (ethane-1,2-diamine)platinum(II) complexes connected by a polymethylene chain were reported. The influence of ester or amide function at the 9-position of the acridine moiety and the influence of the length of polymethylene chain having $n=2$ to $n=6$ in relation to the cytotoxic activity were also investigated.

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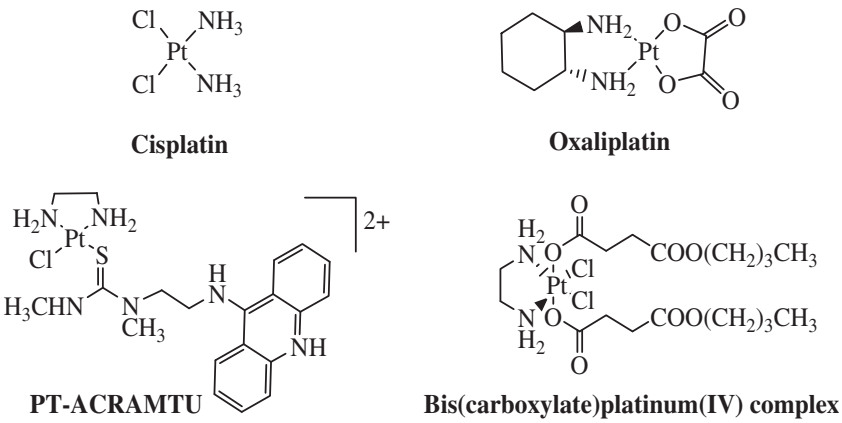


Fig. 1. Chemical structures of anticancer platinum complexes either used in clinic: cisplatin, oxaliplatin ; or under preclinical assessment: PT-ACRAMTU, bis(carboxylate)platinum(IV) complex.

2. Experimental section

2.1. Material and methods

All column chromatography was performed with Merck neutral aluminum oxide 90 standardized (63–200 μm) or silica gel (Acros organic, 60 Å, 35–70 μm). All thin layer chromatography was performed on Fluka aluminum oxide plates (with fluorescent indicator 254 nm) or Merck silica gel 60 F_{254} plates. Melting points were determined on a Reichert-Jung-Koffler apparatus and were not corrected. NMR spectra (300 MHz for ^{1}H, 75 MHz for ^{13}C, and 128 MHz for ^{195}Pt) were recorded on Bruker Avance 300 and 600 instruments using the indicated solvents. Chemical shifts were reported in ppm (δ). High resolution mass spectrometry (HRMS) was obtained with a Brucker MicroTOF-Q spectrometer. Elemental analyses were performed with an Elemental Analyser Thermo electron Flash EA 1112 at the "*Plateforme d'Analyse Chimique et de Synthèse Moléculaire de l'Université de Bourgogne (PACSMUB)*."

2.2. Synthesis

2.2.1. Synthesis of alcohols 2a–j

A mixture of acridine-9-carboxylic acid hydrate (**1**) (97%, 0.50 g, 2.17 mmol), thionyl chloride (10 mL) and dry *N*,*N*-dimethylformamide (one drop) was heated to reflux for 1 h. The thionyl chloride was then removed in vacuo. For 2a–e, the residue was dissolved in dry dichloromethane (10 mL), the solution cooled to 0 °C, a solution of aminoalcohol (2.82 mmol) and triethylamine (0.90 mL, 6.51 mmol) in dry dichloromethane (10 mL) was added under N_2 and stirred to room temperature for 2 h. For 2f–j, the residue was dissolved in diol (52.0 mmol) and stirred to 100 °C for 2 h. The reaction was diluted with dichloromethane (30 mL), washed with 10% sodium bicarbonate solution (2 × 20 mL) and dried (Na_2SO_4). The solvent was removed in vacuo to provide crude product which was purified on aluminum oxide eluting with 96% dichloromethane and 4% methanol (for 2a–e) or on aluminum oxide eluting with 98% dichloromethane and 2% methanol (for 2f–j).

2a: Yield : 65%. mp 244–246 °C. ^{1}H NMR ($CDCl_3$, 300 MHz) δ 8.25 (d, 2H, J = 8.5 Hz), 8.11 (d, 2H, J = 8.5 Hz), 7.81 (m, 2H), 7.61 (m, 2H), 4.02 (m, 2H), 3.91 (m, 2H). ^{13}C NMR (DMSO, 75 MHz) δ 166.2, 148.2, 142.6, 130.7, 129.2, 126.7, 125.9, 121.9, 59.8, 42.1.

2b: Yield : 88%. mp 212–216 °C. ^{1}H NMR (CD_3OD, 300 MHz) δ 8.17 (d, 2H, J = 9 Hz), 8.07 (d, 2H, J = 9 Hz), 7.89 (m, 2H), 7.66 (m, 2H), 3.74 (m, 4H), 1.99 (p, 2H, J = 7 Hz). ^{13}C NMR (CD_3OD, 75 MHz) δ 169.3, 149.6, 143.8, 132.3, 129.6, 128.2, 126.6, 123.7, 60.6, 38.2, 33.2.

2c: Yield : 46%. mp 139–141 °C. ^{1}H NMR ($CDCl_3$, 300 MHz) δ 7.98 (d, 2H, J = 9 Hz), 7.92 (d, 2H, J = 8 Hz), 7.65 (m, 2H), 7.46 (m, 2H), 3.59 (m, 4H), 1.76 (m, 2H), 1.63 (m, 2H). ^{13}C NMR ($CDCl_3$, 75 MHz) δ 167.3, 148.0 141.7, 130.6, 128.7, 126.7, 125.2, 122.2, 61.6, 39.9, 29.8, 26.0.

2d: [21] Yield : 47%. mp 169–171 °C.

2e: [22]Yield : 80%. mp 108–110 °C.

2f: [23]Yield : 54%. mp 190–192 °C (lit.[23] 184–186 °C).

2g: [24]Yield : 69%. mp 133–135 °C (lit.[24] 133–135 °C).

2h: Yield : 72%. mp 96–98 °C. ^{1}H NMR ($CDCl_3$, 300 MHz) δ 8.27 (d, 2H, J = 9 Hz), 8.02 (d, 2H, J = 9 Hz), 7.81 (m, 2H), 7.61 (m, 2H), 4.69 (t, 2H, J = 7 Hz), 3.74 (t, 2H, J = 7 Hz), 2.01 (m, 2H), 1.75 (m, 2H). ^{13}C NMR ($CDCl_3$, 75 MHz) δ 167.6, 148.6, 137.2, 130.4, 129.8, 127.2, 125.1, 122.3, 66.3, 62.0, 29.1, 25.3.

2i: Yield 45%. mp 219–221 °C. ^{1}H NMR ($CDCl_3$, 300 MHz) δ 8.25 (d, 2H, J = 9 Hz), 8.00 (d, 2H, J = 9 Hz), 7.78 (m, 2H), 7.57 (m, 2H), 4.50 (2H, J = 7 Hz), 3.67 (t, 2H, J = 7 Hz), 1.94 (m, 2H), 1.67 (m, 4H). ^{13}C NMR ($CDCl_3$, 75 MHz) δ 167.6, 148.7, 137.2; 130.3, 129.9, 127.1, 125.1, 122.3, 66.3, 62.5, 32.2, 28.5, 22.3.

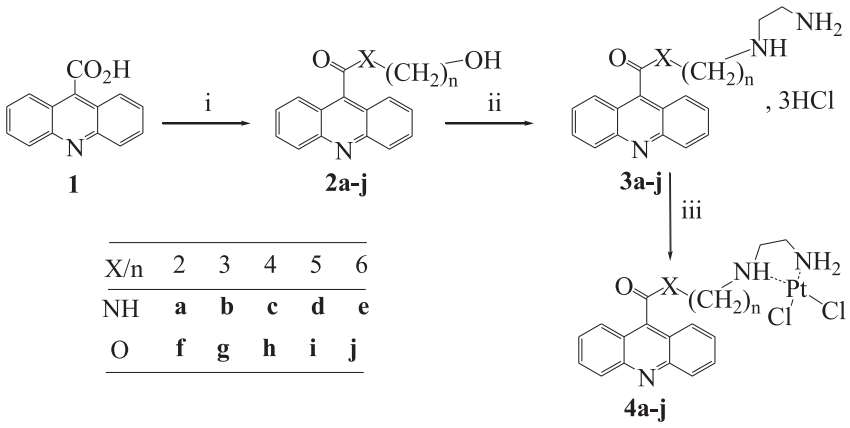
2j: Yield 56%. mp 55–57 °C. ^{1}H NMR ($CDCl_3$, 300 MHz) δ 8.27 (d, 2H, J = 9 Hz), 8.02 (d, 2H, J = 9 Hz), 7.80 (m, 2H), 7.60 (m, 2H), 4.65 (t, 2H, J = 7 Hz), 3.65 (t, 2H, J = 7 Hz), 1.90 (p, 2H, J = 7 Hz), 1.48 (m, 6H). ^{13}C NMR ($CDCl_3$, 75 MHz) δ 167.6, 148.6, 137.3; 130.4, 129.9, 127.1, 125.1, 122.3, 66.4, 62.6, 32.6, 28.7, 25.8, 25.4.

2.2.2. Synthesis of ligands 3a–j[1]

The above alcohols 2a–j (0.55 mmol) were dissolved in dry pyridine (5 mL) and methanesulfonylchloride (0.1 mL, 1.29 mmol) was added. The solution was stirred at 20 °C under N_2 for 1 h then chilled on ice and treated with water (0.16 mL), followed by 1,2 diaminoethane (1.5 mL). The mixture was stirred 20 h at 20 °C and then the solvents were removed. The residue was dissolved in dichloromethane (30 mL), and the organic phase was washed with 1 M Na_2CO_3 (50 mL) and water (50 mL). After drying over Na_2SO_4, the solvent was removed and the residue was chromatographed on silica eluting with MeOH/NH_4OH (97/3, v/v). The pure product was converted to the trihydrochloride.

3a: Yield 20%. mp 220–222 °C.^{1}H NMR (D_2O, 300 MHz) δ 8.30 (m, 6H), 7.96 (m, 2H), 4.08 (t, 2H, J = 7 Hz), 3.58 (m, 4H), 3.47 (m,

[1] Although the elemental analysis values for "C" are somewhat unsatisfactory for most of the compounds, the spectroscopic analysis data (^{1}H, ^{13}C, ^{195}Pt NMR, HRMS presented in the supporting information) reasonably support the formula.)



X/n	2	3	4	5	6
NH	a	b	c	d	e
O	f	g	h	i	j

Scheme 1. Reagents and conditions : (i) 1) $SOCl_2$, DMF, Δ, 2) $NH_2(CH_2)_nOH$, CH_2Cl_2, NEt_3, room temperature or $OH(CH_2)_nOH$, 100 °C; (ii) 1) MsCl, Pyridine, Room temperature, 2) $NH_2(CH_2)_2NH_2$, room temperature, 3) HCl 1 N in diethyl ether; (iii) H_2O, Na_2CO_3, K_2PtCl_4, room temperature.

2H). ^{13}C NMR (D_2O, 75 MHz) δ 167.8, 155.5, 142.1, 136.1, 128.9, 125.8, 122.2, 119.4, 46.7, 44.5, 36.4, 35.4. Anal. Calc. for $C_{18}H_{20}N_4O.3HCl.3H_2O$ requires: C, 45.82; H, 6.20; N, 11.87. Found: C, 46.02; H, 5.99; N, 11.74.

3b: Yield 55%. mp 199–201 °C. ^{1}H NMR (D_2O, 300 MHz) δ 8.23 (m, 6H), 7.93 (m, 2H), 3.82 (t, 2H, J = 7 Hz), 3.51 (m, 6H), 2.27 (p, 2H, J = 7 Hz). ^{13}C NMR (D_2O, 75 MHz) δ 165.7, 149.9, 139.5, 137.8, 129.4, 126.1, 121.8, 119.9, 46.1, 44.4, 37.5, 35.7, 25.6. Anal. Calc. for $C_{19}H_{22}N_4O.3HCl.3H_2O$ requires: C, 46.97; H, 6.43; N, 11.53. Found: C, 47.10; H, 6.59; N, 11.97.

3c: Yield 39%. mp 130–132 °C. ^{1}H NMR (CD_3OD, 300 MHz) δ 8.21 (d, 2H, J = 9 Hz), 8.10 (d, 2H, J = 9 Hz), 8.02 (m, 2H), 7.82 (m, 2H), 3.82 (m, 2H), 3.48 (m, 2H), 3.27 (m, 4H), 2.00 (m, 4H). ^{13}C NMR (CD_3OD, 75 MHz) δ 169.7, 148.5, 142.2, 132.6, 128.7, 128.5, 125.7, 122.8, 47.6, 41.5, 39.7, 38.2, 27.0, 25.5. Anal. Calc. for $C_{20}H_{24}N_4O.3HCl.2H_2O$ requires: C, 49.85; H, 6.48; N, 11.63. Found: C, 49.01; H, 6.43; N, 11.41.

3d: Yield 25%. mp 186–188 °C. ^{1}H NMR (D_2O, 300 MHz) δ 8.31 (m, 6H), 7.97 (m, 2H), 3.72 (t, 2H, J = 7 Hz), 3.41 (m, 4H), 3.18 (m, 2H), 1.84 (m, 4H), 1.59 (p, 2H, J = 7 Hz). ^{13}C NMR (D_2O, 75 MHz) δ 166.1, 150.7, 140.0, 137.9, 129.3, 126.1, 122.4, 119.9, 48.0, 44.0, 40.1, 35.4, 27.8, 25.2, 23.3. Anal. Calc. for $C_{21}H_{26}N_4O.3HCl.2H_2O$ requires: C, 50.87; H, 6.71; N, 11.30. Found: C, 48.40; H, 6.79; N, 11.83.

3e: Yield 40%. mp 137–139 °C. ^{1}H NMR (D_2O, 300 MHz) δ 8.16 (m, 6H), 7.87 (m, 2H), 3.68 (t, 2H, J = 7 Hz), 3.43 (m, 4H), 3.17 (t, 2H, J = 7 Hz), 1.78 (m, 4H), 1.51 (m, 4H). ^{13}C NMR (D_2O, 75 MHz) δ 165.7, 150.7, 139.7, 137.9, 129.3, 126.1, 122.1, 119.8, 48.2, 44.1, 40.3, 35.4, 28.0, 25.8, 25.5, 25.3. Anal. Calc. for $C_{22}H_{28}N_4O.3HCl.1.5H_2O$ requires: C, 52.75; H, 6.84; N, 11.19. Found: C, 51.43; H, 6.95; N, 11.11.

3f: Yield 20%. mp 214–216 °C. ^{1}H NMR (D_2O, 300 MHz) δ 8.26 (m, 6H), 7.89 (m, 2H), 5.10 (t, 2H, J = 7 Hz), 3.71 (t, 2H, J = 7 Hz), 3.46 (m, 2H), 3.34 (m, 2H). ^{13}C NMR (D_2O, 75 MHz) δ 164.7, 145.7, 139.6, 138.0, 129.6, 126.1, 122.2, 119.8, 63.0, 46.3, 44.6, 35.3. Anal. Calc. for $C_{18}H_{19}N_3O_2.3HCl.3H_2O$ requires: C, 45.73; H, 5.97; N, 8.89. Found: C, 47.01; H, 6.04; N, 8.99.

3g: Yield 29%. mp 167–169 °C. ^{1}H NMR (D_2O, 300 MHz) δ 8.11 (m, 4H), 8.01 (d, 2H, J = 9 Hz), 7.77 (m, 2H), 4.83 (t, 2H, J = 7 Hz), 3.39 (m, 4H), 3.27 (t, 2H, J = 7 Hz), 2.34 (p, 2H, J = 7 Hz). ^{13}C NMR (D_2O, 75 MHz) δ 165.0, 146.1, 139.2, 137.9, 129.5, 125.9, 121.7, 119.7, 65.0, 45.0, 44.3, 35.4, 24.8. Anal. Calc. for $C_{19}H_{21}N_3O_2.3HCl.3H_2O$ requires: C, 46.88; H, 6.21; N, 8.63. Found: C, 45.13; H, 6.02; N, 8.74.

3h: Yield 25%. mp 205–207 °C. ^{1}H NMR (D_2O, 300 MHz) δ 8.23 (m, 6H), 7.90 (m, 2H), 4.81 (m, 2H), 3.37 (m, 4H), 3.19 (t, 2H, J = 7 Hz), 2.01 (p, 2H, J = 7 Hz), 1.90 (p, 2H, J = 7 Hz). ^{13}C NMR (D_2O, 75 MHz) δ 165.7, 146.8, 139.8, 137.9, 129.5, 126.1, 122.1, 119.8, 67.7, 47.6, 44.1, 35.4, 24.9, 22.3. Anal. Calc. for $C_{20}H_{23}N_3O_2.3HCl.3H_2O$ requires: C, 47.96; H, 6.44; N, 8.39. Found: C, 48.14; H, 6.32; N, 8.51.

3i: Yield 31%. mp 208–210 °C. ^{1}H NMR (D_2O, 300 MHz) δ 8.20 (m, 4H), 8.14 (d, 2H, J = 9 Hz), 7.86 (m, 2H), 4.79 (m, 2H), 3.37 (m, 4H), 3.11 (t, 2H, J = 7 Hz), 1.95 (p, 2H, J = 7 Hz), 1.78 (p, 2H, J = 7 Hz), 1.53 (p, 2H, J = 7 Hz). ^{13}C NMR (D_2O, 75 MHz) δ 165.9, 146.7, 139.8, 137.7, 129.4, 126.0, 122.0, 119.9, 68.3, 47.9, 44.0, 35.4, 27.2, 25.2, 22.2. Anal. Calc. for $C_{21}H_{25}N_3O_2.3HCl.H_2O$ requires: C, 52.67; H, 6.31; N, 8.78. Found: C, 50.91; H, 6.38; N, 9.02.

3j: Yield 31%. mp 139–141 °C. ^{1}H NMR (D_2O, 300 MHz) δ 8.15 (m, 4H), 8.03 (d, 2H, J = 9 Hz), 7.80 (m, 2H), 4.72 (t, 2H, J = 7 Hz), 3.40 (m, 4H), 3.11 (t, 2H, J = 7 Hz), 1.90 (p, 2H, J = 7 Hz), 1.71 (p, 2H, J = 7 Hz), 1.48 (m, 4H). ^{13}C NMR (D_2O, 75 MHz) δ 165.6, 146.7, 139.4, 137.8, 129.4, 125.9, 121.7, 119.7, 68.7, 48.1 44.1, 35.4, 27.5, 25.4, 25.3, 24.7. Anal. Calc. for $C_{22}H_{27}N_3O_2.3HCl.4H_2O$ requires: C, 48.31; H, 7.00; N, 7.68. Found: C, 46.44; H, 7.06; N, 7.88.

2.2.3. Synthesis of complexes 4a–j[1]

The trihydrochloride 3a–j (0.20 mmol) was added to a solution of K_2PtCl_4 (84 mg, 0.20 mmol) in water (4 mL). The pH was adjusted to 8 with 2 M sodium bicarbonate solution and the mixture was stirred in the dark for 24 h. A solution of 5% aqueous KCl (20 mL) was then added, and the mixture was stirred for 90 min. The resulting precipitate was collected, washed several times with water, acetone and dried to give pure product 4a–j.

4a: Yield 69%. mp 221–223 °C. HRMS calcd. for $C_{18}H_{20}Cl_2N_4OPt$ $[M+Na]^+$ 596.0555 found 596.05613. ^{195}Pt NMR (DMF, 600 MHz) not obtained. Anal. Calc. for $C_{18}H_{20}Cl_2N_4OPt.H_2O$ requires: C, 36.50; H, 3.74; N, 9.46. Found: C, 35.03; H, 3.64; N, 9.02.

4b: Yield 70%. mp >260 °C. HRMS calcd. for $C_{19}H_{22}Cl_2N_4OPt$ $[M+Na]^+$ 610.06963 found 610.07147. ^{195}Pt NMR (DMF, 128 MHz) δ −2342. Anal. Calc. for $C_{19}H_{22}Cl_2N_4OPt.2H_2O$ requires: C, 36.55; H, 4.20; N, 8.97. Found: C, 35.50; H, 4.02; N, 8.69.

4c: Yield 70%. mp 250–252 °C. HRMS calcd. for $C_{20}H_{24}Cl_2N_4OPt$ $[M+Na]^+$ 624.08687 found 624.08651. ^{195}Pt NMR (DMF, 128 MHz) δ −2341. Anal. Calc. for $C_{20}H_{24}Cl_2N_4OPt.3H_2O$ requires: C, 36.59; H, 4.61; N, 8.53. Found: C, 34.95; H, 4.48; N, 8.83.

4d: Yield 71%. mp 254–256 °C. HRMS calcd. for $C_{21}H_{26}Cl_2N_4OPt$ $[M+Na]^+$ 638.10253 found 638.10332. ^{195}Pt NMR (DMF,

128 MHz) δ −2339. Anal. Calc. for $C_{21}H_{26}Cl_2N_4OPt.0.5H_2O$ requires: C, 40.33; H, 4.35; N, 8.96. Found: C, 38.51; H, 4.56; N, 9.36.

4e: Yield 72%. mp 248–250 °C. HRMS calcd. for $C_{22}H_{28}Cl_2N_4OPt$ $[M+Na]^+$ 652.11670 found 652.11796. ^{195}Pt NMR (DMF, 128 MHz) δ −2341. Anal. Calc. for $C_{22}H_{28}Cl_2N_4OPt.3H_2O$ requires: C, 38.60; H, 5.01; N, 8.18. Found: C, 37.45; H, 4.91; N, 8.12.

4f: Yield 43%. mp 239–241 °C. HRMS calcd. for $C_{18}H_{19}Cl_2N_3O_2Pt$ $[M+Na]^+$ 597.03958 found 597.03897. ^{195}Pt NMR (DMF, 128 MHz) δ −2343. Anal. Calc. for $C_{18}H_{19}Cl_2N_3O_2Pt.2H_2O$ requires: C, 35.36; H, 3.79; N, 6.87. Found: C, 33.68; H, 3.63; N, 6.57.

4g: Yield 46%. mp 207–209 °C. HRMS calcd. for $C_{19}H_{21}Cl_2N_3O_2Pt$ $[M+Na]^+$ 611.05524 found 611.05716. ^{195}Pt NMR (DMF, 128 MHz) δ −2343. Anal. Calc. for $C_{19}H_{21}Cl_2N_3O_2Pt.H_2O$ requires: C, 37.57; H, 3.82; N, 6.92. Found: C, 36.06; H, 3.72; N, 6.94.

4h: Yield 68%. mp 163–165 °C. HRMS calcd. for $C_{20}H_{23}Cl_2N_3O_2Pt$ $[M+Na]^+$ 625.07090 found 625.07319. ^{195}Pt NMR (DMF, 128 MHz) δ −2342. Anal. Calc. for $C_{20}H_{23}Cl_2N_3O_2Pt$ requires: C, 39.81; H, 3.84; N, 6.96. Found: C, 40.35; H, 3.68; N, 7.17.

4i: Yield 60%. mp 156–158 °C. HRMS calcd. for $C_{21}H_{25}Cl_2N_3O_2Pt$ $[M+Na]^+$ 639.08656 found 639.08972. ^{195}Pt NMR (DMF, 128 MHz) δ −2338. Anal. Calc. for $C_{21}H_{25}Cl_2N_3O_2Pt$ requires: C, 40.85; H, 4.08; N, 6.81. Found: C, 42.64; H, 4.04; N, 6.96.

4j: Yield 52%. mp 129–131 °C. HRMS calcd. for $C_{22}H_{27}Cl_2N_3O_2Pt$ $[M+Na]^+$ 653.10221 found 653.10475. ^{195}Pt NMR (DMF, 128 MHz) δ −2338. Anal. Calc. for $C_{22}H_{27}Cl_2N_3O_2Pt.H_2O$ requires: C, 40.67; H, 4.50; N, 6.47. Found: C, 41.34; H, 4.35; N, 6.66.

2.3. Cell lines and culture conditions

Human colon cancer cell lines HCT 116, SW480 and HT-29 were obtained from the American Type Culture Collections (Manassas, VA, United States), and were cultured in RPMI 1640 medium (Biowhittaker, France) supplemented with 10% fetal bovine serum (Biowhittaker, France) in a 5% CO_2 atmosphere. All cell lines were maintained as exponentially growing monolayers in mycoplasma free culture condition checked by polymerase chain reaction (PCR) analysis (PCR Mycoplasma Test Kit I/C, PromoKine, PromoCell France).

2.4. Assay of cytotoxicity in cancer cell lines

The newly synthetized acridine-based tethered (ethane-1,2-diamine) derivatives connected by a polymethylene spacer (3a–j), cisplatin (Sigma-Aldrich, France), and oxaliplatin (Sanofi-Aventis, France) were diluted in sterile physiological serum (Aguettant Co., France), whilst new acridine-based tethered (ethane-1,2-diamine)Pt(II) complexes connected by a polymethylene chain (4a–j) were diluted into dimethylformamide (DMF) (Sigma, France). In cell culture, maximum concentration of DMF did not exceed 3% in the medium.

HCT 116, SW480 and HT-29 were seeded in 96-well plates at a density of 20,000 cells per well. At sub-confluence, cells were treated for 72 h by increasing concentrations (from 0 to 500 μM) of the different compounds. After incubation, cells were washed in PBS 1×, fixed in pure ethanol, stained with crystal violet (1%), and eluted in 33% acetic acid. The intensity of coloration was determined by the measurement of absorbance by spectrophotometry (UVM 340, Bioserv) at λ = 570 nm. Each concentration measurement was conducted in *triplicate* from three independent experiments. Results were expressed as concentration-response curves, representing the percentage of cytotoxicity according to the concentration of the drug. From these curves, the 50% Inhibitory Concentration (IC_{50}), representing the concentration which inhibits 50% of cell growth, was calculated after linearization.

Table 1
NMR ^{195} Pt of platinum complexes 4a–j.

Compounds X = NH	^{195}Pt NMR δ (ppm)	Compounds X = O	^{195}Pt NMR δ (ppm)
4a, n = 2	Not obtained	4f, n = 2	−2343
4b, n = 3	−2342	4g, n = 3	−2343
4c, n = 4	−2341	4h, n = 4	−2342
4d, n = 5	−2339	4i, n = 5	−2338
4e, n = 6	−2341	4j, n = 6	−2338

3. Results and discussion

3.1. Preparation and spectroscopic properties

Synthesis of alcohols was carried out as shown in Scheme 1. Acridine-9-carboxylic acid **1** was coupled selectively with appropriate aminoalcohol or diol via the thionyl chloride. To avoid dimerization with diols, it was necessary to heat at 100 °C during 2 h. The resulting alcohols 2a–j were activated with methanesulfonyl chloride and treated with an excess of 1,2-diaminoethane to give the free instable bases of the ligands 3a–j. After quick chromatography purification on silica gel, these diamines were isolated as trihydrochloride salts 3a–j by treatment of HCl 1 M in diethyl ether. Each ligand was characterized by ^{1}H and ^{13}C NMR spectroscopic methods. The platinum complexes 4a–j were prepared by addition of an aqueous solution of K_2PtCl_4 to a solution of the ligand trihydrochlorides 3a–j in water adjusted to basic pH by addition of sodium bicarbonate. Pure products were isolated directly by precipitation from the reaction mixture.

In each complex, ^{195}Pt NMR confirmed Pt coordination to the diamine function. The ^{195}Pt chemical shift values (Table 1) are characteristic of platinum(II) complexes with a diamine associated with two chloride atoms. The HRMS were consistent with the proposed empirical formulas. For compound 4a, ^{195}Pt NMR signal was not obtained, probably because of poor solubility but the presence of platinum in the chemical structure of compound 4a was clearly confirmed by accurate mass measurement.

Table 2
Cytotoxicity of acridine-based derivates platinated (4a–j) or not (3a–j), cisplatin and oxaliplatin, and DMF in human colon HCT116, SW480 and HT29 cells after a 72 h-treatment. Results are expressed as IC_{50}, each value representing the mean ± SD of at least 3 different experiments (statistics: acridine compounds versus cisplatin or oxaliplatin: ** : $p<0.01$; *** $p<0.001$) (*nd* = not determinable).

Compounds	IC_{50} (μM)		
	HCT116	SW480	HT29
3a	*nd*	*nd*	*nd*
3b	*nd*	*nd*	*nd*
3c	*nd*	*nd*	*nd*
3d	*nd*	*nd*	*nd*
3e	*nd*	*nd*	*nd*
4a	*nd*	*nd*	*nd*
4b	*nd*	*nd*	*nd*
4c	*nd*	232.3 ± 15.8	*nd*
4d	*nd*	229.6 ± 24.6	*nd*
4e	*nd*	*nd*	*nd*
3f	352.2 ± 13.2 *	465.6 ± 14.5	*nd*
3g	126.3 ± 9.2 **	108.2 ± 5.6	114.3 ± 6.1 **
3h	153.1 ± 6.1 **	113.4 ± 7.4	201.4 ± 4.5 **
3i	51.8 ± 4.6 ***	34.5 ± 3.2	49.8 ± 3.1 ***
3j	37.4 ± 7.1 ***	17.3 ± 6.2	46.8 ± 7.1 ***
4f	*nd*	*nd*	76.3 ± 6.2 ***
4g	242.0 ± 10.9 **	*nd*	193.2 ± 4.2 **
4h	198.7 ± 9.8 **	231.1 ± 10.6	187.3 ± 7.4 **
4i	249.8 ± 12.2 **	*nd*	243.6 ± 6.0
4j	128.6 ± 7.9 **	93.4 ± 6.6	190.3 ± 7.8 **
Cisplatin	453.2 ± 6.9	*nd*	373.2 ± 6.4
oxaliplatin	478.3 ± 9.3	*nd*	308.1 ± 2.4
DMF	372.3 ± 8.9	352.8 ± 6.9	313.9 ± 7.1

3.2. Cytotoxicity in cancer cell lines

Acridine-based derivatives platinated or not were tested in three human colic cancer cell lines HCT 116, SW480 and HT-29, as colorectal cancer is a clinical indication of Pt compounds. Oxaliplatin was used as the reference molecule as referred to clinical protocols of CRC, and cisplatin as the reference of Pt derivate family. Despite differences in mechanisms of action and DNA-Pt adducts formation, cisplatin and oxaliplatin displayed similar cytotoxic properties in each cell line tested in our experimental conditions (Table 2). Indeed, our cytotoxic assay was performed under stringent conditions, as treatments were administrated in subconfluent cells. According to this protocol, the two series of acridine-based complexes (3a–j and 4a–j) displayed various cytotoxic properties (Table 2 and Fig. 2) in the three colic cancer cell lines, which are differently sensitive to the reference molecules. SW480 was quite resistant to cisplatin and oxaliplatin compared to HT-29 and HCT 116 (Table 2). HT-29 was the most sensitive cell line, with oxaliplatin disclosing more cytotoxic effect than cisplatin. HCT 116 exhibited intermediate sensibility, with similar IC_{50} for both molecules (Table 2).

This study reported, for the first time, the synthesis and *in vitro* evaluation of series of compounds containing the acridine-9-carboxamide chromophore (3a–e and 4a–e). Unfortunately, acridine-9-carboxamide tethered (ethane-1,2-diamine)Pt(II) complexes (4a–e) did not display significant cytotoxicity, which remained lower than cisplatin and oxaliplatin, especially in HCT 116 and HT-29 cells (Table 2). The non platinated counterparts (3a-e) were totally inactive. Such findings corroborate previous testing of a cisplatin-type complex tethered to acridine intercalator via one methylene chain (at the 9-position of acridine skeleton) [25]. Such compound demonstrated only low antitumor activity against P388 cells transplanted into mice. From our results, we could suggest that short linkage connecting the acridine chromophore to Pt-moiety was probably not the major parameter determining poor biological effect as compounds with hexamethylene linker did not always exhibit higher cytotoxicity. Thus, in such series of compounds, the size of the spacer link might not modulate the noncovalent intercalation, as well as selectivity of platination. However, different series of acridine-2- and -4-carboxamide linked analogues of (ethane-1,2-diamine)Pt(II) complexes have also been synthetized, and the platinum complexes were generally more cytotoxic *in vitro* than nonplatinated analogues [13,26]. Acridine-4-carboxamide compounds were more cytotoxic than acridine-2-carboxamide tethered Pt(II) complexes in P388 leukemia cells *in vitro* [26] ; and acridine-4-carboxamide complexes with the shorter linker chain lengths were generally the most active [13] in different cell lines sensitive or resistant to cisplatin [13,26]. In spite of that, addition of 9-amino substituent on acridine-4-carboxamide led to decreased cytotoxic activities in HeLa cells [27].

Interestingly, the replacement of amide by ester functionalities in the series of novel acridine-9-carboxylate tethered (ethane-1,2-diamine) complexes platinated (4f–j) or not (3f–j) and connected by a polymethylene chain increased their cytotoxic effect which was higher than cisplatin or oxaliplatin in the three colic HCT 116, SW480 and HT-29 cell lines (Table 2). The influence of the polymethylene linker in platinated complexes was dependent on the cell line: in HCT 116 and SW480, 4j, having the hexamethylene chain, remained the most cytotoxic, whilst in HT-29, the most sensitive cell line to reference Pt compounds, 4f, with the dimethylene linker was the most active (Table 2). A relationship between cytotoxicity and polymethylene chain length in platinum compounds was also reported by Silva et al. [28]. Furthermore, the non platinated ligands (3f–j) displayed powerful cytotoxic effect compared to cisplatin and oxaliplatin, with a gradual effect according to the

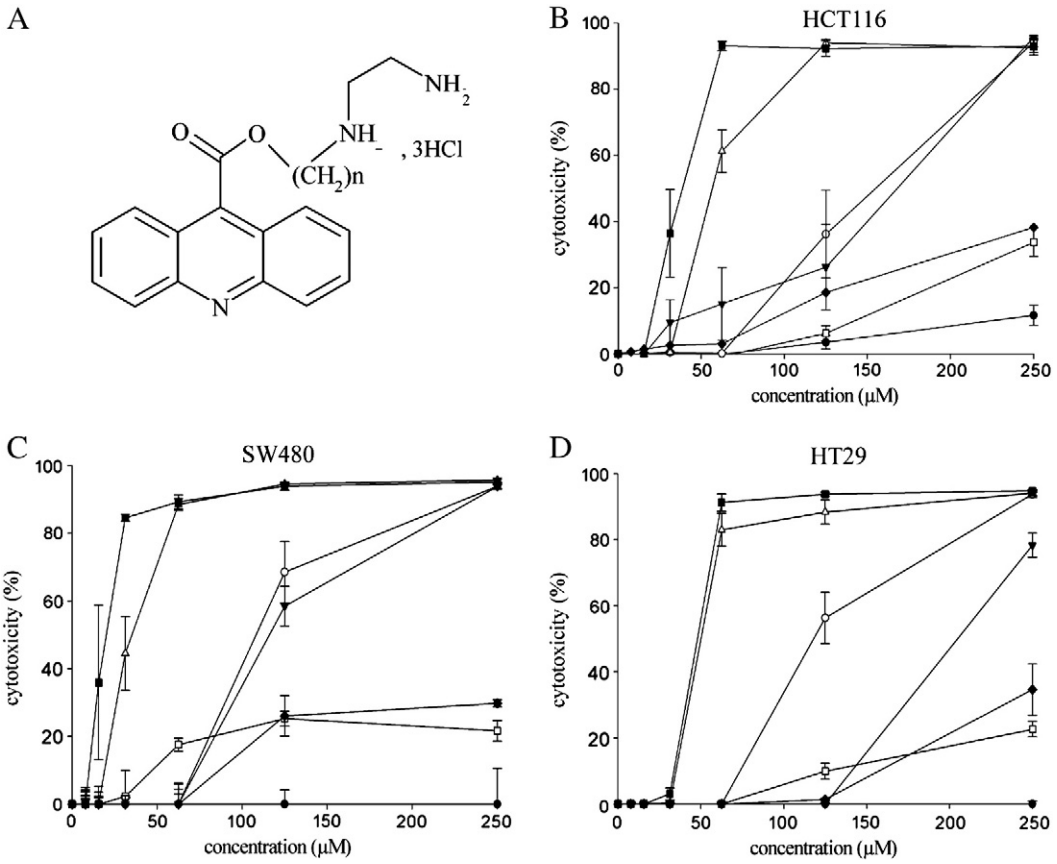


Fig. 2. Concentration-response curves of cytotoxicity of acridine-9-carboxylate tethered (ethane-1,2-diamine) derivates connected by di- to hexamethylene chain (A) 3f (●) 3 g (○) 3 h (▼) 3i (△) 3j (■), and of reference molecules cisplatin (□) and oxaliplatin (♦) in human colic HCT116 (B), SW480 (C) and HT29 (D) cell lines after 72 h of drug incubation. Cytotoxicity values are represented by the mean ± SD of 3 independent experiments.

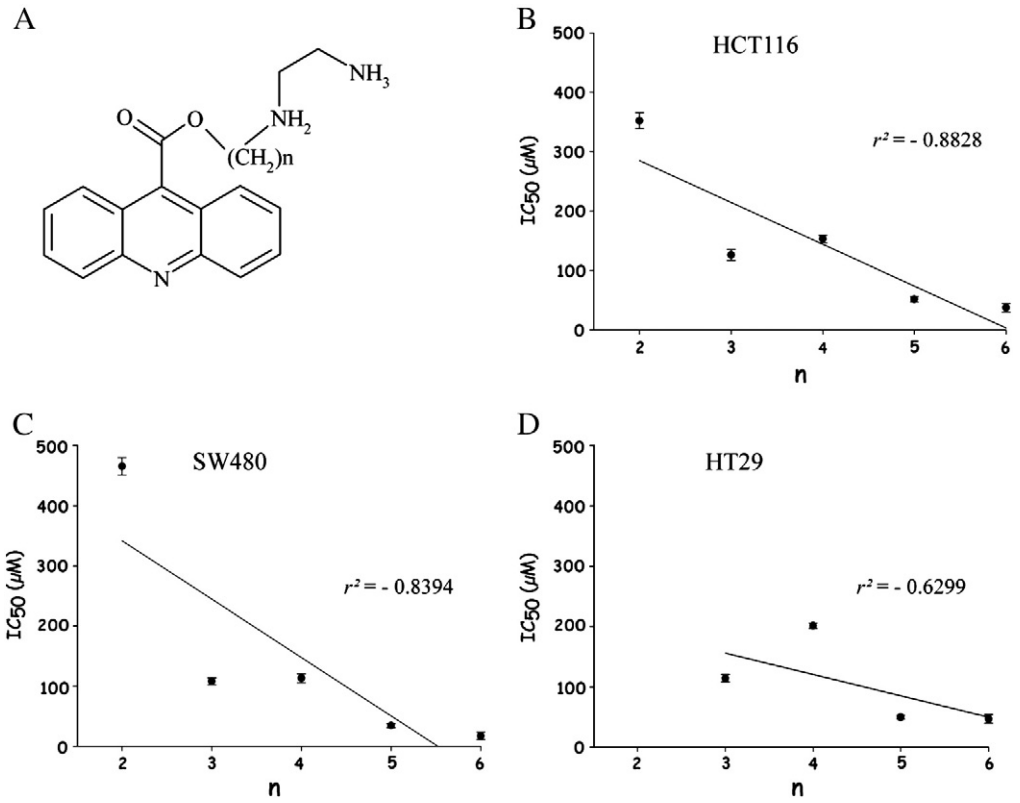


Fig. 3. Relationship between polymethyleme chain length (n) of acridine-9-carboxylate tethered (ethane-1,2-diamine) derivates connected by di- to hexamethylene chain (A) and IC_{50} values calculated from cytotoxic assay after 72 hours of drug incubation in human colic HCT116 (B), SW480 (C) and HT29 (D). Pearson's correlation coefficients were indicated on each graph. IC_{50} values are represented by the mean ± SD of 3 independent experiments.

size of the polymethylene spacer (Table 2 and Fig. 2). Cytotoxicity increased with the length of the polymethylene spacer, as highlighted by the negative correlation between the number of carbon in the alkyl chain and IC_{50} of compounds in all cell lines ($r^2 = -0.8828$; $r^2 = -0.8394$; $r^2 = -0.6299$ in HCT 116, SW480, and HT-29 respectively) (Fig. 3). Likewise, cytotoxic properties were high whatever the sensitivity of the cell line studied (Table 2). In final, theses new derivatives with acridine-9-carboxylate chromophore could potentially generate promising anticancer drugs. Nevertheless, no synergy between platination and intercalation were described in acridine-9-carboxylate tethered (ethane-1,2-diamine)platinum (II) complexes compared to unplatinated ligands. Physical and molecular analyses will be conducted to understand such phenomenon at the DNA level.

4. Conclusions

Two series of new acridine-based tethered (ethane-1,2-diamine) platinum(II) complexes connected by an appropriate polymethylene chain were prepared in a three step synthesis, starting from **1**. In human colic cancer cell lines HCT 116, SW480 and HT-29, series of complexes bearing the acridine-9-carboxamide chromophore displayed either no cytotoxic effect for non-platinated derivatives (3a–e), either cytotoxic properties similar or less potent than cisplatin and oxaliplatin for platinated complexes (4a–e). Interestingly, derivatives bearing the acridine-9-carboxylate chromophore (3f–j and 4f–j) were more active: platinated complexes (4f–j) exhibited higher cytotoxicity than reference molecule cisplatin and oxaliplatin, whilst unplatinated acridine-9-carboxylate intercalators (3f–j) exhibited tremendous cytotoxicity, with increasing intensity from compounds connected to a polymethylene linker having n = 2 to n = 6. These experiments suggest that acridine-9-substituted chromophore could be of great interest, especially with acridine-9-carboxylate functionality. Future investigations will examine interactions at the DNA levels and antitumor activity *in vivo*.

5. Abbreviations

CRC	Colorectal cancer
DMF	Dimethylformamide
HRMS	High resolution mass spectrometry
IC_{50}	50% inhibitory concentration
PCR	Polymerase chain reaction

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Appendix A. Supplementary data

Supplementary data to this article can be found online at doi:10.1016/j.jinorgbio.2012.02.006.

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