European Journal of Medicinal Chemistry 69 (2013) 719–727

Contents lists available at ScienceDirect

European Journal of Medicinal Chemistry

journal homepage: http://www.elsevier.com/locate/ejmech

Original article

cis-Dichloroplatinum(II) complexes tethered to dibenzo[*c*,*h*][1,6] naphthyridin-6-ones: Synthesis and cytotoxicity in human cancer cell lines *in vitro*

Nicolas Desbois [a,**], David Pertuit [b], Johnny Moretto [a], Claire Cachia [c], Bruno Chauffert [d], Florence Bouyer [a,*]

[a] *INSERM UMR U866 "Lipides/Nutrition/Cancer", Université de Bourgogne, UFR Médecine, 7 bd Jeanne d'Arc, BP 89700, 21079 Dijon Cedex, France*
[b] *EA 3660 Unité de Molécules d'Intérêt Biologique, UFR Pharmacie, 7 bd Jeanne d'Arc, BP 89700, 21079 Dijon Cedex, France*
[c] *Université de Bourgogne, UFR Pharmacie, 7 bd Jeanne d'Arc, BP 89700, 21079 Dijon Cedex, France*
[d] *Service d'Oncologie Médicale, CHU Amiens Sud, Av Laennec, 80054 Amiens Cedex 1, France*

ARTICLE INFO

Article history:
Received 16 May 2013
Received in revised form
10 September 2013
Accepted 16 September 2013
Available online 26 September 2013

Keywords:
Topoisomerase I targeting agents
Cisplatin
5*H*-Dibenzo[*c*,*h*][1,6]naphthyridin-6-one
Cytotoxicity
Colorectal cancer cell

ABSTRACT

A novel family of cisplatin-type complexes tethered to dibenzo[*c*,*h*][1,6]naphthyridin-6-one topoisomerase inhibitor *via* a polymethylene chain and their nonplatinated counterparts were prepared. Their potential cytotoxicity was assessed in three human colorectal cancer cell lines HCT 116, SW480 and HT-29 and compared to the reference molecules cisplatin and oxaliplatin. Platinated compounds were poorly active whilst nonplatinated dibenzo[*c*,*h*][1,6]naphthyridin-6-one moieties exhibited higher cytotoxic properties than cisplatin and oxaliplatin whatever the length of the polymethylene chain; molecules containing the tri- and hexamethylene chain length were the most cytotoxic.

1. Introduction

Platinum (Pt) derivatives represent an old family of anticancer drugs, that still play a pivotal role in the treatment of many solid tumors, including lung, ovarian, colon and bladder cancers [1–3]. The transition metal complex *cis*-diaminedichloroplatinum(II), also known as cisplatin (Fig. 1), consists of the leader molecule. Oxaliplatin (Fig. 1), a third-generation derivative with antitumor activity against cisplatin resistant tumor cells, represents the reference Pt compound used in the management of colorectal cancer [4–6]. In cancer cells, Pt drugs target DNA to establish coordination bonds especially at the N-7 of guanine base of DNA [7–9]. These so-called Pt-DNA adducts generate both intra- and interstrand crosslinks which, in final, lead to inhibition of DNA replication and RNA transcription [8–10]. In clinical practice, Pt drug efficacy could be restricted by intrinsic toxicities or resistance occurrence [11–13]; and despite the thousands of Pt derivates already synthetized, only a few Pt drugs are available in clinic.

Several strategies have been intensively explored to develop new compounds, especially by combining in a same molecule a Pt moiety to another entity exhibiting cytotoxic properties [14]. Such approach could have several advantages: to combine 2 modes of action which could help to circumvent drug resistance; to decrease toxicity of each drug used separately and to increase DNA targeting by incorporation of Pt into suitable "carrier" molecules, such as acridine derived-pharmacophore [15,16], anthraquinone [17], bis-naphthalimide [18], camptothecin [19]….

Our approach was focused on developing Pt compounds coupled to a cytotoxic moiety exhibiting topoisomerase I inhibiting properties. Human DNA topoisomerases are nuclear enzymes that control DNA topology, allowing, for example, DNA supercoiling relaxation during replication and transcription [20,21]. A few inhibitors of topoisomerases I are commercially available in clinic, especially those derived from camptothecin, a cytotoxic quinoline alkaloid extracted from a Chinese tree *Camptotheca acuminata*. Camptothecin exhibited too cytotoxic properties to be used in clinic, but

* Corresponding author. Tel.: +33 03 80 39 34 50.
** Corresponding author. Tel.: +33 03 80 39 32 52.
E-mail addresses: nicolas.desbois@u-bourgogne.fr (N. Desbois), florence.bouyer@u-bourgogne.fr (F. Bouyer).

0223-5234/$ – see front matter © 2013 Elsevier Masson SAS. All rights reserved.
http://dx.doi.org/10.1016/j.ejmech.2013.09.037

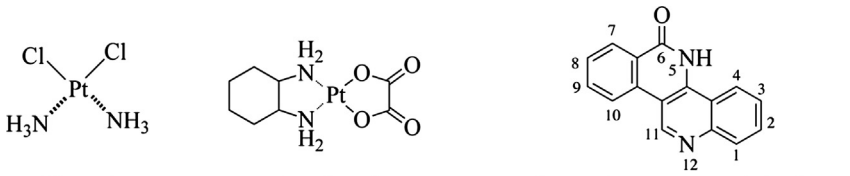


Fig. 1. The structure of the cisplatin, oxaliplatin and dibenzo[*c*,*h*]naphthyridin-6-one derivative.

2 analogs, irinotecan and topotecan, have been commercially approved [22–24]. However, these two camptothecin-derived topoisomerase 1 inhibitors displayed two major limits [23]: they suffer from partial hydrolytic inactivation due to the presence of a δ-lactone in the E-ring of their molecular structure. And, they are substrates for efflux pumps such as MDR1 (*Multi Drug Resistance 1*) and BCRP (*Breast Cancer Resistance Protein*) which play a major role in anticancer drug resistance. Since, many non-camptothecin and novel camptothecin derived-topoisomerase I inhibitors have been developed [25–28].

Thus, we described novel compounds associating a Pt moiety with a promising non-camptothecin topoisomerase I inhibitor fragment. Many families of such inhibitors have already been identified, such as indolocarbazoles [29], benzophenazines [30], benz[α]acridines [31], or benzo[*c*]phenanthridines [32]. Previous studies outlined the good correlation between topoisomerase I targeting activity and cytotoxicity of *5H*-dibenzo[*c*,*h*]naphthyridin-6-one derivatives [33,34] (Fig. 1).

We designed cisplatin type complexes tethered to promising topoisomerase inhibitor dibenzo[*c*,*h*]naphthyridin-6-one derivative by a variable polymethylene linker (Fig. 2) and their potential cytotoxic properties were assessed in a panel of human colorectal cancer cell lines, as clinical protocols of colorectal cancer commonly include Pt or anti-topoisomerase drugs [5,6]. Cytotoxicity was discussed according to the size of the alkyl chain bond and to the presence or absence of the platinum fragment.

2. Results and discussion

2.1. Preparation and spectroscopic properties

The compounds described in this study and the methods of synthesis are outlined in Schemes 1 and 2.

Reaction of 4-chloro-6,7-methylenedioxyquinoline [35] (**1**) with the corresponding amines **3a**–**e** yielded the 4-substituted quinolines **2a**–**e** respectively (Scheme 1). Phenol was utilized to form a phenolic intermediate of heightened reactivity to 4-chloroquinoline **1**. Treatment of commercially 2-bromo-5-nitrobenzoic acid with thionyl chloride provided the acid chloride **4** which was treated with **2a**–**e** to yield the benzamide derivatives **5a**–**e**. Initially, Sharma [36] protocol using 2-iodo-5-nitrobenzoic acid was applied, but unfortunately, this method was not reproducible in our condition: the iodine atom was more bulky than the bromine atom. The Heck coupling reaction of amide **5a**–**e** with $Pd(OAc)_2$, a phosphine ligand (PBu_3), and a base (Ag_2CO_3) in DMF reflux afforded **6a**–**e** formation in good yields (36–68%). Deprotection of diboc derivatives **6a**–**e** with trifluoroacetic acid provided **7a**–**e**. Intermediate TFA salts which were converted with HCl 1 N diethylether to **7a**–**e** 3HCl for biological study.

The Pt complexes **8a**–**e** (Scheme 2) were prepared by addition of an aqueous solution of K_2PtCl_4 to a basic solution of **7a**–**e** ligands and the products were isolated directly from the reaction mixture. In each complex, coordination of Pt to diamine was confirmed by ElectroSpray Ionization (ESI) mass spectrometry (HR-MS), with the appearance in both spectra of ionic pattern corresponding to $[M + Na]^+$.

2.2. Cytotoxicity in cancer cell lines

Cytotoxicity of 5*H*-dibenzo[*c*,*h*][1,6]naphthyridin-6-one-based Pt(II) complexes (**8a**–**e**) and their nonplatinated counterparts (**7a**–**e**) was performed in three human colic cancer cell lines HCT 116, HT-29 and SW480, as colorectal cancer is a clinical indication of Pt compounds [5,6]. Based on clinical trials, oxaliplatin in combination with 5-FU and folinic acid, has been approved for many years for the treatment of advanced metastatic colorectal cancer. Considering that topoisomerase I inhibitors represent also firmly established first-line treatment drugs of some chemotherapeutic protocols of colorectal cancer, we were interesting in synthesizing new compounds combining the Pt moiety to a topoisomerase I inhibitor carrier, anticipating that such compounds would localize in the vicinity of DNA and would carry out powerful cytotoxic effects. In the literature, the potential benefit of the concomitant use of a topoisomerase inhibitor and a Pt drug has already been underlined in different types of cancer [37,38]. However, the efficacy of such association, tested in human colorectal cell lines, was dependant of the schedule of administration applied [39]. At the molecular level, Pt-DNA adducts could act as endogenous DNA topoisomerase I poisons to enhance the stability of covalent Topoisomerase I-DNA complexes [40].

In our biological evaluation, cytotoxicity of newly synthesized compounds was thus compared to the approved Pt drug in clinic, oxaliplatin, but also to the reference Pt drug, cisplatin. In our experimental conditions, oxaliplatin was more cytotoxic than cisplatin in the three cell lines tested, as highlighted by IC_{50} values (Table 1). High IC_{50} values could be attributed to stringent conditions of cytotoxic assay, as drug treatment was applied on subconfluent cells. The platinated **8a**–**e** and nonplatinated **7a**–**e** derivates of 2,3-methylenedioxy-8-nitro-5*H*-dibenzo[*c*,*h*][1,6] naphthyridin-6-one displayed very different cytotoxic properties (Table 1; Figs. 3 and 4) in the three colic cancer cell lines. Unfortunately, the platinated complexes **8a**–**e** did not exhibit significant cytotoxicity, which remained much lower than **7a**–**e** and oxaliplatin and cisplatin in the three cell lines studied, especially in HCT 116 (Table 1 and Fig. 3). **8a**–**e** displayed cytotoxic properties 20

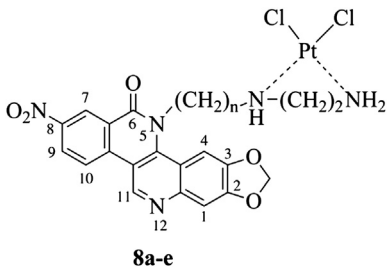
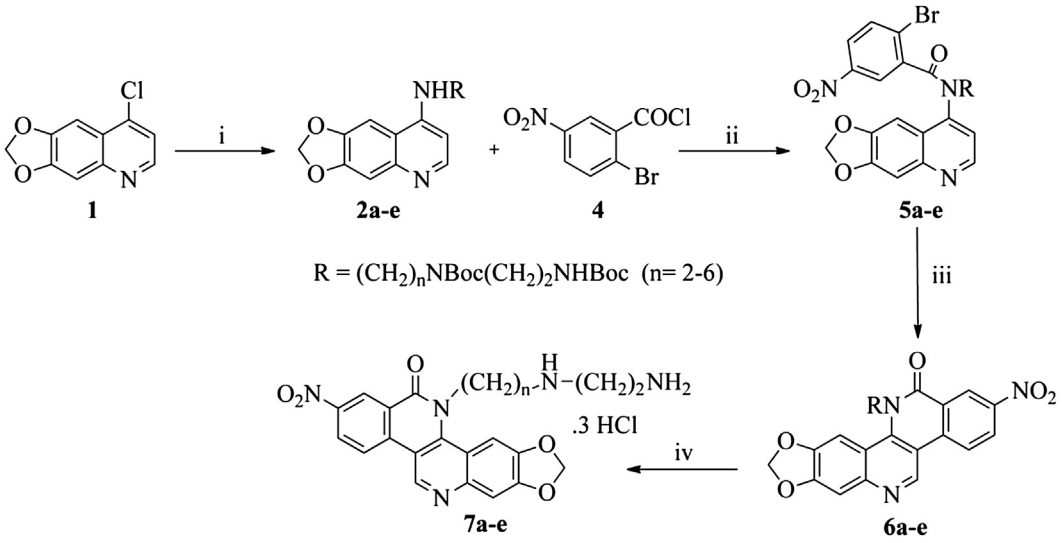


Fig. 2. Compounds of interest.



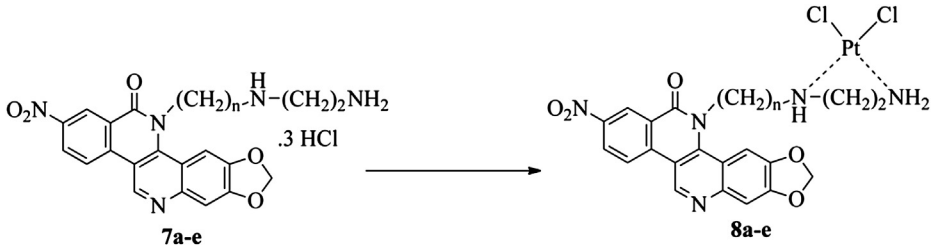
Scheme 1. Reagents and conditions: (i) a) phenol, Δ; b) $NH_2(CH_2)_nNBoc(CH_2)_2NHBoc$ (**3a**–**e**), 110 °C; (ii) CH_2Cl_2, NEt_3; (iii) $Pd(OAc)_2$, PBu_3, Ag_2CO_3, DMF, Δ; (iv) a) TFA, 0 °C then RT; b) HCl 1 N/diethylether.

times lower than **7a**–**e**, suggesting that, in cell culture medium, the Pt drugs were not cleaved to generate the 5*H*-dibenzo[*c*,*h*][1,6]naphthyridin-6-one derivative and the Pt moiety. In addition, the presence of a polymethylene spacer in platinated complexes did not play a role in the cytotoxic potential of the complexes. The absence of significant activity may imply that the (*N*-(aminoalkyl)-(ethane-1,2-diamine))Pt(II) linker in 5-position interfered with dibenzo[*c*,*h*][1,6]naphthyridin-6-one targeting topoisomerase I activity, and in final failed to exhibit high cytotoxicity. Other hypotheses could involve lower cellular uptake of platinated complexes, or disturbance of cell death mechanism by steric hindrance. Thus, instead of combining 2 modes of action, the presence of the two different entities – the topoisomerase I inhibiting and the Pt moiety – in a same complex was detrimental to its cytotoxic activity. Nevertheless, drugs combining a Pt moiety to a bioactive carrier ligand did not always support enhanced cytotoxic properties. Previous testing of cisplatin-type complexes tethered to a substituted acridine chromophore containing a carboxamide functionality [15,16] also displayed low cytotoxicity in human cancer cells, including human colorectal cancer cell lines. However, several Pt complexes of differently substituted acridine-4-carboxamide [41,42] or acridine-9-carboxylate chromophore were reported as promising anticancer drugs active in different cell lines sensitive or resistant to cisplatin [41,42] including human colon cell lines HCT 116, SW480 and HT-29 [15]. Recently, the conjugation of Pt to a 7-substituted camptothecin moiety generated compounds with growth inhibitory activity against a panel of human tumor cancer cell lines, and with appreciable antitumor activity in mice bearing nonsmall cell lung cancer H460 tumor xenograft [19]. Interestingly, such hybrid compounds cumulated both properties of single molecules: the topoisomerase I poisoning activity and the DNA-platination [19], corroborating the idea of combining several mechanisms of action in a unique derivative.

In spite of that, nonplatinated derivates of 2,3-methylenedioxy-8-nitro-5*H*-dibenzo[*c*,*h*][1,6]naphthyridin-6-one (**7a**–**e**) displayed potent cytotoxic properties, as highlighted by IC_{50} values, which could be 5–10 times lower than reference compounds as observed in HCT 116 cells (Table 1 and Fig. 4). The powerful cytotoxic properties of the substituted 5*H*-dibenzo[*c*,*h*][1,6]naphthyridin-6-one were previously described by LaVoie and co-workers who extensively investigated such compounds in term of cytotoxicity, topoisomerase I targeting activity [33,43–45] and antitumor activity *in vivo*, mainly in athymic nude mice bearing human xenografts [33,46–48]. For many 5*H*-dibenzo[*c*,*h*][1,6]naphthyridin-6-one derivatives, cytotoxicity correlated with their topoisomerase I inhibitory activity [33,35,36,46,49,50], the ceto-group in position 6 playing a key role in enhanced topoisomerase I targeting and cytotoxic activities [33].

5*H*-Dibenzo[*c*,*h*][1,6]naphthyridin-6-one derivatives were potentially efficacious in the treatment of colorectal cancer, as ARC-111, a well-known and extensively studied derivative, was highly potent in scid (severe combined immunodeficient) mice carrying human HCT-8 colon adenocarcinoma tumor xenografts [48]; and Genz-644282, selected for phase I clinical trial, exhibited potent cytotoxicity towards human colorectal cancer cells HCT 116 and HT-29, and significative antitumor activity in many human colon carcinoma tumor xenografts (HCT 116, HT-29, HCT-15, DLD-1) [47].

The originality of **7a**–**e** leads in the chemical substituent in position 5, as a polymethylene spacer ($n = 2$–6) connected the 2,3-methylenedioxy-8-nitro-5*H*-dibenzo[*c*,*h*][1,6]naphthyridin-6-one



Scheme 2. Reagents and conditions: a) $Na_2CO_3 \cdot 2N$, b) K_2PtCl_4, H_2O, c) KCl 5%.

Table 1
Determination of IC_{50} values assessed by cytotoxic assay of naphthyridin-6-one-based derivatives platinated (**8a**–**e**) or not (**7a**–**e**), the vehicle DMF, and the reference drugs, cisplatin and oxaliplatin, in human cancer colorectal HCT 116, HT-29 and SW480 cell lines after a 72 h-treatment. Each IC_{50}value represented the mean ± standard deviation of at least 3 different experiments (*nd* = not determinable). Statistical analyses compared IC_{50} values of newly synthetized drugs versus cisplatin (*) or oxaliplatin (†) (*/†: $p < 0.05$; **/††: $p < 0.01$; ***/†††: $p < 0.001$).

Compounds	IC_{50} (μM)		
	HCT 116	HT-29	SW480
7a	24.97 ± 2. 73 (**) (††)	43.02 ± 3.83 (***) (††)	40.70 ± 4.42 (†)
7b	12.16 ± 0.58 (**) (††)	22.20 ± 2.19 (**) (††)	30.13 ± 5.34 (†)
7c	30.71 ± 11.51 (**) (††)	41.20 ± 4.34 (***) (††)	43.74 ± 2.22 (*) (†)
7d	25.15 ± 2.64 (**) (††)	47.60 ± 1.92 (***) (††)	50.73 ± 4.52
7e	15.44 ± 4.71 (**) (††)	30.84 ± 5.51 (**) (††)	24.00 ± 0.28 (**) (†)
8a	387.68 ± 11.74	319.57 ± 22.28	440.22 ± 50.33
8b	*nd*	428.26 ± 9.87	460.14 ± 8.87
8c	*nd*	446.38 ± 13.29	*nd*
8d	*nd*	384.06 ± 34.46	*nd*
8e	491.07 ± 3.16	*nd*	364.13 ± 34.34
Cisplatin	208.50 ± 9.97	188.86 ± 13.07	185.76 ± 65.82
Oxaliplatin	126.19 ± 25.33	143.16 ± 33.96	58.64 ± 11.02
DMF	*nd*	418.48 ± 7.99	409.42 ± 7.32

moiety to the 5-[*N*-[aminoalkyl]-ethane-1,2-diamine] linear chain. The ability of an alkylamino group on the side chain attached to the N-5 position to generate powerful cytotoxic compounds was expected, even though molecules combining such 5-substituent to an 8-nitro group have never been explored before. Actually, the presence of a N-5-alkylamino group was already associated with potent topoisomerase 1 targeting and cytotoxic activities [33,35]. On the contrary, additional substituents on the carbon directly attached to the N-5 position [33] has been shown to decrease topoisomerase I targeting activity and cytotoxicity, as well as increased steric bulk in close proximity to the N-5 heteroatom [33,35,46,49]. Recently, a series of dibenzo[*c*,*h*][1,6]naphthyridin-6-one derivatives with varied ethylenediamine substituent in position 5- were proved to be more active when both amino group were tertiary [50]. In our study, we demonstrated that nonsubstituted amino group in substituent in position-5 could also retain high cytotoxic properties.

Moreover, we clearly showed that the length of the polymethylene linker at the 5-position influence the cytotoxicity of the 2,3-methylenedioxy-8-nitro-5*H*-dibenzo[*c*,*h*][1,6]naphthyridin-6-one derivates (**7a**–**e**). The propylene and hexamethylene spacers generated the most powerful compounds whatever the cell line tested (Fig. 5). High cytotoxicity of compounds **7b** and **e** came out in nonplatinated derivatives compared to their platinated counterparts. These data are quite new, as until now, the high cytotoxic dibenzo[*c*,*h*][1,6]naphthyridin-6-one derivatives were characterized by compounds connected at the 5-position, to a two-carbon linker to an alkylamine [33]. Even though a complete series of compounds bearing a polymethylene linker ($n = 2$–6) was not investigated in the literature, partial data derived from ARC-111 analogs corroborated our findings: insertion of a methylene group into the side chain in position-5 of ARC-111 – resulting in a trimethylene substituent – did allow for retention of high topoisomerase I and cytotoxic activity [33], as well as compounds containing 6 atoms linear alkyl side from the N-5 position [33]. The high cytotoxicity of the derivative connected to the hexamethylene linker could be due to the substantial conformational flexibility of the linker that would not interfere with the cytotoxic activity, and potentially with the topoisomerase 1-targeting activity of the dibenzo[*c*,*h*][1,6]naphthyridin-6-one chromophore, in which the ceto-group in position 6 is highly involved.

Compared to the literature, especially to ARC-111, **7a**–**e** displayed another distinctive feature. The presence of a nitro substituent in the 8-position supplied derivatives with potent cytotoxic properties. Such findings were in agreement with previous work performed on ARC-111, in which the replacement of the 8,9-dimethoxy groups with a 8,9-dinitro substituent allowed for retention of potent topoisomerase I activity and cytotoxicity [34]. The nature of the substituent in position 8- and 9- of the dibenzo [*c*,*h*][1,6]naphthyridin-6-one significantly influenced the cytotoxicity of compounds. Thus, in ARC-111 analogs, the replacement of 8-nitro by 8-amino, 8-arylamine [36] or by a 8,9-diethoxy [46] substituent decreased cytotoxic and topoisomerase I activities.

Further works will assess the topoisomerase I targeting activity of **7a**–**e**, as such inhibitory activity could be expected from data of the literature. Optimization of the nitro group in position 8 and/or 9 will also be performed in a way to design compounds with enhanced cytotoxicity.

3. Conclusion

Two series of substituted dibenzo[*c*,*h*][1,6]naphthyridin-6-one derivatives were synthetized either platinated (**8a**–**e**) or not (**7a**–**e**). Platinated complexes **8a**–**e** did not exhibit significant cytotoxic properties in human colic cancer cell lines HCT 116, SW480 and HT-29. But, nonplatinated compounds **7a**–**e** displayed higher cytotoxic effect than **8a**–**e** and reference molecules, cisplatin and oxaliplatin. Their cytotoxicity was influenced by the length of the polymethylene linker ($n = 2$–6), compounds bearing $n = 3$ (**7b**) and $n = 6$ (**7e**), disclosing the most powerful capacities. In final, **7a**–**e** could potentially generate promising anticancer drugs.

Future investigations will examine whether topoisomerase I inhibitory activity could contribute to the observed cytotoxic activity of these compounds, and whether changes in 8- or/and 9-substituents could optimize cytotoxicity. Alternatively, conformational structures of **8a**–**e** will be examined to determine the causes of low cytotoxicity of Pt complexes.

4. Experimental

4.1. Chemistry

4.1.1. Materials 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 $60F_{254}$ plates. Melting points were determined on a Reichert–Jung–Koffler apparatus and were not corrected. NMR spectra (300 MHz for ^{1}H, and 75 MHz for ^{13}C) were recorded on a Bruker Avance 300 and 600 instruments using the indicated solvents. Chemical shifts were reported in ppm (δ). All the coupling constants (J) are in Hertz. Accurate mass measurements (HR-MS) were obtained with a LTQ Orbitrap XL (THERMO) instrument in ESI mode. Elemental analyses were performed with an Elemental Analyser Thermo electron Flash EA 1112. Infrared spectra were recorded on IR FT BRUKER Vertex 70v.

The compound 4-chloro-6,7-methylenedioxyquinoline (**1**) was prepared according to reported methods [35]. The compounds *N*,*N*′-(*tert*-butoxycarbonyl)-*N*-[aminoalkyl]-ethane-1,2-diamines (**3a**–**e**) were prepared by a method described by Holmes et al. [41] that consisted in modification of the diboc procedure to avoid monoboc product: for that, a mixture of di-*tert*-butyldicarbonate and

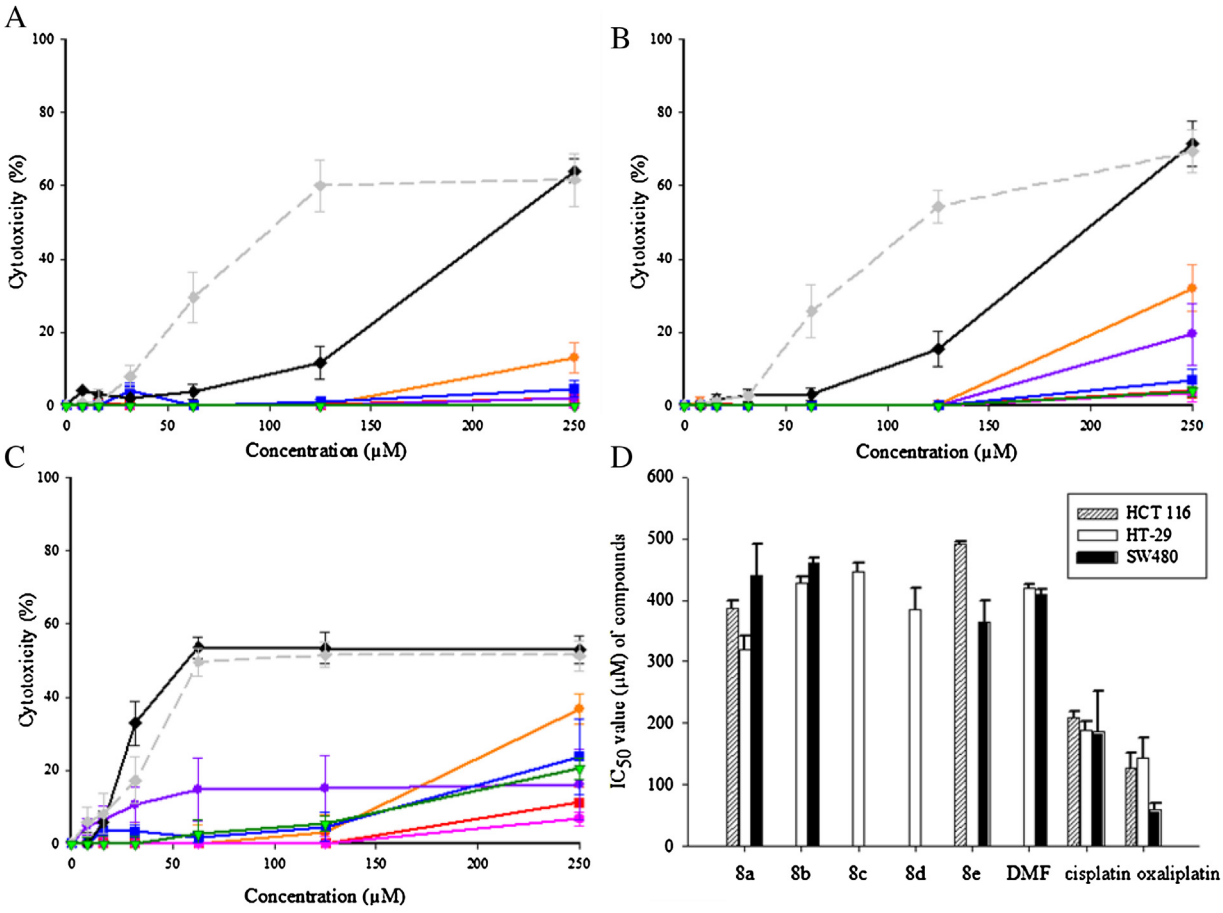


Fig. 3. Concentration–response curves of cytotoxicity of platinated complexes (**8a**–**e**) connected by a di- to hexamethylene chain **8a** (●) **8b** (■) **8c** (⬢) **8d** (●) **8e** (■), and of their vehicle DMF (▼), as well as of the reference molecules cisplatin (♦) and oxaliplatin (♦) in human cancer colorectal HCT 116 (A), HT-29 (B) and SW480 (C) cell lines after a treatment of 72 h. Cytotoxicity values are represented by the mean ± standard deviation of 3 independent experiments. IC_{50} values of each compound evaluated in HCT 116 (▨), HT-29 (□) and SW480 (▬) are reported in (D).

appropriate *N*-[[(benzyloxycarbonyl)amino]-alkyl]-ethane-1,2-diamine in dry dichloromethane was stirred for 5 days.

*4.1.2. Synthesis of 4-[N,N′-(tert-butoxycarbonyl)-N-[aminoalkyl]-ethane-1,2-diamine]-6,7-methylenedioxyquinolines (**2a**–**e**)*

A mixture of 4-chloro-6,7-methylenedioxyquinoline (**1**) (1.35 g, 6.50 mmol) in phenol (3.23 g, 34.4 mmol) was heated to reflux for 3 h. The bath temperature was lowered to 110 °C, and the appropriate *N,N′*-(*tert*-butoxycarbonyl)-*N*-[aminoalkyl]-ethane-1,2-diamine (**3**) (7.82 mmol) was added. The mixture was stirred at this temperature for 18 h.

The residue was partitioned between dichloromethane (100 mL) and 10% aqueous NaOH (100 mL). The aqueous layer was extracted with dichloromethane. The organic phase was dried (Na_2SO_4) and evaporated to dryness. The crude product was purified on aluminum oxide eluting dichloromethane. **2a**: Yield: 78%. mp 60–62 °C. ^{1}H NMR ($CDCl_3$, 300 MHz) δ 8.55 (d, 1H, J = 5.5 Hz), 7.26 (s, 1H), 7.13 (s, 1H), 6.39 (br, 1H), 6.24 (d, 1H, J = 5.5 Hz), 6.03 (s, 2H), 3.28 (m, 8H), 1.43 (m, 18H), ^{13}C NMR ($CDCl_3$, 75 MHz) δ 157.8, 156.0, 149.9, 149.7, 148.8, 146.7, 146.3, 114.2, 106.3, 101.4, 97.6, 96.7, 81.2, 79.6, 53.4, 46.5, 43.8, 39.3, 28.4 ($6CH_3$). **2b**: Yield: 54%. mp 110–112 °C. ^{1}H NMR ($CDCl_3$, 300 MHz) δ 8.33 (d, 1H, J = 5.5 Hz), 7.26 (m, 2H), 6.32 (d, 1H, J = 5.5 Hz), 6.12 (br, 1H), 6.04 (s, 2H), 3.31 (m, 8H), 1.88 (m, 2H), 1.50 (s, 9H), 1.43 (s, 9H); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 156.3, 156.0, 149.9, 149.5, 148.8, 146.7, 146.5, 114.5, 106.3, 101.4, 98.1, 96.8, 80.6, 79.5, 53.4, 46.8, 44.4, 39.4, 28.4 ($6CH_3$, $1CH_2$). **2c**: Yield: 33%. Brown oil. ^{1}H NMR ($CDCl_3$, 300 MHz) δ 8.37 (d, 1H, J = 5.5 Hz), 7.28 (s, 1H), 7.26 (s, 1H), 6.34 (d, 1H, J = 5.5 Hz), 6.05 (s, 2H), 3.30 (m, 8H), 1.72 (m, 4H), 1.46 (s, 9H), 1.43 (s, 9H); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 156.0 (2C), 150.0, 149.9, 148.3, 147.2, 146.8, 114.2, 106.1, 101.5, 98.5, 96.7, 80.2, 79.2, 52.6, 46.8, 43.2, 39.7, 28.4 ($6CH_3$), 27.3, 26.5. **2d**: Yield: 80%. mp 64–66 °C. ^{1}H NMR ($CDCl_3$, 300 MHz) δ 8.38 (d, 1H, J = 5.5 Hz), 7.35 (s, 1H), 7.28 (s, 1H), 7.04 (br, 1H), 6.36 (d, 1H, J = 5.5 Hz), 6.06 (s, 2H), 3.28 (m, 8H), 1.72–1.79 (m, 6H), 1.46 (s, 9H), 1.43 (s, 9H); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 155.9 (2C), 149.9, 149.4, 148.7, 146.7, 146.2, 114.1, 106.4, 101.5, 98.6, 95.9, 79.9 (2C), 53.0, 46.6, 43.1, 39.6, 27.8–28.6 ($6CH_3$, $3CH_2$). **2e**: Yield: 85%. mp 92–94 °C. ^{1}H NMR ($CDCl_3$, 300 MHz) δ 8.34 (d, 1H, J = 5.5 Hz), 7.31 (s, 1H), 7.26 (s, 1H), 6.34 (d, 1H, J = 5.5 Hz), 6.05 (s, 2H), 3.28 (m, 8H), 1.41–1.72 (m, 24H); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 156.3, 155.9, 149.9, 149.3, 148.9, 146.7, 146.3, 114.1, 106.5, 101.5, 98.5, 95.8, 79.8, 79.2, 53.7, 46.4, 42.9, 39.6, 26.3–28.4 ($6CH_3$, $4CH_2$).

*4.1.3. Synthesis of N-(6,7-methylenedioxyquinolin-4-yl)-N-[N,N′-(tert-butoxycarbonyl)-N-[aminoalkyl]-ethane-1,2-diamine]-2-bromo-4-nitrobenzamides (**5a**–**e**)*

A solution of commercially 2-bromo-5-nitrobenzoic acid (0.56 g, 2.27 mmol) in thionyl chloride (10 mL) was refluxed with stirring under nitrogen for 3 h. The mixture was evaporated. The solution of crude chloride acid **4** in dry dichloromethane (29 mL) was added to a solution of appropriate 4-[*N,N′*-bis (*tert*-butoxycarbonyl)-*N*-[aminoalkyl]-ethane-1,2-diamine]-6,7-methylenedioxyquinoline (**2**) (0.60 g,

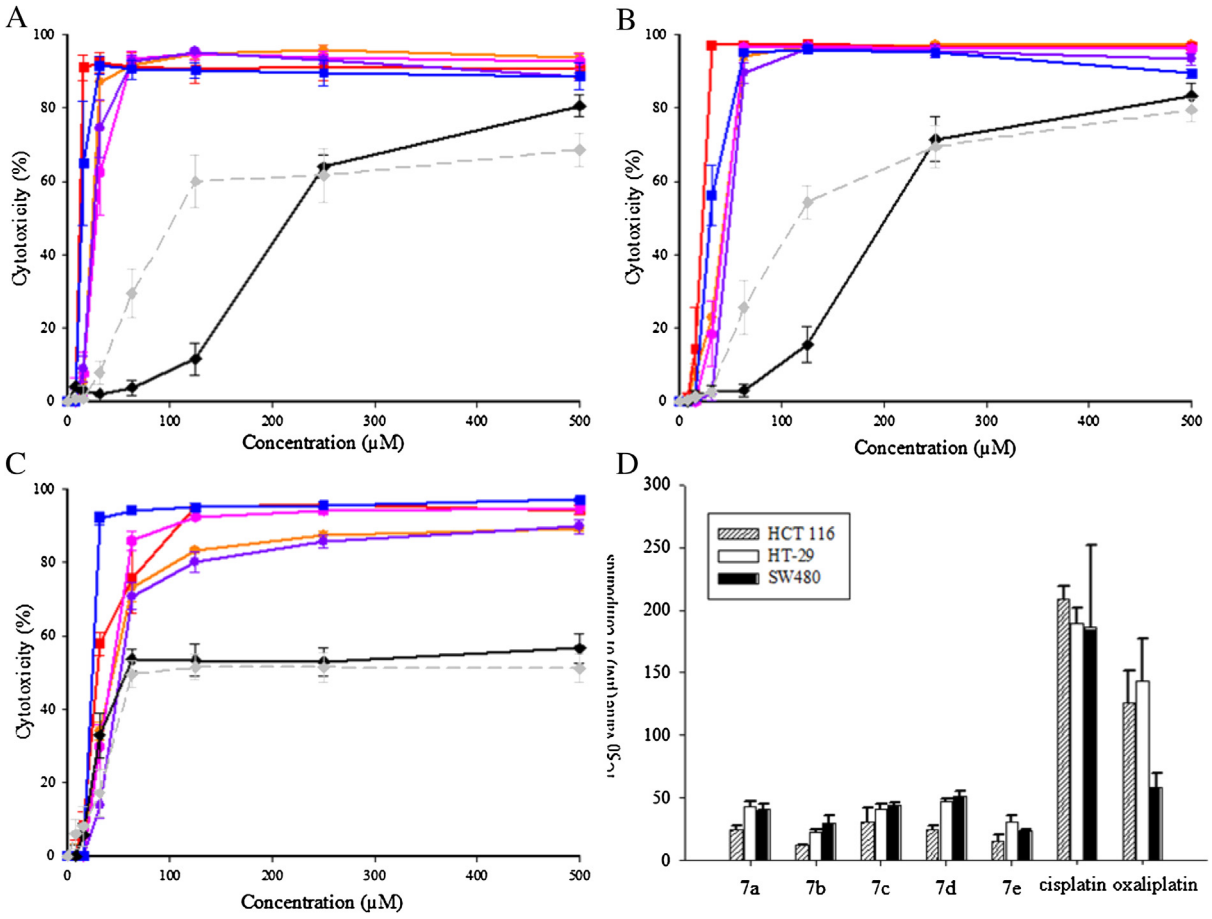


Fig. 4. Concentration–response curves of cytotoxicity of substituted *5H*-dibenzo[*c,h*][1,6]naphthyridin-6-ones trihydrochlorides (**7a**–**e**) connected by a di- to hexamethylene chain **7a** (●) **7b** (■) **7c** (⬣) **7d** (●) **7e** (■), and of reference molecules cisplatin (♦) and oxaliplatin (♦) in human cancer colorectal HCT 116 (A), HT-29 (B) and SW480 (C) cell lines after a treatment of 72 h. Cytotoxicity values are represented by the mean ± standard deviation of 3 independent experiments. IC_{50} values of each compound evaluated in HCT 116 (▨), HT-29 (□) and SW480 (▬) are reported in (D).

1.19 mmol) and triethylamine (1.32 mL, 9.52 mmol) in dry dichloromethane (10 mL). The mixture was refluxed with stirring under nitrogen for 16 h. At room temperature, a saturated aqueous Na_2CO_3 solution (50 mL) was added and the aqueous layer was extracted with dichloromethane (3 × 40 mL). The combined organic phases were dried (Na_2SO_4) and evaporated to dryness. The crude mixture was chromatographed on silica in 2:1 ethyl acetate-cyclohexane. **5a**: Yield: 26%. mp 90–92 °C. ^{1}H NMR ($CDCl_3$, 300 MHz) δ 8.43 (d, 1H, J = 5.5 Hz), 7.76 (m, 2H), 7.52 (m, 1H), 7.21–7.26 (m, 3H), 6.13 (m, 2H), 4.61 (m, 1H), 3.52 (m, 1H), 3.17–3.35 (m, 6H), 1.36 (m, 18H), ^{13}C NMR ($CDCl_3$, 75 MHz) δ 166.8, 156.0 (2C), 151.5, 148.6, 148.2, 148.0, 146.0, 144.4, 138.4, 134.1, 127.1, 124.8, 123.1, 122.1, 119.5, 106.7, 102.4, 97.8, 80.5, 79.4, 60.5, 47.3, 45.4, 39.4, 28.5 ($6CH_3$). **5b**: Yield: 65%. mp 70–72 °C. ^{1}H NMR ($CDCl_3$, 300 MHz) δ 8.53 (d, 1H, J = 5.5 Hz), 7.78 (m, 2H), 7.56 (d, 1H, J = 9 Hz), 7.32 (s, 1H), 7.26 (m, 2H), 6.19 (m, 2H), 4.49 (m, 1H), 3.31 (m, 1H), 3.25 (m, 6H), 1.72 (m, 2H), 1.43 (m, 18H), ^{13}C NMR ($CDCl_3$, 75 MHz) δ 166.4, 155.9 (2C), 151.5, 149.1, 148.5, 148.2, 146.0, 144.1, 138.8, 134.0, 127.3, 124.7, 122.3 (2C), 119.1, 106.7, 102.5, 97.5, 80.2 (2C), 60.4, 46.8 (2C), 39.5, 28.4 (6C). **5c**: Yield: 59%. mp 66–68 °C. ^{1}H NMR ($CDCl_3$, 300 MHz) δ 8.52 (d, 1H, J = 5.5 Hz), 7.80 (m, 2H), 7.56 (d, 1H, J = 9 Hz), 7.33 (s, 1H), 7.26 (m, 2H), 6.19 (m, 2H), 4.52 (m, 1H), 3.43 (m, 1H), 3.25 (m, 6H), 1.45–1.75 (m, 4H), 1.43 (m, 18H), ^{13}C NMR ($CDCl_3$, 75 MHz) δ 166.3, 155.4 (2C), 151.6, 149.8 (2C), 148.1, 146.0, 144.2, 134.0, 130.6, 127.3, 124.7, 122.3, 120.9, 119.0, 106.6, 102.5, 97.4, 79.9 (2C), 60.4, 48.6, 46.0, 39.5, 28.4 (6C), 25.2, 21.1. **5d**: Yield: 50%. mp 47–49 °C. ^{1}H NMR ($CDCl_3$, 300 MHz) δ 8.52 (d, 1H, J = 5.5 Hz), 7.79 (m, 2H), 7.56 (d, 1H, J = 9 Hz), 7.31 (s, 1H), 7.28 (s, 1H), 7.19 (d, 1H, J = 5.5 Hz), 6.19 (m, 2H), 4.47 (m, 1H), 3.41 (m, 1H), 3.25 (m, 6H), 1.52–1.78 (m, 6H), 1.43 (m, 18H), ^{13}C NMR ($CDCl_3$, 75 MHz) δ 166.2, 156.0 (2C), 151.5, 149.7, 148.5, 148.1, 145.9, 144.2, 138.9, 134.1, 127.3, 124.7, 122.3, 121.9, 119.0, 106.6, 102.4, 97.5, 79.7 (2C), 60.4, 48.9, 47.5, 39.5, 29.7, 28.4 (7C), 27.6, 24.0. **5e**: Yield: 72%. mp 42–44 °C. ^{1}H NMR ($CDCl_3$, 300 MHz) δ 8.52 (d, 1H, J = 5.5 Hz), 7.79 (m, 2H), 7.57 (d, 1H, J = 9 Hz), 7.32 (s, 1H), 7.28 (s, 1H), 7.20 (d, 1H, J = 5.5 Hz), 6.19 (m, 2H), 4.47 (m, 1H), 3.40 (m, 1H), 3.23 (m, 6H), 1.52–1.75 (m, 8H), 1.43 (m, 18H), ^{13}C NMR ($CDCl_3$, 75 MHz) δ 166.2, 155.9 (2C), 151.5, 149.7, 148.5, 148.1, 145.9, 144.3, 138.9, 134.0, 127.3, 124.7, 122.4, 121.9, 119.1, 106.7, 102.4, 97.5, 79.7 (2C), 60.4, 49.0, 46.4, 39.6, 28.4 (7C), 27.8, 26.6, 26.4.

*4.1.4. Synthesis of 2,3-methylenedioxy-8-nitro-5-[N,N′-(tert-butoxycarbonyl)-N-[aminoalkyl]-ethane-1,2-diamine]-5H-dibenzo[c,h][1,6]naphthyridin-6-ones (**6a**–**e**)*

To a solution of appropriate *N*-(6,7-methylenedioxyquinolin-4-yl)-*N*-[*N,N′*-(*tert*-butoxycarbonyl)-*N*-[aminoalkyl]-ethane-1,2-diamine]-2-bromo-4-nitrobenzamide (**5**) (0.60 mmol) in dry DMF (11 mL) were successively added $Pd(OAc)_2$ (26.9 mg, 0.12 mmol), Bu_3P (0.06 mL, 0.24 mmol) and Ag_2CO_3 (0.33 g, 1.20 mmol). The mixture was refluxed for 45 min and the reaction mixture was diluted with ether and the precipitate was removed by filtration. The filtrate was diluted with dichloromethane (30 mL) and washed with water (3 × 30 mL) and brine (30 mL). The organic layer was then dried with anhydrous sodium sulfate, filtered and evaporated.

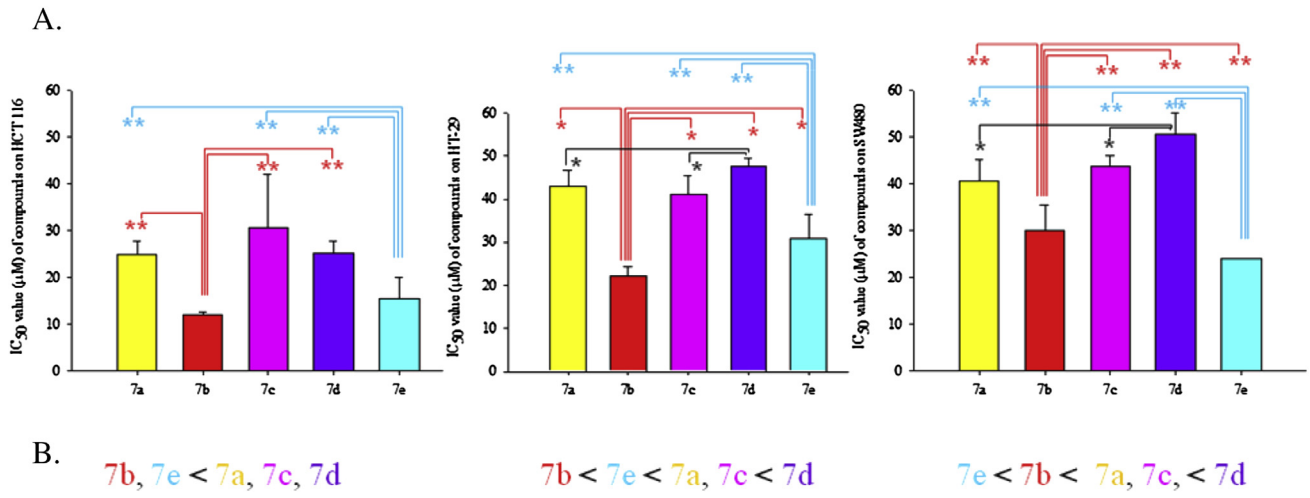


Fig. 5. Influence of the polymethylene linker chain on IC_{50} values of substituted *5H*-dibenzo[*c,h*][1,6]naphthyridin-6-ones trihydrochlorides (**7a**–**e**) in human cancer colorectal HCT 116, HT-29 and SW480 cell lines after a 72 h-treatment. Statistics: *: $p < 0.05$; **: $p < 0.01$ (A). Ranking of IC_{50} values from the most to the less powerful derivatives according to the size of the polymethylene linker; < expressed significant differences between compounds (B).

The mixture was purified using column chromatography (SiO_2, AcOEt).

6a: Yield: 63%. mp 230–232 °C. ^{1}H NMR ($CDCl_3$, 300 MHz) δ 9.37 (s, 1H), 9.26 (s, 1H), 8.54 (d, 1H, $J = 9$ Hz), 8.46 (d, 1H, $J = 9$ Hz), 7.40 (m, 2H), 6.16 (s, 2H), 4.74 (t, 2H, $J = 7$ Hz), 3.04–3.40 (m, 6H), 1.34 (m, 18H), ^{13}C NMR ($CDCl_3$, 75 MHz) δ 163.5, 156.1 (2C), 151.5, 148.5, 148.4, 147.2, 143.6, 143.3, 137.8, 127.4, 125.5, 124.8, 122.9, 114.4, 110.6, 106.7, 102.7, 101.3, 80.7 (2C), 50.3, 47.4, 45.6, 39.3, 28.4 (6C). **6b**: Yield: 56%. mp 109–111 °C. ^{1}H NMR ($CDCl_3$, 300 MHz) δ 9.45 (s, 1H), 9.31 (s, 1H), 8.58 (d, 1H, $J = 9$ Hz), 8.49 (d, 1H, $J = 9$ Hz), 7.53 (m, 2H), 6.20 (s, 2H), 4.48 (t, 2H, $J = 7$ Hz), 3.27–3.40 (m, 6H), 2.32 (t, 2H, $J = 7$ Hz), 1.42 (m, 18H), ^{13}C NMR ($CDCl_3$, 75 MHz) δ 163.0, 155.9 (2C), 151.1, 148.8, 148.1, 147.0, 143.9, 143.1, 137.5, 127.2, 125.5, 124.8, 122.8, 114.3, 110.3, 107.3, 102.6, 100.6, 80.2 (2C), 49.1, 46.4, 44.6, 39.3, 29.7, 28.3 (6C). **6c**: Yield: 36%. mp 163–165 °C. ^{1}H NMR ($CDCl_3$, 300 MHz) δ 9.46 (s, 1H), 9.33 (s, 1H), 8.59 (d, 1H, $J = 9$ Hz), 8.50 (d, 1H, $J = 9$ Hz), 7.51 (s, 1H),7.48 (s, 1H), 6.21 (s, 2H), 4.50 (t, 2H, $J = 7$ Hz), 3.23 (m, 6H), 1.66–2.08 (m, 4H), 1.41 (m, 18H), ^{13}C NMR ($CDCl_3$, 75 MHz) δ 163.0, 156.1 (2C), 151.3, 148.3, 148.1, 146.9, 143.7, 143.1, 137.3, 127.3, 125.3, 124.7, 122.8, 114.3, 110.5, 106.7, 102.6, 100.4, 80.2 (2C), 50.7, 46.9, 46.4, 39.1, 28.1 (6C), 26.0 (2C). **6d**: Yield: 62%. mp 189–191 °C. ^{1}H NMR ($CDCl_3$, 300 MHz) δ 9.42 (s, 1H), 9.29 (s, 1H), 8.56 (d, 1H, $J = 9$ Hz), 8.47 (d, 1H, $J = 9$ Hz), 7.50 (s, 1H), 7.45 (s, 1H), 6.20 (s, 2H), 4.45 (t, 2H, $J = 7$ Hz), 3.24 (m, 6H), 1.66–2.08 (m, 6H), 1.40 (m, 18H), ^{13}C NMR ($CDCl_3$, 75 MHz) δ 163.0, 156.1 (2C), 151.1, 148.5, 148.1, 147.1, 143.8, 143.2, 137.4, 127.2, 125.5, 124.8, 122.8, 114.4, 110.4, 106.9, 102.6, 100.6, 80.0 (2C), 51.0, 47.4, 46.2, 39.2, 29.7, 28.5, 28.3 (6C), 24.0. **6e**: Yield: 68%. mp 72–74 °C. ^{1}H NMR ($CDCl_3$, 300 MHz) δ 9.43 (s, 1H), 9.30 (s, 1H), 8.57 (d, 1H, $J = 9$ Hz), 8.48 (d, 1H, $J = 9$ Hz), 7.52 (s, 1H), 7.45 (s, 1H), 6.21 (s, 2H), 4.45 (t, 2H, $J = 7$ Hz), 3.24 (m, 6H), 1.66–2.07 (m, 6H), 1.41 (m, 18H), ^{13}C NMR ($CDCl_3$, 75 MHz) δ 163.0, 156.1 (2C), 151.2, 148.8, 148.1, 147.1, 144.0, 143.3, 137.6, 127.2, 125.6, 124.9, 122.8, 114.4, 110.3, 107.3, 102.6, 100.7, 79.9 (2C), 51.1, 47.4, 46.9, 39.7, 28.8, 28.6, 28.5 (7C), 26.6, 26.4.

*4.1.5. Synthesis of 2,3-methylenedioxy-8-nitro-5-[N-[aminoalkyl]-ethane-1,2-diamine]-5H-dibenzo[c,h][1,6]naphthyridin-6-ones trihydrochlorides (**7a**–**e**)*

Boc deprotection of **6** was attained by dropwise addition of trifluoroacetic acid (TFA) (1.2 mL, 16.2 mmol) at 0 °C to a solution of **6** (0.15 mmol) in distilled CH_2Cl_2 (5 mL). The mixture was stirred at room temperature under nitrogen for 1 h. The solvents were then removed by rotary evaporation. The crude product was purified using column chromatography (SiO_2, CH_2Cl_2/MeOH/NH_4OH, 90/15/3). The instable product was diluted with dry dichloromethane (2 mL) and treated with an anhydrous 1 N hydrochloric acid–ether solution (2 mL). The solution was concentrated under *vacuo* started again with dry ether (3 mL). The suspension was stirred overnight and filtered. **7a**: Yield: 49%. mp 223–225 °C. ^{1}H NMR (DMSO, 300 MHz) δ 9.94 (s, 1H), 9.62 (se, 2H), 9.00 (m, 2H), 8.67 (d, 1H, $J = 8$ Hz), 9.37 (se, 3H), 7.78 (s, 1H), 7.75 (s, 1H), 6.41 (s, 2H), 5.00 (t, 2H, $J = 7$ Hz), 3.44 (m, 2H), 3.13 (m, 4H), ^{13}C NMR (DMSO, 75 MHz) δ 162.4, 152.3, 148.6, 147.1, 145.1, 141.1, 140.4, 137.1, 127.6, 125.1, 125.0, 123.2, 114.5, 111.6, 103.6, 102.0, 101.8, 49.9, 46.9, 44.2, 35.1. HR-MS (ESI) m/z = 422.1450 $[M + H]^+$ 422.1459 calcd for $C_{21}H_{20}N_5O_5$. Anal. Calc. for $C_{21}H_{19}N_5O_5 \cdot 3HCl \cdot 2H_2O$ requires: C, 44.50; H, 4.62; N, 12.36. Found: C, 46.30; H, 4.72; N, 12.17. **7b**: Yield: 80%. mp 240–242 °C. ^{1}H NMR (D_2O, 300 MHz) δ 9.30 (s, 1H), 8.87 (s, 1H), 8.54 (d, 1H, $J = 8$ Hz), 8.38 (d, 1H, $J = 8$ Hz), 7.38 (s, 1H), 7.24 (s, 1H), 6.33 (s, 2H), 4.43 (m, 2H), 3.24–3.46 (m, 6H), 2.44 (m, 2H), ^{13}C NMR (DMSO, 75 MHz) δ 162.2, 152.8, 149.0, 147.2, 145.9, 140.3, 139.3, 136.4, 127.6, 125.2, 124.8, 123.1, 114.5, 110.9, 104.0, 101.6, 99.6, 48.3, 44.4, 44.0, 35.2, 24.8. HR-MS (ESI) m/z = 436.1608 $[M + H]^+$ 436.1616 calcd for $C_{22}H_{22}N_5O_5$. Anal. Calc. for $C_{22}H_{21}N_5O_5 \cdot 3HCl \cdot OEt_2 \cdot 2H_2O$ requires: C, 47.68; H, 5.85; N, 10.69. Found: C, 48.96; H, 6.09; N, 10.21. **7c**: Yield: 85%. mp 259–261 °C. ^{1}H NMR (D_2O, 300 MHz) δ 9.44 (s, 1H), 8.92 (s, 1H), 8.60 (d, 1H, $J = 8$ Hz), 8.43 (d, 1H, $J = 8$ Hz), 7.53 (s, 1H), 7.37 (s, 1H), 6.38 (s, 2H), 4.42 (t, 2H, $J = 7$ Hz), 3.42 (m, 4H), 3.20 (t, 2H, $J = 7$ Hz), 2.08 (m, 2H), 1.78 (m, 2H), ^{13}C NMR (D_2O, 75 MHz) δ 163.6, 153.9, 149.7, 147.4, 146.7, 139.7, 138.6, 135.8, 128.2, 124.5, 123.9, 123.7, 114.8, 110.8, 104.5, 101.3, 99.8, 51.0, 47.4, 44.0, 35.3, 25.0, 22.9. HR-MS (ESI) m/z = 450.1752 $[M + H]^+$ 450.1772 calcd for $C_{23}H_{24}N_5O_5$. Anal. Calc. for $C_{23}H_{23}N_5O_5 \cdot 3HCl \cdot H_2O$ requires: C, 47.89; H, 4.89; N, 12.14. Found: C, 47.74; H, 4.73; N, 11.98. **7d:** Yield: 50%. mp 208–210 °C. ^{1}H NMR (D_2O, 300 MHz) δ 9.46 (s, 1H), 8.92 (s, 1H), 8.58 (d, 1H, $J = 8$ Hz), 8.43 (d, 1H, $J = 8$ Hz), 7.55 (s, 1H), 7.39 (s, 1H), 6.37 (s, 2H), 4.38 (t, 2H, $J = 7$ Hz), 3.38 (m, 4H), 3.11 (t, 2H, $J = 7$ Hz), 2.00 (m, 2H), 1.73 (m, 2H), 1.42 (m, 2H). ^{13}C NMR (D_2O, 75 MHz) δ 163.6, 154.1, 149.8, 147.5, 147.2, 139.4, 139.1, 135.6, 128.2, 124.5, 124.0, 123.9, 114.9, 110.7, 104.6, 101.5, 99.4, 51.6, 47.8, 44.0, 35.3, 27.3, 25.0, 22.8. HR-MS (ESI) m/z = 469.1919 $[M + H]^+$ 469.1929 calcd for $C_{24}H_{26}N_5O_5$. Anal. Calc. for $C_{24}H_{25}N_5O_5 \cdot 3HCl \cdot 2H_2O$ requires: C, 47.37; H, 5.30; N, 11.50. Found: C, 47.80; H, 5.15; N, 11.21. **7e**: Yield: 64%. mp 241–243 °C. ^{1}H NMR (D_2O, 300 MHz) δ 9.35 (s, 1H), 8.83 (s, 1H), 8.57 (d, 1H, $J = 9$ Hz),

8.38 (d, 1H, $J = 9$ Hz), 7.43 (s, 1H), 7.34 (s, 1H), 6.42 (s, 2H), 4.28 (t, 2H, $J = 7$ Hz), 3.47 (m, 4H), 3.18 (t, 2H, $J = 7$ Hz), 1.96 (m, 2H), 1.77 (m, 2H), 1.43 (m, 4H), ^{13}C NMR (DMSO, 75 MHz) δ 162.3, 152.2, 148.5, 147.0, 145.0, 143.9, 141.9, 136.7, 127.6, 124.9, 124.8, 123.1, 114.2, 110.5, 103.9, 102.8, 101.2, 50.9, 46.8, 44.1, 35.5, 25.7, 25.6, 25.5, 25.3 HR-MS (ESI) $m/z = 478.2063$ $[M + H]^+$ 478.2085 calcd for $C_{25}H_{28}N_5O_5$. Anal. Calc. for $C_{25}H_{27}N_5O_5 \cdot 3HCl \cdot H_2O$ requires: C, 49.64; H, 5.33; N, 11.58. Found: C, 50.50; H, 5.14; N, 11.21.

4.1.6. Synthesis of (2,3-methylenedioxy-8-nitro-5-[N-[aminoalkyl]-ethane-1,2-diamine]-5H-dibenzo[c,h][1,6]naphthyridin-6-ones) dichloroplatinums (II) (**8a**–**e**)

To a solution of K_2PtCl_4 (84 mg, 0.20 mmol) in water (4 mL), the trihydrochloride **7** (0.20 mmol) was added. The pH was adjusted to 8 with 2 N 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 **8**. **8a**: Yield 76%. mp >260 °C. IR: 1653 (CO), 1457 (NO_2), 1336 (NO_2) cm^{-1}. ^{1}H NMR (DMSO, 300 MHz) δ 9.69 (s, 1H), 9.00 (s, 1H), 8.96 (m, 1H), 8.62 (m, 1H), 7.70 (s, 1H), 7.50 (s, 1H), 6.31 (s, 2H), 4.55 (m, 2H), 2.50–3.33 (m, 6H). HR-MS (ESI) $m/z = 709.0274$ $[M + Na]^+$ 709.0305 calcd for $C_{21}H_{19}Cl_2N_5NaO_5Pt$. Anal. Calc. for $C_{21}H_{19}Cl_2N_5O_5Pt \cdot CH_3COCH_3$ requires: C, 38.67; H, 3.38; N, 9.39. Found: C, 38.16; H, 3.48; N, 9.20. **8b**: Yield 67%. mp >260 °C. IR: 1655 (CO), 1458 (NO_2), 1336 (NO_2) cm^{-1}. ^{1}H NMR (DMSO, 300 MHz) δ 9.67 (s, 1H), 9.00 (s, 1H), 8.94 (m, 1H), 8.60 (m, 1H), 7.67 (s, 1H), 7.48 (s, 1H), 6.31 (s, 2H), 4.37 (m, 2H), 2.50–3.33 (m, 6H), 2.21 (m, 2H). HR-MS (ESI) $m/z = 723.0470$ $[M + Na]^+$ 723.0461 calcd for $C_{22}H_{21}Cl_2N_5NaO_5Pt$. Anal. Calc. for $C_{22}H_{21}Cl_2N_5O_5Pt \cdot 2\times\ CH_3COCH_3$ requires: C, 41.13; H, 4.07; N, 8.57. Found: C, 41.71; H, 3.93; N, 8.73. **8c**: Yield 66%. mp >260 °C. IR: 1656 (CO), 1458 (NO_2), 1337 (NO_2) cm^{-1}. ^{1}H NMR (DMSO, 300 MHz) δ 9.63 (s, 1H), 8.97 (s, 1H), 8.94 (m, 1H), 8.58 (m, 1H), 7.63 (s, 1H), 7.45 (s, 1H), 6.31 (s, 2H), 4.48 (m, 2H), 2.50–3.33 (m, 6H), 1.84 (m, 4H). HR-MS (ESI) $m/z = 737.0637$ $[M + Na]^+$ 737.0618 calcd for $C_{23}H_{23}Cl_2N_5NaO_5Pt$. Anal. Calc. for $C_{23}H_{23}Cl_2N_5O_5Pt \cdot H_2O$ requires: C, 37.66; H, 3.44; N, 9.55. Found: C, 37.66; H, 3.27; N, 9.23. **8d**: Yield 43%. mp >260 °C. IR: 1654 (CO), 1458 (NO_2), 1337 (NO_2) cm^{-1}. ^{1}H NMR (DMSO, 300 MHz) δ 9.69 (s, 1H), 9.01 (s, 1H), 8.97 (m, 1H), 8.63 (m, 1H), 7.67 (s, 1H), 7.49 (s, 1H), 6.31 (s, 2H), 4.48 (m, 2H), 2.50–3.33 (m, 6H), 1.90 (m, 4H), 1.55 (m, 2H). HR-MS (ESI) $m/z = 751.0810$ $[M + Na]^+$ 751.0774 calcd for $C_{24}H_{25}Cl_2N_5NaO_5Pt$. Anal. Calc. for $C_{24}H_{25}Cl_2N_5O_5Pt$ requires: C, 39.52; H, 3.45; N, 9.60. Found: C, 41.81; H, 3.41; N, 9.21. **8e**: Yield 69%. mp >260 °C. IR: 1660 (CO), 1457 (NO_2), 1335 (NO_2) cm^{-1}. ^{1}H NMR (DMSO, 300 MHz) δ 9.69 (s, 1H), 9.01 (s, 1H), 8.96 (m, 1H), 8.62 (m, 1H), 7.68 (s, 1H), 7.50 (s, 1H), 6.32 (s, 2H), 4.47 (m, 2H), 2.50–3.33 (m, 6H), 1.91 (m, 2H), 1.76 (m, 2H), 1.48 (m, 4H). HR-MS (ESI) $m/z = 765.0947$ $[M + Na]^+$ 765.0931 calcd for $C_{25}H_{27}Cl_2N_5NaO_5Pt$. Anal. Calc. for $C_{25}H_{27}Cl_2N_5O_5Pt \cdot H_2O$ requires: C, 39.43; H, 3.84; N, 9.20. Found: C, 39.44; H, 3.73; N, 8.88.

4.2. Cell lines, growth and cytotoxic assays

The three human colorectal cancer cell lines HCT 116, SW480 and HT-29 were obtained from the American Type Culture Collections (Manassas, VA, United States). They were cultured in RPMI 1640 medium (Biowhittaker, France) supplemented with 10% fetal bovine serum (Biowhittaker, France) in a 5% CO_2 atmosphere. Experiments were carried out on exponentially growing monolayer cells which were mycoplasma free. The absence of mycoplasma was checked by polymerase chain reaction (PCR) analysis (PCR Mycoplasma Test Kit I/C, PromoKine, PromoCell France).

For cytotoxic assays, molecules were prepared as followed: the substituted 5*H*-dibenzo[*c*,*h*][1,6]naphthyridin-6-one derivatives containing a polymethylene spacer in position −5 (**7a**–**e**), cisplatin (Sigma–Aldrich, France), and oxaliplatin (Sanofi-Aventis, France) were diluted in sterile physiological serum (Aguettant Co., France). The 5*H*-dibenzo[*c*,*h*][1,6]naphthyridin-6-one-based tethered [*N*-(aminoalkyl)-(ethane-1,2-diamine)]Pt(II) complexes connected by a polymethylene chain with $n = 2$ to 6 (**8a**–**e**) were diluted into dimethylformamide (DMF) (Sigma, France), that's why the vehicle DMF was also tested alone. However, in any case, the maximal concentration of DMF in cell culture medium did not exceed 3%.

Cytotoxic assays were conducted on HCT 116, SW480 and HT-29 in 96-well plates. Subconfluent cells were treated for 72 h by increasing concentrations from 0 to 500 μM of the newly synthetized or reference molecules. After treatment, cytotoxicity was assessed by crystal violet staining. Cells were washed in Phosphate Buffered Saline (PBS) 1×, fixed in pure ethanol, stained with 1% crystal violet, and eluted in 33% acetic acid. The intensity of coloration was determined by the measurement of absorbance by spectrophotometry (UVM 340, Bioserv) at $\lambda = 570$ 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. The 50% Inhibitory Concentration (IC_{50}), representing the concentration which inhibits 50% of cell growth, was calculated for each compound.

Statistical analyses were performed to examine the significance of differences in IC_{50} values between newly synthetized derivatives and reference Pt drugs (cisplatin and oxaliplatin) using the Mann–Whitney test. Moreover, in each cell line, comparison of IC_{50} according to the length of the polymethylene linker was assessed using Kruskal–Wallis rank sum test. Differences were considered statistically significant when p-value was <0.05.

Acknowledgments

The Nievre committee of the French National League against cancer supported this work. We are really thankful to Marie-José Penouilh for the HR-MS mass spectra and Marcel Soustelle for elemental analyses at the "*Welience, Pôle Chimie Moléculaire de l'Université de Bourgogne* (*WPCM*)".

Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.ejmech.2013.09.037.

References

[1] A.M. Florea, D. Busselberg, Metals and breast cancer: risk factors or healing agents? J. Toxicol. 2011 (2011) 159619.
[2] I. Kostova, Platinum complexes as anticancer agents, Recent Pat. Anticancer Drug Discov. 1 (2006) 1–22.
[3] D. Lebwohl, R. Canetta, Clinical development of platinum complexes in cancer therapy: an historical perspective and an update, Eur. J. Cancer 34 (1998) 1522–1534.
[4] B. Desoize, C. Madoulet, Particular aspects of platinum compounds used at present in cancer treatment, Crit. Rev. Oncol. Hematol. 42 (2002) 317–325.
[5] R.J. Cersosimo, Management of advanced colorectal cancer, part 1, Am. J. Health Syst. Pharm. 70 (2013) 395–406.
[6] J.J. Marin, F. Sanchez de Medina, B. Castano, L. Bujanda, M.R. Romero, O. Martinez-Augustin, R.D. Moral-Avila, O. Briz, Chemoprevention, chemotherapy, and chemoresistance in colorectal cancer, Drug Metab. Rev. 44 (2012) 148–172.
[7] M.D. Hall, M. Okabe, D.W. Shen, X.J. Liang, M.M. Gottesman, The role of cellular accumulation in determining sensitivity to platinum-based chemotherapy, Annu. Rev. Pharmacol. Toxicol. 48 (2008) 495–535.
[8] L. Kelland, The resurgence of platinum-based cancer chemotherapy, Nat. Rev. Cancer 7 (2007) 573–584.
[9] D. Wang, S.J. Lippard, Cellular processing of platinum anticancer drugs, Nat. Rev. Drug Discov. 4 (2005) 307–320.

[10] Y.P. Ho, S.C. Au-Yeung, K.K. To, Platinum-based anticancer agents: innovative design strategies and biological perspectives, Med. Res. Rev. 23 (2003) 633–655.
[11] B. Chauffert, M. Correia, C. Sergent, Update on mechanisms of drug resistance, Bull. Cancer 86 (1999) 97–103.
[12] P. Heffeter, U. Jungwirth, M. Jakupec, C. Hartinger, M. Galanski, L. Elbling, M. Micksche, B. Keppler, W. Berger, Resistance against novel anticancer metal compounds: differences and similarities, Drug Resist. Updates 11 (2008) 1–16.
[13] D.J. Stewart, Mechanisms of resistance to cisplatin and carboplatin, Crit. Rev. Oncol. Hematol. 63 (2007) 12–31.
[14] N. Margiotta, N. Denora, R. Ostuni, V. Laquintana, A. Anderson, S.W. Johnson, G. Trapani, G. Natile, Platinum(II) complexes with bioactive carrier ligands having high affinity for the translocator protein, J. Med. Chem. 53 (2010) 5144–5154.
[15] F. Bouyer, J. Moretto, D. Pertuit, A. Szollosi, M.A. Lacaille-Dubois, Y. Blache, B. Chauffert, N. Desbois, Synthesis, cytotoxicity and structure-activity relationships between ester and amide functionalities in novel acridine-based platinum(II) complexes, J. Inorg. Biochem. 110 (2012) 51–57.
[16] M. Carland, M.J. Grannas, M.J. Cairns, V.J. Roknic, W.A. Denny, W.D. McFadyen, V. Murray, Substituted 9-aminoacridine-4-carboxamides tethered to platinum(II)diamine complexes: chemistry, cytotoxicity and DNA sequence selectivity, J. Inorg. Biochem. 104 (2010) 815–819.
[17] D. Gibson, K.F. Gean, R. Ben-Shoshan, A. Ramu, I. Ringel, J. Katzhendler, Preparation, characterization, and anticancer activity of a series of cis-PtCl2 complexes linked to anthraquinone intercalators, J. Med. Chem. 34 (1991) 414–420.
[18] J.M. Perez, I. Lopez-Solera, E.I. Montero, M.F. Brana, C. Alonso, S.P. Robinson, C. Navarro-Ranninger, Combined effect of platination and intercalation upon DNA binding of novel cytotoxic Pt-bis(naphthalimide) complexes, J. Med. Chem. 42 (1999) 5482–5486.
[19] R. Cincinelli, L. Musso, S. Dallavalle, R. Artali, S. Tinelli, D. Colangelo, F. Zunino, M. De Cesare, G.L. Beretta, N. Zaffaroni, Design, modeling, synthesis and biological activity evaluation of camptothecin-linked platinum anticancer agents, Eur. J. Med. Chem. 63C (2013) 387–400.
[20] S.J. Brill, S. DiNardo, K. Voelkel-Meiman, R. Sternglanz, Need for DNA topoisomerase activity as a swivel for DNA replication for transcription of ribosomal RNA, Nature 326 (1987) 414–416.
[21] K.C. Neuman, Single-molecule measurements of DNA topology and topoisomerases, J. Biol. Chem. 285 (2010) 18967–18971.
[22] K. Legarza, L.X. Yang, New molecular mechanisms of action of camptothecin-type drugs, Anticancer Res. 26 (2006) 3301–3305.
[23] Y. Xu, M.A. Villalona-Calero, Irinotecan: mechanisms of tumor resistance and novel strategies for modulating its activity, Ann. Oncol. 13 (2002) 1841–1851.
[24] Y. Pommier, Topoisomerase I inhibitors: camptothecins and beyond, Nat. Rev. Cancer 6 (2006) 789–802.
[25] S. Basili, S. Moro, Novel camptothecin derivatives as topoisomerase I inhibitors, Expert Opin. Ther. Pat. 19 (2009) 555–574.
[26] G.L. Beretta, V. Zuco, P. Perego, N. Zaffaroni, Targeting DNA topoisomerase I with non-camptothecin poisons, Curr. Med. Chem. 19 (2012) 1238–1257.
[27] C. Sheng, Z. Miao, W. Zhang, New strategies in the discovery of novel non-camptothecin topoisomerase I inhibitors, Curr. Med. Chem. 18 (2011) 4389–4409.
[28] Y. Pommier, DNA topoisomerase I inhibitors: chemistry, biology, and interfacial inhibition, Chem. Rev. 109 (2009) 2894–2902.
[29] Y. Yamashita, N. Fujii, C. Murakata, T. Ashizawa, M. Okabe, H. Nakano, Induction of mammalian DNA topoisomerase I mediated DNA cleavage by antitumor indolocarbazole derivatives, Biochemistry 31 (1992) 12069–12075.
[30] N. Vicker, L. Burgess, I.S. Chuckowree, R. Dodd, A.J. Folkes, D.J. Hardick, T.C. Hancox, W. Miller, J. Milton, S. Sohal, S. Wang, S.P. Wren, P.A. Charlton, W. Dangerfield, C. Liddle, P. Mistry, A.J. Stewart, W.A. Denny, Novel angular benzophenazines: dual topoisomerase I and topoisomerase II inhibitors as potential anticancer agents, J. Med. Chem. 45 (2002) 721–739.
[31] D. Makhey, C. Yu, A. Liu, L.F. Liu, E.J. LaVoie, Substituted benz[*a*]acridines and benz[*c*]acridines as mammalian topoisomerase poisons, Bioorg. Med. Chem. 8 (2000) 1171–1182.
[32] Y.L. Janin, A. Croisy, J.F. Riou, E. Bisagni, Synthesis and evaluation of new 6-amino-substituted benzo[*c*]phenanthridine derivatives, J. Med. Chem. 36 (1993) 3686–3692.
[33] A.L. Ruchelman, S.K. Singh, A. Ray, X.H. Wu, J.M. Yang, T.K. Li, A. Liu, L.F. Liu, E.J. LaVoie, 5*H*-Dibenzo[*c*,*h*]1,6-naphthyridin-6-ones: novel topoisomerase I-targeting anticancer agents with potent cytotoxic activity, Bioorg. Med. Chem. 11 (2003) 2061–2073.
[34] S.K. Singh, A.L. Ruchelman, T.K. Li, A. Liu, L.F. Liu, E.J. LaVoie, Nitro and amino substitution in the D-ring of 5-(2-dimethylaminoethyl)-2,3-methylenedioxy-5*H*-dibenzo[*c*,*h*][1,6]naphthyridin-6-ones: effect on topoisomerase-I targeting activity and cytotoxicity, J. Med. Chem. 46 (2003) 2254–2257.
[35] A.L. Ruchelman, P.J. Houghton, N. Zhou, A. Liu, L.F. Liu, E.J. LaVoie, 5-(2-Aminoethyl)dibenzo[*c*,*h*][1,6]naphthyridin-6-ones: variation of *n*-alkyl substituents modulates sensitivity to efflux transporters associated with multidrug resistance, J. Med. Chem. 48 (2005) 792–804.
[36] L. Sharma, Y.C. Tsai, A.A. Liu, L.F. Liu, E.J. LaVoie, Cytotoxicity and TOP1-targeting activity of 8- and 9-amino derivatives of 5-butyl- and 5-(2-*N*,*N*-dimethylamino)ethyl-5*H*-dibenzo[*c*,*h*][1,6]naphthyridin-6-ones, Eur. J. Med. Chem. 44 (2009) 1471–1476.
[37] T. Sunami, K. Nishio, F. Kanzawa, K. Fukuoka, S. Kudoh, J. Yoshikawa, N. Saijo, Combination effects of TAS-103, a novel dual topoisomerase I and II inhibitor, with other anticancer agents on human small cell lung cancer cells, Cancer Chemother. Pharmacol. 43 (1999) 394–401.
[38] E. Wasserman, W. Sutherland, E. Cvitkovic, Irinotecan plus oxaliplatin: a promising combination for advanced colorectal cancer, Clin. Colorectal Cancer 1 (2001) 149–153.
[39] C. Plasencia, A. Abad, E. Martinez-Balibrea, M. Taron, Antiproliferative effects of ZD0473 (AMD473) in combination with 5-fluorouracil or SN38 in human colorectal cancer cell lines, Invest. New Drugs 22 (2004) 399–409.
[40] R.C. van Waardenburg, L.A. de Jong, M.A. van Eijndhoven, C. Verseyden, D. Pluim, L.E. Jansen, M.A. Bjornsti, J.H. Schellens, Platinated DNA adducts enhance poisoning of DNA topoisomerase I by camptothecin, J. Biol. Chem. 279 (2004) 54502–54509.
[41] R.J. Holmes, M.J. McKeage, V. Murray, W.A. Denny, W.D. McFadyen, cis-Dichloroplatinum(II) complexes tethered to 9-aminoacridine-4-carboxamides: synthesis and action in resistant cell lines in vitro, J. Inorg. Biochem. 85 (2001) 209–217.
[42] H.H. Lee, B.D. Palmer, B.C. Baguley, M. Chin, W.D. McFadyen, G. Wickham, D. Thorsbourne-Palmer, L.P. Wakelin, W.A. Denny, DNA-directed alkylating agents. 5. Acridinecarboxamide derivatives of (1,2-diaminoethane)dichloroplatinum(II), J. Med. Chem. 35 (1992) 2983–2987.
[43] A.L. Ruchelman, S.K. Singh, X. Wu, A. Ray, J.M. Yang, T.K. Li, A. Liu, L.F. Liu, E.J. LaVoie, Diaza- and triazachrysenes: potent topoisomerase-targeting agents with exceptional antitumor activity against the human tumor xenograft, MDA-MB-435, Bioorg. Med. Chem. Lett. 12 (2002) 3333–3336.
[44] A.L. Ruchelman, J.E. Kerrigan, T.K. Li, N. Zhou, A. Liu, L.F. Liu, E.J. LaVoie, Nitro and amino substitution within the A-ring of 5*H*-8,9-dimethoxy-5-(2-*N*,*N*-dimethylaminoethyl)dibenzo[*c*,*h*][1,6]naphthyridin-6-ones: influence on topoisomerase I-targeting activity and cytotoxicity, Bioorg. Med. Chem. 12 (2004) 3731–3742.
[45] A.L. Ruchelman, S. Zhu, N. Zhou, A. Liu, L.F. Liu, E.J. LaVoie, Dimethoxy benzo[*i*]phenanthridine-12-carboxylic acid derivatives and 6*H*-dibenzo[*c*,*h*][2,6]naphthyridin-5-ones with potent topoisomerase I-targeting activity and cytotoxicity, Bioorg. Med. Chem. Lett. 14 (2004) 5585–5589.
[46] W. Feng, M. Satyanarayana, L. Cheng, A. Liu, Y.C. Tsai, L.F. Liu, E.J. LaVoie, Synthesis of *N*-substituted 5-[2-(*N*-alkylamino)ethyl]dibenzo[*c*,*h*][1,6]naphthyridines as novel topoisomerase I-targeting antitumor agents, Bioorg. Med. Chem. 16 (2008) 9295–9301.
[47] L.S. Kurtzberg, S. Roth, R. Krumbholz, J. Crawford, C. Bormann, S. Dunham, M. Yao, C. Rouleau, R.G. Bagley, X.J. Yu, F. Wang, S.M. Schmid, E.J. Lavoie, B.A. Teicher, Genz-644282, a novel non-camptothecin topoisomerase I inhibitor for cancer treatment, Clin. Cancer Res. 17 (2011) 2777–2787.
[48] T.K. Li, P.J. Houghton, S.D. Desai, P. Daroui, A.A. Liu, E.S. Hars, A.L. Ruchelman, E.J. LaVoie, L.F. Liu, Characterization of ARC-111 as a novel topoisomerase I-targeting anticancer drug, Cancer Res. 63 (2003) 8400–8407.
[49] W. Feng, M. Satyanarayana, Y.C. Tsai, A.A. Liu, L.F. Liu, E.J. Lavoie, Facile formation of hydrophilic derivatives of 5*H*-8,9-dimethoxy-5-[2-(*N*,*N*-dimethylamino)ethyl]-2,3-methylenedioxydibenzo[*c*,*h*][1,6]naphthyridin-6-one (ARC-111) and its 12-aza analog via quaternary ammonium intermediates, Bioorg. Med. Chem. Lett. 18 (2008) 3570–3572.
[50] W. Feng, M. Satyanarayana, Y.C. Tsai, A.A. Liu, L.F. Liu, E.J. LaVoie, Novel topoisomerase I-targeting antitumor agents synthesized from the *N*,*N*,*N*-trimethylammonium derivative of ARC-111, 5*H*-2,3-dimethoxy-8,9-methylenedioxy-5-[(2-*N*,*N*,*N*-trimethylammonium)ethyl]dibenzo[*c*,*h*][1,6]naphthyridin-6-one iodide, Eur. J. Med. Chem. 44 (2009) 3433–3438.

List of captions

BCRP: Breast Cancer Resistance Protein
DMF: dimethylformamide
DMSO: dimethylsulfoxide
ESI: electrospray ionization
HR-MS: high resolution-mass spectrometry
IC_{50}: 50% inhibitory concentration
MDR1: multi drug resistance 1
mp: melting point
NMR: nuclear magnetic resonance
PBS: phosphate buffered saline
PCR: polymerase chain reaction
Pt: platinum
Scid: severe combined immuno deficiency
TFA: trifluoroacetic acid