Available online at www.sciencedirect.com
ScienceDirect
European Journal of Medicinal Chemistry 43 (2008) 2505–2517
EUROPEAN JOURNAL OF MEDICINAL CHEMISTRY
http://www.elsevier.com/locate/ejmech

Original article

Novel imidazo[1,2-*a*]naphthyridinic systems (part 1): Synthesis, antiproliferative and DNA-intercalating activities

Mounir Andaloussi [a], Emmanuel Moreau [a,*], Nicolas Masurier [a], Jacques Lacroix [b], René C. Gaudreault [b], Jean-Michel Chezal [a], Anas El Laghdach [c], Damien Canitrot [a], Eric Debiton [a], Jean-Claude Teulade [a], Olivier Chavignon [a,**]

[a] *Univ Clermont 1, UFR Pharmacie, Clermont-Ferrand F-63001, France*
[b] *Unité des Biotechnologies et de Bioingénierie, Centre de recherche, C.H.U.Q., Hôpital Saint-François d'Assise, Université Laval, Québec G1L 3L5, Canada*
[c] *Department of Chemistry, Faculty of Sciences of Tetouan, M'Hannech II, BP 2121 Tétouan, Morocco*

Received 13 September 2007; received in revised form 18 January 2008; accepted 8 February 2008
Available online 29 February 2008

Abstract

Novel imidazo[1,2-*a*]naphthyridinic systems **6a**−**15a** and **6b**−**15b** were obtained from Friedländer's reaction in imidazo[1,2-*a*]pyridine series. Most of the compounds were evaluated for their antitumor activity in the NCIs in vitro human tumor cell line screening panel. Among them, pentacyclic derivatives **13b** and **14a** exhibited in vitro activity comparable to anticancer agent such as amsacrine. Their mechanism of cytotoxicity action was unrelated to poisoning or inhibiting abilities against topo1. On the contrary, we highlighted a direct intercalation of the drugs into DNA by electrophoresis on agarose gel.

Keywords: Imidazo[1,2-*a*]pyridine; Imidazonaphthyridine; Imidazonaphthonaphthyridine; Benzoimidazonaphthyridine; Friedländer's reaction; DNA-intercalator

1. Introduction

The discovery and development of novel therapeutic agents for the treatment of malignancy are one of the most important goals in medicinal chemistry. One interesting group of chemotherapeutic agents used in cancer therapy comprises molecules that target directly DNA, mediated through their interaction with topoisomerase enzymes. During the past decades, numerous studies have demonstrated DNA recognizing molecules that act as anticancer agents, including three major classes such as DNA-alkylating drugs (e.g., nitrogen mustards [1], cisplatin [2], nitrosoureas [3]), topoisomerase inhibitors (e.g., F11782 [4], irinotecan [5], or topotecan [6]) and DNA-intercalators (e.g., anthracyclines [7], anthraquinones [8], or acridine [9]). Anthracyclines' [7] derivatives and amsacrine [10] are now being used as topo2 inhibitor for treatment in a wide range of tumors. Campthotecin [11] is known as a potent pentacyclic topo1 inhibitor. Even though they are known utility in clinical oncology, these data have been exploited by medicinal chemists to create new categories of DNA-targeted and/or topoisomerase-targeted anticancer drugs. NCS314622 [12] was the first indenoisoquinoline [13−15] compound found to have antitumor activity, and subsequent studies have shown that a large number of indenoisoquinoline derivatives are topo1 poisons. The natural product BE-13793c [16] was the first indolocarbazole compound reported to poison topo1, and there are at least two indolocarbazoles NB506 [17] and J10788 [18] currently undergoing clinical trials.

This background about DNA-targeted and/or topoisomerase-targeted compounds led us to speculation about whether chromophores such as pyridine or quinoline connected to

* Corresponding author. Tel.: +33 4 73 17 80 13; fax: +33 4 73 17 80 12.
** Corresponding author. Tel.: +33 4 73 17 80 09; fax: +33 4 73 17 80 12.
E-mail addresses: emmanuel.moreau@u-clermont1.fr (E. Moreau), olivier.chavignon@u-clermont1.fr (O. Chavignon).

0223-5234/$ - see front matter © 2008 Elsevier Masson SAS. All rights reserved.
doi:10.1016/j.ejmech.2008.02.017

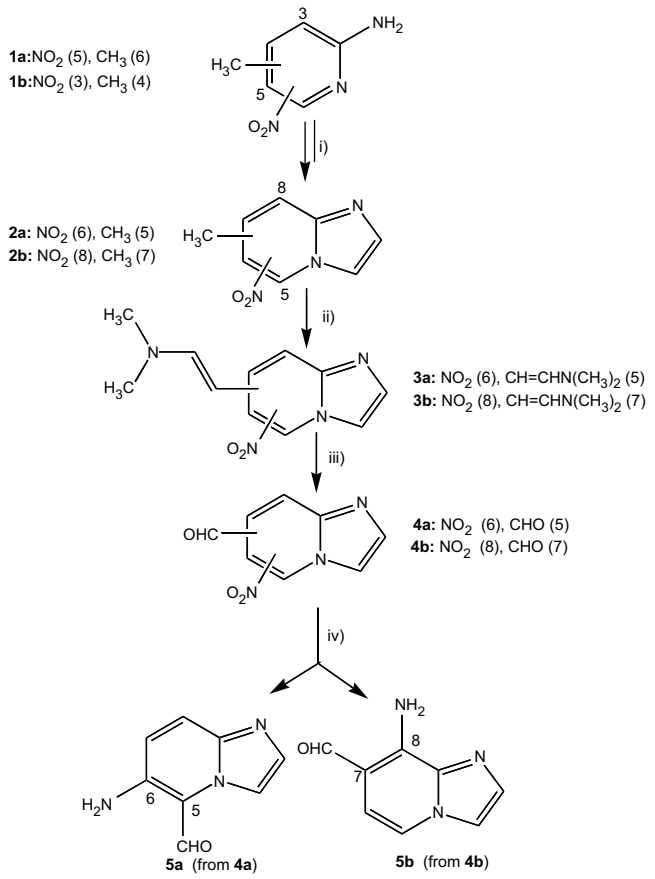
imidazo[1,2-*a*]pyridine (IP) [19–24] scaffold could be able to intercalate with DNA and/or to interact with topoisomerase enzymes. So, we report here our efficient synthesis and biological study that look at a panel of 20 IP derivatives including tricyclic, tetracyclic and pentacyclic systems.

2. Results and discussion

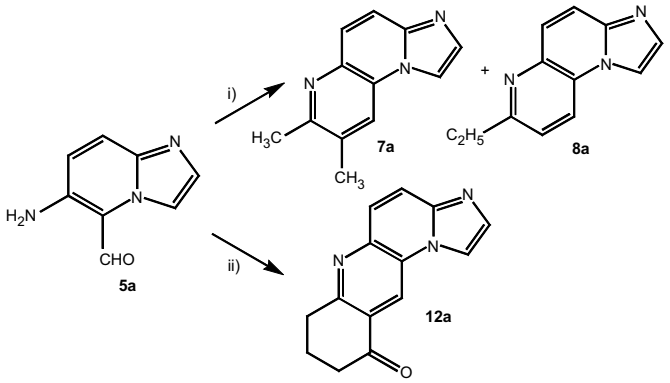
2.1. Chemistry

The preparation of the imidazonaphthyridinic system in IP series was achieved via a Friedländer's reaction [25] occurring between the *o*-aminated aldehydes **5**(**a**,**b**) and ketones such as acetone, butanone, acetophenone, cyclohexanone, ethyl 4-oxocyclohexane carboxylate, 1,3-cyclohexandione, α- or β-tetralone.

The synthesis of compounds **5**(**a**,**b**) is described in Scheme 1. For example, compound **5a** was prepared from 2-amino-6-methyl-5-nitropyridine **1a** and chloroacetaldehyde in anhydrous ethanol. The resulting imidazopyridine **2a** [26] was condensed with *N*,*N*-dimethylformamide dimethyl acetal in anhydrous DMF [27] to give the enamine **3a**, characterized as the *E*-isomer ($J = 13.5$ Hz). The conversion of the enamine intermediate **3a** into to the corresponding aldehyde **4a** was performed using $NaIO_4$, leading to the *o*-nitro aldehyde **4a** in 51% yield. The reduction of **4a** using Fe/HCl in a mixture EtOH/H_2O (10:1) [28] afforded the *o*-aminated aldehyde **5a** in 71% yield.



Scheme 1. Access to *o*-aminated aldehydes **5**(**a**,**b**). Reagents and conditions: (i) $ClCH_2CHO$, EtOH, Δ; (ii) DMF/DMA, DMF, Δ; (iii) $NaIO_4$, THF, H_2O, rt; (iv) Fe/HCl (12 N), EtOH, H_2O, Δ.



Scheme 2. A representative synthesis of imidazopyridine derivatives **7a**, **8a** and **12a** from **5a** according to modified Friedländer's reaction. Reagents and conditions: (i) EtOH/KOH 10%, Δ (method A) and butanone; (ii) AcOH, Δ (method B) and 1,3-cyclohexadione.

Compounds **6a**–**15a** and **6b**–**15b** were prepared according to modified Friedländer's reaction, starting from 6-aminoIP-5-carbaldehyde **5a** or 8-aminoIP-7-carbaldehyde **5b**, respectively, and the ketones aforementioned.

For example, the preparation of imidazo[1,2-*a*][1,5]naphthyridines **6a**–**9a** was carried out using anhydrous EtOH/KOH 10% in presence of either acetone, butanone or acetophenone. While acetone and acetophenone afforded compounds **6a** [29] (65%) and **9a** (31%) imidazonaphthyridinic systems, respectively, butanone afforded the dimethylated derivative **7a** and the ethylated derivative **8a** in 22% and 5% yield, respectively.

This protocol is also effective for the synthesis of benzo[*g*]imidazo[1,2-*a*][1,5]naphthyridines from cyclohexanone and ethyl 4-oxocyclohexane to give **10a** (32%) and **11a** (21%), respectively. In contrast, treatment of **5a** with 1,3-cyclohexadione in alkali medium gave no trace of the desired tetracyclic system **12a**. After prolonged reaction time, unchanged starting material was recovered together with an unidentified material. Finally, compound **12a** might be prepared in 30% yield when the reaction took place in acetic acidic medium.

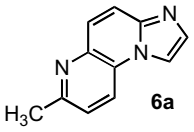
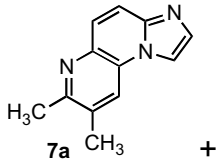
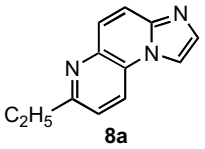
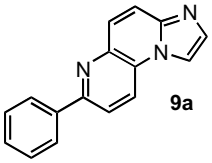
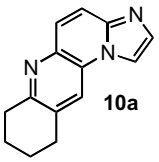
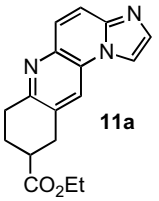
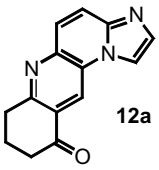
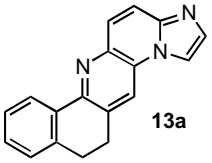
Imidazo[1,2-*a*]naphtho[1,5]naphthyridines **13a**–**15a** were prepared from α- or β-tetralone, respectively. Under alkaline conditions the α-tetralone gave the one-single dihydro compound **13a**, while the β-tetralone yielded compounds **14a** (40%) and **15a** (28%), which were easily isolated by column chromatography. It is of interest that the use of acidic conditions afforded only compound **14a** in 27% yield.

The preparation of compounds **6b**–**15b** was carried out using similar experimental procedures (see Scheme 2 and Section 4).

2.2. Tumor cell growth inhibition activity

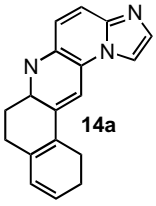
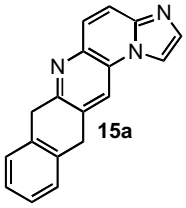
The tumor cell growth inhibition of angular **6a**–**9a**, **6b**–**9b** (tricyclic system), **10a**–**12a**, **10b**–**12b** (tetracyclic system), and **13a**, **14a**, **13b**–**15b** (pentacyclic system) was assessed

Table 1
Access to imidazopyridine derivatives from *o*-aminoaldehyde **5a** and ketones according to modified Friedländer's reaction: EtOH/KOH 10%, Δ (method A) or AcOH, Δ (method B)

Entry	Ketones	Products (from **5a**)	Time (h)	Method	Yield (%)
1	Acetone	 **6a**	1.5	A	65
2	Butanone	 **7a** +	2	A	22
		 **8a**	2	A	5
3	Acetophenone	 **9a**	1	A	31
4	Cyclohexanone	 **10a**	1	A	32
5	Ethyl 4-oxocyclohexane carboxylate	 **11a**	1	A	21
6	1,3-Cyclohexandione	 **12a**	1	B	30
7	α-Tetralone	 **13a**	1	A	26

(*continued on next page*)

Table 1 (*continued*)

Entry	Ketones	Products (from **5a**)	Time (h)	Method	Yield (%)
		 **14a** +	1	A	40
				B	27
8	β-Tetralone	 **15a**	1	A	28

on HT-1080 (fibrosarcoma), HT-29 (colon carcinoma), M-21 (skin melanoma) and MCF-7 (breast carcinoma) cells.

Imidazonaphthyridines **6a**–**8a** and **6b**–**8b** were inactive on tumor cell growth at the concentrations tested. Interestingly, compounds **9a** and **9b** that are substituted by a phenyl ring on the fused-pyridinic moiety have a significant impact on tumor cell proliferation in all cell lines tested ($GI_{50} \sim 50$ μM versus $GI_{50} > 100$ μM for compounds **6a**–**8a** and **6b**–**8b**). In contrast, the tetracyclic compounds **10a**–**12a**, **10b** and **12b** had no impact on tumor cell proliferation at the exception of compound **11b** having a GI_{50} ranging from 48.4 to 60.5 μM. In addition, the pentacyclic derivatives **13a**, **14a** and **13b**–**15b** exhibited the highest GI_{50} ranging from 2.2 to 64.8 μM (Tables 1 and 2). Interestingly, compound **14a** seems to exhibit some specificity towards breast-derived cells such as MCF-7 where it showed a GI_{50} at least fourfold higher than in any other tumor cell line. Data showed that compound **13b** exhibited GI_{50} ranging from 6.6 to 13.4 μM. In comparison, their geometric isomers **14b** and **13a** were less active suggesting that the geometric orientation of the fused tetralone-pyridinic moiety seems an important parameter to consider with respect to cell growth inhibition.

2.3. Electrophoresis gel assay

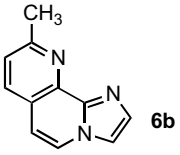
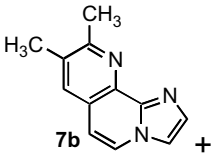
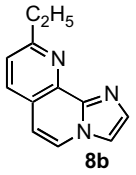
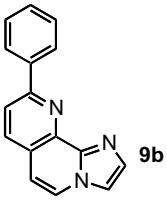
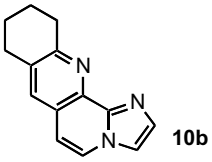
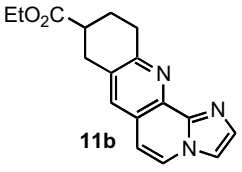
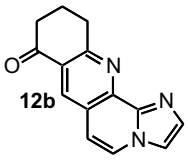
Compounds **13b** and **14a** that have exhibited significant tumor cell growth inhibition has been evaluated also for their DNA-targeting properties. These latter might be evaluated by different methods: DNA intercalation might be studied by NMR spectroscopy with a synthetic oligodeoxynucleotide [30]; poisoning, inhibition of topoisomerase or intercalation of DNA might be interpreted by agarose-gel electrophoresis [31–33]. Therefore, these methods minimize possible misinterpretation of data. For example, intercalation could be misinterpreted as inhibition, inhibition of relaxation activity might be misinterpreted as a lack of intercalation, and enzyme-independent nicking of the plasmid could be misinterpreted as poisoning. In this study, we have then chosen to use a recent method based on the alteration of the electrophoretic mobility of pGEM®-9Zf(−) DNA plasmid by the action of human topoisomerase 1 (topo1) [34]. This method offered the advantage of validating a screening assay for the detection of compounds with the ability to inhibit topo1 both by inhibition of relaxation activity and by empoisoning. The assay could be distinguished between both types of inhibition and, in most cases, clearly separated these phenomena from intercalation, thus helping to clarify the role of each in topo1 activity.

Fig. 1 shows the relationship between the migration distance of the plasmidic DNA and the concentration of the drug required to produce a maximal intercalation of the plasmids into DNA strands or the maximal inhibition of topo1 activity. All possible forms of plasmid DNA (supercoiled plasmid (sc)), nicked plasmid in which one strand of the double strand is intact and the other cleaved (Nk), fully relaxed plasmid with both strands intact but free of supercoiled (Rel) and relaxed topoisomers (Rn) were separated. DNA was relaxed with topo1 in presence or absence of **14a** and **13b**. The DNA relaxation products were then resolved by gel electrophoresis on agarose containing ethidium bromide to stain the DNA. Campthotecin (20 μM) was used as a positive control. The drug strongly stabilized the cleaved DNA–topo1 complex (Fig. 1, lane 13) such as a poison of topo1. The effect of ethidium bromide (BET) at 0.4 μM (Fig. 1, lane 14) on the migration of the relaxed topoisomers produced by topo1 provided a reference for the quantitative evaluation of the DNA intercalation by the tested compounds. Compounds **13b** and **14a** were incubated with the plasmid and then relaxed with topo1 (Fig. 1, lanes 5–8 and 9–12, respectively). As shown in lanes 5–8, increasing concentrations of **14a** altered the relaxed topo1 migration. Indeed, at 50 μM, the latter exhibited an action similar to BET. For compound **13b**, the same effect was obtained at 40 μM.

2.4. DNA cell cycle analysis

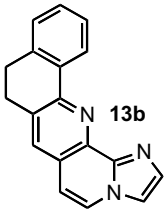
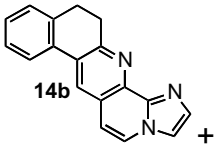
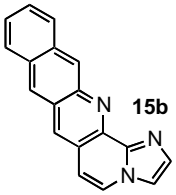
Compounds **13b** and **14a** were further evaluated to decipher more precisely the mechanism(s) of action underlying their

Table 2
Access to imidazopyridine derivatives from *o*-aminoaldehyde **5b** and ketones according to modified Friedländer's reaction: EtOH/KOH 10%, Δ (method A) or AcOH, Δ (method B)

Entry	Ketones	Products (from **5b**)	Time (h)	Method	Yield (%)
1	Acetone	 **6b**	2	A	47
2	Butanone	 **7b** +	2	A	49
		 **8b**	2	A	16
3	Acetophenone	 **9b**	2	A	53
4	Cyclohexanone	 **10b**	1.5	A	40
5	Ethyl 4-oxocyclohexane carboxylate	 **11b**	1	A	34
6	1,3-Cyclohexandione	 **12b**	1.5	B	33

(*continued on next page*)

Table 2 (*continued*)

Entry	Ketones	Products (from **5b**)	Time (h)	Method	Yield (%)
7	α-Tetralone	 **13b**	1	A	40
		 **14b** +	1	A	20
				B	21
8	β-Tetralone	 **15b**	1	A	15

antiproliferative effect and their inhibitory effect on the cell cycle. To that end, flow cytometry analyses were performed for compounds **13b** and **14a** at 6.6 and 2.2 μM, respectively (GI_{50}). Exponentially growing MCF-7 cells were treated with DMSO (control), **14a**, **13b** or an acridine analogue such as amsacrine for 4, 24 and 48 h as described in Section 4.

Flow cytometry analysis revealed that **13b** and **14a** noticeably arrested the cell cycle in the G_2/M phase. Compound **13b**, at 6.6 μM, after 24 h of incubation showed that, the percentage of the cells in the G_2/M phase is seven times higher in controls (no drug). At a concentration of 2.2 μM, compound **14a** induced an accumulation of cells in G_2/M phase was upraised twofold compared to controls (no drug). Then, FACS data of compounds **14a** and **13b** seem to have the same profile than the *m*-Amsa (Fig. 2). This latter was known to be composed by two functional domains: the acridine chromophore constitutes the DNA-binding domain, and the aniline group represents the topoisomerase 2-binding domain. So, these polycyclic derivatives of acridine scaffold, in which one or more fused heterocycles have been added to the tricyclic chromophore, open new fields in the search for active molecules [35].

3. Conclusion

In summary, we have developed a novel synthetic approach of 20 angular imidazonaphthyridinic compounds using a modified Friedländer's method and we have assessed their

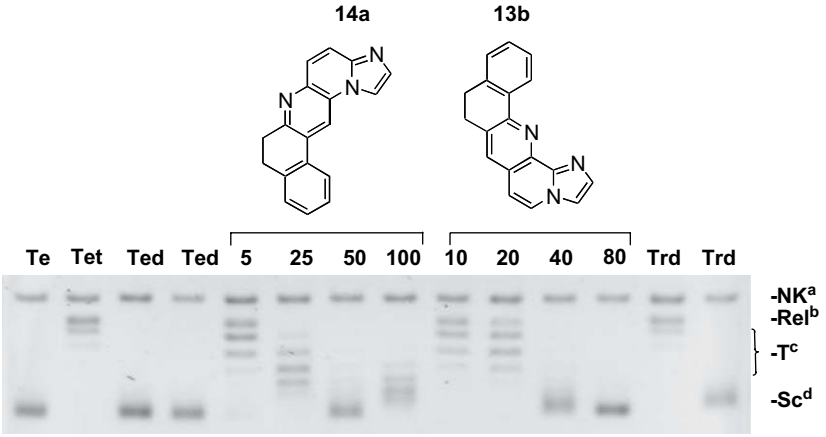


Fig. 1. Electrophoresis on agarose gel comparing the effect of CPT, BET, compounds **14a** and **13b** on the relaxation plasmid DNA by human topoisomerase 1. (a) DNA nicked; (b) DNA relaxed; (c) relaxed topoisomers; (d) DNA supercoiled. Lane 1 (Te): ADN supercoiled (sc). Lane 2 (Tet): ADN sc, topo1. Lane 3 (Ted1): ADN sc, **14a** (100 μM). Lane 4 (Ted2): ADN sc, **13b** (80 μM). Lanes 5–8: ADN sc, topo1 and **14a** (5–100 μM). Lanes 9–12: ADN sc, topo1, **13b** (10–80 μM); Lane 13 (Trd1): ADN sc, topo1 and CPT (20 μM). Lane 14 (Trd2): ADN sc, topo1 and BET (0.4 μM).

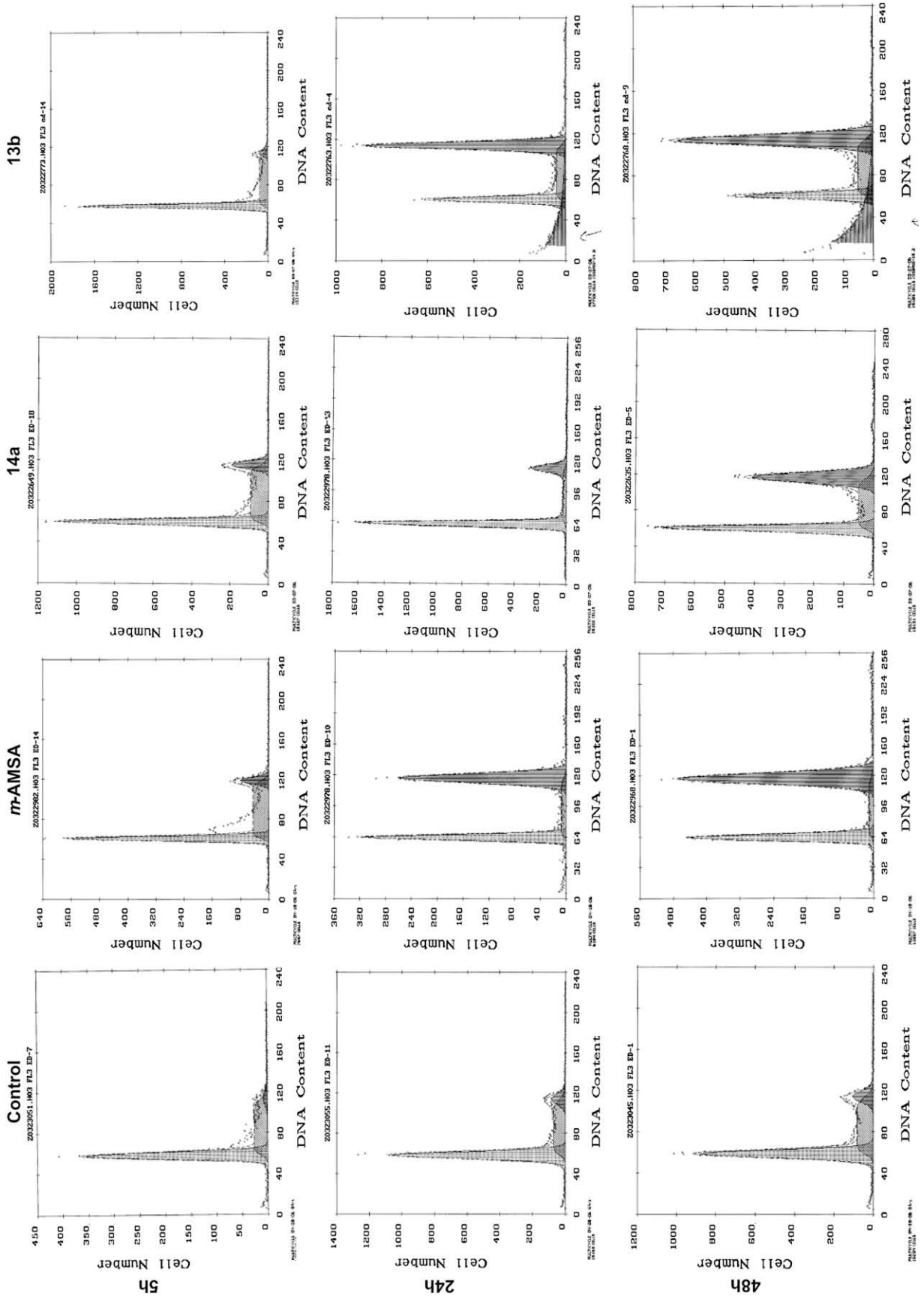


Fig. 2. Quantification of the FACS analyses for *m*-Amsa and compounds **14a** and **13b**.

Table 3
Antiproliferative activity (GI_{50} (μM)) of compounds **6–15**

Compound[a]	GI_{50} (μM)[b]			
	HT-1080	HT-29	M-21	MCF-7
m-Amsa	1.6	16.8	49.6	5.6
6a	>100	>100	>100	>100
7a	>100	>100	>100	>100
8a	>100	>100	>100	>100
9a	43.3	55.8	70.1	51.8
10a	>100	>100	>100	>100
11a	>100	>100	>100	>100
12a	>100	>100	>100	>100
13a	32.7	27.8	52	20.9
14a	13.3	37.9	38.1	2.2
15a	Nd[c]	Nd[c]	Nd[c]	Nd[c]
6b	>100	>100	>100	>100
7b	>100	>100	>100	>100
8b	>100	>100	>100	>100
9b	57.7	48.4	56.5	60.5
10b	>100	>100	>100	>100
11b	64.9	79.6	80.3	57.7
12b	>100	>100	>100	>100
13b	13.4	7.2	8.1	6.6
14b	41.4	49.2	64.8	46.5
15b	20.7	18.3	25.2	21.5

[a] All compounds were purified by column chromatography and their molecular structure confirmed by ^{1}H NMR and ^{13}C NMR.
[b] Drug concentration required to inhibit tumor cell proliferation by 50%.
[c] Nd: not determined.

antiproliferative activities on four different cell lines along with their DNA-intercalating properties and their topoisomerase inhibition potency (Table 3). It appears that tricyclic and tetracyclic systems were inactive on tumor cell growth at the concentrations tested, excepted for compounds **9a** and **9b** substituted by a phenyl group. In comparison, pentacyclic nucleus such as compounds **13b** and **14a** showed significant cytotoxic activity. Their mechanism of cytotoxicity action seems unrelated to poisoning or inhibiting abilities against topo1. On the contrary, we highlighted a direct intercalation of the drugs into DNA strands by electrophoresis on agarose gel. Our structural studies to date of DNA-targeted and/or topoisomerase-targeted chromophore in IP series provided a new class DNA recognizing derivatives. As a continuation of this findings which provide guidance for the development of new DNA-binding chromophores, we will study other chromophores connected to the imidazo[1,2-*a*]pyridine skeleton and the results of further optimization will be reported in due course.

4. Experimental

4.1. Chemistry

Melting points were determined on an Electrothermal IA9300 (capillary) and are not corrected. IR spectra were recorded on an FTIR Nicolet Impact 410. ^{1}H NMR (400 MHz) spectra were recorded on a Bruker Avance 400 spectrometer using $CDCl_3$ as solvent unless specified otherwise. ^{13}C NMR were also recorded on a Bruker Avance 400 spectrometer. For ^{13}C NMR data, a sign (−) indicate a secondary or quaternary carbon. MS spectral analyses were performed on a Hewlett–Packard 5985b mass spectrometer. All air-sensitive reactions were run under anhydrous argon atmosphere.

4.1.1. General procedure for the synthesis of compounds 2(*a*,*b*)

To a solution of 2-amino-6-methyl-5-nitropyridine **1a** or 2-amino-4-methyl-3-nitropyridine **1b** (3.10 g, 20.25 mmol) dissolved in ethanol (50 mL), chloroacetaldehyde (50% in water) (11.30 g, 72.43 mmol) was slowly added and the mixture was refluxed for 7 h. After cooling to room temperature, the solvent was removed under reduced pressure and 60 mL of water were added. The resulting mixture was alkalinized with Na_2CO_3 and extracted with CH_2Cl_2 (3 × 100 mL). The combined organic extracts were dried over Na_2SO_4, filtered and concentrated under reduced pressure. The crude product was purified by chromatography (alumina, CH_2Cl_2/EtOH, 99:1, v/v).

*4.1.1.1. 5-Methyl-6-nitroimidazo[1,2-a]pyridine (**2a**) [26].* Yield 98%. M.p. 153–154 °C; IR (KBr, cm^{-1}) 1556, 1320; ^{1}H NMR δ 3.00 (s, 3H, CH_3), 7.60 (d, 1H, $J = 9.0$ Hz, Ar), 7,72 (s, 1H, Ar), 7.83 (m, 2H, Ar); ^{13}C NMR δ 16.7, 112,6, 115.4, 120.4, 135.9 (−), 136.9, 137.7 (−), 145.0 (−); MS: (*m*/*z*) 177 (M^+, 100) 160 (62), 132 (57), 104 (61), 77(83), 51 (67).

*4.1.1.2. 7-Methyl-8-nitroimidazo[1,2-a]pyridine (**2b**).* Yield 97%. M.p. 151–152 °C; IR (KBr, cm^{-1}) 2923, 1530; ^{1}H NMR δ 2.50 (s, 3H, CH_3), 6.73 (d, 1H, $J = 7.0$ Hz, Ar), 7.65 (s, 1H, Ar), 7.69 (s, 1H, Ar), 8.16 (d, 1H, $J = 7$ Hz, Ar); ^{13}C NMR δ 17.7, 113.7, 114.7, 127.6, 129.5 (−), 134.8, 138.3 (−), 138.4 (−); MS: (*m*/*z*) 177 (M^+, 100), 160 (37), 132 (24), 104 (28), 77 (44).

4.1.2. General procedure for the synthesis of compounds 3(*a*,*b*)

To a solution of the nitro compound **2a** or **2b** (4.0 g, 22.60 mmol) dissolved in anhydrous DMF (105 mL), DMF/DMA (5.40 mL, 40.38 mmol) was added. The reaction mixture was heated to 130 °C (external temperature) for 2 h. After cooling to room temperature, the solvent was evaporated and 50 mL of water were added. The resulting mixture was extracted with CH_2Cl_2 (3 × 100 mL). The combined organic layers were dried over Na_2SO_4, filtered and concentrated under reduced pressure to afford **3a** or **3b** that was used without further purification.

*4.1.2.1. (E)-5-(N,N-Dimethylaminoethenyl)-6-nitroimidazo-[1,2-a]pyridine (**3a**).* Yield 74%. M.p. 144–146 °C; IR (KBr, cm^{-1}) 1626, 1258; ^{1}H NMR δ 3.05 (s, 6H, CH_3), 5.83 (d, 1H, $J = 13.5$ Hz, H-vinyl), 7.20 (d, 1H, $J = 9.5$ Hz, Ar), 7.40 (d, 1H, $J = 13.5$ Hz, H-vinyl), 7.63 (m, 1H, Ar), 7.83 (d, 1H, $J = 9.5$ Hz, Ar), 7.93 (m, 1H, Ar); ^{13}C NMR δ 53.5,

84.4, 110.9, 114.3, 122.4, 132.8 (−), 135.0, 139.5 (−), 146.0 (−), 149.4; MS: (*m/z*) 177 (M^+, 78), 104 (86), 77 (100).

*4.1.2.2. (E)-7-(N,N-Dimethylaminoethenyl)-8-nitroimidazo-[1,2-a]pyridine (**3b**).* Yield 74%. M.p. 197−198 °C; IR (KBr, cm^{-1}) 1643, 1530, 1298; ^1H NMR δ 2.96 (s, 6H, CH_3), 5.64 (d, 1H, $J = 13.0$ Hz, H-vinyl), 6.81 (d, 1H, $J = 7.5$ Hz, Ar), 7.10 (d, 1H, $J = 13.0$ Hz, H-vinyl), 7.38 (s, 1H, Ar), 7,48 (s, 1H, Ar), 7,80 (d, 1H, $J = 7.5$ Hz, Ar); ^{13}C NMR δ 41.0, 89.0, 107.9, 112.8, 126.9, 130.6 (−), 133.6, 133.8, 140.3 (−), 146.8; MS: (*m/z*) 232 (M^+, 61), 215 (52), 200 (100), 185 (45), 174 (42), 157 (76), 131 (39).

*4.1.3. General procedure for the synthesis of compounds **4(a,b)***

To a solution of **3a** or **3b** (3.38 g, 14.56 mmol) dissolved in THF/H_2O (1/1, v/v) (336 mL), sodium periodate (9.0 g, 42.07 mmol) was added and the reaction mixture was stirred at room temperature for 3 h. The resulting mixture was extracted with CH_2Cl_2 (3 × 100 mL). The combined organic layers were dried over Na_2SO_4, filtered and concentrated *in vacuo*. The resulting product was used without further purification.

*4.1.3.1. 6-Nitroimidazo[1,2-a]pyridine-5-carbaldehyde (**4a**).* Yield 51%. M.p. 126−127 °C; IR (KBr, cm^{-1}) 1343, 1299; ^1H NMR δ 7.97 (m, 2H, $J = 9.5$ Hz, Ar), 8.05 (s, 1H, Ar), 9.10 (s, 1H, Ar), 10.50 (s, 1H, CHO); ^{13}C NMR δ 109.2, 117.7, 121.7, 135.2 (−), 136.0, 138.6 (−), 146.2 (−), 185.9; MS: (*m/z*) 86 (M^+, 64), 83 (38), 84 (100), 71 (61), 57 (68).

*4.1.3.2. 8-Nitroimidazo[1,2-a]pyridine-7-carbaldehyde (**4b**).* Yield 91%. M.p. 133−134 °C; IR (KBr, cm^{-1}) 3425, 2924, 1605, 1540; ^1H NMR δ 7.42 (d, 1H, $J = 7.0$ Hz, Ar), 7.91 (s, 1H, Ar), 8.00 (s, 1H, Ar), 8.40 (d, 1H, $J = 7.0$ Hz, Ar), 10.20 (s, 1H, CHO); ^{13}C NMR δ 108.6, 116.5, 123.3, 128.6, 136.6 (−), 138.6 (−), 141.6 (−), 185.0; MS: (*m/z*) 191 (M^+,41), 161 (60), 117 (73), 105 (74), 90 (85), 63 (100).

*4.1.4. General procedure for the synthesis of compounds **5(a,b)***

To a solution of compound **4a** or **4b** (2.90 g, 15.18 mmol) dissolved in a mixture of ethanol/water (100 mL, 10:1, v/v), powder of iron (6.20 g, 110.71 mmol) and five drops of hydrochloric acid (37%) were added slowly. The reaction mixture was refluxed for 1 h. After cooling at room temperature, the reaction mixture was filtered on Celite®, then the filtrate was basified with a saturated solution of Na_2CO_3. The resulting mixture was extracted with CH_2Cl_2 (3 × 100 mL). The combined organic layers were dried over Na_2SO_4, filtered and concentrated *in vacuo*. The crude product was purified by chromatography on alumina eluted with CH_2Cl_2/EtOH (98:2, v/v).

*4.1.4.1. 6-Aminoimidazo[1,2-a]pyridine-5-carbaldehyde (**5a**).* Yield 71%. M.p. 131−132 °C; IR (KBr, cm^{-1}) 1634, 1524; ^1H NMR δ 6.65 (d, 1H, $J = 9.5$ Hz, Ar), 7.55 (s, 1H, Ar), 7.65 (d, 1H, $J = 9.5$ Hz, Ar), 7.95 (s, 1H, Ar), 10.42 (s, 1H, CHO); ^{13}C NMR δ 112.5 (−), 113.0, 119.2, 125.6, 132.6 (−), 136.4, 147.1 (−), 180.9; MS: (*m/z*) 161 (100), 149 (17), 133 (67), 117 (50), 105 (76), 90 (87), 79 (50), 63 (73).

*4.1.4.2. 8-Aminoimidazo[1,2-a]pyridine-7-carbaldehyde (**5b**).* Yield 74%. M.p. 151−152 °C; IR (KBr, cm^{-1}) 1623; ^1H NMR δ 6.84 (d, 1H, $J = 7.0$ Hz, Ar), 7.45 (d, 1H, $J = 7.0$ Hz, Ar), 7.53 (s, 1H, Ar), 7.56 (s, 1H, Ar), 9.80 (s, 1H, CHO); ^{13}C NMR δ 107.3 (−), 113.7, 115.0, 115.9, 133.4, 137.5 (−), 141.6 (−), 191.2; MS: (*m/z*) 161 (M^+, 72), 133 (100), 106 (68), 79 (29).

*4.1.5. General procedure for the synthesis of compounds **6(a,b)−15(a,b)***

Method A. To a solution of **5a** or **5b** (0.25 g, 1.55 mmol), dissolved in dry ethanol (30 mL) was added a dry solution of EtOH/KOH 10% (1.7 mL) and the appropriated ketone (3.40 mmol) was added. The reaction mixture was degassed under dry argon, refluxed (TLC monitoring). After cooling at room temperature, the reaction mixture was concentrated under reduced pressure and the crude product was purified by chromatography on alumina eluted with CH_2Cl_2/EtOH (98:2, v/v) to give the desired Friedländer's derivatives.

Method B. To a solution of the appropriated ketone (1.40 mmol) and acetic acid (0.8 mL), a solution of compound **5a** (or **5b**) (0.20 g, 1.24 mmol) dissolved in acetic acid (1.20 mL) was added dropwise, and the mixture refluxed (TLC monitoring). After cooling to room temperature, the solvent was evaporated and then 20 mL of water were added. The resulting mixture was basified with a saturated solution of Na_2CO_3 (pH = 8) and extracted with CH_2Cl_2 (3 × 50 mL). The combined organic layers were dried over Na_2SO_4, filtered and concentrated *in vacuo*. The crude product was purified by chromatography on alumina eluted with CH_2Cl_2/EtOH (98:2, v/v) to afford the Friedländer's derivatives.

*4.1.5.1. 7-Methylimidazo[1,2-a][1,5]naphthyridine (**6a**).* Compound **6a** was synthesized from **5a** and acetone using method A. Reaction time: 1.5 h. Yield 65%. M.p. 131−133 °C; ([29]: 132−134 °C).

*4.1.5.2. 7,8-Dimethylimidazo[1,2-a][1,5]naphthyridine (**7a**) and 7-ethylimidazo[1,2-a][1,5]naphthyridine (**8a**).* Compounds **7a** and **8a** were synthesized from **5a** and butanone using method A. Reaction time: 2 h. The purification by chromatography gave, first: compound (**7a**). Yield 22%. M.p. 177−178 °C; IR (KBr, cm^{-1}) 1518, 1312; ^1H NMR δ 2.42 (s, 3H, CH_3), 2.58 (s, 3H, CH_3), 7.60 (m, 3H, Ar), 7.80 (s, 1H, Ar), 7.89 (s, 1H, Ar); ^{13}C NMR δ 19.9, 22.8, 111.3, 119.5, 123.0, 127.0, 127.5 (−), 132.1 (−), 133.2, 138.2 (−), 143.4 (−), 155.7 (−); MS: (*m/z*) 197 (M^+, 100), 196 (30), 182 (10), 155 (12), 101 (10), 77 (11), 63 (11). And second, compound (**8a**). Yield 5%; IR (CCl_4, cm^{-1}) 2925, 1549; ^1H NMR δ 1.40 (t, 3H, $J = 7.5$ Hz, CH_3), 3.05 (q, 2H, $J = 7.5$ Hz, CH_2), 7.40 (d, 1H, $J = 8.5$ Hz, Ar), 7.60−7.73 (m, 3H, Ar), 8.02 (s, 1H, Ar), 8.13

(d, 1H, $J = 8.5$ Hz, Ar); ^{13}C NMR δ 14.2, 31.7 (−), 111.6, 120.8, 122.0, 123.4, 127.5, 127.8, 133.4, 140.6 (−), 143.6 (−), 161.7 (−); MS: (*m/z*) 196 (M^+, 100), 181 (11), 169 (19), 155 (6), 142 (8), 129 (6), 115 (8).

*4.1.5.3. 7-Phenylimidazo[1,2-a][1,5]naphthyridine (**9a**).* Compound **9a** was synthesized from **5a** and acetophenone using method A. Reaction time: 1 h. Yield 31%. M.p. 130–131 °C; IR (KBr, cm^{-1}) 1455, 1308; ^{1}H NMR δ 7.41–7.51 (m, 3H, Ar), 7.72 (s, 1H, Ar), 7.76 (m, 2H, Ar), 7.90 (m, 2H, Ar), 8.00 (m, 3H, Ar); ^{13}C NMR δ 111.7, 119.9, 121.0, 123.4, 127.1 (2*C), 127.8, 128.9 (2*C), 129.4, 133.5, 138.4 (−), 140.8 (−), 143.4 (−), 154.9 (−); MS: (*m/z*) 245 (M^+, 100), 244 (28).

*4.1.5.4. 7,8,9,10-Tetrahydrobenzo[g]imidazo[1,2-a][1,5]-naphthyridine (**10a**).* Compound **10a** was synthesized from **5a** and cyclohexanone using method A. Reaction time: 1 h. Yield 32%. M.p. 100–102 °C; IR (KBr, cm^{-1}) 1521, 1317; ^{1}H NMR δ 1.75–1.85 (m, 4H, CH_2), 2.85–2.94 (m, 4H, CH_2), 7.65–7.72 (m, 3H, Ar), 7.92 (s, 1H, Ar), 8.62 (s, 1H, Ar). ^{13}C NMR δ 22.5 (−), 23.0 (−), 29.2 (−), 33.0 (−), 111.5, 119.9, 122.6, 127.2, 127.5 (−), 132.9, 133.0 (−), 138.6 (−), 143.4 (−), 156.0 (−); MS: (*m/z*) 223 (M^+, 100), 222 (40), 195 (16).

*4.1.5.5. Ethyl 8,9,10,11-tetrahydrobenzo[g]imidazo[1,2-a]-[1,5]naphthyridine-9-carboxylate (**11a**).* Compound **11a** was synthesized from the addition of **5a** to ethyl 4-oxocyclohexane carboxylate using method A. Reaction time: 1 h. Yield 21%. M.p. 128–129 °C. IR (KBr, cm^{-1}) 1711, 1309; ^{1}H NMR δ 1.15 (t, 3H, $J = 7.0$ Hz, CH_3), 2.20–2.30 (m, 2H, CH_2), 2.90 (m, 1H, CH), 3.05–3.23 (m, 4H, 2*CH_2), 4.20 (q, 2H, $J = 7.0$ Hz, CH_2), 7.51 (s, 1H, Ar), 7.54 (m, 2H, Ar), 7.80 (s, 1H, Ar), 7.85 (s, 1H, Ar); ^{13}C NMR δ 14.3, 25.8 (−), 31.4 (−), 31.5 (−), 39.2, 60.2 (−), 111.6, 120.3, 122.8, 127.1, 127.6 (−), 130.8 (−), 133.1, 139.1 (−), 143.4 (−), 154.6 (−), 174.5 (−); MS: (*m/z*) 295 (M^+, 74), 222 (38), 221 (100), 220 (48), 207 (9).

*4.1.5.6. 8,9-Dihydrobenzo[g]imidazo[1,2-a][1,5]naphthyridine-10(7H)-one (**12a**).* Compound **12a** was synthesized from **5a** and 1,3-cyclohexadione using method B. Reaction time: 1 h. Yield 30%. M.p. 231–232 °C; IR (KBr, cm^{-1}) 2945, 1680, 1316; ^{1}H NMR δ 2.30 (m, 2H, CH_2), 2.83 (t, 2H, $J = 6.5$ Hz, CH_2), 3.31 (t, 2H, $J = 6.5$ Hz, CH_2), 7.73 (s, 1H, Ar), 7.79 (m, 2H, Ar), 8.16 (s, 1H, Ar), 8.82 (s, 1H, Ar); ^{13}C NMR δ 21.9 (−), 32.6 (−), 38.7 (−), 112.9, 115.2 (−), 121.7, 123,9, 126.5, 128.0 (−), 134.0, 143.1 (−), 143.8 (−), 160.4 (−), 197.3 (−); MS: (*m/z*) 237 (M^+, 100), 209 (35), 208 (28), 181 (42), 180 (30), 56 (23).

*4.1.5.7. 11,12-Dihydroimidazo[1,2-a]naphtho[1,2-g][1,5]-naphthyridine (**13a**).* Compound **13a** was synthesized from **5a** and α-tetralone using method A. Reaction time: 1 h. Yield 26%. M.p. 194–195 °C; IR (KBr, cm^{-1}) 1301, 1100; ^{1}H NMR δ 3.02 (m, 2H, CH_2), 3.15 (m, 2H, CH_2), 7.23–7.40 (m, 3H, Ar), 7.66 (s, 1H, Ar), 7.80 (m, 2H, Ar), 7.95 (s, 1H, Ar), 8.00 (s, 1H, Ar), 8.45 (d, 1H, $J = 7$ Hz, Ar); ^{13}C NMR δ 27.9 (−), 28.8 (−), 111.5, 119.0, 121.6, 125.5, 127.4, 127.7, 127.9, 129.6, 132.6 (−), 133.3 (−), 133.4 (−), 133.6, 138.2 (−), 139.4 (−), 143.9 (−), 150.6(−) and one carbon was not observed; MS: (*m/z*) 271 (M^+, 100), 270 (65), 135 (12).

*4.1.5.8. 7,8-Dihydroimidazo[1,2-a]naphtho[2,1-g][1,5]naphthyridine (**14a**) and 7,12-dihydroimidazo[1,2-a]naphtho[2,3-g]-[1,5]naphthyridine (**15a**).* Compounds **14a** and **15a** were synthesized from **5a** and β-tetralone. Reaction time: 1 h. The purification by chromatography gave first, compound (**14a**). Yield 40% (method A), 27% (method B). M.p. 214–215 °C; IR (KBr, cm^{-1}) 1519, 1320; ^{1}H NMR δ 2.82 (t, 2H, $J = 7.5$ Hz, CH_2), 2.96 (t, 2H, $J = 7.5$ Hz, CH_2), 7.10 (m, 3H, Ar), 7.43 (m, 3H, Ar), 7.57 (d, 1H, $J = 7.5$ Hz, Ar), 7.85 (s, 1H, Ar), 8.11 (s, 1H, Ar). ^{13}C NMR δ 28.5 (−), 31.8 (−), 111.5, 116.6, 120.3, 124.4, 126.9, 127.4, 128.5 (−), 128.6, 129.3, 129.6 (−), 131.9 (−), 133.5, 137.5 (−), 139.2 (−), 143.5 (−), 156.1 (−); MS: (*m/z*) 271 (M^+, 100), 270 (65) and second, compound (**15a**). Yield 28% (method A). M.p. 207–209 °C; IR (KBr, cm^{-1}) 2810, 1680; ^{1}H NMR δ 2.97 (s, 2H), 3.96 (t, 2H), 6.65 (d, 1H, $J = 7$ Hz), 7.26 (m, 2H), 7.49 (m, 3H), 7.70 (s, 1H), 7.88 (s, 1H), 8.55 (s, 1H); ^{13}C NMR δ 29.7, 36.6, 109.0, 111.4, 117.4, 119.2, 120.3, 122.9, 127.3, 127.9, 132.5, 132.7, 135.4, 135.2, 139.0, 142.1, 143.0, 148.7; MS: (*m/z*) 271 (M^+, 100), 270 (70), 229 (30), 135 (15).

*4.1.5.9. 9-Methylimidazo[1,2-h][1,7]naphthyridine (**6b**).* Compound **6b** was synthesized from **5b** and acetone using method A. Reaction time: 2 h. Yield 47%. M.p. 163–164 °C; (Lit. [29]: 166–168 °C).

*4.1.5.10. 8,9-Dimethylimidazo[1,2-h][1,7]naphthyridine (**7b**) and 9-ethylimidazo[1,2-h][1,7]naphthyridine (**8b**).* Compounds **7b** and **8b** were synthesized from **5a** and butanone using method A. Reaction time: 2 h. The purification by chromatography gave first, compound (**7b**). Yield 49%. M.p. 263–265 °C; IR (KBr, cm^{-1}) 1514, 1324; ^{1}H NMR δ 2.45 (s, 3H, CH_3), 2.76 (s, 3H, CH_3), 6.94 (d, 1H, $J = 7.0$ Hz, Ar), 7.62 (s, 1H, Ar), 7.67 (s, 1H, Ar), 7.72 (s, 1H, Ar), 7.94 (d, 1H, $J = 7.0$ Hz, Ar); ^{13}C NMR δ 19.6, 23.5, 111.4, 114.6, 122.2, 123.3 (−, 131.9 (−), 132.3, 134.6, 138.3 (−), 143.3 (−), 159.8 (−); MS: (*m/z*) 197 (M^+, 100), 196 (45), 182 (7) and second, compound (**8b**). Yield 16%; IR (CCl_4, cm^{-1}) ν 1549, 1252; ^{1}H NMR δ 1.41 (t, 3H, $J = 7.5$ Hz, CH_3), 3.01 (q, 2H, $J = 7.5$ Hz, CH_2), 6.95 (d, 1H, $J = 7.0$ Hz, Ar), 7.30 (d, 1H, $J = 8.0$ Hz, Ar), 7.61 (s, 1H, Ar), 7.68 (s, 1H, Ar), 7.90 (m, 2H, Ar); ^{13}C NMR δ 14.1, 32.8 (−), 111.7, 114.8, 122.1, 122.8 (−), 123.1, 132.4, 135.0, 140.0 (−), 142.9 (−), 165.3 (−); MS: (*m/z*) 197 (M^+, 77), 196 (100), 169 (43).

*4.1.5.11. 9-Phenylimidazo[1,2-h][1,7]naphthyridine (**9b**).* Compound **9b** was synthesized from **5b** and acetophenone using method A. Reaction time: 2 h. Yield 53%. M.p.

140–142 °C; IR (KBr, cm^{-1}) 2361, 1317; ^1H NMR δ 6.81 (d, 1H, $J = 7.0$ Hz, Ar), 7.41–7.44 (m, 3H, Ar), 7.51 (s, 1H, Ar), 7.65 (s, 1H, Ar), 7.77–7.90 (m, 3H, Ar), 8.25 (d, 2H, $J = 8.0$ Hz, Ar); ^{13}C NMR δ 111.4, 115.0, 119.6, 123.5, 123.6 (−), 127.4 (2^*C), 128.6 (2^*C), 129.5, 132.3, 135.3, 138.4 (−), 140.1 (−), 142.8 (−), 157.3 (−); MS: (*m/z*) 245 (M^+, 100), 244 (70).

*4.1.5.12. 8,9,10,11-Tetrahydrobenzo[b]imidazo[1,2-h][1,7]-naphthyridine (**10b**).* Compound **10b** was synthesized from **5b** and cyclohexanone using method A. Reaction time: 1.5 h. Yield 40%. M.p. 203–204 °C; IR (KBr, cm^{-1}) 1634, 1320; ^1H NMR δ 1.57 (m, 2H, CH_2), 1.66 (m, 2H, CH_2), 2.95 (t, 2H, $J = 5.0$ Hz, CH_2), 3.20 (t, 2H, $J = 5.0$ Hz, CH_2), 6.90 (d, 1H, $J = 7.0$ Hz, Ar), 7.60 (s, 1H, Ar), 7.62 (s, 2H, Ar), 7.88 (d, 1H, $J = 7.0$ Hz, Ar); ^{13}C NMR δ 22.6 (−), 22.9 (−), 29.0 (−), 33.2 (−), 111.4, 114.6, 123.0, 132.3, 132.5 (−), 134.1, 138.3 (−), 142.9 (−), 159.8 (−) and one carbon was not observed; MS: (*m/z*) 223 (M^+, 100), 222 (60), 207 (10), 195 (11).

*4.1.5.13. Ethyl 8,9,10,11-tetrahydrobenzo[b]imidazo[1,2-h][1,7]naphthyridine-9-carboxylate (**11b**).* Compound **11b** was synthesized from **5b** and ethyl-4-oxocyclohexane carboxylate using method A. Reaction time: 1 h. Yield 34%. M.p. 126–127 °C; IR (KBr, cm^{-1}) 3092, 1714; ^1H NMR (CD_3OD) δ 1.30 (t, 3H, $J = 7.0$ Hz, CH_3), 1.90 (m, 2H, CH_2), 2.20 (m, 2H, CH_2), 2.90 (m, 3H, CH, CH_2), 3.05 (m, 4H), 4.20 (q, 2H, $J = 7.0$ Hz, CH_2), 7.15 (d, 1H, $J = 7.0$ Hz, Ar), 7.63 (s, 1H, Ar), 7.92 (m, 2H, Ar), 8.22 (d, 1H, $J = 7.0$ Hz, Ar); ^{13}C NMR (CD_3OD) δ 14.6, 26.6 (−), 32.0 (−), 32.6 (−), 40.3, 61.9 (−), 113.4, 117.2, 125.0, 125.3 (−), 130.7, 133.0 (−), 136.1, 138.1 (−), 143.1 (−), 159.7 (−), 176.2 (−); MS: (*m/z*) 295 (M^+, 100), 203 (10), 176 (5), 122 (10).

*4.1.5.14. 10,11-Dihydrobenzo[b]imidazo[1,2-h][1,7]naphthyridine-8(9H)-one (**12b**).* Compound **12b** was synthesized from **5b** and 1,3-cyclohexadione using method B. Reaction time: 1.5 h. Yield 33%. M.p. 259–260 °C; IR (KBr, cm^{-1}) 2945, 1684, 1593; ^1H NMR δ 2.27 (m, 2H, CH_2), 2.80 (m, 2H, CH_2), 3.40 (m, 2H, CH_2), 7.10 (d, 1H, $J = 7.0$ Hz, Ar), 7.69 (s, 1H, Ar), 7.77 (s, 1H, Ar), 8.00 (d, 1H, $J = 7.0$ Hz, Ar), 8.66 (s, 1H, Ar); ^{13}C NMR δ 21.8 (−), 33.3 (−), 38.9 (−), 112.3, 116.1, 123.3 (−), 124.2, 127.2 (−), 133.5, 134.5, 142.1 (−), 142.4 (−), 164.3 (−), 197.7 (−); MS: (*m/z*) 237 (M^+, 100), 208 (72), 181 (43), 140 (23), 104 (24), 63 (20).

*4.1.5.15. 8,9-Dihydroimidazo[1,2-h]naphtho[1,2-b][1,7]naphthyridine (**13b**).* Compound **13b** was synthesized from **5b** and α-tetralone using method A. Reaction time: 1 h. Yield 40%. M.p. 135–137 °C; IR (KBr, cm^{-1}) 1400, 726; ^1H NMR δ 2.97 (m, 2H, CH_2), 3.07 (m, 2H, CH_2), 6.90 (d, 1H, $J = 7.0$ Hz, Ar), 7.20 (d, 1H, $J = 7.0$ Hz, Ar), 7.35 (m, 2H, Ar), 7.56 (s, 1H, Ar), 7.67 (s, 1H, Ar), 7.72 (s, 1H, Ar), 7.89 (d, 1H, $J = 7.0$ Hz, Ar), 8.79 (d, 1H, $J = 7.0$ Hz, Ar); ^{13}C NMR δ 28.0 (−), 28.6 (−), 111.6, 114.8, 123.5, 124.2 (−), 127.2, 127.4, 127.8, 129.8, 132.1 (−), 132.3, 133.3, 134.2 (−), 138.7 (−), 139.4 (−), 143.3 (−), 153.8 (−); MS: (*m/z*) 271 (M^+, 100), 270 (64), 229 (11), 135 (15).

*4.1.5.16. 12,13-Dihydroimidazo[1,2-h]naphtho[2,1-b][1,7]-naphthyridine (**14b**) and imidazo[1,2-h]naphtho[2,3-b][1,7]-naphthyridine (**15b**).* Compounds **14b** and **15b** were synthesized from **5b** and β-tetralone. Reaction time: 1 h. The purification by chromatography gave first, compound (**14b**). Yield 20% (method A), 21% (method B). M.p. 139–141 °C. IR (KBr, cm^{-1}) 1406, 1325; ^1H NMR δ 3.01 (t, 2H, $J = 7.0$ Hz, CH_2), 3.43 (t, 2H, $J = 7.0$ Hz, CH_2), 7.07 (d, 1H, $J = 7.5$ Hz, Ar), 7.23–7.35 (m, 3H, Ar), 7.63 (s, 1H, Ar), 7.70 (s, 1H, Ar), 7.84 (d, 1H, $J = 7.5$ Hz, Ar), 7.98 (d, 1H, $J = 7.5$ Hz, Ar), 8.28 (s, 1H, Ar). ^{13}C NMR δ 29.0 (−), 32.8 (−), 112.0, 115.3, 123.6, 124.2, 124.3, 127.3, 128.4, 128.6, 128.8, 129.7 (−), 132.5 (−), 132.7, 137.6 (−), 138.9 (−), 143.0 (−), 160.1 (−) and one carbon was not observed; MS: (*m/z*) 271 (M^+, 100), 270 (76), 269 (22) and second, compound (**15b**). Yield: 15% (method A); M.p. 180–181 °C; IR (KBr, cm^{-1}) 1508, 1398, 1332; ^1H NMR (DMSO-d_6) δ 7.37 (d, 1H, $J = 7.0$ Hz, Ar), 7.62 (m, 2H, Ar), 7.66 (s, 1H, Ar), 8.07 (s, 1H, Ar), 8.21–8.32 (m, 3H, Ar), 8.85 (s, 1H, Ar), 8.94 (s, 1H, Ar), 9.07 (s, 1H, Ar); ^{13}C NMR (DMSO-d_6) δ 111.9, 118.1, 123.3 (−), 124.4, 125.1 (−), 126.2, 126.6, 126.7, 127.2, 128.1, 128.3, 131.3 (−), 131.5, 134.0 (−), 135.0, 141.3, 142.2 (−), 143.9 (−); MS: (*m/z*) 269 (M^+, 100), 242 (27), 214 (10), 57 (11).

4.2. Pharmacology

4.2.1. Cell culture and growth inhibition assay

The tumor cell proliferation assay of compounds **6a**,**b**–**15a**,**b** was assessed using the procedure described by the National Cancer Institute [36]. 96-well microtiter plates were seeded with 100 μL of tumor cells suspended in high glucose DMEM supplemented with 5% (v/v) defined bovine calf serum iron (Hyclone, Dresden, Germany). Plates were incubated at 37 °C in a moisture-saturated atmosphere containing 5% CO_2 for 24 h. Freshly solubilized drugs in DMSO were diluted in fresh medium and aliquots of 100 μL containing sequential dilution of drugs were added to the wells. Final drug concentrations ranged from 10 to 0.3 μM. DMSO concentration was maintained lower than 0.5% to avoid solvent's cytotoxicity. Plates were incubated for 48 h. Assays were stopped by addition of cold trichloroacetic acid to the wells (final concentration was 10%), followed by incubation for 60 min at 4 °C. Plates were washed five times with tap water. Sulforhodamine B solution (50 μL) at 0.1% (w/v) in 1% acetic acid was added to each well, and plates were incubated for 15 min at room temperature. After staining, unbound dye was removed by washing five times with 1% acetic acid. Bound stain was solubilized with 10 mM Tris base, and the absorbance was read using a μQuant Universal Microplate Spectrophotometer (Biotek, Winooski, VT) at 585 nm. The results were compared with those of a control reference plate fixed on the treatment day and the growth inhibition percentage was calculated for

each drug contact period. The experiments were performed at least twice in triplicate. The GI_{50} assay was considered valid when the variability among data for a given set of conditions, within the same experiment, was less than 10% with respect to the mean value.

4.2.2. Plasmid DNA unwinding assay

pGEM®-9Zf(−) DNA plasmid (2.9 kb; Promega, Madison, WI, USA) was purchased at a concentration of 1 μg/ml and was stored frozen until use, at which time it was diluted in reaction buffer [consisting of 50 mM Tris/HCl (pH 7.5), 20 mM KCl, 1 mM EDTA, 0.3 mg/ml BSA and 1 mM DTT (dithiothreitol)]. Human topo1 (Calbiochem, Darmstadt, Germany) in solution at 2 units/μl (1 unit is the amount of enzyme needed to relax 0.5 μg of plasmid in 30 min at 37 °C) was used without further purification. Proteinase K, Tris (Trismabase), EDTA, DNase free BSA, TBE 10×, ethidium bromide (95%), SDS 20%, loading buffer, DMSO (HPLC grade), DTT, $CaCl_2$, CPT, BET and *m*-Amsa were obtained from Sigma–Aldrich.

The assay for DNA unwinding and topo1 poisoning has been adapted from the method described by Webb [34]. Briefly, supercoiled pGEM DNA was diluted into relaxation buffer to a concentration of 25 ng/μl. To 75 ng of supercoiled plasmid dilution, 3 μL of test compound solutions, control compound or blank solution was added. The volume of solution was completed to 11 μL with the relaxed buffer. Immediately prior use, the test and the control compounds were diluted in DMSO. The concentration of DMSO was maintained below 1%. Samples were incubated for 20 min at room temperature, then 2 μL of topo1 solution was added. This solution was incubated further in the dark at 37 °C for 2 h with gentle shaking. The reaction was stopped by addition of 1.25 μL of a 20% (w/v) SDS (sodium dodecyl sulfate) solution followed by 2.5 μL of proteinase K (1 mg/ml). Protein digestion was carried out in total darkness for 1 h at 45 °C, afterward 3 μL of electrophoresis loading buffer was added. From this point, the samples were treated as described above. Samples were analyzed by gel electrophoresis using 1% (w/v) agarose gels containing 0.1% (w/v) SDS. The presence of SDS during electrophoresis improves band resolution, allowing for more reliable measurement of topoisomerase bands. Electrophoresis was carried out using TBE buffer in a horizontal electrophoresis apparatus for, typically, 16 h at 1.25 V/cm. The gels were stained in 1 μg/ml ethidium bromide/TBE solution for 30 min. The stained gels were re-inserted in the electrophoresis apparatus and electrophoresed again for 2 h at 2 V/cm in TBE buffer containing ethidium bromide at 1 μg/ml. The second electrophoresis ensures a clear separation of nicked, covalently closed relaxed and linearized plasmid bands. The gel was visualized under UV illumination.

4.2.3. Flow cytometric analyses

MCF-7 cells were incubated with compounds **14a**, **13b** or *m*-Amsa for (4, 24 and 48 h). The flow cytometry analysis of cell DNA content was performed using an Epics XL (Coulter, Hialeah, FL) after propidium iodide labeling of cells. Fluorescence attributable to PI was determined using excitation by an argon laser, operating at 488 nm and a power output of 15 MW. Cell distribution was calculated using the Multicycle software program (Phoenix, Flow Systems, San Diego, CA) and expressed by the ratio of tested compound (**14a** or **13b**)/control or *m*-Amsa/control in MCF-7 cells.

Acknowledgments

We thank Mrs. Lise Vigouroux for her technical help in performing the DNA-intercalating experiments.

References

[1] P. Belmont, M. Jourdan, M. Demeunynck, J.-F. Constant, J. Garcia, J. Lhomme, J. Med. Chem. 42 (1999) 5153–5159.
[2] E. Budzisza, U. Krajewskab, M. Rozalskib, A. Szulawskac, M. Czyzc, B. Nawrotd, Eur. J. Pharm. 502 (2004) 59–65.
[3] E.D. Batts, C. Maisel, D. Kane, L.L.P. Fu, T. O'Brien, S. Remick, N. Bahlis, S.L. Gerson, Cancer Chem. Pharm. 60 (2007) 415–421.
[4] D. Perrin, B. Van Hille, J.-M. Barret, A. Kruczynski, C. Etie'Vant, T. Imbert, B.T. Hill, Biochem. Pharm. 59 (2000) 807–819.
[5] O.H. Temmink, E.K. Hoebe, M. Fukushima, G. Peters, Eur. J. Cancer 43 (2007) 175–183.
[6] M.T. Tomicic, M. Christmann, B. Kaina, Cancer Res. 65 (2005) 8920–8926.
[7] R.F. Battisti, Y. Zhong, L. Fang, S. Gibbs, J. Shen, J. Nadas, G. Zhang, D. Sun, Mol. Pharm. 4 (2007) 140–153.
[8] K. Pors, S.D. Shnyder, P.H. Teesdale-Spittle, J.A. Hartley, M. Zloh, M. Searcey, L.H. Patterson, J. Med. Chem. 49 (2006) 7013–7023.
[9] J.R. Goodell, A.A. Madhok, H. Hiasa, D.M. Ferguson, Bioorg. Med. Chem. 14 (2006) 5467–5480.
[10] K.-M. Chen, Y.-W. Sun, Y.-W. Tang, Z.-Y. Sun, C.-H. Kwon, Mol. Pharm. 2 (2005) 118–128.
[11] C. Losasso, E. Cretaio, K. Palle, L. Pattarello, M.-A. Bjornsti, P. Benedetti, J. Biol. Chem. 282 (2007) 9855–9864.
[12] M. Cushman, L. Cheng, J. Org. Chem. 43 (1978) 3781–3783.
[13] M. Cushman, M. Jarayaman, J.A. Vroman, A.K. Fukunaga, B.M. Fox, G. Kohlhagen, D. Strumberg, Y. Pommier, J. Med. Chem. 43 (2000) 3688–3698.
[14] A. Morrell, M.S. Placzek, J.D. Steffen, S. Antony, K. Agama, Y. Pommier, M. Cushman, J. Med. Chem. 50 (2007) 2040–2048.
[15] A. Morrell, M. Placzek, S. Parmley, S. Antony, T.S. Dexheimer, Y. Pommier, M. Cushman, J. Med. Chem. 50 (2007) 4419–4430.
[16] M. Ohkubo, K. Kojiri, H. Kondo, S. Tanaka, H. Kawamoto, T. Nishimura, I. Nishimura, T. Yoshinari, H. Arakawa, H. Suda, H. Morishima, S. Nishimura, Bioorg. Med. Chem. Lett. 9 (1999) 1219–1224.
[17] B. Pilch, E. Allemand, M. Facompre, C. Bailly, J.-F. Riou, J. Soret, J. Tazi, Cancer Res. 61 (2001) 6876–6884.
[18] H.I. Hurwitz, R.B. Cohen, J.P. McGovren, S. Hirawat, W.P. Petros, Y. Natsumeda, T. Yoshinari, Cancer Chemother. Pharmacol. 59 (2007) 139–147.
[19] A. Favier, M. Blackledge, J.-P. Simorre, S. Crouzy, V. Dabouis, A. Gueiffier, D. Marion, J.-C. Debouzy, Biochemistry 40 (2001) 8717–8726.
[20] J.-M. Chezal, E. Moreau, G. Delmas, A. Gueiffier, Y. Blache, G. Grassy, C. Lartigue, O. Chavignon, J.-C. Teulade, J. Org. Chem. 66 (2001) 6576–6584.
[21] K.F. Byth, C. Geh, C.L. Forder, S.E. Oakes, A.P. Thomas, Mol. Cancer Ther. 5 (2006) 655–664.
[22] D. Cai, K.F. Byth, G.I. Shapiro, Cancer Res. 66 (2006) 435–444.
[23] M. Hayakawa, K.-I. Kawaguchi, H. Kaizawa, T. Koizumi, T. Ohishi, M. Yamano, M. Okada, M. Ohta, S.-I. Tsukamobi, F.I. Raynaud, P. Parker, P. Workmun, M.D. Waterfiled, Bioorg. Med. Chem. 15 (2007) 5837–5844.

[24] H.-J. Lee, J.S. Kim, M.-E. Suh, H.-J. Park, S.K. Lee, H.-K. Rhee, H.-J. Kim, E.-K. Seo, C. Kim, C.-O. Lee, H.-Y.P. Choo, Eur. J. Med. Chem. 42 (2007) 168–174.
[25] N. Desbois, J.-M. Chezal, F. Fauvelle, J.-C. Debouzy, C. Lartigue, A. Gueiffier, Y. Blache, E. Moreau, J.-C. Madelmont, O. Chavignon, J.-C. Teulade, Heterocycles 65 (2005) 1121–1137.
[26] R. Jacquier, H. Lopez, G. Maury, J. Heterocycl. Chem. 10 (1973) 755–762.
[27] J.K. Son, J.K. Son, Y. Jahng, Heterocycles 62 (2002) 1109–1115.
[28] E. Moreau, S. Fortin, M. Desjardins, J.L.-C. Rousseau, E. Petitclerc, R.C. Gaudreault, Bioorg. Med. Chem. 13 (2005) 6703–6712.
[29] J.-M. Chezal, E. Moreau, O. Chavignon, V. Gaumet, J. Métin, Y. Blache, A. Diez, X. Fradera, J. Luque, J.-C. Teulade, Tetrahedron 58 (2002) 295–307.
[30] J.-C. Debouzy, S. Crouzy, V. Dabouis, A. Gueiffier, B. Brasme, C. Bachelet, A. Favier, J.-P. Simorre, L. Mazet, A. Peinnequin, Arch. Biochem. Biophys. 2 (1999) 202–205.
[31] B.L. Staker, M.D. Feese, M. Cushman, Y. Pommier, D. Zembower, L. Stewart, A.B. Burgin, J. Med. Chem. 48 (2005) 2336–2345.
[32] E. Marco, W. Laine, C. Tardy, A. Lansiaux, M. Iwao, F. Ishibashi, C. Bailly, F. Gago, J. Med. Chem. 48 (2005) 3796–3807.
[33] P. Yang, Q. Yang, X. Quian, J. Cui, Bioorg. Med. Chem. 13 (2005) 5909–5914.
[34] M.R. Webb, S.E. Ebeler, Biochem. J. 384 (2004) 527–541.
[35] M. Demeunynck, Expert Opin. Ther. Patents 14 (2004) 55–70.
[36] NCI/NIH Developmental Therapeutics Program, Human tumor cell line screen. Available from: http://dtp.nci.nih.gov/branches/btb/ivclsp.html.