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Synthesis and antiproliferative evaluation of new isomeric ellipticine quinones

[Síntesis y evaluación de la actividad antiproliferativa de nuevas elipticinquinonas isomericas]

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Abstract: The synthesis of new isomeric ellipticine quinones **3a-c** and their *in vitro* antiproliferative activities on cancer cell lines is reported. The designed *N*-heterocyclic quinones **3a-c** were synthesized through a three step sequence which involves: a) one-pot preparation of 4-methoxycarbonyl-3,4-dimethylisoquinoline-5,8-quinone **1** from 2,5-dihydroxyacetophenone, methyl aminocrotonate and silver (II) oxide; b) regioselective amination of **1** with arylamines to give aminoquinones **2a-c** and c) palladium-catalyzed intramolecular oxidative coupling of 7-aminoisoquinoline-5,8-quinones **2a-c**. The *in vitro* antiproliferative activity of the new angular quinones was evaluated againts one normal cell line (lung fibroblasts) and gastric, lung and bladder cancer cell lines in 72-h drug exposure assays. The new compounds displayed similar or higher antiproliferative activity with respect to their quinone precursors **2a-c**. The isomeric ellipticine quinone **2b** appears as the more active member on bladder cancer cell line (IC_{50}: 2.4 µM), comparable to etoposide used as anticancer reference drug.

Keywords: Ellipticine; quinones; oxidative coupling; antiproliferative activity

Resumen: Se describe la síntesis de las nuevas quinonas **3a-c**, isoméricas de elipticina, y sus actividades antiproliferativas *in vitro* en líneas de células de cáncer. Las quinonas *N*-heterocíclicas **3a-c** se sintetizaron a través de una secuencia que involucra: a) preparación de 4-metoxicarbonil-3,4-dimetlisoquinolin-5,8-quinone **1** a partir de 2,5-dihidroxiacetofenona, aminocrotonato de metilo y óxido de plata (I); b) aminación regioselectiva de **1** con arilaminas para producir las aminoquinonas **2a-c** y c) acoplamiento oxidante intramolecular de 7-aminoisoquinolin-5,8-quinonas **2a-c** catalizado con paladio. La actividad antiproliferative *in vitro* de los nuevos compuestos fue evaluada en una línea celular normal (fibroblastos de pulmón) y líneas de células de cáncer gástrico, pulmón y vejiga en ensayos de exposición de 72 horas a la droga. Las quinonas **3a-c** exhiben interesantes propiedades antiproliferativas destacando la elipticinquinona isomérica **2b** en células de cáncer de vejiga (IC50: 2.4 µM) comparado con etopósido usada como droga anticancer de referencia. Los nuevos compuestos mostraron actividades antiproliferativa similar o mayor respecto de las correspondientes quinonas precursoras **2a-c.** La elipticin quinona isomérica **2b** corresponde al miembro más activo en células de cáncer de vejiga (IC_{50}: 2.4 µM), comparable a la del etopósido, usada como droga anticáncer de referencia.

Palabras clave: Elipticina; quinonas; acoplamiento oxidante; actividad antiproliferativa.

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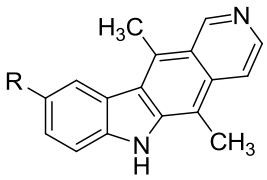
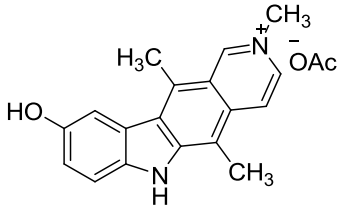
INTRODUCTION

Quinone moieties are present in many drugs such as doxorubicin, mitomycin, mitoxantrone, and saintopin, which are used clinically in the therapy of solid cancers. Anticancer quinones are currently the focus of intensive research because of their biological activity and complex modes of action, which differ depending on their particular structure. A number of natural and synthetic heterocyclic quinones have important biological activities such as antitumoral, antiprotozoan, and antibiotic activities (Tsizin *et al*., 1978; Bass *et al*., 2013). Many of these compounds possess antineoplastic chemotherapeutic properties (Kock *et al*., 2005). Among them, carbazolequinone alkaloids (Figura 1) exhibit notable biological properties such as cardiotonic, antituberculosis, and neuronal cell-protecting activities (Shin-Ya *et al*., 1997; Kazumi *et al*., 1989; Knölker *et al*., 2003; Choi *et al*., 2006; Choi *et al*., 2008). Pyrido- and quinolinocarbazole alkaloids (Figure 1) are also well-known for their wide range of potent biological activities (Gribble *et al*., 1990; Knölker *et al*., 2008). Ellipticine (5,11-dimethyl-6H-pyrido[4,3- b]carbazole) and 9-methoxyellipticine (Figure 1) were isolated from the leaves of *Ochrosia elliptica Labill* by Goodwin (Goodwin *et al*., 1959). The biological activity of ellipticine was considered to be based mainly on DNA intercalation and topoisomerase II inhibition. The first clinical success of celiptium (Figura 1) (Juret *et al*., 1978; Paoletti *et al*., 1980; Dodion *et al*., 1982; Juret *et al*., 1982; Clarysse *et al*., 1984) led to extensive studies into the synthesis of ellipticinium derivatives, and several of these progressed to clinical trials (Rouesse *et al*., 1985; Ohashi & Oki, 1996). Since the commercialization of some ellipticine derivatives and their successful clinical uses prompted tremendous development in the chemistry and biology of pyridocarbazole alkaloids.

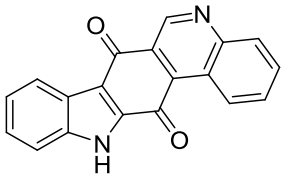
Figure 1
Structure of ellipticine and some analogues



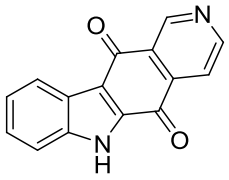
R = H, Ellipticine
R = OMe, 9-Methoxyellipticine



Celiptium



Calothrixin B



Ellipticine quinone

Ellipticine quinone (Gribble *et al*., 1984; Kecha *et al*., 1985; Bennasar *et al*., 2005) is a pivotal synthetic intermediate in the early Gribble syntheses of ellipticines that shows antitumor activity (Bernardo *et al*., 2004).

The only known quinolino[4,3-*b*]carbazole alkaloid, calothrixin B (Figure 1) (7H-indolo[3,2-j]phenanthridine-7,13(12H)-dione), was first obtained from a blue-green algae Calothrix cyanobacteria in 1999 (Rickards *et al*., 1999). It is a pentacyclic quinone that exhibits antimalarial activity as well as activity against human HeLa cancer cells and inhibition of RNA polymerase (Chen *et al*., 2003; Khan *et al*., 2009).

Based on the above precedents and recent results in the high yield synthesis of antiproliferative phenylaminoisoquinoline-5,8-quinones endowed with *in vitro* topoisomerase I inhibition ability (Valderrama *et al*., 2009; Monsalve *et al*., 2012), we were interested to synthesize new isomeric ellipticine quinones to evaluate their in vitro antiproliferative activity on a panel of three cancer cell lines.

MATERIALS AND METHODS

General

All reagents and solvents were commercially available reagent grade. Melting points were determined on a Stuart Scientific SMP3 apparatus and are uncorrected. ^{1}H-NMR spectra were recorded on Bruker AM-200 and AM-400 instruments in $CDCl_3$ + DMSO-d_6. ^{13}C-NMR spectra were obtained at 50 and 100 MHz in $CDCl_3$ + DMSO-d_6. Chemical shifts are expressed in δ ppm downfield relative to TMS, and the coupling constants (J) are reported in Hertz. The HRMS were obtained on a Thermo Finnigan spectrometer, model MAT 95XP. Silica gel Merck 60 (70–230 mesh) was used for preparative column chromatography, and TLC aluminum foil $60F_{254}$ for analytical TLC.

Chemistry

Synthesis of 1,3-Dimethy-l-4-methoxycarbonyl-isoquinoline-5,8-quinone 1:

A suspension of 2,5-dihydroxyacetophenone (1.0 mmol), silver(II) oxide (2.2 mmol), and $MgSO_4$ (1g) and dichloromethane (25 mL) was stirred for 1 h. Silver (II) oxide (2.2 mmol) was added to the mixture and the stirring was continued for 90 min. The mixture was filtered and the solvent was removed to yield crude quinone **1** (231 mg, 94%) which was chromatographed on silica gel (9:1 dichloromethane/ethyl acetate) to yield pure quinone **1** (74%).

Synthesis of 7-aminoisoquinolinequinone derivatives 2a-c:

A suspension of quinone **1** (1 mmol), the required arylamine (2 mmol), $CeCl_3.7H_2O$ (0.05 mmol), and ethanol (20 mL) was left with stirring at rt after completion of the reaction as indicated by TLC. The reaction mixture was partitioned between chloroform/water, the organic extract was washed with water (2 x 15 mL), dried over Na_2SO_4, and evaporated under reduced pressure. The residue was column chromatographed over silica gel (CH_2Cl_2/ethyl acetate 90:10) to yield the corresponding aminoquinones **2a**, **2b** and **2c** in 93%, 89% and 90% yield respectively. The spectral properties of the compounds **2a-c** (^{1}RMN, ^{13}C RMN) were comparable with those previously described for these compounds (Valderrama *et al*., 2006).

Synthesis of pyrido[3,4-b]carbazole-5,11-dione derivatives **3a-c**:

A suspension of the aminoisoquinolinequinone **2a**, **2b** or **3c** (1 mmol), $Pd(OAc)_2$ (1,2 mmol) and glacial acetic (5mL) was refluxed under nitrogen atmosphere after completion of the reaction as indicated by TLC. The reaction mixture was cooled, neutralized with solid sodium hydrogencarbonate and filtered. The filtrate was diluted with water (20 mL) and then extracted with ethyl acetate (2 x 15 mL). The organic extract was chromatographed on silica gel (CH_2Cl_2) to give the pyrido[3,4-*b*]carbazole-5,11-dione derivatives **3a-c**.

Methyl 1,3-dimethyl-5,11-dioxo-10,11-dihydro-5H-pyrido[3,4-b]carbazole-4-carboxylate 3a:

Orange solid (60% yield), mp 108.5-110°C; IR: ν_{max} 3317 (NH), 1725 (C=O), 1652 (C=O quinone); ^{1}H RMN (400 MHz, $CDCl_3$ + DMSO-d_6): δ 2.62 (s, 3H, 3-Me), 3.08 (s, 3H, 1-Me), 4.10 (s, 3H, CO_2Me), 7.43 (m, 2H, arom.), 7.50 (d, *J* = 8.0 Hz, 1H, arom.), 8.26 (d, *J* = 8.0 Hz, 1H, arom.), 9.62 (s, 1H, NH). ^{13}C NMR (400 MHz, $CDCl_3$ + DMSO-d_6): δ 22.7, 26.0, 53.0, 113.8, 116.9, 121.7, 123.2, 124.1, 124.3, 125.8, 127.2, 138.0, 138.5, 139.5, 160.0, 160.6, 169.6, 178.7, 178.7. HRMS (APCI): [M+] calcd for $C_{19}H_{14}N_2O_4$: 334.09536; found: 334.09484.

Methyl 7-methoxy-1,3-dimethyl-5,11-dioxo-10,11-dihydro-5H-pyrido[3,4-b]carbazole-4-carboxylate 3b:

Red solid (53% yield), mp 233-234°C; IR: ν_{max} 3283 (NH), 1726 (C=O), 1667 and 1657 (C=O quinone); ^{1}H RMN (400 MHz, $CDCl_3$ + DMSO-d_6): δ 2.61 (s, 3H, 3-Me), 3.02 (s, 3H, 1-Me), 3.89 (s, 3H, OMe), 4.10 (s, 3H, CO_2Me), 7.06 (d, *J* = 9.0 Hz, 1H, arom), 8.38 (d, *J* = 9.0 Hz, 1H, arom), 9.45 (s, 1H, NH). ^{13}C NMR (400 MHz, $CDCl_3$ + DMSO-d_6): δ 22.8, 30.9, 53.4, 55.8, 103.2, 114.2, 116.9, 119.9, 121.9, 125.3, 126.0, 132.6, 137.3, 139.3, 157.9, 160.5, 160.9, 169.7, 178.1, 178.8. HRMS (APCI): [M+] calcd for $C_{20}H_{16}N_2O_5$: 364.10593; found: 364.10522.

Methyl 6,9-dimethoxy-1,3-dimethyl-5,11-dioxo-10,11-dihydro-5H-pyrido[3,4-b]carbazole-4-carboxylate 3c:

Purple solid (49% yield), mp 245.5 - 247.5 °C; IR: ν_{max} 3424 (NH), 1727 (C=O), 1668 and 1659 (C=O quinone); ^{1}H RMN (400 MHz, $CDCl_3$ + DMSO-d6): δ 2.94 (s, 3H, 3-Me), 3.33 (s, 3H, 1-Me), 3.83 (s, 3H, OMe), 3.90 (s, 3H, OMe), 3.93 (s, 3H, CO_2Me), 6.67 (d, *J* = 8.6 Hz, 1H, arom), 6.88 (d, *J* = 8.6 Hz, 1H, arom), 8.26 (s, 1H, NH). ^{13}C NMR (400 MHz, $CDCl_3$ + DMSO-d6): δ 22.1, 26.0, 53.0, 56.5, 56.7, 104.9, 108.1, 116.3, 125.9 (2C), 130.9, 138.5 (2C), 142.1, 149.3, (2C), 159.4, 159.9, 169.2, 176.6, 178.1. HRMS (APCI): [M+] calcd for $C_{21}H_{18}N_2O_6$: 394.11649; found: 394.11533.

Electrochemical Measurement (Prieto *et al*., 2007)

Cyclic voltammograms of compounds were obtained on a Bioanalytical Sytem BAS CV-50W electrochemical analyzer. A small capacity measuring cell was equipped with a platinum disc as working electrode, an Ag/10 nM Ag (MeCN) reference electrode for non aqueous solvent, with a platinum wire auxiliary electrode, a mechanical mini-stirrer, and a capillary to supply an inert argon atmosphere. A 0.1 M solution of tetrabutyl-ammonium tetrafluoroborate in acetonitrile was used as supporting electrolyte.

Anticancer assay (Rodríguez *et al.*, 1999)

The cell lines used in this work were obtained from the American Type Culture Collection (ATCC, Manasas, VA, USA). They included MRC-5 normal human fibroblasts (CC-171), AGS human gastric adenocarcinoma cells (CRL-1739), SK-MES-1 human lung cancer cells (HTB-58, and J82 human bladder carcinoma cells (HTB-1). After the arrival of the cells, they were proliferated in the corresponding culture medium as suggested by the ATCC. The cells were stored in medium containing 10% glycerol in liquid nitrogen. The viability of the cells after thawing was higher than 90% assessed by trypan blue exclusion test. Cells were sub-cultured once a week and medium was changed every two days. Cells were grown in the following media: MRC-5, SK-MES-1, and J82 in MEM, and AGS cells in Ham F-12. The MEM medium contained 2 mM $_L$-glutamine, 1 mM sodium pyruvate, and 1.5 g/L sodium hydrogencarbonate. Ham F-12 was supplemented with 2 mM $_L$-glutamine and 1.5 g/L sodium hydrogencarbonate. All media were supplemented with 10% heat-inactivated FBS, 100 IU/mL penicillin, and 100 µg/mL streptomycin in a humidified incubator with 5% CO_2 in air at 37 °C. For the experiments, cells were plated at a density of 50,000 cells/mL in 96-well plated. One day after seeding, the cells were treated with the medium containing the compounds at concentrations ranging from 0 to 100 µM during 3 days, and finally the MTT reduction assay was carried out. The final concentration of MTT was 1 mg/mL. The compounds were dissolved in DMSO (1% final concentration) and complete medium. Untreated cells (medium containing 1% DMSO) were used as controls. Each experiment was carried out in sextuplicate.

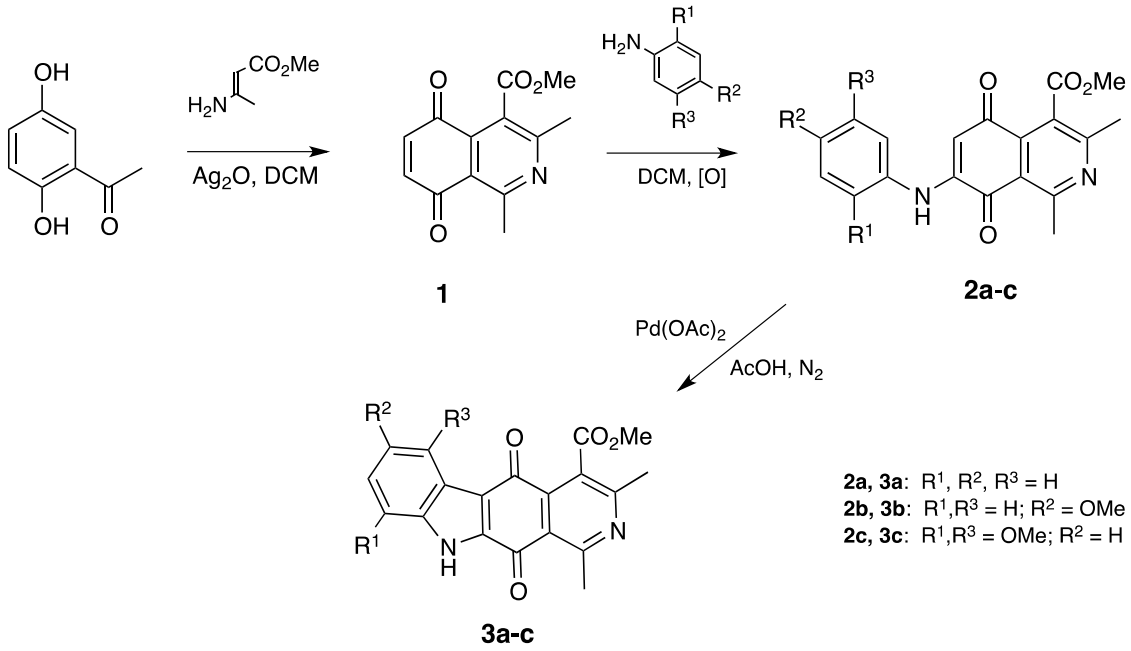
RESULTS AND DISCUSSION

We explore the access to isomeric ellipticine quinones from phenylaminoisoquinolinequinones by using a well-documented carbazolequinone synthetic method based on the palladium-catalyzed intramolecular oxidative coupling reaction of arylamino-1,4-quinones (Akerman *et al.*, 1975). This method has been successfully employed for the synthesis of ellipticine and related alkaloids from diarylamines (Miller & Mook, 1980; Motoi *et al*., 1991; Knölker & Fröhener, 1998). The entry to the target isomeric ellipticine quinones **3a-c** was planned from aminoisoquinoline-5,8-quinones **2a-c** by using the palladium-catalyzed oxidative coupling reaction. The elected aminoisoquinoline-5,8-quinones **2a-c** were prepared from isoquinolinequinone **1** which in turn was synthesized through a previously reported one pot procedure from 2,5-dihydroacetophenone, methyl aminocrotonate and silver (II) oxide (Valderrama *et al.*, 2006). Scheme 1 outlined the reaction sequence to prepare the ellipticine quinones **3a-c**.

The oxidative cyclization of **2a** to pyridocarbazolquinone **3a** was examined by using stoichiometric amounts of $Pd(OAc)_2$ in glacial acetic acid at reflux. After several trials the cyclization products **3a-3c** were isolated in 60, 53 and 49% yields respectively.

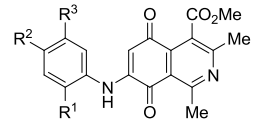
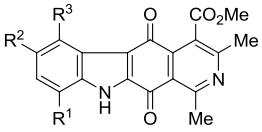
The structure of the new compounds **3a-c** were fully established by mean of their $^1H/^{13}C$ NMR and high resolution mass spectra.

Scheme 1
Synthesis of isomeric ellipticine quinones 3a-c



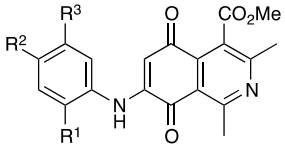
The redox potentials of compounds **3a-c** were measured by cyclic voltammetry in acetonitrile as solvent, at room temperature, using a platinum electrode and 0.1 M tetraethylammonium tetrafluoroborate as the supporting electrolyte (Prieto *et al*., 2007). All compounds show two one-electron reduction waves to form the corresponding anion-radical and dianion. The first half-wave potential values, $E^{I}_{1/2}$, evaluated from the voltammograms, are summarized in Table 1. The data indicate that the $E^{I}_{1/2}$, values for the first electron, which is related with the formation of the semiquinone radical anion, are in the potential range 578-624 mV. Comparison of the half wave potentials of **3a** and **3b** with those of their respective precursors **2a** and **2b** indicate that reduction of the products are located at a more positive region with respect to their precursors. The results revealed that the donor-acceptor interactions between the isoquinolinequinone nucleus (acceptor) and the arylamine group (donor) in **2a** and **2b** is more favorable than that of the acceptor with the fused indole fragment in compounds **3a** and **3b**. In the case of quinone **3c** and its precursor **2c** it was observed that the interaction of the acceptor with their respective donors is more favorable in **3c** than **2c**.

Table 1
Electrochemical potentials of compounds 2a-c and 3a-c.

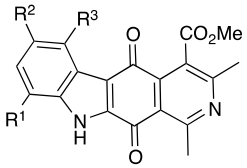
Structure	Compound	R^1	R^2	R^3	$-E^I_{1/2}$ (mV)
	**2a**	H	H	H	592
	2b	H	OMe	H	622
	2c	OMe	H	OMe	573
	**3a**	H	H	H	578
	3b	H	OMe	H	588
	3c	OMe	H	OMe	624

The newly synthesized isomeric ellipticine quinones **3a-c** were evaluated for their *in vitro* anticancer activities against human normal cell: MRC-5 human lung fibroblasts and three human tumor cells: AGS gastric adenocarcinoma, SK-MES-1 lung cancer, and J82 bladder carcinoma, in 72 h drug exposure MTT assays. Etoposide, a clinically used anticancer agent, was used as a positive control. The antiproliferative activity of the compounds was measured using a conventional microculture tetrazolium reduction assay (Scudiero *et. al.*, 1988; Van de Loosdrecht *et al.*, 1994). The antiproliferative activities by each of the heterocyclic quinones are expressed in terms of IC_{50} (Table 2). The previously reported antiproliferative activity of arylaminoisoquinolinequinone **2a-c** were included in Table 2 together with those of their cyclization products **3a-c** to get information on the differences in the antiproliferative activity as consequence of the eventual redox cycling and/or DNA-binding biological mechanism.

Table 2
Antiproliferative activity of isomeric ellipticine quinones 3a-c and its precursors 2a-c



2a-c



3a-c

N°	R^1	R^2	R^3	IC_{50} (μM)[a] MRC-5[b]	AGS[c]	SK-MES-1[d]	J82[e]
2a	H	H	H	5.6	2.1	4.2	5.8
2b	H	OMe	H	9.0	2.3	7.2	7.4
2c	OMe	H	OMe	31.3	19.9	>100	>100
3a	H	H	H	2.4	1.2	3.9	2.9
3b	H	OMe	H	2.5	2.4	7.5	2.4
3c	OMe	H	OMe	>100	38.6	33.9	>100
-	Etoposide			3.9 ± 0.2	0.36 ± 0.02	2.5 ± 0.2	2.8 ± 0.2

[a] **Data represent mean average values for six independent determinations.**
[b] **Human lung fibroblasts cells.**
[c] **Human gastric adenocarcinoma cell line.**
[d] **Human lung cancer cell line.**
[e] **Human bladder carcinoma cell line.**

The screening showed that compounds **3a-c** exhibit significant antitumor activity in the range IC_{50}: 1.2-38.9 µM. As indicated in Table 2, the antitumor activity of compounds **3a** and **3b** on bladder cancer cells were comparable to that shown by the reference drug etoposide (IC_{50} = 2.8 µM). Comparison between the IC_{50} and $E^{I}_{1/2}$ values, indicate that for compounds **3a** and **3b**, the more positive the $E_{1/2}$ (respect to compounds **2a** and **2b**) the stronger the antitumor promoting effect on AGS, J82 and SK-MES-1 cell lines. On the contrary, **3b** shows less cytotoxic activity in all the cell lines compared to **2b**. Analyses of the data revealed that the first reduction potential $E_{1/2}$ is an important parameter determining the antitumoral activity on AGS gastric adenocarcinoma, SK-MES-1 lung adenocarcinoma and J82 bladder carcinoma cell lines.

CONCLUSIONS

In conclusion, we have described preliminary results on the synthesis and antiproliferative evaluation of three new isomeric ellipticine quinones The new quinones expressed moderate to high *in vitro* antiproliferative activity against three human cancer cell lines: AGS (gastric), SK-MES-1 (lung) and J82 (bladder) cell lines. Compound **3b** appears as a promising active compound against bladder cancer cell line, with IC_{50} value at 2.4 µM, comparable to that of the anti-cancer agent etoposide.

ACKNOWLEDGEMENTS

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