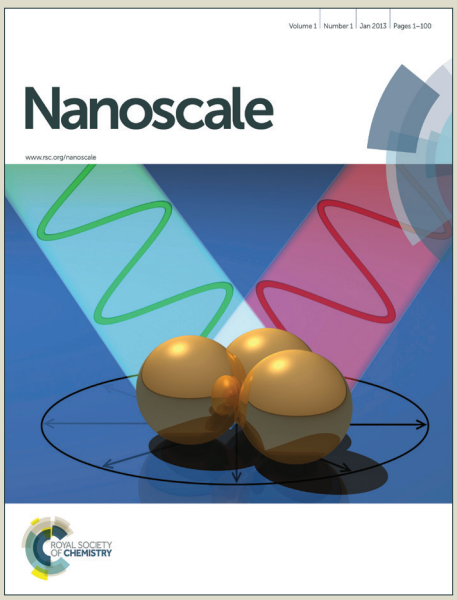
Nanoscale

Accepted Manuscript



ROYAL SOCIETY OF CHEMISTRY

www.rsc.org/nanoscale

Investigations in dendrimer space reveal solid and liquid tumor growth-inhibition by original phosphorus-based dendrimers and corresponding monomers and dendrons with ethacrynic acid motifs

Nabil El Brahmi[a,b], Serge M. Mignani*[,c] Joachim Caron[a,b], Saïd El Kazzouli[b], Mosto M. Bousmina[b], Anne-Marie Caminade[a], Thierry Cresteil[d], Jean-Pierre Majoral*[,a]

[a] *Laboratoire de Chimie de Coordination du CNRS, 205 route de Narbonne, BP 44099, 31077 Toulouse Cedex 4. France.*
[b] *Euro-Mediterranean University of Fez, Route de Sidi Hrazem, Fès Shore, 30070 Fès, Morocco.*
[c] *Université Paris Descartes, PRES Sorbonne Paris Cité, CNRS UMR 860, Laboratoire de Chimie et de Biochimie pharmacologiques et toxicologique, 45, rue des Saints Pères, 75006 Paris, France.*
[d] *IPSIT, IFR141, Université Paris Sud, 5 rue Jean Baptiste Clément, 92290 Chatenay-Malabry, France.*

ABSTRACT

The well-known reactive diuretic Ethacrynic acid (**EA**, Edecrin), showing low antiproliferative activities, was chemically modified and grafted on phosphorus dendrimers and corresponding simple branched phosphorus dendrons-like affording original nanodevices showing moderate to strong antiproliferative activities against liquid and solid tumor cell lines, respectively.

KEYWORDS

Ethacrynic acid, phosphorus dendrimers, branched phosphorus dendrons-like, antiproliferative activity, tumor cell lines

1. Introduction

Ethacrynic acid (**EA**, Edecrin) is a well-known reactive diuretic used in the treatment of high blood pressure and swelling caused by diseases such as congestive heart failure, liver failure, and kidney failure.[1] It has proven particularly effective in patients with refractory edema or with edematous states accompanied by azotemia or electrolyte disturbances, and may also effectively reduce elevated intraocular pressure, highlighting its potential usefulness in the treatment of glaucoma.[2] **EA** mainly acts by inhibiting sodium reabsorption along the ascending loop of Henle, producing a transient slight increase in both glomerular filtration rate and renal plasma flow, which is then followed by their decrease, diuresis and dehydratation.[3]

As an unsaturated ketone derivative (a Michael acceptor) of an aryloxyacetic acid, **EA** reacts with nucleophiles, such as thiols for instance, which add to the α,β-unsaturated carbonyl unit at the β-carbon position of the **EA**. Interestingly, **EA** binds competitively to and potently inhibits at the H-site of glutathione S-transferase P1-1 (GSTP1-1, GSTpi), which is overexpressed in a variety of cancer cells.[4] This enzyme catalyzes the conjugation of reduced glutathione with a broad range of substrates, including chemotherapeutic agents, and acts as a detoxification enzyme. A high concentration (>50μM) of **EA** has been shown to inhibit cell growth and induce apoptosis in several cancer cells. This would suggest that GSTP1-1 inhibitors like **EA** could have therapeutic potentials in cancer [4,5] by; 1) reversing drug resistance; 2) sensitizing to chemotherapeutic agents; and 3) inducing malignant cell death directly. In addition, **EA** induces cell death through the oxidative stress resulting

from the depletion of glutathione (GSH) as well as through the activation of the MAPK pathway. It has been shown to potentiate the cytotoxic effect of chemotherapeutic agents such as cisplatin, chlorambucil, melphalan, mitomycin C, and doxorubicin *in vitro,*[6] and lenalidomide and thalidomide *in vivo*[7], and is now the focus of a Phase I clinical trial in combination with, as the alkylating agent, triethylene-thiophosphoric acid triamide (Thiotepa) for advanced cancer treatment (malignant solid tumors).[8]

Interestingly, **EA** is efficacious in primary cultures derived from patients with chronic lymphocytic leukemia (CLL) through the inhibition of the Wnt/β-catenin signaling pathway.[9] Based on these studies, I. G. H. Schmidt-Wolf and co-workers studied the *in vivo* antitumor effect of orally administered **EA** at high dose (135mg/mouse/day corresponding to ~5.4g/kg/day) in a murine myeloma model (BALB/c mice).[10] After 60-day treatment at this dose, tumor growth had significantly reduced *versus* control. However, a lower dose of **EA** (75mg/mouse/day) alone showed an insufficient anti-tumor effect that was similar to that achieved using the thalidomide analog lenalidomide (~200mg/mouse/day). This immunomodulatory agent is commonly used in patients with myeloma for its antiangiogenic and antineoplastic properties. Combination of lenalidomide (~200mg/mouse/day) and **EA** (75mg/mouse/day) permitted sufficient reduction of tumor growth.

Several **EA** analogs have been synthesized with the principal aim of improving the physicochemical properties (PK/PD behavior) of **EA** and ultimately enhancing its antiproliferative activity and capacity to inhibit GSTpi activity. An overview of the main modifications performed is presented in Figure 1, an example of which is modification of the carboxylic acid function (pKa ~2.8) which conferred poor cell penetration.

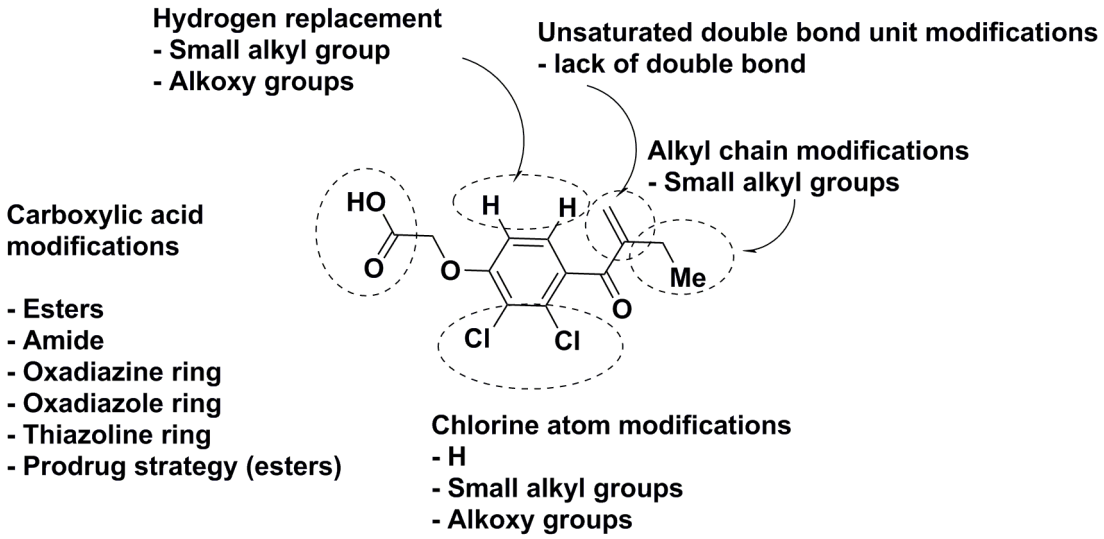


Figure 1. Overview of possible modifications on ethacrynic acid skeleton

The structural modifications implemented to enhance the antiproliferative activity of **EA** concerned either the carboxylic functional group or the unsaturated double bond unit.

a) Carboxylic group modifications: formation of simple amides[11], aryl amides[11,12], alkyl aryl amides[11,12], formation of oxadiazole[13] and thiazole analogs.[14] Both the modification of the carboxylic function into esters - with small alkyl groups - and alkyl chain in α–position of the double bond by linear alkyl chains have been done.[15] Esters formed from atenolol or timolol in a prodrug approach have also been described as potential novel antiglaucoma agents.[16]

b) Modifications of the unsaturated double bond unit: replacement of the double bond by a simple alkyl chain and introduction of either small alkoxy groups or small alkyl groups in place of the two chlorine atoms or of the hydrogen atoms on the phenyl ring of **EA**.[17] These simplified **EA** derivatives displayed either antiproliferative or anti-metastatic activities in wound healing assays though never both. For instance, the simplified **EA** derivative *para*-acyled *m*-cresol inhibited migration in two different tumor cell lines, C4-2B prostate cancer and Hs578Ts breast cancer, with a half-maximum inhibitory concentration (IC_{50}) of respectively 10µM and 80µM, though displaying no antiproliferative activity. Similarly, 2-(2,6-dimethoxy-4-propionylphenoxy)acetic acid inhibited the migration of human MCF-7/AZ breast cancer cells by ~50%, without any antiproliferative activity.

Simple **EA** modifications such as the replacement of the carboxylic function by various esters, thiazole or oxadiazole heterocycles, produced moderate to good antiproliferative activities against several tumor cell lines such as CLL, human lung adenocarcinoma A549, human breast cancer MCF7, T47D and MDA-MB-231, human leukemia HL-60, human prostate carcinoma PC-3, androgen-independent prostate cancer DU145.[13-15] The analogs displayed a much improved average antiproliferative activity with an IC_{50} ranging from 1 to 20µM, by comparison with the ~50µM for **EA**. Among them, the most promising **EA** derivatives are within the oxadiazole series and are shown in the Figure 2. G-S. Zhao, X-L. Guo et al.[13] designed compounds **1** and **2** which displayed interesting *in vitro* antiproliferative activities against several tumor cell lines (HL-60, PC-3, DU145, T47D, MCF-7 and MDA-MB-231) with concentration inhibiting half of cell growth (GI_{50}) ranging from 1.4 to 4µM, and from 1.5 to 4.5µM, respectively. Interestingly, both oxadiazole derivatives showed ~2 times less potency against human mammary epithelial MCF10A cells and both inhibited GSTP1-1 activity at the µM range (IC_{50} ~3-4µM). Compound **1** administered intravenously into nude mice at a dose of 8mg/kg, inhibited *in vivo* growth of SW620 human colon cancer xenografts affording ~44% reduction of tumor volume after 17 days. These effects were associated with S-phase arrest and the induction of cell apoptosis through an increased ratio of cellular Bax/bcl-2 expression, the release of cytochrome-c and activation of caspase-3. A similar *in vivo* antitumor effect was found by administration of 5-Fu (15 mg/kg).[13b]

The **EA** amide derivatives **3** and **4** (Figure 2) have been shown to display growth inhibiting activity against CLL, with an EC_{50} of ~2µM *versus* 10µM for **EA**. Similar to **EA**, both compounds inhibited the Wnt signaling pathway with an IC_{50} of 4µM.[12] Other **EA** analogs bearing ester functions have also been described. The most potent anticancer compounds are the compounds **5** and **6** which showed a GI_{50} of 5.2µM and 3.9µM, respectively, against human leukemia HL-60 cells.[15]

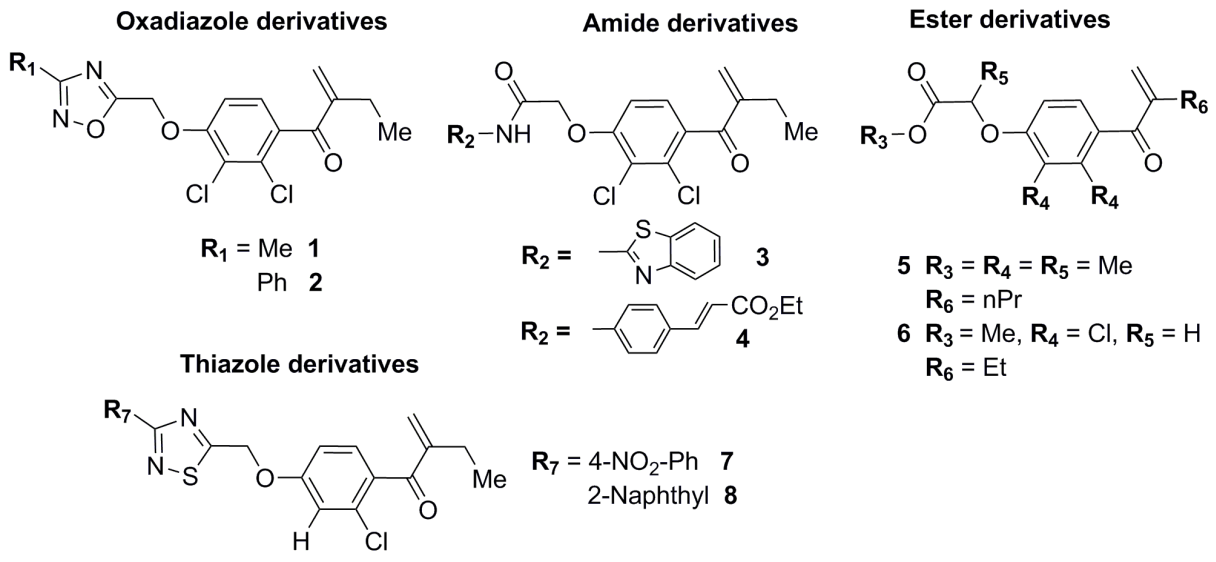


Figure 2. Structure of several EA derivatives used as anticancer compounds

Replacement of the carbonyl function by a thiazole ring has also been highlighted as an approach to improve the ability to inhibit GSTpi activity.[14] For these authors, the most potent compounds have been **7** and **8**, which inhibited GSTpi respectively at concentrations of 5µM and ~10µM, and HL-60 cell proliferation with an IC_{50} of ~1µM (Figure 2). In addition, E. G. Yang et al. highlighted that **EA** could inhibit the hypoxia inducible factor (HIF) pathway by disrupting HIF-1α interaction with CH1/p300 at a dose of ~9µM (IC_{50}).[18] The blockade of the HIF pathway resulted in the down-regulation of VEGF expression and thus represents an interesting new strategy to tackle tumor angiogenesis and metastasis.

Though rare, malignant pleural mesothelioma (MPM) has poor prognosis and is a major concern to its resistance to chemotherapy and radiation treatment. The incidence of this aggressive tumor estimated to double over the next 20 years in many countries.[19] Platinum analogs, doxorubicin and some antimetabolites (methotrexate, raltitrexed, pemetrexed) have shown modest single-agent activity, though their combination offers better results.[19c] For instance, pemetrexed and cisplatin has been shown to improve survival as well as lung function and symptom control by comparison with cisplatin alone. The combination of pemetrexed and carboplatin is also an alternative effective therapy. D. Osella et al. described the synthesis and the antiproliferative activities of Pt(II) and Pt(IV) complexes (**9** and **10**) containing two EA moieties as leaving groups to decrease the intrinsic resistance of Pt complexes related to the action of GST (Figure 3).[20] These two complexes showed poorer cytotoxic activities *versus* cisplatin either alone or in combination with **EA**, yet improved activity compared to carboplatin alone against three primary cell lines derived from pleural effusion of previously untreated patients suffering from MPM and one cisplatin-resistant cell line. Cellular GST activity remained unchanged, while GSH level increased.

Furthermore, several **EA RAPTA** derivatives (for instance **11** and **12**) have been synthesized and shown to be excellent GST P1-1 inhibitors (Figure 3).[21]

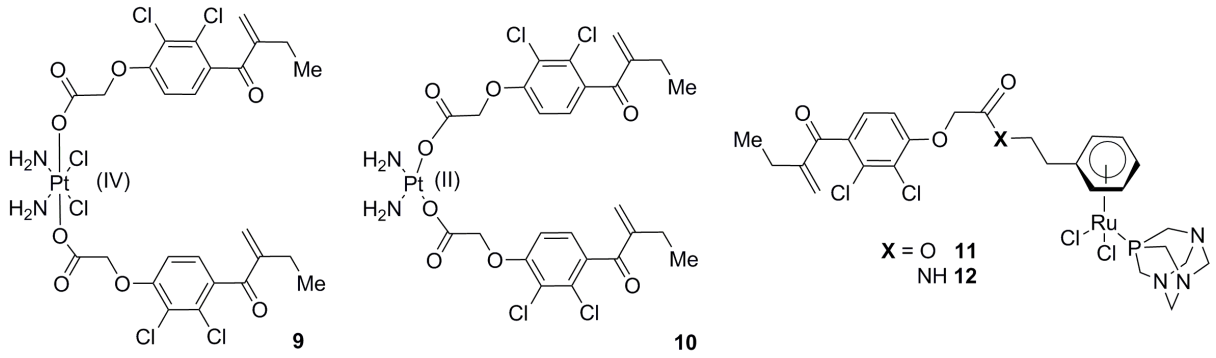


Figure 3. Pt(II), Pt(IV) and Ru ethacrynic acid complexes

With the above mentioned encouraging results in mind, we decided to head out in a new exploratory direction and to prepare original **EA** derivatives using dendrimers[22] and dendrons[23] as scaffolds in the development of new anticancer agents.

Dendrimer and dendron nanostructures represent ideal delivery vehicles and hold great promise for the future in nanomedicine. Dendrimers (from the Greek words “dendri” meaning tree and “meros” meaning part) are a family of nanosized macromolecules, characterized by a highly homostructurally branched 3D-architecture and compact spherical geometry in solution. The fine-tuning of the chemical modifications made to dendrimers in turn modifies dendrimer composition, architecture and properties both *in vitro* and *in vivo*, such as their biocompatibility with cells and tissues and their

PK/PD behavior. As reviewed by Gajbhiye et al.[22e], dendrimers can be employed as drugs *per se* in different therapeutic fields, as anticancer agents, anti-prions, anti-Alzheimer's agents, anti-coagulants, antidotes, antioxidants, anti-inflammatories etc, although to date, very few dendrimers have reached clinical trial phase. Several reviews including ours provide an overview of the main biological applications of dendrimers in oncology.[24] In a nutshell, several types of dendrimers have been developed, such as PAMAM, PPI, carbosilane, triazine, polyether, polylysine, viologen, and phosphorus dendrimers. Among the latter type, J-P. Majoral and A-M. Caminade et al. reported the preparation of different polycationic phosphorus dendrimers that were biologically active against, for instance, the prion peptide PrP 185-208 (Creutzfeld-Jakob disease), the Aβ 1-28 peptide (Alzheimer's disease) or for diagnosis purpose. Polyanionic phosphorus dendrimers have been shown to dramatically amplify the number of natural killer (NK) cells (first line of human immune defense) among the peripheral blood mononucleated cells (PBMCs) as well as reduce inflammation and osteoclastogenesis.[25]

We recently introduced the "dendrimer space concept" as a new paradigm for medicinal chemists to envisage and find original drug-based dendrimers.[26] Inspired by the concepts of druglikeness' and 'druggability' – both terms fully integrated practically into the drug discovery process – the dendrimer space thus defines a new 'druggable' cluster included in the vast volume of chemical space.

Herein, we report the synthesis and characterization of novel and biocompatible multivalent phosphorus dendrimers - whole or highly fragmented (called dendrons-like) - that have been covalently grafted with EA moieties on their surface, and their antiproliferative activity against cancer cell lines as KB and HL-60 cells lines and quiescent endothelial progenitor cells EPC.

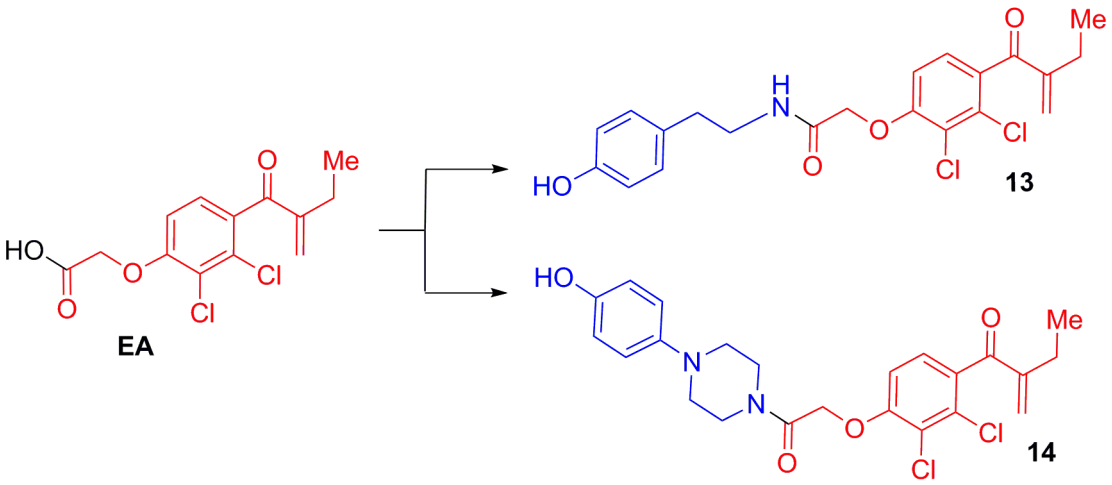
2. Results and Discussion

With the antiproliferative activity (*vide supra*) of **EA** in mind, we wished to find and to develop original macromolecular **EA** derivatives. To this end, we focused our attention on grafting **EA** moieties on phosphorus dendrimers and to corresponding simple branched phosphorus derivatives. To the best of our knowledge, no dendrimer or dendron bearing **EA** moieties have been described to date, and our approach represents a novel route towards new anticancer agents.

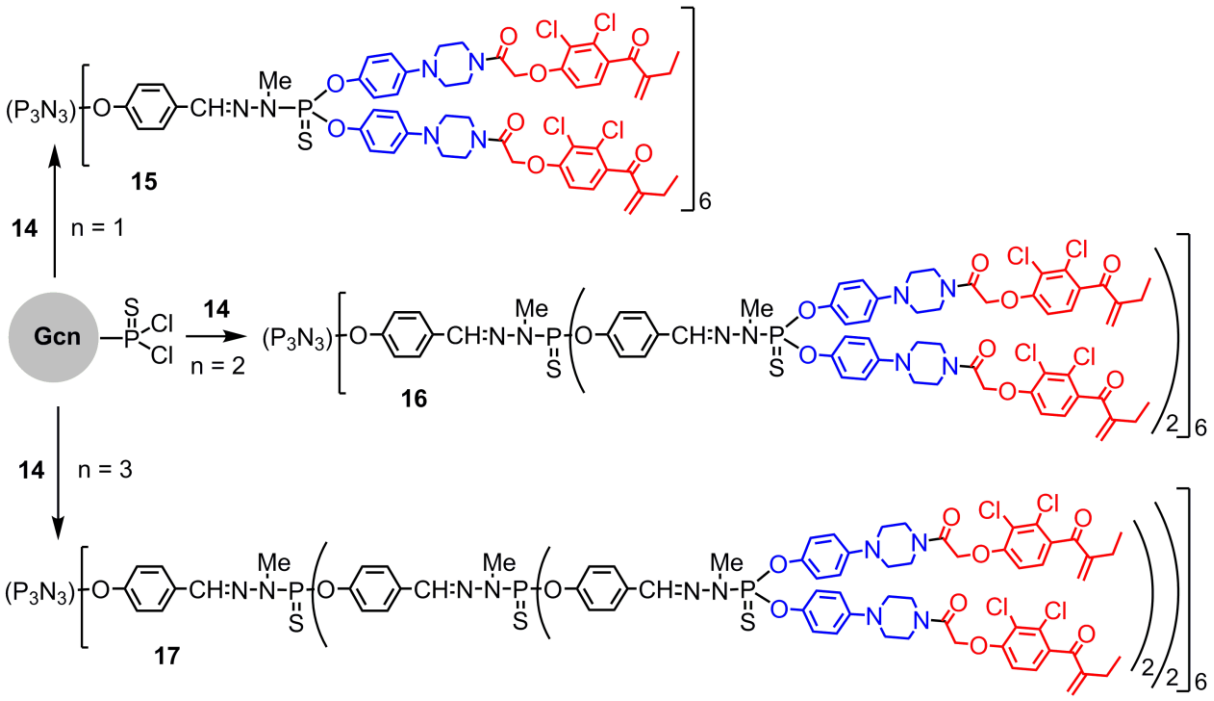
First the novel **EA** amide derivatives **13** and **14** (Scheme 1) resulting from a peptide coupling reaction between **EA** and either tyramine or phenolpiperazine were prepared (cf. supplementary material). This choice was based on the good antiproliferative activities (*vide supra*) of **3** and **4,** each one of them bearing an amido linkage (Figure 2). The presence of the phenol function on **13** and **14** was mandatory since it allows the specific nucleophilic substitution of this group with thio or oxo phosphoryl di- or trichloride derivatives as well as with the terminal hydrazido thiophosphoric dichloride groups linked at the surface of phosphorus dendrimers of generation 1 to 3, with almost quantitative yields. A total of 13 monomers and dendritic **EA** derivatives were thus prepared (Schemes 1-3). The full structure of compound **17** is depicted in Figure 4.

Interestingly, using MTS assays, compared to the modest antiproliferative activity shown by **EA** against KB and HL-60 cancer cell lines (IC_{50s} of ~10-40µM), the **EA** derivatives **13** and **14** displayed potent antiproliferative activity against both cell lines with IC_{50s} of ~400nM and ~800nM, respectively. Similarly, while **EA** displayed no antiproliferative activity against EPC quiescent cells (endothelial progenitor cells, Cyprinus carpio) (IC_{50} > 100µM), **13** and **14** showed low potency with IC_{50s} ~3µM. The 'safety ratio' for **13** and **14**, defined as IC_{50} EPC/IC_{50} KB or HL-60, was ~5-9 for **13** and ~4-8 for **14**, respectively, suggesting their specific action on rapidly proliferating cells, for example, cancer cells (table 1).

These promising results prompted us to compare against **EA** alone the antiproliferative activity on solid and liquid tumor cells of different generations (G_n = 1-3) of phosphorus dendrimers with 12 to 48 **EA** moieties on their surface, and their corresponding branched based phosphates and thiophosphates bearing either three or two **EA** moieties. These results revealed preliminary structure-activity relationships between cell growth inhibition and the structure and composition of dendrimers, or simplified dendron-based phosphate or thiophosphate. In addition, we tested the most potent derivative on quiescent EPC cells in order to define a "safety ratio". All antiproliferative results and total of **EA** monomers and dendritic derivatives prepared are shown in Table 1.



Scheme 1. Synthesis of the **EA** derivatives **13** and **14** from **EA** and tyramine or phenolpiperazine



Scheme 2. Schematic representation of the dendrimers of generation 1 - 3 decorated with 12, 24 or 48 **EA** derivatives.

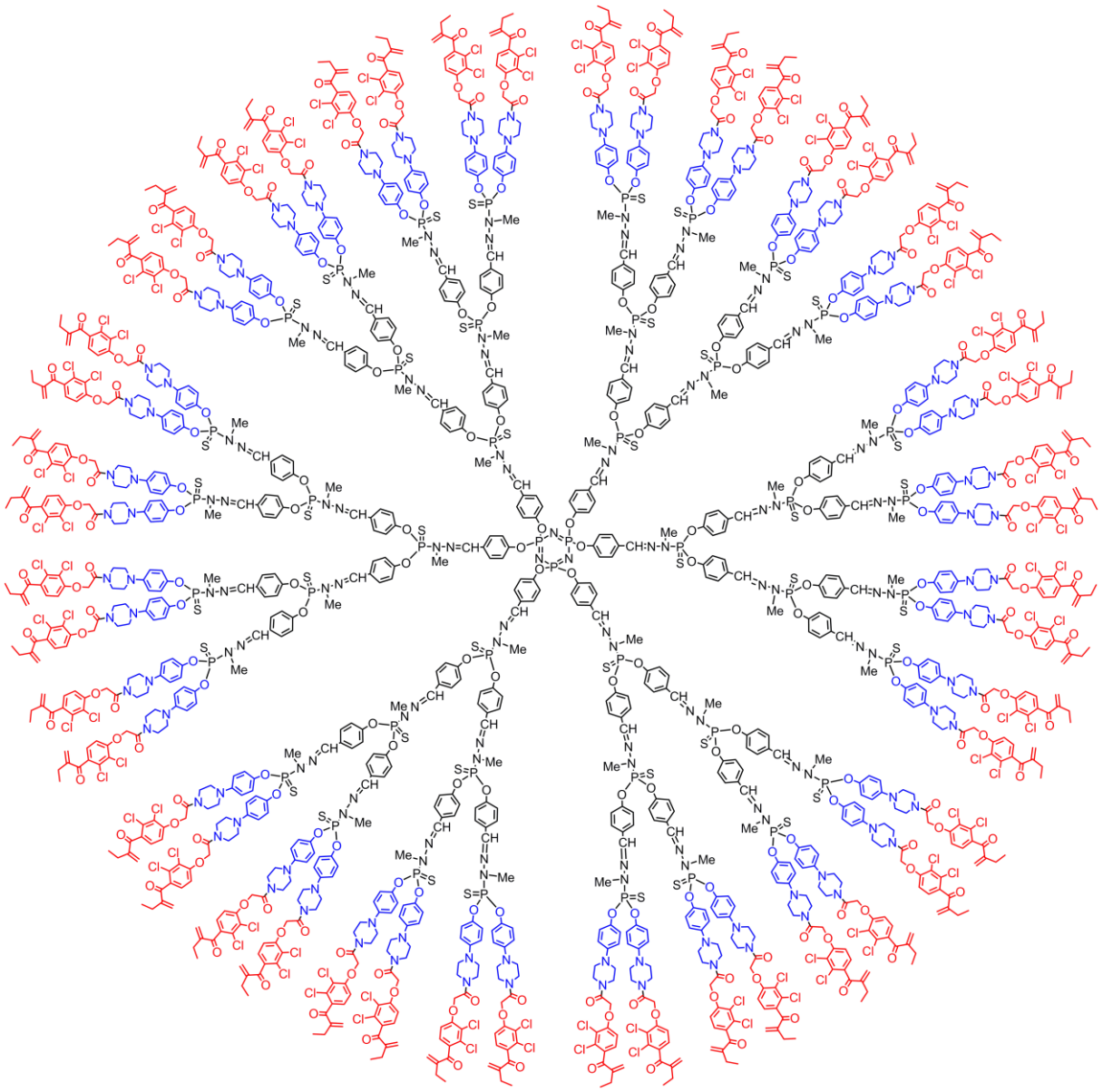
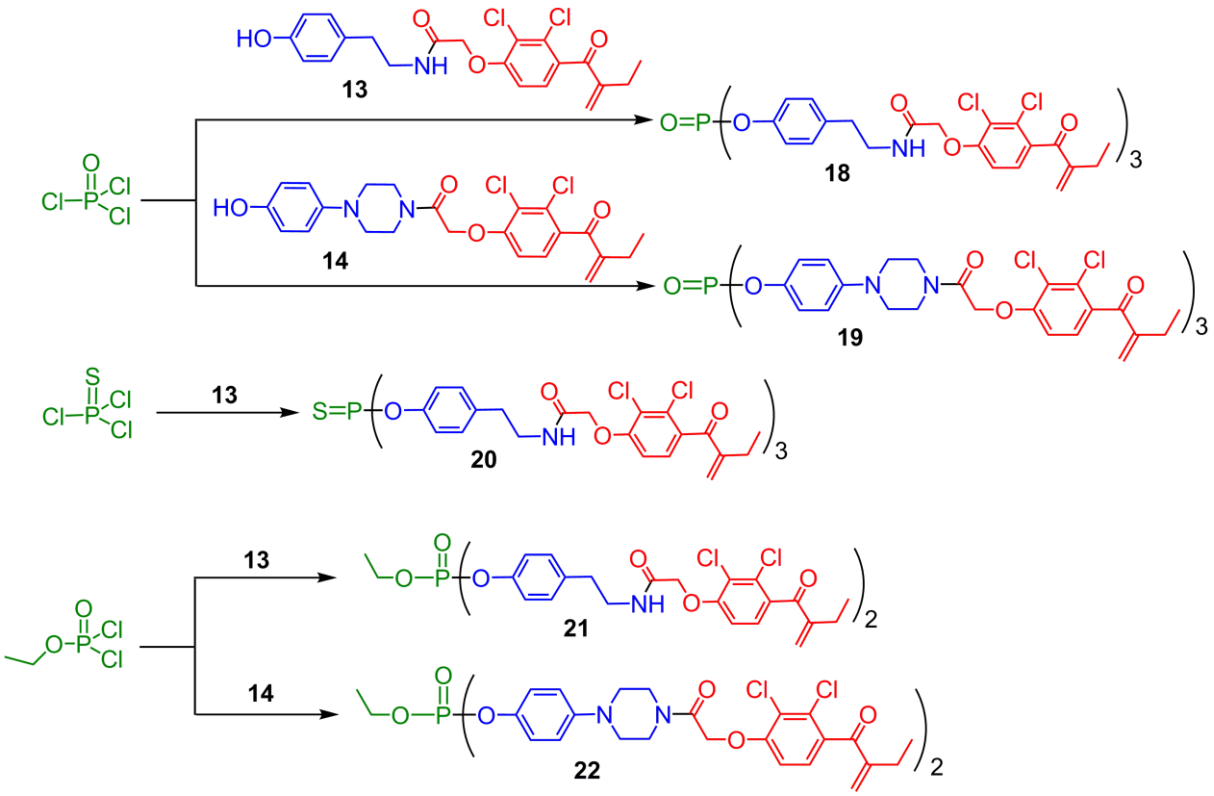


Figure 4. Full structure of compound **17**.



Scheme 3. Synthesis of dendron-like structures

Cpds	Inhibition of cell proliferation at 1μM (%) (at 10μM)			IC_{50}* (μM)		
	KB	HL-60	EPC	KB	HL-60	EPC
EA	0±1% (28±14%)	0±10% (0±7%)	20±2% (17±3%)	11±2	37±1	>100
13	65±4% (100±1%)	35±5% (100±1%)	40±5% (96±1%)	0.40±0.06	0.72±0.07	3.6±0.1
14	95±1% (100±1%)	54±8% (100±1%)	0±1% (97±1%)	0.42±0.02	0.84±0.14	3.2±0.1
15	30±4% (91±4%)	11±2% (75±3%)	0±1% (0±10%)	1.2±0.1	6±1	>100
16	61±4% (93±1%)	17±1% (72±1%)	0±4% (0±4%)	0.7±0.1	5±1	>100
17	78±10% (93±2%)	3±6% (66±1%)	0±1% (0±10%)	0.10±0.03	4±3	>100
18	0±14% (0±8%)	0±12% (0±9%)				
19	4±2% (0±5%)	0±4% (0±4%)				
20	0±8% (6±14%)	0±6% (0±9%)	0±7% (0±1%)	>100	>100	>100
21	80±2% (100±1%)	88±2% (100±1%)	10±11% (32±13%)	0.35±0.01	1.2±0.1	17±5
22	0±10% (19±14%)	43±1% (83±3%)	9±6% (13±7%)	8.5±1.5	6±1	>20

*Half maximal inhibitory concentration. Results are given as the mean ±SE of three individual determinations.

Table 1. Antiproliferative activities of monomeric, branched or dendritic ethacrynic acid derivatives

Dendrons-like derivatives

None of the dendrons with three branches each bearing one **EA** moiety, that is to say **18**, **19** or **20** displayed antiproliferative activity whatever the nature of the branch (4-(2-aminoethyl)phenoxyl or 4-(piperazin-1-yl)phenoxyl) and phosphate or thiophosphate as scaffolds.

Interestingly, the replacement of one branch bearing the **EA** derivative moiety of the inactive compound **18** by a simple ethoxy group affording the compound **21**, strongly increased the antiproliferative potency against KB with an IC_{50} of 340nM. However, **21** showed low antiproliferative activity against HL-60 (IC_{50} = 2.8µM) and the EPC cell line (IC_{50}≈10µM). Nevertheless a 'safety ratio' of ~30 supported the potential safety of this compound (Table 1). Surprisingly, the replacement of the 2-phenylethanamine chain of **21** by a 1-phenylpiperazine linkage (compound **22**) decreased the antiproliferative activity against KB by ~25 times and against HL60 by ~2 times. Antiproliferative activity against EPC cell line remained low (IC_{50} >20µM).

Phosphorus dendrimers

Spurred on by these encouraging results - precisely the high antiproliferative activity of **21** bearing an NH-amido linkage - we prepared the corresponding dendrimers of generation 1, 2 and 3 incorporating 12, 24 or 48 **EA** derivatives on their surface and 12, 24 and 48 amido units (NH-C=O). Unfortunately, these dendrimers soluble as 10 mM stock solution in DMSO were strongly insoluble and precipitate in the aqueous culture medium, and consequently could not be accurately tested. Therefore, the antiproliferative activity of the analogous dendrimers (generation 1-3) but owing the piperazine linkages (dendrimers **15**, **16** and **17**) were tested against both KB and HL-60 cell lines. None of these dendrimers proved problematic with regards to solubility during the MTS assays.

At 1 µM, dendrimers **15**, **16** and **17** displayed moderate-to-good inhibitory activity (30-78%) against KB, and low inhibitory activity against HL-60 (3-11%). The corresponding values for IC_{50} ranged from 1.2 µM to 120 nM for KB, and were all ~4 µM for HL-60. Taken together, these results suggest a greater potency of all dendrimers studied against solid tumors as compared to that against liquid tumors (HL-60).

As shown in Figure 5, we found a good linear curve fit between the growth inhibitory effects (IC_{50}) against the KB cell line and the number of **EA** moieties (y = -29.76 × (Nbr of terminal groups on the dendrimer) + 1500, R^2 = 0.98). Consequently, each terminal group – or several of them - participated proportionally to the global activity of the dendrimer. Interestingly, all these phosphorus dendrimers whatever their generation Gn displayed low inhibitory activity (IC_{50} > 100µM against quiescent EPC cells.

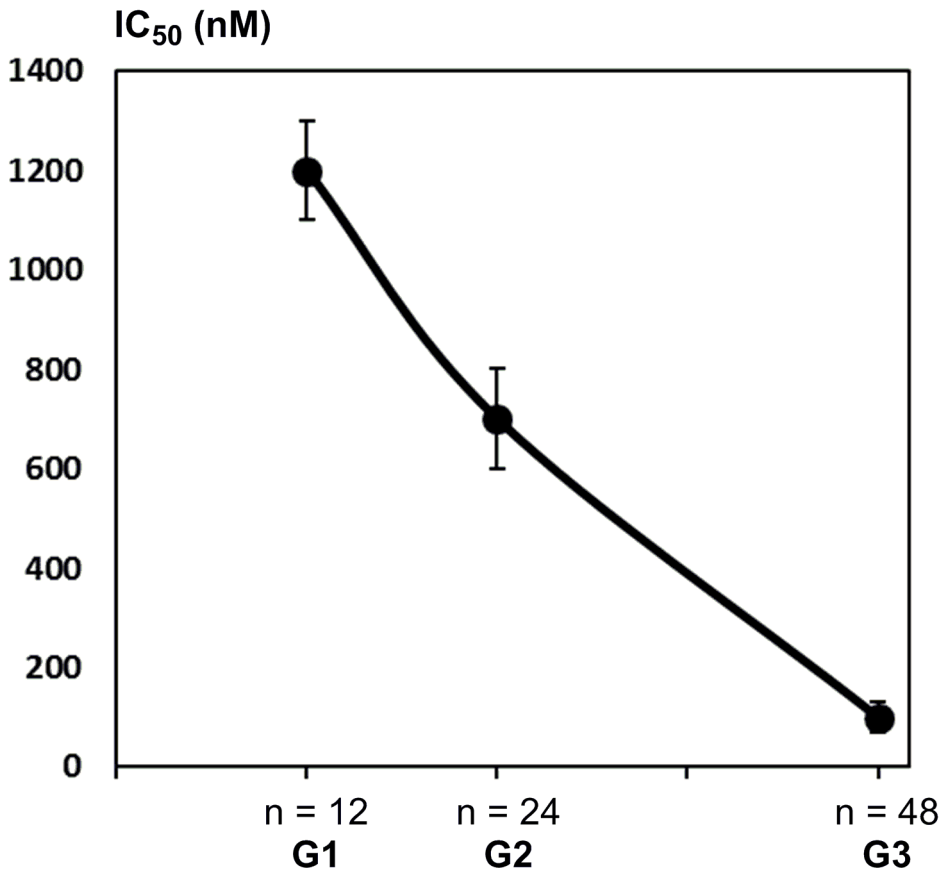


Figure 5. Antiproliferative activity (IC_{50} nM) of dendrimers (**15** (n = 12, G1), **16** (n = 24, G2) and **17** (n = 48, G3)) in KB cells versus number of terminal EA moieties

3. Experimental section

The preparation of the macromolecular **EA** derivatives starts from the simple and useful condensation of new phenol substituted by **EA** derivatives **13** and **14** (Scheme 2) with either phosphorus dendrimer skeleton bearing several - the number depending of the generation of the dendrimer - phosphonothioic dichloride moieties or phosphoryl or thiophosphoryl (di) or (tri)chloride (scheme 2). (cf supplementary information for synthesis and characterization of all these molecular and macromolecular ethacrynic derivatives). Antiproliferative activity was tested as previously described. [27]

4. Conclusion

In conclusion, we prepared a series of original **EA**-derivative based on both phosphorus dendrimers and corresponding simple branched phosphorus dendrons-like. Interestingly, in the dendrimer series, the dendrimer **17** (generation 3) displayed potent antiproliferative activity (IC_{50} ~120nM) against the solid tumor KB cell line and low activity against the liquid HL-60 cell line. Tests against EPC cells revealed a very good 'safety ratio'.

Within the corresponding series of prepared dendrons, the dendron **21** showed good antiproliferative activity (IC_{50} ~340nM) against the solid tumor KB cell line, and again low activity against liquid HL-60 cell line (~8 times lower); the 'safety ratio' was also very good against EPC cells.

Taken together, these first results prompted us to pursue our effort to prepare novel series of **EA** derivatives – grafted on dendrimers or not - as potent anticancer agents. Up-to-date, **EA** derivatives displayed low antiproliferative potency. Consequently, **17** and **22** represent good starting points for the development of new anticancer agents.
In addition, considering the generally successful clinical translation of dendrimers/dendrons with good pharmacodynamic and pharmacokinetic behavior (PK/PD), we anticipate that these dendrimers and dendrons are constituting a new group of antitumor candidates.

Notes and references

1. a) G. Zhao, T. Yu, R. Wang, X. Wang and Y. Jing, *Bioorg. Med. Chem*., 2005, **13**, 4056-4062 b) G. Zhao, C. Liu, R. Wang, D. Song, X. Wang, H. Lou and Y. Jing, *Bioorg. Med. Chem*., 2007, **15**, 2701-2707 and references cited therein

2. a) J. F. Maher and G. E. Schreiner, *Ann. Intern. Med*., 1965, **62**, 5-29 b) S. L. Fine and R. I. Levy, *N. Engl. J. Med.*, 1965, **273**, 583-586 c) W. J. Schneider and E. L. Becker, *Arch. Intern. Med*., 1966, **117**, 715-717 and references cited therein

3. M. Burg and N. Green, *Kidney International*, 1973, **4**, 301–308 and references cited therein

4. a) L. A. Cazenave, J. A. Moscow, C. E. Myers and K. H. Cowan, *Cancer Treat. Res*., 1989, **48**, 171-187 b) S. Awasthi, S. K. Srivastava, F. Ahmad, H. Ahmad and G. A. Ansari, *Biochem. Biophys. Acta*, 1993, **1164**, 173-178 c) A. J. Oakley, J. Rossjohn, M. Lo Bello, A. M. Caccuri, G. Federici and M. W. Parker, *Biochemistry,* 1997, **36**, 576-585 d) S. Goto, T, Iada, S. Cho, M. Oka, S. Kohno and T. Kondo, *Free Radical Res*., 1999, **31**, 549-558 e) D. M. Towsend and K. D. Tew, *Oncogene*, 2003, **22**, 7369-7375 and references cited therein

5. a) J. A. Moscow, A. J. Townsend and K. H. Cowan KH, *Mol. Pharmacol.*, 1989, **36**, 22–28 b) V. Adler, Z. Yin, S. Y. Fuchs, M. Benezra, L. Rosario, K. D. Tew, M. R. Pincus, M. Sardana, C. J. Henderson, C. R. Wolf, R. J. Davis and Z. Donai, *EMBO J.,* 1999, **18**, 321–334 c) T. Wang, P. Arifoglu, Z. Ronai and K. D. Tew, *J. Biol. Chem*., 2001, **276**, 20999–21003 d) R. Wang, C. Li, D. Song, G. Zhao and Y. Jing, *Cancer Res.,* 2007, **67**, 7856-7864 and references cited therein

6. a) K. D. Tew, A. M. Bomber and S. J. Hoffman, *Cancer Res*, 1988, **48**, 3622-3625 b) J. Hansson, K. Berhane, V. M. Castro, U. Jungnelius and B. Mannervik, *Cancer Res*., 1991, **51**, 94-98

7. M. Schmidt, Y. Kim, S-M. Gast, T. Endo, D. Lu, D. Carson and I. G-H. Schmidt-Wolf, *In vivo*, 2011, **25**, 325-334

8. P. J. O'Dwyer, F. LaCreta, S. Nash, P. W. Tinsley, R. Schilde, M. L. Clappe, T. K. Tew, L. Panting, S. Litwin, R. L. Comis and R. F. Ozols, *Cancer Res*., 1991, **51**, 6059-6065

9. D. Lu, J. X. Liu, T. Endo, H. Zhou, S. Yao, K. Willert, I. G. H. Schmidt-Wolf, T. J. Kipps and D. A. Carson, PLoS ONE, available at www.plosone, 2009, 4, e8294 [accessed on September 2014]

10. Y. Kim, S-M. Gast, T. Endo, D. Lu, D. Carson and I. G. H. Schmidt-Wolf, *Leukemia Research*, 2012, **36**, 598-600

11. a) L-W. Chang, K. Pei, S-W. Chen, H-L. Huang, K-J. Lin, T-C. Yen and C-S. Yu, *Chem. Pharm. Bull*., 2009, **57**, 714-718 b) Y-H. Su, L-W. Chiang, K-C. Jeng, H-L. Huang, J-T. Chen, W-J. Lin, C-W. Huang and C-S. Yu, *Bioorg. Med. Chem. Letters*, 2011, **21**, 1320-1324

12. G. Jin, D. Lu, S. Yao, C. N. Wu, J. X. Liu, D. A. Carson and H. B. Cottam, *Bioorg. Med. Chem. Letters*, 2009, **19**, 606-609

13. a) X. Yang, G. Liu, H. Li, Y. Zhang, D. Song, C. Li, R. Wang, B. Liu, W. Liang, Y. Jing and G. Zhao, *J. Med. Chem*., 2010, **53**, 1015-1022, b) P. Zhang, J-H. Chen, X. dong, M-T. Tang, L-Y. Gao, G-S. Zhao, L-G. Yu and X-L. Guo, *Biom. Pharm*., 2013, **67**, 58-65 c) G. Liu, R. Wang, Y. Wang, P. Li, G. Zhao, L. Zhao and Y. Jing, *Mol. Cancer Ther*., 2013, **12**, 1837-1847

14. T. Li, G. Liu, H. Li, X. Yang, Y. Jing and G. Zhao, *Bioorg. Med. Chem.,* 2012, **20**, 2316-2322

15. G. Zhao, C. Liu, R. Wang, D. Song, X. Wang, H. Lou and Y. Jing, *Bioorg. Med. Chem.*, 2007, **15**, 2701-2707

16. G. Cynkowska, T. Cynkowski, A. A. Al-Ghananeem, H. Guo, P. Ashton and P. A. Crooks, *Bioorg. Med. Chem. Letters*, 2005, **15**, 3524-3527

17. a) R. F. J. Janser, R. K. Meka, Z. E. Bryant, E. A. Adogla, E. K. Vogel, J. L. Wharton, C. M. Tilley, C. N. Kaminski, S. L. Ferrey, S. Van slambrouck, W. F. A. Steelant and I. Janser, *Bioorg. Med. Chem. Letters*, 2010, **20**, 1848-1850 b) Z. E. Bryant, R. F. J. Janser, M. Jabarkhail, M. S. Candelaria-Lyons, B. B. Romero, S. Van slambrouck, W. F. A. Steelant and I. Janser, *Bioorg. Med. Chem. Letters*, 2011, **21**, 912-915

18. Y-R. Na, K-C. Han, H. Park and E. G. Yang, *Biochem. Biophys. Res. Comm*., 2013, **434**, 879-884

19. a) R. Ismail-Khan, L. A. Robinson, C. C Williams, C. R. Garrett, G. Bepler, and G. R. Simon, *Cancer Control*, 2006, **13**, 255-263 b) A. A. Mujoomdar, T. R. Tilleman, W. G. Richards, R. Bueno and D. J. Sugarbaker, *J. Thorac. Cardiovasc. Surg.,* 2010, **140**, 352-355 c) R. A. Stahel, W. Weder, Y. Lievens and E. Felip, *Annals of Oncology*, 2010, **21**, 126-128 and references cited therein

20. I. Zanellato, I. Bonarrigo, M. Sardi, M. Alessio, E. Gabano, M. Ravera and D. Osela, *ChemMedChem*, 2011, **12**, 2287-2293

21. G. Suss-Fink, *Dalton Trans.*, 2010, **39**, 1673-1688 and references cited therein

22. a) J. B. Wolinsky and M. W. Grinstaff, *Adv. Drug. Deliv. Rev*., 2008, **60**, 1037-1055 b) C. Villalonga-Barber, M. Micha-Screttas, B. R. Steele, A. Georgopoumos and C. Demetzos, *Curr. Top. Med. Chem*., 2008, **8**, 1294-1309 c) S. H. Medina, and M. E. H. El-Sayed, *Chem. Rev*., 2009, **109**, 3141-3157 d) M. A. Mintzer, and M. W. Grinstaff, *Chem. Soc. Rev*., 2010, **40**, 173-190 e) A. R. Menjoge, R. M. Kannan, and D. A. Tomalia, *Drug Discov. Today*, 2010, **15**, 171-185 f) D. Astruc, E. Boisselier, and C. Omelas, *Chem. Rev.,* 2010, **110**, 1857-1959 and references cited therein

23. a) A. Barnard, P. Posocco, S. Pricl, M. Calderon, R. Haag, M. E. Hwang, V. W. T. Shum, D. W. Pack, and D. K. Smith, *J. Am. Chem. Soc*., 2011, **133**, 20288-20300 b) J. Khandare, M. Calderon, N. M. Dagia, and R. Haag, *Chem. Soc. Rev*., 2012, **41**, 2824-2848 and references cited therein

24. a) S. El Kazzouli, S. Mignani, M. Bousmina and J-P. Majoral, *New J. Chem*., 2012, **36**, 227-240 b) A-M. Caminade, C. O., Turrin and J-P. Majoral, *New J. Chem.,* 2010, **34**, 1512-1524 c) E. R. Gillies, and J. M. Fréchet, *Drug Discovery Today*, 2005, **10**, 35-43 d) B. K. Nanjwade, H. M. Bechra, G. K. Derkar, F. V. Manvi and V. K. Nanjwade, *Eur. J. Pharm. Sci.*, 2009, **38**, 185-196 e) J. B. Wolinsky, and M. W. Grinstaff, *Adv. Drug Delivery Rev.,* 2008, **60**, 1037-1055 f) L. M. Kaminskas, V. M. McLeod, C. J. H. Porter, and B. J. Boyd, *Mol. Pharm.,* 2012, **9**, 355-373 g) S. Mignani, S. El Kazzouli, M. Bousmina, and J-P. Majoral, *Adv. Drug Delivery Rev.*, 2013, **65**, 1316-1330 h) P. Kesharwani, R. K. Tekade, and N. K. Jain, *Biomaterials,* 2014, **35**, 5539-5548 i) S. Mignani, S. El Kazzouli, M. Bousmina, and J-P. Majoral, *Chem. Rev.*, 2014, **114**, 1327-1342 j) D. Shcharbin, A. Janaszewska, B. Klajnert-Maculewicz, B. Ziemba, V. Dzmitru, I. Halets, S. Loznikova, N. Shcharbina, K. Milowska, M. Ionov, A. Shakhbazau, and M. Bryszewska, J*. Control. Release,* 2014, **181**, 40-52 and reference herewith

25.a) T. Wasiak, M. Ionov, K. Nieznanski, H. Nieznanska, O. Klementieva, M. Granell, J. Cladera, J. P. Majoral, A. M. Caminade and B. Klajnert, *Mol. Pharmaceutics*, 2012, **9**, 458–469; ;b) S. Mignani and J. P. Majoral, *New J. Chem*., 2013, **37**, 3337-3357; c) E. Blattes, A. Vercellone, H. Eutamene, C. O. Turrin, V. Theodorou, J. P. Majoral, A. M. Caminade, J. Prandi, J. Nigou and G. Puzo, *Proceedings Nat. Acad. Sci. USA*, 2013, **22** , 8795-8800; d) M. Poupot, L. Griffe, P. Marchand, A. Maraval, O. Rolland, L. Martinet, F. E. L'Faqihi-Olive, C. O. Turrin, A. M. Caminade, J. J. Fournié, J-P. Majoral and R. Poupot, *FASEB J.*, 2006, 2**0**, 2339–2335; e) E. Trevisiol, V. Le Berre-Anton, J. Leclaire, G. Pratviel, J. P. Majoral, A. M. Caminade J. M. François, and B. Meunier *New. J. Chem*. 2003, **27**, 1713-1719

26. S. Mignani, S. El Kazzouli, M. Bousmina, and J. P.Majoral, *Prog. Polym. Sci*., 2013, **38**, 993-1008

27. N. El Brahmi, S. El Kazzouli, S. Mignani, E. El Mokhtar, G. Aubert, R. Laurent, A.M. Caminade, M. Bousmina, T. Cresteil, and J.P. Majoral. *Mol. Pharmaceutics*, 2013, **10**, 1459-1464