Antitumor activity of EP80061, a small-glyco drug in preclinical studies

 # 5459

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Introduction

Heparan sulphate (HS) containing proteoglycans regulates the activity of many proteins (growth factors, cytokines, adhesion molecules...) involved in pathologies like cancer, inflammation, cardiovascular, metabolic and neuro-degenerative diseases, as well as in viral infection.

HS mimicking oligosaccharides can interfere with protein/HS interactions and thus modulate the resulting biological effects. Endotis' platform for the development of anticancer "small-glyco drugs" aims at identifying HS-mimetic oligosaccharides with inhibitory activity on several growth factors and proteins (VEGF-A, FGF-2, PDGF-B, SDF-1α, heparanase...) involved in tumour growth and metastasis. A library of fully synthetic oligosaccharides of different sizes containing various substitutions were evaluated *in vitro* for their affinity for these targets, as well as for their efficacy on cell proliferation, migration, endothelial tubule formation and heparanase inhibition.

Based on these assays, compounds exhibiting significant inhibitory activities were selected and their antitumoral properties was evaluated *in vivo*. This strategy led to EP80061 as a lead candidate for further developments of small-glyco drugs as anticancer compounds.

Conclusion & Perspectives

The data presented here showed that fully synthetic oligosaccharides, mimicking natural HS, can interfere with the activity of several growth factors and chemokine involved in tumour growth and metastasis. They also showed that the introduction of chemical substitutions improved their inhibitory properties and led to target selectivity.

The optimization of the first series of EP compounds allowed the identification of potent inhibitors of *in vitro* tubules formation and heparanase activity. Moreover, the lead-candidate EP80061 demonstrated strong anti-tumoral and anti-metastatic effects in mouse and rat models.

We are now pursuing the optimization of EP compounds to improve their selectivity for anti-cancer targets and, to this aim, we are currently developing target-specific *in vitro* assays.

We intend in the future, to open Endotis' platform to other therapeutic areas by developing "small-glyco drugs" selective for the HS-binding targets implicated in inflammation, neurodegenerative and infections pathologies.

In vitro Results

First Series of EP Compounds

Biacore

Material & Methods

- Heparin was immobilized on a Biacore sensorchip.
- EP compounds (various doses) were co-incubated with a fixed concentration of the target (FGF-2, PDGF-B, VEGF-A, SDF1-a) for 30 minutes.
- The mixture was then injected onto the streptavidin (control reference) and heparin surfaces.
- Percentages of inhibition were calculated from the binding of free target on heparin and IC_{50} were determined.

Results

Oligosaccharide size-dependent effect on the HS-mimetic affinity for growth factor or chemokine.

Compounds		Targets			
		FGF2	PDGF-B	VEGF-A	SDF1-α
Number	Nature	IC50 (nM)			
EP99	Natural dp8	638	12,900	366	3,600
EP02	Modified dp2	2,460	20,400	5,300	>30,000
EP40	Modified dp4	187	1,350	310	3,600
EP60	Modified dp6	4.5	85	5.2	43
EP13	Modified dp8	2.4	10.9	4.6	23

Selectivity of the HS-mimetic affinity for growth factor or chemokine by introduction of specific chemical substitutions.

Compounds		Targets			
		FGF2	PDGF-B	VEGF-A	SDF1-α
Number	Nature	IC50 (nM)			
EP99	Natural dp8	638	12,900	366	3,600
EP70	Modified dp8	2,012	255	18.6	176
EP90	Modified dp6	419	624	247	2,900
EP52	Modified dp6	167	194	59	865
EP63	Modified dp4	211	897	427	3,000

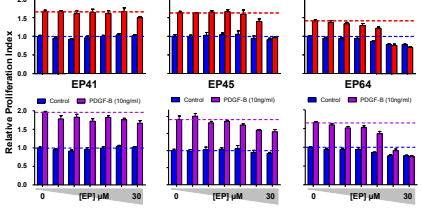
Proliferation

Material & Methods

- Normal Human Dermal Fibroblast were starved for 24 h in FGF-2-deprived medium containing only 50% of the recommended serum concentration.
- Cells were then stimulated with the corresponding growth factor for a 24 h period in presence of increasing concentrations of EP compounds (0; 0.1; 0.3; 1; 3; 10; 30 µM).
- The total number of cells was measured using the CellTiter 96 AQ_{ueous} one solution cell proliferation assay (Promega) and cell viability was assessed by the Trypan Blue method (>95%).

Results

EP compounds promoted inhibition of FGF-2 and/or PDGF-B-induced cell proliferation. Different level of inhibition efficacy and target selectivity were observed. None of the compounds inhibited VEGF-A-induced proliferation of Human Umbilical Vein Endothelial Cells (data not shown).



First Optimization of EP Compounds

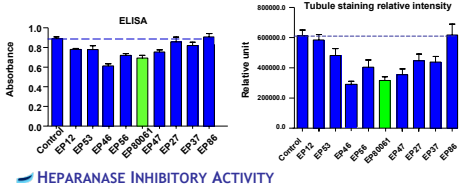
Angiokit Assays

Material & Methods (TCS CellWorks)

- 24- and 96-well AngioKits (human angiogenesis model) were purchased from TCS CellWorks.
- Culture medium (containing growth factors, reference inhibitors when appropriate, etc... supplemented with 30µM EP compound) was replaced at day 1 (plate delivery), day 4 and day 7.
- At day 10, anti-CD31 ELISA and tubule staining procedures were performed.

Results

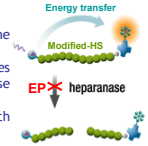
Several optimized EP compounds inhibited tubules formation, mimicking an anti-angiogenic activity. In particular, EP80061 promoted 23% inhibition of endothelial cells detection by ELISA and 48% inhibition of tubules staining. The number and size of tubules, as well as the number of junctions were significantly reduced by EP80061 (data not shown). In these conditions, cell viability was superior to 95%.



Heparanase Inhibitory Activity

Material & Methods (Cisbio Bioassay)

- The assay is based on FRET technology and on the ability of heparanase to degrade HS.
- Briefly, an intact modified-HS substrate promotes energy transfer of fluorescence. When heparanase cleaves the substrate, the energy transfer is lost.
- Addition of EP compound interfered with heparanase activity and IC_{50} were determined.



Results

Optimized EP compounds displayed a relevant anti-heparanase activity. Suramin, the well-known inhibitor of heparanase was less efficient than EP compounds in the assay. In particular, EP80061 was a ~20-fold more potent inhibitor than Suramin.

Compounds	IC_{50}* (nM) Mean ± SD
Suramin	922
EP12	640 ± 104
EP53	149 ± 107
EP46	70 ± 14
EP56	218 ± 32
EP47	138 ± 13
EP80061	43 ± 18
EP27	138 ± 54
EP37	266 ± 18
EP86	192 ± 1

(*) : Estimated from regression

In vivo Results Using EP80061

A-673 SC Tumor Model

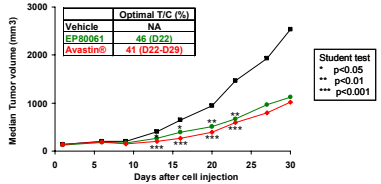
Material & Methods

- Tumor cell line: human A-673 rhabdomyosarcoma
- Animals: female Swiss nu/nu (Charles river, France)
- Drug administration: *bolus* IP, daily from the day after cells injection
- Tumor induction: SC inoculation of 10^7 A-673 cells to mice
- Reference substance : Avastin®.

Groups	Number of mice	Treatment	Dose (mg/kg)	Adm. route	Treatment schedule
1	10	Vehicle	-	IP	Q1Dx28
2	10	EP80061	30	IP	Q1Dx28
3	10	Avastin®	5	IP	Q3Dx10

Results

EP80061 and Avastin® displayed a significant antitumor activity in the SC A-673 tumor bearing Swiss Nude mice model.



B16-F10 IV Tumor Model

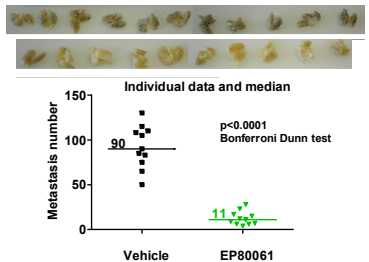
Material & Methods

- Tumor cell line: murine B16-F10 melanoma
- Animals: female C57BL/6J mice (Charles river, France)
- Drug administration: *bolus* IV, simultaneously to cells injection
- Tumor induction: IV inoculation of 10^5 B16-F10 cells to mice
- At D14, mice were sacrificed. Lungs were fixed in Tellyenisniczky solution. Number of metastasis in lungs were counted.

Groups	Number of mice	Treatment	Dose (mg/kg)	Adm. route	Treatment schedule
1	11	Vehicle	-	IP	[Q1Dx5]x2
2	10	EP80061	30	IP	[Q1Dx5]x2

Results

EP80061 (30 mg/kg) induced a very potent anti-metastatic effect on B16-F10 dissiminated tumor model in C57Bl/6 mice.



MAT 13762 IV Tumor Model

Material & Methods

- Tumor cell line: rat MAT 13762 mammary adenocarcinoma
- Animals: male Fischer 344 rats (Charles river, France)
- Drug administration: bolus IP, simultaneously to cells injection
- Tumor induction: IV inoculation of 10^5 MAT 13762 cells to rat
- At D13, rats were sacrificed. Number of metastasis in lungs were counted.

Groups	Number of rat	Treatment	Dose (mg/kg)	Adm. route	Treatment schedule
1	7	Vehicle	-	IP	Q1Dx1
2	9	EP80061	30	IP	Q1Dx1

Results

EP80061 (30 mg/kg) induced a very potent anti-metastatic effect on MAT13762 dissiminated tumor model in Fischer 344 rat.