The PG500 Series: Novel Heparan Sulfate Mimetics as Potent Angiogenesis and Heparanase Inhibitors for Cancer Therapy.

1,4Dredge K, 1Hammond E, 1Davis K, 1Li CP, 1Liu L, 1Johnstone K, 1Handley P, 1Wimmer N, 2Gonda T, 1Gautam A, 1,3Ferro V and 1Bytheway I

1Drug Design Group, Progen Pharmaceuticals Ltd, PO Box 2403, Toowong, Brisbane, Qld 4066, Australia.

2Molecular Oncogenesis Group, Diamantina Institute, University of Queensland, Australia.

3Present address: School of Physical and Chemical Sciences, Queensland University of Technology, Brisbane Qld 4001, Australia.

4Corresponding author – keithd@progen-pharma.com, tel: +61 7 38423340, fax: +61 7 37209624.

Keywords

Heparan sulfate, angiogenesis, heparanase, cancer therapy.

Abstract

Heparan sulfate mimetics, which we have called the PG500 series, have been developed to target the inhibition of both angiogenesis and heparanase activity. This series extends the technology underpinning PI-88, a mixture of highly sulfated oligosaccharides which reached Phase III clinical development for hepatocellular carcinoma. Advances in the chemistry of the PG500 series provide numerous advantages over PI-88. These new compounds are fully sulfated, single entity oligosaccharides attached to a lipophilic moiety, which have been optimized for drug development. The rational design of these compounds has led to vast improvements in potency compared to PI-88, based on in vitro angiogenesis assays and in vivo tumor models. Based on these and other data, PG545 has been selected as the lead clinical candidate for oncology and is currently undergoing formal preclinical development as a novel treatment for advanced cancer.
Introduction

It is now widely recognized that tumors are critically dependent on angiogenesis [1, 2], i.e. the growth of new blood vessels from those already surrounding a tumor. Thus, the inhibition of angiogenesis has become an important therapeutic strategy for cancer [3]. Cell surface/extracellular matrix (ECM) heparan sulfate (HS) glycosaminoglycans are complex polysaccharides that are ubiquitous in nature and play important roles in the regulation of several aspects of cancer biology, including angiogenesis, tumor progression and metastasis [4, 5]. Several growth factors, their receptors, extracellular matrix molecules and enzymes bind to specific sites on the HS sugar chain to facilitate cellular and biochemical responses. HS mimetics function by blocking these interactions and inhibiting processes crucial to tumor progression and is therefore a promising approach for new cancer therapeutics [4, 6, 7]. This has been illustrated by the sulfated oligosaccharide PI-88, which reached Phase III clinical development for hepatocellular carcinoma.

The PG500 series represents a collection of new HS mimetics based on anomerically pure, fully sulfated, mono-, di-, tri-, tetra- and pentasaccharide glycosides modified by the addition of an aglycon at the non-reducing end of the molecule. The aglycones are primarily lipophilic groups chosen specifically to improve the biological activities, for example efficacy and pharmacokinetic properties. PG500 series compounds are believed to interfere with two important processes in tumor development, namely angiogenesis via inhibition of vascular endothelia growth factor (VEGF), fibroblast growth factor-1 (FGF-1), fibroblast growth factor-1 (FGF-2) and metastasis, via inhibition of heparanase activity. The recent discovery that heparanase acts as a signaling molecule for VEGF induction [8] and induces VEGF-C to facilitate tumor lymphangiogenesis [9] also implicates heparanase in the development of tumor vascularization. Furthermore, enhanced heparanase expression in human tumors correlates with metastatic potential, tumor vascularity and reduced postoperative survival of cancer patients, attributed to enzymatic and nonenzymatic activities of the heparanase protein. Urinary and plasma levels of heparanase are elevated in cancer patients and suppressed in response to effective anticancer treatments. Thus, the enzyme is a promising target for anticancer drug development [10].

This study illustrates the improvements in biological activity that was observed during the discovery phase of the PG500 series program to design a HS mimetic. Ultimately, this has led to the emergence of a series of compounds that may be classified as new chemical entities (NCE’s) on a single carbon backbone with potent anti-angiogenic, anti-metastatic and antitumor activity. This is due to the dual mode of action as a heparanase inhibitor and an angiogenesis inhibitor with low anti-coagulant activity in comparison to PI-88 or heparin.
Material and Methods

Cell lines, cell culture, reagents and compounds
Human umbilical vein endothelial cells (HUVEC) were maintained in EBM-2 media supplemented with 2% FBS and Singlequots™ (Lonza, Walkersville, MD). HT29 tumor cells were cultured in RPMI1640 cell culture medium (Invitrogen, Mt Waverley, Australia), foetal bovine serum (Invitrogen), penicillin/streptomycin (Sigma-Aldrich, Castle Hill, Australia) at vivoPharm Pty, Australia. B16 tumor cells were cultured in DMEM - High Glucose (Gibco, Mt Waverley, Australia), 10% foetal bovine serum (Hyclone, Logan, UT, USA) and 1x Penicillin/Streptomycin/Glutamine (Gibco) at the Diamantina Institute, University of Queensland, Australia. PG500 series compounds were synthesized by Progen Pharmaceuticals Ltd. and dissolved using phosphate buffered saline (PBS), pH 7.2 and stored at 4°C for the duration of each experimental period.

BIACore binding assays
Binding affinities of ligands binding at the HS-binding site of the growth factors FGF-1, FGF-2 and VEGF were measured using a surface plasmon resonance solution affinity assay performed on a BIACore 3000 (BIACore, Uppsala, Sweden) operated using the BIACore Control Software, as previously described [11, 12].

Anticoagulant Assays
The anticoagulant activity of these compounds was investigated using the activated partial thromboplastin time or APTT test (which assesses effects on the intrinsic coagulation pathway), and the Heptest (which assesses the inhibition of exogenous Factor Xa by plasma Antithrombin III). Both tests were conducted in the laboratory of Job Harenberg, University of Mannheim, Germany according to conventional medical laboratory protocols.

Fondaparinux Heparanase assay
Heparanase activity was determined by measuring the cleavage of fondaparinux [13]. The newly formed reducing disaccharide can be detected by reacting with the mono-tetrazolium salt WST-1 (Auspep, Melbourne, Australia). Recombinant human heparanase was expressed in insect cells [14]. Assays were set up in a volume of 100 μL comprising 40 mM sodium acetate buffer, pH 5.0, 100 μM Fondaparinux and varying concentrations of inhibitor in 96 well plates (Costar 9018, Corning) pre-coated with BSA. Purified recombinant heparanase (2.55 nM) was then added to start the assay. The plate was incubated at 37 °C for 24 h and the assay stopped by the addition of WST-1 solution (100 μL of 1.69 mM WST-1 in 0.1 M NaOH). After sealing the plates with adhesive film, reaction of the product disaccharide with WST-1 occurred at 60 °C for 60 min. The absorbance was then measured at 584 nm using a microplate reader (FluoStar, BMG) and the amount of product disaccharide quantified using D-galactose as standard. D-galactose was shown to yield the same absorbance on a per molar basis when reacted with WST-1 as the product
disaccharide. The IC₅₀ value for each compound was determined and converted to a Kᵢ using Cheng and Prusoff, 1973 [15] and the Kₘ of heparanase for fondaparinux, which was determined to be 33 ± 6 µM.

**Growth factor-induced endothelial cell proliferation assay**

Growth factor-induced endothelial cell proliferation using either HUVECs or dHMVECs was induced using FGF-1, FGF-2 or VEGF in the absence or presence of various concentrations of PG500 series compounds ranging from 0.01-50µM, depending on the individual experiment. Briefly, 100µL cells at a concentration of 2x10⁵/µL, 50µL of growth factor (typically 10ng/mL for FGF-2 and VEGF and 25ng/mL for FGF-1) and 50µL of medium or PG500 series compounds were added to each well for 72 hours. Twenty microlitres of the CellTitre 96™ Aqueous One Solution Cell Proliferation Assay (Promega) was added for the final 2-4h prior to reading the absorbance at 490nm to obtain OD values. Once these values were subtracted from the blanks (media only), the data was imported into BIACORE software to determine the IC₅₀ values for each curve. IC₅₀ values have been measured to indicate the inhibitory effect of each compound on either FGF1-, FGF2- or VEGF-induced endothelial cell proliferation.

**Tube formation assay**

HUVECs in the fourth or fifth passage at 70-80% confluence were harvested and resuspended in Clonetics endothelial growth medium (EGM2) containing all supplements as directed by manufacturer, except heparin, at a cell density of 4 x 10⁵ cells per mL. For each set of triplicate wells, 200µL of cells (4x10⁵/mL) were treated with an equal volume of compound to obtain final concentrations of 10, 50 or 100µM. A 100µL aliquot of cells was then plated onto 96-well plates precoated with growth factor reduced Matrigel™ (BD Biosciences, San Jose, CA) and incubated for 18-22 h. Tube formation was examined by phase-contrast microscopy and images were collected using an Olympus C5050 digital camera. Tube formation inhibition was quantitated manually from images by recording the total number of nodes connecting 3 or more tubules. Results are expressed as percentage inhibition compared to control. Untreated HUVECs were used as a control for normal cell growth and tube formation in Matrigel™.

**Rat aortic assay for angiogenesis**

The rat aortic assay was performed using a modified protocol of the method originally described [16]. Briefly, 180µL of Matrigel™ (BD Biosciences) was pipetted into 48-well tissue culture plates (Nunc). The plates were incubated at 37°C for 30 minutes to allow Matrigel™ to solidify. Aortic segments were placed on top on the Matrigel™ in the centre of each well before being sealed with an additional 60µL of Matrigel™. Each well was supplemented with 1mL media containing control or PG500 series compounds at concentrations of 1, 10 and/or 50µM. Media was replenished every 24h and on day 6 microvessels were scored using two assessors and employing a scoring system from 0-5 where 0 = no microvessels to 5 = diffuse angiogenesis as previously described [17]. In some experiments, the viability of the tissue was assessed following treatment
with a compound by washing out compound and adding complete media with VEGF (10ng/mL) for an additional 72h.

**Tumor models**

HT29 colorectal cells were grown to no more than 70-80% confluency prior to harvest. Adherent cells were harvested by trypsinisation and washed in HBSS. Following a total of three washes, the cells were resuspended in HBSS and adjusted to a final cell concentration of 2 x $10^7$/mL. Prior to inoculation the injection site, on the dorsal right flank, was liberally swabbed with alcohol and 100μL of cells (2 x10⁶ cells) were injected. The treatment of mice commenced with an average tumor volume of approximately 155mm³. Tumors were measured in two dimensions (length and width) and the tumor volume (mm³) calculated using the equation:

$$V = \text{length} \times \text{width}^2 \times \pi/6$$

B16 melanoma cells were grown to no more than 70-80% confluency prior to harvest. Adherent cells were harvested using EDTA and washed in HBSS. Following a total of three washes, the cells were resuspended in HBSS and adjusted to a final cell concentration of 5 x $10^6$/mL. Prior to inoculation the injection site, on the neck scruff, was liberally swabbed with alcohol and 100μL of cells (5 x10⁷ cells) were injected subcutaneously for the solid tumor studies. For the experimental metastasis model, the final cell concentration was 2 x $10^6$/mL and 100μL of cells (2 x10⁵ cells) were injected intravenously via tail vein injection (Day 0). Treatment in the experimental metastasis model commenced on Day 0, or in the solid tumor model, on Day 3 following tumor cell inoculation. Compounds were injected via the subcutaneous route in a volume of injection of 100μL PBS at doses stated in figure legends. Clinical observation was performed daily to monitor the animals’ health and bodyweight measurements taken throughout the study.

**Statistical Analysis**

Data from the B16 melanoma metastatic model was analyzed by a one-way analysis of variance (ANOVA) followed by a post hoc Dunnett’s test (Graphpad Instat, v3.0). Data are presented as mean ± SEM. Differences with a p value of less than 0.05 were considered statistically significant. Data from the HT29 xenograft model were analyzed by ANOVA (all pairwise multiple comparison procedure and multiple comparison versus control group – Holm-Sidak Method) using SigmaStat v3.0. A p value of less than 0.05 was considered significant.
Results

The compounds were assessed initially for their affinity for the angiogenic growth factors FGF-1, FGF-2, and VEGF, and for their ability to inhibit heparanase activity (Table 1). The PG500 compounds show high affinity for the growth factors in the BIAcore assays although the affinities are generally lower than the values observed for PI-88. This is most likely a consequence of the lipophilic aglycones and their ability to influence the binding properties of these compounds. Nonetheless, these findings were critical as they provided confirmation that the essential chemical alterations on the carbohydrate portion of each compound did not adversely affect the heparan mimetic properties of these agents. Importantly, it also became evident that most PG500 series compounds, particularly those containing a larger lipophilic moiety, had lower APTT and Heptest results, thus reducing the anticoagulant activity compared to PI-88.

<table>
<thead>
<tr>
<th>Compound</th>
<th>$K_a$ (nM)</th>
<th>$K_i$ (nM)</th>
<th>Anticoagulant Activity</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>FGF-1</td>
<td>FGF-2</td>
<td>VEGF</td>
</tr>
<tr>
<td>PG517</td>
<td>0.4</td>
<td>129</td>
<td>21</td>
</tr>
<tr>
<td>PG518</td>
<td>0.2</td>
<td>160</td>
<td>18</td>
</tr>
<tr>
<td>PG536</td>
<td>0.6</td>
<td>160</td>
<td>22</td>
</tr>
<tr>
<td>PG537</td>
<td>0.4</td>
<td>108</td>
<td>40</td>
</tr>
<tr>
<td>PG545</td>
<td>8.0</td>
<td>390</td>
<td>29</td>
</tr>
<tr>
<td>PG546</td>
<td>9.7</td>
<td>390</td>
<td>95</td>
</tr>
<tr>
<td>PG547</td>
<td>50.0</td>
<td>610</td>
<td>790</td>
</tr>
<tr>
<td>PG554</td>
<td>24.8</td>
<td>547</td>
<td>190</td>
</tr>
<tr>
<td>PG561</td>
<td>32.2</td>
<td>530</td>
<td>380</td>
</tr>
<tr>
<td>PG562</td>
<td>4.9</td>
<td>311</td>
<td>14</td>
</tr>
<tr>
<td>PI-88</td>
<td>0.2</td>
<td>95</td>
<td>2</td>
</tr>
</tbody>
</table>

The biological benefits of the PG500 series really became apparent in cell-based screens employed to mimic various processes involved in angiogenesis, presumably due to the introduction of the lipophilic moieties in the molecules. The first of these assays focuses on the ability of these compounds to inhibit the proliferation of endothelial cells in response to angiogenic growth factors. The selected PG500 series compounds all inhibited FGF1, FGF2 and VEGF-induced endothelial cell proliferation using HUVECs (Figure 1). The IC$_{50}$ values of some PG500 series compounds (e.g. PG545) in growth factor-induced HUVEC proliferation assays are up to approximately 20-fold (for FGF-2) or 40-fold (for FGF-1 and VEGF) lower compared to PI-88.

The second model, the tube formation assay, considers the ability of the compounds to disrupt endothelial cell organization and in vitro angiogenesis [18]. In this assay, a number of PG500 series compounds (PG536, PG537, PG545, and PG562) inhibited tube formation over 90% at
10μM (Figure 2a). At this concentration, the inhibition by these compounds has been significantly improved compared with PI-88 and earlier compounds in the series such as PG517 or PG518 [19]. Figure 2b illustrates the relative potencies of PG517 and PG545 at 10μM compared to vehicle. A third cell-based model, the rat aortic assay for angiogenesis, is a well known ex vivo assay for the testing of putative anti-angiogenic agents [18]. The most potent compounds in this model emerged as PG536, PG545 and PG546, all inhibiting angiogenesis over 80% when administered daily at 10μM for 6-8 days (Figure 3a). This potent inhibition by compounds such as PG545 and PG546 is illustrated in Figure 3b. Although PI-88 inhibits up to 60% at 10μM, increasing the concentration up to 50μM did not lead to greater inhibition (data not shown). In studies which assessed tissue viability following 6 days of treatment with PG500 series compounds, microvessels emerged in most cultures treated with VEGF for 72 hours (data not shown), indicating that the compounds do not induce a toxic effect on the tissue.

To demonstrate the effectiveness of these agents in tumor models, we selected one experimental metastatic model (B16 melanoma), previously found to be responsive to PI-88 treatment, and two solid tumor models that previously demonstrated resistance to PI-88 treatment. The B16 melanoma model is a syngeneic mouse model which was employed as the first in vivo screen by the inoculation of tumor cells via an intravenous route for the experimental metastasis model or subcutaneously for the solid tumor model. In both models, PG500 compounds were dosed for twelve consecutive days. As shown in Figure 4a, selected compounds in this test PG545, PG546 and PG547, potently inhibited the development of metastatic nodules when administered at 10mg/kg. The reduction in the number of metastatic nodules on the lung by treatment with PG545 is exemplified by Figure 4b. Previously, it was found that 30mg/kg daily treatment with PI-88 also led to similar findings in this model (Figure S1, supplementary data). In the solid tumor B16 model (resistant to PI-88), tumor development during the treatment period was potently inhibited by the selected compounds PG545, PG546 and PG547 (Figure 5a). Of these compounds demonstrating efficacy, PG545 was selected for further testing in a xenograft model (HT29 colon) and found to significantly inhibit the tumor development compared to saline control (Figure 5b). In addition, the chemotherapeutic agent 5-fluorouracil (5-FU) was also used as a positive control in the study.
**Discussion**

This study details the rationale for the development of a new series of potent heparan sulfate mimetics; collectively known as the PG500 series. In a distinct departure from PI-88 from which they owe their parentage, the candidate compounds are now fully synthetic and fully sulfated single entities that exhibit potent antitumor properties comparable to or better than PI-88 or other known sulfated polysaccharides (e.g. heparin). For these reasons outlined above, further development of compounds, particularly PG545, is currently underway.

BIACore binding data, which probes for HS binding site activity, indicated low nanomolar binding of PG500 series compounds to HS-binding proteins FGF1, FGF2 and VEGF which are important in tumor angiogenesis. The potency with which these compounds inhibit the activity of heparanase, which is critical for the remodeling of the extracellular matrix, extends the potential use of these compounds. The real departure in this technology becomes apparent, however, in cell-based assays where, particularly the later compounds in the series, potently inhibit growth factor-induced endothelial cell proliferation - the first important cellular process in angiogenesis. The effects of the PG500 series on subsequent processes in angiogenesis have been studied by the tube formation assay and the rat aortic assay for angiogenesis, both of which are routinely used to identify compounds as potent anti-angiogenic molecules. It is also important to note that the PG500 series compounds do not induce cytotoxicity at concentrations tested in the models described. For example, the lethal concentration required to kill 50% of endothelial cells (LC50) for PG545 is 78µM (Table S1, supplementary data) which is over 80-fold higher than its IC50 concentration in the endothelial cell proliferation assay. Previous data indicated that PG500 series compounds can induce anti-angiogenic activity in *in vivo* models of angiogenesis [19].

In order to determine whether such activity would be applicable to a clinical cancer setting, we investigated the ability of some of these PG500 series compounds (PG545, PG564 and PG547) to inhibit tumour development *in vivo* in the B16 solid tumour and metastatic model. In the solid tumour model, which is resistant to PI-88, tumour development was reduced by daily administration of all three compounds. Further investigations will assess the duration that such an effect can be maintained in the presence or absence of compound. In addition, the intravenous injection of B16 melanoma cells via the tail vein colonize in the lungs of mice which can be utilized as an *in vivo* screen for putative anti-metastatic activity and in which PI-88 has been found to be active (Figure S1, supplementary data). Again, all three compounds tested significantly reduced the number of metastatic nodules in this model which may be indicative of a class effect of HS mimetics/heparanase inhibitors. A recent paper using the B16 experimental metastasis model found that the tyrosine kinase inhibitor sunitinib actually accelerated metastasis in all mice when the drug was administered 7 days prior to tumor implantation or in half the mice when administered for 7 days post-implantation, thus not producing a survival advantage [20]. Similar data was noted using an anti-VEGFR2 antibody in the RIP1-Tag2 model of pancreatic neuroendocrine cancer (PNET) with the authors concluding that such undesirable therapy-
triggered change in the natural history of the treated tumors is convergent with emerging examples involving other targeted therapy-driven changes [21]. Based on these recent publications, the results with PG500 series compounds in the experimental metastasis model could prove to be a new ally for cancer therapy.

PG545 was then tested in the HT29 colon xenograft model and found to significantly inhibit tumour development comparable with the standard of care chemotherapeutic agent 5-fluorouracil (5-FU). The fact that administration of these agents, and in particular PG545, to tumor-bearing animals led to significant tumour growth inhibition strongly supports the further development of these HS mimetics for the treatment of cancer. It is of interest to note that the enhanced potency of PG500 series compounds in in vitro angiogenesis assays provides a plausible explanation for the improved efficacy observed with these compounds in solid tumor models, known to be resistant to PI-88 treatment. Moreover, the similar effects of PG500 series compounds to PI-88 on the enzymatic activity of heparanase in vitro seems to correlate with the efficacy of all compounds in a metastatic model of melanoma. The relative role of heparanase and angiogenesis in forming a particular tumor microenvironment is a key field of intensive research which will likely facilitate the development of such drug candidates to the clinic.

In summary, we have demonstrated that the PG500 series of heparan sulfate mimetics potently block the enzymatic activity of heparanase, angiogenesis and tumor development. The inhibition of heparanase activity is a considerably important attribute and one which will require the development of a sophisticated in vivo model to fully appreciate its relative contribution to the anti-tumor effects of these compounds. Taken together, the data demonstrates that the administration of these agents to tumor-bearing animals significantly reduce metastatic nodule formation and solid tumor development. Based on these data and other considerations associated with the manufacture, chemistry and biology of these compounds, PG545 has been identified as the lead clinical candidate for further development as a new class of anti-cancer agent.
Acknowledgements

The authors acknowledge Dr. Ralf Brandt (vivoPharm, Adelaide, Australia) for the HT29 data, Job Harenberg (University of Mannheim, Germany) for the anticoagulant data and Bruce Wyse (Tetra-Q, Brisbane, Australia) for supply of the rat aortic tissue.
References

15. Cheng Y, Prusoff WH (1973) Relationship between the inhibition constant (K1) and the concentration of inhibitor which causes 50 per cent inhibition (I50) of an enzymatic reaction. Biochem Pharmacol 22:3099-3108
Legends

Figure 1: Concentration that inhibits 50% (IC$_{50}$) values for selected PG500 series compounds in a growth factor - induced HUVEC proliferation assay. Enhanced anti-proliferative activity was particularly evident in compounds from PG536 onwards generating approximately 20-fold (for FGF1), 10-fold (for FGF-2) or 20-fold (for VEGF) improvements compared to PI88, the IC$_{50}$ values of which have been published elsewhere [19].

Figure 2: Inhibition of HUVEC tube formation by selected PG500 series compounds at 10µM (Figure 2a). In particular, PG536, PG537, PG545 and PG562 inhibit tube formation over 80% at 10µM. By contrast, PI-88 at 10µM inhibits tube formation by 29% as previously reported by our group [19]. Some representative images of control, PG517 (10µM) and PG545 (10µM) are shown in Figure 2b.

Figure 3: Inhibition of angiogenesis by selected PG500 series compounds in the rat aortic assay (Figure 3a). Some photomicrographs are also shown to illustrate the activity of PG545 and PG546 in this model (Figure 3b). In some experiments designed to examine the reversibility of the antiangiogenic effect at the end of the treatment period (which was typically six days), aortic segments receiving fresh medium containing VEGF (10ng/mL) frequently developed new microvessels within 72 h (data not shown).

Figure 4: PG500 series compounds significantly inhibit tumor metastases in the B16 melanoma model. The compounds PG545, PG546 and PG547 were administered subcutaneously once daily for 12 days at 10 mg/kg/day after tumor cells were inoculated intravenously via tail vein injection at Day 0. Data expressed as mean number of metastases with SEM (Figure 4a). ** = P<0.01 versus control as analysed by a one-way ANOVA followed by a Dunnett’s post hoc test. An example of metastatic nodules in lungs from control and PG545-treated animals are shown in Figure 4b.

Figure 5: PG500 series compounds inhibit tumor progression in solid tumor models. In the B16 melanoma model, PG545, PG546 and PG547 were administered subcutaneously once daily for 12 days at 15 mg/kg/day, 3 days after tumor challenge at Day 0. Potent inhibition of tumor growth was evident in each treatment group. In the HT29 xenograft model, PG545 was shown to significantly inhibit tumor development (P<0.01) when administered daily at 5mg/kg/day. Data expressed as mean tumor volume with SEM (Figure 4b). Statistical analysis was conducted using a one way ANOVA followed by a Dunnett’s post hoc test.
Figure 1
Figure 2

(a) % Inhibition of Tube Formation

(b) Control, PG517, PG545
Figure 3

(a) Graph showing the percentage inhibition of microvesSEL sprouting.

(b) Images of control, PG545, and PG546 conditions.
Figure 4

a

![Bar graph showing the number of features for Control, PG545, PG546, and PG547. The Control group has a significantly higher number of features compared to the other groups, indicated by asterisks.]({"caption" : "Bar graph showing the number of features for Control, PG545, PG546, and PG547. The Control group has a significantly higher number of features compared to the other groups, indicated by asterisks."})

b

![Images showing control and PG545 conditions. The control image shows several black spots, while the PG545 image shows a noticeable change in color.]({"caption" : "Images showing control and PG545 conditions. The control image shows several black spots, while the PG545 image shows a noticeable change in color."})
Figure 5

a) Drug treatment

- Control
- PG545 (15 mg/kg)
- PG546 (15 mg/kg)
- PG547 (15 mg/kg)

b) Treatment day

- Control
- PG545 (5 mg/kg, qd)
- 5-FU (75 mg/kg, 1x weekly)

P<0.01