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(54) Title: METHOD OF TREATMENT AND DEVICE

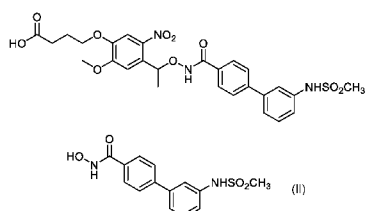


Figure 7

(57) Abstract: A method and an angioplasty balloon catheter used in a method of preventing or minimizing incidence of neointimal hyperplasia (NIH) in a blood vessel of an animal following angioplasty treatment. The method comprising locating the angioplasty balloon catheter at a vessel site where angioplasty treatment is desired, wherein a balloon component of the catheter is at least partially coated on an external surface thereof with a photo-labile drug / linker conjugate of Formula I; wherein the balloon is inflated at the site to initiate release of the drug / linker conjugate to vessel wall at the site and an optical fiber light diffuser, connected by optical fiber to a source of UV light at a wavelength of from about 350 nm to about 380 nm, is located within the balloon to emit said UV light, wherein UV light emission is of suitable power and for sufficient duration to cleave the drug / linker conjugate and activate an effective amount of the drug of Formula II to prevent or minimize NIH following the angioplasty treatment at the site.

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Method of treatment and device

Field of the invention

[0001] The present invention relates to a method of preventing or minimizing the incidence of neointimal hyperplasia (NIH) in a blood vessel of an animal, particularly a human, following angioplasty treatment for vascular disease. The invention also relates to a drug eluting angioplasty balloon catheter suitable for use in such a method.

Background of the invention

[0002] Vascular injury induced by percutaneous transluminal angioplasty (PTA) techniques results in the therapeutic benefit of increased lumen diameter. However, as a consequence, a balance between adequate healing of the affected vessel and prevention of restenosis, is required. Restenosis is the result of several mechanical and biological processes occurring at the time of injury, including vessel recoil and excessive neointimal proliferation¹ – referred to as neointimal hyperplasia (NIH). Stenting in combination with balloon angioplasty techniques reduces vessel recoil and drug eluting stents (DES) effectively, although incompletely, reduce neointima (NI) formation². The anatomic restrictions associated with vascular stenting, particularly in the periphery, together with the non-homogenous distribution of drug release from DES, with about 85% of the stented vessel *not* being covered by stent struts, leads to low drug levels on much of the surrounding tissue, thus reducing the anti-neointimal hyperplasia (NIH) effect. In addition, an *in situ* device is at risk of fracture and high drug concentrations in focal stent strut/vessel contact areas results in delayed/incomplete endothelialization (healing), which is associated with increased inflammatory and (late) thrombotic risk and the need for long-term dual anti-platelet therapy³⁻⁴.

[0003] The observation by Axel and colleagues of single dose exposure of vascular smooth muscle cells to the anti-proliferative agent paclitaxel (PTX) resulting in *long lasting* inhibition of cellular proliferation and migration⁵ has in recent times been translated into long lasting inhibition of restenosis subsequent to short-term, lesion specific intra-mural exposure to high concentrations of PTX via angioplasty balloon delivery, heralding the advent of the drug eluting balloon catheter (DEBc)⁶. The theoretical advantages of DEB over DES,

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including homogenous drug delivery to vessel wall, immediate release (no agent *in situ*), reduced inflammation/thrombosis and anti-platelet requirements has facilitated promulgation of this new technology⁴. In addition, the relatively poor performance of stenting in occlusive peripheral artery disease (PAD) emphasizes the need for novel catheter based therapies for the management of this condition. Initial reports of use of a PTX-coated DEBc in PAD identified significant reductions of 60+% in NIH, reduced late lumen loss and reduced target-lesion revascularization⁷ with several subsequent studies demonstrating improved outcomes, reduced need for revascularization and improved safety over uncoated conventional PTA for treatment of *de-novo* lesions and in the setting of in-stent restenosis⁸⁻¹⁰. Importantly DEBc have also been demonstrated to be superior to PTA in the management of diabetic critical leg ischemia¹¹. In addition, based on meta-analysis of studies of femoro-popliteal PAD and results of studies in coronary vessels, DEBc afford improvements in outcomes over DES¹²⁻¹⁴. These observations combined with improved cost effectiveness of DEBc over DES¹⁵ afford improved treatment options in management of NIH.

[0004] Currently PTX coated DEBc are the only commercially available devices able to deliver this novel treatment modality, although pre-clinical studies using sirolimus and zotarolimus-coated balloons have also been identified to reduce NIH^{16,17}. Recent pre-clinical and clinical reports suggest concerns with PTX mediated cytotoxicity to vascular endothelial cells from increased necrosis and apoptosis, together with reduced endothelial migration resulting in potentially higher rates of inflammation and vessel thrombosis^{18,19}. Other deleterious effects observed include luminal obstruction and, importantly, inflammation from PTX-based particulate matter discharged into the bloodstream at the time of balloon deployment^{6,20-23}. These results, together with recent withdrawal of the IN.PACT Amphirion PTX DEBc from the market based on safety concerns raised by results from the IN.PACT DEEP trial²⁴, suggest that notwithstanding the potentially superior efficacy of DEB catheters in the management of NIH an opportunity exists to improve on current DEB technology.

[0005] The underlying molecular mechanisms responsible for the NIH vascular smooth muscle cell (VSMC) phenotype are not completely understood, however gene array studies identify a potentially complex interplay between many candidate genes, suggesting a coordinated response governed by a hierarchical transcriptional mechanism^{25,26}. Epigenetic

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transcriptional regulation affords one potential mechanism for coordinating the control of complex gene regulatory events such as those identified in NIH²⁶. Histone modification by acetylation and deacetylation results in significant transcriptional control over multiple genes with phenotypic outcomes including inhibition of cell apoptosis and growth arrest²⁷. Pharmacological inhibition of histone deacetylation using histone deacetylase inhibitors (HDACi) may offer potential for inhibition of NIH, and this approach has been used therapeutically with efficacy and low toxicity in the treatment of hematological malignancies²⁸. *In vitro* studies identify Trichostatin A, a member of the HDACi class of agents, as able to modulate VSMC proliferation²⁹ whilst additional recent *in vivo* studies demonstrate HDACi-mediated attenuation of NIH^{26,30}. Indeed studies suggest HDACi therapy may have a unique capacity to attenuate restenosis by inhibition of NIH together with anti-inflammatory/thrombotic effects³¹, significant enhancement of vascular endothelial cell tissue-type plasminogen activator (t-PA) expression³² and lack of toxicity²⁸ combining to reduce the risk of thrombotic re-occlusion and promoting healing (re-endothelialization) post intervention.

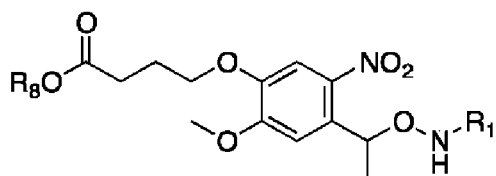
[0006] International patent publication no. WO2004/098495 discloses the use of a medical device coated with an HDACi compound drug for treating NIH. However, a problem associated with devices such as these, which is consistent with the problem associated with the PTX coated DEBc referred to above, is that there is no control over release of the drug, such that it can cause adverse effects away from the intended site of therapy. International patent publication no. WO1996/023543 and US patent no. 5,470,307 both disclose a photo-labile drug-linked balloon catheter for use in treatment of coronary conditions, where the drug is chemically bound to the balloon surface by a photo-labile linker. The drug is therefore released from the balloon only upon illumination from a light source of the appropriate wavelength and power to cleave the drug from the photo-labile linker. This approach is still problematic as a significant quantity of active drug is released into the bloodstream, having the potential to exert unwanted effects away from the site of intended therapy.

[0007] A light activated version of the HDACi compound, suberoylanilide hydroxamic acid (SAHA), has been described³⁶ wherein selective release of the active agent by mild UV irradiation regulates inflammation and immunity in a subpopulation of macrophages.

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Similarly, HDACi compounds linked to a photo-switchable azobenzene moiety have been disclosed³⁷ as potential anti-tumour agents, and in European patent publication no 2970107 HDACi compounds linked to photo-switchable diazo compounds with a substituted or unsubstituted aryl or heteroaryl ring are disclosed as modulators of protein function.

[0008] A photo-labile version of the HDACi compound hydroxamic acid is disclosed in International patent publication no. WO2013/057186 for release of hydroxamic acid from a solid support in the context of chemical synthesis or for drug activity screening, wherein the photo-linker is based on the o-nitroveratryl group, and has the following structure:



wherein R₁ is a protecting group, such as Boc, Fmoc, Alloc, Cbz, Bn and R₈ is hydrogen or C₁-C₈ alkyl.

[0009] Previous studies by the present inventors have identified a novel HDACi compound, 3'-methanesulfonylamino-biphenyl-3-hydroxamic acid, (referred to herein as MCT-3) with significant *in-vivo* anti-NIH activity and low systemic toxicity³³.

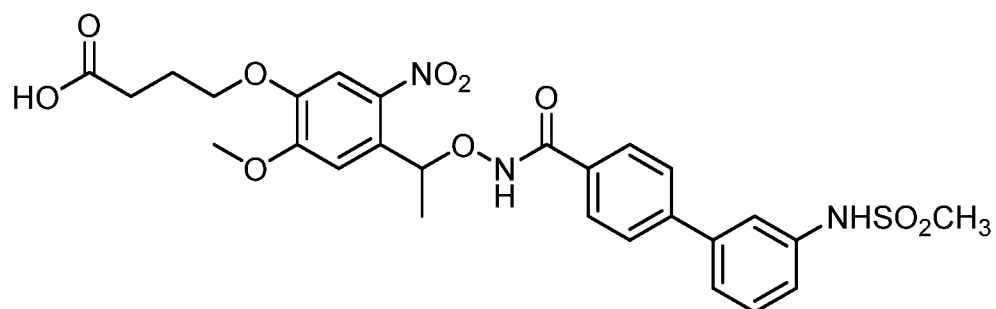
[0010] It is with the above background and problems associated with known approaches to preventing or minimizing NIH following PTA in mind that the present invention has been conceived.

Summary of the invention

[0011] The present inventors have now conceived and demonstrated efficacy of a novel approach to preventing or minimizing incidence of NIH in a blood vessel of an animal following angioplasty treatment, that involves coating, but not chemically binding, the balloon component of an angioplasty balloon catheter with a specific photo-labile drug / linker conjugate, wherein the photo-labile drug / linker conjugate is released into the vessel wall at the site of balloon deployment when the balloon is inflated. The drug is cleaved from the

linker and thereby activated at its intended site of action by UV light irradiation of the appropriate wavelength and power from an optical fiber located within the balloon that is connected to a UV light source. The specific drug selected is demonstrated to exhibit favorable activity in preventing or at least minimizing NIH without significant adverse effects. Further, the approach adopted by the present inventors of coating the catheter balloon rather than chemically binding the drug to it, such that UV activation only takes place following balloon inflation initiated drug release, minimizes collateral deployment toxicity. In addition, the specific photo-labile linker selected is shown to facilitate sufficient cleavage of the conjugate and activation of the drug to elicit its intended anti-NIH effect at the site of intended action, using the UV light power available via optical fiber transmission to the balloon catheter platform.

[0012] According to one aspect of the present invention there is provided a method of preventing or minimizing incidence of neointimal hyperplasia (NIH) in a blood vessel of an animal following angioplasty treatment, comprising locating an angioplasty balloon catheter at a vessel site where angioplasty treatment is desired, wherein a balloon component of the catheter is at least partially coated on an external surface thereof with a photo-labile drug / linker conjugate of Formula I:



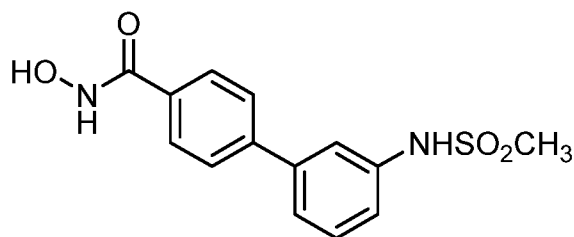
Formula I

;

wherein the balloon is inflated at the site to initiate release of the drug / linker conjugate to vessel wall at the site and an optical fiber connected to a source of UV light at a wavelength of from about 350 nm to about 380 nm is located within the balloon to emit said UV light, wherein UV light emission is of suitable power and for sufficient duration to cleave the drug /

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linker conjugate and activate an effective amount of the drug of Formula II to prevent or minimize NIH following the angioplasty treatment at the site:



Formula II

[0013] According to another aspect of the present invention there is provided an angioplasty balloon catheter for preventing or minimizing incidence of neointimal hyperplasia (NIH) in a blood vessel of an animal following angioplasty treatment, wherein the balloon component of the catheter is at least partially coated on an external surface thereof with a photo-labile drug / linker conjugate of Formula I, wherein the catheter comprises or engages with an optical fiber connected to a source of UV light at a wavelength of from about 350 nm to about 380 nm, the fiber being located within the balloon to emit said UV light of suitable power and for sufficient duration to cleave the drug / linker conjugate and activate an effective amount of the drug of Formula II at a site of angioplasty treatment, to prevent or minimize NIH following angioplasty treatment at the site.

Brief description of the figures

[0014] The invention will be described by way of example only with reference to the following figures, wherein:

[0015] Figure 1 shows a bar graph of Intima:Media ratio (IMR) in ligated left internal carotid artery 8 weeks post ligation and 4 weeks post intervention for the following treatment conditions: Con = no intervention; PABA = plain balloon angioplasty; PTX = 3 μ g/mm² paclitaxel coated DEBc; MCT-3 = 3 μ g/mm² MCT-3 coated DEBc (n = 4-8) (** p <0.01, *** p <0.001).

[0016] Figure 2 shows a graph the ratio of ligated LICA to contralateral unligated RICA vessel Ki67, PCNA, p21^{WAF1/CIP1}, IL-6 and MCP-1 mRNA expression in PABA treated,

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MCT-3 DEBc $3\mu\text{g}/\text{mm}^2$ and PTX DEBc $3\mu\text{g}/\text{mm}^2$ treated vessel sections 8 weeks post ligation and 4 weeks post intervention, demonstrating a significant anti-proliferative and anti-inflammatory molecular signature. $*p<0.05$ vs PABA, $n=4-8$).

[0017] Figure 3 shows the chemical structure and UV365nm activation profile of conjugated MCT-3 / photo-labile linker, wherein (3A) is the chemical structure of conjugated MCT-3 demonstrating production of activated MCT-3 upon UV365nm exposure; (3B) shows the ESI-MS of conjugated MCT-3 (*) m/z 588.50; and (3C) shows the ESI-MS of native MCT-3 (†), m/z 307.35 produced from conjugated MCT-3 with subsequent 2 minute and $40\text{mW}/\text{cm}^2$ UV365nm exposure (view at 200%).

[0018] Figure 4 shows toxicity and potency of conjugated MCT-3, wherein (4A) shows a bar graph of percentage cellular toxicity of umbilical vein endothelial cells (HUVEC) treated under the following conditions: untreated (Control) and 24hr treated 1.0mmol/l conjugate-MCT-3 (cMCT-3) pre-UV irradiation; 1.0mmol/l conjugate-MCT-3 post 4 min UV 365 nm irradiation (cMCT-3+UV); 1.0mmol/l PTX (PTX); and 1.0mmol/l MCT-3; demonstrating significantly reduced conjugate-MCT-3 cellular toxicity in comparison to MCT-3 and PTX ($*p<0.05$ vs. 1.0mmol/l MCT-3 and 1.0mmol/l PTX. ($n=3-4$)) and no discernable toxicity impact attributable to the UV irradiation itself. Figure (4B) shows a Western Blot of histone H3 activity (blue arrow-head) in HL-60 cells, where Con = untreated; 3E=24hrs treated with elute ($10.0\mu\text{M}$) from conjugate-MCT-3 coated DEB (**no** UV365nm), MCT-3=24hrs treated with $10.0\mu\text{M}$ MCT-3, 3E+UV=24hrs treated with elute ($10.0\mu\text{M}$) from conjugate-MCT-3 coated DEB (with UV365nm, 4 minutes, 65mW). Figure (4C) shows an HPLC trace (280nm) of ethanol extracted tissue from target vessel post Epi-Solve UV365nm activation for 4 minutes, 65mW (view at 200%), where c-MCT-3 is conjugated MCT-3, MCT-3 is native MCT-3 generated *in-situ* post UV irradiation. The concentration of c-MCT-3 is in the order of 60 mM to 100mM and the concentration of MCT-3 generated *in-situ* in the target vessel tissue is in the order of 2% of that of the conjugated MCT-3, namely around 1 to 2 mM, which is a more than adequate concentration to effect the desired pharmacological activity.

[0019] Figure 5 is a still photograph from a video showing ultra-sonic spray coating of an angioplasty balloon catheter that has been coated with MCT-3 dissolved in a solution of

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ethanol and Ultravist excipient (1:1 mix). The video shows that a fine and even particulate coating is achieved with this application technology (view at 250%).

[0020] Figure 6 shows a photograph *in situ* of a 500µm fiber-optic light diffuser custom manufactured by Medlight S.A. to deliver UV365nm, wherein the light diffuser is located within an Abbott Armada 35 catheter balloon via the guidewire port.

[0021] Figure 7 shows a photograph of the device of the invention in operation, where A depicts the Prizmatix UV365nm light source, which is connected to a SMA905 connector, custom fibre-optic from Medlight and c-MCT-3 coated balloon catheter; and inset B shows a three times magnified image of the UV illuminated c-MCT-3 coated balloon (view 200%).

[0022] Figure 8 shows photographs of exteriorised ovine right internal carotid artery (RICA) with inflated conjugate MCT-3 coated DEB of the invention *in situ*, pre (A) and during (B) UV365nm light activation (view 200%).

Detailed description of the invention

[0023] Throughout this specification, unless the context requires otherwise, the word "comprise", or variations such as "comprises" or "comprising", will be understood to imply the inclusion of a stated integer or group of integers but not the exclusion of any other integer or group of integers.

[0024] The reference to any prior art in this specification is not, and should not be taken as, an acknowledgment or any form of suggestion that that prior art forms part of the common general knowledge in Australia.

[0025] All references cited in this document are included as part of the present disclosure by way of reference in their entirety.

[0026] Throughout this specification the terms "photo-labile drug / linker conjugate", "MCT-3 conjugate", "conj-MCT-3", "conjugate-MCT-3" and "conjugate" refer to the conjugate compound of Formula I, which comprises chemically bound MCT-3 (Formula II) and photo-labile linker, which can be prepared as outlined in detail in Example 1.

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[0027] The MCT-3 conjugate is cleaved into its component MCT-3 and linker components by UV irradiation and it is in this context that the linker and the drug / linker conjugate are referred to as being photo-labile. This cleavage of the MCT-3 conjugate is also referred to herein as activation of MCT-3, given that in its conjugated form the MCT-3 conjugate is substantially inactive and following UV initiated cleavage of the conjugate the MCT-3 is converted to a state where it is able to exhibit the desired inhibition of NIH activity.

[0028] In one aspect the present invention relates to a conventional balloon angioplasty catheter that is coated with the photo-labile drug/linker conjugate of Formula I. Importantly, the conjugate is coated onto all or at least part of the exterior surface of the balloon component of the catheter; the exterior surface being that surface of the ball that comes into contact with the vessel wall when the catheter is deployed and inflated. Without wishing to be bound by theory, it is understood by the present inventors that the conjugate or at least a proportion of the conjugate coated onto the balloon surface is released from the surface and enabled to come into intimate contact with the vessel wall at the time the balloon is inflated. The conjugate is then absorbed or taken up into the vessel wall, which may be assisted by including with the conjugate on the balloon coating one or more vessel avid agent/s as excipients in the coating material used to coat the balloon. Vessel avid excipients are agents that exhibit an appropriate safety profile and are taken up or absorbed across the vessel wall, likely into the extracellular region of the vessel wall. Examples of vessel avid agents include contrast media such as Ultravist®-370 (Bayer), an iopromide containing non-ionic and water soluble x-ray contrast medium. Other suitable vessel avid agents include hydrophilic carriers such as urea, shellac and butyryl trihexyl citrate and lipophilic lubricants including talc, magnesium stearate and calcium or ammonium salts.

[0029] The material used to coat the catheter balloon may include other excipients or indeed other therapeutically active agents. Examples of other therapeutic agents include other HDAC inhibitors such as trichostatin-A, suberoylanilide hydroxamic acid (SAHA), trapoxin, butyric acid, MS-27-275, oxamflatin, apicidin, depsipeptide, and depudecin. chemotherapeutic agents, such as paclitaxel, bleomycin, doxorubicin, adriamycin, 5FU, neocarcinostatin; other agents suitable for arresting the proliferation of smooth muscle cells such as rapamycin (sirolimus), zotarolimus, antiplatelet and anticoagulant agents, receptor blockers, growth

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factors and other hormones, anticoagulants, including heparin, hirudin, hirulog, tissue plasminogen activator, and fibrinogen; anti-inflammatory agents, such as steroids, ibuprofen, aspirin, somatostatin, angiopeptin, and anti-inflammatory peptide 2; cytotoxins, including colchicine, dexamethasone, doxorubicin, methotrexate, and psoralen; antibiotics; enzymes and enzyme inhibitors, including urokinase, 2,4-dinitrophenol, and thiol protease inhibitors; and immuno-suppressive agents such as tacrolimus, everolimus and cyclosporine. Such other therapeutic agents may also be bound to a photo-labile linker so as to be activated in a similar manner to MCT-3 conjugate.

[0030] Each excipient incorporated into the coating material must be pharmaceutically "acceptable" in the sense of being compatible with the other ingredients of the composition and not injurious to the subject. Examples of other excipients that can be incorporated into the coating material include buffers, diluents, solvents, emulsifiers, solubilizers, stabilizers, suspending agents, tonicity agents and viscosity altering agents. If necessary, pharmaceutical additives, such as antioxidants, aromatics, colorants, preservatives and radioactive or phosphorescent labelling or imaging agents may also be added. Further details of conventional pharmaceutical excipients and other additives are provided in Remington's Pharmaceutical Sciences, 18th Edition, Mack Publishing Co., Easton, PA, USA, the disclosure of which is included in its entirety by way of reference. Some agents included into the coating material may be present for the purpose of assisting application of the agent to the surface, such as to assist in passage through a spray coating device, to modify surface tension, to encourage even surface coating or to dissolve materials to be deposited on the balloon surface. For example, volatile solvents such as C₁ to C₆, preferably C₁ to C₄ alcohols, such as methanol or ethanol, may be used to assist in surface application of the material, while subsequently being eliminated from the surface by evaporation.

[0031] The balloon catheters of the present invention may be coated using a variety of techniques, including dipping, spraying, painting, printing, for example, of the coating material that incorporates the conjugate and any optional excipients, other therapeutic agents and/or agents to assist coating onto the balloon surface. In one embodiment, the balloon is spray coated and in one example the balloon is coated using an ultrasonic coating technique.

[0032] MCT-3 concentration on the surface of the balloon may conveniently be in the range of from about $0.1 \mu\text{g}/\text{mm}^2$ to about $100 \mu\text{g}/\text{mm}^2$, such as from about $0.5 \mu\text{g}/\text{mm}^2$ to about $50 \mu\text{g}/\text{mm}^2$, or from about $1 \mu\text{g}/\text{mm}^2$ to about $20 \mu\text{g}/\text{mm}^2$. In preferred aspects of the invention the MCT-3 concentration deposited on the balloon surface is from about $1 \mu\text{g}/\text{mm}^2$ to about $10 \mu\text{g}/\text{mm}^2$, from about $2 \mu\text{g}/\text{mm}^2$ to about $5 \mu\text{g}/\text{mm}^2$ or about $3 \mu\text{g}/\text{mm}^2$. For the purpose of determining the appropriate concentration of MCT-3 conjugate within the coating material account must be taken of the amount of MCT-3 within the conjugate solution and the surface area of the balloon to be coated can be calculated based on the surface area of the balloon approximating that of a cylinder ($2\pi rh$). The total quantity of MCT-3 required to coat each balloon at the desired concentration can then be readily calculated taking into account the coating density (dictated for example by ultrasonic spray coater flow rates and passage times in the case of spray coating or coating material viscosity in the case of dip coating methods).

[0033] Even though only relatively small percentages (for example about 0.1 % to about 5% or about 0.5% to about 4% or about 1% to about 3% or about 2%) of MCT-3 conjugate are converted to MCT-3 *in situ* at the site of desired MCT-3 action due to the relatively low UV energy delivered to the balloon catheter, low concentrations of MCT-3 at the vascular surface will suffice to effect the desired activity. For example, concentrations of MCT-3 at the vascular surface of about 0.5 mM to about 5 mM, about 1 mM to about 4mM, such as about 2 mM or about 3mM will be effective in inhibiting NIH.

[0034] In one embodiment of the present invention, the balloon is coated with the coating material in layers to thereby increase the effective surface concentration of the conjugate, or alternatively to add components in a layered approach, such as to facilitate release of components at different time points. For instance, the exterior surface of the balloon (e.g., the portion which, when deployed in a subject, contacts the subject's vasculature), may be coated with a biodegradable carrier that is removed during passage of the catheter to the site of intended deployment. Similarly, to protect the external surface of the balloon from unintended release of the conjugate an external sheath can be provided over the device that is removed at the time of deployment, which is routine in the field. In the case of adoption of a protective sheath the time between sheath removal and balloon inflation must be minimized to avoid premature shedding of the drug into the blood stream at the site prior to balloon inflation.

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[0035] As outlined above, a particular advantage associated with the present invention is that drug conjugate inadvertently released from the balloon site into the blood stream or away from the site of deployment of the balloon is inactive and will therefore no exhibit unintended activity away from the intended site of therapy. In order to cleave the MCT-3 conjugate from the linker to thereby activate the MCT-3 drug, UV light irradiation is required. Without wishing to be bound by theory it is understood that the MCT-3 conjugate is taken up or absorbed into the vessel wall, whereby UV irradiation at the site of balloon deployment penetrates into the vessel wall to thereby activate the drug at the intended site of action.

[0036] The required UV irradiation can readily be delivered via an optical fiber light diffuser that is connected by (including being integral to) an optical fiber to a source of UV light, wherein the optical fiber can readily penetrate through the vessels of the patient's body to the site of deployment of the balloon catheter and into the balloon catheter itself, for example via the guide wire port of the catheter. In use the catheter is guided into position adjacent to an area to be treated using, for example, a guide wire, and the balloon is then inflated by introducing a fluid (gas, liquid or liquid gas blend) into the inflation port of the catheter, so as to contact and dilate the surrounding tissue. Following inflation of the balloon, radiation from an irradiation source is delivered via one or more light diffusers connected to the optical fiber/s which extend through the terminal end of the catheter into the balloon. A diffusive radio-opaque tip is optionally attached to the terminal end of the optical fiber to form a light diffuser, through which the radiation is delivered and scattered throughout the balloon. The light delivered through the balloon subsequently causes photolytic cleavage of the MCT-3 conjugate to activate MCT-3 in, or at least in close vicinity to, the vessel wall.

[0037] For example the MCT-3 conjugate can be cleaved by exposure to UV irradiation at wavelengths of from about 350 nm to about 380 nm, from about 360 nm to about 370 nm, from about 362 nm to about 357 nm, from about 363 nm to about 368 nm, from about 364 to about 366 nm or at about 365 nm. For the purpose of UV initiated cleavage of the MCT-3 conjugate the UV light emission power is, for example, from about 5 mW/cm² to about 100 mW/cm² for a duration of from about 2 seconds to about 10 minutes, such as from about 20 mW/cm² to about 80 mW/cm² for a duration of from about 30 seconds to about 5 minutes, from about 30 mW/cm² to about 50 mW/cm² for a duration of from about 60 seconds to about

3 or 4 minutes, or from about 35 mW/cm² to about 45 mW/cm² for a duration of from about 1.5 minutes to about 2.5 minutes, or about 40 mW/cm² for a duration of about 2 minutes. The duration of illumination and power of UV light irradiation adopted can readily be optimized by a skilled person to ensure activation of the desired dose of active MCT-3 at the site of balloon deployment.

[0038] The UV irradiation is generated by a UV light source, which may, for example, comprise a laser generator tuned to the desired wavelength of irradiation. Intermediate linkages to dye filters may be utilized to screen out transmitted energy at unused or antagonistic wavelengths (particularly cytotoxic or cytogenic wavelengths), and secondary emitters may be utilized to optimize the light energy at the principle wavelength of the laser source or the geometry of the catheter system. Radiation to promote activation of MCT-3 from the MCT-3 conjugate can be provided by a variety of sources including, but not limited to, non-coherent UV light sources and excimer sources. In one embodiment the components utilised are a portable Prizmatix Silver-LED high power UV365nm light source with SMA905 fibre-optic connector, which delivers >65mW UV365nm to a 500µm fibre-optic light diffuser custom manufactured by Medlight S.A., Switzerland via the guidewire port to an Abbott Armada 35 catheter balloon coated with MCT-3 conjugate. A customized catheter can also readily be manufactured utilizing routine techniques, which includes an integral optical fiber, instead of requiring a separate optical fiber to be passed through the guide wire lumen of a conventional catheter assembly.

[0039] Typical features of balloon catheters well known in the art include a catheter body which may include a covering enclosing the conduit, wherein the covering is fabricated from a suitable polymer material as described herein to permit transmission of adequate light energy to accomplish the photolytic cleavage reaction and release the active MCT-3. The catheter may include a fiber optic conduit disposed partially within or between the catheter body and covering, with the dilation balloon surrounding the catheter body and fluidly communicating with the lumen such that the dilation balloon may be selectively inflated or distended, and wherein the dilation balloon therefore defines the surface on which the MCT-3 conjugate is applied. As used herein, the term catheter comprises a generally tubular medical device for insertion into vessels, particularly blood vessels. The design, fabrication, construction, or

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assembly of conventional catheters may be utilized to provide certain capabilities required by the procedure being performed and to accomplish adjunct functions unrelated to the delivery or application of the drug to the remotely located tissue site. Conventional catheter designs that may be adapted for the site-specific application or delivery of drug according to the methods described herein include percutaneous transluminal angiography (PTA) catheters, percutaneous transluminal coronary angioplasty (PTCA) catheters, vascular and peripheral vascular catheters, thrombectomy catheters and embolectomy catheters, with dilation capabilities. As is well understood in the art, the catheters of the present invention may be manufactured from a variety and/or a combination of biocompatible and non-biocompatible materials, including, without limitation, polyester, Gortex, polytetrafluoroethylene (PTFE), polyethylene, polypropylene, polyurethane, silicon, steel, stainless steel, titanium, Nitinol or other shape memory alloys, copper, silver, gold, platinum, Kevlar fiber, and carbon fiber. Where non-biocompatible materials may come into contact with a subject's anatomy, the components made from the non-biocompatible materials may be covered or coated with a biocompatible material.

[0040] According to the present invention the therapeutic methods described herein can be performed on an animal subject or patient and in this context the animal may be an experimental animal (eg. mouse, rat, guinea pig, rabbit), a companion animal (eg. cat, dog), an agricultural animal (eg. horse, cattle, sheep, donkey, goat, pig), a reptile, avian or captive wild animal. Preferably the subject animal is a mammal and most preferably the animal is a human. As will be well understood the appropriate coating concentration of the drug conjugate on the balloon surface and drug activation level by UV irradiation will depend upon issues such as the age, weight, height, gender, particular vessel location of intended treatment and general state of health of the subject and can, based upon such factors, be readily determined by a skilled medical practitioner.

[0041] For example, blood vessels in which the procedures according to the present invention may be performed upon include arteries such as central arteries including coronary arteries, the aorta, carotid artery, subclavian artery, axillary artery, common and internal iliac arteries, and peripheral arteries such as femoral artery, brachial artery, anterior tibial artery, dorsalis pedis artery and arch of foot artery as well as arterioles and veins, in particular major

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veins such as the vena cava, pulmonary vein, femoral vein, internal and external jugular veins, renal vein, subclavian vein, axillary vein, cephalic vein, superior mesenteric vein, basilic vein, femoral vein and great saphenous vein. For example, methods of the invention may be performed on veins in the case of venous bypass grafts, which can subsequently develop NIH. Methods of the invention may also be performed to prevent fistula NIH and on arteriovenous fistulas, which are created by joining arteries and veins, such are extensively used for hemodialysis and subject to varying rates of NIH.

[0042] The treatment according to the present invention is effect to prevent or at least minimize the incidence of NIH in a blood vessel at the site of, and following an angioplasty procedure. As demonstrated in the examples, a comparison of the effect upon NIH can readily be made following treatment with a balloon according to the present invention against the effect of treatment with an equivalent uncoated balloon catheter. For example, in an experimental animal patient the effect can be monitored by analysis of treated and control blood vessels post-mortem, for example by fixing vessel section and measuring neointimal area. In the case of a human patient such analysis may be undertaken by imaging of the vessel wall post therapy using conventional angiography techniques, ultrasound imaging and contrast agents.

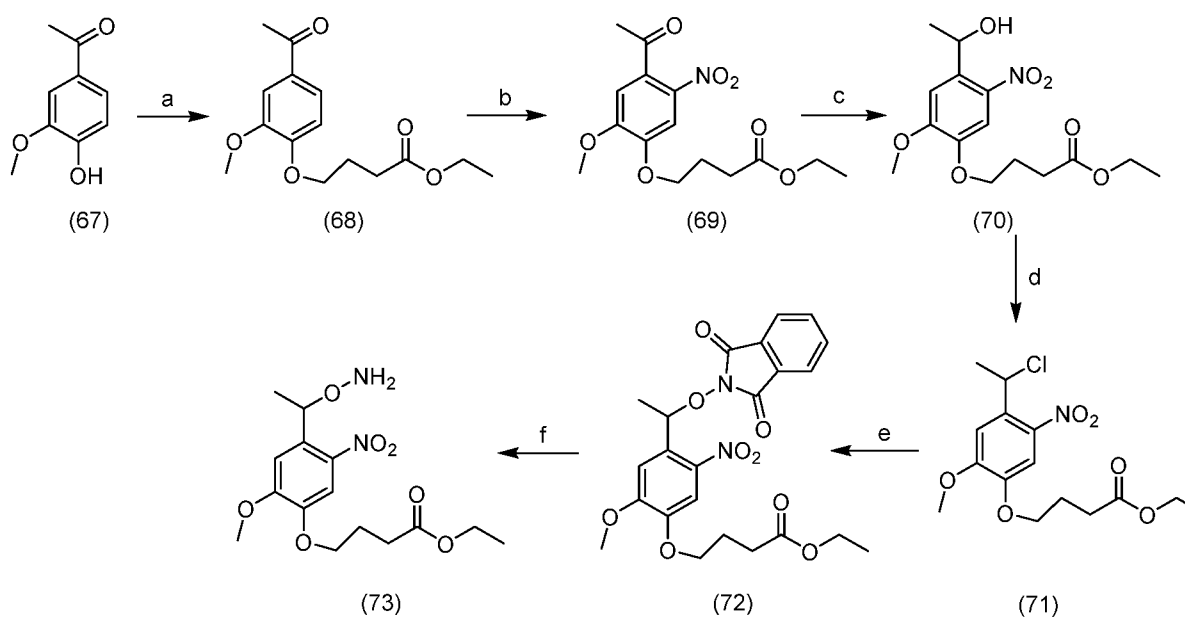
[0043] Those skilled in the art will appreciate that the invention described herein is susceptible to variations and modifications other than those specifically described. It is to be understood that the invention includes all such variations and modifications. The invention also includes all of the steps, features, compositions and compounds referred to or indicated in this specification, individually or collectively, and any and all combinations of any two or more of said steps or features.

[0044] The invention will now be described with reference to the following Examples. These Examples are not to be construed as limiting the invention in any way.

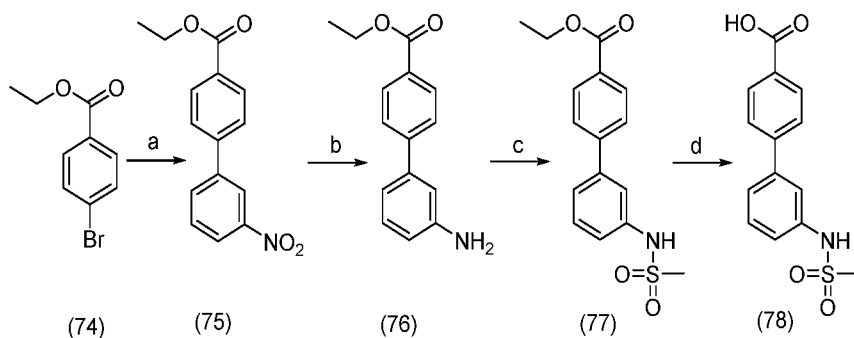
Examples

Example 1 – Chemical synthesis of metacept-3 / photo-labile linker conjugate

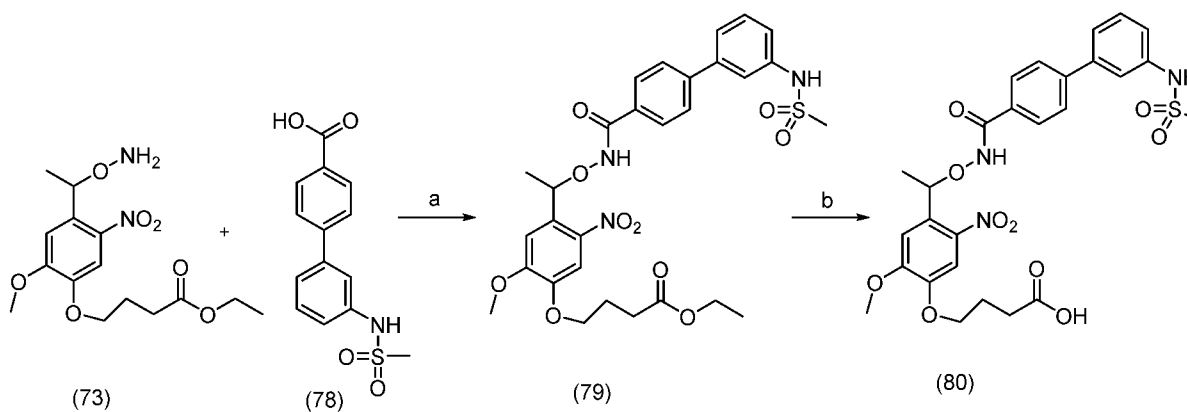
Scheme-1: Schematic representation of hydroxylamine (intermediate-I) (73).



[0045] Reagents and reaction conditions: a) K_2CO_3 , Ethyl-4-bromobutyrate, DMF, $50^\circ C$ o/n. b) acetic anhydride, HNO_3 , 3hrs. c) $NaBH_4$, MeOH, 4 hrs. d) $SOCl_2$, DCM, 3 hrs. e) *N*-hydroxyphthalimide, TEA, DMF, $60^\circ C$. f) $NH_2NH_2 \cdot H_2O$, EtOH.

Scheme-2: synthesis of Metaccept-3 carboxylic acid (intermediate-II) (78).

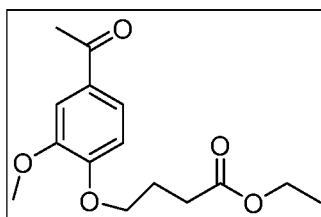
[0046] Reagents and reaction conditions: a) 3-nitrophenyl boronic acid, Na_2CO_3 , 10% Pd/C, EtOH, reflux, 24hrs. b) H_2 , 10% Pd/C, EtOAc, 48 hrs. c) MeSO_2Cl , pyridine, RT, 12 hrs. d) NaOH (aq), MeOH, RT, 3 hrs.

Scheme-3: Schematic representation of Metaccept-3 / photo-labile linker conjugate (80).

[0047] Reagents and reaction conditions: a) PyBOP, NMM, DMF, o/n. b) Phosphate buffer, dioxane, Novozyme-435.

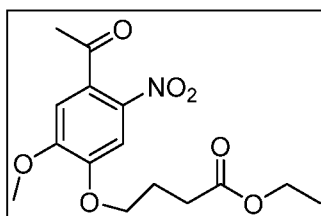
*Experimental procedures*Synthesis of Hydroxalamine (Intermediate-I)

(68) - Ethyl 4-(4-acetyl-2-methoxyphenoxy) butanoate



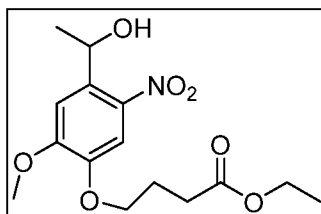
[0048] Ethyl 4-bromobutyrate (8.6 mL, 60 mmol) was added to the suspension of acetovanillone (10.0 g, 60 mmol), K_2CO_3 (12.4 g, 90 mmol) in DMF. The reaction mixture was stirred at room temperature for 24 hours, and then K_2CO_3 was filtered off. After filtration, water was added to the reaction mixture and extracted with ethyl acetate. Organic layer repeatedly washed with ethyl acetate, dried over sodium sulphate, and recrystallised from ethyl acetate and hexane. Required ketoester was obtained in quantitative yields. (16.8g)

(69) Ethyl 4-(4-acetyl-2-methoxy-5-nitrophenoxy) butanoate



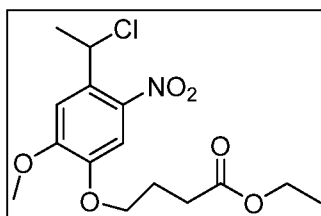
[0049] A solution of compound 68 (10.0 g, 35.7 mmol) in acetic anhydride (30 mL) was added dropwise to the mixture of 70% HNO_3 (200 mL) and acetic anhydride (40 mL) at 0 °C. The resultant reaction mixture was stirred at same temperature for 3 hours. After completion of the reaction by TLC, reaction mixture was immediately poured into ice-cold water and the precipitate was immediately collected by filtration. The precipitate was washed repeatedly with water and dried under vacuum to afford 10.8 g required compound in 82% yield (9.5 g) as a pale yellow solid.

(70) Ethyl 4-(4-(1-hydroxyethyl)-2-methoxy-5-nitrophenoxy) butanoate



[0050] Sodium borohydride (1.2 g, 31.7 mmol) was slowly added in portion wise to the solution of compound 69 (4.0 g 12.3 mmol) at 0 °C. After complete addition of the reagent, the reaction mixture was warmed to room temperature. Reaction mixture was stirred for 3 hrs, the reaction mixture was quenched with sat.ammonical solution. Reaction mixture was partitioned between water and ethyl acetate and organic layer was extracted and dried over sodium sulphate. The crude compound was recrystallised from ethyl acetate and hexane.(Yield 4.0 g)

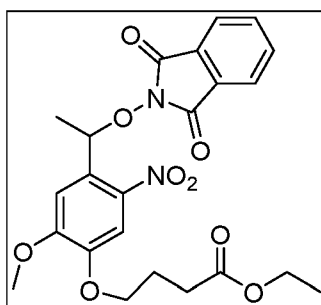
(71) Ethyl 4-(4-(1-chloroethyl)-2-methoxy-5-nitrophenoxy) butanoate



[0051] Thionyl chloride (15 mL) was added to the solution of hydroxy compound 70 (3.0 g, 9.17 mmol) in dichloromethane at 0 °C. The reaction mixture was allowed to warm to room temperature and stirred for 3 hours. Solvent was removed under vacuum and co-evaporated 3 times with toluene. Crude compound was purified by column chromatography using ethyl acetate and hexane as an eluent to obtain pale yellow coloured solid (yield 90%, 2.84 g).

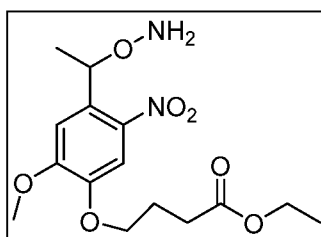
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(72) Ethyl 4-(4-(1-((1,3-dioxoisindolin-2-yl)oxy)ethyl)-2-methoxy-5-nitrophenoxy)butanoate



[0052] Compound 71 (2.0 g, 5.7 mmol) and N-hydroxyphthalimide (1.1 g, 6.8 mmol) were dissolved in DMF and triethyl amine (2 mL) was added. The reaction mixture was stirred at 60 °C for 12 hrs before being cooled to room temperature. Water added to the reaction mixture and extracted with ethyl acetate. Organic layer was dried over sodium sulphate, concentrated in vacuo, and purified by column chromatography to obtain yellow coloured solid (yield 70%).

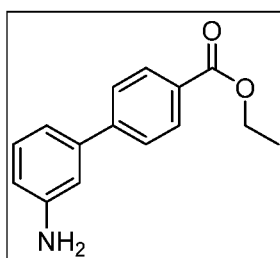
(73) Ethyl 4-(4-(1-(aminooxy)ethyl)-2-methoxy-5-nitrophenoxy)butanoate



[0053] Hydrazine hydrate (0.45 mL, 65%) was added to the compound 72 (1.0 g, 2.1 mmol) in ethanol (15 mL). The reaction mixture was refluxed for 2 hrs and the resulting white precipitate was removed by filtration. Filtrate was concentrated to give required hydroxylamine as a yellow coloured liquid.

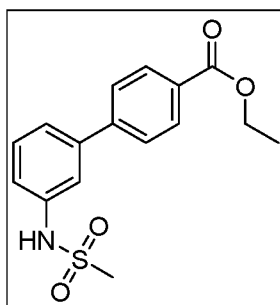
Synthesis of 3'-(methylsulfonamido)-[1,1'-biphenyl]-4-carboxylic acid – (Intermediate II)

(76) Methyl 3'-amino-[1,1'-biphenyl]-4-carboxylate



[0054] Sodium carbonate (1.1 g, 10 mmol) and palladium on charcoal (480 mg, 10% wt Pd, 0.45 mmol) added to the solution of 3-nitrophenylboronic acid (1.5 g, 9 mmol) and Ethyl 4-bromobenzoate (2.0 g, 9.5 mmol) in ethanol. The resultant reaction mixture was refluxed for 28 hrs under inert atmosphere and cooled to room temperature. After removing the Sodium carbonate by filtration, the crude reaction mass was dissolved in ethyl acetate and reduced to amine in the presence of 10% Pd/C, under hydrogen atmosphere for 24 hrs. After complete conversion of the starting material to amine, the suspension was filtered through celite bed, diluted with water, and extracted with ethyl acetate. The crude reaction mixture was purified by column chromatography to obtain required compound in 80% yield as yellow coloured liquid (1.7 g).

(77) Methyl 3'-(methylsulfonamido)-[1,1'-biphenyl]-4-carboxylate

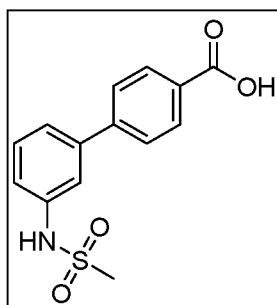


[0055] To a suspension of compound 68 (1.0 g, 3.3 mmol) in DMF, K₂CO₃ (678 mg, 4.9 mmol) was added. After stirring for 30 min, benzyl bromide (0.43 mL, 3.6 mmol) was added

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and stirred overnight. The reaction mixture was diluted with water, extracted with ethyl acetate, and recrystallised from ethyl acetate and hexane. (Yield 90%, 1.2 g).

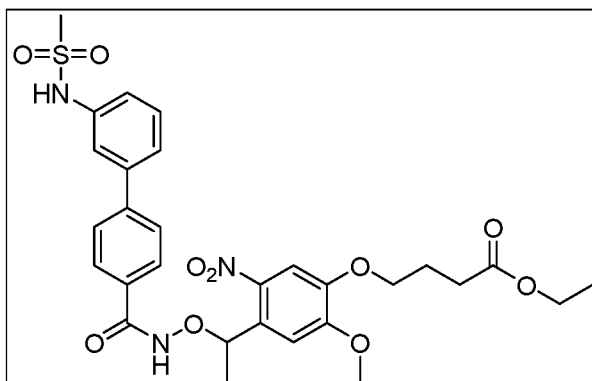
(78) 3'-(methylsulfonamido)-[1,1'-biphenyl]-4-carboxylic acid



[0056] Solution of compound 77 (500 mg, 1.26 mmol) dissolved in mixture of methanol and THF (2:1) was added aqueous sodium hydroxide (1.5 mL). Resultant reaction mixture was refluxed for 3 hrs and cooled to room temperature. Alkaline reaction mixture was acidified with 1M HCl and extracted with ethyl acetate to obtain required compound in quantitative yields (450 mg).

Synthesis of Metaccept-3 / photo-labile linker conjugate

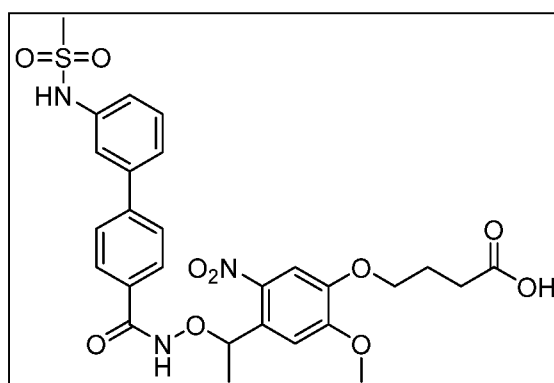
(79) Ethyl 4-(2-methoxy-4-(1-(((3'-(methylsulfonamido)-[1,1'-biphenyl]-4-carbonyl)-12-azanyl)oxy)ethyl)-5-nitrophenoxy)butanoate



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[0057] PyBOP (520 mg, 1.0 mmol) and NMM (0.33 ml, 3mmol) was added to the solution of compound 78 (370 mg, 1.27 mmol) in DMF. After stirring the reaction mixture for 5 min compound 73 (340 mg, 1.0 mmol) in DMF was added. The reaction mixture was allowed to react overnight and partitioned between water and ethyl acetate. The organic layer was back extracted for three times and the organic layer was dried over sodium sulphate. The crude compound was purified by column chromatography to obtain required hydroxamate in 80% yield (625 mg).

(80) 4-(2-methoxy-4-(1-((3'-(methanesulfonylamino)-[1,1'-biphenyl]-4-carboxamido)oxy)ethyl)-5-nitrophenoxy)butanoic acid



[0058] Compound 79 (290 mg, 0.51 mmol) was dissolved in the mixture of dioxane and phosphate buffer (5:1) 5 mL and Novozyme 435 (200 mg) was added. The reaction mixture was shaken for 5 days at 30 °C. After 5 days of stirring, enzyme was filtered off and crude compound was recrystallised from ethyl acetate and hexane to obtain required MCT-3 / photo-labile linker conjugate as a pale yellow coloured solid in 70 % yield (191 mg).

Example 2 – Synthesis of MCT-3

[0059] MCT-3 (3'-methanesulfonylamino-biphenyl-3-hydroxamic acid) (molecular weight 306 Da), a derivative of oxamflatin, was synthesised³⁴ to 95+% purity.

Example 3 - Production of Drug Eluting Balloon Catheters

MCT-3 coated DEBc

[0060] MCT-3 eluting balloon angioplasty catheters were produced using plain balloon angioplasty catheters provided by Medtronic Australasia Pty. Plain balloon catheters ranged from 3.0-6.0mm in balloon diameter and 40-80mm in balloon length. A Sonotek Ultrasonic Spray Coater (CSIRO Biomedical Translational Facility (BMTF)/Monash Medtech and Bio21 Institute, The University of Melbourne) at a flow rate 0.05-0.1ml/min and pressure 3.0psi and a 12-24sec passage time was used to deliver a 65.0mM/l solution of MCT-3 (400mg MCT-3 dissolved initially in 1.0ml DMSO then in 19.0ml of a 50:50 mix of 100% ethanol and Ultravist®-370 (Bayer) (an iopromide containing non-ionic and water soluble x-ray contrast medium) to the surface of plain balloon catheter balloons to give a final coating concentration of 3.0µg/mm².

[0061] The surface area of each balloon coated was calculated based on the surface area of the balloon approximating that of a cylinder ($2\pi rh$). The total quantity of MCT-3 required to coat each balloon at 3.0ug/mm² was calculated and delivered to the surface of the angioplasty balloon catheter using predetermined ultrasonic spray coater flow rates, passage times and MCT-3 drug concentration (65.0mmol/l).

[0062] The spray coating process does not impact upon biological activity of MCT-3.

PTX coated DEBc

[0063] PTX coated DEBc were provided by Medtronic Australasia Pty. The IN.PACT Admiral 130cm in length, 40-60mm balloon, 5.0mm balloon diameter coated with 3.0µg/mm² paclitaxel were used in *in vivo* studies.

MCT-3 / photo-labile linker conjugate coated DEBc

[0064] The MCT-3 / photo-labile linker conjugate coated DEBc (conjugate coated DEBc, as shown in Figure 5) is produced using the same ultrasonic coating technique optimised

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during production of MCT-3 coated DEBc outlined above, in collaboration with the CSIRO, Manufacturing Division, Clayton, Melbourne. MCT-3 / photo-labile linker conjugate dissolved in a 1:1 ethanol (100%):Ultravist solution generates an even, fine particulate finish with complete evaporation of ethanol at coating completion.

[0065] A portable Prizmatix Silver-LED high power UV365nm light source with SMA905 fibreoptic connector delivers >65mW UV365nm to a 500µm fibre-optic light diffuser custom manufactured by Medlight S.A., Switzerland via guidewire port to an Abbott Armada 35 catheter balloon (Figure 6). The complete device with all components connected and illuminating UV light is depicted in Figure 7. Four minute, UV365nm balloon surface activation of 3.0µg/mm² conj-MCT-3 delivers 1.3mmol/l surface-active MCT-3 (Figure 3) and gave rise to significant HDAC inhibitory activity (Figure 4).

Example 4 - *In vivo* studies

[0066] An ovine model of vascular injury induced neointimal hyperplasia was established to evaluate the effect of MCT-3 coated DEBc in comparison to uncoated and PTX coated DEBc. The partial ligation method of flow induced vascular remodelling (35) was adapted for use in our ovine system.

[0067] Sheep were anaesthetised using 5 mg/kg of propofol delivered using an 18G Jelco catheter placed in the foreleg. The animals were intubated and anaesthesia was maintained with gaseous isoflurane at a flow rate of 2.5% with positive ventilation. Following stable anaesthesia, a sterile surgical site was prepared on the left side of the neck. The left internal carotid artery (LICR) was exposed by blunt dissection and partially ligated with a silk suture. The incision was then closed, again with a silk suture (Dynek) and the animals recovered. At this point animals were assigned to either the MCT-3-coated, PTX-coated or control uncoated (PABA) group. 28 days' post partial ligation of the LICR the treated group of animals had either a MCT-3 or PTX DEBc positioned and inflated in the region of LCA ligation for 120 seconds whilst the control group had a plain, uncoated angioplasty balloon (PABA) inflated in the same region of LICA ligation, also for 120 seconds. Subsequently both MCT-3 or PTX

and plain angioplasty balloons devices were withdrawn and the introducer sheath removed from the vessel together with purse string suture closure. The skin incision was closed and the animals recovered. Following 28 days, the animals were euthanized and the region of interest (ligated LICA blood vessel) collected, together with the contralateral un-ligated right internal carotid artery (RICA) serving as an internal control, for histopathological analysis.

Carotid artery neointimal hyperplasia testing model

[0068] Carotid arteries were fixed with 10% neutral buffered formaldehyde solution for 48hrs and were then embedded in paraffin. Carotid artery sections (4µm) from both ligated and contralateral arteries were stained with Hematoxylin and Eosin (H&E). Briefly, after dewaxing with xylene and rehydration through a graded series of ethanol washes staining was performed for 5 minutes at 25°C. Sections were rinsed under running tap water for 30 seconds, differentiated in acid alcohol for 1 second and then rinsed under running tap water for 30 seconds. They were then blued in Scott's tap water for 3-10 seconds, rinsed under running tap water for 30 seconds and stained with alcoholic eosin for 5 minutes. Subsequently, slides were incubated in absolute alcohol x3 for 2 minutes each, cleared in xylene x3 for 2 minutes each and mounted with Permount. H&E stained sections were imaged using an Olympus BX50 microscope and images scanned using the Aperio Scanscope AT Turbo scanner. Image J was then used to measure the medial and neointimal areas of each section with morphometric analysis performed by an investigator who was kept blind to the experimental procedure. The intimal and medial area of each group, and their ratio were calculated by the following protocol: Intima area = (equals) inner elastic membrane surrounding area – (minus) lumen area; media area = (equals) outer elastic membrane surrounding area – (minus) inner elastic membrane surrounding area.

Evaluation of Carotid artery neointimal hyperplasia

[0069] In order to evaluate the *in vivo* effects of the MCT-3 device an ovine partial carotid ligation model was established as outlined above. Radiological and gross macroscopic evaluation identified development of a significant stenosis at the site of partial carotid artery

ligation with resultant proximal neointima formation compared to the contralateral un-ligated vessel in control animals as assessed by Haematoxylin and Eosin and VVG staining.

[0070] Deployment of the MCT-3-coated device 28 days after partial carotid ligation in the ovine model resulted in significant attenuation of neointima (NI) formation compared with uncoated balloon angioplasty deployment (Figure 1). In addition MCT-3 attenuated NI formation more effectively than PTX coated DEBc (also Figure 1).

Protocol for gene expression signature in-vivo analysis

[0071] Extraction of mRNA from formalin fixed paraffin embedded (FFPE) sections from index and contralateral carotid artery sections from untreated, MCT-3 and PTX treated animals was undertaken utilising the Qiagen RNeasy FFPE RNA kit according to the manufacturer's instructions.

[0072] RT-PCR: Reverse Transcription mRNA was performed using a 20 µl reaction mix containing dNTPs (100mM), MultiScribe Reverse Transcriptase (50U/µl), RT buffer (10X), RNase Inhibitor (20U/µl), nuclease free water (Invitrogen; Thermo Fisher Scientific, Inc, Waltham, MA, USA), primer and mRNA samples.

[0073] Real-time PCR: Real-time analysis of mRNA expression was performed in duplicate using Sensifast SYBR No-Rox kit (Bioline, London, UK). Reaction volumes of 20 µl contained 2x Sensifast SYBR no-rox mix, nuclease-free water and RT reaction product. Each PCR run also included wells of no template control (NTC). A melting point dissociation curve generated by the instrument (Applied Biosystems 7500 Real-Time PCR System; Thermo Fisher Scientific, Inc, Waltham, MA, USA) was used to confirm that only a single product was present. The fluorescence data were quantitated using the threshold cycle (C_T) value. Data was normalise to Actin and presented as the mean fold change compared with the pre-treatment screen sample.

Primer sequences for PCNA, Ki67, p21WAF1/CIP1, IL-6, MCP-1 and Actin

[0074] Proliferating cell nuclear antigen (PCNA) fwd 5' AGG-CGC-TTA-AGG-ATC-TCA-T 3', rev 5' GAA-GGG-TTA-GCT-GCA-CCA-AG 3'; Ki67 fwd 5' ACA-GCT-AGA-ACA-TGG-CGA-TG 3', rev 5' GGA-AAT-CCA-GGT-GAC-TTG-CT 3'; p21CIP1/WAF1 fwd 5' CCA-GAC-CAG-CAT-GAC-AGA-TTT-C 3', rev 5' GCT-TCC-TCT-TGG-AGC-AGA-TCA-G 3'; IL-6 fwd 5' GCT-GCT-CCT-GGT-GAT-GAC-TTC 3', rev 5' GGT-GGT-GTC-ATT-TTT-GAA-ATC-TTC-T 3'; and MCP-1 fwd 5' GCT-GTG-ATT-TTC-AAG-ACC-ATC-CT 3', rev 5' GGC-GTC-CTG-GAC-CCA-TT3' and Actin fwd 5' AAG-AAG-AAA-TTG-CCG-CCC-TCG 3', rev 5' TAA-GGG-TTA-GGA-TGC-CAC-GCT-T 3'

Evaluation of gene expression signature in-vivo

[0075] Evaluation of gene expression signatures from partially ligated carotid arteries treated with plain balloon angioplasty, MCT-3 DEB or PTX DEB was performed. Proliferative gene expression profiling demonstrated attenuation of Ki67 and PCNA expression together with augmentation of p21^{CIP1/WAF1} expression in MCT-3 treated blood vessels over plain balloon angioplasty treatment (Figure 2), which is consistent with a significant anti-proliferative and anti-inflammatory molecular signature.

Example 5 - In vitro studies

Cell culture and toxicity studies

[0076] HL60 cells were cultured in RPMI-1640 (Gibco; Thermo Fisher Scientific, Inc., Waltham, MA, USA) containing 10% heat-inactivated fetal calf serum (Thermo Fisher Scientific, Inc.) and kept in a 5% CO₂ incubator at 37 °C. The agents added to plates for 24 h were as follows: MCT-3, MCT-3 conjugate without UV exposure and MCT-3 conjugate with UV exposure and were used at a final concentration of 10.0 µM.

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[0077] Human umbilical vein endothelial cells (HUVECs) [Lonza (CC-2519, pooled donor), Basel, Switzerland] were maintained in Media-199 (Sigma, USA) supplemented with penicillin/streptomycin, 20% fetal calf serum (FCS), 20 µg/mL endothelial cell growth factor (Sigma) and 20 µg/mL heparin and kept in a 5% CO₂ incubator at 37 °C and used for cell cytotoxicity studies from MCT-3, MCT-3 conjugate and PTX DEBc eluted samples.

[0078] MCT-3, MCT-3 conjugate and PTX were added to 1x10⁵ HUVEC at a final concentration of 1.0mmol/l for 24hrs. Cell viability was assessed using 0.4% trypan blue staining immediately after culture. Black staining cells were considered as non-viable cells and unstained bright cells as viable. All experiments were repeated a minimum of 3 times with averages displayed graphically. Results demonstrating reduced cell death resulting from MCT-3 conjugate in comparison to MCT-3 and PTX treated cells are shown in Figure 4A.

Western Blot H3 acetylation

[0079] HL60 cells were treated with elute captured following washing of uncoated DEBc (Con) or DEBc coated with MCT-3 or MCT-3 conjugate with (3E+UV) and without (3E) UV exposure, where washed was with phosphate-buffered saline (PBS). Protein extracts were obtained from HL60 cells using extraction buffer with protease inhibitors (Roche) and PhosSTOP (Roche). Protein concentrations were determined using Bradford protein assay (BioRad). Protein (25 µg per lane) was loaded onto a 15 % precast SDS polyacrylamide electrophoresis gel (BioRad). After electrophoresis (100 V, 90 min), separated proteins were transferred (15 mA, 60 min) to polyvinylidene difluoride membrane (BioRad). Nonspecific binding sites were blocked with 5 % non-fat milk (GE Healthcare, USA) for 120 min at room temperature, and blots were then incubated with antibodies against p21CIP1/WAF1 (sc-397; SantaCruz Biotechnology), or Histone H3 Acetyl overnight at 4 °C. Anti-rabbit horseradish-peroxidase-conjugated IgG (1:1000; DakoCytomation) was used to detect the binding of its correspondent antibody. Blots were also re-probed for β-actin (Cell Signalling) to ensure equal protein loading. Protein expressions were detected with ECL Advanced Western Blotting Detection Kit (GE Healthcare) and quantified using Quantity One (v.4.6.7) analysis software (BioRad).

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[0080] The comparable levels of histone H3 production in the HL-60 cells exposed to elute from MCT-3 DEBc and MCT-3 conjugate coated DEBc following UV exposure demonstrate the biological efficacy of the UV initiated cleavage of the conjugate in inducing HDACi activity (Figure 4B).

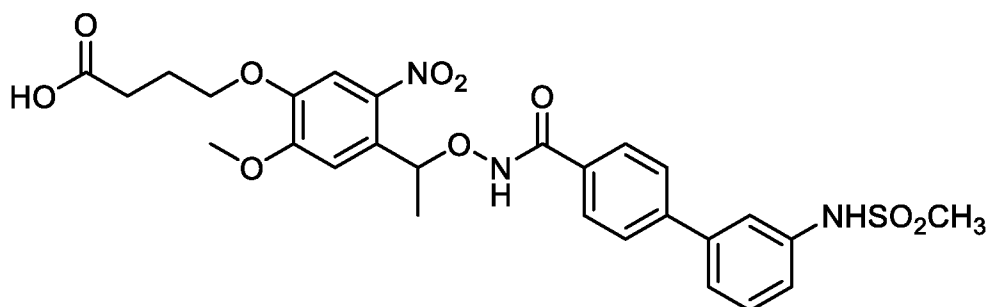
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THE CLAIMS DEFINING THE INVENTION ARE AS FOLLOWS:

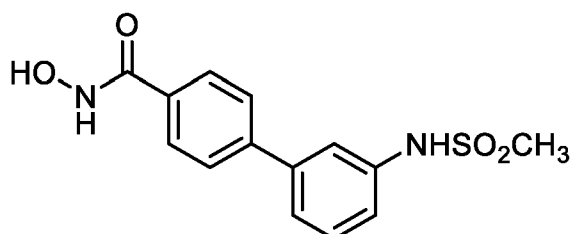
1. A method of preventing or minimizing incidence of neointimal hyperplasia (NIH) in a blood vessel of an animal following angioplasty treatment comprising locating an angioplasty balloon catheter at a vessel site where angioplasty treatment is desired, wherein a balloon component of the catheter is at least partially coated on an external surface thereof with a photo-labile drug / linker conjugate of Formula I:



Formula I

;

wherein the balloon is inflated at the site to initiate release of the drug / linker conjugate to vessel wall at the site and an optical fiber light diffuser, connected by optical fiber to a source of UV light at a wavelength of from about 350 nm to about 380 nm, is located within the balloon to emit said UV light, wherein UV light emission is of suitable power and for sufficient duration to cleave the drug / linker conjugate and activate an effective amount of the drug of Formula II to prevent or minimize NIH following the angioplasty treatment at the site:



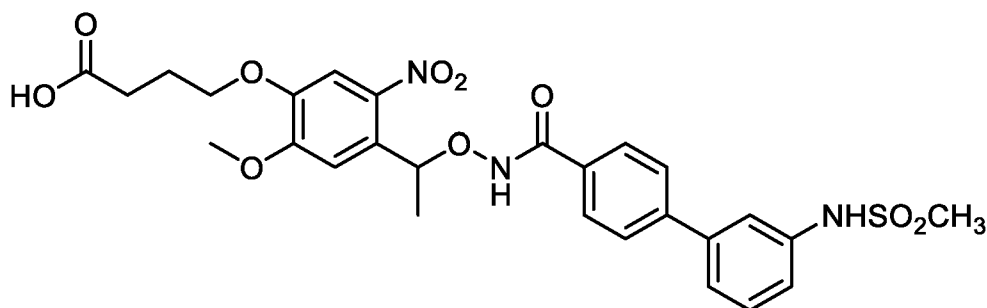
Formula II

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2. The method of claim 1 wherein the balloon component of the catheter is at least partially coated with the photo-labile drug / linker conjugate in combination with one or more excipients and/or other active agents.
3. The method of claim 2 wherein the one or more excipients comprise a vessel wall avid agent.
4. The method of claim 3 wherein the vessel wall avid agent comprises iopromide.
5. The method of any one of claims 1 to 4 wherein the UV light is at a wavelength of from about 360 nm to about 370 nm.
6. The method of any one of claims 1 to 4 wherein the UV light is at a wavelength of about 365 nm.
7. The method of any one of claims 1 to 6 wherein the blood vessel is an artery.
8. The method of claim 7 wherein the artery is a coronary artery.
9. The method of claim 7 wherein the artery is a peripheral artery.
10. The method of any one of claims 1 to 9 wherein UV light emission power is from about 5 mW/cm² to about 100 mW/cm² for a duration of from about 2 seconds to about 10 minutes.
11. The method of any one of claims 1 to 9 wherein UV light emission power is from about 30 mW/cm² to about 50 mW/cm² for a duration of from about 60 seconds to about 4 minutes.
12. The method of any one of claims 1 to 11 wherein the animal is a human.

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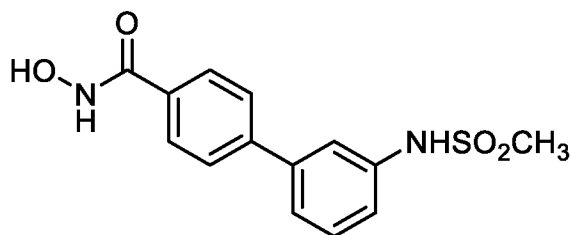
13. An angioplasty balloon catheter for preventing or minimizing incidence of neointimal hyperplasia (NIH) in a blood vessel of an animal following angioplasty treatment, wherein the balloon component of the catheter is at least partially coated on an external surface thereof with a photo-labile drug / linker conjugate of Formula I:



Formula I

;

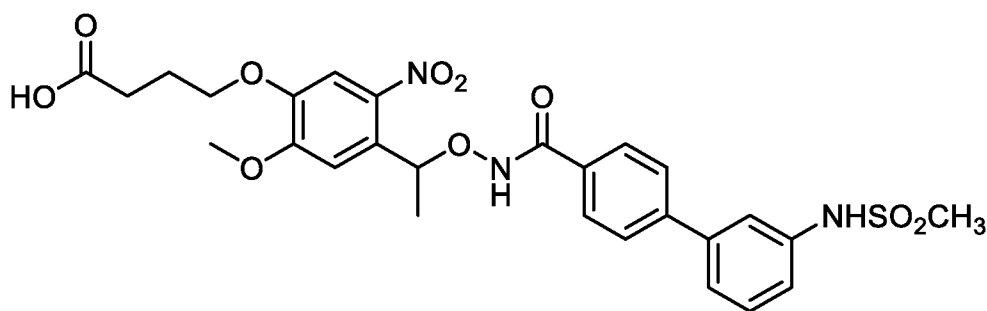
wherein the catheter comprises or engages with an optical fiber light diffuser connected by optical fiber to a source of UV light at a wavelength of from about 350 nm to about 380 nm, the light diffuser being located within the balloon to emit said UV light of suitable power and for sufficient duration to cleave the drug / linker conjugate and activate an effective amount of the drug of Formula II at a site of angioplasty treatment, to prevent or minimize NIH following angioplasty treatment at the site:



Formula II

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14. The angioplasty balloon catheter of claim 13 wherein the balloon component of the catheter is at least partially coated with the photo-labile drug / linker conjugate in combination with one or more excipients and/or other active agents.
15. The angioplasty balloon catheter of claim 14 wherein the one or more excipients comprise a vessel wall avid agent.
16. The angioplasty balloon catheter of claim 15 wherein the vessel wall avid agent comprises iopromide.
17. The angioplasty balloon catheter of any one of claims 13 to 16 wherein the UV light is at a wavelength of from about 360 nm to about 370 nm.
18. The angioplasty balloon catheter of any one of claims 13 to 16 wherein the UV light is at a wavelength of about 365 nm.
19. An angioplasty balloon catheter assembly for preventing or minimizing incidence of neointimal hyperplasia (NIH) in a blood vessel of an animal following angioplasty treatment, wherein the balloon component of the catheter is at least partially coated on an external surface thereof with a photo-labile drug / linker conjugate of Formula I:

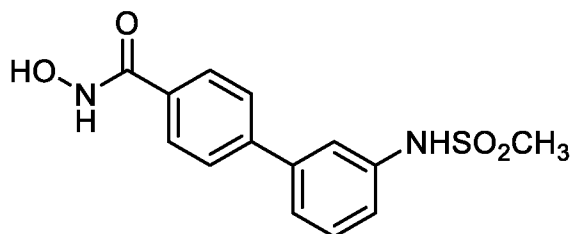


Formula I

;

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wherein an optical fiber light diffuser is located within said catheter and within the balloon; the assembly further comprising a source of UV light at a wavelength of from about 350 nm to about 380 nm and an optical fiber that transmits the UV light from the source to the light diffuser, which emits the UV light of suitable power and for sufficient duration to cleave the drug / linker conjugate and activate an effective amount of the drug of Formula II at a site of angioplasty treatment, to prevent or minimize NIH following angioplasty treatment at the site:



Formula II

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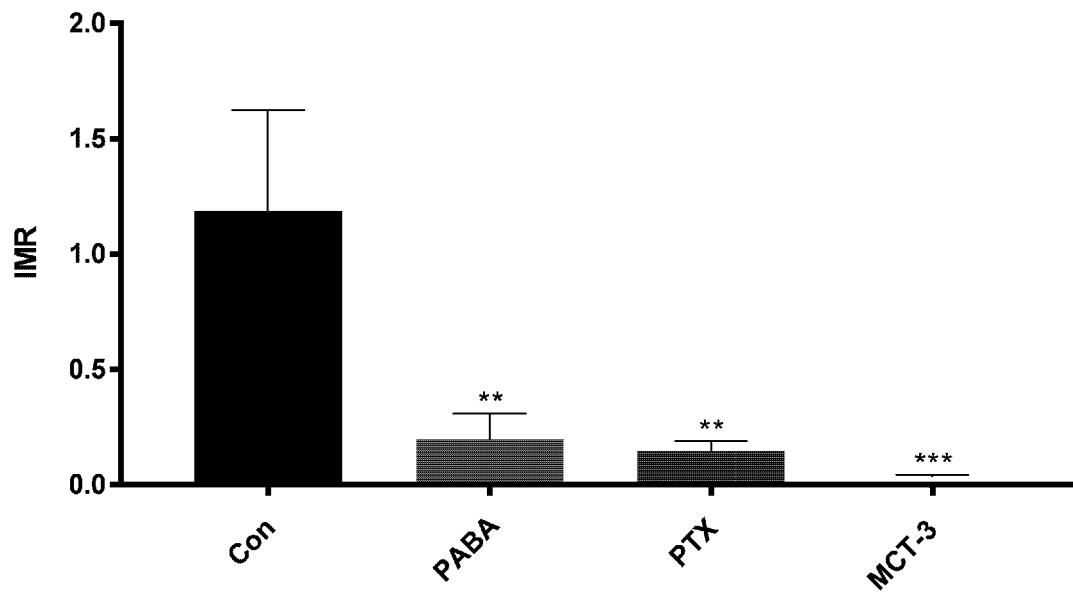


Figure 1

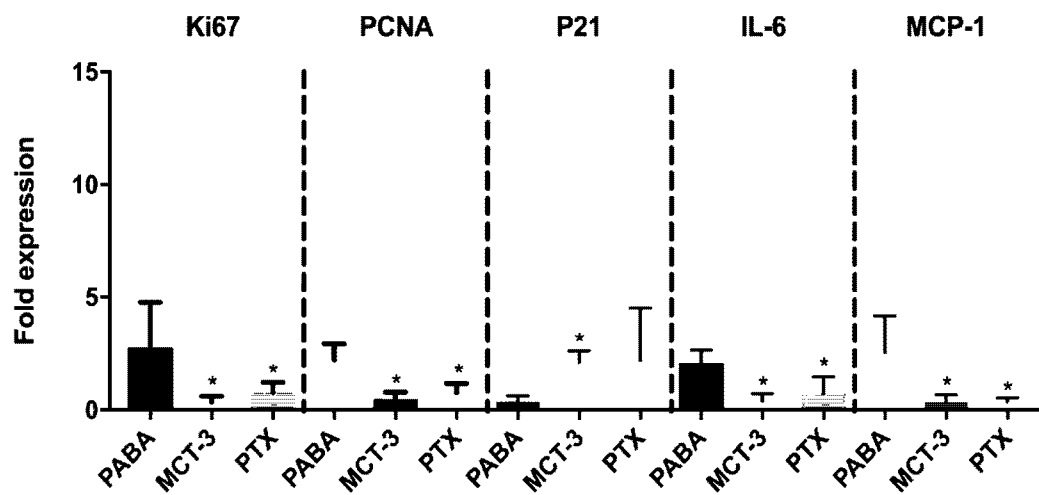
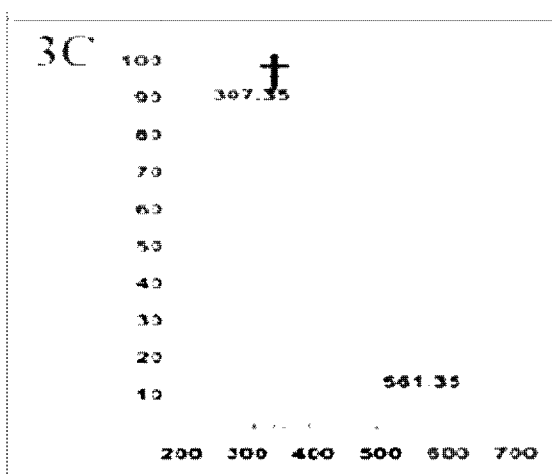
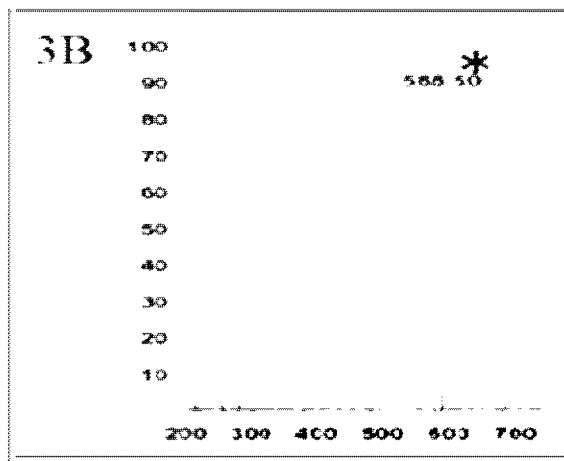
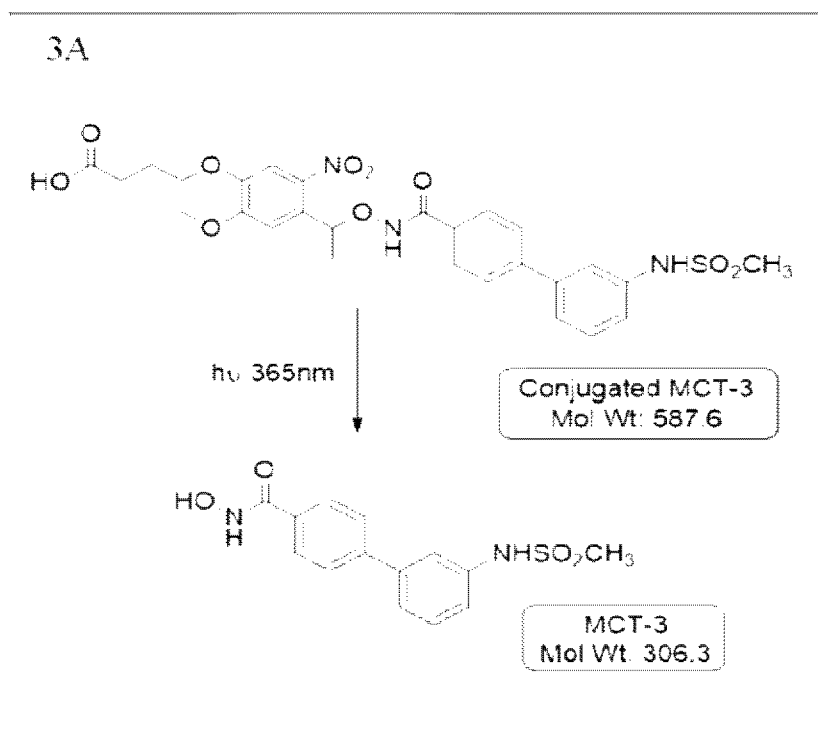


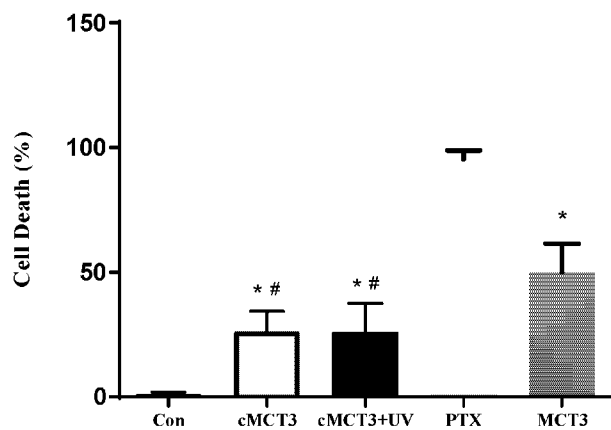
Figure 2



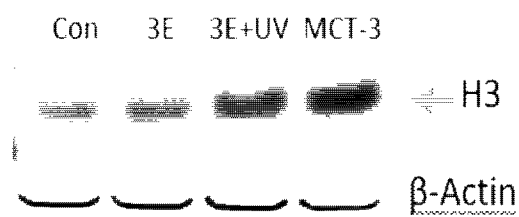
Figures 3A to C

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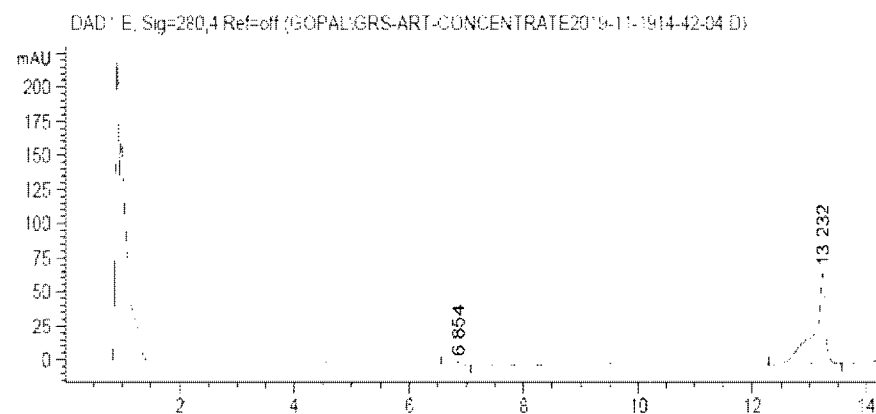
4A



4B



4C



Figures 4A to C

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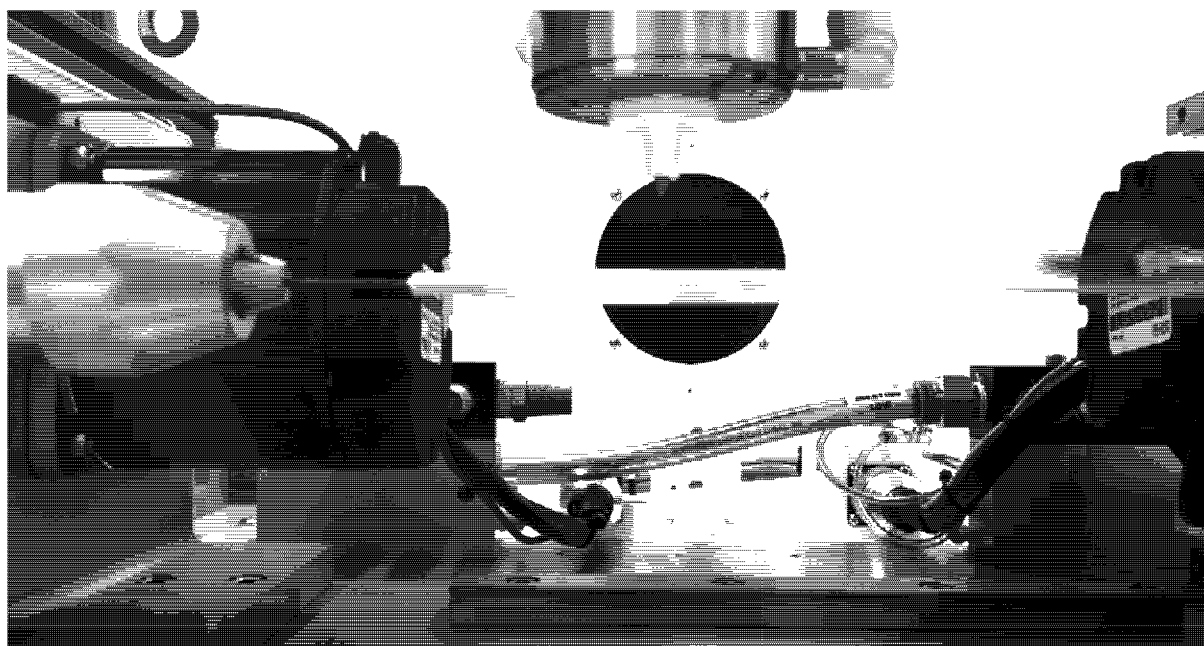


Figure 5

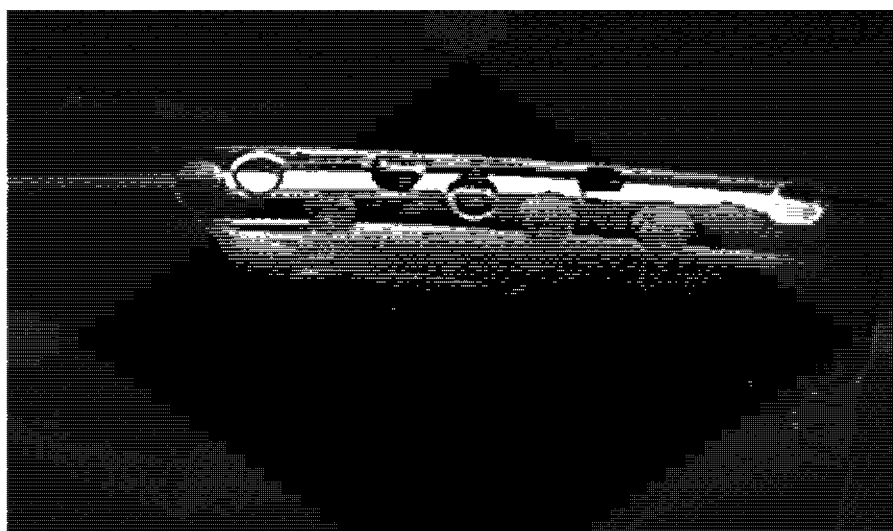


Figure 6

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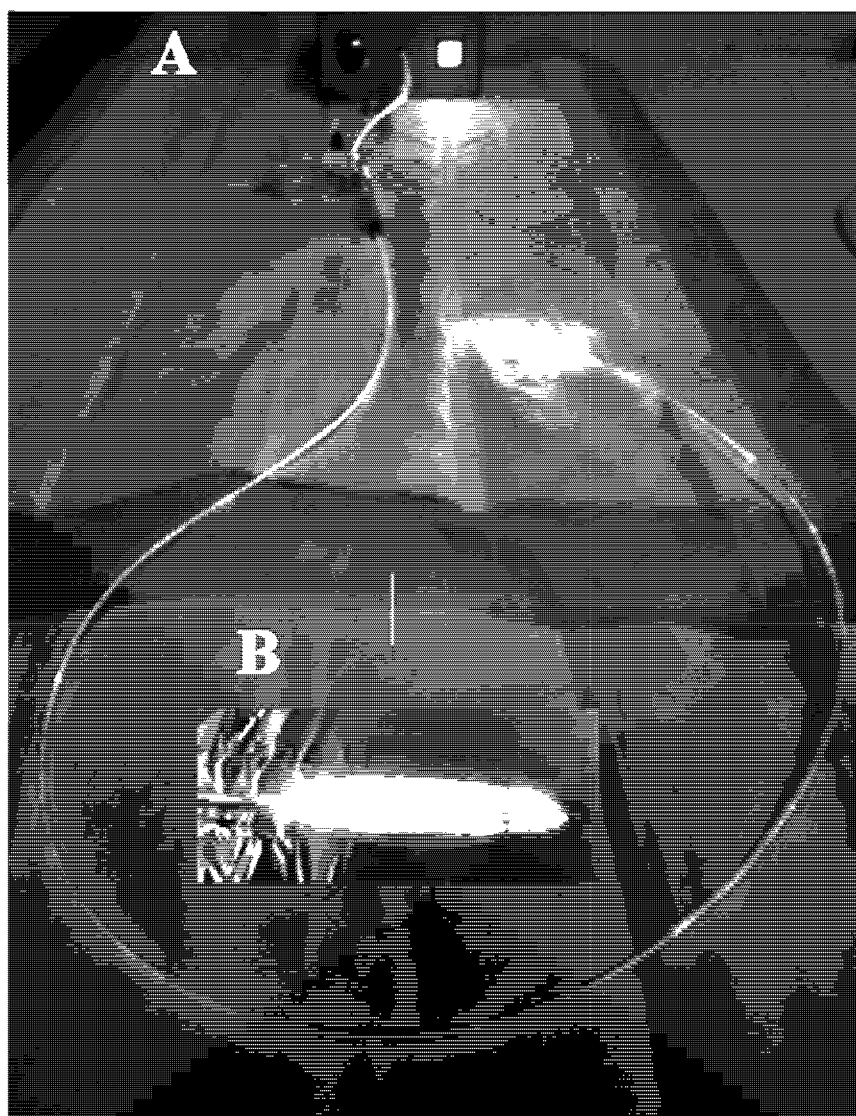
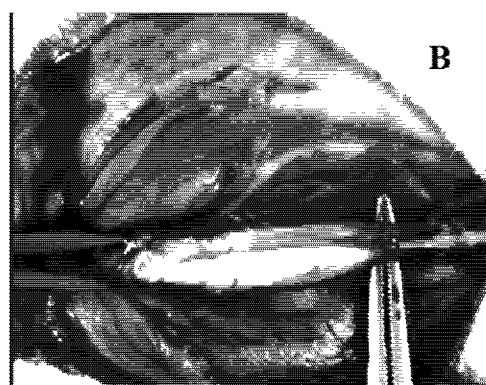
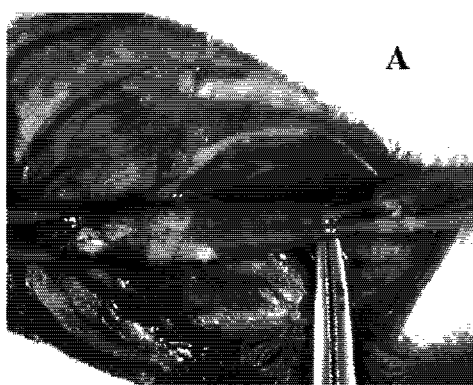


Figure 7

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Figures 8A and B

INTERNATIONAL SEARCH REPORT

International application No.

PCT/AU2020/050009

A. CLASSIFICATION OF SUBJECT MATTER

A61K 31/18 (2006.01) A61P 9/10 (2006.01) A61K 47/69 (2017.01) A61K 31/165 (2006.01) A61K 47/54 (2017.01)
C07C 311/08 (2006.01) C07C 311/15 (2006.01) C07C 311/31 (2006.01) C07C 311/17 (2006.01) A61M 25/10 (2013.01)

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

Databases: PATENTSCOPE, GOOGLE; EPOQUE: WPIAP, EPODOC; STN: CAPLUS, BIOSIS, MEDLINE, EMBASE, REGISTRY

Search terms: 3'-methanesulfonylamino-biphenyl-3-hydroxamic acid, ethyl 4-(4-acetyl-2-methoxy-5-nitrophenoxy)butanoate, photolabile, balloon catheter, neointimal hyperplasia and similar terms

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
	Documents are listed in the continuation of Box C	



Further documents are listed in the continuation of Box C



See patent family annex

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"A" document defining the general state of the art which is not considered to be of particular relevance	"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"D" document cited by the applicant in the international application	"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"E" earlier application or patent but published on or after the international filing date	"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	"&" document member of the same patent family
"O" document referring to an oral disclosure, use, exhibition or other means	
"P" document published prior to the international filing date but later than the priority date claimed	

Date of the actual completion of the international search
17 March 2020

Date of mailing of the international search report
17 March 2020

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INTERNATIONAL SEARCH REPORT		International application No.
C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT		PCT/AU2020/050009
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	WO 2005/025557 A1 (MONASH UNIVERSITY) 24 March 2005 see whole document, in particular the abstract, claims and pages 26-27 of D1	
A	DEAR, A.E et al.: "Conformational analogues of Oxamflatin as histone deacetylase inhibitors", Organic & Biomolecular Chemistry, (2006) vol. 4 pages 3778-3784 see whole document, in particular page 3778 and Fig.1 of D2	
A	WO 2005/061448 A1 (MONASH UNIVERSITY) 07 July 2005 see whole document, in particular the abstract and page 28	

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INTERNATIONAL SEARCH REPORT Information on patent family members		International application No. PCT/AU2020/050009	
This Annex lists known patent family members relating to the patent documents cited in the above-mentioned international search report. The Australian Patent Office is in no way liable for these particulars which are merely given for the purpose of information.			
Patent Document/s Cited in Search Report		Patent Family Member/s	
Publication Number	Publication Date	Publication Number	Publication Date
WO 2005/025557 A1	24 March 2005	WO 2005025557 A1	24 Mar 2005
WO 2005/061448 A1	07 July 2005	WO 2005061448 A1	07 Jul 2005
End of Annex			
Due to data integration issues this family listing may not include 10 digit Australian applications filed since May 2001. Form PCT/ISA/210 (Family Annex)(July 2019)			