Bioorganic & Medicinal Chemistry Letters 30 (2020) 127291

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry Letters

journal homepage: www.elsevier.com/locate/bmcl

Synthesis and biological evaluation of a novel photo-activated histone deacetylase inhibitor

Gopal R. Sama[a], Hongbin Liu[b], Simon Mountford[c], Phillip Thompson[c], Andrea Robinson[a], Anthony E. Dear[b,*]

[a] Department of Chemistry, Monash University, Melbourne, Victoria, Australia
[b] Department of Medicine, Eastern Health Clinical School, Monash University, Melbourne, Victoria, Australia
[c] Monash Institute of Pharmaceutical Sciences, Melbourne, Victoria, Australia

ARTICLE INFO

Keywords:
Metacept-3
Caged metacept-3
HDACi
DEBc
Photolytic release of HDACi

ABSTRACT

Hydroxamic acid-based histone deacetylase inhibitors (HDACi) are a class of epigenetic agents with potentially broad therapeutic application to several disease states including post angioplasty mediated neointimal hyperplasia (NIH). Precise spatiotemporal control over the release of HDACi at the target blood vessel site is required for the safe and successful therapeutic use of HDACi in the setting of drug eluting balloon catheter (DEBc) angioplasty treatment of NIH. We aimed to develop and characterise a novel photoactive HDACi, as a potential coating agent for DEBc.

Metacept-3 **1** was caged with a photo-labile protecting group (PPG) to synthesise a novel UV365nm active HDACi, caged metacept-3 **15**. Conversion of caged metacept-3 **15** to active/native metacept-3 **1** by UV365nm was achievable in significant quantities and at UV365nm power levels in the milliwatt (mW) range.

In vitro evaluation of the biological activity of pre and post UV365nm activation of caged metacept-3 **15** identified significant HDACi activity in samples exposed to short duration, mW range UV365nm. Toxicity studies performed in human umbilical vein endothelial cells (HUVEC's) identified significantly reduced toxicity of caged metacept-3 **15** pre UV365nm exposure compared with native metacept-3 **1** and paclitaxel (PTX).

Taken together these findings identify a novel photo-activated HDACi, caged metacept-3 **15**, with pharmacokinetic activation characteristics and biological properties which may make it suitable for evaluation as a novel coating for targeted DEBc angioplasty interventions.

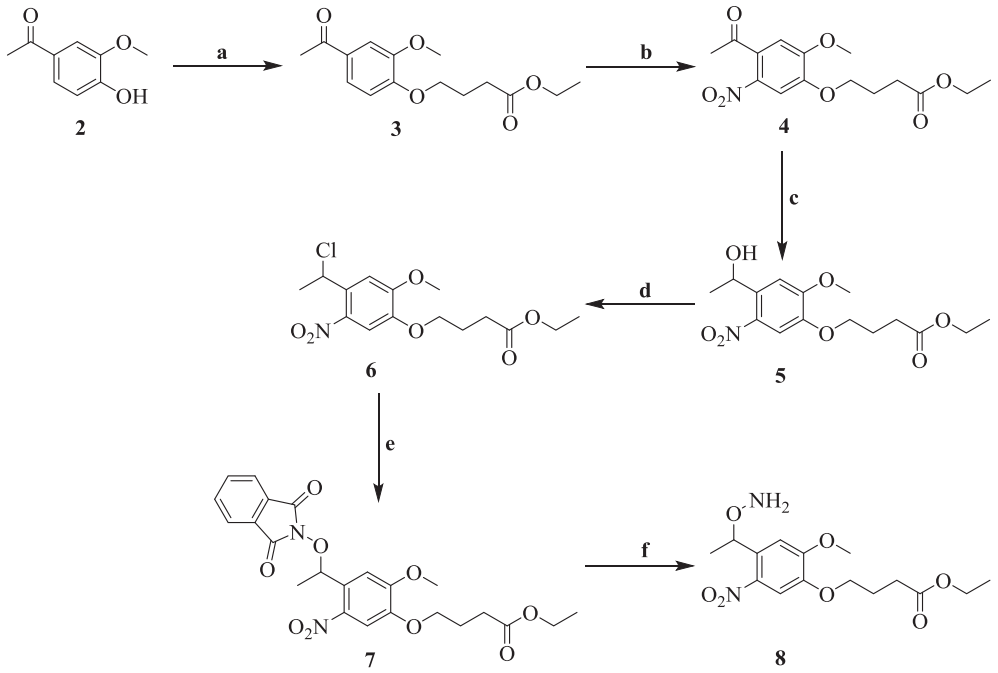
Inhibition of histone deacetylase (HDAC) activity leads to the accumulation of acetylated histones and other cellular proteins with resultant modulation of gene expression and associated cellular differentiation, growth arrest, apoptosis together with additional cellular processes.[1–4] As such, HDACi have emerged as promising candidates for a number of diverse disease states including cancer and inflammatory conditions including cardiovascular, gastrointestinal and neurological disorders.[3,4]

The HDACi metacept-3 **1**, a derivative of the hydroxamic acid HDACi oxamflatin,[5] has recently been evaluated *in vitro* and *in vivo* demonstrating attenuation of vascular smooth muscle cell migration, proliferation, and inhibition of neointimal hyperplasia (NIH) in a murine model.[6] Exploration in an ovine model as to the potential therapeutic efficacy of metacept-3 **1** as an alternative DEBc coating for the treatment of NIH, prompted by safety concerns in relation to current PTX coatings,[7] demonstrated potentially promising therapeutic results[8] and whilst metacept-3 **1** has exhibited low systemic toxicity[6] local vascular endothelial cell toxicity remained problematic particularly at higher concentrations associated with DEBc-mediated drug delivery.[8]

To circumvent potential local and deployment-mediated systemic toxicity in the setting of metacept-3 **1** coated DEBc angioplasty design of an inert, photo-inducible formulation of metacept-3 **1** able to be rapidly released upon light exposure once in contact with the vessel intima was investigated. Metacept-3 **1** was caged with a 2-nitrobenzyl based photolabile protecting group enabling UV365nm photo-activation. Quantification of UV365nm-mediated conversion of caged metacept-3 **15** to native metacept-3 **1** was determined together with biological activity of post UV365nm activated caged metacept-3 **15** and cellular toxicity.

* Corresponding author at: Department of Medicine, Eastern Health Clinical School, Monash University, Level 2, 5 Arnold Street Box Hill 3128, Melbourne, Victoria, Australia.
E-mail address: Anthony.dear@monash.edu (A.E. Dear).

https://doi.org/10.1016/j.bmcl.2020.127291
Received 2 April 2020; Received in revised form 21 May 2020; Accepted 26 May 2020
Available online 30 May 2020
0960-894X/



Scheme 1. Synthesis of hydroxylamine **8** fragment. *Reagents and reaction conditions:* **a**) K_2CO_3, ethyl-4-bromobutyrate, DMF, 50 °C, 15 h; **b**) acetic anhydride, HNO_3, 0 °C, 3 h; **c**) $NaBH_4$, MeOH, rt, 3 h; **d**) $SOCl_2$, DCM, rt, 3 h; **e**) *N*-Hydroxyphthalimide, TEA, DMF, 60 °C, 12 h; **f**) $NH_2NH_2{\cdot}H_2O$, EtOH, reflux, 2 h.

The caging of metacept-3 **1** was achieved using a convergent synthetic strategy. Starting from commercially available acetovanillone **2**, hydroxylamine fragment **8** was synthesized over six steps in 35% overall yield (Scheme 1). The ketoester **3** was synthesized by activation of acetovanillone **2** with K_2CO_3, followed by the addition of ethyl-4-bromobutyrate. The ketoester **3** was subjected to an aromatic substitution reaction in the presence of a nitration mixture consisting of HNO_3 and acetic anhydride at 0 °C to yield compound **4**. Reduction of the ketone to the secondary alcohol **5** was achieved with $NaBH_4$ in MeOH. Following reduction, o-nitrobenzyl chloride **6** was synthesized in the presence of thionyl chloride and later subjected to a nucleophilic substitution reaction with *N*-hydroxyphthalimide to furnish the imide-protected hydroxylamine **7**. Finally, deprotection of **7** with hydrazine hydrate in EtOH gave the first fragment, hydroxylamine **8**, as a yellow oil in accordance with previously published methods[9–12] (see Supporting information).

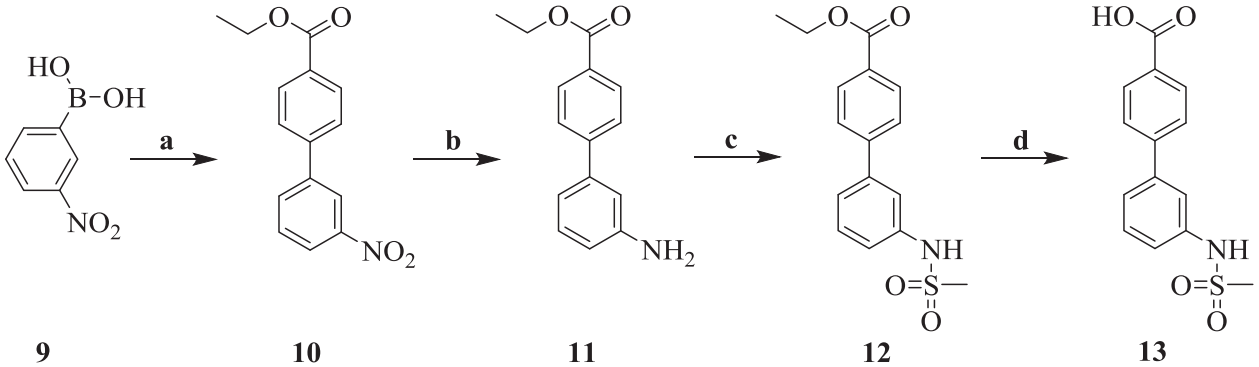
The second fragment, metacept-3 carboxylic acid **13**, was synthesised according to a previous literature report (Scheme 2).[5] Biphenyl nitro compound **10** was synthesized using a Suzuki coupling of 3-nitrophenyl boronic acid **9** and 4-bromomethyl ester in the presence of Na_2CO_3 and 10% Pd(0)/C catalyst. Subsequently, the crude compound **10** was reduced to the amine **11** in the presence of freshly added 10% Pd(0)/C catalyst under a hydrogen atmosphere. The amine **11** was then treated with mesyl chloride to give sulfonamide ester **12**. Finally, saponification of the ester in the presence of aq. NaOH produced the target sulfonamide carboxylic acid **13** in good yield.[5]

The two fragments, o-nitrohydroxylamine **8** and biphenyl carboxylic acid **13**, were coupled in the presence of PyBOP and NMM in DMF to furnish the caged metacept-3 ester **14** (Scheme 3).[10] Finally, the target caged metacept-3 **15** was obtained by hydrolysis of the ester with Novozyme-435 in phosphate buffer and dioxane (1:5 mixture).[10]

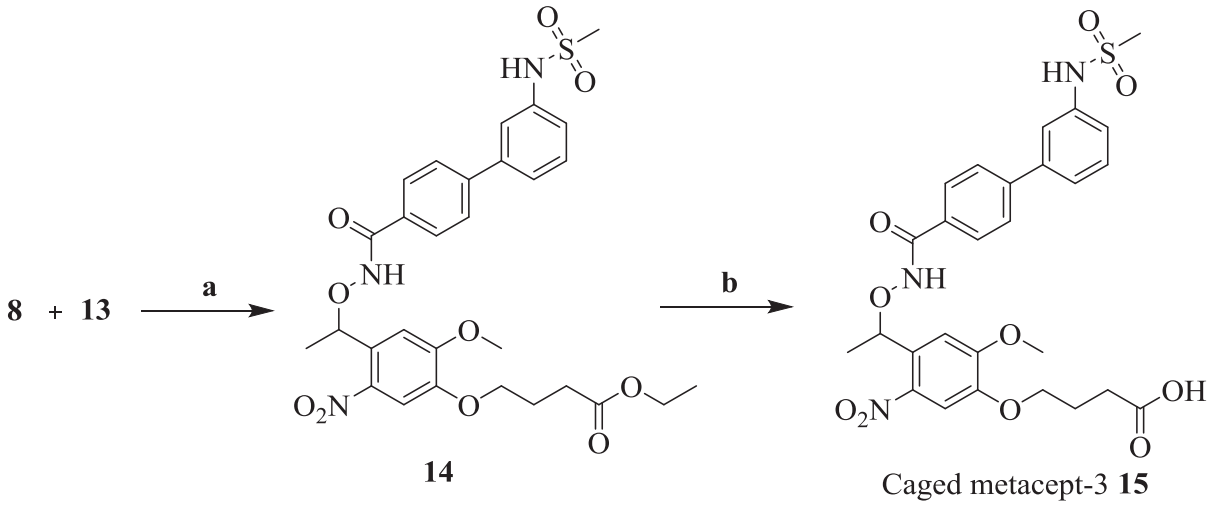
Human umbilical vein endothelial cells (HUVECs) ((Lonza (CC-2519, pooled donor), Basel, Switzerland)) were maintained in Media-199 (Sigma, USA) supplemented with penicillin/streptomycin, 20% foetal calf serum (FCS), 20 μg/mL endothelial cell growth factor (Sigma) and 20 μg/mL heparin and kept in a 5% CO_2 incubator at 37 °C.

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% CO_2 incubator at 37 °C.

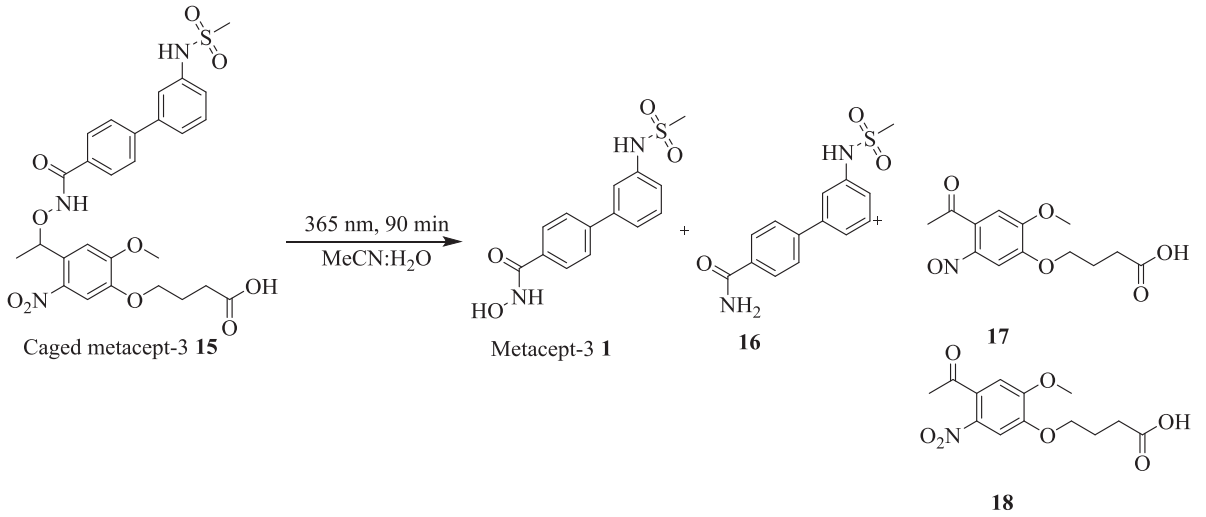
The agents (caged metacept-3 **15**, native metacept-3 **1** and PTX) were dissolved in 100% DMSO and added to plates at a final



Scheme 2. Synthesis of metacept-3 carboxylic acid **13**. *Reagents and reaction conditions:* **a**) Ethyl 4-bromobenzoate, Na_2CO_3, 10% Pd/C, EtOH, reflux, 24 h; **b**) H_2, 10% Pd/C, EtOAc, 48 h; **c**) $MeSO_2Cl$, pyridine, rt, 12 h; **d**) 1 M NaOH (aq), MeOH, rt, 3 h.



Scheme 3. Synthesis of the metacept-3 photolabile linker **15**. *Reagents and reaction conditions*: **a**) PyBOP, NMM, DMF, rt, 15 h; **b**) Phosphate buffer, dioxane, Novozyme-435, 30 °C, 5 days.



Scheme 4. Photolysis of caged metacept-3 **15** at 365 nm UV light.

concentration of 1.0 mmol/L for 24 h.

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. Cell viability at 24hrs (%) = (the total number of viable cells at 24 h-the total number of viable cells at the beginning of the experiment/the total number of viable cells at the beginning of the experiment). All experiments were repeated a minimum of 3 times with averages displayed graphically.

HL60 cells treated with 10.0 μM caged metacept-3 **15** (with and without UV treatment), eluted from the surface of a coated DEBc, and metacept-3 **1** for 24 h were washed with phosphate-buffered saline (PBS). Protein extracts were obtained from HL60 cells using extraction buffer with protease inhibitors (Roche) and PhosSTOP (Roche). Histones were isolated by acid extraction. Briefly, cells were lysed in ice-cold lysis buffer (10 mmol/L HEPES (pH 7.9), 1.5 mmol/L $MgCl_2$, 10 mmol/L KCl, 0.5 mmol/L DTT, and 1.5 mmol/L phenylmethylsulfonyl fluoride), with protease inhibitors (ABCAM) and 5 mol/L H_2SO_4 was added. After incubation on ice for 1 h the suspension was centrifuged and the supernatant was harvested, mixed with acetone at a ratio of 9:1, and incubated at −20 °C overnight. After centrifugation, the pellet was washed with 70% ethanol, air dried, and the acid-soluble histone fraction dissolved in H_2O. 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 a 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 Histone H3 Acetyl (Santa Cruz Biotechnology Inc, USA) 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 expression was detected with ECL Advanced Western Blotting Detection Kit (GE Healthcare) and quantified using Quantity One (v.4.6.7) analysis software (BioRad).

Results of *in vitro* studies were expressed as means ± standard error of the mean (SEM), and analyzed using GraphPad Prism 5 software (GraphPad Software, Inc., La Jolla, CA, USA), using unpaired t-tests for two-group comparisons and one-way analysis of variance and Dunnett's post hoc (ANOVA) for three or more group comparisons. P-value of < 0.05 was considered to be statistically significant.

Caged metacept-3 analogue **15** was subjected to photolysis at UV365 nm using a UVP® CL-1000® Ultraviolet Crosslinker (5.0 cm distance from light source to sample) to study the photolytic release of

Table 1
Caged metacept-3 analogue **15** photolysis results.

Time interval	Caged metacept-3 **15** %	Metacept-3 **1** %
Initial	100	0
30 min	53.3	46.7
45 min	18	82
60 min	8.4	91.6
75 min	2.4	97.6
90 min	0.7	99.3

the target hydroxamic acid (metacept-3, **1**) in a polar solvent (Scheme 4). Initially, a 4.26 mM solution of caged metacept-3 analogue **15** was prepared in a MeCN and water (1:3) solvent system and irradiated with an 8-Watt UV365nm light. Subsequent HPLC analysis of the photolytic reaction aliquots collected at 30, 45, 60, 75, and 90 min after UV exposure revealed formation of the required hydroxamic acid **1** as the major product (Scheme 4, Table 1). The analysis showed that 47% of the available metacept-3 **1** (*m/z* 307.0751, calculated for $(C_{14}H_{15}N_2O_4S)^+$), peak at ~6.6 min) was released after 30 min of exposure to UV light and the remaining 53% of the caged metacept-3 **15** was unchanged.

For every 15 min, half of the starting material **15** was cleaved and after 90 min, 99% of the caged metacept-3 analogue **15** had undergone photolysis. Significantly, negligible amount of amide formation (i.e. formation of **16**, peak at ~8.3 min) was observed (for chromatograms see supporting information).

Subsequently, the caged metacept-3 **15** photolysis experiment was repeated using a 85 μM concentration in a DMSO, EtOH and water (4:4:92 v/v/v) solvent mixture. Aliquots of samples were withdrawn at 2, 4 and 6-minute time intervals after exposure at 5.0 cm to UV365nm, 100mW (OmniCure S2000 Spot UV Curing System). In contrast to the earlier experiment, an increase in the hydroxamic acid compound (metacept-3 **1**, peak at 6.8 min) was observed and the photolysis was faster. After 6 min of irradiation, only 8.2% of the caged metacept-3 **15** standard (Peak at 14.6 min) remained unchanged. In addition, an increase in the concentration of carboxamide **16** (peak at 8.2 min) was observed and may reflect the effects of using a different solvent to undertake these studies (see Supporting information for chromatograms).

On decreasing the concentration of the caged metacept-3 standard **15** from 4.26 mM to 85 μM, approximately 62% of metacept-3 **1** was observed after only 2 min of UV exposure with 91% of the caged metacept-3 undergoing photolysis after 6 min of exposure to the 365 nm UV light (Table 2).

Human umbilical vein endothelial cells (HUVECs) were treated with 1.0 mmol/L solution of metacept-3 **1**, 1.0 mmol/L solution of caged metacept-3 analogue **15** and 1.0 mmol/L solution of PTX. A significant reduction in cellular toxicity was observed after 24 h of treatment with caged metacept-3 analogue **15** when compared to native metacept-3 **1** and PTX (Fig. 1). We have previously investigated MCT-3-mediated concentration dependent effects on HUVEC cell viability[8] and specifically investigated the highest (1.0 mmol/L) concentration of all drugs assayed in order to demonstrate the maximum concentration dependent differences in cell viability attributable to each compound.

Caged metacept-3 **15** was coated to the surface of an inflated angioplasty balloon at a dose of 3.0ug/mm2 using a pre-programmed SonoTek Exactacoat Ultrasonic Spray Coating System with an AccuMist ultrasonic nozzle as previously described[8] and exposed to UV365nm light (65mW) for 4 min. Western blot experiments using a HL-60 cell line were performed to evaluate compound release and subsequent HDAC inhibitory behaviour as measured by level of histone H3 acetylation (blue arrowhead). HDACi activity of caged metacept-3 analogue **15** (10.0 μM) eluted from coated DEBs without UV exposure (caged metacept-3 **15** without UV) demonstrated no significant increase in histone H3 acetylation compared to control (untreated cells). Caged metacept-3 **15** (10.0 μM) eluted from coated DEBs with UV exposure (Caged metacept-3 **15** + UV) demonstrated increased histone H3 acetylation (blue arrowhead) similar to levels identified with metacept-3 **1**. This experiment provides support for the release of metacept-3 **1** after photolysis in its bioactive form and preservation of its biological activity as a histone deacetylase inhibitor (HDACi) of similar potency to metacept-3 **1** (Fig. 2). B-Actin levels indicate equal protein loading. A replicate western blot is available in Supporting information Fig. 3.

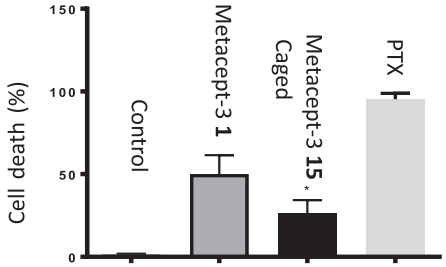


Fig. 1. Effect of caged metacept-3 **15** pre-UV activation, metacept-3 **1** and PTX on HUVEC toxicity (*$p < 0.05$ vs 1.0 mmol/L metacept-3 **1** and 1.0 mmol/L PTX). Control = Untreated cells.

Photo-activation of small molecules with potentially significant pharmacological and therapeutic activity may aid in provision of targeting treatment strategies particularly in the setting of novel DEBc coatings. In order to investigate the potential therapeutic utility of photo-activation of the HDACi metacept-3 **1** a caged metacept-3 analogue **15** was synthesised using a convergent synthetic approach. Two fragments were synthesised, *O*-substituted hydroxylamine intermediate **8** from acetovanillone **2,** and metacept-3 carboxylic acid intermediate **13** from 3-nitrophenyl boronic acid **9**. These two intermediates were then coupled to give caged metacept-3 ester **14** and enzymatic hydrolysis of ester group furnished the target caged metacept-3 **15**. The caged metacept-3 analogue **15**, and all intermediates leading to its synthesis, were fully characterised. Photolysis HPLC studies confirmed the formation of metacept-3 **1** after UV 365 nm light-induced cleavage from the linker. Significant reduction in the toxicity in HUVEC cell lines was observed for the caged metacept-3 analogue **15** compared to metacept-3 **1** and PTX. Metacept-3 **1** formed after photolysis of the caged metacept-3 analogue **15** was found to induce acetylated histone H3 activity *in vitro*, with levels comparable to native metacept-3 **1**, thereby confirming its HDACi activity and therapeutic potential.

Previous studies have identified UV and visible light-mediated synthesis and activation of HDACi.[13–15] To our knowledge this is the first study investigating photo-activation of an hydroxamate HDACi, using the convergent synthetic strategy previously outlined, for the specific purposes of DEBc-mediated vascular targeting. Given the relatively short clinical time frames associated with DEB intervention (< 3–4 min) identification of a low toxicity, time efficient, photo-activatable agent such as caged metacept-3 analogue **15** may afford an improvement to current DEB coatings which have been associated with both local and systemic toxicity issues.[7,16] Future studies will investigate the viability of using the caged metacept-3 analogue **15** as an alternative DEBc coating in *in vitro* and *in vivo* models.

Table 2
Caged metacept-3 **15** photolysis results in DMSO/EtOH/H2O.

Time interval	Caged metacept-3 **15**	Metacept-3 **1**	Carboxamide **16**
2 Minutes	28.9	61.6	9.3
4 Minutes	11.1	79	9.8
6 Minutes	8.2	81.4	10.2

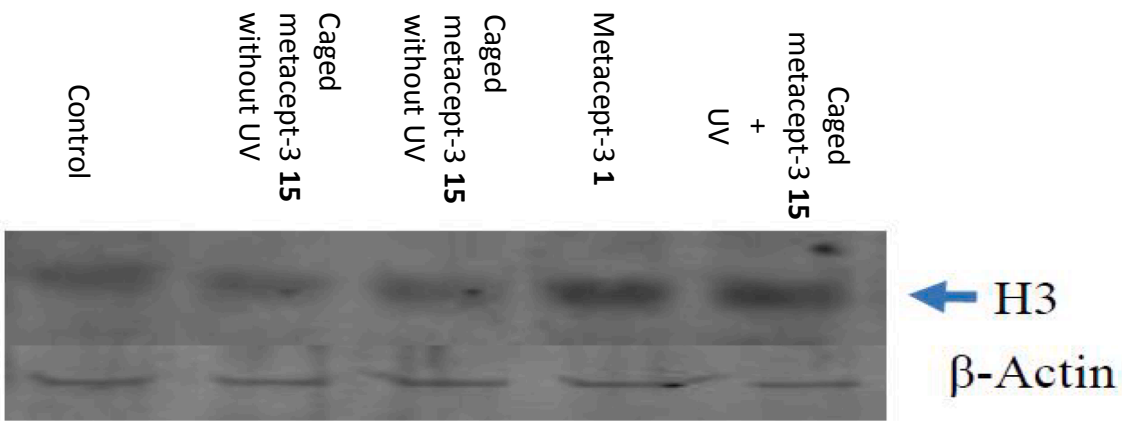


Fig. 2. Effect of caged metacept-3 **15** with and without UV treatment on histone H3 expression in HL-60 cells.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgements

Gopal Reddy Sama is grateful to Monash University for the award of Monash Graduate Scholarship (MGS), Monash International Postgraduate Research Scholarship (MIPRS) and the School of Chemistry for Dean's Postgraduate Research Scholarship (DPRS) and Dean's International Postgraduate Research Scholarship (DIPRS).

Professor Helmut Thissen and Mr Paul Pasic, CSIRO Manufacturing, Melbourne, Victoria, Australia for assistance with ultrasonic coating.

This work was supported, in part, by the Eastern Health Foundation Research Grants Program.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.bmcl.2020.127291.

References

1. Cress WD, Seto E. Histone deacetylases, transcriptional control, and cancer. *J Cell Physiol.* 2000;184:1–16.
2. Gregoretti IV, Lee YM, Goodson HV. Molecular evolution of the histone deacetylase family: functional implications of phylogenetic analysis. *J Mol Biol.* 2004;338:17–31.
3. Bolden JE, Peart MJ, Johnstone RW. Anticancer activities of histone deacetylase inhibitors. *Nat Rev Drug Discov.* 2006;5:769–784.
4. Porter NJ, Mahendran A, Breslow R, Christianson DW. Unusual zinc-binding mode of HDAC6-selective hydroxamate inhibitors. *Proc Natl Acad Sci USA.* 2017;114:13459–13464.
5. Dear AE, Liu HB, Mayes PA, Perlmutter P. Conformational analogues of Oxamflatin as histone deacetylase inhibitors. *Org Biomol Chem.* 2006;4:3778–3784.
6. Rahmatzadeh M, Liu HB, Krishna SM, et al. A novel agent with histone deacetylase inhibitory activity attenuates neointimal hyperplasia. *Cardiovasc Drugs Ther.* 2014;28:395–406.
7. Katsanos K, Spiliopoulos S, Kitrou P, Krokidis M, Karnabatidis D. Risk of death following application of paclitaxel-coated balloons and stents in the femoropopliteal artery of the leg: a systematic review and meta-analysis of randomized controlled trials. *J Am Heart Assoc.* 2018;7:e011245.
8. Liu HB, Byrne M, Perlmutter P, et al. A novel epigenetic drug eluting balloon angioplasty device: evaluation in a large animal model of neointimal hyperplasia. *Cardiovasc Drugs Ther.* 2019;33:687–692.
9. Qvortrup K, Nielsen TE. In-Bead Screening of Hydroxamic Acids for the Identification of HDAC Inhibitors. *Angew Chem.* 2016;55:4472–4475.
10. Qvortrup K, Petersen RG, Dohn AO, Møller KB, Nielsen TE. Solvent-controlled chemoselectivity in the photolytic release of hydroxamic acids and carboxamides from solid support. *Org Lett.* 2017;19:3263–3266.
11. Qvortrup K, Komnatnyy VV, Nielsen TE. A photolabile linker for the solid-phase synthesis of peptide hydrazides and heterocycles. *Org Lett.* 2014;16:4782–4785.
12. Qvortrup K, Nielsen TE. A photolabile linker for the solid-phase synthesis of 4-substituted NH-1,2,3-triazoles. *Chem Commun.* 2011;47:3278–3280.
13. Sun S, Oliveira BL, Jiménez-Osés G, Bernardes GJL. Radical-mediated thiol-ene strategy: photoactivation of thiol-containing drugs in cancer cells. *Angew Chem Int Ed Engl.* 2018;57:15832–15835.
14. Szymanski W, Ourailidou ME, Velema WA, Dekker FJ, Feringa BL. Light-controlled histone deacetylase (HDAC) inhibitors: towards photopharmacological chemotherapy. *Chemistry.* 2015;21:16517–16524.
15. Ieda N, Yamada S, Kawaguchi M, Miyata N, Nakagawa H. (7-Diethylaminocoumarin-4-yl)methyl ester of suberoylanilide hydroxamic acid as a caged inhibitor for photocontrol of histone deacetylase activity. *Bioorg Med Chem.* 2016;24:2789–2793.
16. Montgomery A, Kushner J, Altman D. Cutaneous polyarteritis nodosa resulting from a paclitaxel-eluting balloon angioplasty. *JAAD Case Rep.* 2017;4:50–52.