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CITATION

Griffith, Darren; Morgan, Maria P.; Marmion, Celine J. (2009): A novel anti-cancer bifunctional platinum drug candidate with dual DNA binding and histone deacetylase inhibitory activity.. Royal College of Surgeons in Ireland. Journal contribution. https://hdl.handle.net/10779/rcsi.10991441.v1

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A novel anti-cancer bifunctional platinum drug candidate with dual DNA binding and histone deacetylase inhibitory activity†

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Received (in Cambridge, UK) 14th August 2009, Accepted 14th September 2009
First published as an Advance Article on the web 12th October 2009
DOI: 10.1039/b916715c

Published on 12 October 2009 on http://pubs.rsc.org | doi:10.1039/B916715C
Downloaded by Royal College of Surgeons - Ireland on 10 January 2013

The successful design and synthesis of a novel multifunctional platinum drug candidate with DNA binding, histone deacetylase inhibitory activity and enhanced selectivity for cancer cells are described.

Nearly 50% of all cancer therapies are platinum (Pt)-based,[1] yet surprisingly to date only three Pt drugs have been approved for world-wide clinical use, namely cisplatin, carboplatin and oxaliplatin.[1,2] The cytotoxicity of Pt drugs is attributed to their ability to bind DNA nucleobases and induce apoptosis.[2] Despite their enormous success, their widespread application and efficacy are hindered by toxic side effects, their limited activity against many human cancers and their susceptibility to acquired drug resistance.[2] The need to overcome these drawbacks has stimulated the search for new molecular targets in addition to DNA which may present unique opportunities for therapeutic exploitation. The recent correlation between the inhibition of enzymes that regulate chromatin structure/function and tumour growth suppression has, for example, validated chromatin control as a promising new molecular target in contemporary medical oncology.

Chromatin is a complex structure that plays a key role as an epigenetic regulator of gene expression in eukaryotic cells. The fundamental repeating unit of chromatin is the nucleosome consisting of core histones around which DNA coils.[3] Some histone residues protrude the nucleosome and are subject to numerous enzyme-catalysed post-translational modifications.[4,5] Histone acetyltransferases (HAT's) and histone deacetylases (HDAC's) are two such enzymes that work in harmony to acetylate and deacetylate core histone lysine residues, respectively. Histone acetylation leads to an open chromatin structure that upregulates transcription whereas deacetylation leads to a condensed structure and transcriptional repression.[6] Inhibition of one or other of these enzyme classes can therefore dramatically affect chromatin structure and thus function. HAT's and HDAC's have therefore emerged as novel molecular targets for which inhibitors are sought that could reprogram transcription and inhibit tumour cell growth and progression.

A range of structurally diverse HDAC inhibitors (HDACi's) have been shown to cause cell cycle arrest, differentiation and/or apoptosis of tumour cells[7,8] and several of these are now undergoing clinical trials.[9] Suberoylanilide hydroxamic acid (SAHA, vorinostat), Fig. 1, is the first FDA-approved pan-HDACi to enter the clinic[10] as a treatment for cutaneous T-cell lymphoma. Crystal structures of human HDAC's with SAHA bound[11–13] show the hydroxamic acid coordinated to the active-site zinc (Zn) ion. Several HDACi's have thus been designed based on these data in which their structural motif consists of a metal binding group, a linker domain (that mimics the C_α functional group of lysine) that occupies the enzyme's narrow channel and a cap group which interacts with residues on the enzyme surface.

We devised a strategy to derivatise SAHA in such a way so as to facilitate its binding to Pt while not compromising its HDAC inhibitory activity. We anticipated that the novel bifunctional Pt–HDACi conjugate should bind DNA in much the same way as classical Pt drugs but because of its additional functionality it might be active against a broader spectrum of human cancer cells and/or cells that have acquired resistance to Pt-based regimes. An important property of some HDACi's such as SAHA is their selectivity for tumour cells over normal cells.[6,14] Because Pt drugs react indiscriminately in the body giving rise to many of their drawbacks, we speculated that the presence of the inhibitor, with its known affinity for tumour cells, might also confer selectivity to the drug candidate thereby reducing the non-selective toxicity of classical Pt drugs as well as potentially offering an advantage over concurrent administration of classical Pt drugs with HDACi's.[15] Despite the prevalence of Pt-based therapeutics, there have been no Pt or indeed metal drug candidates reported to date with dual DNA binding and HDAC inhibitory properties. Herein we describe the synthesis, characterisation and pharmacological evaluation of a novel Pt drug candidate with such dual functionality.

We derivatised SAHA by adding a malonic acid substituent to its cap group or phenyl ring to give malSAHA (**2**), Scheme 1. We anticipated that Pt would bind malSAHA in an *O*,*O*′-bidentate mode in much the same way as cyclobutane-1,1-dicarboxylate binds to Pt in carboplatin[2]

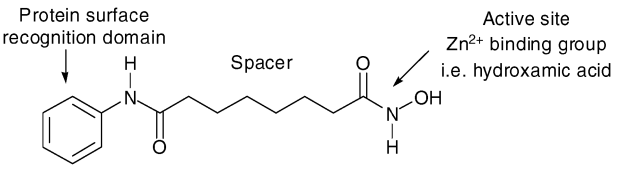
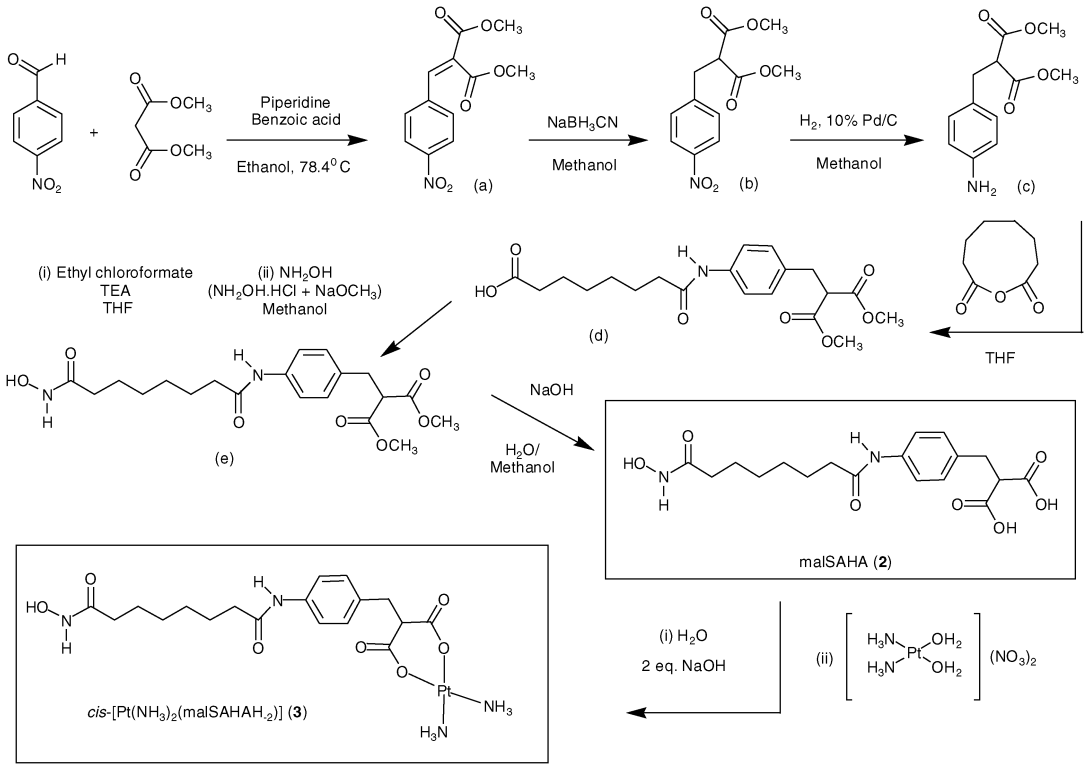


Fig. 1 Structure of suberoylanilide hydroxamic acid (**1**).

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† Electronic supplementary information (ESI) available: Experimental procedures and pharmacological evaluation. See DOI: 10.1039/b916715c

Published on 12 October 2009 on http://pubs.rsc.org | doi:10.1039/B916715C
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Scheme 1 Synthesis of malSAHA (**2**) and its Pt complex *cis*-[$Pt^{II}(NH_3)_2$(malSAHAH_{-2})] (**3**).

but leaving the linker domain unchanged and the hydroxamic acid moiety free to bind the HDAC active-site Zn^{2+} ion. Although hydroxamic acids are known to be strong metal ion chelators,[16,17] we previously reported that they have a low affinity for Pt.[18–20] We were therefore confident that the Pt would bind selectively to the malonato substituent. We also believed it important that the two functional entities of the Pt–HDACi conjugate, upon reaching the nucleus, would separate to allow each to work independently of the other. Carboplatin has been shown to undergo hydrolysis *via* a classical ring opening process followed by hydration and consequent displacement of cyclobutane-1,1-dicarboxylate, to give DNA binding adducts such as $[Pt(NH_3)_2(H_2O)_2]^{2+}$.[21,22] We envisaged that our Pt–malSAHA conjugate should act in much the same way as carboplatin releasing the malSAHA derivative thus free to inhibit HDAC's and the resulting Pt moiety free to bind DNA nucleobases.

The new ligand malSAHA (**2**) was synthesised according to Scheme 1.† The synthetic protocol for generating Pt–dicarboxylato complexes is well established.[23] Reaction of the disodium salt of malSAHA with *cis*-$[Pt^{II}(NH_3)_2(H_2O)_2]^{2+}$, generated from the reaction of iodoplatin and silver nitrate, gave *cis*-$[Pt^{II}(NH_3)_2$(malSAHAH_{-2})] (**3**), Scheme 1, in good yield (66%) and excellent purity.† *cis*-$[Pt(NH_3)_2$(malH_{-2})] (where mal is malonic acid) was also synthesised as a reference standard for biological tests.†

A well established electrophoretic technique was used to investigate the effect of *cis*-$[Pt^{II}(NH_3)_2$(malSAHAH_{-2})] on DNA supercoiling, where changes in DNA mobility are taken as evidence of a direct metal–DNA interaction leading to DNA unwinding.[24] Fig. 2 shows an agarose gel in which increasing amounts of *cis*-$[Pt^{II}(NH_3)_2$(malSAHAH_{-2})] have bound to negatively supercoiled (SC), closed circular pUC19 plasmid DNA. The rate of migration of the SC band decreases until it co-migrates with the open circular (OC) relaxed band as seen in lane 9. This DNA mobility shift confirms binding of the Pt complex **3** to nucleotides causing unwinding of the DNA. Similar effects are shown for cisplatin, Fig. 2, lanes 14–16.[25]

The ability of malSAHA and *cis*-$[Pt(NH_3)_2$(malSAHAH_{-2})] as well as known HDAC inhibitors (trichostatin A and SAHA) to inhibit HDAC1 was investigated in triplicate using Cayman's HDAC1 Inhibitor Screening Assay Kit, Table 1. This is a cell-free assay which utilises an acetylated lysine substrate incubated with human recombinant HDAC1. The IC_{50} value for malSAHA (142 ± 29 nM) compares favourably with that found for SAHA (83 ± 23 nM) which suggests that

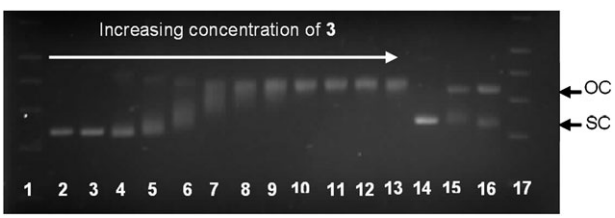


Fig. 2 Unwinding of closed circular supercoiled pUC19 plasmid DNA by **3**. The top bands correspond to the open circular (OC) form of plasmid DNA and the bottom bands to closed, negatively supercoiled (SC) plasmid DNA. pUC19 DNA (30 μM) was incubated for 72 hours with 0, 1, 10, 20, 30, 40, 50, 60, 70, 80, 90, and 100 μM of **3** (lanes 2–13) and 0, 30 and 40 μM cisplatin (lanes 14–16). DNA ladder (lanes 1 and 17).

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Published on 12 October 2009 on http://pubs.rsc.org | doi:10.1039/B916715C
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Table 1 IC_{50} values (nM) against HDAC1 at 200 μM

	IC_{50}/nM
Trichostatin A	8 ± 0.3
SAHA	83.5 ± 23.1
MalSAHA	142 ± 29.8
cis-[$Pt^{II}(NH_3)_2$(malSAHAH$_{-2}$)]	1143 ± 83.2

the presence of the malonate substituent in malSAHA does not adversely affect its HDAC1 inhibitory activity. *cis*-[$Pt^{II}(NH_3)_2$(malSAHAH$_{-2}$)] exhibits HDAC1 inhibitory activity at low micromolar concentrations, despite the fact that we would not expect the complex to significantly hydrolyse in this cell-free assay.

Finally, the cytotoxicities of *cis*-[$Pt^{II}(NH_3)_2$(malSAHAH$_{-2}$)], malSAHA, SAHA, *cis*-[$Pt(NH_3)_2$(malH$_{-2}$)] and cisplatin were studied by means of a colorimetric cell proliferation microculture assay (MTS assay) against cisplatin-sensitive and cisplatin-resistant ovarian cancer cell lines A2780P and A2780cisR, respectively, and the non-tumorigenic, normal human dermal fibroblast cells, NHDF, Table 2. *cis*-[$Pt^{II}(NH_3)_2$(malSAHAH$_{-2}$)], while having a similar cytotoxicity (IC_{50} 9 ± 3 μM) as compared to cisplatin (IC_{50} 2.9 ± 0.1 μM) against A2780P, differs greatly to cisplatin in that it is significantly less toxic to NHDF's (IC_{50} 83 ± 7.6 μM *versus* IC_{50} 10 ± 1.8 μM). Whilst we did not observe any synergistic effect or improved efficacy against the cisplatin-sensitive or cisplatin-resistant cell lines, nevertheless we did observe marked selectivity for tumour cells relative to the non-tumorigenic, normal cells. *cis*-[$Pt^{II}(NH_3)_2$(malSAHAH$_{-2}$)] offers a distinct advantage therefore over treatments involving cisplatin alone as the non-toxic malSAHA may act as a Trojan horse delivering the DNA binding agent Pt^{II} and the HDACi, malSAHA, to their target sites, thereby reducing non-specific effects. We also observed that *cis*-[$Pt^{II}(NH_3)_2$(malH$_{-2}$)] is nearly twofold less cytotoxic compared to *cis*-[$Pt^{II}(NH_3)_2$(malSAHAH$_{-2}$)]. The presence of the HDACi, malSAHA in *cis*-[$Pt^{II}(NH_3)_2$(malSAHAH$_{-2}$)], is thus enhancing its cytotoxicity.

There is an unmet medical need for cancer therapeutics that overcome the shortcomings of existing therapies. Pt drugs, while ranking among the most successful cancer chemotherapeutic agents, have considerable drawbacks. The correlation between HDAC inhibition and suppression of tumour growth has brought the design of HDACi to the forefront of oncology research and is validating chromatin control as an important cancer target. We have combined both approaches and have developed *cis*-[$Pt^{II}(NH_3)_2$(malSAHAH$_{-2}$)], the first Pt complex of its kind with dual DNA binding and HDAC inhibitory activity. The structure of this lead compound will be optimised to further enhance its efficacy and safety profile with a view to generating a new class of cancer chemotherapeutics beyond those currently in use.

Table 2 IC_{50} values (μM) obtained for the test compounds against the ovarian cancer cell lines A2780P and A2780cisR and the non-tumorigenic normal cell line NHDF for 72 hours

	A2780P	A2780cisR	NHDF
Cisplatin	2.9 ± 0.1	28.5 ± 1.5	10 ± 1.8
SAHA	3.5 ± 0.1	3.5 ± 0.1	4.5 ± 0.3
MalSAHA	205 ± 44.2	258 ± 15.9	335 ± 3.1
cis-[$Pt^{II}(NH_3)_2$(malSAHAH$_{-2}$)]	9 ± 3.1	70 ± 3.5	83 ± 7.6
cis-[$Pt(NH_3)_2$(malH$_{-2}$)]	16 ± 4.3	81 ± 5.8	48 ± 2.2

This material is based upon works supported by the Science Foundation Ireland under Grant No. [07/RFP/CHEF570]. We also gratefully acknowledge the Programme for Research in Third Level Institutions (PRTLI), administered by the HEA for funding. We also thank colleagues in EU COST D39 for fruitful discussions.

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