Available online at www.sciencedirect.com
ScienceDirect
Bioorganic & Medicinal Chemistry 15 (2007) 6126–6134
Bioorganic & Medicinal Chemistry

Norcantharimides, synthesis and anticancer activity: Synthesis of new norcantharidin analogues and their anticancer evaluation

Timothy A. Hill,[a] Scott G. Stewart,[a] Stephen P. Ackland,[b] Jayne Gilbert,[b] Benjamin Sauer,[a] Jennette A. Sakoff[b] and Adam McCluskey[a,*]

[a]*Chemistry, Chemistry Building, The University of Newcastle, University Drive, Callaghan, NSW 2308, Australia*
[b]*Department of Medical Oncology, Newcastle Mater Misericordiae Hospital, Waratah, NSW 2298, Australia*

Received 20 March 2007; revised 12 June 2007; accepted 13 June 2007
Available online 20 June 2007

Abstract—A range of amines was reacted with norcantharidin (**2**) to provide the corresponding norcantharimides (**9**–**43**). Treatment of norcantharidin with allylamine afforded the corresponding allyl-norcantharimide (**20**) which was amenable to epoxidation (mCPBA, **22**) and subsequent ring opening ($MeOH/H^+$; **23**) or alternatively, osmylation (OsO_4/NMO; **24**). These simple synthetic modifications of **2** facilitated the development of a novel series of norcantharimides displaying modest to good broad spectrum cytotoxicity against HT29 and SW480 (colorectal carcinoma); MCF-7 (breast adenocarcinoma); A2780 (ovarian carcinoma); H460 (lung carcinoma); A431 (epidermoid carcinoma); DU145 (prostate carcinoma); BE2-C (neuroblastoma); and SJ-G2 (glioblastoma). Analogues possessing a C_{10}, C_{12} or C_{14} alkyl chain or a C_{12} linked bis-norcantharimide displayed the highest levels of cytotoxicity.

1. Introduction

More than five decades of research effort in cancer drug discovery and development have provided less than 100 approved products for the treatment of malignancy.[1] Although major advances have been made in the chemotherapeutic management of some patients, particularly in haematologic malignancies, one-half of all cancer patients either do not respond to therapy, relapse following initial response or ultimately die from their metastatic disease. For such patients a better systemic therapy offers the only chance for prolonged survival or ultimately a cure. Many mechanisms of tumour cell resistance to conventional agents involve alterations in DNA cell cycling events. New agents that specifically target cell cycle events have the capacity to overcome resistance to chemotherapy, or possibly induce tumour cell death when used alone. This requires continued research to discover novel therapeutic products that can be used in combination with biologic agents and immune therapies to eradicate systemic disease not curable by surgery or irradiation. There is, understandably, considerable pressure to develop new treatments and new therapeutic approaches to the treatment of cancer.

Over the past decade we have been exploring the connection between protein phosphatase inhibition (PP1 and PP2A) and anticancer activity.[2–6] In particular we have focused on the synthetic modification of cantharidin (**1**) and norcantharidin (**2**) (Chart 1). Clinical evaluations of **1** have indicated promise in the treatment of liver tumours and the KB cell line at low concentrations.[7–10] A direct outcome of our early work in this area was the discovery that various norcantharimides displayed modest anticancer activity and as such they have proved to be interesting lead compounds in the search for new anticancer agents. Others, notably Lin et al., have also reported the synthesis and anticancer activity of the cantharimides, and in doing so reduced toxicity whilst maintaining biological activity.[11,12] However, we felt that given the known nephrotoxicity of cantharidin,

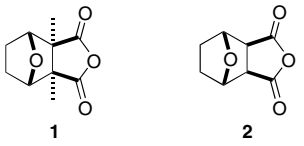


Chart 1.

Keywords: Cantharidin; Norcantharidin; Norcantharimides; Cytotoxicity; Anticancer.
* Corresponding author. Tel.: +61 249 216486; fax: +61 249 215472; e-mail: Adam.McCluskey@newcastle.edu.au

0968-0896/$ - see front matter
doi:10.1016/j.bmc.2007.06.034

1, and the relative ease of synthesis associated with preparation of large quantities of norcantharidin, **2**, that the norcantharimides also presented an ideal opportunity to develop a library of potential anticancer agents.[3,6]

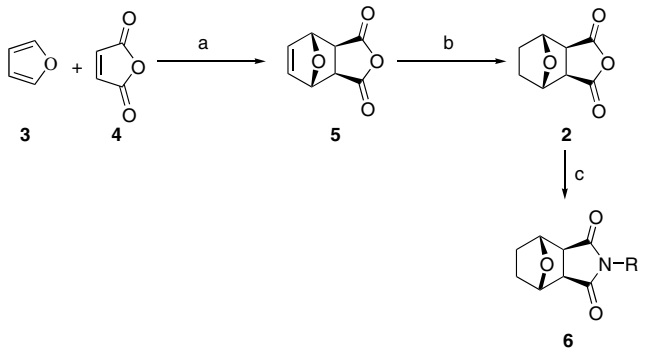
2. Results and discussion

The key starting material in this work was the readily synthesisable 5,6-dehydronorcantharidin, **5**, which was prepared on a large scale through exo-selective cycloaddition of the relatively cheap furan, **3**, and maleic anhydride. Subsequent hydrogenation of **5** (H_2, 40 psi, 10% Pd–C) following a modified procedure of Eggelte et al. provided the starting norcantharidin (**2**) in excellent yields.[13]

Treatment of **2** with an appropriate primary amine and NEt_3 facilitated a condensation reaction to provide a series of cyclic imides (**6**, Scheme 1, compounds **9**–**43**, Tables 1 and 2) similar to our procedure for the synthesis of related amino acid substituted *N*-derivatives.[3]

Our approaches to medicinal chemistry have relied on the rapid and robust generation of small-targeted compound libraries for bioassay.[3–6] Thus, simple reflux afforded the desired analogues in good to excellent yields (30–90%, Tables 1 and 2), contrasting the requirement for high pressure approaches with the cantharimide analogues reported by Lin et al.[11] Reaction yields were essentially as anticipated with variations reflecting the nucleophilicity of the parent amine.

We first examined a series of simple alkyl norcantharimide analogues, developed based on linear alkyl amines (Table 1). The data arising from subsequent cell survival screening against a panel of nine human cancer cell lines routinely grown in our laboratory: HT29 and SW480 (colorectal carcinoma); MCF-7 (breast adenocarcinoma); A2780 (ovarian carcinoma); H460 (lung carcinoma); A431 (epidermoid carcinoma); DU145 (prostate carcinoma); BE2-C (neuroblastoma); and SJ-G2 (glioblastoma) are shown in Table 1. Note that cantharidin (**1**) and norcantharidin (**2**) are regularly run in our laboratories as comparative controls.

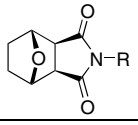
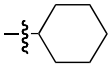
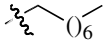
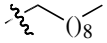
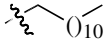
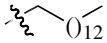
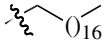
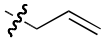
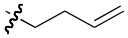


Scheme 1. Reagents and conditions: (a) rt, 48 h, ether; (b) 4 atm H_2, 10% Pd–C, acetone, 3-days; (c) RNH_2, $PhCH_3$ reflux, 36 h.

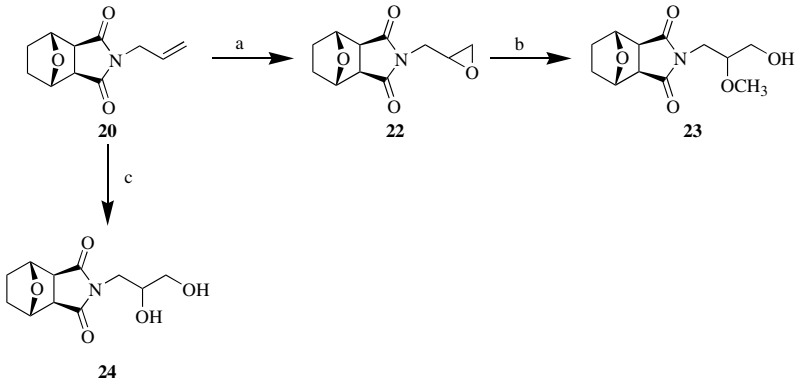
As can be seen both **1** and **2** are potent, broad spectrum anticancer agents with **1** being typically 10-fold more potent than **2**. With the alkyl-substituted norcantharimides **9**–**19**, modest to poor activity is observed. The only instances of noteworthy cytotoxicity are observed with **16**, **17** and **18**, all of which possess a long alkyl chain (C_{10}, C_{12} and C_{14}, respectively). Presumably these alkyl chains assist in penetration of the cell membrane making these analogues bio-available. Shorter (**9**–**15**), longer (**18**), branched or cyclic alkyl groups display no activity. Notwithstanding this, the data obtained suggest that **16**–**18** have potential as anticancer agents. The introduction of a terminal double bond, **20** and **21**, has essentially no impact on activity (but does allow easy functionalisation—see Scheme 2), although the allyl substitution is more active than the butenyl analogue. This differential reactivity is most likely an artefact of the greater stability/reactivity of the allyl group with the potential delocalisation of any charge back to the norcantharimide nitrogen. This stabilisation is not possible with the corresponding butenyl substituent. It is also of note that the introduction of the terminal double bond returns a modest increase in activity relative to the parent propyl analogue **10**. Both **10** and **20** show very modest levels of activity against SW480 (**10**, 45%; **20**, 50% at 100 μM, respectively) and MCF-7 (**20**, $GI_{50} = 80 \pm 12$ μM) cells lines, promising but of no great worth at this stage.

Suitably encouraged by that simple norcantharimides displayed reasonable cytotoxicity, we next examined the introduction of higher levels of functionality. In this instance we limited our explorations to terminally substituted analogues. Commencing with the easily functionalised allyl unit of **20**, mCPBA mediated epoxidation gave **22**, which underwent a subsequent (1*S*)-(+)-10-camphorsulfonic acid mediated methanolysis to the methoxy alcohol **23**. Alternatively osmylation with OsO_4 in the presence of NMO afforded the diol **24** (Scheme 2). These modification of the allyl substituent gave rise to the most interesting structure activity data thus far. Cytotoxicity screening showed the parent epoxide **22** possessed very low levels of cytotoxicity (ca. 25% at 100 μM across all cell lines evaluated). The methoxy alcohol **23** displays lower levels of cytotoxicity than epoxide **22**. Notwithstanding this introduction of a diol unit (**24**) gave an analogue displaying considerably higher levels of cytotoxicity, than **22**, with GI_{50} values determined for four cell lines (SW480, MCF-7, A2780 and BE2-C with GI_{50}'s of 62 ± 2, 46 ± 4, 59 ± 2 and 70 ± 4 μM, respectively) and also modest levels of activity at the remaining five cell lines (ca. 45% at 100 μM). Removal of the secondary alcohol (**24** and **25**) results in a significant reduction of cytotoxicity (ca. 20% at 100 μM across all cell lines evaluated), strongly suggesting that both –OH groups are required for cytotoxicity. Indeed the requirement for two free –OH groups is confirmed by the lack of activity associated with analogues **25**–**29**. Encouraged by the increase in potency observed via the introduction of oxygen bearing functionallity we next sought to introduce such functionallity via a terminal carboxylate. Disappointingly these carboxylate analogues, **30** and **31**, failed to elicit any noteworthy levels

Table 1. Cytotoxicity of cantharidin (1), norcantharidin (2) and norcantharimides (**9**–**21**) in a panel of human cancer cell lines

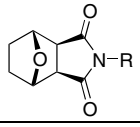
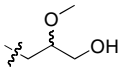
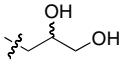
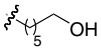
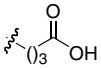
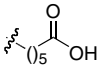
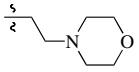
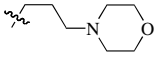
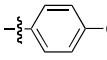
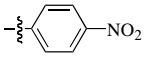
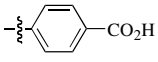
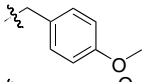
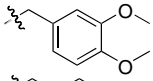
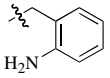
Compound[b]	R	Tumour cell line[a]								
		HT29 Colon	SW480 Colon	MCF-7 Breast	A2780 Ovarian	H460 Lung	A431 Skin	DU145 Prostate	BE2-C Neuronal	SJ-G2 Brain
										
1		3.2 ± 0.1	4.5 ± 0.3	7.5 ± 0.4	4.4 ± 0.3	3.3 ± 0.2	2.9 ± 0.2	2.1 ± 0.3	3.7 ± 0.6	1.7 ± 0.1
2		57 ± 5	44 ± 6	68 ± 4	38 ± 1	45 ± 3	31 ± 1	28 ± 3	43 ± 6	23 ± 3
9		*<10*	*<10*	*<10*	*<10*	*<10*	*<10*	*<10*	*<10*	*<10*
10		16 ± 11	45 ± 44	*<10*	*<10*	*<10*	*<10*	*<10*	*<10*	*<10*
11		*<10*	*<10*	*<10*	*<10*	*<10*	*<10*	*<10*	*<10*	*<10*
12		*<10*	*<10*	*<10*	*<10*	*<10*	*<10*	*<10*	*<10*	*<10*
13		*<10*	*<10*	*<10*	*<10*	*<10*	*<10*	*<10*	*<10*	*<10*
14		*<10*	*<10*	*<10*	*<10*	*<10*	*<10*	*<10*	*<10*	*<10*
15		*70 ± 5*	*65 ± 4*	*88 ± 18*	*50 ± 10*	*<10*	*61 ± 3*	*44 ± 11*	*68 ± 16*	*54 ± 11*
16		*20 ± 1*	*59 ± 0*	*44 ± 4*	*44 ± 7*	*>100*	*52 ± 5*	*83 ± 4*	*39 ± 4*	*72 ± 2*
17		*25 ± 4*	*55 ± 2*	*52 ± 8*	*40 ± 4*	*53 ± 4*	*35 ± 4*	*66 ± 4*	*40 ± 6*	*58 ± 2*
18		19 ± 0	52 ± 4	43 ± 7	35 ± 4	66 ± 7	47 ± 3	73 ± 2	50 ± 4	49 ± 5
19		*<10*	*<10*	*<10*	*<10*	*<10*	*<10*	*<10*	*<10*	*<10*
20		*19 ± 9*	*50 ± 26*	*96 ± 14* *80 ± 12*	*67 ± 12*	*20 ± 8*	*32 ± 5*	*15 ± 17*	*62 ± 5*	*2 ± 29*
21		*<10*	*<10*	*<10*	*<10*	*<10*	*<10*	*<10*	*<10*	*<10*

Cytotoxicity levels are first expressed as % inhibition at 100 μM drug concentration (*in italics*) and if potent, as Growth Inhibition, GI_{50}, μM.
[a] Data were calculated after 72 h of continuous drug exposure, values are means (±SEM) of 3–6 experiments.
[b] Compound, compound number.



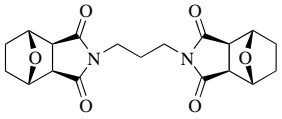
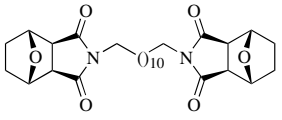
Scheme 2. Reagents and conditions: (a) mCPBA, rt 16 h; (b) (1*S*)-(+)-10-camphorsulfonic acid/MeOH; (c) OsO_4/NMO, acetone/H_2O, 80 °C 16 h.

Table 2. Cytotoxicity of norcantharimides (**22–43**) in a panel of human cancer cell lines

Compound[b]	R	Tumour cell line[a]								
		HT29 Colon	SW480	MCF-7 Breast	A2780 Ovarian	H460 Lung	A431 Skin	DU145 Prostate	BE2-C Neuronal	SJ-G2 Brain
										
22		*25 ± 3*	*30 ± 6*	*10 ± 16*	*40 ± 6*	*17 ± 5*	*40 ± 9*	*22 ± 5*	*20 ± 13*	*25 ± 4*
23		*12 ± 4*	*<10*	*33 ± 3*	*<10*	*<10*	*<10*	*<10*	*<10*	*<10*
24		*39 ± 12*	*69 ± 29*	*>100*	*>100*	*32 ± 11*	*60 ± 19*	*67 ± 24*	*73 ± 7*	*27 ± 5*
		>100	*62 ± 2*	*46 ± 4*	*59 ± 2*	*>100*	*>100*	*>100*	*70 ± 4*	*>100*
25		*31 ± 12*	*18 ± 3*	*45 ± 22*	*15 ± 2*	*16 ± 3*	*<10*	*<10*	*<10*	*<10*
26		*21 ± 4*	*16 ± 2*	*51 ± 13*	*18 ± 2*	*14 ± 1*	*6 ± 3*	*<10*	*22 ± 9*	*12 ± 5*
27		*<10*	*<10*	*<10*	*<10*	*<10*	*<10*	*<10*	*<10*	*<10*
28		*<10*	*<10*	*<10*	*<10*	*<10*	*<10*	*<10*	*<10*	*<10*
29		*<10*	*<10*	*<10*	*<10*	*<10*	*<10*	*<10*	*<10*	*<10*
30		*<10*	*<10*	*<10*	*<10*	*<10*	*<10*	*<10*	*<10*	*<10*
31		*14 ± 7*	*11 ± 1*	*47 ± 35*	*52 ± 29*	*12 ± 2*	*11 ± 7*	*12 ± 6*	*22 ± 21*	*<10*
32		*29 ± 5*	*29 ± 1*	*12 ± 4*	*7 ± 2*	*18 ± 0*	*30 ± 5*	*3 ± 5*	*39 ± 8*	*3 ± 2*
33		*<10*	*<10*	*<10*	*<10*	*<10*	*<10*	*<10*	*<10*	*<10*
34		*<10*	*<10*	*<10*	*<10*	*<10*	*<10*	*<10*	*<10*	*<10*
35		*63 ± 4*	*78 ± 24*	*>100*	*90 ± 11*	*50*	*25 ± 3*	*24 ± 3*	*54 ± 9*	*42 ± 6*
36		*47 ± 8*	*21 ± 3*	*22 ± 4*	*15 ± 3*	*12 ± 1*	*27 ± 0*	*<10*	*14 ± 6*	*44 ± 4*
37		*60 ± 4*	*44 ± 11*	*>100*	*53 ± 11*	*37 ± 0*	*13 ± 4*	*13 ± 1*	*45 ± 5*	*23 ± 7*
38		*<10*	*<10*	*<10*	*<10*	*<10*	*<10*	*<10*	*<10*	*<10*
39		*45 ± 4*	*24 ± 4*	*64 ± 10*	*20 ± 1*	*23 ± 6*	*14 ± 5*	*<10*	*29 ± 2*	*11 ± 3*
40		*45 ± 8*	*29 ± 9*	*71 ± 22*	*29 ± 11*	*31 ± 15*	*<10*	*12 ± 6*	*11 ± 5*	*<10*
41		*33 ± 10*	*13 ± 4*	*41 ± 26*	*18 ± 5*	*21 ± 7*	*<10*	*<10*	*13 ± 1*	*<10*

(*continued on next page*)

Table 2 (*continued*)

Compound[b]	R	Tumour cell line[a]								
		HT29 Colon	SW480	MCF-7 Breast	A2780 Ovarian	H460 Lung	A431 Skin	DU145 Prostate	BE2-C Neuronal	SJ-G2 Brain
42		*10 ± 7*	*16 ± 5*	*52 ± 41*	*31 ± 26*	*18 ± 7*	*10 ± 5*	*13 ± 5*	*10 ± 8*	*10 ± 8*
43		8.3 ± 0.7	24 ± 4	18 ± 0	19 ± 1	31 ± 7	18 ± 4	60 ± 6	17 ± 4	43 ± 10

Cytotoxicity levels are first expressed as % inhibition at 100 μM drug concentration (*in italics*) and if potent, as Growth Inhibition, GI_{50}, μM.
[a] Data were calculated after 72 h of continuous drug exposure, values are means (±SEM) of 3–6 experiments.
[b] Compound, compound number.

of cytotoxicity. However the hexanoic acid substituted **31** does show improved activity against MCF-7 and A2780 cell lines when compared with the analogous hexyl alcohol **26** and hexyl substituted **13** suggesting that the introduction of a terminal acid moiety is beneficial to cytotoxicity. The introduction of a morpholine substituent, **32**–**34**, had no effect on activity, which is contray to our findings with the corresponding ring opened analogues.[14]

Given our lack of improvement via terminally substituted linear or branched chains and the reported activity of the corresponding aromatic substituted cantharimides by Lin,[11,12] we synthesised a range of the equivalent phenyl substituted norcantharimides (Table 2, **35**–**38**).[3] In all instances we observed no noteworthy cytotoxicity at 100 μM drug dose (our primary screen prior to GI_{50} determination). Interestingly our most active aniline derived analogues, **35** and **37**, correspond well with the only active cantharimide aniline analogue reported by Lin.[11,12] However, Lin's cantharidin analogue is significantly more potent against the cell lines examined (HL-60 IC_{50} = 8.4 μM; and Hep G2 IC_{50} = 69 μM) a pattern which is also seen when comparing the parent cantharidin (**1**) and norcantharidin (**2**). In our hands no beneficial effect was observed by generation of a small series of benzyl substituted analogues (**39**–**41**), with no appreciable activity noted in all cases. As we were unable to increase the potency of our norcantharimides via the introduction of an aromatic ring, we directed our synthetic efforts towards linear, norcantharimide dimers of the type recently reported by Noda et al.[15]

Treatment of 1,3-diaminopropane and 1,12-diaminododecane with 0.5 equiv of **2** under standard conditions afforded bis-norcantharimides **42** and **43**. Bis-norcantharimide **43** with a dodecyl linker proved to be the most cytotoxic of all the analogues generated herein with broad spectrum cytotoxicity in excess of that observed for norcantharidin (**2**) with GI_{50} values ranging from 8.3 ± 0.7 to 60 ± 6 μM across the panel of cell lines examined.

3. Conclusions

Simple modification of the parent norcantharidin (**2**) has allowed the development of a new series of norcantharimides with modest to good cytotoxicities. The most potent analogues synthesised contained either a C_{10}, C_{12}, C_{14} alkyl chain (analogues **16**–**18**), a 1,2-diol moiety (**23**) or a dodecyl-linked second norcantharimide moiety (**43**). Of the analogues generated the dodecyl-linked bis-norcantharimide (**43**) was the most potent analogue displaying μM potent cytotoxicities against all the cell lines examined at levels that improve on the lead norcantharidin (**2**).

4. Experimental

4.1. Materials and methods

All reagents were of commercial quality and were used as received (Aldrich). Solvents were dried and purified using standard techniques. Reactions were monitored by TLC, on aluminium plates coated with silica gel with fluorescent indicator (Merck 60 F_{254}).

Unless otherwise noted, NMR spectra were recorded in $CDCl_3$ at 300 MHz for ^1H and at 75 MHz for ^{13}C (Bruker Advance 300MX). GCMS was performed using a Shimadzu GCMS-QP2010. The instrument uses a quadrupole mass spectrometer and detects samples via electron impact ionisation (EI) or chemical ionisation using methane (CI). The University of Wollongong Biomolecular Mass Spectrometry Laboratory analysed samples for HRMS. The spectra were run on the VG

Autospec-oa-tof tandem high resolution mass spectrometer using CI +ve (Chemical Ionisation), with methane as the carrier gas and PFK as the reference.

4.1.1. Cell culture and stock solutions. Stock solutions were prepared as follows and stored at −20 °C: Cantharidin (Biomol, USA) as a 30 mM solution in dimethylsulfoxide (DMSO); norcantharidin as a 30 mM solution in water and norcantharidin analogues as 40 mM solutions in DMSO. All cell lines were cultured at 37 °C, under 5% CO_2 in air, and were maintained in Dulbecco's modified Eagle's medium (Trace Biosciences, Australia) supplemented with 10% foetal bovine serum, 10 mM sodium bicarbonate penicillin (100 IU/ml), streptomycin (100 μg/ml) and glutamine (4 mM).

4.1.2. In vitro growth inhibition assay. Cells in logarithmic growth were transferred to 96-well plates. Cytotoxicity was determined by plating cells in duplicate in 100 mL medium at a density of 2500–4000 cells/well. On day 0 (24 h after plating) when the cells were in logarithmic growth, 100 μL medium with or without the test agent was added to each well. After 72-h drug exposure growth inhibitory effects were evaluated using the MTT (3-[4,5-dimethyltriazol-2-yl]-2,5-diphenyl-tetrazolium bromide) assay and absorbance read at 540 nm. Percentage growth inhibition was determined at a fixed drug concentration of 100 μM. A value of 100% is indicative of total cell growth inhibition. Those analogues showing appreciable percentage growth inhibition underwent further dose–response analysis allowing for the calculation of a GI_{50} value. This value is the drug concentration at which cell growth is 50% inhibited based on the difference between the optical density values on day 0 and those at the end of drug exposure.[2]

4.1.3. Chemistry. General synthetic procedure for the synthesis of norcantharimides: Amine (1 equiv, 2.97 mmol) was added to a magnetically stirred solution of norcantharidin **2** (1.0 g, 2.97 mmol) and triethylamine (1.5 mL) in toluene (15 mL). This solution was refluxed for 36 h before being cooled, diluted with EtOAc (45 mL), washed with $NaHCO_3$ (2× 5 mL, saturated solution), dried ($MgSO_4$), filtered and concentrated under reduced pressure. The resulting crude norcantharimide was either recrystallised from EtOAc/Hexane or subjected to flash chromatography (~40% EtOAc/Hexane) to afford norcantharimides **9**–**35** (~7–98% yield, depending on amine).

4.1.4. 4-Ethyl-10-oxa-4-azatricyclo[5.2.1]decane-3,5-dione (9). Isolated as a white solid, 81%, mp 166–169 °C. ^{1}H NMR (DMSO-d_6): 1.09 (3H, t, J = 7.21 Hz), 1.55 (2H, m), 1.80 (2H, m), 2.80 (2H, s), 3.46 (2H, q, J = 7.1 Hz), 4.81 (2H, dd, J = 2.4, 3.1 Hz). ^{13}C NMR (DMSO-d_6): 12.9, 28.5, 33.9, 49.9, 79.0, 176.9. HR-MS *m*/*z*: (calcd for $C_{11}H_{15}NO_3$: 209.10519).

4.1.5. 4-Propyl-10-oxa-4-azatricyclo[5.2.1]decane-3,5-dione (10). Isolated as a white solid, 80%, mp 79–80 °C. ^{1}H NMR ($CDCl_3$): δ 0.87 (3H, t, J = 7.3 Hz), 1.55–1.61 (4H, m), 1.83–1.86 (2H, m), 2.84 (2H, s, 2H), 3.44 (2H, t, J = 7.3 Hz), 4.86 (2H, br s). ^{13}C NMR ($CDCl_3$): δ 10.5, 20.3, 28.0 (2C), 40.0, 49.3, 78.4, 176.6 (2C). HR-MS *m*/*z*: (calcd for $C_{11}H_{15}NO_3$: 209.10519).

4.1.6. 4-Butyl-10-oxa-4-azatricyclo[5.2.1]decane-3,5-dione (11). Isolated as a white solid, 93%, mp 82–83 °C. ^{1}H NMR ($CDCl_3$): δ 0.90 (3H, t, J = 7.2 Hz), 1.28 (2H, sep, J = 7.8 Hz), 1.49–1.59 (4H, m), 1.82–1.85 (2H, m), 2.83 (2H, s), 3.45 (2H, t, J = 7.3 Hz), 4.85 (2H, q, J = 0.7 Hz). ^{13}C NMR ($CDCl_3$): δ 12.9, 19.3, 28.0 (2C), 29.0, 38.2, 49.3 (2C) 78.4, 176.6 (2C). HR-MS *m*/*z*: (calcd for $C_{12}H_{17}NO_3$: 223.12084).

4.1.7. 4-Sec-butyl-10-oxa-4-azatricyclo[5.2.1]decane-3,5-dione (12). Isolated as a white solid, 98%, mp: 44–46 °C. ^{1}H NMR (DMSO-d_6): 0.79 (3H, t, J = 7.5 Hz), 1.30 (3H, d, J = 7.0 Hz), 1.64–1.52 (2H, m), 1.80–1.92 (4H, m), 2.77 (2H, m), 4.03 (1H, m), 4.82 (2H, s). ^{13}C NMR (DMSO-d_6): 10.9, 17.5, 25.9, 28.6, 49.5, 50.1, 79.2, 177.5. HR-MS *m*/*z*: (calcd for $C_{12}H_{17}NO_3$: 223.12084).

4.1.8. 4-Hexyl-10-oxa-4-azatricyclo[5.2.1]decane-3,5-dione (13). Isolated as a colourless oil, 87%. ^{1}H NMR ($CDCl_3$): δ 0.85 (4H, m), 1.25 (8H, m), 1.57 (4H, m), 1.84 (2H, m), 2.84 (2H, s), 3.43 (2H, t, J = 7.1 Hz), 4.85 (2H, q, J = 2.4 Hz). ^{13}C NMR ($CDCl_3$): δ 13.4, 21.9, 25.7, 26.9, 28.0, 30.7, 38.5, 49.3, 78.5, 176.7. HR-MS *m*/*z*: (calcd for $C_{14}H_{21}NO_3$: 251.15214).

4.1.9. 4-Cyclohexyl-10-oxa-4-azatricyclo[5.2.1]decane-3,5-dione (14). Isolated as a white solid, 82%, mp 97–99 °C. ^{1}H NMR (DMSO-d_6): 1.16 (4H, m), 1.53 (6H, m), 1.77 (4H, m), 2.05 (2H, m), 2.73 (2H, s), 3.86 (1H, m), 4.78 (2H, m). ^{13}C NMR (DMSO-d_6): 25.0, 25.8, 28.5, 28.6, 49.4, 51.9, 79.1, 177.3. HR-MS *m*/*z*: (calcd for $C_{14}H_{19}NO_3$: 249.13649).

4.1.10. 4-Octyl-10-oxa-4-azatricyclo[5.2.1]decane-3,5-dione (15). Isolated as a white solid, 72%, mp 36–37 °C. ^{1}H NMR ($CDCl_3$): δ 0.85 (3H, t, J = 6.9 Hz), 1.24 (10H, s), 1.52–1.59 (4H, m), 1.81–1.84 (2H, m), 2.82 (2H, s), 3.43 (2H, t, J = 7.5 Hz), 4.84 (2H, q, J = 1.0 Hz). ^{13}C NMR ($CDCl_3$): δ 13.4 , 22.0, 21.9, 26.1, 27.0, 28.0 (2C), 28.4, 28.5, 31.1, 38.5, 49.3 (2C) 78.4, 176.5 (2C). HR-MS *m*/*z*: (calcd for $C_{16}H_{25}NO_3$: 279.18344).

4.1.11. 4-Decyl-10-oxa-4-azatricyclo[5.2.1]decane-3,5-dione (16). Isolated as a white solid, 68%, mp 30–31 °C. ^{1}H NMR ($CDCl_3$): δ 0.88 (3H, t, J = 6.7 Hz), 1.26 (14H, s), 1.52–1.59 (4H, m, 4H), 1.82–1.85 (2H, m), 2.81 (2H, s), 3.44 (2H, t, J = 7.3 Hz), 4.84 (2H, t, J = 2.1 Hz). ^{13}C NMR ($CDCl_3$): δ 14.0, 22.7, 26.7, 27.6, 28.7 (2C), 29.1, 29.3, 29.5, 32.0, 39.2, 50.5 (2C), 79.1 (2C), 177.2 (2C). HR-MS *m*/*z*: (calcd for $C_{18}H_{29}NO_3$: 307.21474).

4.1.12. 4-Dodecyl-10-oxa-4-azatricyclo[5.2.1]decane-3,5-dione (17). Isolated as a white solid, 82%, mp 35–37 °C. ^{1}H NMR ($CDCl_3$): δ 0.87 (4H, m), 1.24 (20H, s), 1.85–1.82 (2H, m), 1.60–1.50 (4H, m), 2.83 (2H, s), 3.44 (2H, t, J = 7.3 Hz), 4.85 (2H, q, J = 2.3 Hz). ^{13}C NMR ($CDCl_3$): δ 13.5, 22.1, 26.1, 27.0, 28.0, 28.5, 28.8, 28.9, 29.0, 38.5, 49.3, 78.5, 176.7. HR-MS *m*/*z*: (calcd for $C_{20}H_{33}NO_3$: 335.24604).

4.1.13. 4-Tetradecyl-10-oxa-4-azatricyclo[5.2.1]decane-3,5-dione (18). Isolated as a white solid, 79%, mp 45–46 °C. ^{1}H NMR ($CDCl_3$): δ 0.86 (2H, m), 1.23 (20H, s), 1.56 (2H, m), 1.83 (2H, quin, J = 4.5 Hz), 2.82 (2H, s), 3.42 (2H, t, J = 7.3 Hz), 4.84 (2H, q, J = 2.2 Hz). ^{13}C NMR ($CDCl_3$): δ 13.5, 22.1, 26.1, 28.0, 28.5, 29.0, 29.1, 38.5, 49.3, 78.5, 176.7. HR-MS *m/z*: (calcd for $C_{22}H_{37}NO_3$: 363.27734).

4.1.14. 4-Octadecyl-10-oxa-4-azatricyclo[5.2.1]decane-3,5-dione (19). Isolated as a white solid, 80%, mp 62–64 °C. ^{1}H NMR ($CDCl_3$): δ 1.58 (2H, m), 1.83–1.91 (4H, m), 2.86 (2H, s), 2.33 (2H, *t*, J = 7.4 Hz), 3.54 (2H, t, J = 6.8 Hz), 4.86 (2H, q, J = 2.3 Hz). ^{13}C NMR ($CDCl_3$): δ 22.0, 28.0, 30.4, 37.5, 49.3, 78.5, 176.7, 177.1. HR-MS *m/z*: (calcd for $C_{26}H_{45}NO_3$: 419.33994).

4.1.15. 4-Allyl-10-oxa-4-azatricyclo[5.2.1]decane-3,5-dione (20). Isolated as a white solid, 75%, mp 116–117 °C. ^{1}H NMR ($CDCl_3$): δ 1.55–1.62 (2H, m), 1.85–1.88 (2H, m), 2.89 (2H, s), 4.08 (2H, dd, J = 4.0, 1.3 Hz), 4.88–4.90 (2H, m), 5.16–5.22 (2H, m), 5.71–5.76 (1H, m). ^{13}C NMR (DMSO-d_6): δ 28.0 (2C), 40.3, 49.4, 78.4, 116.9, 129.8, 176.0 (2C). HR-MS *m/z*: (calcd for $C_{11}H_{13}NO_3$: 207.08954).

4.1.16. 4-But-3-enyl-10-oxa-4-azatricyclo[5.2.1]decane-3,5-dione (21). Isolated as a white solid, 31%, mp 64–65 °C. ^{1}H NMR (DMSO-d_6): δ 1.61 (4H, br s), 2.17 (2H, q, J = 6.9 Hz), 3.00 (2H, s), 3.37 (2H, t, J = 6.9 Hz), 4.66 (2H, s), 4.99 (2H, m), 5.65 (1H, m). ^{13}C NMR (DMSO-d_6): δ 28.8 (2C), 32.2, 38.2, 50.2 (2C), 79.2, 79.2, 117.9, 135.5, 170.1 (2C). HR-MS *m/z*: (calcd for $C_{12}H_{15}NO_3$: 221.10519).

4.1.17. 4-Oxiranylmethyl-10-oxa-4-azatricyclo[5.2.1]decane-3,5-dione (22). *m*-Chloroperbenzoic acid (2.15 g, 77% in water, 9.66 mmol) was added in one portion to cooled and magnetically stirred solution at 0 °C of allyl-*N*-norcantharimide **20** (1.0 g, 4.83 mmol) in CH_2Cl_2 (20 mL). The resulting solution was warmed to room temperature, stirred for 16 h before being diluted with CH_2Cl_2 (30 mL) and washed with $NaHCO_3$ (3× 10 mL, sat solution). The organic layer was dried (Na_2SO_4), filtered and concentrated under reduced pressure to afford a white solid. Flash chromatography (50% EtOAc/Hexane) afforded the norcantharimide epoxide (820 mg) as a white solid, 76%, mp 83–84 °C. ^{1} H NMR ($CDCl_3$): δ 1.53–1.59 (2 H, m), 1.79–1.82 (2H, m), 2.51–2.54 (1H, m), 2.68 (1H, t, J = 4.1 Hz), 2.86 (2H, s), 3.04–3.06 (1H, m), 3.55 (1H, dd, J = 14, 4.6 Hz), 3.64 (1H, dd, J = 14, 4.6 Hz), 4.80–4.82 (2H, m). ^{13}C NMR ($CDCl_3$): δ 27.9 (2C), 39.8 (2C), 45.2, 47.8, 49.3, 49.4 (2C) 78.4, 176.3 (2C). HR-MS *m/z*: (calcd for $C_{11}H_{13}NO_4$: 223.08446).

4.1.18. 4-(3-Hydroxypropyl)-10-oxa-4-azatricyclo[5.2.1]-decane-3,5-dione (25). Isolated as a white solid, 80%, mp 164–166 °C. ^{1}H NMR ($CDCl_3$): δ 1.61 (2H, m), 1.84 (2H, m), 2.89 (2H, s), 3.01 (1H, br s), 3.64 (2H, q, J = 3.6 Hz), 3.70 (2H, t, J = 4.8 Hz), 4.86 (2H, q, J = 2.1 Hz). ^{13}C NMR ($CDCl_3$): δ 27.9, 41.3, 49.4, 59.5, 78.6, 177.1. HR-MS m/z: (calcd for $C_{10}H_{13}NO_4$: 211.08446).

4.1.19. 4-(6-Hydroxyhexyl)-10-oxa-4-azatricyclo[5.2.1]-decane-3,5-dione (26). Isolated as a white solid, 62%, mp 56–57 °C. ^{1}H NMR ($CDCl_3$): δ 1.27 (4H, m), 1.48 (2H, m), 1.57 (2H, m), 2.15 (2H, m), 2.82 (2H, s), 3.41 (2H, m), 3.54 (2H, m), 4.80 (2H, m). ^{13}C NMR ($CDCl_3$): δ 24.5, 25.6, 26.8, 27.9, 31.8, 38.3, 49.3, 61.9, 78.5, 176.8. HR-MS *m/z*: (calcd for $C_{14}H_{21}NO_4$: 267.14706).

4.1.20. 4-(2-Hydroxy-1-methylethyl)-10-oxa-4-azatricyclo[5.2.1]decane-3,5-dione (27). Isolated as a yellow solid, 22%, mp 104–107 °C. ^{1}H NMR (DMSO-d_6): 1.28 (3H, d, J = 7.1 Hz), 1.58 (2H, m), 1.84 (m), 2.14 (1H, s), 2.83 (2H, s), 3.70 (1H, m), 3.80 (1H, m), 4.26 (1H, m), 4.84 (2H, m). ^{13}C NMR (DMSO-d_6): 13.8, 28.5, 28.6, 49.6, 49.7, 50.4, 63.5, 79.3, 177.9. HR-MS *m/z*: (calcd for $C_{11}H_{15}NO_4$: 225.10011).

4.1.21. Synthesis of 4-(2-hydroxy-1,1-dimethylethyl)-10-oxa-4-azatricyclo[5.2.1.02,6]decane-3,5-dione (28). Isolated as a pale yellow solid, 46%, mp 134–136 °C. ^{1}H NMR (DMSO-d_6): δ 1.39 (6H, s), 1.52 (2H, m), 1.78 (2H, m), 2.72 (2H, s), 3.52 (1H, br s), 3.71 (2H, s), 4.78 (2H, dd, J = 2.3, 3.1 Hz). ^{13}C NMR (DMSO-d_6): δ 22.1, 28.4, 49.6, 63.0, 68.9, 79.5, 179.1. HR-MS *m/z*: (calcd for $C_{12}H_{17}NO_4$: 239.11576).

4.1.22. Synthesis of 4-(1-hydroxymethylpropyl)-10-oxa-4-azatricyclo[5.2.1]decane-3,5-dione (29). Isolated as a pale yellow oil, 49%. ^{1}H NMR (DMSO-d_6): δ 0.79 (3H, t, J = 7.4 Hz), 1.38–1.79 (6H, m), 2.81 (2H, q, J = 11.6, 7.0 Hz), 3.01 (1H, br s), 3.63 (1H, m), 3.87 (1H, m), 4.05 (1H, m), 4.79 (2H, s). ^{13}C NMR (DMSO-d_6): δ 10.3, 20.7, 28.4, 28.5, 49.4, 49.6, 56.5,62.1, 79.2, 178.2. HR-MS *m/z*: (calcd for $C_{12}H_{17}NO_4$: 239.11576).

4.1.23. 4-(3,5-Dioxo-10-oxa-4-azatricyclo[5.2.1]dec-4-yl)-butyric acid (30). Isolated as a white solid, 45%, mp 162–164 °C. ^{1}H NMR ($CDCl_3$): δ 1.58 (2H, m), 1.83–1.91 (4H, m), 2.33 (2H, t, J = 7.4 Hz), 2.86 (2H, s), 3.54 (2H, t, J = 6.8 Hz), 4.86 (2H, q, J = 2.3 Hz). ^{13}C NMR ($CDCl_3$): δ 22.0, 28.0, 30.4, 37.5, 49.3, 78.5, 176.7, 177.1. HR-MS *m/z*: (calcd for $C_{12}H_{15}NO_5$: 253.09502).

4.1.24. 6-(3,5-Dioxo-10-oxa-4-azatricyclo[5.2.1]dec-4-yl)-hexanoic acid (31). Isolated as a white solid, 56%, mp 110–112 °C. ^{1}H NMR ($CDCl_3$): δ 1.30 (2 H, m), 1.60 (6H, m), 1.83 (2H, m), 2.32 (2H, t, J = 7.44), 2.86 (2H, s), 3.45 (2H, t, J = 7.23), 4.86 (2H, q, J = 2.2 Hz). ^{13}C NMR ($CDCl_3$): δ 23.5, 25.4, 26.6, 28.0, 33.2, 38.2, 49.3, 78.5, 176.8, 178.6. HR-MS *m/z*: (calcd for C1 $4H_{19}NO_5$: 281.12632).

4.1.25. 4-(2,3-Dihydroxypropyl)-10-oxa-4-azatricyclo[5.2.1]-decane-3,5-dione (23). OsO_4 (120 mg of a 2.5% solution in *t*-BuOH, 0.012 mmol, 0.5 mol%) was added dropwise to a magnetically stirred solution of allyl substituted **20** (500 mg, 2.41 mmol), *N*-methylmorpholine-*N*-oxide (310 mg, 2.65 mmol) in acetone/water (5:2 mL). The resulting solution was heated at 80 °C for 16 h before being diluted with ether (100 mL) and washed with water (3× 20 mL). The organic layer was concentrated under reduced pressure to afford a brown solid. Flash chromatography (70% EtOAc/hexanes) afforded a pale white solid

which was recrystallised from EtOAc to afford a white crystalline solid, 34%. ^{1}H NMR (DMSO-d_6): δ 1.62 (4H, s), 3.00 (2H, s), 3.23–3.32 (2H, m), 3.63 (1H, q, J = 5.7 Hz), 4.51 (1H, t, J = 5.7 Hz), 4.66 (2H, s), 4.73 (1H, d, J = 4.6 Hz). ^{13}C NMR (DMSO-d_6): 27.8 (2C), 41.8, 49.3 (2C) 63.9, 67.9, 78.3 (2C), 177.4. HR-MS m/z: (calcd for $C_{11}H_{15}NO_5$: 241.09502).

4.1.26. 4-(3-Hydroxy-2-methoxypropyl)-10-oxa-4-azatricyclo[5.2.1]decane-3,5-dione (24). (1*S*)-(+)-10-Camphorsulfonic acid (15 mg, 0.06 mmol) was added to a magnetically stirred solution of epoxide **22** (220 mg, 0.98 mmol) in methanol (4 mL). The resulting solution was warmed to 35 °C and stirred for 16 h before being concentrated under reduced pressure. The resulting clear oil was subjected to flash chromatography (70% EtOAc/Hexanes) to afford a white solid, 78%, mp 95–96 °C. ^{1}H NMR (DMSO-d_6): δ 1.60–1.63 (2H, m), 1.85–1.88 (2H, m), 2.91 (2H, s), 3.33–3.39 (2H, m), 3.38 (3H, s), 3.60–3.68 (2H, m), 3.94–3.96 (1H, m), 4.88–4.89 (2H, m). ^{13}C NMR (DMSO-d_6): δ 28.0 (2C), 41.7, 49.4 (2C), 58.7, 67.7, 73.5, 78.6 (2C), 177.0 (2C). HR-MS m/z: (calcd for $C_{12}H_{17}NO_5$: 255.11067).

4.1.27. Synthesis of 4-morpholin-4-yl-10-oxa-4-azatricyclo[5.2.1]decane-3,5-dione (32). Isolated as a yellow solid, 43%, mp 169–171 °C. ^{1}H NMR (DMSO-d_6): δ 1.53 (2H, m), 1.78 (2H, m), 2.73 (2H, s), 3.18 (4H, t, J = 4.5 Hz), 3.71 (4H, t, J = 4.7 Hz), 4.78 (2H, m); ^{13}C NMR (DMSO-d_6): δ 28.5, 47.9, 51.2, 66.7, 79.1, 175.1. HR-MS m/z: (calcd for $C_{12}H_{16}N_2O_4$: 252.1101).

4.1.28. Synthesis of 4-(2-morpholin-4-ylethyl)-10-oxa-4-azatricyclo[5.2.1.02,6]decane-3,5-dione (33). Isolated as an orange brown solid, 37%, mp 109–111 °C; ^{1}H NMR (DMSO-d_6): 1.56 (2H, m), 1.80 (2H, m), 2.43-2.48 (6H, m), 2.84 (2H, s), 3.55 (2H, t, J = 4.5 Hz), 3.60 (4H, t, J = 4.5 Hz), 4.82 (2H, m). ^{13}C NMR (DMSO-d_6): 28.6, 36.6, 49.9, 53.4, 55.1, 67.0, 79.0, 177.1. HR-MS m/z: (calcd for $C_{14}H_{20}N_2O_4$: 280.14231).

4.1.29. Synthesis of 4-(3-morpholin-4-ylpropyl)-10-oxa-4-azatricyclo[5.2.1.02,6]decane-3,5-dione (34). Isolated as an orange/brown oil, 7%. ^{1}H NMR (DMSO-d_6): δ 1.55 (2H, m), 1.70 (2H, s), 1.81 (2H, m), 2.81 (2H, s), 2.30 (2H, t), 2.37 (4H, m), 3.48 (2H, t, J = 4.5 Hz), 3.65 (4H, t, J = 4.7 Hz), 4.82 (2H, m). ^{13}C NMR (DMSO-d_6): δ 24.2, 28.5, 37.2, 49.9, 51.3, 53.4, 55.8, 66.8, 79.0, 177.2. HR-MS m/z: (calcd for $C_{15}H_{22}N_2O_4$: 294.15796).

4.1.30. 4-Phenyl-10-oxa-4-azatricyclo[5.2.1]decane-3,5-dione (35). Isolated as a bone coloured solid, 79%, mp 171–172 °C. ^{1}H NMR: ($CDCl_3$): δ 1.63–1.65 (2H, m), 1.87–1.90 (2H, m), 3.01 (2H, s), 4.97 (2H, q, J = 1.1 Hz), 7.25–7.28 (2H, m), 7.42-7.45 (2H, m). ^{13}C NMR: ($CDCl_3$): 28.1 (2C), 49.5 (2C), 78.9, 125.0 (2C), 128.1, 128.5, 175.7. HR-MS m/z: (calcd for $C_{14}H_{13}NO_3$: 243.08954).

4.1.31. 4-(4-Hydroxyphenyl)-10-oxa-4-azatricyclo[5.2.1]-decane-3,5-dione (36). Isolated as a white solid, 45%, 172–173 °C. ^{1}H NMR ($CDCl_3$): δ 1.56–1.62 (4H, m), 3.01 (2H, s), 4.98 (2H, q, J = 0.9 Hz), 6.86 (2H, d, J = 8.1 Hz), 7.11 (2H, d, J = 8.1 Hz). ^{13}C NMR (DMSO-d_6): δ 28.1 (2C), 49.4, 78.9, 115.4 (2C), 127.4 (2C), 181.5 (2C). HR-MS m/z: (calcd for $C_{14}H_{13}NO_4$: 259.08446).

4.1.32. 4-(4-Nitrophenyl)-10-oxa-4-azatricyclo[5.2.1]decane-3,5-dione (37). Isolated as an orange yellow solid,76%, mp 207–209 °C. ^{1}H NMR ($CDCl_3$): δ 1.66–1.69 (2H, m), 1.90–1.94 (2H, m), 3.07 (2H, s), 4.99 (2H, q, J = 0.9 Hz), 7.55 (2H, d, J = 7.1 Hz), 8.29 (2H, d, J = 7.1 Hz). ^{13}C NMR ($CDCl_3$): δ 28.0 (2C), 49.5 (2C), 79.1, 123.7, 126.4, 136.8, 146.5, 174.9. HR-MS m/z: (calcd for $C_{14}H_{12}N_2O_5$: 288.07462).

4.1.33. 4-(3,5-Dioxo-10-oxa-4-azatricyclo[5.2.1]dec-4-yl) benzoic acid (38). Isolated as a white solid, 36%, mp 275–277 °C. ^{1}H NMR ($CDCl_3$): 1.63 (2H, m), 1.88 (2H, m), 3.00 (2H, s), 4.96 (2H, q, J = 1.9 Hz), 7.30 (2H, d, J = 8.1 Hz), 8.13 (2H, d, J = 8.4 Hz). ^{13}C NMR ($CDCl_3$): 28.0, 49.5, 78.9, 125.3, 129.8, 132.2, 134.3, 160.1, 175.3. HR-MS m/z: (calcd for $C_{15}H_{13}NO_5$: 287.07937).

4.1.34. 4-Benzyl-10-oxa-4-azatricyclo[5.2.1]decane-3,5-dione (39). Isolated as a white solid, 91%, mp 97–100 °C. ^{1}H NMR (DMSO-d_6): δ 1.21 (2H, m), 1.54 (2H, m), 1.77 (2H, m), 2.81 (2H, s), 4.56 (2H, s), 4.81 (2H, m), 7.22 (5H, m). ^{13}C NMR (DMSO-d_6): δ 28.4, 42.3, 49.8, 78.9, 127.5, 127.8, 128.4, 135.3, 176.7. HR-MS m/z: (calcd for $C_{16}H_{17}NO_4$: 287.11576).

4.1.35. 4-(4-Methoxybenzyl)-10-oxa-4-azatricyclo[5.2.1]-decane-3,5-dione (40). Isolated as a white solid, 82%, mp 83–85 °C. ^{1}H NMR ($CDCl_3$): δ 1.60 (2H, m), 1.85 (2H, m), 2.84 2H, s), 3.77 (3H, s), 4.56 (2H, s), 4.87 (2H, q, J = 2.1 Hz), 6.81 (2H, d J = 6.7 Hz), 7.25 (2H, d, J = 6.6 Hz). ^{13}C NMR ($CDCl_3$): δ 28.0, 41.4, 49.5, 54.6, 78.5, 113.4, 127.3, 129.1, 158.7, 176.2. HR-MS m/z: (calcd for $C_{16}H_{17}NO_4$: 287.11576).

4.1.36. 4-(3,4-Dimethoxybenzyl)-10-oxa-4-azatricyclo[5.2.1]-decane-3,5-dione (41). Isolated as a white solid, 76%, mp 155–157 °C. ^{1}H NMR ($CDCl_3$): δ 1.60 (2H, m), 1.84 (2H, m), 2.86 (2H, s), 3.83 (3H, s), 3.84 (3H, s), 4.56 (2H, s), 4.87 (2H, q, J = 2.4 Hz), 6.77 (1H, m), 6.89 (2H, m). ^{13}C NMR ($CDCl_3$): δ 28.0, 41.7, 49.5, 55.3, 78.5, 110.7, 110.9, 120.1, 127.6, 148.1, 148.5, 176.3. HR-MS m/z: (calcd for $C_{17}H_{19}NO_5$: 317.12632).

4.1.37. 4-(2-Aminobenzyl)-10-oxa-4-azatricyclo[5.2.1]-decane-3,5-dione (42). Isolated as a yellow solid, 43%, mp 159–160 °C. ^{1}H NMR (DMSO-d_6): δ 1.54–1.46 (4H, m), 2.74 (2H, s), 3.84 (2H, s), 4.71 (2H, s), 6.54 (1H, t), 6.68 (1H, d), 7.09–7.00 (2H, m). ^{13}C NMR (DMSO-d_6): δ 29.0, 53.7, 79.8, 115.3, 116.1, 118.7, 128.9, 129.8, 146.8, 174.1. HR-MS m/z: (calcd for $C_{15}H_{16}N_2O_3$: 272.11609).

4.1.38. Bis-3,6-epoxycyclohexane-1,2-dicarboximido)-trimethylene (bis-norcantharimide-propyl linker) (43). Isolated as a white solid, 80%. ^{1}H NMR (DMSO-d_6): δ 1.58 (2H, quin), 1.85 (4H, m), 2.86 (2H, s), 3.43 (2H, m),

4.86 (2H, m). ^{13}C NMR (DMSO-d_6): δ 24.7, 28.0, 35.6, 49.4, 78.5, 176.5. HR-MS *m/z*: (calcd for $C_{19}H_{22}N_2O_6$: 374.14779).

4.1.39. Bis-3,6-epoxycyclohexane-1,2-dicarboximido)-dodecylmethyl(bis-norcantharimide-dodecyl linker) (44). Isolated as a white solid, 62%. ^{1}H NMR ($CDCl_3$): δ 1.22 (10H, s), 1.59–1.51 (4H, m), 1.85 (2H, m), 2.84 (2H, s), 3.43 (2H, t, $J = 7.3$ Hz), 4.85 (2H, q, $J = 2.2$ Hz). ^{13}C NMR ($CDCl_3$): δ 26.0, 27.0, 28.5, 28.8, 28.9, 38.5, 49.3, 78.5, 176.6. HR-MS *m/z*: (calcd for $C_{28}H_{40}N_2O_6$: 500.28864).

Acknowledgment

This work received financial support from the Hunter Medical Research Institute and the Biotechnology Innovation Fund, AusIndustry, Australia.

References and notes

1. http://www.fda.gov/cder/cancer/approved.htm.
2. McCluskey, A.; Bowyer, M. C.; Collins, E.; Sim, A. T. R.; Sakoff, J. A.; Baldwin, M. L. *Bioorg. Med. Chem. Lett.* **2000**, *10*, 1687–1690.
3. McCluskey, A.; Walkom, C.; Bowyer, M. C.; Ackland, S. P.; Gardiner, E.; Sakoff, J. A. *Bioorg. Med. Chem. Lett.* **2001**, *11*, 2941–2946.
4. Sakoff, J. A.; Ackland, S. P.; Baldwin, M. L.; Keane, M. A.; McCluskey, A. *Invest. New Drugs* **2002**, *20*, 1–11.
5. McCluskey, A.; Ackland, S. P.; Bowyer, M. C.; Baldwin, M. L.; Garner, E.; Walkom, C. C.; Sakoff, J. A. *Bioorg. Chem.* **2003**, *31*, 66–77.
6. Hart, M. E.; Chamberlin, A. R.; Walkom, C.; Sakoff, J. A.; McCluskey, A. *Bioorg. Med. Chem. Lett.* **2004**, *14*, 1969–1973.
7. Decker, R. H. *J. Invest. Dermatol.* **1963**, *42*, 465–471.
8. Li, Y. M.; Casida, J. E. *Proc. Natl. Acad. Sci. U.S.A.* **1992**, *89*, 11867–11870.
9. Honkanen, R. E. *FEBS Lett.* **1993**, *330*, 283–285.
10. Graziano, M. J.; Waterhouse, A. L.; Casida, J. E. *Biochem. Biophys. Res. Commun.* **1987**, *149*, 79–81.
11. Lin, L.-H.; Huang, H.-S.; Lin, C.-C.; Lee, L. W.; Lin, P.-Y. *Chem. Pharm. Bull.* **2004**, *52*, 855–857.
12. Lin, P.-L.; Shi, S.-J.; Shu, H.-L.; Chen, H.-F.; Lin, C. C.; Liu, P.-C.; Wang, L. W. *Bioorg. Chem.* **2000**, *28*, 266–272.
13. Egglette, T. A.; de Koning, H.; Huisman, H. O. *Tetrahedron Lett.* **1973**, *29*, 2445–2447.
14. Hill, T. A.; Stewart, S. G.; Sauer, B.; Gilbert, J.; Ackland, S. P.; Sakoff, J. A.; McCluskey, A. *Bioorg. Med. Chem. Lett.* **2007**, *17*, 3392–3397.
15. Nakatani, T.; Jinpo, K.; Noda, N. *Chem. Pharm. Bull.* **2007**, *55*, 92–94.