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Norcantharimide analogues possessing terminal phosphate esters and their anti-cancer activity

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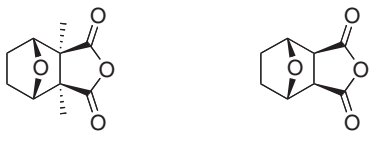
ABSTRACT

A family of norcantharidin analogues possessing a terminal alcohol (ethanol, propanol, butanol, pentanol, hexanol and cyclohexanol) moiety were treated with either chlorodiethyl, chlorodiphenyl or chloro-bis-trichloroethyl-phosphate to afford highly focused libraries of the corresponding phosphate esters. Subsequent biological screening against a panel of nine human cancer cell lines identified a trend between the ease of phosphate unmasking (phosphate ester hydrolysis) and cell death. The most potent analogues possessed either a diphenyl or a bis-trichloroethyl moiety. The effect of alkyl spacer was also examined with the hexyl analogues typically more potent. 4-Aza-4-(3-{bis(2,2,2-trichloroethyl)phosphate}propyl)-10-oxatricyclo[5.2.1.0]decane-3,5-dione (**10b**) was the most potent analogue synthesised with an average GI_{50} of 11 μM across a panel of nine human carcinoma cell lines: colon carcinoma (HT29 and SW480); breast carcinoma (MCF-7); ovarian carcinoma (A2780); lung carcinoma (H460); skin carcinoma (A431); prostate carcinoma (DU145); neuronal carcinoma (BE2-C) and brain carcinoma (SJ-G2). This represents a fivefold improvement in anti-proliferative activity relative to the lead, norcantharidin.

1. Introduction

Cantharidin (**1**) and norcantharidin (**2**) have a long history of use as anti-proliferative agents (Fig. 1).[1] This use originates from the Chinese traditional medicine, *Mylabris* from the dried bodies of blister beetles, of which cantharidin is the active component. *Mylabris* has been utilized in the treatment of various cancerous growths and is reported as a safe and effective treatment of molluscum contagiosum.[2–4] Cantharidin is a small molecule amenable to rapid structural modifications; lipophilic not requiring an active transport mechanism to enter cells; it is not a substrate for p-glycoprotein; it does not induce myelosuppression and indeed has been shown to induce haematopoiesis in animal and human studies. However cantharidin does possess liver and renal toxicities.[5–12] Fortunately, the removal of the 2,3-dimethyl substituents furnishing norcantharidin in addition to simplifying the chemistry, appears to overcome these toxicity issues. Norcantharidin is reported to induce apoptosis in human cervical,[13,14] oral,[15,16] melanoma,[17] ginival,[15] bladder,[18] adenocystic carcinoma,[19] glioblastoma,[20] bone,[21] leukaemia,[21] ovarian,[22] liver,[23,24,21] gall bladder[25] and colon[26–29] cancer cell lines, however so far the main application is for hepatoma.[30,31]

Numerous research groups have reported various structural modifications of norcantharidin, ones in which protein phosphatase inhibition is maintained and others in which this activity is removed, but anti-proliferative activity is maintained.[2–4,29,31–43] As is routine in our laboratory we conducted both protein phosphatase 1 and 2A inhibition studies, and in keeping with our earlier reports relating to similar cantharimides, all analogues herein were devoid of any protein phosphatase inhibitory properties. These cantharimide analogues are exemplified by the generic (**3**). In our own laboratory we have developed a series of bis-norcantharidimides with modest levels of activity, and N-aromatic variants with poor to modest activity. N-aliphatic and N-aliphatic-functional analogues are much less studied. Indeed in our most recent report we noted that that introduction of a free terminal –OH moiety (**4**) gave rise to a modest level of cytotoxicity (Fig. 2).[43]



1 2

Figure 1. Chemical structures of cantharidin and norcantharidin.

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Interestingly in subsequent SAR development, modifications of the terminal –OH were unsuccessful in increasing anti-proliferative activity. Methylation of the C2′-OH abolished what little activity that was present, whilst isosteric modifications of –OH to –COOH, morpholino, ethylmorpholino, etc. all proved ineffective.[43] Notwithstanding this, the fact that the incorporation of a second norcantharidin unit did increase activity suggested that modifications of this N-R substituent was possible. Herein we report an elegantly simple series of modifications that furnish considerable enhancements in anti-proliferative activity.

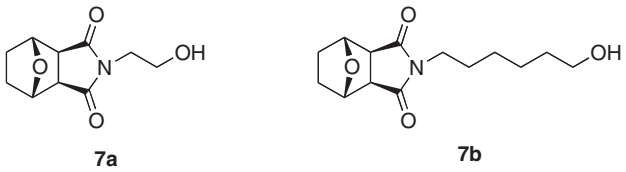


Figure 3. Chemical structures of *N*-alcohol substituted lead norcantharimides **7a** and **7b**.

2. Results

Our current investigations commenced with the synthesis of the *N*-ethanol and *N*-hexanol analogues (**7a**, X = CH_2CH_2; R_1 = OH; **7b** X = $CH_2(CH_2)_5$; R_1 = OH; Fig. 3) as shown in and Scheme 1.

As anticipated screening against our panel of cancer cell lines afforded no activity (GI_{50} >100 μM) in all cases. As we have previously shown that simple modifications of this terminal group did not increase activity, but the introduction of a second group (OH) did, we decided to explore the introduction of a more highly functionalized end group. At this time we were also exploring the introduction of phosphates and related groups in an allied program, and hence it seemed logical to explore a similar series of analogues here. Thus, treatment of **7a,b** with diethylchlorophosphate in the presence of *n*-butyltin oxide and triethylamine gave the desired terminally substituted phosphate analogue (**8a**, X = CH_2CH_2; R_1 = $P(O)(OEt)_2$ and **8b**, X = $-(CH_2)_6-$; R_1 = $P(O)(OEt)_2$).

As can be seen from Table 1, the introduction of the terminal diethyl phosphate to **7a** with the synthesis of **8a** brought immediate rewards with an increase from no activity at 100 μM drug concentration to an average GI_{50} value of 29 μM across the nine carcinoma cell lines examined. This represents an improvement over the parent norcantharidin across the same panel of cancer cell lines (average GI_{50} = 42 μM) and equipotent with the most potent of our previously reported nocantharimides (bis-3,6-epoxycyclohexane-1,2-dicarboximido)-trimethylene (bis-norcantharimide-propyl linker) average GI_{50} = 26 μM).[43] Interestingly the same substitution with the hexyl analogue **8b** showed no discernable increase in anti-proliferative activity, possibly an artefact of the increased chain length. Hence in this study we sought to investigate the effect of the chain length X and also the nature of the phosphate ester substituents.

The development of the required focused compound libraries was as per Scheme 1. Analogues with variations in chain length X and differentially substituted phosphates were synthesised in good yield (37–83%) and subsequently evaluated for their anti-proliferative effects. The outcome of this screening is shown in Table 2.

As noted previously, the introduction of a diethylphosphate moiety confers favourable anti-proliferative properties to **8a**, but continued exploration of the alkyl spacer with this series of compounds through propyl to hexyl (**8b**–**e**) renders these analogues inactive. Introduction of a conformationally restricted short linker in the guise of a 1,2-disubstituted cyclohexane (**8f**) results in a modest reduction of the anti-proliferative activity noted with **8a** (average GI_{50} values of 47 and 29 μM respectively).

Having established the favourable outcome with the diethyl phosphate analogues, we next turned our attention to variations of the phosphate ester moiety. We thus examined the commercially available diphenyl (**9**) and trichloroethyl (**10**) phosphates. On examination of the cytotoxicity screening outcomes (Table 2) it is clear that the diphenylphosphates (**9a**–**f**) on the whole display an improved anti-proliferative profile. Only the short alkyl spacer analogues (**9a** and **9c**) are bereft of activity. The butyl (**9d**), pentyl (**9e**) and hexyl (**9b**) display activities varying from equipotent with **2** to a fivefold improvement returning average GI_{50} values of 24, 48, and 12.5 μM respectively. The 1,2-disubstituted cyclohexane (**9f**) is almost twice as potent as the parent **2** with an average GI_{50} of 27 μM. Only the ethyl spacer based trichloroethyl phosphate (**10a**) analogue, in this family, is inactive with all others (**10b**–**f**) returning modest to good anti-proliferative activity. The most potent analogue in this last series is the hexyl spacer (**10b**, average GI_{50} = 11 μM), followed by the pentyl (**10e**, average GI_{50} = 20 μM), the butyl (**10d**, average GI_{50} = 24 μM), the 1,2-disusbstituted cyclohexyl (**10f**, average GI_{50} = 55 μM) and the propyl (**10c**, average GI_{50} = 67 μM). Excepting **10a** and **10c**, all trichloroethyl phosphate analogues are more potent than **2**. Interestingly, the hexyl spacer analogue of the diphenyl and trichloroethyl series displayed comparable anti-proliferative activity between the two series and the greatest potency within each of these series.

The results shown in Table 2 display two clear trends. The first is that with the exception of the small linker (ethyl **8a** and cyclohexyl **8f**) analogues the ethyl ester protection results in a lack of bioactivity. This in itself is not surprising. Simple alkyl esters are more resistant to hydrolysis than aromatic or trihaloalkyl phosphates and so it may be expected that the possibly bioactive 'naked' phosphate form of the molecule is not reached.[44] In general both the phenyl and bis(trichloroethyl) analogues show far better bioactivity relative to the simple alkyl ester analogues which can be explained by this ease of hydrolysis.[45,46] Why the shorter chain leads to a increase in anti-proliferative activity is not clear, especially as is the case of the ethyl linker, where the diphenyl (**9a**) and bis(trichloroethyl) (**10a**) show no discernable activity. The second trend is the effect of linker length. For both the diphenyl (**9**) and bis(trichloroethyl) (**10**) series the optimal chain length appears to lie with the hexyl chain, even though this result is not mirrored in the diethyl series. In general none of the compounds tested displayed any major specificity towards one cancer line over another suggesting the target is fairly generic to all cancer forms.

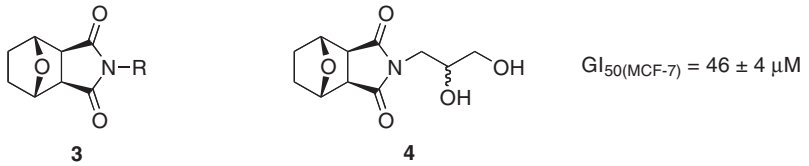
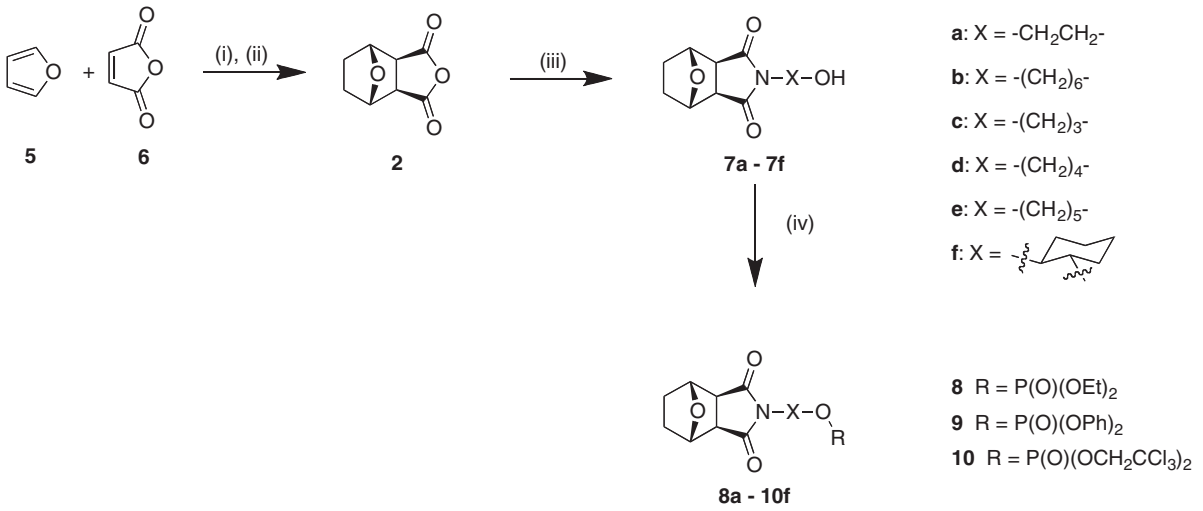
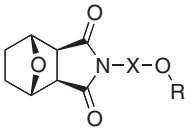


Figure 2. Chemical structure of generic cantharimides (**3**) and a glycerol substituted norcantharimide (**4**) displaying modest levels of cytotoxicity against the MCF-7 breast cancer cell line (from Ref. 43).



Scheme 1. Reagents and conditions: (i) Et_2O, rt, 48 h; (ii) H_2, 10% Pd–C, rt, EtOH; (iii) H_2N–X–OH, Δ, 18 h; (iv) $ClP(O)(OR)_2$, *n*-Bu_2O, Et_3N, rt.

Table 1
Anti-proliferative effects (GI_{50} values in μM) of norcantharidin (**2**), lead *N*-alcohols (**7a**, **7b**) and the lead phosphate esters (**8a**, **8b**) against a panel of human cancer cell lines



Compound	X	R	HT29[a]	SW480[a]	MCF-7[b]	A2780[c]	H460[d]	A431[e]	DU145[f]	BE2-C[g]	SJ-G2[h]
2	—	—	57 ± 5	44 ± 6	68 ± 4	38 ± 1	45 ± 3	31 ± 1	28 ± 3	43 ± 6	23 ± 3
7a	CH_2CH_2	H	>100	>100	>100	>100	>100	>100	>100	>100	>100
7b	$-(CH_2)_6-$	H	>100	>100	>100	>100	>100	>100	>100	>100	>100
8a	CH_2CH_2	$P(O)(OEt)_2$	33 ± 1.0	18 ± 0.4	28 ± 1.3	15 ± 0.6	49 ± 5.0	27 ± 1.5	21 ± 3.3	—[i]	43 ± 4.1
8b	$-(CH_2)_6-$	$P(O)(OEt)_2$	>100	>100	>100	>100	>100	>100	>100	>100	>100

[A] Colon carcinoma.
[B] Breast carcinoma.
[C] Ovarian carcinoma.
[D] Lung carcinoma.
[E] Skin carcinoma.
[F] Prostate carcinoma.
[G] Neuronal carcinoma.
[H] Brain carcinoma.
[i] Not determined.

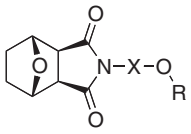
To confirm the importance of the norcantharidin scaffold, an analogue of one of our more active compounds, (**9d**), having a simple pthalimido group (**12**) was synthesised as shown in Scheme 2. This was subjected to the same biological testing and the results are shown in Table 3. This phenyl ester was inactive across all cell lines and clearly shows the crucial influence of the norcantharidin on anti-proliferative activity.

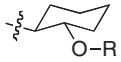
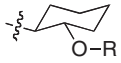
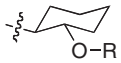
Phosphate esters have been introduced in the past to increase bioactivity by either acting as a prodrug.[43] The problem therein is the addition of a 'naked' phosphate group, which is negatively charged, results in a molecule with low membrane permeability. Masking this group as an ester prodrug can increase cell permeability, affording greater cellular uptake followed by esterase action to release the bioactive compound. This effect is the most likely cause of the increased bioactivity as the other probable fate of these types of molecules is phosphate cleavage by a phosphatase enzyme to yield the hydroxyl substituted base scaffolds (**7**). These types of molecules have been tested in the past and found to be inactive.[43]

3. Conclusion

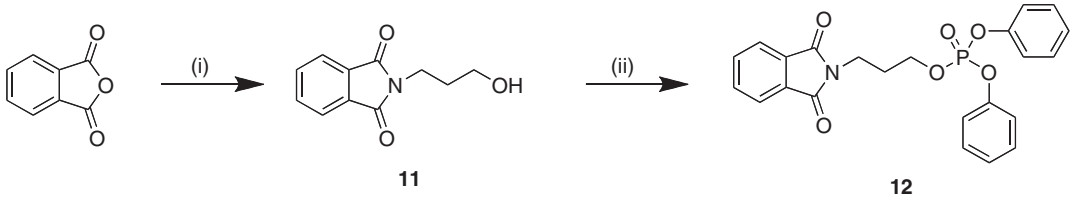
A series of norcantharidin (**2**) analogues containing a protected phosphate tail at varying distance from the tricylic scaffold were synthesized in four steps in modest yields. Biological screening of these analogues against a panel of nine human cancer cell lines: human colon carcinoma cell lines (HT29 and SW480); human breast carcinoma cell line (MCF-7); human ovarian carcinoma cell line (A2780); human lung carcinoma cell line (H460); human skin carcinoma cell line (A431); human prostate carcinoma cell line (DU145); human neuroblastoma cell line (BE2-C); human glioblastoma cell line (SJ-G2) resulted in the identification of a new series of functionalised norcantharidin analogues with enhanced broad spectrum anti-proliferative activity. Multiple analogues are at least equipotent with norcantharidin and a number displayed about a fivefold increase in cell death. The relative ease of phosphate ester hydrolysis correlates well with the observed anti-proliferative activity. Of the phosphates examined the diphenyl and bis-trichloroethyl analogues

Table 2
Anti-proliferative effects (GI_{50} values in μM) of terminal phosphate esters **8a–10f** against a panel of human cancer cell lines



Compound	X	R	HT29[a]	SW480[a]	MCF-7[b]	A2780[c]	H460[d]	A431[e]	DU145[f]	BE2-C[g]	SJ-G2[h]
8a	$-CH_2CH_2-$	$P(O)(OEt)_2$	33 ± 1.0	18 ± 0.4	28 ± 1.3	15 ± 0.6	49 ± 5.0	27 ± 1.5	21 ± 3.3	—[i]	43 ± 4.1
8b	$-(CH_2)_6-$	$P(O)(OEt)_2$	>100	>100	>100	>100	>100	>100	>100	>100	>100
8c	$-(CH_2)_3-$	$P(O)(OEt)_2$	—[i]	—[i]	—[i]	—[i]	—[i]	—[i]	—[i]	—[i]	—[i]
8d	$-(CH_2)_4-$	$P(O)(OEt)_2$	>100	>100	>100	>100	>100	>100	>100	>100	>100
8e	$-(CH_2)_5-$	$P(O)(OEt)_2$	>100	>100	>100	>100	>100	>100	>100	>100	>100
8f		$P(O)(OEt)_2$	57 ± 1.7	33 ± 3.2	50 ± 3.2	22 ± 2.9	89 ± 1.0	47 ± 2.9	36 ± 3	31 ± 4.5	60 ± 1.7
9a	$-CH_2CH_2-$	$P(O)(OPh)_2$	>100	>100	>100	>100	>100	>100	>100	>100	>100
9b	$-(CH_2)_6-$	$P(O)(OPh)_2$	5.4 ± 0	14 ± 0.33	9.1 ± 1	11 ± 0.8	25 ± 3.7	13 ± 0	7.0 ± 0.2	14 ± 0.3	14 ± 0
9c	$-(CH_2)_3-$	$P(O)(OPh)_2$	>100	>100	>100	>100	>100	>100	>100	>100	>100
9d	$-(CH_2)_4-$	$P(O)(OPh)_2$	14 ± 0.2	28 ± 1.2	19 ± 1	17 ± 1.0	64 ± 4.8	21 ± 1.9	13 ± 0.6	25 ± 3.2	17 ± 2.0
9e	$-(CH_2)_5-$	$P(O)(OPh)_2$	42 ± 3.1	50 ± 1.2	31 ± 1.3	46 ± 2.3	63 ± 1.3	45 ± 2.0	52 ± 1.0	56 ± 2.1	50 ± 2.9
9f		$P(O)(OPh)_2$	11 ± 1.1	26 ± 1.5	14 ± 0.6	24 ± 1.3	48 ± 3.1	26 ± 0.7	35 ± 1.3	28 ± 0.9	31 ± 1.0
10a	$-CH_2CH_2-$	$P(O)(OCH_2Cl_3)_2$	>100	>100	>100	>100	>100	>100	>100	>100	>100
10b	$-(CH_2)_6-$	$P(O)(OCH_2Cl_3)_2$	11 ± 0.3	10 ± 0.7	12 ± 0.9	7.7 ± 0.3	13 ± 0.3	12 ± 0.3	13 ± 0.9	9.3 ± 0.1	14 ± 0.3
10c	$-(CH_2)_3-$	$P(O)(OCH_2Cl_3)_2$	57 ± 4	72 ± 5	60 ± 6	47 ± 9	78 ± 8	80 ± 7	>100	50 ± 3	58 ± 3
10d	$-(CH_2)_4-$	$P(O)(OCH_2Cl_3)_2$	16 ± 1.5	32 ± 1.7	28 ± 0	26 ± 1.0	56 ± 1.0	31 ± 1.9	49 ± 0.3	40 ± 2	48 ± 0.3
10e	$-(CH_2)_5-$	$P(O)(OCH_2Cl_3)_2$	15 ± 0.9	17 ± 0.3	16 ± 0.2	16 ± 0.9	29 ± 0.7	18 ± 1.0	26 ± 1	22 ± 0.7	19 ± 1.7
10f		$P(O)(OCH_2Cl_3)_2$	56 ± 9.2	69 ± 8.6	54 ± 12.0	39 ± 6.4	>100	48 ± 4.6	69 ± 8.1	51 ± 7.6	45 ± 5.8

[A] Colon carcinoma.
[B] Breast carcinoma.
[C] Ovarian carcinoma.
[D] Lung carcinoma.
[E] Skin carcinoma.
[F] Prostate carcinoma.
[G] Neuronal carcinoma.
[H] Brain carcinoma.
[i] Not determined.



Scheme 2. Reagents and conditions: (i) $H_2N-(CH_2)_4-OH$, toluene, Δ, MW, 20 min; (ii) $ClP(O)(OPh)_2$, *n*-Bu_2O, Et_3N, rt.

displayed the highest level of cell death. In general the more labile phosphate esters six carbons from the norcantharidin scaffold were the most active. The lack of activity with the pthalimido analogue (**12**) demonstrates the pivotal nature of the norcantharidin skeleton to the observed anti-proliferative activity.

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). Electrospray Mass spectra were recorded on a Shimadzu LCMS 2010 EV using a mobile phase of 1:1 acetonitrile/H_2O with 0.1% formic acid.

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).

Table 3
Anti-proliferative comparison (GI_{50} values in μM) of the norcantharidin ester **9d** versus an analogous phthlamido ester against a panel of human cancer cell lines

Compound		HT29[a]	SW480[a]	MCF-7[b]	A2780[c]	H460[d]	A431[e]	DU145[f]	BE2-C[g]	SJ-G2[h]
9d	**9d**	14 ± 0.2	28 ± 1.2	19 ± 1	17 ± 1.0	64 ± 4.8	21 ± 1.9	13 ± 0.6	25 ± 3.2	17 ± 2.0
12	**12**	>100	>100	>100	>100	>100	>100	>100	>100	>100

[A] Colon carcinoma.
[B] Breast carcinoma.
[C] Ovarian carcinoma.
[D] Lung carcinoma.
[E] Skin carcinoma.
[F] Prostate carcinoma.
[G] Neuronal carcinoma.
[H] Brain carcinoma.

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 μL 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-dimethyltiazol-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.[43]

4.2. General synthesis

4.2.1. General Method A

4.2.1.1. Formation of the N-substituted norcantharidin analogues (7a–f). *4.2.1.1.1. Illustrative synthesis of 4-aza-4-(2-hydroxyethyl)-10-oxatricyclo[5.2.1.0]decane-3,5-dione (**7a**)[43].* To norcantharidin (**2**) (168 mg, 1 mmol) in toluene (10 mL) was added 2-aminoethanol (67 mg, 1.1 mmol, 1.1 equiv) and triethylamine (202 mg, 2.0 equiv). The resultant solution was heated at reflux for 18 h and evaporated to dryness. The desired compound (**7a**) was obtained via flash column chromatography using EtOAc as the solvent as a white solid (73%), mp 164–166 °C.

^{1}H NMR ($CDCl_3$): δ 4.86, dd, *J* = 2.1 Hz, 2H; 3.70, t, *J* = 4.8 Hz, 2H; 3.64, q, *J* = 3.6 Hz, 2H; 3.01 br s, OH; 2.89, s, 2H; 1.84, m, 2H; 1.61, m, 2H.

^{13}C NMR ($CDCl_3$): 177.1, 78.6, 59.5, 49.4, 41.3, 27.9.

MS(ES) *m/z*: 212 (M+H).

4.2.2. General Method B

4.2.2.1. Formation of the phosphate esters (8a–10f). *4.2.2.1.1. Illustrative synthesis of 4-aza-4-(2-{diethylphosphate}ethyl)-10-oxatricyclo[5.2.1.0]decane-3,5-dione (**8a**).* To a solution of 4-aza-4-(2-hydroxyethyl)-10-oxatricyclo[5.2.1.0]decane-3,5-dione (**7a**) (211 mg, 1 mmol) in anhydrous CH_2Cl_2 (10 mL) was added *n*-Bu_2SnO (50 mg, 0.2 mmol, 0.2 equiv) and the resulting mixture stirred at room temperature for 30 min under a N_2 atmosphere. Next diethyl chlorophosphate (172.5 mg, 1 mmol, 1.0 equiv) and triethylamine (500 μL, 3.6 mmol, 3.6 equiv) was then added and the mixture stirred for 18 h. The reaction was then quenched with H_2O (10 mL). The organic layer was then separated, dried ($MgSO_4$) and evaporated to dryness. The crude product was then purified via flash column chromatography using 4:1 EtOAc/hexanes as the solvent to yield the title compound (**8a**) as a white solid (70%), mp 172–174 °C.

^{1}H NMR ($CDCl_3$): δ 4.85, dd, *J* = 3.11, 2.23 Hz, 2H; 4.12, m, 2H; 4.07, m, 4H; 3.75, t, *J* = 5.36 Hz, 2H; 2.92, s, 2H; 1.85, m, 2H; 1.60, m, 2H; 1.31, t, *J* = 7.1 Hz, 6H.

^{13}C NMR ($CDCl_3$): δ 176.4, 78.3, 63.5, 62.5, 49.6, 38.5, 28.0, 15.5 ppm.

MS(EI) *m/z*: 347.

HR-MS (ESI, M+H) *m/z*: calcd for $C_{14}H_{23}NO_7P$: 348.12121; found 348.12122.

*4.2.2.1.2. 4-Aza-4-(6-hydroxyhexyl)-10-oxatricyclo[5.2.1.0]decane-3,5-dione (**7b**).* Synthesised as described in *Method A* from norcantharidin (**2**) and 6-aminohexanol to yield the title compound (**7b**) as a colourless oil (45%).

^{1}H NMR ($CDCl_3$): δ 4.68, dist t, 2H; 3.40, t, *J* = 6.5 Hz, 2H; 3.26, t, *J* = 7.2 Hz, 2H; 3.08, br s, OH; 2.73, s, 2H; 1.69, m, 2H; 1.45, m, 2H; 1.38, m, 4H; 1.15, m, 4H.

^{13}C NMR ($CDCl_3$): δ 177.2, 78.7, 61.9, 49.5, 38.5, 32.0, 28.2, 27.0, 25.9, 24.8 ppm.

MS(ES) *m/z*: 268 (M+H).

HR-MS (ESI, M+H) *m/z*: calcd for $C_{14}H_{22}NO_4$: 268.15488; found 268.15496.

*4.2.2.1.3. 4-Aza-4-(3-hydroxypropyl)-10-oxatricyclo[5.2.1.0]decane-3,5-dione (**7c**).* Synthesised as described in *Method A* from norcantharidin (**2**) and 3-aminopropanol to yield the title compound (**7c**) as a colourless oil (80%).

^{1}H NMR ($CDCl_3$): δ 4.83, dist t, 2H; 3.62, t, *J* = 6.5 Hz, 2H; 3.48, q, *J* = 6.5 Hz, 2H; 2.73, s, 2H; 1.82, m, 2H; 1.73, m, 2H; 1.65, m, 2H.

^{13}C NMR ($CDCl_3$): δ 177.8, 79.0, 58.5, 49.8, 35.2, 30.2, 28.4 ppm.

MS(ES) *m/z*: 226 (M+H).

HR-MS (ESI, M+H) *m/z*: calcd for $C_{11}H_{16}NO_4$: 226.10793; found 226.10797.

*4.2.2.1.4. 4-Aza-4-(4-hydroxybutyl)-10-oxatricyclo[5.2.1.0]decane-3,5-dione (**7d**).* Synthesised as described in *Method A* from norcantharidin (**2**) and 4-aminobutanol to yield the title compound (**7d**) as a pale yellow oil (61%).

^{1}H NMR ($CDCl_3$): δ 4.77, dist t, 2H; 3.54, t, J = 6.3 Hz, 2H; 3.42, t, J = 6.9 Hz, 2H; 2.80, s, 2H; 2.43, br s, OH; 1.76, m, 2H; 1.55, m, 4H; 1.45, m, 2H.

^{13}C NMR ($CDCl_3$): δ 177.2, 78.9, 61.8, 49.7, 38.6, 29.4, 28.4, 23.9 ppm.

MS(ES) *m/z*: 240 (M+H).

HR-MS (ESI, M+H) *m/z*: calcd for $C_{12}H_{18}NO_4$: 240.12358; found 240.12359.

*4.2.2.1.5. 4-Aza-4-(5-hydroxypentyl)-10-oxatricyclo[5.2.1.0]decane-3,5-dione (**7e**).* Synthesised as described in *Method A* from norcantharidin (**2**) and 5-aminopentanol to yield the title compound (**7e**) as a white solid (70%). mp 96–98 °C.

^{1}H NMR ($CDCl_3$): δ 4.86, br s, OH; 4.66, s, 2H; 3.54, t, J = 6.5 Hz, 2H; 3.46, t, J = 7.0 Hz, 2H; 3.14, s, 2H; 1.79, m, 2H; 1.72, m, 2H; 1.53, m, 4H; 1.25, m, 2H.

^{13}C NMR ($CDCl_3$): δ 180.3, 79.1, 61.1, 49.3, 38.4, 30.3, 27.4, 26.0, 21.8 ppm.

MS(ES) *m/z*: 254.1 (M+H).

HR-MS (ESI, M+H) *m/z*: calcd for $C_{13}H_{20}NO_4$: 254.13923; found 254.13919.

*4.2.2.1.6. 4-Aza-4-(2-hydroxycyclohexyll)-10-oxatricyclo[5.2.1.0]decane-3,5-dione (**7f**).* Synthesised as described in *Method A* from norcantharidin (**2**) and 2-aminocyclohexanol to yield the title compound (**7f**) as a white solid (70%), mp 172–174 °C.

^{1}H NMR ($CDCl_3$): δ 4.81, dist t, 2H; 3.91, m, 1H; 3.65, m, 1H; 3.06, br s, OH; 2.76, s, 2H; 2.19, m, 2H; 1.99, m, 2H; 1.79, m, 2H; 1.55, m, 6H.

^{13}C NMR ($CDCl_3$): δ 177.3, 79.2, 69.5, 50.8, 49.6, 45.9, 34.6, 28.6, 26.4, 8.6 ppm.

MS(ES) *m/z*: 266 (M+H).

HR-MS (ESI, M+H) *m/z*: calcd for $C_{14}H_{20}NO_4$: 266.13923; found 266.13940.

*4.2.2.1.7. 4-Aza-4-(6-{diethylphosphate}hexyl)-10-oxatricyclo[5.2.1.0]decane-3,5-dione (**8b**).* Synthesised as described in *Method B* from 4-aza-4-(6-hydroxyhexyl)-10-oxatricyclo[5.2.1.0]decane-3,5-dione (**7e**) and diethyl chlorophosphate to yield the title compound (**8e**) as a clear oil (63%).

^{1}H NMR ($CDCl_3$): δ 4.85, dist t, 2H; 4.10, app quintet, J = 7.1 Hz, 4H; 4.04, app quintet, J = 6.8 Hz, 2H; 3.45, t, J = 7.2 Hz, 2H; 2.89, s, 2H; 1.83, m, 2H; 1.63, m, 4H; 1.43, m, 2H; 1.36, m, 4H; 1.34, t, J = 7.1 Hz, 6H.

^{13}C NMR ($CDCl_3$): δ 177.0, 78.7, 67.1, 63.3, 49.5, 38.5, 29.7, 28.3, 27.0, 25.8, 24.6, 15.8 ppm.

MS(EI) *m/z*: 402.

HR-MS (ESI, M+H) *m/z*: calcd for $C_{18}H_{31}NO_7P$: 404.18381; found: 404.18389.

*4.2.2.1.8. 4-Aza-4-(4-{diethylphosphate}butyl)-10-oxatricyclo[5.2.1.0]decane-3,5-dione (**8d**).* Synthesised as described in *Method B* from 4-aza-4-(4-hydroxybutyl)-10-oxatricyclo[5.2.1.0]decane-3,5-dione (**7d**) and diethyl chlorophosphate to yield the title compound (**8d**) as a pale yellow oil (37%).

^{1}H NMR ($CDCl_3$): δ 4.80, br s, 2H; 4.05, q, J = 7.2 Hz, 4H; 3.98, m, 2H; 3.44, dist t, 2H; 2.82, s, 2H; 1.78, m, 2H; 1.56, m, 6H; 1.28, t, J = 7.2 Hz, 6H.

^{13}C NMR ($CDCl_3$): δ 177.1, 79.0, 66.7, 63.7, 49.8, 38.3, 28.5, 27.3, 23.7, 16.1 ppm.

MS(EI) *m/z*: 375.

HR-MS (ESI, M+H) *m/z*: calcd for $C_{16}H_{27}NO_7P$: 376.15251; found 376.15255.

*4.2.2.1.9. 4-Aza-4-(5-{diethylphosphate}pentyl)-10-oxatricyclo[5.2.1.0]decane-3,5-dione (**8e**).* Synthesised as described in *Method B* from 4-aza-4-(5-hydroxypentyl)-10-oxatricyclo[5.2.1.0]decane-3,5-dione (**7e**) and diethyl chlorophosphate to yield the title compound (**8e**) as a clear oil (60%).

^{1}H NMR ($CDCl_3$): δ 4.81, br s, 2H; 4.05, m, 4H; 3.95, m, 2H; 3.41, t, J = 7.2 Hz, 2H; 2.82, s, 2H; 1.78, m, 2H; 1.56, m, 8H; 1.30, t, J = 7.2 Hz, 6H.

^{13}C NMR ($CDCl_3$): δ 177.1, 79.0, 66.1, 63.6, 49.8, 38.7, 29.6, 28.5, 27.0, 22.5, 16.1 ppm.

MS(ES) *m/z*: 390 (M+H).

HR-MS (ESI, M+H) *m/z*: calcd for $C_{17}H_{29}NO_7P$: 390.16816; found 390.16823.

*4.2.2.1.10. 4-Aza-4-(2-{diethylphosphate}cyclohexyl)-10-oxatricyclo[5.2.1.0]decane-3,5-dione (**8f**).* Synthesised as described in *Method B* from 4-aza-4-(2-hydroxycyclohexyll)-10-oxatricyclo[5.2.1.0]decane-3,5-dione (**7f**) and diethyl chlorophosphate to yield the title compound (**8f**) as an off white semi solid (45%).

^{1}H NMR ($CDCl_3$): δ 4.72, dist t, 2H; 4.15, m, 4H; 3.83, m, 1H; 3.56, m, 1H; 2.70, s, 2H; 2.18, m, 2H; 1.94, m, 2H; 1.72, m, 2H; 1.49, m, 4H; 1.28, t, J = 7.1 Hz, 6H; 12.3, m, 2H.

^{13}C NMR (75 MHz, $CDCl_3$): δ 177.2, 79.0, 69.0, 65.1, 50.6, 49.3, 34.3, 28.4, 26.2, 15.8 ppm.

MS(ES) *m/z*: 402 (M+H).

HR-MS (ESI, M+H) *m/z*: calcd for $C_{18}H_{29}NO_7P$: 402.16816; found 402.16819.

*4.2.2.1.11. 4-Aza-4-(2-{diphenylphosphate}ethyl)-10-oxatricyclo[5.2.1.0]decane-3,5-dione (**9a**).* Synthesised as described in *Method B* from 4-aza-4-(2-hydroxyethyl)-10-oxatricyclo[5.2.1.0]decane-3,5-dione (**7a**) and diphenyl chlorophosphate to yield the title compound (**9a**) as a colourless oil (54%).

^{1}H NMR ($CDCl_3$: δ 7.28, m, 4H; 7.11, m, 6H; 4.78, dd, J = 2.7 Hz, 2.3 Hz, 2H; 4.33, dt, J = 8.7, 5.4, 5.5 Hz, 2H; 3.75, t, J = 5.4 Hz, 2H; 2.76, s, 2H; 1.73, m, 2H; 1.47 (m, 2H).

^{13}C NMR: δ 176.4, 149.8, 129.3, 124.9, 119.5, 78.3, 63.9, 49.4, 38.2, 29.7 ppm.

MS(ES) *m/z*: 444 (M+H).

HR-MS (ESI, M+H) *m/z*: calcd for $C_{22}H_{23}NO_7P$: 444.12121; found 444.12119.

*4.2.2.1.12. 4-Aza-4-(6-{diphenylphosphate}hexyl)-10-oxatricyclo[5.2.1.0]decane-3,5-dione (**9b**).* Synthesised as described in *Method B* from 4-aza-4-(6-hydroxyhexyll)-10-oxatricyclo[5.2.1.0]decane-3,5-dione (**7b**) and diphenyl chlorophosphite to yield the title compound (**9b**) as a colourless oil (75%).

^{1}H NMR ($CDCl_3$): δ 7.28, m, 5H; 7.15, m, 5H; 4.80, dist t, 2H; 4.20, ABq, J = 6.7 Hz, 1H; 4.16, ABq, J = 6.7 Hz, 1H; 3.39, d, J = 7.2 Hz, 2H; 2.79, s, 2H; 1.79, m, 2H; 1.55, m, 6H; 1.23, m, 4H.

^{13}C NMR ($CDCl_3$): δ 177.1, 150.6, 129.7, 125.2, 120.0, 79.0, 69.1, 49.8, 38.7, 29.8, 28.5, 27.3, 26.0, 24.8 ppm.

MS(ES) *m/z*: 500 (M+H).

HR-MS (ESI, M+H) *m/z*: calcd for $C_{26}H_{31}NO_7P$: 500.18381; found 500.18389.

*4.2.2.1.13. 4-Aza-4-(3-{diphenylphosphate}propyl)-10-oxatricyclo[5.2.1.0]decane-3,5-dione (**9c**).* Synthesised as described in *Method B* from 4-aza-4-(3-hydroxypropyl)-10-oxatricyclo[5.2.1.0]decane-3,5-dione (**7c**) and diphenyl chlorophosphate to yield the title compound (**9c**) as a colourless oil (83%).

^{1}H NMR ($CDCl_3$): δ 7.28, m, 3H; 7.16, m, 6H; 4.79 (dd, J = 3.1, 2.2 Hz, 2H), 4.20, dd, J = 13.6, 6.2 Hz, 2H; 3.53, t, J = 7.0 Hz, 2H; 2.79, s, 2H; 1.91, m, 2H; 1.76, m, 2H; 1.50, m, 2H.

^{13}C NMR ($CDCl_3$): δ 176.8, 150.2, 129.6, 125.2, 117.8, 79.8, 66.5, 49.6, 35.3, 28.3 ppm.

MS(ES) *m/z*: 458 (M+H).

HR-MS (ESI, M+H) *m/z*: calcd for $C_{23}H_{25}NO_7P$: 458.13686; found 458.13690.

*4.2.2.1.14. 4-Aza-4-(4-{diphenylphosphate}butyl)-10-oxatricyclo[5.2.1.0]decane-3,5-dione (**9d**).* Synthesised as described in *Method B* from 4-aza-4-(4-hydroxybutyl)-10-oxatricyclo[5.2.1.0]

decane-3,5-dione (**7d**) and diphenyl chlorophosphate to yield the title compound (**9d**) as a colourless oil (70%).

^{1}H NMR ($CDCl_3$): δ 7.30, m, 5H; 7.17, m, 5H; 4.80, dist t, 2H; 4.20, dist t, 2H; 3.44, dist t, 2H; 2.80, s, 2H; 1.76, m, 2H; 1.59, m, 6H.

^{13}C NMR ($CDCl_3$): δ 177.0, 150.5, 129.7, 125.2, 120.1, 79.0, 68.4, 49.9, 38.1, 28.5, 27.2, 23.6 ppm.

MS(ES) *m/z*: 472 (M+H).

HR-MS (ESI, M+H) *m/z*: calcd for $C_{24}H_{27}NO_7P$: 472.15251; 472.15262.

*4.2.2.1.15. 4-Aza-4-(5-{diphenylphosphate}pentyl)-10-oxatricyclo[5.2.1.0]decane-3,5-dione (**9e**).* Synthesised as described in *Method B* from 4-aza-4-(5-hydroxypentyl)-10-oxatricyclo[5.2.1.0] decane-3,5-dione (**7e**) and diphenyl chlorophosphite to yield the title compound (**9e**) as a pale yellow oil (72%).

^{1}H NMR ($CDCl_3$): δ 7.29, m, 5H; 7.14, m, 5H; 4.79, dist t, 2H; 4.19, ABq, J = 6.5 Hz, 1H; 4.15, ABq, J = 6.5 Hz, 1H; 3.38, d, J = 7.2 Hz, 2H; 2.78, s, 2H; 1.76, m, 2H; 1.66, m, 2H, 1.53, m, 4H; 1.28, m, 2H.

^{13}C NMR ($CDCl_3$): δ 177.0, 150.5, 129.7, 125.2, 120.0, 79.0, 68.9, 49.8, 38.5, 29.5, 28.5, 26.9, 22.4 ppm.

MS(ES) *m/z*: 486 (M+H).

HR-MS (ESI, M+H) *m/z*: calcd for $C_{25}H_{29}NO_7P$: 486.16816; found 486.16818.

*4.2.2.1.16. 4-Aza-4-(2-{diphenylphosphate}cyclohexyl)-10-oxatricyclo[5.2.1.0]decane-3,5-dione (**9f**).* Synthesised as described in *Method B* from 4-aza-4-(2-hydroxycyclohexyll)-10-oxatricyclo[5.2.1.0]decane-3,5-dione (**7f**) and diphenyl chlorophosphate to yield the title compound (**9f**) as a white solid (70%), mp: 110–112 °C.

^{1}H NMR ($CDCl_3$): δ 7.30, m, 5H; 7.17, m, 5H; 4.79, dist t, 2H; 4.53, m, 1H; 3.90, m, 1H; 2.74, s, 2H; 2.17, m, 4H; 1.78, m, 2H; 1.60, m, 6H.

^{13}C NMR ($CDCl_3$): δ 177.1, 150.7, 129.7, 125.2, 120.1, 79.2, 78.0, 50.0, 49.5, 32.4, 28.5, 26.1 ppm.

MS(ES) *m/z*: 498 (M+H).

HR-MS (ESI, M+H) *m/z*: calcd for $C_{26}H_{29}NO_7P$: 498.16816; found 498.16820.

*4.2.2.1.17. 4-Aza-4-(2-{bis(2,2,2-trichloroethyl)phosphate}ethyl)-10-oxatricyclo[5.2.1.0]decane-3,5-dione (**10a**).* Synthesised as described in *Method B* from 4-aza-4-(2-hydroxyethyl)-10-oxatricyclo[5.2.1.0]decane-3,5-dione (**7a**) and bis(2,2,2-trichloroethyl) chlorophosphate to yield the title compound (**10a**) as a colourless oil (60%).

^{1}H NMR ($CDCl_3$): δ 5.25, s, 2H; 4.80, dd, J = 3.0, 2.2 Hz, 2H, dd; 4.57, ABq, J = 9.4, 4.7 Hz, 4H; 4.28, ABq, J = 8.8, 5.1 Hz, 2H; 3.75, t, J = 4.8, 4.8 Hz, 2H; 1.75, m, 2H; 1.55, m, 2H.

^{13}C NMR ($CDCl_3$): 176.4, 78.4, 64.3, 59.7, 49.5, 38.2, 27.9, 13.6.

MS(ES) *m/z*: 551 (M+H).

HR-MS (ESI, M+H) *m/z*: calcd for $C_{14}H_{17}Cl_6NO_7P$: 551.88738; found 551.88729.

*4.2.2.1.18. 4-Aza-4-(6-{bis(2,2,2-trichloroethyl)phosphate}hexyl)-10-oxatricyclo[5.2.1.0]decane-3,5-dione (**10b**).* Synthesised as described in *Method B* from 4-aza-4-(6-hydroxyhexyll)-10-oxatricyclo[5.2.1.0]decane-3,5-dione (**7b**) and bis(2,2,2-trichloroethyl)chlorophosphate to yield the title compound (**10b**) as a pale yellow oil (61%).

^{1}H NMR ($CDCl_3$): δ 4.84, br s, 2H; 4.63, d, J = 6.3 Hz, 4H; 4.24, ABq, J = 6.7 Hz, 1H; 4.19, ABq, J = 6.7 Hz, 1H; 3.46, d, J = 7.1 Hz, 2H; 2.86, s, 2H; 1.83, m, 2H; 1.74, m, 2H; 1.56, m, 4H; 1.42, m, 2H; 1.30, m, 2H.

^{13}C NMR ($CDCl_3$): δ 177.1, 94.6, 78.9, 69.3, 49.7, 38.6, 29.7, 28.5, 27.1, 25.8, 24.6 ppm.

MS(ES) *m/z*: 610 (M+H).

HR-MS (ESI, M+H) *m/z*: calcd for $C_{18}H_{25}Cl_6NO_7P$: 607.94998; found 607.95003.

*4.2.2.1.19. 4-Aza-4-(3-{bis(2,2,2-trichloroethyl)phosphate}propyl)-10-oxatricyclo[5.2.1.0]decane-3,5-dione (**10c**).* Synthesised as described in *Method B* from 4-aza-4-(3-hydroxypropyl)-10-oxatricyclo[5.2.1.0]decane-3,5-dione (**7c**) and bis(2,2,2-trichloroethyl)chlorophosphate to yield the title compound (**10c**) as a colourless oil (71%).

^{1}H NMR ($CDCl_3$): δ 4.83, dd, J = 3.1, 2.2 Hz, 2H; 4.62, dABq, J = 11.2 Hz, 6.6 Hz, 4.14, dt, J = 6.5 Hz, 6.5 Hz, 2H; 3.58, t, J = 6.7 Hz, 2H; 2.84, s, 2H; 1.97, m, 2H; 1.78, m, 2H; 1.57, m, 2H.

^{13}C NMR ($CDCl_3$): δ 176.5, 94.1, 78.5, 76.5, 65.8, 49.3, 34.7, 28.7, 27.4 ppm.

MS(ES) *m/z*: 566 (M+H).

HR-MS (ESI, M+H) *m/z*: calcd for $C_{15}H_{19}Cl_6NO_7P$: 565.90303; found 565.90309.

*4.2.2.1.20. 4-Aza-4-(4-{bis(2,2,2-trichloroethyl)phosphate}butyl)-10-oxatricyclo[5.2.1.0]decane-3,5-dione (**10d**).* Synthesised as described in *Method B* from 4-aza-4-(4-hydroxybutyl)-10-oxatricyclo[5.2.1.0]decane-3,5-dione (**7d**) and bis(2,2,2-trichloroethyl) chlorophosphate to yield the title compound (**10d**) as a pale yellow oil (76%).

^{1}H NMR ($CDCl_3$): δ 4.77, dist t, 2H; 4.57, d, J = 6.6 Hz, 2H; 4.56, d, J = 6.6 Hz, 2H; 4.15, dist t, 2H; 3.44, dist t, 2H; 2.81, s, 2H; 1.77, m, 2H; 1.64, m, 4H; 1.56, m, 2H.

^{13}C NMR ($CDCl_3$): δ 176.9, 94.6, 94.5, 78.8, 68.4, 49.6, 37.8, 28.3, 26.8, 23.3 ppm.

MS(ES) *m/z*: 584 (M+H).

HR-MS (ESI, M+H) *m/z*: calcd for $C_{16}H_{21}Cl_6NO_7P$: 579.91868; found 579.91872.

*4.2.2.1.21. 4-Aza-4-(5-{bis(2,2,2-trichloroethyl)phosphate}pentyl)-10-oxatricyclo[5.2.1.0]decane-3,5-dione (**10e**).* Synthesised as described in *Method B* from 4-aza-4-(5-hydroxypentyl)-10-oxatricyclo[5.2.1.0]decane-3,5-dione (**7e**) and bis(2,2,2-trichloroethyl) chlorophosphate to yield the title compound (**10e**) as a white solid (52%), mp: 82–84 °C.

^{1}H NMR ($CDCl_3$): δ 4.79, dist t, 2H; 4.57, d, J = 6.5 Hz, 2H; 4.56, d, J = 6.5 Hz, 2H; 4.17, ABq, J = 7.1 Hz, 1H; 4.12, ABq, J = 7.1 Hz, 1H; 3.41, d, J = 7.1 Hz, 2H; 2.79, s, 2H; 1.77, m, 2H; 1.70, m, 2H; 1.55, m, 4H; 1.32, m, 2H.

^{13}C NMR ($CDCl_3$): δ 177.0, 94.7, 94.6, 78.9, 69.1, 49.8, 38.4, 29.4, 28.5, 26.8, 22.3 ppm.

MS(ES) *m/z*: 596 (M+H).

HR-MS (ESI, M+H) *m/z*: calcd for $C_{17}H_{23}Cl_6NO_7P$: 593.93433; found 593.93441.

*4.2.2.1.22. 4-Aza-4-(2-{bis(2,2,2-trichloroethyl)phosphate}cyclohexyl)-10-oxatricyclo[5.2.1.0]decane-3,5-dione (**10f**).* Synthesised as described in *Method B* from 4-aza-4-(2-hydroxycyclohexyll)-10-oxatricyclo[5.2.1.0]decane-3,5-dione (**7f**) and bis(2,2,2-trichloroethyl)chlorophosphate to yield the title compound (**10f**) as a white solid (58%), mp: 181–184 °C.

^{1}H NMR ($CDCl_3$): δ 4.83, dist t, 2H; 4.62, d, J = 6.3 Hz, 4H; 4.53, m, 1H; 3.95, m, 1H; 2.79, s, 2H; 2.26, m, 4H; 1.83, m, 2H; 1.60, m, 6H.

^{13}C NMR ($CDCl_3$): δ 177.1, 94.5, 79.2, 78.4, 49.9, 49.6, 32.5, 28.6, 26.1 ppm.

MS(ES) *m/z*: 671 (M+HCOOH+H_2O).

HR-MS (ESI, M+H) *m/z*: calcd for $C_{18}H_{22}Cl_6NO_7P$: 604.92650; found 604.92657.

4.2.2.2. *N*-(4-Hydroxybutyl)phthalimide (11). A solution of phthalic anhydride (50 mg, 3.4 mmol) and 4-aminobutanol (300 mg, 3.4 mmol) in toluene (5 mL) was heated under microwave irradiation (200 W, 100 °C) for 20 min. After cooling the solvent was removed in vacuo and the resultant residue dissolved in ethyl acetate (20 mL). After washing with water (2 × 20 mL) the organic layer was dried ($MgSO_4$), filtered and evaporated in vacuo to yield a pale yellow oil that solidified on standing (0.68 g, 92%), mp: 52–54 °C.

^{1}H NMR ($CDCl_3$): δ 7.57, m, 1H; 7.51, m, 2H; 3.48, m, 2H; 3.38, s, OH; 1.58, m, 2H; 1.43, m, 2H.

^{13}C NMR ($CDCl_3$): δ 167.7, 133.2, 131.2, 122.4, 61.0, 37.0, 29.1, 24.4 ppm.

MS(ES) *m/z*: 220 (M+H).

HR-MS (ESI, M+H) *m/z*: calcd for $C_{12}H_{13}NO_3$: 219.08954; found: 219.08957.

4.2.2.3. 4-(1,3-Dioxoisoindolin-2-yl)butyl diphenylphosphate (12). Synthesised as described in *Method B* from *N*-(4-hydroxybutyl)phthalimide (**11**) and diphenyl chlorophosphate to yield the title compound (**13**) as a clear oil (56%).

^{1}H NMR ($CDCl_3$): δ 7.82, m, 2H; 7.68, m, 2H; 7.32, m, 5H; 7.16, m, 5H; 4.27, m, 2H; 3.67, m, 2H; 1.74, m, 4H.

^{13}C NMR ($CDCl_3$): δ 168.1, 150.4, 133.8, 131.9, 129.6, 125.1, 123.0, 119.9, 68.4, 37.0, 27.4, 24.5 ppm.

MS(ES) *m/z*: 452 (M+H).

HR-MS (ESI, M+H) *m/z*: calcd for $C_{24}H_{22}NO_6P$: 451.11847; found 451.11849.

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