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Original article

Synthesis and anticancer activity of a series of norcantharidin analogues

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ABSTRACT

Cantharidin (**1**) and norcantharidin (**2**) display high levels of anticancer activity against a broad range of tumour cell lines. Synthetic manipulation of norcantharidin yields (3*S*,3a*R*,4*S*,7*R*,7a*S*)-3-hydroxyhexahydro-4,7-epoxyisobenzofuran-1(3*H*)-one (**3**), which also displays a high level of anticancer activity against tumour cells but interestingly, shows selectivity towards HT29 (colon; $GI_{50} = 14$ μM) and SJ-G2 (glioblastoma; $GI_{50} = 15$ μM) cell lines. Substitution at the hydroxyl group of the cyclic lactone within (**3**) produces a diasteromeric pair of products that have no difference in cytotoxicity over the cell lines tested. Incorporation of an isopropyl tail at this position (**16**) produced the most promising compound of this series to date, with strong selectivity towards HT29 (colon; $GI_{50} = 19$ μM) and SJ-G2 (glioblastoma; $GI_{50} = 21$ μM) cell lines but completely void of any activity against the remaining tumour cell lines ($GI_{50} > 100$ μM), as per the parent molecule. We also discovered that the introduction of a terminal phosphate moiety (**28**) at the same position produced a different trend in cytotoxicity with strong activity in BE2-C (neuroblastoma; $GI_{50} = 9$ μM) cells; suggestive of an alternate mode of action.

1. Introduction

Cantharidin, *exo*,*exo*-bicyclo[2.2.1]heptane 2,3-dicarboxylic acid anhydride (**1**) is found in approximately 1500 species of Mylabris (blister beetles) (Fig. 1). The dried body of blister beetles can comprise up to 6% by weight of cantharidin. Upon contact with the skin, cantharidin induces rapid blistering, giving rise to their common name, blister beetles. For the past 2000 years cantharidin in the form of Mylabris has been used in Chinese traditional medicine. Cantharidin has been used for the treatment of a number of conditions, but arguably the most important is its reported use as a treatment of hepatoma and oesophageal carcinoma with the first recorded incidence of this usage in 1264. In Western society cantharidin is known as the active ingredient of the purported aphrodisiac "Spanish fly" and used topically for the treatment of warts. Generally, however Western medicine has decreed cantharidin too toxic for internal use due to the induction of nephrotoxicity. Interestingly cantharidin does not induce myleosuppression, a toxicity commonly seen with most anticancer chemotherapies. Such toxicity is dose limiting and significantly reduces the efficacy of chemotherapy treatment [1–4].

Norcantharidin (**2**), the demethylated analogue of cantharidin also possesses anticancer activity and stimulates the bone marrow, however, the nephrotoxicity associated with cantharidin treatment is absent (Fig. 1) [1,5]. Norcantharidin is active *in vitro* against several tumour cell lines including cervical, hepatoma, ovarian, laryngocarcinoma, colon, osteocarcinoma, and leukaemia cell lines [1,5,6]. However, our own studies have shown norcantharidin to be approximately 10 fold less cytotoxic than cantharidin in many of these cell lines. Norcantharidin has also been used *in vivo* in the treatment of primary hepatoma, oesophageal, gastric and cardiac carcinomas [1].

The anticancer activity of cantharidin (**1**) and norcantharidin (**2**) is thought to come from the inhibition of protein phosphatase 1 (PP1) and protein phosphatase 2A (PP2A), two phosphatases that are known to be involved in many different cellular processes, including cell cycle progression and control of mitotic events [2,7–10].

While there are many other naturally derived compounds known to inhibit PP1 and PP2A, fostriecin (**4**) is most important as it displays broad spectrum anticancer activity and has undergone phase I clinical trial assessment. These trials however, produced poor results because of its susceptibility to oxidative degradation resulting in a short plasma half life of 30 min [8,11–14].

In an effort to mimic some of these desired attributes shown by fostriecin, synthetic modification of norcantharidin to yield (3*S*,3a*R*,4*S*,7*R*,7a*S*)-3-hydroxyhexahydro-4,7-epoxyisobenzofuran-1(3*H*)-one (Novo-6; **3**), a cyclic lactone that shares some structural similarity with fostriecin was examined (Fig. 2). The introduction of a cyclic lactone moiety adversely affects the ability of this new lead,

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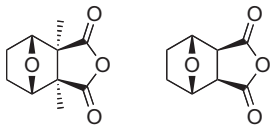
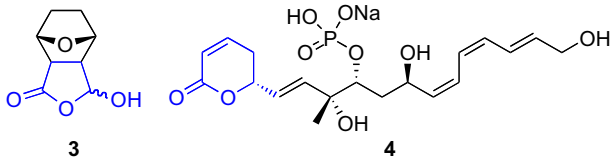


1 2

Fig. 1. Chemical structure of cantharidin (**1**) and norcantharidin (**2**).



3 4

Fig. 2. Chemical structure of (3*S*,3a*R*,4*S*,7*R*,7a*S*)-3-hydroxyhexahydro-4,7-epoxyisobenzofuran-1(3*H*)-one (**3**) and fostriecin (**4**).

3, to ring open to the dicarboxylic acid rendering it PP1 and PP2A inactive. This is in keeping with our and Bertini et al. reports on the importance of anhydride ring opening in relation to the PP1 and PP2A inhibition by the (nor)cantharidin series of compounds [15,16]. This new lead compound is also applicable for further chain extension, in an effort to produce more stable cytotoxic derivatives based on fostriecin.

Previous efforts using the norcantharidin backbone in producing cytotoxic compounds has had limited success with few exceptions [6,17–28]. Our initial screening of **3** showed a surprising degree of colon cancer cell line (HT29) selectivity [6,29], we now show that (**3**) is also potent in glioblastoma cells (SJ-G2) (Table 1).

We thus set about examining the effect of varying the side chain and the cytotoxicity of this class of norcantharidin analogues [6,17].

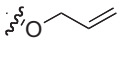
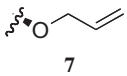
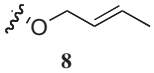
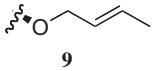
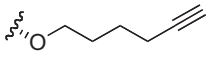
2. Chemistry

The synthesis of (3*S*,3a*R*,4*S*,7*R*,7a*S*)-3-hydroxyhexahydro-4,7-epoxyisobenzofuran-1(3*H*)-one (**3**) from its precursor (3a*R*,4*S*,7-*R*,7a*S*)-3a,4,7,7a-tetrahydro-4,7-epoxyisobenzofuran-1,3-dione (**5**) proceeded as a one step reduction in wet ethanol in the presence of catalytic Pd/C under 4 bar of H_2 as shown in Scheme 1. Subsequent filtration through a celite bed and recrystallisation afforded (3*S*,3a*R*,4*S*,7*R*,7a*S*)-3-hydroxyhexahydro-4,7-epoxyisobenzofuran-1(3*H*)-one (**3**) in moderate to good yields (40–65%). While this is a robust method for the synthesis of **3**, the isolated yield was variable, a consequence of changes in the water content of the hydrogenation solvent.

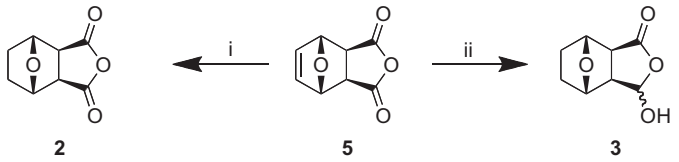
With the cyclic lactone (**3**) in hand, we initially investigated the development of three focused libraries by treatment of **3** with corresponding commercially available alcohols that consisted of either unsaturated (Library A), straight chained (Library B) or branched (Library C) tails as shown in Scheme 2.

We have previously shown that modification in this manner of the norcantharidin backbone can be achieved via an S_N2 directed substitution reaction between an alcohol and (**3**) in the presence of catalytic pTsOH at reflux with the alcohol acting as both the reagent and solvent for the reaction. However this synthetic route was only effective with low boiling point alcohols that could be separated from the desired analogues *in vacuo*. Hence, a new methodology was employed where a solution of **3**, the desired alcohol and pTsOH in THF was heated under microwave radiation (see experimental) to afford the desired norcantharidin analogue with only residual

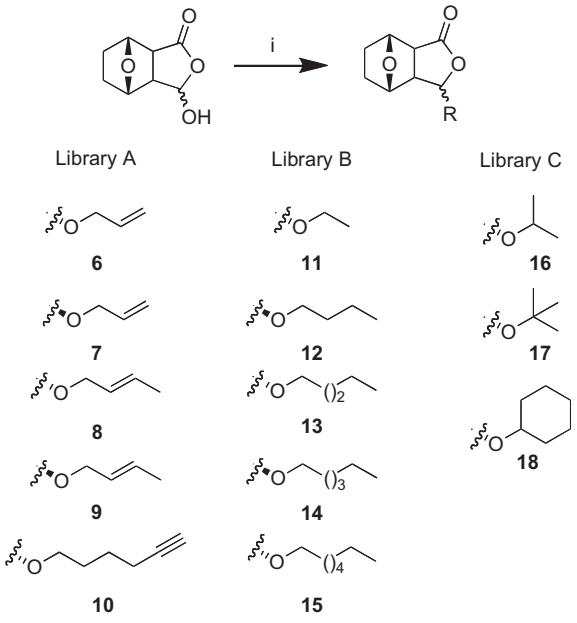
Table 1
Anticancer activity of norcantharidin (**2**), (3*S*,3a*R*,4*S*,7*R*,7a*S*)-3-hydroxyhexahydro-4,7-epoxyisobenzofuran-1(3*H*)-one (**3**) and unsaturated analogues (**6**–**10**). Values in are GI_{50} (μM) concentrations.

Compound	HT29[a]	SJ-G2[b]	BE2-C[c]	SW480[a]	MCF-7[d]	A2780[e]	H460[f]	A431[g]	DU145[h]
Norcantharidin **2**	23 ± 4	23 ± 5	36 ± 0	41 ± 4	34 ± 3	27 ± 1	>100	72 ± 3	90 ± 10
Novo-6 **3**	14 ± 3	15 ± 4	28 ± 2	>100	>100	>100	>100	>100	>100
 **6**	nd	nd	nd	nd	>100	nd	>100	>100	>100
 **7**	nd	nd	>100	>100	>100	>100	>100	>100	>100
 **8**	42 ± 5	55 ± 3	84 ± 5	93 ± 8	77 ± 10	57 ± 5	>100	>100	>100
 **9**	31 ± 4	35 ± 4	63 ± 11	>100	>100	>100	>100	>100	>100
 **10**	51 ± 1	55 ± 0	>100	>100	>100	>100	>100	>100	>100

nd = not determined.
[a] Colon carcinoma.
[b] Glioblastoma.
[c] Neuroblastoma.
[d] Breast carcinoma.
[e] Ovarian carcinoma.
[f] Lung carcinoma.
[g] Skin carcinoma.
[h] Prostate carcinoma.



Scheme 1. Reagents and conditions: (i) dry acetone, 4 bar H_2, 10% Pd/C 48 h; (ii) Wet EtOH, 4 bar H_2, 10% Pd/C, 48 h.

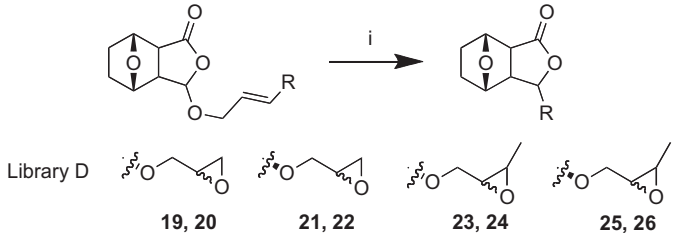


Scheme 2. Reagents and conditions. (i) ROH, cat. p-TsOH, THF, 80 °C, MW – see experimental for details.

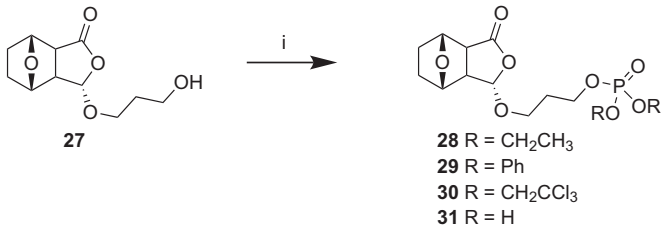
amounts of the used alcohol remaining which was removed by subsequent chromatography.

Analogues with unsaturated tails were also modified to incorporate an epoxide ring. This was carried out in an attempt to achieve some of the broader spectrum anticancer activity shown by other small molecule natural products such as Fostriecin. Reaction of the unsaturated analogues (**6**–**9**) with *m*-chloroperbenzoic acid as shown in Scheme 3, produced epoxide analogues (Library D) in moderate to excellent overall yield (26–85%).

Analogues with a terminal phosphate moiety were also synthesised in a final attempt to mimic the biological activity associated with Fostriecin. Reaction of a terminal hydroxyl norcantharidin analogue (**27**) with various chlorophosphates in the presence of dibutyltin oxide produced the desired analogues (Library E) in good yields (50–78%). Further synthetic manipulation of analogue (**30**) produced a final analogue with the phosphate esters cleaved resulting in a terminal free phosphoric acid moiety (**31**) (Scheme 4).



Scheme 3. Reagents and conditions. (i) m-CPBA, CH_2Cl_2, 0 to rt °C, 16 h.



Scheme 4. Reagents and conditions: (i) Bu_2SnO, CH_2Cl_2, Cl—P(OR)$_2$, TEA.

3. Cytotoxicity

We are interested in the development of anti-cancer agents that show specificity towards a particular cancer type, such as **3**, which displayed high levels of colon cancer specificity, as well as agents that show a broad range of toxicities across an array of cancer cell lines. We have a particular interest in cancers with poor prognosis and have developed an in-house panel of cancer cell lines to identify compounds that may prove beneficial in the treatment of such cancers. With the desired analogues synthesized, we examined their cytotoxicity against a panel of nine human tumour cell lines: HT29 and SW480 (colon carcinoma), MCF-7 (breast carcinoma), A2780 (ovarian carcinoma), H460 (lung carcinoma), A431 (skin carcinoma), DU145 (prostate carcinoma), BE2-C (neuroblastoma) and SJ-G2 (glioblastoma). All analogues were initially screened at a dose of 100 μM and those that induced appreciable growth inhibition underwent full dose response analysis in order to obtain a GI_{50} value.

4. Results and discussion

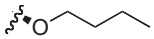
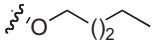
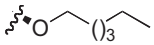
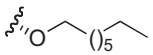
Evaluation of the cytotoxicity data indicated that no particular library of analogues produced significantly different levels of activity than any other. However, there were some compounds that showed comparable activity to the parent norcantharidin derivative (3*S*,3a*R*,4*S*,7*R*,7a*S*)-3-hydroxyhexahydro-4,7-epoxyisobenzofuran-1(3*H*)-one (**3**) or in some cases greater activity in particular cell lines.

The first library assessed, Library A, consisting of compounds with unsaturation within the newly formed tail produced some interesting data in its own right (Table 1). With these compounds being the first to be synthesized, both possible isomers were isolated and separately tested to determine if there was a difference in cytotoxicity attributed to the molecular orientation of the stereocentre. Both isomers comprising of an allyl tail (**6**, **7**) produced a similar growth inhibition profile with slight preference towards the HT29 and SJG2 cells lines, however, neither analogue displayed noteworthy potency.

The second pairing of compounds consisting of a crotyl tail (**8**, **9**) showed initial promise with percentage inhibition at 100 μM deemed high enough to proceed to the second level of screening and obtain GI_{50} values. Interestingly again, both compounds showed similar activity and preference towards HT29 (colon) and SJ-G2 (glioblastoma) cells with GI_{50} values of (42 ± 5 and 55 ± 3 μM) (**8**) and (31 ± 4 and 35 ± 4 μM) (**9**) respectively with activity being completely void against the other cell lines tested. The final compound of Library A (**10**) consisting of a hexyl carbon chain and a terminal alkyne showed minimal activity, suggesting a tail of this length is not advantageous; however, this analogue still presented with some selectivity towards the HT29 and SJ-G2 cells.

The introduction of simple aliphatic chains produced a completely different trend in the cytotoxicity data (Table 2). As previous data suggested, there was no significant difference in cytotoxicity between the *R* and *S* isomers of any one compound,

Table 2
Anticancer activity of straight chain analogues (**11**–**15**). Values in are GI_{50} (μM) concentrations.

Compound	HT29[a]	SJ-G2[b]	BE2-C[c]	SW480[a]	MCF-7[d]	A2780[e]	H460[f]	A431[g]	DU145[h]
 **11**	77 ± 12	49 ± 15	>100	>100	>100	>100	>100	>100	>100
 **12**	50 ± 6	32 ± 2	93 ± 7	>100	>100	>100	>100	>100	>100
 **13**	32 ± 3	37 ± 6	38 ± 2	39 ± 4	27 ± 6	20 ± 1	>100	68 ± 5	>100
 **14**	nd	nd	>100	>100	>100	>100	>100	>100	>100
 **15**	40 ± 7	45 ± 10	51 ± 4	49 ± 8	36 ± 5	28 ± 2	>100	72 ± 3	86 ± 8

nd = not determined.
[a] Colon carcinoma.
[b] Glioblastoma.
[c] Neuroblastoma, MCF-7.
[d] Breast carcinoma.
[e] Ovarian carcinoma.
[f] Lung carcinoma.
[g] Skin carcinoma.
[h] Prostate carcinoma.

thus only the major isomer, formed in excess, was isolated and screened. Compounds (**11**) and (**12**) comprising of ethyl and butyl chains respectively showed limited cytotoxicity along with (**14**), having a hexyl tail. This is consistent with results shown by (**10**), indicating a six carbon chain at this position of these analogues is detrimental to activity and is causing an unfavourable interaction within the binding pocket. However, (**13**) and (**15**) with pentyl and octyl chains produced comparable cytotoxicity to (**2**) across all cell lines tested, showing no selectivity towards HT29 (colon) or SJ-G2 (glioblastoma) cells as previously observed with these derivatives.

Adding alcohols with varying degrees of branching again produced interesting results. Analogue **16** consisting of an isopropyl tail produced the most notable data of the series. This analogue showed similar potency to the lead (**3**) and maintained selectivity against HT29 (colon) and SJ-G2 (glioblastoma) cells, with GI_{50}s of 19 ± 0.9 and 21 ± 2.1 μM respectively (Table 3). A similar selectivity profile was observed with further branching to the tertiary butyl tail as with **17** and cyclohexyl **18**; however potency was compromised.

The effect of introducing an epoxide ring via modification of derivatives (**6**–**9**) was also carried out to produce compounds (**19**–**26**) (Table 4). In each case, two products were isolated and tested separately with almost identical ^{1}H and ^{13}C NMR spectra. Numerous attempts at crystallisation of the formed clear oils were unsuccessful and as a result, the absolute stereochemistry of each isolated product is still unknown. Although, none of these analogues, irrespective of stereocentre configuration, displayed noteworthy potency in growth inhibition the selectivity towards the HT29 and SJ-G2 cells was still apparent.

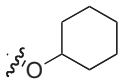
The final structural modification assessed was the introduction of terminal phosphate moieties to the tail of novo analogues via modification of a novo analogue with a terminal hydroxyl group (**27**) (Table 5). All esterifications proceeded in good yields. Interestingly **28** produced a different trend in cytotoxicity with significant growth inhibition in BE2-C cells (GI_{50} = 9.2 μM) through to a negligible effect in H460 cells (GI_{50} > 100 μM). This is in contrast to analogues **29** and **30** that maintained the selectivity towards HT29 and SJ-G2 cells, albeit at relatively low potency. We also examined the effect of a free terminal phosphoric acid moiety. Accordingly we generated analogue **31**; however this analogue displayed low levels of cytotoxicity being active against only the HT29 (GI_{50} = 42 ± 4 μM), MCF-7 (45 ± 5 μM) and A2780 (33 ± 4 μM) cell lines. This is in keeping with previous findings in our laboratory that long alkyl chains afford broad spectrum activity in the ring-opened norcantharidin analogues and the norcantharimides [22,23,30].

5. Conclusion

A series of norcantharidin cyclic lactone derivatives were prepared, by microwave assisted organic synthetic approaches, in moderate to good yields and their ability to inhibit the growth of a panel of human cancer cell lines was examined. Interestingly, there was no observed difference in cytotoxicity across any of the cancer cell lines tested between the two possible diastereoisomers produced for each analogue during synthesis.

Overall the most interesting data collected was attributed to the isopropyl substituted derivative **16**, with strong selectivity shown towards HT29 and SJ-G2 cell lines returning GI_{50} values of 19 ± 0.9 and 21 ± 2.1 μM respectively while being completely inactive against all other cell lines. We also discovered a new cytotoxicity trend following the introduction of a terminal phosphoric acid moiety giving rise an analogue with strong selectivity towards BE2-C cells and the greatest anti-growth activity (GI_{50} 9 μM) observed in this study. These analogues add considerably to the SAR

Table 3
Anticancer activity of branched analogues (**16**–**18**). Values in are GI_{50} (μM) concentrations.

Compound	HT29[a]	SJ-G2[b]	BE2-C[c]	SW480[a]	MCF-7[d]	A2780[e]	H460[f]	A431[g]	DU145[h]
 **16**	19 ± 1	21 ± 3	44 ± 5	94 ± 1	>100	>100	>100	>100	>100
 **17**	52 ± 5	48 ± 7	>100	>100	>100	>100	>100	>100	>100
 **18**	45 ± 4	41 ± 11	>100	>100	>100	>100	>100	>100	>100

nd = not determined.
[a] Colon carcinoma.
[b] Glioblastoma.
[c] Neuroblastoma, MCF-7.
[d] Breast carcinoma.
[e] Ovarian carcinoma.
[f] Lung carcinoma.
[g] Skin carcinoma.
[h] Prostate carcinoma.

surrounding this class of compounds and hold promise for future development of novel anti-cancer agents.

6. Experimental

6.1. Biology

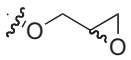
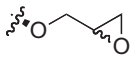
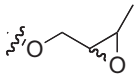
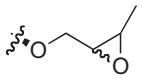
6.1.1. Cell culture and stock solutions

Stock solutions were prepared as follows and stored at −20 °C: Cantharidin (Biomol, USA) as a 30 mM solution in dimethylsulphoxide (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).

6.1.2. In vitro growth inhibition assay

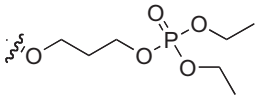
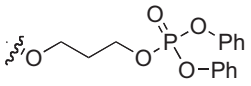
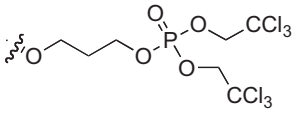
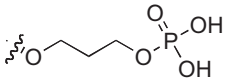
Cells in logarithmic growth were transferred to 96-well plates. Cytotoxicity was determined by plating cells in duplicate in 100 mL medium at a density of 2500–4000 cells/well. On day 0, (24 h after plating) when the cells were in logarithmic growth, 100 μL medium with or without the test agent was added to each well. After 72 h drug exposure growth inhibitory effects were evaluated using the MTT (3-[4,5-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

Table 4
Anticancer activity of epoxide analogues (**19**–**26**). Values recorded are the percentage growth inhibition at 100 μM compound concentration.

Compound		HT29[a]	SJ-G2[b]	BE2-C[c]	SW480[a]	MCF-7[d]	A2780[e]	H460[f]	A431[g]	DU145[h]
	**19**	69 ± 1	75 ± 1	42 ± 5	32 ± 1	37 ± 2	46 ± 4	11 ± 3	35 ± 2	33 ± 2
	20	52 ± 2	59 ± 3	42 ± 4	16 ± 3	43 ± 4	83 ± 7	7 ± 3	18 ± 2	21 ± 4
	**21**	68 ± 2	73 ± 1	35 ± 5	23 ± 5	33 ± 2	42 ± 4	7 ± 2	22 ± 3	25 ± 2
	22	74 ± 1	76 ± 2	38 ± 3	28 ± 3	40 ± 2	42 ± 3	14 ± 1	28 ± 3	30 ± 2
	**23**	75 ± 1	88 ± 2	54 ± 13	41 ± 12	61 ± 13	55 ± 16	24 ± 5	35 ± 6	37 ± 3
	24	68 ± 3	79 ± 1	53 ± 21	37 ± 13	46 ± 12	58 ± 16	9 ± 4	17 ± 2	20 ± 2
	**25**	70 ± 2	80 ± 2	23 ± 5	17 ± 0	27 ± 2	26 ± 3	9 ± 1	18 ± 0	17 ± 5
	26	nd	nd	nd	nd	nd	nd	nd	nd	nd

nd = not determined.
[a] Colon carcinoma.
[b] Glioblastoma.
[c] Neuroblastoma, MCF-7.
[d] Breast carcinoma.
[e] Ovarian carcinoma.
[f] Lung carcinoma.
[g] Skin carcinoma.
[h] Prostate carcinoma.

Table 5
Anticancer activity of terminal phosphate esters and phosphoric acid analogues (**28**–**31**). Values in are GI_{50} (μM) concentrations.

Compound	HT29[a]	SJ-G2[b]	BE2-C[c]	SW480[a]	MCF-7[d]	A2780[e]	H460[f]	A431[g]	DU145[h]
 **28**	17 ± 1	43 ± 3.4	9 ± 0.2	9 ± 7	20 ± 1	22 ± 2	>100	87 ± 12	84 ± 3
 **29**	40 ± 4	40 ± 10	>100	>100	>100	>100	>100	>100	>100
 **30**	37 ± 10	41 ± 10	>100	>100	89 ± 6	81 ± 1	>100	>100	>100
 **31**	42 ± 4	57 ± 2	67 ± 4	65 ± 4	45 ± 5	33 ± 4	6 ± 3	52 ± 2	65 ± 6

nd = not determined.
[a] Colon carcinoma.
[b] Glioblastoma.
[c] Neuroblastoma, MCF-7.
[d] Breast carcinoma.
[e] Ovarian carcinoma.
[f] Lung carcinoma.
[g] Skin carcinoma.
[h] Prostate carcinoma.

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 [29].

6.2. Chemistry

6.2.1. General experimental – general methods

All reagents were purchased from Sigma–Aldrich, Matrix Scientific or Lancaster Synthesis and were used without purification. With the exception of THF (anhydrous > 99%) obtained from Sigma–Aldrich, all solvents were re-distilled from glass prior to use.

^1H and ^{13}C NMR spectra were recorded on a Bruker Avance™ AMX 300 MHz spectrometer at 300.1315 and 75.4762 MHz, respectively. Chemical shifts (δ) are reported in parts per million (ppm) measured relative to the internal standards, and coupling constants (J) are expressed in Hertz (Hz). 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.

Melting points were recorded on a Stuart Scientific melting point apparatus (UK) and are uncorrected. Thin layer chromatography (TLC) was performed on Merck silica gel 60 F_{254} pre-coated aluminium plates with a thickness of 0.2 mm. Column chromatography was performed under 'flash' conditions on Merck silica gel 60 (230–400 mesh) or using the Biotage SP4 flash purification system with a 100 g pre-packed snap column.

A CEM Discover® BenchMate microwave (120 °C, 200 W, 1 h) was used to perform several refluxes. Hydrogenations were performed using the H-Cube® continuous-flow hydrogenation reactor utilizing a palladium-carbon (CatCart®) catalyst, a flow rate of 1 ml/min, 40 bar of pressure and column temperature 40 °C.

6.2.2. General microwave procedure

An alcohol derivative (1.07 mmol, 1 eq) was added to a magnetically stirred solution of (3*S*,3a*R*,4*S*,7*R*,7a*S*)-3-hydroxyhexahydro-4,7-epoxyisobenzofuran-1(3*H*)-one (1.18 mmol, 1.05 eq) (**3**) in anhydrous THF (5 mL). To this, a catalytic amount of 4-toluene sulfonic acid (10 mg) was added and the resulting solution was treated with microwave radiation at 80 °C for 1 h at 150 W. The resulting solution was subjected to silica chromatography (2:8 EtOAc:hexane) to afford the desired derivative.

6.2.3. (3S,3aR,4S,7R,7aS)-3-(Allyloxy)hexahydro-4,7-epoxyisobenzofuran-1(3H)-one (6) and (3R,3aR,4S,7R,7aS)-3-(allyloxy)hexahydro-4,7-epoxyisobenzofuran-1(3H)-one (7)

Synthesised as per the general microwave procedure above from **3** and allyl alcohol to afford the title compounds (6:1) as a white solid (R_f 0.81) (26%), m.p. 36–38 °C; ^1H NMR ($CDCl_3$) (300 MHz): δ 5.89 (m, 1H), 5.30 (d, J = 1.7 Hz, 1H), 5.28 (m, 2H), 4.83 (d, J = 4.6 Hz, 1H), 4.69 (d, J = 4.6 Hz, 1H), 4.30 (m, 1H), 4.07 (m, 1H), 2.92 (d, J = 8.0 Hz, 1H), 2.53 (dd, J = 1.7, 8.0 Hz, 1H), 1.85–1.46 (m, 4H). ^{13}C NMR ($CDCl_3$) (75 MHz): δ 175.5, 132.3, 118.0, 105.8, 79.6, 79.0, 69.7, 50.3, 49.7, 28.0, 27.3; IR (KBr) ν_{max}/cm^{-1}: 2988 (C–H), 1747 (C=O), 1638 (C=C), 1150 (C–O); m/z (APCI M + H) 211. HRMS calculated for M + H; $C_{11}H_{15}O_4$; 211.0965.

6.2.4. (3R,3aR,4S,7R,7aS)-3-(Allyloxy)hexahydro-4,7-epoxyisobenzofuran-1(3H)-one (7)

Isolated as a white crystalline solid (R_f 0.62) (8%), m.p. 100 °C; ^1H NMR ($CDCl_3$) (300 MHz): δ 5.87–5.60 (m, 1H), 5.56 (d, J = 6.8 Hz,

1H), 5.37–5.23 (m, 2H), 5.13 (d, J = 4.8 Hz, 1H), 4.92 (d, J = 4.8 Hz, 1H), 4.46–4.40 (m, 1H), 4.17–4.10 (m, 1H), 2.89 (d, J = 8.4 Hz, 1H), 2.72–2.68 (m, 1H), 1.86–1.71 (m, 2H), 1.57–1.43 (m, 2H). ^{13}C NMR ($CDCl_3$) (75 MHz): δ 174.8, 132.5, 117.7, 102.4, 78.7, 76.0, 70.8, 51.1, 46.6, 27.6, 27.5; IR (KBr) ν_{max}/cm^{-1}: 2988 (C–H), 1747 (C=O), 1638 (C=C), 1150 (C–O); m/z (APCI M + H) 211. HRMS calculated for M + H; $C_{11}H_{14}O_4$; 211.0965.

*6.2.5. (3S,3aR,4S,7R,7aS)-3-((E)-But-2-en-1-yloxy)hexahydro-4,7-epoxyisobenzofuran-1(3H)-one (**8**) and (3R,3aR,4S,7R,7aS)-3-((E)-but-2-en-1-yloxy)hexahydro-4,7-epoxyisobenzofuran-1(3H)-one (**9**)*

Synthesised as per the general microwave procedure above from **3** and but-2-en-1-ol to afford the title compounds (6:1) as a white crystalline solid (R_f 0.81) (42%), m.p. 78 °C; ^{1}H NMR ($CDCl_3$) (300 MHz): δ 5.78 (dq, J = 15.2, 6.4 Hz, 1H), 5.55 (dt, 15.2, 6.0 Hz, 1H), 5.30 (d, J = 1.1 Hz, 1H), 4.83 (d, J = 4.5 Hz, 1H), 4.69 (d, J = 4.5 Hz, 1H), 4.24 (dd, J = 6.0, 0.8 Hz, 1H), 4.01 (dd, J = 7.1, 0.8 Hz, 1H), 2.91 (d, J = 7.9 Hz, 1H), 2.50 (dd, J = 7.9, 1.1 Hz, 1H), 1.80–1.45 (m, 4H), 1.73 (d, J = 6.4 Hz, 3H). ^{13}C NMR ($CDCl_3$) (75 MHz): δ 175.5, 131.2, 125.0, 105.5, 79.7, 79.0, 69.6, 50.4, 49.8, 28.0, 27.2, 17.3; IR (KBr) ν_{max}/cm^{-1}: 2985 (C–H), 1752 (C=O), 1617 (C=C), 1153 (C–O); m/z (APCI M + H) 225. HRMS calculated for M + H; $C_{12}H_{16}O_4$; 225.1121.

Isolated as a white solid (R_f 0.62) (9%), m.p. 116–118 °C; ^{1}H NMR ($CDCl_3$) (300 MHz): δ 5.76 (dq, J = 15.3, 6.4 Hz, 1H), 5.57 (m, 1H), 5.56 (d, J = 6.8 Hz, 1H), 5.11 (d, J = 4.8 Hz, 1H), 4.90 (d, J = 4.8 Hz, 1H), 4.35 (m, 1H), 4.06 (m, 1H), 2.88 (d, J = 8.3 Hz, 1H), 2.67 (dd, J = 6.8, 8.3 Hz, 1H), 1.85–1.38 (m, 4H), 1.73 (d, J = 6.4 Hz, 3H). ^{13}C NMR ($CDCl_3$) (75 MHz): δ 175.0, 130.6, 125.3, 102.1, 78.7, 76.0, 70.6, 51.2, 46.6, 27.6, 27.5, 17.2; IR (KBr) ν_{max}/cm^{-1}: 2985 (C–H), 1752 (C=O), 1617 (C=C), 1153 (C–O); m/z (APCI M + H) 225. HRMS calculated for M + H; $C_{12}H_{17}O_4$; 225.1121.

*6.2.6. (3S,3aR,4S,7R,7aS)-3-(Hex-5-yn-1-yloxy)hexahydro-4,7-epoxyisobenzofuran-1(3H)-one (**10**)*

Synthesised as per the general microwave procedure above from **3** and hex-5-yn-1-ol to afford the title compound as a clear oil (80%); ^{1}H NMR (Acetone-d_6) (300 MHz): δ 5.32 (d, J = 1.5 Hz, 1H), 4.73 (d, J = 4.2 Hz, 1H), 4.65 (d, J = 4.2 Hz, 1H), 3.79–3.71 (m, 1H), 3.62–3.55 (m, 1H), 3.00 (d, J = 7.9 Hz, 1H), 2.53 (dd, J = 7.9, 1.5 Hz, 1H), 2.36–2.31 (m, 1H), 2.23–2.14 (m, 3H), 2.08–2.02 (m, 1H), 1.74–1.51 (m, 6H). ^{13}C NMR (Acetone-d_6) (75 MHz): δ 175.2, 106.6, 83.2, 79.5, 78.8, 68.7, 68.1, 50.0, 49.2, 27.8, 27.5, 27.0, 24.4, 17.0; IR (film) ν_{max}/cm^{-1}: 3325 (C≡C–H), 2933 (C–H), 2145 (C≡C), 1779 (C=O), 1124 (C–O); m/z (APCI M + H) 251. HRMS calculated for M + H; $C_{14}H_{19}O_4$; 251.1278.

*6.2.7. (3S,3aR,4S,7R,7aS)-3-Ethoxyhexahydro-4,7-epoxyisobenzofuran-1(3H)-one (**11**)*

Synthesised as per the general microwave procedure above from **3** and ethanol to afford the title compound as a white solid (80%), m.p. 71–72 °C; ^{1}H NMR ($CDCl_3$) (300 MHz): δ 5.27 (d, J = 1.5 Hz, 1H), 4.84 (d, J = 4.7 Hz, 1H), 4.70 (d, J = 4.7 Hz, 1H), 3.87 (m, 1H), 3.60 (m, 1H), 2.91 (d, J = 8.0, 1H), 2.50 (dd, J = 1.5,8.0 Hz, 1H), 1.81–1.76 (m, 2H), 1.60–1.50 (m, 2H), 1.23 (t, J = 7.1 Hz, 3H). ^{13}C NMR ($CDCl_3$) (75 MHz): δ 175.6, 106.7, 79.7, 79.0, 64.9, 50.5, 49.8, 28.1, 27.3, 14.4; IR (KBr) ν_{max}/cm^{-1}: 2979 (C–H), 2881 (C–H), 1752 (C=O), 1190 (C–O); m/z (APCI M + H) 199. HRMS calculated for M + H; $C_{10}H_{15}O_4$; 199.0965.

*6.2.8. (3S,3aR,4S,7R,7aS)-3-Butoxyhexahydro-4,7-epoxyisobenzofuran-1(3H)-one (**12**)*

Synthesised as per the general microwave procedure above from **3** and butan-1-ol to afford the title compound as pale yellow solid (23%), m.p. 46–47 °C; ^{1}H NMR ($CDCl_3$) (300 MHz): δ 5.26 (d, J = 1.7 Hz, 1H), 4.84 (d, J = 4.5 Hz, 1H), 4.70 (d, J = 4.5 Hz, 1H), 3.80 (m, 1H), 3.53 (m, 1H), 2.91 (d, J = 8.0 Hz, 1H), 2.50 (dd, J = 1.7, 8.0 Hz, 1H), 1.79–1.33 (m, 8H), 0.92 (t, J = 7.3 Hz, 3H). ^{13}C NMR ($CDCl_3$) (75 MHz): δ 175.9, 107.0, 79.7, 79.0, 69.2, 50.4, 49.8, 30.9, 28.0, 27.3, 18.6, 13.2; IR (KBr) ν_{max}/cm^{-1}: 2952 (C–H), 2870 (C–H), 1772 (C=O), 1122 (C–O); m/z (APCI M + H) 227. HRMS calculated for M + H; $C_{12}H_{19}O_4$; 227.1278.

*6.2.9. (3S,3aR,4S,7R,7aS)-3-(Pentyloxy)hexahydro-4,7-epoxyisobenzofuran-1(3H)-one (**13**)*

Synthesised as per the general microwave procedure above from **3** and pentan-1-ol to afford the title compound as a yellow oil (53%); ^{1}H NMR ($CDCl_3$) (300 MHz): δ 5.22 (d, J = 1.7 Hz, 1H), 4.79 (d, J = 4.6 Hz, 1H), 4.66 (d, J = 4.6 Hz, 1H), 3.75 (m, 1H), 3.48 (m, 1H), 2.88 (d, J = 7.9 Hz, 1H), 2.46 (dd, J = 1.7, 7.9 Hz, 1H), 1.75–1.25 (m, 10H), 0.86 (t, J = 6.3 Hz, 3H). ^{13}C NMR ($CDCl_3$) (75 MHz): δ 175.6, 106.9, 79.7, 79.0, 69.5, 50.4, 49.8, 28.5, 28.0, 27.5, 27.2, 21.8, 13.4; IR (film) ν_{max}/cm^{-1}: 2938 (C–H), 1777 (C=O), 1122 (C–O); m/z (APCI M + H) 241.0. HRMS calculated for M + H; $C_{13}H_{21}O_4$; 241.1434.

*6.2.10. (3S,3aR,4S,7R,7aS)-3-(Hexyloxy)hexahydro-4,7-epoxyisobenzofuran-1(3H)-one (**14**)*

Synthesised as per the general microwave procedure above from **3** and hexan-1-ol to afford the title compound as a yellow oil (31%); ^{1}H NMR ($CDCl_3$) (300 MHz): δ 5.24 (d, J = 1.8 Hz, 1H), 4.82 (d, J = 4.7 Hz, 1H), 4.68 (d, J = 4.7 Hz, 1H), 3.78 (m, 1H), 3.50 (m, 1H), 2.90 (d, J = 8.0 Hz, 1H), 2.48 (dd, J = 1.8, 8.0 Hz, 1H), 1.85–1.22 (m, 12H), 0.88 (t, J = 6.6 Hz, 3H). ^{13}C NMR ($CDCl_3$) (75 MHz): δ 175.5, 106.9, 79.7, 79.0, 69.5, 50.4, 49.8, 28.8, 28.0, 27.3, 25.0, 21.9, 20.4, 13.4; IR (film) ν_{max}/cm^{-1}: 2932 (C–H), 1780 (C=O), 1124 (C–O); m/z (APCI M + H) 255. HRMS calculated for M + H; $C_{14}H_{23}O_4$; 255.1591.

*6.2.11. (3S,3aR,4S,7R,7aS)-3-(Octyloxy)hexahydro-4,7-epoxyisobenzofuran-1(3H)-one (**15**)*

Synthesised as per the general microwave procedure above from **3** and octan-1-ol to afford the title compound as a dark yellow oil (26%); ^{1}H NMR ($CDCl_3$) (300 MHz): δ 5.25 (d, J = 1.7 Hz, 1H), 4.83 (d, J = 4.6 Hz, 1H), 4.69 (d, J = 4.6 Hz), 3.80 (m, 1H), 3.51 (m, 1H), 2.90 (d, J = 8.0 Hz, 1H), 2.49 (dd, J = 1.7, 8.0 Hz, 1H), 2.04–1.25 (m, 16H), 0.88 (t, J = 6.2 Hz, 3H). ^{13}C NMR ($CDCl_3$) (75 MHz): δ 175.4, 106.9, 79.7, 79.0, 69.5, 50.4, 49.8, 31.2, 28.8, 28.7, 28.6, 28.0, 27.3, 25.4, 22.1, 13.5; IR (film) ν_{max}/cm^{-1}: 2938 (C–H), 1782 (C=O), 1123 (C–O); m/z (APCI M + H) 283. HRMS calculated for M + H; $C_{16}H_{27}O_4$; 283.1904.

*6.2.12. (3S,3aR,4S,7R,7aS)-3-Isopropoxyhexahydro-4,7-epoxyisobenzofuran-1(3H)-one (**16**)*

Synthesised as per the general microwave procedure above from **3** and isopropanol to afford the title compound as a white solid (49%), m.p. 74–76 °C; ^{1}H NMR ($CDCl_3$) (300 MHz): δ 5.36 (d, J = 1.6 Hz, 1H), 4.83 (d, J = 4.3 Hz, 1H), 4.68 (d, J = 4.3 Hz, 1H), 3.98 (sept, J = 6.2 Hz, 1H), 2.91 (d, J = 8.0 Hz, 1H), 2.47 (dd, J = 1.6, 8.0 Hz, 1H), 1.79–1.75 (m, 2H), 1.59–1.46 (m, 2H), 1.22–1.21 (m, 6H). ^{13}C NMR ($CDCl_3$) (75 MHz): δ 175.7, 105.2, 79.7, 79.0, 71.5, 50.8, 50.0, 28.1, 27.6, 22.7, 21.0; IR (KBr) ν_{max}/cm^{-1}: 2964 (C–H), 2984 (C–H), 1732 (C=O), 1124 (C–O); m/z (APCI M + H) 212. HRMS calculated for M + H; $C_{11}H_{17}O_4$; 213.1121.

*6.2.13. (3S,3aR,4S,7R,7aS)-3-(Tert-butoxy)hexahydro-4,7-epoxyisobenzofuran-1(3H)-one (**17**)*

Synthesised as per the general microwave procedure above from **3** and *tert*-butanol to afford the title compound as a white solid (76%), m.p. 127–128 °C; ^{1}H NMR ($CDCl_3$) (300 MHz): δ 5.47 (d, J = 1.3 Hz, 1H), 4.83 (d, J = 3.9 Hz, 1H), 4.64 (d, J = 3.5 Hz, 1H), 2.88 (d, J = 8.0 Hz, 1H), 2.45 (dd, J = 1.3, 7.0 Hz, 1H), 1.76–1.27 (m, 13H). ^{13}C NMR ($CDCl_3$) (75 MHz): δ 175.7, 102.2, 79.6, 79.0, 76.9 51.7, 50.0, 28.2, 28.0, 27.1; IR (KBr) ν_{max}/cm^{-1}: 2985 (C–H), 2878 (C–H), 1759

(C=O), 1121 (C–O); *m/z* (APCI M + H) 227. HRMS calculated for M + H; $C_{12}H_{19}O_4$; 227.1278.

6.2.14. (3S,3aR,4S,7R,7aS)-3-(Cyclohexyloxy)hexahydro-4,7-epoxyisobenzofuran-1(3H)-one (**18**)

Synthesised as per the general microwave procedure above from **3** and cyclohexanol to afford the title compound as a yellow solid (26%), m.p. 100–102 °C; ^{1}H NMR ($CDCl_3$) (300 MHz): δ 5.41 (d, *J* = 1.8 Hz, 1H), 4.84 (d, *J* = 4.5 Hz, 1H), 4.68 (d, *J* = 4.5 Hz, 1H), 3.65 (m, 1H), 2.92 (d, *J* = 8.0 Hz, 1H), 2.49 (dd, *J* = 1.8, 8.0 Hz, 1H), 1.85–1.22 (m, 14H). ^{13}C NMR ($CDCl_3$) (75 MHz): δ 175.9, 105.1, 79.7, 79.0, 50.8, 50.0, 32.7, 30.1, 28.1, 27.2, 24.9, 23.3; IR (KBr) ν_{max}/cm^{-1}: 2972 (C–H), 2853 (C–H), 1748 (C=O), 1193 (C–O); *m/z* (APCI M + H) 253. HRMS calculated for M + H; $C_{14}H_{21}O_4$; 253.1434.

6.3. General epoxidation procedure

m-Chloroperbenzoic acid (0.74 g, 77% in water, 3.31 mmol, 2 eq) was added to a magnetically stirred solution of the corresponding unsaturated Novo derivative (1.66 mmol, 1 eq) in anhydrous DCM (10 mL) at 0 °C. The resulting solution was warmed to room temperature, and stirred for 16 h before being diluted with CH_2Cl_2 (20 mL) and washed with $NaHCO_3$ (3 × 10 mL, sat solution). The organic layer was collected, dried over $MgSO_4$, filtered and concentrated under reduced pressure. The formed clear oil was subjected to silica chromatography (EtOAc:hexanes 3:7) to afford both isomers of the desired epoxide.

6.3.1. (3S,3aR,4S,7R,7aS)-3-(Oxiran-2-ylmethoxy)hexahydro-4,7-epoxyisobenzofuran-1(3H)-one (**19**, **20**)

Synthesised as per the general epoxidation procedure above from (**6**) to afford the title compounds as clear oils (1:1) combined yield (74%); ^{1}H NMR ($CDCl_3$) (300 MHz) (**19**): δ 5.31 (d, *J* = 1.5 Hz, 1H), 4.82 (d, *J* = 4.6 Hz, 1H), 4.70 (d, *J* = 4.6 Hz, 1H), 4.07 (dd, *J* = 2.7, 11.4 Hz, 1H), 3.39 (dd, *J* = 7.1, 11.4 Hz, 1H), 3.16 (m, 1H), 2.91 (d, *J* = 7.9 Hz, 1H), 2.82 (t, *J* = 4.4 Hz, 1H), 2.58 (m, 2H), 1.82–1.69 (m, 2H), 1.59–1.46 (m, 2H). ^{13}C NMR ($CDCl_3$) (75 MHz): δ 175.3, 106.6, 79.5, 79.0, 70.5, 50.3, 49.7, 49.6, 43.9, 28.0, 27.2; IR (film) ν_{max}/cm^{-1}: 2992 (C–H), 1742 (C=O), 1230 (C–O), 1150 (C–O); *m/z* APCI (M + H) 227.

^{1}H NMR ($CDCl_3$) (300 MHz) (**20**): δ 5.27 (d, *J* = 1.6 Hz, 1H), 4.80 (d, *J* = 4.5 Hz, 1H), 4.68 (d, *J* = 4.5 Hz, 1H), 3.78 (m, 2H), 3.14 (m, 1H), 2.89 (d, *J* = 7.9 Hz, 1H), 2.79 (t, *J* = 4.5 Hz, 1H), 2.66 (m, 1H), 2.52 (dd, *J* = 1.6, 7.9 Hz, 1H), 1.79–1.66 (m, 2H), 1.57–1.43 (m, 2H). ^{13}C NMR ($CDCl_3$) (75 MHz): δ 175.3, 106.6, 79.6, 79.0, 68.5, 50.2, 49.6, 49.3, 43.7, 28.0, 27.2; IR (film) ν_{max}/cm^{-1}: 2992 (C–H), 1742 (C=O), 1230 (C–O), 1150 (C–O); *m/z* APCI (M + H) 227. HRMS calculated for M + H; $C_{11}H_{15}O_4$; 227.0914.

6.3.2. (3R,3aR,4S,7R,7aS)-3-(Oxiran-2-ylmethoxy)hexahydro-4,7-epoxyisobenzofuran-1(3H)-one (**21**, **22**)

Synthesised as per the general epoxidation procedure above from (**7**) to afford the title compounds as clear oils (1:1) combined yield (68%); ^{1}H NMR ($CDCl_3$) (300 MHz) (**21**): δ 5.59 (d, *J* = 6.8 Hz, 1H), 5.12 (d, *J* = 4.7 Hz, 1H), 4.91 (d, *J* = 4.7 Hz, 1H), 4.12 (dd, *J* = 2.6, 11.4 Hz, 1H), 3.40 (dd, *J* = 7.4, 11.4 Hz, 1H), 3.26 (m, 1H), 2.87 (m, 2H), 2.73 (dd, *J* = 6.8, 8.2 Hz, 1H), 2.58 (m, 1H), 1.86–1.71 (m, 2H), 1.57–1.40 (m, 2H). ^{13}C NMR ($CDCl_3$) (75 MHz): δ 174.7, 103.3, 78.7, 76.9, 76.5, 76.0, 72.0, 51.1, 49.8, 46.6, 43.8, 28.8, 27.6, 27.4; IR (film) ν_{max}/cm^{-1}: 2994 (C–H), 1742 (C=O), 1230 (C–O), 1150 (C–O); *m/z* APCI (M + H) 227.

^{1}H NMR ($CDCl_3$) (300 MHz) (**22**): δ 5.52 (d, *J* = 6.8 Hz, 1H), 5.03 (d, *J* = 3.8 Hz, 1H), 4.87 (d, *J* = 3.8 Hz, 1H), 3.90 (m, 2H), 3.18 (m, 1H), 2.75 (m, 4H), 1.75–1.69 (m, 2H), 1.55–1.32 (m, 2H). ^{13}C NMR ($CDCl_3$) (75 MHz): δ 174.7, 103.4, 78.7, 76.0, 68.7, 51.0, 49.4, 46.5, 43.7, 27.5, 27.4; IR (film) ν_{max}/cm^{-1}: 2994 (C–H), 1742 (C=O), 1230 (C–O), 1150 (C–O); *m/z* APCI (M + H) 227. HRMS calculated for M + H; $C_{11}H_{15}O_5$; 227.0914.

6.3.3. (3S,3aR,4S,7R,7aS)-3-((3-Methyloxiran-2-yl)methoxy) hexahydro-4,7-epoxyisobenzofuran-1(3H)-one (**23**, **24**)

Synthesised as per the general epoxidation procedure above from (**8**) to afford the title compounds as clear oils (1:1) combined yield (85%); ^{1}H NMR ($CDCl_3$) (300 MHz) (**23**): δ 5.30 (d, *J* = 1.6 Hz, 1H), 4.82 (d, *J* = 4.6 Hz, 1H), 4.70 (d, *J* = 4.6 Hz, 1H), 4.01 (dd, *J* = 2.9, 11.3 Hz, 1H), 3.42 (dd, *J* = 6.8, 11.3 Hz, 1H), 2.89 (m, 3H), 2.56 (dd, *J* = 1.6, 7.9 Hz, 1H), 1.85–1.70 (m, 2H), 1.59–1.46 (m, 2H), 1.32 (d, *J* = 5.0 Hz, 3H). ^{13}C NMR ($CDCl_3$) (75 MHz): δ 175.4, 106.8, 79.7, 79.0, 70.1, 56.7, 51.9, 50.3, 49.6, 28.0, 27.2, 16.6; IR (film) ν_{max}/cm^{-1}: 2985 (C–H), 1752 (C=O), 1226 (C–O), 1153 (C–O); *m/z* (APCI M + H) 241.

^{1}H NMR ($CDCl_3$) (300 MHz) (**24**): δ 5.27 (d, *J* = 1.6 Hz, 1H), 4.82 (d, *J* = 4.6 Hz, 1H), 4.70 (d, *J* = 4.6 Hz, 1H), 3.80 (m, 2H), 2.93 (m, 3H), 2.52 (dd, *J* = 1.6, 7.9 Hz, 1H), 1.84–1.70 (m, 2H), 1.59–1.45 (m, 2H), 1.32 (d, *J* = 5.2 Hz, 3H). ^{13}C NMR ($CDCl_3$) (75 MHz): δ 175.3, 106.6, 79.7, 79.0, 68.1, 56.3, 51.5, 50.2, 49.7, 28.0, 27.2, 16.6; ν_{max}/cm^{-1}: 2985 (C–H), 1752 (C=O), 1226 (C–O), 1153 (C–O); *m/z* (APCI M + H) 241. HRMS calculated for M + H; $C_{12}H_{17}O_5$; 241.1071.

6.3.4. (3R,3aR,4S,7R,7aS)-3-((3-Methyloxiran-2-yl)methoxy) hexahydro-4,7-epoxyisobenzofuran-1(3H)-one (**25**, **26**)

Synthesised as per the general epoxidation procedure above from (**9**) to afford the title compounds as clear oils (1:1) combined yield (26%); ^{1}H NMR ($CDCl_3$) (300 MHz) (**25**): δ 5.52 (d, *J* = 6.8 Hz, 1H), 5.05 (d, *J* = 4.4 Hz, 1H), 4.89 (d, *J* = 4.4 Hz, 1H), 4.03 (dd, *J* = 3.5, 12.0 Hz, 1H), 3.84 (dd, *J* = 2.7, 12.0 Hz, 1H), 3.09 (m, 1H), 2.89 (m, 2H), 2.70 (dd, *J* = 6.8, 8.2 Hz, 1H), 1.84–1.69 (m, 2H), 1.56–1.41 (m, 2H), 1.34 (d, *J* = 5.3 Hz, 3H). ^{13}C NMR ($CDCl_3$) (75 MHz): δ 174.8, 103.4, 78.7, 76.0, 68.2, 56.5, 51.4, 51.0, 46.6, 27.6, 27.4, 16.7; IR (film) ν_{max}/cm^{-1}: 2985 (C–H), 1752 (C=O), 1226 (C–O), 1153 (C–O); *m/z* (APCI M + H) 241.

^{1}H NMR ($CDCl_3$) (300 MHz) (**26**): 5.50 (d, *J* = 6.8 Hz, 1H), 5.05 (d, *J* = 4.4 Hz, 1H), 4.89 (d, *J* = 4.4 Hz, 1H), 3.79 (m, 2H), 2.98 (m, 3H), 2.70 (dd, *J* = 6.8, 8.2 Hz, 1H), 1.84–1.68 (m, 2H), 1.56–1.41 (m, 2H), 1.34 (d, *J* = 5.2 Hz, 3H). ^{13}C NMR ($CDCl_3$) (75 MHz): 174.7, 103.2, 78.7, 76.1, 66.3, 56.1, 51.1, 51.0, 46.6, 27.6, 27.4, 16.7; IR (film) ν_{max}/cm^{-1}: 2985 (C–H), 1752 (C=O), 1226 (C–O), 1153 (C–O); *m/z* (APCI M + H) 241. HRMS calculated for M + H; $C_{12}H_{17}O_5$; 241.1071.

6.3.5. (3S,3aR,4S,7R,7aS)-3-Propan-3-oloxyhexahydro-4,7-epoxyisobenzofuran-1(3H)-one (**27**)

1,3-Propanediol (11.8 mmol, 10 eq) was added to a magnetically stirred solution of (3*S*,3a*R*,4*S*,7*R*,7a*S*)-3-hydroxyhexahydro-4,7-epoxyisobenzofuran-1(3*H*)-one (1.18 mmol, 1 eq) in anhydrous THF (5 mL). To this, a catalytic amount of 4-toluene sulfonic acid (10 mg) was added and the resulting solution was treated with microwave radiation at 80 °C for 1 h at 150 W. The resulting solution was subjected to silica chromatography (2:8 EtOAc:Hexane) to afford the desired analogue as a clear oil (80%); ^{1}H NMR ($CDCl_3$) (300 MHz): δ 5.22 (d, *J* = 1.5 Hz, 1H), 4.74 (d, *J* = 4.5 Hz, 1H), 4.64 (d, *J* = 4.5 Hz, 1H), 3.84 (m, 1H), 3.64 (m, 3H), 2.86 (d, *J* = 7.9 Hz, 1H), 2.70 (br, 1H), 2.44 (dd, *J* = 1.5, 7.9 Hz, 1H), 1.74 (m, 4H), 1.48 (m, 2H); ^{13}C NMR ($CDCl_3$) (75 MHz): δ 177.8, 107.1, 79.7, 79.0, 66.6, 58.9, 50.3, 49.8, 31.6, 28.0, 27.2; IR (film) ν_{max}/cm^{-1}: 3535 (O–H), 2943 (C–H), 1773 (C=O), 1039 (C–O); *m/z* (APCI M + H) 229. HRMS calculated for M + H; $C_{11}H_{17}O_5$; 229.1071.

6.4. General phosphoesterification procedure

Dibutyltin oxide (0.19 mmol, 0.2 eq) was added to a magnetically stirred solution of (3*S*,3a*R*,4*S*,7*R*,7a*S*)-3-propan-3-oloxyhexahydro-4,7-epoxyisobenzofuran-1(3*H*)-one (**27**) (0.95 mmol, 1 eq) in

anhydrous CH_2Cl_2 (10 mL) and stirred for 30 min at room temperature under a nitrogen atmosphere. To this, the desired chlorophosphate (0.95 mmol, 1 eq) and triethylamine (3.41 mmol, 3.6 eq) were added and left to stir for a further 18 h at room temperature under a nitrogen atmosphere. The resulting solution was then quenched with water (15 mL). The organic layer was collected, dried over $MgSO_4$, filtered and concentrated under reduced pressure. The formed clear oil was subjected to silica chromatography (EtOAc:hexanes 1:1) to afford the desired phosphate analogue.

*6.4.1. Phosphoric acid diethyl ester 3-(5-oxo-4,10-dioxa-tricyclo [5.2.1.0]dec-3-yloxy)-propyl ester (**28**)*

Synthesised using the general procedure as above, from diethylchlorophosphate to afford (**28**) as a clear oil (50%); ^{1}H NMR ($CDCl_3$) (300 MHz): δ 5.22 (d, J = 1.4 Hz, 1H), 4.78 (d, J = 4.3 Hz, 1H), 4.66 (d, J = 4.3 Hz, 1H), 4.09 (m, 6H), 3.86 (m, 1H), 3.62 (m, 1H), 2.86 (d, J = 7.9 Hz, 1H), 2.46 (dd, J = 1.4, 7.9 Hz, 1H), 1.92 (m, 2H), 1.73 (m, 2H), 1.52 (m, 2H), 1.30 (t, J = 7.1 Hz); ^{13}C NMR ($CDCl_3$) (75 MHz): δ 175.4, 106.9, 79.6, 79.0, 65.1, 63.4, 63.3, 63.2, 50.3, 49.6, 29.6, 28.0, 27.2, 15.6, 15.5; IR (film) ν_{max}/cm^{-1}: 2943 (C–H), 1773 (C=O), 1269 (P=O), 1038 (C–O); m/z (APCI M + H) 365. HRMS calculated for M + H; $C_{15}H_{26}O_8P$; 365.1360.

*6.4.2. Phosphoric acid 3-(5-oxo-4,10-dioxa-tricyclo[5.2.1.0]dec-3-yloxy)-propyl ester diphenyl ester (**29**)*

Synthesised using the general procedure as above, from diphenylchlorophosphate to afford (**29**) as a clear oil (61%); ^{1}H NMR ($CDCl_3$) (300 MHz): δ 7.34 (m, 4H), 7.20 (m, 6H), 5.16 (d, J = 1.7 Hz, 1H), 4.81 (d, J = 4.8 Hz, 1H), 4.60 (d, J = 4.8 Hz, 1H), 4.33 (m, 2H), 3.86 (m, 1H), 3.59 (m, 1H), 2.86 (d, J = 7.9 Hz, 1H), 2.44 (dd, J = 1.7, 7.9 Hz, 1H), 1.97 (m, 2H), 1.72 (m, 2H), 1.48 (m, 2H); ^{13}C NMR ($CDCl_3$) (75 MHz): δ 175.4, 150.1, 129.3, 124.9, 119.7, 106.8, 79.6, 79.0, 65.1, 64.8, 50.2, 49.6, 29.1, 28.0, 27.3; IR (film) ν_{max}/cm^{-1}: 2961 (C–H), 1774 (C=O), 1590 (Ar), 1489 (Ar), 1222 (P=O), 1190 (C–O); m/z (ACPI M + H) 461. HRMS calculated for $C_{23}H_{26}O_8P$; 461.1360.

*6.4.3. Phosphoric acid 3-(5-oxo-4,10-dioxa-tricyclo[5.2.1.0]dec-3-yloxy)-propyl ester bis-(2,2,2-trichloro-ethyl) ester (**30**)*

Synthesised using the general procedure as above, from bis(2,2,2-trichloroethyl)phosphochloridate to afford (**30**) as a clear oil (78%); ^{1}H NMR ($CDCl_3$) (300 MHz): δ 5.28 (d, J = 1.7 Hz, 1H), 4.85 (d, J = 4.7 Hz, 1H), 4.72 (d, J = 4.7 Hz, 1H), 4.66 (m, 4H), 4.34 (m, 2H), 3.94 (m, 1H), 3.71 (m, 1H), 2.92 (d, J = 7.9 Hz, 1H), 2.51 (dd, J = 1.7, 7.9 Hz, 1H), 2.06 (m, 2H), 1.81 (m, 2H), 1.55 (m, 2H); ^{13}C NMR ($CDCl_3$) (75 MHz): δ 175.9, 107.3, 94.7, 94.6, 80.2, 79.6, 66.1, 66.0, 65.3, 50.9, 50.2, 30.1, 30.0, 28.6, 27.8; IR (film) ν_{max}/cm^{-1}: 2954 (C–H), 1775 (C=O), 1235 (P=O), 918 (C–Cl); m/z (APCI M + H) 571. HRMS calculated for $C_{15}H_{20}Cl_6O_8P$; 568.9021.

*6.4.4. Phosphoric acid mono-[3-(5-oxo-4,10-dioxa-tricyclo[5.2.1.0] dec-3-yloxy)-propyl] ester (**31**)*

Freshly prepared activated Zn–Cu couple consisting of ~16% Cu (0.88 mmol, 2 eq) and acetylacetone (4.38 mmol, 10 eq) was added to a magnetically stirred solution of phosphoric acid 3-(5-oxo-4,10-dioxa-tricyclo[5.2.1.0^{2,6}]dec-3-yloxy)-propyl ester bis-(2,2,2-trichloro-ethyl) ester (**30**) (0.44 mmol, 1 eq) in anhydrous DMF (3 mL) under a nitrogen atmosphere. The resultant solution was heated for 2 h at 55 °C during which the red Zn–Cu couple dissolved and turned green. At the conclusion of this period, Chelex resin (10 mL settled volume in 15 mL water: 30 mL MeOH) was added and stirred for a further hour. The reaction was filtered through celite and washed with a small amount of water. The solvent was then removed *in vacuo* to afford the desired phosphate analogue (**31**) as a clear oil (68%); ^{1}H NMR (D_2O) (300 MHz): δ 5.49 (d, J = 1.6 Hz, 1H), 4.82 (d, J = 4.8 Hz, 1H), 4.78 (d, J = 4.8 Hz, 1H), 3.86–3.61 (m, 4H), 3.15 (d, J = 8.1 Hz, 1H), 2.64 (dd, J = 1.6, 8.1 Hz, 1H), 1.75–1.72 (m, 2H), 1.66–1.49 (m, 4H); ^{13}C NMR (D_2O) (75 MHz): δ 176.5, 106.9, 79.6, 78.4, 65.2, 63.8, 51.3, 49.8, 29.9, 28.7, 27.9; IR (thin film) cm^{-1}: 3510 (O–H), 1773 (C=O), 1243 (P=O), 1082 (C–O); LRMS (APCI M-2): 306. HRMS calculated for $C_{11}H_{18}O_8P$; 309.0734.

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Appendix A. Supplementary material

Supplementary material associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.ejmech.2012.06.010.

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