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FULL PAPER

Synthesis and cytotoxicity of octahydroepoxyisoindole-7-carboxylic acids and norcantharidin-amide hybrids as norcantharidin analogues

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Abstract: Octahydroepoxyisoindole analogues (**7a-n**) of norcantharidin were accessed through a Diels-Alder reaction of an amine substituted furan with maleic anhydride and subsequent reduction of the bicycle[2.2.1]heptane olefin. Despite retention of the carboxylate and the ether bridgehead known to impart cytotoxic activity with norcantharidin, none of these analogues displayed noteworthy cytotoxicity against the 11 cell lines examined herein: HT29 (colon); MCF-7 (breast); A2780 (ovarian); H460 (lung); A431 (skin); Du145 (prostate); BE2-C (neuroblastoma); SJ-G2 and U87 (glioblastoma); MIA (pancreatic); and SMA (spontaneous murine astrocytoma). The incorporation of an amino substituted system post synthesis of norcantharidin afforded facile access to an acid / amide substituted norcantharidin analogues **13-26**. Of these only **13c**, **24-26** displayed sufficient activity at the initial 25 μM compound screening dose to warrant full growth inhibition evaluation. Common to these analogues was the presence of a 4-biphenyl moiety, and in particular 3-(2-(furan-2-ylmethyl)-3-(4-biphenylamino)-3-oxopropylcarbamoyl)-7-oxabicyclo[2.2.1]heptane-2-carboxylic acid **13c** and 3-(2-(pyrrole-2-ylmethyl)-3-(4-biphenylamino)-3-oxopropylcarbamoyl)-7-oxabicyclo[2.2.1]heptane-2-carboxylic acid **24** displayed high levels of cytotoxicity returning GI_{50} values of 15 nM (HT29) to 2.9 μM (U87) and 17 nM (SMA) to 2.8 μM (U87) respectively. These represent the most cytotoxic norcantharidin analogues reported to date.

Introduction

Adult members of the Meloidae family of Coleoptera (beetles) deter attacks from many predators by discharging droplets of cantharidin-laden blood reflexively from their hind leg joints.[1,2] These cantharidin (**1**) rich secretions are also used as a copulation gift to protect the fertilised eggs from predation (Figure 1).[3,4]

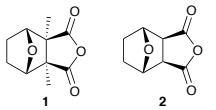


Figure 1. Chemical structures of cantharidin (**1**) and the demethylated analogue norcantharidin (**2**).

The dried body of the Mylabris beetle which contains cantharidin, has a long history of use in Chinese traditional medicine for treatment of dermal conditions and tumours with the first chemotherapeutic application reported in 1264.[4] Cantharidin and the demethylated analogue norcantharidin (**2**) are potent inhibitors of the serine/threonine protein phosphatases, especially protein phosphatases 1 and 2A (PP1 and PP2A) (Figure 1).[5,6] The interplay between protein kinases, which predominately phosphorylate serine and threonine residues, and phosphatases, which remove phosphate moieties, modulates the vast majority of cellular signal transduction events including neurotransmission, muscle contraction, glycogen synthesis, T-cell activation, and cell proliferation.[5,6]

Nonetheless, clinical development of cantharidin has been curtailed by its nephrotoxicity, and current use in Western medicine has been limited to topical applications.[7,8] However, the demethylated norcantharidin (**2**) displays no such nephrotoxicity issues.[9]

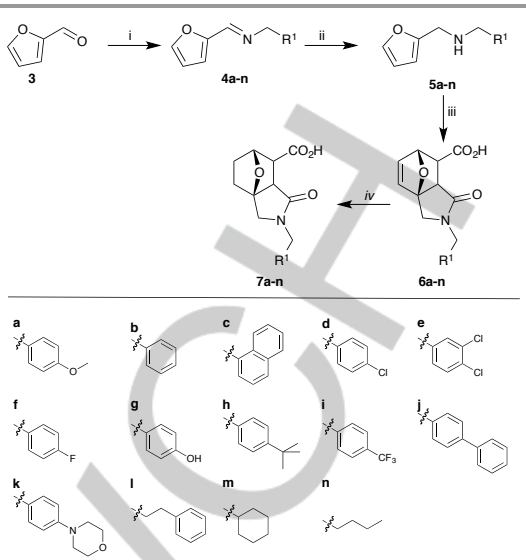
Rapid access to norcantharidin is afforded via a room temperature Diels-Alder reaction of furan and maleic anhydride followed by hydrogenation of the 5,6-olefin. This active anhydride is then amenable to a range of other synthetic transformations. Over the past decade, we and others have exploited the facile access to the 7-oxa-bicyclo[2.2.11]heptane scaffold in drug discovery programs geared towards developing cytotoxic agents.[10-18] Norcantharidin analogues also show promise against *Plasmodium falciparum*,[19] and *Haemonchus contortus*.[20]

Members of the norcantharidin class of compounds includes the benzoyloxymethyl-substituted norcantharidins,[21] the acid amides,[22-24] anhydride modified ethers,[25,26] norcantharimides,[13,27-29] the bis-norcantharimides,[24,30] the tetracyclic norcantharimides,[13] along with the tetracyclic-bis-norcantharimides.[30]

Having recently developed an expedient flow chemistry approach to substituted furans, which gave rise to a series of analogues with moderate to good levels of cytotoxicity across a panel of cancer cell lines,[31] we rationalised that incorporation of these furans with maleic anhydride might afford access to novel norcantharidin analogues with enhanced cytotoxicity. The outcomes of these investigations are reported herein.

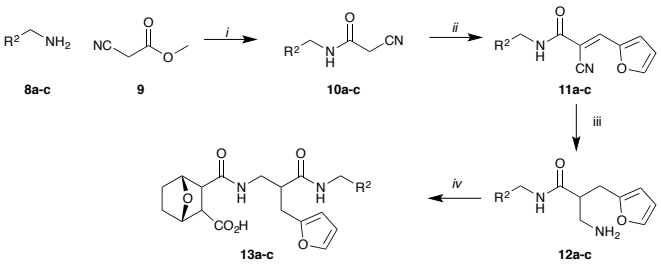
Results and Discussion

Thus far few modifications of the norcantharidin scaffold have resulted in retention or enhancement of the cytotoxicity associated with this family of compounds. It is known that removal of the 7-O atom and modifications to the 5,6-bridge of the bicyclo[2.2.1] moiety reduce analogue potency.[32,33] Based on our prior development of norcantharidin analogues we envisaged two potentially facile routes to modified norcantharidins; *Route A* would afford a family of octahydroepoxyisoindole-7-carboxlic acids (**7a**-**n**) (Scheme 1),[34] and *Route B* would result in the incorporation of furanyl amines into a second series of novel norcantharidin analogues (**13a**-**c**, Scheme 2).[35] *Route B* offered the possibility of developing chimeric molecules combining features from our prior reports of cytotoxic norcantharidin analogues and cytotoxic acrylamide analogues.[36-38] We first explored the synthesis of a focused library of octahydroepoxyisoindole-7-carboxylic acids (**7a**-**n**). Synthesis commenced with treatment of furfural (**3**) with a range of amines (see Table 1 for details) to afford imines (**4a**-**n**) which were subjected to H-cube flow reduction using 10%-Pd/C and 50 bar H_2 at a 1.0 mL min^{-1} flow rate and this effected smooth conversion to the furanyl amines **5a**-**n** in good to excellent yields. Diels-Alder addition with maleic anhydride at room temperature gave the initial Diels-Alder product that underwent an intramolecular amine attack of the anhydride to afford the hexahydroepoxyisoindole-7-carboxylic acids **6a**-**n**, which were readily reduced using 10%-Pd/C and 50 bar H_2 at a 1.0 mL min^{-1} flow rate to give a focused library of octahydroisoindolene-7-carboxylic acids, **7a**-**n**. Given our interest in the potential cytotoxicity of norcantharidin analogues we screened this focused library against our panel of eleven cancer cell lines: HT29; MCF-7 (breast); A2780 (ovarian); H460 (lung); A431 (skin); Du145 (prostate); BE2-C (neuroblastoma); SJ-G2 and U87 (glioblastoma); MIA (pancreatic); SMA (spontaneous murine astrocytoma).[31] In this instance, no analogue displayed noteworthy cytotoxicity at the initial 25 μM screening concentration. This suggests that although the oxctahydroisoindolene-7-carboxylic acids (**7a**-**n**) are structurally related to norcantharidin, and are interesting scaffolds, they are not promising for the development of cytotoxic agents. As such no analogue proceeded to a full dose-response evaluation, as such exploration of this family of compound ceased at this point, and our attention turned to alternative analogues. These findings are in keeping with prior reports of a highly prescriptive norcantharidin analogue cytotoxicity pharmacophore.[32,33,39,40]



Scheme 1. Reagents and conditions. *Route A*: (i) furan-2-carbaldehyde (**3**), amine in MeOH [0.05 M], rt, 0.5 h; (ii) imine (**4a-n)** in MeOH [0.05 M], H-Cube Pro™, 10% Pd/C, 50 bar H_2, 50 °C, with a flow rate of 1.0 mL.min^{-1}; (iii) maleic anhydride, diethyl ether, rt, 24 h; (iv) hexahydroisoindole-7-carboxylic acid (**6a-n**) in MeOH [0.05 M], H-Cube Pro™, 10% Pd/C, 50 bar H_2, 50 °C, with a flow rate of 1.0 mL.min^{-1}.

Accordingly, we turned our attention to the synthesis of a series of acid-amide based norcantharimides. In designing these proposed analogues, we were aware of recent efforts that coupled norcantharidin with cis-platin to produce hybrid drugs with good efficacy, this suggested that additional norcantharidin-hybrids may also elicit a favourable cytotoxic response.[41] As we have previously developed a series of highly cytotoxic acrylamides,[36-38] we chose to explore the development of norcantharidin-acrylamide like chimeric compounds. To this end, and with regard to the side chains installed in the isoindolene analogues above, we synthesized a library of furan-based cyanoamides (**13a**-**c**) (Scheme 2).

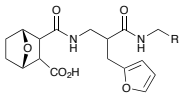


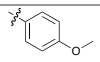
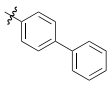
Scheme 2. Reagents and conditions: *Route B*: (i) MeOH, μw, 200 W, 120 °C, 0.5 h; (ii) cyanoamide (**10a-c**), piperidine (cat.), EtOH, rt, 1 h; (iii) acrylamide (**11a-c**) in MeOH [0.05 M], RaNi, 10 bar H_2, 50 °C, with a flow rate of 1.0 mL.min^{-1}; (iv) acetone, rt, 4 h.

Three amines were selected in the development of the first norcantharidin based library, **13a**-**c**: 4-methyoxybenzylamine (**8a**), 4-methylbenzylamine (**8b**) and 4-phenylbenzylamine (**8c**). Treatment of **8a**-**c** with methyl cyanoacetate under microwave irradiation afforded cyanoamides (**10a**-**c**) in good yields. Subsequent Knoevenagel condensation with furan gave **11a**-**c**; selective flow reduction of the olefin and cyano moieties installed the primary amines in **12a**-**c**,[35] which on stirring with norcantharidin gave the norcantharidin-amide analogues **13a**-**c**. The ^{1}H and ^{13}C NMR spectra of **13a**-**c** showed clear evidence of

diastereomers, but given the early stage of this study, no attempt was made to separate. We also note that in two separate studies on norcantharidin analogues, no discernible difference was noted between purified stereoisomers.[42,43] These three compounds, **13a-c**, comprise Library B and the outcomes of the initial cytotoxicity screening against our panel of eleven cell lines at 25 μM compound concentration is presented in Table 1.

Table 1. Evaluation of the cytotoxicity of 7-oxa-bicyclo[2.2.1]heptane derivatives **13a-c** (Library B) against a panel of eleven cancer cell lines. Values are the percentage of growth inhibition at 25 μM drug concentration.

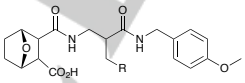


R	HT29[a]	U87[b]	MCF-7[c]	A2780[d]	H460[e]	A431[f]	Du145[g]	BE2-C[h]	SJ-G2[b]	MIA[i]	SMA[j]
 **13a**	14 ± 7	21 ± 4	33 ± 3	14 ± 5	<10	<10	25 ± 4	24 ± 6	20 ± 4	11 ± 2	10 ± 8
 **13b**	<10	26 ± 4	16 ± 5	<10	<10	<10	17 ± 0.4	16 ± 6	21 ± 7	<10	<10
 **13c**	88 ± 4	81 ± 9	57 ± 2	58 ± 2	67 ± 3	82 ± 1	60 ± 5	56 ± 4	43 ± 10	87 ± 2	71 ± 7

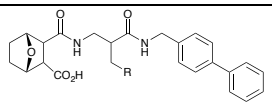
[a] HT29 (colon carcinoma); [b] U87 and SJ-G2 (glioblastoma); [c] MCF-7 (breast carcinoma); [d] A2780 (ovarian carcinoma); [e] H460 (lung carcinoma); [f] A431(skin carcinoma); [g] Du145 (prostate carcinoma); [h] BE2-C (neuroblastoma); [i] MIA (pancreatic carcinoma); [j] SMA (spontaneous murine astrocytoma).

Of the three analogues in Library B, only biphenyl substituted **13c** showed promising levels of cytotoxicity with >80% growth inhibition against HT29, U87 and A431 cell lines and modest activity (40-80% growth inhibition) against the remaining cell lines. Based on this promising activity we expanded the scope of the furan moieties, which were accessed from the corresponding furan aldehydes as per Scheme 2. The detail of the additional furan analogues and outcomes of the cytotoxicity screening of these analogues, Library C **14**-**19**, is presented in Table 2. This library spanned modified furans and exemplars of the parent amines **8a**-**c**.

Table 2. Evaluation of the cytotoxicity of 7-oxa-bicyclo[2.2.1]heptane derivatives **14**-**19** (Library C) against a panel of eleven cancer cell lines. Values are the percentage of growth inhibition at 25 μM drug concentration.



R	HT29[a]	U87[b]	MCF-7[c]	A2780[d]	H460[e]	A431[f] GI_{50} (μM)	Du145[g]	BE2-C[h]	SJ-G2[b]	MIA[i]	SMA[j]
 **14**	30 ± 7	22 ± 4	<10	29 ± 1	18 ± 2	36 ± 16	<10	<10	20 ± 3	14 ± 8	10 ± 11
 **15**	12 ± 5	26 ± 3	25 ± 4	13 ± 4	<10	<10	22 ± 4	15 ± 1	17 ± 6	<10	<10
 **16**	10 ± 3	30 ± 3	31 ± 5	13 ± 3	<10	<10	23 ± 2	12 ± 3	24 ± 3	11 ± 5	<10
 **17**	28 ± 2	19 ± 1	1 ± 11	27 ± 2	16 ± 1	32 ± 21	6 ± 5	<10	23 ± 4	11 ± 10	10 ± 9

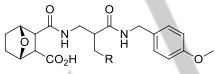
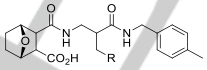
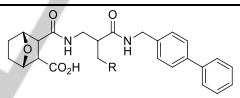
											
 **18**	26 ± 6	21 ± 10	15 ± 4	39 ± 2	27 ± 7	38 ± 19	9 ± 5	<10	36 ± 5	15 ± 4	35 ± 19
 **19**	36 ± 7	18 ± 5	23 ± 4	38 ± 6	19 ± 5	43 ± 15	5 ± 21	<10	<10	10 ± 3	37 ± 16

[a] HT29 (colon carcinoma); [b] U87 and SJ-G2 (glioblastoma); [c] MCF-7 (breast carcinoma); [d] A2780 (ovarian carcinoma); [e] H460 (lung carcinoma); [f] A431(skin carcinoma); [g] Du145 (prostate carcinoma); [h] BE2-C (neuroblastoma); [i] MIA (pancreatic carcinoma); [j] SMA (spontaneous murine astrocytoma).

As can be seen from the data presented in Table 2, no potency enhancement was observed, with **14-19** displaying lower growth inhibition than the lead **13c** across all cell lines evaluated. This was most evident with **18** and **19**, based on the most active parent amine **8c**. These data suggest that additional substituents at the furan moiety are detrimental to growth inhibition in this panel of cell lines.

As modifications to the furan moiety adversely affected the observed cytotoxicity we next examined the effect of bioisosteric modifications of the furan moiety through the synthesis of selected pyrrole and thiophene analogues. Synthesis was conducted as per Scheme 2 commencing from pyrrole and thiophene carboxaldehydes. This gave Library D comprising analogues **20-26**, and the cytotoxicity screening results are shown in Table 3.

Table 3. Evaluation of the cytotoxicity of 7-oxa-bicyclo[2.2.1]heptane derivatives **20-26** (Library D) against a panel of eleven cancer cell lines. Values are the percentage of growth inhibition at 25 μM drug concentration.

R	HT29[a]	U87[b]	MCF-7[c]	A2780[d]	H460[e]	A431[f] GI_{50} (μM)	Du145[g]	BE2-C[h]	SJ-G2[b]	MIA[i]	SMA[j]
											
 **20**	<10	27 ± 2	11 ± 4	<10	<10	<10	<10	<10	16 ± 2	<10	<10
 **21**	28 ± 7	18 ± 5	15 ± 9	31 ± 4	18 ± 10	36 ± 18	13 ± 10	<10	22 ± 4	10 ± 7	12 ± 12
											
 **22**	31 ± 6	27 ± 8	18 ± 4	28 ± 4	18 ± 10	41 ± 15	<10	<10	20 ± 7	<10	20 ± 7
 **23**	29 ± 8	20 ± 5	<10	31 ± 3	20 ± 3	34 ± 15	12 ± 2	<10	22 ± 2	12 ± 1	10 ± 7
											
 **24**	86 ± 3	77 ± 7	62 ± 3	57 ± 3	71 ± 3	81 ± 2	65 ± 5	54 ± 4	59 ± 4	87 ± 3	70 ± 7
 **25**	79 ± 2	74 ± 4	60 ± 2	60 ± 1	57 ± 2	86 ± 1	62 ± 2	77 ± 2	38 ± 2	85 ± 1	69 ± 2
 **26**	92 ± 2	>100	54 ± 4	64 ± 6	78 ± 3	47 ± 11	14 ± 5	>100	>100	79 ± 7	73 ± 8

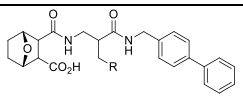
[a] HT29 (colon carcinoma); [b] U87 and SJ-G2 (glioblastoma); [c] MCF-7 (breast carcinoma); [d] A2780 (ovarian carcinoma); [e] H460 (lung carcinoma); [f] A431(skin carcinoma); [g] Du145 (prostate carcinoma); [h] BE2-C (neuroblastoma); [i] MIA (pancreatic carcinoma); [j] SMA (spontaneous murine astrocytoma).

The pyrrole (**20**, **22**) and thiophene (**21**, **23**) analogues developed from cyanoamides **10a** and **10b**, were essentially inactive across the cell line panel examined. Analogues **24-26** based on the biphenyl cyanoamide **10c**, returned more promising levels of cytotoxicity with pyrrole (**24**) at 54-87%; thiophene (**25**) at 38-86%; and indole (**26**) at 47-100% growth

inhibition. Attempts to enhance the cytotoxicity of thiophene based analogues through the introduction of a 4-triifluoromethyl or a diphenylmethamine moiety was unsuccessful.

From the analogues developed herein, the biphenyl substituted **13c** and **24-26** showed sufficient activity to warrant full dose response evaluation and this data is presented in Table 4.

Table 4. Evaluation of the cytotoxicity (GI_{50} (μM)) of 7-oxa-bicyclo[2.2.1]heptane derivatives **13a** and **24-26** against a panel of eleven cancer cell lines.



R	HT29[a]	U87[b]	MCF-7[c]	A2780[d]	H460[e]	A431[f] GI_{50} (μM)	Du145[g]	BE2-C[h]	SJ-G2[b]	MIA[i]	SMA[j]
 **13c**	0.015 ± 0.004	2.9 ± 1.3	0.027 ± 0.001	0.13 ± 0.09	1.6 ± 0.71	0.045 ± 0.02	0.026 ± 0.003	0.029 ± 0.01	-[k]	0.056 ± 0.022	0.11 ± 0.091
 **24**	0.056 ± 0.01	2.8 ± 1.6	0.036 ± 0.007	0.062 ± 0.02	0.2 ± 0.11	0.065 ± 0.02	0.029 ± 0.007	0.051 ± 0.01	-	0.10 ± 0.003	0.017 ±0.003
 **25**	0.39 ± 0.11	5.1 ± 1.0	0.49 ± 0.20	4.8 ± 4.1	12 ± 4.1	1.4 ± 0.26	0.79 ± 0.08	1.0 ± 0.20	31 ± 2.5	1.0 ± 0.14	1.7 ± 0.36
 **26**	15 ± 0.000	34 ± 0.58	26 ± 2.0	23 ± 2.3	16 ± 0.33	30 ± 0.67	30 ± 0.33	17 ± 0.67	13 ± 0.88	20 ± 1.2	18 ± 3.7

[a] HT29 (colon carcinoma); [b] U87 and SJ-G2 (glioblastoma); [c] MCF-7 (breast carcinoma); [d] A2780 (ovarian carcinoma); [e] H460 (lung carcinoma); [f] A431(skin carcinoma); [g] Du145 (prostate carcinoma); [h] BE2-C (neuroblastoma); [i] MIA (pancreatic carcinoma); [j] SMA (spontaneous murine astrocytoma); [k] not determined

Analysis of the data presented in Table 4 reveals that these norcantharidin analogues display modest (**26**, 15 – 34 μM) to excellent levels of cytotoxicity (**13c**, **24** and **25**). Furan (**13c**) and pyrrole (**24**) are highly cytotoxic with GI_{50} values ranging from 15 nM (HT29) to 2.9 μM (U87) and 17 nM (SMA) to 2.8 μM (U87) respectively. Only modest activity was noted against the SJ-G2 cell line. The isosteric manipulation of the furan 'O' to the pyrrole 'NH' is well tolerated, but the thiophene 'S' (**25**) displayed poorer activity (>10 fold less active) than the furan and pyrrole analogues **13c** and **24**. However, the introduction of the additional phenyl moiety with indole **26** had a detrimental effect on cytotoxicity. This is consistent with the effects of additional substituents on the furan core observed earlier (Table 2) suggesting a size restriction in this region.

Cantharidin reduces cell viability in a time, concentration and cell line dependent manner. Clinically cantharidin stimulates the production of white blood cells by bone marrow contrasting most other anticancer drugs. It is widely held that cantharidin, and its analogues, elicit their anticancer effects through the inhibition of PP2A. Others and we have shown that cantharidin analogues transiently accelerate cells through the S-phase of the cycle leading, resulting in cell defects and ultimately cell death. Within the known series of cantharidin analogues, those possessing at least one carboxylic acid moiety are known to inhibit both PP1 and PP2A, and while not the focus of this body of work, we have previously reported that analogues similar to those described herein are potent PP1 and PP2A inhibitors, e.g. **27** and **28** (Figure 2). The cytotoxicity data determined with the norcantharidin analogues reported herein is consistent with the inhibition of protein phosphatases (but not confirmed by PP1 and PP2A inhibition studies), and consistent with all other literature reports to this effect.[39,40]

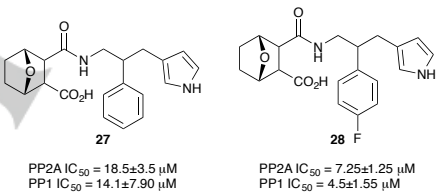


Figure 2. Example norcantharidin analogues **27** and **28** with the PP1 and PP2A inhibition data shown.

Conclusions

Rapid modification of the norcantharidin scaffold is achievable, but not all modifications give rise to notable levels of cytotoxicity. The incorporation of furanyl amine moieties prior to the Diels-Alder addition of maleic anhydride afforded a family of octahydroepoxyisoindoles (**7a-n**) that while retaining the oxa-bicyclo bridgehead and free carboxylate, known to impart cytotoxicity in related norcantharidin analogue, the inclusion of the fused lactam abolished cytotoxicity. However, the ease of synthetic access to this rigid scaffold may be of interest in other medicinal chemistry programs. Flow reduction of a family of cyanoamides to the corresponding amines (**11a-c**), and their subsequent reaction with norcantharidin gave rise to a novel series of norcantharidin analogues. The initial library development and screening revealed the presence of a terminal 4-biphenyl moiety (**13c**) imparted good levels of cytotoxicity, but that additional substituents on the pendant furan moiety were detrimental to activity. Isosteric modification of the furan moiety gave rise to the pyrrole (**24**), thiophene (**25**) and indole (**26**) analogues, which proceeded to full dose-response evaluation. Of these four norcantharidin analogues, **13c** and **24** showed highly promising levels of cytotoxicity, typically in the sub 100 nM potency range except against U87, A2780, H460 and SMA for

13c; and U87 and H460 for **24**. Current focus is directed towards isomer isolation which appears less onerous than initially anticipated. While analogues such as **24** contain 5 chiral centres, current analysis suggests a degree of stereoselectivity is imparted throughout the synthetic protocol. For example, RP-HPLC analysis of a racemic sample of **24** indicated the presence of only 3 diastereomers which are readily separable via preparative HPLC (Supporting Information). Combined, these represent the most cytotoxic norcantharidin analogues reported thus far, and they are promising leads for further development.

Experimental Section

In vitro growth inhibition assay

All test agents were prepared as stock solutions (20 mM) in dimethyl sulfoxide (DMSO) and stored at -20 °C. All cell lines were cultured in a humidified atmosphere 5% CO_2 at 37 ºC. The cancer cell lines were maintained in Dulbecco's modified Eagle's medium (DMEM) (Trace Biosciences, Australia) supplemented with 10% foetal bovine serum, 10 mM sodium bicarbonate, penicillin (100 IU/mL), streptomycin (100 µg/mL), and glutamine (2 mM). 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-dimethylthiazol-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 25 µM. A value of 100% is indicative of complete 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.[27]

All reagents were purchased from Sigma Aldrich and were used without purification, with the exception of furfural, which was distilled from glass prior to use. Solvents were bulk, and distilled from glass prior to use.

^{1}H and ^{13}C NMR spectra were recorded on a Brüker Advance™ AMX 400 MHz spectrometer at 400.1 and 100.1 MHz, respectively. Chemical shifts (δ) are reported in parts per million (ppm) measured to relative the internal standards. 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 acetonitirle:H_2O with 0.1 % formic acid. Gas chromatography-mass spectrometry (GC-MS) was performed on a Shimadzu GC-MS QF2010 EI/NCI System equipped with a ZB-5MS capillary column of 5% phenylarylene stationary phase.

Melting points were recorded on a BUCHI Melting Point M-565. IR spectra were recorded on a PerkinElmer Spectrum Two™ FTIR Spectrometer. Thin layer chromatography (TLC) was performed on Merck 60 F_{254} pre-coated aluminium plates with a thickness of 0.2 mm. Microwave irradiations were conducted using a CEM Discover® Benchmate microwave, and hydrogenations were performed either using a ThalesNano H-Cube™ or a ThalesNano H-CubePro™ continuous-flow hydrogenation reactor. All reactions were passed through the H-Cube™ reactor once, unless otherwise specified.

Synthesis detail for the precursor compounds **5a**-**5n**, **10a**-**10e**, **11a**-**11r** and **12a**-**12r**, and final compounds **7a**-**n** is provided in the supporting material.

3-(2-(Furan-2-ylmethyl)-3-(4-methoxybenzylamino)-3-oxopropylcarbamoyl)-7-oxabicyclo[2.2.1]heptane-2-carboxylic acid (13a).[4]

3-Amino-2-(furan-2-ylmethyl)-*N*-(4-methoxybenzyl)propanamide (**12a**) and norcantharidin (**2**) (1.4 equivalents) were dissolved separately in acetone (2 x 10 mL) and the solutions were added together and the reaction was left to stir at room temperature for 4 hours. The solution was filtered and washed with cold acetone and dried under suction to afford **13a** as a white solid (0.30 g, 78 %); mp 168–170 °C. LRMS (ESI$^-$) m/z 455 [M-H]$^-$. HRMS calc'd for $C_{24}H_{27}N_2O_7$, [M-H]$^-$ 455.1824; Found 455.1830. ^{1}H NMR (DMSO-d_6, 400 MHz): δ 11.91 (br s, OH), 8.21 (t, *J* = 5.4 Hz, NH), 7.49 (d, *J* = 1.0 Hz, NH), 7.40 (t, *J* = 5.9 Hz, NH), 7.07 (d, *J* = 8.6 Hz, 2H), 6.83 (d, *J* = 8.6 Hz, 2H), 6.34 (dd, *J* = 3.0, 1.9 Hz, 1H), 6.08 (d, *J* = 2.9 Hz, 1H), 4.73 (d, *J* = 3.7 Hz, 1H), 4.40 (d, *J* = 3.8 Hz, 1H), 4.22 (dd, *J* = 14.9, 6.0 Hz, 1H), 4.11 (dd, *J* = 14.9, 5.6 Hz, 1H), 3.72 (s, 3H), 3.24 – 3.18 (m, 1H), 3.07–2.99 (m, 1H), 2.83 (s, 2H), 2.77–2.68 (m, 3H), 1.57–1.40 (m, 4H). ^{13}C NMR (DMSO-d_6, 101 MHz) δ 173.0, 172.7, 171.3, 158.6, 153.7, 141.9, 131.7, 128.9, 114.0, 110.8, 106.6, 79.3, 77.2, 55.5, 53.6, 52.0, 45.2, 42.0, 41.3, 29.2, 28.8, 28.6. IR (cm^{-1}) 3295 (NH), 3091 (OH), 2985, 2937 (CH), 1692 (CO), 1651 (C=C), 1562, 1514 (NH bend), 1302 (CH_3), 1247 (CO), 818 (*p*-Ph).

3-(2-(Furan-2-ylmethyl)-3-(4-methylbenzylamino)-3-oxopropylcarbamoyl)-7-oxabicyclo[2.2.1]heptane-2-carboxylic acid (13b).[4]

Synthesised as described as for **13a** from 3-amino-*N*-(4-methylbenzylamino)-2-((5-methylfuran-2-yl)methyl)propanamide (**12b**) and norcantharidin (**2**) to afford **13b** as a white solid (diastereomers collected in a 1:1 ratio) (0.42 g, overall yield 60%); mp 166–167 °C. LRMS (ESI$^-$) m/z 439 [M-H]$^-$. HRMS calc'd for $C_{24}H_{27}N_2O_6$, [M-H]$^-$ 439.1875; Found 439.1878. ^{1}H NMR (DMSO-d_6, 400 MHz): δ 11.92 (br s, OH), 8.37 (t, *J* = 5.6 Hz, NH), 8.22 (t, *J* = 5.5 Hz, NH)*, 7.62 (t, *J* = 5.6 Hz, NH), 7.42 (t, *J* = 5.4 Hz, NH)*, 7.12–7.01 (m, 4H), 6.34 (s, 1H), 6.10 (dd, *J* = 12.0, 2.7 Hz, 1H), 4.72 (d, *J* = 10.0 Hz, 1H), 4.41 (s, 1H), 4.27–4.13 (m, 2H), 3.25–3.21 (m, 2H), 3.06–3.03 (m, 2H), 2.85–2.68 (m, 5H), 2.26 (s, 3H), 1.56–1.40 (m, 4H). ^{13}C NMR (DMSO-d_6, 101 MHz,) δ 173.0, 172.9*, 172.9, 172.8*, 171.3, 171.2*, 153.7, 153.7*, 141.9, 141.9*, 136.8, 136.7*, 136.1, 136.0*, 129.2, 127.6*, 110.8, 106.5, 106.6*, 79.3, 79.2*, 77.2, 77.2*, 53.6, 53.3*, 52.0, 51.7*, 45.2, 45.0*, 42.3, 41.3, 31.2, 29.4, 29.2*, 28.9, 28.8*, 28.8, 28.6*, 21.1. IR (cm^{-1}) 3306 (NH), 3001 (OH), 2984, 2947, 2919, 2871 (CH), 1729 (CO), 1692 (CO), 1644 (C=C), 1537 (NH bend), 1381 (CH_3), 1251 (C-O), 1180 (C-O), 835 (*p*-C Ph), 738 (CH_2 bend). *Diastereomers peaks.

3-(2-(Furan-2-ylmethyl)-3-(4-biphenylamino)-3-oxopropylcarbamoyl)-7-oxabicyclo[2.2.1]heptane-2-carboxylic acid (13c).

Synthesised as described as for **13a** from 3-amino-*N*-(4-biphenylamino)-2-((5-methylfuran-2-yl)methyl)propanamide (**12c**) and norcantharidin (**2**) to afford **13c** as a white solid (diastereomers collected in a 1:1 ratio) (0.04 g, overall yield 44%); mp 180 °C. LRMS (ESI$^-$) m/z 501 [M-H]$^-$. HRMS calc'd for $C_{29}H_{29}N_2O_6$, [M-H]$^-$ 501.2031; Found 501.2039. ^{1}H NMR (DMSO-d_6, 400 MHz): δ 11.89 (br s, 1H), 8.48 (t, *J* = 5.8 Hz, 1H), 8.33 (t, *J* = 5.7 Hz, 1H), 7.64 (d, *J* = 8.2 Hz, 2H), 7.57 (d, *J* = 7.9 Hz, 2H), 7.52 (d, *J* = 1.0 Hz, 1H), 7.45 (d, *J* = 7.7 Hz, 2H), 7.35 (d, *J* = 7.4 Hz, 1H), 7.25 (t, *J* = 8.0 Hz, 2H), 6.39–6.33 (m, 1H), 6.13 (dd, *J* = 11.2, 2.9 Hz, 1H), 4.73 (d, *J* = 9.1 Hz, 1H), 4.46–4.42 (m, 1H), 4.34–4.22 (m, 2H), 3.25 (d, *J* = 6.7 Hz, 1H), 3.09–3.02 (m, 1H), 2.80 (dd, *J* = 24.0, 14.1 Hz, 5H), 1.56–1.43 (m, 4H). ^{13}C NMR (DMSO-d_6, 101 MHz): δ 173.1, 173.0, 171.3, 153.6, 141.9, 140.5, 139.2, 139.0, 129.4, 128.2, 127.8, 127.0, 126.9, 110.8, 106.6, 79.2, 77.3, 53.6, 53.3*, 51.7, 45.2, 45.0*, 42.2, 41.3, 29.4, 28.8*. IR (cm^{-1}) 3306 (NH), 3022 (OH), 2988, 2984, 2915, 2870 (CH), 1698 (CO), 1642 (C=C), 1555, 1490 (NH bend), 1243, 1206 (CO), 1178 (CO), 806 (*p*-Ph). *Diastereomers peaks.

3-[(5-Methylfuran-2-yl)methyl]-3-(4-methylbenzylamino)-3-oxopropylcarbamoyl)-7-oxabicyclo[2.2.1]heptane-2-carboxylic acid (14).

Synthesised as described as for **13a** from 3-amino-*N*-(4-methoxybenzylamino)-2-((5-methylfuran-2-yl)methyl)propanamide (**12d**) and norcantharidin (**2**) to afford **14** as a white solid (diastereomers collected in a 1:3 ratio) (0.09 g, overall yield 29%); mp 163–165 °C. LRMS (ESI$^+$) m/z 471 [M+H]$^+$. HRMS calc'd for $C_{25}H_{31}N_2O_7$, [M-H]$^+$ 471.2126; Found 471.2138. ^{1}H NMR (DMSO-d_6, 400 MHz): δ11.90 (br s, 1H), 8.34 (t, *J* = 5.7 Hz, NH)*, 8.19 (t, *J* = 5.8 Hz, NH), 7.59 (t, *J* = 5.5 Hz, NH)*, 7.38

(t, *J* = 5.8 Hz, NH), 7.09 (dd, *J* = 8.4, 6.4 Hz, 2H), 6.83 (d, *J* = 8.6 Hz, 2H), 5.97–5.90 (m, 2H), 4.75–4.69 (m, 1H), 4.40 (d, *J* = 3.7 Hz, 1H), 4.25–4.08 (m, 2H), 3.72 (s, 3H), 3.25–3.18 (m, 1H), 3.06–2.98 (m, 1H), 2.86–2.61 (m, 5H), 2.18 (s, 3H), 1.59–1.39 (m, 4H). ^{13}C NMR (DMSO-d_6, 101 MHz): 173.0, 172.9*, 172.8*, 171.3, 171.2*, 158.6, 151.8, 151.8*, 150.2, 131.9, 131.7*, 128.9, 114.0, 107.2, 106.7, 79.3, 77.2, 55.5, 53.6, 53.3*, 52.0, 51.7*, 45.1, 44.9*, 42.0, 41.3, 29.3, 29.2*, 28.8, 28.7*, 13.7. IR (cm^{-1}) 3285 (NH), 3090 (OH), 2978, 2941 (CH), 1682 (CO), 1648 (C=C), 1562, 1512 (NH bend), 1301 (CH_3), 1233 (CO), 816 (*p*-C Ph). *Diastereomer peaks.

3-[(5-(Hydroxymethyl)furan-2-yl)methyl]-3-(4-methyoxybenzylamino)-3-oxopropylcarbamoyl)-7-oxabicyclo[2.2.1]heptane-2-carboxylic acid (15).

Synthesised as described as for **14** from 3-amino-2-((5-(hydroxymethyl)furan-2-yl)methyl)-*N*-(4-methoxybenzylamino)propanamide (**12e**) and norcantharidin (**2**) to afford **15** as a white solid (diastereomers collected in a 7:3 ratio) (0.10 g, overall yield 44%); mp 132–136 °C. LRMS (ESI$^-$) m/z 485 [M-H]$^-$. HRMS calc'd for $C_{25}H_{29}N_2O_8$, [M-H]$^-$ 485.1929; Found 485.1940. ^{1}H NMR (DMSO-d_6, 400 MHz): δ 11.96 (br s, 1H), 8.36 (t, *J* = 5.8 Hz, NH), 8.21 (t, *J* = 5.8 Hz, NH)*, 7.62 (t, *J* = 5.6 Hz, NH), 7.40 (t, *J* = 5.8 Hz, NH)*, 7.09 (dd, *J* = 8.5, 6.6 Hz, 2H), 6.83 (d, *J* = 8.6 Hz, 2H), 6.13 (d, *J* = 2.9 Hz, 1H), 6.01 (dd, *J* = 13.4, 3.0 Hz, 1H), 5.10 (br s, 1H), 4.72 (dd, *J* = 12.4, 3.1 Hz, 1H), 4.40 (d, *J* = 3.6 Hz, 1H), 4.31 (s, 2H), 4.25–4.10 (m, 2H), 3.26–3.21 (m, 1H), 3.08–3.01 (m, 1H), 2.84–2.62 (m, 5H), 2.09 (s, 3H), 1.58–1.40 (m, 4H). ^{13}C NMR (DMSO-d_6, 101 MHz) δ 173.0, 172.9, 172.7*, 171.3, 158.6, 154.3, 152.9, 152.9*, 131.8, 131.7*, 128.9, 114.0, 108.0, 107.0, 80.1, 79.3, 79.2*, 77.2, 56.1, 55.5, 53.6, 53.3*, 52.0, 51.7*, 51.2*, 45.0, 44.8*, 42.0, 41.3*, 31.2, 29.4, 29.2*, 28.9, 28.7*, 27.9*. IR (cm^{-1}) 3200 (NH), 3056 (OH), 2988, 2949, 2933, 2912 (CH), 1735 (CO), 1641 (C=C), 1565 (NH bend), 1322 (CH_3), 1249 (CO), 820 (*p*-C Ph). *Diastereomer peaks.

3-[2-(Benzofuran-2-yl)methyl]-3-(4-methoxybenzylamino)-3-oxopropylcarbamoyl)-7-oxabicyclo[2.2.1]heptane-2-carboxylic acid (16).

Synthesised as described as for **14** from 3-amino-2-(benzofuran-2-ylmethyl)-*N*-(4-methoxybenzylamino)propanamide (**12f**) and norcantharidin (**2**) to afford **16** as a white solid (diastereomers collected in a 2:3 ratio) (0.07 g, overall yield 36%); mp 186–188 °C. LRMS (ESI$^-$) m/z 505 [M-H]$^-$. HRMS calc'd for $C_{28}H_{29}N_2O_7$, [M-H]$^-$ 505.1980; Found 505.1982. ^{1}H NMR (DMSO-d_6, 400 MHz): δ 11.91 (br s, 1H), 8.41 (t, *J* = 5.9 Hz, NH), 8.26 (t, *J* = 5.9 Hz, NH)*, 7.69 (t, *J* = 5.8 Hz, 1H), 7.51 (t, *J* = 4.5 Hz, NH), 7.55–7.51 (m, 1H), 7.25–7.17 (m, 2H), 6.96 (dd, *J* = 12.4, 8.6 Hz, 2H), 6.64 (dd, *J* = 8.6, 6.7 Hz, 2H), 6.56 (d, *J* = 10.5 Hz, 1H), 4.73 (dd, *J* = 13.1, 2.8 Hz, 1H), 4.43 (d, *J* = 4.0 Hz, 1H), 4.30–4.19 (m, 1H), 4.08 (qd, *J* = 5.6, 5.2 Hz 1H), 3.67 (d, *J* = 1.8 Hz, 3H), 3.28 (d, *J* = 6.3 Hz, 1H), 3.14–3.05 (m, 1H), 2.99–2.80 (m, 5H), 1.57–1.39 (m, 4H). ^{13}C NMR (DMSO-d_6, 101 MHz) δ 173.0, 172.9*, 172.6, 172.5*, 171.4, 171.3*, 158.5, 157.4, 157.3*, 154.5, 131.7, 131.5*, 129.0, 128.7, 123.8, 123.0, 120.9, 113.9, 111.2, 103.7, 79.3, 79.2*, 77.2, 77.2*, 55.4, 53.6, 53.3*, 52.0, 51.7*, 45.0, 44.8*, 41.9, 41.4*, 29.4, 29.3, 29.1*, 28.9, 28.8*. IR (cm^{-1}) 3280 (NH), 3096 (OH), 2987, 2944, 2920 (CH), 1689 (CO), 1644 (C=C), 1560, 1512 (NH bend), 1312 (CH_3), 1255 (CO), 820 (*p*-C Ph). *Diastereomer peaks.

3-[(5-(Hydroxymethyl)furan-2-yl)methyl]-3-(4-methylbenzylamino)-3-oxopropylcarbamoyl)-7-oxabicyclo[2.2.1]heptane-2-carboxylic acid (17).

Synthesised as described as for **14** from 3-amino-*N*-(4-methylbenzylamino)-2-((5-methylfuran-2-yl)hydroxymethyl)propanamide (**12g**) and norcantharidin (**2**) to afford **17** as a pale yellow solid (diastereomers collected in a 1:1 ratio) (0.18 g, overall yield 58%); mp 129–132 °C. LRMS (ESI$^+$) m/z 471 [M+H]$^+$. HRMS calc'd for $C_{25}H_{29}N_2O_7$, [M-H]$^-$ 471.1980; Found 471.1994. ^{1}H NMR (DMSO-d_6, 400 MHz): δ 11.90 (br s, 1H), 8.37 (t, *J* = 5.8 Hz, NH), 8.23 (t, *J* = 5.8 Hz, NH)*, 7.64 (t, *J* = 5.7 Hz, NH), 7.42 (t, *J* = 5.8 Hz, NH)*, 7.07 (q, *J* = 8.1 Hz, 4H), 6.14 (d, *J* = 2.6 Hz, 1H), 6.02 (dd, *J* = 13.8, 2.9 Hz, 1H), 5.10 (br s, 1H), 4.75–4.70 (m, 1H), 4.41 (d, *J* = 3.0 Hz, 1H), 4.31 (s, 2H), 4.26–4.14 (m, 2H), 3.26–3.19 (m, 1H), 3.05 (dt, *J* = 12.9, 6.4 Hz, 1H), 2.86–2.66 (m, 5H), 2.26 (s, 3H), 1.56–1.41 (m, 4H). ^{13}C NMR (DMSO-d_6, 101 MHz): 173.0, 172.9*, 172.8*, 171.3, 171.2*, 154.3, 152.9, 152.9*, 136.8, 136.7*, 136.1, 136.1*, 129.2, 127.6, 107.9, 107.0, 79.3, 79.2*, 77.2, 77.2*, 56.1, 53.6, 53.3*, 51.9, 51.6*, 45.0, 44.8*, 42.3, 41.3*, 31.2, 29.4, 29.2*, 28.9, 28.8*, 28.7*, 21.1. IR (cm^{-1}) 3250 (NH), 3091 (OH), 2987, 2955, 2916 (CH), 1698 (CO), 1645 (C=C), 1566, 1512 (NH bend), 1320 (CH_3), 1247 (CO), 808 (*p*-C Ph). *Diastereomer peaks.

3-(5-(Hydroxymethyl)furan-2-yl)methyl)-3-(4-biphenylamino)-3-oxopropylcarbamoyl)-7-oxabicyclo[2.2.1]heptane-2-carboxylic acid (18).

Synthesised as described as for **14** from 3-amino-*N*-(4-biphenylamino)-2-((5-methylfuran-2-yl)hydroxymethyl)propanamide (**12h**) and norcantharidin (**2**) to afford **18** as a pale brown solid (diastereomers collected in a 1:1 ratio) (0.10 g, overall yield 38%); mp 151–153 °C. LRMS (ESI$^-$) m/z 531 [M-H]$^-$. HRMS calc'd for $C_{30}H_{31}N_2O_7$, [M-H]$^-$ 531.2137; Found 531.2146. ^{1}H NMR (DMSO-d_6, 400 MHz): δ 11.92 (br s, 1H), 8.47 (t, *J* = 5.9 Hz, 1H), 8.32 (t, *J* = 5.8 Hz, 1H)*, 7.63 (d, *J* = 8.0 Hz, 2H), 7.57 (d, *J* = 7.8 Hz, 2H), 7.45 (d, *J* = 7.8 Hz, 2H), 7.35 (t, *J* = 7.3 Hz, 1H), 7.26 (t, *J* = 7.6 Hz, 2H), 6.16 (d, *J* = 3.0 Hz, 1H), 6.04 (dd, *J* = 12.7, 3.0 Hz, 1H), 4.76–4.71 (m, 1H), 4.46–4.41 (m, 1H), 4.36–4.22 (m, 4H), 3.27–3.21 (m, 1H), 3.09 (dd, *J* = 12.7, 6.5 Hz, 1H), 2.87–2.66 (m, 5H), 1.54–1.39 (m, 4H). ^{13}C NMR (DMSO-d_6, 101 MHz): δ 173.1, 173.0*, 172.9, 172.9*, 171.3, 171.2*, 154.4, 152.9, 152.9*, 140.5, 140.5*, 139.2, 139.1, 139.0*, 129.4, 128.2, 127.8, 127.0, 126.9*, 108.0, 107.1, 79.3, 79.3*, 77.2, 77.2*, 56.2, 53.6, 53.3*, 51.9, 51.6*, 45.1, 44.9*, 42.3, 41.4*, 31.2, 29.4, 29.2*, 28.9, 28.8*, 28.8*. IR (cm^{-1}) 3308 (NH), 3021 (OH), 2988, 2947, 2919 (CH), 1722 (CO), 1690 (CO), 1649 (C=C), 1537 (NH bend), 1381 (CH_3), 1251 (CO), 1120 (CO), 835 (*p*-C Ph), 740 (CH_2).*Diastereomer peaks.

3-[2-(Benzofuran-2-yl)methyl]-3-(4-biphenylamino)-3-oxopropylcarbamoyl)-7-oxabicyclo[2.2.1]heptane-2-carboxylic acid (19).

Synthesised as described as for **14** from 3-amino-*N*-(4-biphenylamino)-2-((5-methylfuran-2-yl)benzofuran)propanamide (**12i**) and norcantharidin (**2**) to afford **19** as pale brown solid (diastereomers collected in a 1:1 ratio) (0.03 g, overall yield 21%); mp 154–157 °C. LRMS (ESI$^-$) m/z 551 [M-H]$^-$. HRMS calc'd for $C_{33}H_{31}N_2O_6$, [M-H]$^-$ 551.2188; Found 551.2196. ^{1}H NMR (DMSO-d_6, 400 MHz): δ 8.57 (t, *J* = 5.9 Hz 1H), 8.42 (t, *J* = 5.9 Hz 1H)*, 7.71 (t, *J* = 5.2 Hz 1H), 7.56 (t, *J* = 6.9 Hz, 3H), 7.46 (dd, *J* = 13.9, 7.1 Hz, 3H), 7.36 (t, *J* = 6.5 Hz, 3H), 7.27–7.18 (m, 2H), 7.12 (dd, *J* = 11.5, 8.2 Hz, 2H), 6.60 (d, *J* = 8.9 Hz, 1H), 4.74–4.69 (m, 1H), 4.51–4.43 (m, 1H), 4.42–4.32 (m, 1H), 4.24–4.11 (m, 1H), 3.13 (d, *J* = 6.5 Hz, 1H), 3.02–2.79 (m, 6H), 1.60–1.35 (m, 4H).^{13}C NMR (DMSO-d_6, 101 MHz): δ 173.1, 173.1*, 172.8, 172.7*, 171.5, 171.4*, 157.4, 154.5, 140.4, 140.4*, 139.1, 138.9, 138.9*, 138.9*, 129.4, 129.0, 128.0, 127.8, 126.9, 126.8*, 123.9, 123.0, 121.0, 111.2, 103.8, 79.6, 79.2*, 79.2*, 77.3, 77.3*, 53.6, 53.4*, 52.2, 51.9*, 49.9, 45.1, 44.9*, 42.2, 41.4*, 29.4, 29.2*, 28.9, 28.8*, 28.4*. IR (cm^{-1}) 3306 (NH), 3020 (OH), 2984, 2947, 2919, 2871 (CH), 1730 (CO), 1680 (C=C), 1537 (NH bend), 1253 (CO), 835 (*p*-C Ph). *Diastereomer peaks.

3-(2-(Pyrrole-2-ylmethyl)-3-(4-methoxybenzylamino)-3-oxopropylcarbamoyl)-7-oxabicyclo[2.2.1]heptane-2-carboxylic acid (20).[4]

2-Cyano-3-(pyrrole-2-yl)-N-(4-methoxybenzyl)acrylamide (**11j**) (0.14 g, 0.5 mmol) was dissolved in sufficient EtOH:EtOAc (1:1) (10 mL) to form a 0.05 M solution. This solution was hydrogenated using the H-Cube Pro™ with a RaNi catalyst, 70 bar H_2 pressure, 70 °C and a flow rate of 1 mL.min^{-1}. The solvent was removed *in vacuo* and to afford 2-((1*H*-pyrrol-2-yl)methyl)-3-amino-*N*-(4-methoxybenzyl)propanamide (**12j**) as a yellow oil (diastereomers collected in a 1:1 ratio) (0.11 g, 77%). The crude product and norcantharidin (**2**) (0.20 g, 1.8 mmol) were dissolved separately in acetone (2 x 10 mL) and the solutions were added together and the reaction was left to stir at room temperature for 4 hours. The solution was filtered and washed with cold acetone and dried under suction to afford **20** as a white solid (0.06 g, overall yield 38%); mp 166–167 °C. LRMS (ESI$^-$) m/z 454 [M-H]$^-$. HRMS calc'd for $C_{24}H_{28}N_3O_6$, [M-H]$^-$ 545.1984; Found 545.1991. ^{1}H NMR (DMSO-d_6, 400 MHz): δ 11.89 (br s, OH), 10.39 (s, NH), 8.20 (t, *J* = 5.5 Hz, NH), 8.07 (t, *J* = 5.3 Hz, NH)*, 7.50 (t, *J* = 5.4 Hz, NH), 7.29 (t, *J* = 5.3 Hz, NH)*, 7.07 (d, *J* = 8.4 Hz, 2H), 6.82 (d, *J* = 8.5 Hz, 2H), 6.55 (s, 2H), 5.90 – 5.75 (m, 2H), 4.71 (s, 1H), 4.39 (s, 1H), 4.23–4.13 (m, 2H), 3.71 (s, 3H), 3.21–3.15 (m, 1H), 3.07 (dd, *J* = 12.0, 5.7 Hz, 1H), 2.86–2.61 (m, 5H), 1.55–1.39 (m, 4H). ^{13}C NMR (DMSO-d_6, 101 MHz): δ 174.0, 173.0, 172.8*, 171.2, 142.1, 142.0*, 140.5, 139.4, 139.1*, 139.0*,

129.4, 128.4, 128.3, 128.2*, 127.8, 127.2, 127.0, 126.9*, 126.0, 124.5, 79.3, 79.2*, 77.2, 53.7, 53.3*, 51.8, 48.4, 48.1*, 42.2, 41.4*, 31.7, 30.4, 29.4, 29.2, 28.9*, 26.8, 22.5, 14.3. IR (cm^{-1}) 3432 (NH), 3306 (NH), 3074 (OH), 2992, 2937, 2882, 2834 (CH), 1685 (CO), 1653 (CO), 1635 (C=C), 1538, 1513 (NH bend), 1302 (CH_3), 1245, 1223 (C-O), 806 (*p*-C Ph). *Diastereomer peaks.

3-(2-(Thiophene-2-ylmethyl)-3-(4-methoxybenzylamino)-3-oxopropylcarbamoyl)-7-oxabicyclo[2.2.1]heptane-2-carboxylic acid (21).[4]

Synthesised as described as for **20** from 3-amino-*N*-(4-methoxybenzylamino)-2-((5-methylthiophene-2-yl)methyl)propanamide (**12k**) and norcantharidin (**2**) to afford **21** as a white solid (diastereomers collected in a 1:1 ratio) (0.1 g, overall yield 41%); mp 158–159 °C. LRMS (ESI^-) m/z 471 $[M-H]^-$. HRMS calc'd for $C_{24}H_{27}N_2O_6S$, $[M-H]^-$ 471.1595; Found 471.1608. ^1H NMR (DMSO-d_6, 400 MHz): δ 11.89 (br s, 1H), 8.33 (t, *J* = 4.7 Hz, NH), 8.20 (t, *J* = 5.6 Hz, NH)*, 7.58 (t, *J* = 5.9 Hz, NH), 7.40 (t, *J* = 6.2 Hz, NH)*, 7.30 (d, *J* = 5.2 Hz, 1H), 7.25 (t, *J* = 6.0 Hz, 1H)*, 7.17 (d, *J* = 8.5 Hz, 2H), 7.03 (t, *J* = 8.2 Hz, 2H), 6.98–6.87 (m, 1H), 6.90–6.74 (m, 3H), 4.72 (d, *J* = 9.9 Hz, 1H), 4.41 (d, *J* = 2.8 Hz, 1H)*, 4.37 (d, *J* = 4.0 Hz, 1H)*, 4.23–4.09 (m, 2H), 3.71 (s, 3H), 3.26–3.17 (m, 1H), 3.05 (dd, *J* = 12.8, 7.5 Hz, 1H), 3.01–2.93 (m, 1H), 2.93–2.66 (m, 4H), 1.55–1.38 (m, 4H). ^{13}C NMR (DMSO-d_6, 101 MHz): 172.9, 172.8, 171.2, 158.5, 142.1, 142.0*, 131.8, 129.0*, 128.9, 127.2, 126.0, 124.4, 114.0, 79.2, 77.3, 55.5, 53.4, 48.0, 42.0, 41.3, 30.4, 29.4, 28.9, 22.5. IR (cm^{-1}) 3310 (NH), 3062 (OH), 2986, 2963, 2920, 2874 (CH), 1695 (CO), 1646 (C=C), 1545, (NH bend), 1382 (CH_3), 1244 (CO), 816 (*p*-C Ph). * Diastereomer peaks.

3-(2-(Pyrrole-2-ylmethyl)-3-(4-methylbenzylamino)-3-oxopropylcarbamoyl)-7-oxabicyclo[2.2.1]heptane-2-carboxylic acid (22).

Synthesised as described as for **20** from 3-amino-*N*-(4-methylbenzylamino)-2-((5-methylpyrrole-2-yl)methyl)propanamide (**12l**) and norcantharidin (**2**) to afford **22** as a pale brown solid (diastereomers collected in a 1:1 ratio) (0.02 g, overall yield 21%); mp 173–176 °C. LRMS (ESI^-) m/z 438 $[M-H]^-$. HRMS calc'd for $C_{24}H_{28}N_3O_5$, $[M-H]^-$ 438.2034; Found 438.2040. ^1H NMR (DMSO-d_6, 400 MHz): δ 10.39 (s, 1H), 8.23 (t, *J* = 5.9 Hz, 1H), 8.10 (t, *J* = 5.7 Hz, 1H), 7.53 (t, *J* = 5.8 Hz, 1H), 7.32 (t, *J* = 5.9 Hz, 1H)*, 7.09–7.01 (m, 4H), 6.56 (d, *J* = 1.5 Hz, 1H), 5.89 (dd, *J* = 5.3, 2.6 Hz, 1H), 5.77 (d, *J* = 9.0 Hz, 1H), 4.76–4.71 (m, 1H), 4.67 (d, *J* = 2.6 Hz, 1H)*, 4.44–4.39 (m, 1H), 4.28–4.16 (m, 2H), 3.23–3.18 (m, 1H), 3.10–3.02 (m, 1H), 2.81 (ddd, *J* = 24.3, 22.1, 12.4 Hz, 5H), 2.26 (s, 3H), 1.57–1.45 (m, 4H). ^{13}C NMR (DMSO-d_6, 101 MHz): δ 173.5, 173.4*, 173.0, 171.3, 171.2*, 136.9, 136.7*, 136.1, 136.0*, 129.5, 129.2, 127.6, 116.4, 107.7, 105.7, 79.3, 79.2*, 77.2, 77.2*, 53.7, 53.4*, 52.2, 52.0*, 51.7*, 46.7, 46.5*, 42.3, 41.3, 31.2, 29.3, 29.2*, 29.1, 28.9*, 28.8, 28.5*, 28.4*, 21.1. IR (cm^{-1}) 3304 (NH), 3031 (OH), 2986, 2967, 2920 (CH), 1735 (CO), 1691 (CO), 1649 (C=C), 1540 (NH bend), 1388 (CH_3), 1252 (CO), 1145 (CO), 835 (*p*-Ph). *Diastereomer peaks.

3-(2-(Thiophene-2-ylmethyl)-3-(4-methylbenzylamino)-3-oxopropylcarbamoyl)-7-oxabicyclo[2.2.1]heptane-2-carboxylic acid (23).[4]

Synthesised as described as for **20** from 3-amino-*N*-(4-methylbenzylamino)-2-((5-methylthiophene-2-yl)methyl)propanamide (**12m**) and norcantharidin (**2**) to afford **23** as a white solid (diastereomers collected in a 1:1 ratio) (0.13 g, overall yield 38%); mp 161–163 °C. LRMS (ESI^-) m/z 455 $[M-H]^-$. HRMS calc'd for $C_{24}H_{27}N_2O_5S$, $[M-H]^-$ 455.1646; Found 455.1650. ^1H NMR (DMSO-d_6, 400 MHz): δ 11.91 (br s, OH), 8.33 (t, *J* = 5.8 Hz, NH)*, 8.2 (t, *J* = 5.8 Hz, NH), 7.61 (t, *J* = 5.7 Hz, NH)*, 7.41 (t, *J* = 5.8 Hz, NH), 7.32 (dd, *J* = 5.1, 0.8 Hz, 1H), 7.05 (d, *J* = 7.9 Hz, 2H), 6.98 (t, *J* = 8.5 Hz, 2H), 6.94–6.90 (m, 1H), 6.83 (dd, *J* = 8.9, 2.9 Hz, 1H), 4.75–4.69 (m, 1H)*, 4.42 (d, *J* = 3.3 Hz, 1H), 4.27–4.11 (m, 2H), 3.27–3.19 (m, 1H), 3.09–2.95 (m, 2H), 2.89 (dd, *J* = 12.3, 6.9 Hz, 1H), 2.84–2.67 (m, 2H), 2.51–2.49 (m, 2H), 2.25 (s, 3H), 1.57–1.40 (m, 4H). ^{13}C NMR (DMSO-d_6, 101 MHz): δ 172.9, 172.8*, 172.7, 171.2*, 171.1, 142.1, 142.0*, 136.8, 136.6*, 136.1, 136.0*, 129.1, 127.6, 127.2*, 126.0, 124.4, 80.1, 79.3, 79.2*, 77.2, 77.2*, 53.6, 53.3*, 51.9, 51.7*, 51.2, 48.3, 48.0*, 42.3, 41.3*, 30.4, 30.2*, 29.4, 29.2*, 28.9, 28.8*, 21.1. IR (cm^{-1}) 3310 (NH), 3062 (OH), 2986, 2963, 2920, 2874 (CH), 1695 (CO), 1646 (C=C), 1545, (NH bend), 1382 (CH_3), 1244 (CO), 816 (*p*-C Ph). *Diastereomer peaks.

3-(2-(Pyrrole-2-ylmethyl)-3-(4-biphenylamino)-3-oxopropylcarbamoyl)-7-oxabicyclo[2.2.1]heptane-2-carboxylic acid (24).

Synthesised as described as for **12a** from 3-amino-*N*-(4-biphenylamino)-2-((5-methylpyrrole-2-yl)methyl)propanamide (**12n**) and norcantharidin (**2**) to afford **24** as a white solid (diastereomers collected in a 7:3 ratio) (0.04 g, 38%); mp 194 °C. LRMS (ESI^-) m/z 500 $[M-H]^-$. HRMS calc'd for $C_{29}H_{30}N_3O_5$, $[M-H]^-$ 500.2191; Found 500.2200. ^1H NMR (DMSO-d_6, 400 MHz): δ 11.85 (s, 1H), 10.42 (s, 1H), 8.33 (t, *J* = 5.8 Hz, 1H), 8.19 (t, *J* = 5.9 Hz, 1H), 7.63 (d, *J* = 7.4 Hz, 2H), 7.56 (d, *J* = 8.1 Hz, 2H), 7.46 (t, *J* = 7.6 Hz, 2H), 7.35 (t, *J* = 7.3 Hz, 1H), 7.23 (t, *J* = 7.3 Hz, 2H), 6.57 (d, *J* = 1.4 Hz, 1H), 5.90 (dd, *J* = 5.3, 2.6 Hz, 1H), 5.79 (d, *J* = 8.6 Hz, 1H), 4.73 (dd, *J* = 11.3, 2.5 Hz, 1H), 4.44 (s, 1H), 4.36–4.22 (m, 2H), 3.25–3.20 (m, 1H), 3.12–3.03 (m, 1H), 2.87–2.60 (m, 5H), 1.54–1.39 (m, 4H). ^{13}C NMR (DMSO-d_6, 101 MHz): δ 172.6, 172.5*, 171.9, 170.2, 170.1*, 139.4, 138.2, 138.0*, 137.9, 128.4, 128.3, 127.2, 126.7, 125.9, 125.8, 115.4, 106.6, 104.6, 78.2, 76.2, 52.3, 50.9, 50.7*, 45.5, 41.2, 40.3, 28.3, 27.8*, 27.4. IR (cm^{-1}) 3303 (NH), 3022 (OH), 2991, 2960, 2918, 2855 (CH), 1712 (CO), 1686 (C=C), 1533, 1484 (NH bend), 1242, 1212 (C-O), 1165 (C-O), 801 (*p*-Ph). *Diastereomer peaks.

3-(2-(Thiophene-2-yl)methyl)-3-(4-biphenylamino)-3-oxopropylcarbamoyl)-7-oxabicyclo[2.2.1]heptane-2-carboxylic acid (25).[4]

Synthesised as described as for **20** from 3-amino-*N*-(4-biphenylamino)-2-((5-methylthiophene-2-yl)methyl)propanamide (**12o**) and norcantharidin (**2**) to afford **25** as a white solid (diastereomers collected in a 1:1 ratio) (0.23g, overall yield 46%); mp 171–174 °C. LRMS (ESI^-) m/z 471 $[M-H]^-$. HRMS calc'd for $C_{29}H_{29}N_2O_5S$, $[M-H]^-$ 471.1803; Found 471.1805. ^1H NMR (DMSO-d_6, 400 MHz): δ 11.89 (br s, OH), 8.44 (t, *J* = 5.3 Hz, NH), 8.31 (t, *J* = 5.6 Hz, NH)*, 7.63 (d, *J* = 7.9 Hz, 2H), 7.61 (d, *J* = 2.1 Hz, 1H)*, 7.59 (d, *J* = 2.3 Hz, 1H), 7.54 (d, *J* = 8.2 Hz, 2H), 7.46 (d, *J* = 7.6 Hz, 2H), 7.35 (dd, *J* = 8.8, 6.2 Hz, 2H), 7.18 (t, *J* = 8.6 Hz, 2H), 6.97–6.91 (m, 1H), 6.85 (dd, *J* = 8.7, 3.2 Hz, 1H), 4.73 (d, *J* = 11.1 Hz, 1H), 4.50–4.40 (m, 1H), 4.37–4.09 (m, 2H). 3.25 (d, *J* = 7.0 Hz, 1H), 3.08 (dd, *J* = 8.0, 5.2 Hz, 1H), 2.96–2.71 (m, 5H), 1.46–1.37 (m, 2H), 1.27–1.17 (m, 2H). ^{13}C NMR (DMSO-d_6, 101 MHz): δ 174.0, 173.0, 172.8, 171.2, 142.1, 142.02, 140.5, 139.4, 139.1, 139.0, 129.4, 128.4, 128.3, 128.2, 127.8, 127.2, 127.0, 126.9, 126.0, 124.5, 79.3, 79.2, 77.2, 53.7, 53.3, 51.8, 48.4, 48.1, 42.2, 41.4, 31.7, 30.4, 29.4, 29.2, 28.9, 26.8, 22.5, 14.3. IR (cm^{-1}) 3316 (NH), 3070 (OH), 3033, 2985, 2923, 2876 (CH), 1691 (CO), 1646 (C=C), 1534, 1487 (NH bend), 1242 (CO), 819 (*p*-C Ph). *Diastereomer peaks.

3-[2-(1H-indol-3-yl)methyl]-3-(4-biphenylamino)-3-oxopropylcarbamoyl)-7-oxabicyclo[2.2.1]heptane-2-carboxylic acid (26).

Synthesised as described as for **20**, using EtOH:DMF (8:2) as the solvent for the reduction, from 3-amino-*N*-(4-biphenylamino)-2-((5-methylpyrrole-2-yl)1*H*-indol-3-yl)propanamide (**12p**) and norcantharidin to afford **26** as a white solid (diastereomers collected in a 2:1 ratio) (0.02 g, 23%); mp 160–161 °C. LRMS (ESI^-) m/z 550 $[M-H]^-$. HRMS calc'd for $C_{33}H_{32}N_3O_5$, $[M-H]^-$ 550.2347; Found 550.2355. ^1H NMR (DMSO-d_6, 400 MHz): δ 10.83 (s, 1H), 8.39 (t, *J* = 6.0 Hz, 0.3H)*, 8.25 (t, *J* = 6.0 Hz, 0.7H)*, 7.62 (d, *J* = 7.5 Hz, 2H), 7.57 – 7.54 (m, 1H), 7.51 – 7.44 (m, 4H), 7.41 – 7.34 (m, 3H), 7.14 – 7.04 (m, 4H), 6.97 (t, J = 7.4 Hz, 1H), 4.74 – 4.72 (m, 1H), 4.43 – 4.42 (s, 1H), 4.36 – 4.15 (m, 2H), 3.32 – 3.29 (m, 1H, obscured), 3.14 – 3.09 (m, 1H), 2.94 – 2.77 (m, 5H), 1.54 – 1.39 (m, 4H). ^{13}C NMR (DMSO-d_6, 101 MHz): δ 173.6*, 173.5, 172.54, 172.48*, 170.8, 170.7*, 140.04*, 140.02, 138.8*, 138.7, 138.42, 138.39*, 136.2, 128.9, 127.6, 127.34*,127.31, 127.26, 126.5, 126.4, 123.1, 120.8, 118.6*, 118.5, 118.1, 111.82, 111.80*, 111.23, 111.21*, 78.9, 76.8, 53.3, 52.9*, 51.7, 51.4*, 46.7, 46.5*, 41.7, 41.2*, 41.1, 28.9*, 28.7, 28.44*, 28.38, 25.9*, 25.7 IR (cm^{-1}) 3350 (NH), 3012 (OH), 2990, 2956, 2921, 2843 (CH), 1699 (CO), 1653 (C=C), 1579, 1495 (NH bend), 1236, 1199 (C-O), 1168 (C-O), 800 (*p*-Ph). *Diastereomer peaks.

3-[2-(Thiophen-2-yl)methyl]-3-(4-(trifluoromethyl)benzyl)amino)-3-oxopropylcarbamoyl)-7-oxabicyclo[2.2.1]heptane-2-carboxylic acid (27).

Synthesised as described as for **20** from 3-amino-*N*-(4-(trifluoromethyl)benzyl amino)-2-((5-methylthiophene-2-

yl)methyl)propanamide (**12q**) and norcantharidin (**2**) to afford **27** as a white solid (diastereomers collected in a 1:1 ratio) (0.12 g, 57%); mp 190–193 °C. LRMS (ESI^-) m/z 509 $[M-H]^-$. HRMS calc'd for $C_{24}H_{25}F_3N_2O_5S$, $[M-H]^-$ 509.1364; Found 509.1369. ^1H NMR (DMSO-d_6, 400 MHz): δ 11.89 (s, OH), 8.52 (t, *J* = 5.8 Hz, NH), 8.38 (t, *J* = 5.7 Hz, NH)*, 7.67 (t, *J* = 5.8 Hz, NH), 7.59 (d, *J* = 8.1 Hz, NH), 7.46 (t, *J* = 6.1 Hz, NH)*, 7.33 (dd, *J* = 5.1, 1.1 Hz, 1H), 7.27 (dd, *J* = 11.0, 8.4 Hz, 2H), 6.97–6.90 (m, 1H), 6.83 (dd, *J* = 8.5, 3.0 Hz, 1H), 4.73 (dd, *J* = 11.0, 3.1 Hz, 1H), 4.43 (s, 1H), 4.37–4.25 (m, 2H), 3.28–3.23 (m, 1H), 3.11–3.04 (m, 1H), 3.03–2.79 (m, 5H), 1.58–1.41 (m, 4H). ^{13}C NMR (DMSO-d_6, 101 MHz): δ 173.1, 172.9, 171.3, 171.2*, 142.0, 142.0, 128.4, 128.2, 128.2*, 127.2, 126.1, 125.4, 125.4*, 124.5, 79.8, 79.3, 79.2*, 77.3, 53.7, 53.3*, 52.0, 51.7*, 48.4, 48.2*, 42.2, 41.4, 30.4, 29.3, 29.2, 28.9*. IR (cm^{-1}) 3301 (NH), 3056 (OH), 2988, 2975, 2903, 2892 (CH), 1734 (CO), 1642 (C=C), 1524 (NH bend), 1246 (CO), 1170 (CO), 804 (*p*-C Ph). *Diastereomer peaks.

3-[2-(Thiophen-2-yl)methyl]-3-((2,2-diphenylethyl)amino)-3-oxopropylcarbamoyl)-7-oxabicyclo[2.2.1]heptane-2-carboxylic acid (28).

Synthesised as described as for **20** from 3-amino-*N*-(2,2-diphenylethyl)amino)-2-((5-methylthiophene-2-yl)methyl)propanamide (**12r**) and norcantharidin (**2**) to afford **28** as a white solid (0.05g, 26%); mp 162–164 °C. LRMS (ESI^-) m/z 531 $[M-H]^-$. HRMS calc'd for $C_{30}H_{31}N_2O_5S$, $[M-H]^-$ 531.1959; Found 531.1969. ^1H NMR (DMSO-d_6, 400 MHz): δ 11.85 (s, 1H), 8.00 (t, *J* = 5.2 Hz, NH), 7.40 (t, *J* = 5.4 Hz, NH), 7.29–7.15 (m, 11H), 6.85 (dd, *J* = 4.9, 3.5 Hz, 1H), 6.71 (d, *J* = 2.7 Hz, 1H), 4.71 (d, *J* = 3.4 Hz, 1H), 4.44 (d, *J* = 4.0 Hz, 1H), 4.16 (t, *J* = 7.7 Hz, 1H), 3.73 (dd, *J* = 13.5, 7.2 Hz, 1H), 3.64 (dd, *J* = 12.9, 5.6 Hz, 1H), 3.12 (dd, *J* = 12.7, 6.3 Hz, 1H), 2.92 (dd, *J* = 13.3, 5.9 Hz, 1H), 2.86–2.71 (m, 4H), 1.56–1.39 (m, 4H). ^{13}C NMR (DMSO-d_6, 101 MHz): δ 173.1, 173.0, 171.0, 143.5, 142.1, 128.8, 128.3, 127.2, 126.7, 125.8, 124.3, 79.0, 77.3, 53.4, 51.9, 50.5, 47.7, 43.8, 41.0, 30.1, 29.3, 29.0. IR (cm^{-1}) 3306 (NH), 3012 (OH), 2998, 2985, 2965, 2918, 2884, 2834 (CH), 1712 (CO), 1655 (C=C), 1564 (NH bend), 1243 (CO), 1175 (CO), 800 (*p*-C Ph).

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Keywords: anti-tumour agent • norcantharidin chimera • structure-activity relationship • Knoevenagel condensation • growth inhibition

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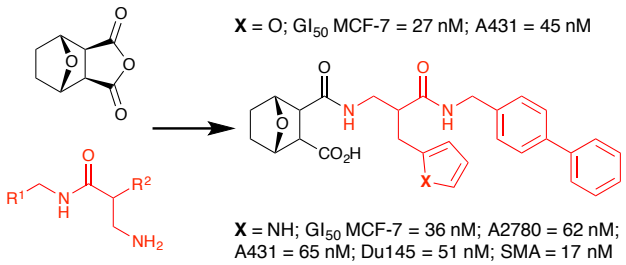

Norcantharidin acrylonitrile hybrids yields cytotoxic chimeric molecules with excellent activity against breast (MCF-7), skin (A431), prostate (Du145), neuroblastoma (BE2-C) and pancreatic (MIA) carcinomas and against SMA (spontaneous murine astrocytoma).