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Synthesis of cytotoxic spirocyclic imides from a biomass-derived oxanorbornene

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Dedicated to Professor Richard J. K. Taylor in appreciation of his tenure as editor in chief of Tetrahedron.

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ABSTRACT

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Keywords: Cytotoxicity Drug design Active surfactants Cantharidin Spirocyclic *N*-Substituted derivatives of cantharimide and norcantharimide represent a promising but underutilized motif for therapeutic applications. Herein, we report a divergent strategy for the preparation of secondary amides and norcantharimide resembling spirocyclic imides from a biomass-derived oxanorbornene and assess their biological activity. Computational modelling suggests these compounds fall perfectly within lead-like chemical space (200 Da < RMM < 350 Da, -1 < AlogP < 3), with the spirocyclic imides preferred due to their lack of reactive functionalities. Biological analysis of the spirocyclic imides revealed that the compounds displayed antiproliferative activity against a range of human cancer cells (A549, HCT 116, OVCAR-3, MDA-MB-231, MCF7 and PC-3) with the *N*-octyl derivative displaying the greatest potential as a potent broad-spectrum anticancer drug. Dose-response curves for the *N*-octyl spirocyclic imide found EC₅₀ values of 56–95 μ M dependent on the cell line, with highest activity against human colorectal carcinoma cells (HCT 116).

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1. Introduction

Cyclic imides represent an important structural motif that has found widespread use across biological, medicinal and polymer chemistry.^{1,2} They are prevalent in natural products and therapeutics that have a wide range of biological activities; finding potential as sedatives,³ hypnotics,⁴ anxiolytics,⁵ antiinflammatories,⁶ antivirals,⁷ antibacterials,⁸ antimicrobials⁹ and carcinostatics.¹⁰ Notable therapeutic examples include thalidomide **1**, phensuximide **2**, lenalidomide **3** and fluorouracil **4** (**Figure 1**).

Cantharidin 5, *exo,exo-2,3,-*dimethyl-7-oxabicyclo[2.2.1] heptane-2,3,dicarboxylic acid anhydride, and the cyclic imide analogue cantharimide 6 are natural products found within the Mylabris genus of the Meloidae family of Chinese blister beetles;¹¹ the dried bodies of which have been widely utilized within Chinese traditional medicine for over 2000 years for a range of ailments.¹² Of particular interest is the cytotoxicity of cantharidin 5, first reported in 1264,¹² which has since been found to be active against a number of human cancer cell lines, particularly liver and esophagus carcinoma.¹³ Cantharidin has also been identified to induce haematopoiesis in both humans and animals, as well as to be a potent and selective inhibitor of the serine/threonine protein phosphatases PP1 and PP2A.14,15 Unfortunately, cantharidin is also known to possess a number of severe side effects including hepato- and nephrotoxicity, thus limiting its application to date to a topical treatment for molluscum contagiosum.^{16,17}

Figure 1. Chemical structures of thalidomide **1**, phensuximide **2**, lenalidomide **3**, fluorouracil **4** and the cantharidin family **5–8**, cantharidin **5**, cantharimide **6**, norcantharidin **7**, and norcantharimide **8**.

In comparison, the demethylated species norcantharidin **7** also demonstrates similar anticancer activity to the parent compound, however without the associated toxicity.¹⁶ Its synthesis is likewise readily accessible through the Diels-Alder [4+2] cycloaddition between furan and maleic anhydride, followed by subsequent reduction of the alkene.¹⁸ This has led to numerous investigations into synthetic analogues of cantharidin **5**, with several groups reporting structure-activity relationships (SAR) for cytotoxicity and PP1/PP2A inhibition following modification of the 7-oxabicylo[2.2.1]heptyl ring system^{19–23} or ring-opening the anhydride.^{24–29} However, these synthetic analogues have seldom shown cytotoxicity comparable to cantharidin **5**, suggesting only limited modification of the parent compound is tolerated.

Rather surprisingly, derivatization of cantharidin **5** or norcantharidin **7** to the corresponding *N*-substituted imides, through condensation of the anhydride with an amine, has seen minimal investigation, despite a few encouraging reports.^{13–15,23,30–35} For example, potential for broad spectrum cytotoxicity against a range of cell lines has been highlighted a number of times,^{13,15,31,32,34} with Lin *et al.* demonstrating that *N*-thiazolylcantharimides were more potent than cantharidin against the human hepatocellular carcinoma cell lines SK-Hep-1 and Hep 3B.³⁰ McCluskey *et al.* have also found that norcantharimides

substituted with either D- or L-histidine are more potent than norcantharidin 7 and equipotent to cantharidin 5 for the inhibition of PP1 and PP2A.¹⁴ Finally, Gasser *et al.* has shown that *N*-substituted norcantharimides display unique potential as



nematocides for the treatment of *Haemonchus contortus*.³³ We therefore believe substantial scope exists for exploring the potential of novel cyclic imides based on *N*-substituted cantharimide **6** and norcantharimide **8** for therapeutic applications.

Ourselves, along with Pehere et al., have previously reported, independently and simultaneously, the synthesis of 2-[(1*S**,5*S**,7*S**)-4-oxo-3,10-dioxatricyclo-[5.2.1.0^{1,5}]dec-8-en-5yl] acetic acid 12, through the tandem Diels-Alder cycloaddition and lactonization reaction of furfuryl alcohol **10** and itaconic anhydride **9** (Scheme 1).^{36,37} Oxanorbornene **12** is isolated as a single species, due to the lactonization step only being favorable when the [4+2] cycloaddition yields the prox-exo intermediate 11, which drives ring-opening of the anhydride and subsequent crystallization of 12 from the dynamic reaction mixture as a racemic mixture.³⁷ This direct and atom economical reaction therefore presents a highly efficient route to yield a functionalized oxanorbornene. Furthermore, itaconic anhydride 9 and furfuryl alcohol 10 are readily available from biomass; the former through the simultaneous decarboxylation and dehydration of citric acid (ca. 1,000,000 tons per annum),³⁸ the latter by hydrogenation of furfural, which is produced through the acid-catalyzed dehydration of pentoses (ca. 200,000 tons per annum).39

Scheme 1. Preparation of oxanorbornene 12 *via* the [4+2] cycloaddition and intramolecular lactonization of furfuryl alcohol 10 and itaconic anhydride 9.





Scheme 2. A. Previous work on the synthesis of bio-based ROMP polymers from oxanorbornene lactone 12 and oxanorbornene lactam 15. B. This work on the divergent synthesis of secondary amides and spirocyclic imides from oxanorbornene 12.

Subsequently, we have been interested in exploring derivatives of oxanorbornene 12 as novel reactive monomers for the synthesis of sustainable polymers via ring-opening metathesis polymerization (ROMP). We have reported a number of examples utilizing ester and tertiary amide derivatives 13 for the synthesis of various homo- and copolymers 14 derived from biomass using Grubbs 2nd generation catalyst (Scheme 2).^{36,40,41} These polymerizations were found to be well-controlled, but slow, which was attributed to the endo-orientation of the carboxylic acid derivative within 13. In more recent work, structurally similar N-substituted lactams 15 possessing an exoorientated carboxylic acid were found to display well-controlled and extremely rapid polymerizations in the presence of Grubbs 3rd generation catalyst.⁴² During the course of these investigations, whilst attempting to produce secondary amide derivatives of 12 for ROMP, we observed a cyclization that yielded a mixture of two species; the desired secondary amide and a spirocyclic imide (Scheme 2). Inspired by the structural similarity between the spirocyclic imide and the aforementioned cantharimide 6 and norcantharimide 8, we were motivated to explore this avenue further. Herein, we present a divergent strategy for the selective preparation of secondary amides 19a-e (pathway A) and spirocyclic imides 21a-e (pathway B) based on the 7-oxabicylo[2.2.1]heptyl ring system, from oxanorbornene 12. A computational study of these compounds by lead-likeness and molecular analysis (LLAMA) suggested that spirocyclic imides 21a-e represent highly novel and lead-like compounds, and as such they were subsequently investigated for their biological activity against a range of human cancer cell lines.

2. Results and Discussion

Initially, amide synthesis was performed in a similar manner to that reported previously, with oxanorbornene 12 first converted to the acid chloride intermediate, using oxalyl chloride and catalytic DMF (**Scheme 3**). The acid chloride was easily isolated, redissolved in fresh anhydrous CH_2Cl_2 , and reacted with *N*-butylamine in the presence of two equivalents of triethylamine, to account for the formation of the hydrochloric acid by-product. This yielded the desired secondary amide **20a**



along with spirocyclic imide **22**. Compounds **20a** and **22** were separable by flash column chromatography, allowing them to be studied independently.

Scheme 3. Synthesis of *N*-butyl secondary amide **20a** and spirocyclic imide **22** by the amidation of **12** by oxalyl chloride and *N*-butylamine.

The respective structures of 20a and 22 were determined using a combination of electrospray ionization-mass spectroscopy (ESI-MS), FTIR and 2D ¹H-¹³C heteronuclear multiple bond correlation NMR spectroscopy (Figure 2). ESI-MS showed that the two species had identical molecular weights, suggesting they were structural isomers. FTIR then clearly identified a sharp peak at 3396 cm⁻¹ in 22, which was absent in 20a, most likely corresponding to the O-H stretch of an alcohol. This strongly implied that compound 22 was the result of an intramolecular cyclization, which led to the ring-opening of the lactone moiety. Finally, ¹H-¹³C HMBC NMR spectroscopy of the two compounds showed that the butyl protons alpha to the nitrogen (yellow dot) had a clear correlation to both carbonyl groups (grey dot) in 22 (Figure 2 spectrum B), rather than just the one as observed in the secondary amide 20a (Figure 2 spectrum A). This confirmed the spirocyclic imide structure for 22, which can be formed by an intramolecular 5-exo-trig cyclization of secondary amide 20a. The structures of both N-butyl secondary amide 20a and spirocyclic imide 22 were then subsequently confirmed by X-ray crystallography (CCDC 2000921 & 2000922).

Intrigued by the unusual nature of spirocyclic compound **22**, we were encouraged to investigate this transformation further in an effort to selectively produce either compound and study their applications. Furthermore, the synthesis of cyclic imides is traditionally performed through the dehydrative condensation of an anhydride with an amine or the cyclisation of an amic-acid, which usually requires either high temperature, a Lewis acid catalyst or the use of stoichiometric acidic reagents.⁴³ More recently, there has been great interest in direct routes to cyclic imides using readily available starting materials due to the therapeutic potential of this class of compounds.^{4,43–45} The intramolecular 5-*exo*-trig cyclization of a secondary amide onto a lactone under homogeneous basic conditions, as reported here, provides a mild and highly efficient method for the synthesis of cyclic imides.⁴⁶



Figure 2. ¹H-¹³C HMBC NMR spectroscopy experiments for A. *N*-butyl secondary amide 20a and B. *N*-butyl spirocyclic imide 22, and associated X-ray crystal structures.

In an effort to selectively synthesize either the N-butyl secondary amide 20a or the spirocyclic imide 22, the quantity of triethylamine was adjusted during the amidation step of the reaction (Figure S101). When varying the quantity of triethylamine from 0.8–4.0 equivalents relative to oxanorbornene 12, it was found by ¹H NMR spectroscopy of the reaction mixture that either species could be formed, with 0.8 equivalents giving exclusively the N-butyl secondary amide 20a whilst 4.0 equivalents produced exclusively the N-butyl spirocyclic imide 22. This was rationalized on the basis that amides are typically poor nucleophiles for N-attack due to delocalization of the lone pair, which results in O-attack being preferred.⁴⁷ In contrast, ambident amide nucleophiles have been shown to prefer N-attack over *O*-attack.⁴⁸ Deprotonation of the amide would therefore favor N-attack through the now nucleophilic nitrogen leading to formation of the spirocyclic product 22, whilst under neutral or acidic conditions ring-opening of the lactone is disfavored, leading to formation of the secondary amide 20a. The N-butyl secondary amide 20a was then exposed to 2.0 equivalents of triethylamine to see if it could promote formation of the imide 22 (Figure S102). It was observed that 20a was indeed converted to 22, with optimal formation of the imide found after 7 hours (20a 4%; 22 85%; 10 and 23 11%), and complete consumption of the amide observed after 24 hours (20a 0%; 22 41%; 10 and 23 59%). This experiment reaffirmed our hypothesis that



deprotonation of the amide is necessary to form imide 22 whilst also highlighting its inherent instability in solution, with clear degradation products observed to form over the course of the reaction. The degradation products were subsequently identified to be furfuryl alcohol 10 and 1-butyl-3-methylene-pyrrolidine-2,5,-dione 23, formed through a retro-Diels-Alder reaction, which is possible following ring-opening of the lactone moiety (Scheme 4).

Scheme 4. Intramolecular 5-*exo*-trig cyclization of **20a** under basic conditions to form **22** and subsequent retro-Diels-Alder, which yields the degradation products furfuryl alcohol **10** and 1-butyl-3-methylene-pyrrolidine-2,5-dione **23**.

On the basis of the above studies, we realized that hydrogenation of the oxanorbornene unit within structures 20a and 22 would prevent the retro-Diels-Alder reaction and, in the case of the spirocyclic imide, provide a motif that bears a striking resemblance to the natural products cantharimide 6 and norcantharimide 8, suggesting a potential therapeutic application. Buoyed by this we set about investigating derivatization of oxanorbornene 12 by amidation and hydrogenation (Scheme 5).



This can be done by either first hydrogenating 12 to produce the oxanorbornane 18 which can then be functionalized with the desired amine **19a–e** (pathway A), or by producing the desired secondary amide 20a-e derivatives which are then hydrogenated 21a-e (pathway B). Rather surprisingly, we found that it was possible to selectively form either the secondary amides 19a-e or the spirocyclic imides 21a-e depending on the method used, providing a simple and divergent route to either species from oxanorbornene 12. In the case of pathway A, oxanorbornene 12 was first hydrogenated using 10 wt% palladium on carbon, vielding the corresponding oxanorbornane 18 in an excellent 93% yield. Amidation was then performed using a series of alkyl and aryl amines according to the procedure described previously, producing secondary amides 19a-e in moderate to excellent yields (33–77%). Notably, pyridine was used as the base rather than triethylamine due to its lower basicity (pK_{aH} pyridine: 5.23; NEt₃: 10.65, both in H_2O) and less than two equivalents were used to prevent formation of spirocyclic species.⁴⁹ Alternatively, by pathway B, amidation of oxanorbornene 12 could be used to selectively produce the secondary amides 20a-e in moderate to excellent yields (31-86%). Hydrogenation of 20a-e then resulted in a tandem cyclisation to produce spirocyclic imides 21a-e in excellent yields (76-98%). A possible explanation for this is that the hydrogenation conditions result in the formation of palladium hydrides which are sufficiently basic to deprotonate the amide, facilitating the cyclisation. As described previously, spirocyclic imides 21a-e could be easily differentiated from secondary amides 19a-e through a combination of ESI-MS, FTIR spectroscopy and ¹H-¹³C HMBC NMR spectroscopy (see supporting information), and the structure of **21d** was confirmed by X-ray crystallography (CCDC 2000923).

Scheme 5. Synthesis of secondary amides **19a–e** and spirocyclic imides **21a–e** by divergent amidation/hydrogenation strategy from oxanorbornene **12** and the X-ray crystal structure of **21d**.

In view of the resemblance of these compounds to cantharimide 6 and norcantharimide 8, we were intrigued as to



whether these compounds held potential as lead-like targets for therapeutic applications. Lead-likeness and molecular analysis (LLAMA) is an open-access, web-based tool developed by the University of Leeds for the decoration and assessment of small molecule scaffolds for their lead-likeness and novelty.50 Analyzing secondary amides 19a-e and spirocyclic imides 21a-e by LLAMA, it was found that all compounds lay within lead-like space on the basis of their relative molecular mass (RMM) (200 Da < RMM < 350 Da) and predicted lipophilicity (-1 < AlogP < 3) as shown in Figure 3. However, whilst spirocyclic imides **21a-e** were assigned minimal lead-likeness penalties (0-1: due to the absence of an aromatic ring in 21d,e), secondary amides 19ae were assigned higher penalties (5-6) due to the presence of the undesirable lactone functionality and the absence of an aromatic ring in **19d**,e. The compounds were then analyzed for novelty against the ZINC database, a repository of over twenty million commercially available molecules.⁵¹ In both cases, the secondary amides 19a-e and the spirocyclic imides 21a-e were found to possess a 0.0% likeness to a random 2% of the database. Finally, PMI analysis found that the majority of the compounds lie along the rod-like to disc-like axis. Overall, these results strongly suggest that the spirocyclic imides 21a-e represent highly novel and promising lead-like compounds for further consideration as therapeutics.

Figure 3. A. Lead-likeness and B. PMI analysis plots for secondary amides 19a-e and spirocyclic imides 21a-e. Blue region represents the 3D region of PMI space where I1 + I2 > 1.2.

Previously, cantharidin and norcantharidin analogues have shown promising anticancer activities against a range of cancer cell types, both *in vitro* and *in vivo*.^{14,29,32,52,53} Based on this, we screened the racemic spirocyclic imides 21a-e, for their activity against six human cancer cell lines. For this we selected A549 human lung carcinoma cells, HCT 116 colorectal carcinoma cells, OVCAR-3 human ovarian adenocarcinoma cells, MDA-MB-231 human breast adenocarcinoma cells, MCF7 human breast adenocarcinoma cells and PC-3 human prostate adenocarcinoma cells to represent a range of cell types. For the initial investigation, we performed a broad assessment of the potential of all five molecules to inhibit cancer cell growth at two moderate fixed concentrations (100 µM and 200 µM) and over three time points: 24, 48 and 72h. Cell growth was monitored as a function of relative cell viability, and the results are shown in Table 1, Figure 4A and Figures S103–S108.

The data in Table 1 shows that after 72h of exposure, promisingly, all compounds had a negative impact on the growth of all studied cell lines. Figures S103-S108 confirm that these results have both a concentration- and time-dependent effect. In particular, HCT 116, A549, OVCAR-3 and MCF7 cells were most susceptible to compounds 21a-e after 72h of exposure, while PC-3 and MDA-MB-231 cells showed more resistance. Furthermore, it was apparent that compound **21c** consistently exhibited the highest potency from all the compounds assayed. In HCT 116 cells, at 200 µM, almost complete cell death was achieved, with similar efficacy in A549 and MCF7 cells, suggesting that compound 21c has potential as a potent, broadspectrum anticancer agent. When analyzing the biological results in comparison with the chemical structures of each compound, whereby 21c followed by 21b were the most toxic, it was apparent that the presence of a long alkyl chain (C8 and C6 chains respectively) increased the anticancer effect. This was unexpected, with 21a-c in fact receiving penalties during the LLAMA simulation as a result of the prediction placing importance on the presence of aromatic moieties. Instead, the three less active compounds had either a short alkyl chain (**21a**, C4) or aryl/aryloxy side-groups (**21d**,e).

	HCT 116		A549		PC-3		OVCAR-3		MCF7		MDA-MB-231	
	100 µM	200 µM	100 µM	200 µM	100 µM	200 µM	100 µM	200 µM	100 µM	200 µM	100 µM	200 µM
21a	79 ±2	27 ±5	83 ± 8	46 ± 7	82 ± 8	62 ± 5	69 ± 5	59 ± 9	68 ± 12	46 ± 5	72 ± 4	64 ±6
21b	61 ±8	23 ± 2	74 ±5	43 ±5	69 ±6	55 ±9	64 ±7	47 ±9	51 ±10	35 ± 7	77 ± 4	59 ± 3
21c	31 ± 4	5 ±2	33 ± 5	18 ±7	61 ± 4	40 ±9	35 ± 2	16 ± 3	29 ± 9	10 ±5	62 ± 5	40 ±11

 63 ± 8

73 ± 7

77 + 5

63 ± 9

61 ± 2

57 ± 10

71 + 12

 67 ± 10

48 + 5

 39 ± 4

75 + 4

76 ± 9

 65 ± 5

63 ± 8

Table 1. Cytotoxicity of compounds **21a–e** in the cancer cell panel. Cytotoxicity is expressed as the percentage of relative metabolic activity (compared to untreated cells) at 100 μ M and 200 μ M drug concentration after 72 h of continuous drug exposure, presented as mean \pm S.D (n=5)



Figure 4. A. Representative cytotoxicity profiles of compounds 21a–e in HCT 116 cells over 24, 48 and 72h as assessed by the PrestoBlue metabolic assay, B. Dose-response curves of compound 21c in all cell lines. Data are presented as mean \pm S.D (n=4). C. DLS traces of compounds 21a–e showing the presence of well-defined nanoaggregates in milliQ water (concentration 125 µg/mL).

Given the structural similarity of the two most active compounds to traditional surfactant molecules, *i.e.* the presence of a polar head group and aliphatic sidechain, it was hypothesized that this could be the cause of the enhanced cytotoxicity of **21b-c** over the other lead-like compounds. Many surfactants have toxicological effects on cells as a result of hydrocarbon chain insertion into lipid bilayers, causing perturbation of cell membranes and lysis, which is typically a function of hydrocarbon chain length and critical micelle/aggregation concentration (CMC/CAC).^{54,55} The lactate dehydrogenase (LDH) cytotoxicity colorimetric assay was employed to assess this, as a reliable method for measuring release of the cytosolic enzyme LDH into the cell culture medium upon any damage to the cell plasma membrane. Surprisingly, no LDH release was observed after 48h incubation at the highest concentration of 200 µM in A549 cells as a representative experiment (**Figure S109**), indicating that no cell membrane lysis was occurring. To further verify this result, as it is reported that surfactant toxicity is significantly heightened at concentrations lower than the CMC/CAC, the propensity of compounds 21a-e to first selfassemble, and then the concentration at which this occurred, was assessed using a dynamic light scattering (DLS) method.⁵⁶ In accordance with our hypothesis, all compounds were confirmed to form nanoaggregates in aqueous solution of a size range 100-120 nm (Figure 4C), and with CAC values dependent upon sidechain chemistry (Figure S110, Table S1). The calculated CAC values confirmed that the LDH assay had been performed both above and below CAC values, and therefore that there was no influence of this parameter on targeted toxicity against cell membranes. Interestingly, given the important role of surfactants for the formulation of poorly soluble drugs,⁵⁷ these findings crucially highlight the potential of these compounds as "active"

21d

21e

74 + 3

 72 ± 4

42 + 2

49 ± 6

78 + 2

78 ± 5

36 + 7

46 ± 6

72 + 9

83 ± 5

surfactants for formulation of a second drug, in order to achieve combination therapies, target different mechanisms of action and prevent drug resistance.⁵⁸

Finally, on the basis of these positive observations and due to the potency of **21c**, further biological analysis was performed to determine the EC_{50} concentration in all the tested cell lines. EC_{50} values were determined after 48h incubation and the results are shown in Figure 4, Figures S111-S116 and Table 2. All values were less than 100 μ M and followed the same trend as the initial cytotoxicity assays, whereby the lowest EC50 values were observed in HCT 116 cells (56 µM). Overall, the biological assays, in agreement with the LLAMA predictions, confirmed that molecules based on the spirocyclic imide scaffold described here show potential as active agents or excipients for anticancer applications. While the final EC_{50} values were modest in comparison to traditional anticancer drugs (typical $EC_{50} < 10$ µM), the compounds assessed in this work can be considered as unoptimized and preliminary candidates in terms of their introduced functionalities.⁵⁹ Future work will endeavor to further explore the possibility to introduce different sidechain substituent chemistries in an effort to enhance the biological activity of these interesting compounds, assess their range of therapeutic potential such as within antimicrobial applications, as well as investigating their potential for the codelivery of other active agents by exploiting their inherent propensity to self-assemble into nanoparticles.

Table 2. Calculated EC₅₀ values of compound 21c.

	НСТ 116	A549	PC-3	OVCAR- 3	MCF7	MDA-MS- 231
ЕС50 (µМ)	56±3	83±3	95±5	79±14	79±8	80±22

3. Conclusion

Functionalization of biomass derived oxanorbornene 12 has led to a divergent strategy for the preparation of a range of new spirocyclic imides 21a-e and secondary amides 19a-e. Computational modelling identified that these compounds represent highly novel and lead-like structures for the development of clinical candidates, with the spirocyclic imides 21a-e preferred due to their lack of reactive functionalities. Accordingly, the spirocyclic imides 21a-e were analyzed for their biological activity using cytotoxicity assays against a range of human cancer cell lines. Overall, all compounds showed some degree of antiproliferative effect, with 21c presenting the greatest potential as a potent broad spectrum anticancer agent. The compounds demonstrated a propensity to self-assemble into nanoaggregates, indicating promising application potential as "active" surfactants for formulation of a second drug. This study justifies further investigation into the therapeutic potential of these unusual species.

4. Experimental Section

4.1. General Experimental

All reagents and solvents were purchased from commercial suppliers and used as sold, excluding furfuryl alcohol which was distilled prior to use. Anhydrous CH₂Cl₂ was obtained from an Inert Technologies PureSolv EN 1-4 enclosed solvent purification system. All air- and water-sensitive reactions were carried out in oven-dried glassware under either a nitrogen or argon atmosphere. Analytical TLC was performed on aluminum

backed plates pre-coated (0.25 mm) with Merck KGaA silica gel 60 F_{254} . Compounds were visualized by exposure to UV-light (254 nm) and stained using KMNO₄ or phosphomolybdic acid followed by heating. Flash column chromatography was performed using Fluorochem silica gel LC60A (40–60 µm). All mixed solvent eluents are reported as v/v solutions. Brine refers to a saturated solution of aqueous sodium chloride.

¹H and ¹³C-Nuclear magnetic resonance (NMR) spectra were acquired using either a JEOL ECS 400 MHz spectrometer or a Bruker DPX-400 400 MHz spectrometer. ¹H spectra were referenced internally to the residual protic solvent resonance (CHCl₃ = 7.27 ppm, DMSO-d₆ = 2.50 ppm and CH₃OH = 3.31 ppm). ¹³C-Spectra were referenced internally to the solvent resonance (CDCl₃ = 77.16 ppm, CD₃OD = 49.00 ppm and DMSO-d₆ = 39.52 ppm). ¹H-NMR coupling constants are reported in Hertz (Hz). Coupling constants are reported using the following notation, or combination of; s = singlet, br = broad, d = doublet, t = triplet, q = quartet, quin = quintet, sex = sextet, sept = septet, oct = octet, non = nonet and m = multiplet. Assignment of signals in ¹H and ¹³C-spectra was determined using ¹H-¹H COSY, DEPT-135, ¹H-¹³C HMQC and HMBC experiments where appropriate.

High resolution mass spectra (HRMS) were recorded using electrospray ionization (ESI) on a Bruker micrOTOF mass spectrometer in tandem with an Agilent series 1200 liquid chromatography system or on a Waters Xevo G2-XS Quadrupole time-of-flight mass spectrometer. All Infra-Red (IR) data was obtained using a Perkin-Elmer Spectrum Two or Spectrum 400 FT-IR spectrometer or an Agilent Technologies Cary 630 FTIR spectrometer. Absorbances are reported using the following notation; w = weak, m = medium and s = strong. Melting points were determined using either a Stuart SMP3, SMP20 or an SRS OptiMelt MPA100 hot stage apparatus and were not corrected. Dynamic light scattering measurements were recorded using a Malvern Panalytical Zetasizer Nano S fitted with a 633 nm He-Ne laser operating at 4 mW. Measurements were performed at 25 °C using a scattering angle of 173° (backscattering), and the results analyzed using Malvern Panalytical Zetasizer software version 7.13.

Diffraction data were collected at 110 K (195 K for 20a) on an Oxford Diffraction SuperNova diffractometer with Cu-Ka radiation ($\lambda = 1.54184$ Å) using an EOS CCD camera. The crystal was cooled with an Oxford Instruments Cryojet. Diffractometer control, data collection, initial unit cell determination, frame integration and unit-cell refinement was carried out with "Crysalis". Face-indexed absorption corrections were applied using spherical harmonics, implemented in SCALE3 ABSPACK scaling algorithm. OLEX2 was used for overall structure solution, refinement and preparation of computer graphics and publication data. Within OLEX2, the algorithm used for structure solution was SHELXT, chargeflipping. Refinement by full-matrix least-squares used the SHELXT algorithm within OLEX2. Hydrogen atoms were placed using a "riding-mode" and included in the refinement at calculated positions. Structures for 22, 20a and 21d were deposited in the CCDC (CCDC 2000921-3). 60

LLAMA was used to determine if compounds resided in a region of chemical space within which they have a greater potential for development into clinical candidates, as defined by Nadin *et al.*⁶¹ Structural novelty was then assessed against the Murcko framework, where all side chain functionality was removed reducing the molecule to its ring systems and the linkers between them, with and without the alpha atoms.⁶² These frameworks were then assessed against a random 2% of the

ZINC database.⁵¹ Finally, the molecules were analyzed by principal moments of inertia (PMI) analysis, in order to determine shape distribution. Thus, the system randomly generated a number of 3D conformers of each molecule and minimized their energy, selecting the lowest energy conformer for further analysis. The moments of inertia were then calculated along the X, Y, and Z-axis, with the I1 coordinates calculated by dividing inertia(X) by inertia(Z) and I2 by dividing inertia(Y) by inertia(Z). The resulting PMI plot of I1 against I2 provides an indication of whether the molecule is rod (I1=1.0, I2=0.0), disc (I1=0.5, I2=0.5) or sphere (I1=1.0, I2=1.0)-like in nature (**Figure 3**).

4.2. Compound Data

2-[(15*,55*,75*)-4-Oxo-3,10-dioxatricyclo-[5.2.1.0^{1,5}]dec-8en-5-yl] acetic acid (12): Acid 12 was prepared from itaconic anhydride and furfuryl alcohol using either of the procedures described below. Solvent Free: Itaconic anhydride (3.0 g, 27 mmol, 1.0 eq.) was suspended in furfuryl alcohol (2.3 mL, 27 mmol, 1.0 eq.), the slurry obtained was allowed to stir at ambient temperature. After circa 5 h, the suspension had thickened to a paste could no longer be stirred. The reaction mixture was left for a further 19 h until a tan solid had formed. The crude material was then purified by recrystallization from acetone to give the target acid 12 as an off-white crystalline solid (3.8 g, 68%). Reaction solvent: Itaconic anhydride (25.0 g, 223 mmol, 1.0 eq.) and furfuryl alcohol (19.4 mL, 223 mmol, 1.0 eq.) were suspended in acetonitrile (12 mL) and the slurry allowed to stir at ambient temperature, after 24 h a white suspension had formed. The solid was removed by filtration and the filtrate was concentrated in vacuo. The concentrated filtrate was suspended in EtOAc (100 mL) and filtered. The obtained solids were combined and recrystallized from acetone, to give the target acid 12 as an off-white crystalline solid (20.0 g, 43%). m.p. 130.9-131.5 °C; IR (Neat) v_{max} 3100 (m), 1776 (s) and 1733 (s) cm⁻¹; ¹H NMR (400 MHz, CD₃OD): $\delta = 6.59$ (dd, J = 5.9, 1.7 Hz, 1H, 1-H), 6.55 (d, J = 5.9 Hz, 1H, 2-H), 5.02 (dd, J = 4.7, 1.6 Hz, 1H, 6-H), 4.92 (d, J = 10.8 Hz, 1H, 7-H), 4.54 (d, J = 10.8 Hz, 1H, 7-H), 2.44–2.35 (m, 3H, 5/9-H), 1.55 (d, J = 12.3 Hz, 1H, 5-H); ¹³C NMR (100 MHz, CD₃OD): δ = 180.0 (8), 173.0 (10), 139.3 (1), 131.5 (2), 95.6 (3), 80.1 (6), 70.0 (7), 53.3 (4), 40.7 (9), 37.7 (5); HRMS (ESI) m/z calculated for C₁₀H₁₀NaO₅ 233.0420 (M+Na)⁺, found 233.0417, 1.2 ppm error. Data is consistent with previously reported characterization data.³⁶

General procedure for the synthesis of secondary amides from 12. Acid 12 (2.0 g, 9.5 mmol, 1.0 eq.) was suspended in anhydrous CH₂Cl₂ (5 mL) under an atmosphere of nitrogen. The suspension was cooled to 0 °C and oxalyl chloride 2.0 M solution in CH₂Cl₂ (12 mL, 24 mmol, 2.5 eq.) was added dropwise over 10 minutes, followed by DMF (4 drops). The suspension was stirred at ambient temperature until a solution was obtained, after which it was concentrated in vacuo, to yield the crude acid chloride as a light brown solid. The crude material was redissolved in fresh anhydrous CH₂Cl₂ (10 mL) and cooled to 0 °C. Pyridine (0.77 mL, 9.5 mmol, 1.0 eq.) was then added dropwise, followed by the primary amine (9.5 mmol, 1.0 eq.) in CH₂Cl₂ (10 mL). The solution was allowed to stir at ambient temperature overnight before diluting with CH2Cl2 (30 mL) and H₂O (50 mL). The organic layer was separated and further washed with 1M HCl_(aq) (50 mL), H₂O (50 mL) and brine (50 mL). The organic layer was then dried (MgSO₄), filtered and concentrated in vacuo.

N-Butyl-2-[(1*S**,5*S**,7*S**))-4-oxo-3,10-dioxatricyclo

[5.2.1.0^{1,5}]dec-8-en-5-yl]acetamide (20a): Acid 12 (2.0 g, 9.5 mmol, 1.0 eq.) and butylamine (0.94 mL, 9.5 mmol, 1.0 eq.) were

coupled according to the general procedure for the synthesis of secondary amides. The crude material was then purified by flash column chromatography (80:20, EtOAc:PE) to give the target compound 20a as a white solid (1.7 g, 86%). Crystals suitable for X-ray analysis were grown by slow evaporation of an acetone solution. $R_{\rm F} = 0.18$ (80:20, EtOAc:PE); m.p. 83.2–83.7 °C; IR (Neat) v_{max} 3240 (m), 3078 (w), 2961 (m), 2876 (w), 1768 (s), 1631 (s) and 1562 (s) cm⁻¹; ¹H NMR (400 MHz, CDCl₃): $\delta = 6.60$ (d, J = 5.9 Hz, 1H, 2-H), 6.50 (dd, J = 5.9, 1.8 Hz, 1H, 1-H), 6.34 (br, 1H, N-H), 5.01 (dd, J = 4.6, 1.7 Hz, 1H, 6-H), 4.84 (d, J =10.7 Hz, 1H, 7-H), 4.58 (d, J = 10.7 Hz, 1H, 7-H), 3.18 (dt, J =7.1, 2.5 Hz, 2H, 11-H), 2.49 (dd, J = 11.9, 4.8 Hz, 1H, 5-H), 2.33 (d, J = 14.3 Hz, 1H, 9-H), 2.25 (d, J = 14.3 Hz, 1H, 9-H), 1.50-1.40 (m, 2H, 12-H), 1.37-1.27 (m, 2H, 1×5-H/2×13-H), 0.89 (t, J = 7.26, 3H, 14-H); ¹³C NMR (100 MHz, CDCl₃): δ = 179.3 (8), 168.5 (10), 137.3 (1), 131.5 (2), 94.5 (3), 78.5 (6), 69.7 (7), 52.8 (4), 42.7 (9), 39.6 (11), 36.6 (5), 31.4 (12), 20.1 (13), 13.8 (14); HRMS (ESI) m/z calculated for C₁₄H₁₉NNaO₄ 288.1202 (M+Na)⁺, found 288.1206, 1.5 ppm error.

N-Hexyl-2-[(1*S**,5*S**,7*S**))-4-oxo-3,10-dioxatricyclo

[5.2.1.0^{1,5}]dec-8-en-5-yl]acetamide (20b): Acid 12 (2.0 g, 9.5 mmol, 1.0 eq.) and hexylamine (1.3 mL, 9.5 mmol, 1.0 eq.) were coupled according to the general procedure for the synthesis of secondary amides. The crude material was then purified by flash column chromatography (70:30-100:0, EtOAc:PE) to give the target compound **20b** as a yellow crystalline solid (0.87 g, 31%). $R_{\rm F} = 0.19$ (70:30, EtOAc:PE); m.p. 61.6–62.4 °C; IR (Neat) $v_{\rm max}$ 3303 (m), 2927 (m), 2855 (m), 1767 (s), 1669 (m), 1649 (s) and 1541 (s) cm⁻¹; ¹H NMR (400 MHz, CDCl₃): $\delta = 6.60$ (d, J = 5.7Hz, 1H, 2-H), 5.67 (dd, J = 5.7, 1.6 Hz, 1H, 1-H), 6.39–6.32 (br, 1H, N-H), 5.01 (dd, J = 4.6, 1.6 Hz, 1H, 6-H), 4.84 (d, J = 10.5 Hz, 1H, 7-H), 4.57 (d, J = 10.5 Hz, 1H, 7-H), 3.18 (dt, J = 7.1, 6.8 Hz, 2H, 11-H), 2.49 (dd, J = 12.1, 4.6 Hz, 1H, 5-H), 2.32 (d, J = 14.6 Hz, 1H, 9-H), 2.25 (d, J = 14.6, 1H, 9-H), 1.52–1.41 (m, 2H, 12-H), 1.33–1.20 (m, 7H, 5-H/13-H/14-H/15-H), 0.86 (t, J = 7.2 Hz, 3H, 16-H); ¹³C NMR (100 MHz, CDCl₃): δ = 179.3 (8), 168.5 (10), 137.3 (1), 131.5 (2), 94.5 (3), 78.5 (6), 69.7 (7), 52.8 (4), 42.7 (9), 39.9 (11), 36.6 (5), 31.5 (15), 29.3 (12), 26.6 (13), 22.6 (14), 14.1 (16); HRMS (ESI) m/z calculated for C₁₆H₂₃₋ $NNaO_4$ 316.1519 (M+Na)⁺, found 316.1525, -1.9 ppm error.

N-Octyl-2-[(1*S**,5*S**,7*S**))-4-oxo-3,10-dioxatricyclo

[5.2.1.0^{1,5}]dec-8-en-5-yl]acetamide (20c): Acid 12 (2.0 g, 9.5 mmol, 1.0 eq.) and octylamine (1.6 mL, 9.5 mmol, 1.0 eq.) were coupled according to the general procedure for the synthesis of secondary amides. The crude material was then purified by flash column chromatography (90:10, EtOAc:PE) to give the target compound **20c** as a pale yellow crystalline solid (2.1 g, 69%). $R_{\rm F}$ = 0.3 (90:10, EtOAc:PE); m.p. 75.8–76.2 °C; IR (Neat) v_{max} 3302 (m), 2918 (m), 2851 (m), 1767 (s), 1669 (m), 1649 (s) and 1543 (s) cm⁻¹; ¹H NMR (400 MHz, CDCl₃): $\delta = 6.60$ (d, J = 6.1 Hz, 1H, 2-H), 6.50 (dd, J = 6.1, 1.9 Hz, 1H, 1-H), 6.37–6.28 (br, 1H, N-H), 5.01 (dd, J = 4.8, 1.6 Hz, 1H, 6-H), 4.84 (d, J = 10.8 Hz, 1H, 7-H), 4.57 (d, J = 10.8 Hz, 1H, 7-H), 3.18 (dt, J = 12.3, 6.3 Hz, 2H, 11-H), 2.49 (dd, J = 12.3, 4.8 Hz, 1H, 5-H), 2.33 (d, J = 14.6 Hz, 1H, 9-H), 2.24 (d, J = 14.6 Hz, 1H, 9-H), 1.51–1.40 (m, 2H, 12-H), 1.34-1.17 (m, 11H, 5-H/13-H/14-H/15-H/16-H/17-H), 0.86 (t, J = 7.1 Hz, 3H, 18-H); ¹³C NMR (100 MHz, CDCl₃): $\delta = 179.3$ (8), 168.5 (10), 137.3 (1), 131.6 (2), 94.5 (3), 78.5 (6), 69.7 (7), 52.8 (4), 42.7 (9), 40.0 (11), 36.6 (5), 31.9 (12), 29.4 (13), 29.3 (14/15), 26.9 (16), 22.7 (17), 14.2 (18); HRMS (ESI) m/z calculated for C₁₈H₂₇NNaO₄ 344.1832 (M+Na)⁺, found 344.1837, -0.9 ppm error.

N-Benzyl-2-[(1S*,5S*,7S*))-4-oxo-3,10-

dioxatricyclo[5.2.1.0^{1,5}]dec-8-en-5-yl]acetamide (20d): Acid 12

(2 g, 9.52 mmol, 1.0 eq.) and benzylamine (1.04 mL, 9.52 mmol, 1.0 eq.) were coupled according to the general procedure for the synthesis of secondary amides. The crude material was then purified by flash column chromatography (70:30-90:10, EtOAc:PE) to give the target compound 20d as a white crystalline solid (1.67 g, 58%). $R_{\rm F} = 0.3$ (80:20, EtOAc:PE); m.p. 106.8–107.4 °C; IR (Neat) υ_{max} 3286 (m), 1765 (s), 1636 (s) and 1547 (s) cm⁻¹; ¹H NMR (400 MHz, CDCl₃): $\delta = 7.31-7.25$ (m, 2H, ArH), 7.24-7.18 (m, 3H, ArH), 6.75-6.66 (br, 1H, N-H), 6.54 (d, *J* = 5.8 Hz, 1H, 2-H), 6.45 (dd, *J* = 5.8, 1.6 Hz, 1H, 1-H), 4.95 (dd, J = 4.8, 1.6 Hz, 1H, 6-H), 4.75 (d, J = 10.6 Hz, 1H, 7-H), 4.51 (d, J = 10.6 Hz, 1H, 7-H), 4.37 (dd, J = 14.6, 5.8 Hz, 1H, 11-H), 4.30 (dd, J = 14.6, 5.8 Hz, 1H, 11-H), 2.41 (dd, J =12.0, 4.9 Hz, 1H, 5-H), 2.32 (d, J = 14.8 Hz, 1H, 9-H), 2.23 (d, J = 14.8 Hz, 1H, 9-H), 1.25 (d, J = 12.0 Hz, 1H, 5-H); ¹³C NMR $(100 \text{ MHz}, \text{CDCl}_3): \delta = 179.2 \ (8), \ 168.6 \ (10), \ 137.8 \ (1), \ 137.4$ (ArC), 131.4 (2), 128.8 (ArCH), 127.8 (ArCH), 94.5 (3), 78.5 (6), 69.6 (7), 52.8 (4), 43.9 (11), 42.5 (9), 36.6 (5); HRMS (ESI) m/z calculated for C₁₇H₁₇NNaO₄ 322.1050 (M+Na)⁺, found 322.1049, 0.3 ppm error.

N-(4-Methoxybenzyl)-2-[(1*S**,5*S**,7*S**))-4-oxo-3,10

dioxatricyclo[5.2.1.0^{1,5}]dec-8-en-5-yl]acetamide (20e): Acid 12 (0.50 g, 2.4 mmol, 1.0 eq.) and 4-methoxybenzylamine (0.31 mL, 2.4 mmol, 1.0 eq.) were coupled according to the general procedure for the synthesis of secondary amides. The crude material was then purified by flash column chromatography (80:20, EtOAc:PE) to give the target compound 20e as a crystalline solid (0.24 g, 31%). $R_{\rm F} = 0.25$ (80:20, EtOAc:PE); m.p. 150.3-150.9 °C; IR (Neat) v_{max} 3259 (m), 3088 (w), 2954 (w), 1758 (s), 1635 (m), 1561 (m) and 1508 (m) cm⁻¹; ¹H NMR (400 MHz, DMSO-d₆): $\delta = 8.41$ (t, J = 5.8 Hz, 1H, N-H), 7.20– 7.15 (m, 2H, ArH), 6.90–6.85 (m, 2H, ArH), 6.55 (dd, J = 5.8, 1.7 Hz, 1H, 1-H), 6.52 (d, *J* = 5.8 Hz, 1H, 2-H), 5.00 (dd, *J* = 4.6, 1.7 Hz, 1H, 6-H), 4.74 (d, J = 10.6 Hz, 1H, 7-H), 4.47 (d, J =10.6 Hz, 1H, 7-H), 4.15 (d, J = 5.8 Hz, 2H, 11-H), 3.72 (s, 3H, 12-H), 2.21 (s, 2H, 9-H), 2.17 (dd, J = 11.9, 4.7 Hz, 1H, 5-H), 1.51 (d, J = 11.9 Hz, 1H, 5-H); ¹³C NMR (100 MHz, DMSO-d₆): $\delta = 177.7$ (8), 168.0 (10), 158.3 (ArC), 137.5 (1), 131.0 (2), 128.7 (ArCH), 113.68 (ArCH), 94.0 (9), 78.0 (6), 68.2 (7), 55.1 (12), 52.2 (4), 41.7 (11), 40.6 (9), 36.1 (5); HRMS (ESI) m/zcalculated for C₁₈H₂₀NO₅ 330.1338 (M+H)⁺, found 330.1341, -0.9 ppm error.

(1S*,2S*,4R*)-1'-Butyl-1-(hydroxymethyl)spiro[7-

hept-2-ene-6,3'-pyrrolidine]-2',5'-dione oxabicyclo[2.2.1] (22): Acid 12 (2.0 g, 9.5 mmol, 1.0 eq.) was suspended in anhydrous CH₂Cl₂ (5 mL) under an atmosphere of nitrogen. The suspension was cooled to 0 $^{\circ}\mathrm{C}$ and oxalyl chloride 2.0 M solution in CH2Cl2 (12 mL, 24 mmol, 2.5 eq.) was added dropwise over 10 minutes, followed by DMF (4 drops). The suspension was stirred at ambient temperature until a solution was obtained, after which it was concentrated in vacuo, to yield the crude acid chloride as a light brown solid. The crude material was redissolved in fresh anhydrous CH2Cl2 (10 mL) and cooled to 0 °C and a solution of butylamine (4.7 mL, 48 mmol, 5.0 eq.) in CH₂Cl₂ (10 mL) was added dropwise. The solution was allowed to stir at ambient temperature overnight before diluting with CH₂Cl₂ (30 mL) and H₂O (50 mL). The organic layer was separated and further washed with 1M HCl_(aq) (50 mL), H₂O (50 mL) and brine (50 mL). The organic layer was then dried (MgSO₄), filtered and concentrated in vacuo. The crude material was purified by flash column chromatography (80:20, EtOAc:PE) to give the target spirocyclic imide 22 as a white crystalline solid (839 mg, 33%). Crystals suitable for X-ray analysis were grown by slow evaporation of an acetone solution. $R_{\rm F} = 0.25$ (80:20, EtOAc:PE); m.p. 69.2–70.0 °C; IR (Neat) $v_{\rm max}$

3396 (m), 3024 (w), 2946 (w), 2874 (m), 1767 (m), 1674 (s) and 1685 (s) cm⁻¹; ¹H NMR (400 MHz, CDCl₃): δ = 6.65 (dd, *J* = 5.7, 1.7 Hz, 1H, 1-H), 6.48 (d, *J* = 5.7 Hz, 1H, 2-H), 5.13 (dd, *J* = 4.8, 1.7 Hz, 1H, 6-H), 4.02 (d, *J* = 11.6 Hz, 1H, 7-H), 3.90 (dd, *J* = 11.6, 4.1 Hz, 1H, 7-H), 3.50 (t, *J* = 7.4 Hz, 2H, 11-H), 2.72– 2.61 (m, 2H, 5-H/9-H), 2.47–2.37 (m, 2H, OH/9-H), 1.59–1.48 (m, 3H, 12-H/5-H), 1.37–1.25 (m, 2H, 13-H), 0.91 (t, *J* = 7.3 Hz, 3H, 14-H); ¹³C NMR (100 MHz, CDCl₃): δ = 180.4 (8), 176.2 (10), 140.3 (1), 135.1 (2), 92.8 (3), 79.1 (6), 60.3 (7), 49.9 (4), 44.0 (5), 40.1 (9), 39.0 (11), 29.7 (12), 20.1 (13), 13.7 (14); HRMS (ESI) *m*/*z* calculated for C₁₄H₁₉NNaO₄ 288.1214 (M+Na)⁺, found 288.1206, -2.7 ppm error.

General procedure for the hydrogenation of secondary amides to imides. Secondary amide (1.9 mmol, 1.0 eq.) was dissolved in MeOH (1 mL) under nitrogen, the addition of EtOAc (1 mL) may be necessary in some cases to obtain a solution. In a separate flask Pd/C (10 wt%) was suspended in MeOH (1 mL) under nitrogen. H₂ was bubbled through the suspension of Pd/C in MeOH for 10 minutes. The solution of secondary amide was then added to the catalyst suspension. The reaction mixture was allowed to stir at room temperature for 16 hours under H₂ (1 bar). The reaction mixture was then passed through a pad of celite® and concentrated *in vacuo*.

(1S*,2S*,4R*)-1'-Butyl-1-(hydroxymethyl)spiro[7-

oxabicyclo[2.2.1]heptane-2,3'-pyrrolidine]-2',5'-dione (21a): Butyl amide 20a (0.10 g) was hydrogenated according to the general procedure for the hydrogenation of secondary amides. The crude material was purified using flash column chromatography (80:20-90:10, EtOAc:PE) to give the target compound **21a** as a clear oil (0.77 g, 76%). $R_{\rm F} = 0.33$ (90:10, EtOAc:PE); IR (Neat) v_{max} 3460 (w), 2958 (w), 2874 (w), 1771 (w) and 1685 (s) cm⁻¹; ¹H NMR (400 MHz, CDCl₃): δ = 4.72 (t, J = 5.3 Hz, 1H, 6-H), 3.93 (d, J = 5.3 Hz, 2H, 7-H), 3.49 (t, J = 7.3 Hz, 2H, 11-H), 2.97 (d, J = 17.9 Hz, 1H, 9-H), 2.71 (d, J = 17.9 Hz, 1H, 9-H), 2.52 (ddd, J = 12.1, 5.5, 2.6 Hz, 1H, 5-H), 2.02-1.91 (m, 2H, 1-H), 1.78-1.62 (m, 3H, 2-H, OH), 1.59-1.49 (m, 3H, 12-H/5-H), 1.37–1.23 (m, 2H, 13-H), 0.91 (t, J = 7.3 Hz, 3H, 14-H); ¹³C NMR (100 MHz, CDCl₃): $\delta = 180.5$ (8), 175.7 (10), 90.4 (3), 77.4 (6), 62.7 (7), 52.6 (4), 45.3 (5), 39.2 (9), 38.8 (11), 31.3 (2), 29.7 (12/1), 20.1 (13), 13.7 (14); HRMS (ESI) m/z calculated for C₁₄H₂₁NNaO₄ 290.1363 (M+Na)⁺, found 290.1368, -2.5 ppm error.

(1S*,2S*,4R*)-1'-Hexyl-1-(hydroxymethyl)spiro[7-

oxabicyclo[2.2.1] heptane-2,3'-pyrrolidine]-2',5'-dione (21b): Hexyl amide 20b (400 mg) was hydrogenated according to the general procedure for the hydrogenation of secondary amides. The crude material was purified by flash column chromatography (70:30, EtOAc:PE) to give the target compound 21b as a clear oil (378 mg, 94%). $R_{\rm F} = 0.32$ (80:20, EtOAc:PE); IR (Neat) $v_{\rm max}$ 3460 (w), 2931 (m), 2859 (w), 1771 (m) and 1686 (s) cm⁻¹; ¹H NMR (400 MHz, CDCl₃): δ = 4.68 (t, J = 5.2 Hz, 1H, 6-H), 3.89 (s, 2H, 7-H), 3.44 (t, J = 7.5 Hz, 2H, 11-H), 2.96 (d, J = 18.4 Hz, 1H, 9-H), 2.68 (d, J = 18.4 Hz, 1H, 9-H), 2.48 (ddd, J = 11.9, 5.2, 2.5 Hz, 1H, 5-H), 2.34-2.18 (br, 1H, OH), 2.02-1.82 (m, 2H, 1-H/2-H), 1.75-1.60 (m, 2H, 1-H/2-H), 1.58-1.47 (m, 3H, 5-H/12-H), 1.30–1.19 (m, 6H, 15-H/14-H/13-H), 0.84 (t J = 7.2 Hz, 3H, 16-H); ¹³C NMR (100 MHz, CDCl₃): δ = 180.5 (8), 175.8 (10), 90.4 (3), 77.1 (6), 62.6 (7), 52.6 (4), 45.2 (5), 39.2 (9), 39.0 (11), 31.4 (12), 31.2 (1), 29.6 (2), 27.5 (13), 26.5 (14), 22.6 (15), 14.1 (16); HRMS (ESI) m/z calculated for C₁₆H₂₅NNaO₄ 318.1676 $(M+Na)^+$, found 318.1676, 0.1 ppm error.

(1S*,2S*,4R*)-1-(Hydroxymethyl)-1'-octyl-spiro[7-

oxabicyclo[2.2.1] heptane-2,3'-pyrrolidine]-2',5'-dione (21c): Octyl amide 20c (0.10 g) was hydrogenated according to the

general procedure for the hydrogenation of secondary amides. The crude material was purified using flash column chromatography (70:30, EtOAc:PE) to give the target compound **21c** as a clear oil (0.098 g, 98%). $R_{\rm F} = 0.26$ (70:30, EtOAc:PE); IR (Neat) v_{max} 3452 (w), 2926 (m), 2856 (m), 1771 (w) and 1688 (s) cm⁻¹; ¹H NMR (400 MHz, CDCl₃): $\delta = 4.69$ (t, J = 4.9 Hz, 1H, 6-H), 3.90 (s, 2H, 7-H), 3.45 (t, J = 7.3 Hz, 2H, 11-H), 2.97 (d, J = 18.3 Hz, 2H, 9-H), 2.69 (d, J = 18.3 Hz, 1H, 9-H), 2.52-2.45 (m, 1H, 5-H), 2.13–2.04 (br, 1H, OH), 2.01–1.88 (m, 2H, 1-H), 1.77-1.61 (m, 2H, 2-H), 1.58-1.47 (m, 3H, 5-H/12-H), 1.30-1.18 (m, 10H, 13-H/14-H/15-H/16-H/17-H), 0.85 (t, J = 6.3 Hz, 3H, 18-H); ¹³C NMR (100 MHz, CDCl₃): $\delta = 180.5$ (8), 175.8 (10), 90.4 (3), 77.4 (6), 62.7 (7), 52.6 (4), 45.3 (5), 39.2 (9), 39.1 (11), 31.9 (31.3), 29.6 (1), 29.3 (2), 29.2 (14), 27.6 (15), 26.9 (16), 22.7 (17), 14.2 (18); HRMS (ESI) m/z calculated for C₁₈H₂₉₋ $NNaO_4$ 346.1989 (M+Na)⁺, found 346.1992, -0.5 ppm error.

(1S*,2S*,4R*)-1'-Benzyl-1-(hydroxymethyl)spiro[7-

oxabicyclo[2.2.1] heptane-2,3'-pyrrolidine]-2',5'-dione (21d): Benzyl amide 20d (400 mg) was hydrogenated according to the general procedure for the hydrogenation of secondary amides. The crude material was purified using flash column chromatography (80:20-90:10, EtOAc:PE) to give the target compound 21d as a clear oil (381 mg, 95%). Crystals suitable for X-ray analysis were grown by slow evaporation of an acetone solution. $R_{\rm F} = 0.36$ (90:10, EtOAc:PE); IR (Neat) $v_{\rm max}$ 3490 (m), 2978 (w), 1772 (m) and 1692 (s) cm⁻¹; ¹H NMR (400 MHz, $CDCl_3$): $\delta = 7.40-7.35$ (m, 2H, Ar-H), 7.31-7.23 (m, 3H, Ar-H), 4.67 (t, J = 5.7 Hz, 1H, 6-H), 4.65 (d, J = 14.1 Hz, 1H, 11-H), 4.60 (d, J = 14.1 Hz, 1H, 11-H), 3.80–3.67 (m, 2H, 7-H), 2.99 (d, J = 18.4 Hz. 1H, 9-H), 2.70 (d, J = 18.4. Hz, 1H, 9-H), 2.49 (ddd, J = 12.1 Hz, 5.7, 2.4 Hz, 1H, 5-H), 2.03–1.86 (m, 2H, 2-H/1-H), 1.77–1.57 (m, 3H, 2-H/3-H/OH), 1.53 (d, J = 12.1 Hz, 1H, 5-H); ¹³C NMR (100 MHz, CDCl₃): $\delta = 180.2$ (8), 175.3 (10), 136.0 (ArC), 129.0 (ArCH), 128.7 (ArCH), 128.1 (ArCH), 90.3 (3), 77.2 (6), 62.9 (7), 52.9 (4), 44.9 (5), 42.6 (11), 39.3 (9), 31.3 (2), 29.9 (1); HRMS (ESI) m/z calculated for C₁₇H₁₉NNaO₄ 324.1206 $(M+Na)^+$, found 324.1206, 0.2 ppm error.

(1*S**,2*S**,4*R**)-1'-(4-Methoxybenzyl)-1-(hydroxymethyl)spiro[7-oxabicyclo[2.2.1]heptane-2,3'-

pyrrolidine]-2',5'-dione (21e): 4-Methoxybenzyl amide 20e (0.10 g) was hydrogenated according to the general procedure for the hydrogenation of secondary amides. The crude material was purified using flash column chromatography (80:20, EtOAc:PE) to give the target compound 21e as a white crystalline solid (0.098 g, 98%). $R_{\rm F} = 0.29$ (80:20, EtOAc:Hexane); IR (Neat) $v_{\rm max}$ 3416 (w), 3312 (w), 2943 (w), 2835 (w), 1758 (w), 1672 (s), 1609 (m), 1508 (s) cm⁻¹; ¹H NMR (400 MHz, CDCl₃): $\delta = 7.35$ – 7.30 (m, 2H, Ar-H), 6.83–6.78 (m, 2H, Ar-H), 4.67 (t, J = 5.3 Hz, 1H, 6-H), 4.59 (d, J = 14.0 Hz, 1H, 11-H), 4.54 (d, J = 14.0 Hz, 1H, 11-H), 3.79–3.68 (m, 2H, 7-H), 3.75 (s, 3H, 12-H), 2.97 (d, J = 18.2 Hz, 1H, 9-H), 2.68 (d, J = 18.2 Hz, 1H, 9-H), 2.48 (ddd, J = 12.0, 5.5, 2.5 Hz, 1H, 5-H), 2.02–1.93 (m, 1H, 1-H), 1.93–1.86 (m, 1H, 2-H), 1.71–1.58 (m, 1H, 1-H/2-H), 1.52 (d, J = 12.0 Hz, 1H, 5-H); ¹³C NMR (100 MHz, CDCl₃): δ = 180.2 (8), 175.4 (10), 159.3 (ArC), 130.4 (ArCH), 128.2 (ArC), 113.9 (ArCH), 90.3 (3), 77.2 (6), 62.8 (7), 55.3 (12), 52.9 (4), 44.8 (5), 42.0 (11), 39.2 (9), 31.2 (2), 29.9 (1); HRMS (ESI) m/z calculated for C₁₈H₂₂NO₅ 332.1504 (M+H)⁺, found 332.1498, 1.8 ppm error.

2-[(1*S**,5*S**,7*R**)-**4-**Oxo-**3**,10-dioxatricyclo[5.2.1.0^{1,5}]decan-**5-**yl]acetic acid (18): Acid 12 (1.0 g, 4.8 mmol, 1.0 eq.) was dissolved in MeOH (5 mL) under nitrogen. In a separate flask Pd/C (10 wt%) was suspended in MeOH (1 mL) under nitrogen. H_2 was bubbled through the suspension of Pd/C in MeOH for 10 minutes. The solution of secondary amide was then added to the catalyst suspension. The reaction mixture was allowed to stir at room temperature for 16 hours under H₂ (1 bar). The reaction mixture was then passed through a pad of celite® and concentrated *in vacuo* to give the target compound **18** as a white powder (0.93 g, 93%). m.p. 184.6–185.2 °C; IR (Neat) v_{max} 2991 (m), 1713 (s) cm⁻¹; ¹H NMR (400 MHz, CD₃OD): $\delta = 4.59$ (d, J = 10.6 Hz, 1H, 7-H), 4.53 (t, J = 5.4 Hz, 1H, 6-H), 4.48 (d, J = 10.6 Hz, 1H, 7-H), 2.88 (d, J = 12.5, 5.1, 2.3 Hz, 1H, 5-H), 2.04–1.89 (m, 2H, 1-H/2-H), 1.80 (d, J = 12.5 Hz, 1H, 5-H), 1.77–1.67 (m, 2H, 1-H/2-H), 1.80 (d, J = 12.5 Hz, 1H, 5-H), 1.77–1.67 (m, 2H, 1-H/2-H); ¹³C NMR (100 MHz, CD₃OD): $\delta = 181.9$ (8), 173.3 (10), 93.4 (3), 77.7 (6), 70.6 (7), 54.5 (4), 45.9 (5), 40.1 (9), 29.7 (1), 25.2 (2); HRMS (ESI) *m*/*z* calculated for C₁₀H₁₃O₅ 213.0769 (M+H)⁺, found 213.0763, 2.8 ppm error.

General procedure for the synthesis of hydrogenated secondary amides. Hydrogenated acid 18 (0.5 g, 2.4 mmol, 1.0 eq.) was suspended in anhydrous CH₂Cl₂ (5 mL) under an atmosphere of nitrogen. The suspension was cooled to 0 °C and oxalyl chloride 2.0 M solution in CH₂Cl₂ (3.0 mL, 5.9 mmol, 2.5 eq.) was added dropwise over 10 minutes, followed by DMF (2 drops). The suspension was stirred at ambient temperature until a solution was obtained, after which it was concentrated in vacuo, to yield the crude acid chloride as a light brown solid. The crude material was redissolved in fresh anhydrous CH₂Cl₂ (5 mL) and cooled to 0 °C. Pyridine (0.19 mL, 2.4 mmol, 1.0 eq.) was then added dropwise, followed by the primary amine (2.4 mmol, 1.0 eq.) in CH₂Cl₂ (5 mL). The solution was allowed to stir at ambient temperature overnight before diluting with CH₂Cl₂ (30 mL) and H₂O (50 mL). The organic layer was separated and further washed with 1M HCl_(aq) (50 mL), H₂O (50 mL) and brine (50 mL). The organic layer was dried (MgSO₄), filtered and concentrated in vacuo.

N-Butyl-2-[(1S*,5S*,7R*)-4-oxo-3,10-dioxatricyclo

[5.2.1.0^{1,5}]decan-5-yl]acetamide (19a): Hydrogenated acid 18 (0.50 g, 2.4 mmol, 1.0 eq.) and butylamine (0.24 mL, 2.4 mmol, 1.0 eq.) were coupled according to the general procedure for the synthesis of hydrogenated secondary amides. The crude material was then purified by flash column chromatography (80:20, EtOAc:PE) to give the target compound 19a as a white solid (0.21 g, 33%). $R_{\rm F} = 0.27$ (80:20, EtOAc:PE); m.p. 58.1–58.5 °C; IR (Neat) v_{max} 3319 (w), 2958 (w), 2868 (w), 1765 (s), 1635 (s), 1553 (m) cm⁻¹; ¹H NMR (400 MHz, CDCl₃): $\delta = 6.26$ (br. s, 1H, N-H), 4.55 (t, J = 5.5 Hz, 1H, 6-H), 4.50 (s, 2H, 7-H), 3.25-3.12 (m, 2H, 11-H), 2.65 (d, J = 15.0 Hz, 1H, 9-H), 2.41 (d, J = 15.0 Hz, 1H, 9-H), 2.42–2.37 (m, 1H, 5-H), 2.24 (ddd, J = 12.2, 8.8, 3.2 Hz, 2-H), 2.02–1.92 (m, 1H, 1-H), 1.75 (t, J = 12.2, 5.5 Hz, 1H, 2-H), 1.61 (d, J = 12.5 Hz, 1H, 5-H), 1.61–1.55 (m, 1H, 1-H), 1.50-1.40 (m, 2H, 12-H), 1.36-1.27 (m, 2H, 13-H), 0.89 (t, J = 7.4 Hz, 3H, 14-H); ¹³C NMR (100 MHz, CDCl₃): $\delta = 180.6$ (8), 168.5 (10), 92.3 (3), 76.3 (6), 69.8 (7), 54.2 (4), 44.6 (5), 41.4 (9), 39.6 (11), 31.4 (12), 29.0 (1), 24.6 (2), 20.1 (13), 13.8 (14); HRMS (ESI) m/z calculated for C₁₄H₂₂NO₄ 268.1555 (M+H)⁺, found 268.1549, 2.2 ppm error.

N-Hexyl-2-[(1*S**,5*S**,7*R**)-4-oxo-3,10-dioxatricyclo [5.2.1.0^{1,5}]decan-5-yl]acetamide (19b): Hydrogenated acid 18 (0.50 g, 2.4 mmol, 1.0 eq.) and hexylamine (0.31 mL, 2.4 mmol, 1.0 eq.) were coupled according to the general procedure for the synthesis of hydrogenated secondary amides. The crude material was then purified by flash column chromatography (70:30, EtOAc:PE) to give the target compound 19b as a yellow oil (0.43 g, 61%). $R_F = 0.27$ (70:30, EtOAc:PE); IR (Neat) v_{max} 3312 (w), 2950 (m), 2924 (m), 1762 (s), 1643 (s), 1538 (s) cm⁻¹; ¹H NMR (400 MHz, CDCl₃): $\delta = 6.28$ (br. s, 1H, N-H), 4.54 (t, J = 5.3 Hz, 1H, 6-H), 4.49 (s, 2H, 7-H), 3.24–3.11 (m, 2H, 11-H), 2.65 (d, J

= 14.6 Hz, 1H, 9-H), 2.41 (d, J = 14.6 Hz, 1H, 9-H), 2.41–2.37 (m, 1H, 5-H), 2.23 (ddd, J = 12.5, 8.9, 3.1 Hz, 1H, 2-H), 2.01–1.91 (m, 1H, 1-H), 1.79–1.70 (m, 1H, 2-H), 1.60 (d, J = 12.7 Hz, 1H, 5-H), 1.60–1.55 (m, 1H, 1-H), 1.49–1.40 (m, 2H, 12-H), 1.32–1.22 (m, 6H, 13-H/14-H/15-H), 0.85 (t, J = 6.7 Hz, 3H, 16-H); ¹³C NMR (100 MHz, CDCl₃): $\delta = 180.6$ (8), 168.5 (10), 92.3 (3), 76.2 (6), 69.7 (7), 54.2 (4), 44.6 (5), 41.3 (9), 39.9 (11), 31.5 (13), 29.3 (12), 29.0 (1), 26.6 (14), 24.55 (2), 22.6 (15), 14.1 (16); HRMS (ESI) *m*/*z* calculated for C₁₆H₂₆NO4 296.1870 (M+H)⁺, found 296.1862, 2.7 ppm error.

N-Octyl-2-[(1S*,5S*,7R*)-4-oxo-3,10-dioxatricyclo

[5.2.1.0^{1,5}]decan-5-vl]acetamide (19c): Hydrogenated acid 18 (0.50 g, 2.4 mmol, 1.0 eq.) and octylamine (0.39 mL, 2.4 mmol, 1.0 eq.) were coupled according to the general procedure for the synthesis of hydrogenated secondary amides. The crude material was then purified by flash column chromatography (70:30, EtOAc:PE) to give the target compound 19c as a yellow waxy solid (0.51 g, 67%). R_F = 0.3 (70:30, EtOAc:PE); m.p. 53.4–54.0 °C; IR (Neat) v_{max} 3394 (w), 2917 (m), 2850 (m), 1754 (s), 1665 (s) and 1523 (m) cm⁻¹; ¹H NMR (400 MHz, CDCl3): $\delta = 6.30$ (br. s, 1H, N-H), 4.54 (t, J = 5.2 Hz, 1H, 6-H), 4.49 (s, 2H, 7-H), 3.21–3.11 (m, 2H, 11-H), 2.65 (d, J = 14.5 Hz, 1H, 9-H), 2.40 (d, J = 14.5 Hz, 1H, 9-H), 2.40–2.35 (m, 1H, 5-H), 2.22 (ddd, J =12.3, 8.6, 3.1 Hz, 1H, 2-H), 2.01-1.90 (m, 1H, 1-H), 1.79-1.69 (m, 1H, 2-H), 1.60 (d, J = 12.5 Hz, 1H, 5-H), 1.60–1.54 (m, 1H, 1-H), 1.49-1.39 (m, 2H, 12-H), 1.31-1.17 (m, 10H, 13-H/14-H/15-H/16-H/17-H), 0.84 (t, J = 6.6 Hz, 3H, 18-H); ¹³C NMR $(100 \text{ MHz}, \text{CDCl}_3): \delta = 180.6 (8), 168.5 (10), 92.25 (3), 76.2 (6),$ 69.7 (7), 54.2 (4), 44.6 (5), 41.4 (9), 39.9 (11), 31.9 (13), 29.4 (4), 29.3 (12/15) 29.0 (1), 26.9 (16), 24.6 (2), 22.7 (17), 14.2 (18); HRMS (ESI) m/z calculated for C₁₈H₃₀NO₄ 324.2184 (M+H)⁺, found 324.2175, 2.8 ppm error.

N-Benzyl-2-[(1S*,5S*,7R*)-4-oxo-3,10-dioxatricyclo

[5.2.1.0^{1,5}]decan-5-yl]acetamide (19d): Hydrogenated acid 18 (0.50 g, 2.4 mmol, 1.0 eq.) and benzylamine (0.26 mL, 2.4 mmol, 1.0 eq.) were coupled according to the general procedure for the synthesis of hydrogenated secondary amides. The crude material was then purified by flash column chromatography (75:25, EtOAc:PE) to give the target compound 19d as a white crystalline solid (0.55 g, 77%). $R_{\rm F} = 0.28$ (75:25, EtOAc:PE); m.p. 122.2-122.7 °C; IR (Neat) v_{max} 3326 (w), 2961 (w), 1765 (s), 1635 (s) and 1538 (s) cm⁻¹; ¹H NMR (400 MHz, CDCl₃): $\delta =$ 7.27–7.15 (m, 5H, ArH), 6.75–6.69 (br. s, 1H, N-H), 4.45 (t, J = 5.3 Hz, 1H, 6-H), 4.38 (s, 2H, 7-H), 4.31 (dd, J = 14.9, 6.1 Hz, 1H, 11-H), 4.26 (dd, J = 14.9, 6.1 Hz, 1H, 11-H), 2.62 (d, J = 14.0 Hz, 1H, 9-H), 2.34 (d, J = 14.0 Hz, 1H, 9-H), 2.27 (ddd, J = 12.5, 5.2, 2.4 Hz, 1H, 5-H), 2.10 (ddd, J = 12.4, 8.7, 3.2 Hz, 1H, 1-H), 1.93-1.82 (m, 1H, 2-H), 1.70-1.60 (m, 1H, 1-H), 1.53 (d, J = 12.5 Hz, 1H, 5-H), 1.53–1.47 (m, 1H, 2-H); ¹³C NMR (100) MHz, $CDCl_{3}$: $\delta = 180.5$ (8), 168.6 (10), 137.8 (ArC), 128.7 (ArCH), 127.8 (ArCH), 127.6 (ArCH), 92.2 (3), 76.2 (6), 69.6 (7), 54.0 (4), 44.6 (5), 43.8 (11), 41.1 (9), 29.0 (2), 24.5 (1); HRMS (ESI) m/z calculated for C₁₇H₂₀NO₄ 302.1399 (M+H)⁺, found 302.1392, 2.3 ppm error.

N-(4-Methoxybenzyl)-2-[($1S^*, 5S^*, 7R^*$)-4-oxo-3,10-dioxatricyclo[5.2.1.0^{1.5}]decan-5-yl]acetamide

dioxatricyclo[5.2.1.0^{1.5}]decan-5-yl]acetamide (19e): Hydrogenated acid 18 (0.50 g, 2.4 mmol, 1.0 eq.) and 4methoxybenzylamine (0.31 mL, 2.4 mmol, 1.0 eq.) were coupled according to the general procedure for the synthesis of hydrogenated secondary amides. The crude material was then purified by flash column chromatography (80:20, EtOAc:PE) to give the target compound 19e as a white powder (0.45 g, 58%). $R_{\rm F} = 0.29$ (80:20, EtOAc:PE); m.p. 160.7–161.4 °C; IR (Neat) $v_{\rm max}$ 3259 (w), 2954 (w), 1762 (s), 1635 (m), 1568 (m) and 1508 (m) cm⁻¹; ¹H NMR (400 MHz, CDCl₃): $\delta = 8.49$ (t, J = 5.7 Hz, 1H, N-H), 7.21–7.14 (m, 2H, ArH), 6.91–6.84 (m, 2H, ArH), 4.48 (t, J = 5.4 Hz, 1H, 6-H), 4.37 (d, J = 10.4 Hz, 1H, 7-H), 4.33 (d, J = 10.4 Hz, 1H, 7-H), 4.22–4.12 (m, 2H, 11-H), 3.73 (s, 3H, 12-H), 2.66 (d, J = 14.4 Hz, 1H, 9-H), 2.46 (d, J = 14.4 Hz, 1H, 9-H), 2.10–2.03 (m, 1H, 5-H), 2.02–1.94 (m, 1H, 1-H), 1.85–1.76 (m, 1H, 2-H), 1.72 (d, J = 12.1 Hz, 1H, 5-H), 1.64–1.54 (m, 2H, 1-H/2-H); ¹³C NMR (100 MHz, CDCl₃): $\delta = 179.4$ (8), 168.4 (10), 158.3 (ArC), 131.0 (ArC), 128.7 (ArCH), 113.7 (ArCH), 91.8 (3), 75.5 (6), 68.5 (7), 55.1 (12), 53.2 (4), 44.4 (5), 41.7 (11), 39.9 (9), 28.5 (2), 24.2 (1); HRMS (ESI) *m/z* calculated for C₁₈H₂₂NO₅ 332.1498 (M+H)⁺, found 332.1498, 2.7 ppm error.

4.3. Cell culture and stock solutions

A549 human lung carcinoma cells were obtained from the American Type Culture Collection (ATCC; Middlesex UK) and used in a passage window of 25. HCT 116 colorectal carcinoma cells, OVCAR-3 human ovarian adenocarcinoma cells, MDA-MB-231 human breast adenocarcinoma cells, MCF7 human breast adenocarcinoma cells and PC-3 human prostate adenocarcinoma cells were obtained from AMS Biotechnology (Europe) Limited (Abingdon, UK) and used in a passage window of 10. Cells were cultured in F-12K Medium (A549, PC-3), McCoy's 5A Medium (HCT 116), RPMI-1640 Medium (MDA-MB-231, OVCAR-3) or Minimum Essential Medium (MCF7) (Sigma-Aldrich) supplemented with 10% (ν/ν) FBS (Sigma-Aldrich) and at 37 °C with 5% CO₂. Stock solutions of compounds were prepared as 40 mM solutions in DMSO and stored at 4 °C.

4.4. Cytocompatibility evaluation in cancer cell panel

The cytotoxicity of the compounds was assessed using the PrestoBlueTM Cell Viability assay at two fixed concentrations of 100 µM and 200 µM at three time points of 24, 48 and 72 h. Cells were seeded into 96 well plates at a density of 3×10^3 cells per well and allowed to adhere in growth media overnight at 37 °C and 5% CO₂. Cell media were discarded and replaced with fresh media (100 µL) containing the appropriate compound and concentration. Triton X-100 applied at 0.5% (v/v) in culture medium was used as a cell death (positive) control and culture medium containing 0.5% DMSO was used as a negative control. After 24, 48 or 72 h exposure, the medium was removed and replaced with 100 µL fresh medium containing 10% (v/v) PrestoBlue reagent for 60 minutes. Cell viability was then assessed by measuring resultant fluorescence readings at 544/620 nm $(\lambda_{ex}/\lambda_{em})$. Relative metabolic activity was calculated by setting values from the negative control as 100% and positive control values as 0% metabolic activity.

Compound 21c underwent further dose-response analysis in all cell lines to calculate the EC₅₀ value. Cells were seeded into 96 well plates at a density of 3×10^3 cells per well and allowed to adhere in growth media overnight at 37 °C and 5% CO2. Cell media were discarded and replaced with fresh media (100 µL) containing 17c (5-250 µM). Triton X-100 applied at 0.5% (v/v) in culture medium was used as a cell death (positive) control and culture medium containing 0.5% DMSO was used as a negative control. After 48 h exposure, the medium was removed and replaced with 100 µL fresh medium containing 10% (v/v) PrestoBlue reagent for 60 minutes. Cell viability was then assessed by measuring resultant fluorescence readings at 544/620 nm ($\lambda_{ex}/\lambda_{em}$). Relative metabolic activity was calculated by setting values from the negative control as 100% and positive control values as 0% metabolic activity. EC50 values were calculated using the dose-response fitting function in OriginPro software.

Bibliography

- Hargreaves, M. K.; Pritchard, J. G.; Dave, H. R. Cyclic Carboxylic Monoimides. *Chem. Rev.* 1970, 70 (4), 439–469. https://doi.org/10.1021/cr60266a001.
- Guo, X.; Facchetti, A.; Marks, T. J. Imide- and Amide-Functionalized Polymer Semiconductors. *Chem. Rev.* 2014, *114* (18), 8943–9021. https://doi.org/10.1021/cr500225d.
- Franks, M. E.; Macpherson, G. R.; Figg, W. D. Thalidomide. Lancet 2004, 363 (9423), 1802–1811. https://doi.org/10.1016/S0140-6736(04)16308-3.
- Espinosa-Jalapa, N. A.; Kumar, A.; Leitus, G.; Diskin-Posner, Y.; Milstein, D. Synthesis of Cyclic Imides by Acceptorless Dehydrogenative Coupling of Diols and Amines Catalyzed by a Manganese Pincer Complex. J. Am. Chem. Soc. 2017, 139 (34), 11722–11725. https://doi.org/10.1021/jacs.7b08341.
- Wu, Y.-H.; Rayburn, J. W.; Allen, L. E.; Ferguson, H. C.; Kissel, J. W. Psychosedative Agents. 2. 8-(4-Substituted 1-Piperazinylalkyl)-8-Azaspiro[4.5]Decane-7,9-Diones. J. Med. Chem. 1972, 15 (5), 477–479. https://doi.org/10.1021/jm00275a009.
- Abdel-Aziz, A. A.-M.; ElTahir, K. E. H.; Asiri, Y. A. Synthesis, Anti-Inflammatory Activity and COX-1/COX-2 Inhibition of Novel Substituted Cyclic Imides. Part 1: Molecular Docking Study. *Eur. J. Med. Chem.* 2011, 46 (5), 1648–1655. https://doi.org/10.1016/j.ejmech.2011.02.013.
- Verschueren, W. G.; Dierynck, I.; Amssoms, K. I. E.; Hu, L.; Boonants, P. M. J. G.; Pille, G. M. E.; Daeyaert, F. F. D.; Hertogs, K.; Surleraux, D. L. N. G.; Wigerinck, P. B. T. P. Design and Optimization of Tricyclic Phtalimide Analogues as Novel Inhibitors of HIV-1 Integrase. *J. Med. Chem.* 2005, *48* (6), 1930– 1940. https://doi.org/10.1021/jm049559q.
- Zhang, Y.-Y.; Zhou, C.-H. Synthesis and Activities of Naphthalimide Azoles as a New Type of Antibacterial and Antifungal Agents. *Bioorg. Med. Chem. Lett.* 2011, 21 (14), 4349– 4352. https://doi.org/10.1016/j.bmcl.2011.05.042.
- Zentz, F.; Valla, A.; Le Guillou, R.; Labia, R.; Mathot, A.-G.; Sirot, D. Synthesis and Antimicrobial Activities of N-Substituted Imides. *Farm.* 2002, *57* (5), 421–426. https://doi.org/10.1016/S0014-827X(02)01217-X.
- Machado, K. E.; Oliveira, K. N. de; Santos-Bubniak, L.; Licínio, M. A.; Nunes, R. J.; Santos-Silva, M. C. Evaluation of Apoptotic Effect of Cyclic Imide Derivatives on Murine B16F10 Melanoma Cells. *Bioorg. Med. Chem.* 2011, 19 (21), 6285–6291. https://doi.org/10.1016/j.bmc.2011.09.008.
- Puerto Galvis, C. E.; Vargas Méndez, L. Y.; Kouznetsov, V. V. Cantharidin-Based Small Molecules as Potential Therapeutic Agents. *Chem. Biol. Drug Des.* **2013**, *82* (5), 477–499. https://doi.org/10.1111/cbdd.12180.
- Wang, G.-S. Medical Uses of Mylabris in Ancient China and Recent Studies. J. Ethnopharmacol. 1989, 26 (2), 147–162. https://doi.org/10.1016/0378-8741(89)90062-7.
- Kok, S. H. L.; Chui, C. H.; Lam, W. S.; Chen, J.; Lau, F. Y.; Wong, R. S. M.; Cheng, G. Y. M.; Lai, P. B. S.; Leung, T. W. T.; Yu, M. W. Y.; Tang, J. C. O.; Chan, A. S. C. Synthesis and Structure Evaluation of a Novel Cantharimide and Its Cytotoxicity on SK-Hep-1 Hepatoma Cells. *Bioorg. Med. Chem. Lett.* 2007, *17* (5), 1155–1159. https://doi.org/10.1016/j.bmcl.2006.12.039.
- McCluskey, A.; Walkom, C.; Bowyer, M. C.; Ackland, S. P.; Gardiner, E.; Sakoff, J. A. Cantharimides: A New Class of Modified Cantharidin Analogues Inhibiting Protein Phosphatases 1 and 2A. *Bioorg. Med. Chem. Lett.* **2001**, *11* (22), 2941–2946. https://doi.org/10.1016/S0960-894X(01)00594-7.
- Robertson, M. J.; Gordon, C. P.; Gilbert, J.; McCluskey, A.; Sakoff, J. A. Norcantharimide Analogues Possessing Terminal Phosphate Esters and Their Anti-Cancer Activity. *Bioorg. Med. Chem.* 2011, *19* (18), 5734–5741. https://doi.org/10.1016/j.bmc.2011.01.031.
- Massicot, F.; Dutertre-Catella, H.; Pham-Huy, C.; Liu, X.-H.; Duc, H. T.; Warnet, J.-M. In Vitro Assessment of Renal Toxicity and Inflammatory Events of Two Protein Phosphatase Inhibitors Cantharidin and Nor-Cantharidin*. *Basic Clin. Pharmacol. Toxicol.* 2005, *96* (1), 26–32. https://doi.org/10.1111/j.1742-7843.2005.pto960104.x.
- Tromovitch, T. A. Cantharadin. JAMA J. Am. Med. Assoc. 1971, 215 (4), 640. https://doi.org/10.1001/jama.1971.03180170074022.

- Grogan, C. H.; Rice, L. M. Bicyclic Imides and Isoindolines 1. J. Med. Chem. 1963, 6 (6), 802–805. https://doi.org/10.1021/jm00342a042.
- Tatlock, J. H.; Angelica Linton, M.; Hou, X. J.; Kissinger, C. R.; Pelletier, L. A.; Showalter, R. E.; Tempczyk, A.; Ernest Villafranca, J. Structure-Based Design of Novel Calcineurin (PP2B) Inhibitors. *Bioorg. Med. Chem. Lett.* **1997**, 7 (8), 1007– 1012. https://doi.org/10.1016/S0960-894X(97)00141-8.
- Laidley, C. W.; Dauben, W. G.; Guo, Z. R.; Lam, J. Y. L.; Casida, J. E. 2-Carboxymethylendothal Analogues as Affinity Probes for Stabilized Protein Phosphatase 2A. *Bioorg. Med. Chem.* **1999**, 7 (12), 2937–2944. https://doi.org/10.1016/S0968-0896(99)00239-4.
- Essers, M.; Wibbeling, B.; Haufe, G. Synthesis of the First Fluorinated Cantharidin Analogues. *Tetrahedron Lett.* 2001, 42 (32), 5429–5433. https://doi.org/10.1016/S0040-4039(01)01056-5.
- Baba, Y.; Hirukawa, N.; Tanohira, N.; Sodeoka, M. Structure-Based Design of a Highly Selective Catalytic Site-Directed Inhibitor of Ser/Thr Protein Phosphatase 2B (Calcineurin). J. Am. Chem. Soc. 2003, 125 (32), 9740–9749. https://doi.org/10.1021/ja034694y.
- Thaqi, A.; Scott, J. L.; Gilbert, J.; Sakoff, J. A.; McCluskey, A. Synthesis and Biological Activity of Δ-5,6-Norcantharimides: Importance of the 5,6-Bridge. *Eur. J. Med. Chem.* 2010, 45 (5), 1717–1723. https://doi.org/10.1016/j.ejmech.2010.01.004.
- McCluskey, A.; Taylor, C.; Quinn, R. J.; Suganuma, M.; Fujiki, H. Inhibition of Protein Phosphatase 2A by Cantharidin Analogues. *Bioorg. Med. Chem. Lett.* **1996**, 6 (9), 1025–1028. https://doi.org/10.1016/0960-894X(96)00166-7.
- Enz, A.; Zenke, G.; Pombo-Villar, E. 7-Oxa[2.2.1]Bicycloheptane-2,3-Dicarboxylic Acid Derivatives as Phosphatase Inhibitors. *Bioorg. Med. Chem. Lett.* **1997**, 7 (19), 2513–2518. https://doi.org/10.1016/S0960-894X(97)10005-1.
- Sodeoka, M.; Baba, Y.; Kobayashi, S.; Hirukawa, N. Structure-Activity Relationship of Cantharidin Derivatives to Protein Phosphatases 1, 2A1, and 2B. *Bioorg. Med. Chem. Lett.* 1997, 7 (14), 1833–1836. https://doi.org/10.1016/S0960-894X(97)00316-9
- McCluskey, A.; Keane, M. A.; Mudgee, L.-M.; Sim, A. T. R.; Sakoff, J.; Quinn, R. J. Anhydride Modified Cantharidin Analogues. Is Ring Opening Important in the Inhibition of Protein Phosphatase 2A? *Eur. J. Med. Chem.* 2000, *35* (10), 957–964. https://doi.org/10.1016/S0223-5234(00)00186-0.
- Hart, M. E.; Chamberlin, A. R.; Walkom, C.; Sakoff, J. A.; McCluskey, A. Modified Norcantharidins: Synthesis, Protein Phosphatases 1 and 2A Inhibition and Anticancer Activity. *Bioorg. Med. Chem. Lett.* 2004, *14* (8), 1969–1973. https://doi.org/10.1016/j.bmcl.2004.01.093.
- Hill, T. A.; Stewart, S. G.; Gordon, C. P.; Ackland, S. P.; Gilbert, J.; Sauer, B.; Sakoff, J. A.; McCluskey, A. Norcantharidin Analogues: Synthesis, Anticancer Activity and Protein Phosphatase 1 and 2A Inhibition. *ChemMedChem* 2008, *3* (12), 1878–1892. https://doi.org/10.1002/cmdc.200800192.
- Pen-Yuan, L.; Sheng-Jie, S.; Hsien-Liang, S.; Hsue-Fen, C.; Chiung-Chang, L.; Pong-Chun, L.; Leng-Fang, W. A Simple Procedure for Preparation of N-Thiazolyl and N-Thiadiazolylcantharidinimides and Evaluation of Their Cytotoxicities against Human Hepatocellular Carcinoma Cells. *Bioorg. Chem.* 2000, 28 (5), 266–272. https://doi.org/10.1006/bioo.2000.1178.
- Lin, L.-H.; Huang, H.-S.; Lin, C.-C.; Lee, L.-W.; Lin, P.-Y. Effects of Cantharidinimides on Human Carcinoma Cells. *Chem. Pharm. Bull. (Tokyo).* 2004, *52* (7), 855–857. https://doi.org/10.1248/cpb.52.855.
- Hill, T. A.; Stewart, S. G.; Ackland, S. P.; Gilbert, J.; Sauer, B.; Sakoff, J. A.; McCluskey, A. Norcantharimides, Synthesis and Anticancer Activity: Synthesis of New Norcantharidin Analogues and Their Anticancer Evaluation. *Bioorg. Med. Chem.* 2007, *15* (18), 6126–6134. https://doi.org/10.1016/j.bmc.2007.06.034.
- Campbell, B. E.; Tarleton, M.; Gordon, C. P.; Sakoff, J. A.; Gilbert, J.; McCluskey, A.; Gasser, R. B. Norcantharidin Analogues with Nematocidal Activity in Haemonchus Contortus. *Bioorg. Med. Chem. Lett.* 2011, 21 (11), 3277–3281. https://doi.org/10.1016/j.bmcl.2011.04.031.
- Wu, J.-Y.; Kuo, C.-D.; Chu, C.-Y.; Chen, M.-S.; Lin, J.-H.; Chen, Y.-J.; Liao, H.-F. Synthesis of Novel Lipophilic N-Substituted Norcantharimide Derivatives and Evaluation of Their Anticancer Activities. *Molecules* 2014, 19 (6), 6911–6928. https://doi.org/10.3390/molecules19066911.

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- Foster, R. W.; Benhamou, L.; Porter, M. J.; Bučar, D.-K.; Hailes, H. C.; Tame, C. J.; Sheppard, T. D. Irreversible Endo -Selective Diels-Alder Reactions of Substituted Alkoxyfurans: A General Synthesis of Endo -Cantharimides. *Chem. Eur. J.* 2015, *21* (16), 6107–6114. https://doi.org/10.1002/chem.201406286.
- Bai, Y.; De bruyn, M.; Clark, J. H.; Dodson, J. R.; Farmer, T. J.; Honoré, M.; Ingram, I. D. V.; Naguib, M.; Whitwood, A. C.; North, M. Ring Opening Metathesis Polymerisation of a New Bio-Derived Monomer from Itaconic Anhydride and Furfuryl Alcohol. *Green Chem.* 2016, *18* (14), 3945–3948. https://doi.org/10.1039/C6GC00623J.
- Pehere, A. D.; Xu, S.; Thompson, S. K.; Hillmyer, M. A.; Hoye, T. R. Diels–Alder Reactions of Furans with Itaconic Anhydride: Overcoming Unfavorable Thermodynamics. *Org. Lett.* **2016**, *18* (11), 2584–2587. https://doi.org/10.1021/acs.orglett.6b00929.
- Berovic, M.; Legisa, M. Citric Acid Production. In *Biotechnology* Annual Review; Elsevier, 2007; Vol. 13, pp 303–343. https://doi.org/10.1016/S1387-2656(07)13011-8.
- Moreau, C.; Belgacem, M. N.; Gandini, A. Recent Catalytic Advances in the Chemistry of Substituted Furans from Carbohydrates and in the Ensuing Polymers. *Top. Catal.* 2004, 27 (1–4), 11–30. https://doi.org/10.1023/B:TOCA.0000013537.13540.0e.
- Bai, Y.; Clark, J. H.; Farmer, T. J.; Ingram, I. D. V; North, M. Wholly Biomass Derivable Sustainable Polymers by Ring-Opening Metathesis Polymerisation of Monomers Obtained from Furfuryl Alcohol and Itaconic Anhydride. *Polym. Chem.* 2017, 8, 3074–3081. https://doi.org/10.1039/c7py00486a.
- Lawrenson, S. B.; Hart, S.; Ingram, I. D. V; North, M.; Parker, R. R.; Whitwood, A. C. Ring-Opening Metathesis Polymerization of Tertiary Amide Monomers Derived from a Biobased Oxanorbornene. ACS Sustain. Chem. Eng. 2018, 6 (8), 9744– 9752. https://doi.org/10.1021/acssuschemeng.8b00779.
- Blanpain, A.; Clark, J. H.; Farmer, T. J.; Guo, Y.; Ingram, I. D. V.; Kendrick, J. E.; Lawrenson, S. B.; North, M.; Rodgers, G.; Whitwood, A. C. Rapid Ring Opening Metathesis Polymerization of Monomers Obtained from Biomass Derived Furfuryl Amines and Maleic Anhydride. *ChemSusChem* 2019, *12* (11), 2393–2401. https://doi.org/10.1002/cssc.201900748.
- Zhang, J.; Senthilkumar, M.; Ghosh, S. C.; Hong, S. H. Synthesis of Cyclic Imides from Simple Diols. *Angew. Chem. Int. Ed.* 2010, 49 (36), 6391–6395. https://doi.org/10.1002/anie.201002136.
- Ito, R.; Umezawa, N.; Higuchi, T. Unique Oxidation Reaction of Amides with Pyridine- N -Oxide Catalyzed by Ruthenium Porphyrin: Direct Oxidative Conversion of N -Acyl- 1 -Proline to N -Acyl- 1 -Glutamate. J. Am. Chem. Soc. 2005, 127 (3), 834–835. https://doi.org/10.1021/ja045603f.
- Driller, K. M.; Klein, H.; Jackstell, R.; Beller, M. Iron-Catalyzed Carbonylation: Selective and Efficient Synthesis of Succinimides. *Angew. Chem. Int. Ed.* 2009, 48 (33), 6041–6044. https://doi.org/10.1002/anie.200902078.
- Pal'chikov, V. A.; Tarabara, I. N.; Omel'chenko, I. V.; Shishkin, O. V.; Kas'yan, L. I. Reaction of N-Substituted Exo-2-Hydroxy-5-Oxo-4-Oxatricyclo[4.2.1.03,7]Nonane-Endo-9-Carboxamides with Acetic Acid. *Russ. J. Org. Chem.* 2010, *46* (6), 823–829. https://doi.org/10.1134/S1070428010060096.
- Kaiser, D.; Bauer, A.; Lemmerer, M.; Maulide, N. Amide Activation: An Emerging Tool for Chemoselective Synthesis. *Chem. Soc. Rev.* 2018, 47 (21), 7899–7925. https://doi.org/10.1039/C8CS00335A.
- Breugst, M.; Tokuyasu, T.; Mayr, H. Nucleophilic Reactivities of Imide and Amide Anions. J. Org. Chem. 2010, 75 (15), 5250– 5258. https://doi.org/10.1021/jo1009883.
- Tshepelevitsh, S.; Kütt, A.; Lõkov, M.; Kaljurand, I.; Saame, J.; Heering, A.; Plieger, P. G.; Vianello, R.; Leito, I. On the Basicity of Organic Bases in Different Media. *Eur. J. Org. Chem.* 2019, 2019 (40), 6735–6748. https://doi.org/10.1002/ejoc.201900956.
- Colomer, I.; Empson, C. J.; Craven, P.; Owen, Z.; Doveston, R. G.; Churcher, I.; Marsden, S. P.; Nelson, A. A Divergent Synthetic Approach to Diverse Molecular Scaffolds: Assessment of Lead-Likeness Using LLAMA, an Open-Access Computational Tool. *Chem. Commun.* 2016, *52* (45), 7209–7212. https://doi.org/10.1039/C6CC03244C.
- Irwin, J. J.; Sterling, T.; Mysinger, M. M.; Bolstad, E. S.; Coleman, R. G. ZINC: A Free Tool to Discover Chemistry for Biology. J. Chem. Inf. Model. 2012, 52 (7), 1757–1768. https://doi.org/10.1021/ci3001277.
- 52. Tarleton, M.; Gilbert, J.; Sakoff, J. A.; McCluskey, A. Synthesis and Anticancer Activity of a Series of Norcantharidin Analogues.

Eur. J. Med. Chem. **2012**, *54*, 573–581. https://doi.org/10.1016/j.ejmech.2012.06.010.

- Kok, S.; Chui, C.; Lam, W.; Chen, J.; Lau, F.; Wong, R.; Cheng, G.; Tang, W.; Cheng, C.; Tang, J.; Chan, A. Mechanistic Insight into a Novel Synthetic Cantharidin Analogue in a Leukaemia Model. *Int. J. Mol. Med.* 2006, *18* (2), 375–379. https://doi.org/10.3892/ijmm.18.2.375.
- Lu, B.; Vayssade, M.; Miao, Y.; Chagnault, V.; Grand, E.; Wadouachi, A.; Postel, D.; Drelich, A.; Egles, C.; Pezron, I. Physico-Chemical Properties and Cytotoxic Effects of Sugar-Based Surfactants: Impact of Structural Variations. *Colloids Surfaces B Biointerfaces* 2016, *145*, 79–86. https://doi.org/10.1016/j.colsurfb.2016.04.044.
- Perinelli, D. R.; Cespi, M.; Casettari, L.; Vllasaliu, D.; Cangiotti, M.; Ottaviani, M. F.; Giorgioni, G.; Bonacucina, G.; Palmieri, G. F. Correlation among Chemical Structure, Surface Properties and Cytotoxicity of N-Acyl Alanine and Serine Surfactants. *Eur. J. Pharm. Biopharm.* **2016**, *109*, 93–102. https://doi.org/10.1016/j.ejpb.2016.09.015.
- Partearroyo, M. A.; Ostolaza, H.; Goñi, F. M.; Barberá-Guillem, E. Surfactant-Induced Cell Toxicity and Cell Lysis. *Biochem. Pharmacol.* 1990, 40 (6), 1323–1328. https://doi.org/10.1016/0006-2952(90)90399-6.
- Lawrence, M. J. Surfactant Systems: Their Use in Drug Delivery. *Chem. Soc. Rev.* **1994**, *23* (6), 417. https://doi.org/10.1039/cs9942300417.
- Ma, L.; Kohli, M.; Smith, A. Nanoparticles for Combination Drug Therapy. ACS Nano 2013, 7 (11), 9518–9525. https://doi.org/10.1021/nn405674m.
- Florento, L.; Matias, R.; Tuaño, E.; Santiago, K.; dela Cruz, F.; Tuazon, A.; Florento, L. M. Comparison of Cytotoxic Activity of Anticancer Drugs against Various Human Tumor Cell Lines Using In Vitro Cell-Based Approach. *Int. J. Biomed. Sci.* 2012, 8 (1), 76–80.
- Groom, C. R.; Bruno, I. J.; Lightfoot, M. P.; Ward, S. C. The Cambridge Structural Database. *Acta. Cryst.* 2016, *B72* (2), 171– 179. https://doi.org/10.1107/S2052520616003954.
- Nadin, A.; Hattotuwagama, C.; Churcher, I. Lead-Oriented Synthesis: A New Opportunity for Synthetic Chemistry. *Angew. Chem. Int. Ed.* 2012, *51* (5), 1114–1122. https://doi.org/10.1002/anie.201105840.
- Bernis, G. W.; Murcko, M. A. The Properties of Known Drugs. 1. Molecular Frameworks. J. Med. Chem. 1996, 39 (15), 2887–2893. https://doi.org/10.1021/jm9602928.

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Declaration of interests

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The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: