A Flow Chemistry Approach to Norcantharidin Analogues

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Received January 14, 2011: Revised March 18, 2011: Accepted March 29, 2011

Abstract: Acid-ester and acid-amide norcantharidin derivatives are prepared using a 'one-pot' synthetic procedure utilizing the ThalesNano H-cube™ flow hydrogenator. Traditionally, rapid library generation and reaction scale up of these analogues was limited by the batch wise hydrogenation of 5,6-dehydronorcantharidin. This was resolved with the use of flow chemistry. With no associated scale up issues, a method was devised to produce norcantharidin, along with acid-ester and acid-amide analogues on any scale necessary for biological screening.

Keywords: Cantharidin, Norcantharidin, Flow hydrogenation, Flow chemistry, Protein phosphatase inhibition.

INTRODUCTION

The process of drug development is a long and arduous one. From lead discovery and compound library development the synthetic chemistry requirements change from the production of milligram to grams and at time kilograms quantities of active compound(s) [1]. This places considerable pressure on the robustness of the chemistry utilised [2]. One of our teams primary areas of research has been in the development of small molecule inhibitors, based on cantharidin (**1**) and norcantharidin (**2**) (Fig. **1**), of the serine / threonine protein phosphatases 1 and 2A (PP1 and PP2A) [3-5].

1 **2**

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

Our initial interest was sparked by the structural simplicity of **1** and **2** relative to the archetypal protein phosphatase inhibitors such as okadaic acid (**3**) and microcystin-LR (**4**) (Fig. **2**) [6,7]. We, and others, have developed a number of highly focused norcantharidin derived compound libraries [4,8-11]. Our efforts in this area have been rewarded by the development of new classes of inhibitor with a broad range of protein phosphatase inhibition levels and PP1/PP2A selectivity [4]. We have also explored the effect of these inhibitors potential as anti-malarial, anti-parasitic and anticancer drugs [4,12].

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While simple approaches to multiple analogues of **2** are available commencing with the Diels–Alder addition of furan and maleic anhydride followed by hydrogenation to **2** (Scheme **1**). The direct synthesis of **1** is more complex due to the presence of two methyl groups preventing simple Diels-Alder approaches [13], but its total synthesis has been greatly accelerated by the use of high pressure chemistry approaches [14]. Additionally the presence of these methyl groups has been deemed responsible for the known nephrotoxicity of **1**. Hence **2** is the more promising lead compound in a drug development program as it retains most of the PP1 / PP2A inhibition and anti-cancer properties of **1** including the lack of myelosuppresion, with greatly reduced nephrotoxicity [3-5].

Notwithstanding the ease of synthesis of **2**, the need for hydrogenation in the second synthetic step stalls rapid library development in a standard laboratory environment. Typically this step is limited to a batch wise approach, with the quantity of each batch limited by the volume of the hydrogenating system. This also introduces batch-to-batch variations in the amount and quality of **2** produced. This is a disadvantage not only in regards to library development but to reaction scale up.

In efforts to accelerate both analogue development and reaction scale up, our team has invested heavily in flow chemistry technology *via* the Australian Cancer Research Foundation Centre for Kinomics. Having access to the ThalesNano H-cube™ (H-cube) flow hydrogenator we turned our attention to the synthesis of, initially, norcantharidin (**2**) and later the 'one-pot' synthesis of norcantharidin analogue libraries.

Typically our batch hydrogenation of **5** to **2** is conducted using a Parr hydrogenator: 10% Pd-C, 4 bar H_2, 24 hours on a 5 g scale. Our initial evaluation of this reaction under flow hydrogenation conditions was conducted with a 0.05M solution of **5** in acetone at 1 mL/min at 50 ºC and 50 bar H_2 pressure [15]. These conditions were chosen as being at the mid-range of the capability of the H-cube (upper limit is 100 ºC, 100 bar H_2 and a flow rate of 3 mL/min) [16]. In this in-

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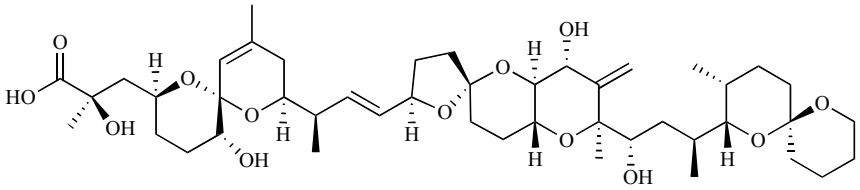

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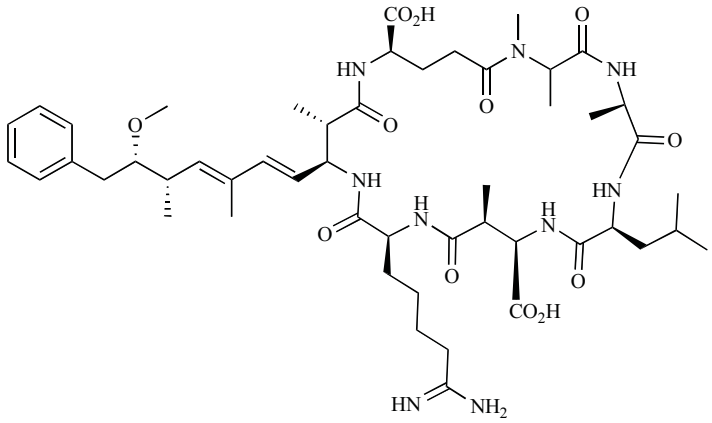

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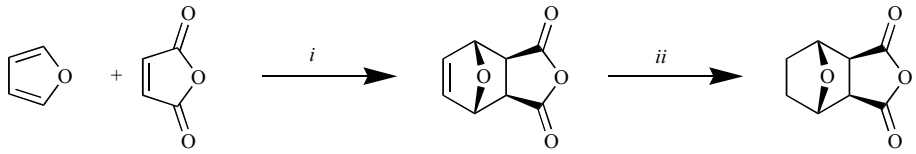
Fig. (2). Okadaic acid (**3**) and microcystin LR (**4**), two archetypal members of the okadaic acid class of compounds.



Scheme 1. Reagents and Conditions: *i*) Et_2O, rt, 24-48 hrs; (ii) Dry Acetone, 10% Pd-C; H_2, 4 Bar, 24 hrs, rt.

stance we observed 100 % conversion of **5** to **2** (Table **1**, entry 1). Subsequent optimisation studies commenced with changes in the H_2 pressure, and as can be seen from the data presented in Table **1**, all conditions excepting entry 2, effected complete conversion of **5** to **2**. Controlled H_2 mode at 0 bar (atmospheric pressure), at room temperature, only afforded a 60% conversion at 1 mL/min.

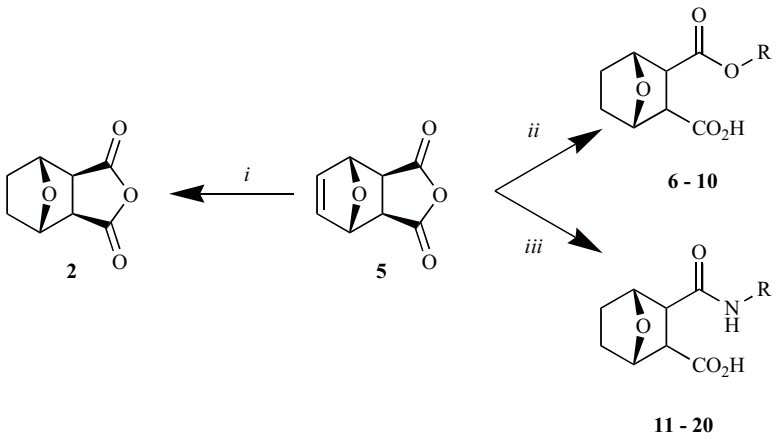
The continuous production of **2** from **5** using the conditions outlined in Table **1**, entry 1, affords the desired compound at a rate of 12g/day which compares very favourably with 5g/day *via* our batch hydrogenation approaches. Additionally, hydrogen is produced "on demand" from deionized water and the catalyst cartridges are supplied pre-packaged, which eliminates the safety concerns associated with flammable gases and active catalyst disposal are negated [16,17].

In previous studies we have shown that simple stirring of **2** in the presence of amines or alcohols afforded excellent yields of ring opened acid-amides or acid-ester analogues [8,18]. Having successfully converted our batch hydrogenation synthesis of **2** to a flow approach, we examined the possibility of coupling this process in a 'one-pot' manner with the aforementioned reactions with simple aliphatic alcohols. We rationalised that such an approach would permit access to the required quantities of analogues for biological screening in a rapid manner, and facilitate compound scale up should the biological data suggest a favourable outcome [19].

Thus we modified the synthetic procedure used for the flow synthesis of **2** from **5** with the simple incorporation of

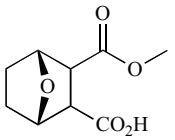
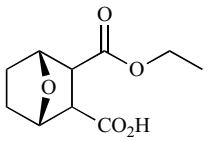
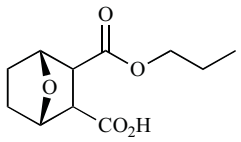
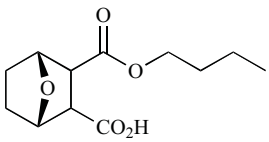
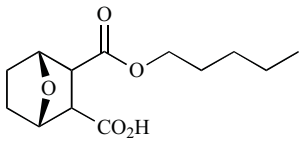
Table 1. Optimisation of the Flow Hydrogenation of 5,6-dehydronorcantharidin (5) to Norcantharidin (2)

Entry	Catalyst	H_2 Pressure (bar)	Temperature (°C)	Yield (%)
1	10% Pd/C	50	50	100
2	10% Pd/C	0 (atmospheric pressure)	rt	60
3	10% Pd/C	30	30	100
4	10% Pd/C	40	40	100



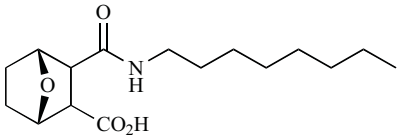
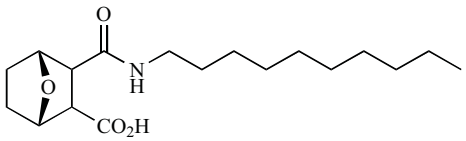
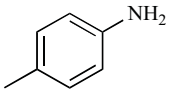
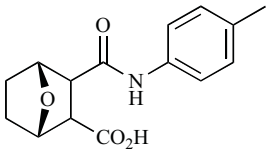
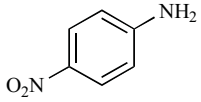
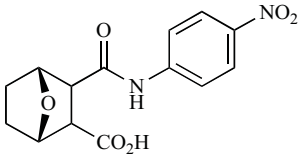
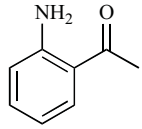
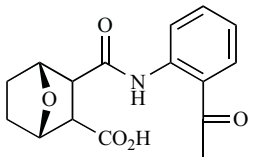
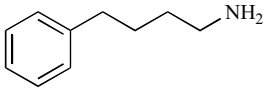
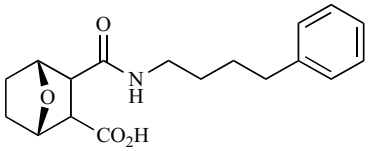
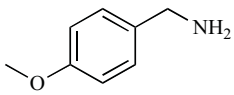
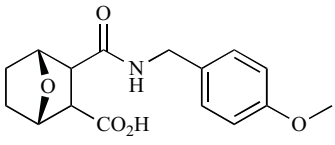
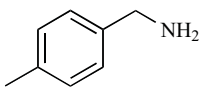
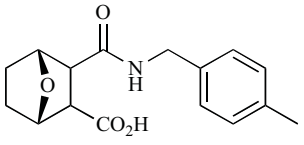
Scheme 2. Reagents and Conditions: i) 0.05M solution of **5** in acetone, 50 bar, 50°C, 1 mL/min, 10% Pd/C; (ii) 0.05M solution of **3** in ROH, 50 bar, 50°C, 1 mL/min, 10% Pd/C; (iii) 0.055M (or 0.03M) solution of RNH_2 in acetone added to 0.05M solution of **3** in acetone, 50 bar, 50°C, 1 mL/min, 10% Pd/C.

Table 2. A 'One-Pot' Coupled Flow Hydrogenation and Ring Opening Synthesis of Norcantharidin Ring Opened Acid-Esters and Acid-Amides (Protein Phosphatase Inhibition Data Taken from Refs. [8, 15, 18])

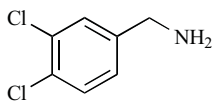
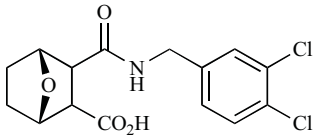
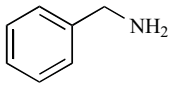
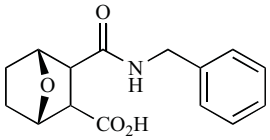
Entry	Alcohol	Product	Yield (%)	PP1 IC_{50} (μM)	PP2A IC_{50} (μM)
1	CH_3OH	 **6**	100	4.71	0.41
2	CH_3CH_2OH	 **7**	100	2.96	0.45
3	$CH_3(CH_2)_2OH$	 **8**	100	4.82	0.47
4	$CH_3(CH_2)_3OH$	 **9**	0%	>100	>100
5	$CH_3(CH_2)_4OH$	 **10**	0%	>100	>100

either an alcohol or an amine into the reagent stream that contained **5**. A series of solutions of **5** (0.05M) in methanol (Table **2**, entry 1), ethanol (Table **2**, entry 2) and propanol (Table **2**, entry 3) were subjected to flow hydrogenation conditions of 1 mL/min reagent flow, 50 bar H_2 at 50ºC. The outcomes of these reactions are tabulated in Table **2**.

Table 3. A 'One-Pot' Coupled Flow Hydrogenation and Ring Opening Synthesis of Norcantharidin Ring Opened Acid-Amides (Protein Phosphatase Inhibition Data Taken from Ref. [8, 15, 18])

Entry	Amine	Product	Yield (%)	PP1 IC_{50} (μM)	PP2A IC_{50} (μM)
1	$CH_3(CH_2)_7\mathbf{NH_2}$	 **11**	63	56 ± 9	25 ± 5
2	$CH_3(CH_2)_9\mathbf{NH_2}$	 **12**	58	25 ± 7	8.6 ± 1.5
3		 **13**	0%	n.d.[a]	n.d.
4		 **14**	0%	74 ± 13	23 ± 1.5
5		 **15**	0%	n.d.	n.d.
6		 **16**	55	(68 ± 3)[b]	(87 ± 2)[b]
7		 **17**	100	35 ± 5	13 ± 1
8		 **18**	100[c]	n.d.	n.d.

(Table 3). Contd…..

Entry	Amine	Product	Yield (%)	PP1 IC_{50} (μM)	PP2A IC_{50} (μM)
9		 **19**	52	>100	>100
10		 **20**	91	35 ± 0	12 ± 0

[a]n.d. = not determined; [b]percentage inhibition at 100 μM drug concentration; [c]reagents were recycled through the catalyst bed until MS showed complete consumption of the starting anhydride **5**.

The data presented in Table **2** shows that for short chain alcohols the ring opening of the anhydride by the alcohol and reduction of the 5,6-double bond of **5** occurs in an excellent yield. The longer chain alcohols failed to give any observable product under these conditions (Table **2**, entries 4 and 5), nor as 0.05M solutions in dry acetone (data not shown). We have previously noted that the reaction of **2** with longer chain alcohols is sluggish and difficult to drive to completion this is reflected when conducting a two-step process *via* flow hydrogenation [18].

In the development of our protein phosphatase inhibitor libraries we have previously reported that the acid amide analogue is more potent than the equivalent acid ester analogue [18,20]. Thus, an acetone solution of **5** (0.05M) and an amine (0.055M, see Table **3** for details) were subjected to flow hydrogenation conditions of 1 mL/min reagent flow, 50 bar H_2 at 50ºC. The outcomes of these reactions are tabulated in Table **3**.

As we had observed no conversion to the acid ester with issues with long chain alcohols above, we first evaluated the addition of octyl and decylamine to **5** (Table **3**, entries 1 and 2). As can be seen the addition proceeded smoothly with yields of 63% and 58% respectively. While quantitative conversion was not obtained, the outcome was more favourable than that noted with alcohols. This is most likely a function of the amine versus alcohol nucleophilicity. In a more thorough investigation of this flow hydrogenation/nucleophilic anhydride ring opening reaction sequence we specifically targeted products that were of interest to our protein phosphatase inhibition program [3-5, 8, 12, 18, 20].

The attempted addition of three anilines (Table **3**, entries 7-9) failed. For example, under the conditions used, addition of the 0.055 M aniline solution to the 0.05 M solution of **5** resulted in the immediate precipitation of the (1*S*,4*R*)-3-(anilino)-7-oxabicyclo[2.2.1]hept-5-ene-2-carboxylic acid. The precipitation of products during a flow chemistry transformation is a limitation of this technology, but one that is simply overcome by a 'solvent-switch' approach [21]. Solvent switching was not required in this instance with dilution of the feeder solution to 0.03 M (in acetone) sufficient to prevent the product from precipitating and thus, with 4-methylbenzylamine afforded quantitative conversion to the desired product (Table **3**, entry 8). This process was repeated for the other amines that precipitated and also effected quantitative conversion to the desired products (data not shown). The other amines evaluated (Table **3**, entries 6, 7, 9 and 10) all proceed to afford the desired products in good to excellent yields (52-100%).

An additional advantage flow chemistry here was the ability to loop the reagents stream, i.e. in the event of incomplete conversion the post-catalyst eluant was delivered back to the reagent vessel and the reaction continued until complete conversion observed (by MS). The effect of such reagent looping is shown with **17** (Table **3**, entry 7).

In conclusion herein we have reported the combined reduction and nucleophilic anhydride ring opening of a focused library of norcantharidin analogues. This reaction sequence works best with amine nucleophiles but due care and consideration must be given to the product solubility as flow chemistry has a low tolerance for materials that precipitate in the reaction lines.

This two-step procedure was conducted in excellent yields, typically quantitative, allowing for the rapid development of novel potentially biologically active analogues. The flow chemistry approaches developed herein are independent of scale and facilitate both the initial screening stages of a drug design and discovery program, and the increase in compound quantity required as such a program progresses through to in cell and animal studies.

EXPERIMENTAL

General Experimental

All starting materials were purchased from Aldrich Chemical Co. and Lancaster Synthesis. Solvents were bulk, and distilled from glass prior to use. Reaction progress was monitored by TLC, on aluminium plates coated with silica gel with fluorescent indicator (Merck 60 F_{254}) and flash chromatography was conducted utilising SNAP Biotage KP-SIL columns. ^1H and ^{13}C spectra were recorded on a Bruker

Advance AMX 300 MHz spectrometer at 300.13 and 75.48 MHz, respectively. Chemical shifts are relative to TMS as internal standard. All compounds returned satisfactory Mass spectra were obtained using a micromass liquid chromatography Z-path (LCZ) platform spectrometer. Mass to charge ratios (*m/z*) are stated with their peak intensity as apercentage in parentheses. All mass spectra were obtained *via* the ES method thus fragmentation patterns were not observed. The University of Wollongong, Australia, Biomolecular Mass Spectrometry Laboratory, analysed samples for HRMS. The spectra were run on a micromass QTof2 spectrometer using polyethylene glycol or polypropylene glycol as the internal standard.

In a Typical Synthesis of Acid-Ester Analogues [18]

A mixture of 5,6-dehydronorcantharidin (**5**) (0.05 M in methanol) was stirred until dissolved. The corresponding solution was then passed through the H-cube flow hydrogenation apparatus under the conditions of 50 bar, 50°C, 1 mL/min, controlled H_2, 10% Pd/C. The reaction eluant was collected, and the solvent removed *in vacuo* to leave a white solid which did not require further purification.

(1S,4R)-3-(Methoxycarbonyl)-7-oxabicyclo[2.2.1]heptane-2-carboxylic acid (6)

^{1}H NMR (acetone-d_6) (300 MHz): δ 4.78-4.77 (m, 2H), 3.55 (s, 3H), 3.10-3.00 (m, 2H), 1.70-1.55 (m, 4H); ^{13}C NMR (acetone-d_6) (75 MHz): δ 170.9, 170.7, 77.8, 77.4, 51.5, 50.9, 50.1, 28.2, 27.2; MP 140-141°C [18].

(1S,4R)-3-(Ethoxycarbonyl)-7-oxabicyclo[2.2.1]heptane-2-carboxylic acid (7)

^{1}H NMR ($CDCl_3$) (300 MHz): δ 8.88 (bs, 1H), 4.93 (d, *J* = 2.9 Hz, 1H), 4.87 (d, *J* = 2.9 Hz, 1H), 4.11 (q, *J* = 7.1 Hz, 2H), 3.03-2.95 (m, 2H), 1.83-1.79 (m, 2H), 1.55-1.48 (m, 2H), 1.22 (t, *J* = 7.1 Hz, 3H); ^{13}C NMR ($CDCl_3$) (75 MHz): δ 175.7, 170.3, 78.0, 77.8, 60.5, 51.8, 51.7, 28.5, 28.4, 13.4; MP 112-113°C [18].

In a Typical Synthesis of Acid-Amide Analogues [8,20]

A mixture of 5,6-dehydronorcantharidin (**5**) (0.05M in acetone) was stirred until dissolved. To this, a solution of amine (0.055M in acetone) was added and the resulting solution was passed through the H-cube flow hydrogenation apparatus under the conditions of 50 bar, 50°C, 1 mL/min, controlled H_2, 10% Pd/C. The reaction was collected, and the solvent removed *in vacuo* to leave an off white precipitate. This was purified by either adding ether (10 mL) and collecting the observed precipitate under suction or subjected to flash silica chromatography (~5% MeOH/DCM) to afford the desired norcantharidin acid amide.

(1S,4R)-3-(4-Methoxybenzylcarbamoyl)-7-oxabicyclo[2.2.1]heptane-2-carboxylic acid (17)

^{1}H NMR (DMSO-d_6) (300 MHz): δ 7.68 (t, *J* = 5.5 Hz, 1H), 7.17 (d, *J* = 8.7 Hz, 2H), 6.83 (d, *J* = 8.7 Hz, 2H), 4.69 (s, 1H), 4.46-4.44 (m, 1H), 4.19 (dd, *J* = 14.9, 5.5 Hz, 1H), 4.01 (dd, *J* = 14.9, 5.5 Hz, 1H), 3.71 (s, 3H), 2.82 (d, *J* = 9.8 Hz, 1H), 2.72 (d, *J* = 9.8 Hz, 1H), 1.71-1.38 (m, 4H); ^{13}C NMR (DMSO-d_6) (75 MHz): δ 173.1, 171.2, 158.0, 131.3, 128.4, 113.5, 78.6, 77.5, 54.9, 53.9, 44.9, 28.7, 27.9, 21.1; MP 143-144°C [8].

(1S,4R)-3-(Benzylcarbamoyl)-7-oxabicyclo[2.2.1]heptane-2-carboxylic acid (20)

^{1}H NMR (DMSO-d_6) (300 MHz): δ 7.97 (t, *J* = 5.7 Hz, 1H), 7.62-7.59 (m, 1H), 7.43-7.37 (m, 2H), 7.31-7.23 (m, 2H), 4.73 (s, 1H), 4.49 (d, *J* = 4.1 Hz, 1H), 4.28-4.14 (m, 2H), 2.95 (d, *J* = 9.6 Hz, 1H), 2.85 (d, *J* = 9.6 Hz, 1H), 1.63-1.41 (m, 4H); ^{13}C NMR (DMSO-d_6) (75 MHz): δ 173.0, 172.2, 130.9, 128.1, 127.6, 126.2, 78.9, 77.1, 53.1, 51.9, 42.4, 28.0, 27.7; MP 170°C [8].

ACKNOWLEDGEMENTS

The authors acknowledge the financial support of the Australian Research Council and the Australian Cancer Research and Ramaciotti Foundations. MT acknowledges the UNI-PRS postgraduate funding from the University of Newcastle. KAY acknowledges John Morris Scientific (Australia) for generous scholarship provision. SNM and EU acknowledge the Lambton High School Work Experience release program.

REFERENCES

[1] DiMasi, J. A. The value of improving the productivity of the drug development process: faster times and better decisions. *Pharmacoecon*. **2002**, *20*, 1-10.

[2] Colombo, M.; Peretto, I. Chemistry strategies in early drug discovery: an overview of recent trends. *Drug Discov. Today* **2008**, *13*, 677-684.

[3] McCluskey, A.; Sim, A. T. R.; Sakoff, J. A. Serine-threonine protein phosphatase inhibitors: development of potential therapeutic strategies. *J. Med. Chem.* **2002**, *45*, 1151-1175

[4] McCluskey, A.; Sakoff, J. A. Small Molecule inhibitors of serine / threonine protein phosphatases. *Mini Rev. Med. Chem.* **2001**, *1*, 43-55

[5] Sakoff, J. A.; McCluskey, A. Protein phosphatase inhibition: structure based design. Towards new therapeutic agents. *Cur. Pharm. Des.* **2004**, *10*, 1139-1159.

[6] Bagu, J. R.; Sykes, B. D.; Craig, M. M.; Holmes, C. F. B. A molecular basis for different interactions of marine toxins with protein phosphatase-1. Molecular models for bound motuporin, microcystins, okadaic acid, and calyculin A. *J. Biol. Chem.* **1997**, *272*, 5087-5097.

[7] Sheppeck, J. E.; Gauss, C. M.; Chamberlin, A. R. Inhibition of the Ser-Thr phosphatases PP1 and PP2A by naturally occurring toxins. *Bioorg. Med. Chem.* **1997**, *5*, 1739-1750.

[8] 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*, 1878-1892

[9] Tatlock, J. H.; Linton, A.; Hou, X. J.; Kissinger, C. R.; Pelletier, L. A.; Showalter, R. E.; Tempczyk, A.; Villafranaca, J. E. Structure-based design of novel calcineurin (PP2B) inhibitors *Bioorg. Med. Chem. Lett*. **1997**, *7*, 1007-1012

[10] Sodeoka, M.; Baba, Y.; Kobayashi, S.; Hirukawa, N. Structure-activity relationship of cantharidin derivatives to protein phosphatases 1, $2A_1$, and 2B *Bioorg. Med. Chem. Lett*. **1997**, *7*, 1833-1836

[11] 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*, 2937-2944.

[12] Bajsa, J.; McCluskey, A.; Gordon, C. P.; Stewart, S. G.; Hill, T. A.; Sahu, R.; Duke, S. O.; Tekwani, B. L. The antiplasmodial activity of norcantharidin analogues that may act by protein phosphatase inhibition. *Bioorg. Med. Chem. Lett*. **2010**, *20*, 6688-6695.

[13] Stork, G.; van Tamelen, E. E.; Friedman, L. J.; Burgstahler, A. W. A Stereospecific Synthesis of Cantharidin *J. Am. Chem. Soc.* **1953**, *75*, 384–392.

[14] Dauben, W. G.; Kessel, C. R.; Kazuo, H.; Takemura, K. H. Simple, efficient total synthesis of cantharidin *via* a high-pressure Diels-Alder reaction. *J. Am. Chem. Soc.* **1980**, *102*, 6893–6894.

[15] Sakoff, J. A.; Ackland, S. P.; Baldwin, M. L.; Keane, M. A.; McCluskey, A. Anticancer activity and protein phosphatase 1 and 2A inhibition of a new generation of cantharidin analogues. *Investig. New Drugs* **2002**, *20*, 1-11.

[16] www.thalesnano.com accessed 16th December 2010.

[17] Clapham, B.; Wilson, N. S.; Michmerhuizen, M. J.; Blanchard, D. P.; Dingle, D. M.; Nemcek, T. A.; Pan, J. Y.; Sauer, D. R. Construction and validation of an automated flow hydrogenation instrument for application in high-throughput organic chemistry. *J. Comb. Chem.* **2008**, *10,* 88-93.

[18] McCluskey, A.; Bowyer, M. C.; Collins, E.; Sim, A. T. R.; Sakoff, J. A.; Baldwin, M. L. Anhydride modified cantharidin analogues: synthesis, inhibition of protein phosphatases 1 and 2A and anticancer activity. *Bioorg. Med. Chem. Lett.* **2000**, *10*, 1687-1690.

[19] In a typical library development study 25 mg of compound is required for complete chemical characterisation and biological evaluation. At 1 mL/min a 0.05M solution reaction produces ca 10 mg/min (assumes average MW of product ~ 350 and a 60% purified yield). Thus a 5 minute flow chemistry run affords ca 50 mg of pure analogue. The equivalent batch process requires 24 hrs.

[20] 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*, 1969-1973.

[21] Baxendale, I. R.; Deeley, J.; Griffiths-Jones, C. M.; Ley, S. V.; Saaby, S.; Geoffrey K. Tranmer, G. K. A flow process for the multi-step synthesis of the alkaloid natural product oxomaritidine: a new paradigm for molecular assembly. *Chem. Commun*. **2006**, 2566-2568.