Pergamon

Bioorganic & Medicinal Chemistry Letters 12 (2002) 391–393

BIOORGANIC &
MEDICINAL
CHEMISTRY
LETTERS

The First Two Cantharidin Analogues Displaying PP1 Selectivity

Adam McCluskey,[a,*] Mirella A. Keane,[a] Cecilia C. Walkom,[a] Michael C. Bowyer,[b]
Alistair T. R. Sim,[c] David J. Young[d] and Jennette A. Sakoff[e]

[a]*Medicinal Chemistry Group, School of Biological and Chemical Sciences, The University of Newcastle, University Drive, Callaghan, Newcastle, NSW 2308, Australia*
[b]*School of Science & Technology, Central Coast Campus, The University of Newcastle, PO Box 127, NSW, Australia*
[c]*Discipline of Medical Biochemistry, Faculty of Medicine and Health Sciences, The University of Newcastle, University Drive, Callaghan, Newcastle, NSW 2308, Australia*
[d]*Faculty of Science and Technology, Griffith University, Brisbane, QLD 4111, Australia*
[e]*Department of Medical Oncology, Newcastle Mater Misericordiae Hospital, NSW 2298, Australia*

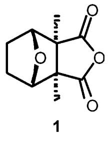
Received 12 July 2001; accepted 12 November 2001

Abstract—High pressure Diels-Alder reactions of furan and dimethylmaleate, and thiophene and maleimide resulted in two cantharidin analogues, **3** and **6** possessing PP1 selectivity (>40- and >30-fold selectivity) over PP2A. Both compounds exhibited moderate PP1 activity, **3** IC_{50} 50 µM and **6** IC_{50} 12.5 µM. Interestingly, the corresponding mono-ester derivatives of **3** showed no such selectivity. © 2002 Elsevier Science Ltd. All rights reserved.

The serine/threonine protein phosphatases, in particular protein phosphatases 1 (PP1) and 2A (PP2A) via reversible phosphorylation modulate numerous cellular signal transduction events, moderating such diverse functions as neurotransmission, muscle contraction, glycogen synthesis, T-cell activation and cell proliferation.[1–5] Knowledge of structure and subsequent correlation of binding function for both PP1 and PP2A would, therefore, provide a vital link toward understanding signal transduction mechanisms.

Okadaic acid (PP1 $IC_{50} = 60$ nM, PP2A $IC_{50} = 1$ nM), calyculin A (PP1 $IC_{50} = 0.5–1.0$ nM, PP2A $IC_{50} = 2.0$ nM), microcystin-LR and tautomycin are representative of the structurally diverse group of compounds comprising the okadaic acid class of compounds. All are potent protein phosphatase 1 and 2A inhibitors.[6] Recently Chamberlin et al.[7] have used molecular modelling to reconcile the apparent anomalies between the structural diversity of the okadaic acid class of compounds and the similarities in the binding at both PP1 and PP2A. The okadaic acid class of compounds are potent and competitive inhibitors of both PP1 and PP2A.

Cantharidin (**1**) represents the simplest known inhibitor of the serine/threonine protein phosphatases 1 and 2A.[8] Cantharidin shows moderate PP2A selectivity (PP2A $IC_{50} = 0.16$ µM; PP1 $IC_{50} = 1.7$ µM; PP2A selectivity = 10.6). To date, all cantharidin analogues have, at best, maintained similar levels of PP2A selectivity and potency.[8] Only fostriecin exhibits any degree of PP2A selectivity (PP2A IC_{50} 3.4 nM; IC_{50} PP1 130 µM; PP2A selectivity >40, 000).[9]



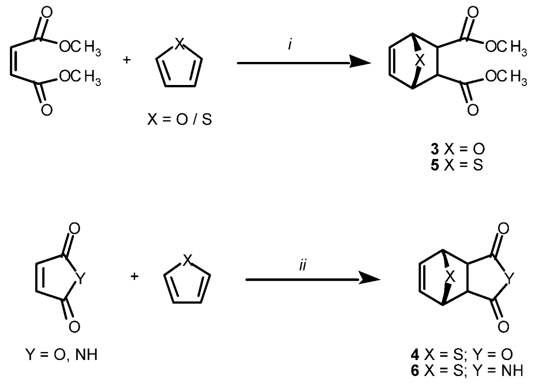
1

Phosphatidic acid,[10] tautomycin[11] and the modified microcystin analogues recently reported by Chamberlin are the only inhibitors that we are aware of exhibiting selectivity towards PP1.[12] However, with the exception of phosphatidic acid, PP1 selectivity is typically <10-fold. As part of an on-going study we have been investigating the effect of anhydride and bridgehead modification on the ability of cantharidin analogues to selectivity inhibit PP1 or PP2A.[13] As has been the case with other researchers, we have typically met limited success,[14] although we have recently reported the development of the cantharimides as potent inhibitors of both PP1 and PP2A.[15] Herein we report the fortuitous discovery of the first two-cantharidin analogues to display PP1 selectivity.

---

*Corresponding author. Tel.: +61-249-21-6486; fax: + 61-249-21-5472; e-mail: amcclusk@mail.newcastle.edu.au

PII: S0960-894X(01)00777-6



Scheme 1. Reagents and conditions: (i) dimethyl maleate (1 equiv), furan or thiophene (2 equiv), CH_2Cl_2, 40 °C, 17 kbar, 71 h; (ii) maleic anhydride or maleimide (1 equiv), furan or thiophene (2 equiv), CH_2Cl_2, 40 °C, 17 kbar, 71 h.

Results and Discussion

Chemistry

With the exception of norcantharidin (**2**), compounds were synthesised via high pressure Diels–Alder reaction (17 kbar, 71 h, 40 °C) (Scheme 1). In a typical reaction, thiophene (0.084 g, 0.011 mol) and maleimide (0.489 g, 0.005 mol) were dissolved in distilled dichloromethane (1.5 mL) in a sealed plastic tube and subjected to 17 kbar at 40 °C for 71 h. In the first instance, crude **6** was isolated as a yellow precipitate, subsequent recrystallisation ($CHCl_3$) afforded exo-**6**.[16] Kotsuki et al.[17] have previously reported the high-pressure synthesis of **4** and **5** (15 kbar and 100 °C for 3 h). In our hands the same transformation was effected in higher yield (ca. 60% vs ca. 40%) by increasing the reaction time (71 h) and at lower temperature (40 °C). We have also found it possible to synthesise **5** in $LiClO_4$–Et_2O mixtures (14%). This material was identical to that produced by the high-pressure reaction.[17]

^1H NMR indicated the major products isolated in all cases were the *exo* isomers, for example with **3** the C_2 and C_3 protons appear as a doublet ($J = 1$ Hz), considering the dihedral angle, this is a good indication of an *exo* configuration, and is in agreement with literature.[18] With **3** recrystallisation (diethylether) afforded only the *exo* isomer.

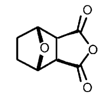
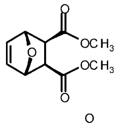
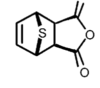
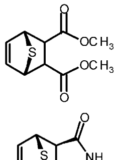
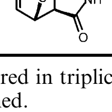
Careful selection of diene and ene allowed synthesis of analogues possessing modified anhydrides (Scheme 1): a maleimide linkage (**6**), a bis-methyl ester (**3** and **5**), and compounds with a 7-*S* moiety replacing the conventional 7-*O* substituent (**4**–**6**).

Biology

The cantharidin analogues, **3**–**6**, were screened for their ability to inhibit protein phosphatases 1 and 2A, with preliminary screening conducted at 100 μM.[13] Norcantharidin (**2**) was included as an internal standard to ensure the relative validity of our protocol. As can be seen from Table 1, **2** displayed the PP1 selectivity and potency typically reported,[8,13–15] however **3** (PP1 = 46%, and PP2A = 6%) and **6** (PP1 = 87%, and PP2A = 30%) at 100 μM showed interesting selectivity towards PP1 as opposed to the inherent PP2A selectivity of previous cantharidin analogues.[8]

Further examination of **3** and **6** allowed determination of IC_{50} values; **3** PP1 $IC_{50} = 50$ μM, PP2A $IC_{50} > 2000$ μM; and **6** PP1 $IC_{50} = 12.5$, PP2A $IC_{50} = 426$ μM. Thus, both of these compounds are > 40- and > 30-fold PP1 selective (vs PP2A). This represents the synthesis of the most PP1 selective cantharidin analogues to date. It is noteworthy that the increase in selectivity did not arise from an increased potency at PP1, but at the expense of inhibition of PP2A, with an IC_{50} > 2000 for **3** and an IC_{50} of 426 μM for **6**.

Table 1. Inhibition of PP1 and PP2A by selected cantharidin analogues

Compd no.	Compd	Inhibition of PP1 (%)[a]	IC_{50} (μM)	Inhibition of PP2A (%)[a]	IC_{50} (μM)	PP1 Selectivity
2		90	2.4	97	2.1	0.875
3		46	50	6	> 2000	> 40
4		0	ND	0	ND	ND
5		3	ND	3	ND	ND
6		87	12.5	30	426	34

[a] Inhibition measured in triplicate at 100 μM;
ND, not determined.

Simple bioisosteric replacement of 7-*S* for 7-*O* had a pronounced impact upon protein phosphatase inhibition. In this context, the 7-*S* analogue of **2** (analogue **4**) displayed no PP1 or PP2A inhibition. An essentially identical result was obtained for the 7-*S* analogue of **3** (analogue **5**). This again reinforces the belief that the 7-*O* substituent, or a substituent capable of being a hydrogen-donor and/or acceptor is crucial for inhibition of PP1 and/or PP2A.[19] On this basis we suggest that the maleimide NH of **6** probably binds in the 7-*O* region and the 7-*S* presents itself to one of the metals known to reside in the active site of the protein. With **3** the presence of both methyl esters appears paramount for PP1 selectivity, as we have previously shown that the corresponding mono-esters, whilst potent PP1 and PP2A inhibitors, do not display PP1 selectivity.[14]

Conclusions

We have generated, in a simple one-pot procedure, the first two cantharidin analogues exhibiting selectivity towards PP1. As a result of the findings presented herein we are currently exploring **3** and **6** as potential leads in the development of more potent and selective PP1 inhibitors. Such analogues, potentially, will be of use in evaluating the roles of PP1 and PP2A in signal transduction studies.

Acknowledgements

We gratefully acknowledge financial assistance from Hunter Medical Research (Australia), The National Health & Medical Research Council, the University of Newcastle and Clive & Vera Ramaciotti Foundation. A.M. also wishes to thank Professor A.R. Chamberlin for helpful discussions.

References and Notes

1. Cohen, P.; Cohen, P. T. W. *J. Biol. Chem.* **1989**, *264*, 21435.
2. Cohen, P. *The Structure and Regulation of Protein Phosphatases*; Raven: New York, 1990.
3. Shenolinkar, S.; Nairn, A. C. *Adv. Second Mess. Phosp. Res.* **1991**, *1*, 231.
4. Krebs, E. G. *Angew. Chem., Int. Ed. Engl.* **1993**, *32*, 1122.
5. Fischer, E. *Angew. Chem., Int. Ed. Engl.* **1992**, *31*, 1130.
6. Sheppeck, J. E.; Gauss, C.-M.; Chamberlin, A. R. *Bioorg. Med. Chem.* **1997**, *5*, 1739.
7. Gauss, C.-M.; Sheppeck, J. E.; Nairn, A. C.; Chamberlin, A. R. *Bioorg. Med. Chem.* **1997**, *5*, 1751.
8. McCluskey, A.; Sakoff, J. A. *Mini Rev. Med. Chem.* **2001**, *1*, 43.
9. Walsh, A. H.; Chen, A. T.; Honaken, R. E. *FEBS Lett.* **1997**, *416*, 230.
10. Kishikawa, K.; Chalfant, C. E.; Perry, D. K.; Bielawaska, A.; Hannun, Y. A. *J. Biol. Chem.* **1999**, *274*, 21335.
11. Sheppeck, J. E.; Guass, C.-M.; Chamberlin, A. R. *Bioorg. Med. Chem.* **1997**, *5*, 1739.
12. Aggen, J. B.; Humphrey, J. M.; Gauss, C. M.; Huang, H. B.; Nairn, A. C.; Chamberlin, A.R *Bioorg. Med. Chem.* **1999**, *7*, 543.
13. McCluskey, A.; Keane, M. A.; Mudgee, L.-M.; Sim, A. T. R.; Sakoff, J.; Quinn, R. J. *Eur. J. Med. Chem.* **2000**, *35*, 957.
14. McCluskey, A.; Bowyer, M. C.; Collins, E.; Sim, A. T. R.; Sakoff, J. A.; Baldwin, M. L. *Bioorg. Med. Chem. Lett.* **2000**, *10*, 1687.
15. McCluskey, A.; Bowyer, M. C.; Walkom, C.; Ackland, S. P.; Gardiner, E.; Sakoff, J. A. *Bioorg. Med. Chem. Lett.* **2001**, *11*, 2941.
16. Synthesis of *exo, exo*-10-thia-4-azatricyclo[5.2.1.0]dec-8-ene-2,5-dione (**6**). Thiophene (0.084 g, 0.011 mol) and maleimide (0.489 g, 0.005 mol) were mixed at room temperature in distilled dichloromethane (1.5 mL). The homogeneous solution was then subjected to 17 kbar pressure at 40 °C for a total of 71 h. After the pressure was released the yellow precipitate that had formed was collected and recrystallised ($CHCl_3$), yielded 0.109 g, 60%, mp 153–156 °C. ^1H NMR ($CDCl_3$) δ 2.25 (s), 3.05 (s, 2H), 3.95 (m, 2H), 6.35 (dd, 2H, $J=2$, 3 Hz). ^{13}C NMR (CDCl3) δ 40.65, 53.13, 135.64, 173.17. Analysis (calcd %) C 53.02; H 3.89; N 7.73; (found %) C 53.37; H 3.82; N 8.01. All compounds returned satisfactory spectroscopic and analysis data.
17. Kotsuki, H.; Nishizawa, H.; Kitagawa, S.; Masamitsu, O.; Yamasaki, N.; Matsuoka, K.; Tokoroyama, T. *Bull. Chem. Soc. Jpn.* **1979**, *52*, 544.
18. Kotsuki, H.; Nizhizawa, H.; Ochi, M.; Matsukoa, K. *Bull. Chem. Soc. Jpn.* **1982**, *55*, 496.
19. McCluskey, A.; Taylor, C.; Quinn, R. J.; Suganuma, M.; Fujiki, H. *Bioorg. Med. Chem. Lett.* **1996**, *6*, 1025.