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# Design of novel and potent cPLA$_2\alpha$ inhibitors containing an α-methyl-2-ketothiazole as a metabolically stable serine trap

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ABSTRACT

We report the design of novel, potent cPLA$_2\alpha$ inhibitors that possess an α-methyl-2-ketothiazole that acts as a serine-reactive moiety. We describe the optimization of the series for potency and metabolic stability towards ketone reduction. This was achieved by attenuating the reactivity of the ketone using a combination of electronic and steric effects.



Cytosolic phospholipase A$_2\alpha$ is an intracellular enzyme (cPLA$_2\alpha$, group IVA phospholipase) which generates free arachidonic acid by selectively cleaving the *sn*-2 position of arachidonyl-glycophospholipids.[1] Further metabolism of arachidonic acid leads to a variety of inflammatory mediators such as leukotrienes, prostaglandins, thromboxanes and acetylation of the lyso glycerophosphatidyl choline generates platelet activating factor (PAF).[2] cPLA$_2\alpha$ is therefore considered as playing a key role in a number of inflammatory conditions and as such as a potential target for the treatment of a number of diseases such as rheumatoid arthritis, asthma and osteoarthritis.[3] Inhibitors of cPLA$_2\alpha$ are therefore still of great interest as potential new therapeutic agents.[4]

We have previously described a series of potent cPLA$_2\alpha$ inhibitors containing a 1,3-dioxypropan-2-one group as a serine-reactive moiety,[5] typified by compound **1** (Fig. 1). We have demonstrated by NMR studies, analogous to those performed on the trifluoromethylketone-arachidonyl (TFMA) cPLA$_2\alpha$ inhibitor[6] that the ketone in compound **1** interacts with a serine residue on the enzyme to form a complex containing an ionized hemiketal.[7] However, we have also observed that these molecules are highly metabolized and in particular the ketone, which is electrophilic, undergoes rapid reduction to the inactive hydroxyl derivative, rendering these compounds unsuitable as drug candidates or even as tool

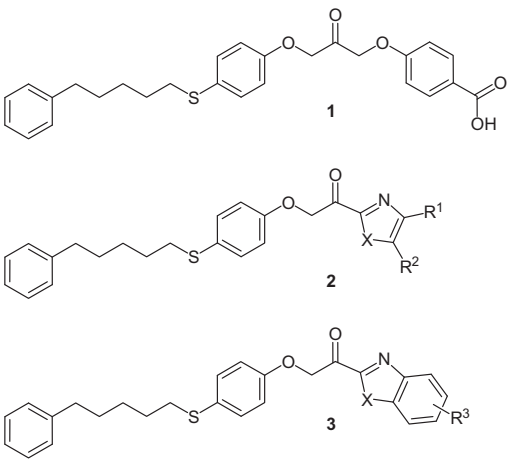


**Figure 1.** Novel series of cPLA$_2\alpha$ inhibitors.

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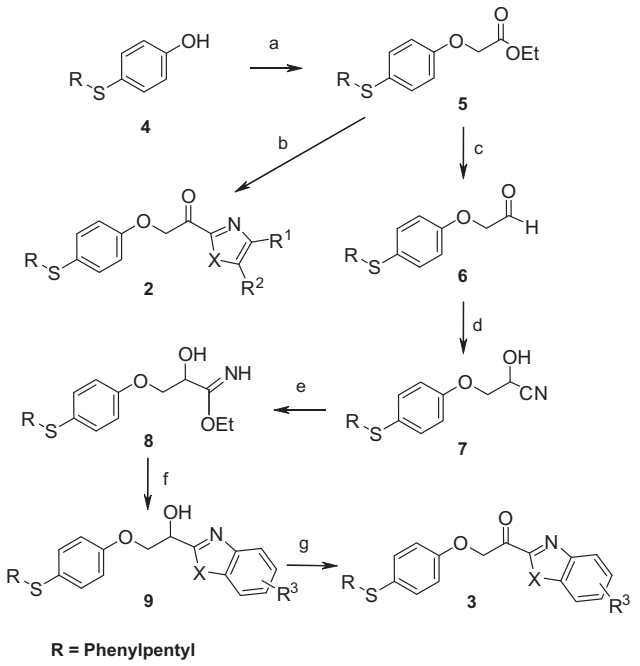



**Scheme 1.** Reagents and conditions: (a) ethyl bromoacetate, $Cs_2CO_3$, DMF, rt, 88%; (b) 2-lithiated heterocycle, THF, −78 °C to rt, 50–80%; (c) dibal, THF, −78 °C to rt, 83%; (d) acetonecyanohydrin, TEA, rt, 60%; (e) HCl, EtOH, rt, >95%; (f) 1,2-diaminobenzene or 2-aminophenol, MeCN, reflux, 45–70%; (g) Dess–Martin reagent, rt, 50–70%.

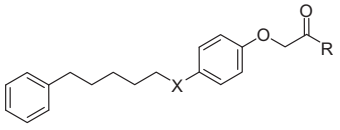
compounds for use in in vivo models of inflammation. Our efforts in the area were therefore directed to improving the metabolic stability of these compounds and render them more drug-like. Our approach was to explore a number of modifications to the ketone in **1**, designed to maintain its ability to interact with the catalytic serine residue in the enzyme,[8] while making it resistant to reduction.

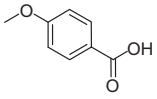
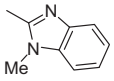
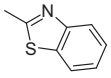
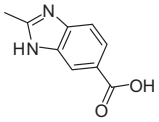
After a review of the options, we were attracted to some keto-heterocycles, as these have been used in a variety of amidase, protease and lipase inhibitors.[9] Furthermore, when incorporated into some human neutrophil elastase (HNE) inhibitors, they have been reported to possess good in vivo pharmacokinetic (PK) properties.[10]

The initial design strategy was to use compound **1** as a template in which to incorporate various keto-heterocycles. In the 1,3-dioxypropan-2-one series of $cPLA_2$ inhibitors,[5] we established that structural modifications close to the ketone were allowed only at the benzoic acid end of the molecule. Introduction of keto-heterocycles into this part of the molecule was therefore proposed and the structures **2** and **3** were explored as potential $cPLA_2\alpha$ inhibitors (Fig. 1). The synthetic routes used to prepare such heterocyclic derivatives are outlined in Scheme 1.

The ester **5** was readily synthesized and proved a versatile intermediate. This ester reacted with a variety of lithiated heterocycles to afford the ketones **2** directly.[11] Interestingly, very little double addition to the ester was observed. Some of the bicyclic derivatives **3** such as the benzothiazoles, were also amenable to synthesis by this approach. Benzoxazole and benzimidazole derivatives were synthesized from ester **5** by first converting it to the

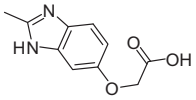
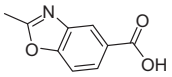
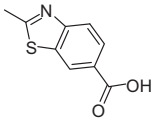
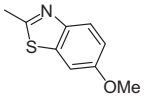
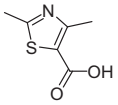
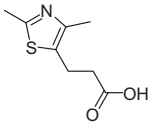
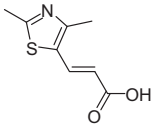
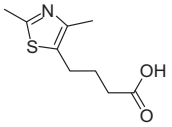
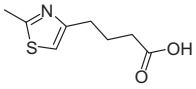
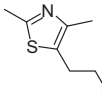
**Table 1**
$cPLA_2\alpha$ inhibition and metabolic stability of keto-heterocycles



| Compd | R | X | $cPLA_2$ $IC_{50}$[a] (μM) | HL60 cells $IC_{50}$[a] (μM) | % loss ketone[b] | $Cl_{int}$ rat heps[c] | $K_{hyd}$[d] |
|---|---|---|---|---|---|---|---|
| **1** |  | S | 0.08 | 1.5 | nt | nt | nt |
| **10** |  | S | 1 | >10 | 74 | nt | <0.1 |
| **11** |  | S | >10 | nt | 62 | nt | nt |
| **12** |  | S | >10 | nt | nt | nt | <0.1 |
| **13** |  | S | >10 | nt | nt | nt | nt |
| **14** |  | S | 1 | nt | 30 | nt | 0.48 |
| **15** |  | S | 2.7 | 3.7 | nt | nt | nt |

(*continued on next page*)

**Table 1** (*continued*)

| Compd | R | X | cPLA$_2$ IC$_{50}$[a] (μM) | HL60 cells IC$_{50}$[a] (μM) | % loss ketone[b] | Cl$_{int}$ rat heps[c] | $K_{hyd}$[d] |
|---|---|---|---|---|---|---|---|
| **16** |  | S | >10 | >10 | nt | nt | nt |
| **17** |  | S | 0.14 | 3.6 | 86 | 35 | 5 |
| **18** |  | S | 0.08 | 1.6 | 100 | 28 | nt |
| **19** |  | S | >10 | nt | 30 | nt | nt |
| **20** |  | S | 0.3 | 1.1 | 44 | 11 | 0.61 |
| **21** |  | S | 0.5 | 0.5 | 54 | nt | nt |
| **22** |  | S | 4.3 | 3 | nt | nt | nt |
| **23** |  | S | 0.08 | 0.5 | 44 | nt | <0.1 |
| **24** |  | O | 2 | 6 | 40 | nt | nt |
| **25** |  | S | 0.28 | 1.3 | 21 | 31 | <0.1 |

[a] Values are means of at least two experiments. nt = not tested.
[b] Loss of parent ketone by reduction in rat liver S9 fraction.
[c] Intrinsic clearance in rat hepatocytes: μL/min/$10^{-6}$ cells.
[d] $K_{hyd}$: Equilibrium constant for hydration of the ketone in DMSO/water.

imidate **8** by standard methods. This reacted with a variety of 1,2-diaminobenzenes or 2-amino-phenols to afford the hydroxy intermediates **9** which were oxidized to the required keto derivatives **3**.[16] A number of these hydroxy-heterocyclic derivatives **9**, and the equivalent hydroxy derivatives of **2**, were tested for cPLA$_2\alpha$ activity throughout the course of this work and in all cases exhibited no inhibition of the enzyme. Table 1 summarizes the initial set of compounds made.

The key properties tabulated include cPLA$_2\alpha$ potencies in a cell-free enzyme assay and a cellular assay and metabolic stability to ketone reduction. The enzyme assay measured inhibition of cleavage of a soluble fluorogenic substrate[12] by recombinant enzyme as previously described.[5a] The cell assay measured inhibition of [$^3$H]-arachidonate release following A23187 challenge of DMSO differentiated HL60 cells.[5a] Ketone stability to metabolic reduction was initially assessed by an in vitro assay using rat liver S9 fraction which measured the disappearance of the parent ketone and appearance of the hydroxy metabolite.[13] The intrinsic clearance in rat hepatocytes was subsequently measured for some key compounds.[14] Firstly, a range of unsubstituted mono- and bi-cyclic heterocyclic derivatives, **10–14** were evaluated. All had disappointing levels of activity compared to compound **1**. The effect of incorporating an acidic substituent onto the heterocycle, to mimic the acid found in **1**, was then explored. Encouragingly, addition of the acid group afforded compounds **15–20** with interesting levels of potency, and analogues **17** and **18** possessing similar potency to that seen in compound **1**. However, the intrinsic clearance of both compounds in rat hepatocytes was high (35 and 28, respectively) and this appeared to be due to ketone reduction, as both compounds were extensively reduced in the rat liver S9 assay. Compound **17** was assessed in a rat in vivo PK study which confirmed its poor metabolic profile, exhibiting very high clearance (Cl >200) and very short plasma half-life ($T$½ <0.2 h). Although we had designed a new series of potent cPLA$_2\alpha$ inhibitors, we had not made any progress

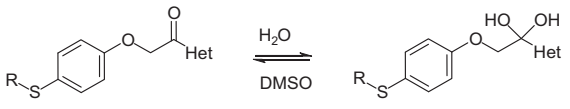



**Figure 2.** Assessing ketone reactivity by K hydration.

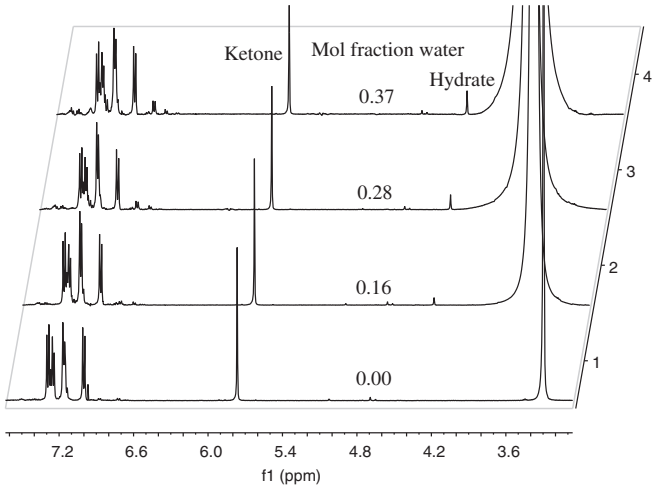



**Figure 3.** Detail of the NMR spectrum of compound **14** and its hydrate.

over the 1,3-dioxypropan-2-one series in terms of improved PK properties.

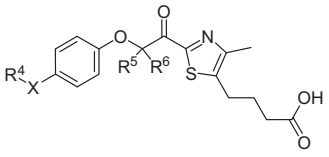
At this stage of the program the design strategy was re-assessed and it was decided to optimize potency in a keto-heterocyclic series in which the ketone had low electrophilicity and hence potentially better metabolic stability. To this end a method was needed to assess the electrophilicity of the ketone in various keto-heterocycles. It had been observed that the ketone in **1** was reactive towards water and readily formed a hydrate. A convenient NMR method was developed for measuring the equilibrium constant for this hydration process ($K_{hyd}$) for the various ketone containing compounds (Fig. 2).[15]

The $K_{hyd}$ was initially measured for a small group of keto-heterocyclic derivatives **10**, **12**, **14** and **17** to see how it varied and is summarized in Table 1. Figure 3 shows the hydration of compound **14** as a typical example and how the amount of hydrate present increases dependent on the mole fraction of water in the sample. In order to determine the ratio of ketone to hydrate it was most convenient to integrate the methylene singlet at 5.78 ppm (ketone) and 4.34 ppm (hydrate), to define the $K_{hyd}$.[15] These data were used to rank ketone electrophilicity and to prioritize the choice of keto-heterocycles to optimize.

The data show that the least reactive ketones towards hydration are in the monocyclic imidazole and thiazole compounds **10** and **12**. Interestingly, the addition of a fused phenyl ring onto **10**, to give the benzothiazole **14**, led to a more reactive ketone. The benzoxazole derivative **17** has an even more electrophilic ketone, presumably a result of a combination of the fused phenyl ring and an electron-withdrawing acid substituent. The chosen strategy, based on these data, was to attach an acid onto the thiazole **10** using an alkyl chain to try and increase potency by optimizing the interaction of the acid with the enzyme. Benzo-fusion was not used as this had led to increased ketone electrophilicity in **14**. The chain length connecting the acid to the thiazole ring was varied in **20**, **21** and **23**, and the linking chain was rigidified with a double bond in **22**. The position of attachment to the thiazole ring was also explored in **24**, as were other interactive groups such as hydroxyl **25**. It was encouraging to see that activity was found in these compounds **20–25**. Both the chain length of the acid and its position on the ring influenced activity, indicating a specific interaction with the enzyme. This led to compound **23** which combined excellent potency with a measured $K_{hyd}$ which was very much lower than that of **17**. However, rather disappointingly the liver S9 assay showed that the ketone in **23** was still being reduced. This compound was also assessed in a rat in vivo PK study, and although the clearance had been reduced compared to **18**, (71 vs >200) the plasma half-life was still very short ($T½$ ~0.2 h). It was concluded that clearance needed to be reduced even further in this series.

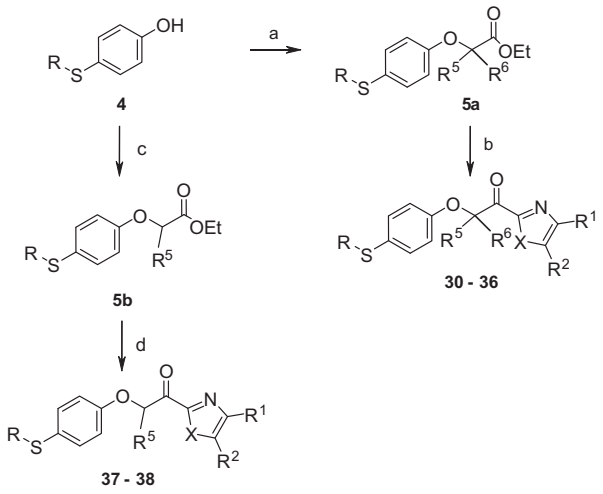
Analogues **26–29**, in which the phenylpentylthio group was replaced with other groups, were briefly investigated, to see if these would influence metabolism (Table 2). More polar groups such as

**Table 2**
cPLA$_2\alpha$ inhibition and metabolic stability of keto-thiazoles




| Compd | $R^5$ | $R^6$ | R4-X- | cPLA$_2$ IC$_{50}$[a] (μM) | HL60 cells IC$_{50}$[a] (μM) | % loss of ketone[b] | Cl$_{int}$ rat heps[c] |
|---|---|---|---|---|---|---|---|
| **26** | H | H | Ph(CH$_2$)5–O | 0.24 | 1.2 | 17 | 20 |
| **27** | H | H | PhCH$_2$S | 3 | 2 | nt | nt |
| **28** | H | H | DecylSO$_2$ | 0.3 | 0.2 | 33 | 41 |
| **29** | H | H | Decyl–S | 0.04 | 0.23 | 13 | nt |
| **30** | Me | H | Ph(CH$_2$)5–S | 0.2 | 0.7 | 0 | nt |
| **31** | Me | Me | Ph(CH$_2$)5–S | 6.7 | >10 | nt | nt |
| **32** | Et | H | 4-Chlorobenzyl–S | 7.9 | nt | nt | nt |
| **33** | Isopropyl | H | Ph(CH$_2$)5–S | 4.4 | 5.6 | nt | nt |
| **34** | Phenyl | H | Ph(CH$_2$)5–S | 3.6 | 3.2 | nt | nt |
| **35** | Me | H | Ph(CH$_2$)5–O | 0.7 | 2 | nt | 10 |
| **36** | Me | H | 4-Cl-benzyl–S | 1.5 | 1.5 | nt | nt |
| **37** | Me (S) | H | Ph(CH$_2$)5–S | >1 | nt | nt | nt |
| **38** | Me (R) | H | Ph(CH$_2$)5–S | 0.2 | nt | nt | 8 |

[a] Values are means of at least two experiments. nt = not tested.
[b] Loss of parent ketone by reduction in rat liver S9 fraction.
[c] Intrinsic clearance in rat hepatocytes μL/min/10$^{-6}$ cells.




**Scheme 2.** Reagents and conditions: (a) ethyl bromo-$R^5$,$R^6$-acetate, $Cs_2CO_3$, DMF, rt, 40–70%; (b) 2-lithiated thiazole, THF, −78 °C to rt, 30–70%; (c) ethyl 2-(*S*)-hydroxypropionate or ethyl 2-(*R*)-hydroxypropionate, DEAD, $PPh_3$, THF, 0 °C to rt, 60% (d) 2-lithiated thiazole, THF, −78 °C to rt, 55%.

**Table 3**
Rat in vivo PK properties of ketothiazole derivatives

| Compd | Cl (mL/min/Kg) | $V_{ss}$ (L/Kg) | *T* ½ (h) |
|---|---|---|---|
| **17** | >200 | nd | <0.2 |
| **23** | 71 | 0.4 | 0.2 |
| **35** | 10.5 | 0.35 | 1.25 |
| **36** | 10.5 | 0.55 | 1.15 |

Compounds dosed intra-venously to rats at 2 mg/Kg.
nd = not defined, due to extremely high Cl.

phenylpentyloxy **26** maintained reasonable potency, but benzylthio **27** led to much lower potency. The more lipophilic decylsulphonyl **28** and decylthio groups **29** gave very potent compounds, exhibiting some of the best enzyme and cell potencies observed in this series (~200 nM). However, all these compounds were still reduced by liver S9 and two that were assessed in rat hepatocytes, **26** and **28**, also had high intrinsic clearances of 20 and 41, respectively. Despite the lower ketone reactivity, the compounds still had poor metabolic stability and were not suitable for in vivo studies. Since the ketone reactivity to hydration had been reduced as much as could be measured, other approaches were considered to further stabilize these ketones to reduction.

It was decided to evaluate the effect of steric bulk close to the ketone on its stability. Compounds with a range of substituents on the α-position of the ketone **30–36** were evaluated (Table 2). The synthetic route to these α-substituted keto-thiazoles is outlined in Scheme 2.[16] Interestingly, only compound **30**, which bears a single methyl group α- to the ketone, retained good potency. Incorporating two methyl groups or bulkier groups α- to the ketone led to a large drop in activity. Gratifyingly, the α-methyl substituent had also improved the stability of the ketone to reduction and in the rat liver S9 assay no loss of compound **30** was observed. Some less lipophilic analogues, **35** and **36**, were also made and they maintained reasonable levels of potency against $cPLA_2\alpha$. These were evaluated in rat in vivo PK studies and both had low in vivo clearance (Cl ~10) and improved plasma half-lives (*T*½ ~1.2 h). This represents a 10-fold improvement over the non-methylated derivatives such as **23** (Table 3). These results encouraged us to make the two enantiomers of compound **30** by the route outlined in Scheme 2.

It was gratifying to see that the activity resided in only one of the enantiomers **38**. The intrinsic clearance in rat hepatocytes of this compound was also measured and it was found to be as low as that seen for its close analogue **35**.

We have demonstrated in compounds such as **35, 36** and **38** that a ketone-based serine trap can be made metabolically stable to reduction, while maintaining good levels of enzyme inhibition. This was achieved by attenuating the reactivity of the ketone using a combination of electronic and steric effects. The balance of potency and PK properties for these compounds make them suitable for use in in vivo models of inflammation to help elucidate the role of $cPLA_2\alpha$ in various disease states. The metabolic stability of these derivatives to other metabolic pathways needs to be assessed and in combination with further improvements in their potency and physical properties, may lead to compounds which exhibit a much more drug-like profile.

## References and notes


1. (a) Clark, J. D.; Schievella, A. R.; Nalesfki, E. A.; Lin, L. L. *J. Lipid Mediat. Cell Signal.* **1995**, *12*, 83; (b) Six, D. A.; Dennis, E. A. A. *Biochim. Biophys. Acta* **2000**, *1488*, 1.
2. (a) Leslie, C. C. *J. Biol. Chem.* **1997**, *272*, 16709; (b) Funk, C. D. *Science* **2001**, *294*, 1871; (c) Leslie, C. C. *Prostaglandins Leukot. Essent. Fatty Acids* **2004**, *70*, 373.
3. (a) Lehr, M. *Anti-Inflammatory Anti-Allergy Agents Med. Chem.* **2006**, *5*, 149; (b) Clark, J. D.; Tam, S. *Expert Opin. Ther. Pat.* **2004**, *14*, 937; (c) Linkous, A.; Yazlovitskaya, E. *Cell. Microbiol.* **2010**, *12*, 1369.
4. (a) McKew, J. C.; Foley, M. A.; Thakker, P.; Behnke, M. L.; Lovering, F. E.; Sum, F.-W.; Tam, S.; Wu, K.; Shen, M. W. H.; Zhang, W.; Gonzalez, M.; Liu, S.; Mahadevan, A.; Sard, H.; Khor, S. P.; Clark, J. D. *J. Med. Chem.* **2006**, *49*, 135; (b) McKew, J. C.; Lovering, F.; Clark, J. D.; Bemis, J.; Xiang, Y.; Shen, M.; Zhang, W.; Alvarez, J. C.; Joseph-McCarthy, D. *Bioorg. Med. Chem. Lett.* **2003**, *13*, 4501; (c) Kirincich, S. J.; Xiang, J.; Green, N.; Tam, S.; Yang, H. Y.; Shim, J.; Shen, M. W. H.; Clark, J. D.; McKew, J. C. *Bioorg. Med. Chem. Lett.* **2009**, *17*, 4383; (d) McKew, J. C.; Lee, K. L.; Shen, M. W. H.; Thakker, P.; Foley, M. A.; Behnke, M. L.; Hu, B.; Sum, F.-W.; Tam, S.; Hu, Y.; Chen, L.; Kirincich, S. J.; Michalak, R.; Thomason, J.; Ipek, M.; Wu, K.; Wooder, L.; Ramarao, M. K.; Murphy, E. A.; Goodwin, D. G.; Albert, L.; Xu, X.; Donahue, F.; Ku, M. S.; Keith, J.; Nickerson-Nutter, C. L.; Abraham, W. M.; Williams, C.; Hegen, M.; Clark, J. D. *J. Med. Chem.* **2008**, *51*, 3388; (e) Kokotos, G.; Six, D. A., et al *J. Med. Chem.* **2004**, *47*, 3615; (f) Six, D. A. et al *Bioorg. Med. Chem. Lett.* **2006**, *16*, 2798.
5. (a) Connolly, S.; Bennion, C.; Botterell, S.; Croshaw, P. J.; Hallam, C.; Hardy, K.; Hartopp, P.; Jackson, C. G.; King, S. J.; Lawrence, L.; Mete, A.; Murray, D.; Robinson, D. H.; Smith, G. M.; Stein, L.; Walters, I.; Wells, E.; Withnall, W. J. *J. Med. Chem.* **2002**, *45*, 1348; (b) Walters, I.; Bennion, C.; Connolly, S.; Croshaw, P. J.; Hardy, K.; Hartopp, P.; Jackson, C. G.; King, S. J.; Lawrence, L.; Mete, A.; Murray, D.; Robinson, D. H.; Stein, L.; Wells, E.; Withnall, W. J. *Bioorg. Med. Chem. Lett.* **2004**, *14*, 3645.
6. Trimble, L. A.; Street, I. P.; Perrier, H.; Tremblay, N. M.; Weech, P. K.; Bernstein, M. A. *Biochemistry* **1993**, *32*, 12560.
7. Mete, A. Poster presented at "XVth International Symposium on Medicinal Chemistry", Edinburgh, UK, 1998.
8. (a) Dessen, A.; Tang, J.; Schmidt, H.; Stahl, M.; Clark, J. D.; Seehra, J.; Somers, W. S. *Cell* **1999**, *97*, 349; (b) Sharp, J. D.; Pickard, R. T.; Chiou, X. G.; Manetta, J. V.; Kovacevic, S.; Miller, J. R.; Varshavsky, A. D.; Roberts, E. F.; Strifler, B. A. *J. Biol. Chem.* **1994**, *269*, 23250; (c) Burke, J. E.; Dennis, E. A. *J. Lipid Res.* **2009**, S237.
9. (a) Edwards, P. D.; Meyer, E. F., Jr.; Vijayalakshmi, J.; Tuthill, P. A.; Andisik, D. A.; Gomes, B.; Strimpler, A. *J. Am. Chem. Soc.* **1992**, *114*, 1854; (b) Edwards, P. D.; Wolanin, D. J.; Andisik, D. W.; Davis, M. W. *J. Med. Chem.* **1995**, *38*, 76; (c) Edwards, P. D.; Zottola, M. A.; Davis, M. W.; Williams, J.; Tuthill, P. A. *J. Med. Chem.* **1995**, *38*, 3972; (d) Maryanoff, B. E.; Costanzo, M. J. *Bioorg. Med. Chem. Lett.* **2008**, *16*, 1562. and references therein; (e) Boger, D. L.; Miyauchi, H.; Hedrick, M. P. *Bioorg. Med. Chem.* **2001**, *9*, 647.
10. (a) Ohmoto, K. *J. Med. Chem.* **2000**, *43*, 4927; (b) Ohmoto, K. *Bioorg. Med. Chem.* **2001**, *9*, 1307; (c) Odagaki, Y.; Ohmoto, K.; Matsuoka, S.; Hamanaka, N.; Nakai, H.; Toda, M.; Katsuya, Y. *Bioorg. Med. Chem. Lett.* **2001**, *16*, 1562; (d) Ferraris, D. et al *Bioorg. Med. Chem. Lett.* **2004**, *14*, 5579.
11. Chikashita, H.; Ishibaba, M.; Ori, K.; Ito, K. *Bull. Chem. Soc. Jpn.* **1988**, *61*, 3637.
12. Huang, Z.; Laliberte, F.; Tremblay, N. M.; Weech, P. K.; Street, I. P. *Anal. Biochem.* **1994**, *222*, 110.
13. Mandagere, A. K.; Thompson, T. N.; Hwang, K. K. *J. Med. Chem.* **2002**, *45*, 304.
14. Soars, M. G.; Burchell, B.; Riley, R. J. *J. Pharmacol. Exp. Ther.* **2002**, *301*, 382.
15. Approximately 8 mg of compound was divided between four NMR tubes and dissolved in 0.45 ml dry DMSO-$d_6$. To each tube was added either 0, 20, 40 or 60 μl water (or deuterium oxide). The samples were mixed and equilibrated for

5 days at room temperature. The ratio of hydrate to ketone present in each sample was determined by accurate integration of the proton NMR spectrum. This ratio was plotted against the mol fraction of water (or deuterium oxide) present and Khyd was then determined from the slope. Additional measurements suggested that the isotope effect on the equilibrium constant was very small and that values obtained from water or deuterium oxide measurements could be used interchangeably.

16. Connolly, S.; Mete, A. WO Patent 2000034254; *Chem. Abstr*. **2000**, *133*, 30725.