Pergamon

Bioorganic & Medicinal Chemistry Letters 9 (1999) 2849–2854

BIOORGANIC & MEDICINAL CHEMISTRY LETTERS

GENERATION OF AN UGI LIBRARY OF PHOSPHATE MIMIC-CONTAINING COMPOUNDS AND IDENTIFICATION OF NOVEL DUAL SPECIFIC PHOSPHATASE INHIBITORS

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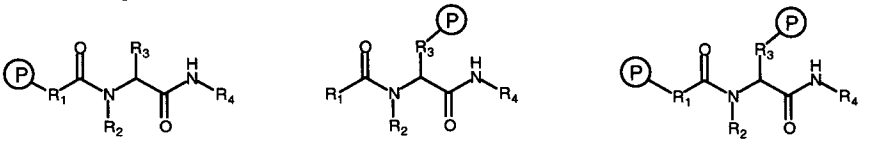
Received 16 June 1999; accepted 31 August 1999

Abstract: The four-component Ugi reaction was utilized to prepare a library of dipeptidic compounds in order to explore the binding requirements of the key cell cycle phosphatase, Cdc25. Several phosphate surrogates were incorporated into the Ugi product to mimic either the mono- or bis-phosphorylated substrate. © 1999 Elsevier Science Ltd. All rights reserved.

The dual specificity phosphatase, Cdc25, is an essential activator of the cell cycle at either the G1/S[1] or G2/M[2] transition of the cell cycle. Cdc25 regulates these checkpoints by controlling the dephosphorylation of adjacent pTpY residues on complementary CDK-cyclin complexes.[3] It is believed that inhibitors of Cdc25 have antitumor properties.[4]

Protein tyrosine phosphatases are characterized by a large regulatory N-terminal domain and a small C-terminal domain that contains the catalytic core. Recently, the X-ray crystal structure of the catalytic domain of human Cdc25A was solved to 2.5 Å resolution.[5] Other than the signature HC-X5-R phosphate binding loop, which shields the catalytically important Cys430 in the active site, the overall structure of this enzyme is distinct from other known PTPases. In particular, the active site of Cdc25 is notable for its shallow substrate binding pocket. This may be necessary for the dual specific phosphatases[6] to accomodate phosphorylated tyrosine as well as serine/threonine residues in the active site. The shallow binding groove may also explain the dearth of information available concerning the substrate specificity of Cdc25, since phosphorylated Cdc2 fragments as well as synthetic phosphopeptides are known to be extremely poor substrates for this enzyme.[7]

We were interested in designing mechanism-based active site inhibitors of Cdc25. To gain a better understanding of the structural requirements for small molecule binding in the active site, we designed a library of small molecules which would probe the phosphate binding site of the enzyme. In this paper we report the application of the Ugi four-component condensation reaction to prepare libraries containing known phosphate mimics to explore the interactions in the active site.



Ⓟ = **Phosphate Mimic**

Figure 1

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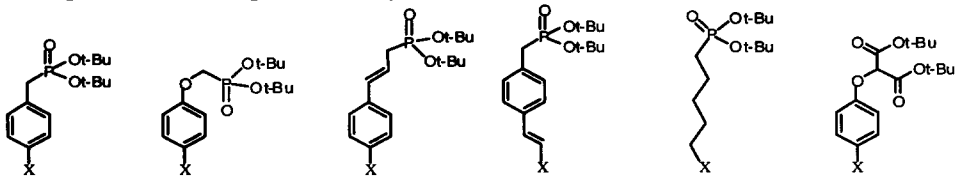
PII: S0960-894X(99)00497-7

The Ugi reaction is a four-component condensation reaction that has been widely used to prepare combinatorial libraries in either solid or solution phase.[8] While the Ugi protocol has been used previously to prepare PTP1B inhibitors,[9] we envisioned the use of phosphate analogs such as phosphonates and malonates to anchor these molecules in the active site of the enzyme. The remaining components of the Ugi reaction would then be used to probe the binding interactions in the vicinity of the active site. By incorporating the phosphate mimics into either the acid or aldehyde components of the Ugi reaction, these surrogates could be positioned at either the amino terminal or the central portion of the dipeptide product to probe different regions of the active site (Figure 1). An additional feature of this strategy is that utilization of phosphate mimics in both the acid and aldehyde components would lead to the synthesis of "bis-phosphorylated products" that would have the potential to mimic the bisphosphate binding motif inherent in the natural substrate. We envisioned that these libraries would not only probe the sidechain interactions that are important for Cdc25 binding, but would also provide insight into the design of nonhydrolyzable phosphate surrogates.

Figure 2. Phosphate mimic components of Ugi reaction.



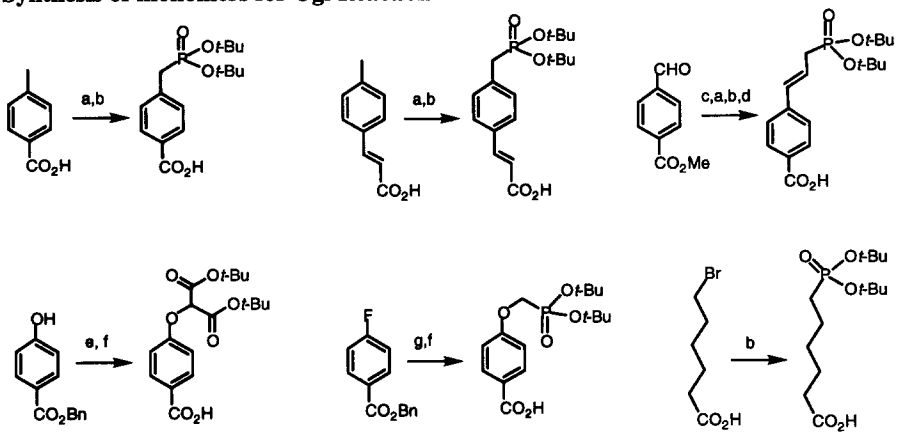
1a: X = CO_2H **1b**: X = CHO **2a**: X = CO_2H **2b**: X = CHO **3**: X = CO_2H **4**: X = CO_2H **5a**: X = CO_2H **5b**: X = CHO **6a**: X = CO_2H **6b**: X = CHO

Components for the Ugi reaction were selected to probe both the steric and electronic requirements of the active site (Figure 2). For example, monomers **1–3** were chosen to explore the chain length connecting the phosphate mimic to the aromatic ring. Conversely, **4** retains the positioning of the phosphonate moiety from the aromatic ring but increases the distance between the aromatic ring and the peptide backbone, while **5** explores the effect of an aliphatic phosphonate. Finally, **6** was prepared based on Burke's report of the effective use of malonates as phosphate replacements in PTP1B inhibitors.[10] The synthesis of these carboxylic acid components is shown in Figure 3. The corresponding aldehydes **1b**, **5b**, and **6b** were prepared from the carboxylic acids using known methods,[11] while aldehyde **2b** was prepared from 4-fluorobenzaldehyde using the same procedure as that used for the acid.[12] All the products were prepared as *t*-butyl esters of carboxylic and phosphonic acids to facilitate the Ugi reaction and were deprotected with TFA/$CHCl_3$ prior to biochemical analysis.

A small set of diverse reagents was chosen for preparation of the Ugi library based on reactivity, and steric and electronic factors (Table 1). Three different libraries were prepared using either the phosphoacid mimic, the phosphoaldehyde component, or both the phosphoacid and aldehyde fragments. The phosphoacid library contained 2400 members: six phosphate mimics, ten amines, eight aldehydes, and five isonitriles. The phosphoaldehyde library contained 1280 analogs: four phosphate mimics, ten amines, eight acids, and four isonitriles. Finally, the library containing two mimics numbered 640 compounds: four phosphoacid mimics, ten

amines, four phosphoaldehyde mimcs, and four isonitriles. The aldehydes and acids shown in Table 1 were used in the phosphoacid and phosphoaldehyde mimic libraries, respectively, while the amines and isonitriles were common to all libraries.[13]

Figure 3. Synthesis of monomers for Ugi Reaction

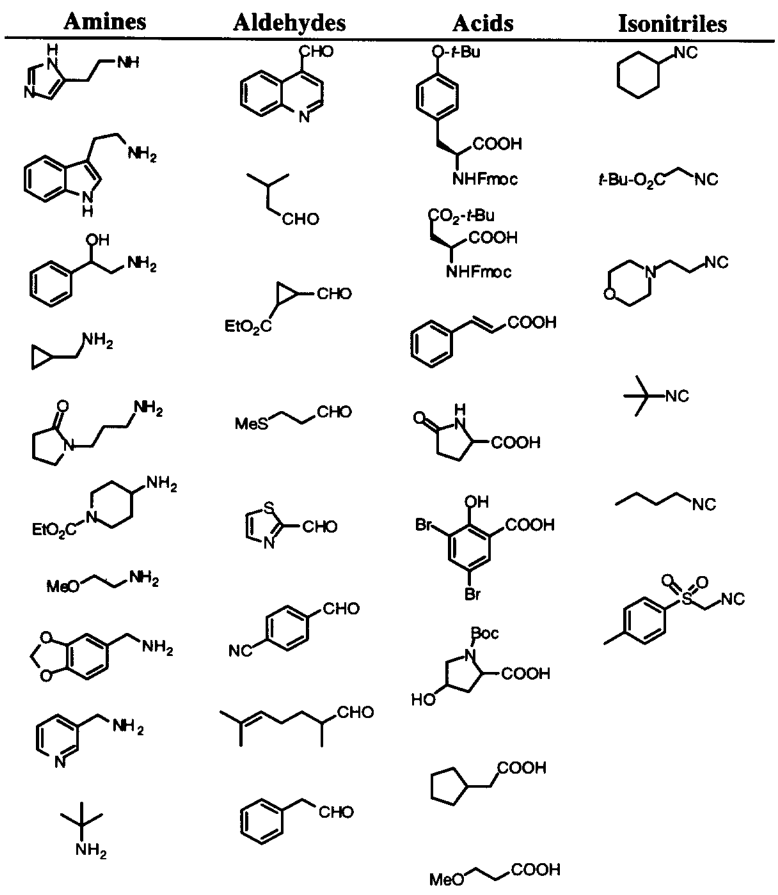


a. NBS, benzoyl peroxide, CCl_4, reflux. b. HP(O)(O-t-Bu)$_2$, NaH, DMF, 0-25 °C. rt. c. CH_3CHPPh_3, THF, rt. d. LiOH, MeOH/H_2O. e. di-*t*-Butyl diazomalonate, $Rh_2(OAc)_4$, toluene, reflux. f. 10%Pd/C, H_2 (40 psi), MeOH. g. HP(O)(O-*t*-Bu)$_2$, NaH, $(CH_2O)_n$, DMF, 0-25 °C.

The crude libraries were initially only screened for inhibition of Cdc25,[14] while the resynthesized actives were also assayed against VHR[15] (a dual specificity phosphatase) and PTP1B[16] (a tyrosine phosphatase). The initial screening of these Ugi products revealed certain structural motifs that were conserved in all three libraries. Some of the favorable side-chain interactions include aromatic groups at the acid position, R_1, bicyclic aromatics such as indole and methylenedioxyphenyl at the amino position, R_2, and aromatic and extended hydrophobic groups at the aldehyde position, R_3. Consistent with the X-ray crystal structure of the catalytic site, these results would suggest that the active site of Cdc25A is quite lipophilic with the exception of the essential phosphate binding site. The most active compounds were resynthesized, purified, and their mode of inhibition was characterized. Some of the more potent analogs and their biological activity against Cdc25A, VHR, and PTP1B are shown in Table 2. [17]

The results shown indicate that some of these compounds indeed inhibit Cdc25A in the low micromolar range. In some cases encouraging levels of selectivity against VHR and PTP1B were also observed. Surprisingly, the presentation of the phosphate mimics in the context of the Ugi product (i.e., N-terminal or central) does not seem to be particularly important. However, we were encouraged that compounds **13–15**, which mimic the bisphosphorylated motif of the natural substrate seem, to be better inhibitors of the enzyme.

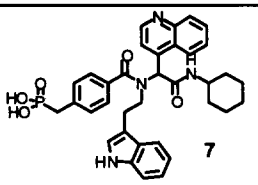
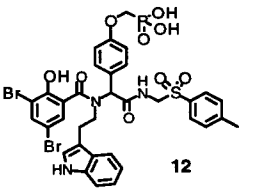
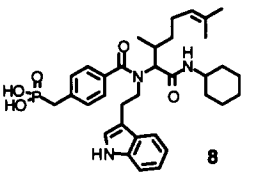
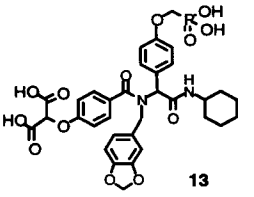
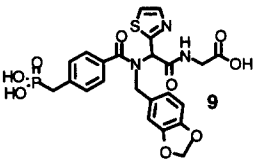
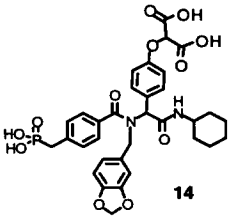
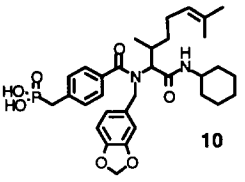
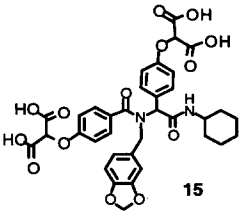
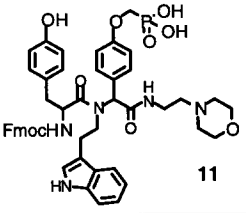
Table 1. Components of the Ugi Library.

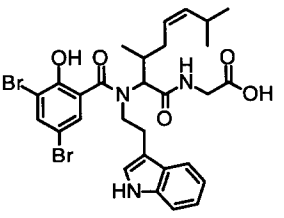


Mechanistic analysis of these compounds revealed a noncompetitive mode of inhibition, suggesting that these compounds are not interacting at the active site of the enzyme. Lack of active site interaction is also supported by compounds such as **16**, which is devoid of a phosphate surrogate, and **17**, which is an analog of **8** containing an α,α-difluoromethylenephosphonate[18] moiety. Both displayed equivalent activity [**16** (15 μM) and **17** (27 μM)] and mode of inhibition to other members of our library against Cdc25A despite the apparent lack of a critical binding element in one case and the addition of a unit that has historically led to improved binding over methylenephosphonates[19] in the other.

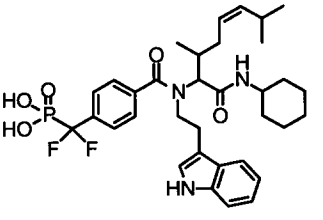
In light of these results, it is somewhat surprising that the bis-mimic compounds were the most potent inhibitors. Examination of the published X-ray crystal structure of Cdc25A reveals a number of exposed basic residues on the surface of the protein, which could account for sequestration of these highly charged inhibitors. In particular, a hydrophobic pocket 18.5 Å from the active site, which contains two non-ion paired guanidino sidechains pertaining to Arg-445 and Arg-449, is one possible candidate for a binding site.

Table 2. Activity (μM) for resynthesized hits from Ugi libraries.

Structure	Cdc2	VH	PTP1	Structure	Cdc2	VH	PTP1
 7	16	87	>160	 12	10	>120	95
 8	23	17	61	 13	0.7	5.0	82
 9	35	>9	>96	 14	3.0	11	107
 10	23	19	73	 15	3.0	9.0	89
 11	8.0	6.1	16				



16



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In summary, we have prepared three Ugi libraries that incorporate phosphate mimics in either the amino terminal, central, or both terminal and middle portions of the dipeptidic product. Our premise was that the phosphate mimic would be sufficient to anchor these compounds in the active site of the enzyme. Thus, we would be able to probe binding determinants in the vicinity of the active site and gain a better understanding of the elements critical for inhibitor design. While our initial hypothesis of finding active site directed competitive inhibitors of Cdc25A was not realized, since all of these compounds displayed a noncompetitive mode of inhibiton, we have discovered a novel class of potent non-competitive phosphatase inhibitors. Current efforts to map the binding site of these non-competitive inhibitors will be reported in due course.

Acknowledgement: We would like to thank Dr. Krishna Murthi and Patrick Bureau for their assistance in the preparation of Ugi monomers from Table 1.

References and Notes

1. (a) Hoffmann, I.; Draetta, G.; Karsenti, E. *EMBO J.* **1994**, *13*, 4302. (b) Jinno, S.; Suto, K.; Nagata, A.; Igarashi, M.; Kanaoka, Y.; Nojima, H.; Okayama, H. *EMBO J.* **1994**, *13*, 1549.
2. (a) Hoffmann, I.; Clarke, P. R.; Marcote, M. J.; Karsenti, E.; Draetta, G. *EMBO J.* **1993**, *12*, 53. (b) Glaktionov, K.; Lee, A. K.; Eckstein, J.; Draetta, B.; Meckler, J.; Loda, M.; Beach, K. *Science* **1995**, *269*, 1575.
3. (a) Honda, R.; Ohba, Y.; Nagata, A.; Okayama, H.; Yasuda, H. *FEBS Lett.* **1993**, *318*, 331. (b) Strausfeld, U.; Labbe, J. C.; Fesquet, D.; Cavadore, J. C.; Picard, A.; Sadhu, K.; Russell, P.; Doree, M. *Nature* **1991**, *351*, 242.
4. Weinert, T. *Science* **1997**, *277*, 1450 and references cited therein.
5. Fauman, E. B.; Cogswell, J. P.; Lovejoy, B.; Rocque, W. J.; Holmes, W.; Montana, V. G.; Piwnica-Worms, H.; Rink, M. J.; Saper, M. A. *Cell* **1998**, *93*, 617.
6. Hofmann, K.; Bucher, P.; Kajava, A. V. *J. Mol. Biol.* **1998**, *282*, 195.
7 Gautier, J.; Solomon, M. J.; Booher, R. N.; Bazan, J. F.; Kirschner, M. W. *Cell*, **1991**, *67*, 197.
8. (a) Ugi, I.; Goebel, M.; Gruber, B.; Heilingbrunner, M.; Heiß, C.; Horl, W.; Kern, O.; Starnecker, M.; Domling, A. *Res. Chem. Intermed.* **1996**, *22*, 625. (b) Mjalli, A. M. M.; Sarshar, S.; Baiga, T. J. *Tetrahedron Lett.* **1996**, *37*, 2943.
9. Li, Z.; Yeo, S. L.; Pallen, C. J.; Ganesan, A. *Bioorg. Med. Chem. Lett.* **1998**, *8*, 2443.
10. Ye, B.; Akamatsu, M.; Shoelson, S. E.; Wolf, G.; Giorgetti-Peraldi, S.; Yan, X.; Roller, P. P.; Burke, T. R., Jr. *J. Am. Chem. Soc.* **1995**, *38*, 4270.
11. Nahm, S.; Weinreb, S. M. *Tetrahedron Lett.* **1981**, *22*, 3815.
12. Berge, J.; Moore, G. N. *Synlett* **1994**, 187.
13. Ugi reactions were run as 1 M EtOH solutions containing a 1:1:1:1 ratio of each component and heated to 50 °C for 48 h. Product purity was estimated by TLC for every reaction and HPLC for 10% of the set. Products were estimated to be 30–95% pure. Acid labile groups were removed with 1:1 TFA/$CHCl_3$. Library products were tested without purification. Resynthesized actives were prepared as above and purified using silica gel chromatography with EtOAc/hexanes as eluent. All purified compounds gave satisfactory NMR and analytical data.
14. Acid mimic library products were screened in duplicate at a single concentration of 200 μM, while the aldehyde and bis mimic libraries were screened at 40 μM. Wells which showed >50% inhibition versus control and reproduced within a factor of three in %inhibition were treated as real, all others were treated as false positives and counted as zero inhibition.
15. Yuvaniyama, J.; Denu, J. M.; Dixon, J. E.; Saper, M. A. *Science* **1996**, *272*, 1328.
16. Jia, Z.; Barford, D.; Flint, A. J.; Tonks, N. K. *Science* **1995**, *268*, 1754.
17. The assay for Cdc25 is described in: Eckstein, J.; Beer-Romero, P.; Berdo, I. *Protein Sci.* **1996**, *5*, 5. The assays for PTP1B and VHR are described in: Gottlin, E. B.; Xu, X.; Epstein, D. M.; Burke, S. P.; Eckstein, J. W.; Ballou, D. P.; Dixon, J. E. *J. Biol. Chem.* **1996**, *271*, 27445.
18. Burke, T. R., Jr.; Smyth, M. S.; Nomizu, M.; Otaka, A.; Roller, P. P. *J. Org. Chem.* **1993**, *58*, 1336.
19. Burke, T. R., Jr.; Kole, H. K.; Roller, P. P. *Biochem. Biophys. Res. Commun.* **1994**, *204*, 129.