Pergamon

Bioorganic & Medicinal Chemistry Letters 9 (1999) 2053–2058

BIOORGANIC & MEDICINAL CHEMISTRY LETTERS

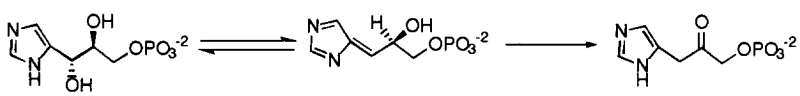
DESIGN AND SYNTHESIS OF β-CARBOXAMIDO PHOSPHONATES AS POTENT INHIBITORS OF IMIDAZOLE GLYCEROL PHOSPHATE DEHYDRATASE

Barbara A. Schweitzer,* Paul J. Loida, Rebecca L. Thompson-Mize, Claire A. CaJacob and Shridhar G. Hegde
Monsanto Company, 800 N. Lindbergh Blvd, St. Louis, MO 63167, U.S.A

Received 24 March 1999; accepted 4 June 1999

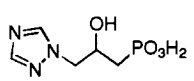
Abstract: We describe the synthesis and enzymatic activity of a library of β-carboxamido phosphonates as inhibitors of imidazole glycerol phosphate dehydratase (IGPD). Biological results suggest the presence of an enzymatic interaction site not previously observed for other inhibitors of IGPD.

Imidazole glycerol phosphate dehydratase (IGPD), an enzyme in the histidine biosynthetic pathway, catalyzes the conversion of imidazole glycerol phosphate (IGP), **1**, to imidazole acetol phosphate (IAP), **3**. The histidine biosynthetic pathway is an attractive target for herbicide discovery since it is absent in mammals, yet is critical for plant growth. Independent biorational design of inhibitors of IGPD has previously been undertaken by several laboratories; several such inhibitors have shown promise as nonselective herbicides.[1–4]



1 **2** **3**

Most reported inhibitors are transition state mimics, designed to resemble the proposed diazafulvene reaction intermediate **2**, and as such they typically contain three proposed binding groups: (1) a nitrogen containing heterocyclic ring (2) a hydrogen bond donor/acceptor and (3) a phosphate or phosphonate.[1–4] The most active reported inhibitor is compound **4** (K_i = 0.6 nM), in which the imidazole ring of the substrate has been replaced with a triazole and the phosphate replaced with a phosphonate.[5] Although most investigators have focused on substitutions of these three groups, their major modification or deletion drastically reduces activity. For example, replacement of the phosphate with a malonate results in a decrease in activity of several orders of magnitude,[1] while replacement of the imidazole with a pyrimidine or pyridine results in even weaker activity.[6]



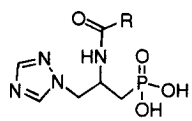
4

0960-894X/99/$ - see front matter
PII: S0960-894X(99)00338-8

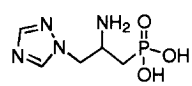
We sought to probe additional interactions besides those of the three postulated binding groups in order to generate novel classes of molecules with enhanced IGPD binding affinity. To this end we have synthesized a library of triazole carboxamide phosphonic acids (**5**) that has allowed us to probe additional sites of interaction within the enzyme without significantly altering the three postulated interacting groups.

Since no crystal structure data are available for the IGPD active site, the selection of R groups in **5** relied on computational chemistry. Our initial effort focused on probing sufficient structural diversity space to enhance the chances of finding R groups with potential for binding. Cerius[2] software (Version 3.5, 1997, MSI, San Diego, CA) was used to conduct a principal components analysis (PCA) of public and proprietary databases to assess diversity. We identified ten structures with diverse R groups from the principal components analysis for initial synthesis and in vitro assay. A second focused library was then designed and synthesized, which consisted of molecules closely resembling active analogs from the initial library.

The carboxamides **5** were synthesized by one of two routes that employed solution-phase chemistry and solid phase-reagent capture resins. We found that it was critical to eliminate all traces of the starting amine **7**, since subsequent hydrolysis of the latter affords **6**, which is an effective inhibitor (K_i = 15 nM) of IGPD,[3] and difficult to separate from the desired product. The amine phosphonate diester **7** was synthesized and treated with a variety of acids or acid chlorides, followed by hydrolysis of the phosphonate diester to afford the desired carboxamide **5** (Scheme 1).



5



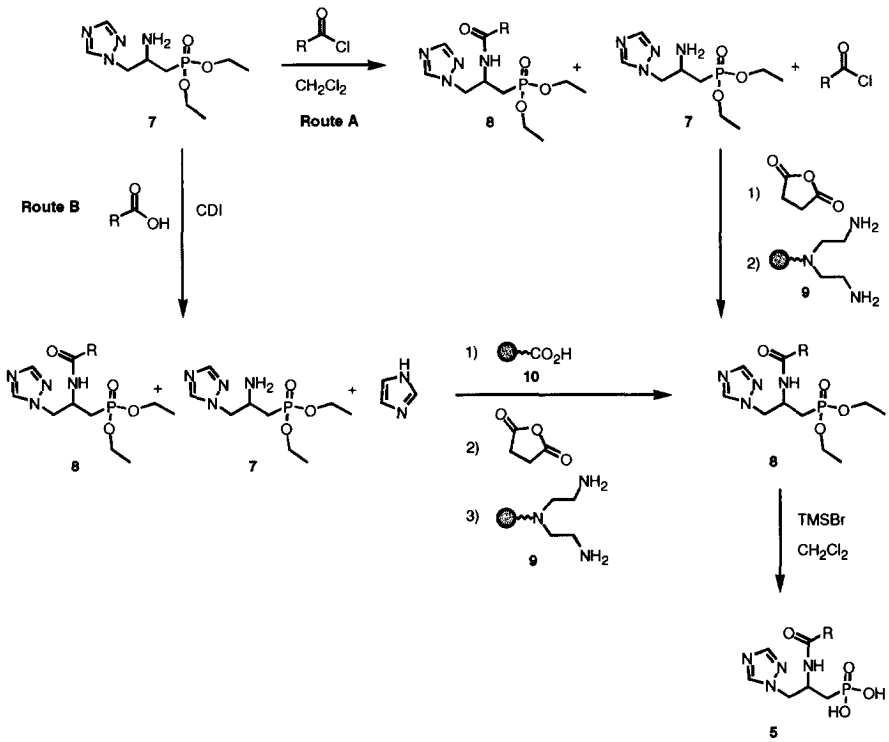
6

Reactions were very efficient in most cases, with good yields and little or no side product. In some cases purification of the intermediate diester **7** or of the phosphonic acid **5** was necessary to obtain acceptable purity for biological assays.[7] Coupling of the amine with an acid chloride afforded the desired carboxamide (Scheme 1-Route A). Succinic anhydride in combination with an amine resin **9**[8] was effective in removing unreacted amine and acid chloride from the reaction mixture. The desired carboxamide **5** can also be obtained through coupling of the amine with an acid using CDI as the coupling agent (Scheme 1-Route B). Carboxylic acid resin **10**[9] was used to remove the imidazole by-product from the reaction, followed by treatment with the succinic anhydride/amine resin **9** combination to remove unreacted amine as above. ^{1}H NMR, ^{31}P NMR spectroscopy, HPLC, and MS were used to characterize the intermediate diesters and the subsequent phosphonic acids.

Inhibition constants were measured using the fungal form of the enzyme from *Cryptococcus neoformans*.[10] Inhibition data were determined using a previously reported endpoint assay (IC_{50})[11] or a continuous coupled assay (K_i).[12] The stability of the compounds in the enzyme assay was verified by incubating a subset of the

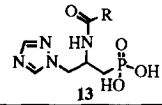
compounds with the assay components and analyzing by LC-MS for presence of carboxamide and starting amine. The carboxamides are stable under the assay conditions. In our assay the parent amine displayed an IC_{50} of 230 nM and a K_i of 45 nM, which is consistent with previously published data.[3] Our carboxamides displayed a mixed type of inhibition that is also consistent for some inhibitors of this type.[2] Inhibition constants of the library ranged from 80 to 11,000 nM and tracked well with IC_{50} values, which ranged from 0.38 to 50 μM. Enzyme inhibition data is shown in Table 1.

SCHEME 1

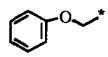
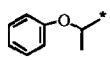
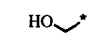
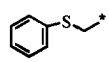
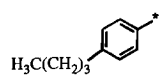
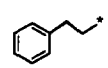
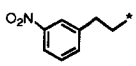
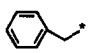
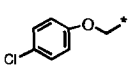
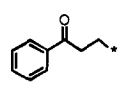
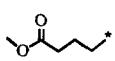
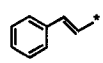
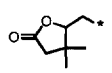


The assay data indicated that we had succeeded in identifying new potent inhibitors of IGPD. For example, the phenoxyacetamide **5a** displayed an inhibition constant of 80 nM. These results were very interesting in light of the fact that this and other carboxamides increase the molecular weight by over one-half and also add substantial steric bulk when compared to previously reported inhibitors. Our results thus suggest the presence of an additional, possibly lipophilic, site of interaction between the inhibitor and IGPD. More than 70% of the compounds tested from the more focused library were potent inhibitors of IGPD ($IC_{50} \leq 2$ mM). From the data presented above it appears that a linker of two methylene units provides optimum spacing between the carbonyl moiety of the carboxamide and bulky hydrophobic substituents such as a phenyl group. Activities of compounds with greater (**5y**) or fewer (**5i** and **5cc**) methylene units are diminished.

Table 1. IGPD inhibition by **13**.



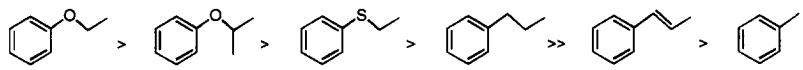
13

Compd	R	IC_{50}	K_i (μM)	K_{is} (μM)	Compd	R	IC_{50}	K_i (μM)	K_{is} (μM)
5a		0.38	0.08	0.27	**5p**		4.8	1.13	5.0
5b		1.5	0.23	1.22	**5q**		4.6	1.15	5.8
5c[a]		0.80	nm[b]	nm	**5r**	CH_3CH_2	6.1	1.20	9.0
5d		0.91	0.23	1.23	**5s**		4.5	1.30	5.5
5e		0.92	0.25	1.26	**5t**		4.1	1.30	5.9
5f		0.96	0.27	1.14	**5u**		4.0	1.45	5.6
5g	$CH_3(CH_2)_3CH_2$	0.84	0.34	1.22	**5v**		10	2.20	17
5h[a]		1.5	nm	nm	**5w**		16	3.50	22
5i		2.7	0.34	3.9					
5j		2.0	0.55	2.12	**5x**		17	4.00	19
5k		3.5	0.56	2.4	**5y**[a]		20	nm	nm
5l		2.8	0.70	3.95	**5z**		21	4.50	25
5m[a]		2.9	nm	nm	**5aa**[a]		>50	nm	nm
5n	CH_3	3.9	0.80	6.1	**5bb**		>50	7.50	33
5o		3.1	0.88	3.7	**5cc**		>50	10.00	100

* Indicates point of attachment. [a]Prepared by Route B. [b]Not measured

The nature of the linker unit may play a critical role in maximizing the interaction between the protein and the ligands. For example, substitution of the linker unit with a methyl group (**5b**) decreases activity by more than a factor of two. Electronic effects within the linker can also have a deleterious effect on activity. Replacement of the oxygen in the linker with a sulfur (**5e**) or carbon (**5f**) atom caused activity to decrease by over twofold.

Introduction of one degree of unsaturation into the linker (**5z**) also caused activity to drop dramatically (i.e. by greater than one order of magnitude). This is not surprising when one considers the effect this substitution has on the orientation of the hydrophobic group.



5a **5b** **5e** **5f** **5z** **5cc**

It is also interesting to note that aromatic rings connected to the linker give the maximum activity, as compared with smaller substituents such as five-membered rings or smaller aliphatic groups. This observation suggests that the phenyl ring fills the volume of the cavity in the enzyme more efficiently than the five-membered rings or aliphatic groups. Alternatively, the phenyl ring may be involved in a π–π interaction with an aromatic residue of the protein.

In summary, we have demonstrated that the carboxamide phosphonates represent a novel class of IGPD inhibitors displaying significant structural differences from previously synthesized compounds. The structure–activity relationships and biological activity of these compounds provide useful information for the design of additional inhibitors. This information is also likely to aid structure-based design efforts once the X-ray crystal structure of the enzyme becomes available.

Acknowledgments: We thank Mr. Frederico Bernal for preparation of some of the starting materials and the members of the computational modeling team for assistance with design and analysis of the library. We also thank Mr. Robert Chott for LC-MS analysis of the library and Drs. Paula Miller and Eugene Logusch for many helpful discussions. Finally, we thank Prof. V. Jo Davisson for his gift of the IGPD plasmid.

References and Notes

1. Lindell, S. D.; Earnshaw, C. G.; Wright, B. J.; Carver, D. S.; O'Mahony, M. J.; Saville-Stones, E. A. *Bioorg. Med. Chem. Lett.* **1996**, *6*, 547.
2. Mori, I.; Fonne-Pfister, R.; Matsunaga, S.-i.; Tada, S.; Kimura, Y.; Iwasaki, G.; Mano, J.-i.; Hatano, M.; Nakano, T.; Koizuni, S.-I; Scheidegger, A.; Hayakawa, K.; Ohta, D. *Plant Physiol.* **1995**, *107*, 719.
3. Cox, J. M.; Hawkes, T. R.; Bellini, P.; Ellis, R. M.; Barrett, R.; Swanborough, J. J.; Russell, S. E.; Walker, P. A.; Barnes, N. J.; Knee, A. J.; Lewis, T.; Davies, P. R. *Pestic. Sci.* **1997**, *50*, 297.
4. Norcross, R. D.; von Matt, P.; Kolb, H. C.; Bellus, D. *Tetrahedron* **1997**, *53*, 10289.
5. Hawkes, T. R.; Cox, J. M.; Barnes, N. J.; Beautement, K.; Edwards, L. S.; Kipps, M. R.; Langford, M. P.; Lewis, T.; Ridley, S. M.; Thomas, P. G. *Brighton Crop Prot. Conf.--Weeds* **1993**, 739.
6. Unpublished results.

7. Compounds **5d**, **5v**, and **5aa** were purified using a Waters Delta Prep 3000 equipped with a Dynamax C-18 column, 25 cm, 21.4 cm id, 8 mm particle size. Acetonitrile and water (+0.1% TFA) was used with a gradient of 0–30% acetonitrile over 20 min and a flow rate of 12 mL/min.
8. Parlow, J. J.; Mischke, D. A.; Woodard, S. S. *J. Org. Chem.* **1997**, *62*, 5908.
9. Flynn, D. L.; Crich, J. Z.; Devraj, R. V.; Hockerman, S. L.; Parlow, J. J.; South, M. S.; Woodard, S. *J. Am. Chem. Soc.* **1997**, *119*, 4874.
10. The gene for *Cryptococcus neoformans* IGPD was obtained as plasmid pHIS3-T7 from V. Jo Davisson. Protein was overexpressed in and purified from E. coli as previously described. See Parker, A. R. PhD Thesis, Purdue University, 1995.
11. IGPD activity was determined by monitoring the production of imidazoleacetol phosphate by its UV absorbance at 290 nm in the presence of base. (See Martin, R. G.; Baerberich, M. A.; Ames, B. N.; Davis, W. W.; Goldberger, R. F.; Yourno, J. D. In *Methods in Enzymology* **1971**, *17B*, 3-44). The reaction contained 100 mM HEPES pH 8.1, 100 mM $MnCl_2$, 350 mM IGP and 1.5 mg IGPD *E. coli* cell free extract (overexpressing IGPD[10]) in a volume of 300 mL. The reaction was initiated with IGP, allowed to proceed for 16 min and stopped by addition of 60 mL of 6 N NaOH. After 30 min the absorbance at 290 nm was determined relative to a blank which lacked IGP. Inhibitors were preincubated with IGPD for 30 min and a control with no IGP was used to correct for absorbance due to inhibitors. The IC_{50} was determined by plotting the data in a dose–response curve. (See Copeland, R. A. In *Enzymes: A Practical Introduction to Structure, Mechanism, and Data Analysis*; VCH: Weinheim, 1996). The IC_{50} represents the means of duplicate determinations and were within 15%.
12. A continuous, coupled assay that has been reported previously was used for determining K_m and K_i. (See Parker, A. R.; Moore, J. A.; Schwab, J. M.; Davisson, V. J. *J. Am. Chem. Soc.* **1195**, *117*, 10605). The reaction was maintained at 37 °C and contained 50 nM IGPD, 8–1000 mM IGP for K_m determination and 0.05–20 mM inhibitor for K_i evaluation.