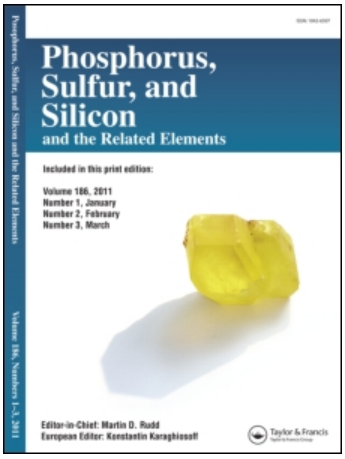


Phosphorus, Sulfur, and Silicon and the Related Elements
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713618290

New 4-(α-Hetero-Phosphonomethyl) Pyrrole 2-Carboxylates are EPSP Synthase Inhibitors

James A. Sikorski; Mark L. Peterson; Susan D. Corey; Jose L. Font; Mark C. Walker

To cite this Article Sikorski, James A. , Peterson, Mark L. , Corey, Susan D. , Font, Jose L. and Walker, Mark C.(1999) 'New 4-(α-Hetero-Phosphonomethyl) Pyrrole 2-Carboxylates are EPSP Synthase Inhibitors', Phosphorus, Sulfur, and Silicon and the Related Elements, 144: 1, 617 — 620
To link to this Article: DOI: 10.1080/10426509908546320
URL: http://dx.doi.org/10.1080/10426509908546320

Phosphorus, Sulfur and Silicon, 1999, Vol. 144-146, pp. 617-620
Reprints available directly from the publisher
Photocopying permitted by license only

New 4-(α-Hetero-Phosphonomethyl) Pyrrole 2-Carboxylates are EPSP Synthase Inhibitors

JAMES A. SIKORSKI, MARK L. PETERSON, SUSAN D. COREY, JOSE L. FONT and MARK C. WALKER

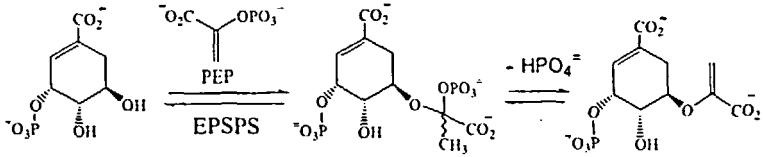
Monsanto Company, St. Louis, Missouri 63198 USA

The preparation of several new EPSP synthase inhibitors is described where a pyrrole scaffold replaces the complex shikimate ring in S3P.

Keywords: glyphosate; EPSP synthase; inhibitor; pyrrole; S3P

INTRODUCTION

EPSP (5-enolpyruvoylshikimate 3-phosphate) synthase (EPSPS, E.C. 2.5.1.19) has generated considerable interest in our laboratory as an enzyme for new inhibitor design since it functions as the biological target for the commercially successful herbicide, glyphosate.[1] EPSPS catalyzes an unusual transfer reaction of the carboxyvinyl portion of phosphoenolpyruvate (PEP) regiospecifically to the 5-OH of shikimate 3-phosphate (S3P) forming EPSP and inorganic



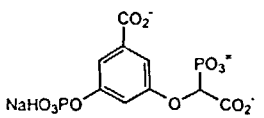
S3P 1 EPSP

phosphate (P_i).[1,2] This enzyme proceeds through a single, kinetically competent,[3] tightly bound[4] tetrahedral intermediate **1**. While structural analogs of **1** are potent EPSPS bisubstrate inhibitors,[5,6] we have recently identified 4-(α-heterophosphonomethyl)pyrrole 2-carboxylates[7] as simplified, synthetically accessible scaffolds which can replace the complex shikimate ring in these systems.

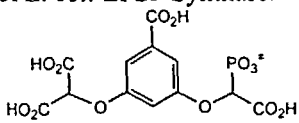
AROMATIC EPSPS INHIBITORS

A predictive 3-D model for **1** was developed from NMR conformational analysis of bound substrates.[8,9] Since the enzyme-bound shikimate ring adopts an unusually flattened conformation,[9] the aromatic tetrahedral intermediate mimic **2** functions as a reasonably potent EPSPS inhibitor, where the simplified benzene ring acts as a suitable substitute for the more highly functionalized shikimate ring.[10] Analogs have also been prepared in which the labile 3-phosphate functionality was replaced with either a malonate ether group (**3**)[11,12] or a hydroxy-malonate moiety.[8] The greater potency observed for **2** and **3** versus their 5-phenol analogs (**4**,**5**) is directly attributable to the charged phosphonoacetate ether moiety at the 5-position which demonstrated the need to access the PEP binding site in these systems. While **2** and **3** were low micromolar inhibitors of EPSPS, their potencies failed to match the low nanomolar levels frequently observed with 3-phosphate containing shikimate inhibitors and stimulated a search for another scaffold.

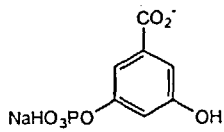
Table 1. Aromatic Inhibitors of *E. coli* EPSP Synthase.



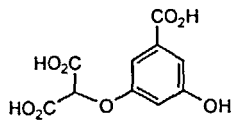
2. $K_{i(app)} = 0.16 \pm 0.04\ \mu M$



3. $K_{i(app)} = 1.3 \pm 0.2\ \mu M$



4. $IC_{50} = 7.9$ mM



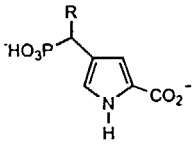
5. $IC_{50} = 10$ mM

PYRROLE-BASED EPSPS INHIBITORS

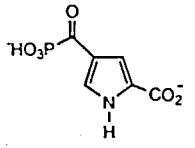
The phenolic S3P analogs **4-5** displayed relatively weak affinity for enzyme compared to S3P (K_d = 7 ± 1.2 μM).[13] Consequently, the lack of potency in the aromatic tetrahedral intermediate mimics **2-3** might be due to a less than optimal fit within the S3P subsite. Molecular modeling studies suggested that a smaller heteroaromatic ring might provide a better template.[7] This led to the design and synthesis of the 4-(α-hydroxy-phosphonomethyl)pyrrole 2-carboxylate **6** which was found to be a surprisingly good EPSPS inhibitor ($K_{i(app)}$ = 14.5 ± 0.7 μM), competitive with S3P. [7] As such, **6** compares quite favorably with either 5-deoxy-S3P[14] or S3P[13] in its ability to bind at the S3P subsite. Moreover, the potency of **6** is also orders of magnitude better than the phenolic S3P analogs **4-5**. Remarkably, the highly functionalized shikimate ring in S3P may be replaced with a simple achiral pyrrole surrogate and still retain potent interactions with this enzyme.

These results led us to prepare the related analogs **7-9** (Table 2) to investigate whether other phosphate mimics could be incorporated at the pyrrole 4-position. We also prepared the sensitive *N*-hydroxymethyl analog **10** since molecular modeling experiments suggested that **10** might function as either a substrate for EPSPS or a potential irreversible inactivator through loss of formaldehyde at the active site. Similarly, the *N*-phosphonomethyl derivatives **11-12** were prepared as possible bisubstrate inhibitors which have the potential to extend **6** and **7** into the PEP binding site.

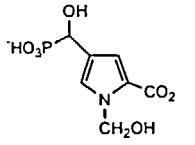
Table 2. New Pyrrole-Based Inhibitors of *E. coli* EPSP Synthase.



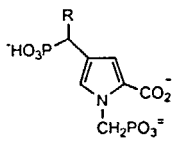
6, R = OH
7, R = H
8, R = NH_2



9



10



11, R = OH
12, R = H

CONCLUSIONS

The activities of pyrrole **6** and analogs **7-12** demonstrate the scope and limitations in replacing the highly functionalized and chiral shikimate ring in S3P with a simple, synthetically accessible, achiral, heterocyclic scaffold.

References

[1] Franz, J. E.; Mao, M. K.; Sikorski, J. A. in *Glyphosate: A Unique Global Herbicide,* (American Chemical Society Monograph, Washington, D.C., 1997).

[2] Anderson, K. S.; Johnson, K. A. *Chem. Rev.*, **90,** 1131 (1990).

[3] (a) Anderson, K. S.; Sikorski, J. A.; Benesi, A. J.; Johnson, K. A. *J. Am. Chem. Soc.*, **110,** 6577 (1988). (b) Anderson, K. S.; Sikorski, J. A.; Johnson, K. A. *Biochemistry,* **27,** 7395 (1988).

[4] (a) Anderson, K. S.; Johnson, K. A. *J. Biol. Chem.*, **265,** 5567 (1990). (b) Cleland, W. W. *Biochemistry,* **29,** 3194 (1990).

[5] Walker, M. C.; Jones, C. R.; Somerville, R. L.; Sikorski, J. A. *J. Am. Chem. Soc.*, **114,** 7601 (1992).

[6] Alberg, D. G.; Lauhon, C. T.; Nyfeler, R.; Fässler, A.; Bartlett, P. A. *J. Am. Chem. Soc.*, **114,** 3535 (1992).

[7] Peterson, M. L.; Corey, S. D.; Font, J. L.; Walker, M. C.; Sikorski, J. A. *Bioorg. Med. Chem. Lett.*, **6,** 2853 (1997).

[8] Shah, A.; Font, J. L.; Miller, M. J.; Ream, J. E.; Walker, M. C.; Sikorski, J. A. *Bioorg. Med. Chem.*, **5,** 323 (1997).

[9] Leo, G. C.; Castellino, S.; Sammons, R. D.; Sikorski, J. A. *Bioorg. Med. Chem. Lett.*, **2,** 151 (1992).

[10] Miller, M. J.; Ream, J. E.; Walker, M. C.; Sikorski, J. A. *Bioorg. Med. Chem.*, **2,** 331 (1992).

[11] Miller, M. J.; Braccolino, D. S.; Cleary, D. G.; Ream, J. E.; Walker, M. C.; Sikorski, J. A. *Bioorg. Med. Chem. Lett.*, **4,** 2605 (1994).

[12] Miller, M. J.; Cleary, D. G.; Ream, J. E.; Snyder, K. R.; Sikorski, J. A. *Bioorg. Med. Chem.*, **3,** 1685 (1995).

[13] Anderson, K. S.; Sikorski, J. A.; Johnson, K. A. *Biochemistry,* **27,** 1604 (1988).

[14] Pansegrau, P. D.; Anderson, K. S.; Widlanski, T.; Ream, J. E.; Sammons, R. D.; Sikorski, J. A.; Knowles, J. R. *Tetrahedron Lett.*, **32,** 2589 (1991).