Sulfoxide Functionality as a Potential Carbonium Ion Mimic in Enzyme Inhibitor Design. Synthesis of a Novel Inhibitor of 5-Enolpyruvylshikimate-3-phosphate Synthase

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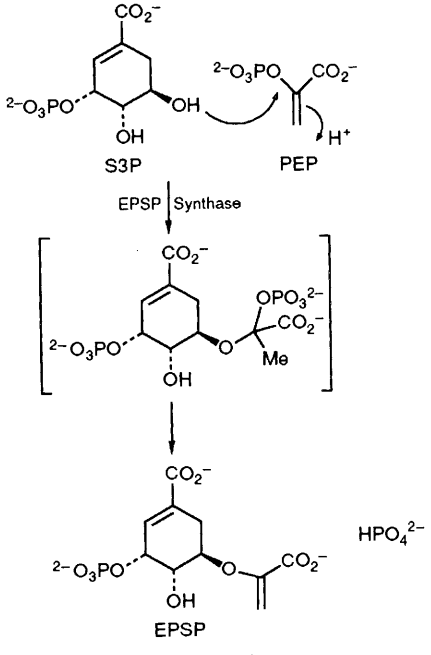
A novel 5-enolpyruvylshikimate-3-phosphate synthase (EPSP) inhibitor has been prepared by employing a sulfoxide moiety to mimic the carbocationic centre of the phosphoenolpyruvate (PEP) carbonium ion intermediate.

One strategy for designing an enzyme inhibitor is to synthesize a chemical entity that mimics the high-energy intermediate involved in the reaction mechanism. In this paper we report the discovery of a new inhibitor of 5-enolpyruvylshikimate-3-phosphate synthase (EPSP synthase, E.C. 2.5.1.19), which employs a sulfoxide functionality to mimic the carbocationic centre of the transient species involved in the enzymatic reaction.

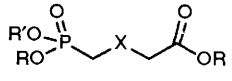
EPSP synthase is an enzyme in the shikimate acid pathway[1] found only in plants, fungi and bacteria and is the target of glyphosate **1**, the active ingredient in one broad spectrum herbicide. The enzyme catalyses a unique transfer of the carboxyvinyl group from phosphoenolpyruvate (PEP) to shikimate-3-phosphate (S3P) to yield 5-enolpyruvyl-shikimate-3-phosphate (EPSP), a key intermediate in the biosynthesis of phenylalanine, tyrosine, tryptophan and other important secondary metabolites. Recent investigations[2] of this unique enzyme reaction confirmed an addition–elimination mechanism, in which the 5-OH of S3P adds to PEP to give a tetrahedral intermediate, followed by the extrusion of the phosphate group to give the product EPSP (Scheme 1). This and other studies[3] implicate the involvement of the carbonium ion of PEP as the transient intermediate during the addition reaction (see Fig. 1). An ammonium functionality can mimic a carbonium ion in enzyme inhibitor design.[4] Glyphosate is a competitive inhibitor of PEP[5] in this reaction probably because its protonated nitrogen atom resembles the transient carbocationic centre of PEP. Intrigued by this hypothesis and the opportunity to discover novel inhibitors of EPSP synthase, we synthesized the sulfoxide analogue of glyphosate. Like the ammonium function the sulfoxide moiety is tetrahedral, but the sulfur–oxygen bond is highly polarized[6] and the high pK_a[7] of its oxygen favours a substantial positive charge character at the sulfur atom, making it a possible mimic for the carbocationic centre of the PEP. Furthermore, the availability of hydrogen bonding for the sulfinyl group at the enzyme active site would further strengthen this mimicking ability of the sulfoxide moiety.



Scheme 1

Sulfide **6** was prepared *via* the condensation of diethyl mercaptomethylphosphonate[8] with ethyl bromoacetate.† Acid hydrolysis (HCl) of **6** provided **7**; **9** was obtained by the oxidation of **7** with 2 equiv. of *m*-chloroperbenzoic acid followed by saponification. Synthesis of the sulfoxide **8** was difficult as it is unstable under most reaction conditions. Indeed, the free triacid form of the sulfoxide decomposes rapidly, probably as a result of a Pummerer rearrangement and/or disportionation.‡ The target **8**§ was synthesized by neutralizing **7** with 2 equiv. of sodium hydroxide followed by the oxidation with 1 equiv. of sodium metaperiodate in water at room temperature for 3 h. Compound **3** was synthesized by the reaction of *N*-hydroxyglycine with phosphorous acid and formaldehyde in aqueous HCl.[9] Synthesis of **5** has been described previously.[10]

Enzyme assays were performed by incubating the compound at 30 °C with the *Escherichia coli* EPSP synthase in the presence of S3P and [1-^{14}C] PEP at pH 7.0. The extent of conversion of [^{14}C] PEP to enol-labelled [^{14}C] EPSP was determined quantitatively by HPLC radioassay.[11] The results are described in Table 1. Sulfoxide **8** is indeed a potent inhibitor of EPSP synthase. Like glyphosate, the inhibition appears competitive with respect to PEP.[12] This result is significant considering that an overwhelming number of synthetic PEP analogues and known competitive inhibitors of other pyruvate utilizing enzymes are inactive against EPSP synthase.[13] Also, very few glyphosate analogues inhibit the



1; R = R′ = H X = NH
2; R = R′ = H X = NMe
3; R = R′ = H X = NOH
4; R = R′ = H X = O
5; R = R′ = H X = CH_2
6; R = R′ = Et X = S
7; R = R′ = H X = S
8; R = Na, R′ = H X = SO
9; R = R′ = H X = SO_2

Table 1 Inhibition of EPSP synthase

Inhibitor	K_i/μmol dm^{-3}
1 (Glyphosate)	0.25
2	200
3	0.8
8	3.2
4, 5, 7, 9	nil (>12 000)

† Alternatively, **6** can be synthesized from the coupling of ethyl thioglycolate and diethyl chloromethyl phosphonate.

‡ The multiple products resulted from acidification of sulfoxide **8** were not characterized. Other mode of decomposition (as suggested by one referee) such as decarboxylation involving the sulfoxide moiety in a six-membered cyclic transition state is also possible.

§ Sulfoxide **8** was a solid and melted (decomposed) slowly above 170 °C. ^{1}H NMR (D_2O): δ 4.5–4.0 (AB quartet, *J* 15 Hz, 2H), δ 3.4–3.6 (m, 2H); ^{31}P NMR (D_2O): δ 8.13.

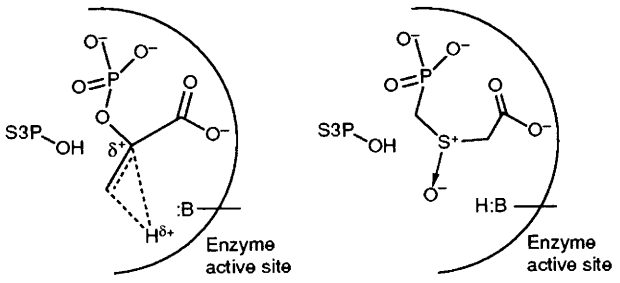


Fig. 1

enzyme significantly. For example, closely related derivatives such as **4**, **5** and **6** are all inactive. Although the sulfoxide **8** is one order of magnitude weaker than glyphosate **1**, the EPSP synthase exhibits an extreme steric requirement at the enzyme active site (*cf.* **1** *vs.* 2) making direct comparison between them inappropriate. The K_i of **8** is closer to *N*-hydroxy glyphosate **3**, a more appropriate ammonium species for contrasting their carbonium mimicking abilities. Furthermore, the sulfoxide **8** assayed is a racemic mixture and presumably the K_i of the pure enantiomer with the correct juxtaposition would be much higher. The resemblance of the sulfoxide **8** to the transient PEP carbonium ion at the active site is illustrated in Fig. 1. The fact that the sulfone **9** (which has a more covalent S–O linkage and lesser ability to hydrogen bond with a proton donor compared with the sulfinyl group[14]) is void of activity further underscores the uniqueness of the sulfoxide functionality to mimic the carbonium ion intermediate. Moreover, the sulfoxide **8** also exhibits significant biological activities *in vivo*.[15] To our knowledge, this is the first time a sulfoxide moiety has been successfully employed to mimic a carbocationic centre of a reaction intermediate in an enzyme-catalysed reaction and it, therefore, provides a new tool in the design of enzyme inhibitors.

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12 Detail biochemical results will be reported elsewhere.
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15 The phytotoxicity of the sulfoxide **8** was evaluated using a derooted sorghum seedling bioassay modified from that described by Kiltz *et al.* for buckwheat (B. Labər, H. Kiltz and N. Amrhein, *Z. Naturforsch., Teil C*, 1986, 41, 49.). It exhibited 1–10 × less activity compared with glyphosate for inhibition of anthocyanin biosynthesis, transpiration rate, leaf injury and inhibition of root formation.