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RATIONAL HERBICIDE DESIGN BY INHIBITION OF TRYPTOPHAN BIOSYNTHESIS

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Abstract: Compounds designed to mimic the tryptophan synthase α subunit reactive intermediate were found to be potent inhibitors of the enzyme. These compounds are herbicidal and the herbicidal mode of action was demonstrated to be due to disruption of tryptophan biosynthesis.

Keywords: Herbicides, Enzyme Inhibitors, Biosynthesis, Phosphonic Acid and Derivatives

Introduction

Economic and environmental factors drive the demand for new herbicides that are highly potent, crop selective, and have low or nonexistent mammalian toxicity. New herbicidal classes acting on novel target sites will be required to meet these expectations.[1] Inhibition of amino acid biosynthesis is a demonstrated mode of action for several important classes of herbicides[2]. Glyphosate, a major herbicide in economic terms, inhibits biosynthesis of phenylalanine, tyrosine, and tryptophan.[3] Enzymes involved specifically in tryptophan biosynthesis are absent in animals and have been proposed as potential herbicide targets,[4] but no examples have been published. In this report, we describe potent in vitro inhibitors of tryptophan synthase, the final enzyme in the tryptophan biosynthesis pathway and evidence that they are herbicidal through their inhibition of tryptophan biosynthesis.

Several reasons led to our selection of tryptophan synthase (E.C.4.2.1.20) as a potential herbicide target. Higher plants have relatively low reserves of tryptophan and of the mRNA for tryptophan synthase, suggesting a relatively low capacity for tryptophan synthesis. All organisms that synthesize tryptophan are known to do so by a single route, such that inhibiting this one pathway should dramatically reduce tryptophan levels. Tryptophan synthase is also one of the most thoroughly studied enzymes of those involved in the biosynthesis of amino acids.[5] Extensive work has been published on bacterial tryptophan synthase detailing its purification,[6] biochemistry,[7] inhibition[8] and the effect of structural modifications on the activity.[9] Tryptophan synthase (TS) is composed of four subunits forming an $\alpha 2\beta 2$ complex, which catalyzes the final two reactions in tryptophan biosynthesis. The α subunit catalyzes a retroaldol reaction where indoleglycerol-3-phosphate (IGP) is cleaved to yield indole and D-glyceraldehyde-3-phosphate (GAP). Indole from the α subunit reaction is channeled via a 25 angstrom tunnel to the β subunit active site. The β subunit catalyzes the condensation of L-serine and indole to form tryptophan. The three dimensional structure of tryptophan synthase has been determined for the bacterial enzyme by X-ray both in the absence and presence of the inhibitor indolepropanol-

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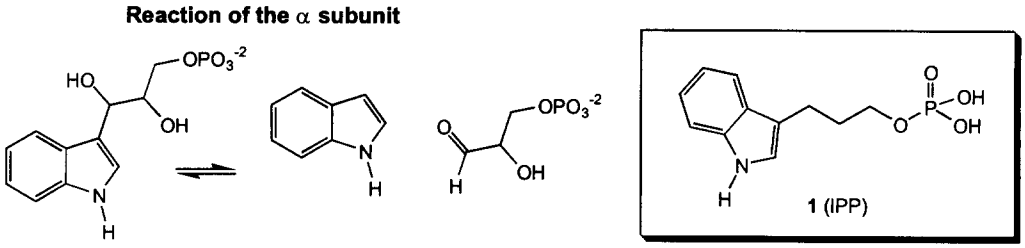
3-phosphate (IPP).[10,11] From the beginning of the project, we applied this structural information to inhibitor design.

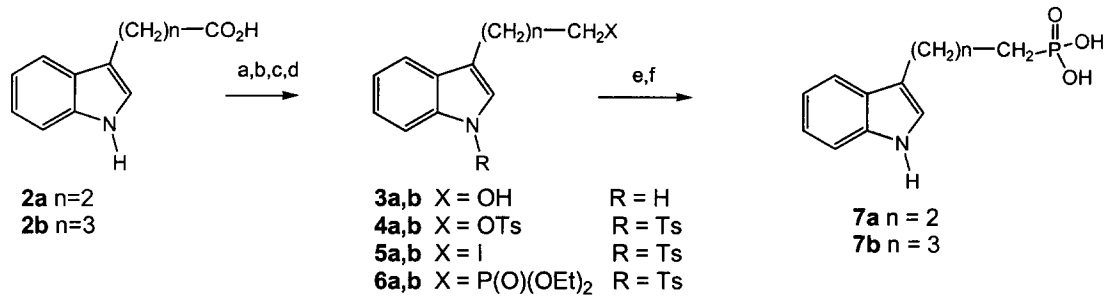
Figure 1



Results and Discussion

The starting point for our inhibitor design strategy was the known inhibitor, indole-3-propanol phosphate (IPP) **1**.[12] This compound is an inhibitor of the TSα subunit reaction with a K_i of 15 μM. Due to metabolic instability of phosphates in biological systems, we evaluated the phosphonate isosteres **7a** and **7b**. These were prepared by the sequence shown in Scheme 1. Reduction of 3-indole-propionic acid, **2a,** and 3-indole-butyric acid, **2b,** with LAH provided the primary alcohols **3a** and **3b**. These were converted to ditosylated derivatives **4a** and **4b** by treatment with 2 equiv each of sodium hydride and tosyl chloride. Conversion to the primary iodide followed by treatment with triethylphosphite yielded the desired phosphonate esters **6a** and **6b**. Removal of the protecting groups gave the desired phosphonates **7a** and **7b**.

Scheme 1

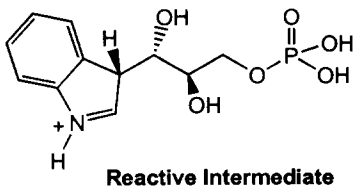


Reagents and conditions: (a) LAH; (b) NaH, TsCl; (c) NaI; (d) $P(OEt)_3$; (e) 20% KOH; (f) TMSBr.

Phosphonates **7a** and **7b** were tested both in vitro for inhibition of the TSα subunit reaction (determined using the *Salmonella typhimurium* holoenzyme) and in vivo for herbicidal activity on whole plants. Both phosphonates are inhibitors of TSα with similar potency (**7a** I_{50} = 12.5 μM; **7b** I_{50} = 20 μM) to IPP **1** (I_{50} = 5

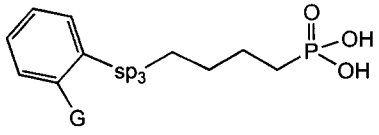
μM). This result mirrors the effect of chain length on inhibition levels shown by a set of phosphate analogs prepared by Kirschner.[12]

Although the phosphonate analog (**7b**) of IPP demonstrated that a phosphonate group can be used as a phosphate bioisostere, the level of enzymatic inhibition was modest and the compound displayed very little herbicidal activity (slight postemergence growth inhibition, later outgrown). We speculated that an improvement in the level of enzymatic activity to 1 μM or less would be required for useful herbicidal activity in this series. To produce stronger inhibitors, we targeted compounds with shapes complementary to the reactive intermediate of the TSα subunit reaction. In the enzymatic reaction, the C-3 position of the indole ring is protonated giving a reactive intermediate **8** containing a sp_3 atom at C-3. The generic target structure **9** was chosen because it incorporates a sp_3 atom that mimics the C-3 position of the reaction intermediate. In this generic the C-2 atom of the indole ring was removed. This was done to simplify the synthesis and to allow for compounds with more conformational flexibility.



Reactive Intermediate

8

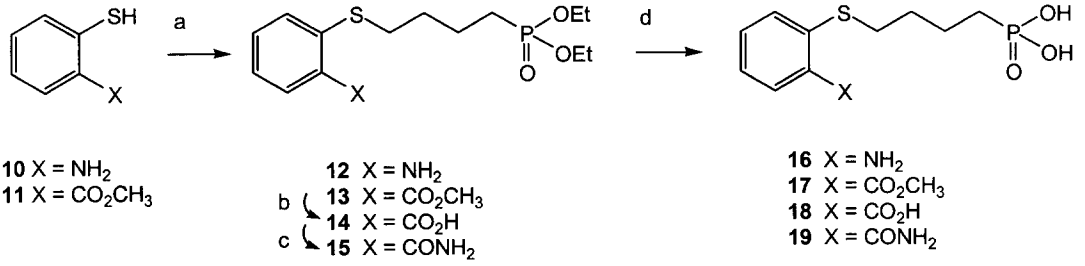


G = hydrogen bond donor

9

The first set of derivatives targeted were a series of aryl alkylphosphonate sulfides (sp_3 = S) bearing either a carboxamide or amine *ortho* to the sulfur atom. The synthesis of these compounds is described in Scheme 2. The chemistry is very straightforward and high yielding. The key reactions are an arylmercaptide addition to diethyl 4-bromobutylphosphonate[13] followed by TMSBr cleavage of the esters.[14]

Scheme 2

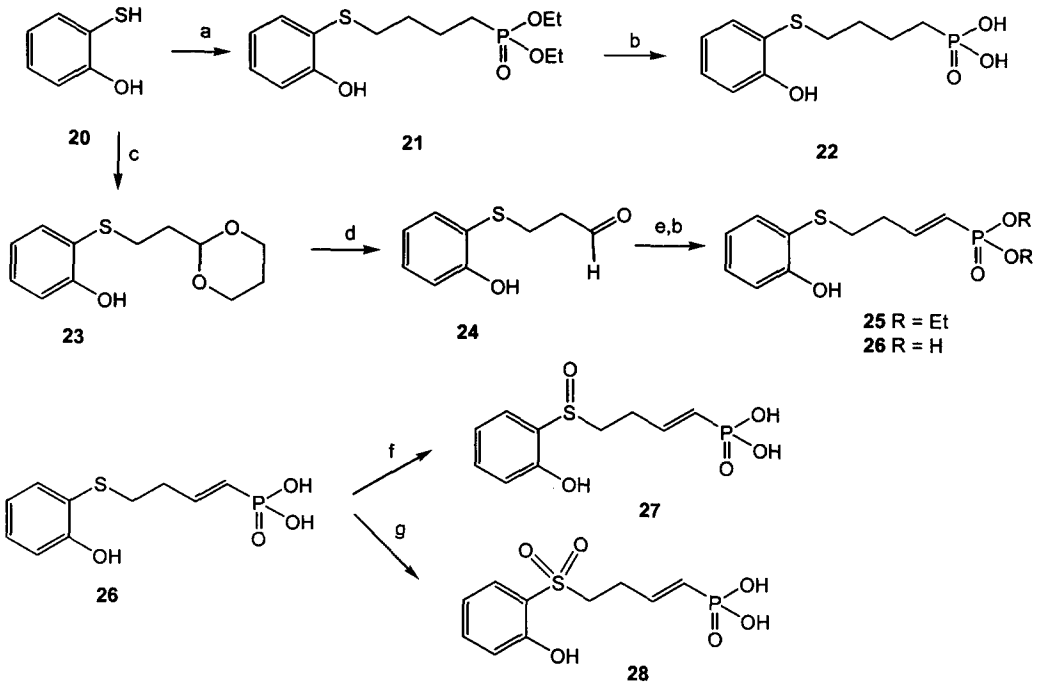


Reagents and conditions:(a) $Br(CH_2)_4P(O)(OEt)_2$, TEA; (b) NaOH; (c) $SOCl_2$, NH_4OH; (d) TMSBr.

The four phosphonic acids (**16, 17, 18,** and **19**) shown in Scheme 2 were tested in the in vitro TSα enzyme assay. Although compounds **17-19** were inactive, the *ortho*-amino compound **16** had very good enzymatic

activity (I_{50} = 400 nM). In addition, this compound, and especially its related salts and esters, displayed greenhouse herbicidal activity. When sprayed postemergence, the compounds were herbicidal (65 to 90% injury) on lambsquarter and pigweed. The discovery of the good enzymatic and herbicidal activity of the arylsulfide **16**, prompted the synthesis of additional analogs. Scheme 3 shows the synthesis of several *ortho*-hydroxyphenylsulfides. The α,β unsaturated compound **26** was made by treatment of aldehyde **24** with the anion of tetraethyl methylenediphosphonate.[15] This Wittig reaction afforded the *trans* olefin selectively. The sulfoxide and sulfone derivatives were prepared by oxidation of phosphonic acid. Purification of these very polar compounds required C-18 reverse-phase chromatography.

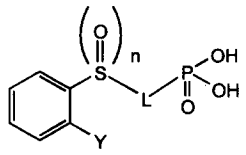
Scheme 3



Reagents and conditions: (a) $Br(CH_2)_4P(O)(OEt)_2$, TEA; (b) TMSBr; (c) TEA, 2-(2-chloroethyl)-1,3-dioxane; (d) HCl; (e) n-BuLi, $CH_2(P(=O)(OEt)_2)_2$; (f) 1 equiv mCPBA; (g) 2 equiv mCPBA

Table 1 compiles the biological activity for the arylsulfide phosphonates. The herbicidal activities of several *ortho*-hydroxyphenyl sulfides are significantly improved compared to **16**. Only postemergence herbicidal activity is observed. It is interesting to note that while both the sulfoxide and sulfone are good enzyme inhibitors, the sulfoxide shows much better herbicidal activity. Introduction of rigidity in the linking chain in the form of a double bond, compound **26**, led to greater biological activity.

Table 1 Arylsulfide Phosphonate Inhibitors of TSα as Herbicides.



Compound	n	L	Y	I_{50} TSα (μM)*	Herbicidal activity**
16	0	$-(CH_2)_4-$	NH_2	0.400	+
22	0	$-(CH_2)_4-$	OH	0.130	+++
26	0	$-CH_2CH_2CH=CH-$	OH	0.570	++++
27	1	$-(CH_2)_4-$	OH	0.440	+++
28	2	$-(CH_2)_4-$	OH	0.360	inactive

**Postemergence application at 4 kg/ha, rated 2 weeks after treatment. Inactive = no herbicidal activity; + = at least 65% injury on one species; ++, +++, and ++++ = at least 80% injury on one, two, and three or more species.

Plants treated with herbicidal tryptophan synthase inhibitors show symptoms typical of a herbicide whose mode of action is the inhibition of amino acid biosynthesis. The herbicidal activity is slow to develop, beginning with growth cessation followed by chlorosis or mottling, and sometimes by necrosis. Compounds are active postemergence at 1 to 4 kg/ha, with dicots generally more sensitive than monocot species. A patent has issued describing further the herbicidal activity of these compounds.[16] The most active compound in the arylsulfide phosphonate series was the α,β unsaturated compound **26**. No synergy has been observed with other herbicides known to inhibit amino acid synthesis enzymes such as glyphosate, HOE-704 or glufosinate.

The mode of action was substantiated in vivo using reversal studies of the herbicidal effects on *Arabidopsis thaliana*. Seed was germinated on an agar medium in a standard 96-well microtiter plate. Each row of wells contained an *ortho*-hydroxyphenylsulfide herbicide in a series of dilutions. Every second row contained the herbicide plus 0.1 mM L-tryptophan. Seedlings that received herbicide and no tryptophan were stunted, chlorotic or severely injured. With the simultaneous addition of tryptophan, the herbicidal activity was completely eliminated (Table 2). This dramatic reversal of the herbicidal effect by tryptophan was observed for several *ortho*-hyroxyphenylsulfides including: **22**, **26,** and **27**, conclusively demonstrating that the herbicidal activity is due to inhibition of tryptophan biosynthesis.

Table 2 *Arabidopsis* Reversal Studies in Agar

	Inhibitor Concentration (μM)							
Compound	1000	500	250	125	63	31	16	8
22	7C*	6C	6C	5C	3	3	0	0
22+Trp*0.1 mM	0	0	0	0	0	0	0	0
26	6C	6C	6C	6C	6C	5C	5C	5C
26+Trp 0.1 mM	0	0	0	0	0	0	0	0
27	6C	6C	6C	6C	6C	6C	5C	5C
27+Trp 0.1 mM	0	0	0	0	0	0	0	0

* Zero to 9 scale, with zero representing no injury and 9 complete kill; C- chlorotic seedlings

Summary

The discovery of the 4-(arylthio)-butylphosphonate series has demonstrated that novel herbicides can be found in the tryptophan biosynthetic pathway. Tryptophan synthase is a target of the herbicides, and some herbicidal inhibitors are described with I_{50}'s in the range of 0.13 to 0.57 μM. Compounds in this series are postemergence herbicides with greater potency on broadleaf plants than on grasses. Tryptophan supplementation studies in agar have demonstrated that disruption of the supply of tryptophan is the mode of action for these compounds. Because of this, there is now a strong rationale for finding additional inhibitors of tryptophan synthase and other enzymes in the tryptophan pathway.

References and Notes

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