JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

Use of Resistant ACCase Mutants To Screen for Novel Inhibitors against Resistant and Susceptible Forms of ACCase from Grass Weeds

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The aryloxyphenoxypropionic acid (AOPP) and cyclohexanedione (CHD) herbicides inhibit the first committed enzyme in fatty acid biosynthesis, acetyl CoA carboxylase (ACCase). The frequent use of AOPP and CHD herbicides has resulted in the development of resistance to these herbicides in many grass weed species. New herbicides that inhibit both the susceptible and resistant forms of ACCase in grass weeds would have obvious commercial appeal. In the present study, an attempt was made to identify molecules that target both the herbicide-sensitive and -resistant forms of ACCase. Seven experimental compounds, either CHD-like or AOPP−CHD hybrids, were synthesized and assayed against previously characterized susceptible and resistant forms of ACCase. All seven compounds inhibited ACCase from sensitive biotypes of *Setaria viridis* and *Eleusine indica* (I_{50} values from 6.4 to >100 μM) but were not particularly potent compared to some commercialized herbicides (I_{50} values of 0.08−5.6 μM). In almost all cases, the I_{50} values for each compound assayed against the resistant ACCases were higher than those against the corresponding sensitive ACCase, indicating reduced binding to the resistant ACCases. One compound, a CHD analogue, was almost equally effective against the resistant and susceptible ACCases, although it was not a very potent ACCase inhibitor per se (I_{50} of 51 and 76 μM against susceptible ACCase from *S. viridis* and *E. indica*, respectively). The AOPP−CHD hybrid molecules also inhibited some of the resistant ACCases, with I_{50} values ranging from 6.4 to 50 μM. These compounds may be good leads for developing ACCase inhibitors that target a wider range of ACCase isoforms, including those found in AOPP- and CHD-resistant weed biotypes.

KEYWORDS: Structure−activity relationships; acetyl coenzyme A carboxylase; ACCase; aryloxyphenoxypropionic acid; cyclohexanedione; herbicide resistance

INTRODUCTION

The aryloxyphenoxypropionate (AOPP) and cyclohexanedione (CHD) herbicides inhibit the enzyme acetyl CoA carboxylase (ACCase; EC 6.4.1.2), which catalyzes the conversion of acetyl CoA to malonyl CoA (*1*, *2*). Inhibition of ACCase leads to inhibition of acyl lipid biosynthesis, eventually resulting in death of the plant (*1*). In general, ACCase from grasses is susceptible to inhibition by AOPP and CHD herbicides, whereas dicot ACCase is tolerant to these herbicides (*3*). These herbicides inhibit the eukaryotic form of ACCase found in the plastids of most grass species, but not the prokaryotic form, which is the major form present in dicots (*4*). This is the primary mechanism of selectivity of these herbicides between grasses and dicots. Tolerance of these herbicides in some grasses, including some cereal crops, is conferred by enhanced metabolism of the herbicides to inactive compounds (*5*).

It has been suggested that these two classes of herbicides share a common binding site on the target enzyme, ACCase. Double inhibition studies with AOPP and CHD herbicides and malonyl CoA and CoA suggest that the two groups of herbicides overlap in their binding to the target enzyme and that they compete with malonyl CoA for binding at this site (*6*). Further studies have shown that sethoxydim, a CHD, and haloxyfop,

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10.1021/jf034738m CCC: $27.50
Published on Web 07/08/2004

Table 1. I_{50} Values and R/S I_{50} Ratios for ACCase from Herbicide-Sensitive and -Resistant Biotypes of Green Foxtail (S1, Sensitive; R1 and R2, Resistant) and Goosegrass (S3, Sensitive; R3, Resistant), Assayed in Vitro with Known ACCase Inhibitors (*17*, *21*, *23*)

	green foxtail						goosegrass		
	I_{50} (μM)			I_{50} (μM)			I_{50} (μM)		
herbicide	S1	R1	R1/S1	S1	R2	R2/S1	S3	R3	R3/S3
diclofop	0.7	28	47						
fenoxaprop	0.6	28	48	4.4	30	6.9	1.0	25	25
fluazifop							5.6	>500	>90
quizalofop	0.08	4.7	60	2.1	8.9	4.2			
clethodim	0.6	18	31	3.2	7.8	2.4	1.5	6.6	4.4
sethoxydim	1.7	85	50	7.7	3260	420	3.8	77	20
tralkoxydim	0.3	9.4	31						

an AOPP, are linear, noncompetitive inhibitors with various substrates of ACCase and that the transcarboxylase reaction (transfer of CO_2 from the biotin prosthetic group to form malonyl CoA) is inhibited by AOPP and CHD herbicides (*3*). Double inhibition studies also suggest that the hydrophobic oxime region of CHD herbicides overlaps with the hydrophobic aryloxyphenoxy region of AOPP herbicides at the binding site on the enzyme (*7*). Their results indicated that the CoA binding site was distinct from the herbicide binding site and suggested synthesis of CoA conjugates of herbicides to further study substrate and herbicide binding sites on ACCase. The conjugates diclofopyl-CoA, haloxyfopyl-CoA, and quizalofopyl-CoA were prepared and found to inhibit rat liver ACCase more than the parent acids, thus confirming that CoA and herbicide binding sites are different (*8*).

The frequent use of AOPP and CHD herbicides has resulted in the development of resistance to these herbicides in many grass weed species in North and Central America, Europe, and Australia (*9*). The species in which resistance has developed include wild oat (*Avena fatua*) (*10*, *11*), green foxtail (*Setaria viridis*) (*12*), giant foxtail (*Setaria faberi*) (*13*), annual ryegrass (*Lolium rigidium*) (*14*−*16*), goosegrass (*Eleusine indica*) (*17*), and maize (*Zea mays*) (*18*). In most cases, resistance is due to alteration of the target enzyme, ACCase, making it less sensitive to inhibition by these herbicides (*11*, *17*−*24*).

The results of enzyme inhibition studies suggest several distinct mutations in the ACCase gene, conferring different levels of resistance to various ACCase inhibitors. For example, ACCase from one biotype of green foxtail (referred to as R1 in this study) was highly resistant to a broad spectrum of AOPP and CHD herbicides, with R/S I_{50} ratios ranging from 31 for clethodim to 60 for quizalofop (*21*). (For convenience, published I_{50} values for various herbicide−ACCase combinations are summarized in **Table 1**.) In contrast, a second biotype of green foxtail (referred to as R2 in this study) was very resistant to sethoxydim (R/S I_{50} ratio of 420) but only marginally resistant to other AOPP and CHD herbicides (*23*; **Table 1**). This is similar to resistance in a sethoxydim-resistant maize line (*18*), also used in this study (designated R4). A third pattern of resistance was observed in a biotype of goosegrass (referred to as R3 in this study); it was highly resistant to fluazifop, moderately resistant to fenoxaprop and sethoxydim, but only marginally resistant to clethodim (R/S I_{50} ratios of >90, 24, 20, and 4, respectively) (*17*; **Table 1**). ACCase from the corresponding susceptible biotypes (S1 for green foxtail, S3 for goosegrass, and S4 for maize) was sensitive to inhibition by the AOPP and CHD herbicides tested (**Table 1**; *17*, *18*, *21*, *23*).

More recently, cDNA fragments encoding the carboxyltransferase domain of the multidomain plastid ACCase from herbicide-resistant maize and from herbicide-sensitive and -resistant annual ryegrass were cloned and sequenced (*25*). A leucine residue was found in ACCases from herbicide-resistant plants at a position occupied by isoleucine in all ACCases from sensitive grasses studied. Leucine is also present at the equivalent position in herbicide-resistant ACCases from other eukaryotes. It was also shown that a single isoleucine to leucine replacement at an equivalent position changes the wheat plastid ACCase from sensitive to resistant. These results have revealed an important mutation conferring herbicide-resistant ACCase. As of yet, structural information of the complete multidomain plastid ACCase is not available.

New herbicides that inhibit both the susceptible and resistant forms of ACCase in grass weeds would have obvious commercial appeal. In an attempt to identify molecules that target both the herbicide-sensitive and -resistant forms of ACCase, seven experimental compounds, either CHD-like or AOPP−CHD hybrids (**Figure 1**), were synthesized and assayed against previously characterized susceptible and resistant forms of ACCase. In addition, one compound (**IV**) was also assayed against resistant and susceptible maize ACCase.

MATERIALS AND METHODS

Synthesis of Compounds. *Compound I.* Wittig reaction of 3-ethylthiobutyraldehyde (*26*) and 1-triphenylphosphoranylidene-2-propanone in refluxing dichloromethane gave 7-ethylthio-3-hepten-2-one (*Z*/*E*, 1:14), which upon treatment with dimethyl 2-methoxymalonate and sodium methoxide in methanol yielded 5-(2-ethylthiopropyl)-4-methoxy-4-methylcarboxy-1,3-cyclohexanedione. Decarbomethoxylation (KOH/LiOH, then acidification), O-acylation with propionyl chloride and triethylamine, and rearrangement (acetone cyanohydrin, triethylamine) gave 5-(2-ethylthiopropyl)-4-methoxy-2-propanoyl-1,3-cyclohexanedione, which on treatment with O-allylhydroxylamine hydrochloride (triethylamine in ethanol) yielded **I**.

Compounds II and III. In an analogous manner, condensation of dimethyl 2-fluoromalonate with 6-(5-chloro-2-pyridylthio)-3-hexen-2-one (sodium methoxide in xylene) followed by decarbomethoxylation, O-propanoylation, acetone cyanohydrin-catalyzed rearrangement, and reaction with either O-allylhydroxylamine or O-ethylhydroxylamine gave **II** and **III**, respectively.

Compound IV. Reaction of 2,4,6-trimethylphenylacetyl chloride and *N*-methylhydroxylamine (triethylamine in THF/H_2O) gave the corresponding *N*-methylhydroxamic acid, which was O-alkylated with ethyl 2-bromo-2-methylpropionate (potassium carbonate in DMF). Treatment of the product with potassium *t*-butoxide in toluene and acidification gave **IV**.

Compounds V and VI. The syntheses of these oxazinediones were achieved by methodology previously described (*27*). Thus, O-acylation of 2,6,6-trimethyl-2*H*-1,2-oxazine-3,5(4*H*, 6*H*)-dione with 2-[4-(4-trifluoromethylphenoxy)phenoxy]propionyl chloride or 2-[4-(5-trifluoromethyl-2-pyridyloxy)phenoxy]propionyl chloride followed by acetone cyanohydrin-catalyzed rearrangement gave **V** and **VI**, respectively.

Compound VII. In an analogous manner, acylation of 5,5-dimethyl-1,3-cyclohexanedione with 2-[4-(4-trifluoromethylphenoxy)phenoxy]-propionyl chloride and subsequent rearrangement gave **VII**.

All of the preceding compounds were purified and were completely characterized by proton nuclear magnetic resonance (NMR) and mass spectrometry (MS).

Plant Material. The sources of the resistant and susceptible seed used in this study have been described previously (*12*, *17*, *18*). Seeds of all biotypes were germinated and grown in vermiculite in a growth chamber at 20/15 °C (day/night) in a 16-h photoperiod at 75% relative humidity. Young leaf tissue was collected from plants at the 2−3 leaf stage, frozen in liquid nitrogen, and stored at −80 °C.

ACCase Extraction and Assay. ACCase was extracted as previously described (*23*). Three grams of frozen tissue was homogenized in 30

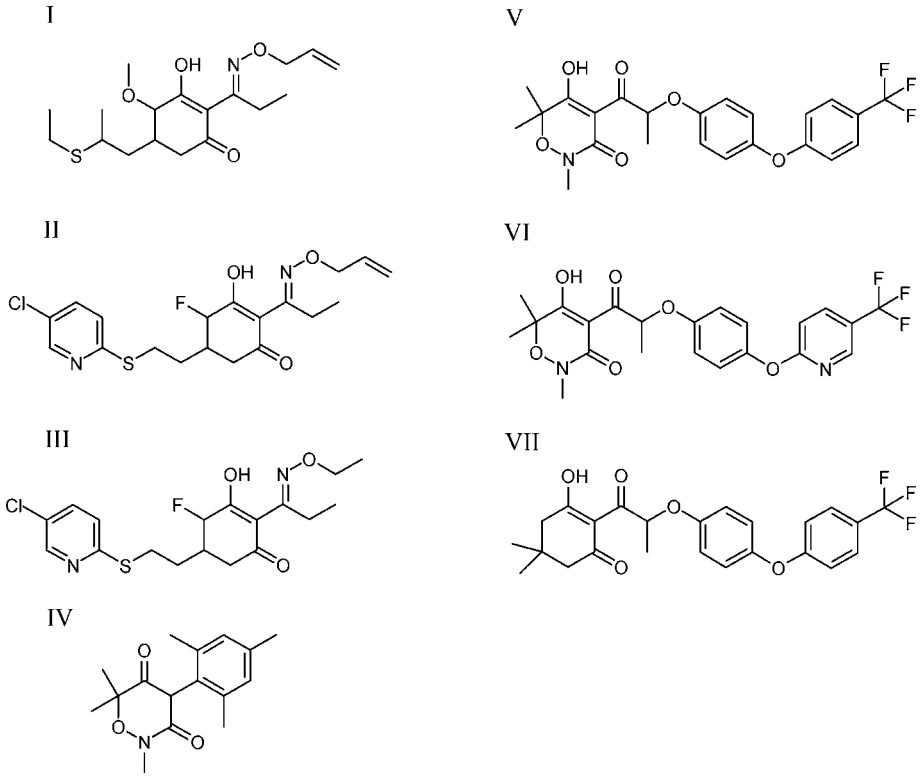

Figure 1. Chemical structures of experimental compounds used in the study. Compounds **I**–**III** are based on a CHD core structure; compounds **IV**–**VII** are CHD–AOPP hybrids.

mL of extraction buffer [100 mM Tricine (pH 7.5), 20% (v/v) glycerol, 50 mM KCl, 0.2 mM phenylmethylsulfonyl fluoride, 5 mM dithiothreitol] and centrifuged at 27 000*g* for 15 min. The supernatant was brought to 45% ammonium sulfate saturation and stirred for 30 min at 4 °C, followed by a 30 min centrifugation at 27 000*g*. The supernatant was discarded, and the pellet was resuspended in 700 μL of elution buffer [100 mM Tricine (pH 8.3), 10% (v/v) glycerol, 50 mM KCl, 1 mM dithiothreitol] and desalted on a Sephadex G-25 column, preequilibrated with elution buffer.

ACCase was assayed as previously described (*11*). The enzyme extract was incubated at 32 °C in assay buffer [20 mM Tricine−KOH (pH 8.3), 10 mM KCl, 5 mM ATP, 2 mM $MgCl_2$, 0.2 mg (w/v) BSA, 2.5 mM dithiothreitol, 3.7 mM $NaHCO_3$ (including 0.185 MBq of $NaH^{14}CO_3$)], along with appropriate concentrations of compounds **I**−**VII**. Acetyl CoA (0.25 mM final concentration) was added to initiate the reaction, and after 10 min, concentrated HCl was added to stop the reaction. Aliquots (50 μL) of the assay solutions were transferred onto 2.2-cm filter paper disks and dried under an infrared lamp. The acid- and heat-stable products were then quantified by liquid scintillation spectroscopy. Three samples per biotype were assayed in triplicate for each concentration of a compound. I_{50} values were calculated from the linear equation using the two concentrations bracketing 50% inhibition.

RESULTS

The specific activities of ACCase extracted from the resistant biotypes were not different from those of their respective susceptible counterparts (green foxtail, 77−79 nmol CO_2 fixed min^{-1} mg^{-1} protein; goosegrass, 12−19 nmol C fixed min^{-1} g f wt^{-1}). This is in agreement with previous results (*17*, *21*, *23*).

Inhibition of Susceptible ACCase by Experimental Compounds. Compounds **I**−**VII** inhibited ACCase from the sensitive biotypes of green foxtail (S1) and goosegrass (S3) (**Figures 2** and **3**; **Table 2**). However, the extent of inhibition varied among the compounds. Compound **V** was the most potent inhibitor of S1 ACCase, with an I_{50} of 0.9 μM, whereas compounds **I** and **IV** were the least potent (I_{50} = 54 and 51 μM, respectively) (**Table 2**). Compounds **III** and **V** were the most potent against the S3 goosegrass ACCase (I_{50} = 4.3 and 4.6 μM, respectively), and compound **IV** the least inhibitory (I_{50} = 76 μM), with the I_{50} values of other compounds falling between these extremes. Compound **IV** inhibited the susceptible maize (S4) ACCase but was much less potent than two known ACCase inhibitors, sethoxydim and fluazifop (**Table 3**).

Inhibition of Resistant ACCase by Experimental Compounds. The activities of the same experimental compounds were tested against ACCase from resistant biotypes of green foxtail (two different biotypes), goosegrass, and maize. In almost all cases, the I_{50} values obtained with the resistant ACCase were higher than with the corresponding sensitive ACCase (**Tables 2** and **3**), indicating reduced binding of these compounds to the resistant ACCases. In the case of ACCase from green foxtail biotype R1, the I_{50} values in most cases were considerably higher than those for the corresponding sensitive ACCase, S1 (see **Table 2**, R1/S1 I_{50} ratios of 1.3−17). Only with compound **IV** were the I_{50} values similar in both the resistant and susceptible biotypes (R1/S1 I_{50} ratio = 1.3). However, compound **IV** was a weak inhibitor of the sensitive ACCase in general. Similar results were obtained with ACCase from resistant green foxtail biotype R2, with only compounds **I** and **IV** showing similar inhibitory activity against ACCase from R2 and the corresponding S biotype (R2/S1 I_{50} ratios = 1.7 and 1.0, respectively; **Table 2**). However, as indicated previously, the I_{50} values for compounds **I** and **IV** against the sensitive ACCase were relatively high.

Slightly different results were obtained when the test compounds were assayed against ACCase from the resistant goosegrass biotype (R3). In this case, the R/S I_{50} ratios were very low (1.0−1.7) for compounds **V**, **VI**, and **VII** and slightly higher (3.0) for compound **IV** (**Table 2**). Again, the I_{50} value for compound **IV** against the S ACCase was relatively high (76 μM).

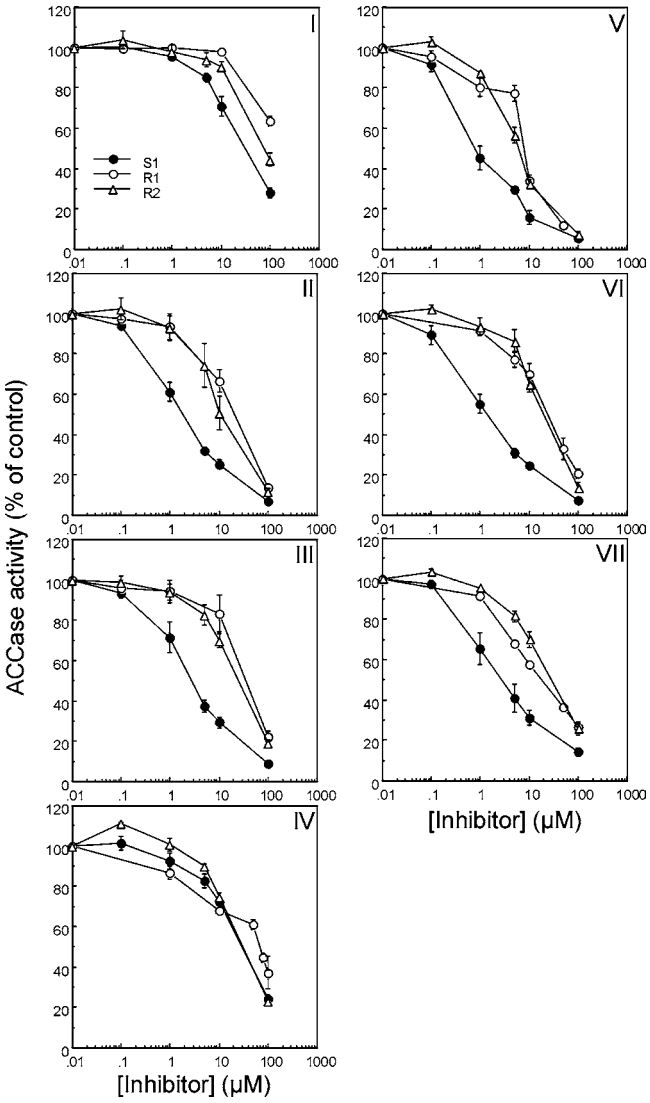

Figure 2. Inhibition of ACCase from susceptible (S1) and resistant (R1 and R2) biotypes of green foxtail. Vertical bars represent ± standard error (SE).

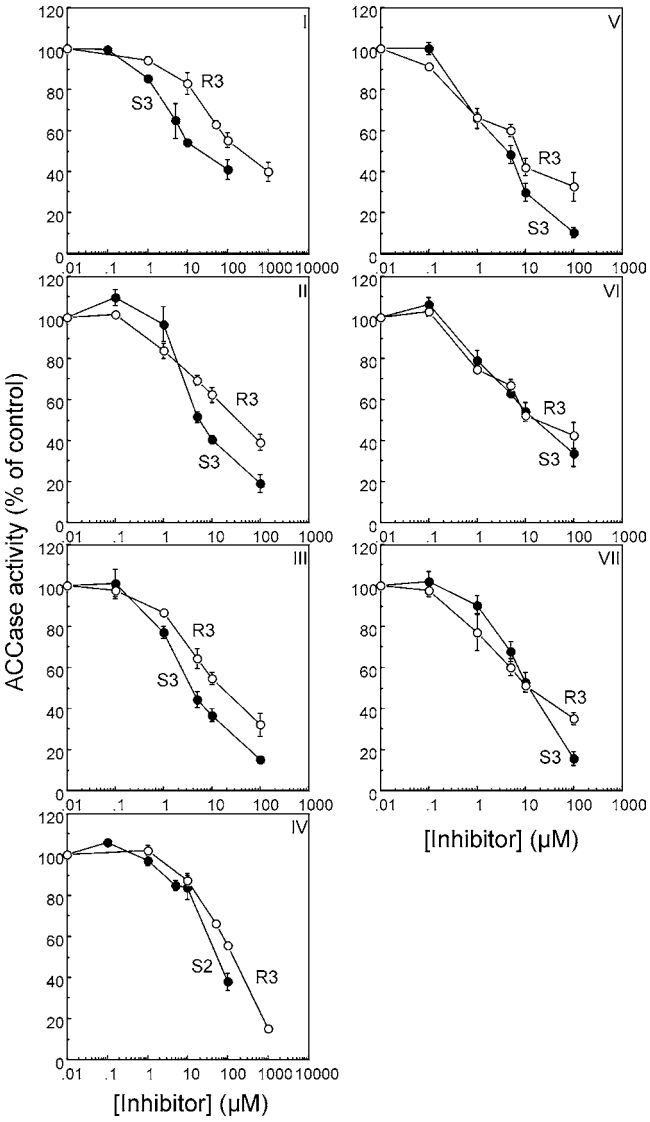

Figure 3. Inhibition of ACCase from susceptible (S3) and resistant (R3) biotypes of goosegrass. Vertical bars represent ±SE.

As expected, ACCase from the susceptible maize line (S4) was sensitive to sethoxydim and fluazifop, whereas that from the resistant maize line (R4) was resistant to inhibition by these herbicides, especially to sethoxydim (**Table 3**). Although compound **IV** was not a particularly potent inhibitor of susceptible maize ACCase, it was a stronger inhibitor of the resistant maize ACCase than either sethoxydim or fluazifop (**Table 3**). This resulted in a much lower R/S I_{50} ratio for compound **IV** than for either of these two known herbicides.

DISCUSSION

The ideal ACCase inhibitor from a study such as this would have two essential characteristics: inhibition of a range of "sensitive" ACCases from different grass species and equal inhibition of "resistant" ACCases from weeds that have evolved resistance to AOPP and CHD herbicides. In other words, the ideal candidate would be a potent inhibitor of a wide range of ACCase isoforms and not be readily desensitized by frequent mutations that give rise to resistant forms of ACCase.

In general, the experimental compounds tested were not particularly potent ACCase inhibitors compared to some commercialized herbicides (compare the S1 and S3 I_{50} values in **Table 1** with those in **Table 2**). In only a few cases were the I_{50} values of the experimental compounds in the low micromolar range. Therefore, none of these compounds would satisfy the primary criterion for ideal activity described above. However, some did inhibit ACCase significantly at relatively low concentrations (e.g., compound **V**), indicating that they are at least useful leads in new inhibitor development. This is not surprising,

Table 2. I_{50} Values and R/S I_{50} Ratios for ACCase Herbicide-Sensitive and -Resistant Biotypes of Green Foxtail (S1, R1, R2) and Goosegrass (S3, R3), Assayed in Vitro with Experimental ACCase Inhibitors

	green foxtail					goosegrass		
	I_{50} (μM)			I_{50} (μM)		I_{50} (μM)		
compound	S1	R1	R1/S1	R2	R2/S1	S3	R3	R3/S3
I	54	>100	>1.9	89	1.7	38	407	11
II	2.5	38	15	12	4.6	5.7	58	10
III	3.5	59	17	45	13	4.3	29	6.6
IV	51	67	1.3	54	1.0	76	227	3.0
V	0.9	8.1	9.0	6.4	7.1	4.6	7.8	1.7
VI	1.8	32	18	36	20	28	31	1.1
VII	3.5	24	6.9	50	14	17	16	1.0

Table 3. I_{50} Values and R/S I_{50} Ratios for ACCase from Wild-Type (S4) and Sethoxydim-Resistant (R4) Biotypes of Maize, Assayed in Vitro

	I_{50} (μM)		
inhibitor	S4	R4	R4/S4
compound **IV**	18	40	2.2
sethoxydim	0.9	>100	>111
fluazifop	1.5	55	37

given that all the experimental compounds are closely related to known classes of ACCase inhibitors.

The more important question in this study addressed the second criterion, that is, would these compounds be equally potent against resistant ACCase isoforms? This goal might be more achievable if there was only one resistant form of ACCase. However, at least four different patterns of resistance to ACCase inhibitors have been observed in different weed biotypes in which resistance is conferred by an altered form of ACCase (*28*). These include high-level resistance to sethoxydim and low-level resistance to other AOPP and CHD herbicides, high-level resistance to fluazifop and low-level resistance to other AOPP and CHD herbicides, relatively high-level resistance to all AOPP and CHD herbicides, and resistance to AOPP but not to CHD herbicides. Collectively, these results suggest that at least four different forms of ACCase occur in different resistant weed populations. Therefore, the new molecules sought must inhibit a range of ACCase isoforms with different structural, steric, or electrostatic properties in or around the herbicide binding domain.

Only one compound, number **IV**, had somewhat similar activity against the resistant and susceptible forms of ACCase used in this study. Unfortunately, this compound had inherently low activity even against the susceptible ACCase isoforms (I_{50} values of 18−76 μM; **Tables 2** and **3**), which would preclude it from further development as a herbicide. However, it has value as a new lead to develop a broad-based inhibitor for all isoforms of ACCase. For this compound, meeting the second requirement for comparable activity against resistant and susceptible ACCases is negated by its low inhibitory activity in general. The results do suggest, however, that it may be possible to identify new inhibitors that bind equally to resistant and susceptible forms of the enzyme. The challenge is to identify molecules that do so in the low micromolar range. Although it is based on a CHD core and is homologous to the 3-aryltetramic class of ACCase inhibitors (*29, 30*), compound **IV** lacks the aliphatic substituents of more typical CHDs and 3-aryltetramic acids. Further structural modifications to this molecule, bringing it closer to other CHDs and 3-aryltetramic acids, may lead to a more potent molecule that would inhibit the S and R ACCases at the same low concentration.

Derivatives of CHD herbicides (based on modification of the alkyl chains) have been used to study their effect on whole plants, de novo fatty acid biosynthesis, and inhibition of ACCase in order to better understand structure−activity relationships of CHD herbicides (*31*). Several new compounds were 2 orders of magnitude more effective than known CHD herbicides in vitro; however, these compounds were not very effective at the whole-plant level. Any new inhibitors developed through a rational design approach must also meet other critical requirements for biological activity, including adequate foliar penetration, transport through the plant, and stability in the plant tissue.

A novel class of cyclic triketones were synthesized by attaching the cyclic portion of CHD oximes to AOPP herbicides (*32*). The compounds thus produced, which included compound **VII**, had 100× and 1−10× greater binding affinity with ACCase than CHD and AOPP herbicides, respectively. However, the authors suggested that these compounds probably would not overcome target-site-based herbicide resistance based on an altered herbicide binding site.

Since resistant ACCases often remain relatively susceptible to one or more AOPP or CHD herbicides (see **Table 1** for examples), it is possible that AOPP−CHD hybrid molecules may be more potent inhibitors of resistant forms of ACCase. The AOPP−CHD hybrid structures used in this study (compounds **V**, **VI**, and **VII**) were relatively potent inhibitors of the S1 and S3 ACCases (**Table 2**). In particular, compound **V** demonstrated consistently high activity against the susceptible ACCases S1 and S3 and was also a relatively strong inhibitor of the three resistant ACCases against which it was assayed (R1, R2, R3; **Table 2**). Compounds **VI** and **VII** were equally potent inhibitors of the R and S ACCases from goosegrass. In contrast, while they were active against ACCase from the susceptible green foxtail biotype (S1), they were less effective against the resistant green foxtail ACCases (**Table 2**).

Although compound **V** shares some structural similarities with fluazifop, it was much more potent against the resistant goosegrass ACCase than fluazifop (I_{50} values of 7.8 and >500 μM for compound **V** and fluazifop, respectively; **Tables 1** and **2**). Presumably, the CHD moiety in the hybrid structure contributes to the high potency of this compound. Although this compound did not satisfy the criteria for high overall activity, it is also a potential lead for further synthesis and evaluation.

Based on amino acid sequence comparisons of plastidic ACCase from herbicide-sensitive and -resistant plants, it has become clear that an Ile to Leu change close to the highly conserved motif of the carboxyltransferase domain of ACCase plays an important role in the development of resistance to ACCase inhibitor herbicides. Zhang and Devine (*33*) reported such a mutation in the plastidic ACCase from herbicide-resistant green foxtail, and Zagnitko et al. (*25*) reported a similar mutation in maize and annual ryegrass. The latter authors suggested that this mutation changes the interaction of the herbicides with the enzyme without compromising the enzyme activity. More recently, Délye et al. (*34*) reported that an Ile to Asn substitution was responsible for resistance in blackgrass. We can speculate that this change, and possibly other amino acid substitutions not yet identified, may reduce binding of some inhibitors more than others, depending on how the altered charge and steric parameters change the affinity of the inhibitors for the binding niche on the enzyme. However, without knowing the three-dimensional structure of the binding niche, it is difficult to say more about the effect of specific mutations on inhibitor interaction with the enzyme.

None of the experimental compounds examined in this work was completely effective against the range of ACCase enzymes tested. However, the activity of compound **V** suggests it may be possible to develop potent ACCase inhibitors that are effective against both susceptible and resistant forms of the enzyme. The inclusion of herbicide-resistant target enzymes in screening protocols is one approach to identifying new molecules with broad-based potency against isoforms of target enzymes for which there are known differences in susceptibility.

ABBREVIATIONS USED

ACCase, acetyl coenzyme A carboxylase; AOPP, aryloxyphenoxypropionic acid; CHD, cyclohexanedione.

ACKNOWLEDGMENT

We acknowledge chemical synthetic assistance from Weiming Fan, Arthur Steiger, and Stefan Blarer and technical assistance from Corinne Biesenthal and Susan Marles. We dedicate this work to F. Dana Hess, whose enthusiasm and intellect inspired us all.

LITERATURE CITED

(1) Burton, J. D.; Gronwald, J. W.; Somers, D. A.; Connelly, J. A.; Gengenbach, B. G.; Wyse, D. L. Inhibition of plant acetyl-CoA carboxylase by the herbicides sethoxydim and haloxyfop. *Biochem. Biophys. Res. Commun.* **1987**, *148*, 1039−1044.

(2) Rendina, A. R.; Felts, J. M. Cyclohexanedione herbicides are selective and potent inhibitors of acetyl-CoA carboxylase from grasses. *Plant Physiol.* **1988**, *86*, 983−986.

(3) Burton, J. D.; Gronwald, J. D.; Keith, R. A.; Somers, D. A.; Gengenbach, B. G.; Wyse, D. L. Kinetics of inhibition of acetyl-coenzyme A carboxylase by sethoxydim and haloxyfop. *Pestic. Biochem. Physiol.* **1991**, *39*, 100−109.

(4) Konishi, T.; Sasaki, Y. Compartmentalization of two forms of acetyl-CoA carboxylase in plants and the origin of their tolerance towards herbicides. *Proc. Natl. Acad. Sci. U.S.A.* **1994**, *91*, 3598−3601.

(5) Devine, M. D.; Shimabukuro, R. H. Resistance to acetyl coenzyme A carboxylase inhibiting herbicides. In *Herbicide Resistance in Plants*; Powles, S. B., Holtum, J. A. M., Eds.; Lewis: Boca Raton, FL, 1994.

(6) Rendina, A. R.; Beaudoin, J. D.; Craig-Kennard, A. C.; Breen, M. K. Kinetics of inhibition of acetyl-CoA carboxylase by aryloxyphenoxypropionate and cyclohexanedione graminicides. *Proceedings of the Brighton Crop Protection Conference on Weeds*, 1989; pp 163−172.

(7) Rendina, A. R.; Craig-Kennard, A. C.; Beaudoin, J. D.; Breen, M. K. Inhibition of acetyl-coenzyme A carboxylase by two classes of grass-selective herbicides. *J. Agric. Food Chem.* **1990**, *38*, 1282−1287.

(8) Taylor, W.; Hixon, M.; Chi, H.; Marsillii, E.; Rendina, A. R. Inhibition of acetyl-coenzyme A carboxylase by coenzyme A conjugates of grass selective herbicides. *Pestic. Sci.* **1995**, *43*, 177−180.

(9) Devine, M. D.; Eberlein, C. V. Physiological, biochemical and molecular aspects of herbicide resistance based on altered target sites. In *Herbicide Activity*: *Toxicology, Biochemistry and Molecular Biology*; Roe, R. M., Burton, J. D., Kuhr, R. J., Eds.; IOS Press: Amsterdam, 1997.

(10) Heap, I. M.; Murray, B. G.; Loeppky, H. A.; Morrison, I. N. Resistance to aryloxyphenoxypropionate and cyclohexanedione herbicides in wild oat (*Avena fatua*). *Weed Sci.* **1993**, *41*, 232−238.

(11) Seefeldt, S. S.; Fuerst, E. P.; Gealy, D. R.; Shukla, A.; Irzyk, G. P.; Devine, M. D. Mechanisms of resistance to diclofop of two wild oat (*Avena fatua*) biotypes from the Willamette Valley of Oregon. *Weed Sci.* **1996**, *44*, 776−781.

(12) Heap, I. M.; Morrison, I. N. Resistance to aryloxyphenoxypropionate and cyclohexanedione herbicides in green foxtail (*Setaria viridis*). *Weed Sci.* **1996**, *44*, 25−30.

(13) Stoltenberg, D. E.; Wiederholt, R. J. Giant foxtail (*Setaria faberi*) resistant to aryloxyphenoxypropionate and cyclohexanedione herbicides. *Weed Sci.* **1995**, *43*, 527−535.

(14) Heap, I. M.; Knight, R. Variation in herbicide cross-resistance among populations of annual ryegrass (Lolium rigidum) resistant to diclofop-methyl. *Aust. J. Agric. Res.* **1990**, *41*, 121−128.

(15) Preston, C.; Tardif, F. J.; Christopher, J. T.; Powles, S. B. Multiple resistance to dissimilar herbicide chemistries in a biotype of *Lolium rigidum* due to enhanced activity of several herbicide degrading enzymes. *Pestic. Biochem. Physiol.* **1996**, *54*, 123−134.

(16) Tardif, F. J.; Powles, S. B. Herbicide multiple-resistance in a *Lolium rigidum* biotype is endowed by multiple mechanisms: isolation of a subset with resistant acetyl-CoA carboxylase. *Physiol. Plant.* **1994**, *91*, 488−494.

(17) Leach, G. E.; Devine, M. D.; Kirkwood, R. C.; Marshall, G. Target enzyme-based resistance to acetyl-coenzyme A carboxylase inhibitors in *Eleusine indica*. *Pestic. Biochem. Physiol.* **1995**, *51*, 129−136.

(18) Parker, W. B.; Marshall, L. C.; Burton, J. D.; Somers, D. A.; Wyse, D. L.; Gronwald, J. W.; Gengenbach, B. G. Dominant mutations causing alterations in acetyl-coenzyme A carboxylase confer tolerance to cyclohexanedione and aryloxyphenoxypropionate herbicides in maize. *Proc. Natl. Acad. Sci. U.S.A.* **1990**, *87*, 7175−7179.

(19) Gronwald, J. W.; Eberlein, C. V.; Betts, K. J.; Baerg, R. J.; Ehlke, N. J.; Wyse, D. L. Mechanism of diclofop resistance in an Italian ryegrass (*Lolium multiflorum* Lam.) biotype. *Pestic. Biochem. Physiol.* **1992**, *44*, 126−139.

(20) Tardif, F. J.; Holtum, J. A. M.; Powles, S. B. Occurrence of a herbicide-resistant acetyl-coenzyme A carboxylase mutant in annual ryegrass (*Lolium rigidum*) selected by sethoxydim. *Planta* **1993**, *190*, 176−181.

(21) Marles, M. A. S.; Devine, M. D.; Hall, J. C. Herbicide resistance in *Setaria viridis* conferred by a less sensitive form of acetyl coenzyme A carboxylase. *Pestic. Biochem. Physiol.* **1993**, *46*, 7−14.

(22) Maneechote, C.; Holtum, J. A. M.; Preston, C.; Powles, S. B. Resistant acetyl-CoA carboxylase is a mechanism of herbicide resistance in a biotype of *Avena sterilis* ssp. ludoviciana. *Plant Cell Physiol.* **1994**, *35*, 627−635.

(23) Shukla, A.; Leach, G. E.; Devine, M. D. High-level resistance to sethoxydim conferred by an alteration in target enzyme, acetyl-CoA carboxylase, in *Setaria faberi* and *Setaria viridis*. *Plant Physiol. Biochem.* **1997**, *35*, 803−807.

(24) Shukla, A.; Dupont, S.; Devine, M. D. Resistance to ACCase-inhibitor herbicides in wild oat: Evidence for target site-based resistance in two biotypes from Canada. *Pestic. Biochem. Physiol.* **1997**, *57*, 147−155.

(25) Zagnitko, O.; Jelenska, J.; Tevzadze, G.; Haselkorn, R.; Gornicki, P. An isoleucine/leucine residue in the carboxyltransferase domain of acetyl-CoA carboxylase is critical for interaction with aryloxyphenoxypropionate and cyclohexandione inhibitors. *Proc. Natl. Acad. Sci. U.S.A.* **2001**, *98*, 6617−6622.

(26) Luo, T. Nouvelles 2-[1-(oxyamino)-alkylidene]-5-(2-methylthiopropyl)-cyclohexane-1,3-diones herbicides et leurs applications. French Patent 2531953, February 24, 1984; *Chem. Abstr.* **1984**, *101*, 130291.

(27) Lee, S.-F. Heterocyclic Diones as Pesticides and Plant Growth Regulators. European Patent Application 0394889, October 10, 1990; *Chem. Abstr.* **1991**, *114*, 143431.

(28) Devine, M. D. Mechanisms of resistance to acetyl-CoA carboxylase inhibitors: A review. *Pestic. Sci.* **1997**, *51*, 259−264.

(29) Fischer, R.; Konze, J.; Santel, H.-J. Bicyclic 3-aryl tetramic acid herbicides. A new type of ACCase inhibitor. *8th IUPAC International Congress of Pesticide Chemistry, Washington, DC, July 4−9, 1994*; Abstract 120.

(30) Krauskopf, B.; Luerssen, K.; Santel, H.-J.; Schmidt, R. R.; Wachendorff-Neumann, U.; Fischer, R.; Erdelen, C.; Insecticidal, acaricidal and herbicidal 1-H-3-arylpyrrolidine-2,4-dione derivatives. U.S. Patent 5,258,527, Nov 11, 1993.

(31) Maier, A.; Golz, A.; Lichtenthaler, H. K.; Meyer, N.; Retzlaff, G. Studies on the effect of different cyclohexane-1,3-diones on de-novo fatty acid biosynthesis in Poaceae. *Pestic. Sci.* **1994**, *42*, 153−161.

(32) Rendina, A. R.; Campopiano, O.; Marsilii, E.; Hixon, M.; Chi, H.; Hagenah, J. A.; Taylor, W. S. Overlap between herbicidal inhibitors of acetyl-coenzyme A carboxylase: Enhanced binding of cyclic triketones, a novel class of graminicide. *Pestic. Sci.* **1995**, *43*, 368−371.

(33) Zhang, X. Q.; Devine, M. D. A possible point mutation of plastidic ACCase gene conferring resistance to sethoxydim in green foxtail (*Setaria viridis*). *Weed Sci. Soc. Am. Abstr*. **2000**, *40*, 33.

(34) Délye, C.; Zhang, X. Q.; Chalopin, C.; Michel, S.; Powles, S. B. An Isoleucine Residue within the Carboxyl-Transferase Domain of Multidomain Acetyl-Coenzyme A Carboxylase Is a Major Determinant of Sensitivity to Aryloxyphenoxypropionate But Not to Cyclohexanedione Inhibitors. *Plant Physiol.* **2003**, *132*, 1716−1723.

Received for review July 7, 2003. Revised manuscript received June 4, 2004. Accepted June 5, 2004. This research was supported by a grant from the Natural Sciences and Engineering Research Council of Canada to M.D.D., with additional funds from the former Sandoz Agro Inc. (now Syngenta Inc.).

JF034738M