Vinylogous Sulfonylureas: A New Class of Acetohydroxyacid Synthase Inhibitors Incorporating a Large Bridging Moiety

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Herbicide, Sulfonylurea Analogue, Acetohydroxyacid Synthase (= Acetolactate Synthase) Inhibitor

Several acrylic acid derivatives incorporating elements of sulfonylurea structure around a central vinylogous core were synthesized and found to be moderate inhibitors of the enzyme, acetohydroxyacid synthase (AHAS). Some compounds showed post-emergence herbicidal activity against mustard. Biological activity was found to be markedly influenced by the nature of the 2-arylcarbamoyl group. When this group contained a phenyl ring the compounds were inactive. A 4,6-dimethoxypyrimidin-2-ylcarbamoyl function resulted in the best activity but this activity was highly dependent on the nature of the 4,6-substituents on the pyrimidine ring, with 4,6-dimethylpyrimidines showing very little activity. The structures of the most active compound, ethyl 3-(2-chlorophenyl)sulfonylamino-3-methylthio-2-[(4,6-dimethoxypyrimidin-2-yl)carbamoyl]acrylate, and an inactive analogue, 4,6-dimethylpyrimidin-2-yl-3-(2-chlorophenyl)sulfonylamino-3-methylthio-2-cyanoacrylate, were determined by X-ray crystallography. The resulting structures showed several conformational differences which may play an important role in inhibitor binding to AHAS and the resultant herbicidal activity.

Introduction

The commercially important sulfonylureas [1] and imidazolinone [2] herbicides owe their phytotoxicity to inhibition of acetohydroxyacetic acid synthase (AHAS, EC 4.1.3.18, also known as acetolactate synthase). Since the discovery of the mode of action of these compounds a plethora of AHAS inhibitors has emerged including triazolopyrimidine sulfonamides [3], pyrimidinyl salicylates [4], pyrimidinyloxyalkanoates [5], phthalyl anilides [6] and certain quinones [7]. Although some structural elements, such as an arylsulfonyl group, a pyrimidinyl ring or an acidic function, recur in these compounds, there do not seem to be any "essential elements" required for activity.

AHAS catalyses the first step in the biosynthesis of the branched chain amino acids. It has binding sites for substrates (one molecule of pyruvate and either a second molecule of pyruvate or a molecule of α-ketobutyrate), cofactors (FAD and TPP) and for feedback inhibitors (valine and leucine or isoleucine). Because this formerly obscure enzyme is now of commercial significance, its binding properties and the interactions of substrates, cofactors and inhibitors have been studied intensively. However, detailed knowledge of the herbicide-binding site, particularly for the plant enzyme, is still limited. Partial overlap of various herbicide-binding sites with each other and other binding sites on the enzyme has been inferred from kinetic studies [8], equilibrium dialysis [9], binding studies with 3-bromopyruvate [8] and studies of cross-resistance between different inhibitors, including valine and leucine, with enzymes from various herbicide-resistant mutants [10–14]. However, the currently held view is that the herbicide-binding site is largely extraneous to the other binding sites on the enzyme [8]; the main evidence in support of this hypothesis being the isolation of herbicide-tolerant enzymes from mutant plants that are indistinguishable from wild type plants in all aspects except for herbicide resistance. An interesting sequence homology between AHAS and pyruvate

Abbreviations: FAD, flavin adenine dinucleotide; TPP, thiamine pyrophosphate.

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oxidase lead Schloss to postulate that the AHAS herbicide-binding site is an evolutionary vestige of a quinone-binding site [7]. It is therefore tempting to consider an intriguing parallel between the AHAS herbicide site and the herbicide-binding site on the D1 peptide of PS II which is a functional quinone-binding site and which is also subject to inhibition by a wide range of chemically diverse inhibitors [7].

The cyanoacrylate herbicides [15, 16] may be thought of as "vinylogous" derivatives of urea PS II herbicides (Fig. 1). The notion of a commonality between the PS II site and the AHAS site lead to extrapolation of this idea to the sulfonylureas and to the synthesis and testing of a series of "vinylogous" sulfonylureas (VSUs). The compounds synthesized incorporated the peripheral structural features of chlorsulfuron, with a 2-chlorophenylsulfonylamino group on the "left-hand" end of the molecule and a pyrimidinyl or triazinylcarbamoyl group on the "right-hand" side. A few phenylcarbamoyl analogues were synthesized to confirm a lack of activity in the absence of a pyrimidine or triazine in this position. In order to explore structure activity relationships within this group of compounds and to further our understanding of the requirements of the binding site, X-ray crystallographic studies of inhibitor structure were performed.

Materials and Methods

Chemical syntheses

The synthesis of the vinylogous sulfonylureas (**1**) was based on literature procedures as shown in Fig. 2. The synthesis of the most active compound **1c** is given below. Synthesis of N-phthalylvaline anilides (**2**) was performed as described previously [6]. Novel compounds were characterized by proton and carbon-13 NMR spectra. Satisfactory microanalyses were obtained for all novel compounds (except **1d** and **1l**).

Synthesis of ethyl 3-(2-chlorophenyl)sulfonylamino-3-methylthio-2-[(4,6-dimethoxypyrimidin-2-yl)carbamoyl]acrylate (**1c**)

2-Amino-4,6-dimethoxypyrimidine [17] (3 g) and anhydrous sodium acetate (2 g) were suspended in dry acetone (20 ml) and ethyl malonyl chloride (2.6 ml) was added dropwise. The mixture was

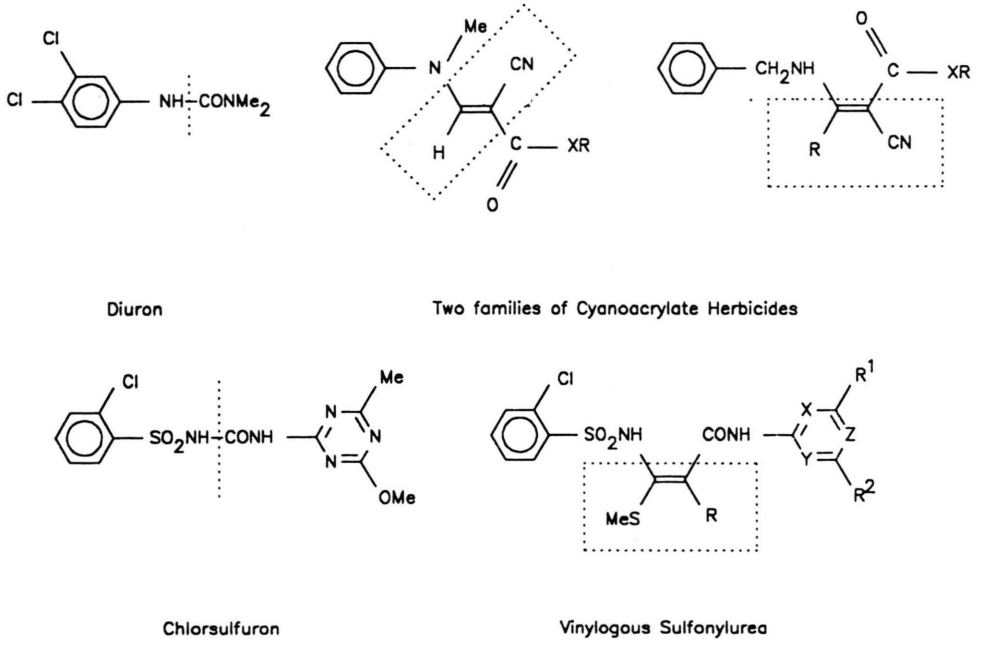


Fig. 1. Cyanoacrylate PS II herbicides may be thought of as derived from diuron after insertion (along dotted line) of a vinylogous bridge (outlined by a dotted box); vinylogous sulfonylureas may be thought of as chlorsulfuron containing a similar insertion.

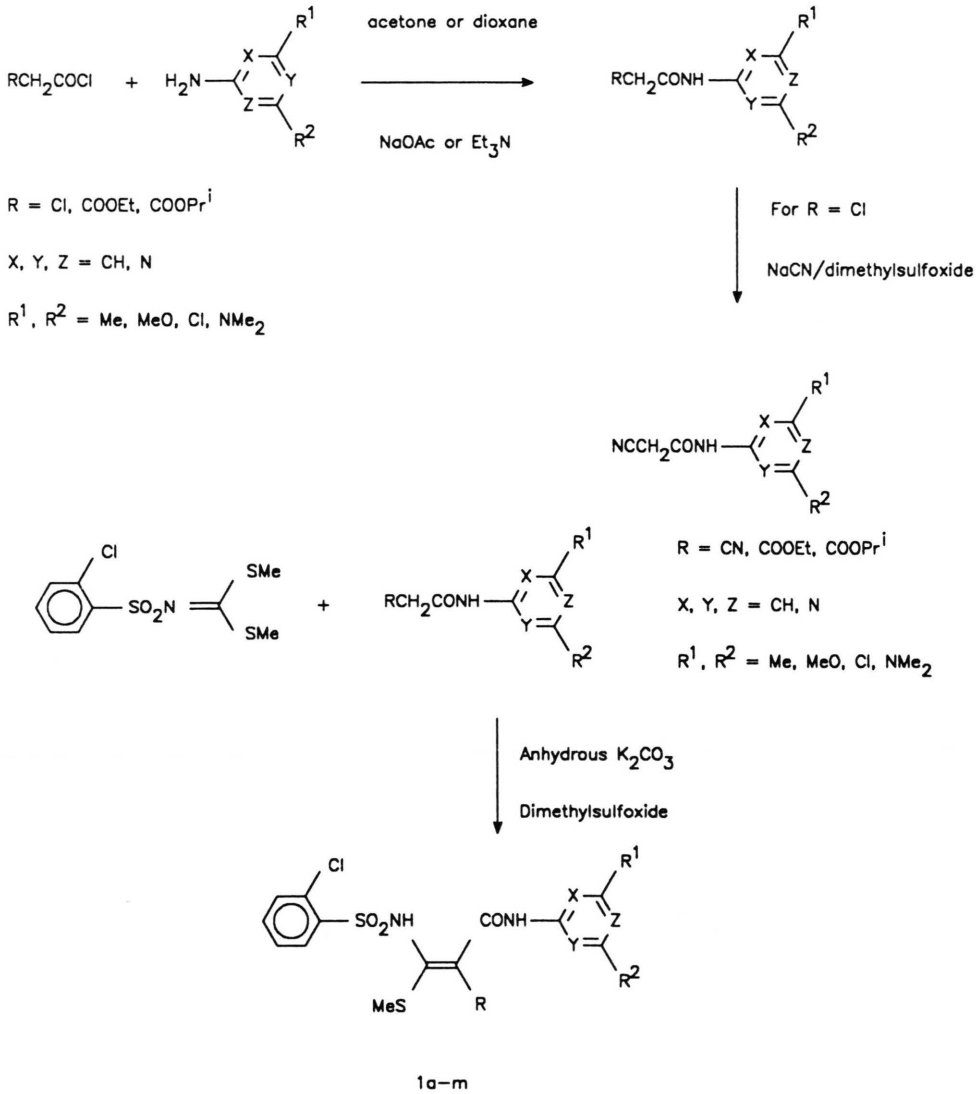


Fig. 2. Scheme for synthetic route to vinylogous sulfonylureas (**1**).

boiled under reflux protected from moisture for 30 min, then cooled, poured into a saturated aqueous solution of sodium chloride (200 ml) and extracted with two portions (100 ml) of methylene chloride. The combined organic extracts were filtered dry and evaporated to give a brown oil (5.4 g). The crude product was purified by chromatography on silica gel eluting with methylene chloride and then 10% acetone in methylene chloride. The eluant fractions containing ethyl 4,6-dimethoxypyrimidin-2-ylcarbamoylacetate (as determined by thin layer chromatography) were combined and evaporated to give a pale oil which solidified on standing. The solid was crystallized from ethyl acetate (1.6 g, yield 30%), m.p. 83–85 °C.

$C_{11}H_{15}N_3O_5$

Calcd	C 49.1	H 5.6	N 15.6%,
Found	C 49.0	H 5.7	N 15.7%.

The method of Tominaga [19] was used to condense the above product (5 mmol, 1.3 g) with N-bis(methylthio)methylene-2-chlorophenylsulfonamide (synthesized using a standard procedure

[18]) (5 mmol, 1.5 g) in dry dimethyl sulfoxide (10 ml) with anhydrous potassium carbonate (6.5 mmol, 0.9 g) as base. The mixture was stirred, protected from moisture, at room temperature overnight, then poured into water and acidified with concentrated hydrochloric acid. The product was extracted into methylene chloride, the organic layer was separated, filtered dry and evaporated and the residue purified by chromatography on silica gel eluting ethyl 3-(2-chlorophenyl)sulfonylamino-3-methylthio-2-[(4,6-dimethoxypyrimidin-2-yl)carbamoyl]acrylate (**1c**) (yield 22% after chromatography with methylene chloride just ahead of remaining ester starting material). The product was crystallized from ethyl acetate:light petroleum, b.p. 40–60 °C, m.p. 140–143 °C.

$C_{19}H_{21}ClN_4O_7S_2$

Calcd C 44.1 H 4.1 N 10.8%,
Found C 43.8 H 4.0 N 10.5%.

Attempts to improve the yields of this synthesis were unsuccessful.

Inhibition of AHAS

Assay of inhibitor activity, expressed as pI_{50} ($-\log_{10}$ of the concentration of inhibitor giving 50% of the control reaction) was performed as described previously [6].

Glass-house assays for herbicidal activity

For post-emergence herbicide assays, test compounds (18 mg) were dissolved in 10 ml acetone. The solution was diluted to 20 ml with 0.02% aqueous Tween 20 and sprayed onto trays containing 7–10 day old seedlings of mustard (*Brassica napus* L.), peas (*Pisum sativum* L.), linseed (*Linum usitassimum* L.), barley (*Hordeum vulgare* L.) and ryegrass (*Lolium perenne* L.) grown in potting mix. This gave a dose rate equivalent to 4 kg ha^{-1}. After 14 days the herbicidal effects on each species were evaluated visually using a ten point score, where 10 denotes complete kill.

X-ray crystallography

Compounds for X-ray crystallography were recrystallized from aqueous ethanol. Structures were determined for **1c, 1j** and **2b.** Details of coordinates have been lodged with the Cambridge Crystallographic Database.

Results and Discussion

AHAS inhibition

The AHAS inhibition activities of the vinylogous sulfonylureas synthesized are given in Table I. The ethyl 4,6-dimethoxypyrimidin-2-ylcar-

Table I. Physical and biological data for vinylogous sulfonylureas (**1**) (for structures see Fig. 2).

Compound							Physical parameters				Herbicidal scores at 4 kg ha^{-1}		
No.	R	R^1	R^2	X	Y	Z	m.p. [°C]	H 5[1]	Solvent[2]	pI_{50} for AHAS	M[3]	L	P
1a	COOEt	Me	Me	N	N	CH	166–170	6.72	EtOAc:petrol	5.1	0	0	0
1b	COOEt	Me	MeO	N	N	CH	174–175	6.25	$CHCl_3$:petrol	6.0	8	0	9
1c	COOEt	MeO	MeO	N	N	CH	140–143	5.73	EtOAc:petrol	7.1	10	1	7
1d	COOEt	Me	MeO	N	N	N	128–130	*	EtOH	5.2	0	0	0
1e	COOEt	Me	NMe_2	N	N	CH	175–176	6.1	MeOH	4.5	1	0	3
1f	COOEt	MeO	MeO	CH	CH	CH	138–139	**	EtOH	NA[4]	1	3	2
1g	$COOPr^i$	Me	Me	N	N	CH	159–161	6.71	EtOAc:petrol	4.8	0	0	0
1h	$COOPr^i$	Me	MeO	N	N	CH	172–173	6.22	$CHCl_3$:petrol	6.0	2	0	0
1i	$COOPr^i$	MeO	MeO	N	N	CH	138–140	5.73	EtOAc	5.5	2	0	0
1j	CN	Me	Me	N	N	CH	188–191	7.32	CH_3CN	NA	0	0	0
1k	CN	Me	MeO	N	N	CH	193–195	6.88	DMF:EtOH	6.0	0	1	0
1l	CN	MeO	MeO	N	N	CH	240 (d)	5.79	CH_3CN	5.1	0	0	0
1m	CN	MeO	MeO	CH	CH	CH	183–184	*	MeOH	NA	0	0	0

[1] H 5 is ^1H NMR shift value δ ($CDCl_3$) ppm for **1a–1i,** δ [$(CD_3)_2SO$] for **1j–1m** of H 5 in pyrimidine amide ring (* denotes triazine amide, ** phenylamide).
[2] Recrystallization solvent: DMF, dimethylformamide; EtOAc, ethyl acetate; petrol, light petroleum b.p. 40–60 °C.
[3] Plants tested: M, mustard (*Brassica napus* L.); L, Linseed (*Linum usitassimum* L.); P, peas (*Pisum sativum* L.).
[4] NA denotes not active at concentrations < or = 10^{-4} M.

bamoylacrylate ester (**1c**) was found to be the most active compound, with a pI_{50} value of 7.1; about 10-fold less active than chlorsulfuron, which had a pI_{50} of 7.9 in the same assay. The corresponding 4,6-dimethylpyrimidin-2-ylcarbamoylacrylate ester (**1a**) was 100-fold less active (pI_{50} = 5.1). The AHAS inhibiting activity of the vinylogous sulfonylureas is extremely sensitive to the nature of the substituents on the pyrimidine ring. It was found that for the ethyl ester series R=COOEt) the observed pI_{50} values are inversely proportional to the value obtained for the proton NMR shift of the H 5 proton in the pyrimidine ring (Fig. 3). This effect is not so marked for the isopropyl ester series (R=COOPr^i) because the activity of **1i** is unexpectedly low. The upfield (lower) shift values for H 5 reflect a greater electron density in the pyrimidine ring, suggesting that this feature contributes strongly to effective binding of these inhibitors to the AHAS molecule. In the vinylogous sulfonylurea series, as with the classical sulfonylureas, phenylcarbamoyl analogues (**1f** and **1m**) of pyrimidinylcarbamoyl compounds were not active as inhibitors of the enzyme. In contrast, N-phthalyl-valine anilides showed similar (low) AHAS inhibition activity (Table II) to analogous pyrimidin-2-ylamides, and variation in the phenyl or pyrimidine ring substitution (methyl *vs* methoxy) had no discernable effect on activity.

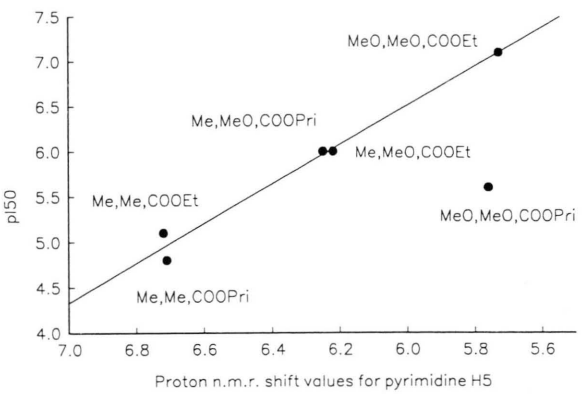
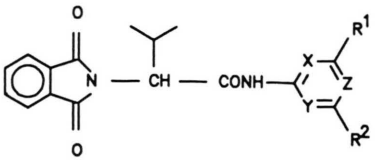


Fig. 3. Relationship of pI_{50} values of some vinylogous sulfonylureas (**1**) for AHAS inhibition with ^{1}H NMR shift values [δ ($CDCl_3$)] of pyrimidine H 5 protons. Legends give R^1, R^2 and Y for each data point. The equation of regression line is $pI_{50} = -2.18\ (\pm 0.16)\ \delta + 19.59\ (\pm 1.03)$; correlation coefficient $r^2 = 0.98$ (point for MeO, MeO, COOPr^i omitted because it is an obvious outlier).

Table II. Structure, melting points and AHAS inhibition values for some N-phthalyl-L-valine-amides (**2**).



No.	R^1	R^2	X	Y	Z	m.p. [°C]	pI_{50}
2a	Me	Me	N	N	CH	171–172	4.6
2b	MeO	MeO	N	N	CH	144–146	4.9
2c	Me	Me	CH	CH	CH	168–170	4.9
2d	MeO	MeO	CH	CH	CH	154–155	4.9

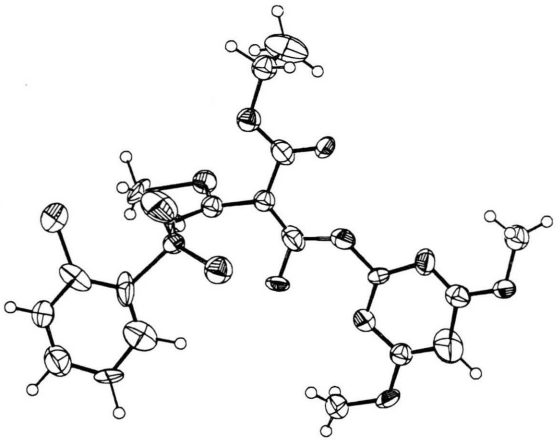
Herbicidal activity

Vinylogous sulfonylureas exhibited post-emergence herbicidal activity with mustard the most sensitive species tested. The symptoms observed (immediate growth retardation followed, for the more active compounds, by plant death after about 2 weeks) are similar to those observed for other AHAS-inhibiting herbicides. No activity against barley or ryegrass was detected except for some growth-retarding effects. Levels of herbicidal activity on mustard reflected the pI_{50} values obtained for AHAS inhibition and, as expected, the nature of the pyrimidine substituents exerted a dramatic effect on the herbicidal efficacy of the compounds. The most active compound, the 4,6-dimethoxypyrimidinyl ester **1c,** showed growth-retarding effects at spray rates down to 16 g ha^{-1}. This compound **1c** showed herbicidal effects (80% plants killed) at spray rates down to 125 g ha^{-1}, whereas the 4-methoxy-6-methyl compound **1b** required a spray rate of 4 kg ha^{-1} (a 32-fold increase in concentration) before a similar effect was observed, and the 4,6-dimethyl analogue **1a** showed virtually no herbicidal effects. In a series of benzene sulfonylurea herbicides the herbicidal activity was reported [20] to be greatest for the 4-methoxy-6-methylpyrimidin-2-yl compound, followed by the 4,6-dimethyl and then the 4,6-dimethoxypyrimidinyl analogues. In a herbicidal series of pyrimidinyl salicylates [4], the 4,6-dimethylpyrimidine is less herbicidal than the 4-methoxy-6-methyl and 4,6-dimethoxypyrimidines, but the differences are negligible. Similarly, for series of 2-(pyrimidin-2-yloxy)-alkanoic acids [5], the nature of the pyrimidinine substituents (methyl or methoxy)

had no discernible effect on herbicidal activity. Therefore it appears that the vinylogous sulfonylureas are unusual among AHAS inhibitor herbicides in that the substituents on the pyrimidine ring exert a marked effect on activity. The N-phthalylvaline amides are not herbicidal.

X-ray structure analysis

The crystal structrures of the vinylogous sulfonylureas examined (**1c** and **1j**) showed several features of possible relevance to inhibitor binding (Fig. 4, 5 and 7). The configuration of the substituents was clearly established and it was found, surprisingly perhaps, that the small substituents



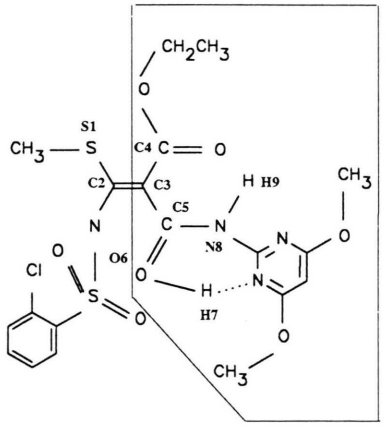
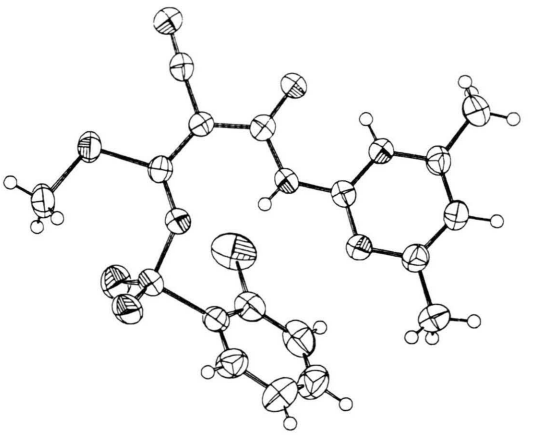


Fig. 4. X-ray crystal structure of **1c** showing restricted planar region (outlined by box), *cis* double bond carbonyl system C2–C3–C5–O6, *trans* amide O6–C5–N8–H9, the displaced proton (H7), and assumed hydrogen bonding (dotted line).



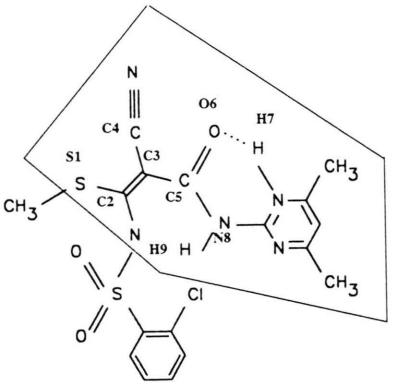


Fig. 5. X-ray crystal structure of **1j** showing extended planar region (outlined by box), *trans* double bond carbonyl system C2–C3–C5–O6 and *trans* amide O6–C5–N8–H9.

(the methylthio group and either the ethoxycarbonyl group or the nitrile function) are in a *cis* conformation. Another unusual feature noted in the crystal structures of both compounds was that the acidic proton (H7) was not observed associated with the sulfonylamino function. For the nitrile **1j** (Fig. 5) this proton was observed transferred to the planar pyrimidineamide portion of the molecule, but for the ester **1c** (Fig. 4) it was not detected.

Although the X-ray structure of chlorsulfuron does not appear to have been published, Camilleri *et al.* infer from an NMR study [21] that this molecule has a planar conformation stabilized by intramolecular hydrogen bonding. This observation implies that the amide is in an unusual *cis* confor-

mation. For both vinylogous sulfonylureas examined, the pyrimidinylcarbamoyl portion of the molecule is also planar, but the amide (O6–C5–N8–H9 in both structures) is in the *trans* conformation. Interestingly though, this conformation is stabilized in **1j** by intramolecular hydrogen bonding which is evident between the displaced acidic proton (H7) and the amide carbonyl oxygen. However, hydrogen bonding may not be required for planarity, as the unrelated N-phthalylvaline anilide (**2b**) (Fig. 6) also has the pyrimidine ring coplanar with the amide system in the absence of hydrogen bonding. There are several major differences observed between the crystal structures of the active compound **1c** and the inactive analogue **1j.** Although both compounds show the amide function in the *trans* conformation, **1c** shows a *cis* arrangement of the carbonyl (C5–O6) bond and the double bond (C2–C3) whereas **1j** has the corresponding bonds in the *trans* conformation. The compounds also differ markedly in the extent of electron delocalization as demonstrated by coplanarity. In compound **1j** the torsion angle

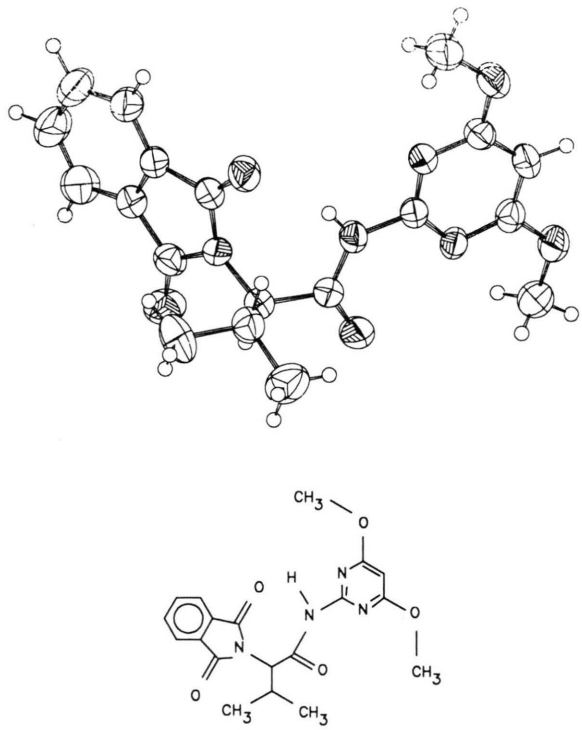


Fig. 6. X-ray crystal structure of N-phthalyl-valine 4,6-dimethoxypyrimidin-2-ylamide **2b.**

Fig. 7. Crystal structures of **1c** and **1j** viewed approximately perpendicularly to the pyrimidinylcarbamoyl plane to show the relative orientations of the 2-chlorophenyl rings.

S1–C2–C3–C4 is −5.9° and the length of the double bond 1.414(3) Å, showing coplanarity of the bonds adjacent to the double bond and a shorter C2–C3 bond compared with a torsion angle for S1–C2–C3–C4 in **1c** of −79.6° and a bond length for C2–C3 of 1.496(4) Å. It would appear that the coplanar pyrimidinylcarbamoyl system is more localized in the active compound **1c,** even though the presence of electron donating methoxy groups on the pyrimidine might have been expected to exert the opposite effect. The differences between torsion angles for S1–C2–C3–C4 are translated into differences between the overall orientations of the 2-chlorophenyl ring to the pyrimidinylcarbamoyl system, as shown in the view of the two compounds seen in Fig. 7.

Conclusions

Compared with the classic sulfonylureas, the vinylogous sulfonylureas (VSUs) exhibit moderate AHAS inhibition or herbicidal activity. Although the peripheral groups were chosen to mimic chlorsulfuron, it is surprising that these much larger molecules show similar biological activity and retain some features usually associated with sulfonylureas, such as a loss of activity when the pyrimidine ring is replaced by a phenyl ring. The most interesting feature of the series is the marked variation in biological activity with changes in the pyrimidine substitution. The correlation between activity and the proton NMR shift of the pyrimidine H5 proton suggests that the activity of this class of compounds is influenced by the electron density in the pyrimidine ring. The VSUs are larger than the classical sulfonylureas, so it is likely that only a portion of the vinylogous sulfonylurea molecule can occupy the chlorsulfuron-binding site, and it is probable that the pyrimidine portion of the molecule is important for binding. There-

fore it is possible that other related compounds with different substituents in the "right-hand" side, not tested in this study, may also show herbicidal activity. It is also likely that only certain conformations of VSUs can bind, and that the conformation adopted by **1c** allows binding whereas that adopted by the inactive **1j** does not. It is interesting that the initial concept, that of introducing a large-bridging group into a sulfonylurea structure in parallel with the structural relationship between the cyanoacrylates and ureas such as diuron, yielded active compounds. However, at this stage, it would only be speculation to infer that this indicated a similarity between the two binding sites, possibly based on their quinone-binding properties. It is most likely that the observed activity of the VSUs is merely testament to the ability of the enzyme to interact with a very large variety of chemically diverse inhibitors.

Acknowledgements

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