Famoxadone: the discovery and optimisation of a new agricultural fungicide†

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Abstract: Famoxadone (3-anilino-5-methyl-5-(4-phenoxyphenyl)-1,3-oxazolidine-2,4-dione), is a new agricultural fungicide recently commercialized by DuPont under the trade name Famoxate®. Famoxadone is a member of a new class of oxazolidinone fungicides that demonstrate excellent control of plant pathogens in the Ascomycete, Basidiomycete, and Oomycete classes that infect grapes, cereals, tomatoes, potatoes and other crops. DuPont's entry into the oxazolidinone area resulted from the procurement of 5-methyl-5-phenyl-3-phenylamino-2-thioxo-4-oxazolidinone (1) from Professor Detlef Geffken, then at the University of Bonn. An extensive analog program was initiated immediately after the fungicidal activity of 1 was discovered through routine greenhouse testing. The discovery program in the oxazolidinone area eventually culminated in the advancement of famoxadone to commercial development in the early 1990s. The synthesis of various oxazolidinone ring systems and the development of the structure-activity relationships that led to the discovery of famoxadone are described.

Keywords: oxazolidinone; oxazolidinedione; famoxadone; discovery; fungicide; chemistry

1 INTRODUCTION

The discovery of efficacious and environmentally benign agrochemicals is becoming increasingly more challenging as business and regulatory pressures continue to rise. A discovery strategy that has proven successful for DuPont, as well as for other agricultural chemical companies, is the procurement and screening of novel compounds from academic and industrial sources around the world. This strategy has provided a large and diverse array of novel compounds at a relatively low cost.

DuPont's entry into the oxazolidinone area resulted from the procurement of 5-methyl-5-phenyl-3-phenylamino-2-thioxo-4-oxazolidinone (Fig 1; **1**) from Professor Detlef Geffken, then at the University of Bonn, Germany. Greenhouse testing of **1** in fungicide screens showed 100% preventive control of tomato late blight (*Phytophthora infestans* (Mont) de Bary) at 200 mg litre^{-1}, and 97% preventive control of grape downy mildew (*Plasmopara viticola* Berl and de Toni) at 10 mg litre^{-1}. Preventive control of these Oomycete pathogens was a key fungicide product concept for DuPont at the time, and the potency of the lead compound was extremely intriguing. Vigorous chemical optimization programs ensued in both the DuPont and Geffken laboratories, and new syntheses were developed to facilitate the preparation of closely related analogs, as well as to modify the core oxazolidinone ring. The mode of action of the oxazolidinones was determined to be inhibition of mitochondrial electron transport at complex III. *In vitro* assays were developed which supplemented data provided from greenhouse and field evaluations, and they greatly enhanced the optimization effort. After three years of work and the preparation of over 700 analogs, famoxadone was advanced to commercial development.[1–4] Not only was the discovery program successful in ultimately bringing a product to market,

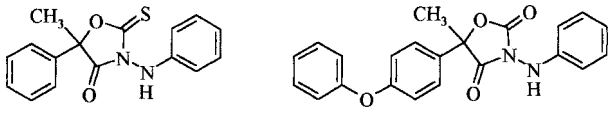

Figure 1. Lead compound from D Geffken (**1**) and famoxadone.

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it also exemplified a true collaboration between DuPont and the laboratory of Professor Geffken.

2 EXPERIMENTAL METHODS

2.1 Chemicals

Most chemical starting materials were obtained from Aldrich Chemical (Milwaukee, WI, USA), Sigma Chemical (St Louis, MO, USA), VWR (Trenton, NJ, USA) or Fluka Chemical (Buchs, Switzerland).

2.2 Chemical methods

Melting points were determined on a Thomas Hoover Unimelt capillary melting point apparatus. [^{1}H]NMR spectra were recorded on a Varian EM 390 (90 MHz) or a Varian XL-200 spectrometer (200 MHz) using tetramethylsilane as an internal standard, in either deuterochloroform or deuterodimethyl sulfoxide solution (chemical shifts in δppm). IR spectra were recorded on a Mettler FP61 or a Perkin Elmer 1600 series FTIR. Thin-layer chromatograms were developed on glass pre-coated with silica gel F_{254}. The spots were revealed under UV light or with iodine.

2.2.1 Synthesis of famoxadone

A mixture of ethyl 2-(4-phenoxyphenyl)lactate (0.052 mol), 1,1′-carbonyldiimidazole (0.06 mol) and dry dichloromethane (100 ml) was agitated at 25 °C for 19 h. Water (30 ml) was then added and the mixture was agitated for 15 min. Acetic acid (5 ml) and phenylhydrazine (0.069 mol) were then added, and the mixture agitated at 25 °C for 24 h, after which water (100 ml) was added followed by hydrochloric acid to lower the pH to 2; the aqueous layer was then removed. After washing the dichloromethane layer with water (50 ml), the solvent was removed under vacuum and the oily residue was mixed with hexane (150 ml) and ethyl acetate (15 ml), heated to 65 °C, cooled to 20 °C, and then filtered. The solids were washed with a mixture of ethyl acetate (20 ml) and hexane (80 ml) and then dried to give the product (0.041 mol). Mp 140–142 °C; [^{1}H]NMR ($CDCl_3$) δ: 7.55 (m, 2H); 7.40–7.10 (m, 8H); 7.07 (m, 4H); 6.75 (d, 2H); 6.07 (s, 1H); 1.99 (s, 3H).

2.3 Greenhouse testing

Aqueous suspensions of test compounds were sprayed onto grape or tomato seedlings to the point of run-off and afterwards the plants were placed in a drying chamber. The following day the seedlings were inoculated with a spore suspension of *P viticola*, (the causal agent of grape downy mildew) or *Ph infestans* (the causal agent of tomato late blight) and then incubated in a water-saturated atmosphere for 24 h at 20 °C. The plants were transferred to another growth chamber for 6 days of incubation at 20 °C. Grape plants were then incubated for 24 h in a water-saturated atmosphere at 20 °C, after which disease ratings were made on both grape and tomato plants. Percentage control was calculated by comparing the percentage disease estimates for the treated plants with those of the untreated controls. On average, 98% and 85% of disease were present on untreated control tomato and grape plants, respectively.

Percentage disease control data in Tables 2–6 and 8 are mean values from multiple tests conducted during the course of this work. Each test was performed with three replicates. The average standard error measurement (SEM) percentage values for the tests performed during this period are listed in Table 1.

2.4 Mitochondrial preparations

All buffers and purification procedures described in this section were at 0–4 °C.

2.4.1 Rat heart mitochondria

Rat hearts were isolated using an established procedure at Haskell Laboratory (Newark, DE, USA). Immediately after extraction the hearts were placed in sodium chloride solution (20 g litre^{-1}) and then washed with buffer A, which contained sucrose (250 mM), Hepes–sodium hydroxide (20 mM) and EDTA (0.1 mM; pH 7.5). The hearts were minced with a razor blade and homogenized in buffer A (1 + 4, w/v) using a Dounce homogenizer with a loosely fitting pestle, followed by a ground-glass pestle. The homogenate was centrifuged for 10 min at 900 *g*. The supernatant was collected and centrifuged for 40 min at 30 000 *g*. The resulting submitochondrial pellet was homogenized in buffer A to 20 mg of protein ml^{-1}, flash frozen in liquid nitrogen and stored at −80 °C until used.

2.4.2 Phytophthora mitochondria

Ph infestans sporangia were isolated by washing V8 agar plates with cold water and filtering the washings through glass wool. Sporangia were incubated at 12 °C for 1–4 h and were observed under a microscope to determine when zoospores were being released. When many zoospores were observed, aliquots of culture medium were mixed with buffer A (1 + 1 by volume). Following two 30-s bursts in a mini-bead beater, the lysate was centrifuged for 10 min at 600 *g*. The supernatant was centrifuged for 20 min at 20 000 *g* and the mitochondrial pellet was suspended in a minimal volume of buffer A. Mitochondria were used for inhibition studies within 1–2 h of isolation.

Table 1. Average standard error measurement (SEM) percentage values for disease control data

	SEM (±) for	
Data mean range	Ph infestans	P viticola
0–10	5.1	9.8
45–55	16.7	17.3
75–85	9.2	10.4
85–95	5.8	6.9
95–100	1.8	1.4

2.5 Determination of enzyme inhibition

Reaction mixtures (1 ml) were held in quartz cuvettes containing potassium phosphate (0.1 M) and cytochrome *c* (10 μM) at pH 7.5 at 25 °C. Submitochondria (5–20 μg of protein) was added to the reaction mixture 10 min before the addition of inhibitor ethanol (5 μl) which was immediately followed by the addition of NADH (100 nmol) in ethanol (10 μl). The reaction (NADH to O_2) was monitored continuously for 1 min at 340 nm using an HP 8542A diode array spectrophotometer. Linear initial rates were fitted to a line using the spectrophotometer's software. Inhibition constants were determined by fitting rates to eqn (1), where v is the observed initial rate, V is the uninhibited rate, I is the inhibitor concentration and IC_{50} is the concentration of inhibitor which gives 50% inhibition.

$$v = \frac{V}{1 + I/IC_{50}} \quad (1)$$

3 RESULTS AND DISCUSSION

3.1 Mode of action

Famoxadone is a potent inhibitor of mitochondrial electron transport blocking the function of ubiquinol:cytochrome *c* oxidoreductase (bc_1, complex III).[5,6] Inhibitors at this site have been classified into three groups according to their interactions with the bc_1 cytochrome. Oxazolidinones belong to group I, whose other members include myxothiazol, strobilurins and stigmatellins. Whereas all of these materials bind in the Q_o domain of the enzyme complex, oxazolidinones are chemically distinct and represent a new class within group I.

Biological effects observed for oxazolidinones were consistent with inhibition of mitochondrial function. When zoospores of the plant pathogen *Ph infestans* were treated with famoxadone, oxygen consumption ceased and the zoospores lost motility within seconds.[6] Within minutes the zoospores disintegrated.[6] When mycelia of *Ph infestans* were treated with an oxazolidinone, protein and nucleic acid biosyntheses were curtailed.[6]

3.2 Synthesis

3.2.1 Synthesis routes to oxazolidinones

The synthesis of various oxazolidinone ring systems had already been reported in the literature when DuPont became interested in this class of chemistry.[7–14] The syntheses used to prepare 3-amino-oxazolidinones are illustrated in Fig 2.[10,11] Treatment of *N*-methyl-2-hydroxyhydroxamic acid **2** with 1,1′-carbonyldiimidazole (CDI) afforded the dioxazinedione **3a**. Cyclization could also be accomplished using phosgene and triethylamine, albeit in lower yield. Reaction of the hydroxamic acid **2** with 1,1′-thiocarbonyldiimidazole (TCDI) gave the corresponding thioxodioxazinone **3b**. The dioxazinedione (**3a** or **3b**) was then treated with a monosubstituted hydrazine to give the 3-amino-oxazolidinone (**4a** or **4b**, respectively). The five-membered ring structure was confirmed by [^{1}H]NMR and IR analyses, and none of the isomeric six-membered ring oxadiazinones were observed.[15,16]

These syntheses were quite general and afforded appropriately high yields of oxazolidinones, but the multi-step preparation of the starting hydroxamic acids limited our progress. A shorter route was envisaged involving treatment of 2-hydroxy hydrazides with a carbonylating agent, as illustrated in Fig 3. The glycolic acid hydrazide **5a** reacted smoothly with TCDI to form the five-membered ring 2-thioxo-4-oxazolidinone **6a** in good yield. The reaction of mandelic acid hydrazide **5b** with TCDI did not proceed as cleanly and a number of products were formed. However, the reaction with CDI produced mainly the desired 2,4-oxazolidinedione **6b**. Hydrazides bearing a tertiary alcohol, such as **5c**, reacted in an entirely different manner. Treatment with TCDI afforded the oxadiazol-2(3*H*)-one **7** in high yield without any of the desired 2-thioxo-4-oxazolidinone **1**. Apparently, in this case, acylation of the aniline nitrogen proceeded faster than acylation of the hydroxyl group, and subsequent cyclization led to the five-membered ring oxadiazol-2(3*H*)-one.[17] This synthetic limitation was a significant set-back, since the 5,5-disubstituted compounds had significantly

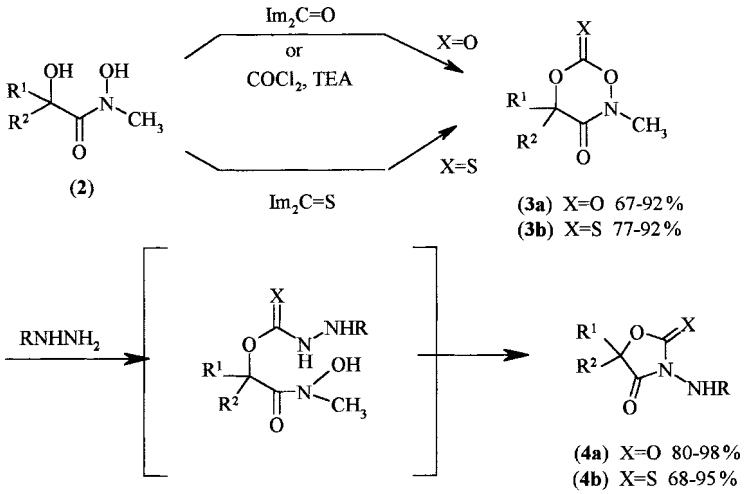


Figure 2. Literature syntheses of 3-amino-oxazolidinones.

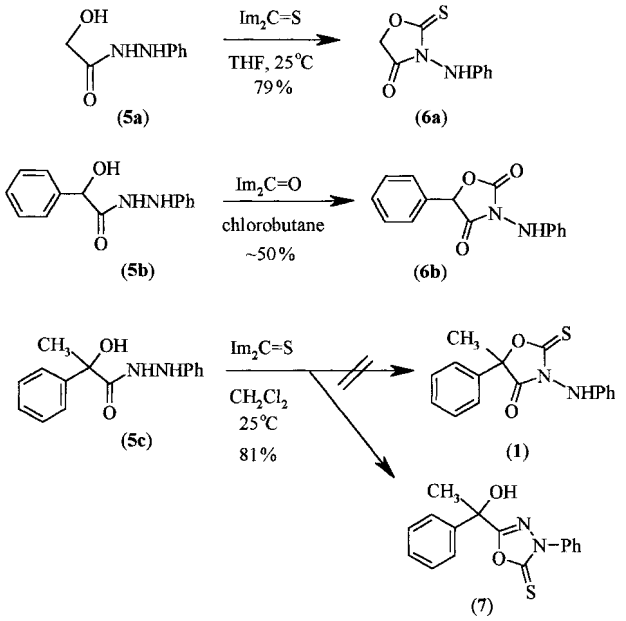


Figure 3. 3-Phenylamino-oxazolidinones from 2-hydroxy hydrazides.

higher fungicidal activity and, therefore, an alternative synthetic route was pursued.

An efficient 'one-pot' synthesis of 5,5-disubstituted 2-thioxo-4-oxazolidinones was developed starting from readily available 2-hydroxy esters (Fig 4). Sequential treatment of the ester **8** with potassium *tert*-butoxide, carbon disulfide, ethyl chloroformate and finally phenylhydrazine afforded the 2-thioxo-4-oxazolidinone in good to excellent yield. The procedure was fairly general and the product could often be purified without chromatography. The only disubstituted 2-hydroxy esters which worked poorly in this reaction were those bearing strongly electron-withdrawing groups (eg $R^1 = CF_3$), powerful electron-donating groups (eg $R^2 = 4\text{-}(CH_3)_2N\text{–Ph}$), or those with sterically hindered hydroxyl groups (eg $R^2 = 2,5\text{-}(CH_3)_2\text{–Ph}$).

Attempts to prepare the corresponding oxazolidinediones using the same methodology were unsuccessful. Treatment of the 2-hydroxy ester **8** with a base, carbonyl sulfide instead of carbon disulfide and, finally, an *S*-alkylating agent, failed to afford the thiolcarbamate. Carbonyl sulfide was apparently insufficiently electrophilic to react with the tertiary alkoxide.

2,4-Oxazolidinediones could, however, be obtained in high yield by hydrolysis of the corresponding 2-thioxo-4-oxazolidinone with aqueous potassium per-

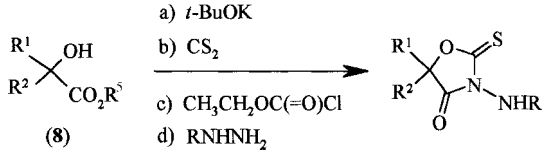


Figure 4. One-pot synthesis of 5,5-disubstituted 2-thioxo-4-oxazolidinones.

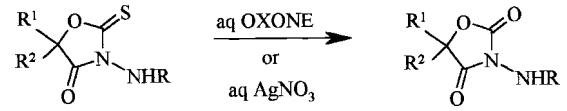


Figure 5. Conversion of thioxo-oxazolidinones to oxazolidinediones.

oxymonosulfate (Oxone®) or aqueous silver nitrate[7,18] (Fig 5). Not only did this procedure provide a route to 2,4-oxazolidinediones in high yield, but it also generated both the oxo- and thioxo-compounds for biological evaluation from a single synthetic sequence.

Based on considerations of biological activity, cost and waste generation, interest shifted from the 2-thioxo-4-oxazolidinones to the 2,4-oxazolidinediones. It therefore became desirable to develop a synthesis that provided these compounds directly, and avoided the inefficiency of introducing a sulfur atom and then subsequently removing it. Biological results also suggested that the 5-alkyl-5-aryl-3-phenylamino-oxazolidinediones were the most desirable for maximum fungicidal activity, and therefore efforts were directed towards compounds with this substitution pattern.

A phosgene-based route was attempted first (Fig 6). Treatment of the 2-hydroxy ester **8** with phosgene and *N,N*-diethylaniline (DEA) with a catalytic amount of pyridine produced the chloroformate **9**. The chloroformate was not purified but treated directly with phenylhydrazine and *N,N*-diisopropylethylamine. Cyclization of the resulting carbazate was slow at room temperature, and therefore the reaction was heated to reflux in the presence of triethylamine (TEA). Yields were generally low, several by-products were observed (eg the acrylate **10**) and unreacted starting material was generally recovered despite efforts to drive the phosgene reaction to completion. We now believe that starting material was in fact consumed, and the intermediate chloroformate **9** decomposed to the tertiary chloride under the reaction conditions; the tertiary chloride then hydrolyzed back to the starting 2-hydroxy ester during the aqueous work-up.

The need for a higher-yielding route to 2,4-oxazolidinediones led to the development of the route illustrated in Fig 7. Treatment of the 2-hydroxy ester **8** with CDI formed the acylimidazole **11**, which was not isolated but treated directly with phenylhydrazine and acetic acid (HOAc) to give the 2,4-oxazolidinedione. In general, yields were good and the products did not require chromatography for purification. Acetic acid appeared to be critical to the success of the reaction because without it no product was observed. It was postulated that acetic acid protonated the imidazolyl ring and thereby activated the carbonyl to nucleophilic attack. Stronger acids, such as hydrochloric acid, gave no reaction, presumably due to irreversible protonation of the phenylhydrazine, rendering it non-nucleophilic.

With a general, direct and high-yielding route to oxazolidinediones now available, a variety of R^1 and R^2 substituents could be surveyed quickly and easily. A

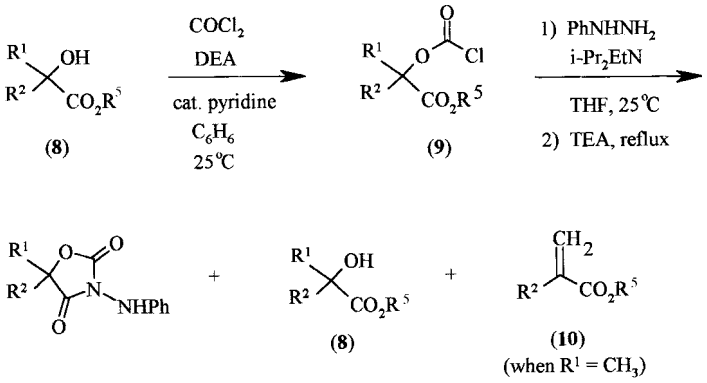


Figure 6. Preparation of 2,4-oxazolidinediones using phosgene.

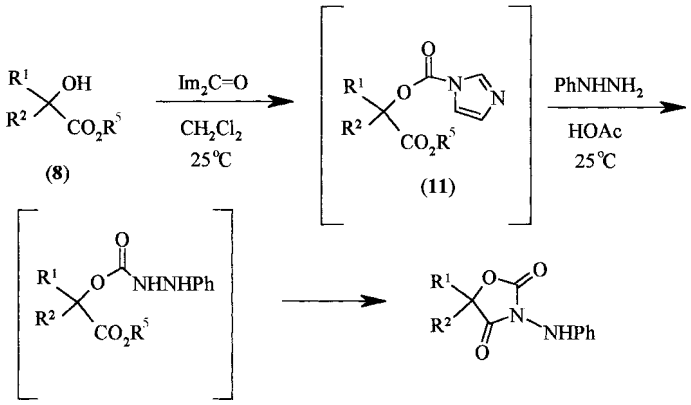


Figure 7. Preparation of 2,4-oxazolidinediones using 1,1′-carbonyldiimidazole (CDI).

wide assortment of 2-hydroxy esters was all that was required. As illustrated in Fig 8, 2-hydroxy esters are common building blocks and readily accessible by a number of synthetic routes.[19]

3.2.2 Chiral oxazolidinones

The majority of compounds prepared during the course of the optimization program were racemates possessing a single stereogenic center at C-5 of the oxazolidinone ring. An obvious early question was whether some, or all, of the biological activity resided in one of the two enantiomers. Milligram quantities of the two enantiomers of the original 2-thioxo-4-oxazolidinone (**1**) were obtained and greenhouse testing indicated that most of the fungicidal activity resided in the (*S*)-enantiomer. Later in the discovery program, multigram quantities of (*S*)-famoxadone were frequently required and a more efficient synthetic sequence was needed. A route incorporating classical resolution of 2-(4-phenoxy-phenyl)-lactic acid (**12**) proved to be the most expeditious (Fig 9). A variety of commercial homochiral amines was tested and quinidine found to be optimal in terms of selectivity and crystallinity of only one of the diasteromeric salt products. Treatment of the 2-hydroxy acid **12** with 0.55 equivalents of quinidine followed by separation, esterification and cyclization with CDI and phenylhydrazine afforded (*R*)- and (*S*)-famoxadone.

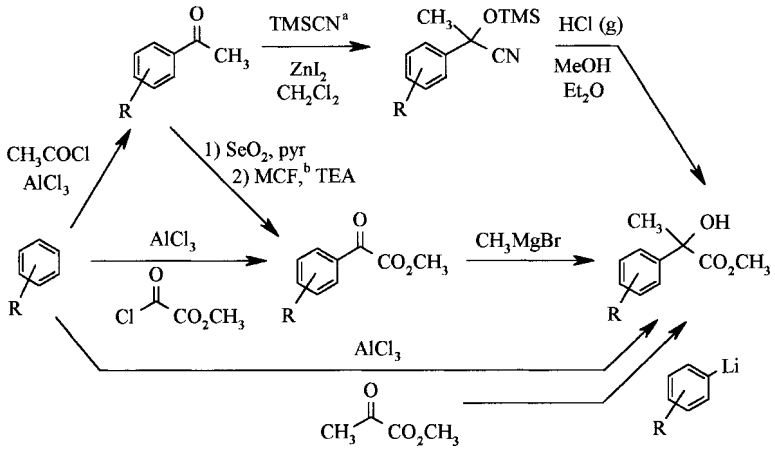


Figure 8. Methods for preparing 2-(substituted phenyl)lactate esters. [a]TMSCN = trimethylsilyl cyanide. [b]MCF = methyl chloroformate.

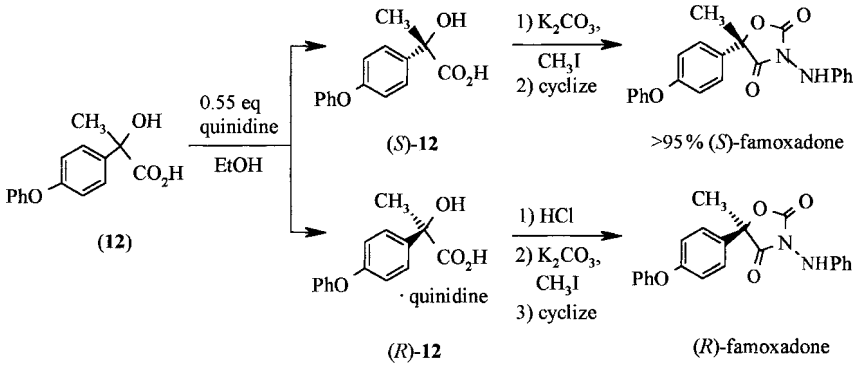


Figure 9. Preparation of (*R*)- and (*S*)-famoxadone by classical resolution.

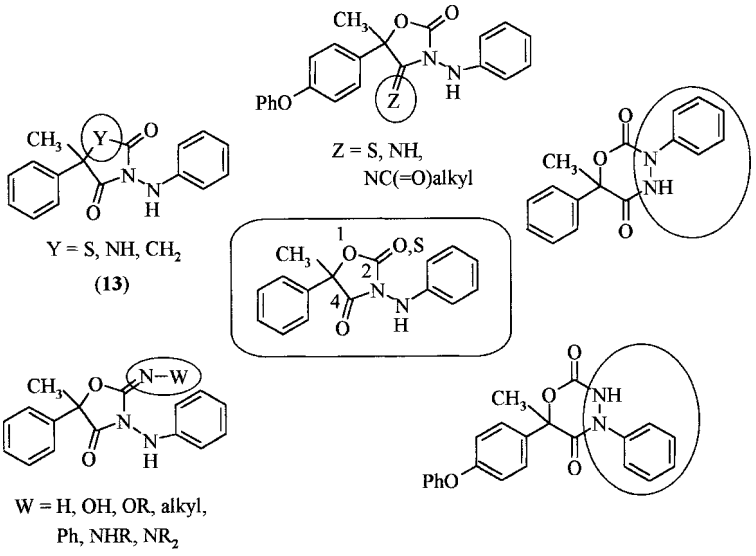


Figure 10. Ring analogs of 2-thioxo-4-oxazolidinone and 2,4-oxazolidinediones.

3.2.3 Ring analogs

Variations of the central 2-thioxo-4-oxazolidinone or 2,4-oxazolidinedione ring were also investigated as part of the optimization program (Fig 10).[20] Modifications included replacement of oxygen at the 1-position with sulfur, nitrogen or carbon; preparation of 2-imino analogs such as imines (=NR), oximes (=NOR) and hydrazones (=NNR_2),[21] formation of 4-thiono (=S) and 4-imino (=NR) derivatives,[22] and the synthesis of two 6-member ring analogs. The chemistry and biological activity of most of these analogs will be the subject of future publications. The one modification that is discussed in greater detail below is that of the 1-position replacement analogs (**13**).

Figure 11 illustrates the preparation of the sulfur analog **13a**. Treatment of methyl atrolactate (**14**) with sulfonyl chloride and catalytic DMF afforded the tertiary chloride **15** with a large amount of the corresponding acrylate as a by-product. The two were not separated but the mixture was treated with the phenyl thiolcarbazate salt **16** and heated to give the thio analog **13a**. However, the yield was low and a large amount of the methyl thiol carbazate **17** was also

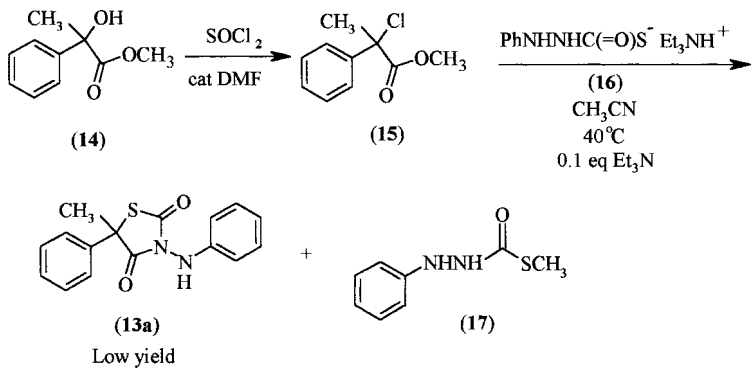


Figure 11. Synthesis of ring analog **13a** with sulfur at the 1-position instead of oxygen.

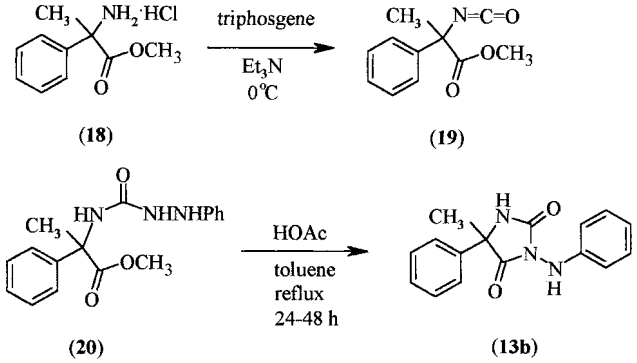


Figure 12. Preparation of hydantoin ring analog **13b**.

isolated. As expected, the carbonyl stretching frequencies in **13a** were lower than in the oxazolidinedione, due to the change in ring geometry caused by the larger sulfur atom.

The 1-NH ring analog **13b** was also prepared as shown in Fig 12. Treatment of the amino ester **18** with triphosgene afforded the isocyanate **19**. Subsequent condensation with phenylhydrazine afforded the *N*-anilinourea **20**, which was then cyclized with acetic acid and heat to give the desired hydantoin **13b**. An attempt to methylate **13b** with methyl trifluoromethyl sulfonate failed to give either the *N*- or *O*-methyl derivative. The 1-NCH_3 derivative of the hydantoin derived from the mandelate ester was ultimately prepared (ie the analog lacking the 5-methyl group) by a different chemical route.

The final analog prepared in this series was the *N*-anilino succinimide **13c** wherein the 1-oxygen was replaced with CH_2 (Fig 13). Condensation of acetophenone with ethyl cyanoacetate afforded the cinnamate **21** as a mixture of (*E*)- and (*Z*)-isomers. Conjugate addition of cyanide followed by decarboxylation afforded the nitrile **22**, which was then saponified with potassium hydroxide to afford the diacid **23**. Activation of the acid with acetyl chloride followed by reaction with phenyl hydrazine afforded the open-chain hydrazide, which was then cyclized with heat to give the succinimide **13c**.

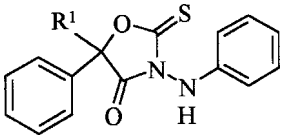
3.3 Structure–activity relationships

3.3.1 Effect of various substituents on the 2,4-oxazolidinedione ring

Over 700 analogs were prepared in the oxazolidinone area. The following structure-activity relationships were established based on testing in greenhouse fungicide screens.

The data in Table 2 illustrate the structure-activity relationships for R^1, which is methyl in compound **1**, and clearly indicates that steric bulk has a significant effect on biological activity. For example, changing R^1 from methyl to ethyl reduced preventive control of *Ph infestans* and *P viticola* eightfold and fourfold, respectively (**1** vs **25**). The $R^1 = n$-butyl compound **27** showed no activity against these diseases at the rates indicated. Reducing the size of R^1 from methyl (**1**) to hydrogen (**24**) also lowered activity. These data and others suggested that a methyl group was the optimal substituent at this position.

Table 2. Effect of various R^1 groups on preventive control of *Phytophthora infestans* on tomatoes and *Plasmopara viticola* on grapes



		Disease control (%)	
Compound	*R^1*	Ph infestans[a]	P viticola[b]
1	CH_3	100	97
24	H	86	8
25	CH_3CH_2	33	23
26	$CH_2=CHCH_2$	0	41
27	$CH_3(CH_2)_2CH_2$	0	0

[a] 200 mg litre^{-1}.
[b] 10 mg litre^{-1}.

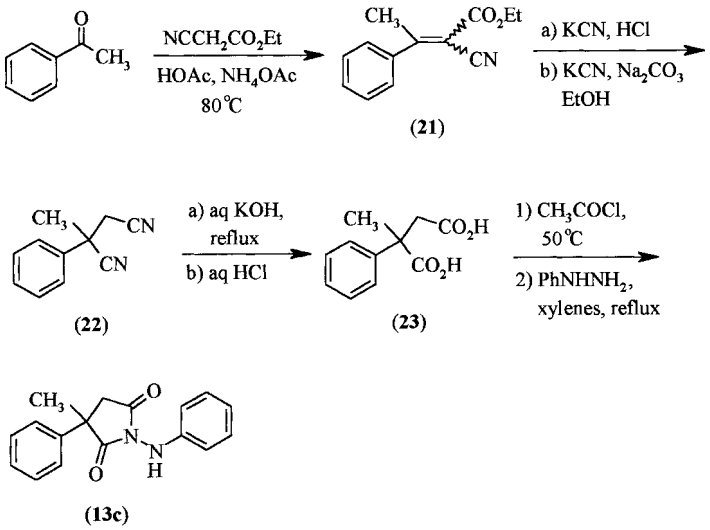
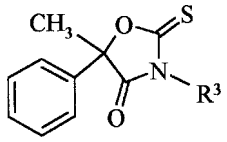


Figure 13. Synthesis of succinimide ring analog **13c**.

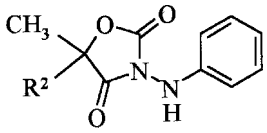
Table 3. Effect of various R^3 groups on preventive control of *Phytophthora infestans* on tomatoes and *Plasmopara viticola* on grapes



Compound	R^3	Disease control (%) Ph infestans[a]	P viticola[b]
1	PhNH	100	100
28	Ph	0	15
29	$PhCH_2$	0	43
30	$PhCH_2NH$	15	5
31	(Cyclohexyl)NH	0	65
32	(2-Pyridyl)NH	25	68
33	(2-CH_3-Ph)NH	92	100
34	(2-Cl-Ph)NH	38	99
35	(3-CH_3-Ph)NH	90	91
36	(3-F-Ph)NH	100	100
37	(3-CH_3O-Ph)NH	94	97
38	(4-F-Ph)NH	97	100
39	(4-CH_3O-Ph)NH	0	73
40	(4-CF_3-Ph)NH	0	89

[a] 200 mg litre^{-1}.
[b] 40 mg litre^{-1}.

Table 5. Effect of R^2 on preventive control of *Phytophthora infestans* on tomatoes and *Plasmopara viticola* on grapes



Compound	R^2	Disease control (%) Ph infestans[a]	P viticola[b]
44	$CH_3(CH_2)_4CH_2$	100	94
45	Cyclohexyl	48[b]	29
46	Ph	88	48
47	2-F-Ph	100	100
48	2-CH_3-Ph	17	44[a]
49	3-Br-Ph	100[c]	97
50	3-Cl-Ph	100	86
51	3-CH_3-Ph	42	8
52	3-CF_3O-Ph	60	6
53	4-F-Ph	93	56
54	4-Cl-Ph	76	17
55	4-CH_3O-Ph	15	25
56	4-CF_3O-Ph	97	100
Famoxadone	4-PhO-Ph	95	100
57	2,4-diF-Ph	100	100

[a] 200 mg litre^{-1}.
[b] 40 mg litre^{-1}.
[c] Data for *Ph infestans* on potatoes.

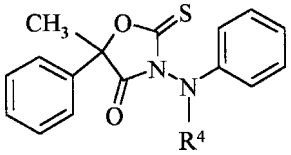
The effect of varying R^3, the group attached to the oxazolidinone ring nitrogen atom which is NHPh in **1**, was also investigated (Table 3). The data for compounds **28**–**32** suggest that an anilino or substituted anilino moiety at this position is critical for good activity. Two oxazolidinones with R^3 = OPh were also prepared and these too were significantly less active, especially against *Ph infestans*. Data for compounds **33**–**40** indicate that fungicidal activity generally decreased, sometimes dramatically, when substituents of any type were introduced onto the phenyl ring. The only substituted compounds that appeared to have comparable activity were the 2-CH_3, 3-F and 4-F compounds **33**, **36** and **38**; however, additional testing at lower concentrations showed that they too were less active than **1**. As with R^1, the optimal substituent was the one in the lead compound (**1**).

Somewhat surprisingly, a hydrogen atom on the exocyclic nitrogen was not essential for fungicidal activity. As indicated in Table 4, the *N*-methyl compound **41** was also active, although less so than **1**. The *N*-ethyl and *N*-acetyl compounds, **42** and **43** respectively, were significantly less active, as were oxazolidinones with larger *N*-alkyl groups. Once again, the substituent in the lead compound **1** (R^3 = H) was best at this position.

A diverse group of substituents could be tolerated at the R^2 position, as indicated in Table 5.[23,24] Aryl groups were preferred but the alkyl-substituted compounds **44** and **45** were also active. In general, *ortho*-substitution on the R^2 phenyl ring was detrimental to activity (eg see **48**). Only a fluorine atom at this position improved fungicidal control (see **47**). Oxazolidinones with a bromine or chlorine atom at the 3-position were more active than the unsubstituted compound (**49**, **50** vs **46**), but other *meta*-substituted compounds were generally less active. A wide variety of substituents could be tolerated at the *para*-position and many groups improved activity. Two of the most active compounds were the 2,4-difluoro- and 4-phenoxyoxazolidinones, **57** and famoxadone, respectively. Famoxadone was preferred due to its lower estimated manufacturing cost and superior residual control properties.

Once it was determined that a 4-phenoxy substituent was desirable, a wide variety of substituted-

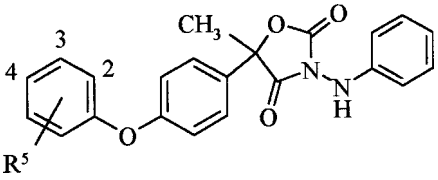
Table 4. Effect of R^4 on preventive control of *Phytophthora infestans* on tomatoes and *Plasmopara viticola* on grapes



Compound	R^4	Disease control (%) Ph infestans[a]	P viticola[b]
1	H	100	100
41	CH_3	65	82
42	CH_3CH_2	24	NT[c]
43	$CH_3C(=O)$	23	45

[a] 200 mg litre^{-1}.
[b] 40 mg litre^{-1}.
[c] NT = Not tested.

Table 6. Effect of R^5 on preventive control of *Phytopthora infestans* on tomatoes and *Plasmopara viticola* on grapes



		% Disease control	
Compound	*R^5*	Ph infestans[a]	P viticola[b]
58	2-*i*-Pr	0	84
59	2-Cl	98	68
60	2-F	100	82
61	3-F	98	97
62	3-Cl	92	100
63	3-PhO	64	100[c]
64	3-*t*-Bu	74	100[d]
65	4-Cl	97	74
66	4-Br	97	77
67	4-CH_3O	68	99
68	4-Ph	0	96[a,b,c]
69	4-*t*-Bu	0	21

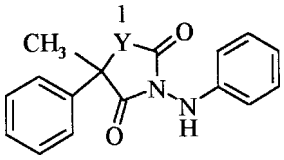
[a] 200 mg litre^{-1}.
[b] 2 mg litre^{-1}.
[c] 5 mg litre^{-1}.
[d] 40 mg litre^{-1}.

phenoxy compounds were studied (Table 6). Good activity was maintained even with large groups in the 3-position, as illustrated by **63** and **64**. Substitution in the 4-position was more limited. The 4-Ph and 4-*tert*-Bu compounds **68** and **69** were inactive against *Ph infestans* at 200 mg litre^{-1}, although the 4-Br compound **66** was quite active. In summary, some of the substituted compounds were nearly as active as famoxadone, but none were significantly more active or possessed a desirable attribute which was lacking in the unsubstituted material, and all of the substituted compounds would have been more expensive to prepare commercially.

3.3.2 *Biological activity of the enantiomers*

The enantiomers of the lead compound (**1**) and famoxadone were tested for intrinsic activity using *in vitro* assays comprised of submitochondrial particles isolated from rat heart and *Ph infestans* (Table 7). In both compounds, the (*S*)-enantiomer was superior to the (*R*)-enantiomer for inhibition of mitochondrial electron transport, and (*S*)-famoxadone was approximately tenfold more potent than (*S*)-**1**. As one might expect, the racemic mixtures were approximately half as active as the (*S*)-enantiomers, and the (*R*)-enantiomers were significantly less active. The greenhouse fungicide data on *Ph infestans* and *P viticola* were consistent with the mitochondria data. The (*S*)-enantiomers were the more active isomers, and the racemic mixtures were approximately half as active. The future commercialization of (*S*)-famoxadone as a potential renewal strategy for famoxadone is being considered.

Table 8. Activity of 1-oxygen ring analogs **13** for preventive control of *Phytophthora infestans* on tomatoes and *Plasmopara viticola* on grapes



		% Disease control	
Compound	*Y*	Ph infestans[a]	P viticola[b]
46	O	88	48
13a	S	15	0
13b	NH	77	0
13c	CH_2	35[c]	0

[a] 200 mg litre^{-1}.
[b] 40 mg litre^{-1}.
[c] Data for *Ph infestans* on potatoes.

3.3.3 *Biological evaluation of the ring analogs*

The biological activities for the 1-oxygen ring analogs **13** are shown in Table 8. Preventive control of *Ph infestans* was lower for all three analogs **13a, 13b** and **13c** relative to the 2,4-oxazolidinedione (**46**), and

Table 7. Selectivity of enantiomers of **1** and famoxadone for inhibition of electron transport in submitochondria and for greenhouse control of *Plasmopara viticola*

	Electron transport IC_{50} (ng ml^{-1}) (±SEM)		*Disease control of*
Compound	*Rat heart*	Ph infestans	P viticola *ED_{90} (μg ml^{-1})*[a]
(*R, S*)-**1**	45 (±3)[b]	13 (±2)	10
(*R*)-**1**	1400 (±20)	450 (±14)	80
(*S*)-**1**	17 (±2)	3.8 (±0.9)	3
(*R, S*)-Famoxadone	4.5 (±1.4)[c]	—	3
(*R*)-Famoxadone	200 (±11)	—	10
(*S*)-Famoxadone	2.2 (±0.6)	—	0.4

[a] ED_{90} values were estimated from DuPont plant disease control reports. Dose rates used were 200, 40, 10, 2 and 0.4 mg litre^{-1}.
[b] Mean and standard deviation from five determinations.
[c] Mean and standard deviation from 11 determinations.

there was no preventive control of *P viticola* at 40 mg litre^{-1}. However, other ring analogs illustrated in Fig 10 possessed significant fungicidal activity and will be discussed at a future date. In fact, fenamidone the emerging fungicide from Aventis, can be considered a ring analog of the 2,4-oxazolidinediones.

In summary, the structure-activity relationships indicated that R^1 and R^3 were sensitive to structural variation, whereas compounds with a wide variety of R^2 groups were fungicidally active. In the end, a 4-phenoxyphenyl group was found to be optimal at this position. This project ultimately led to the advancement of famoxadone to commercial development for the control of fungal infections of grapes, cereals, tomatoes, potatoes, and other crops. Additional work in the area, eg analogs with heterocyclic substituents and process development studies,[19] will be the subject of future publications.

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