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The Synthesis of Novel Acetolactate Synthase Inhibitors, N-(Asymmetrically Disubstituted Phosphoryl)-N′-(4,6-Dimethoxypyrimidin-2-yl) Ureas*

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ABSTRACT: *In view of the isosterism of the sulfonyl group(-SO_2-) and the phosphoryl group, two new types of compounds N-(N-aryl-O-alkyl phosphoryl)-N′-(4,6-dimethoxypyrimidin-2-yl) ureas* **(2)** *and N-(N-aryl-N-alkylphosphoryl)-N′-(4,6-dimethoxypyrimidin-2-yl) ureas* **(3)** *were designed and synthesized by treating N-(arylaminochlorophosphoryl)-N′-(4,6-dimethoxypyrimidinyl-2-) ureas* **(4)** *with alcohols or amines. Compounds* **4** *were obtained by treating dichlorophosphoryl isocyanate with 4,6-dimethoxy-2-aminopyrimidine and then with aromatic amines. The enzyme tests in vitro indicated that compounds* **2** *and* **3** *were two novel classes of acetolactate synthase (ALS) inhibitors and also showed that phosphoryl groups [-P(O)(OR)-, R = alkyl] and [-P(O)(NHR), R = alkyl] were likely to be good bioisosteres of the sulfonyl group (-SO_2-) in the sulfonylureas.* © 1999 John Wiley & Sons, Inc. Heteroatom Chem 10:237–241, 1999

INTRODUCTION

Acetolactate synthase (ALS) is the first common enzyme in the biosynthetic pathway to the branched-chain amino acids valine, leucine, and isoleucine. It is a very effective target site for herbicidal action and the selective target between plants and mammals.

Sulfonylurea herbicides represent a new class of herbicides discovered in the mid-1970s [1], whose mode of action has been established as the inhibition of ALS [2]. Much attention has been focused on these types of compounds, because they have high potent activity, a high degree of selectivity, and excellent environmental safety [3]. Numerous modifications of the structures of this class of herbicides have been reported [4]. However, the skeletal structure (-SO_2NHCONH-) of sulfonylurea herbicides is still kept in these modifications, and the shortcoming of a long residual time in soil of the sulfonylurea compounds cannot be overcome.

We have paid attention to the similarity between sulfonyl (-SO_2-) and phosphoryl groups [-P(O)R-, R = OR′, NHR′, etc.]. Their close homolgy in terms of size, bond angle, bond length, and configuration suggest that they have a good degree of isosterism. Recently, the literature [5,6] proved that the sulfonyl group was a bioisostere of the phosphoryl group and useful in bioisosteric replacement of the phosphoryl group.

To develop new ALS inhibitors, and also to overcome the defect, we have modified the skeletal structure -SO_2NHCONH- of the herbicidal sulfonylurea in view of isosterism and have designed two kinds of compounds **2** and **3** evolved from the sulfonylurea compounds **1** by replacing the sulfonyl group (-SO_2-) by the -P(O)(OR)- or the -P(O)(NHR)- group. The family of compounds **1** is one kind of the sulfo-

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nylurea compounds having excellent herbicidal activity [7].

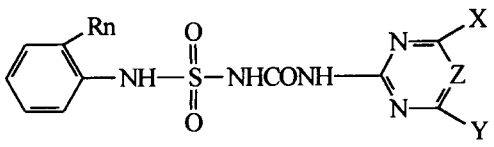
SYNTHESIS

The synthetic method of compounds **2** and **3** is shown in the scheme. Dichlorophosphoryl isocyanate [$Cl_2P(O)NCO$] was synthesized by treating H_2NCO_2Et with PCl_5 in boiling benzene [8], and this compound was then treated with 4,6-dimethoxy-2-aminopyrimidine below 0°C in THF to obtain N-dichlorophosphoryl-N′-4,6-dimethoxy pyrimidin-2-yl) urea **(4).** Compound **4** was allowed to react with aromatic amines at room temperature to give compounds **5a–k,** which were then treated with alcohols and amines, repectively, in the presence of triethylamine to give the target compounds **2a–p** and **3a–c.**

Compounds **2** amd **3** were characterized by elemental analyses (Table 1), ^{1}H NMR, IR spectroscopy (Table 2), and MS.

As shown in Table 2, in the ^{1}H NMR spectra of compounds **2,** there are three peaks for the three NH groups at low field. The peaks of the two NH groups bonded to the P atom are split into doublets owing to the P–H coupling. The ^{1}H NMR spectra of compounds **3** show that there are four peaks for the NH groups at low field. The peaks of the NH groups bonded to the P atom are split into doublets owing to the P–H coupling, and the peak of the NH group bonded to the P atom and the methyl group is split into a multiplet owing to the double coupling of the P–H and H–H.

The IR spectra of compounds **2** show the NH peaks at about 3380 and 3240 cm^{-1}, the carbonyl peak at about 1700 cm^{-1} and the strong absorption bands of P=O at about 1240 cm^{-1}. In the IR spectra of **3,** the NH peaks appear at about 3380 and 3220 cm^{-1}, the carbonyl C=O peak at about 1670 cm^{-1}, and the strong absorption band of P=O at about 1200 cm^{-1}.



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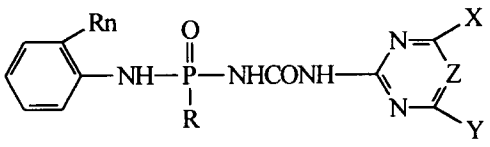


2 R = OR' ; **3** R = NHR'; R'= CH_3,C_2H_5,$(CH_3)_2CH$

SCHEME 1

TABLE 1 The Physical Data of Compounds **2** and **3**

					Elemental Analyses (*%*) *Calcd* (*Found*)		
No.	*Rn*	*R*	*MP* (°*C*)	*Yield* (*%*)	C	H	N
2a	H	Me	103–105	63.6	46.08(45.77)	4.87(4.90)	19.40(19.07)
2b	H	Et	126–127	68.4	47.19(47.24)	5.22(5.25)	18.38(18.37)
2c	2-Cl	Me	120–122	64.1	42.06(41.84)	4.39(4.23)	17.52(17.43)
2d	2-Cl	Et	156–158	75.8	43.81(43.82)	4.23(4.57)	16.75(16.58)
2e	2-Cl	i-Pr	170–171	50.8	44.80(44.70)	5.07(4.89)	16.31(16.30)
2f	3-Cl	Me	96–97	56.3	42.19(41.84)	3.91(4.23)	17.28(17.43)
2g	4-Cl	Me	121–122	46.9	41.85(41.84)	4.10(4.23)	17.45(17.43)
2h	4-Cl	Et	135–137	54.8	43.70(43.32)	4.38(4.57)	16.65(16.85)
2i	2-CH_3	Me	107–109	61.2	47.40(47.24)	5.28(5.25)	18.01(18.37)
2j	3-CH_3	Me	92–93	59.7	47.38(47.24)	5.14(5.25)	18.18(18.37)
2k	4-CH_3	Me	101–102	62.2	47.40(47.24)	4.98(5.25)	18.34(18.37)
21	4-CH_3O	Me	88–90	73.1	45.29(45.34)	5.04(5.04)	17.43(17.63)
2m	2-NO_2	Me	161–162	47.8	41.82(41.78)	4.23(4.13)	20.05(20.39)
2n	2-NO_2	Et	185–186	50.4	42.18(42.25)	4.54(4.46)	19.50(19.72)
2o	4-NO_2	Me	165–166	48.5	40.62(40.78)	4.13(4.13)	19.98(20.39)
2p	2-Br	Me	151–152	44.9	37.72(37.67)	3.66(3.81)	15.66(15.70)
3a	2-Cl	Me	163–165	58.3	41.97(41.95)	4.33(4.49)	20.74(20.97)
3b	4-CH_3O	Me	131–132	56.8	40.64(40.87)	4.56(4.38)	23.87(23.84)
3c	2-NO_2	Me	111–112	51.4	45.61(45.45)	5.18(5.30)	20.99(21.21)

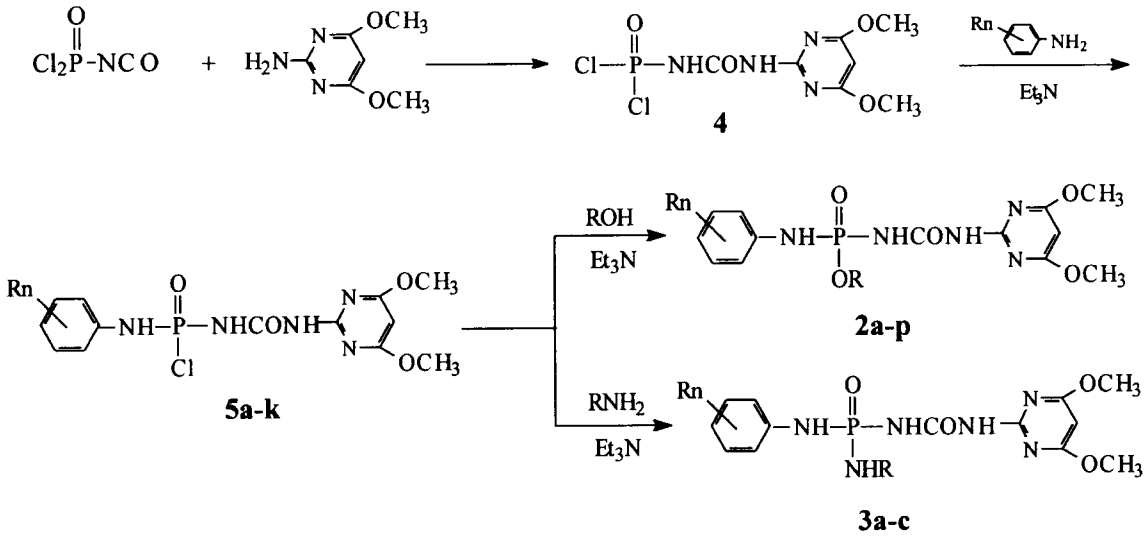
TABLE 2 The ^{1}H NMR, IR Data of Compounds **2** and **3**

No.	*^{1}H NMR (δ, $CDCl_3$ as solvent)*	*IR(cm^{-1})*
2a	3.80 (d, 3H, CH_3O, J = 9.60), 3.89 (s, 6H, $2CH_3O$), 5.58 (s, 1H, CH), 6.70–7.45 (m, 7H, C_6H_5, 2NH), 9.20 (br, 1H, NH)	3360, 3246 (NH) 1698 (C=O) 1215 (P=O)
2b	1.30 (t, 3H, CH_3, J = 6.90), 3.87 (s, 6H, $2CH_3O$), 4.10 (m, 2H, CH_2), 5.56 (s, 1H, CH), 6.70–7.40 (m, 7H, C_6H_5, 2NH), 9.20 (br, 1H, NH)	3367 3278 (NH) 1704 (C=O) 1241 (P=O)
2c	3.78 (d, 3H, CH_3O, J = 9.66), 3.88 (s, 6H, $2CH_3O$), 5.60 (s, 1H, CH), 6.70–7.30 (m, 5H, C_6H_4, NH), 7.75 (d, 1H, NH, J = 7.96), 9.24 (br, 1H, NH)	
2d	1.25 (t, 3H, CH_3, J = 7.01), 3.86 (s, 6H, $2CH_3O$), 4.16 (m, 2H, CH_2), 5.67 (s, 1H, CH), 6.80 (br, 1H, NH), 6.91–7.33 (m, 4H, C_6H_4), 7.75 (d, 1H, NH, J = 8.19), 9.24 (br, 1H, NH)	3359, 3201 (NH) 1680 (C=O) 1238 (P=O)
2e	1.20 (d, 6H, $2CH_3$, J = 2.14), 3.86 (s, 6H, $2CH_3O$), 4.90 (m, 1H, OCH), 5.67 (s, 1H, CH), 6.80–7.30 (m, 5H, C_6H_5, NH), 7.73 (d, 1H, NH, J = 8.04), 9.24 (br, 1H, NH)	
2f	3.80 (d, 3H, CH_3O, J = 9.87), 3.88 (s, 6H, $2CH_3O$), 5.60 (s, 1H, CH), 6.70 (br, 1H, NH), 6.90–7.30 (m, 4H, C_6H_4), 7.62 (d, 1H, NH, J = 8.10), 9.30 (s, 1H, NH)	
2g	3.80 (d, 3H, CH_3O, J = 9.63), 3.89 (s, 6H, $2CH_3O$), 5.60 (s, 1H, CH), 6.80–7.50 (m, 6H, C_6H_4, 2NH), 9.24 (s, 1H, NH)	3350, 3238 (NH) 1689 (C=O) 1210 (P=O)
2h	1.30 (t, 3H, CH_3, J = 7.13), 3.86 (s, 6H, $2CH_3O$), 4.10 (m, 2H, CH_2), 5.60 (s, 1H, CH), 6.70–7.40 (m, 6H, C_6H_4, 2NH), 9.30 (br, 1H, NH)	
2i	2.26 (s, 3H, CH_3), 3.80 (d, 3H, CH_3O, J = 9.65), 3.87 (s, 6H, $2CH_3O$), 5.60 (s, 1H, CH), 6.60–7.35 (m, 6H, C_6H_4, 2NH), 9.20 (s, 1H, NH)	3360, 3219 (NH) 1696 (C=O) 1208 (P=O)
2j	2.28 (s, 3H, CH_3), 3.79 (d, 3H, CH_3O, J = 9.84), 3.86 (s, 6H, $2CH_3O$), 5.60 (s, 1H, CH), 6.60–7.40 (m, 6H, C_6H_4, 2NH), 9.24 (s, 1H, NH)	381, 3206 (NH) 1698 (C=O) 1192 (P=O)
2k	2.28 (s, 3H, CH_3), 3.80 (d, 3H, CH_3O, J = 9.90), 3.88 (s, 6H, $2CH_3O$), 5.60(s, 1H, CH), 6.65–6.90 (br, 2H, 2NH), 7.06–7.31 (dd, 4H, C_6H_4, J = 7.93), 9.20 (s, 1H, NH)	3350, 3198 (NH) 1706 (C=O) 1190 (P=O)
2l	3.60–3.90 (m, 12H, $4CH_3O$), 5.60 (s, 1H, CH), 6.60–7.35 (m, 6H, C_6H_4, 2NH), 9.05 (br, 1H, NH)	
2m	3.80 (d, 3H, CH_3O, J = 9.78), 3.88 (s, 6H, $2CH_3O$), 5.60 (s, 1H, CH), 6.80–7.60 (m, 6H, C_6H_4, 2NH), 9.05 (br, 1H, NH)	3369, 3235 (NH) 1701 (C=O) 1199 (P=O)
2n	1.30 (t, 3H, CH_3, J = 6.96), 3.80 (s, 6H, $2CH_3O$), 4.10 (m, 2H, CH_2), 5.60 (s, 1H, CH), 6.60–7.40 (m, 6H, C_6H_4, 2NH), 9.05 (br, 1H, NH)	3380, 3234 (NH) 1700 (C=O) 1248 (P=O)
2o	3.80 (d, 3H, CH_3O, J = 9.66), 3.89 (s, 6H, $2CH_3O$), 5.60 (s, 1H, CH), 6.60–7.45 (m, 6H, C_6H_4, 2NH), 9.20 (br, 1H, NH)	
2p	3.78 (d, 3H, CH_3O, J = 9.84), 3.86 (s, 6H, $2CH_3O$), 5.60 (s, 1H, CH), 6.65–8.05 (m, 6H, C_6H_4, 2NH), 9.24 (br, 1H, NH)	3359, 3230 (NH) 1705 (C=O) 1198 (P=O)
3a	2.80 (d, 3H, CH_3, J = 3.50), 3.84 (s, 6H, $2CH_3O$), 5.67 (s, 1H, CH), 6.92–7.35 (m, 4H, C_6H_4). 7.60–8.00 (m, 3H, 3NH), 9.35 (d, 1H, NH, J = 9.98)	3384, 3198 (NH) 1662 (C=O) 1190 (P=O)
3b	2.81 (d, 3H, CH_3, J = 3.98), 3.84 (s, 6H, $2CH_3O$), 5.67 (s, 1H, CH), 6.65–7.10 (m, 4H, C_6H_4), 7.65 (m, 1H, NH), 7.90 (d, 1H, NH, J = 8.08), 8.10 (d, 1H, NH, J = 8.24), 9.35 (d, 1H, NH, J = 10.4)	3383, 3218 (NH) 1654 (C=O) 1198 (P=O)
3c	2.80 (d, 3H, CH_3, J = 3.82), 3.85 (s, 6H, $2CH_3O$), 5.67 (s, 1H, CH), 6.65–7.30 (m, 5H, C_6H_5, NH), 7.54 (br, 1H, NH), 7.77 (d, 1H, NH, J = 8.16), 8.15 (d, 1H, NH, J = 8.10), 9.30 (d, 1H, NH, J = 10.9)	

The mass spectra of compounds **2** and **3** showed no molecular ion peaks as they were easily cleaved to produce ion peak *m/e* $[M\text{-}ROH]^+$ for **2** and $[M\text{-}RNH_2]^+$ for **3**. Their base peaks were $[ArNH_2]^+$ or 243[(O)PNHC(O)NR, R = 4,6-dimethoxy-pyrimidin-2-yl].

MODE OF ACTION

The enzyme test in vitro was used to research ALS-inhibition activity of compounds **2** and **3.** ALS was partly purified from Chlorosis seedlings of Pea (*Pisum sativum L.*) [2]. ALS assays were carried out in a volume of 0.5 mL at 30°C. The final reaction



Rn = H, 2-Cl, 3-Cl, 4-Cl, 2-Me, 3-Me, 4-Me, 4- MeO, 2-NO_2, 4-NO_2, 2-Br; R=Me, Ei, i-Pr.

SCHEME 2

mixture contained 20 mM K_2HPO_4, 20 mM $MgCl_2$, 10 μM FAD, and various concentrations of the sample. Assays were initiated by adding enzyme (100 μL) and terminated by adding 50 μL of 6 N H_2SO_4. Acetolactate was determined as described by Ray [2]. The acidified reaction mixture was heated for 15 minutes at 60°C, after which 0.5 mL of 0.5% w/v creatine was added. Next, 0.5 mL of 5% w/v α-naphthol, freshly prepared in 2.5 N NaOH, was added, and the solution was heated for an additional 15 minutes at 60°C. The absorbances of the solution were then determined at 525 nm.

The results showed that compounds **2** and **3** could reduce the level of acetolactate synthesized and had excellect ALS-inhibiting activity. At the same concentration (50 or 100 ppm), the inhibiting effects of compounds **2** and **3** were equal to or more than that of the contrast compound DPX–4189, which is a commercial herbicide and has high potent ALS-inhibiting activity. For example, the inhibiting effects of compounds **2b, 2e,** and **3c** were, respectively, 1.2021, 0.9431, and 1.0376 times at 100 ppm and 0.9099, 0.9772, and 1.0832 times at 50 ppm as great as that of DPX–4189.

These results show that compounds **2** and **3** affected the same pharmacological target ALS as the sulfonylurea compounds, which indicated that compounds **2** and **3** were two novel classes of ALS inhibitors. They also indicated that phosphoryl groups [-P(O)(OR)-, R = alkyl] and [-P(O)(NHR), R = alkyl], likely to be good bioisosteres of the sulfonyl group (-SO_2-) in the sulfonylurea.

EXPERIMENTAL

Instruments

Elemental analyses were performed with a CHN CORDERD MT-3 elementary analyzer. ^{1}H NMR spectra were recorded with a Bruker AC-P200 spectrometer. TMS was used as an internal standard for ^{1}H NMR spectroscopy. MS was measured on a VG ZAB-HS instrument.

General Procedure for the Synthesis of N-Dichlorophosphoryl-N′-(4,6-dimethoxy-pyrimidin-2-yl) Urea **(4).** To a solution of 15.5 g (0.1 mol) of 4,6-dimethoxy-2-aminopyrimidine and 40 mL of anhydrous tetrahydrofuran was added dropwise a solution of 16.0 g (0.1 mol) of dichlorophosphoryl isocyanate and 20 mL of anhydrous tetrahydrofuran at less than 35°C. The mixture was stirred for 2 hours at room temperature and then filtered. After removal of the solvent under reduced pressure, a solid was obtained and recrystallized from petroleum ether; yield 94.0%, mp >220°C (beginning to decompose at 220°C), ^{1}H NMR (δ): 9.20–8.90 (br, 2H, 2NH), 5.60 (s, 1H, CH), 3.81 (s, 6H, $2CH_3O$).

General Procedure for the Synthesis of N-(Arylamino-chlorophosphoryl)-N′-(4,6-dimethoxy-pyrimidin-2-yl) Urea **(5a–k).** To a solution of 3.15 g (0.01 mol) of compound **4** and 30 mL of anhydrous tetrahydrofuran was added dropwise a solution of 0.01 mol of aromatic amine, 0.011 mol of triethylamine, and 20 mL of additional tetrahydrofuran at 0–5°C. The mix-

ture was stirred for 4 hours at room temperature and reacted for 2 hours at 35–40°C and then filtered. After removal of the solvent under reduced pressure, the residue was purified on a silicone-gel column with the eluant of petroleum/acetone (4/1) to give compounds **5.** The structures of **5** were characterized by ^{1}H NMR spectroscopy, and some were characterized by elemental analyses. Their melting points and yields: **5a** (R = H): 141–143°C, 86.1%; **5b** (R = 2-Cl): 135–137°C, 78.3%; **5c** (R = 3-Cl): 130–133°C, 91.3%; **5d** (R = 4-Cl): 136–139°C, 76.1%; **5e** (R = 2-CH_3): 140–142°C, 73.9%; **5f** (R = 3-CH_3): 144–146°C, 82.6%; **5g** (R = 4-CH_3): 140–142°C, 73.9%; **5h** (R = 4-CH_3O): 111–112°C, 82.3%; **5i** (R = 2-NO_2): 124–126°C, 76.0%; **5j** (R = 4-NO_2): 137–139°C, 88.0%; **5k** (R = 2-Br): 139–142°C, 77.8%.

General Procedure for the Synthesis of N-(N-Aryl-O-Alkyl Phosphoryl)-N′-(4,6-dimethoxy-pyrimidin-2-yl) Ureas **(2a–p)** *and N-(N-Aryl- N-alkyl phosphoryl)-N′-(4,6-dimethoxy-pyrimidin-2-yl) Ureas* **(3a–c).** To a solution of 0.01 mol of compound **5** and 25 mL of tetrahydrofuran was added dropwise a solution of 0.011 mol of alcohol or amine, 0.011 mol of triethylamine, and 20 mL of tetrahydrofuran with stirring at 0–5°C. The mixture was stirred for 1 hour at 0–5°C and 2 hours at room temperature and then filtered. After removal of the solvent, the residue was purified on a silicone-gel column with the eluant of petroleum/acetone (3/1) to give compounds **2a–p** or **3a–c**.

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