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Synthesis and Evaluation of New Ethyl 2,3-Dihydro-3-oxoisothiazolo[5,4-*b*]pyridine-2-alkanoate Derivatives as Potential Herbicides

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Abstract: Several ethyl 2,3-dihydro-3-oxoisothiazolo[5,4-*b*]pyridine-2-alkanoate derivatives were synthesized as herbicides. Only 5-methyl derivatives inhibited both hypocotyl and root growth in the lettuce (*Lactuca sativa* L.) seedling test at 100 mg litre^{-1}. Only ethyl propionate and valerate derivatives showed significant inhibition at 0·1 mg litre^{-1}, whereas ethyl acetate or butyrate derivatives were inactive. Contrary to unoxidized derivatives, the inhibitory effect of 1-oxide and 1,1-dioxide derivatives was strongly dependent on concentration; ethyl 2,3-dihydro-5-methyl-3-oxoisothiazolo[5,4-*b*]pyridine-2-propionate 1,1-dioxide inhibited 100% of germination at 100 mg litre^{-1} and 45% of lettuce seedling growth at 0·1 mg litre^{-1}. Quantitative structure–inhibition of growth relationship analysis carried out by adaptive least-squares (ALS) method gave a good correlation with small and hydrophobic 5-substituents as well as with odd carbon-chain ethyl alkanoates in position 2. Active compounds did not show auxin-like activity from 0·1 to 100 mg litre^{-1}.

Key words: 3-Oxoisothiazolo[5,4-*b*]pyridines, synthesis, herbicides, plant-growth-inhibitors, SAR analysis

1 INTRODUCTION

The isothiazolo[5,4-*b*]pyridin-3(2*H*)-one system has aroused great interest in pharmacy,[1] but it has been studied very little in agronomy. Only a few derivatives have been reported as insecticides and fungicides.[2] However, our preliminary molecular modelling studies from Sybyl software[3] showed that some 3-oxoisothiazolo[5,4-*b*]pyridine-2-alkanoic acid derivatives (Fig. 1) match well with the auxin binding site recently proposed.[4] In this sense, 3-oxo-1,2-benzisothiazole-2-alkanoic acid analogues were claimed as new root auxin-like compounds (anti-auxins),[5] and related 2-oxothiazolo[4,5-*b*]pyridine-3(2*H*)-acetic acid derivatives presented auxin-like herbicidal activity (Fig. 2).[6] On the other hand, a few of the proposed compounds in Fig. 1 also could be rigid bioisosteric analogs of abscisic acid (ABA) mimics[7] displayed in Fig. 2.

In order to explore plant-growth-regulating activity of the new ethyl 2,3-dihydro-3-oxoisothiazolo[5,4-*b*] pyridine-2-alkanoate derivatives, three regions around

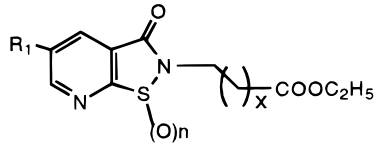

Fig. 1. R_1 = H, NO_2, CH_3, C_6H_5, NH_2. n = 0, 1, 2, x = 0, 1, 2,3.

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(I) (II)

Fig. 2. (I) ABA mimics. (II) Auxin-like herbicides.

the nucleus were selected, as shown in Fig. 1. Substituents in position 5 correspond to R_1 and influence the chemical stability of the system and, consequently, its metabolism. The substituents selected for R_1 (-H, $-NO_2$, $-CH_3$, $-C_6H_5$ and $-NH_2$) are suitable for preliminary biological screenings, since they have a broad range of electronic[8] and hydrophobic[9] properties. The influence of chain length of the ethyl alkanoates in position 2 on plant-growth-regulating activity was analysed by varying x ($x = 0, 1, 2, 3$). Unsubstituted derivatives in position 2 (compounds **2**, **9** and **15**) as well as ethyl α-propionate derivatives (compounds **4** and **11**) were additionally studied. On the other hand the region that surrounds position 1 was analysed through sulfur oxidation states ($n = 0, 1, 2$).

The analysis of the quantitative structure–activity relationship (QSAR) of these derivatives, classified by inhibition of hypocotyl and root elongation of lettuce seedlings into three biological classes (active, moderately active or inactive), was carried out by the adaptive least-squares (ALS) method.[10] The ALS method does not assume any particular distribution of the data and can be considered a non-parametric pattern classifier.

2 EXPERIMENTAL METHODS

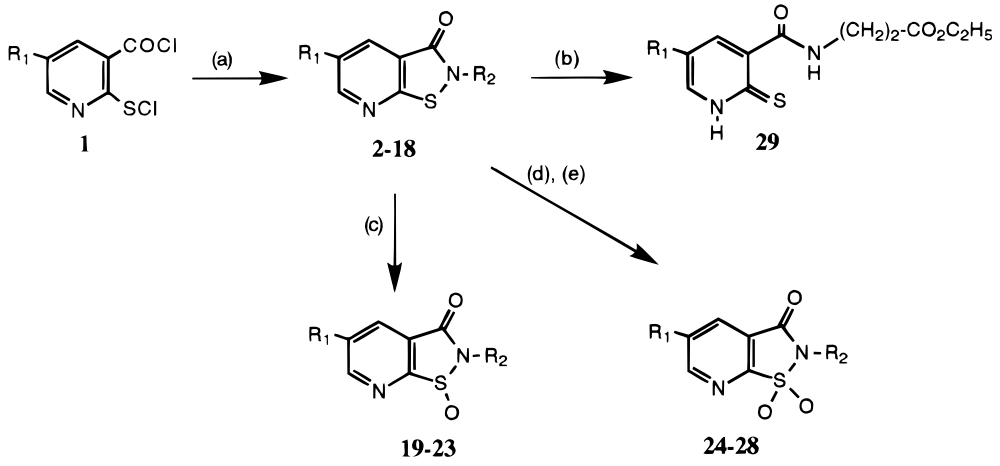
2.1 Chemistry

New ethyl 2,3-dihydro-3-oxoisothiazolo[5,4-*b*]pyridine-2-alkanoates were prepared following the procedures described in Fig. 3. Yields, recrystallization solvents and melting points are given in Table 1. The structures of the synthesized compounds were supported by their infrared and proton magnetic resonance spectra, as well as by elemental CHNS analyses. Relevant spectroscopic data are shown in Table 2.

2.1.1 *Synthesis of isothiazolo[5,4-b]pyridin-3(2H)-ones* (*2–17*)

According to a previously described procedure,[1] 1,2-dihydro-2-thioxo-3-pyridinecarboxylic acids (5·0 mmol) were treated with $SOCl_2$ in boiling xylene to give corresponding acid chlorides. To a stirred mixture of above crude acid chlorides in dry chloroform (30 ml), a catalytic amount of iodine was added and then dry chlorine gas was passed through the solution for 45 min at −19°C. The solvent was removed under vacuum and the respective crude sulphenyl chloride (**1**) was dissolved in dioxane (10 ml). To the resulting mixture, a freshly prepared solution of ethyl aminoalkanoate hydrochloride (20 mmol) and sodium hydroxide (0·80 g, 20 mmol) in water (20 ml) was added dropwise with stirring at 0°C. Stirring was continued at room temperature for a further 3 h. Subsequently, water (70 ml) was added and then hydrochloric acid (1 M) to bring the pH to 6. The resulting solid material was collected and recrystallized from solvents indicated in Table 2. An additional amount of isothiazolo[5,4-*b*]pyridine was obtained by extraction of the aqueous filtrate with dichloromethane (3 × 50 ml).

Compounds **2**, **9** and **15** were obtained passing ammonia through a solution of the respective crude sul-



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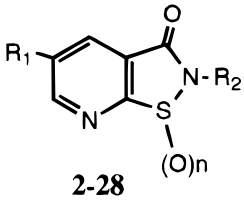
19-23 24-28

Fig. 3. Synthesis of isothiazolo[5,4-*b*]pyridines and their *S*-oxides. *Reagents and conditions*: (a) R_2-NH_2, dioxane, NaOH, r.t; or NH_3, dioxane; (b) $NaHSO_3$ (aq), C_2H_5OH, r.t; (c) $KHSO_5$ (oxone), CH_3OH, H_2O, r.t; (d) m-CPBA (2 eq.), CH_2Cl_2, r.t; (e) NaOH (aq), TBAB, r.t.

TABLE 1
Synthesized Isothiazolo[5,4-*b*]pyridin-3(2*H*)-one Derivatives



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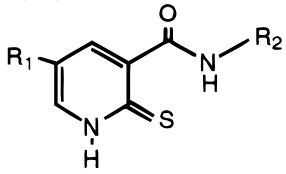

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Compound	Structure			Yield (%)	Solvent recrystn[a]	m.p.(°C)
	R_1	R_2	n			
2	CH_3	H	0	80	A	>240
3	CH_3	$CH_2CO_2C_2H_5$	0	61	B	146–148[1]
4	CH_3	$CH(CH_3)CO_2C_2H_5$	0	40	A	85–86
5	CH_3	$(CH_2)_2CO_2C_2H_5$	0	35	A	143–144
6	CH_3	$(CH_2)_3CO_2C_2H_5$	0	35	A	70–71
7	CH_3	$(CH_2)_4CO_2C_2H_5$	0	30	D	38–40
8	H	$(CH_2)_2CO_2C_2H_5$	0	77	D	103–105[11]
9	NO_2	H	0	60	A	>240
10	NO_2	$CH_2CO_2C_2H_5$	0	80	B	196–198[1]
11	NO_2	$CH(CH_3)CO_2C_2H_5$	0	85	B	140–141
12	NO_2	$(CH_2)_2CO_2C_2H_5$	0	72	B	171–173
13	NO_2	$(CH_2)_3CO_2C_2H_5$	0	67	B	130–132
14	NO_2	$(CH_2)_4CO_2C_2H_5$	0	46	B	119–121
15	C_6H_5	H	0	65	A	194–195
16	C_6H_5	CH_2-$CO_2C_2H_5$	0	60	E	167–169[1]
17	C_6H_5	$(CH_2)_2CO_2C_2H_5$	0	60	C	87–88
18	NH_2	$(CH_2)_2CO_2C_2H_5$	0	62	F	163–165
19	CH_3	$CH_2CO_2C_2H_5$	1	90	C	86–88
20	CH_3	$(CH_2)_2CO_2C_2H_5$	1	90	D	75–77
21	CH_3	$(CH_2)_3CO_2C_2H_5$	1	91	D	69–71
22	CH_3	$(CH_2)_4CO_2C_2H_5$	1	90	D	53–55
23	H	$(CH_2)_2CO_2C_2H_5$	1	85	B	52–54[11]
24	CH_3	CH_2-$CO_2C_2H_5$	2	85	C	78–80
25	CH_3	$(CH_2)_2CO_2C_2H_5$	2	84	D	73–75
26	CH_3	$(CH_2)_3CO_2C_2H_5$	2	85	D	88–90
27	CH_3	$(CH_2)_4CO_2C_2H_5$	2	85	D	65–67
28	H	$(CH_2)_2CO_2C_2H_5$	2	81	B	87–89[11]
29	CH_3	$(CH_2)_2CO_2C_2H_5$	—	90	A	65–67

[a] A: ethanol, B: isopropanol, C: ethyl acetate, D: ethyl acetate + cyclohexane (2 + 1 by volume), E: cyclohexane, F: toluene.

fenyl chloride (**1**) in dioxane for 30 min. The solvent was removed under vacuum and then water (50 ml) and hydrochloric acid (35%) were added to bring the pH to 2. The solid was filtered and recrystallized from ethanol.

2.1.2 Ethyl 5-amino-2,3-dihydro-3-oxoisothiazolo[5,4-b]pyridine-2-propionate (***18***)

A suspension of **12** (1·0 g, 3·4 mmol), iron (1·16 g, 20 mmol) and ammonium chloride (0·53 g, 10 mmol) in ethanol + water (1 + 1 by volume; 100 ml) was refluxed for 3 h. The resulting warm suspension was filtered and the solid material was extracted with boiling ethanol. Combined filtrates and extracts were concentrated to about 50 ml by evaporation in vacuum. The crystalline product obtained on cooling was collected by filtration and recrystallized from ethanol. The solid, a mixture of *N*-(2-(ethoxycarbonyl)ethyl)-5-amino-2-thioxo-1,2-dihydro-3-pyridinecarboxamide and ethyl 5-amino-2,3-dihydro-3-oxoisothiazolo[5,4-*b*]pyridine-2-propionate, was suspended in carbon tetrachloride (10 ml) and bromine (0·79 g, 5 mmol) was then added. The mixture was stirred at room temperature for 5 h and the solid material was collected by filtration, treated with a solution of sodium hydrogen carbonate (10 ml, 2 M), and extracted with chloroform (3 × 40 ml). The organic layer was separated, the solvent removed under vacuum and the solid was recrystallized from toluene to give **18** (0·56 g; 62%).

TABLE 2
Spectroscopic Data of the Isothiazolo[5,4-*b*]pyridin-3(2*H*)-one Derivatives

	IR ν (cm^{-1})[a]			*[^1H]NMR δ (ppm)*[b]		
Compound	*-SO_{n-}*	*-COO-*	*-CON-*	*4-H*	*5-H*	*6-H*
2	—	—	1650	ND[c]	—	ND[c]
3	—	1730	1660	8·17	—	8·75*
4	—	1770	1680	8·06	—	8·58
5	—	1710	1650	8·12*	—	8·71*
6	—	1740	1660	8·05	—	8·58
7	—	1720	1660	8·08	—	8·57
8	—	1725	1665	8·25	7·33	8·73
9	—	—	1660	8·90*	—	9·50*
10	—	1735	1670	8·90*	—	9·65*
11	—	1750	1675	8·90*	—	9·80*
12	—	1730	1670	8·90	—	9·50
13	—	1730	1670	9·02	—	9·50
14	—	1730	1670	9·02	—	9·58
15	—	—	1670	8·50*	—	9·14*
16	—	1730	1660	8·52	—	9·05
17	—	1730	1660	8·41	—	8·96
18	—	1730	1645	7·49	—	8·26
19	1128	1750	1710	8·09	—	8·76
20	1107	1730	1710	8·01	—	8·69
21	1111	1737	1710	8·05	—	8·70
22	1115	1730	1706	8·03	—	8·71
23	1107	1730	1711	8·25	7·66	8·90
24	1336, 1157	1763	1745	8·17	—	8·81
25	1336, 1148	1735	1735	8·12	—	8·76
26	1330, 1148	1735	1735	8·12	—	8·77
27	1336, 1146	1735	1735	8·12	—	8·77
28	1325, 1170	1744	1732	8·36	7·76	8·98
29	—	1730	1645	7·56	—	8·64

[a] Using KBr tablets.
[b] Spectra were recorded in deuterochloroform except for (*) marked shifts which were recorded in hexadeuterodimethyl sulfoxide.
[c] Not determined.

2.1.3 Synthesis of isothiazolo[5,4-b]pyridin-3(2H)-one 1-oxides (***19–23***)

To a stirred suspension of the corresponding isothiazolo[5,4-*b*]pyridin-3(2*H*)-one (**3**, **5–8**) (9·0 mmol) in methanol + water (1 + 1 by volume; 30 ml) at 20°C, oxone® (0·83 g, 13·5 mmol of $KHSO_5$) was added in small portions according to a previously described procedure.[11] When the reaction had been completed (reaction monitored by thin layer chromatography, TLC), the reaction mixture was poured into water (100 ml) and extracted with dichloromethane (3 × 50 ml). Organic layers were dried over sodium sulfate and evaporated. The residue was recrystallized to give the 1-oxides (**19–23**) (Table 2).

2.1.4 Synthesis of isothiazolo[5,4-b]pyridin-3-(2H)-one 1,1-dioxides (***24–28***)

To a suspension of the corresponding isothiazolo[5,4-*b*] pyridin-3-(2*H*)-one **3**, **5–8**) (4·0 mmol) in dichloromethane (10 ml), a solution of 98% *m*-CPBA (1·44 g, 8·2 mmol) in dichloromethane (10 ml) was added dropwise with stirring at 25°C. After the isothiazolo[5,4-*b*] pyridine was completely consumed (reaction monitored by TLC), solid tetrabutylammonium bromide (TBAB, 0·10 g) and sodium hydroxide (2·1 ml, 4 M) were slowly added to the stirred mixture at 25°C. When the sulfoxide was completely consumed, the mixture was separated after addition of water (20 ml) and the organic layer was washed with water (2 × 20 ml) and dried (sodium sulfate). The solvent was removed under vacuum, the residue was chromatographed on a silica gel column using ethylacetate + cyclohexane (2 + 1 by volume) as eluent and recrystallized from solvents indicated in Table 2.

2.1.5 N-(*2-(ethoxycarbonyl)ethyl)-1,2-dihydro-5-methyl-2-thioxo-3-pyridinecarboxamide* (***29***)

A solution of **5** (1·30 g, 5 mmol) in ethanol (20 ml) was treated with an aqueous solution of sodium hydrogen sulfite (10 ml, 1 M). The mixture was stirred at room

temperature for 30 min and then the solvent was removed under vacuum. The solid residue was washed with water, collected by filtration and recrystallized from ethanol to give (1·18 g; 90%).

2.2 Biological assays

Three different bioassays were carried out to evaluate the plant-growth-regulating activity of the synthesized compounds. In all cases compounds were dissolved in 7·6 mM potassium phosphate buffer, pH 7·2 + acetone (99 + 1 by volume) and this solvent system was used as the control.

2.2.1 Assay of lettuce seed germination and root and hypocotyl elongation

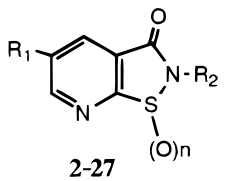
Groups of 10 seeds of *Lactuca sativa* L. cv. Crispilla were placed in Petri dishes (9 cm) containing pads of filter paper (two discs of Whatman No. 1), and 4 ml of test solution was added. Each product was tested at 0·1, 1, 10 and 100 mg litre^{-1}. Seeds were maintained at 26°C in darkness. After four days, root and hypocotyl lengths were measured to the nearest millimetre. All treatments were replicated three times. As the same results were obtained in roots and hypocotyls, Table 3 summarizes the effect of the different products on the whole seedlings expressed as percentage of the control. Average seedling length (±SE) of the control was 6·6(±0·1) cm.

2.2.2 Pea stem bioassay

Different compounds were tested for auxinic activity in the pea stem bioassay.[12] Seeds of *Pisum sativum* L. cv. Frilene were grown in perlite + vermiculite (2 + 1 by mass) in total darkness at 26°C for seven days. When the fourth internode was less than 2 mm long, 6 mm

TABLE 3

Effect of Isothiazolo[5,4-*b*]pyridin-3(2*H*)-ones Derivatives on the Growth of *Lactuca sativa* Seedings



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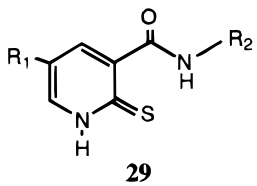

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	Growth of L. Sativa (% *of control*)						
	Structure			*Concentration (mg litre^{-1})*			
Compound	R_1	R_2	*n*	*0·1*	*1*	*10*	*100*
2	CH_3	H	0	N[a]	N	N	N
3	CH_3	$CH_2CO_2C_2H_5$	0	N	N	N	86
4	CH_3	$CH(CH_3)CO_2C_2H_5$	0	N	N	N	86
5	CH_3	$(CH_2)_2CO_2C_2H_5$	0	80	72	67	66
6	CH_3	$(CH_2)_3CO_2C_2H_5$	0	N	N	N	78
7	CH_3	$(CH_2)_4CO_2C_2H_5$	0	40	43	41	39
8	H	$(CH_2)_2CO_2C_2H_5$	0	N	N	N	N
9	NO_2	H	0	N	N	N	N
10	NO_2	$CH_2CO_2C_2H_5$	0	N	N	N	N
11	NO_2	$CH(CH_3)CO_2C_2H_5$	0	N	N	N	N
12	NO_2	$(CH_2)_2CO_2C_2H_5$	0	N	N	N	N
13	NO_2	$(CH_2)_3CO_2C_2H_5$	0	N	N	N	N
14	NO_2	$(CH_2)_4CO_2C_2H_5$	0	N	N	N	N
15	C_6H_5	H	0	N	N	N	N
16	C_6H_5	$CH_2CO_2C_2H_5$	0	N	N	N	N
17	C_6H_5	$(CH_2)_2CO_2C_2H_5$	0	N	N	N	N
18	NH_2	$(CH_2)_2CO_2C_2H_5$	0	N	N	N	N
19	CH_3	$CH_2.CO_2C_2H_5$	1	N	N	N	N
20	CH_3	$(CH_2)_2CO_2C_2H_5$	1	N	91	60	60
24	CH_3	$CH_2CO_2C_2H_5$	2	N	N	N	N
25	CH_3	$(CH_2)_2CO_2C_2H_5$	2	55	55	10	0
26	CH_3	$(CH_2)_3CO_2C_2H_5$	2	N	N	N	N
27	CH_3	$(CH_2)_4CO_2C_2H_5$	2	90	78	74	67
29	CH_3	$(CH_2)_2CO_2C_2H_5$	—	N	N	N	N

[a] N = not significantly different from control at the 5% significance level.

segments were cut from the apical end of the third internode. The excised segments were washed in water for 30 min at 25°C with orbital shaking and placed in sterile plastic Petri dishes (9 cm diameter, six segments per dish) containing 10 ml of test solution. Each product was tested on three individual samples at 0·01, 0·1, 1, 10 and 100 mg litre^{-1}. Petri dishes were incubated in darkness at 26°C for 24 h. Measurement of the final segment lengths with an estimation to 0·01 mm was facilitated by using an optical microscope fitted with a video monitor. All manipulations, except the measurement itself, were carried out in dim red light. Table 4 shows the average elongation of treated segments expressed as percentage of the control. Average elongation ($\pm$SE) of the control pea stem was 0·62($\pm$0·04) mm.

2.2.3 Stomatal conductance bioassay

This bioassay was used to detect abscisic acid (ABA)-type activities.[13] French bean seeds (*Phaseolus vulgaris* L. cv. Tendergreen) were sown in 1-litre plastic pots in perlite + vermiculite (2 + 1 by mass). The plants were grown for 15 days under a 16 : 8 h light : dark regime with day/night temperature and relative humidity of 28/20°C and 60/80%, respectively, in a controlled environmental chamber. The light was provided by a combination of incandescent bulbs and cool-white fluorescent lamps (Sylvania VHO), with a light intensity of 400 μmol m^{-2} s^{-1} active radiation. The plants were watered with Hoagland solution.[14]

For this bioassay, the first trifoliate leaf was used. The leaves were excised under water and immediately fed with water for 2 h. Water was then changed for test solution and leaves kept in this solution for 60 min, allowing intact leaves to take in the test solution through the petiole. Stomatal conductance measurements were performed during the latter period at 0, 10, 20, 30, 45 and 60 min with a Delta-T AP4 porometer. Leaves were illuminated at 350 μmol m^{-2} s^{-1} during feeding. Compound **5** and ABA were tested at 0·1, 1, 10 and 100 mg litre^{-1} in triplicate. Stomatal resistance of the control was 2·02($\pm$0·41) s cm^{-1} at 60 min.

2.3 Calculations

2.3.1 Observed activity

From the lettuce assay, the growth rates were calculated as a percentage of the averaged length of treated plant hypocotyls and roots of those of the control. Activities (L) were classified in three categories for the ALS method:

Activity class 2 for active inhibitory compounds, when the percentage change at a concentration of 0·1 mg litre^{-1} was statistically significant compared to that of the control.

Activity class 1 for moderately active inhibitory compounds, when percentage change compared to that of the control was only statistically significant at 100 mg litre^{-1}.

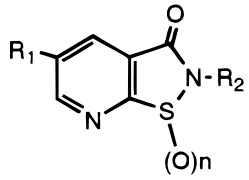
Activity class 0 for inactive compounds, when no statistically significant difference compared to the control was observed in the range 0·1–100 mg litre^{-1}.

2.3.2 Substituent parameters

Electronic (σ), hydrophobic (π) and steric (MR) substituent constants serve as general descriptors for the R_1

TABLE 4

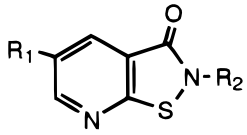
Effect of Isothiazolo[5,4-*b*]pyridin-3(2*H*)-ones, on Elongation of Pea Stem Segments



	Average elongation of stem (% of control)[a]					
	Structure			*Concentration (mg litre^{-1})*		
Compound	R_1	R_2	*n*	*0·01*	*1*	*100*
AIA				131*	171*	240*
5	CH_3	$(CH_2)_2CO_2C_2H_5$	0	122	107	71*
7	CH_3	$(CH_2)_4CO_2C_2H_5$	0	106	115	93
8	H	$(CH_2)_2CO_2C_2H_5$	0	102	107	93
17	C_6H_5	$(CH_2)_2CO_2C_2H_5$	0	100	121	109
25	CH_3	$(CH_2)_2CO_2C_2H_5$	2	99	88	91
27	CH_3	$(CH_2)_4CO_2C_2H_5$	2	118	126*	92
28	H	$(CH_2)_2CO_2C_2H_5$	2	101	97	93

[a] * = difference from control statistically significant at 5% level.

TABLE 5
Structural Features, Inhibitory Activity (L) and ALS Recognition (L_{rec}) and Prediction (L_{pred}) Analysis of Ethyl 2,3-Dihydro-3-oxoisothiazolo[5,4-*b*]pyridine-2-alkanoate Derivatives



Compound	R_1	R_2	σ	π	*MR*	I_e	I_p	*L*	L_{rec}	L_{pred}
2	CH_3	H	−0·17	0·56	5·7	0	0	0	0	1
3	CH_3	$CH_2CO_2C_2H_5$	−0·17	0·56	5·7	1	1	1	1	1
4	CH_3	$CH(CH_3)CO_2C_2H_5$	−0·17	0·56	5·7	1	1	1	1	1
5	CH_3	$(CH_2)_2CO_2C_2H_5$	−0·17	0·56	5·7	1	0	2	2	1
6	CH_3	$(CH_2)_3CO_2C_2H_5$	−0·17	0·56	5·7	1	1	1	1	1
7	CH_3	$(CH_2)_4CO_2C_2H_5$	−0·17	0·56	5·7	1	0	2	2	1
8	H	$(CH_2)_2CO_2C_2H_5$	0·00	0·00	1·0	1	0	0	1	1
9	NO_2	H	0·78	−0·28	7·4	0	0	0	0	0
10	NO_2	$CH_2CO_2C_2H_5$	0·78	−0·28	7·4	1	1	0	0	0
11	NO_2	$CH(CH_3)CO_2C_2H_5$	0·78	−0·28	7·4	1	1	0	0	0
12	NO_2	$(CH_2)_2CO_2C_2H_5$	0·78	−0·28	7·4	1	0	0	0	0
13	NO_2	$(CH_2)_3CO_2C_2H_5$	0·78	−0·28	7·4	1	1	0	0	0
14	NO_2	$(CH_2)_4CO_2C_2H_5$	0·78	−0·28	7·4	1	0	0	0	0
15	C_6H_5	H	−0·01	1·96	25·4	0	0	0	0	0
16	C_6H_5	$CH_2CO_2C_2H_5$	−0·01	1·96	25·4	1	1	0	0	0
17	C_6H_5	$(CH_2)_2CO_2C_2H_5$	−0·01	1·96	25·4	1	0	0	0	0
18	NH_2	$(CH_2)_2CO_2C_2H_5$	−0·66	−1·23	5·4	1	0	0	0	0

physicochemical properties. Structural modifications on substituents in position 2 of the isothiazolo[5,4-*b*] pyridin-3(2*H*)-one system were represented by two presence–absence independent variables I_e and I_p : I_e = 1, when the substituent has an ester group; I_p = 1, when the alkanoate has an even number of carbon atoms measured from heterocyclic system to ester group. Additionally, an indicator variable (I_n) which shows the number of oxygen atoms attached in position 1, was analysed. Hammett's constant[8] (σ), hydrophobic character[9] (π) and molar refractivity (MR)[15] were taken from the literature (Table 5).

All combinations of independent variables described in the above paragraph were tried in order to obtain the best possible correlation. The data processing was done on a Macintosh IIci machine. Subsequently the ALS iteration was performed, and the best discriminant function was selected according to the Spearman rank correlation coefficient, as will be detailed below.

2.3.3 *ALS method*

The ALS method includes an error-correcting feedback algorithm and the details have been described elsewhere.[10,16] The equation (discriminant function) is formulated by a feedback adaptation procedure in a linear form as eqn (1)

$$L = w_0 + w_1 x_1 + w_2 x_2 + \cdots + w_p x_p \qquad (1)$$

where L is the discriminant score for the classification, x_k ($k = 1, 2, \ldots p$) is the kth descriptor for the structure and w_k ($k = 1, 2, \ldots p$) is the weight coefficient. The value of w_k is determined by the least squares adaptation using the starting score a_j ($j = 1, 2, \ldots m$ in the 'm' group case) and the correction term C_i. In this study the algorithm was carried out using the computer program Data Desk.[17]

Moriguchi and coworkers[10] proposed a 'ridit' as a standard numerical score for ordered categories. The choice of ridit as the numerical score is based on the assumption that only the potency order groups are reliable, i.e. quantitative differences in potency between the different groups and between the different compounds within a group are uncertain in the data to be analysed. It is defined in eqn (2)

$$a_j = \left[2\left(n_j + 2 \sum_{i=1}^{j-1} n_i \right) \Big/ n \right] - 2 \qquad (2)$$

where a_j is the ridit for group 'j' and n_i and n_j are the size of groups 'i' and 'j' respectively. From eqn (2) the mean value of a_j over 'n' compounds becomes zero and $a_1 = -1$ and $a_2 = +1$ for two groups of the same size.

The procedure begins with the setting of forcing factors S_i, which are taken to be $S_i = a_j$. By use of S_i in place of L in eqn (1), the ordinary least-squares estimate w_k ($k = 1, 2, \ldots p$) is used as the initial weight vector. Then L_i for each substance is calculated from eqn (1).

All substances are classified on the basis of the values of L_i and the cutoff point by $b_j = 0{\cdot}5(a_j + a_{j+1})$ as follows: if $L_i \leqslant b_1$ assigns the ith substance to class 0; if $b_1 < L_i \leqslant b_2$, assigns ith the substance to class 1 and if $L_i > b_2$, assigns the ith substance to class 2.

At iteration 2 and thereafter, the forcing factor S_i is adapted as $S_i = L_i$ (when the ith substance is correctly classified) or $S_i = L_i - C_i$ (when misclassified), where the sign '$-$' is chosen to correspond with $S_i - L_i$. The correction term C_i for the misclassified compound 'i' at each iteration is given as eqn (3), where the b_j cutoff point is nearer to L_i.[16] From the newly adapted S_i, the least-squares estimate of w_k is computed and the new L_i is calculated from eqn (1).

$$C_i = 0{\cdot}1/[0{\cdot}1 + (0{\cdot}45 + |L_i - b_j|)^2] \qquad (3)$$

The adaptation is repeated until all substances are correctly classified or repeated a maximum of 15 times, and the best discriminant function is selected.

The results of the ALS calculation were validated by the leave-one-out prediction. The measure of the predictive ability is obtained by leaving out one compound and using the remaining compounds as the training set. The discriminant function developed from the training set is used to predict the potency class of the compound left out. This procedure is continued until each compound of the data set has been left out of the training set once. The predictive results were given as the misclassified number and the Spearman rank correlation coefficient for the overall leave-one-out classification.

3 RESULTS AND DISCUSSION

Isothiazolo[5,4-*b*]pyridin-3(2*H*)-ones (**2**–**17**) were synthesized in a single step by reacting 2-chlorothio-3-pyridinecarbonyl chloride derivatives[1] (**1**) with amines as described in Fig. 3. The 5-amino derivative **18** was obtained by reduction of compound **12** with iron. The oxidation of isothiazolo[5,4-*b*]pyridin-3(2*H*)-ones (**3**, **5**–**8**) to their 1-oxides (**19**–**23**) was accomplished with 1·5 equivalents of $KHSO_5$ in the form of oxone® dissolved in 50% aqueous methanol at 20°C, giving good yields (≈90%) in 1 h. Isothiazolo[5,4-*b*]pyridin-3(2*H*)-one 1,1-dioxides (**24**–**28**) were prepared in a single pot from isothiazolo[5,4-*b*]pyridin-3(2*H*)-ones (**3**, **5**–**8**). When compounds **3**, **5**–**8** had been reacted with 98% m-CPBA (1 : 2·2 equiv.) in dichloromethane, the subsequent addition of aqueous sodium hydroxide (1 : 2·1 equiv) and TBAB (cat) to the reaction mixture at room temperature gave 1,1-dioxides (**24**–**28**) in good yields (≈85%). This procedure gave similar yields and was simpler than the two-pot oxone®/NaOCl method recently reported.[11]

As seen from Table 3, only 5-methyl derivatives of ethyl 2,3-dihydro-3-oxoisothiazolo[5,4-*b*]pyridin-2-alkanoates (**3**–**7**) showed statistically significant inhibition of *L. sativa* growth at 100 mg litre^{-1}. The ester group seems to be necessary to produce activity, so the 5-methyl-2-unsubstituted compound (**2**) was inactive. The 3-oxoisothiazolo[5,4-*b*]pyridine nucleus is also necessary for inhibitory activity; thus 3-pyridinecarboxamide (**29**), obtained by reduction of the 5-methyl-2-propionate derivative (**5**), was inactive. Chain length of the ethyl alkanoate was also another important inhibitory factor. Ethyl 5-methyl-2-propionate (**5**) and valerate (**7**) derivatives inhibited lettuce growth at 0·1 mg litre^{-1}. However, ethyl 5-methyl-2-acetate (**3**) and butyrate (**6**) derivatives lost activity at 10 mg litre^{-1}. Ethyl α-2,3-dihydro-3-oxoisothiazolo[5,4-*b*]pyridine-2-propionate (**4**) was also inactive at 10 mg litre^{-1}, and its behaviour was more like that of acetate (**3**) than β-propionate (**5**).

On the other hand, ethyl 2,3-dihydro-5-methyl-3-oxoisothiazolo[5,4-*b*]pyridin-2-alkanoate 1-oxides (**19** and **20**) and 1,1-dioxides (**24**–**27**) differed notably from the unoxidized analogues (**3**, **5**–**7**): only ethyl propionate (**20** and **25**) and valerate (**27**) derivatives inhibited lettuce growth at 100 mg litre^{-1}, and their resulting inhibition was more dependent on the concentration than in the case of **5** and **7**. The most active of the 1,1-dioxides was the ethyl propionate (**27**), whereas, for unoxidized derivatives, it was the ethyl valerate (**7**).

The above qualitative conclusions were refined by means of a QSAR analysis. When the previous ALS discriminant method was applied on tested 3-oxoisothiazolo[5,4-*b*]pyridine derivatives (**2**–**27**, Table 3), the oxidation indicator variable I_n was not significant statistically. Thus 1-oxides and 1,1-dioxides were discarded for the final analysis to prevent statistical redundancy. Compounds analysed by ALS are listed in Table 5 along with descriptors and activity ratings (Section 2.3). The resulting discriminant function is expressed as eqn (4), where the figure in parentheses beside coefficients is the contribution index (|coef| × SD of descriptor),[10] 'n' stands for the number of compounds, 'n_{mis}' is the number misclassified and the figure in parentheses after the value of 'n_{mis}' is the number misclassified by two categories and 'R_s' is the Spearman rank coefficient correlation.[18]

$$L = 0{\cdot}392 + 1{\cdot}516(1{\cdot}367)\pi - 0{\cdot}142(1{\cdot}100)MR + 1{\cdot}224(0{\cdot}481)I_e - 0{\cdot}454(0{\cdot}230)I_p \qquad (4)$$

$n = 17$ recognition: $n_{mis} = 1$ (0), $R_s = 0{\cdot}907$ ($p = 0{\cdot}000$)

prediction: $n_{mis} = 4$ (0), $R_s = 0{\cdot}760$ ($p = 0{\cdot}002$)

On the basis of eqn (4) for the inhibition of lettuce growth of compounds **2**–**18**, hydrophobic ($\pi > 0$) and small ($MR \approx 1$) R_1 substituents will have favourable effects on activity. Also eqn (4) confirms that an ester group ($I_e = 1$) and a chain with an odd number of carbons ($I_p = 0$) are preferred for inhibition. In this

equation the scores were $a_1 = -0{\cdot}5882$, $a_2 = 1{\cdot}1765$, $a_3 = 1{\cdot}7647$, and cutoff points were $b_1 = 0{\cdot}2941$ and $b_2 = 1{\cdot}4706$. The recognition of eqn (4) was very good ($n_{mis} = 1$) and leave-one-out prediction gave a good significance level of 0·2%. Misclassified compounds were by only one category (Table 5). Oxidized compounds, which were not included in the analysis, also followed the tendency suggested by eqn (4).

The most active compounds in the lettuce test, the 5-methyl 2-propionate (**5**) and 2-valerate (**7**) derivatives, the corresponding 1,1-dioxides (**25** and **27**), as well as the related 5-H derivatives (**8** and **28**) and the unoxidized 5-phenyl-2-propionate (**17**) derivative were investigated on cell elongation in pea stem bioassay in order to ascertain potential auxinic activity. Table 4 shows that pea segments have a high degree of response to auxin (AIA) giving a large increase in length at 0·01 mg litre^{-1} or higher concentrations. Tested compounds did not show auxin-like activity from 0·01 to 100 mg litre^{-1}. Weak or marginal elongation was observed at ≤ 1 mg litre^{-1}. Additionally, compound **5**, analogous to the ABA-mimic displayed in Fig. 2, was assayed by the stomatal conductance test and compared with the ABA effect. No significant differences were observed between the control and compound **5** in the 0·1 to 100 mg litre^{-1} range, although ABA increased stomatal resistance six-fold at 100 mg litre^{-1}.

Unlike 3-oxo-1,2-benzisothiazole anti-auxin analogues[5] ethyl 2,3-dihydro-3-oxoisothiazolo[5,4-*b*] pyridin-2-alkanoate derivatives inhibited root growth, and their activity depended on the number of carbon atoms in the alkyl chain. The absence of auxin or anti-auxin activities in ethyl 2,3-dihydro-3-oxoisothiazolo[5,4-*b*]pyridin-2-alkanoate derivatives provides new structural features to refine existing models of receptors.[4,19] The findings described above indicate that derivatives **7** and **25** can be leads to a promising series of herbicidal compounds despite this activity not being related to the auxinic mode of action.

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