

Synthesis and Biological Activity of Urea and Thiourea Derivatives from 2-Aminoheterocyclic Compounds

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ABSTRACT

Thirty-eight N-substituted-N'-(2-thiazolyl and furfuryl)ureas and thioureas were prepared by reaction of 2-aminothiazole and 2-furfurylamine with the appropriate iso(thio)cyanate. All compounds were tested for herbicidal activity and selectivity on seedlings of wheat (a monocotyledonous plant) and cucumber (a dicotyledonous plant). Only one compound (**1**) out of 14 ureas was characterized by considerable herbicidal activity against the wheat seedlings and two compounds (**1** and **2**) - towards the cucumber seedlings. The phenylurea derivative of 2-aminothiazole (**1**) was 1.7-fold more and the 3-chlorophenylurea derivative of 2-furfurylamine (**23**) was equally as active as the standard diuron with respect to selective herbicidal activity. Among 24 thioureas, four compounds (**15**, **16**, **17**, and **18**) to displayed the highest selective herbicidal activity and two other compounds (**19** and **33**) were almost equal to diuron activity. Selective herbicidal ratio (SHR) represents the degree of herbicidal effect of the investigated compounds compared to diuron at both test objects. Four compounds (**16**, **17**, **18**, and **23**) possessed SHR << 100 in the wheat seedlings

while in the cucumber seedlings they had SHR >> 100. Therefore these compounds were substantially more active herbicides to the wheat seedlings as compared to diuron. The cytokinin-like activity of the synthesized compounds was also investigated in terms of betacyanin synthesis and radish cotyledon enlargement. The urea derivatives exhibited mostly high cytokinin-like activity but their activity remained lower than those of kinetin and N-phenyl-N'-(4-pyridyl)urea. The N-(3-fluorophenyl)-N'-(2-thiazolyl)urea (**2**) possessed the greatest activity at 10 μ M while the corresponding compound with 3-chlorophenyl (**4**) was the most active cytokinin-like substance in the whole concentration range tested. Attention was also given to structure-activity relationships for the screened compounds. In general, the ureas and thioureas containing a 2-thiazole ring were more active than those containing a 2-furfuryl residue.

Key words: Cytokinin; Herbicide; Synthesis; Thiourea; Urea

INTRODUCTION

Urea and thiourea derivatives containing 5-member heterocyclic rings have played an important role in developing useful agrochemicals and pharmacolog-

ical agents, for example, ureidothiazoles (C: CX.S.CY:N) where Y = NRCONMeR' are effective herbicides for a broad spectrum of weeds (Dowding and Leeds 1971). N-Methylfurfurylurea herbicides gave selective weed control in cereals, such as oats and wheat, as well as cotton and beans (Sasse and others 1969). Thiazolylthiourea derivatives exhibited fungicidal and nematocidal activity (Kumar and others 1989), as well as acaricidal and insecticidal activity (Kano and others 1984).

1, 2,3 -Thiadiazolyl ureas (N:N.S.CX:C) where X = NR₁CONR₂R₃ are proven plant defoliant (Arndt and others 1977). The defoliating effects appear to be limited to plants of the same family as cotton, because concentrations of up to 100 µm failed to cause leaf drop in several of the dicotyledonary species (A. G. Schering, personal communication). The N-phenyl-N'-(1,2,3-thiadiazol-5-yl)urea (thidiazuron, Dropp) belongs to this group of compounds and later on its high cytokinin activity was detected (Mok and others 1982).

N-Methyl-N-(2-thiazolyl)-N'-alkyl substituted thioureas are plant growth regulators that inhibit stem elongation of rice and kidney bean plants without phytotoxicity (Teruhisa and others 1972). The thiazolylthioureas possess antituberculous (Gheorghiu and others 1962), antiviral (Sumiyuki and Masayoshi 1967), antimalarial (Burkard and others 1972) and antileukemic (Zee-Cheng and Cheng 1979) activities as well.

Substituted pyrazolyl-phenyl ureas have been synthesized and their plant growth regulating properties were demonstrated (Creuzburg and others 1977). These substances were more active cytokinins than N,N'-diphenylurea (DPU). Newly synthesized derivatives of 3(5)-acylaminopyrazole showed potent inhibiting activity on cell proliferation of different tumor forms (Pevarello and others 2001).

N-[2-(2-oxo-1-imidazolidinyl)ethyl]-N'-phenylurea (ethylenediurea EDU) has displayed cytokinin-like activity in the tobacco callus bioassay (Lee and Chen 1982) and has induced cellular defenses against ozone damage in a number of plant species (Lee and Bennett 1985). Among some synthetic EDU analogues, the greatest stimulating activity on the antioxidant enzymes in human cells was shown by compounds with a chlorine atom at the *para* position of the phenyl ring (Kerr and Boswell 1992).

4-Aminomethyl derivatives of 2-methyl-5-hydroxybenzimidazole have been reported as stimulators of plant growth (Smirnov and others 1984). Among these compounds, 2-methyl-4-[dimethylaminomethyl]-5-hydroxybenzimidazole dihydrochloride (Ambiol) functions as an antioxidant

(Santracek and Krepelka 1988) and increases growth of two dicotyledons under drought conditions (Darlington and others 1996).

The cyclocondensation products of N-(5-aryl-1,3,4-oxadiazol-2-yl)-N'-(4-fluorophenyl) ureas are more potent fungicides than their parent ureas (Mishra and others 2000).

Aromatic ureas characterized by the presence of a 2-substituted and 2,6-disubstituted-4-pyridyl moiety have even higher cytokinin activity than *trans*-zeatin (Okamoto and others 1983). Our earlier studies on the biological effects of N-phenylureas containing pyridyl residues indicated that these substances are potential herbicides and plant growth regulators (Vassilev and others 1984; Yonova and others 1989).

In view of the above articles it is apparent that the presence of a 5- or 6-member heterocyclic nucleus in the urea and/or thiourea molecules manifests important and multiple biological effects, and is the basis for target-oriented synthesis.

In our continuing search for biologically active compounds, we decided to synthesize a series of N-aryl and alkyl-substituted-N'-(2-furfuryl and thiazolyl) ureas and thioureas and to evaluate their plant growth regulating activity, focusing mainly on the herbicidal, root growth inhibitory and stimulatory, and cytokinin-like activities.

MATERIALS AND METHODS

Synthesis

All melting points were measured on a Boetius micro melting point apparatus and are uncorrected. IR spectra in KBr were recorded on a Philips Analytical PU9800 FTIR spectrophotometer; ν_{\max} (cm⁻¹) is given for the main absorption bands. ¹H NMR and ¹³C NMR spectra were recorded on a Bruker WM-250 spectrometer using tetramethylsilane (TMS) as an internal reference at 250 and 62.8 MHz, respectively; chemical shifts are expressed in δ (parts per million) values. All new compounds gave satisfactory elemental analytical results for C, H and N, and were performed on a Yanaco CHN CORDER MT-3 instrument.

Ureas and thioureas were generated cleanly, and in high yield, by condensation of 2-aminothiazole and 2-furfurylamine with the corresponding iso(thio)cyanates. All crude products were purified by the recrystallization from ethanol.

Preparation of 2-thiazolyl- and 2-furfuryl- ureas. To a cooled solution of 2-aminothiazole or 2-furfurylamine (10 mmol) in dry benzene (20 mL) was added dropwise appropriate isocyanate (10 mmol)

in dry benzene (5 mL) with good stirring for 30 min. The mixture was stirred for about 1 h maintaining the reaction temperature below 10°C and left overnight at room temperature. The solid products obtained were filtered, washed with ice-cold ethanol and vacuum-dried at room temperature. The reactions with 2-furfurylamine were strongly exothermic with fast crystallization of the reaction products. *Aryl(2-thiazolyl)thioureas*, *aryl and alkyl(2-furfuryl)thioureas* were obtained by the same procedure but the reaction solvent was ethanol.

Preparation of alkyl and cyclohexyl(2-thiazolyl)thioureas. A mixture of 2-aminothiazole (6 mmol) and appropriate alkyl and cyclohexylisothiocyanate (6 mmol) was heated on a steam bath for 1 h. Then the solution was cooled to room temperature and the crystalline products precipitated out.

Biological Evaluations

Determination of Herbicidal, Root Growth Inhibitory and Stimulatory Activities. All the synthesized chemicals were dissolved in aqueous solutions with the Tween-80 emulsifier (0.02% v/v) and were tested in the concentration range 1–1000 μM .

Root Growth Assay. Twenty-five seeds of wheat (*Triticum aestivum* L. cv. Sadovo-1) and cucumber (*Cucumis sativus* L. cv. Bistrensky) were placed in 10 cm Petri dishes on filter paper moistened with 5 ml of test solution or water containing 0.02% Tween-80 (control). Petri dishes were kept in the dark at $25 \pm 1^\circ\text{C}$ for 96 h. Then, the length of the primary root of each plantlet was measured (in mm) and expressed as a percentage of the control root length. When the relative value varied from 0 to 30%, a herbicidal efficiency was manifested. If it varied from 31 to 99% an inhibiting effect was evident and a value above 100% demonstrated the stimulating effect of the tested compound. Two experiments with six replications per variant were performed.

Results for the selective herbicidal activity are expressed as relative herbicidal efficiency (RHE), defined as the ratio of the herbicidal efficiency (HE) of each compound at 1000 μM concentration on the cucumber seedlings to that on the wheat seedlings. The concentration that inhibited root growth to 50% (ID_{50}) was determined by interpolation of % inhibition against the log of the test concentrations. A selective herbicidal ratio (SHR) (ID_{50} of test compound divided in ID_{50} of diuron in μM) was also calculated. This index represents the degree of herbicidal effect of the compounds investigated compared to diuron for both wheat and cucumber.

Determination of Cytokinin-like Activity. All investigated compounds were tested in the concentration range 1000–0.001 μM .

Betacyanin Assay. The *Amaranthus* bioassay according to Biddington and Thomas (1973) was used with minor modifications. *Amaranthus caudatus* L. cv. Viridis seeds were germinated for 72 h in darkness at 25°C. Twenty explants in each replicate, consisting of the upper portion of the hypocotyl plus the cotyledons were placed in 6 cm Petri dishes containing 2 ml incubation medium (10 mM Na-K-phosphate buffer, pH = 6.3, 5 μM L-tyrosine and 5 mM potassium nitrate) or the test solutions prepared in the same medium. After a 20-h incubation at 25°C in the dark, betacyanin was extracted with 2 ml of distilled water by means of two cycles of freezing and thawing. The quantity of betacyanin was determined by difference spectroscopy ($A_{542} - A_{620}$) in a Specol 11 spectrometer and expressed in percentages of the control. Experiments were done three times with six replicates.

Cotyledon Enlargement Bioassay (Letham 1971). Radish seeds (*Raphanus sativus* L. cv. Red with white root) were germinated in darkness at 28°C for 72 h. Ten cotyledons per replicate were excised and transferred to 6 cm Petri dishes containing 3 ml of test solution freshly prepared in 2 mM K-phosphate buffer (pH 6.0). After exposure for 72 h to 60 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ of fluorescent light, the cotyledons were blotted and weighed. Two experiments with six replicates were averaged and standard deviations were calculated.

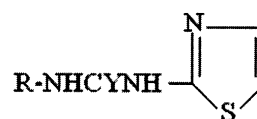
Statistical Analysis

The Fisher's least significant difference-test was used for statistical analysis. Differences between control and treatment means were considered significant at $p \leq 0.05$ and $p \leq 0.01$ (Steel and Torrie 1960).

Results

Synthesis

Nineteen N-aryl and alkyl-N'-(2-thiazolyl)ureas and thioureas were synthesized.



Thiazolylureas, Y = O and

Table 1. Characteristics of the Newly Synthesized Urea and Thiourea Derivatives of 2-aminothiazole and 2-furfurylamine

Chem. category / Comp d		Yield (%) ^a	M.p. (°C)	Molecular formula	Analysis (C, H, N) calcd / found
Thiazolylurea	2	93	245-50s	C ₁₀ H ₈ FN ₃ OS	50.63 / 50.59, 3.38 / 3.30, 17.72 / 17.42
	3	98	242-48s	C ₁₀ H ₈ FN ₃ OS	50.63 / 50.43, 3.38 / 3.14, 17.72 / 17.84
	4	70	155-6	C ₁₀ H ₈ ClN ₃ OS	47.34 / 47.41, 3.16 / 3.26, 16.57 / 16.39
	6	quant	160-1	C ₁₀ H ₁₅ N ₃ OS	53.33 / 53.23, 6.67 / 6.74, 18.67 / 18.31
Thiazolylthiourea	12	70	226	C ₁₀ H ₇ Cl ₂ N ₃ S ₂	39.60 / 39.72, 2.31 / 2.24, 13.86 / 13.41
	14	60	168-9	C ₁₁ H ₁₁ N ₃ S ₂	53.01 / 52.84, 4.42 / 4.30, 16.87 / 16.60
	17	64	190	C ₆ H ₉ N ₃ S ₂	38.50 / 38.62, 4.81 / 4.75, 22.46 / 22.56
	18	90	140	C ₈ H ₁₃ N ₃ S ₂	44.65 / 44.79, 6.05 / 6.15, 19.53 / 19.58
	19	60	163-4	C ₁₀ H ₁₅ N ₃ S ₂	49.79 / 49.61, 6.22 / 6.19, 17.43 / 17.65
Furfurylurea	21	82	128	C ₁₂ H ₁₁ FN ₂ O ₂	61.54 / 61.44, 4.70 / 4.52, 11.97 / 12.05
	22	89	167	C ₁₂ H ₁₁ FN ₂ O ₂	61.54 / 61.71, 4.70 / 4.82, 11.97 / 11.68
	23	87	142-3	C ₁₂ H ₁₁ ClN ₂ O ₂	57.49 / 57.67, 4.39 / 4.12, 11.18 / 10.94
	24	95	93	C ₈ H ₁₁ ClN ₂ O ₂	47.41 / 47.57, 5.43 / 5.59, 13.83 / 13.70
	25	95	139-40	C ₁₂ H ₁₈ N ₂ O ₂	64.86 / 64.43, 8.11 / 7.95, 12.61 / 12.32
	26	quant	186-7	C ₁₆ H ₁₄ N ₂ O ₂	72.18 / 71.90, 5.26 / 5.18, 10.53 / 10.70
Furfurylthiourea	28	88	96-8	C ₁₂ H ₁₁ FN ₂ OS	57.60 / 57.49, 4.40 / 4.16, 11.20 / 11.06
	31	85	133	C ₁₂ H ₁₀ Cl ₂ N ₂ OS	47.84 / 47.68, 3.32 / 3.31, 9.30 / 8.98
	33	98	85	C ₁₃ H ₁₄ N ₂ OS	63.41 / 63.13, 5.69 / 5.53, 11.38 / 11.45
	34	99	140	C ₁₆ H ₁₄ N ₂ OS	68.08 / 67.86, 4.96 / 5.05, 9.93 / 10.19
	35	84	88	C ₇ H ₁₀ N ₂ OS	49.41 / 49.70, 5.88 / 5.82, 16.47 / 16.20
	37	86	65-6	C ₁₀ H ₁₆ N ₂ OS	56.60 / 56.83, 7.55 / 7.49, 13.21 / 12.93
	38	96	95-6	C ₁₂ H ₁₈ N ₂ OS	60.50 / 60.31, 7.56 / 7.59, 11.76 / 11.84

a) Yield of crude products.

s) Melting points with sublimation.

R = **1** (phenyl, Ph), **2** (3-fluorophenyl, 3-FPh), **3** (4-fluorophenyl, 4-FPh), **4** (3-chlorophenyl, 3-ClPh), **5** (2-chloroethyl, 2-ClEt), **6** (cyclohexyl, CH), **7** (1-naphthyl, 1-N)

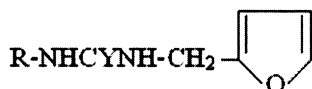
Thiazolylthioureas, Y = S and

R = **8** (Ph), **9** (4-FPh), **10** (4-chlorophenyl, 4-ClPh), **11** (4-bromophenyl, 4-BrPh), **12** (2, 4-dichlorophenyl, 2,4-Cl₂Ph), **13** (4-tolyl, 4-Tol), **14** (benzyl, Bz), **15** (2-naphthyl, 2-N), **16** (methyl, Me), **17** (ethyl, Et), **18** (n-butyl, Bu), **19** (CH).

Nineteen N-aryl and alkyl-N'-(2-furfuryl)ureas and thioureas were obtained.

Furfurylureas, Y = O and

R = **20** (Ph), **21** (3-FPh), **22** (4-FPh), **23** (3-ClPh), **24** (2, 2-ClEt), **25** (CH), **26** (1-N)



Furfurylthioureas, Y = S and

R = **27** (Ph), **28** (4-FPh), **29** (4-ClPh), **30** (4-BrPh), **31** (2,4-Cl₂Ph), **32** (4-Tol), **33** (Bz), **34** (2-N), **35** (Me), **36** (Et), **37** (Bu), **38** (CH).

Table 1 summarizes the physical properties and the CHN-contents of the 22 newly synthesized

compounds, which are undescribed in the literature. The yields of N-(4-halogenphenyl)-N'-(2-thiazolyl)thioureas were considerably higher (>90%) than those described in the literature (<50%) (Durga and Ramesh 1974).

The structures of the new compounds were confirmed with spectral data (¹H NMR, ¹³C NMR, IR and elemental analysis) to be N,N'-disubstituted ureas/thioureas. The IR spectra showed the presence of a secondary amino group (3076–3180 cm⁻¹) and a carbonyl (1740–1745 cm⁻¹) or thiocarbonyl group (1060–1160 cm⁻¹), which are typical for the urea/thiourea structure. The NMR spectra of the next compounds were: N-(4-fluorophenyl)-N'-(2-thiazolyl)urea (**3**) - ¹H NMR (DMSO-*d*₆) δ 10.54 (bs, 1H), 8.98 (major) and 8.67 (minor, 1H), 7.58–7.06 (m, 4H); ¹³C NMR (DMSO-*d*₆) δ 159.8, 152.0, 137.1, 135.1, 132.2 (d, J = 492.9 Hz), 120.7 (d, J = 7.5 Hz), 115.6 (d, J = 22.4 Hz), 112.5; N-phenyl-N'-(2-furfuryl)urea (**20**) - ¹H NMR (DMSO-*d*₆) δ 8.49 (s, 1H), 7.39–6.90 (td, J = 7.3 Hz, 5H), 6.52 (t, J = 5.7 Hz, 1H), 6.39 (dd, J = 3.1 Hz and 2.5 Hz, 1H), 6.25 (d, J = 3.1 Hz, 1H), 4.29 (d, J = 5.7 Hz, 2H); ¹³C NMR (DMSO-*d*₆) δ 155.3, 153.5, 142.4, 140.6, 129.0, 121.6, 118.1, 110.8, 106.8, 36.5.

Table 2. Herbicidal and Growth-Regulating Activities of Compounds 1–38

Chem. Category/Compd	Relative root length (%)								RHE ^a × 100	
	Wheat, μM				Cucumber, μM					
	1000	100	10	1	1000	100	10	1		
Diuron		9.6	72	99	104	23	83	98	98	240
Thiazolylurea/	1	4.3	76	82	84	18	62	91	106	419
“	2	22	56	73	99	14	53	96	111	64
“	3	33	71	84	90	23	68	91	109	70
“	4	16	55	58	61	23	56	89	106	144
“	5	37	88	89	86	79	112	114	103	214
“	6	16	95	107	108	23	79	106	91	144
“	7	87	95	92	92	79	102	102	89	91
Thiazolylthiourea/	8	52	95	101	108	47	91	89	99	90
“	9	55	95	90	99	53	85	99	102	96
“	10	27	70	87	104	42	74	83	99	156
“	11	52	76	96	93	53	83	97	99	102
“	12	42	80	90	99	62	92	109	91	148
“	13	33	76	104	95	56	80	97	102	170
“	14	55	96	99	111	76	86	103	99	138
“	15	4.7	98	105	119	17	92	96	105	362
“	16	9.9	46	73	86	36	86	121	123	364
“	17	4.1	43	83	76	41	109	129	136	1000
“	18	12	53	50	68	41	92	92	117	342
“	19	23	62	74	80	52	91	129	126	226
Furfurylurea/	20	24	83	107	96	52	83	96	99	217
“	21	21	76	105	119	27	89	97	97	129
“	22	25	80	107	108	42	74	94	102	168
“	23	22	53	79	98	55	77	99	89	250
“	24	77	113	117	120	80	96	102	96	104
“	25	27	79	104	102	55	83	94	95	204
“	26	84	107	111	108	99	109	106	106	118
Furfurylthiourea/	27	58	89	108	114	61	100	106	108	105
“	28	19	95	114	107	35	92	100	106	184
“	29	56	101	114	109	41	85	98	97	73
“	30	67	84	108	113	68	86	98	102	101
“	31	41	86	111	111	64	80	100	102	156
“	32	73	107	123	114	82	92	97	105	112
“	33	19	95	108	101	45	102	106	103	237
“	34	58	84	113	120	79	95	100	106	136
“	35	64	122	116	101	62	86	97	92	97
“	36	43	98	105	110	62	80	88	92	144
“	37	25	90	119	124	36	102	103	97	144
“	38	27	89	114	122	38	92	97	100	141

Control (0 μM treatments) values of the length of wheat and cucumber roots were respectively, 67.5 ± 8.9 mm and 66 ± 5.7 mm. Treatment means were significantly different by LSD - for wheat seedlings. LSD 5% = 13.12 and LSD, 1% = 17.40, for cucumber seedlings LSD 5% = 15.06 and LSD 1% = 20.00
^aRHE is described in the text.

Herbicidal, Root Growth Inhibitory and Stimulatory Activities

The herbicidal activity of the test compounds was recorded using mostly 1000 μM concentrations. A standard, commercial herbicide Diuron (N-(3,4-dichlorophenyl)-N',N'-dimethylurea), was also tested

under similar conditions for comparison. Results of the growth-regulating assay are presented in Table 2.

Some urea and thiourea derivatives applied at lower concentrations (100, 10 and 1 μM) tended to transform inhibitory into stimulatory activity compared to the control. The thiazolylthiourea derivatives predominantly stimulated the growth of

Table 3. Herbicidal Activity of the More Active Compounds

Compound	Dose giving 50% herbicidal effect, μM		SHR ^a \times 100	
	Wheat	Cucumber	Wheat	Cucumber
Diuron	350	550	100	100
1	350	300	100	54
2	175	105	50	19
3	550	400	157	73
4	125	200	36	36
15	500	550	143	100
16	85	725	24	132
17	85	850	24	154
18	106	800	30	145
19	300	1050	86	191
21	450	650	128	118
23	108	2500	31	454
37	600	800	171	145

^aSHR is described in the text.

cucumber roots while the furfurylurea and thiourea derivatives stimulated wheat roots.

In general, the urea and thiourea derivatives of the 2-aminothiazole had greater herbicidal activity than the 2-furfurylamine derivatives. Only one compound (**1**) out of 14 tested N-aryl-N'-(2-furfuryl and thiazolyl) ureas had rather high activity against wheat (4.3%) and two compounds (**1** and **2**) towards cucumber (18% and 14%) in relation to diuron (wheat 9.6% and cucumber 23%).

The phenylurea derivative of 2-aminothiazole (**1**) also had high selective herbicidal activity (RHE = 419 compared to diuron RHE = 240) and the 3-chlorophenylurea derivative of 2-furfurylamine (**23**) had selective herbicidal activity (RHE = 250) equal to that of diuron.

It was of particular importance to obtain the relative order of herbicidal (or root growth inhibitory) activity of the tested compounds against both test objects as a function of the nature and structure of each N substituent. Those of the thiazolylurea derivatives were:

wheat: Ph \gg 3-ClPh = CH > 3-FPh > 4-FPh > 2-ClEt \gg 1-N

cucumber: 3-FPh > Ph > 4-FPh = 3-ClPh = CH \gg 2-ClEt = 1-N

It is evident that unsubstituted phenyl had significant herbicidal activity against wheat while 3-fluorophenyl had greater herbicidal activity towards cucumber.

The relative orders of the furfurylurea derivatives' herbicidal activity were:

wheat: 3-FPh > 3-ClPh > Ph > 4-FPh > CH \gg 2-ClEt > 1-N and

cucumber: 3-FPh > 4-FPh > Ph > 3-ClPh = CH > 2-ClEt > 1-N.

In both plants herbicidal activity was determined by the 3-fluorophenyl substituent. Herbicidal activity did not result from the 2-chloroethyl and 1-naphthyl substituents in either of the two urea groups.

Among the 24 N-aryl and alkyl-N'-(2-furfuryl and thiazolyl) thioureas, four compounds (**15**, **16**, **18** and especially **17**) had much higher selective herbicidal activity compared to diuron (RHE = 362, 364, 342 and 1000, respectively), and another two compounds (**19** and **33**) were almost equal to diuron. Compounds **15** and **17** exhibited higher herbicidal effects than diuron on wheat (4.7% and 4.1%, respectively relative to 9.6% for diuron).

The relative orders of herbicidal activity for the furfurylthiourea derivatives were:

wheat: 4-FPh = Bz > Bu > CH > 2,4-Cl₂Ph > Et > 4-ClPh > Ph = 2-N > Me > 4-BrPh > 4-Tol

cucumber: 4-FPh > Bu > CH > 4-ClPh > Bz > Ph > Me = Et > 2,4-Cl₂Ph > 4-BrPh > 2-N > 4-Tol

The herbicidal activity of this series of compounds against both test plants was attributed to the presence of the 4-fluorophenyl substituent whereas in the compounds containing 4-chloro- or bromophenyl, substituents were inhibitory but not herbicidal. In the series of the alkylthio derivatives, herbicidal activity increased with the increasing length of the alkyl chain, which is in accordance with the conclusion that there is a direct proportion between the herbicidal activity and the lipophilic character of the compounds, resulting in faster penetration of the membrane in order to reach the binding site (Mallipudi and others 1994).

The relative orders of herbicidal activity for the thiazolylthiourea derivatives were:

wheat: Et > 2-N > Me > Bu > CH > 4-ClPh > 4-Tol > 2,4-Cl₂Ph > Ph = 4-BrPh > 4-FPh = Bz

cucumber: 2-N > Me > Et = Bu > 4-ClPh > Ph > CH > 4-BrPh = 4-FPh > 4-Tol > 2,4-Cl₂Ph > Bz

The highest herbicidal activity was manifested by all the alkyl- and the 2-naphthyl-thiazolylthioureas. Total herbicidal activity with well pronounced selectivity in wheat was exhibited by N-(2-naphthyl)-N'-(2-thiazolyl)thiourea (**15**) - 4.7% towards wheat and 16.7% towards cucumber. The N-ethyl-N'-(2-thiazolyl) thiourea (**17**) exerted the highest herbicide selectivity towards wheat at the 1000 μM concentration, and the greatest stimulation of cucumber root growth at the 10 and 1 μM concentrations, 129% and 136%, respectively.

Table 4. Effects of Urea and Thiourea Derivatives of 2-aminothiazole on *Amaranthus* Betacyanin Synthesis (A) and *Raphanus* Cotyledon Enlargement (R)

Chem. Category	Compds No	Cytokinin-like activity at concentrations in μM						
		0.001	0.01	0.1	1	10	100	1000
Thiazolylurea	1	A, 104	100	124	141	186	184	73
		R, 108	109	123	125	123	131	77
"	2	A, 102	100	123	144	199	183	137
		R, 118	111	125	126	131	118	61
"	3	A, 97	97	109	138	162	163	139
		R, 95	107	113	109	114	107	62
"	4	A, 126	145	149	159	160	137	113
		R, 111	115	117	120	130	146	87
"	5	A, 101	110	102	99	103	117	157
		R, 97	105	104	97	105	108	102
"	6	A, 96	97	96	94	99	107	99
		R, 107	105	111	127	152	166	171
"	7	A, 100	107	110	112	113	105	88
		R, 107	105	110	112	113	105	88
Thiazolylthiourea	8	A, 99	102	108	118	150	177	164
		R, 105	111	108	105	108	109	73
"	9	A, 107	101	108	133	170	200	141
		R, 108	109	110	107	102	93	80
"	10	A, 95	95	98	98	121	177	145
		R, -	-	-	106	99	101	63
"	11	A, 92	92	95	103	124	163	159
		R, -	-	-	118	114	102	79
"	12	A, 104	101	96	106	116	153	145
		R, 103	105	96	101	112	176	169
"	13	A, 91	88	88	88	88	110	128
		R, 99	98	100	102	109	123	64
"	14	A, 99	98	100	102	109	123	64
		R, -	-	-	98	103	103	59
"	15	A, 101	90	101	100	102	117	127
		R, 96	99	91	100	95	105	102
"	16	A, 100	95	108	107	117	138	149
		R, 94	91	96	99	102	119	120
Standard	DPU	A, 104	106	112	122	141	168	181
		R, 91	97	91	94	102	109	82
"	4PU	A, 136	135	160	237	277	257	162
		R, 101	109	102	134	109	112	84
"	TDZ	A, 210	218	227	225	210	206	138
		R, 117	116	141	178	256	-	-
"	Kinetin	A, 117	116	141	178	256	-	-
		R, 116	106	109	115	133	-	-

Results represent the yield of the pigment and the weight of 10 cotyledons in percentage of the control.

Control: $Abs_{542nm} - Abs_{620nm} = 0.1568 \pm 0.0028$; Weight $\cdot 10^{-1}$ cotyledons = 0.2262 ± 0.0048 g.

A, LSD 5% = 0.0181; LSD 1% = 0.0241; R, LSD 5% = 0.0569; LSD 1% = 0.0756.

The herbicidal activity of the more active compounds, expressed by ID_{50} , and their selective herbicidal ratios in relation to diuron in both plants are present in Table 3. The compounds **16**, **17**, **18** and particularly **23** possessed $SHR \ll 100$ in wheat seedlings while in cucumber seedlings they had $SHR \gg 100$. Therefore these compounds were substantially more herbicidally active than diuron against wheat seedlings. Of great interest was the comparison of the selective herbicidal ratios of compounds **4** and **23**, which differ only in the het-

erocyclic ring. Both compounds had similar activities towards wheat and very different activity towards cucumber. This appears to be due to the different nature of the 5-member heterocyclic ring present in these compounds.

Cytokinin-like Activity

The known phenylurea cytokinins thiazuron (TDZ) N-phenyl-N'-(4-pyridyl urea (4PU) and N,N'-diphenylurea (DPU) were used as standards to

Table 5. Effects of Urea and Thiourea Derivatives of 2-furfurylamine on *Amaranthus* Betacyanin Synthesis (A) and *Raphanus* Cotyledon Enlargement (R)

Chem. Category/Compd	Cytokinin-like activity at concentrations in μM						
	0.001	0.01	0.1	1	10	100	1000
Furfurylurea/ 20	A, 97	99	105	100	102	110	115
" 21	A, 89	97	105	100	116	134	109
" 22	R, –	–	–	100	98	93	53
" 23	A, 116	105	107	113	106	119	114
" 24	R, 93	105	103	94	94	100	59
" 25	A, 108	110	109	102	127	135	131
" 26	R, 105	92	102	106	101	108	59
" 27	A, 108	101	99	104	117	107	110
" 28	A, 102	103	90	90	95	103	136
" 29	A, 91	85	91	89	93	88	89
Furfurylthiourea/ 30	A, 97	92	93	98	94	100	105
" 31	A, 96	94	96	95	92	97	94
" 32	A, 97	100	98	92	97	101	105
" 33	A, 96	108	101	99	97	97	96
" 34	A, 95	91	89	84	83	89	91
" 35	A, 85	87	88	96	88	81	100
" 36	A, 93	90	97	93	94	93	84
" 37	A, 110	103	103	96	110	106	99
" 38	A, 96	96	100	104	102	106	140
" 39	A, 103	97	99	99	101	112	158
" 40	A, 110	98	107	95	104	118	113
" 41	A, 97	101	99	94	98	99	95
Standard/ DPU	A, 104	106	112	122	141	168	181
" 42	R, 91	97	91	94	102	109	82
" 43	A, 136	135	160	237	277	257	162
" 44	R, 101	109	102	134	109	112	84
" 45	A, 210	218	227	225	210	206	138
" Kinetin	A, 117	116	141	178	256	–	–
" 46	R, 116	106	109	115	133	–	–

Results represent the yield of the pigment and the weight of 10 cotyledons in percentage of the control. Control: $Abs_{542nm} - Abs_{620nm} = 0.1608 \pm 0.0034$; Weight $\cdot 10^{-1}$ cotyledons = 0.2262 ± 0.0048 g. A, LSD 5% = 0.0193; LSD 1% = 0.0255; R, LSD 5% = 0.0569; LSD 1% = 0.0756.

investigate the cytokinin-like activity of the urea and thiourea derivatives of 2-aminothiazole and 2-furfurylamine in an *Amaranthus* bioassay. Only 4PU and DPU were tested as well in a *Raphanus* cotyledon enlargement bioassay.

The *Amaranthus* betacyanin bioassay is highly specific and sensitive so it is especially suitable for the study of structural variation. In fact, it has been used to analyze quantitative structure-activity relationships for the cytokinin activity of adenine derivatives (Iwamura and others 1979), substituted imidazoles (Cavender and others 1988), ethynylimidazo[4,5-b]pyridine and its riboside (Nishikawa and others 2000).

We investigated cytokinin-like activity of all 38 compounds using the *Amaranthus* bioassay. The screening results are summarized in Tables 4 and 5. In general, the ureas and the thioureas containing a

2-thiazole ring were more active than those containing a 2-furfuryl residue. The urea derivatives exhibited higher cytokinin-like activity than the thioureas.

Among the arylthiazolyl-ureas, compounds **1** and **2** possessing phenyl and 3-fluorophenyl substituents manifested the highest cytokinin-like activity at 10 μM (186% and 199%) and 100 μM (184% and 183%, respectively). The 3-chlorophenyl-analogue (**4**) maintained high levels of cytokinin-like activity (145–160%) within the concentration range of 0.01–10 μM . The presence of a fluorine in the *para*-position of the phenyl ring (**3**) decreased the activity compared to that of the 3-fluorophenyl-analogue (**2**) with 37% at 10 μM and 20% at 100 μM . Compound **7**, containing 1-naphthyl substituent, induced the betacyanin synthesis in parallel to the concentration applied up to 1000 μM (171%).

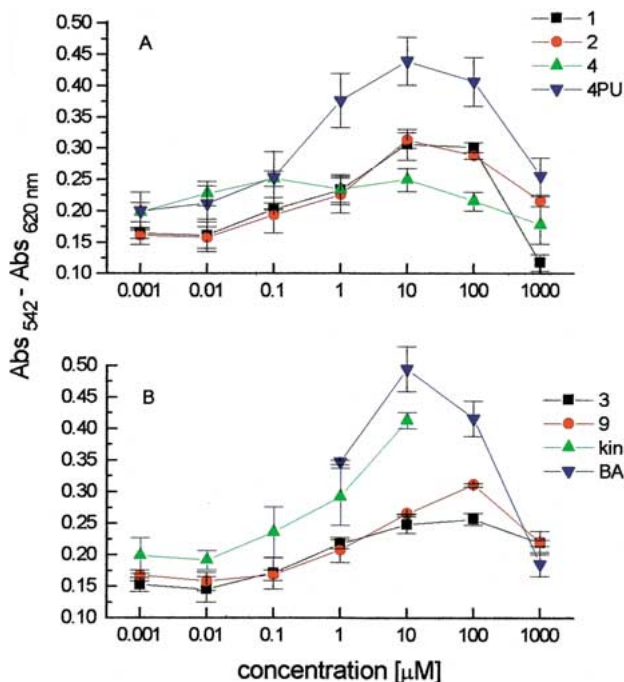


Figure 1. Dose-response graphs of *Amaranthus caudatus* cotyledons exposed to some active thiazole derivatives and standards. Each data point is the mean of 18 replicates.

N-substitution of other groups such as 2-chloroethyl (**5**) and markedly cyclohexyl (**6**) for the phenyl ring afforded less active compounds.

The arylthiazolyl-thioureas displayed cytokinin-like activity at the optimal concentration of 100 μM. N-Phenyl- (**8**), 4-halogenphenyl- (**9,10,11**), 4-methylphenyl- (**13**), and 2,4-dichlorophenyl- (**12**)-substituents resulted in high cytokinin-like activity. The activity of thioureas containing 4-halogenphenyl-substituents decreased proportionally to the electronegativity of the halogen. The 4-fluorophenyl-substituent was the most active, in fact the thiazolylthiourea with this substituent (**9**) was by far the most active compound at the 100 μM concentration (200%) compared to the corresponding urea derivative (**3**) (163%). It had similar activity to thiazuron at the same concentration (206%).

The generally acknowledged high cytokinin activity of kinetin led us to synthesize the furfurylureas. However the furfurylureas had only moderate cytokinin-like activity and the aryl (furfuryl)thioureas were completely inactive. Only N-(3-fluoro- and chlorophenyl)-N'-(2-furfuryl) ureas (**21** and **23**) possessed moderate cytokinin-like activity at the optimal concentration of 100 μM while the same derivatives with a thiazole nucleus

had significantly higher activities at the lower optimal concentration of 10 μM.

Alkylthiourea derivatives of 2-aminothiazole and 2-furfurylamine were characterized by moderate cytokinin-like activity at the 1000 μM concentration. Evidently, the aromatic moiety plays a very important role in enhanced activity. Cytokinin-like activity was the highest with the n-butyl substituent in the thiazolylthiourea series (**18**, 149%) whereas in the furfurylthiourea series it was highest with the ethyl substituent (**36**, 158%).

A comparison of the cytokinin-like activity of more active thiazole derivatives (**1**, **2**, **3**, **4** and **9**), 4PU (urea standard), kinetin and N⁶-benzyladenine (BA) (purine standards) in the *Amaranthus* bioassay is presented in Figure 1. The slopes of the dose-response curves of compounds **1**, **2**, **3** and **4** were similar to that of the curve of 4PU and the purine cytokinins. The curve of compound **9** had a somewhat similar slope and a maximum at a 10-fold higher concentration as compared to the active urea compounds.

The second cytokinin bioassay — radish cotyledon enlargement — was applied to only 14 compounds that had already shown high (or moderate) cytokinin-like activity in a betacyanin bioassay. The highest activity was shown by compound **4** at 100 μM (146%) whereas the optimal concentration of the same substance in the *Amaranthus* bioassay was 10 μM (160%). It promoted both the cytokinin-specific reactions — betacyanin synthesis from 0.001 to 10 μM and enlargement of radish cotyledons — from 0.001 to 100 μM. The lowest concentration in which compound **2** stimulated betacyanin synthesis was 0.1 μM whereas the growth of radish cotyledons was stimulated by the same substance at a lower concentration (0.001 μM). It exerted a maximum effect (131%) on the radish cotyledons at a higher concentration (10 μM), similar to that of kinetin (133%) at the same concentration.

There was no general correlation in the cytokinin-like activity of the 14 compounds and the standards DPU, 4PU and kinetin between betacyanin synthesis in *Amaranthus caudatus* L. and cotyledon enlargement in *Raphanus sativus* L. The results obtained with compounds **1**, **2**, **3**, **4**, **8**, **9–11**, **21**, **23** in the two bioassay systems used in this study were particularly interesting. They exhibited high cytokinin-like activity in the *Amaranthus* betacyanin synthesis test and little activity in the radish cotyledon enlargement test. These results were similar to those discussed by Kefford and others (1966) for the urea derivatives, Iwamura and others (1979) for the adenine derivatives, Cavender and others

(1988) for the substituted imidazoles, and Nishikawa and others (2000) for the ethynylimidazo[4,5-b]pyridines. Some tested compounds showed different levels of cytokinin activity (from agonistic to antagonistic) in various bioassay systems, indicating the species difference of receptor molecules. This suggests the possibility of designing compounds with highly selective cytokinin activities. Compounds **3**, **8**, **9**, and **10** which promoted high *Amaranthus* betacyanin synthesis but had little activity in the other test system, may fall in this category.

Discussion

The structural requirement for high biological activity in ureas/thioureas includes the presence of an intact urea/thiourea bridge connecting the heterocyclic nucleus (5- or 6-member) to a hydrophobic group typically an aromatic ring (Okamoto and others 1983; Mok and others 1982; Basarab and others 1991).

The urea and thiourea derivatives of 2-aminothiazole and 2-furfurylamine were prepared to examine the substituent effect at the one nitrogen atom of the urea/thiourea bridge and to compare the effect of the two attached 5-member heterocyclic nuclei on herbicidal and cytokinin-like activities.

R - NHCYNH - Thiazolyl / Furfuryl

R = aryl, alkyl, 2-chloroethyl, cyclohexyl; Y = O and S.

In general, aryl substituents contributed greater activity than alkyl, 2-chloroethyl, or cyclohexyl substituents. Aromatic phenyl ring substituents also played an important role in activity, and a variety of substituents maintained good herbicidal and cytokinin-like activity. The optimization in the benzene ring showed that compounds with an electronegative nonpolar substituent (F, Cl, Br) at the *meta* or *para* positions had the highest activity. In a series of halogenphenyl-ureas and thioureas, *meta*- and *para*-fluorophenyl derivatives had the highest herbicidal activity whereas *meta*- and *para*-chlorophenyl and *para*-bromophenyl derivatives appeared to have a modest effect or resulted in a complete loss of herbicidal activity. Among fluorophenyl(thiazolyl and furfuryl)ureas, those having a fluorine in the *meta*-position of the aromatic ring tended to exhibit high cytokinin-like activity. The unique ability of *meta*-fluorine substitution to increase cytokinin activity has been observed previously (Isogai 1981; Mok and others 1982; Henrie and others 1988). The *para*-fluorophenyl substituent in the thiazolylthioureas resulted in high cytokinin-like activity but not

herbicidal activity while in the furfurylthioureas the same substituent resulted in high herbicidal activity. Therefore, the 4-fluorophenyl substituent selectively influenced herbicidal or cytokinin-like activities depending on the type of heterocyclic ring.

The presence of two chlorine atoms on the benzene ring, such as the 2,4-dichlorophenyl substituent, did not significantly affect herbicidal and cytokinin-like activities of the thiazolyl- and furfurylthioureas. The introduction of a methyl group at the *para*-position (4-tolyl substituent), the steric size of which is considered to be similar to that of a chlorine atom, retained the same level of cytokinin-like activity but not of herbicidal activity. Therefore, 4-methylphenyl is a bioisosteric analogue of 4-chlorophenyl but only with regard to cytokinin-like activity. The benzyl substituent only in the furfurylthioureas had good herbicidal activity against wheat which might be attributed to a more symmetric structure of these compounds. The 2-naphthyl substituent had the most pronounced effect on herbicidal activity of the thiazolylthioureas and a moderate effect on cytokinin-like activity of the thiazolyl- and furfurylthioureas.

Alkyl (methyl, ethyl, butyl) derivatives of the thiazolylthioureas were at least 5 times more active herbicides than aryl derivatives. It is known that the ureas undergo base-induced hydrolysis via an elimination-addition mechanism. Goure and others (1991) found that arylureas are stable for several hours in pH 3–4 buffer when analyzed by HPLC but are unstable at pH 6 and above. The acidity of the N-H proton increased due to the presence of the electron-withdrawing benzene ring in arylureas and decreased in alkyl-substituted ureas. In our case, the increased molecular stability of alkylureas at pH 6 probably determined their high herbicidal activity.

Herbicidal and cytokinin-like activities were very sensitive to the type of heterocycle. As is well-known, among phenylheterocyclic ureas, pyridyl- and thiadiazolylureas are two major classes of urea derivatives that possess diverse physiological activity, especially cytokinin activity equivalent to or exceeding that of the most active adenine cytokinins in tobacco and bean callus bioassays (Takahashi and others 1978; Mok and others 1982). On the other hand, phenylureas containing an imidazol ring had no chlorophyll retention activity in excised wheat leaves, although the imidazol ring is a quite highly active phytohormone in many bioactive compounds (Cavender and others 1988).

Relationships between thiazolyl- and furfuryl-derivatives and activity showed that the thiazol ring provided significantly enhanced activity whereas the furan ring had little effect. The substitution of

oxygen for sulfur in the heterocyclic ring is a known example for bioisosterism (Chen and others 2000). Therefore the presence of the nitrogen atom in a thiazol ring could play an important role in the biological activity. On the other hand, the furan ring is separated from the urea/thiourea bridge by one methylene group whereas the thiazol ring is attached directly. The quantitative structure-activity relationships of sulfonylurea herbicides have shown that sulfonylureas with favorable herbicidal activity are compounds with a heterocycle, such as triazine or pyridine, attached to an unmodified sulfonylurea bridge (Qian 1999).

Dayan and others (2000) have demonstrated that the new urea derivatives of 4-carboxymethyl-5-isopropyl-thiazole were herbicidally active (at 100 μM) and appeared to act via inhibition of the photosynthetic electron transport system similar to the phenylurea herbicide diuron. The authors show that the most active urea-substituted thiazole contains *para*-chlorophenyl side chain and the relative efficacy increases in the order of *ortho*, *meta* and *para* arrangement.

The similar slopes of the dose - response curves of active thiazole compounds compared to those of 4PU, BA, and kinetin (Figure 1) substantiated the proposal that our compounds realize their cytokinin-like action in a manner similar to the active urea and purine cytokinins. Studies determining cytotoxicity to mammalian cells for thiazolylthioureas showed that they have low cytotoxicity (Irem and others 1990).

In conclusion, the ureas and thioureas containing thiazole nuclei were found to possess high biological activity. The presence of fluoro- or chloro-phenyl substituents in these compounds increased the cytokinin-like activity and alkyl groups markedly increased the herbicidal activity.

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