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Original Article

Synthesis and Phytotoxic Activities of *N*-Substituted Phenyl Isothiazolone Derivatives*

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A series of 3(2*H*)-oxo-*N*-(substituted phenyl)-4,5,6,7-tetrahydro-1,2-benzisothiazoles (**2**) were obtained *via* four reaction steps starting from 2-chlorocyclohexene-1-carboxylic acid. The sulfur atom of 3-oxo-4,5,6,7-tetrahydro-1,2-benzisothiazoles (**2**) was oxidized with an equimolecular amount of 3-chloroperbenzoic acid (3-CPBA) in chloroform to give the corresponding 3-oxo-4,5,6,7-tetrahydro-1,2-benzisothiazole-1-oxides (**3**). Oxidation of **2** with two moles of 3-CPBA afforded 3-oxo-4,5,6,7-tetrahydro-1,2-benzisothiazole-1,1-dioxides (**4**). Phytotoxic activities of the compounds (**2**–**4**) synthesized were assayed by inhibition of protoporphyrinogen-IX oxidase isolated from corn as well as by growth inhibition, chlorophyll decrease and peroxidative destruction of cell membranes of the green microalga *Scenedesmus acutus*. Among the compounds (**2**–**4**), **4** showed the strongest activities according to all phytotoxic parameters, exhibiting phytotoxicities characteristic of peroxidizing herbicides. 3(2*H*)-oxo-2-[4-chloro-3-(isopropoxycarbonyl)phenyl]-4,5,6,7-tetrahydro-1,2-benzisothiazole-1,1-dioxide (**4h**) was the strongest of the compounds **4** tested.

Key words: 3(2*H*)-oxo-*N*-(substituted phenyl)-4,5,6,7-tetrahydro-1,2-benzisothiazoles, 3-oxo-4,5,6,7-tetrahydro-1,2-benzisothiazole-1-oxides, 3-oxo-4,5,6,7-tetrahydro-1,2-benzisothiazole-1,1-dioxides, protoporphyrinogen-IX oxidase, peroxidizing activity.

INTRODUCTION

In our previous papers,[1-3] we have reported phytotoxic activities of several cyclic imides (**1**), competitively inhibiting protoporphyrinogen-IX oxidase (Protox) from corn as well as simultaneously causing growth inhibition, chlorophyll decrease and peroxidative destruction of cellular membranes as could be observed with the green microalga *Scenedesmus acutus*. Cyclic imides have been found to be extremely effective as low use-rate (a few g/ha) herbicides, after an adequate structural modification of the imide moiety.[4,5] Triazoline, pyrazole, 1,2,4-triazine and thiadiazole rings were favorably used to modify the imide moiety in the molecular design of such herbicides, although the thiazole and isothiazole moieties were scarcely applied in the design.

In this paper, we made structural modifications of the herbicidally active tetrahydrophthalimides **1** to obtain 3-oxo-*N*-substituted phenyl-4,5,6,7-tetrahydro-1,2-benzisothiazoles (**2**–**4**) in order to determine the structure-activity relationship of the newly designed compounds. Thus, novel isothiazol-3(2*H*)-ones (**2**–**4**) were synthesized and assayed for their peroxidizing phytotoxicity.

* This paper is dedicated to Prof. Dr. P. Böger in appreciation of his efforts to strengthen the Japanese-German relationship in herbicide biochemistry research.

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MATERIALS AND METHODS

1. Chemicals

Melting points were determined on a Yanagimoto micro-melting point apparatus and are uncorrected. IR spectra and ^{1}H- and ^{13}C-NMR spectra were recorded on a Perkin-Elmer FTIR-1600 spectrophotometer and a JNM EX-400 (400 MHz) spectrometer, respectively. Abbreviations used in this paper are as follows; s, singlet; d, doublet; t, triplet; q, quartet; h, heptet; m, multiplet; br, broad. Preparative high-performance liquid chromatography (HPLC) was carried out with a Kusano Kagaku KHLC-201 instrument using a 300 × 22 mm glass column packed with silica gel. Microanalyses were performed with a Perkin-Elmer PE-2400 elemental analyzer.

Compounds assayed in this paper were synthesized *via* the scheme shown in Fig. 2. Analytical grade chemicals for the phytotoxic assay and for *Scenedesmus acutus* cultivation were purchased from Kanto Chemical Co., Tokyo. All

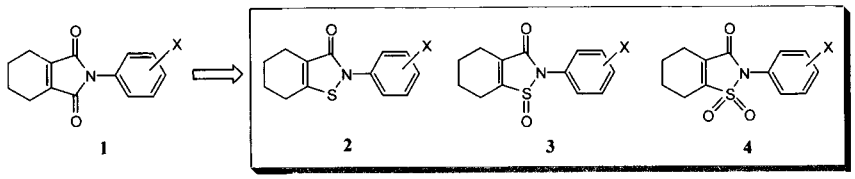


Fig. 1. Target compounds.

other fine chemicals and buffers were from Sigma Chemical Co., USA.

2. Preparation of Compounds

As typical procedures to synthesize 3(2*H*)-oxo-2-(substituted phenyl)-4,5,6,7-tetrahydro-1,2-benzisothiazoles (**2**), the methods used to produce 3(2*H*)-oxo-2-[4-chloro-3-(isopropoxycarbonyl)phenyl]-4,5,6,7-tetrahydro-1,2-benzisothiazole (**2h**) are shown (2.1–2.4), starting from 2-chlorocyclohexene(1)-carboxylic acid (**5**). The oxidation of 3(2*H*)-oxoisothiazoles (**2**) is represented by the synthesis of 3(2*H*)-oxo-2-(4-chlorophenyl)-4,5,6,7-tetrahydro-1,2-benzisothiazole-1-oxide (**3a**) and 3(2*H*)-oxo-*N*-(4-chlorophenyl)-4,5,6,7-tetrahydro-1,2-benzisothiazole-1,1-dioxide (**4a**) in procedure 2.5 and 2.6, respectively.

*2.1. 2-Chloro-N-[4-chloro-3-(isopropoxycarbonyl)phenyl]-cyclohexene(1)-1-carboxamide (**6h**)— General procedure for the preparation of cyclohexene-1-carboxamides (**6a-j**)*

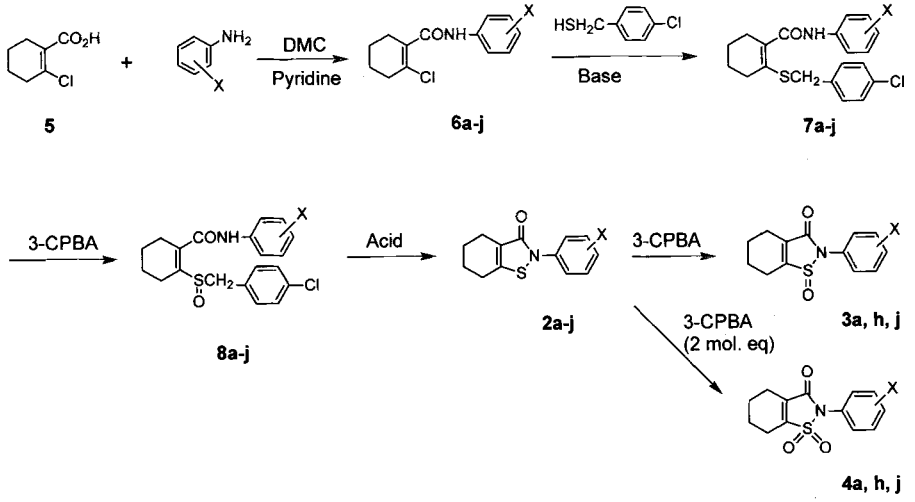
To a stirred solution of **5** (0.22 g, 1.4 mmol), prepared as reported,[6] isopropyl 5-amino-2-chlorobenzoate (0.35 g, 1.6 mmol) and pyridine (0.27 g, 3.4 mmol) in chloroform (20 ml) were added dropwise a solution of 2-chloro-1,3-dimethylimidazolinum chloride (DMC) (0.34 g, 2.0 mmol) in chloroform (20 ml) at 15°C and then stirred at room temperature for 1 hr. The reaction mixture was heated under reflux for 1 hr and then allowed to cool to ambient temperature. The resulting mixture was washed successively with water, 10% aq. $NaHCO_3$, and water, and then dried over Na_2SO_4. After evaporation of the solvent, the residual oil was subjected to preparative HPLC on silica gel, with chloroform as an eluent, to give pure **6h** as a colorless oil (0.25 g, 49%). ^{1}H NMR δ_H ($CDCl_3$): 1.38 (s, J=6.1 Hz, 6H, 2×CH_3), 1.68 (m, 2H, CH_2), 1.74 (m, 2H, CH_2), 2.45 (m, 4H, 2×CH_2), 5.26 (h, J=6.1 Hz, 1H, CH), 7.39 (d, J=8.5 Hz, 1H, Ph), 7.79 (dd, $^1J_{CH}$=8.5 and $^3J_{CH}$=2.7 Hz, 1H, Ph), 7.88 (br, 1H, NH), 7.99 (d, $^3J_{CH}$=2.7 Hz, 1H, Ph). ^{13}C NMR δ_C ($CDCl_3$): 21.4 (t), 21.8 (q), 23.2 (t), 28.2 (t), 34.0 (t), 69.5 (d), 121.8 (d), 123.4 (d), 128.2, 131.2, 131.3 (d), 131.4, 131.8, 136.1, 164.8, 166.0. Found: C, 57.28; H, 5.43; N, 3.81%. Calcd. for $C_{17}H_{19}Cl_2NO_3$: C, 57.32; H, 5.38; N, 3.93%.

*2.2. 2-(4-Chlorobenzylthio)-N-[4-chloro-3-(isopropoxycarbonyl)phenyl]cyclohexene(1)-1-carboxamide (**7h**) — General procedure for the preparation of 2-benzylthiocyclohexen-1-carboxamides (**7a-j**)*

A mixture of **6h** (0.25 g, 0.7 mmol), 4-chlorobenzylmercaptan (0.11 g, 0.7 mmol), K_2CO_3 (0.08 g, 0.8 mmol) and ethanol-water (3 : 1 v/v, 4 ml) was heated under reflux for 2 hr and then allowed to cool to ambient temperature. The separated crystals were isolated by filtration and washed with ethanol to give **7h**. Recrystallization from isopropanol gave colorless needles (0.2 g, 63%); mp 118.3–119.5°C. ^{1}H NMR δ_H ($CDCl_3$): 1.39 (d, J=6.1, 6H, 2×CH_3), 1.66 (m, 2H, 2×CH_2), 2.33 (m, 2H, CH_2), 2.39 (m, 2H, CH_2), 3.86 (s, 2H, SCH_2), 5.27 (h, J=6.1 Hz, 1H, CH), 7.17 (d, J=8.3 Hz, Ph), 7.27 (d, J=8.3, Ph), 7.35 (d, J=8.8 Hz, 1H, Ph), 7.42 (be,1H, NH), 7.65 (dd, $^1J_{CH}$=8.8 and $^3J_{CH}$=2.4 Hz, 1H, Ph), 7.73 (d, $^3J_{CH}$=2.4 Hz, 1H, Ph). ^{13}C NMR δ_C ($CDCl_3$): 21.6 (t), 21.8 (q), 22.9 (t), 28.3 (t), 34.3 (t), 35.8 (t), 69.5 (d), 121.7 (d), 123.4 (d), 127.8, 128.7 (d), 129.9 (d), 131.1 (d), 131.2, 133.0, 133.9, 136.2, 136.3, 136.5, 164.9, 167.4. Found: C, 60.65; H, 5.39; N, 2.91%. Calcd. for $C_{24}H_{25}Cl_2NO_3S$: C, 60.50; H, 5.27; N, 2.93%.

*2.3. 2-(4-Chlorobenzylsulfinyl)-N-[4-chloro-3-(isopropoxycarbonyl)phenyl]cyclohexene(1)-1-carboxamide (**8h**) —General procedure for the preparation of 2-sulfinyl-cyclohexene-1-carboxamides (**8a-j**)*

To a solution of **7h** (0.20 g, 4.2 mmol) in chloroform (10 ml) was added dropwise a solution of 3-chloroperbenzoic acid (3-CPBA, 70%, 0.08 g, 4.6 mmol) in chloroform (5 ml) at 0°C. The reaction mixture was allowed to stand at room temperature for 10 min and then washed with 10% aq. $NaHCO_3$ followed by water and dried over Na_2SO_4. The solvent was evaporated and the crystalline residue was recrystallized from acetonitrile to give compound **8h** as colorless prisms (0.12 g, 57%), mp 168.8–170.4°C. ^{1}H NMR δ_H ($CDCl_3$): 1.40 (s, 6H, 2×CH_3), 1.61 (m, 2H, CH_2), 1.72 (m, 2H, CH_2), 2.44 (m, 4H, 2×CH_2), 4.09 and 4.19 (d, J=12.5 Hz, 2H in total, together with SCH_2), 5.28 (h, J=6.3 Hz, 1H, CH), 7.33 (d, J=8.5 Hz, Ph), 7.33 (d, J=8.5 Hz, 2H, Ph), 7.34 (d, J=8.8 Hz, 1H, Ph), 7.62 (dd, $^1J_{CH}$=8.8 and $^3J_{CH}$=2.7 Hz, 1H, Ph), 7.97 (d, $^3J_{CH}$=2.7 Hz, 1H, Ph), 8.82(br, 1H, NH). ^{13}C NMR δ_C ($CDCl_3$): 20.7 (t), 21.5 (t), 21.6 (t), 21.8 (q), 27.8 (t), 59.1 (t), 65.2 (d), 122.2 (d), 123.6 (d), 128.3, 128.7 (d), 129.4, 131.2 (d), 131.6 (d), 134.3, 136.2, 136.7, 148.6, 164.8, 165.1. Found: C, 58.27; H, 4.98; N, 2.64%. Calcd. for $C_{24}H_{25}Cl_2NO_4S$: C, 58.30; H, 5.10; N,



X: a) 4-Cl; b) 4-CF_3; c) 4-NO_2; d) 2-F,4-Cl; e) 2-F,4-Br; f) 2-CF_3,4-Cl; g) 2-Cl,4-CF_3; h) 3-CO_2*iso*Pr,4-Cl; i) 2-F,4-Cl,5-CO_2*iso*Pr; j) 2-F,4-Cl,5-O-cycloC_5H_9

Fig. 2. Synthesis of 3-oxo-2-phenyl-4,5,6,7-tetahydro-1,2-benzisothiazoles.

2.83%.

*2.4. 3(2H)-Oxo-2-8[4-chloro-3-(isopropoxycarbonyl)phenyl]-4,5,6,7-tetrahydro-1,2-benzisothiazole (**2h**)—General procedure for the preparation of 3-oxo-4,5,6,7-tetrahydro-1,2-benzisothiazoles (**2a-j**)*

To a solution of **8h** (0.1 g) in methanol (5 ml) was added a concentrated HCl solution (1 ml). The mixture was heated under reflex for 1 hr and then evaporated under reduced pressure. The residue was partitioned between 10% Na_2CO_3 and chloroform. The organic phase was washed with water, dried over Na_2SO_4, and then evaporated under reduced pressure to give **2h** as a crystalline powder. Recrystallization from *n*-hexane gave an analytically pure sample of **2h** as colorless needles (0.05 g, 71%), mp 95.5–96.6°C. ^{1}H NMR δ_H ($CDCl_3$): 1.39 [d, J=6.3 Hz, 6H, CH($C\underline{H}_3$)$_2$], 1.84 (q, J=5.9 Hz, 2H, CH_2), 1.93 (q, J=5.9 Hz, 2H, CH_2), 2.43 (t, J=6.1, 2H, CH_2), 2.66 (t, J=6.1 Hz, 2H, CH_2), 5.28 [h, J=6.2 Hz, 1H, $C\underline{H}$(CH_3)$_2$], 7.47 (d, J=8.9 Hz, 1H, Ph), 7.75 (dd, $^1J_{CH}$=8.9 Hz and $^3J_{CH}$=2.7 Hz, 1H, Ph), 8.00 (d, $^3J_{CH}$=2.7 Hz, 1H, Ph). ^{13}C NMR δ_C ($CDCl_3$): 21.4 (t), 21.8 (q), 2.22 (t), 22.5 (t), 24.2 (t), 69.6 (d), 121.8, 125.8 (d), 127.1 (d), 130.8, 131.4, 131.5, 135.9 (d), 148.8, 164.4, 166.7. Found: C, 58.22; H, 5.04; N, 3.97%. Calcd. for $C_{17}H_{18}ClNO_3S$: C, 58.03; H, 5.16; N, 3.98%.

*2.5. 3(2H)-Oxo-2-(4-chlorophenyl)-4,5,6,7-tetrahydro-1,2-benzisothiazole-1-oxide (**3a**) —General procedure for the preparation of 4,5,6,7-tetrahydro-1,2-benzisothiazole-1-oxide (**3a, h, j**)*

To a solution of **2a** (0.1 g, 0.4 mmol) in chloroform (5 ml) was added 3-CPBA (70%, 0.08 g, 0.46 mmol) at 0°C. The reaction mixture was allowed to stand at room temperature for 1 hr. A saturated $NaHCO_3$ solution was added, and the organic phase was separated, washed with water and then dried over Na_2SO_4. The organic phase was evaporated and the crystalline residue was recrystallized from *n*-hexane to give an analytically pure sample of **3a** as colorless prisms (0.08 g, 71%); mp 108.6–109.2°C. ^{1}H NMR δ_H ($CDCl_3$): 1.75 (m, 2H, CH_2), 1.88 (m, 2H, CH_2), 1.93 (m, 2H, CH_2), 2.74 (m, 2H, CH_2), 7.23 (d, J=8.3 Hz, 2H, Ph), 7.39 (d, J=8.3 Hz, 2H, Ph). ^{13}C NMR δ_C ($CDCl_3$): 20.8 (t), 22.0 (t), 25.5 (t), 26.7 (t), 127.7 (d), 129.7 (d), 132.5, 134.1, 135.9, 156.1, 165.7. Found: C, 55.39; H, 4.27; N, 4.68%. Calcd. for $C_{13}H_{12}ClNO_2S$: C, 55.42; H, 4.29; N, 4.93%.

*2.6. 3(2H)-Oxo-N-(4-chlorophenyl)-4,5,6,7-tetrahydro-1,2-benzisothiazole-1,1-dioxide (**4a**) —General procedure for the preparation of 4,5,6,7-tetrahydro-1,2-benzisothiazole-1,2-dioxides (**4a, h, j**)*

To a solution of **2a** (0.1 g, 0.4 mmol) in chloroform (5 ml) was added 3-CPBA (70%, 0.16 g, 0.92 mmol) at 0°C and then the mixture was warmed to room temperature. The reaction mixture was allowed to stand at room temperature for 1 hr. A saturated sodium hydrogen carbonate solution was added, and the organic phase was separated, washed with water, and then dried over Na_2SO_4. The organic phase was evaporated to give the crude product. Recrystallization from *n*-hexane gave an analytically pure sample of **4a** as colorless prisms (0.06 g, 50%); mp 109.2–110.0°C. ^{1}H NMR δ_H ($CDCl_3$): 1.85 (m, 2H, CH_2), 1.92 (m, 2H, CH_2), 2.52 (m, 2H, CH_2), 2.66 (m, 2H, CH_2), 7.40 (d, J=8.8 Hz, 2H, Ph), 7.48 (d, J=8.8 Hz, 2H, Ph). ^{13}C NMR δ_C ($CDCl_3$): 19.2 (t), 20.5 (t), 20.6 (t), 20.9 (t), 127.6, 129.1 (d), 130.0 (d), 135.7, 136.7, 146.2, 159.4. Found: C, 52.74; H, 3.97; N, 4.60%. Calcd. for $C_{13}H_{12}ClNO_3S$: C, 52.44; H, 4.06; N, 4.70%.

3. Phytotoxic Assays

3.1. Determination of protoporphyrinogen-IX oxidase inhibition

Protox inhibition was measured out according to the method of Nicolaus *et al.*[7] Corn seeds (*Zea mays* cv. DK212MF, Yukijirushi-Shubyou Co., Sapporo) were soaked in water for 6 hr and germinated on vermiculite for 6 days in the dark at 30°C. The seedlings were harvested after exposure to light (300 μ Einstein (E)/$m^2 \times$ sec) for 2 hr. After homogenization of the seedlings, purified plastids containing Protox were prepared by three differential centrifugation steps. Protox activity was measured as the formation of protoporphyrin-IX from protoporphyrinogen at 30°C. Protox inhibition was determined after adding the compounds to be assayed to a final assay volume of 3 ml containing 0.1 M tris(hydroxymethyl)aminomethane-HCl (Tris-HCl, pH 7.3), 1 mM ethylenediaminetetraacetic acid (EDTA), 5 mm dithiothreitol (DTT), 0.03% Tween 80 (w/v), 0.3–0.6 mg of etioplast protein (crude Protox) and 2–5 μM protoporphyrinogen-IX. The amount of protoporphyrin-IX formed was measured for 5 min with a Hitachi F-2000 fluorescence spectrophotometer and a thermostated cell holder, using excitation and emission wavelengths of 405 and 633 nm, respectively. The Protox inhibition was estimated 5 min after the addition of various amounts of test compound formulated using DMSO to the assay mixture mentioned above. The molar I_{50} (Protox) value was calculated by the Probit method.[8] The pI_{50} (Protox) was calculated from the equation, pI_{50} (Protox) $= -\log I_{50}$ (Protox).

3.2 Determination of growth inhibition, chlorophyll decrease and ethane formation

The autotrophic culture of the green microalga *Scenedesmus acutus* was carried out according to Watanabe *et al.*[9] Growth inhibition was determined from packed cell volume (pcv) in a graduated microcentrifuge tube 20 hr after incubation with the test compounds. Chlorophyll content was measured spectroscopically in the extraction mixture (methanol: tetrahydrofuran: 5 mM aqueous trifluoroacetic acid = 30 : 16 : 5, v/v/v). Indices of pI_{50} (Growth) and pI_{50} (Chlorophyll) were calculated from I_{50} (Growth) and I_{50} (Chlorophyll) expressed in molar concentrations.

Ethane produced by *S. acutus* during a 20-hr incubation period in the presence of 10^{-5} M of the compounds tested was measured with a gas chromatograph (Shimadzu, GC-8A) equipped with a FID and a headspace. Ethane formation is indicated by the scores, ++++ (12–16 nmol/ml pcv), +++ (7–11 nmol/ml pcv), ++ (3–6 nmol/ml pcv) and + (0–2 nmol/ml pcv). A score of ± means a negligible amount of ethane was formed. The term pcv is packed cell volume of *S. acutus*.

RESULTS AND DISCUSSION

1. Synthesis

The scheme for synthesizing *N*-substituted phenyl 3(*2H*)-oxo-4,5,6,7-tetrahydro-1,2-benzisothiazoles (**2**) is shown in Fig. 2. The reaction of 2-chlorocyclohexene(1)-carboxylic acid (**5**) with substituted aniline was performed under relatively moderate conditions using 2-chloro-1,3-dimethylimidazolinium chloride (DMC) to isolate the desired carboxamides (**6**), giving 26–60% yields after isolation.

The structures of **6** were confirmed on the basis of spectral data, particularly the IR-spectrum (KBr pellet), which showed amide C=O stretching vibration at 1660 cm^{-1}. In the NMR spectra, the amide carbon resonance appeared at δ 165–166 ppm as a singlet and amide proton resonance at δ 7.54–8.45 ppm as a broad singlet.

2-Benzylthiocyclohexene-1-carboxamide compounds (**7**) were synthesized from **6** with 4-chlorobenzylmercaptan in the presence of K_2CO_3, yield 60–84%, and oxidized with 3-CPBA to give 2-sulfinylcyclohexene-1-carboxamides (**8**) in 51–71% yield.

The benzylthio structure of compound **7** was confirmed by ^1H and ^{13}C NMR spectral analysis. The SCH_2 proton signals appeared at δ 3.85–3.92 ppm as a sharp singlet with two-proton intensities. The SCH_2 carbon signal resonated at δ 35.8–36.0 ppm as a triplet and amide carbon signals appeared at δ 166–167 ppm as a singlet.

2-Sulfinylcyclohexen-1-carboxamide **8** showed a strong sulfinyl band at 1025 cm^{-1} in the IR-spectra. The proton of the methylenesulfinyl ($SOCH_2$) group characteristic of the structure of compound **8** resonated at δ 4.09 (d, J=13 Hz) and 4.16 ppm (d, J=13 Hz) in ^1H NMR spectra. The ^{13}C NMR spectra of compound **8** showed methylenesulfinyl carbon resonance at δ 58–60 ppm as a triplet ($^1J_{CH}$=142 Hz) and amide carbon resonance at δ 165–167 ppm as a singlet.

Cyclization of **8** to 3-oxo-4,5,6,7-tetrahydro-1,2-benzisothiazoles (**2**) was completed by heating in HCl aq-ethanol. The yield was 60–89% after isolation.

The structure of 3-oxoisothiazoles **2** was confirmed from the ^{13}C NMR spectra that exhibited a singlet in the carbonyl carbon resonance at δ 167 ppm. In the IR-spectra of **2**, the carbonyl-stretching band of the 3-oxoisothiazole appeared at 1660 cm^{-1} in KBr.

The oxidation reactions of the 3-oxoisothiazole **2** with 3-CPBA were carried out in ethanol. Thus, the oxidation from 3-oxoisothiazoles (**2**) to isothiazole-1-oxides (**3**) was performed in an equimolar mixture of **2** and 3-CPBA. The yield was 36–71% after isolation. The reaction of **2** and 3-CPBA of a molar ratio of **2** : 3-CPBA = 1 : 2 in ethanol gave 3-oxoisothiazole-1,1-dioxides (**4**) in a 34–50% yield.

The structures of 3-oxo-4,5,6,7-tetrahydro-1,2-benzisothiazole compounds **3** and **4** were confirmed on the basis of spectral data, NMR and IR. The 3-oxoisothiazole-1-oxide **3** showed appropriate spectral behavior. In particular, the IR-spectra of **3** showed sulfinylamide at 1102–1104 cm^{-1} and a carboxamide band at 1707–1717 cm^{-1}. The carboxamide carbon resonance appeared at δ 166 ppm as a singlet. Subsequently, 3-oxoisothiazole-1,1-dioxide **4** showed a strong sulfonamide band at 1332–1336 cm^{-1} and 1188–1196 cm^{-1} in the IR-spectra. The sulfonamide carbon signal

Table 1. Spectroscopic and analytical data for products **2**-**4**

	Mp (°C)	Yield (%)	^{1}H NMR(δ, ppm in $CDCl_3$)	^{13}C NMR(δ, ppm in $CDCl_3$)	Formula	Calcd. (Found) % of C	H	N
2a	103-104	77	1.82(q,*J*=6.1,2H), 1.91(q,*J*=6.1,2H), 2.44(t,*J*=6.1,2H), 7.37(d,*J*=7.4,2H), 7.56(d,*J*=7.4,2H)	21.4(t), 22.3(t), 22.6(t), 24.2(t), 121.9, 125.0(d), 129.1(d), 131.8, 135.8, 148.6, 166.8	$C_{13}H_{12}ClNOS$	58.75 (58.70)	4.55 4.53	5.27 5.17)
2b	142-143	62	1.84(q,*J*=5.9,2H), 1.90(q,*J*=5.9,2H), 2.45(t,*J*=6.1,2H), 2.68(d,*J*=5.9,2H), 7.67(d,*J*=9.0,2H), 7.81(d,*J*=9.0,2H)	21.4(t), 22.2(t), 22.5(t), 24.2(t), 121.9(q), 122.1, 122.9(d), 126.2(d), 127.5, 140.5, 149.0, 166.7	$C_{14}H_{12}FNOS$	56.18 (55.89)	4.04 3.96	4.68 4.60)
2c	225-226	62	1.85(q,*J*=5.6,2H), 1.94(q,*J*=5.6,2H), 2.46(t,*J*=6.1,2H), 2.69(t,*J*=6.1,2H),7.93(d,*J*=9.0,2H), 8.28(d,*J*=9.0,2H)	21.3(t), 22.2(t), 22.5(t), 24.3(t), 122.1(d), 122.4, 122.5,124.7(d),144.5, 149.4, 166.8	$C_{13}H_{12}N_2O_3S$	56.51 (56.41)	4.38 4.26	10.14 9.95)
2d	102-103	52	1.83(q,*J*=6.1,2H), 1.92(q,*J*=6.1,2H)), 2.43(t,*J*=6.1,2H), 2.66(t,*J*=6.1,2H),7.21(d,*J*=10.3,1H), 7.25(d,*J*=7.8,1H),	21.5(t), 22.3(t), 22.6(t), 24.5(t), 117.6(d), 120.4, 122.8,124.9(d), 130.4, 134.9, 150.7, 157.7(d), 167.3	$C_{13}H_{11}ClFNOS$	55.02 (54.17)	3.91 3.83	4.91 4.65)
2e	151-152	89	1.83(q,*J*=5.4,2H), 1.91(q,*J*=5.4,2H), 2.45(d,*J*=5.4,2H), 2.67(d,*J*=5.4,2H),7.38(m,3H)	21.5(t), 22.3(t), 22.6(t), 24.5(t), 120.4, 121.1(d), 122.3, 123.3, 127.9(d), 130.7(d), 150.7, 157.6(d), 167.3	$C_{13}H_{12}BrFNOS$	47.57 (47.11)	3.38 3.26	4.27 4.16)
2f	134-135	70	1.85(q,*J*=5.9,2H), 1.93(q,*J*=5.9,2H), 2.44(t,*J*=6.1,2H), 2.68(t,*J*=6.1,2H),7.33(d,*J*=8.5,1H), 7.61(d,*J*=8.5,1H), 7.76(s,1H)	21.4(t),22.2(t),22.5(t),24.5(t), 120.6,121.9(q), 127.7(d), 131.6, 133.0, 133.6(d), 133.7(d), 135.6,151.1, 168.6	$C_{14}H_{11}ClF_3NOS$	50.38 (49.74)	3.32 3.38	4.20 4.01)
2g	117-118	74	1.86(q,*J*=5.9,2H), 1.94(q,*J*=5.9,2H), 2.47(t,*J*=6.1,2H), 2.70(t,*J*=6.1,2H),7.60(m,2H), 7.79(m,1H)	21.4(t), 22.2(t), 22.5(t), 24.5(t), 120.3, 122.7(q), 124.5(d), 127.7(d), 131.3(d), 132.0(q), 134.2, 137.2, 150.9, 167.3	$C_{14}H_{11}ClF_3NOS$	50.38 (50.17)	3.32 3.19	4.20 4.02)
2i	103-104	60	1.35(d,*J*=6.4,6H), 1.73(m,2H), 1.78(m,*J*=5.9,2H), 2.49(m,4H), 5.27(sev.,*J*=6.4,1H), 7.22(d,*J*=10.5,1H), 8.88(d,*J*=8.3,1H)	21.4(q),21.8(q),23.2(t),28.2(t),34.4(t),69.6(d),117.4(d), 124.8(d),127.4, 128.6, 130.9, 132.9, 136.6, 153.6(d), 164.1, 165.5	$C_{17}H_{17}ClFNO_3S$	55.21 (55.46)	4.63 4.85	3.79 3.71)
2j	74-75	80	1.63(m,4H), 1.83(m,4H), 1.84(q,*J*=5.9,2H), 1.90(q, *J*=5.9,2H),2.45(t,*J*=6.0,2H),2.66(t,*J*=6.0,2H), 4.74(m, 1H), 7.05(d,*J*=6.6,1H),7.23(d,*J*=9.5,1H)	21.5(t), 22.2(t), 22.6(t), 23.8(t), 32.6(t), 24.4(t), 81.7(d), 114.9(d),118.1(d), 120.4, 122.3, 123.9, 150.1, 150.6, 150.9(d), 167.3	$C_{18}H_{19}ClFNO_2S$	58.77 (59.09)	5.21 5.24	3.81 3.70)
3h	oil	67	1.38(d,*J*=6.4,2H),1.88(m,2H), 1.98(m,2H), 2.48 (m,2H), 2.78(m,2H),5.27(sev. *J*=6.4,1H), 7.52(m, 2H), 7.88(s,1H)	20.7(t), 21.8(t), 21.8(q), 21.9(t), 22.0(t), 69.7(d), 128.8(d), 129.9(d),131.9, 132.0(d), 132.6, 133.1, 135.8, 156.3, 164.0, 165.5	$C_{17}H_{18}ClNO_4S$	55.51 (55.49)	4.93 4.89	3.81 3.79)
3j	112-113	36	1.63(m,2H), 1.89(m,2H), 1.91(m,4H), 1.91(m,2H), 2.51(m,2H),2.78(m,2H), 4.74(m, 1H),6.94(d,*J*=6.4, 1H), 7.27(d,*J*=9.0,1H)	20.7(t), 22.0(t), 23.8(t), 23.9(t), 32.6(t), 32.7(t), 81.7(d), 116.0 (d),118.2(d), 119.3, 125.2, 135.3, 150.5, 152.5(d), 157.4, 165.5	$C_{18}H_{19}ClFNO_3S$	56.32 (56.29)	4.99 4.92	3.65 3.35)
4h	oil	54	1.38(d,*J*=6.4,6H), 1.85(m,2H), 1.92(m,2H), 2.52(m, 2H), 2.66(m,2H),5.27(sev.*J*=6.4,1H), 7.51(d,*J*=8.6, 1H), 7.58(d,*J*=8.6,1H), 7.90(s,1H)	19.8(t), 20.4(t), 20.5(t), 20.8(t), 21.8(q), 69.8(d), 127.7(d), 130.4,131.1(d), 132.2, 132.3(d), 134.7, 136.1, 146.3, 159.2, 163.7	$C_{17}H_{18}ClNO_5S$	53.19 (54.09)	4.73 4.58	3.65 3.55)
4j	133-134	34	1.63(m,4H),1.84(m,2H), 1.90(m,4H),1.91(m,2H), 2.53(m,2H),2.56(m,2H), 4.75(m,1H), 7.32(d,*J*=8.8), 7.97(d,*J*=7.8, 1H)	19.2(t), 20.4(t), 20.5(t), 20.8(t), 23.4(t), 32.6(t), 81.8(d), 114.4 (d),115.8(d), 118.6, 126.6, 136.1, 147.0, 150.5, 152.2(d), 158.8	$C_{18}H_{19}ClFNO_4S$	54.07 (54.56)	4.79 4.41	3.50 3.24

resonated at δ 159 ppm as a singlet.

2. *Phytotoxic Activities*

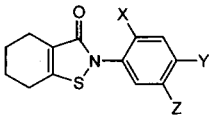
2.1. *Effect of benzene substituents of 3(2H)-oxoisothiazoles (2) on phytotoxicities*

3(*2H*)-Oxo-2-(4-chlorophenyl)-4,5,6,7-tetrahydro-1,2-benzisothiazole **2a** was designed as a structural mimic of a model peroxidizing herbicide, chlorophthalim (**1** : X=4-Cl). Phytotoxic activities, pI_{50} (Protox) at the cell-free level and *in vivo* phytotoxic parameters, pI_{50} (Growth), pI_{50} (Chlorophyll) and pI_{50} (Ethane), were determined for the compound (**2a**) and compared with those of the intermediate **8a** to check the improvement in activity with cyclization. The phytotoxicities, Protox inhibition, growth inhibition and chlorophyll decrease, exhibited by **8a** and **2a** were more than 100 times less extensive than these shown by chlorophthalim, although **2a** was ca. 10 times more active as a phytotoxicant than **8a**. Since, however, the 3(*2H*)-oxoisothiazole **2a** inhibited Protox and generated ethane, **2a** was confirmed as a (weak) peroxidizing compound (see data in Fig. 3). Thus, we then investigated the relationship between substituents at the benzene ring of 3(*2H*)-oxoisothiazoles (**2**) and phytotoxic activitiy (results shown in Table 2). All compounds tested exhibited Protox inhibition, but 3(*2H*)-oxoisothiazoles (**2h**, **2j** and **2i**) disubstituted at the 3- and 4-positions of the benzene ring or trisubstituted at the 2-, 4- and 5-positions were more phytotoxic than the 3(*2H*)-oxoisothiazoles bearing 4-monosubstituted or 2,4-disubstituted phenyl group(s). This finding is similar to the phytotoxicity exhibited by *N*-(3,4-disubstituted phenyl)-3,4,5,6-tetrahydrophthalimide and *N*-(2,4,5-trisubstituted phenyl)-3,4,5,6-tetrahydrophthalimide herbicides,[10] indicating that this series of 3(*2H*)-oxoisothiazoles are also imide type peroxidizers.

2.2. *Peroxidizing phytotoxicities of 3(2H)-oxoisothiazole-1-oxide (3) and 3(2H)-oxo-isothiazole-1,1-dioxide (4) (3-oxoisothiazole mimics of chlorophthalim analogs)*

The 3(*2H*)-oxoisothiazoles (**2**) were readily oxidized to 3(*2H*)-oxoisothiazole-1-oxides (**3**) and 3(*2H*)-oxoisothiazole-1,1-dioxides (**4**) by oxidizing reagents, as mentioned in the section on synthesis 1. Peroxidizing phytotoxicities of the oxidized 3(*2H*)-oxoisothiazole-1-oxides (**3a**, **3h**, **3j**) and 3(*2H*)-oxoisothiazole-1,1-dioxides (**4a**, **4h**, **4j**) were also assayed and compared with those of 3(*2H*)-oxo-isothiazoles (**2a**, **2h** and **2j**). The results are shown in Table 3. All compounds tested inhibited Protox and generated ethane formation, indicating they are peroxidizing compounds.

Table 2. 3(*2H*)-Oxo-2-phenyl-4,5,6,7-tetrahydro-1,2-benzisothiazoles: Substiuents the benzene ring and phytotoxic activities

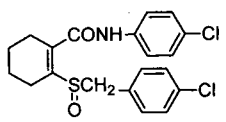


2

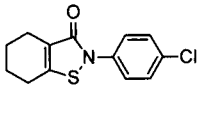
No.	X	Y	Z	pI_{50}		
				Protox	Growth	Chlorophyll
2a	H	Cl	H	4.86	4.69	4.78
2b	H	CF_3	H	4.31	4.13	4.26
2c	H	NO_2	H	4.96	4.63	4.88
2d	F	Cl	H	4.94	4.52	4.69
2e	F	Br	H	4.65	4.60	4.63
2f	CF_3	Cl	H	4.43	4.26	4.35
2g	Cl	CF_3	H	5.02	4.80	4.34
2h	H	Cl	$CO_2CH(CH_3)_2$	5.38	5.09	5.26
2i	F	Cl	$CO_2CH(CH_3)_2$	5.13	5.08	5.14
2j	F	Cl	O-cycloC_5H_9	5.13	4.89	5.02

Among three sets of compounds (Group A, **2a**, **3a** and **4a**; Group B, **2h**, **3h** and **4h**; Group C, **2j**, **3j** and **4j**), 3(*2H*)-oxoisothiazole-1,1-dioxides (**4a**, **4h** and **4j**) were the strongest peroxidizing phytotoxicants, although their activities were about 100 times weaker than those of reference cyclic imides with corresponding *N*-phenyl moieties. No significant difference in phytotoxic activities was observed between 3(*2H*)-oxo-isothiazoles (**2**) and 3(*2H*)-oxoisothiazole-1-oxides (**3**). From these results, we conclude that the 3(*2H*)-oxoisothiazole-1,1-dioxides (**4**) relate in bioisosterism to tetrahydrophthalimide herbicides.[5]

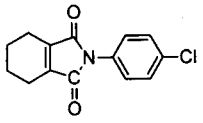
Since 3(*2H*)-oxo-2-(4-chlorophenyl)-1,2-benzisothiazole, the unsaturated benzenoid analog of **2a**, was a very weak Protox inhibitor (11.2% inhibition at 10^{-5} M),[11] an 1,2-unsaturated cyclohexene ring may be essential for peroxidizing activity. Regarding aromatization at the five-



8a



2a

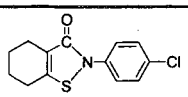
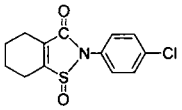
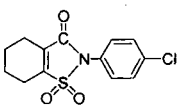
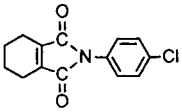
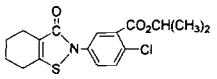
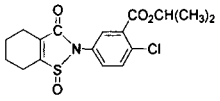
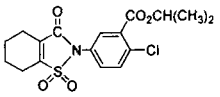
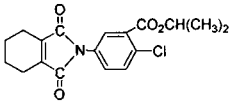
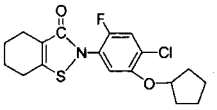
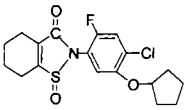
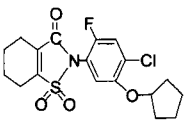
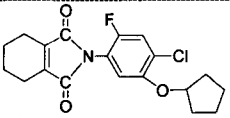


Chlorophthalim

pI_{50}(Protox)	< 4.00	4.86	7.60
pI_{50}(Growth)	< 4.00	4.69	7.00
pI_{50}(Chlorophyll)	< 4.00	4.78	7.10

Fig. 3. Structural modification and phytotoxicity improvement.

Table 3. Phytotoxic activities of compounds designed

Group	No.	Structure	pI_{50} Protox	Ethane	Growth	Chlorophyll
A	**2a**		4.86	+++	4.69	4.78
	3a		4.97	++	4.52	4.67
	4a		5.40	++++	5.07	5.28
	Reference A (Chlorophthalim)		7.60	6.43 ++++	7.00	7.10
B	**2h**		5.38	++	5.09	5.09
	3h		5.14	++	4.29	4.30
	4h		7.08	++++	6.08	6.30
	Reference B*		9.00	6.20 ++++	5.91	6.00
C	**2j**		5.13	+	4.89	5.02
	3j		5.40	+	5.14	5.32
	4j		6.07	++	5.99	5.82
	Reference C*		7.86	++++	7.29	7.40

* Reference compounds B and C were prepared according to patents, GB 2071100 (O. Yamada *et al.* 1981) and WO 92/01671 (K. Hirata *et al.* 1992), respectively.

membered heterocyclic moiety, the aromatized 3(2*H*)-oxoisothiazoles (**2** and **3**) in this study were weaker peroxidizing compounds than the non-aromatized 3(2*H*)-oxoisothiazole-1,1-dioxides (**4**).

However, further molecular modification is required to establish practical herbicides with the 3(2*H*)-oxoisothiazole-1,1-dioxides group.

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