Substituted Thiosemicarbazides and Corresponding Cyclized 1,3,4-Oxadiazoles and Their Anti-inflammatory Activity

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Abstract □ Several 1-(4-biphenoxyacetyl)-4-substituted arylthiosemicarbazides and their corresponding cyclized 2-(4-biphenoxymethyl)-5-arylamino-1,3,4-oxadiazoles were synthesized and characterized by elemental analyses and IR, mass, and nuclear magnetic resonance spectra. All compounds were evaluated for anti-inflammatory activity by determining their ability to provide protection against carrageenin-induced edema in rat paw. The anti-inflammatory activity possessed by substituted thiosemicarbazides [100 mg/kg, intraperitoneal(ip)] ranged from 22 to 68%, whereas substituted 1,3,4-oxadiazoles (100 mg/kg, ip) provided protection of 10–76%. Hydrocortisone (10 mg/kg, ip) and oxyphenbutazone (40 mg/kg, ip), used as standard reference drugs, decreased edema in rat paw by 44.6 and 52.9%, respectively. All compounds (1 mM) possessed antiproteolytic activity that was reflected by their ability to cause in vitro inhibition of trypsin-induced hydrolysis of bovine serum albumin. This inhibition ranged between 43 and 72% for substituted thiosemicarbazides and 30 and 83% for substituted 1,3,4-oxadiazoles.

Search for newer nonsteroidal anti-inflammatory agents has led to the synthesis of substituted thiosemicarbazides and their corresponding cyclized 1,3,4-oxadiazoles.[1] Earlier studies have provided evidence for the anti-inflammatory activity of substituted oxadiazoles[2–4] and that greater activity was possessed by 1,3,4-oxadiazoles compared with 1,2,4-oxadiazoles.[5] Among oxadiazoles evaluated for antispasmodic and anti-inflammatory effectiveness, oxolamine, a 3,5-disubstituted oxadiazole, exhibited significant anti-inflammatory, antipyretic, and antitussive properties.[2] The role of proteolytic enzymes in the inflammatory process has been proposed earlier.[6] Also, anti-inflammatory drugs have been shown to possess antiproteolytic activity.[1,4,7] These observations prompted extension of our earlier studies[1] to synthesize substituted thiosemicarbazides and their corresponding cyclized 1,3,4-oxadiazoles for evaluation of their anti-inflammatory and antiproteolytic properties (Scheme I). The anti-inflammatory activity was determined by the ability of these agents to provide protection against carrageenin-induced edema in rat paw, and their ability to inhibit trypsin-induced hydrolysis of bovine serum albumin reflected the antiproteolytic activity possessed by these compounds.

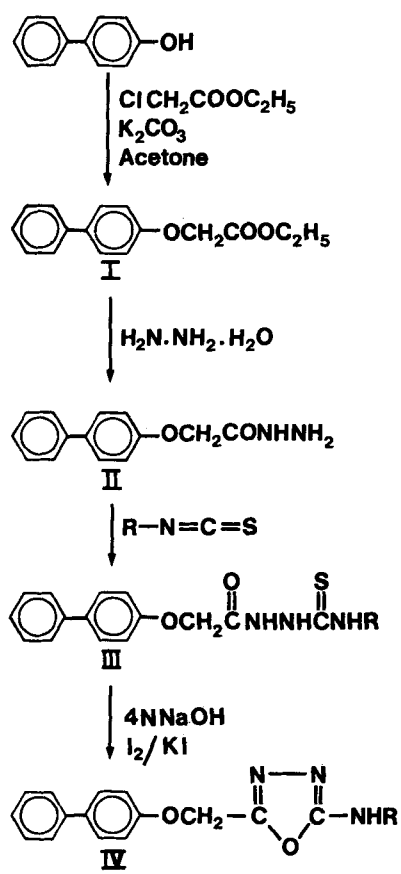
Experimental Section

The various 1-(4-biphenoxyacetyl)-4-substituted thiosemicarbazides (III) and their cyclized 2-(4-biphenoxymethyl)-5-arylamino-1,3,4-oxadiazoles (IV) were synthesized and checked for their purity as a single spot by thin-layer chromatography on Analtech silica gel. Melting points were taken in open capillary tubes and are uncorrected. The IR spectra (KBr) were determined on a Perkin-Elmer 180 spectrometer and are reported in reciprocal centimeters (cm^{-1}). The proton nuclear magnetic resonance (^1H NMR) spectra were recorded on a Varian EM-390 90 MHZ spectrometer as solutions in D_6-dimethyl sulfoxide (D_6-DMSO). The chemical shifts are reported in δ units downfield from the internal reference tetramethylsilane. The C, H, and N analyses were provided by Galbraith Laboratories (Knoxville, TN).

Ethyl 4-Biphenoxyacetate (I)—A mixture of 4-hydroxybiphenyl (0.2 mol), anhydrous potassium carbonate (0.2 mol), and ethyl chloroacetate (0.2 mol) in 200 mL of dry acetone was refluxed on a steam bath under anhydrous condition for 20 h. The mixture was filtered hot, and the excess of solvent was removed from the filtrate by distillation under reduced pressure. The residue was cooled, and the white solid mass which separated out was removed by filtration and was recrystallized from ethanol (mp, 60 °C).

4-Biphenoxyacetyl Hydrazide (II)—A mixture of 0.2 mol of I and 99% hydrazine hydrate (0.3 mol) in 100 mL of ethanol was refluxed on a steam bath for 10 h. The reaction mixture was concentrated to a volume of 50 mL and allowed to cool. The solid mass which separated out on cooling was removed by filtration and washed with small amounts of ice-cold ethanol. The solid mass was dried and recrystallized from 90% ethanol (mp, 160 °C).

1-(4 Biphenoxyacetyl)-4-Substituted Arylthiosemicarbazides (III)—A mixture of 0.01 mol of II and appropriate aryl isothiocyanate (0.01 mol) in 100 mL of 95% ethanol was refluxed on a steam bath for 4 h. The excess of ethanol was removed by distillation under reduced pressure. The solid mass which separated out on cooling was removed by filtration, dried, and recrystallized from ethanol (Table I). The



Scheme I

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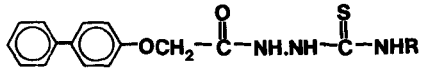
Table I—Physical Constants of 1-(4-Biphenoxyacetyl)-4-substituted Thiosemicarbazides



Compound	R	Melting Point, °C	Yield, %	Molecular Formula	Chemical Shifts in ^{1}H NMR, δ
IIIa	4-ClC_6H_4	200	95	$C_{21}H_{18}N_3O_2SCl$	4.75(s,2H,-OCH_2), 7.0–7.8(m,13H,Ar-H)
IIIb	4-BrC_6H_4	203	90	$C_{21}H_{18}N_3O_2SBr$	4.8(s,2H,-OCH_2), 7.0–7.7(m,13H,Ar-H)
IIIc	4,IC_6H_4	196	88	$C_{21}H_{18}N_3O_2SI$	4.7(s,2H,-OCH_2), 7.1–7.9(m,13H,Ar-H)
IIId	2-$OCH_3C_6H_4$	143	90	$C_{22}H_{21}N_3O_3S$	3.75(s,3H,-OCH_3), 4.8(s,2H,-OCH_2), 7.0–7.8(m,13H,Ar-H)
IIIe	4-$OCH_3C_6H_4$	177	80	$C_{22}H_{21}N_3O_3S$	3.8(s,3H,-OCH_3), 4.7(s,2H,-OCH_2), 7.1–7.9(m,13H,Ar-H)
IIIf	2-$OC_2H_5C_6H_4$	162	90	$C_{23}H_{23}N_3O_3S$	—[a]
IIIg	4-$OC_2H_5C_6H_4$	180	94	$C_{23}H_{23}N_3O_3S$	—

[a] —, Not determined.

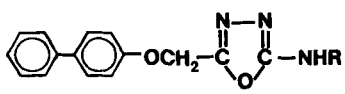
presence of the characteristic bands of C=O (attached to nitrogen), C=S (flanked by nitrogen), and NH groups at 1684, 1506, and 3344 cm^{-1}, respectively, in their IR spectra and recognition of the characteristic peaks in NMR spectra provided support for the structure of substituted thiosemicarbazides.

2-(4-Biphenoxymethyl)-5-Arylamino-1,3,4-Oxadiazoles (IV)—The appropriate **III** (0.01 mol) was suspended in 300 mL of ethanol and to this, 5 mL of NaOH (5 N) was gradually added with cooling and shaking. To the clear solution, iodine in KI solution (5%) was gradually added with stirring until the color of iodine persisted at room temperature. The mixture was refluxed on a steam bath and more KI solution was carefully added until a permanent tinge of excess iodine was obtained. The reaction mixture was gradually poured over crushed ice (500 g), and the solid mass which separated out was removed by filtration, washed with water, dried, and washed with warm carbon disulphide. The crude substituted 1,3,4-oxadiazoles were crystallized from ethanol in the presence of bone charcoal. The substituted 1,3,4-oxadiazoles (Table II) exhibited characteristic bands for C=N (1630 cm^{-1}), NH (3500 cm^{-1}),-C–O–C- (1042 cm^{-1}), and the penta atomic ring (1389 cm^{-1}) in their IR spectra (KBr). Further support for their structure was achieved by the recognition of the characteristic peaks in their NMR spectra.

Carrageenin-Induced Edema Test—Adult albino rats of either sex weighing 100–120 g were divided in groups of six. The rats were treated intraperitoneally (ip) with substituted thiosemicarbazides (**IIIa–g**) and 1,3,4-oxadiazoles (**IVa–g**) 1 h before the injection of carrageenin. The control group received an equivalent amount of dimethyl formamide (DMF) used as the solvent to dissolve the test compounds. A 0.5-mL aliquot of a freshly prepared 1% suspension of carrageenin in 0.9% saline was then injected into the planter aponeurosis of the right hind paw[8] of each rat. The rats in the standard reference group received hydrocortisone (10 mg/kg, ip) or oxyphenbutazone (40 mg/kg, ip) dissolved in 0.9% saline. The increase in the paw volume was measured by the micropipette method[8] before and 4 h after the administration of carrageenin. The anti-inflammatory activity of the test compounds and the standard reference drugs was determined with the following formula: % anti-inflammatory activity = $1 - (1 - V_c/V_t)$ 100, where V_c represents mean increase in paw volume in the control group of rats and V_t represents mean increase in paw volume in rats treated with the test compounds. Statistical analyses were carried out with the t test, comparing the mean changes in V_c with mean changes in V_t.

Assay of Antiproteolytic Activity—The in vitro inhibition of trypsin-induced hydrolysis of bovine serum albumin (BSA) by **III** and **IV** was used as an index to determine antiproteolytic activity. The reaction mixture consisted of 0.05 M tris buffer (pH 8.2), 0.075 mg of crystalline trypsin (1 g sufficient to hydrolyse 250 g of caesin), 3.3 mM BSA (substrate), and water in a total volume of 1 mL. The test compounds were dissolved in DMF and were used at a final concentration of 1 mM. An equivalent amount of DMF, added to the control tubes, had no effect on the activity of trypsin. All test compounds were pre-incubated with trypsin for 10 min prior to the addition of BSA,

Table II—Physical Constants of 2-(4-Biphenoxymethyl)-5-arylamino-1,3,4-oxadiazoles



Compound	R	Melting Point, °C	Yield, %	Molecular Formula	Chemical Shifts in ^{1}H NMR, δ
IVa	4-ClC_6H_4	204	60	$C_{21}H_{16}N_3O_2Cl$	5.2(s,2H,-OCH_2), 6.6–7.7(m,13H,Ar-H)
IVb	4-BrC_6H_4	210	69	$C_{21}H_{16}N_3O_2Br$	5.3(s,2H,-OCH_2), 6.6–7.7(m,13H,Ar-H)
IVc	4-IC_6H_4	216	62	$C_{21}H_{16}N_3O_2I$	5.3(s,2H,-OCH_2), 6.8–7.8(m,13H,Ar-H)
IVd	2-$OCH_3C_6H_4$	165	42	$C_{22}H_{19}N_3O_3$	3.8(s,3H,-OCH_3), 5.2(s,2H,-OCH_2), 6.9–7.8(m,13H,Ar-H)
IVe	4-$OCH_3C_6H_4$	184	53	$C_{22}H_{19}N_3O_3$	3.7(s,3H,-OCH_3), 5.3(s,2H,-OCH_2), 6.9–7.9(m,13H,Ar-H)
IVf	2-$OC_2H_5C_6H_4$	158	50	$C_{23}H_{21}N_3O_3$	—[a]
IVg	4-$OC_2H_5C_6H_4$	152	55	$C_{23}H_{21}N_3O_3$	—

[a] —, Not determined.

and the reaction mixture was further incubated for 5 min. The reaction was stopped by the addition of 0.5 mL of trichloroacetic acid (15%, w/v). The acid-soluble products of protein breakdown, obtained after centrifugation, were determined to calculate the activity of trypsin.[4,7] A 0.5-mL aliquot of the acid-soluble supernatant solution was added to 5 mL of a freshly prepared mixture of 8% Na_2CO_3 solution and a solution containing $CuSO_4$ (0.064%) and sodium-potassium tartrate (0.12%) in equal volumes. The mixture was allowed to stand at room temperature for 10 min, and 0.5 mL of Folin-Ciocalteau reagent was added. After 30 min, the amounts of the protein breakdown products as a reflection of the activity of trypsin were determined by measuring the absorbance of the mixture at 750 nm against the reagent blank in a spectrophotometer.[7] The percent decrease in the breakdown products of BSA with **III** and **IV** was used to evaluated the ability of these compounds to inhibit the activity of trypsin during hydrolysis of BSA, and such an inhibitory effect reflected their anti-proteolytic activity. In these experiments, sodium salicylate (1 mM) was used as a reference drug for comparison of the antiproteolytic activity of the test compounds.

Results and Discussion

The anti-inflammatory activity of substituted thiosemicarbazides **IIIa–IIIg** at ip doses of 100 mg/kg is reflected by their ability to provide 22–68% protection against carrageenin-induced edema in rat paw (Table III) The maximum protection of 68% was observed with 1-(4-biphenoxyacetyl)-4-(2-methoxyphenyl)-thiosemicarbazide (**IIId**), whereas 1-(4-biphenoxyacetyl)-4-(4-bromophenyl)-thiosemicarbazide (**IIIb**) showed the least anti-inflammatory activity of 22%. The anti-inflammatory activity possessed by the corresponding cyclized oxadiazoles (**IVa–IVg**) at the same ip dose of 100 mg/kg ranged from 10 to 76%. The maximum activity of 76% was observed with 2-(4-biphenoxymethyl)-5-(2-methoxyphenyl)amino-1,3,4-oxadiazole (**IVd**), whereas 2-(4-biphenoxymethyl)-5(4-bromophenyl) amino-1,3,4-oxadiazole (**IVb**) showed the lowest protection of 10%. The anti-inflammatory activities possessed by standard reference drugs, hydrocortisone (10 mg/kg, ip) and oxyphenbutazone (40 mg/kg, ip) were 45 and 53%, respectively. All compounds possessed antiproteolytic activity, and the degree of inhibition of the in vitro activity of trypsin during hydrolysis of BSA at a final concentration of 1 mM ranged from 43 to 72% for substituted thiosemicarbazides (**IIIa–IIIg**) and 30 to 87% for 1,3,4-oxadiazoles (**IVa–IVg**). In comparison, sodium salicylate (1 mM) showed 55% inhibition of the activity of trypsin activity (Table III).

These investigations have failed to provide a fixed pattern of the effects of cyclization on the changes in the anti-inflammatory and antiproteolytic activity of substituted thiosemicarbazides and their corresponding cyclized oxadiazoles. The increase in the anti-inflammatory activity of some thiosemicarbazides (**IIId, IIIe**) during cyclization to oxadiazoles (**IVd, IVe**) was associated with the decrease in their antiproteolytic properties. A similar relationship, in the reverse direction, was observed during cyclization of **IIIc, IIIf,** and **IIIg** into **IVc, IVf,** and **IVg**; that is, a decrease in the anti-inflammatory activity of oxadiazoles (**IVc, IVf, IVg**) compared with thiosemicarbozides (**IIIc, IIIf, IIIg**) was associated with the increase in antiproteolytic activity. The cyclization of **IIIa** to **IVa** and **IIIb** to **IVb**, however, was an exception because an increase or a decrease in the anti-inflammatory activity of these compounds was associated with a similar increase or decrease in their antiproteolytic property.

The results of the present study indicated that the higher activity possessed by **IIIc, IIId, IVa,** and **IVd** cannot account for their greater effectiveness compared with the standard anti-inflammatory drugs because the test compounds were used at the higher ip dose of 100 mg/kg compared with the lower ip doses of hydrocortisone (10 mg/kg) and oxyphenbutazone (40 mb/kg) used to determine their ability to provide protection against carrageenin-induced edema in rats. Thus, none of the test compounds was found to possess greater anti-inflammatory activity than that possessed by hydrocortisone and oxyphenbutazone. On the other hand, the greater in vitro inhibition of the activity of trypsin during hydrolysis of BSA by eight of the test compounds (Table III) compared with the standard reference sodium salicylate clearly indicates that such an inhibition of trypsin activity cannot account for the cellular mechanism of action for the anti-inflammatory activity possessed by substituted thiosemicarbazides and their cyclized 1,3,4-oxadiazoles.

Table III—Anti-Inflammatory and Antiproteolytic Properties of 1-(4-Biphenoxyacetyl)-4-substituted Thiosemicarbazides (III) and Their Corresponding Cyclized 2-(4-Biphenoxymethyl)-5-arylamino-1,3,4-oxadiazoles (IV)

Compound	Anti-inflammatory Activity (100 mg/kg, ip)[a]			Antiproteolytic Activity, % Protection (1 mM)[b]
	Mean Increase in Paw Volume, mL[c]	Protection, %	p[d]	
Control	0.50 ± 0.04	—	—	—
IIIa	0.37 ± 0.01	26.0	<0.02	47.6 ± 0.2
IIIb	0.39 ± 0.01	22.0	<0.05	43.0 ± 0.2
IIIc	0.27 ± 0.02	46.0	<0.001	50.4 ± 0.4
IIId	0.16 ± 0.01	68.0	<0.001	72.4 ± 0.5
IIIe	0.34 ± 0.03	32.0	<0.01	65.7 ± 0.4
IIIf	0.36 ± 0.03	28.0	<0.02	50.5 ± 0.2
IIIg	0.30 ± 0.03	40.0	<0.001	55.6 ± 0.3
Control	0.59 ± 0.04	—	—	—
IVa	0.23 ± 0.04	61.0	<0.001	82.7 ± 0.3
IVb	0.53 ± 0.03	10.2	>0.1	30.1 ± 0.3
IVc	0.45 ± 0.03	23.7	<0.05	54.5 ± 0.4
IVd	0.14 ± 0.03	76.3	<0.001	65.6 ± 0.4
IVe	0.37 ± 0.03	37.3	<0.01	55.4 ± 0.4
IVf	0.45 ± 0.02	23.7	<0.05	55.6 ± 0.3
IVg	0.36 ± 0.04	39.0	<0.01	57.7 ± 0.3

[a] The experimental procedures are as described in the text; the mean increases in paw volume in rats treated with hydrocortisone (10 mg/kg, ip) or oxyphenbutazone (40 mg/kg, ip) observed in these experiments were 0.28 ± 0.2 and 0.24 ± 0.03 mL, respectively; these results indicate percent protections of 44.6 ($p < 0.001$) by hydrocortisone and 52.9 ($p < 0.001$) by oxyphenbutazone. [b] Assay procedures and the content of the reaction mixture are as described in the text; each experiment was done in triplicate and the mean values ± standard error of the mean were calculated from two separate experiments; the percent protection observed with sodium salicylate (1 mM) was 54.8 ± 0.4. [c] Expressed as mean ± standard error. [d] Single-tailed *t* test.

References and Notes

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