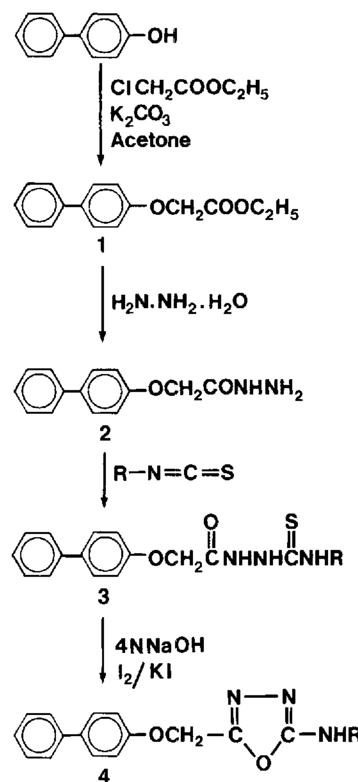
Anti-inflammatory Activity of Substituted 1,3,4-Oxadiazoles

KRISHNA RAMAN*, SURENDRA S. PARMAR*‡x, AND STEVEN K. SALZMAN*

Received January 27, 1989, from the *Research Department, Division of Medical Sciences, Alfred I. duPont Institute of the Nemours Foundation, P.O. Box 269, 1600 Rockland Road, Wilmington, DE 19899, and the ‡Department of Physiology, University of North Dakota, School of Medicine, Grand Forks, ND 58202. Accepted for publication April 21, 1989.

Abstract □ Various 2-(4-biphenoxymethyl)-5-arylamino-1,3,4-oxadiazoles were synthesized by cyclization of the corresponding 1-(4-biphenoxyacetyl)-4-substituted thiosemicarbazides. These compounds were characterized by their elemental analyses and infrared, mass, and nuclear magnetic resonance spectral data. All substituted thiosemicarbazides (100 mg/kg, ip) and cyclized substituted oxadiazoles (100 mg/kg, ip) possessed anti-inflammatory activity, as reflected by their ability to provide protection against carrageenin-induced edema in the rat paw which ranged from 28 to 68% and 36 to 76%, respectively. Cyclization of the substituted thiosemicarbazides, in general, resulted in an increase in the anti-inflammatory activity of their corresponding substituted oxadiazoles, with the exception of those containing 2,4-dimethyl and 3,4-dimethyl substituents in their molecular structure. Hydrocortisone (10 mg/kg, ip) and oxyphenbutazone (40 mg/kg, ip) were used as the standard reference drugs and these provided 45 and 53% protection, respectively. All compounds (1 mM) possessed antiproteolytic activity and the in vitro inhibition of trypsin-induced hydrolysis of bovine serum albumin ranged from 13 to 75% for substituted thiosemicarbazides and 39 to 70% for substituted oxadiazoles. There was no relationship between the anti-inflammatory activity of substituted thiosemicarbazides and substituted oxadiazoles and their antiproteolytic effectiveness. The low toxicity of these compounds was reflected by their high approximate LD_{50} values, ranging from 500 to 1000 mg/kg.

Earlier reports in the literature indicate that compounds containing an oxadiazole unit in their structure possess anti-inflammatory activity.[1–3] Recent investigations have provided evidence for the ability of oxadiazoles[4–6] to exhibit anti-inflammatory activity. These investigations prompted efforts for the synthesis of substituted oxadiazoles by cyclization of their corresponding precursor substituted thiosemicarbazides, with the aim to obtain newer nonsteroidal anti-inflammatory agents. These compounds were evaluated for their ability to provide protection against carrageenin-induced edema in rat paw and to inhibit trypsin-induced hydrolysis of bovine serum albumin, in an attempt to elucidate their cellular mechanism of action. The toxicity profile of some of these compounds was assessed by determining their approximate LD_{50} values. The synthetic procedures used in the present investigations are outlined in Scheme I.



Scheme I

Experimental Section

The various 2-(4-biphenoxymethyl)-5-arylamino-1,3,4-oxadiazoles were synthesized by cyclization of the corresponding 1-(4-biphenoxyacetyl)-4-substituted thiosemicarbazides by following the steps outlined in Scheme I. The compounds were checked for their purity by thin-layer chromatography on Analtech 2/5 × 10 cm, 250 μm analytical plates coated with silica gel. Melting points were taken in open capillary tubes and are uncorrected. The IR spectra were determined on a Perkin-Elmer 180 spectrometer and are reported in reciprocal centimeters (cm^{-1}). The ^1H NMR spectra were recorded on a Varian EM-390 90 MH_Z spectrometer as solutions in 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).

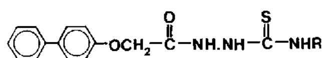
Ethyl-4-phenoxy acetate (1)—This compound was prepared by refluxing a mixture of 4-hydroxybiphenyl (0.2 mol) and ethylchloroacetate (0.2 mol) in 200 mL of dry acetone on a steam bath for 20 h. The mixture was filtered hot and the excess solvent from the filtrate was removed by distillation under reduced pressure. The residue was cooled and the white solid mass which separated out was filtered and recrystallized from ethanol; mp 60 °C.

4-Biphenoxyacetylhydrazide (2)—This compound was prepared by refluxing a mixture of ethyl-4-biphenoxyacetate (2; 0.2 mol) and 99% hydrazine hydrate (0.3 mol) in 100 mL of absolute ethanol on a steam bath for 10 h. The reaction mixture was concentrated to 50 mL by removing ethanol by distillation and allowed to cool. The solid mass which separated out on cooling was collected by filtration, washed with a small amount of ice-cold ethanol, dried, and recrystallized from 90% ethanol to obtain 4-biphenoxyacetylhydrazide; mp 160 °C.

1-(4-Biphenoxyacetyl)-4-Substituted Thiosemicarbazides (3)—These compounds were prepared by refluxing a mixture of 4-biphenoxyacetylhydrazide (2; 0.01 mol) and the appropriate arylisothiocyanate (0.1 mol) in 95% ethanol on a steam bath for 4 h. The excess solvent was removed by distillation under reduced pressure. The solid mass which separated out on cooling was filtered, dried, and recrystallized from ethanol. The substituted thiosemicarbazides thus synthesized are recorded in Table I. These compounds were characterized by their sharp melting points and elemental analyses for their carbon, hydrogen, and nitrogen content. The presence of the characteristic bands of C=O (attached to nitrogen), C=S (flanked by

0022-3549/89/1200-0999$01.00/0
© 1989, American Pharmaceutical Association

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
3a C_6H_5	170	90	$C_{21}H_{19}N_3O_2S$	4.75 (s, 2H, —OCH_2), 7.0–7.7 (m, 14H, Ar-H)
3b 2—$CH_3C_6H_4$	147	85	$C_{22}H_{21}N_3O_2S$	2.3 (s, 3H, —CH_3), 4.8 (s, 2H, —OCH_2), 6.8–7.8 (m, 13H, Ar-H)
3c 3—$CH_3C_6H_4$	144	85	$C_{22}H_{21}N_3O_2S$	2.3 (s, 3H, —CH_3), 4.7 (s, 2H, —OCH_2), 6.8–7.7 (m, 13H, Ar-H)
3d 4—$CH_3C_6H_4$	188	92	$C_{22}H_{21}N_3O_2S$	2.3 (s, 3H, —CH_3), 4.75 (s, 2H, —OCH_2), 7.0–7.7 (m, 13H, Ar-H)
3e 2,4$(CH_3)_2C_6H_3$	135	80	$C_{23}H_{23}N_3O_2S$	2.5 (bs, 6H, CH_3), 4.8 (s, 2H, —OCH_2), 7.0–7.8 (m, 1H, Ar-H)
3f 3,4$(CH_3)_2C_6H_3$	170	80	$C_{23}H_{23}N_3O_2S$	2.4 (bs, 6H, CH_3), 4.7 (s, 2H, —OCH_2), 7.1–7.8 (m, 12H, Ar-H)

nitrogen), and N—H groups at 1684, 1506, and 3344 cm^{-1}, respectively, in their IR spectra and determination of characteristic peaks in NMR spectra provided further support for the structure of these substituted thiosemicarbazides.

2-(4-Biphenoxymethyl)-5-arylamino-1,3,4-oxadiazoles (4)—These compounds were synthesized by cyclization of the appropriate 1-(4-biphenoxyacetyl)-4-substituted thiosemicarbazides (3) according to the method of Silberg and Cosma.[7] The appropriate substituted thiosemicarbazides (3; 0.01 mol) were suspended in ethanol (300 mL) and to this NaOH (4M; 5 mL) was slowly added with cooling and shaking. To the clear solution so obtained, iodine in KI solution (5%) was added gradually with stirring until the color of iodine persisted at room temperature. The mixture was then refluxed on a water bath and more iodine solution was carefully added until a permanent tinge of excess iodine was obtained. The reaction mixture was then poured over crushed ice (500 g) and the solid mass which separated out was filtered, washed with water, dried, and again washed with warm carbon disulphide. The crude product was then recrystallized from dilute ethanol containing charcoal. The various 2-(4-biphenoxymethyl)-5-arylamino-1,3,4-oxadiazoles thus synthesized are recorded in Table II. The presence of the 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 the IR spectrum of 2 provided support for their structure. The determination of characteristic peaks in NMR spectra provided further support for the structure of these compounds.

Carrageenin-Induced Edema Test—Adult albino rats of either sex weighing 100–120 g were used in the investigation. Rats were divided in groups of six, and 0.5 mL of a freshly prepared 1% suspension of carrageenin in 0.9% saline was injected into the planter aponeurosis of the right hind paw[8] 1 h after the administration of the test compounds which were dissolved in DMF. The rats were treated intraperitoneally with 1-(4-biphenoxyacetyl)-4-substituted thiosemicarbazides (**3a**–**3f**) and 2-(4-biphenoxymethyl)-5-arylamino-1,3,4-oxadiazoles (**4a**–**4f**) 1 h before the injection of carrageenin. The control group received an equivalent amount of DMF used as a solvent to dissolve the test compounds. 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 paw volume was measured by the micropipette method[8] before and 4 h after the administration of carrageenin. The anti-inflammatory activity of test compounds and standard reference drugs was determined by using the formula in eq 1:

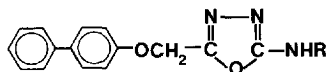
$$\% \text{ anti-inflammatory activity} = \left(1 - \frac{V_t}{V_c}\right) \times 100 \quad (1)$$

where V_t represents the mean increase in paw volume in rats treated with test compounds, and V_c represents the mean increase in paw volume in control group of rats. Statistical analyses were carried out by applying the single-tailed t test, comparing the mean changes in paw volume in the control group to the mean paw volume changes in rats treated with the test compounds (**3a**–**3f**; **4a**–**4f**).

Toxicity Study—The acute toxicity of some of the substituted thiosemicarbazides (3) and substituted oxadiazoles (4) was determined in albino mice. Each group of mice, comprised of four animals, was fasted for 18 h prior to the administration of the test compounds. The various compounds (3 and 4) were administered intraperitoneally in doses of 250, 500, 750, and 1000 mg/kg, and the 24-h mortality was recorded to calculate the approximate LD_{50} values.

Assay of Antiproteolytic Activity—The antiproteolytic activity of substituted thiosemicarbazides (3) and substituted oxadiazoles (4) was determined by assessing their ability to inhibit trypsin-induced hydrolysis of bovine serum albumin (BSA). The reaction mixture consisted of 0.05 M tris-buffer, pH 8.2, 0.075 mg of crystalline trypsin (1 g sufficient to hydrolyze 250 g of casein), 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, was found to have no effect on the activity of trypsin. The test compounds were preincubated with trypsin for 10 min prior to the addition of BSA 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, provided an index of the enzyme activity.[10] Suitable 0.5-mL aliquots of the acid-soluble supernatant solution were 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 absorbance of the mixture was measured at 750 nm against a reagent blank in a Perkin-Elmer spectrophotometer. The percent decrease in breakdown of BSA with substituted thiosemicarbazides (3) and substituted oxadiazoles (4) reflected their antiproteolytic activity.

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
4a C_6H_5	186	61	$C_{21}H_{17}N_3O_2$	5.3 (s, 2H, —OCH_2), 6.6–7.7 (m, 14H, Ar-H)
4b 2-$CH_3C_6H_4$	157	40	$C_{22}H_{19}N_3O_2$	2.3 (s, 3H, —CH_3), 5.3 (s, 2H, —OCH_2), 7.1–7.8 (m, 13H, Ar-H)
4c 3-$CH_3C_6H_4$	179	52	$C_{22}H_{19}N_3O_2$	2.3 (s, 3H, —CH_3), 5.3 (s, 2H, —OCH_2), 7.0–7.8 (m, 13H, Ar-H)
4d 4-$CH_3C_6H_4$	196	60	$C_{22}H_{19}N_3O_2$	2.3 (s, 3H, —CH_3), 5.2 (s, 2H, —OCH_2), 7.1–7.8 (m, 13H, Ar-H)
4e 2,4$(CH_3)_2C_6H_3$	180	46	$C_{23}H_{21}N_3O_2$	2.3 (bs, 6H, —CH_3), 5.3 (s, 2H, —OCH_2), 7.0–7.8 (m, 12H, Ar-H)
4f 3,4$(CH_3)_2C_6H_3$	186	45	$C_{23}H_{21}N_3O_2$	2.4 (bs, 6H, —CH_3), 5.3 (s, 2H, —OCH_2), 6.9–7.8 (m, 12H, Ar-H)

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

Compound	Anti-inflammatory Activity (100 mg/kg, ip)[a]			Approximate LD_{50} Value, mg/kg ip[b]	Antiproteolytic Activity, % Protection (1 mM)[c]
	Mean increase in Paw Volume (mL + SE)	Protection, %	p[d]		
Control	0.50 ± 0.04	—	—	—	—
3a	0.19 ± 0.02	63.9	<0.001	750	41.4 ± 0.4
3b	0.36 ± 0.03	28.0	<0.02	—	33.3 ± 0.5
3c	0.30 ± 0.02	40.0	<0.001	500	55.2 ± 0.6
3d	0.24 ± 0.30	52.6	<0.001	750	75.2 ± 0.6
3e	0.16 ± 0.01	67.7	<0.001	500	31.5 ± 0.3
3f	0.27 ± 0.02	46.4	<0.001	500	13.3 ± 0.3
Control	0.59 ± 0.04	—	—	—	—
4a	0.23 ± 0.04	60.0	<0.001	—	69.8 ± 0.4
4b	0.29 ± 0.01	50.5	<0.001	500	38.9 ± 0.4
4c	0.18 ± 0.03	68.7	<0.001	500	60.4 ± 0.4
4d	0.27 ± 0.01	54.7	<0.001	1000	52.7 ± 0.4
4e	0.41 ± 0.03	29.5	<0.01	1000	61.0 ± 0.3
4f	0.39 ± 0.03	32.6	<0.01	750	57.7 ± 0.3

[a] The experimental procedures are as indicated in the text; the mean increase in paw volume in rats treated with hydrocortisone (10 mg/kg, ip) or oxyphenbutazone (40 mg/kg, ip) observed in these experiments was 0.31 ± 0.03 and 0.29 ± 0.03 mL, respectively; these results have indicated the percent protection of 47.7 ($p < 0.001$) by hydrocortisone and 50.0 ($p < 0.001$) by oxyphenbutazone. [b] The approximate LD_{50} values were determined in mice. [c] Assay procedures and the contents of the reaction mixture are as indicated in the text; each experiment was done in triplicate and the mean values with ± standard error of the mean were calculated from two separate experiments; the percent protection observed with sodium salicylate (1 mM) was 54.4 ± 0.5. [d] Single-tailed *t* test.

Results and Discussion

The various 1-(4-biphenoxyacetyl)-4-substituted thiosemicarbazides (**3a**–**3f**) possessed anti-inflammatory activity. The degree of protection afforded by these substituted thiosemicarbazides (100 mg/kg, ip) against carrageenin-induced edema in rat paw ranged from 28 to 68% (Table III). The abilities of hydrocortisone (10 mg/kg, ip) and oxyphenbutazone (40 mg/kg, ip), used as standard reference drugs, to provide such protection were 48 and 50%, respectively. The maximum protection of 68% was observed with 1-(4-biphenoxyacetyl)-4-(2,4-dimethylphenyl)-thiosemicarbazide (**3e**), while 1-(4-biphenoxyacetyl)-4-(2-methylphenyl)-thiosemicarbazide (**3b**) provided the minimum protection of 28%. The low toxicity of these substituted thiosemicarbazides was reflected by their high approximate LD_{50} values which ranged from 500 to 1000 mg/kg (ip) in mice (Table III). All substituted thiosemicarbazides (1 mM) exhibited antiproteolytic activity which was reflected by their ability to inhibit in vitro trypsin-induced hydrolysis of BSA (Table III). The degree of inhibition of trypsin activity by these substituted thiosemicarbazides ranged between 13 and 72%. Sodium salicylate (1 mM), used as a reference standard drug, produced a 54% decrease in the in vitro hydrolysis of BSA by trypsin (Table III).

The ability of 2-(4-biphenoxymethyl)-5-arylamino-1,3,4-oxadiazoles (**4a**–**4f**) to provide protection against carrageenin-induced edema in rat paw is recorded in Table III. The degree of protection provided by these substituted oxadiazoles (100 mg/kg, ip) ranged between 30 and 76% as compared with hydrocortisone (10 mg/kg, ip) and oxyphenbutazone (40 mg/kg, ip) which showed 48 and 50% reductions, respectively. The low toxicity of some of these substituted oxadiazoles was reflected by their high approximate LD_{50} values of 500–1000 mg/kg. All substituted oxadiazoles (1 mM) possessed antiproteolytic activity and their ability to inhibit in vitro hydrolysis of BSA ranged from 39 to 83%. Sodium salicylate (1 mM), used as a standard reference drug, produced 54% inhibition (Table III).

Among substituted thiosemicarbazides, attachment of a substituent in the phenyl nucleus of the thiosemicarbazide moiety resulted in a decrease in the anti-inflammatory activity, with the exception of 2,4-dimethylphenyl-substituted thiosemicarbazide (**3e**). Cyclization of the substituted thiosemicarbazides into their corresponding substituted oxadiazoles resulted in an increase in anti-inflammatory activity of oxadiazoles containing a monosubstituted phenyl nucleus. No significant change in the anti-inflammatory activity of phenyl-substituted thiosemicarbazide (**3a**) was observed on its cyclization to phenyl-substituted oxadiazole (**4a**). The presence of 2-4-dimethylphenyl (**4e**) and 3,4-dimethylphenyl (**4f**) substituents in the oxadiazoles caused a significant decrease in the anti-inflammatory activity on cyclization from the corresponding substituted thiosemicarbazides (**3e** and **3f**). The competition between two methyl substituents for the specific receptor site(s) could presumably be responsible for such a decrease in the anti-inflammatory activity of dimethyl-substituted oxadiazoles (**4e**, **4f**) in comparison with the phenyl-substituted oxadiazoles (**4b**, **4c**, **4d**). The low approximate LD_{50} values of substituted thiosemicarbazides and substituted oxadiazoles reflect low inherent toxicity of these newer compounds; thus, these compounds warrant further investigations for their use as potential anti-inflammatory drugs. The antiproteolytic activity of substituted thiosemicarbazides (**3a**–**3f**) and their corresponding cyclized oxadiazoles (**4a**–**4f**) failed to provide a correlation between their antiproteolytic activity and anti-inflammatory effectiveness. An increase in antiproteolytic activity was observed with only four compounds (**3a**, **3b**, **3e**, **3f**) during cyclization to corresponding oxadiazoles (**4a**, **4b**, **4e**, **4f**). The anti-inflammatory and antiproteolytic properties of substituted thiosemicarbazides (**3a**–**3f**) and cyclized oxadiazoles (**4a**–**4f**) showed no structure–activity relationship and failed to establish antiproteolytic activity as a cellular basis of their anti-inflammatory property.

References and Notes

1. Palazzo, G.; Travella, M.; Strani, G.; Silverstrini, B. *J. Med. Pharm. Chem.* **1961**, *4*, 351.
2. Najer, H.; Giudicelli, R.; Morel, C.; Menin, J. *Bull. Soc. Chim. France* **1966**, 153.
3. Kishore, V.; Kumar, S.; Parmar, S. S.; Stenberg, V. I. *Res. Commun. Chem. Pathol. Pharmacol.* **1975**, *11*, 581.
4. Dannhardt, G.; Mayer, K. K.; Sommer, I. *Sci. Pharm.* **1984**, *52*, 280.
5. Singh, I. P.; Saxena, A. K.; Shankar, K. *Eur. J. Med. Chem.–Chim. Ther.* **1986**, *21*, 267.
6. Yamamoto, M.; Ori, Y. *Jpn. Kokai Tokkyo Koho JP.* 62 96 480 (87, 96, 480) May 2, 1987; through *Chem. Abstr.* **1987**, *107*, 115596U.
7. Silberg, A.; Cosma, N. *Acad. Repub. Pop. Rom. Filiala Cluj Stud. Cercet. Chim.* **1959**, *10*, 151; through *Chem. Abstr.* **1960**, *54*, 8794g.
8. Winter, C. A.; Risley, E. A.; Nuss, G. W. *Proc. Soc. Exp. Biol.* **1962**, *111*, 544.
9. Smith, C. C. *J. Pharmacol. Exp. Ther.* **1950**, *100*, 408.
10. Chaudhari, A.; Chaturvedi, A. K.; Parmar, S. S.; Brumleve, S. J. *Res. Commun. Chem. Pathol. Pharmacol.* **1974**, *7*, 205.

Acknowledgments

The authors wish to express their sincere thanks to Dr. Charles R. Hartzell for his advice and encouragement. These investigations are supported in part by the Max Baer Grant of the Dakota State Aerie Fraternal Order of Eagles and the Bush Foundation Development Grant to S.S.P.