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

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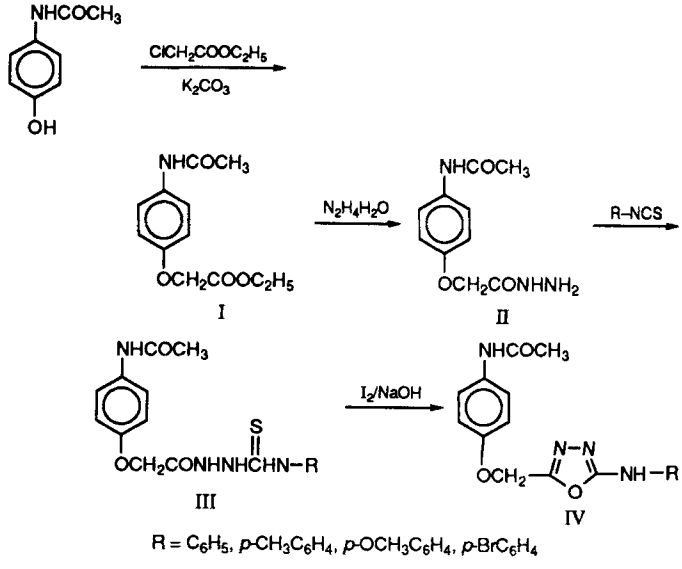
Abstract □ Various 5-[[(acetamidophen-4-yl)oxy]methyl]-2-(*p*-substituted phenylamino)-1,3,4-oxadiazoles (**4a–4d**) were synthesized by cyclization of the corresponding N^1-[[(acetamidophen-4-yl)oxy]acetyl]-N^4-(*p*-substituted phenylamino)-3-thiosemicarbazides (**3a–3d**). All four of the thiosemicarbazides [250 mg/kg, orally (p.o.)] and the corresponding oxadiazoles (250 mg, p.o.) possessed anti-inflammatory activity. In the Carrageenan-induced edema test in rat paw, the activity ranged from 28 to 47% for **3a–3d** and 44 to 63% for **4a–4d**, with indomethacin (10 mg/kg, p.o.), used as the standard reference drug, showing 88.5% protection. The compounds (1 mM) were also tested for the inhibition of bovine serum albumin denaturation, and this activity ranged from 27 to 68%. No correlation was seen between the anti-inflammatory activity of **3a–4d** and the inhibition of denaturation of bovine serum albumin. The low toxicity of these compounds was reflected by their high approximate 50% lethal dose (LD_{50}) values, ranging from 2000 to 2500 mg/kg.

That compounds containing an oxadiazole unit in their structure possess anti-inflammatory activity is well documented.[1-6] These investigations prompted efforts for the synthesis of substituted oxadiazoles by cyclization of their corresponding substituted thiosemicarbazides with the aim to obtain newer nonsteroidal anti-inflammatory agents. These compounds were evaluated for their ability to provide protection against carrageenan-induced edema in rat paw and to inhibit the bovine serum albumin (BSA) denaturation in an attempt to elucidate their cellular mechanism of action. The toxicity profile of some of these compounds was assessed by determining their approximate 50% lethal dose (LD_{50}) values. The synthetic procedures used in the present investigations are outlined in Scheme I.

Experimental Section

Various 5-[[(acetamidophen-4-yl)oxy]methyl]-2-(*p*-substituted phenylamino)-1,3,4-oxadiazoles were synthesized by cyclization of the corresponding N^1-[[(acetamidophen-4-yl)oxy]acetyl]-N^4-(*p*-substituted phenylamino)-3-thiosemicarbazides by following the steps outlined in Scheme 1. Melting points of all the compounds were taken in open capillary tubes and are uncorrected. The UV spectra were obtained on a Perkin-Elmer 1378 spectrophotometer and are recorded in nanometers (nm). The IR spectra were determined on a Perkin-Elmer 221 spectrophotometer and are reported in reciprocal centimeters (cm^{-1}). The ^1H NMR spectra were recorded on a Varian EM-390, 90 MHz spectrophotometer as solutions in DMSO-d_6, and the chemical shifts are reported in units downfield from the internal reference tetramethylsilane (TMS).

Ethyl (*p*-Acetamidophenoxy)acetate (1, Scheme 1)—A mixture of 0.1 mol (15.1 g) of acetamidophenol and 0.15 mol (20.7 g) of anhydrous K_2CO_3 in an excess of super dry acetone (375 mL) was stirred at reflux temperature for 4 h. To the stirred suspension 12.25 g (0.1 mol) of ethylchloroacetate in dry acetone (50 mL) was added in a dropwise manner over a period of 1 h at reflux temperature, and the refluxing continued for 8 h. After keeping the reaction mixture overnight, the excess of solvent was removed, and the residue was poured into crushed ice. The separated white solid was filtered, washed with water crystallized from ethanol; UV: 370 nm; IR (KBr): 3480, 1740 (C=O), 1640, 1060, 600–800; ^1H NMR (DMSO-d_6): 4.98 (s, 2H, OCH_2), 4.42 (q, 2H, $COOCH_2$), 1.42 (t, 3H, CH_3), 3.62 (s, 3H, CH_3), 7.2–7.8 (m, 4H, Ar-H), 9.75 (s, 1H, NH).



Scheme 1

(*p*-Acetamidophenoxy)acetyl Hydrazide (2, Scheme 1)—To a suspension of 1 (0.05 mol, 11.85 g) in ethanol (150 mL), 3.0 g (0.06 mol) of 99% hydrazine hydrate was added, and the reaction mixture was refluxed for 2 h. The resulting clear solution was then added to 200 g of crushed ice and water, and the solid that separated was filtered, washed thoroughly with cold water, dried, and crystallized from ethanol; UV: 289.6 nM; IR (KBr): 3300–3100, 1690, 1650, 1070, 600–800; ^1H NMR (DMSO-d_6): 2.6 (s, 2H, OCH_2), 3.42 (s, 3H, CH_3), 9.8 (s, 1H, NH), 6.9–7.6 (m, 4H, Ar-H), 4.5–5.1 (br, 3H, NH-NH_2), 9.26 (s, 1H, NH).

Anal.—Calcd for $C_{10}H_{13}N_3O_3$; C, H, N.

N^1-[[(Acetamidophen-4-yl)oxy]acetyl]-N^4-(*p*-substituted phenyl)-3-thiosemicarbazides (3a–3d, Table 1)—A mixture of 2- (0.01 mol) and 4-substituted phenyl isothiocyanate (0.01 mol) in 150 mL of absolute ethanol was heated on a water bath for 4 h. The solid, obtained after subsequent concentration and cooling, was filtered, washed with ethanol, dried, and recrystallized from ethanol (**3b–3c**) and dimethylformamide (DMF; **3a**); UV of **3b**: 273.2 nm; IR (KBr) of **3b**: 3400–3100, 1700, 1660, 1080, 1020, 600–800; ^1H NMR (DMSO-d_6) of **3b**: 4.59 (s, 2H, OCH_2), 2.3 (s, 3H, CH_3), 6.8–7.6 (m, 8H, Ar-H), 9.8 (s, 1H, NH), 9.74 (s, 1H, C=S—NH), 9.6 (s, 1H, NH).

Anal.—Calcd for $C_{18}H_{20}N_4O_3S$; C, H, N.

5-[[(Acetamidophen-4-yl)oxy]methyl]-2-(*p*-substituted phenylamino)-1,3,4-oxadiazoles (4a–4d, Table 1)—To a solution of **3** (0.01 mol) in ethanol (30 mL), aqueous NaOH (0.16 mol, 2 N) was added with cooling and shaking. Iodine in KI (aqueous 5%) was then added with shaking at room temperature until the color of iodine persisted. The excess solvent was removed under reduced pressure. The contents were cooled and acidified with acetic acid (10%) to get the corresponding free oxadiazoles (**4a–4d**). The solid was filtered, washed with water and then with CS_2, dried, and recrystallized from ethanol; UV of **4b**: 267.2 nm; IR (KBr) of **4b**: 3300, 3410, 1650, 1070, 600–800; ^1H NMR (DMSO-d_6) of **4b**: 4.5 (s, 2H, OCH_2), 2.0 (s, 3H, CH_3), 2.22 (s, 3H, C=O—CH_3), 9.81 (s, 1H, -NH), 9.94 (s, 1H, -NH), 6.91–8.6 (m, 8H, Ar-H).

Anal.—Calcd for $C_{18}H_{18}N_4O_3$; C, H, N.

Toxicity Study—The acute toxicity of some of the substituted thiosemicarbazides (**3a** and **3c**) and substituted oxadiazoles (**4a** and **4b**) was determined in albino mice according to the Stair Case method.[7] Each group of six mice was fasted for 18 h prior to the administration

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Table 1—Physical Constants of 1–4

Compound	R	R_1	Melting Point, °C	Yield, %	Molecular Formula	Elemental Analysis, % Element	Calcd	Found
1	$-COOC_2H_5$	—[a]	104–105	38	$C_{12}H_{15}NO_4$	C	60.76	60.74
						H	6.33	6.32
						N	5.90	5.91
2	$-CONH-NH_2$	—	194–195	67.2	$C_{10}H_{13}N_3O_3$	C	53.80	53.82
						H	5.83	5.81
						N	18.83	18.82
3a	$-C(=O)-NHNH-C(=S)-NH-C_6H_5$	—	190–191	81.57	$C_{17}H_{18}N_4O_3S$	C	56.99	56.97
						H	5.02	4.04
						N	15.64	15.62
3b	$-C(=O)-NHNH-C(=S)-NH-C_6H_4-CH_3$	—	170–171	83.3	$C_{18}H_{20}N_4O_3S$	C	58.06	58.04
						H	5.37	5.33
						N	15.05	15.01
3c	$-C(=O)-NHNH-C(=S)-NH-C_6H_4-OCH_3$	—	144–145	79.9	$C_{18}H_{20}N_4O_4S$	C	55.67	55.69
						H	5.15	5.18
						N	14.43	14.41
3d	$-C(=O)-NHNH-C(=S)-NH-C_6H_4-Br$	—	122–123	65.5	$C_{18}H_{17}N_4O_3S$	C	46.78	46.76
						H	3.89	3.88
						N	12.84	12.86
4a	—	$-C_6H_5$	223–224	64.8	$C_{17}H_{16}N_4O_3$	C	62.96	62.94
						H	4.92	4.91
						N	17.28	17.29
4b	—	$4-CH_3C_6H_4$	204–206	17.8	$C_{18}H_{18}N_{O3}$	C	63.90	63.93
						H	5.32	5.30
						N	16.56	16.55
4c	—	$4-OCH_3C_6H_4$	155–157	59.3	$C_{18}H_{18}N_4O_4$	C	61.01	61.03
						H	5.08	5.06
						N	15.81	15.83
4d	—	$4-BrC_6H_4$	165–166	61.9	$C_{17}H_{15}N_4O_3$	C	50.74	50.71
						H	3.73	3.72
						N	13.93	13.89

[a] —, Not applicable.

of the test compounds. The various compounds (**3a**, **3c**, **4a**, and **4b**) were administered intraperitoneally in doses of 250, 500, 750, 1000, 2000, and 25 000 mg/kg, and the 24-h mortality was recorded to calculate the approximate LD_{50} values by the probit method.

Inhibition of BSA Denaturation—Compounds **3a–3d** and **4a–4d** were screened for their inhibition of BSA denaturation activity according to the method of Rao and Elias.[8] The test compounds were dissolved in a minimum amount of DMF and diluted with phosphate buffer (0.2 M, pH 7.4). The final concentration of DMF in all solutions was 2%. Test solution (1 mL) containing 1 mM concentration of drug was mixed with 1 mL of 1 mM BSA in phosphate buffer and incubated at 27 °C for 15 min. Denaturation was induced by keeping the reaction mixture at 60 °C in a water bath for 10 min. After cooling, the turbidity was measured at 660 nm. Percent inhibition of BSA denaturation was calculated from control samples to which no drug was added.

Carrageenan-Induced Edema Test—Adult albino rats of either sex weighing 150–240 g were used in the investigation. Rats were divided into groups of six each, and 0.1 mL of a freshly prepared 1% suspension of carrageenan in 0.9% saline was injected into the subplantar region of left hind paw 1 h after the administration of the test compounds which were dissolved in DMF. The rats were treated orally (p.o.) with **3a–3d** and **4a–4d** 1 h before the injection of carrageenan. The control group received an equivalent amount of DMF used as a solvent. The rats in the standard reference group received indomethacin (10 mg/kg, p.o.) dissolved in 0.9% saline. The increase in paw volume was measured by plethysmograph before and 3 h after the administration of carrageenan. The anti-inflammatory activity of test compounds and standard reference drug was determined with the following formula:[9]

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

In eq 1, 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 with the single-tailed t test, comparing the mean changes in paw volume in control to the mean paw volume changes in rats treated with test compounds **3a–3d** and **4a–4d**.

Results and Discussion

Compounds (**3a–3d**) at 250 mg/kg (p.o.) possessed anti-inflammatory activity ranging from 28 to 47% (**3c** and **3a** exhibited 47 and 28%, protection, respectively), and indomethacin, used as a reference standard drug, showed 88.5% activity. The low toxicity of these substituted thiosemicarbazides was reflected by their high approximate LD_{50} values, which ranged from 2000 to 2500 mg/kg [intraperitoneally (i.p.)] in mice (Table 2). All substituted thiosemicarbazides (1 mM) exhibited inhibition of BSA denaturation activity (Table 2) ranging between 28 and 47% (Table 2).

The ability of **4a–4d** at 250 mg/kg (p.o.) to provide protection against carrageenan-induced edema in rat paw is (Table 2) ranged from 44 to 63% compared with indomethacin (10 mg/kg, p.o.), which showed 88.5% reduction. The low toxicity was reflected by their high approximate LD_{50} values (2000–2500 mg/kg). All

Table 2—Anti-Inflammatory and Inhibition of BSA Denaturation of 3a–3d and 4a–4d

Compound	Anti-inflammatory Activity (250 mg/kg, p.o.)[a]			Approximate LD_{50} Value (i.p.); mg/kg[b]	Inhibition of BSA Denaturation Activity, (0.1 mM) % Protection[c]
	Mean Increase in Paw Volume, mL ± SEM	Protection, %	p[d]		
Control	0.58 ± 0.02	—	—	—	—
3a	0.42 ± 0.01	27.5	<0.01	2500	27.1 ± 0.001
3b	0.39 ± 0.01	32.7	<0.01	—	43.4 ± 0.0007
3c	0.31 ± 0.01	46.5	<0.001	2000	39.2 ± 0.0009
3d	0.38 ± 0.01	34.4	<0.001	—	51.0 ± 0.0007
Control	0.57 ± 0.02	—	—	—	—
4a	0.32 ± 0.007	43.8	<0.001	2000	55.4 ± 0.0004
4b	0.21 ± 0.006	63.1	<0.001	2000	42.3 u 0.0001
4c	0.25 ± 0.008	56.14	<0.001	—	66.3 ± 0.0002
4d	0.30 ± 0.004	47.36	<0.001	—	40.2 ± 0.0006

[a] The experimental procedures are as indicated in the text; the mean increase in paw volume in rats treated with indomenthacin (10 mg/kg, p.o.) observed in this experiment was 0.23 ± 0.06 mL; this result has indicated the percent protection of 88.5 ($p < 0.001$) by indomethacin. [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. [d] Single-tailed *t* test.

substituted oxadiazoles (1 mM) possessed inhibition of BSA denaturation activity that ranged from 40 to 67% (Table 2).

Among substituted thiosemicarbazides, attachment of an electron-donating substituent like methoxy or methyl in the phenyl nucleus of the thiosemicarbazide moiety resulted in an increase in anti-inflammatory activity, whereas substituted with bromine in the phenyl nucleus of the thiosemicarbazide moiety resulted in a decrease in the anti-inflammatory activity. Cyclization of the substituted thiosemicarbazides into their corresponding substituted oxadiazoles resulted in an increase in anti-inflammatory activity of oxadiazoles containing a methyl-substituted phenyl nucleus. The inhibition of BSA denaturation activity of **3a–3d** and their corresponding cyclized oxadiazoles **4a–4d** failed to provide a correlation. An increase in the inhibition of BSA denaturation activity was observed with only two compounds (**3a** and **3c**) after cyclization to corresponding oxadiazoles (**4a** and **4c**). The anti-inflammatory and inhibition of BSA denaturation properties of **3a–3d** and **4a–4d** showed no structure–activity relationship and failed to establish inhibition of BSA denaturation activity as a cellular basis of their anti-inflammatory property.

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