Synthesis of 1-(5-Phenyl-2*H*-Tetrazol-2-ylacetyl)-4-substituted Thiosemicarbazides as Possible Antiinflammatory Agents

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A series of 1-(5-phenyl-2*H*-tetrazol-2-ylacetyl)-4-substituted thiosemicarbazids were prepared as possible antiinflammatory agents. Their antiproteolytic activity was reported.

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Several tetrazole derivatives have been reported to be active antiinflammatory agents (1-5). 5-Amino-1-phenyltetrazole has been shown to be useful in patients with rheumatoid arthritis who had previously failed to respond to salicylic acid, phenylbutazone, indomethacin or corticosteroids (6,7). A number of 5-aryl-2-tetrazolylalkanoic acids and certain hydrazine derivatives, like thiosemicarbazides, are known to be good antiinflammatory agents (8-10). These observations prompted the synthesis of a series of 1-(5-phenyl-2*H*-tetrazol-2-ylacetyl)-4-substituted thiosemicarbazides. Furthermore, the role of proteolytic enzymes in the inflammatory process is well known (11,12). The antiprotease property of various antiinflammatory compounds have been shown to reflect their ability to inhibit enzymes that cause the formation of permeability increasing factors (13,14). Therefore, the various compounds synthesized in the present study were evaluated for their antiproteolytic activity. The various 1-(5-phenyl-2*H*-tetrazol-2-ylacetyl)-4-substituted thiosemicarbazides were prepared according to the steps outlined in Scheme 1.

The reaction of benzonitrile (**1**) with sodium azide in the presence of ammonium chloride yielded 5-phenyl-2*H*-tetrazole (**2**). Ethyl (5-phenyl-2*H*-tetrazol-2-yl)acetate (**3**) was obtained by refluxing **2** with ethylbromoacetate in the presence of sodium ethoxide. The acetate **3** was converted into (5-phenyl-2*H*-tetrazol-2-yl)acethydrazide (**4**) by heating with hydrazine hydrate in absolute ethanol. Finally the hydrazide **4** on refluxing with appropriate arylisothiocyanate in absolute ethanol gave the corresponding 1-(5-phenyl-2*H*-tetrazol-2-ylacetyl)-4-substituted thiosemicarbazides (**5-16**).

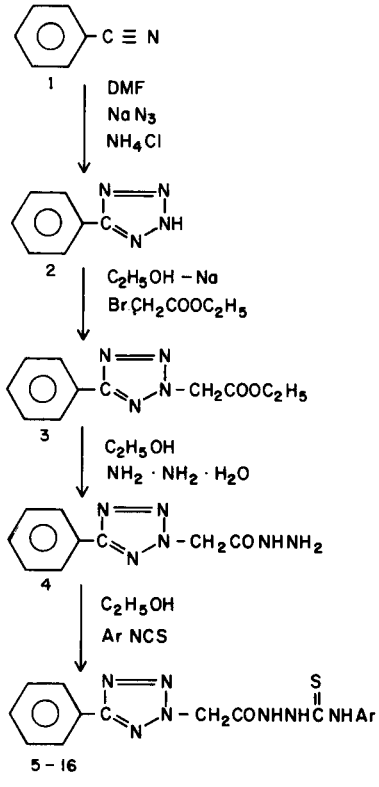
The antiproteolytic activity possessed by these tetrazoles was reflected by their ability to protect trypsin-induced hydrolysis of bovein serum albumin. The degree of protection ranged from 21 to 52% where maximum protection was observed with 1-(5-phenyl-2*H*-tetrazol-2-ylacetyl)-4-(3,4-dimethylphenyl)thiosemicarbazide **16** and minimum protection with 1-(5-phenyl-2*H*-tetrazol-2-ylacetyl)-4-(4-bromophenyl)thiosemicarbazide **13**. In the present study, sodium salicylate afforded 52.6% protection against trypsin-induced hydrolysis of bovine serum albumin.

EXPERIMENTAL

All compounds were analyzed for their carbon, hydrogen and nitrogen contents. Melting points were taken in open capillary tubes with a partial immersion thermometer and are uncorrected. Infrared spectra of these compounds were recorded on Beckman IR-12 spectrometer as a suspension in nujol. Nuclear magnetic resonance spectra were obtained on a Varian Associates EM-390 spectrometer using DMSO-d_6 as a solvent and tetramethylsilane as an internal standard.

5-Phenyltetrazole (**2**).

A mixture of benzonitrile (0.2 mole), sodium azide (0.22 mole) and ammonium chloride (0.22 mole) in DMF was refluxed with stirring on oil bath (127°) for 17 hours (15). The DMF was removed under reduced pressure, the residue was suspended



Scheme – I

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Table I

Physical Constants of 1-(5-Phenyl-2*H*-tetrazol-2-ylacetyl)-4-substituted Thiosemicarbazides (**5-16**)

Compound No.	Ar	Melting Point	Yield %	Molecular Formula	Analysis % Calculated C	Calculated H	Calculated N	Found C	Found H	Found N
5	2-$CH_3C_6H_4$	168	55	$C_{17}H_{17}N_7OS$	55.58	4.63	26.70	55.37	4.52	26.53
6	3-$CH_3C_6H_4$	172	40	$C_{17}H_{17}N_7OS$	55.58	4.63	26.70	55.62	4.45	26.53
7	4-$CH_3C_6H_4$	160	60	$C_{17}H_{17}N_7OS$	55.58	4.63	26.70	55.27	4.68	26.56
8	2-$CH_3OC_6H_4$	177	55	$C_{17}H_{17}N_7O_2S$	53.26	4.43	25.58	53.07	4.19	25.69
9	4-$CH_3OC_6H_4$	170	60	$C_{17}H_{17}N_7O_2S$	53.26	4.43	25.58	53.33	4.51	25.79
10	2-$C_2H_5OC_6H_4$	190	40	$C_{18}H_{19}N_7O_2S$	54.40	4.78	24.68	54.12	4.45	24.56
11	4-$C_2H_5OC_6H_4$	186	50	$C_{18}H_{19}N_7O_2S$	54.40	4.78	24.68	54.51	4.79	24.86
12	4-ClC_6H_4	174	45	$C_{16}H_{14}ClN_7OS$	49.61	3.61	25.32	49.82	3.72	25.35
13	4-BrC_6H_4	178	52	$C_{16}H_{14}BrN_7OS$	44.44	3.24	22.68	44.67	3.39	22.67
14	4-IC_6H_4	189	50	$C_{16}H_{14}IN_7OS$	40.08	2.92	20.45	40.27	2.96	20.56
15	2,4-$(CH_3)_2C_6H_3$	171	60	$C_{18}H_{19}N_7OS$	56.69	4.98	25.72	56.37	4.75	25.73
16	3,4-$(CH_3)_2C_6H_3$	167	70	$C_{18}H_{19}N_7OS$	56.69	4.98	25.72	56.48	4.76	25.74

Table II

Spectral Data of 1-(5-Phenyl-2*H*-tetrazol-2-ylacetyl)-4-substituted Thiosemicarbazides

Compound No.	R	Characteristic Bands in Ir Spectra (cm^{-1}) C=O	NH	Chemical Shifts in Pmr δ (ppm) (a) -CH_2-	Aromatic Protons	-CONHNHCSNH-	R
5	2-CH_3	1700	3300	5.63 (s, 2H)	7.00-8.33 (m, 9H)	9.56-11.00 (b, 3H)	2.16 (s, 3H)
10	2-OCH_2CH_3	1700	3280	5.63 (s, 2H)	6.70-8.26 (m, 9H)	9.00-10.90 (b, 3H)	1.33 (t, 3H) 4.03 (q, 2H)
14	4-I	1695	3280	5.63 (s, 2H)	7.13-8.20 (m, 9H)	9.60-10.73 (b, 3H)	— — —
16	3,4-$(CH_3)_2$	1700	3280	5.63 (s, 2H)	7.00-8.20 (m, 9H)	9.60-10.63 (b, 3H)	2.16 (s, 6H)

(a) Signal multiplicity, s = singlet, b = broad, t = triplet, q = quartet and m = multiplet.

in cold water and was acidified with hydrochloric acid to *p*H 2. The solid product was filtered and recrystallized from ethanol to give 5-phenyltetrazole, m.p. 215°, lit. (15) m.p. 213-215°.

Ethyl (5-Phenyl-2*H*-tetrazol-2-yl)acetate (**3**).

The 5-phenyltetrazol (0.1 mole) was dissolved in a solution of sodium (0.1 g. atom) in 350 ml. of absolute ethanol. The solution was stirred at reflux and the ethyl bromoacetate (0.1 mole) was added in portions. The refluxing was continued for 16 hours. The reaction mixture was then filtered hot and concentrated under reduced pressure. The crude product was filtered and recrystallized from aqueous ethanol, m.p. 83°, lit. (16) m.p. 84-86°; ir (nujol): C=O (1750 cm^{-1}); nmr (DMSO-d_6): δ 1.23 (CH_2CH_3, t, 3H), 4.23 (CH_2CH_3, q, 2H), 5.90 (NCH_2CO, S, 2H), 7.10-8.30 (aromatic, m, 5H).

(5-Phenyl-2*H*-tetrazol-2-yl)acethydrazide (**4**).

A mixture of ethyl (5-phenyl-2*H*-tetrazol-2-yl)acetate (0.1 mole) and 99% hydrazine hydrate (0.15 mole), in 100 ml. of ethanol, was refluxed for 8 hours. The reaction mixture was concentrated and cooled to crystallize out the hydrazide. The hydrazide was filtered and recrystallized from ethanol, m.p. 205°; ir (nujol): C=O (1760 cm^{-1}), and NH_2 (3340); nmr (DMSO-d_6): δ 4.33 ($NHNH_2$, b, 2H), 5.50 ($N\text{-}CH_2CO$, s, 2H), 7.30-8.23 (aromatic, m, 5H) and 9.66 ($CONHNH_2$, b, 1H).

Anal. Calcd. for $C_9H_{10}N_6O$: C, 49.54; H, 4.59; N, 38.53. Found: C, 49.12; H, 4.60; N, 38.32.

1-(5-Phenyl-2*H*-tetrazol-2-ylacetyl)-4-substituted Thiosemicarbazides (**5-16**).

Equimolar quantities of (5-phenyl-2*H*-tetrazol-2-yl)acethydrazide (0.01 mole) and the suitable arylisothiocyanate (0.01 mole) were dissolved in absolute ethanol and the solution was refluxed for 6 hours. The reaction mixture was concentrated under reduced pressure and cooled to crystallize the crude product. The solid mass was filtered and recrystallized from ethanol. The various 1-(5-aryl-2*H*-tetrazol-2-ylacetyl)-4-substituted thiosemicarbazides were characterized by their sharp melting points and elemental analysis (Table I). Furthermore, the characteristic bands in infrared and the chemical shifts of the various protons in proton magnetic resonances spectra of **5, 10, 14,** and **16** supported the structure of **5-16** (Table II).

Assay of Proteolytic Activity of Trypsin.

The antiproteolytic activity of various 1-(5-phenyl-2*H*-tetrazol-2-ylacetyl)-4-substituted thiosemicarbazides (**5-16**) was measured by determining their ability to inhibit trypsin-induced hydrolysis of bovine serum albumin. Compounds were dissolved in dimethylformamide and were used at a final concentration of 1 mM. The acid soluble products of protein breakdown were measured by following the method of Lowry, *et al.* (17). Decrease in the formation of the products of protein breakdown in the presence of **5-16** was used to determine their antiproteolytic activity.

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