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A series of 5-(3,4,5-trimethoxyphenyl)-4-substituted aryl-3-hydrazinocarbonylmethylthio-4*H*-1,2,4-triazoles were synthesized and evaluated for their antiprotolytic and antiinflammatory activities.

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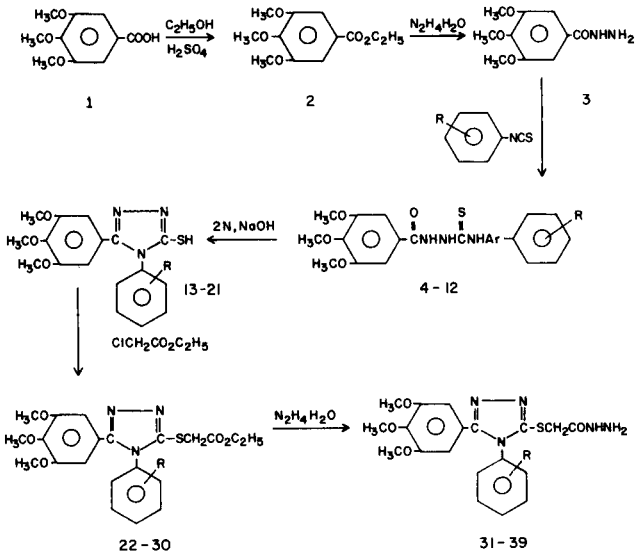
A broad spectrum of pharmacological properties have been demonstrated with the triazole nucleus (2-6). A series of 5-(1-naphthylmethyl)-s-triazoles have been reported to possess significant antiinflammatory activity (7). Earlier studies have indicated that proteolytic enzymes have a role in inflammatory processes (8,9). Some antiinflammatory agents have been reported to exhibit antiproteolytic activity (10). These observations initiated the synthesis of some 5-(3,4,5-trimethoxyphenyl)-4-substituted aryl-3-hydrazinocarbonylmethylthio-4*H*-1,2,4-triazoles and the investigation of their antiproteolytic and antiinflammatory properties. The various substituted-4*H*-1,2,4-triazoles were synthesized according to the steps outlined in Scheme I.



Scheme I

Ethyl-3,4,5-trimethoxybenzoate **2** (11), obtained by refluxing 3,4,5-trimethoxybenzoic acid **1** in absolute ethanol containing a few drops of concentrated sulphuric acid, was converted into 3,4,5-trimethoxybenzoic acid hydrazide **3** (12) in the presence of hydrazine hydrate. The condensation of acid hydrazide **3** with suitable aryl isothiocyanates resulted in the formation of 1-(3,4,5-trimethoxybenzoyl)-4-substituted aryl-3-thiosemicarbazides **4-12**. These thiosemicarbazides **4-12** on refluxing with 2*N* sodium hydroxide solution were cyclized into their corresponding 5-(3,4,5-trimethoxyphenyl)-4-substituted aryl-3-mercapto-4*H*-1,2,4-triazoles **13-21**. Treatment of these triazoles **13-21** with equimolar quantities of ethyl chloroacetate in the presence of anhydrous potassium carbonate resulted the formation of 5-(3,4,5-trimethoxyphenyl)-4-substituted aryl-3-ethoxycarbonylmethylthio-4*H*-1,2,4-triazoles **22-30**. These compounds **22-30** on further treatment with hydrazinehydrate yielded 5-(3,4,5-trimethoxyphenyl)-4-substituted aryl-3-hydrazinocarbonyl-

Table I

Physical Constants of 1-(3,4,5-Trimethoxybenzoyl)-4-substituted Aryl-3-thiosemicarbazides

Compound No.	R	M.p. °C	Yield %	Molecular Formula	Analysis % Calcd. % C	H	N	Found % C	H	N
4	H	188	80	$C_{17}H_{19}N_3O_4S$	56.53	5.26	11.63	56.14	4.91	11.74
5	2-CH_3	182	75	$C_{18}H_{21}N_3O_4S$	57.60	5.60	11.20	57.80	5.32	11.25
6	3-CH_3	185	80	$C_{18}H_{21}N_3O_4S$	57.60	5.60	11.20	57.71	5.84	11.11
7	2-OCH_3	152	85	$C_{18}H_{21}N_3O_5S$	55.24	5.37	10.74	54.95	5.50	11.00
8	4-OCH_3	198	90	$C_{18}H_{21}N_3O_5S$	55.24	5.37	10.74	55.42	5.15	10.62
9	4-Cl	205	85	$C_{17}H_{18}ClN_3O_4S$	51.51	4.54	10.60	51.81	4.78	10.50
10	4-Br	207	70	$C_{17}H_{18}BrN_3O_4S$	46.36	4.09	9.54	46.26	4.22	9.69
11	2,4-$(CH_3)_2$	168	90	$C_{19}H_{23}N_3O_4S$	58.61	5.91	10.79	58.46	6.17	10.44
12	3,4-$(CH_3)_2$	189	85	$C_{19}H_{23}N_3O_4S$	58.61	5.91	10.79	58.57	6.21	10.62

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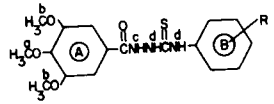
Table II

Spectral Data of 1-(3,4,5-Trimethoxybenzoyl)-4-substituted Aryl Thiosemicarbazides



Compound No.	R	Characteristic Bands in Ir Spectra (cm^{-1})				Pmr Chemical Shifts δ (ppm)						
		C=S	C=C	C=O	-NH-	a	b	Ring A Protons	Ring B Protons	c	d	R
4	H	1100	1610	1640	1570, 3160, 3280	3.70 (s)	3.80 (s)	7.20 (s)	7.00-7.53 (m)	9.73 (s)	9.40-10.60 (b)	--
6	3-CH_3	1095	1615	1645	1570, 3180, 3330	3.70 (s)	3.83 (s)	7.26 (s)	6.80-7.40 (m)	9.73 (s)	9.33-10.63 (b)	2.30 (s)
7	2-OCH_3	1110	1600	1650	1570, 3180, 3340	3.70 (s)	3.83 (s)	7.26 (s)	6.80-7.26 (m)	9.20 (s)	9.50-10.70 (b)	3.76 (s)
10	Br	1100	1615	1650	1570, 3140, 3280	3.70 (s)	3.80 (s)	7.20 (s)	7.40 (s)	9.73 (s)	9.40-10.50 (b)	-

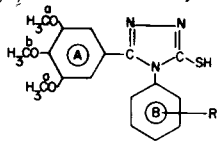
Table III

Physical Constants of 5-(3,4,5-Trimethoxyphenyl)-4-substituted Aryl-3-mercapto-4*H*-1,2,4-triazoles

Compound No.	R	M.p. °C	Yield %	Molecular Formula	Analysis Calcd. %			Found %		
					C	H	N	C	H	N
13	H	210-212	60	$C_{17}H_{17}N_3O_3S$	59.64	4.97	12.28	59.31	4.83	12.33
14	2-CH_3	194	75	$C_{18}H_{19}N_3O_3S$	60.50	5.32	11.76	60.75	5.41	11.56
15	3-CH_3	199-200	60	$C_{18}H_{19}N_3O_3S$	60.50	5.32	11.76	60.62	5.55	11.84
16	2-OCH_3	185	65	$C_{18}H_{19}N_3O_4S$	57.90	5.09	11.23	58.24	5.31	11.47
17	4-OCH_3	252-253	65	$C_{18}H_{19}N_3O_4S$	57.90	5.09	11.23	58.18	5.26	11.11
18	4-Cl	251	70	$C_{17}H_{16}ClN_3O_3S$	53.98	4.23	11.11	54.17	4.05	10.92
19	4-Br	256	73	$C_{17}H_{16}BrN_3O_3S$	48.34	3.79	9.95	48.26	3.99	10.27
20	2,4-$(CH_3)_2$	248	65	$C_{19}H_{21}N_3O_3S$	61.45	5.66	11.32	61.18	5.82	11.55
21	3,4-$(CH_3)_2$	233-234	60	$C_{19}H_{21}N_3O_3S$	61.45	5.66	11.32	61.26	5.74	11.12

Table IV

Spectral Data of 5-(3,4,5-Trimethoxyphenyl)-4-substituted Aryl-3-mercapto-4*H*-1,2,4-triazoles



Compound No.	R	Characteristic Bands in Ir Spectra (cm^{-1}) C=C/C=N	Pmr Chemical Shifts δ (ppm)				
			a	b	Ring A Protons	Ring B Protons	R
13	H	1600	3.56 (s)	3.63 (s)	6.60 (s)	7.20-7.66 (m)	--
15	3-CH_3	1605	3.53 (s)	3.63 (s)	6.60 (s)	7.10-7.53 (m)	2.33 (s)
16	2-OCH_3	1600	3.53 (s)	3.60 (s)	6.60 (s)	6.93-7.60 (m)	3.56 (s)
19	Br	1600	3.60 (s)	3.66 (s)	6.60 (s)	7.23-7.83 (m)	--

Table V

Physical Constants of 5-(3,4,5-Trimethoxyphenyl)-4-substituted Aryl-3-ethoxycarbonylmethylthio-4*H*-1,2,4-triazoles

Compound No.	R	M.p. °C	Yield %	Molecular Formula	Analysis					
					Calcd. %			Found %		
					C	H	N	C	H	N
22	H	137-138	80	$C_{21}H_{23}N_3O_5S$	58.74	5.36	9.79	58.51	5.01	9.91
23	2-CH_3	100	80	$C_{22}H_{25}N_3O_5S$	59.59	5.64	9.48	59.75	5.82	9.52
24	3-CH_3	95-96	75	$C_{22}H_{25}N_3O_5S$	59.59	5.64	9.48	59.81	5.76	9.43
25	2-OCH_3	91-92	85	$C_{22}H_{25}N_3O_6S$	57.51	5.66	9.15	57.91	5.76	9.43
26	4-OCH_3	103	70	$C_{22}H_{25}N_3O_6S$	57.51	5.66	9.15	57.88	5.92	9.37
27	4-Cl	98	85	$C_{21}H_{22}ClN_3O_5S$	54.42	4.75	9.07	54.17	4.45	9.31
28	4-Br	110-111	80	$C_{21}H_{22}VrN_3O_5S$	49.60	4.33	8.26	49.99	4.65	8.58
29	2,4-$(CH_3)_2$	99-100	85	$C_{23}H_{27}N_3O_5S$	60.39	5.90	9.19	60.67	6.22	9.48
30	3,4-$(CH_3)_2$	139-140	75	$C_{23}H_{27}N_3O_5S$	60.39	5.90	9.19	60.67	6.22	9.48

methylthio-4*H*-1,2,4-triazoles **31-39**.

All 5-(3,4,5-trimethoxyphenyl)-4-substituted aryl-3-hydrazinocarbonylmethylthio-4*H*-1,2,4-triazoles **31-39** were investigated for their ability to inhibit the trypsin-induced hydrolysis of bovine serum albumin and to protect the formation of carrageenin-induced edema in rats. All compounds, except **35**, were found to inhibit the bovine serum albumin breakdown caused by trypsin and the drgree of inhibition ranged from 22.2 to 72.2%. The degree of inhibition of **33**, **34**, **36**, **37** and **38** was 39.5, 72.2, 46.5, 50.2 and 44.4%, respectively. All compounds **31-39** exhibited the protection against carrageenin-induced edema and the degree of protection ranged from 6 to 46%. The percent protection observed with **33**, **34**, **35**, **36**, **37** and **38** was 46, 37, 28, 41, 6 and 14%, respectively. These observations have failed to provide a correlationship between the antiproteolytic activity and antiinflammatory activity by these substituted-4*H*-1,2,4-triazoles **31-39**.

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. Infrared spectra were recorded on Beckman Ir-33 in nujol mull. Proton magnetic resonance spectra were obtained on a Varian EM-390 instrument using tetramethylsilane as an internal reference and deuterated dimethylsulphoxide as solvent.

Ethyl 3,4,5-Trimethoxybenzoate (**2**).

A mixture of 3,4,5-trimethoxybenzoic acid **1** (0.3 mole) and 6 ml. of concentrated sulphuric acid in 300 ml. of anhydrous ethanol was refluxed on a steam bath for 20 hours. The reaction mixture was concentrated under reduced pressure and upon cooling, a white solid separated out. The crude product **2** was filtered, washed with cold water, dried and recrystallized from ethanol, m.p. 60° (reported m.p. 53-57°) (11); ir: (nujol mull), C=O (1750 cm^{-1}); pmr δ (carbon tetrachloride): 1.33 (t, 3H, $COOCH_2CH_3$), 4.20 (q, 2H, $COOCH_2CH_3$), 3.73 (S, 3H, 4-OCH_3), 3.80 (S, 6H, 3 and 5-OCH_3), 7.10 (S, 2H, C_6H_2).

3,4,5-Trimethoxybenzoic Acid Hydrazide (**3**).

A solution of **2** (0.25 mole) and hydrazine hydrate (99%, 0.40 mole) in 100 ml. of absolute ethanol was refluxed on a steam bath for 10 hours. The excess of ethanol was removed under reduced pressure. The concentrated solution on cooling gave solid mass of **3** which was filtered, dried and recrystallized from ethanol, m.p. 157° (reported m.p. 158-159°) (12); ir: (nujol mull), C=O (1660 cm^{-1}), NH (3200 cm^{-1}), NH_2 (3340 cm^{-1}); pmr δ (DMSO-d_6): 3.70 (S, 3H, 4-OCH_3), 3.80 (S, 6H, 3 and 5-OCH_3), 7.16 (S, 2H, C_6H_2).

1-(3,4,5-Trimethoxybenzoyl)-4-substituted Aryl-3-thiosemicarbazides (**4-12**).

Equimolar quantities of **3** (0.02 mole) and the appropriate aryl isothiocyanate (0.02 mole) in 25 ml. of absolute ethanol was refluxed on a steam bath for 3-5 hours. Excess of ethanol was removed by distillation under reduced pressure. The crude product which separated out was filtered, dried and recrystallized from ethanol. These thiosemicarbazides **4-12** were characterized by their sharp melting points and elemental analyses (Table I). The infrared and proton magnetic resonance spectral data of **4**, **6**, **7** and **10** (Table II) further supported their structure.

5-(3,4,5-Trimethoxyphenyl)-4-substituted Aryl-3-mercapto-4*H*-1,2,4-triazoles (**13-21**).

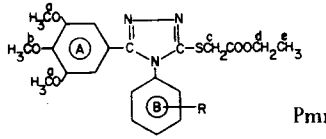
Suitable substituted thiosemicarbazides **4-12** (0.0175 mole) were dissolved in 2*N* sodium hydroxide and the resulting solution was refluxed on free flame for 2-3 hours. After cooling, the reaction mixture was filtered and the filtrate was acidified with dilute hydrochloric acid until complete precipitation occurred. The solid mass which precipitated out was filtered, washed with water, dried and recrystallized from ethanol. The elemental analyses and melting points of **13-21** are recorded in Table III and spectral data, infrared and proton magnetic resonance, of **13**, **15**, **16** and **19** are recorded in Table IV.

5-(3,4,5-Trimethoxyphenyl)-4-substituted Aryl-3-ethoxycarbonylmethylthio-4*H*-1,2,4-triazoles (**22-30**).

A mixture of ethyl chloroacetate (0.015 mole), the appropriate compound **13-21** (0.015 mole) and anhydrous potassium carbonate (0.018 mole) in 60 ml. of dry acetone was refluxed on a steam

Table VI

Spectral Data of 5-(3,4,5-Trimethoxyphenyl)-4-substituted Aryl-3-ethoxycarbonylmethylthio-4*H*-1,2,3-triazoles



Compound No.	R	Characteristic Bands in Ir Spectra (cm^{-1})		Pmr Chemical Shifts δ (ppm)							
		C=C/C=N	C=O	a	b	C	d	e	Ring A Protons	Ring B Protons	R
22	H	1585	1725	3.35 (s)	3.63 (s)	4.10 (s)	4.16 (q)	1.20 (t)	6.60 (s)	7.33-7.66 (m)	--
24	3-CH_3	1600	1740	3.56 (s)	3.63 (s)	4.10 (s)	4.16 (q)	1.20 (t)	6.66 (s)	7.10-7.60 (m)	2.36 (s)
25	2-OCH_3	1600	1735	3.56 (s)	3.63 (s)	4.10 (s)	4.13 (q)	1.20 (t)	6.63 (s)	7.00-7.70 (m)	3.70 (s)
28	4-Br	1585	1740	3.56 (s)	3.63 (s)	4.06 (s)	4.10 (q)	1.20 (t)	6.60 (s)	7.26-7.90 (s)	--

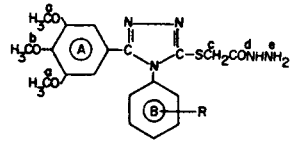
Table VII

Physical Constants of 5-(3,4,5-Trimethoxyphenyl)-4-substituted Aryl-3-hydrazinocarbonylmethylthio-4*H*-1,2,4-triazoles

Compound No.	R	M.p. °C	Yield %	Molecular Formula	Analysis					
					Calcd. %			Found %		
					C	H	N	C	H	N
31	H	201-202	62	$C_{19}H_{21}N_5O_4S$	54.93	5.06	16.86	55.21	5.34	16.94
32	2-CH_3	183-184	60	$C_{20}H_{23}N_5O_4S$	55.94	5.36	16.31	56.18	5.64	16.22
33	3-CH_3	196-197	60	$C_{20}H_{23}N_5O_4S$	55.94	5.36	16.31	56.27	5.19	16.44
34	2-OCH_3	206	58	$C_{20}H_{23}N_5O_5S$	53.70	5.16	15.73	53.47	5.33	15.92
35	4-OCH_3	222	65	$C_{20}H_{23}N_5O_5S$	53.70	5.16	15.73	53.95	4.89	15.57
36	4-Cl	208-210	63	$C_{19}H_{20}ClN_5O_4S$	50.72	4.44	15.57	50.91	4.72	15.81
37	4-Br	205	60	$C_{19}H_{20}BrN_5O_4S$	46.39	4.04	14.19	46.82	3.78	14.36
38	2,4-$(CH_3)_2$	209	65	$C_{21}H_{25}N_5O_4S$	56.88	5.64	15.80	57.16	5.97	16.22
39	3,4-$(CH_3)_2$	218	70	$C_{21}H_{25}N_5O_4S$	56.88	5.64	15.80	57.20	5.71	15.65

Table VIII

Spectral Data of 5-(3,4,5-Trimethoxyphenyl)-4-substituted Aryl-3-hydrazinocarbonylmethylthio-4*H*-1,2,4-triazoles



Compound No.	R	Characteristic Bands in Ir Spectral (cm^{-1})			Pmr Chemical Shifts δ (ppm)							
		C=C/C=N	C=O	NH	a	b	c	d	e	Ring A Protons	Ring B Protons	R
31	H	1580	1660	3210, 3310	3.53 (s)	3.60 (s)	3.90 (s)	9.26 (s)	4.26 (s)	6.60 (s)	7.30-7.66 (m)	--
33	3-CH_3	1580	1660	3240, 3320	3.53 (s)	3.60 (s)	3.86 (s)	9.33 (s)	4.26 (s)	6.66 (s)	7.10-7.60 (m)	2.33 (s)
34	2-OCH_3	1580	1680	3230, 3360	3.53 (s)	3.60 (s)	3.86 (s)	9.30 (s)	4.26 (s)	6.60 (s)	7.00-7.70 (m)	3.66 (s)
37	4-Br	1590	1680	3210, 3320	3.53 (s)	3.60 (s)	3.83 (s)	9.26 (b)	4.26 (b)	6.60 (s)	7.30-7.83 (m)	--

bath for 8 hours. The reaction mixture was filtered and the excess of acetone was removed under reduced pressure to give **22-30**. Finally the crude products **22-30** were recrystallized from ethanol. The various substituted-4*H*-1,2,4-triazoles **22-30** were characterized by their sharp melting points and elemental analyses (Table V). Furthermore, the structure of these compounds was confirmed by the infrared and proton magnetic resonance spectral data of **22**, **24**, **25** and **28** (Table VI).

5-(3,4,5-Trimethoxyphenyl)-4-substituted Aryl-3-hydrazinocarbonylmethylthio-4*H*-1,2,4-triazoles (**31-39**).

To a solution of the appropriate **22-30** (0.01 mole) in 50 ml. of absolute ethanol was added hydrazine hydrate (99%, 0.015 mole) and the mixture was refluxed on a steam bath for 3-4 hours. Excess of ethanol was removed under reduced pressure and the solid thus separated out was filtered, washed with cold water, dried and recrystallized from ethanol. These compounds **31-39** were characterized by their sharp melting points and elemental analyses (Table VII). The presence of the characteristic bands in the infrared and signals of various protons in the proton magnetic resonance spectra of **31**, **33**, **34** and **37** (Table VIII) provided further support for the structure of these compounds.

Assay of Proteolytic Activity of Trypsin.

The antiproteolytic activity of various substituted-4*H*-1,2,4-triazoles **31-39** was measured by determining their ability to inhibit trypsin-induced hydrolysis of bovine serum albumin (10). The test compounds **31-39** were dissolved in dimethylformamide and were used at a final concentration of 0.1 mmole. The acid soluble products of protein breakdown were determined by the method of Lowry, *et al.*, (13). Decrease in the formation of the products of protein breakdown in the presence of the test compounds **31-39** was used to determine their antiproteolytic activity.

Determination of Antiinflammatory Activity.

Carrageenin-induced Edema Method.

The antiinflammatory activity of **31-39** was determined in albino rats following the method reported by Buttle, *et al.*, (14) and modified by Winter, *et al.*, (15). All test compounds **31-39** were injected intraperitoneally at a dose of 100 mg./kg. to evaluate their ability to provide protection against edema induced by the administration of 0.05 ml. of suspension of carrageenin (1%) in 0.9% sodium chloride solution under the planter aponeurosis of hind paw of rats.

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