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Synthesis and Anti-Inflammatory Evaluation of 3-Methylthio-1,2,4-triazines, 3-Alkoxy-1,2,4-triazines, and 3-Aryloxy-1,2,4-triazines

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Abstract □ To develop nonacidic, nonsteroidal anti-inflammatory agents without GI complications, a series of asymmetric triazines was synthesized and evaluated for anti-inflammatory efficacy in the carrageenan-induced pedal edema assay. Toxicity was estimated by determination of approximate LD_{50} values in mice. Twenty-five compounds possessed activity comparable to the standard, indomethacin. Thirteen of the 25 compounds were selected for dose–response evaluation in the carrageenan assay based on their relative toxicity and anti-inflammatory activity. Neurotoxicity of the 13 triazines was estimated by determination of NTD_{50} values in mice. Five of the 13 compounds tested in the dose–response assay were active in terms of anti-inflammatory efficacy (ED_{50} values) and lack of overt neurotoxicity (NTD_{50} values) when compared to indomethacin. To determine the effect of these five developmental triazines on chronic inflammation, they were evaluated in the adjuvant-induced polyarthritis assay. One was comparable to indomethacin in reducing adjuvant-induced inflammation in this assay.

Keyphrases □ Triazines, asymmetric—synthesis, evaluation of anti-inflammatory activity □ Anti-inflammatory agents—asymmetric triazines, synthesis, evaluation of activity □ Polyarthritis, adjuvant induced—assay, asymmetric triazines

In recent years, the literature on nonsteroidal anti-inflammatory agents has increased dramatically. From 1966 to 1976, 807 new compounds from 262 research laboratories were identified as new nonsteroidal anti-inflammatory agents. However, clinical reports were available on only 65 drugs; and of those that have been marketed, only a few have been commercially successful. GI irritation continues to be the principal complication with most developmental, clinical, and commercial nonsteroidal anti-inflammatory drugs.

The occurrence of GI effects in humans has been demonstrated by numerous investigators with administration of various salicylates (1–4), phenylbutazone (5, 6), indomethacin (7), tolmetin (8), naproxen (9), and ibuprofen (10). The ongoing search for novel classes of nonsteroidal anti-inflammatory agents in part reflects the continued inability to separate anti-inflammatory efficacy from GI toxicity. Although considerable controversy concerns the etiology of this toxicity, it generally is agreed that gastric irritation is associated, directly or indirectly, with the acidic nature of these drugs and their metabolites (11, 12).

DISCUSSION

To eliminate GI complications while maintaining anti-inflammatory activity, a series of 81 asymmetric triazines was synthesized and evaluated for potential anti-inflammatory efficacy. The carrageenan-induced pedal edema assay was utilized to detect primary level activity; acute toxicity was estimated by determination of LD_{50} values in mice. A dose–response carrageenan assay and neurotoxicity evaluation were used to determine the ED_{50} and NTD_{50} values of those compounds active at the primary level. Neurotoxicity was estimated by determination of NTD_{50} values in mice. Compounds that were comparable to indomethacin in the secondary stage of evaluation were tested in the adjuvant-induced polyarthritis assay to determine their effect on a chronic inflammatory condition.

Synthesis—3-Methoxy-5-substituted-1,2,4-triazines (III*a*–III*v*) and 3-alkoxy-5-substituted-phenyl-1,2,4-triazines (IV*a*–IV*l*) were synthesized. Melting points and recrystallization solvents are shown in Tables I–III. The 3-methylthio-1,2,4-triazines (II*a*–II*v*) served as common intermediates in the synthesis of all 3-alkoxy- and 3-aryloxy-1,2,4-triazines (III*a*–III*v*, IV*a*–IV*l*, X*a*–X*h*, and XI*a*–XI*i*). The 3-methylthio intermediates were synthesized according to the method of Paudler and Chen (13) with modifications by Heilman *et al.* (14).

Treatment of II with sodium methoxide in refluxing methanol resulted in nucleophilic displacement of the methylthio function to afford the corresponding 3-methoxy-5-substituted-1,2,4-triazines (III*a*–III*v*). Reaction of II with a variety of sodium alkoxides refluxing in dioxane afforded the corresponding 3-alkoxy-5-phenyl-1,2,4-triazines (IV*a*–IV*l*).

5,6-Disubstituted-3-alkoxy-1,2,4-triazines (VII*a*–VII*h* and VIII*a*–VIII*i*) were synthesized. Melting points and recrystallization solvents are shown in Tables IV and V. Cyclization of symmetrical 1,2-diones (V*a*–V*h*) under basic conditions with methylthiosemicarbazide hydrogen iodide afforded the 3-methylthio-5,6-disubstituted-1,2,4-triazines (VI*a*–VI*h*). Nucleophilic displacement of the methylthio group with the appropriate sodium alkoxide produced the desired 3-alkoxy-5,6-disubstituted-1,2,4-triazines (VII*a*–VII*h* and VIII*a*–VIII*i*).

Biology—The effect of the asymmetric triazines on the inflammatory response was evaluated at the primary level in the carrageenan-induced pedal edema assay (15). Carrageenan injected into the plantar tissue of the hindpaw of Sprague–Dawley rats produces an edematous condition, which simulates in part the inflammatory process found in human arthritis (16–18). Nonsteroidal anti-inflammatory drugs such as indomethacin, phenylbutazone, and aspirin inhibit the formation of this edema (19, 20).

Compounds were administered orally, using 0.25% methylcellulose as the vehicle. Five rats were used per dose, with the reported percent reduction in inflammation represented by the average of the reduction produced in the five animals. Compounds were administered at levels expected to be subtoxic by consideration of their approximate, measured LD_{50} values. The LD_{50} values in mice were determined in a standard,

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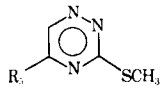
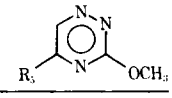
Table I—Melting Points or Boiling Points, Recrystallization Solvents, and Elemental Analyses of 3-Methylthio-5-substituted-1,2,4-triazines (II*a*–II*v*)



Compound	R_5	Melting Point or Boiling Point	Recrystallization Solvent	Elemental Analysis, % Calculated C, H, N	Elemental Analysis, % Found C, H, N
II*a*	Phenyl	99–100°[a]	Hexane	—	—
II*b*	3-Chlorophenyl	79–81°[b]	Hexane	—	—
II*c*	3-Trifluoromethylphenyl	123–125°[b]	Hexane	—	—
II*d*	4-Methylphenyl	160–161°[b]	Methanol	—	—
II*e*	4-Chlorophenyl	165–166°[b]	Methanol	—	—
II*f*	3,4-Dimethoxyphenyl	150–152°	Isopropanol	54.73, 4.98, 15.96	54.56, 5.02, 15.90
II*g*	4-Bromophenyl	153–154°	Heptane	42.55, 2.84, 14.89	42.55, 2.88, 15.03
II*h*	4-Methoxyphenyl	106–107°	Heptane	56.65, 4.72, 18.03	56.32, 4.74, 17.80
II*i*	2,4-Difluorophenyl	79–80°	Hexane	50.21, 2.93, 17.57	50.16, 2.96, 17.87
II*j*	2,4-Dichlorophenyl	106–107°	Heptane	44.12, 2.57, 15.44	44.16, 2.56, 15.50
II*k*	2,5-Dimethoxyphenyl	113–114°	Heptane	54.75, 4.94, 16.97	54.94, 5.02, 16.67
II*l*	4-Ethylphenyl	71–72°	Hexane	62.34, 5.63, 18.18	62.11, 5.66, 18.43
II*m*	4-Morpholinophenyl	127–128°	Heptane	58.33, 5.55, 19.44	57.93, 5.69, 19.77
II*n*	*tert*-Butyl	91–93°/ 0.1 mm Hg	—	52.46, 7.10, 22.95	52.30, 7.21, 22.90
II*o*	Cyclopropyl	44–46°	Hexane	50.27, 5.42, 25.13	50.11, 5.41, 25.07
II*p*	1-Adamantyl	140–141°	Ethyl acetate	64.37, 7.28, 16.09	64.22, 7.42, 16.32
II*q*	2-Furyl	88–90°	Hexane	49.74, 3.63, 21.76	50.01, 3.64, 21.82
II*r*	2-Thienyl	105–106°	Hexane	45.93, 3.35, 20.10	46.04, 3.40, 20.20
II*s*	2-Benzofuryl	134–135°	Heptane	59.26, 3.70, 17.39	59.63, 3.77, 17.87
II*t*	9-Anthracyl	181–183°	Acetone	71.26, 4.32, 13.85	70.79, 4.32, 14.01
II*u*	1-Naphthyl	99–100°	Hexane	66.40, 4.35, 16.60	66.33, 4.40, 16.89
II*v*	2-Naphthyl	117–118°	Heptane	66.40, 4.35, 16.60	66.30, 4.26, 16.64

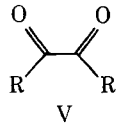
[a] Identical to melting point given in Ref. 13. [b] Identical to melting point given in Ref. 14.

Table II—Melting Points or Boiling Points, Recrystallization Solvents, and Elemental Analyses of 3-Methoxy-5-substituted-1,2,4-triazines (III*a*–III*v*)



Compound	R_5	Melting Point or Boiling Point	Recrystallization Solvent	Elemental Analysis, % Calculated C, H, N	Elemental Analysis, % Found C, H, N
III*a*	Phenyl	75–76°[a]	Heptane	—	—
III*b*	3-Chlorophenyl	93–94°	Hexane	54.18, 3.61, 18.96	54.25, 3.64, 18.90
III*c*	3-Trifluoromethylphenyl	95–97°	Hexane	51.76, 3.14, 16.47	51.80, 3.10, 16.52
III*d*	4-Methylphenyl	139–140°	Benzene	65.67, 5.47, 20.90	65.52, 5.51, 21.01
III*e*	4-Chlorophenyl	131–132°	Benzene	54.18, 3.61, 18.96	54.22, 3.66, 19.04
III*f*	3,4-Dimethoxyphenyl	167–169°	Methanol	58.29, 4.30, 17.00	57.82, 5.38, 16.96
III*g*	4-Bromophenyl	108–109°	Ethyl acetate–hexane	46.11, 3.01, 15.79	46.83, 3.31, 15.98
III*h*	4-Methoxyphenyl	106–107°	Dioxane	60.82, 5.07, 19.35	60.77, 5.16, 19.35
III*i*	2,4-Difluorophenyl	91–93°	Heptane	53.81, 3.94, 18.83	53.41, 4.18, 18.78
III*j*	2,4-Dichlorophenyl	114–115°	Ethyl acetate–hexane	46.87, 2.73, 16.41	46.64, 2.68, 16.49
III*k*	2,5-Dimethoxyphenyl	108–109°	Heptane	58.30, 5.26, 17.00	58.43, 5.33, 16.99
III*l*	4-Ethylphenyl	50–51°	Hexane	66.97, 6.05, 19.53	66.60, 6.07, 19.49
III*m*	4-Morpholinophenyl	122–123°	Ethyl acetate–hexane	61.76, 5.88, 20.59	61.42, 5.91, 20.77
III*n*	*tert*-Butyl	45–46°	Hexane	57.48, 7.78, 25.15	57.52, 7.81, 25.20
III*o*	Cyclopropyl	119–120°/ 1.8 mm Hg	—	55.62, 6.00, 27.80	55.03, 6.04, 27.28
III*p*	1-Adamantyl	101–102°	Heptane	68.57, 7.75, 17.14	68.38, 7.88, 17.14
III*q*	2-Furyl	96–97°	Hexane	54.24, 3.95, 23.73	54.36, 3.99, 24.00
III*r*	2-Thienyl	129–130°	Hexane	49.74, 3.63, 21.76	50.05, 3.75, 21.80
III*s*	2-Benzofuryl	139–140°	Ethyl acetate	63.43, 3.97, 18.50	63.31, 3.99, 18.59
III*t*	9-Anthracyl	195–196°	Methanol	75.16, 4.53, 14.63	74.72, 4.53, 14.58
III*u*	1-Naphthyl	76–77°	Hexane	70.89, 4.64, 17.72	70.40, 4.51, 17.76
III*v*	2-Naphthyl	130–131°	Heptane	70.89, 4.64, 17.72	70.46, 4.69, 17.85

[a] Identical to melting point given in Ref 13.



V

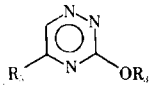
V*a*: R = phenyl
V*b*: R = 4-chlorophenyl
V*c*: R = 4-methylphenyl
V*d*: R = 3,4-dichlorophenyl
V*e*: R = 3,4-dimethoxyphenyl
V*f*: R = 2-furyl
V*g*: R = 2-pyridyl
V*h*: R = methyl

multidimensional observational assay and calculated according to the method of Litchfield and Wilcoxon (21). The anti-inflammatory and LD_{50} results are shown in Table VI.

Based on the anti-inflammatory performance of each compound relative to the indomethacin standard, 25 of the 81 compounds evaluated gave a reduction in edema equal to or greater than the standard. However, test compounds were administered at 200 mg/kg (unless overt toxicity necessitated a lower dose), while indomethacin was administered at 2.5 mg/kg. Of the 25 active triazines, 13 were selected for secondary evaluation based on their relative toxicity and anti-inflammatory activity. Those compounds selected had LD_{50} values greater than 300 mg/kg and produced a percent reduction in inflammation equal to or greater than the standard, which was run simultaneously.

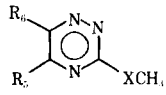
Within the 3-methylthio-5-substituted-1,2,4-triazine series, seven of the 22 compounds screened were active; two were selected for secondary testing (II*c* and II*f*). Among the 3-methoxy-5-substituted-1,2,4-triazines, eight of the 22 compounds evaluated were active; five were selected for further testing (III*a*, III*b*, III*f*, III*n*, and III*o*). In the 3-alkoxy-5-substituted phenyl series, five of the 12 compounds tested were active; four were selected for secondary evaluation (IV*a*, IV*b*, IV*e*, and IV*i*). Within the 3-methylthio- and 3-methoxy-5,6-disubstituted-1,2,4-triazine series, only two of the 16 compounds evaluated were active; one was selected for secondary testing (VII*g*). In the 3-alkoxy-5,6-disubstituted-1,2,4-triazine

Table III—Melting Points or Boiling Points, Recrystallization Solvents, and Elemental Analyses of 3-Alkoxy-5-substituted-phenyl-1,2,4-triazines (IV*a*–IV*l*)



Compound	R_3	R_5	Melting Point or Boiling Point	Recrystallization Solvent	Elemental Analysis, % Calculated C, H, N	Found C, H, N
IV*a*	Ethyl	Phenyl	48–49°	Hexane	64.67, 5.47, 20.89	64.37, 5.40, 20.59
IV*b*	*n*-Propyl	Phenyl	44–45°	Hexane	64.98, 6.05, 19.03	64.62, 6.01, 18.71
IV*c*	*n*-Pentyl	Phenyl	36–38°	Hexane	69.11, 7.05, 17.27	68.92, 7.02, 17.06
IV*d*	Benzyl	Phenyl	78–80°	Heptane	72.99, 4.98, 15.96	73.26, 4.99, 16.26
IV*e*	Allyl	Phenyl	155°/0.3 mm Hg		67.59, 5.29, 19.71	67.78, 5.34, 19.66
IV*f*	4-Methoxyphenyl	Phenyl	149–151°	Ethyl acetate–heptane	68.81, 4.69, 15.05	68.89, 4.75, 14.66
IV*g*	Cyclohexyl	Phenyl	69–71°	Heptane	70.56, 6.71, 16.46	70.31, 6.62, 16.80
IV*h*	*n*-Decyl	Phenyl	46–47°	Hexane	72.80, 8.69, 13.41	73.00, 8.83, 13.12
IV*i*	Ethyl	3-Chlorophenyl	79–81°	Heptane	56.06, 4.28, 17.83	56.16, 4.28, 18.02
IV*j*	Isopropyl	3-Chlorophenyl	73–74°	Heptane	57.72, 4.85, 16.83	57.86, 4.86, 17.18
IV*k*	Phenyl	3-Chlorophenyl	125–127°	Heptane	63.50, 3.55, 14.81	63.43, 3.46, 14.95
IV*l*	Benzyl	3-Chlorophenyl	81–83°	Heptane	64.54, 4.06, 14.11	64.66, 4.16, 14.10

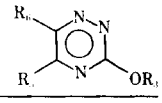
Table IV—Melting Points or Boiling Points, Recrystallization Solvents, and Elemental Analyses of 3-Methylthio- and 3-Methoxy-5,6-disubstituted-1,2,4-triazines (VI*a*–VI*h* and VII*a*–VII*h*)



Compound	X	R_5, R_6	Melting Point or Boiling Point	Recrystallization Solvent	Elemental Analysis, % Calculated C, H, N	Found C, H, N
VI*a*	S	Phenyl	119–120°[a]	Ethyl acetate	—	—
VI*b*	S	4-Chlorophenyl	142–143°	Ethyl acetate	55.17, 3.16, 12.07	54.99, 3.16, 12.11
VI*c*	S	4-Methylphenyl	166–167°	Chloroform	70.36, 5.54, 13.68	70.27, 5.56, 13.52
VI*d*	S	3,4-Dichlorophenyl	127–128°	Hexane–chloroform	46.04, 2.16, 10.17	45.90, 2.12, 10.14
VI*e*	S	3,4-Dimethoxyphenyl	130–131°	Chloroform	60.15, 5.26, 10.53	60.01, 5.42, 10.68
VI*f*	S	2-Furyl	91–92°	Hexane	55.60, 3.47, 16.34	55.48, 3.46, 16.30
VI*g*	S	2-Pyridyl	122–123°	Hexane	59.79, 3.91, 24.91	59.57, 3.90, 25.35
VI*h*	S	Methyl	100–102°/ 0.1 mm Hg	—	46.45, 5.81, 27.10	46.48, 5.95, 26.94
VII*a*	O	Phenyl	78–79°	Hexane	73.00, 4.94, 15.97	72.63, 4.98, 16.39
VII*b*	O	4-Chlorophenyl	146–147°	Methanol	57.83, 3.31, 12.65	57.95, 3.32, 12.77
VII*c*	O	4-Methylphenyl	128–129°	Hexane	74.22, 5.84, 14.43	74.12, 5.73, 14.30
VII*d*	O	3,4-Dichlorophenyl	138–139°	Hexane	48.89, 2.24, 10.47	48.60, 2.24, 10.40
VII*e*	O	3,4-Dimethoxyphenyl	83–84°	Heptane	61.66, 5.48, 10.97	61.64, 5.40, 10.90
VII*f*	O	2-Furyl	105–107°	Hexane	59.26, 3.70, 17.28	59.24, 3.81, 17.35
VII*g*	O	2-Pyridyl	132–133°	Hexane	63.40, 4.15, 26.41	63.47, 4.18, 26.37
VII*h*	O	Methyl	55–57°/ 0.05 mm Hg	—	51.80, 6.47, 30.22	52.05, 6.46, 30.25

[a] Identical to melting point given in M. Gianturco, *Gazz. Chim. Ital.*, **82**, 595 (1952).

Table V—Melting Points or Boiling Points, Recrystallization Solvents, and Elemental Analyses of 3-Alkoxy-5,6-disubstituted-1,2,4-triazines (VIII*a*–VIII*i*)



Compound	R_3	R_5, R_6	Melting Point or Boiling Point	Recrystallization Solvent	Elemental Analysis, % Calculated C, H, N	Found C, H, N
VIII*a*	Ethyl	Phenyl	73–74°	Hexane	73.63, 5.45, 15.15	73.67, 5.40, 15.11
VIII*b*	Isopropyl	Phenyl	96–97°	Heptane	74.20, 5.88, 14.42	73.97, 5.84, 14.39
VIII*c*	Phenyl	Phenyl	123–125°	Ethanol	77.52, 4.65, 12.92	76.97, 4.57, 12.77
VIII*d*	4-Chlorophenyl	Phenyl	162–164°	Heptane	70.10, 3.92, 11.68	70.00, 3.97, 11.61
VIII*e*	4-Methylphenyl	Phenyl	154–156°	Heptane	77.85, 5.05, 12.38	77.78, 5.00, 11.89
VIII*f*	3,4-Dichlorophenyl	Phenyl	133–135°	Hexane–benzene	63.97, 3.32, 10.66	63.98, 3.30, 10.65
VIII*g*	Ethyl	Methyl	74–75°/ 0.07 mm Hg	—	54.90, 7.19, 27.45	55.10, 7.24, 27.40
VIII*h*	Isopropyl	Methyl	75–78°/ 0.05 mm Hg	—	57.48, 7.78, 25.15	57.62, 7.84, 24.90
VIII*i*	Allyl	Methyl	84–86°/ 0.08 mm Hg	—	58.18, 6.66, 25.45	57.94, 6.72, 25.00

series, two of the nine compounds tested were active; one was selected for subsequent evaluation (VIII*i*).

The secondary evaluation consisted of a dose–response carrageenan assay and the determination of the NTD_{50} value in mice, based on the method of Swinyard *et al.* (22). The compounds were administered in the carrageenan assay at multiple doses (*e.g.*, 100, 30, 10, and 3 mg/kg) to obtain a three-point dose–response curve under identical conditions as used in the primary evaluation. If a dose–response relationship was observed, the ED_{50} value was determined according to the method of Litchfield and Wilcoxon (21). The NTD_{50} values were determined to provide additional information concerning the toxicity of the compounds relative to indomethacin.

The results of the secondary testing are shown in Table VII. Based on 50 determinations, indomethacin had a mean ED_{50} value of 2.5 mg/kg and an NTD_{50} value of 112 mg/kg. To compete successfully with this standard, compounds were expected to be less toxic and yet maintain similar levels of efficacy. Therefore, an NTD_{50} value of >300 mg/kg and an ED_{50} value of <30 mg/kg were established as limits for further testing. Five of the 13 compounds subjected to secondary testing fell within these arbitrary bounds (II*f*, III*o*, IV*b*, IV*e*, and VIII*i*).

To determine the effect of these five developmental compounds on a chronic inflammatory condition, each candidate was evaluated in the adjuvant-induced polyarthritis assay (23). An injection of heat-killed *Mycobacterium tuberculosis*, suspended in mineral oil, produced a highly

Table VI—Activity in the Carrageenan-Induced Anti-Inflammatory Assay and LD_{50} Values in Mice of 3-Methylthio- and 3-Alkoxy-1,2,4-triazines

Compound	Reduction, %	Carrageenan Dose, mg/kg	Standard[a] Reduction, %	LD_{50}, mg/kg
II*a*	27	175	35	237
II*b*	17	200	35	300
II*c*	57	200	35	300
II*d*	16	200	33	300
II*e*	22	200	33	300
II*f*	39	200	31	300
II*g*	20	200	20	300
II*h*	18	200	30	300
II*i*	10	150	31	178
II*j*	0	200	20	300
II*k*	37	200	40	300
II*l*	33	200	40	300
II*m*	19	200	31	300
II*n*	57	150	35	178
II*o*	86	200	33	316
II*p*	17	200	20	300
II*q*	39	200	37	316
II*r*	51	200	37	316
II*s*	2	200	20	300
II*t*	0	200	32	300
II*u*	8	200	24	300
II*v*	0	200	26	300
III*a*	86	200	40	300
III*b*	72	200	28	300
III*c*	77	100	35	133
III*d*	0	200	28	300
III*e*	23	200	28	300
III*f*	52	200	35	300
III*g*	33	200	38	300
III*h*	17	200	24	300
III*i*	36	150	37	178
III*j*	16	200	20	300
III*k*	49	200	43	300
III*l*	15	200	26	300
III*m*	14	200	22	300
III*n*	64	200	25	300
III*o*	57	200	33	300
III*p*	30	150	32	178
III*q*	70	175	25	237
III*r*	50	175	29	237
III*s*	12	200	28	300
III*t*	0	200	28	300
III*u*	22	200	28	300
III*v*	7	200	24	300
IV*a*	96	200	42	300
IV*b*	51	200	39	300
IV*c*	29	200	32	300
IV*d*	0	150	32	178
IV*e*	67	200	44	300
IV*f*	15	200	32	300
IV*g*	7	200	24	300
IV*h*	5	200	31	300
IV*i*	71	200	31	300
IV*j*	43	200	43	300
IV*k*	23	200	31	300
IV*l*	18	200	31	300
V*Ia*	0	200	37	300
V*Ib*	7	200	37	300
V*Ic*	0	200	28	300
V*Id*	0	200	28	300
V*Ie*	0	200	37	300
V*If*	2	200	28	300
V*Ig*	19	200	28	300
V*Ih*	54	70	37	100
VII*a*	17	200	31	316
VII*b*	0	200	31	300
VII*c*	12	200	28	300
VII*d*	0	200	28	300
VII*e*	0	200	31	300
VII*f*	5	200	28	316
VII*g*	46	200	42	300
VII*h*	4	40	37	178
VIII*a*	12	200	27	300
VIII*b*	0	200	27	300
VIII*c*	7	200	27	300
VIII*d*	0	200	42	300
VIII*e*	18	200	27	300
VIII*f*	0	200	32	300
VIII*g*	22	200	27	300
VIII*h*	37	200	37	300
VIII*i*	63	200	37	300

[a] Mean percent reduction in inflammation produced by the standard, indomethacin, at a dose of 2.5 mg/kg. An indomethacin standard was run simultaneously with each set of test compounds.

developed arthritic condition after 20 days when injected into the left hindfoot pad of male Wistar–Lewis rats. The developed arthritis was characterized by swelling of all four paws and secondary involvement of the tail and ears (24). The test compounds were administered by gastric intubation in a methylcellulose vehicle on Days 20 and 22. Both left and right hindpaw volumes were recorded daily on Days 20–24. The decrease in mean paw volume per group per day then was calculated as the percent change from Day 20. Efficacy was determined by a percent decrease in the paw volume of test compounds *versus* that of the standard, indomethacin. The test compounds were administered at a dose of 30 mg/kg, and the standard was given at a dose of 1 mg/kg.

The results of this assay are shown in Table VII. Only II*f* was active in the adjuvant-induced polyarthritis assay. This compound appeared to have a rapid onset of action compared to the standard. However, after several days, the activity level began to fall. Additional evaluation is needed to establish whether II*f* possesses a therapeutic advantage over current standards.

Physicochemical Parameters—Since compounds in each series (Tables I–V) differed from others in that series by only one substituent, hydrophobic substituent constants (π values) (25, 26), electronic substituent constants (σ values) (27), and steric substituent constants (E_s values) (28) were utilized to estimate the relative effect of these physicochemical parameters on anti-inflammatory activity. After detailed investigation, it was concluded that within each series, no apparent structure–activity correlation existed between any of these physicochemical parameters and anti-inflammatory activity. Nevertheless, several general trends deserve comment.

First, at the primary screening level, the 5-substituted triazine classes (II*a*–II*v*, III*a*–III*v*, and IV*a*–IV*l*) were inherently more active than the corresponding 5,6-disubstituted triazines (VI*a*–VI*h*, VII*a*–VII*h*, and VIII*a*–VIII*i*). Over 37% of the compounds in the 5-substituted classes were considered active, while only 16% of the compounds in the 5,6-disubstituted classes were active.

Second, the difluorinated triazines (II*i* and III*i*) were among the most toxic derivatives evaluated in this series. Perhaps the toxicity among these fluorinated derivatives is the result of increased susceptibility to nucleophilic attack by the amino and mercapto functions present in DNA

Table VII—ED_{50} Values in Carrageenan-Induced Anti-Inflammatory Assay, NTD_{50} Values in Mice, and Adjuvant-Induced Polyarthritis Activity of Selected 3-Methylthio- and 3-Alkoxy-1,2,4-triazines

Compound	Carrageenan-Induced Anti-Inflammatory Assay		Adjuvant-Induced Polyarthritis Assay[a], % reduction			
	ED_{50}, mg/kg	NTD_{50}, mg/kg	Day 21	Day 22	Day 23	Day 24
II*c*	30	170	NT[b]	NT	NT	NT
II*f*	10	1000	24	19	14	8
III*a*	10	170	NT	NT	NT	NT
III*b*	30	76	NT	NT	NT	NT
III*f*	100	1000	NT	NT	NT	NT
III*n*	100	100	NT	NT	NT	NT
III*o*	30	1000	5	6	0	NT
IV*a*	10	87	NT	NT	NT	NT
IV*b*	30	300	6	0	0	NT
IV*e*	30	300	0	0	0	NT
IV*i*	30	250	NT	NT	NT	NT
VII*g*	30	250	NT	NT	NT	NT
VIII*i*	30	300	4	0	0	NT
Indomethacin	2.5	112	12	19	18	21

[a] All test compounds were administered at 30 mg/kg, while indomethacin was given at 1 mg/kg. [b] Not tested.

and protein structure (29). It may be that the relatively labile fluorine atoms undergo displacement by these functional groups, resulting in interchelation and overt toxic effects.

EXPERIMENTAL[1]

3-Methoxy-5-substituted-1,2,4-triazines (IIIa–IIIv)—In a 1-liter round-bottom flask equipped with condenser and magnetic stirrer was placed the appropriate 3-methylthio-5-substituted-1,2,4-triazine (IIa–IIv) (13,14) dissolved in methanol (500 ml). To this stirred solution was added a molar excess of sodium methoxide, and the mixture was refluxed for 10 hr. (The methanethiol fumes were trapped by a sodium hydroxide scrubber system.) Upon cooling, the mixture was concentrated and treated with hexane–heptane to afford a crystalline product.

Recrystallization from the appropriate solvents (Table II) resulted in the corresponding 3-methoxy-1,2,4-triazine (IIIa–IIIv). The 3-methoxytriazines were characterized by carbon, hydrogen, and nitrogen analyses (Table II) and NMR spectroscopy. This class of triazines was characterized by the following chemical shifts relative to tetramethylsilane in deuterochloroform: δ 4.0–4.2 (s, 3, OCH_3), 7.0–8.2 (m, C-5 aromatic substituent protons), and 9.6–9.8 (s, 1, C-6 triazine proton).

3-Alkoxy-5-substituted-phenyl-1,2,4-triazines (IVa–IVl)—The 3-alkoxy-5-substituted-phenyl-1,2,4-triazines were synthesized in a similar manner as the 3-methoxy-1,2,4-triazines (IIIa–IIIv), except that a molar excess of the desired sodium alkoxide was reacted with the 3-methylthio-1,2,4-triazine (IIa or IIb) and the mixture was refluxed with dioxane. Crude products were recrystallized from the appropriate solvents (Table III) to yield the corresponding 3-alkoxy-1,2,4-triazine (IVa–IVl). The 3-alkoxytriazines were characterized by carbon, hydrogen, and nitrogen analyses (Table III) and NMR spectroscopy; chemical shifts were similar to the 3-methoxytriazines (IIIa–IIIv), but the methoxy shift was replaced by ethyl, *n*-propyl, *n*-pentyl, and higher homologs (Table III).

3-Methylthio-5,6-disubstituted-1,2,4-triazines (VIa–VIh)—In a 1-liter round-bottom flask equipped with a heating mantle, condenser, and magnetic stirrer was placed the appropriate symmetrical 1,2-dione (Va–Vh) dissolved in 80% ethanol (500 ml). To this stirred solution was added an equal molar solution of methylthiosemicarbazide hydrogen iodide and sodium bicarbonate in 80% ethanol. The mixture was refluxed for 10 hr, cooled to ambient temperature, and diluted with water to afford a solid.

The solid material was collected and recrystallized from the appropriate solvents (Table IV) to yield the desired 3-methylthio-5,6-disubstituted-1,2,4-triazine (VIa–VIh). The 3-methylthiotriazines were characterized by carbon, hydrogen, and nitrogen analyses (Table IV) and NMR spectroscopy. This class of triazines was characterized by the following chemical shifts relative to tetramethylsilane in deuterated dimethyl sulfoxide: δ 2.7–2.9 (s, 3, SCH_3) and 7.0–8.2 (m, C-5 and C-6 aromatic substituents).

3-Methoxy-5,6-disubstituted-1,2,4-triazines (VIIa–VIIh)—The 3-methoxy-5,6-disubstituted-1,2,4-triazines (VIIa–VIIh) were synthesized in the same fashion as the corresponding 3-methoxy-5-substituted triazines (IIIa–IIIv). Recrystallization of the solid product from the appropriate solvents (Table IV) afforded the desired 3-methoxy-5,6-disubstituted-1,2,4-triazines (VIIa–VIIh). The carbon, hydrogen, and nitrogen analyses are shown in Table IV; the NMR spectra were identical to those of the 3-methylthio precursor except that the δ 2.7–2.9 (s, 3, SCH_3) signal was replaced by a δ 4.0–4.2 (s, 3, OCH_3) signal further downfield.

3-Alkoxy-5,6-disubstituted-1,2,4-triazines (VIIIa–VIIIi)—The 3-alkoxy-5,6-disubstituted-1,2,4-triazine analogs (VIIIa–VIIIi) were synthesized by the same procedure as the 3-alkoxy-5-substituted-1,2,4-triazines (IVa–IVl). Recrystallization solvents are listed in Table V. The 3-alkoxytriazines (VIIIa–VIIIi) were characterized by carbon, hydrogen, and nitrogen analyses (Table V) and NMR spectroscopy. The 5,6-diphenyl derivatives (VIIIa–VIIIf) produced chemical shifts similar to those of their common precursor VIa, except that the methylthio singlet (δ 2.7–2.9) was replaced by a variety of alkoxy and aryloxy multiplets. The 5,6-dimethyl derivatives (VIIIg–VIIIi) were characterized by the two methyl singlets with δ 2.6–2.5 (s, 3, C-6 methyl) and 2.3–2.5 (s, 3, C-5 methyl) and a variety of multiplets resulting from substituents at C-3.

Carrageenan-Induced Pedal Edema Assay (15)—Male Sprague–Dawley rats, 100–140 g, were used in the pedal edema assay. Five rats were used in each treatment group, the known standard control group, and the vehicle control edema groups. The first and seventh groups were vehicle controls. Therefore, recalibration of the volume differential meter could be done after every 30 animals. All rats were fasted for 2 hr prior to the test, and water was available *ad libitum*.

The experimental drugs and the standard control were given orally and were dissolved or suspended in 0.25% methylcellulose. The volume given was 0.005 cm^3/g of body weight. The edema control groups were administered the vehicle. One hour after the administration of the test compounds, 0.05 cm^3 of a 1% sterile carrageenan solution was injected into the left hindfoot pad of each rat using a 1-cm^3 Cornwall syringe pipet. Three hours after this injection, the paw volumes of the injected paws were measured by means of mercury displacement on a volume differential meter. The apparatus used was a modification of that described by Adamkiewicz *et al.* (30).

The amount of edema was calculated, and the percent reduction of edema from control values was determined. The mean volume ($\pm SD$) of edema based on 50 determinations was 1.24 $\pm$0.226 cm^3. Compounds that reduced edema to a level less than that of indomethacin (dosed at 2.5 mg/kg) were considered active. The ED_{50} values for indomethacin and active test compounds were estimated according to the method of Litchfield and Wilcoxon (21).

Neurotoxicity Determination, NTD_{50} Values (22)—The mean neurotoxic dose was the dose administered orally or intraperitoneally to mice that caused minimal recognizable neurotoxicity in 50% of the animals tested as determined by the following five end-points.

1. Positional sense test: If the hindleg of a normal mouse is lowered gently over the end of a table, it will be lifted quickly back to a normal position. Neurological deficit was indicated by the inability to correct the abnormal position rapidly.
2. Righting test: If a mouse is placed on its back, it will right itself quickly and assume a normal posture. Neurological deficit was indicated by the inability to correct for the abnormal body posture rapidly.
3. Gait and stance test: Neurological deficit was indicated by a circular or zigzag gait, ataxia, abnormal spread of the legs, abnormal body posture, tremor, hyperactivity, lack of exploratory behavior, somnolence, stupor, catalepsy, *etc.*
4. Muscle tone test: Normal animals have a certain amount of skeletal muscle tone that is apparent to the observer on handling. Neurological deficit was indicated by a loss of skeletal muscle tone characterized by hypotonia or flaccidity.
5. Equilibrium test: If a normal mouse is placed on a narrow edge, such as the rim of a cage, it can maintain its equilibrium and walk along the rim. Neurological deficit was indicated by the inability to do so.

Abnormal neurological status disclosed by any of these five tests was taken as the end-point for the NTD_{50} determination. However, if other side effects (*e.g.*, hematuria and hypernea) consistently appeared at doses lower than those causing neurological deficit, they were taken as the end-point.

Adjuvant-Induced Polyarthritis Assay (23, 24)—Five male Wistar–Lewis rats, ~110 g each, were used in each group. On Day 1, 0.1 cm^3 of 3.5-mg/cm^3 suspension of heat-killed *M. tuberculosis* in mineral oil was injected into the left hindfoot pad of each rat. The animals then were kept in cages, with two or three rats per cage for 20 days; food and water were available *ad libitum*. On Day 20, all animals with developed arthritis, *i.e.*, swelling of all four paws and secondary involvement of the tail and ears, were used in the study.

The test compounds and the standard control were dissolved or suspended in methylcellulose and were given orally at doses of 30 and 1 mg/kg, respectively. The volume given was 0.005 cm^3/g of body weight. The control group was administered the vehicle alone. The animals were administered compounds by gavage on Days 20 and 22. The left hindpaw volumes were recorded daily on Days 20–24. The decrease or increase in mean paw volume per group per day was then calculated as the percent change from Day 20. Efficacy was determined by a percent decrease in paw volume of the test compounds compared to indomethocin.

[1] Elemental analyses were performed by the Analytical Group, Diamond Shamrock Corp., Painesville, Ohio. IR spectra were determined on a Perkin-Elmer model 137 spectrophotometer with sodium chloride optics. NMR spectra were recorded on a Varian A56/60D and a Bruker WH-90 spectrometer. Melting points were determined on a Thomas–Hoover capillary melting apparatus and are uncorrected.

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Nonisothermal Kinetics with Programmed Temperature Steps

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Abstract □ Data required for predicting the stability of an active principle in solution can be obtained by two kinetic methods. With the isothermal method, the degradation rate constants are determined at different temperatures, which are kept constant throughout the experiment. With the nonisothermal method, the temperature is increased with time. This paper describes a nonisothermal kinetic method in which the temperature is increased in consecutive equal steps. The results are compared with those obtained by the conventional isothermal method. The values for the activation energy are approximately the same by both methods. Although the technique of nonisothermal kinetics demands sophisticated equipment and high experimental accuracy, it provides a continuous picture over a wide temperature range.

Keyphrases □ Stability—prediction, nonisothermal kinetic method, programmed temperature steps, comparison with isothermal kinetic method □ Drug degradation kinetics—nonisothermal method, programmed temperature steps, comparison with isothermal kinetic method □ Kinetics, degradation—nonisothermal method, programmed temperature steps, comparison with isothermal kinetic method

Degradation kinetics usually are studied under isothermal conditions by determining the reaction rate constant at different temperatures. These temperatures generally are fairly high and are kept constant throughout the experiment; in nonisothermal kinetic studies, the temperature changes continuously with time. Several such methods have been described, with the essential difference between them being the equation for the time–temperature relationship (1–3).

Calculations using a continuous temperature increase were carried out previously *via* a multistep model (3). Study of an experimental model with a stepwise temperature profile then was desired. This paper describes the application of nonisothermal kinetics in which the temperature is raised discontinuously in consecutive equal steps, whose number and duration are predetermined. The results are compared with those obtained in conventional isothermal kinetic studies.

EXPERIMENTAL

The investigation was carried out using an active principle, a substituted benzazepine, as a 0.2% solution in pH 5 buffer–ethanol (60:40). This pH was chosen to give appreciable degradation in a relatively short time[1].

The solution of the active principle was introduced into ampuls, which then were sealed and immersed in a thermostatically controlled bath[2] of glycerol–water. At predetermined times coinciding with the end of a temperature stage, ampuls were removed to determine the remaining amount of active ingredient. This determination was done by high-performance liquid chromatography (HPLC) or by spectrophotometry after separation by TLC followed by elution.

TLC—Plates precoated with silica gel and an indicator[3] were used as the stationary phase. The mobile phase was chloroform—ethanol–concentrated ammonia (90:10:0.8). The plates were developed to 12 cm. UV detection was performed at 254 nm with the use of Dragendorff's reagent and peroxide for visualization of degradation after elution.

The R_f values were 0.5 for the active principle and 0.4, 0.7, and 0.9 for the degradation products. After development, the spot corresponding to the unchanged active principle was eluted[4] with 2.50 ml of solvent

[1] M. O. Baltzer, unpublished results.
[2] Model FP thermostat, Haake.
[3] G 1500/LS 254, Schleicher and Schuell.
[4] Eluchrom apparatus, Camag.

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