Synthesis and Antiinflammatory Activity of Hexahydrothiopyrano[4,3-*c*]pyrazoles and Related Analogues[1]

George C. Rovnyak,*[2a] Robert C. Millonig,*[2b] Joseph Schwartz,[2a] and Virginia Shu[2a]

Organic Chemistry Department and Pharmacology Department, The Squibb Institute for Medical Research, Princeton, New Jersey 08540. *Received March 22, 1982*

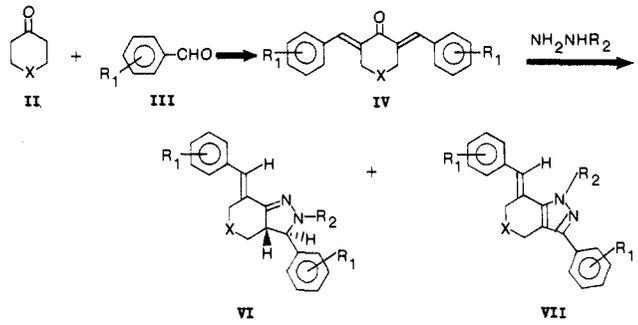
A series of novel hexahydrothiopyrano[4,3-c]pyrazoles and related analogues were prepared and tested for antiinflammatory activity by using the mouse active Arthus reaction and the delayed hypersensitivity skin reaction in guinea pigs as primary screens. The compounds of most interest, **18** and **28**, were further tested in a model of adjuvant-induced arthritis; in this system, both compounds were active when dosed intraperitoneally but failed to produce significant activity when dosed orally at subtoxic doses.

Salicylate preparations and a variety of arylacetic acids, currently used as first-line systemic therapy for inflammatory conditions,[3] all cause varying degrees of gastrointestinal irritation. Although the mechanism by which these agents cause gastrointestinal irritation is more complex than originally thought, it most probably involves the elements of local irritation by high drug concentration as well as inhibition of prostaglandin synthesis.[4,5] In addition, for this class of acidic agents there exists a reasonably good rank-order correlation between prostaglandin synthesis inhibition and their antiinflammatory activity as measured by the carrageenin-induced edema model in the rat hind paw.[5,6] The view has been expressed that the antiinflammatory activity and ulcerogenic side effects of these acidic drugs are intimately related and that any new agent based on this approach (prostaglandin synthesis inhibition, carrageenin edema active) will share the same problem.[7] On the other hand, nonacidic antiinflammatory agents, as a class, are generally much less irritating to the gastrointestinal tract.[8]

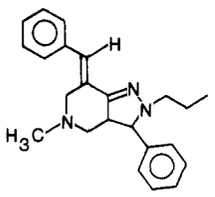
Therefore, we decided to synthesize new, nonacidic compounds and to test these agents for antiinflammatory activity in several immunologically induced models of inflammation that involve some of the histological and biochemical events that are known to occur in various inflammatory conditions in man (for example, polymorphonuclear leukocyte and monocyte infiltration, lysosomal enzyme release, and complement activation). This approach is based on the wealth of evidence that supports the hypothesis that an aberrant immune response is involved in inflammatory conditions such as rheumatoid arthritis.[9]

We chose for screening purposes the mouse active Arthus (MAA) reaction,[10] as an indication of activity against the acute phase of inflammation, and the delayed hypersensitivity skin reaction (DHSR) in guinea pigs,[11] as an indication of activity against the chronic phase of inflammation. In addition, our testing strategy was biased against activity in the carrageenin edema (CE) assay, since this model correlates best, empirically, with the acidic class of antiinflammatory agents, from which we wished to depart.

Scheme I[a]



Others from these laboratories have recently reported[12] that the hexahydropyrazolo[4,3-c]pyridine analogue I



I

possessed antiinflammatory activity comparable to phenylbutazone, as determined in the rat carrageenin edema assay. In addition, this compound was found to possess good activity in the mouse active Arthus (MAA) reaction, although unwanted side effects were observed. Our objective in developing this as an MAA active lead was to prepare analogues that retained good MAA activity, orally, but that possessed little or no CE activity or side effects. We surmised that the basic nitrogen in the six-membered ring of I might be responsible for the CNS effects; thus, we prepared and tested novel thiopyrano[4,3-c]pyrazoles and related analogues of general formula VI. We achieved our objective partially with some of these derivatives, notably **18** and **28**, and these results are the subject of this report.

Chemistry. The general method for preparing the compounds reported here is shown in Scheme I and has

(1) Presented in part at the 17th National Medicinal Chemistry Symposium, Rensselaer Polytechnic Institute, Troy, NY, 1980.
(2) (a) Organic Chemistry Department. (b) Pharmacology Department.
(3) (a) R. P. Evans, *Am. J. Hosp. Pharm.*, **36**, 622 (1979). (b) W. L. Fritz, J. Paxinos, and E. P. Gall, *Drug Ther.*, 8, 36 (1978). (c) W. W. Downie and V. Wright, *Practioner*, **219**, 463 (1977).
(4) (a) K. D. Rainsford, *Drugs Exp. Clin. Res.*, **2**, 121 (1977). (b) K. D. Rainsford *Agents Actions*, 8, 587 (1978).
(5) R. J. Flower, S. Moncada, and J. R. Vane, "The Pharmacological Basis of Therapeutics", 6th ed, A. G. Gilman, L. S. Goodman, and A. Gilman, Eds., New York, 1980, pp. 682–687.
(6) F. A. Kuehl and R. W. Egan, *Science*, **210**, 978 (1980).
(7) K. Brune, M. Glatt, and P. Graf, *Gen. Pharmacol.*, **7**, 27 (1976).
(8) T. Y. Shen, *Drugs Exp. Clin. Res.*, **2**, 1 (1977).
(9) (a) D. A. Willoughby, J. P. Giroud, and G. P. Velo, Eds., "Persepectives in Inflammation—Future Trends and Development", Proceedings of the International Meeting on Future Trends in Inflammation, 3rd, London, Feb 14–18, 1977, University Park Press, Baltimore, 1977. (b) N. J. Zvaifler, *Adv. Immunol.*, **13**, 265 (1973). (c) C. L. Christian and S. A. Paget, "Immunological Diseases", 3rd ed, Vol. II, Max Samter, Ed., 1978, pp 1061–1076.
(10) M. B. Goldlust, T. N. Harrity, and D. M. Palmer, "The Evaluation of Anti-Rheumatic Drugs Using the Cutaneous Arthus Reaction", MTP Press, Lancaster, England, 1978, pp 117–134.
(11) R. J. Wojnar, K. Losee, and R. J. Brittain, *Agents Actions*, **5**, 145 (1975).
(12) J. Krapcho and C. F. Turk, *J. Med. Chem.*, **22**, 207 (1979).

0022-2623/82/1825-1482$01.25/0

been described in detail elsewhere.[13] Briefly, the intermediate ketone II was condensed with 2 equiv of aldehyde III to give the bis(benzylidene) products IV (Table IV). Except as noted in Table IV, the method employing concentrated HCl in ethanol was preferred over the method of Leonard and Choudhury[14] for the preparation of compounds of formula IV. The sulfoxide derivatives **70** and **71** were obtained by sodium metaperiodate oxidation of the corresponding sulfides,[13a] **60** and **63**, respectively. The sulfone analogues **72–78** could be obtained by hydrogen peroxide oxidation either before or after aldol condensation of the aromatic aldehyde with ketone II (X = S or SO_2).

Cyclization of IV with hydrazine or a substituted hydrazine, V,[15] gave the major product VI (Tables I and II), accompanied in some cases by isomeric pyrazole coproducts VII (Table III).[13b]

Biology. Mouse Active Arthus Reaction (MAA).[10] Five weeks prior to challenge, CD-1 mice were sensitized by an intraperitoneal injection of a 1:1 emulsion of Freund's complete adjuvant containing bovine serum albumin (BSA) in saline. At the time of testing, the test compounds were suspended in sterile 1% sodium carboxymethylcellulose in pyrogen-free saline (CMC) and administered in a single intraperitoneal or oral dose 1 h before skin challenge with BSA.

Three and one-half hours after the skin challenge, the animals were sacrificed by exposure to CO_2, and the lesions were cut out, with the actual perimeter of the edema used as a guide. The average lesion weight of the group receiving the test compound was compared with that of the control group, and the percent inhibition by the test compound was calculated and the 50% inhibitory dose determined.

Delayed Hypersensitivity Skin Reaction (DHSR).[11] Male albino guinea pigs, Hartley strain, were sensitized to *Mycobacterium tuberculosis* by the injection of the organism suspended in Freund's incomplete adjuvant into each hind footpad and, subcutaneously, into the back of the neck. Three weeks after sensitization, a skin lesion was produced by the intradermal injection of 1.2 μg of purified protein derivative (PPD). Each test compound, suspended in sesame oil, was administered subcutaneously to three guinea pigs per test group at a dose of 3.1, 6.3, 12.5, or 50.0 mg/kg, 30 min before and 5 h after the injection of the PPD. The activities of the compounds, recorded as the percent inhibition of the average diameter of the erythematous lesion and of the thickness (cellular induration) of the reaction, were measured 24 h after injection of the antigen.

Adjuvant-Induced Arthritis (AA).[16] Adjuvant arthritis was induced in the male Lewis rat. On day 0, 0.50 mg of desiccated *Mycobacterium butyricum* in 0.10 mL of light mineral oil was injected into the plantar surface of the left paw. The test compounds were dissolved or suspended in 1% CMC and administered by the oral route for 21 days, starting on the day prior to adjuvant injection. All animals were sacrificed on day 21. Weights of the injected and noninjected hind paws were measured. The effect of the compounds are reported in terms of its percent inhibition of the locally induced inflammation (injected hind paw) and the systemically induced inflammation (noninjected hind paw).

Carrageenin-Induced Edema (CE).[17] The procedure described by Winter et al. was used. Compounds, dissolved or suspended in 1% CMC, were administered as single oral doses to adult male Charles River Sprague–Dawley (CD) rats, 160–200 g, 2 h prior to injection (footpad) of 0.05 mL of a 1% solution of carrageenin in pyrogen-free saline. Three hours after the injection of carrageenin, the rats were killed and the paws removed and weighed. The contralateral paw served as the control. The average doses of test compound that caused a 50% inhibition of edema (ID_{50}) were determined.

Toxicity. In all test systems, the animals were observed for overt signs of toxicity, such as sedation, hypothermia, and ataxia, although these effects were not quantitated. Selected compounds were examined for acute lethality in mice by the ip and/or po routes, and the results are shown in Table VI.

Discussion

The screening results are given in Tables I–III and show that, generally, these compounds possess more activity in the mouse active Arthus (MAA) model than in the delayed hypersensitivity skin reaction (DHSR).

The results also show that we were largely successful in replacing the NCH_3 group of I by other heteroatom moieties, as the order of activity in the MAA model was generally $SO_2 \simeq NCH_3 > CH_2 > S \simeq O$ (compounds **13**, I, ref 18, **1**, **35**); these and similar comparisons within Table I indicate that the sulfone group seems to be the best substitute for the NCH_3 group of I. Importantly, fewer untoward side effects (such as sedation, hypothermia, and ataxia) were noted in the test animals for the sulfone analogues (SO_2) than for I (and related nitrogen-containing compounds not reported on here).

There appears not to be any significant effect of aromatic substitution on MAA activity (compounds **13–18**). Only two nonbasically substituted compounds, **15** and **18**, possessed significant inhibition in the DHSR model (>40% vs. induration); both of these compounds have electron-donating groups on the aromatic ring.

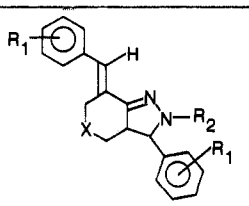
A wide variety of substituents on the dihydropyrazole nitrogen were surveyed; simple alkyl or acyl substitution (**13** or **21**) appears to be optimal for MAA activity. Analogues substituted with basic groups (Table II) generally had greater activity, ip and po, in both the MAA and DHSR models but also showed more side effects when compared with corresponding propyl-substituted analogues (**39** vs. **1**; **45** vs. **13**; **49** vs. **35**). Lethality was observed only for some analogues substituted with basic groups.

The isomeric pyrazole analogues (Table III) possessed activities that were comparable to the corresponding dihydropyrazole analogues (Table I).

Our purposeful departure from the acidic class of nonsteroidal antiinflammatory agents was accompanied, in the present series of compounds, by a general problem of low activity upon oral administration. We prepared a number of structurally modified analogues in an attempt to address this problem. Replacement of the *N*-alkyl substituent by various functional groups failed to achieve the desired effect (e.g., alcohol **8** and acetylenes **22** and **27** had poor activity in the MAA assay, parenterally). The basically

(13) (a) M. S. Puar, G. C. Rovnyak, A. I. Cohen, B. Toeplitz, and J. Z. Gougoutas, *J. Org. Chem.*, **44**, 2513 (1979). (b) G. Rovnyak and V. Shu, *J. Org. Chem.*, **44**, 2518 (1979).
(14) N. J. Leonard and D. Choudhury, *J. Am. Soc.*, **79**, 156 (1957).
(15) The substituted hydrazines were either prepared [see, for example, *Chem. Abstr.*, *59*, 3742*f* (1963); *J. Med. Chem.*, **16**, 302 (1973), and *Can. J. Chem.*, **47**, 1999 (1969)] or were purchased from Aldrich Chemical Co. if available.
(16) M. Rosenthale and C. Nagra, *Proc. Soc. Exp. Biol. Med.*, **125**, 149 (1967).
(17) C. A. Winter, E. A. Risley, and G. W. Nuss, *Proc. Soc. Exp. Biol. Med.*, 111, 544 (1962).
(18) Reference 12; general formula VI, X = CH_2, R′ = CH_3; MAA rating = 2 (ip at 150 mg/kg).

Table I. Nonbasic Side Chain



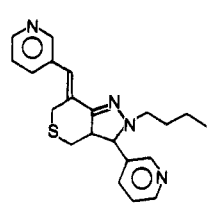
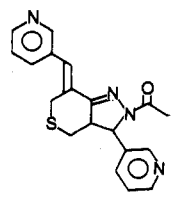
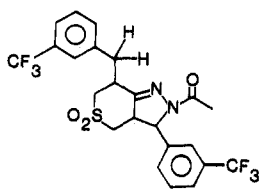
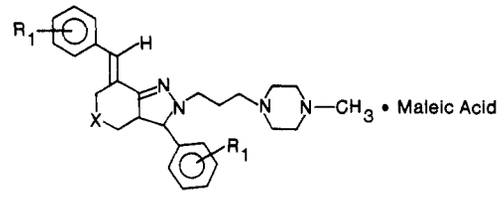
no.	X	R_1	R_2	mp, °C	yield,[a,b] %	ref or recrystn solvent[c]	biol act. MAA[l]	biol act. DHSR[m] (sc)
I	NCH_3	H	n-C_3H_7	106-109	53	12	ip 3	5/18
1	S	H	n-C_3H_7	119.5-122	28	13a	ip 1	1/0
2	S	4-OCH_3	n-C_3H_7	149.5-151.5	35	13b	ip 3, po 0	12/45
3	S	4-Cl	n-C_3H_7	118-122	41	13b	ip 1, po 1	14/36
4	S	3-CF_3	n-C_3H_7	90-93	60	A	ip 2	12/21
5	S	2-CH_3	n-C_3H_7	110-112.5	30	A	ip 2	8/19
6	S	4-CH_3	n-C_3H_7	89-91.5	45	13b	ip 2, po 0	11/33
7	S	H	H	149-155	81	B	ip 3	23/37
8	S	H	CH_2CH_2OH	126-129	54	C	ip 1	4/6
9	S	4-SCH_3	$C(O)CH_3$	183.5-185	56	D[d]	ip 2	2/0
10	SO	H	n-C_3H_7 (isomer A)	176.5-178	31	13a	ip 1	
11	SO	H	n-C_3H_7 (isomer B)	164-166	9	13a	ip 3	
12	SO	4-OCH_3	n-C_3H_7 (isomer A)	196-198	28	E	ip 3	4/12
13	SO_2	H	n-C_3H_7	197-199	40	13a	ip 3, po 1	3/10
14	SO_2	4-Cl	n-C_3H_7	208-210	60	C	ip 3	24/32
15	SO_2	4-OCH_3	n-C_3H_7	195-198	50	C	ip 2, po 0	17/49
16	SO_2	3-CF_3	n-C_3H_7	190-191	73	F	ip 3	8/11
17	SO_2	2-CH_3	n-C_3H_7	170-172	35	C	ip 1, po 2	7/17
18	SO_2	4-CH_3	n-C_3H_7	221-224	57	13b	ip 3 (140), po (>600)	13/52
19	SO_2	4-$C(CH_3)_3$	n-C_3H_7	153-155	49	G	po 1[n]	7/1
20	SO_2	H	H	202-208 dec	90	H	ip 3	3/4
21	SO_2	H	$C(O)CH_3$	218-219.5	92	D[e]	ip 3	8/21
22	SO_2	H	$CH_2C{\equiv}CH$	87-93	48	f	ip 0	4/0
23	SO_2	H	$C(=O)C_6H_5$	272-274	36	I[g]	ip 4, po 1	
24	SO_2	H	$C(=O)C_6H_4Cl$-p	249.5-252	32	I[h]	ip 2	1/20
25	SO_2	3-CF_3	CH_3	211-212	84	C	ip 3	4/0
26	SO_2	3-CF_3	i-C_3H_7	196-198.5	38	13b	ip 2	4/22
27	SO_2	3-CF_3	$CH_2C{\equiv}CH$	148.5-149.5	64	C	ip 2	8/1
28	SO_2	3-CF_3	$C(O)CH_3$	220-222.5	69	D[i]	ip 3 (112), po 1 (>600)	6/15
29	SO_2	4-CH_3	CH_2CF_3	211-213	19	13b	ip 3, po 0[n]	14/11
30	SO_2	4-CH_3	$CH_2C_3H_5$	209-211	68	E	po 0[n]	15/7
31	SO_2	4-CH_3	$CH_2CH_2C_6H_5$	204-206	71	E	po 1[n]	10/0
32	SO_2	4-$C(CH_3)_3$	$CH_2C_3H_5$	148-150	39	G	po 1[n]	15/0
33	SO_2	4-$C(CH_3)_3$	$CH_2CH_2C_6H_5$	178-180	39	E	po (>600)	13/4
34	SO_2	4-Cl	C_6H_5	246.5-249	67	C	po 0	0/8
35	O	H	n-C_3H_7	113.5-115	35	13b	ip 1	9/30
36				125-128	34	J	ip 0	6/12
37				202-203	53	I[j]	ip 0	9/24
38				196-200	68	I[k]	po 2	

Table I (*Continued*)

[a] Yields based on bisaldol ketone 4. [b] All new compounds gave microanalyses that were within 0.3% of theory and infrared and ^{1}H NMR spectra that were in agreement with the assigned structures. [c] Recrystallization solvents: A, methanol-water; B, ethanol, precipitates from reaction mixture; C, acetone-hexane; D, acetic acid-water; E, acetonitrile; F, methanol; G, ethanol; H, methanol, precipitates from reaction mixture; I, chloroform-hexane; J, ethyl acetate-hexane. [d] Prepared by hydrazine ring closure with bisaldol intermediate (product, mp 169-170 °C), followed by acetylation with acetic anhydride in acetic acid. [e] Prepared from compound 20 with acetic anhydride in acetic acid. [f] Chromatography on Woelm neutral alumina I, ether elution. [g] Prepared from compound 20 and benzoyl chloride in benzene. [h] Prepared from compound 20 and *p*-chlorobenzoyl chloride in tetrahydrofuran-benzene. [i] Prepared by hydrazine ring closure with bisaldol intermediate (product, mp 183-185 °C), followed by acetylation with acetic anhydride in acetic acid. [j] Prepared by hydrazine ring closure with bisaldol intermediate (product, mp 146-151 °C), followed by acetylation with acetic anhydride in acetic acid. [k] Prepared from compound 28 by catalytic hydrogenation in ethanol-ethyl acetate using 10% Pd/C. [l] Compounds evaluated in MAA at screening doses of 150 (ip) and 300 mg/kg (po); activity reported on scale of 0 = <20%; 1 = 21-30%; 2 = 31-40%; 3 = 41-60%; 4 = >60% inhibition. ID_{50} (50% inhibitory dose), in parentheses, recorded in milligrams per kilogram. [m] Compounds evaluated in DHSR at two screening doses of 50 mg/kg (sc); activity reported as percent inhibition of erythema/induration of lesion. [n] Screening dose of 150 mg/kg (po).

Table II. Basic Side Chain



no.	X	R_1	mp, [c] °C	yield, [a,b] %	recrystn solvent [d]	biol act. MAA [h]	biol act. DHSR [i] (sc)
39	S	H	179-180 (202-203)	82	A	ip 3	21/6 3
40	S	H (free base)	90.5-92.5	68 [e]	B	ip 3, po 1	11/66
41	S	4-CH_3	174-176	71	A	ip 3 (58), po 2	16/52
42	S	4-OCH_3	170.5-172 (210-212)	73	A	ip 4 (60), po 3	14/34
43	S	2-CH_3	168.5-170 (212-214)	60	A, C	ip 4 (30), po 3 (225)	24/41
44	S	4-S(→O)CH_3	173-175 (194-196)	66	A	ip 3 (190), po 2	11/27
45	SO_2	H	192-195 (197-199)	71	A	ip 4, po 2	7/6
46	SO_2	4-OCH_3	173.5-175 (220-221)	45	A	ip 4 (30), po 1	18/50
47	SO_2	4-OCH_3 (free base)	140-142	88 [f]	D	ip 2 [j]	10/8
48	SO_2	2-CH_3	165.5-167.5 (220.5-221.5)	30	A	ip 4 (<37.5)	14/42
49	O	H	173-175 (192-194)	60	A	ip 4 [k]	9/32
50	O	H (free base)	101-104	16 [g]	E	ip (lethal)	22/51
51	O	4-S(→O)CH_3	172-174 (177-181)	47	A	ip 0	0/0

[a] Yields based on bisaldol ketone 4; purified via the dioxalic acid salt, subsequently converted to free base and dimaleic acid salt (see Experimental Section for general method). [b] All new compounds gave microanalyses that were within 0.3% of theory and IR and ^{1}H NMR spectra that were in agreement with the assigned structures. [c] Melting point of the dioxalic acid salt in parentheses. [d] Recrystallization solvent: A, formation in acetonitrile; B, ether-hexane; C, dimethylformamide-acetonitrile; D, acetone-hexane; E, ether. [e] Prepared from compound 39. [f] Prepared from compound 46. [g] Major portion contained in mother liquor and used for conversion to dimaleic acid salt 49. [h] Compounds evaluated in MAA at screening doses of 150 (ip) and 300 mg/kg (po); activity reported on scale of 0 = <20%; 1 = 21-30%; 2 = 31-40%; 3 = 41-60%; 4 = >60% inhibition. ID_{50} (50% inhibitory dose), in parentheses, recorded in milligrams per kilogram. [i] Compounds evaluated in DHSR at two screening doses of 50 mg/kg (sc); activity reported as percent inhibition of erythema/induration of lesion. [j] Screening dose of 75 mg/kg (ip). [k] Screening dose of 50 mg/kg (ip).

substituted analogues **39–51** had good MAA activity, orally; however, the test animals exhibited lethal, as well as nonlethal, side effects (see Table VI for a comparison of acute LD_{50} values in mice for selected compounds). In addition, replacement of the phenyl ring by a basic pyridyl ring (compounds **36** and **37**) gave analogues with poor MAA activity, parenterally.

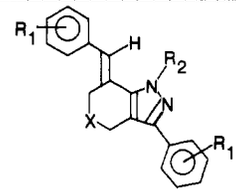
Based on the screening data, two of these compounds, **18** and **28**, were tested in the adjuvant-induced arthritis (AA) model and were compared with more classical anti-inflammatory agents in the carrageenin edema (CE) model. Compound **18** was chosen for its dual activities in both the MAA and DHSR tests. Compound **28**, on the other hand, was active in the MAA test but inactive in the DHSR test. Neither compound showed evidence of side effects in the screening tests. Results are given in Table V.

Neither **18** nor **28** possessed potent activity in the carrageenin-induced edema model (see Table V). This was a favorable finding from our point of view, since this model correlates best with the acidic class of nonsteroidal anti-inflammatory agents, and it was our intention from the inception of this project to hold a bias against such activity.

Despite the generally poor oral activity of compounds **18** and **28** in the MAA model, dosed acutely, we hoped for a better cumulative effect when tested in the chronically dosed rat adjuvant-induced arthritis model. Both **18** and **28** were effective against the development of systemic lesions in adjuvant-induced arthritis when given intraperitoneally; however, compound **18** was ineffective orally up to 150 mg/kg, and compound **28** demonstrated oral activity only at or near toxic dose levels (150–200 mg/kg). Several rats that were dosed orally with **28** at 150 mg/kg were sacrificed in poor condition; upon examination, gastrointestinal erosions were observed.

The evidence in three models (MAA, CE, AA) and in two species (rats and mice) shows that these compounds possess significantly better activity when dosed intraperitoneally than when dosed orally. These observations have

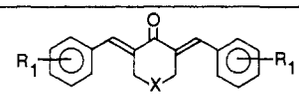
Table III. Isomeric Pyrazoles[a,b]

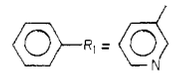


no.	X	R_1	R_2	mp, °C	yield, %	biol. act. MAA[c]	biol. act. DHSR[d] (sc)
52	S	H	n-C_3H_7	115.5-117.5	4	not tested	
53	S	4-Cl	n-C_3H_7	192-194.5	9	ip 3	
54	S	4-OCH_3	n-C_3H_7	131.5-133.5	10	ip 2	15/42
55	S	4-CH_3	n-C_3H_7	140-143	9	ip 2	15/21
56	SO_2	4-CH_3	n-C_3H_7	235-238	3	ip 1[e]	14/25
57	SO_2	4-CH_3	CH_2CF_3	190-192	26	ip 4,[e] po 0[f]	19/23
58	SO_2	3-CF_3	i-C_3H_7	170-172	21	ip 1	5/2
59	O	H	n-C_3H_7	126.5-128.5	10	ip 2	

[a] Obtained as coproducts with corresponding (same X, R_1 and R_2) dihydropyrazole compounds in Table I. [b] Reference 13b. [c] Compounds evaluated in MAA at screening doses of 150 (ip) and 300 mg/kg (po); activity reported on scale of 0 = <20%; 1 = 21-30%; 2 = 31-40%; 3 = 41-60%; 4 = >60% inhibition. [d] Compounds evaluated in DHSR at two screening doses of 50 mg/kg (SC); activity reported as percent inhibition of erythema/induration of lesion. [e] Screening dose of 75 mg/kg (ip). [f] Screening dose of 150 mg/kg (po).

Table IV. Tetrahydro-3,5-bis(phenylmethylene)-4*H*-thiopyran-4-one Derivatives **60-80** (Intermediates Used in the Preparation of Compounds 1-59)[a]



no.	X	R_1	mp, °C	yield, %	solvent of recrystn	ref	formula[b]
60	S	H	150-151	93	EtOH	13b	
61	S	4-Cl	163-165	64	$CHCl_3$/EtOH	13b	
62	S	3,4-Cl_2	151-152.5	68	$CHCl_3$/EtOH	13b	
63	S	4-OCH_3	182.5-184.5	66	EtOH	13b	
64	S	4-CH_3	197.5-200.5	75	$CHCl_3$/EtOH	13b	
65	S	3-CF_3	98-100	62	EtOH	13b	
66	S	2-CH_3	122-124	84	EtOH		$C_{21}H_{20}OS$
67	S	4-SCH_3	205.5-207	69	$CHCl_3$/EtOH		$C_{21}H_{20}OS_3$
68	S	4-S(→O)CH_3	231-235	50[c]	EtOH		$C_{21}H_{20}O_3S_3$
69	S		168-170	71[c]	EtOH		$C_{17}H_{14}N_2OS$
70	SO	H	155-160	89[d]	MeOH	13a	
71	SO	4-OCH_3	192-193.5	73[e]	Me_2CO/MeCN		$C_{21}H_{20}O_4S$
72	SO_2	H	198-200	82	EtOH	13a	
73	SO_2	4-CH_3	198-200	82	EtOH	13b	
74	SO_2	3-CF_3	154.5-155.5	55	$CHCl_3$/Hex	13b	
75	SO_2	4-Cl	210.5-212	77[f]	Me_2CO/Hex		$C_{19}H_{14}Cl_2O_3S$
76	SO_2	4-OCH_3	191-192	60[g]	Me_2CO/Hex	13b	
77	SO_2	2-CH_3	180-181.5	74[h]	Me_2CO/Hex		$C_{21}H_{20}O_3S$
78	SO_2	4-C(CH_3)$_3$	230-233	85	EtOH		$C_{27}H_{32}O_3S$
79	O	H	185-187	52	$CHCl_3$/EtOH	13b	
80	O	4-S(→O)CH_3	225-227	43[c]	$CHCl_3$/EtOH		$C_{21}H_{20}O_4S_2$

[a] General method of preparation given under Experimental Section. [b] New compounds, noted by formula, gave analyses that were within 0.3% of theory. [c] Used piperidine/acetic acid (2:1, v/v) as catalyst. [d] Prepared from compound **60** using sodium metaperiodate in aqueous acetone. [e] Prepared from compound 63 as in footnote *d*. [f] Prepared from compound 61 using 30% H_2O_2 in glacial acetic acid (steam bath) for 15-30 min. [g] Prepared from compound **66** as in footnote *f*.

led us to conclude that these compounds are poorly absorbed from the gastrointestinal tract.

Conclusions

Generally, this series of compounds possesses potent activity in the mouse active Arthus and the adjuvant-induced arthritis reactions when administered by the intraperitoneal route. Some of these compounds (**15**, **18**, **39**, **40**, **41**, **46**, and **50**) also provided significant inhibition of the delayed hypersensitivity skin reaction (primarily the induration phase) when administered by the subcutaneous route.

However, these compounds possessed little or no activity in these systems when administered by the oral route; poor absorption from the gastrointestinal tract is the probable cause for this lack of oral activity.

Compound **28**, administered orally, did produce significant inhibition of the systemic lesions in the adjuvant-induced arthritis model, but this activity occurs at or near toxic dose levels (gross and histological observations indicate lethality is due to gastrointestinal perforations).

We were, thus, successful in achieving several of our objectives: (1) replacement of the NCH_3 group of I by other heteroatoms with retention of MAA and/or DHSR

Table V

no.	dose, mg/kg	local/systemic, % inhibn ip	po
A. Adjuvant-Induced Arthritis			
18	75	28/78	1/0
	150	16/72	17/0
28	75	22/78	0/35
	150	31/97	7/60[a]

no.	ID_{50}, mg/kg ip	po
B. Carrageenin-Induced Edema		
18	84	250
28	>150 (30%)	>200 (37%)

[a] Lethality was observed at this po dose; death was due to gastrointestinal perforations.

activity, (2) reduction of side effects observed in test animals, such as sedation, hypothermia, and ataxia, and (3) low activity in the carrageenin edema assay. However, we were unable to obtain a compound from this series that provided good activity after oral administration of nontoxic doses.

Experimental Section

Melting points were taken on a Thomas-Hoover capillary melting point apparatus and are uncorrected. Proton NMR spectra were obtained on a Perkin-Elmer PE R12B spectrometer operating at 60 MHz and on a Varian T-60 spectrometer, using Me_4Si as an internal standard; Chemical shifts are reported on the δ scale. infrared spectra were obtained on a Perkin-Elmer Model 621 or Infracord spectrometer. All new compounds gave elemental analyses that were within 0.3% of the calculated values.

General Procedure for Preparation of 3,5-Bis(phenylmethylene)-4*H*-thiopyran-4-one Derivatives (60–80, Table IV). A solution of the cyclic ketone II (0.1 mol) and the aromatic aldehyde III (0.2 mol) in 75 mL of ethanol was treated with 6 mL of concentrated hydrochloric acid and heated on a steam bath for several hours. After the solution was cooled, the product was collected and washed (or recrystallized). Additional product can be obtained by concentrating the filtrate, adding another 3–5 mL of concentrated hydrochloric acid, and continued heating. Examples prepared are listed in Table IV. ^1H NMR ($CDCl_3$): the methylene (CH_2X) protons appear as singlets at approximate δ values of 3.9 (X = S), 4.2–4.5 (X = SO), 4.3–4.5 (X = SO_2), 4.95 (X = O), and 4.2 (X = NCH_3). The =CH protons exhibit a broad singlet between δ 7.7 and 8.1. The proton spectra of compounds **60**, **70**, and **72** are described in detail in ref 13a. IR ($CHCl_3$ or Nujol): distinctive absorptions occur in two regions for the α,β-unsaturated carbonyl system at 1650–1680 (C=O) and 1580–1640 (C=C) cm^{-1}. See ref 13 for specific absorptions on some of these compounds.

General Procedure for Preparation of Hexahydrothiopyrano[4,3-*c*]pyrazoles (1–38, Table I, and 52–59, Table III). Equimolar amounts of ketone IV (from Table IV) and hydrazine or a substituted hydrazine[15] in methanol are heated at reflux temperature for 0.5–7 h. After the solution is cooled, the major products of formula VI are obtained. In some cases, pyrazole coproducts VII are also isolated.[13b] Additional data for each product are listed in Tables I and III. ^1H NMR ($CDCl_3$): detailed examination[13a] of compounds **1**, **10**, **11**, and **13** revealed a trans diaxial configuration for the bridgehead and benzyl methine protons; also, the benzylidene (=CH) proton for compounds in Tables I and III has been shown to be cis to the dihydropyrazole (or pyrazole) ring (general formulas VI and VII). Reference 13b describes NMR assignments that distinguishes pyrazole coproducts (Table III) from dihydropyrazole products (Table I). IR (KBr): the conjugated (C=CC=N) system showed the expected absorptions in the 1540–1615 cm^{-1} region. Other absorptions, such as for SO_2 (e.g., 1115, 1315 cm^{-1}) and SO (ca. 1030 cm^{-1}), were consistent with the assigned structures.

Preparation of 2-Acetyl-2,3,3a,4,6,7-hexahydro-3-[4-(methylthio)phenyl]-7-[[4-(methylthio)phenyl]methylene]-thiopyrano[4,3-*c*]pyrazole (9). A mixture of 5.7 g (14.8 mmol) of ketone **67** and 525 mg (16.4 mmol) of anhydrous hydrazine in 200 mL of EtOH/$CHCl_3$ (2:1) was heated at reflux temperature for 1 h. After the mixture was cooled, there was obtained 6 g of crude product; recrystallization from $CHCl_3$/EtOH gave 3.8 g, mp 169–170 °C. This hydrazine cyclization product was acetylated with 5 mL of Ac_2O in 75 mL of HOAc at reflux temperature for 0.5 h. Water (5–10 mL) was added, and the mixture was cooled to 5 °C. The product was collected and washed with HOAc and H_2O to give 3.5 g: mp 183.5–185 °C; IR (KBr) 1595, 1620 (C=CC=N), 1672 cm^{-1} (C=O); NMR ($CDCl_3$) δ 2.35 (s, 3 H, CH_3), 2.46 (s, 6 H, SCH_3), 3.02 (s, 2 H, CH_2), 3.68 (br s, 2 H, CH_2), 4.88 (d, 1 H, J = 8 Hz, CH).

Preparation of 2-Acetyl-2,3,3a,4,6,7-hexahydro-3-phenyl-7-(phenylmethylene)thiopyrano[4,3-*c*]pyrazole 5,5-Dioxide (21). A mixture of 1.6 g (4.73 mmol) of compound **20** in 30 mL of HOAc and 2 mL of Ac_2O was heated on a steam bath for 15 min. Water (50 mL) was added, and the solution was allowed to cool to room temperature. The product was collected, washed with H_2O, and dried in vacuo over P_2O_5 at 50 °C to give 1.65 g of product: mp 218–219.5 °C; IR (KBr), 1570, 1595, 1620 (C=CC=N), 1670 (C=O), 1110, 1310 (SO_2) cm^{-1}; NMR ($CDCl_3$) δ 2.32 (s, 3 H, CH_3), 3.42 (s, 2 H, CH_2), 4.12 (s, 2 H, CH_2), 5.05 (d, 1 H, J = 8.4 Hz, CH), 7.56 (s, 1 H, =CH).

Preparation of 2-Benzoyl-2,3,4a,4,6,7-hexahydro-3-phenyl-7-(phenylmethylene)thiopyrano[4,3-*c*]pyrazole 5,5-Dioxide (23). A mixture of 3.4 g (10 mmol) of compound **20** and 1.7 g (12 mmol) of benzoyl chloride in dry benzene was heated at reflux temperature for 2 h. After the solution was cooled, the crude product was collected and recrystallized from $CHCl_3$/hexane to give 1.6 g of product: mp 272–274 °C; IR (KBr) 1565, 1590 (C=CC=N), 1650 (C=O), 1105, 1305 (SO_2) cm^{-1}.

Preparation of 2-(4-Chlorobenzoyl)-2,3,3a,4,6,7-hexahydro-3-phenyl-7-(phenylmethylene)thiopyrano[4,3-*c*]-pyrazole 5,5-Dioxide (24). A mixture of 5.1 g (15 mmol) of compound **20** and 3.2 g (18 mmol) of *p*-chlorobenzoyl chloride in 200 mL of dry THF/benzene (1:1) was heated at reflux temperature for 2 h. Solvent was removed in vacuo, and the residue was triturated with hexane. The crude product was recrystallized from $CHCl_3$/hexane to give 2.3 g of product: mp 249.5–252 °C; IR (KBr) 1570, 1580, 1595 (C=CC=N), 1655 (C=O), 1110, 1310 (SO_2) cm^{-1}; NMR ($CDCl_3$) δ 3.5 (s, 2 H, CH_2), 4.16 (br d, J = 4.8

Table VI. Therapeutic Ratio of Selected Test Compounds as Compared to Those of Selected Reference Agents

compd	MAA: ID_{50}, mg/kg ip	po	mouse LD_{50}, mg/kg ip	po	therapeutic ratio ip	po
18	140	>600	3400	>4000	24	NC[a]
28	112	>600	>3200	>7200	>29	NC[a]
43	30[b]	225	170	>1200	5.7	>5.3
46	29[b]	234	280	>1200	9.6	>5.1
48	<37.5[c]		130		<3.5	
naproxen	140	411		1200		3.0
indomethacin		517		20		0.04
colchicine	2.4	13.2	3.5	13.5	1.5	1.0
ibuprofen		494		1650		3.4

[a] NC = not calculated. [b] Side effects observed. [c] Lethality observed.

Hz, 2 H, CH_2), 5.31 (d, J = 9.6 Hz, 1 H, CH).

Preparation of 2-Acetyl-2,3,3a,4,6,7-hexahydro-3-[3-(trifluoromethyl)phenyl]-7-[[3-(trifluoromethyl)phenyl]methylene]thiopyrano[4,3-*c*]pyrazole 5,5-Dioxide (28). A mixture of 1.9 g (4.23 mmol) of ketone **74** and 170 mg (5.3 mmol) of anhydrous hydrazine in 100 mL of MeOH was heated at reflux temperature for 0.5 h. Water (20 mL) was added, and the mixture was allowed to cool to 5 °C. Product was collected and dried in vacuo over P_2O_5 at 50 °C to give 1.6 g, mp 183–185 °C.

This hydrazine-cyclized product in 25 mL of HOAc and 2 mL of Ac_2O was heated on a steam bath for 15 min. Water (10 mL) was added, and the mixture was allowed to cool to room temperature. The product was collected, washed with HOAc/H_2O (3:2) and H_2O, and dried in vacuo over P_2O_5 at 50 °C to give 1.56 g: mp 200–202.5 °C; IR (KBr) 1675 (C=O), 1110, 1310 (SO_2) cm^{-1}; NMR ($CDCl_3$) δ 2.39 (s, 3 H, CH_3), 3.53 (s, 2 H, CH_2), 4.14 (s, 2 H, CH_2), 5.12 (d, J = 7.8 Hz, 1 H, CH).

Preparation of 2-Acetyl-2,3,3a,4,6,7-hexahydro-3-(3-pyridinyl)-7-[(3-pyridinyl)methylene]thiopyrano[4,3-*c*]pyrazole (37). A solution 2.94 g (10 mmol) of ketone **69** and 480 mg (15 mmol) of anhydrous hydrazine in 100 mL of $CHCl_3$/MeOH (1:3) was concentrated on a steam bath for 1 h while MeOH was added periodically to maintain volume. After the solution was cooled, the solids were collected and washed with MeOH to give 2.8 g, mp 146–151 °C.

The above hydrazine-cyclized product in 20 mL of HOAc and 2 mL of Ac_2O was heated on a steam bath for 20 min. Water was added, and solvent was removed in vacuo. The residue was crystallized from $CHCl_3$/hexane to give 1.8 g of product: mp 202–203 °C; IR (KBr), 1565, 1580, 1595 (C=CC=N), 1675 (C=O) cm^{-1}; NMR ($CDCl_3$), δ 2.34 (s, 3 H, CH_3), 4.94 (d, J = 9 Hz, 1 H, CH).

Preparation of 2-Acetyl-2,3,3a,4,6,7-hexahydro-3-[3-(trifluoromethyl)phenyl]-7-[[3-(trifluoromethyl)phenyl]methyl]thiopyrano[4,3-*c*]pyrazole 5,5-Dioxide (38). A solution of 1.0 g (1.94 mmol) of compound **28** in 50 mL of EtOH containing 0.5 g of 10% Pd/C was hydrogenated at atmospheric pressure until uptake ceased (52 mL). The catalyst was filtered, and the filtrate was concentrated in vacuo. Chromatography on Baker silica gel and elution with hexane/$CHCl_3$ (100:0 to 0:100) and then MeOH/$CHCl_3$ (5:95) afforded the product, which was recrystallized twice from $CHCl_3$/hexane to give 685 mg of product: mp 196–200 °C; IR (KBr), 1680 (C=O), 1125, 1335, (SO_2) cm^{-1}; NMR ($CDCl_3$) δ 2.35 (s, 3 H, CH_3), 5.0–5.25 (m, 1 H, CH).

General Procedure for Preparation of [3-(*N*-Methylpiperazino)propyl]hexahydrothiopyrano[4,3-*c*]pyrazoles (39-51, Table II). A mixture of 1.0 equiv of ketone IV and 1.1 equiv of 3-(*N*-methylpiperazino)propylhydrazine in chloroform/methanol (1:4) is heated at reflux temperature for 3–6 h. Solvent is removed in vacuo, and the oily residue, dissolved in acetonitrile, is treated with 2.0 equiv of oxalic acid in acetonitrile. After the solution is stirred at room temperature for 0.5 h, the dioxalate salt is collected and, if necessary, recrystallized from dimethylformamide/acetonitrile.

The dioxalate salt is suspended in a mixture of water and chloroform and made basic with solid potassium carbonate. The organic phase is separated, washed with water, dried over anhydrous magnesium sulfate, and concentrated in vacuo. In three cases (**40**, **47**, and **50**), the free base was obtained in crystalline form. The free base, dissolved in acetonitrile, is treated with 2 equiv of maleic acid in acetonitrile to obtain the dimaleate salt (additional data in Table II): NMR (maleate salts, Me_2SO-d_6) δ 7.0–7.2 (s, =CH), 2.60–2.65 (s, NCH_3); aromatic substituent 2.32 (**41**, s, CH_3), 3.75 (**42**, s, OCH_3), 2.28, 2.35 (**43**, s, CH_3), 2.95 (**44**, s, S-CH_3), 3.76 (**46**, s, OCH_3), 2.28, 2.33 (**48**, s, CH_3), 2.74 (**51**, s, S-CH_3); other absorption are complex and not resolved.

Acknowledgment. The authors thank Dr. A. I. Cohen, Dr. M. Puar, and Ms. Mary Young and their associates for the NMR; IR, and microanalytical data. We also thank Dr. M. B. Goldlust and his associates for the mouse active Arthus data and Dr. R. J. Wojnar and his associates for the delayed hypersensitivity skin reaction data. Dr. B. R. Vogt provided helpful guidance and discussion throughout this project.

Selenium Labeling in Nuclear Medicine. 2.[1] D Ring Substituted Estrogens

Samy A. Sadek,[2a,b] Wayne V. Kessler,[2a] Stanley M. Shaw,[2a] John N. Anderson,[2c] and Gordon C. Wolf*[2a,d]

Department of Bionucleonics and School of Health Sciences, Department of Biological Sciences, and Department of Medicinal Chemistry and Pharmacognosy, Purdue University, West Lafayette, Indiana 47907. *Received March 8, 1982*

A series of seven D ring substituted selenium derivatives of estrone/estradiol has been prepared and characterized. Competitive binding studies using the rat uterine cytosol assay are reported for each compound. The most promising agent currently appears to be 17α-[(methylseleno)ethynyl]-17β-estradiol, which possesses 19% of the binding affinity of natural 17β-estradiol.

A radiopharmaceutical for imaging breast tumors would be of immense value in diagnostic nuclear medicine;[3] to date no satisfactory agent is available, though extensive efforts are being expended to this end.[4-7] In considering the rational development of a γ-emitting estrogen, one must examine the biological and chemical consequences of introducing a label. While radioactive halogens (^{123}I or ^{77}Br) offer certain advantages (ease of preparation and desirable radionuclide characteristics), these nuclides also exhibit disadvantages, such as biological instability and short half-lives, which allow little time for synthetic manipulations.

(1) A previous publication has been designated Part 1 in this series: Sadek, S. A.; Shaw, S. M.; Kessler, W. V.; Wolf, G. C. *J. Org. Chem.* **1981**, *46*, 3259.
(2) (a) Department of Bionucleonics and School of Health Sciences. (b) The current work is taken in part from the Ph.D. Thesis of S. A. S., Purdue University, 1982. (c) Department of Biological Sciences. (d) Department of Medicinal Chemistry and Pharmacognosy.
(3) Krohn, K. A. *J. Nucl. Med.* **1980**, *21*, 593.
(4) Longcope, C.; Arunachalam, T.; Rafkin, I.; Caspi, E. *J. Steroid Biochem.* **1981**, *14*, 261.
(5) Gatley, S. J.; Shaughnessy, W. J.; Inhorn, L.; Lieberman, L. M. *J. Nucl. Med.* **1981**, *22*, 459.
(6) Katzenellenbogen, J. A.; Senderoff, S. G.; McElvany, K. D.; O'Brien, H. A., Jr.; Welch, M. J. *J. Nucl. Med.* **1981**, *22*, 42.
(7) Heiman, D. F.; Senderoff, S. G.; Katzenellenbogen, J. A.; Neeley, R. J. *J. Med. Chem.* **1980**, *23*, 994.

0022-2623/82/1825-1488$01.25/0