Synthesis and Anti-inflammatory Activity of Fluorinated Phenyl Styryl Ketones and *N*-Phenyl-5-substituted Aryl-3-*p*-(fluorophenyl) Pyrazolins and Pyrazoles

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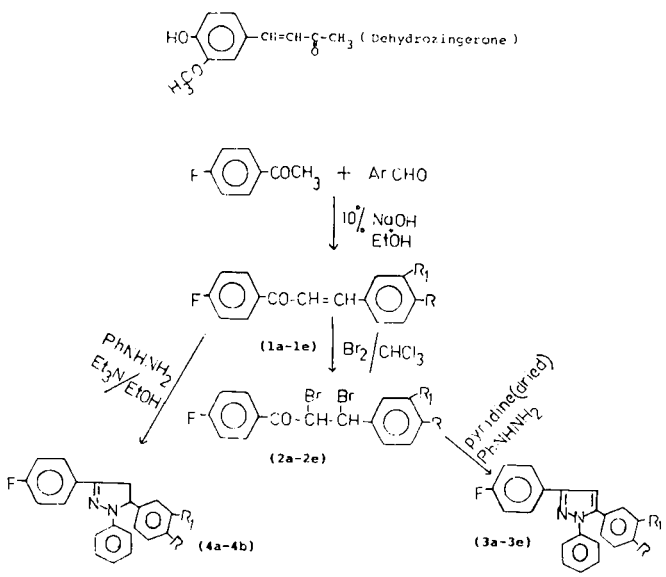
Abstract □ Various *N*-phenyl-5-substituted aryl-3-*p*-(fluorophenyl) pyrazolins and pyrazoles were synthesized by cyclization of the corresponding 4-(fluorophenyl) styryl and 4-(fluorophenyl) dibromostyryl ketones. These compounds were characterized by elemental analysis and UV, infrared, and nuclear magnetic spectral data. All substituted *p*-(fluorophenyl) styryl ketones [250 mg/kg orally (po)] possessed anti-inflammatory activity, as reflected by their ability to provide protection (51–70%) against carrageenin-induced edema in rat paw. Indomethacin (10 mg/kg, po) and dehydrozingerone (70 mg/kg, po), used as standard reference drugs, provided 97 and 60% protection, respectively. All compounds (0.20 mM) showed ability to denature bovine serum albumin, as observed in in vitro inhibition studies. Inhibition ranged from 7 to 59% for substituted *p*-(fluorophenyl) styryl ketones and from 12 to 21% for pyrazoles. No correlation was found between the anti-inflammatory activity of *p*-(fluorophenyl) styryl ketones or substituted pyrazoles and their effectiveness at inhibiting bovine serum albumin denaturation. The low toxicity of *p*-(fluorophenyl) styryl ketones was reflected by the dose that was lethal in 50% of the cases tested (2000–2500 mg/kg).

Protein denaturation is one of the well-documented causes of inflammation.[1–3] Production of autoantigens in certain rheumatic diseases may be due to in vivo protein denaturation.[4] Some anti-inflammatory drugs inhibit protein denaturation.[5] Mizushima[6] and others[7] have used protein denaturation as an in vitro screening model for anti-inflammatory compounds. Compounds containing a phenyl styryl ketone unit inhibit bovine serum albumin (BSA) denaturation[8–10] and possess anti-inflammatory and antimitotic[11] activities. Recent reports[12] reveal that dehydrozingerone (4-hydroxy,3-methoxybenzalacetone), isolated from *Zingiber officinale* (ginger), has anti-inflammatory activity. Saturation of the double bond or variation of the aliphatic part results in loss of the anti-inflammatory activity. The presence of fluorine enhances the therapeutic efficacy and lipid solubility.[13] In view of these observations, we synthesized a number of analogues of dehydrozingerone with fluorine substitution in the aromatic ring and various substitutions in the aliphatic part of the aromatic ring. The phenyl styryl ketones were cyclized to fluoropyrazoles and fluoropyrazolins to obtain more-active compounds. To elucidate cellular mechanisms of action, selected compounds were evaluated for their ability to protect against carrageenin-induced edema in rat paw and to inhibit BSA denaturation. The toxicity profiles of some compounds were assessed by determining the approximate values of the dose that is lethal in 50% of the cases tested (LD_{50} values).

Experimental Section

The various *N*-phenyl-5-substituted aryl-3-*p*-(fluorophenyl)pyrazolins (3) and -pyrazoles (4) were synthesized by cyclization of the corresponding 4-(fluorophenyl) styryl (1) and 4-(fluorophenyl) dibromophenyl styryl (2) ketones (Scheme I). Compound purity was checked by thin-layer chromatography on plates coated with silica gel. Infrared (IR) spectra were determined with sodium chloride optics on a Perkin-Elmer 783 spectrometer. The proton nuclear magnetic resonance (^1H NMR) spectra of compounds dissolved in $CDCl_3$ were recorded on a Perkin-Elmer 783 90-MHz spectrometer. The chemical shifts are reported in parts per million downfield from the internal reference tetramethylsilane.



Scheme I

4-(Fluorophenyl) Styryl Ketones (1)—These compounds were prepared by stirring a mixture of 4-fluoroacetophenone (0.01 mol) and substituted benzaldehydes (0.02 mol) in 20 mL of ethanol and 10% NaOH solution for 0.5 h; the reaction mixture was allowed to stand for 1 h. The precipitated product was filtered and recrystallized from ethanol. The substituted 4-(fluorophenyl) styryl ketones thus synthesized were characterized by their melting points (Table I). The IR spectra showed characteristic bands due to C=O (attached to phenyl), C–F, CH=CH, and aromatic groups at 1680, 1210, 830–980, and 690 cm^{-1}, respectively.

4-(Fluorophenyl) p-Chlorophenylstyryl Ketone (1d)—The general method was used to prepare **1d** in 85% yield: mp, 128–130 °C; ^1H NMR ($CDCl_3$): 6.6–6.8 (2H) and 7.2–8.2 (m, 9H, ArH) ppm.

Anal.—Calc. for $C_{15}H_{10}ClOF$: C, H, N.

4-(Fluorophenyl) p-Dimethylaminophenylstyryl Ketone (1c)—The general method was used to prepare **1c** in 88% yield: mp, 105 °C; ^1H NMR ($CDCl_3$): 6.74–6.84 (2H), 2.8–3.3 [6H, $(CH_3)_2$], and 7.2–8.4 (m, 8H, ArH) ppm.

Anal.—Calc. for $C_{17}H_{16}NOF$: C, H, N.

4-(Fluorophenyl) Dibromostyryl Ketones (2)—These compounds were prepared by vigorous stirring for ~0.5 h of a mixture of 4-(fluorophenyl) styryl ketones (0.01 mol) in chloroform (10 mL) while bromine (0.01 mol) was added in a dropwise manner and allowing the reaction mixture to stand for 1 h. The precipitated product was filtered, washed with ether to remove excess bromine, and recrystal-

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Table I—Characterization of 1–4

Compound	R	R_1	Yield, %	Melting Point, °C	Formula	Analysis, % Calc.	Found
1a	H	H	92	80–81	$C_{15}H_{11}OF$	C 79.6	C 79.2
						H 4.8	H 4.5
1b	OH	OCH_3	83	338–340	$C_{16}H_{13}O_3F$	C 70.5	C 70.3
						H 4.7	H 4.2
1c	$N(CH_3)_2$	H	88	105	$C_{17}H_{16}NOF$	C 75.8	C 75.5
						H 5.9	H 5.4
						N 5.2	N 4.9
1d	Cl	H	85	128–130	$C_{15}H_{10}ClOF$	C 69.2	C 69.0
						H 3.8	H 3.6
2a	H	H	75	166–168	$C_{15}H_{11}Br_2OF$	C 46.6	C 46.2
						H 2.8	H 2.5
2b	OH	OCH_3	66	121	$C_{10}H_{13}Br_2O_3F$	C 44.4	C 44.1
						H 3.0	H 2.7
2c	$N(CH_3)_2$	H	74	116–118	$C_{17}H_{16}NBr_2OF$	C 47.5	C 47.2
						H 3.7	H 3.2
						N 3.2	N 2.8
2d	Cl	H	80	158–159	$C_{15}H_{10}Br_2ClOF$	C 42.8	C 42.5
						H 2.3	H 2.1
3a	H	H	31	136	$C_{21}H_{16}N_2F$	C 79.7	C 79.2
						H 4.7	H 4.3
3b	OH	OCH_3	63	90–92	$C_{27}H_{18}N_2O_2F$	C 70.5	C 70.2
						H 4.8	H 4.2
4a	H	H	23	76	$C_{21}H_{15}N_2F$	C 80.2	C 80.0
						H 4.7	H 4.3
4b	OH	OCH_3	75	88–90	$C_{22}H_{17}N_2O_2F$	C 70.9	C 70.3
						H 4.5	H 4.2
4c	$N(CH_3)_2$	H	36	165–166	$C_{22}H_{20}N_3F$	C 76.5	C 76.1
						H 5.7	H 5.5
						N 12.1	N 11.8
4d	Cl	H	25	94–95	$C_{21}H_{14}N_2ClF$	C 72.4	C 71.9
						H 4.0	H 3.7

lized from ethanol. The substituted 4-(fluorophenyl) dibromostyryl ketones thus synthesized were characterized by their melting points (Table I). The IR spectra showed characteristic bands due to C=O (attached to phenyl), C–F, C–Br, and aromatic groups at 1680, 1210, 560–580, and 690 cm^{-1}, respectively.

***N*-Phenyl-5-substituted Aryl-3-*p*-(fluorophenyl)pyrazolins (3)**—These compounds were synthesized by cyclization of the appropriate 4-(fluorophenyl) styryl ketones. Triethylamine (10 mL) was added to a mixture of 1 (0.01 mol) and phenylhydrazine (0.02 mol) in absolute ethanol (50 mL), and the reaction mixture was refluxed for 8–10 h on a water bath. The contents were cooled, poured onto crushed ice, and kept aside overnight. The resulting precipitate was filtered and recrystallized from ethanol. The *N*-phenyl-5-substituted aryl-3-*p*-(fluorophenyl)pyrazolins thus synthesized were characterized by their melting points. The presence of the characteristic bands for C=N (1490 cm^{-1}), C–F (1220 cm^{-1}), and aromatic ring (690 cm^{-1}) in the IR spectra of 3a provides support for the structure. The method gave *N*-phenyl-5-substituted aryl-3-*p*-(fluorophenyl)pyrazolin (3a) in 31% yield: mp, 136 °C; ^1H NMR ($CDCl_3$): 6.92 (C_4H) and 7.1–8.13 (m, 14H, ArH) ppm.

Anal.—Calc. for $C_{21}H_{16}N_2F$: C, H, N.

***N*-Phenyl-5-substituted Aryl-3-*p*-(fluorophenyl)pyrazoles (4)**—These compounds were synthesized by cyclization of the appropriate 4-(fluorophenyl) dibromostyryl ketones. A mixture of the dibromostyryl ketone (0.01 mol), pyridine (dried; 20 mL), and phenylhydrazine (0.02 mol) was refluxed in an oil bath for ~6–8 h. The cooled product obtained was triturated with glacial acetic acid, and the separated solid was filtered and recrystallized from chloroform. The *N*-phenyl-5-substituted aryl-3-*p*-(fluorophenyl)pyrazoles thus synthesized were characterized by their melting points. The presence of characteristic bands for C=N (1490 cm^{-1}), C–F (1220 cm^{-1}), C=C (1510 cm^{-1}), and aromatic ring (690 cm^{-1}) in the IR spectrum (4a) provides support for the structure. The method gave *N*-phenyl-5-substituted aryl-3-*p*-(fluorophenyl)pyrazole 4a in 23% yield: mp, 76 °C; ^1H NMR ($CDCl_3$): 6.95 (C_4H) and 7.3–7.6 (m, 14H, ArH) ppm.

Anal.—Calc. for $C_{21}H_{15}N_2F$: C, H, N.

N-Phenyl-5-substituted aryl-3-*p*-(fluorophenyl)-4-chloropyrazole (4d) was prepared in 25% yield: mp, 94–95 °C; ^1H NMR ($CDCl_3$): 6.91 (C_4H) and 7.15–8.2 (m, 14H, ArH) ppm.

Anal.—Calc. for $C_{21}H_{14}N_2ClF$: C, H, N.

Inhibition of BSA Denaturation—Inhibition of BSA denaturation was studied according to the method of Elias and Rao.[8] The test compounds were dissolved in a minimum amount of dimethylformamide and diluted with phosphate buffer (0.2 M, pH 7.4). Final concentrations of dimethylformamide in all solutions were <2.5%. The test solution (1 mL), containing different concentrations of drug, was mixed with 1 mL of a 1 mM BSA solution in phosphate buffer and incubated at 27 ± 1 °C for 15 min. Denaturation was induced by keeping the reaction mixture at 60 ± 1 °C in a water bath for 10 min. After the mixture was cooled, turbidity was measured at 660 nm (Spectrocard-108 Systronics spectrometer). The percent inhibition of BSA denaturation was calculated with reference to control samples to which no drug was added. Each experiment was done in triplicate, and the average value is reported. The percent inhibition was determined by using the following formula: Inhibition, % = 100 − 100(*VT*/*VC*). *VT* is the absorbance of the test compound, and *VC* is the absorbance of the control sample.

Carrageenin-Induced Edema Test—Adult albino rats of either sex, weighing 150–200 g, were used. Rats were divided into groups of six. Freshly prepared 1% suspension of carrageenin in 0.9% saline (0.1 mL) was injected into the planter aponeurosis of the left hind paw[14] 1 h after the oral (po) administration of test compounds, which were dissolved in 1 mL of 2% gum acacia. The rats were treated po with 4-(fluorophenyl) styryl ketones (1a and 1c) 1 h before the injection of carrageenin. The control group received an equivalent amount of the gum acacia suspension used to dissolve the test compounds. The rats in the standard reference group received indomethacin (10 mg/kg, po) dissolved in 2% gum acacia. The increase in paw volume was measured by a plethysmograph (mercury displacement is the index of edema and was measured before and 4 h after the administration of carrageenin). The anti-inflammatory activities of test compounds and standard reference drug were determined by using the following formula: Anti-inflammatory Activity, % = 100[(1 − *Vt*)/*Vc*]. *Vt* is the mean increase in paw volume in rats treated with test compounds, and *Vc* is the mean increase in paw volume in the control group of rats. The single-tailed *t* test was

used to compare the mean changes in paw volume in rats in the control group with the mean changes in paw volume in rats treated with the test compounds (1a and 1c).

Toxicity Study—Acute toxicity of some 4-(fluorophenyl) styryl ketones (1), 4-(fluorophenyl) dibromostyryl ketones (2), and pyrazolins and pyrazoles (3 and 4) was determined in albino rats with the Staircase method.[15] Each group of four animals was fasted for 18 h prior to the administration of the test compounds. The test compounds (1–4) were administered intraperitoneally in doses of 2000 and 2500 mg/kg, and the mortality at 24 h was recorded to calculate the approximate LD_{50} values.

Results and Discussion

The 4-(fluorophenyl) styryl ketones **1a–1c** possess anti-inflammatory activity. The degree of protection afforded by **1a–1c** (250 mg/kg, po) against carrageenin-induced edema in rat paw ranged from 57 to 70% (Table II). The abilities of indomethacin (10 mg/kg, po) and dehydrozingerone (70 mg/kg, po), used as standard reference drugs, to provide such protection were 97 and 60%, respectively. The maximum protection (70%) was observed with **1a**, whereas the minimum protection (57%) was observed with **1c**. The low toxicity of **1a–1c** was reflected by their high approximate LD_{50} values, which ranged from 2000 to 2500 mg/kg (intraperitoneal) in rats (Table II). Compounds **1a–1c** (0.20 mM) inhibited BSA denaturation by 7–59% (Table II). Indomethacin and dehydrozingerone (0.20 mM), used as standard reference drugs, inhibited in vitro BSA denaturation by 79 and 71%, respectively (Table II).

The ability of *N*-phenyl-5-substituted aryl-3-*p*-(fluorophenyl)pyrazolins and -pyrazoles (**3a–4c**) at 250 mg/kg (po) to protect against carrageenin-induced edema in rat paw (Table II) ranged from 30 to 47%. This anti-inflammatory activity is much less than those of indomethacin (10 mg/kg, po) and dehydrozingerone (70 mg/kg, po), which gave 97 and 60% protection, respectively. The low toxicity of some substituted pyrazolins and pyrazoles was reflected by their high approximate LD_{50} values of 2000–2500 mg/kg. All substituted pyrazolins and pyrazoles (0.20 mM) inhibited BSA denaturation by 12–45% (Table II).

Table II—Anti-Inflammatory Activity of and Inhibition of BSA Denaturation by 1, 3, and 4

Compound	Mean Increase in Paw Volume ± SE, mL	Anti-inflammatory Activity, % Protection[a]	LD_{50}, mg/kg[b]	Inhibition of BSA Denaturation, % Protection[c]
Control	0.58 ± 0.02	—[d]	—	—
1a	0.17 ± 0.02	70.6 (<0.001)	2500	59.5 ± 0.0092
1b	0.24 ± 0.01	58.2 (<0.001)	2000	22.0 ± 0.0039
1c	0.28 ± 0.01	57.7 (<0.001)	2000	7.6 ± 0.00032
Control	0.59 ± 0.03	—	—	—
3a	0.31 ± 0.04	47.4 (<0.001)	2000	32.6 ± 0.0028
3b	0.36 ± 0.03	38.9 (<0.01)	2000	45.6 ± 0.0036
Control	0.56 ± 0.02	—	—	—
4a	0.38 ± 0.03	32.1 (<0.01)	—	12.6 ± 0.00014
4b	0.34 ± 0.02	39.2 (<0.001)	2000	22.7 ± 0.0016
4c	0.39 ± 0.03	30.3 (<0.01)	—	14.8 ± 0.00018

[a] The mean increases in paw volume in rats treated with indomethacin (10 mg/kg, po) and dehydrozingerone (70 mg/kg, po) were 0.017 ± 0.03 and 0.23 ± 0.03 mL, respectively; these results indicate 97.06% protection ($p < 0.001$) by indomethacin and 60.3% protection ($p < 0.00$) by dehydrozingerone. The test compounds were administered at 250 mg/kg (po). Values in parentheses are p values determined from single-tailed *t* test. [b] Approximate LD_{50} values determined in mice after intraperitoneal administration. [c] Each experiment was done in triplicate, and the mean values ± standard errors of the mean were calculated; the protection observed with indomethacin and dehydrozingerone (0.20 mM) were 79.7 ± 0.034 and 71.3 ± 1.9%, respectively. The test compounds were applied at 0.20 mM. [d] —, Not determined.

Among substituted phenyl styryl ketones, the presence of fluorine in the phenyl nucleus increased the anti-inflammatory activity, except for *p*-(fluorophenyl) *N*-dimethylaminophenylstyryl ketone (**1c**). Cyclization of the substituted phenyl styryl ketones into their corresponding substituted *N*-phenyl-5-substituted aryl-3-*p*-(fluorophenyl)pyrazolins and -pyrazoles decreased the anti-inflammatory activity. No significant change in the anti-inflammatory activity of *N*-dimethylamino-substituted pyrazoles (**4c**) was found. The absence of substituents in the 4-(fluorophenyl) styryl ketone unit significantly increased the anti-inflammatory activity. The high approximate LD_{50} values of substituted 4-(fluorophenyl) styryl ketones and substituted pyrazolins and pyrazoles reflect the low inherent toxicity of these newer compounds. Thus, these compounds warrant further investigations for their use as anti-inflammatory drugs.

The inhibition of BSA denaturation by substituted 4-(fluorophenyl) styryl ketones (**1a–1c**) and their corresponding cyclized pyrazolins and pyrazoles (**3a–4c**) did not correlate with anti-inflammatory effectiveness. An increase in BSA denaturation activity was observed with only **1a** and **3b**. The anti-inflammatory and BSA denaturation activities of **1a–1c** and **3a–4c** showed no structure–activity relationship and failed to establish BSA denaturation activity as the cellular basis for the anti-inflammatory properties.

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