Anti-inflammatory Activity of Quinazolinoformazans

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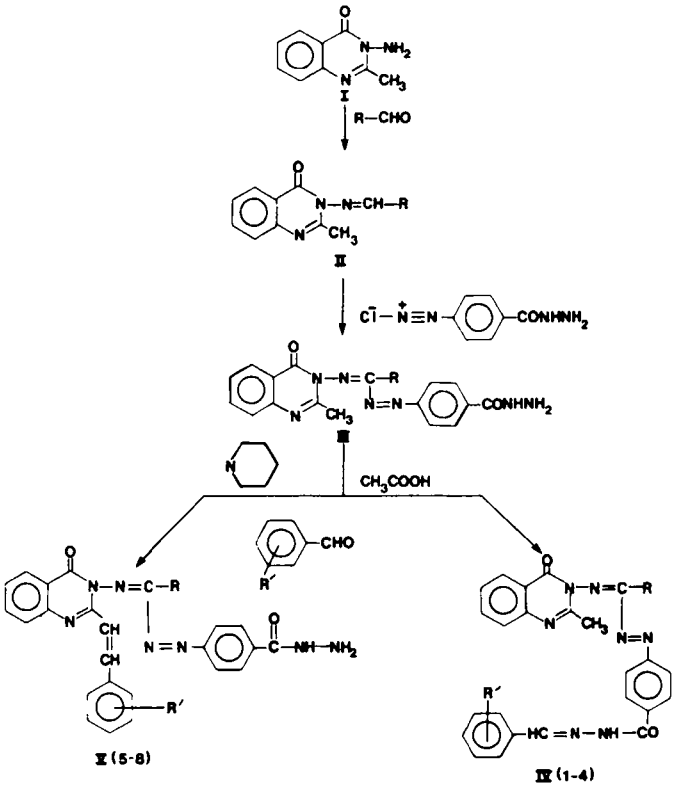
Abstract □ Eight substituted quinazolonoformazans were synthesized and evaluated for anti-inflammatory activity. The degree of protection provided by seven of these compounds, at a dose of 100 mg/kg, po, against carrageenin-induced edema in rat paw ranged from 26 to 57%. The four active substituted quinazolonoformazans (**1**, **2**, **6**, **8**), on further evaluation for antiwrithmogenic activity, provided 10–80% protection against the aconitine-induced writhing response in mice. The ulcerogenic liabilities of two of the most active compounds were also determined. The doses producing ulcers in 50% of the treated rats (UD_{50}) were 155 and 260 mg/kg, ip, for **2** and **8**, respectively. The low toxicities possessed by these substituted quinazolonoformazans were indicated by their LD_{50} values which ranged from 600 to 1300 mg/kg, ip, in mice.

Compounds bearing the quinazolinone moiety are endowed with potent anti-inflammatory effectiveness[1–3] besides other biological activities.[4–6] In spite of the synthesis of a large number of congeners of this heterocyclic nucleus, there still exists a need to prepare quinazolinones possessing different pharmacophores. Earlier studies have provided evidence that introduction of substituents in the quinazolinone nucleus at positions 2 and 3 leads to changes in the anti-inflammatory effectiveness of this class of compounds.[7–9] The anti-inflammatory activity of compounds possessing the formazan moiety in the quinazolinone nucleus, however, has not yet been fully explored. Incorporation of the formazan moiety in different heterocyclic structures has been shown to exhibit anti-inflammatory effectiveness.[10–12]

Thus, to gain further insight into the anti-inflammatory activity of quinazolinone congeners, eight new substituted quinazolinoformazans were synthesized by the incorporation of the formazan moiety at position 3 and the aryl-aldehydic substituent at position 2 of the quinazolinone nucleus. All substituted quinazolinoformazans were evaluated for anti-inflammatory activity against carrageenin-induced edema in rat paw. Furthermore, considering the normal association of pain with inflammation, the antiwrithmogenic activities of four active compounds were determined to provide a possible indication of their analgesic activity[13] by determining their ability to protect aconitine-induced writhing response in mice. In order to evaluate the effectiveness of substituted quinazolinoformazans for possible usefulness as anti-inflammatory drugs, the toxicity of all compounds and the ulcerogenic liability of two of the most active compounds were also determined.

Experimental Section

The various substituted quinazolinoformazans were synthesized by following the steps outlined in Scheme I. The melting points were determined in open capillary tubes and are uncorrected. The purity of the compounds was checked by thin-layer chromatography on silica gel G. The microanalyses for C, H, and N were within ± 0.4% of the calculated values (data may be obtained from author). The IR spectra were recorded in KBr on a Perkin-Elmer 137 Infracord Spectrophotometer (V_{max} in cm^{-1}). The mass spectra were recorded on a JMS 300 double-focusing spectrophotometer fitted with JMSD 2000 data system at 70 ev. The ^1H NMR spectra in $CDCl_3$ or trifluoroacetic acid were recorded on Em-360 spectrometer using TMS as the internal standard (chemical shifts in δ, ppm).



Scheme I

The methods reported earlier were followed to synthesize 3-amino-2-methyl-4(3*H*) quinazolinone (**I**)[14] and 2-methyl-3-(aryl/furyl methylene) amino-4 (3*H*) quinazolinone (**II**).[15] The data for the characterization of compounds **II**, thus prepared are as follows.

2-Methyl-3-(phenylmethylene)-amino-4(3*H*)quinazolinone (II)—Melting point, 180–182 °C; yield 80%; IR: 1680 (cyclic C=O), and 1520 cm^{-1} (C=N).

Anal.—Calc. for $C_{16}H_{13}N_3O$: C,H,N.

2-Methyl-3-(furylmethylene)-amino-4(3*H*)quinazolinone (II)—Melting point, 136–140 °C; yield 60%; IR: 1680 (cyclic C=O), and 1520 cm^{-1} (C=N).

Anal.—Calc. for $C_{14}H_{11}N_3O_2$: C,H,N.

4-[[[[2-Methyl-4-oxo-3(4*H*)quinazolinyl]-imino]arylmethylene]-azo]benzoic Acid Hydrazides (III)—4-Aminobenzoic acid hydrazide (0.02 mol) in glacial acetic acid (2 mL) and HCl (1.5 mL) was diazotized with sodium nitrite (0.2 g in 2 mL) at 0–5 °C. The resultant diazonium chloride solution was added to the solution of 2-methyl-3-(phenylmethylene)-amino-4(3*H*)quinazolinone (**II**; 0.01 mol) or 2-methyl-3-(furylmethylene)-amino-4(3*H*) quinazolinone (**II**;

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0.01 mol) in cold pyridine. The reaction mixture was left overnight at ambient temperature. Thereafter, it was poured into cold water (250 mL) with continuous stirring. The dark colored solid mass which separated out was filtered, washed repeatedly with water, and recrystallized from methanol. The IR peaks at 3450 (NH_2), 1580 (N=N), 1620 (C=N), 1680 (cyclic C=O), and 3055 cm^{-1} (CH_3) were specific to the corresponding groups in their structure. The data for further characterization of compounds **III** thus prepared are as follows.

4-[[[[2-Methyl-4-oxo-3(4*H*)quinazolino]-imino]phenylmethyl]-azo]benzoic Acid Hydrazide (III)—Melting point, 182–185 °C; yield 75%; ^1H NMR ($CDCl_3$): 2.65 (s, 3H, CH_3), 7.2–7.7 (m, 4H, Ar-H), and 8.1–8.45 ppm (m, 4H quinazolinone Ar-H).

Anal.—Calc. for $C_{23}H_{19}N_7O_2$: C,H,N.

4-[[[[2-Methyl-4-oxo-3(4*H*)quinazolino]-imino]furfurylmethyl]-azo]benzoic Acid Hydrazide (III)—Melting point, 165–170 °C; yield 60%.

Anal.—Calc. for $C_{21}H_{17}N_7O_3$: C,H,N.

4-[[[[2-Methyl-4-oxo-3(4*H*)quinazolino]imino]aryl/furfuryl-methyl]-azo]benzoic Acid-(aryl methylene) Hydrazides (IV, 1–4)—Equimolar mixtures of substituted aldehydes and **III** in absolute ethanol containing a few drops of glacial acetic acid were refluxed for 5 h. The excess solvent was removed by distillation. The solid mass which separated out was filtered, washed with petroleum ether, and recrystallized from methanol. The presence of the characteristic bands at 1630 (C=N), 3300 (NH), 1560 (N=N), 3050 (CH_3), and 1730 cm^{-1} (C=O) in their IR spectra provided support for their structure. Further support for their structure was obtained from the ^1H NMR spectrum of **2**, which exhibited signals at 2.65 (s, 3H, CH_3), 8.1–8.45 (m, 4H, quinazoline Ar-H), and 2.3 ppm (s, 6H, Ar-CH), and the mass spectrum of **2**, which was characterized by 100% intensity of the molecular ion peaks at *m/z* 409, 289, 261, 220, 159, 118, and 90.

4-[[[[2-Arylethynyl-4-oxo-3(4*H*)quinazolino]-amino]aryl/furfuryl methyl]-azo]benzoic Acid Hydrazides (V, 5–8)—Equimolar mixtures of substituted aldehydes and **III** were heated in piperidine in an oil bath at 140 °C for 1 h. The jelly-like mass which separated out was filtered and recrystallized from benzene:petroleum ether. The disappearance of a band at 3050 cm^{-1} exhibiting absence of CH_3, and the appearance of the peak at 1610 cm^{-1} representing CH=CH in their IR spectra, confirmed the structure of these compounds. Further support for their structure was provided by the ^1H NMR spectrum of **6**, which showed signals at 2.2 (d, 2H, CH=CH), 8.1–8.45 (m, 4H, quinazoline Ar-H), and 7.2–7.7 ppm (m, 4H, Ar-H), and the mass spectrum of **6**, which exhibited a molecular ion peak at *m/z* 529 and base peak at *m/z* 146, along with other peaks at *m/z* 470, 365, 263, 118, and 90.

Biology—*Carrageenin-Induced Edema Test*—Adult albino rats of either sex weighing 100–120 g were used in this study. Rats were divided in groups of six and 0.5 mL of a freshly prepared 1% suspension of carrageenin in 0.9% saline was injected into the plantar aponeurosis of the right hind paw.[16] The rats were pretreated with substituted quinazolinoformazans suspended in 5% aqueous gum acacia in a dose of 100 mg/kg, po, or with the graded doses of the two active compounds, **2** and **8**. The control group of rats received an equivalent amount of 5% gum acacia, while the rats in the standard reference group received 100 mg/kg, po, of phenylbutazone or varying concentrations of acetylsalicylic acid, sodium salicylate, ibuprofen, and phenylbutazone for the determination of their ED_{50} values. All the test compounds were administered 1 h before the injection of carrageenin. The increase in the rat paw volume was measured by the micropipette method[17] before and 2 h after the administration of carrageenin. The anti-inflammatory activity of substituted quinazolinoformazans and standard reference drugs were determined by using the following formula:

$$\% \text{ Anti-inflammatory Activity} = \left(1 - \frac{V_C}{V_T}\right) \times 100 \quad (1)$$

where V_T represents the mean increase in paw volume in rats treated with the test compounds, and V_C represents mean increase in paw volume in the control group of rats. Statistical analyses were carried out by applying the single-tailed *t* test and by comparing the mean changes in paw volume in the control group with the mean changes in paw volume in rats treated with the test compounds **1–8**.

Antiwrithmogenic Activity—The aconitine-induced writhing response in mice was determined[13,18] to obtain an indication of the possible analgesic activity of substituted quinazolinoformazans, using acetylsalicylic acid as the reference drug for comparative evaluation. The mice (groups of 10 for each dose) were fed with the test compounds 30 min before the ip injection of 20 μg/mouse of aconitine (100% writhmogenic dose). The mice were then observed for any abdominal torsion, stretching of hind legs to the abdominal wall, marked contraction of abdominal area, and the periodic arching of the back to rub the surface on which the mouse was kept. The degree of percent protection by substituted quinazolinoformazans was determined, and this represented an index of their antiwrithmogenic activity.

Ulcerogenic Activity—Adult male and nonpregnant female rats weighing 100–150 g were used for evaluating the ulcerogenic liability of the test compounds.[19] Rats were divided into groups of 10 and were fasted for 24 h prior to the administration of these compounds. Water was allowed ad libitum to the animals. Compounds **2** and **8** and acetylsalicylic acid, sodium salicylate, ibuprofen, and phenylbutazone were administered ip in graded doses. The animals were sacrificed after 8 h, and their duodenum and jejunum were removed and examined under a dissecting microscope for any evidence of shedding of epithelium, petechial and frank hemorrhages, and erosion or discrete ulcer with or without perforation. The presence of any of these criteria was taken as an evidence of the ulcerogenic liability, and the percent ulcerogenic activity was determined from the number of the rats which exhibited ulcers or the signs of ulcerogenic effects.

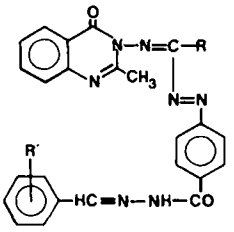
Toxicity Study—The acute toxicities of substituted quinazolinoformazans were determined in mice.[20] Each group of mice, comprised of four animals, was fasted for 18 h prior to the administration of the test compounds. The various test compounds were injected ip and 24 h mortality was recorded to calculate their LD_{50} values.

Results and Discussion

All substituted quinazolinoformazans, with the exception of **5**, possessed anti-inflammatory activity. The degree of protection provided by these compounds (100 mg/kg, po) against carrageenin-induced edema in rat paw ranged from 26 to 57% (Tables I and II). Such protection was comparable to that of 51% observed with phenylbutazone (100 mg/kg, po), which was used as a standard reference drug. It was observed that quinazolinoformazans possessing a methyl substituent at position 2 of the quinazolinone nucleus (Table I), in general, possessed higher anti-inflammatory activity as compared with the corresponding compounds with a substituted aryl-ethenyl moiety (Table II). Contrary to these observations, the maximum protection was observed with **8** which possessed a substituted aryl-ethenyl moiety at position 2 of the quinazolone nucleus. The anti-inflammatory activity reported earlier for substituted formazans,[10] in general, did not indicate greater effectiveness at providing protection against carrageenin-induced edema as compared with the anti-inflammatory activity possessed by the substituted quinazolinoformazans used in the present investigation (Tables I and II). The earlier studies reported protection of 15.6–45.8% by methyl-4-{[aryl(arylazo)methylene]-amino}benzoates and 5.0–41.4% by aryl{[(arylazo)methylene]-amino}benzoic acid,{α-[(substituted phenyl)azo]arylidene}hydrazides against carrageenin-induced edema in rat paw.[10] Although no direct correlation can be established between these groups of compounds possessing the formazan moiety, due to the different molecular structure, it seems likely that the presence of the quinazolinone nucleus may in some way contribute to the greater effectiveness of substituted quinazolinoformazans.

The active compounds, **1**, **2**, **6**, and **8**, providing protection of 35, 46, 42, and 57%, respectively, were further tested for antiwrithmogenic activity in an attempt to gain some idea of their possible analgesic effectiveness.[13,18] These four compounds showed 10–80% protection against aconitine-induced writhing response in mice, whereas acetylsalicylic acid exhibited greater effectiveness and the degree of protection in

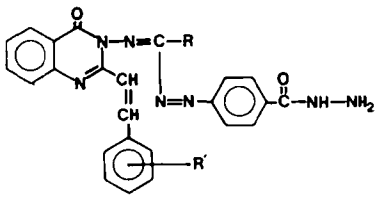
Table I—Anti-inflammatory and Antiwrithmogenic Effectiveness of 4-[[[[2-Methyl-4-oxo-3(4H)quinazolino]-imino]aryl/furfuryl methyl]-azo]-benzoic Acid(aryl methylene) Hydrazides



Compound[a]	R	R'	Melting point, °C	Molecular Formula	Antiedema Effect (100 mg/kg, po)			Antiwrithmogenic Activity (100 mg/kg, po)[b]	LD_{50}, mg/kg, ip
					Mean Increase in Paw Volume, mL ± SE	% Protection	'p' Value	% Protection	
Control	—	—	—	—	0.86 ± 0.03	—	—	—	—
1	C_6H_5	H	110	$C_{30}H_{23}N_7O_2$	0.56 ± 0.07	34.9	0.01	10	750
2	C_6H_5	2-OH	185	$C_{30}H_{23}N_7O_3$	0.46 ± 0.03	46.1	0.001	50	1250
3	2-Furyl	H	200	$C_{28}H_{21}N_7O_3$	0.57 ± 0.05	33.7	0.01	—	750
4	2-Furyl	2-OH	165	$C_{28}H_{21}N_7O_4$	0.64 ± 0.05	25.6	0.05–0.01	—	600
Phenylbutazone	—	—	—	—	0.42 ± 0.04	51.2	0.001	—	—

[a] Microanalyses for C, H, and N were within ±0.4% of the calculated values; the yields of **1**, **2**, **3**, and **4** obtained were 60, 50, 56, and 78%, respectively; biological assay procedures are as described in the text. [b] Acetylsalicylic acid (standard reference drug) showed 80% antiwrithmogenic activity at a dose of 45 mg/kg, po, in mice.

Table II—Anti-inflammatory and Antiwrithmogenic Effectiveness of 4-[[[[2-Arylethynyl-4-oxo-3(4H)quinazolino]-amino]aryl/furfuryl methyl]-azo]-benzoic Acid Hydrazides



Compound[a]	R	R'	Melting point, °C	Molecular Formula	Antiedema Effect (100 mg/kg, po)			Antiwrithmogenic Activity (100 mg/kg, po)[b]	LD_{50}, mg/kg, ip
					Mean Increase in Paw Volume, mL ± SE	% Protection	'p' Value	% Protection	
Control	—	—	—	—	0.86 ± 0.03	—	—	—	—
5	C_6H_5	H	60	$C_{30}H_{23}N_7O_2$	0.85 ± 0.02	1.0	N.S.	—	1000
6	C_6H_5	2-OH	70	$C_{30}H_{23}N_7O_3$	0.50 ± 0.05	41.8	0.001	20	1000
7	2-Furyl	H	175	$C_{28}H_{21}N_7O_3$	0.60 ± 0.03	30.2	0.01	—	1000
8	2-Furyl	2-OH	100	$C_{28}H_{21}N_7O_4$	0.37 ± 0.03	56.9	0.001	80	1300
Phenylbutazone	—	—	—	—	0.42 ± 0.04	51.2	0.001	—	—

[a] Microanalyses for C, H, and N were within ±0.4% of the calculated values; the yields of **5**, **6**, **7**, and **8** were 78, 50, 66, and 48%, respectively; biological assay procedures are as described in the text. [b] Acetylsalicylic acid (standard reference drug) showed 80% antiwrithmogenic activity at a dose of 45 mg/kg, po, in mice.

spite of the use of low dose of 45 mg/kg, po, was 80% (Tables I and II). As is evident from Tables I and II, the LD_{50} values ranging from 600 to 1300 mg/kg, ip, observed in mice, indicate low toxicity of substituted quinazolinoformazans.

In the present study, administration of the two most active compounds, 2 and 8, at the dose levels of 50, 100, and 150 mg/kg, po, provided protection against carrageenin-induced edema of 33.6, 46.1, and 55.2%, respectively, by 2 and 37.0, 56.9, and 68.1%, respectively, by 8. The ED_{50} values were calculated graphically and are 124.2 mg/kg, po, for 2 and 79.4 mg/kg, po, for 8 (Figure 1).

The ulcerogenic liabilities of 2 and 8 were also determined by administration at the dose levels of 100, 200, and 300 mg/kg, ip. At these doses, ulcers were observed in 30, 60, and 90%, respectively, of rats treated with 2, and 20, 40, and 60%, respectively, of rats treated with 8. The doses thus necessary to produce ulcers in 50% of the treated rats (UD_{50}) were graphically calculated and found to be 155 mg/kg, ip, for 2 and 260 mg/kg, ip, for 8 (Figure 1). The comparative evaluation of the ED_{50} values for the anti-inflammatory activity and the UD_{50} values for the ulcerogenic effectiveness of 2 and 8 were undertaken with the standard nonsteroidal anti-inflammatory drugs like acetylsalicylic acid, sodium salicylate, ibuprofen, and phenylbutazone. As is evident from Figure 1, 8 interestingly showed a low ED_{50} value, reflecting a greater anti-inflammatory effectiveness, and a higher UD_{50} value, suggesting a lower ulcerogenic liability than the standard reference drugs used in these investigations.

The results of the present study provide evidence for the significant anti-inflammatory effectiveness of substituted quinazolinoformazans which is comparable to other commonly used nonsteroidal anti-inflammatory drugs.

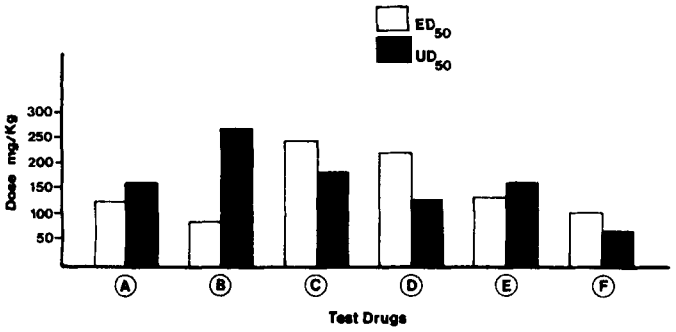


Figure 1—Comparison of the ED_{50} and UD_{50} of **2** and **8** with other nonsteroidal anti-inflammatory drugs: **(A)** compound **2**; **(B)** compound **8**; **(C)** acetylsalicylic acid; **(D)** sodium salicylate; **(E)** ibuprofen; and **(F)** phenylbutazone.

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