Synthesis of 2-Styryl-3,6,8-Trisubstituted Quinazolin 4(3H) ones as Anti-inflammatory agents

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Summary: Sixteen new quinazolinone derivatives have been synthesised by the reaction of 2-styryl-6, 8-disubstituted benzoxazin-4-ones with two different aromatic amines (p-morpholino aniline and 2-aminopyridine) or with two different hydrazides (viz. isonicotinic acid hydrazide and 2,4-dinitrophenyl acetic acid hydrazide) in pyridine. The intermediate 2-styryl 6,8-disubstituted benzoxin-4-ones, were prepared by the reaction of cinnamoyl chloride and 6,8-disubstituted anthranilic acid in dry pyridine. Some of the synthesised title compounds have been found to possess significant anti-inflammatory activity against carrageenin-induced inflammation. An attempt has also been made to establish a definite structure activity relationship. Further, in addition, the compounds were found to be mild central nervous system depressants and were quite non toxic. The structures of the unknown compounds were confirmed by elemental and spectral (I.R. & P.M.R.) analysis.

Quinazolinones are found to be CNS depressants[1,2], anticonvulsants[3], hypnotics and muscle relaxants[4]. Recently, 2-styryl 3,6,8-tri-substituted quinazolinones have been reported to possess a good MAO inhibitor and anticholinergic activity[5]. A number of quinazolones with potent anti-inflammatory and antipyretic activities have also been reported [6,7]. Further substituted-4-quinazolone salicyl esters showed potent anti-inflammatory activity on carrageenin induced oedema, cotton pellet-implantation induced granulation and on formaldehyde induced arthritis in albino rats[8]. Further, different hydrazides, secondary amines and heterocyclic amines are also recognised as having an effective therapeutic index against inflammation[9,10]. On the other hand MAO inhibitors and anticholinergics are recommended for the treatment of various CNS disorders. In addition it is also well known that anticonvulsants, hypnotics and muscle relaxants are the CNS depression agents.

Due to the aforesaid valid findings regarding the effectivity of quinazolinones against various CNS disorders, the ability of the title quinazolinones to inhibit carrageenin induced inflammation was investigated. The gross CNS activity of these compounds was also observed in an attempt to correlate their anti-inflammatory activity with gross CNS disorder (depressant effect).

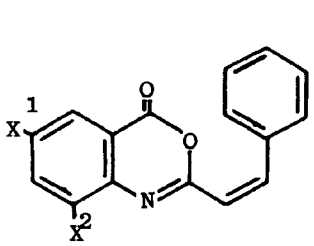
This paper describes the synthesis of title compounds, their toxicity and the action against carrageenin induced oedema in addition with their effectivity on the CNS of albino mice.

The precursors, 2-styryl-6,8-disubstituted-benzoxazin 4(3H)ones (I) were synthesised by the reaction of cinnamoyl chloride with appropriate 3,5-disubstituted anthranilic acid in dry pyridine. In the final step, the cyclo ester grouping of (I) was converted to cycloamidic grouping to get the title compounds, viz. 2-styryl-3,6,8-trisubstituted quinazolin-4(3H)-ones (II) were synthesised by the condensation of appropriate benzoxazinone (I) and different amines or acid hydrazides in pyridine. The structures of all the newly synthesised compounds were confirmed by their sharp melting point, elemental analysis (For C, H&N), I.R.&P.M.R. spectroscopy.

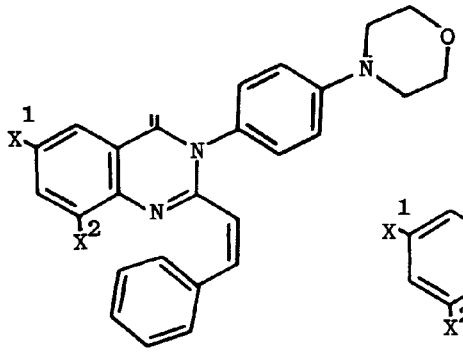
Pharmacological Activity

All the final compounds (II) were screened for their anti-inflammatory activity and gross Central Nervous System (CNS) activity on the brain of albino mice of either sex. The ALD_{50} values were also detremined for all the compounds.

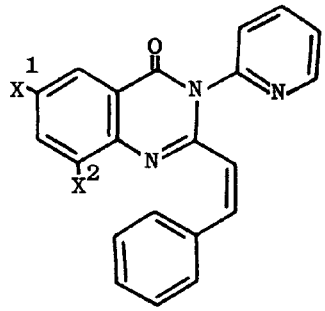
The ALD_{50} (approximate lethal dose in 50% of animals tested) was determined by the method of Weil[11] During the ALD_{50} determination, the compounds were administered intraperitoneally at the doses of 464 & 1000 mg/kg weight of mice, as a gum acacia suspension. The gross behavioural changes on the CNS of the mice were also observed at the same doses, after 1 hr of the compounds administration, in the form of changes in their behavioural activities. Decrease in mobility counts, rate



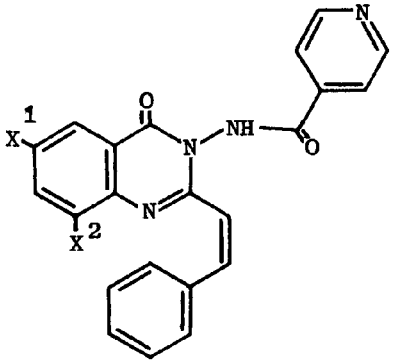
(I)



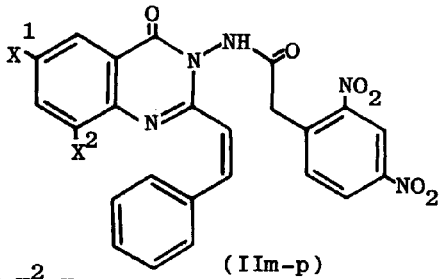
(IIa-d)



(IIe-h)



(IIi-l)



(IIm-p)

Ia, IIa, e, i&m; $X^1=X^2=H$
Ib, IIb, f, j&n; $X^1=Br, X^2=H$
Ie, IIc, g, k&o; $X^1=I, X^2=H$
Id, IId, h, l&p; $X^1=X^2=Br$

of breathing and reactivity to sound and touch, marked the CNS depressant nature of the compounds. For a comparative study, one group of mice was kept as the standard and was given an equal amount of saline water instead of the compound.

Their anti-inflammatory activity on the albino mice was evaluated by adopting the method of Winder[12] by measuring the percentage protection of animals against carrageenin induced oedema.

The compounds were found to be quite non-toxic. In gross CNS observations, all the compounds have been found to be CNS depressants (Table 1), as they decreased the spontaneous motor activity (SMA) and reactivity to sound and touch at the doses of 464, 1000 & 1/5th of ALD_{50} mg/kg (mice). In their anti-inflammatory activity (Table-1), some of the final compounds have shown significant effect against carrageenin induce oedema, whereas, rest of them did not produce any effective inhibition. Indomethacin, which was used for a comparative study, showed 41.6% activity. A study of the anti-inflammatory activity data reveals that;

(i) The presence of bromo group at both the 6 & 8-positions of quinazolinone nucleus, imparts the highest activity in its group of four compounds. On the other hand, the compounds with only one bromo group at position-6 of quinazolinone nucleus have the lowest activity in its group of four compounds with the exception of compound no. IIj.

Table 1.

Gross CNS effect, ALD_{50} and Anti-inflammatory activity of the compounds described in Table 2

Compd.	SMA & Reactivity	ALD_{50} (mg/kg)	Anti-inflamma-Activity (% protection at 1/5th of ALD_{50})
IIa	↓	> 1000	5.1
IIb	↓	> 1000	(-)
IIc	↓	> 1000	37.1
IId	↓	> 1000	45.3
IIe	↓	> 1000	41.4
IIf	↓	> 1000	37.00
IIg	↓	> 1000	39.2
IIh	↓	825	42.9
IIi	↓	681	15.5
IIj	↓	681	27.3
IIk	↓	681	15.2
III	↓	681	28.4
IIm	↓	681	7.1
IIn	↓	> 1000	(-)
IIo	↓	> 1000	5.00
IIp	↓	> 1000	10.9
Indomethacin	–	–	41.6 at 10 mg/kg (mice)

↓ = decreased, (-) - Not effected.

(ii) It is also not worthy that the compounds without any substitution in the quinazolinone ring have better effectivity in comparison with the 6-iodo substituted compounds, with the exception of compounds nos. IIa and IIc, in which the reverse case was noted.

Experimental Procedure

M.ps. were determined in open capillaries, using A.R.H_2SO_4 (Analytical Reagent) bath, and are uncorrected, I.R. spectra, in KBr phase, were recorded on a Perkin-Elmer 157 spectrophotometer (ν max in cm^{-1}). The PMR spectra were recorded in $CDCl_3$ or DMSO on a varian A60D instrument using TMS as internal standard (Chemical shift in δ ppm). The purity of the compounds was checked by TLC using silica-gel-G coated plates (0.25 mm) and the solvent system: benzene-methanol 100:5.

5-Bromo or 3,5-dibromoanthranilic acid[13] *and 5-iodo anthranilic acid*[14]: were prepared by the known procedures.

4-Morpholino aniline[15], *& 2,4-dinitro phenyl acetic acid hydrazide*[16] - were also prepared by known methods.

2-Styryl-6-bromo-benzoxazin-4(3H)-one (Ib) (Table -2): To a solution of 5-bromo anthranilic acid (0.01 mole) in dry pyridine (100 ml), cinnarmoyl chloride (0.013 mole) was added very slowly with constant vigorous shaking. An exothermic reaction which occured was controlled by external cooling. After the addition of all the cinnamoyl chloride, the reaction mixture was allowed to stand at the room temperature for 4 hours. Thereafter it was diluted with water (200 ml) and treated with solid $NaHCO_3$ for removing any unreacted acid and acid chloride. The solid which separated out, was filtered, washed several times with water and dried well in air. Finally it was recrystallised from benzene, m.p. 196°C, yeild 95%.

I.R.: 3050 (Ar–C-H stretch), 1740 (cyclic ester), 1650 (-CH=CH-) & 1620 (C=N) etc; PMR: 7.94 (d, J= 17 Hz, 1H, Ph-CH=), 6.82 to 7.45 (complex multiplet, 8H, Ar-H) & 6.41 (d, J=17 Hz, 1H, N=C=CH=). The coupling constant -J=17 Hz, shows a trans geometry of the vinylic protons. (C, 58.53; H, 3.04; N, 4.31; $C_{16}H_{10}O_2NBr$ requires C, 58.53; H, 3.04; N, 4-26%).

Similarly, other 2-styryl -6,8-disubstituted benzoxazin-4-(3H)-ones(I) were synthesised (Table 2).

2-Styryl-3-(4-morpholinophenyl)-quinazolin-4-(3H)-one (IIa) (Table-2). It was synthesised by refluxing an equimolar (0.0025 mole) mixture of 2-styryl benzoxazin-4(3H)-one and p-morpholinoaniline, in pyridine (20 ml) on a sand bath for 6 hrs with occasional shaking. The reaction mixture was colled at room temperature and poured into a mixture of ice and conc. HCl (50 gm & 20 ml) and left overnight. The solid separated was

Table 2.

Physical data of 2-styryl-6, 8-disubstituted benzoxazin-4(3H)ones (I) and 2-styryl-3,6,8-trisubstituted quinazolin-4(3H)-ones (II)

Compd.*	X^1	X^2	m.p.+ (°C)	Yield (%)	Mol. formula	C(%) Found	C(%) Calc.	H(%) Found	H(%) Calc.	N(%) Found	N(%) Calc.
Ia	H	H	146-48 (Lit. [17] 147-148)	97	$C_{16}H_{11}O_2N$	–	–	–	–	–	–
Ib	Br	H	196	95	$C_{16}H_{10}O_2NBr$	58.49	58.53	2.97	3.04	4.31	4.26
Ic	I	H	186	90	$C_{16}H_{10}O_2NI$	50.92	51.20	2.66	2.66	3.83	3.73
Id	Br	Br	187 (Lit. [17] 185-86)	96	$C16H_9O_2NBr_2$	–	–	–	–	–	–
IIa	H	H	208	79	$C_{26}H_{23}N_3O_2$	76.08	76.28	5.59	5.62	10.41	10.26
IIb	Br	H	140	75	$C_{26}H_{22}N_3O_2Br$	64.09	63.93	5.37	4.50	8.71	8.60
IIc	I	H	213	80	$C_{26}H_{22}N_3O_2I$	58.22	58.31	3.98	4.11	7.86	7.85
IId	Br	Br	220	76	$C_{26}H_{21}N_3O_2Br_2$	54.89	55.02	3.83	3.70	7.51	7.40
IIe	H	H	136	70	$C_{21}H_{15}N_3O$	77.41	77.53	4.72	4.61	13.05	12.92
IIf	Br	H	166	72	$C_{21}H_{14}N_3OBr$	62.17	62.37	3.44	3.46	10.12	10.39
IIg	I	H	150	65	$C_{21}H_{14}N_3OI$	55.82	55.87	2.95	3.10	9.16	9.31
IIh	Br	Br	172	75	$C_{21}H_{13}N_3OBr_2$	51.92	52.17	2.68	2.69	8.69	8.69
IIi	H	H	226	82	$C_{22}H_{16}N_4O_2$	71.69	71.74	4.12	4.34	15.49	15 21
IIj	Br	H	265	75	$C_{22}H_{15}N_4O_2Br$	58.78	59.06	3.15	3.35	12.59	12.52
IIk	I	H	264	80	$C_{22}H_{15}N_4O_2I$	53.41	53.44	2.82	3.03	10.99	11.33
III	Br	Br	270	81	$C_{22}H_{14}N_4O_2Br_2$	50.21	50.19	2.87	2.66	10.63	10.64
IIm	H	H	194	85	$C_{24}H_{17}N_5O_6$	60.87	61.14	3.76	3.60	15.02	14.86
IIn	Br	H	198	80	$C_{24}H_{16}N_5O_6Br$	52.19	52.36	3.01	2.90	12.71	12.72
IIo	I	H	188	85	$C_{24}H_{16}N_5O_6I$	48.61	48.24	3.19	2.68	11.79	11.72
IIp	Br	Br	182	85	$C_{24}H_{15}N_5O_6Br_2$	45.21	45.78	2.29	2.38	10.91	11.12

*Substitution at position-3 in II is as: 4-morpholino–phenyl for IIa-d; pyridine-2yl for IIe-h; isonicotinamido for IIi-l & 2,4-dinitrophenyl acetamido for IIm-p.

+Compounds Ia-d were recrystallised from benzene, IIa-h from ethanol & IIi-p from dioxane.

filtered, washed several times with water and finally recrystallised from ethanol, m.p. 208°C, yield 79%.

I.R.: 3050 & 2830 (Ar & Ali C-H), 1680 (-C=O), 1645 (-CH=CH-), 1620 (C=N), 1520, 1440 & 1380 etc. PMR: 7.95 (d, J=16.9 Hz, 1H, Ph-CH=), 6,81 to 7.65 (complex multiplet, 13H, Ar-H), 6.2 (d, J=16.9 Hz, 1H, N=C-CH=), 3,67 (t, J=6.2 Hz, 4H, $-CH_2-O-CH_2$), 3.09 (t, 5.6 Hz, 4H, $-CH_2-N-CH_2$) (Found C, 76.08; H, 5.59; N, 10.41; $C_{26}H_{23}N_3O_2$ requires C, 76.28; H, 5.62; N, 10.26).

Similarly, other 2-styryl-3,6,8-trisubstituted-quinazolin 4(3H)-ones (II) were synthesised using different (I) and appropriate amines or acid hydrazides in pyridine. Their relevant data are given in Table-2, whereas the spectral data of some of them are as;

IIf I.R.: 3100 (Ar C-H), 1675 (N-C=0), 1650 (-CH=CH-), 1620 & 1590 (C=N), 1420, 1380 etc; PMR: 7.95 (d, J=15 Hz, 1H, Ph-CH=), 6.85 to 7.81 (complex multiplet, 12H, Ar-H) & 6.22 (d, J=15 Hz, 1H, N=C-CH=).

IIk I.R.: 3400 & 3040 (N-H & Ar C-H), 1710 (noncyclic N-C=0), 1690 (cyclic C=0), 1655 (-CH=CM-), 1620 & 1600 (C=N), 1480 & 1420 etc; PMR: 11.31 (s, 1H, N-H), 7.92 (d, J=15.5 Hz, 1H, Ph-CH=), 6.85 to 7.82 (complex multiplet 12 H, Ar-H) & 6.25 (d, J= 15.5 Hz, 1H, N=C-CH=).

IIp I.R. 3400, 3080, 2910 (N-H, Ar & Ali C-H), 1720 (noncyclic N-C=0), 1680 (cyclic N-C=0), 1650 (-CH=CH-) 1600 (C=N), 1530, 1340 etc. PMR: 11.42 (s, 1H, N-H), 7.96 (d, 15 Hz, 1H, Ph-CH=), 6.79 to 7.83 (complex multiplet, 10H, Ar-H), 6.21 (d, J= 15Hz, 1H, N=C-CH=) & 4.65 (s, 2H, $-CH_2-$).

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