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Synthesis and Antihypertensive Activity of 6, 8- Dibromo-3-Phenyl-2-Substituted Styryl-Quinazolin-4(3*h*) ones

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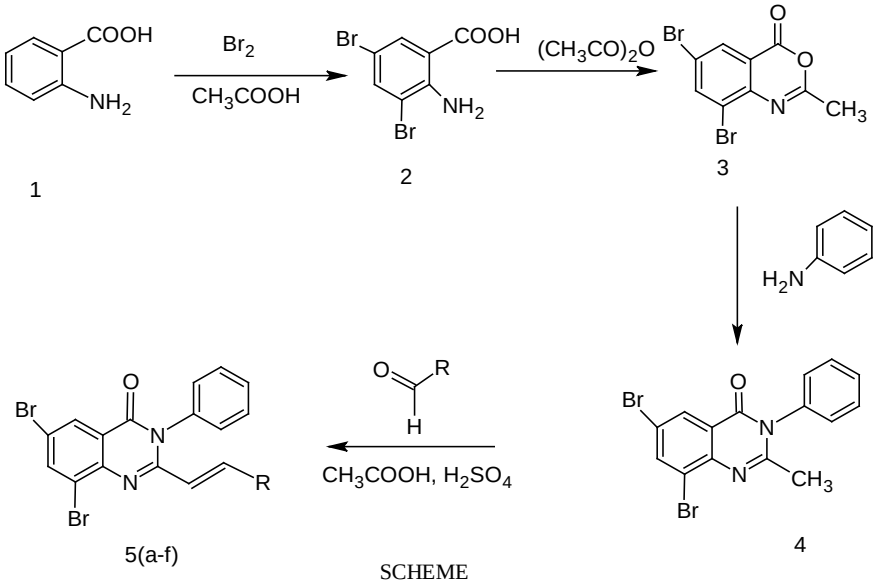
Abstract: A series of 6,8-dibromo-3-phenyl-2-substituted quinazolin-4-(3*H*)ones 5(a-f) have been synthesized from 6,8-dibromo-3-phenyl-2-methyl-quinazolin-4-(3H)one (4) with substituted aromatic aldehydes in presence of glacial acetic acid and concentrated sulphuric acid. The intermediate 6,8-dibromo-2-methyl benzoxazin-4-ones (3). All the newly synthesized compounds have been screened in *Vivo* for their antihypertensive activity. Compounds 5_a, 5_b, 5_e and 5_f were found to be potent members of this series; compound 5_a has exhibited better antihypertensive activity than the standard drug. The structures of the new compounds were established on the basis of their IR, ^{1}HNMR and Mass spectral studies.

Keywords: Anthranilic acid, Acetic anhydride, Quinazoline, Antihypertensive.

INTRODUCTION:

Quinazolinones have been found to exhibit diverse pharmacological activities such as antihypertensive[1], anthemintic[2], anti-inflamatory[3], antitubercular[4], anticonvulsant[5], hypoglycemic[6], antibacterial[7-8], and anti-HIV[9]. Hypertension is still a dangerous disease affecting millions of people all over world. In view of this an attempt has been made to explore quinazolinones as potential antihypertensive agents.

In the present investigation we have reported the synthesis and antihypertensive activity of title compounds 6,8-dibromo-3-phenyl-2-substituted styryl-quinazolin-4(3*H*) ones 5(a-f).(**Scheme 1)**



SCHEME

MATERIALS AND METHODS

The melting points were determined in open capillaries using melting point apparatus and are uncorrected. Purity of the compounds were checked by TLC on pre coated TLC plates having silica gel F28 as adsorbent using pet ether and ethyl acetate (8:2) as mobile phase. the spots were located using UV at 254nm. IR spectra were recorded in KBr on a Shimadzu FTIR 8400 spectrophotometer using KBr pellets techique. ^{1}H NMR spectra were obtained on the AMX-400 liquid state spectrometer at 400 MHz in $CDCl_3$. Mass spectrum were recorded on Shimadzu GCMS spectrometer 210.

Synthesis of 3, 5 dibromo anthranilic acid (2)

To a solution of 40g of anthranilic acid in 500ml of glacial acetic acid, 19ml of bromine was added at a temperature of 16^{o}C. The resulting mixture was extracted with 1 L of water containing 50ml of concentrated HCl. 3,5- Dibromo anthranilic acid precipited out was filtered, washed with distilled water, dried and recrystallised from aqueous ethanol; M.P 225^{o}C; yield 27.86%; Rf =0.13. IR: 686 (C-Br), 1679 (C = O), 3030 (CH of aromatic ring), 3083, 3363 (NH),^{1}H NMR ($CDCl_3$): 7.7-8.2 (s, 2H, Ar H), 6.5 (s, 2H, NH_2).

Synthesis of 6, 8- dibromo-2-methyl-benzoxazin-4-one (3)

A mixture of **(2)** [36g and 13ml of acetic anhydride were taken in a 500ml round bottom flask and refluxed for 1 hr]. the reaction mixture was cooled to get white crystals, which was filtered and washed with distilled water; m.p 164 ^{o}C; yield 64.5%; Rf = 0.8. IR 686 (C – Br), 1753 (C = O), 3030 (CH of aromatic ring). ^{1}H NMR ($CDCl_3$): 2.7 (s, 3H, CH_3), 8.1-8.3(s, 2H, ArH).

Synthesis of 6, 8-dibromo-2-methyl-3-phenyl-quinazolin-4-one (4)

A mixture of **(3)** 24 g (0.075 mole) and aniline (0.086 mole) were heated on a free flame for 15 min. A jelly like mass obtained on cooling was treated with ethanol, to get a solid mass. This crude product was collected by filtration, dried and recrystallised using ethanol; m.p. 200^{o}C; yield 56.6%; Rf = 0.59. IR: 656 (C-Br). 1444, 1587 (C=N, C=C of aromatic ring), 1681 (C = O), ^{1}H NMR ($CDCl_3$):2.2 (s, 3H, CH_3), 7.2-8.3 (m, 7H, ArH).

Synthesis of 6, 8-dibromo-2-substituted styryl-3-phenyl-quinazolin-4one 5(a-f)

Compound (**4)** (0.01 mole) and substituted aldehydes (0.01mole) were solubilised in glacial acetic acid (25ml). To this reaction mixture 5 drops of concentrated sulphuric acid was added and refluxed for 4-16 hr. the reaction mixture was cooled and poured onto ice cold water to get precipitate which is filtered, dried and washed with petroleum ether 40/60 to get pure compounds 5 (a-f).

Antihypertensive activity:

Guinea Pig Ileum model

All the title compounds were screened for antihypertensive activity at 0.4 mg dose using prozosin (α1-receptor antagonist) as the standard. Compound $\mathbf{5_a}$ reduced the contraction of noradrenaline to 100%. Compounds **5f, 5e,** and **5b** reduced the contraction of noradrenaline to 77.8%, 69%, and 55.65% respectively. Compounds $\mathbf{5_c}$ and $\mathbf{5_d}$ did not show activity above 30% blockade.

Effect of Heart Rate in Normotensive Rats:

Heart rate of normotensive rats was recorded on surgivet V3404 ECG/pulse oximeter. Compound **5f, 5b, 5a** and **5e** produced fall in blood pressure by 7.6, 6.2 %, 5.6% and 5.4% respectively. Prozosin produced 6.3% fall in blood pressure. Compounds 5c and 5d were least hypotensive in comparision to prozosin producing fall in blood pressure by 0.4% and 1.3% respectively.

RESULTS:

The structure, physical properties, yield, Rf values, Melting point, IR, Mass and NMR data are as follows.

Table -1: physicochemical properties of the synthesized analogues

Analogues	Substituent (R)	Yield (%)	Rf value	M.P. (^{o}C)
5a	p-methoxy phenyl	40	0.48	228
5b	p-dimethylamino phenyl	29	0.51	243
5c	p-nitro phenyl	23	0.86	197
5d	m-nitro phenyl	25	0.38	169
5e	2-thienyl	29	0.64	215
5f	o-chloro phenyl	30	0.46	168

5_a: 6, 8-dibromo-2-[2-(4-methoxyphenyl) ethenyl]-3-phenyl quinazolin-4(3*H*)-one

6, 8-dibromo-2-methyl-3-phenyl-quinazolin-4one (0.39gm, 0.01 mol), *4*-methoxy benzaldehyde (0.13gm, 0.01 mol). IR: 686 (C-Br). 1301 (CH_3), 1444 (CH_3), 1587, 1444(C = C, C = N of aromatic ring), 3030 (CH of heteroaromatic ring), ^{1}H NMR (CDCl3): 3.5 (s, 3H, OCH_3), 6.15-6.25

(d, 2H, styryl), 7.2-8.4(m, 11H, ArH), MS m/z = 513(M^+).

5_b: 6, 8-dibromo-2(2-[4-(dimethylamino) phenyl] ethenyl-3-phenylquinazolin-4(3*H*)-one

6, 8-dibromo-2-methyl-3-phenyl-quinazolin-4one (0.39gm, 0.01 mol), dimethylamino benzaldehyde (0.14gm, 0.01 mol) IR: 700 (C-Br), 1367 (CH =CH), 1438 (CH_3), 1598 (C = C of aromatic ring). ^{1}H NMR ($CDCl_3$): 3 (s, 6H, $N(CH_3)_2$), 6.05-6.15 (d,2H,styryl), 7.2-8.4(m, 11H, ArH), MS m/z = 526(M^+).

5_c: 6, 8-dibromo-2-[2-(4-nitrophenyl) ethenyl]-3-phenyl quinazolin-4(3*H*)-one

6, 8-dibromo-2-methyl-3-phenyl-quinazolin-4one(0.39gm, 0.01 mol), *4*-nitro benzaldehyde(0.15gm, 0.01 mol) IR: 698 (C-Br).1344 (CH =CH), 1519 (C =C of aromatic ring), 1529 (C-NO_2), ^{1}H NMR ($CDCl_3$): 6.4-6.6 (d, 2H, styryl), 7.2-8.5 (m, 11H, ArH), MS m/z = 528(M^+).

5_d: 6, 8-dibromo-2-[2-(3-nitrophenyl) ethenyl]-3-phenyl quinazolin-4(3*H*)-one

6, 8-dibromo-2-methyl-3-phenyl-quinazolin-4one(0.39gm, 0.01 mol), *3*-nitro benzaldehyde(0.15gm, 0.01 mol) IR: 698 (C-Br).1353 (CH =CH), 1529 (C-NO_2), 1529 (C =C of aromatic ring), ^{1}H NMR ($CDCl_3$): 6.4-6.6 (d, 2H, styryl), 7.2-8.5 (m, 11H, ArH), MS m/z = 528(M^+).

5_e: 6, 8-dibromo-3-phenyl-2-[2-(2 sulfanylphenyl) ethenyl] quinazolin-4(3*H*)-one

6, 8-dibromo-2-methyl-3-phenyl-quinazolin-4one(0.39gm, 0.01 mol), *2*-thiobenzaldehyde(0.13gm, 0.01 mol) IR: 696 (C-S), 696 (C-Br).1398 (CH =CH), 1535 (C = C of aromatic ring), ^{1}H NMR ($CDCl_3$): 6.1-6.2 (d, 2H, styryl), 6.9-8.4 (m, 10H, ArH), MS m/z = 488(M^+).

5_f: 6, 8-dibromo-2-[2-(2-chlorophenyl) ethenyl]-3-phenyl quinazolin-4(3*H*)-one

6, 8-dibromo-2-methyl-3-phenyl-quinazolin-4one(0.39gm, 0.01 mol), *2*-chloro benzaldehyde(0.14gm, 0.01 mol) IR: 698 (C-Br).1346 (CH =CH), 1560 (C = C of aromatic ring), 754 (C-Cl), ^{1}H NMR ($CDCl_3$): 6.3-6.5 (d, 2H, styryl), 7.1-8.6 (m, 10H, ArH), MS m/z = 488(M^+).

Antihypertensive activity:

Guinea Pig Ileum model

All synthesized analogues were screened for antihypertensive activity using Guinea Pig Ileum model against Prozosin as standard drug. The contraction and heights were measured from the base line. Guinea pig of either sex weighing between 250-300g was selected and fasted overnight, being allowed water *ad libitum.* The animals were sacrificed under anesthesia and the lower portion of the ileum was isolated. 3cm long muscles strip was suspended by fixing the tissue to the bottom of the aerator tube with a thread in tyrode solution maintained at 37^{o}C. and the other end was attached to a writing point. The preparation was aerated with oxygen and allowed to stabilize for 90mins. The muscle responses were recorded on a slow moving kymograph using a frontal writing point having a tension of 0.5g weight.

After the stabilization period, the control maximum response for nor adrenaline was recorded and muscle was washed after each response. The antagonist was then injected, allowed to act and then the contractions were elicited using selected dose of nor adrenaline.

Nor adrenaline 0.4mg contracts the ileum to 36mm and results are tabulated **(Table No 2).**

Table No.2 Effect of synthesized analogues on Contraction of the guinea pig ileum

Analogues	Conc^n	Contraction of the muscle in mm.	%Reduction (blockade)
Nor Adr	0.4mg	36	-
5a	0.4mg	0	100%
5b	0.4mg	16	55.6%
5c	0.4mg	26	27.8%
5d	0.4mg	27	25.0%
5e	0.4mg	11	69.0%
5f	0.4mg	08	77.8%
Prozosin	0.4mg	0	100%

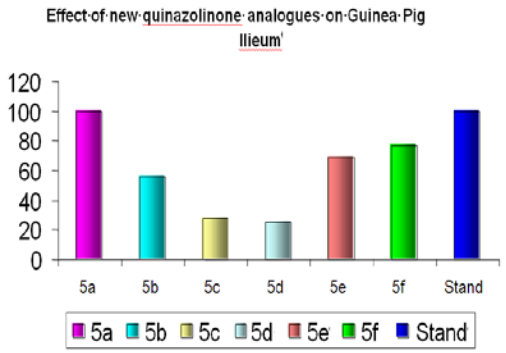


Fig 1. Antihypertensive screening of 6 analogues on Contraction of guinea pig ilieum

Table No.3 Effect of 6 analogues on Heart Rate in Normotensive Rat

Derivatives	Beats per Minute		% Fall in Blood Pressure
	Before Treatment	After Treatment	
5a	276.833±2.982	261.33 ±3.148	5.6%**
5b	275.5± 4.185	258.33±4.061	6.2%*
5c	275.5±3.594	274.166±4.483	0.485%
5d	268.5±4.006	265±3.958	1.3%
5e	289.833±5.689	274±3.276	5.45%*
5f	291.833±4.339	269.5±3.905	7.6%**
Standard	280.5±2.527	262.833±2.574	6.3%**

Note: P ≤ 0.05* considered significant, P ≤ 0.01** very significant,

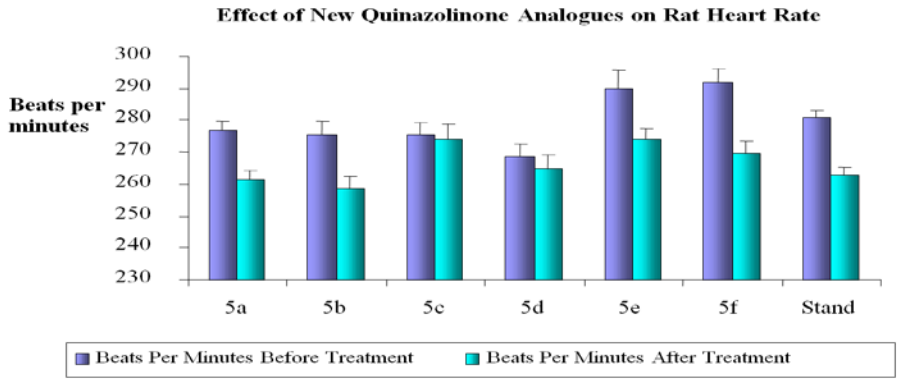


Fig 2: Effect of 6 analogues on Heart Rate in Normotensive Rats.

DISCUSSION:

During the synthesis of intermediate 2 (3, 5 dibromo anthranilic acid) the anthranilic acid could be brominated easily by maintaining the temp at 10^{o}C in the presence of glacial acetic acid with continuous stirring. The mechanism involves free radical substitution of the aromatic ring. The cherry red colour disappeared with the formation of a white coloured precipitate but the TLC plate shows two spots indicating the presence of two products. Mono bromo anthranilic acid is soluble in distilled water containing hydrochloric acid and 3, 5 dibromo anthranilic acid which is a cream white coloured precipitate. The yield of the later compound is more compared to the former one.

In the third step (3) cyclisation was brought about using acetic anhydride. The intermediate 3(6, 8-dibromo-2-methyl-4H-3-1-benzoxazin-4-one) was obtained in needle like crystalline form.

In the fourth step (4) complete quinazolinone molecule (6, 8 dibromo-2-methyl-3-phenyl quinazoline-4(3H)-one could be obtained by a simple heating of aniline and 6, 8-dibromo-2-methyl-4H,3-1-benzoxazin-4-one over a free flame.

The above two steps could not be completed using mono bromo derivatives. Hence the scheme synthesis of mono bromo was dropped.

The synthesis of derivative 1 to 6 was carried out with different substituted benzaldehyde in the presence of glacial acetic acid and 5 drops of sulphuric acid. This is a condensation reaction with the removal of water molecule. The synthesis was favorable due to the presence of two electronegative atoms, which facilitated the removal of proton of the methyl group present in the second place of the quinazolinone molecule.

We failed in the second method using ethanol as a solvent in the presence of few drops of glacial acetic acid and refluxed for about 24hrs.

The literature survey shows that quinazoline 4-one molecule possess hypotensive activity at position 2, 3, 6 substitution. We screened the six derivatives of quinazoline 4-ones and result found that 5a abolished contractions of noradrenaline similar to prozosin. Whereas 5b, 5e, and 5f reduced contractions of noradrenaline

to 55.6%, 69% and 77.8% respectively. But the analogs 5c and 5d did not show activity above 30% blackade. The above results shows electron releasing group shows better activity when compared to electron withdrawing groups.

CONCLUSION:

Compounds **5_a, 5_b, 5_e,** and **5_f** produced significant antihypertensive activity comparable to prazosin. Further structural activity relationship studies have to be carried out on these compounds to get a better antihypertensive activity.

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