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ANTIOXIDANT, ANTIINFLAMMATORY AND ANTIHISTAMINIC ACTIVITIES OF SOME PHENYLPYRAZOLO BENZOTHIAZOLO QUINOXALINE DERIVATIVES

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ABSTRACT

Pharmacologically, phenyl pyrazolo benzothiazolo quinoxaline derivatives are known to exhibit important biological activities like antimicrobial[1], antitubercular[2], anti-inflammatory[3], antioxidant[4], antihistamic[5], antidepressant, hypoglycemic, hypotensive, anticarcenogenic activities[6] etc. In view of these reports, the synthesis of title compounds has been undertaken in the present note and their antioxidant, anti-inflammatory and antihistaminic properties are reported. The structure of chalcones and phenyl pyrazolo benzothiazolo quinoxaline derivatives were confirmed by M. P, TLC and spectral data.

Key words: 2, 3-Diphenyl quinoxaline, 2-Amino benzothiazole, Phenyl pyrazolo benzothiazolo quinoxaline, Antioxidant, Anti-inflammatory, Antihistaminic activity.

INTRODUCTION

2, 3-Diphenyl quinoxaline **(SI)** is fused with 2-amino benzothiazoles **(SII)** by a methylene bridge, which is then allowed for acetylation. The acetylated product **(SIV)** is made to react with different aromatic aldehydes to give chalcones **(SV 1 - SV 5)**. Chalcones refluxed with substituted acid hydrazides to afford different phenyl pyrazolo benzothiazolo quinoxaline derivatives **(SVI 1-SVI 15)**. Pharmacologically, phenyl pyrazolo benzothiazolo quinoxaline derivatives are known to exhibit important biological activities like antimicrobial[1], antitubercular[2], anti-inflammatory[3], antioxidant[4], antihistamic[5], antidepressant, hypoglycemic, hypotensive, anticarcenogenic activities[6] etc. In view of these reports, the synthesis of title compounds has been undertaken in the present note **(Scheme 1)** and their antioxidant, anti-inflammatory and antihistaminic properties are reported.

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EXPERIMENTAL

Materials and methods

The melting point of the compounds were determined on a Thoshniwal electric melting point apparatus and the values were uncorrected. I. R spectra of the compounds were recorded on a Thermo Nicolet Nexus 670-FTIR, IICT, Hyderabad using KBr disc method. ^{1}H NMR spectra were recorded on Avance-300, IICT, Hyderabad using $CDCl_3$ as solvent. Mass spectra were recorded on HITACHI RMU GL, IICT, Hyderabad. All the solvents used were of analytical grade.

Synthesis of 6-((2, 3-diphenylquinoxalin-6yl) methyl)benzo[d]thiazol-2-amine (SIII 7)

General procedure

2, 3-Diphenyl quinoxaline (**SI**) and 2-aminobenzothiazole (**SII**) were prepared following the literature method. **(SI)** and **(SII)** are linked with a methelyne bridge by treating equimolar quantities of **(SI)** and **(SII)** in suitable solvent with 35 parts formaldehyde solution and 35% HCl, stirring for 4 hr. at 70ºCC using magnetic stirrer. Solution was made alkaline using ammonia solution. The product was filtered and recrystallized with aq. ethanol.

Synthesis of 1-(6-((2, 3-diphenylquinoxalin-6-yl) methyl) benzo [d] thiazol – 2 –yl amino) propan-2-one (SIV)[8]

General procedure

A solution of **(SIII**) (0.01M) and chloroacetone (0.01M) were taken into 250 mL round bottom flask. 150 mL of dry acetone and 30g of anhyd. potassium carbonate were added to it and the reaction mixture were refluxed for 6 hr. below 75^{o}CC. Filterate obtained was concentrated under vaccum and recrystallized with aq. ethanol.

(Z)-1-(6-((2, 3-diphenylquinoxalin-6-yl) methyl) benzo [d] thiazol-2-ylamino)-4-phenylbut-3-en-2-one (SV1-SV5)[9]

General procedure

Method of aldol condensation was followed. A solution of NaOH / KOH (8 mL, 10% in water) was added dropwise to a well-stirred solution of **(S IV)** (0.01M) and (0.01M) of appropriate aldehyde in 20 mL ethanol. The reaction mixture was stirred for 24 hr. at cold conditions; then diluted with ice water and acidified with Conc. HCl. The product was filtered and recrystallized with aq. ethanol. The purity of the compound was checked by TLC and melting point.

Synthesis of (3-(6-((2, 3-diphenylquinoxalin-6yl) methyl) benzo [d] thiazol-2-yl ami- no) methyl)-4, 5-dihydro-5-phenylpyrazol-1- yl) (phenyl) methanone (SVI 1–SVI 15)[10]

General procedure

Chalcone (0.01M) and aromatic acid hydrazide (0.02M) were taken in 20 mL glacial acetic acid and refluxed for 10 hr. above 130ºC. The reaction mixture was concentrated and poured in 300 mL of ice-cold water and recrystallized with aq. ethanol. The purity of the compound was checked by TLC and melting point. Physical data are shown in Table 1

Table 1. Physical data of benzothiazolyl 2, 3-diphenyl quinoxaline pyrazoline derivatives

Compd.	X	Ar	Molecular Formula	Melting point range (°C)	% Yield	R_f value
SVI 1	H	C_6H_5	$C_{45}H_{34}N_6OS$	120-122	70	0.89
SVI 2	OH	C_6H_5	$C_{45}H_{34}N_6O_2S$	115-116	67	0.80
SVI 3	F	C_6H_5	$C_{45}H_{33}ClN_6OS$	114-116	66	0.87
SVI 4	Cl	C_6H_5	$C_{45}H_{33}FN_6OS$	112-113	78	0.90
SVI 5	OCH_3	C_6H_5	$C_{46}H_{36}N_6O_2S$	114-116	67	0.86
SVI 6	H	OHC_6H_4	$C_{45}H_{34}N_6O_2S$	120-124	66	0.91
SVI 7	OH	OHC_6H_4	$C_{45}H_{34}N_6O_3S$	119-120	80	0.93
SVI 8	F	OHC_6H_4	$C_{45}H_{33}ClN_6O_2S$	108-110	45	0.90
SVI 9	Cl	OHC_6H_4	$C_{45}H_{33}FN_6O_2S$	112-115	45	0.80
SVI 10	OCH_3	OHC_6H_4	$C_{45}H_{36}N_6O_3S$	110-112	67	0.89
SVI 11	H	ClC_6H_4	$C_{45}H_{33}ClN_6OS$	110-112	56	0.88
SVI 12	OH	ClC_6H_4	$C_{45}H_{33}ClN_6O_2S$	120-122	78	0.82
SVI 13	F	ClC_6H_4	$C_{45}H_{32}Cl_2N_6OS$	130-131	76	0.79
SVI 14	Cl	ClC_6H_4	$C_{45}H_{32}ClFN_6OS$	120-124	56	0.98
SVI 15	OCH_3	ClC_6H_4	$C_{46}H_{35}ClN_6O_2S$	123-126	54	0.80

Pharmacological evaluation

Antioxidant activity[11]

DPPH method : All drugs have been diluted in 95% ethanol to get 250, 100, 50, 25 and 10 µg/mL concentrations. DPPH solution (2µ mol) has been prepared by 95% ethanol. Then 0.5 mL of drug solution and 0.5 mL of DPPH solution (freshly prepared) were added. 0.5 mL of DPPH solution and 0.5 mL of ethanol were used as control. Reaction mixture was allowed for 20 min. UV absorbance was measured at 517 nm. The percentage of scavenging has been calculated by the equation given below. Ascorbic acid was used as standard drug. The results are shown in Table 2.

Anti-inflammatory activity

Carrageenan induced rat hind paw edema method :

Male albino rats weighing between 100 – 200 g, individually housed, provided with adequate food and water. These were divided into various groups. These animals were used for anti-inflammatory studies. Six pyrazoline derivatives were screened for anti-inflammatory activity. The toxicity studies were performed and it was found that no visible toxic symptoms were observed for the first two hours and no death was reported after 24 hours. Among various doses, 2000 mg/kg body weight was observed as safe dose, the $1/10^{th}$ of 2000 mg/kg body weight i. e., 200 mg/kg body weight was fixed as the dose for acute anti-inflammatory screening

The method of Winter et al.[12] was used with slight modification. The apparatus used for the measurement of rat paw volume was that of Buttle. et al., modified by Sharma *et al*. The animals were divided into eight groups of six animals each. One group served as a standard (Ibuprofen) and another group served as control (1% CMC) and rest of the groups were used for the test drugs**.** Food was withdrawn overnight with adequate water before the experiment. The drugs were given orally. After 1 hour, a sub plantar injection of 0.05 mL of 1% Carrageenan was administered. The volume of the injected paw was measured with a plethysmograph immediately. The paw volume was again measured after 3 hours. The average paw volume in a group of drug treated rats were compared with that of a group with vehicle (control group) and the percentage inhibition of edema was calculated using the formula.

$$\% \text{ inhibition} = (1 - Vt / Vc) \times 100$$

where Vt = Mean volume of the test drug, Vc = Mean volume of the control

The results are shown in Table 3.

Table 2. Anti-oxidant activity of benzothiazolyl 2, 3-diphenyl quinoxaline pyrazoline derivatives

Comp	Control	10 mg	25 mg	50 mg	100 mg	250 mg	500 mg	1000 mg
STD	0.1 ± 0.092	0.055 ± 0.019 (45.00)	0.029 ± 0.017 (71.00)	0.020 ± 0.01 (80.00)	0.018 ± 0.009 (82.00)	0.012 ± 0.008 (88.00)	0.010 ± 0.007 (90.00)	0.005 ± 0.005 (95.00)
SVI 1	0.13 ± 0.092	0.12 ± 0.07 (7.60)	0.11 ± 0.06 (15.30)	0.09 ± 0.057 (30.70)	0.089 ± 0.049 (31.53)	0.08 ± 0.02 (38.40)	0.07 ± 0.013 (46.15)	0.06 ± 0.001 (53.80)
SVI 2	0.12 ± 0.092	0.11 ± 0.079 (8.30)	0.09 ± 0.074 (25.00)	0.09 ± 0.071 (25.00)	0.08 ± 0.05 (33.00)	0.07 ± 0.04 (41.60)	0.06 ± 0.03 (50.00)	0.04 ± 0.012 (66.60)
SVI 3	0.11 ± 0.092	0.1 ± 0.08 (9.09)	0.09 ± 0.071 (18.18)	0.06 ± 0.071 (45.45)	0.05 ± 0.05 (4.54)	0.03 ± 0.03 (75.00)	0.02 ± 0.022 (90.00)	0.01 ± 0.02 (0.90)
SVI 4	0.1 ± 0.052	0.09 ± 0.044 (10.00)	0.08 ± 0.04 (20.00)	0.08 ± 0.017 (20.00)	0.07 ± 0.013 (30.00)	0.06 ± 0.011 (40.00)	0.06 ± 0.012 (40.00)	0.05 ± 0.007 (50.00)
SVI 5	0.12 ± 0.112	0.1 ± 0.11 (16.16)	0.07 ± 0.09 (41.60)	0.07 ± 0.07 (41.60)	0.06 ± 0.06 (50.00)	0.05 ± 0.04 (58.30)	0.04 ± 0.03 (66.60)	0.03 ± 0.02 (75.00)
SVI 6	0.14 ± 0.019	0.1 ± 0.044 (28.50)	0.09 ± 0.01 (35.70)	0.06 ± 0.007 (57.14)	0.05 ± 0.0069 (64.20)	0.04 ± 0.005 (71.40)	0.04 ± 0.002 (71.40)	0.03 ± 0.001 (75.00)
SVI 7	0.12 ± 0.0192	0.09 ± 0.018 (25.00)	0.06 ± 0.016 (50.00)	0.05 ± 0.013 (58.30)	0.05 ± 0.003 (58.30)	0.04 ± 0.002 (66.60)	0.03 ± 0.001 (75.00)	0.01 ± 0.001 (91.60)

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Comp	Control	10 mg	25 mg	50 mg	100 mg	250 mg	500 mg	1000 mg
SVI 8	0.13 ± 0.0162	0.1 ± 0.017 (23.00)	0.09 ± 0.012 (30.70)	0.08 ± 0.01 (38.40)	0.08 ± 0.0091 (38.40)	0.07 ± 0.0091 (46.10)	0.06 ± 0.006 (53.80)	0.05 ± 0.004 (61.50)
SVI 9	0.11 ± 0.0192	0.1 ± 0.016 (9.00)	0.1 ± 0.013 (9.00)	0.09 ± 0.015 (18.18)	0.08 ± 0.01 (27.20)	0.08 ± 0.01 (27.20)	0.07 ± 0.009 (36.30)	0.06 ± 0.002 (45.00)
SVI 10	0.1 ± 0.016	0.08 ± 0.015 (20.00)	0.07 ± 0.016 (30.00)	0.06 ± 0.006 (40.00)	0.05 ± 0.005 (50.00)	0.04 ± 0.004 (60.00)	0.03 ± 0.003 (70.00)	0.03 ± 0.002 (70.00)
SVI 11	0.14 ± 0.0192	0.13 ± 0.018 (7.14)	0.12 ± 0.0127 (14.20)	0.11 ± 0.01 (21.40)	0.1 ± 0.09 (28.50)	0.09 ± 0.03 (35.70)	0.08 ± 0.01 (42.80)	0.08 ± 0.009 (42.80)
SVI 12	0.12 ± 0.192	0.09 ± 0.17 (25.00)	0.075 ± 0.012 (37.50)	0.07 ± 0.098 (41.60)	0.065 ± 0.09 (45.80)	0.06 ± 0.05 (50.00)	0.06 ± 0.03 (50.00)	0.03 ± 0.027 (75.00)
SVI 13	0.11 ± 0.0192	0.07 ± 0.017 (39.30)	0.065 ± 0.016 (40.90)	0.06 ± 0.0151 (45.40)	0.06 ± 0.008 (45.40)	0.03 ± 0.009 (72.70)	0.02 ± 0.007 (81.80)	0.009 ± 0.007 (91.80)
SVI14	0.1 ± 0.019	0.06 ± 0.019 (40.00)	0.06 ± 0.009 (40.00)	0.05 ± 0.007 (50.00)	0.03 ± 0.006 (70.00)	0.02 ± 0.003 (80.00)	0.009 ± 0.001 (91.00)	0.005 ± 0.001 (95.00)
SVI 15	0.11 ± 0.192	0.068 ± 0.137 (38.10)	0.06 ± 0.016 (45.40)	0.03 ± 0.01 (72.70)	0.02 ± 0.01 (81.80)	0.01 ± 0.003 (90.90)	0.008 ± 0.003 (92.70)	0.005 ± 0.002 (95.40)

Table 3. Anti-inflammatory studies of benzothiazolyl 2, 3-diphenyl quinoxaline pyrazoline derivatives

Compound	Dose (mg/kg)	Mean edema volume ± S. E. (0-3 hrs)	% Reduction
Control	-	0.40 ± 0.162	-
Ibuprofen	200	0.03 ± 0.15	92.5
SVI 3	200	0.12 ± 0.144[a]	70.0
SVI 7	200	0.09 ± 0.14[a]	77.5
SVI 2	200	0.07 ± 0.13[a]	89.6
SVI 13	200	0.041 ± 0.128[a]	82.5
SVI 14	200	0.035 ± 0.127[a]	91.2
SVI 15	200	0.032 ± 0.122[a]	92.0

One-way ANOVA followed by Schiffe's post hoc test.

Allowance value = 0.239.a = $P < 0.05$ (Vs) control.

Table 4. Antihistaminic studies of benzothiazolyl 2, 3-diphenyl quinoxaline pyrazoline derivatives

Compd.	X	Ar	Mol. formula	Onset of convulsions (s) Mean ± SD	% Protection
SVI 1	H	C_6H_5	$C_{45}H_{34}N_6OS$	999 ± 90	89.1
SVI 2	OH	C_6H_5	$C_{45}H_{34}N_6O_2S$	1035 ± 98	89.5
SVI 3	F	C_6H_5	$C_{45}H_{33}ClN_6OS$	1135 ± 98	90.4
SVI 4	Cl	C_6H_5	$C_{45}H_{33}FN_6OS$	1160 ± 96	90.6
SVI 5	OCH_3	C_6H_5	$C_{46}H_{36}N_6O_2S$	955 ± 91	88.6

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Compd.	X	Ar	Mol. formula	Onset of convulsions (s) Mean ± SD	% Protection
SVI 6	H	OHC_6H_4	$C_{45}H_{34}N_6O_2S$	1070 ± 92	89.9
SVI 7	OH	OHC_6H_4	$C_{45}H_{34}N_6O_3S$	1137 ± 91	90.5
SVI 8	F	OHC_6H_4	$C_{45}H_{33}ClN_6O_2S$	1020 ± 92	89.4
SVI 9	Cl	OHC_6H_4	$C_{45}H_{33}FN_6O_2S$	1020 ± 92	89.4
SVI 10	OCH_3	OHC_6H_4	$C_{45}H_{36}N_6O_3S$	990 ± 96	89.0
SVI 11	H	ClC_6H_4	$C_{45}H_{33}ClN_6OS$	999 ± 92	89.1
SVI 12	OH	ClC_6H_4	$C_{45}H_{33}ClN_6O_2S$	1000 ± 95	89.2
SVI 13	F	ClC_6H_4	$C_{45}H_{32}Cl_2N_6OS$	1022 ± 96	89.4
SVI 14	Cl	ClC_6H_4	$C_{45}H_{32}ClFN_6OS$	1133 ± 92	90.4
SVI 15	OCH_3	ClC_6H_4	$C_{46}H_{35}ClN_6O_2S$	1170 ± 92	90.7
Control				108 ± 12	
CPM				1228 ± 65	92.0

Anti-histaminic activity[13]

Histamine chamber method

In this method, thirty two healthy adult guinea pigs of either sex divided into group of 2 animals each weighing around 400 g, fasted overnight, were kept in histamine chamber and exposed to histamine aerosol (0.5 % aqueous solution of histamine acid phosphate in a Nebulizer) until they collapse. Those that collapse within 2 minutes were revived with fresh air and used for this test. Twelve hours later, the animals were given an oral dose of test compound suspended in 1% acacia solution and after 1 hour for absorption, the guinea pigs were again exposed to the same concentration of histamine aerosol. Those that do not collapse within 6 minutes are deemed protected. Percentage protection has been measured by using the following formula:

$$[1\text{-}T_1 / T_2] \text{ X } 100$$

Where T_1 was the mean of control preconvulsion time in vehicle treated group and T_2 was the mean of control preconvulsion time in drug treated group.. The results are shown in Table 4.

RESULTS AND DISCUSSION

All the synthesized compounds were synthesized through the depicted in the **Scheme I** and confirmed by IR, ^{1}H NMR, Mass spectroscopy. Benzothiazole and diphenyl-quinoxaline were prepared and both were connected with methylene bridge. Chalcones were refluxed with substituted acid hydrazides to afford different phenyl pyrazolo benzothiazolo quinoxaline derivatives **(SVI 1-SVI 15)**. All the compounds shown significant antioxidant activity among them **(SVI -13)** 36.3%, **(SVI -14)** 40% and **(SVI -15)** 38.1% were showed good free radical scavenging activity. In the anti-inflammatory activity compounds **(SVI – 14)** 91.25% and, **(SVI 15)** 92% showed good inhibition of edema volume and compounds **(SVI -3)**, **(SVI -4)**, **(SVI -7)** and **(SVI -15)** showed good % protection of antihistamic activity i. e., 90.4%, 90.6%, 90.5% and 90.7%, respectively.

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