Synthesis and Pharmacological Evaluation of Some Phenylpyrazolo Indoquinoxaline Derivatives

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Abstract: Indoloquinoxalin was fused with 2,3 diphenyl quinoxaline by a methylene bridge which was then allowed for acetylation. The acetylated product was made to react with different aromatic aldehydes to give chalcones. Chalcones refluxed with substituted acid hydrazides to afford different indoloquinoxaline pyrazolines. The structure of chalcones and indoloquinoxaline pyrazolines were confirmed by M.P, TLC and spectral data. All the synthesized compounds were screened for their antioxidant, anti-inflammatory and antihistaminic activities.

Keywords: Indoloquinoxalin, Pyrazoline, Antioxidant, Anti-inflammatory and Antihistaminic activity.

Introduction

Quinoxaline derivatives have been reported to possess a wide variety of biological activities¹⁻³. Notable among these are antioxidant, anti-inflammatory antimicrobial, anti-cancer and antihistaminic activities. Drugs having pyrazoline ring system⁴⁻⁶ are well known for their anti-inflammatory, antioxidant, antihistaminic, antimicrobial, antidepressant, hypoglycemic, hypotensive, anticarcinogenic activities *etc.* In view of the above facts, we report here in the synthesis of some indoloquinoxaline pyrazoline derivatives by condensing indoloquinoxaline chalcones with different aromatic acid hydrazides. All the synthesized compounds (**IQVI 1- IQVI 15**) were screened for their antioxidant, anti-inflammatory and antihistaminic activities. The structure of chalcones and indoloquinoxaline pyrazolines were confirmed by M.P, TLC, Spectral data (Table 1 & 2).

Experimental

The melting point of the compounds was 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. ¹H NMR spectra were recorded on Avance-300, IICT, Hyderabad using CDCl₃ as solvent. Mass spectra were recorded on GCMS-Auto tuning-EI at IICT, Hyderabad. All the solvents used were of analytical grade.

Table 1. Physical data of phenylpyrazolo indoloquinoxaline derivatives

Comp.	X	Ar	Molecular Formula	Melting point range, °C	% Yield	R _f value
IQVI1	H	C ₆ H ₅	C ₅₂ H ₃₇ N ₇ O	119-120	62	0.60
IQVI2	OH	C ₆ H ₅	C ₅₂ H ₃₇ N ₇ O ₂	122-123	45	0.9
IQVI3	F	C ₆ H ₅	C ₅₂ H ₃₆ N ₇ O ₃ F	158-160	75	0.66
IQVI4	Cl	C ₆ H ₅	C ₅₂ H ₃₆ N ₇ OCl	165-166	78	0.7
IQVI5	OCH ₃	C ₆ H ₅	C ₅₃ H ₃₉ N ₇ O ₂	123-125	63	0.82
IQVI6	H	OHC ₆ H ₄	C ₅₂ H ₃₇ N ₇ O ₂	119-120	32	0.88
IQVI7	OH	OHC ₆ H ₄	C ₅₂ H ₃₇ N ₇ O ₃	122-123	57	0.87
IQVI8	F	OHC ₆ H ₄	C ₅₂ H ₃₆ N ₇ O ₂ F	158-160	67	0.8
IQVI9	Cl	OHC ₆ H ₄	C ₅₂ H ₃₆ N ₇ O ₂ Cl	165-166	68	0.89
IQVI10	OCH ₃	OHC ₆ H ₄	C ₅₃ H ₃₉ N ₇ O ₃	112-115	54	0.83
IQVI11	H	ClC ₆ H ₄	C ₅₂ H ₃₆ N ₇ OCl	119-120	58	0.78
IQVI12	OH	ClC ₆ H ₄	C ₅₂ H ₃₆ N ₇ O ₂ Cl	120-123	72	0.85
IQVI13	F	ClC ₆ H ₄	C ₅₂ H ₃₅ N ₇ OClF	158-160	76	0.9
IQVI14	Cl	ClC ₆ H ₄	C ₅₂ H ₃₅ N ₇ OCl ₂	160-163	66	0.89
IQVI15	OCH ₃	ClC ₆ H ₄	C ₅₃ H ₃₈ N ₇ O ₂ Cl	120-123	62	0.75

Table 2. Spectral data of phenyl pyrazolo indoloquinoxaline derivatives

Compd	M ⁺	¹ H NMR, ppm	IR, cm ⁻¹
IQVI1	775,776,777	7.17,7.48-7.95, 7.32,7.48,3.8,3.9,4.9	3030,2945,1675
IQVI2	791,792,793	7.17, 7.48-7.9, 7.32-7.48, 3.8,3.9, 4.8, 5.0	3350,3030,2945,1675
IQVI3	793,794,795,796	7.16, 7.48-7.92, 7.32-7.48, 3.8,3.92, 4.8,6.92	3010,2985,1685
IQVI4	809,810,811,812	7.16, 7.48-7.92, 7.32-7.48,3.8,3.92,4.7,7.06-7.22	3027,2955,1695
IQVI5	805,806,807,808	7.16, 7.48-7.92, 7.32-7.48, 3.8,3.9,3.73,6.72	3010,2975,1663
IQVI6	791,792,793,794	7.15, 7.48-7.92, 7.32-7.48, 3.8,3.92,4.8,5.1	3033,2955,1645
IQVI7	807,808,809,810	7.16, 7.4-7.9, 7.32-7.48,3.8,3.92, 4.8, 6.68-7.78,5.0	3382,3037,2949,1677
IQVI8	809,810,811,812	7.17, 7.48-7.92, 7.32-7.48, 3.82,3.92,4.8,6.14-7.7, 6.98-7.1, 5.2	3387,3036,2945,1675
IQVI9	825,826,827,828	7.16, 7.48-7.9, 7.32-7.4, 3.8,3.92,4.8,6.91-7.78,5.0	3389,3024,2945,1675
IQVI10	821,822,823,824	7.16, 7.48-7.92, 7.32-7.48, 3.8,3.92,4.8,6.72-7.78, 3.73, 5.0	3387,3030,2945,1675
IQVI11	809,810,811,812	7.17, 7.48-7.92, 7.32-7.48, 3.84,3.9,4.8,6.92	3028,2925,1678
IQVI12	825,826,827,828	7.16, 7.48-7.92, 7.32-7.48, 3.8,3.92,4.8,5.0	3037,2948,1679
IQVI13	827,828,829,830	7.15, 7.4-7.92, 7.32-7.48, 3.82,3.92,4.8,6.9-7.0	3072, 2946,1677
IQVI14	843,844,845,846	7.16, 7.48-7.92, 7.32-7.48, 3.8,3.92,4.8,7.6-7.88	3014,2955,1695
IQVI15	839,840,841,842	7.16, 7.48-7.92, 7.32-7.48, 3.8,3.92,4.8,6.72-7.89	3039,2935,1676

Preparation

Synthesis of 2-((2,3-diphenylquinaxaline-6-yl)methyl)-6H-indolo[2,3-b] quinoxaline⁷

Equimolar quantities of 6H-indolo[2,3-b]quinoxalin (**IQ I**) and 2,3-diphenyl quinoxaline (**IQ II**) taken in a suitable solvent with 35 parts formaldehyde solution and 35% HCl, The solution was stirred for 4 h at 70 °C using magnetic stirrer. The purity of the compound was checked by TLC and melting point.

General procedure for Synthesis of 1-(2-((2, 3-diphenylquinoxaline-6-yl) methyl)-6H-indolo [2, 3-b] quinoxaline-6-yl) propan-2-one⁸

2-((2, 3-Diphenylquinaxaline-6-yl)methyl)-6H-indolo[2, 3-b]quinoxaline (**IQ III**) (0.01 M) and chloro acetone (0.01 M) were taken into 250 mL round bottom flask. 150 mL of dry acetone and 30 g of anhydrous potassium carbonate was added to it and the reaction mixture was refluxed for 6 h at 75 °C. Filtrate obtained was concentrated under vacuum. The purity of the compound (**IQ IV**) was checked by TLC and melting point.

General procedure for Synthesis of 1-(2-((2, 3-diphenylquinaxaline-6-yl) methyl)-6H-indolo[2, 3-b]quinaxaline-6-yl)but-3-en-2-one⁹

A solution of NaOH/KOH (8 mL, 10% in water) was added drop wise to a well stirred solution of (0.01 M) 1-(2-((2, 3-diphenylquinaxaline-6-yl) methyl)-6H-indolo[2, 3-b]quinaxaline-6-yl) propan-2-one (**IQ IV**) and 0.01 M appropriate *p*-aryl aldehyde in 20 mL ethanol at cold temperature. The reaction mixture was stirred for 24 h. at room temperature. Then diluted with ice water and acidified with Con.HCl. The product was filtered and recrystallized with aqueous ethanol. The purity of the compounds (**IQV 1-IQV 5**) was checked by TLC and melting point.

General procedure for Synthesis of 4,5-dihydro-5-phenyl-3-((2-((2,3-diphenylquinaxaline-6-yl)methyl)-6H-indolo[2,3-b]quinaxaline-6-yl)pyrazol-1-yl)(phenyl)methanone¹⁰

1-(2-((2, 3-Diphenylquinaxaline-6-yl) methyl)-6H-indolo[2, 3-b]quinaxaline-6-yl)but-3-en-2-one (*i.e.*, Chalcones) (0.01 M) and aromatic acid hydrazide (0.02 M) were taken in 20 mL glacial acetic acid and refluxed for 10 h above 130 °C. The reaction mixture was concentrated and poured in 300 mL of ice-cold water and recrystallized with aq.ethanol. The purity of the compounds (**IQVI 1- IQVI 15**) was checked by TLC and melting point. The physical data are shown in Table 1.

Pharmacological evaluation

Antioxidant activity¹¹

DPPH method

All drugs have been diluted in 95% ethanol to get 1000 µg, 500 µg, 250 µg, 100 µg, 50 µg and 25 µg, 10 µg/mL concentrations. DPPH solution (2 µ.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 (Table 3).

Table 3. Antioxidant activity of phenylpyrazolo indoloquinoline derivatives

Compd	Control	10 µg	25 µg	50 µg	100 µg	250 µg	500 µg	1000 µg
STD	0.1±	0.06±	0.029±	0.020±	0.018±	0.012±	0.010±	0.005±
	0.092	0.017	0.016	0.0141	0.01	0.009	0.008	0.007
IQVI 1	0.12±	0.11±	0.1±	0.091	0.09±	0.088±	0.08±	0.07±
	0.092	0.07	0.06	0.057	0.049	0.02	0.013	0.001
IQVI 2	0.100±	0.089±	0.083±	0.075±	0.059±	0.059±	0.05±	0.042±
	0.092	0.079	0.074	0.071	0.05	0.05	0.03	0.012
IQVI 3	0.11±	0.06±	0.049±	0.03±	0.02±	0.015±	0.0125±	0.011±
	0.092	0.08	0.071	0.071	0.05	0.03	0.022	0.02
IQVI 4	0.11±	0.09±	0.08±	0.071±	0.062±	0.06±	0.05±	0.05±
	0.052	0.044	0.04	0.017	0.013	0.011	0.012	0.007
IQVI 5	0.101±	0.05±	0.05±	0.046±	0.037±	0.032±	0.021±	0.02±
	0.112	0.11	0.11	0.07	0.06	0.04	0.03	0.02
IQVI 6	0.14±	0.102±	0.099±	0.09±	0.09±	0.06±	0.05±	0.05±
	0.019	0.044	0.01	0.007	0.007	0.0069	0.005	0.005
IQVI 7	0.13±	0.12±	0.112±	0.10±	0.09±	0.08±	0.07±	0.06±
	0.0192	0.018	0.016	0.013	0.003	0.002	0.001	0.001
IQVI 8	0.101±	0.102±	0.059±	0.04±	0.028±	0.02±	0.01±	0.009±
	0.0162	0.044	0.012	0.01	0.0091	0.0091	0.006	0.004
IQVI 9	0.13±	0.12±	0.112±	0.10±	0.09±	0.08±	0.08±	0.07±
	0.0192	0.018	0.016	0.015	0.01	0.009	0.009	0.002
IQVI 10	0.11±	0.09±	0.08±	0.05±	0.03±	0.02±	0.019±	0.011±
	0.016	0.015	0.016	0.006	0.005	0.004	0.003	0.002
IQVI 11	0.13±	0.122±	0.088±	0.08±	0.07±	0.04±	0.03±	0.02±
	0.0192	0.018	0.0127	0.012	0.09	0.03	0.01	0.009
IQVI 12	0.101±	0.089	0.069±	0.06±	0.06±	0.041±	0.037±	0.01±
	0.192	±0.17	0.012	0.098	0.09	0.05	0.03	0.027
IQVI 13	0.101±	0.06±	0.059	0.04±	0.03±	0.0162±	0.0153	0.0074±
	0.0192	0.017	±0.016	0.0151	0.008	0.009	0.007±	0.007
IQVI 14	0.12±	0.05±	0.048±	0.04±	0.035±	0.03±	0.01±	0.009±
	0.019	0.019	0.009	0.007	0.006	0.003	0.001	0.001
IQVI 15	0.101±	0.041±	0.04±	0.038±	0.03±	0.02±	0.01±	0.008±
	0.192	0.137	0.016	0.01	0.01	0.003	0.003	0.002
		(59.40)	(60.39)	(62.37)	(70.29)	(80.19)	(90.09)	(92.07)

One-way ANOVA followed by Dunnett's post HOC test; The numbers given in the brackets were the % scavenging.

Anti-inflammatory activity*Carrageenan induced rat hind paw edema method*

The method of Winter *et al.*¹² was used with slight modification. The apparatus used for the measurement of rat paw volume was phlethismograph. 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 h, 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 h. The average paw volume in a group of drug treated rats was compared with that of a group with vehicle (control group) and the percentage inhibition of oedema was calculated using the formula (Table 4).

$$\% \text{ Inhibition} = (1 - V_t / V_c) \times 100$$

V_t = Mean volume of the test drug, V_c = Mean volume of the control.

Table 4. Anti-inflammatory activity of phenyl pyrazolo indoloquininoxaline derivatives

S.No.	Compound	Dose, mg/kg	Mean edema volume ± S.E., 0-3 h	% Reduction
1.	Control		0.40±0.162	
2.	Ibuprofen	200	0.03±0.15	92.5
3.	IQVI3	200	0.24±0.145 ^a	40.0
4.	IQVI5	200	0.26±0.14 ^a	35.0
5.	IQVI8	200	0.25±0.11 ^a	37.5
6.	IQVI13	200	0.09±0.02 ^a	77.5
7.	IQVI14	200	0.075±0.003 ^a	81.2
8.	IQVI15	200	0.04±0.0014 ^a	90.0

One-way ANOVA followed by Schiff's post HOC test. Allowance value = 0.239; $\alpha = P < 0.05$ (Vs) control.

Anti-histaminic activity¹³*Histamine chamber method*

In this method thirty two healthy adult guinea pigs of either sex divided into 8 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 h 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 calculating the time of onset of convulsions (Table 5).

Results and Discussion

All the compounds were synthesized through the depicted in the Scheme 1 and confirmed by IR, ¹H NMR, mass spectroscopy. Indoquinaxoline and diphenyl quinaxoline and both these quinoxalines were connected with methylene bridge, which is then allowed for acetylation. The acetylated product (**IQ IV**) was made to react with different aromatic aldehydes to give chalcones (**IQV 1-IQV 5**). Chalcones refluxed with substituted acid hydrazides to afford different phenylpyrazolo indoloquininoxaline derivatives (**IQVI 1-IQVI 15**). All the compounds shown significant antioxidant activity among them **IQVI-3** 45.45%, **IQVI 5** 50.9%, **IQVI 8** 27.12%, **IQVI13** 40.59%, **IQVI 14** 58.33% and **IQVI 15** 59.40% showed good free radical scavenging activity (Table 3). In the anti-inflammatory activity, compounds **IQVI 13** 77.50% **IQVI 14** 81.25 and **IQVI 15** 90.00% showed good inhibition of edema volume (Table 4)

and compounds **IQVI 3** 90.4%, **IQVI 4** 90.7% **IQVI 8** 90.5% and **IQVI 10** 90.9% showed good % protection of antihistamic activity (Table 5).

Scheme 1

Table 5. Antihistaminic activity of phenylpyrazolo indoloquinoxaline derivatives

Comp	X	Ar	Mol.formula	Onset of Convulsions	% Protection
				(s) Mean±SD	
IQVI 1	H	C ₆ H ₅	C ₅₂ H ₃₇ N ₇ O	994±90	89.1
IQVI 2	OH	C ₆ H ₅	C ₅₂ H ₃₇ N ₇ O ₂	1045±98	89.6
IQVI 3	F	C ₆ H ₅	C ₅₂ H ₃₆ N ₇ O ₃ F	1125±92	90.4
IQVI 4	Cl	C ₆ H ₅	C ₅₂ H ₃₆ N ₇ OCl	1165±96	90.7
IQVI 5	OCH ₃	C ₆ H ₅	C ₅₃ H ₃₉ N ₇ O ₂	945±91	88.5
IQVI 6	H	OHC ₆ H ₄	C ₅₂ H ₃₇ N ₇ O ₂	964±90	89.7
IQVI 7	OH	OHC ₆ H ₄	C ₅₂ H ₃₇ N ₇ O ₃	1074±93	89.9
IQVI 8	F	OHC ₆ H ₄	C ₅₂ H ₃₆ N ₇ O ₂ F	1137±94	90.5
IQVI 9	Cl	OHC ₆ H ₄	C ₅₂ H ₃₆ N ₇ O ₂ Cl	1024±95	89.4
IQVI 10	OCH ₃	OHC ₆ H ₄	C ₅₃ H ₃₉ N ₇ O ₃	1194±96	90.9
IQVI 11	H	ClC ₆ H ₄	C ₅₂ H ₃₆ N ₇ O Cl	980±92	88.9
IQVI 12	OH	ClC ₆ H ₄	C ₅₂ H ₃₆ N ₇ O ₂ Cl	999±95	89.1
IQVI 13	F	ClC ₆ H ₄	C ₅₂ H ₃₅ N ₇ OClF	828±86	86.96
IQVI 14	Cl	ClC ₆ H ₄	C ₅₂ H ₃₅ N ₇ OCl ₂	1012±94	89.33
IQVI 15	OCH ₃	ClC ₆ H ₄	C ₅₃ H ₃₈ N ₇ O ₂ Cl	756±82	85.71
Control				108±12	
CPM				1228±65	91.20

Conclusion

Indoquinoxaline and diphenylquinoxaline both these quinoxalines were connected with methylene bridge, which is then allowed for acetylation. The acetylated product (**IQ IV**) is made to react with different aromatic aldehydes to give chalcones (**IQV 1-IQV 5**). Chalcones refluxed with substituted acid hydrazides to afford different phenyl pyrazolo indoloquinoxaline derivatives (**IQVI 1-IQVI 15**). The synthesized compounds were checked for antioxidant, anti-inflammatory and antihistaminic activity.

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