

SYNTHESIS AND BIOLOGICAL EVALUATION OF SOME INDOQUINOXALINE PYRAZOLINES

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ABSTRACT

Indoquinoxalin (Q-I) is fused with 2,3 diphenyl quinoxaline(Q-II) by a methylene bridge which is then allowed for acetylation. The acetylated product (Q-IV) is made to react with different aromatic aldehydes to give chalcones(QVI-QV5). Chalcones refluxed with substituted acid hydrazides to afford different indoquinoxaline pyrazolines (QVII-QVII5) The structure of chalcones and indoquinoxaline pyrazolines were confirmed by M.P, TLC and Spectral data. All the synthesized compounds were screened for their antioxidant, anti-inflammatory and antihistamic activities.

Key words: *Indoquinoxalin, Pyrazoline, Antioxidant, Anti-inflammatory and Antihistamiine.*

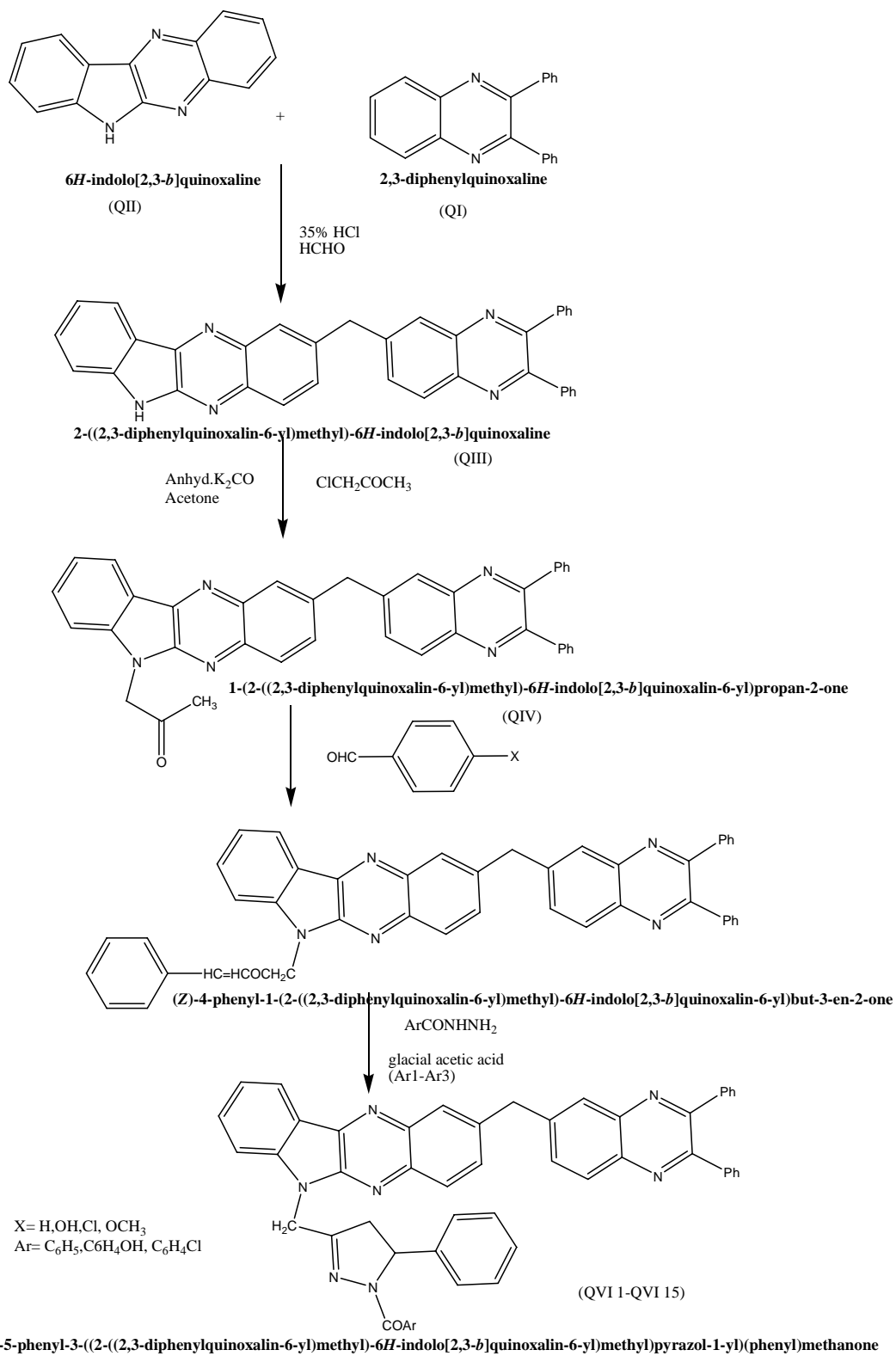
INTRODUCTION

Quinoxaline derivatives have been reported to possess a wide variety of biological activities^{1,2,3}. Notable among these are antioxidant, anti-inflammatory antimicrobial, anti-cancer and antihistamic activities. Drugs having pyrazoline ring system^{4,5,6} are well known for their anti-inflammatory, antioxidant, antihistamic, antimicrobial, antidepressant, hypoglycemic, hypotensive, anticarcinogenic activities etc. In view of the above facts, we report here in the synthesis of some indoquinaxoline pyrazoline derivatives by condensing indoquinoxaline chalcones with different aromatic acid hydrazides. All the synthesized compounds were screened for their antioxidant, anti-inflammatory and antihistamic activities. The structure of chalcones and indoquinoxaline pyrazolines were confirmed by M.P, TLC, Spectral data.

EXPERIMENTAL

The melting point of the compounds was determined on a Thoshniwal electric melting point apparatus and the values were uncorrected. I.R spectrs of the compounds were recorded on a Thermo Nicolet Nexus 670-FTIR, ICT, Hyderabad using KBr disc method. 1H NMR spectra were recorded on Avance-300, ICT, Hyderabad using CDCl₃ as solvent. Mass spectra were recorded on HITACHI RMU GL at 70 eV.

SCHEME



Synthesis of 2-((2,3-diphenylquinaxaline-6-yl)methyl)-6H-indolo[2,3-b] quinaxaline⁷.

Equimolar quantities of 6H-Indolo[2,3-b]quinoxalin (QI) and 2,3-diphenyl quinaxaline(QII) in suitable solvent with 35 parts formaldehyde solution and 35% HCl, stirring for 4 h at 70⁰C using magnetic stirrer. The purity of the compound was checked by TLC and melting point.

Synthesis of 1-(2-((2, 3-diphenylquinaxaline-6-yl) methyl)-6H-indolo [2, 3-b] quinaxaline-6-yl) propan-2one⁸.

2-((2, 3-diphenylquinaxaline-6-yl) methyl)-6H-indolo [2, 3-b] quinaxaline (QIII) (0.01M) and chloroacetone (0.01M) were taken into 250ml round bottom flask. Added to it 150ml of dry acetone and 30g of anhydrous potassium carbonate and the reaction mixture was refluxed for 6h at 75⁰C. Filtrate obtained was concentrated under vacuum. The purity of the compound was checked by TLC and melting point.

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 (8ml, 10% in water) was added dropwise to a well stirred solution of 1-(2-((2, 3-diphenylquinaxaline-6-yl) methyl)-6H-indolo [2, 3-b] quinaxaline-6-yl) propan-2one (QIV) (0.01M) and (0.01M) of appropriate P-aryl aldehyde in 20ml ethanol at room temperature. The reaction mixture was stirred for 24hr. at room temperature. Then diluted with ice water and acidified with Conc.HCl. Filtered the product and recrystallized with aq.ethanol. The purity of the compound was checked by TLC and melting point.

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)(0.01M) and aromatic acidhydrazide(0.02M) were taken in 20ml glacial acetic acid and refluxed for 10h above 130⁰C. The reaction mixture was concentrated and poured in 300ml of ice-cold water and recrystallized with aq.ethanol. The purity of the compound 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 250µg, 100µg, 50µg, 25µg, 10µg/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. 0.5ml 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 3.

Anti-inflammatory activity**Carrageenan induced rat hind paw oedema method:**

Male albino rats weighing between 100 – 200g, individually housed, provided with adequate food and water. They were divided into various groups. These animals were used for antiinflammatory studies. Six pyrazoline derivatives were screened for antiinflammatory activity. The toxicity studies were performed and found, 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 1/10th of 2000 mg/kg body weight i.e., 200mg/kg body weight was fixed as the dose for acute antiinflammatory screening. The method of Winter.*et.al*,¹² (Winter.*et.al*.1962) 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 oedema was calculated using the formula.

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

V_t = Mean volume of the test drug

V_c = Mean volume of the control

The results are shown in table 4.

Anti-histaminic activity¹³

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 calculating the time of onset of convulsions. The results are shown in table 5.

RESULTS AND DISCUSSION

All the synthesized compounds were synthesized through the depicted in the Scheme I and confirmed by IR, 1H-NMR, Mass Spectroscopy. Indo quinaxoline and Diphenyl- quinaxoline and both quinaxalines were connected with methylene bridge were prepared. All the compounds shown significant antioxidant activity among them QVI-13 39.31% and QVI-15 28.21% were showed good free radical scavenging activity. In the antiinflammatory activity compounds QVI 13 83.89% and, QVI 14 80.49% were showed good inhibition of oedema volume and compounds QVI 10, QVI 4 ,QVI 3 were shown good %protection of antihistaminic activity i.e., 90.9%, 90.7%, 90.4% respectively

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Table 1. PHYSICAL DATA OF INDOQUINOXALINE PYRAZOLINE DERIVATIVES

Comp.	X	Ar	Molecular Formula	Melting point range (°C)	% Yield	R _f value
QVI1	H	C ₆ H ₅	C ₅₂ H ₃₇ N ₇ O	119-120	62	0.60
QVI2	OH	C ₆ H ₅	C ₅₂ H ₃₇ N ₇ O ₂	122-123	45	0.9
QVI3	F	C ₆ H ₅	C ₅₂ H ₃₆ N ₈ O ₃	158-160	75	0.66
QVI4	Cl	C ₆ H ₅	C ₅₂ H ₃₆ N ₇ OCl	165-166	78	0.7
QVI5	OCH ₃	C ₆ H ₅	C ₅₃ H ₃₉ N ₇ O ₂	123-125	63	0.82
QVI6	H	OHC ₆ H ₄	C ₅₂ H ₃₇ N ₇ O ₂	119-120	32	0.88
QVI7	OH	OHC ₆ H ₄	C ₅₂ H ₃₇ N ₇ O ₃	122-123	57	0.87
QVI8	F	OHC ₆ H ₄	C ₅₂ H ₃₆ N ₈ O ₄	158-160	67	0.8
QVI9	Cl	OHC ₆ H ₄	C ₅₂ H ₃₆ N ₇ O ₂ Cl	165-166	68	0.89
QVI10	OCH ₃	OHC ₆ H ₄	C ₅₃ H ₃₉ N ₇ O ₃	112-115	54	0.83
QVI11	H	ClC ₆ H ₄	C ₅₂ H ₃₆ N ₇ O Cl	119-120	58	0.78
QVI12	OH	ClC ₆ H ₄	C ₅₂ H ₃₆ N ₇ O ₂ Cl	120-123	72	0.85
QVI13	F	ClC ₆ H ₄	C ₅₂ H ₃₅ N ₈ O ₃ Cl	158-160	76	0.9
QVI14	Cl	ClC ₆ H ₄	C ₅₂ H ₃₅ N ₇ O Cl ₂	160-163	66	0.89
QVI15	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 INDOQUINOXALINE PYRAZOLINE DERIVATIVES

Compound	X	Ar	Mol.formula	M+1	¹ H-NMR (ppm)	IR (cm-1)
QVI1	H	C ₆ H ₅	C ₅₂ H ₃₇ N ₇ O	775,776,777	7.17,7.48-7.95, 7.32,7.48,3.8,3.9,4.9	3030,2945, 1675
QVI2	OH	C ₆ H ₅	C ₅₂ H ₃₇ N ₇ O ₂	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
QVI3	F	C ₆ H ₅	C ₅₂ H ₃₆ N ₇ O ₃ F	793,794,795,79 6	7.16, 7.48-7.92, 7.32- 7.48, 3.8,3.92,4.8,6.92	3010,2985, 1685
QVI4	Cl	C ₆ H ₅	C ₅₂ H ₃₆ N ₇ OCl	809,810,811,81 2	7.16, 7.48-7.92, 7.32- 7.48,3.8,3.92,4.7,7.06- 7.22	3027,2955, 1695
QVI5	OC H ₃	C ₆ H ₅	C ₅₃ H ₃₉ N ₇ O ₂	805,806,807,80 8	7.16, 7.48-7.92, 7.32- 7.48, 3.8,3.9,3.73,6.72	3010,2975, 1663
QVI6	H	OHC ₆ H ₄	C ₅₂ H ₃₇ N ₇ O ₂	791,792,793,79 4	7.15, 7.48-7.92, 7.32- 7.48, 3.8,3.92,4.8,5.1	3033,2955, 1645
QVI7	OH	OHC ₆ H ₄	C ₅₂ H ₃₇ N ₇ O ₃	807,808,809,81 0	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
QVI8	F	OHC ₆ H ₄	C ₅₂ H ₃₆ N ₇ O ₂ F	809,810,811,81 2	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
QVI9	Cl	OHC ₆ H ₄	C ₅₂ H ₃₆ N ₇ O ₂ Cl	825,826,827,82 8	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
QVI10	OC H ₃	OHC ₆ H ₄	C ₅₃ H ₃₉ N ₇ O ₃	821,822,823,82 4	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
QVI11	H	ClC ₆ H ₄	C ₅₂ H ₃₆ N ₇ O Cl	809,810,811,81 2	7.17, 7.48-7.92, 7.32- 7.48, 3.84,3.9,4.8,6.92	3028,2925, 1678
QVI12	OH	ClC ₆ H ₄	C ₅₂ H ₃₆ N ₇ O ₂ Cl	825,826,827,82 8	7.16, 7.48-7.92, 7.32- 7.48, 3.8,3.92,4.8,5.0	3037,2948, 1679
QVI13	F	ClC ₆ H ₄	C ₅₂ H ₃₅ N ₇ OCl F	827,828,829,83 0	7.15, 7.4-7.92, 7.32- 7.48, 3.82,3.92,4.8,6.9- 7.0	3072, 2946,1677
QVI14	Cl	ClC ₆ H ₄	C ₅₂ H ₃₅ N ₇ OCl ₂	843,844,845,84 6	7.16, 7.48-7.92, 7.32- 7.48, 3.8,3.92,4.8,7.6- 7.88	3014,2955, 1695
QVI15	OC H ₃	ClC ₆ H ₄	C ₅₃ H ₃₈ N ₇ O ₂ Cl	839,840,841,84 2	7.16, 7.48-7.92, 7.32- 7.43,8,3.927.89	3039,2935, 1676

Table 3. ANTI OXIDANT ACTIVITY OF INDOQUINOXALINE PYRAZOLINE DERIVATIVES

Com.d	Control	10µg	25µg	50µg	100µg	250µg	500µg	1000µg
STD	0.1± 0.092	0.06± 0.017 (40)	0.029± 0.016 (71)	0.020± 0.0141 (80)	0.018± 0.01 (82)	0.012± 0.009 (88)	0.010± 0.008 (90)	0.005± 0.007 (95)
QVI 1	0.12± 0.092	0.11± 0.07 (7.69)	0.1± 0.06 (15.38)	0.01 0.057 (23)	0.09± 0.049 (30)	0.088± 0.02 (32)	0.08± 0.013 (38.46)	0.07± 0.001 (46.15)
QVI 2	0.100± 0.092	0.089± 0.079 (11.8)	0.083± 0.074 (17.82)	0.075± 0.071 (28.21)	0.059± 0.05 (41.58)	0.059± 0.04 (41.58)	0.061± 0.03 (49.50)	0.042± 0.012 (58.41)
QVI 3	0.11± 0.092	0.093± 0.08 15.45	0.049± 0.071 (60.27)	0.03± 0.071 (72.72)	0.02± 0.05 (81.81)	0.015± 0.03 (85.63)	0.0125± 0.022 (88.63)	0.011± 0.02 (90)
QVI 4	0.11± 0.052	0.09± 0.044 (18.18)	0.08± 0.04 (27.27)	0.071± 0.017 (30.69)	0.062± 0.013 (52.27)	0.06± 0.011 (45.45)	0.05± 0.012 (54.54)	0.05± 0.007 (54.54)
QVI 5	0.101± 0.112	0.075± 0.11 (28.21)	0.07± 0.09 (30.69)	0.071± 0.07 (30.69)	0.0571± 0.06 (43.46)	0.042± 0.04 (58.41)	0.041± 0.03 (59.40)	0.022± 0.02 (78.21)
QVI 6	0.14± 0.019	0.09± 0.044 (18.18)	0.102± 0.01 (26.42)	0.06± 0.007 45.45	0.099± 0.0069 (29.28)	0.05± 0.005 (54.54)	0.088± 0.002 (40.00)	0.08± 0.001 42.85
QVI 7	0.13± 0.0192	0.012± 0.018 (14.28)	0.0142± 0.016 (6.15)	0.10± 0.013 (23.07)	0.09± 0.003 35.71	0.08± 0.002 (38.46)	0.07± 0.001 46.15	0.06± 0.001 52.84
QVI 8	0.101± 0.0162	0.0142± 0.017 (6.15)	0.059± 0.012 (41.58)	0.04± 0.01 (60.39)	0.028± 0.0091 66.36	0.02± 0.0091 (66.36)	0.01± 0.006 (90.00)	0.009± 0.004 (91.08)
QVI 9	0.13± 0.0192	0.0142± 0.016 (6.15)	0.08± 0.013 (20.79)	0.10± 0.015 (23.07)	0.08± 0.01 (27.27)	0.09± 0.01 (30.76)	0.08± 0.009 (38.46)	0.088± 0.002 (40.00)
QVI 10	0.11± 0.016	0.09± 0.015 18.18	0.08± 0.016 (15.38)	0.042± 0.006 (16.81)	0.03± 0.005 (36.36)	0.02± 0.004 (54.54)	0.019± 0.003 (63.63)	0.011± 0.002 (72.72)
QVI 11	0.13± 0.0192	0.122± 0.018 6.15	0.088± 0.0127 (20.00)	0.08± 0.01 (30.76)	0.07± 0.09 (32.30)	0.04± 0.03 (38.46)	0.03± 0.01 (46.15)	0.01± 0.009 (53.84)

QVI 12	0.101± 0.192	0.089 ±0.17 11.88	0.069± 0.012 (23.07)	0.06± 0.098 (31.68)	0.06± 0.09 (40.59)	0.041± 0.05 (49.50)	0.037± 0.03 (49.50)	0.01± 0.027 (60.38)
QVI 13	0.101± 0.0192	0.06± 0.017 (39.62)	0.059 ±0.016 (50.49)	0.04± 0.0151 (60.39)	0.03± 0.008 (70.29)	0.0162± 0.009 (83.96)	0.0153 0.007± (84.85)	0.0074± 0.007 (92.67)
QVI14	0.12± 0.019	0.098± 0.019 (19.00)	0.08± 0.009 (33.88)	0.075± 0.007 (38.01)	0.05± 0.006 (58.67)	0.04± 0.003 (66.94)	0.02± 0.001 (78.51)	0.009± 0.001 (92.56)
QVI 15	0.101± 0.192	0.083± 0.137 (17.82)	0.08± 0.016 (20.79)	0.028± 0.01 (66.36)	0.03± 0.01 (70.29)	0.017± 0.003 (83.16)	0.01± 0.003 (90.09)	0.008± 0.002 (92.07)

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

Table 4. ANTIINFLAMMATORY STUDIES INDOQUINOXALINE PYRAZOLINE DERIVATIVES

S. No.	Compound	Dose (mg/kg)	Mean oedema volume ± S.E. (0-3 hrs)	% Reduction
1.	Control		0.40 ± 0.162	
2.	Ibuprofen	200	0.0416 ± 0.001	92.54
3.	QVI3	200	0.24 ± 0.015 ^a	40.14
4.	QVI5	200	0.26 ± 0.011 ^a	32.89
5.	QVI8	200	0.25 ± 0.017 ^a	40.39
6.	QVI13	200	0.075 ± 0.002 ^a	83.89
7.	QVI14	200	0.09 ± 0.003 ^a	80.49
8.	QVI15	200	0.12±0.0014 ^a	72.79

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

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

Table 5. ANTIHISTAMINIC STUDIES INDOQUINOXALINE PYRAZOLINE DERIVATIVES

Comp.d	X	Ar	Mol.formula	Onset of Convulsions (s) Mean±SD	%Protection
QVI 1	H	C ₆ H ₅	C ₅₂ H ₃₇ N ₇ O	994±90	89.1
QVI 2	OH	C ₆ H ₅	C ₅₂ H ₃₇ N ₇ O ₂	1045±98	89.6
QVI 3	F	C ₆ H ₅	C ₅₂ H ₃₆ N ₇ O ₃ F	1125±92	90.4
QVI 4	Cl	C ₆ H ₅	C ₅₂ H ₃₆ N ₇ OCl	1165±96	90.7
QVI 5	OCH ₃	C ₆ H ₅	C ₅₃ H ₃₉ N ₇ O ₂	945±91	88.5
QVI 6	H	OHC ₆ H ₄	C ₅₂ H ₃₇ N ₇ O ₂	964±90	89.7
QVI 7	OH	OHC ₆ H ₄	C ₅₂ H ₃₇ N ₇ O ₃	1074±93	89.9
QVI 8	F	OHC ₆ H ₄	C ₅₂ H ₃₆ N ₇ O ₂ F	1137±94	90.5
QVI 9	Cl	OHC ₆ H ₄	C ₅₂ H ₃₆ N ₇ O ₂ Cl	1024±95	89.4
QVI 10	OCH ₃	OHC ₆ H ₄	C ₅₃ H ₃₉ N ₇ O ₃	1194±96	90.9
QVI 11	H	ClC ₆ H ₄	C ₅₂ H ₃₆ N ₇ O Cl	980±92	88.9
QVI 12	OH	ClC ₆ H ₄	C ₅₂ H ₃₆ N ₇ O ₂ Cl	999±95	89.1
QVI 13	F	ClC ₆ H ₄	C ₅₂ H ₃₅ N ₇ OClF	828±86	86.96
QVI 14	Cl	ClC ₆ H ₄	C ₅₂ H ₃₅ N ₇ OCl ₂	1012±94	89.33
QVI 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	92.50

(Received: 9 April 2008)

Accepted: 25 April 2008

RJC-172)

**International Conference on Organometallic and
Coordination Chemistry**

Nizhny Novgorod (Russia)

2-8 September 2008

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