International Journal of ChemTech Research
CODEN(USA): IJCRGG ISSN : 0974-4290
Vol.1, No.4, pp 1177-1181, Oct-Dec 2009

Synthesis, Antiinflammatory and Antimicrobial Activities of New Hydrazone and Quinoxaline derivatives

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ABSTRACT: A series of substituted Hydrazone and Quinoxaline derivatives have been synthesized by appropriate methods. All the compounds have been screened for their antibacterial activity against ***staphylococcus aureus*** and ***Escherichia coli***. Some of these compounds have been screened for anti-inflammatory activity against the carageenan induced rat paw edema in albino wistar rats. The structures of the synthesized compounds have been established on the basis of their spectral data.

Key Words: Quinoxaline, Hydrazone carbohydrazide.

INTERODUCTION

The various compounds containing Quinoxaline nucleus has been reported to have various pharmacological activities as anti-microbial[1, 2], anti-inflammatory[3, 4], anti-cancer[5] and anti-HIV[6]. It was anticipated that the combined presence of quinoxaline and 1,3,4- oxadiazole in one framework are reported to have many pharmacological activities as anti-inflammatory[7, 8], anti-microbial[9, 10], anti-cancer[11] and anti-TB[12].

In view of this reaction of diethyl malonate with bromine gave diethyl-dibromomalonate **(I)**. The compound **(I)** on condensation with 4-methyl-o-phenylenediamine in methanol yielded ethyl 2-hydroxy-6-methyl-quinoxaline-3-carboxylate **(II)**. The compound II underwent hydrazinolysis with hydrazine hydrate in methanol yielded 2-hydroxy-6-methyl-quinoxaline-3-carbonyl-hydrazide **(III)**. The compound III on refluxing with different aromatic aldehydes in ethanol furnished N-(2-hydroxy-6-methyl-3-quinoxalinoyl)-N′-(arylidene hydrazine) **(IV-XIII)**. All the compounds synthesized were characterized by their IR and NMR data.

EXPERIMENTAL

The melting points of all compounds were taken in open capillaries & are uncorrected. IR spectra were recorded on Bio-Red FTIR (υ_{max} in cm^{-1}) and ^1H NMR spectra on Bruker 300 MHz instrument in DMSO-d_6/$CDCl_3$ as solvent using TMS as an internal standard. Purity of compounds were checked on silica gel-G plates by TLC.

Synthesis of Diethyl-Dibromomalonate(I): Diethyl malonate (0.66 mole) was taken in a 250 ml conical flask and was placed on a magnetic stirrer. In a separating funnel bromine (0.132 mole) in carbon tetrachloride (30 ml) was taken and was added drop by drop to conical flask containing diethyl malonate with stirring.

It was then refluxed until no more hydrogen bromide was evolved (about 1 hr.). The mixture was cooled and washed five times with 50 ml portion of 5% sodium carbonate solution. The excess of carbon tetrachloride was distilled off.

Synthesis of ethyl 2-hydroxy-6-methyl-quinoxaline-3-carboxylate (II).

The solution of **I** (0.006 mole) and 4-methyl-o-phenylenediamine (0.008 mole;) in methanol (20 ml) was kept for 48 hours. The solid product formed was filtered, washed with cold water and recrystallized from methanol to give **II** as yellow shiny crystals, yield 71.4, mp 170°C. IR: cm^{-1}, 3569 (OH), 1738 (>C=O of ester), 1669 (aromatic ring). ^1H NMR: δ, 1.4 (t, 3H, $-COOCH_2CH_3$), 4.5 (q, 2H, $-COOCH_2CH_3$), 2.5 (s, 3H, CH_3), 7.21-7.26 (m, 2H, Ar-H), 7.8 (d, 1H, Ar-H), 12.2 (d, 1H, OH).

Synthesis of 2-hydroxy-6methyl-quinoxaline-3-carbonyl-hydrazide (III): A mixture of II (0.004 mole) in ethanol (20 ml) and hydrazine hydrate (1 ml) was stirred for one hour on a magnetic stirrer. The resultant yellow compound was filtered, washed with ethanol and recrystallized from ethanol to give III as light yellow crystals, yield 91.3%, mp 248°C. IR: cm^{-1}, 3448 (OH), 3104 (-NH-), 1705 (C=O of amide), 1528 (aromatic ring). ^{1}H NMR: δ, 2.5 (s, 3H, CH_3), 4.6 (s, 1H, -CONH), 7.7 (d, 1H, Ar-H), 7.14-7.22 (m, 2H, Ar-H), 10.1 (s, 2H, NH_2), 12.6 (s, 1H, OH).

Synthesis of Hydrazones from Quinoxaline hydrazides and different aromatic aldehydes (IV-XIII): Compound III (0.002 mole) was dissolved in ethanol (20 ml) and an equimolar quantity of (substituted aromatic aldehydes) (0.002 mole) was added to it. The mixture was refluxed for 4 hours on boiling water bath. The solvent was removed by evaporation and resulting compound was recrystallized from ethanol to give corresponding hydrazone.

(E)-N'-benzylidene-3-hydroxy-7-methylquinoxaline-2-carbohydrazide (IV)

Yield 90%, mp 260°C. IR: cm^{-1}, 3569 (OH), 3142 (-NH-), 1699 (>C=O), 1526 (aromatic ring). ^{1}H NMR: δ, 2.5 (s, 3H, CH_3), 7.17-7.7 (m, 8H, Ar-H), 8.0 (s, 1H, N=CH), 8.3 (s, 1H, NH), 12.1 (s, 1H, OH).

(E)-3-hydroxy-N'-(4-methoxybenzylidene)-7-methylquinoxaline-2-carbohydrazide V.

Yield 89.3%, mp 261-62^{0}C. IR: cm^{-1}, 3451 (OH), 3158 (-NH-), 1699 (>C=O), 1603 (aromatic ring). ^{1}H NMR: δ, 2.5 (s, 3H, CH_3), 3.82 (s, 3H, OCH_3), 6.9-7.8 (m, 7H, Ar-H), 8.2 (s, 1H, N=CH), 8.6 (s, 1H, NH), 12.0 (s, 1H, OH).

(E)-N'-(4-chlorobenzylidene)-3-hydroxy-7-methylquinoxaline-2-carbohydrazide VI

Yield 83.5%, mp 258-61^{0}C. IR: cm^{-1}, 3486 (OH), 3179 (-NH-), 1703 (>C=O), 1530 (aromatic ring). ^{1}H NMR: δ, 2.5 (s, 3H, CH_3), 7.17-7.9 (m, 7H, Ar-H), 8.1 (s, 1H, N=CH), 8.7 (s, 1H, -NH-), 11.3 (s, 1H, OH).

(E)-3-hydroxy-N'-(4-hydroxy-3-methoxybenzylidene)-7-methylquinoxaline-2-carbohydrazide VII

Yield 92.3%, mp 276^{0}C. IR: cm^{-1} 3379 (OH), 3121 (-NH-), 1696 (>C=O), 1513 (aromatic ring). ^{1}H NMR: δ, 2.5 (s, 3H, CH_3), 3.33 (s, 3H, OCH_3), 7.17-7.79 (m, 6H, Ar-H), 8.4 (s, 1H, N=CH), 8.7 (s, 1H, NH), 12.3 (s, 1H, OH), 12.5 (s, 1H, OH).

(E)-3-hydroxy-N'-(2-hydroxybenzylidene)-7-methylquinoxaline-2-carbohydrazide VIII

Yield 85.4%, mp 263-65°C. IR: cm^{-1} 3049 (-NH-), 1691 (>C=O), 1525 (aromatic ring). ^{1}H NMR: δ, 2.5 (s, 1H, CH_3), 6.73-7.8 (m, 7H, Ar-H), 8.3 (s, 1H, N=CH), 8.5 (s, 1H, NH), 11.02 (s, 1H, OH), 12.4 (s, 1H, OH).

(E)-N'-(4-fluorobenzylidene)-3-hydroxy-7-methylquinoxaline-2-carbohydrazide IX

Yield 87.6%, mp 268°C. IR: cm^{-1} 3457 (OH), 3126 (-NH-), 1702 (>C=O), 1545 (aromatic ring). ^{1}H NMR: δ, 2.5 (s, 1H, CH_3), 7.17-7.83 (m, 7H, Ar-H), 8.0 (s, 1H, N=CH), 8.3 (s, 1H, NH), 12.1 (s, 1H, OH).

(E)-N'-(2-chlorobenzylidene)-3-hydroxy-7-methylquinoxaline-2-carbohydrazide X

Yield 80.6%, mp 264-67^{0}C. IR: cm^{-1} 3451 (OH), 3106 (-NH-), 1701 (>C=O), 1529 (aromatic ring). ^{1}H NMR: δ, 2.5 (s, 3H, CH_3), 7.16-8.0 (m, 7H, Ar-H), 8.7 (s, 1H, NH), 8.4 (s, 1H, N=CH), 12.3 (s, 1H, OH).

(E)-3-hydroxy-7-methyl-N'-(3-nitrobenzylidene)quinoxaline-2-carbohydrazide XI

Yield 84.5%, mp 278-79ºC. IR: cm^{-1} 3465 (OH), 3129 (-NH-), 1708 (.C=O), 1529 (aromatic ring). ^{1}H NMR: δ, 2.5 (s, 3H, CH_3), 7.18-8.13 (m, 7H, Ar-H), 8.5 (s, 1H, N=CH), 8.6 (s, 1H, NH), 12.5 (s, 1H, OH).

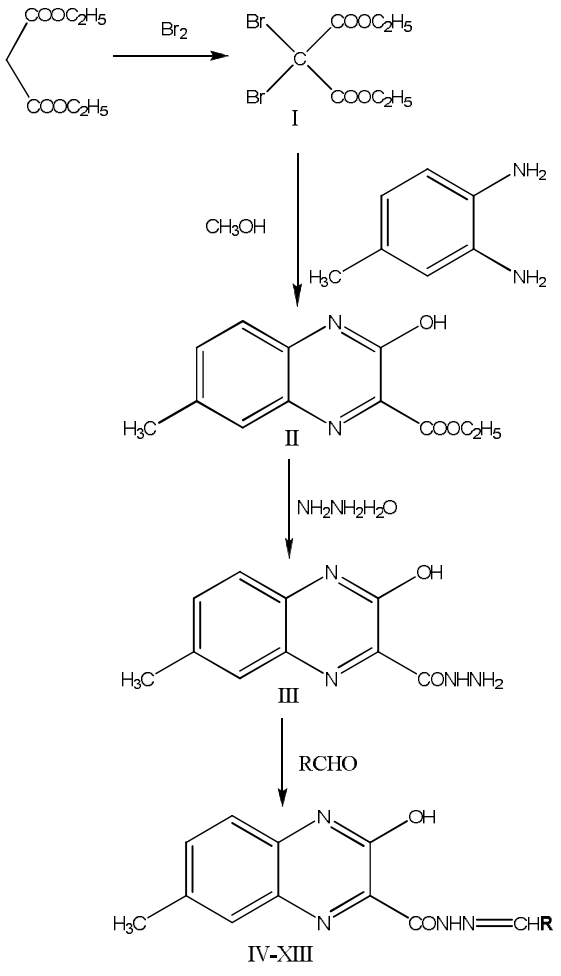
(E)-N'-(4-(dimethylamino)benzylidene)-3-hydroxy-7-methylquinoxaline-2-carbohydrazide XII

Yield 78%, mp 250-51ºC. IR: cm^{-1} 3476 (OH), 3125 (-NH-), 1702 (aromatic ring). ^{1}H NMR: δ, 2.5 (s, 3H, CH_3), 2.99 (s, 6H, -$N(CH_3)_2$), 6.75-7.65 (m, 7H, Ar-H), 8.11 (s, 1H, NH), 8.49 (s, 1H, N=CH).

(E)-3-hydroxy-7-methyl-N'-(4-nitrobenzylidene)quinoxaline-2-carbohydrazide XIII

Yield 81.2%, mp 270-71ºC. IR: cm^{-1} 3451 (OH), 3050 (NH), 1715 (aromatic ring). ^{1}H NMR: δ, 2.5 (s, 3H, CH_3), 7.18-8.34 (m, 7H, Ar-H), 8.4 (s, 1H, N=CH), 8.8 (s, 1H, NH), 12.7 (s, 1H, OH).

SCHEME-



BIOLOGICAL ACTIVITY

Anti-microbial activity: The compounds IV-XIII were screened for their anti-bacterial activity against *Staphylococcus aureus* and *Escherichia coli* at 50µg/ml and 100 µg/ml concentrations by Cup-plate method[13]. Standard anti-bacterial ofloxacin were also tested under similar conditions. Results are presented in **Table II**.

Anti-inflammatory activity: The compounds IV-XIII were tested for their anti-inflammatory activity using carageenan induced in rat paw edema method of winter et ai[14] at an oral dose of 20 mg/kg b.w. in albino rats (weighing 130-200 gm). The percent inhibition of inflammation was calculated by applying formula. Results are presented in **Table III.**

RESULT AND DISCUSSION

Anti-inflammatory activity. We have reported the synthesis of ten new Hydrazones and Quinoxaline **(IV-XIII).** It has been observed that the test compounds **(IV, VIII, IX, X, XII and XIII)** exhibited interesting anti-inflammatory activity, however with a degree of variation. Compound **IX**, **X**, and **XIII** exhibited highly significant anti-inflammatory activity. The activity of these compounds **(XI, X & XIII)** was comparable with that of indomethacin.

Antibacterial activity. All compounds were evaluated for their antibacterial activity against *E. coli, and S. aureus* by using cup plate technique. The % inhibition of zone of each compound is shown in Table-2. The compounds VIII and IX shows good activity on *S. aureus* as compared to that of standard drug ofloxacin. The compounds IX, XIII shows maximum activity against E. coli.

Table 1 - Characterization data of compounds (IV-XIII)

Compound	Yield (%)	M. P. ^{0}C	Mol. formula	Mol. weight
IV	90	260	$C_{17}H_{14}O_2N_4$	306
V	89.3	261-62	$C_{18}H_{16}O_3N_4$	336
VI	83.5	258-61	$C_{17}H_{13}O_2N_4Cl$	375.5
VII	92.3	276	$C_{18}H_{16}O_4N_4$	352
VIII	85.4	263-65	$C_{17}H_{14}O_3N_4$	322
IX	87.6	268	$C_{17}H_{13}O_2N_4F$	324
X	80.6	264-67	$C_{17}H_{13}O_2N_4Cl$	340.5
XI	85.4	278-79	$C_{17}H_{13}O_4N_5$	351
XII	78	250-51	$C_{19}H_{19}O_2N_5$	349
XIII	81.2	270-71	$C_{17}H_{13}O_4N_5$	351

Table 2 - Anti-bacterial activity of the synthesized compounds

	% Inhibition			
	S. aureus		*E. coli*	
Compd	50 µg/ml	100 µg/ml	50 µg/ml	100 µg/ml
IV	39.28	42.85	27.58	31.03
V	35.71	35.71	25.85	30.50
VI	39.28	42.85	28.65	35.55
VII	35.71	39.28	24.13	27.58
VIII	42.85	50.00	27.58	34.48

IX	46.42	60.71	31.03	48.38
X	42.85	42.85	27.58	31.03
XI	32.14	39.28	30.55	40.05
XII	32.14	32.14	26.25	30.50
XIII	28.57	35.71	39.50	45.50

Table 3 – Anti-inflammatory activity of synthesized compound

Compound	Initial paw vol. (Mean ± SEM)	Final paw vol. (Mean ± SEM)	Difference in paw vol. (Mean ± SEM)	% Inhibition
Control	1.107 ± 0.03323	2.367 ± 0.08180	1.260 ± 0.08691	--
Indomethacin	1.117 ± 0.02836	1.410 ± 0.02745	0.2933 ± 0.3422	77
IV	1.123 ± 0.02486	1.733 ± 0.02275	0.6100 ± 0.02221	51.58
V	1.158 ± 0.03341	2.1220 ± 0.06395	0.9633 ± 0.03471	37
VI	1.113 ± 0.02985	2.007 ± 0.08180	0.8933 ± 0.3612	29.36
VII	1.135 ± 0.3557	1.907 ± 0.07719	0.07717 ± 0.06167	38.88
VIII	1.100 ± 0.01414	1.633 ± 0.06004	0.5500 ± 0.05586	56.34
IX	1.137 ± 0.03801	1.600 ± 0.1097	0.4633 ± 0.08077	63.5
X	1.143 ± 0.4587	1.622 ± 0.3135	0.4950 ± 0.04745	62
XI	1.083 ± 0.3712	1.838 ± 0.07778	0.7550 ± 0.09807	40.47
XII	1.068 ± 0.02272	1.708 ± 0.06364	0.6200 ± 0.07165	50.8
XIII	1.073 ± 0.01961	1.550 ± 0.08079	0.5133 ± 0.08077	59.5

Acknowledgment

The authors are thankful to IIT, Delhi for providing NMR spectra of the compounds.

Authors are also thankful to the Head of Pathology Department, Majeedia Hospital, for antimicrobial activity.

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