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SYNTHESIS OF HYDRAZONES DERIVATIVES OF QUINOXALINONE – PROSPECTIVE ANTIMICROBIAL AND ANTIINFLAMMATORY AGENTS

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Abstract: A series of quinoxalinone derivatives was synthesized by the condensation of 1,2-diaminobenzene with α-ketoglutaric acid to yield 3-(3-oxo-3,4-dihydroquinoxalin-2-yl) propionic acid (**2**) and then treated with hydrazine hydrate to yield its hydrazones (**3**). This was further reacted with substituted aromatic aldehydes to produce final compounds (**4a-r**). These hydrazones derivatives were characterized by FT-IR and ^{1}H-NMR data. All the synthesized compounds were evaluated for their antimicrobial and antiinflammatory activity.

Keywords: quinoxalinone, hydrazine hydrate, aromatic aldehydes, antimicrobial, antiinflammatory activity.

Recent advances in targeted therapeutics coupled with new approaches in target identification have accelerated the need to design small compounds with drug like properties (1). Quinoxaline is well known for its broad coverage in the field of medicine as well as for its application in the pharmaceuticals.

Quinoxaline and its derivatives have shown wide range of biological properties such as antimicrobial (2), antibacterial (3), antitubercular (2, 4), antiprotozoal (5) , anti-candida (2, 6), anti-cancer (7), anti-AIDS (8) and antiinflammatory (9) activities.

Quinoxaline derivatives have received much attention in recent years owing to their biological properties. Many scientists report quinoxalinone derivatives as non-classical analogues of the antifolic agents – methotrexate and trimetrexate, as well as the quinoxaline N-oxide with antibacterial activity (10). In connection with these studies we prepared a series of hydrazones derivatives of quinoxalinones which were evaluated for antimicrobial and antiinflammatory activities.

EXPERIMENTAL

The synthetic pathway for preparation of substituted quinoxalines listed in Table 1 is shown in Scheme 1. According to a general classical reaction, the quinoxalin-3-ones (**2**) were obtained by the condensation of appropriately substituted 1,2-diaminobenzene (**1**) with di-keto compounds in refluxing ethanol. Reaction progress was monitored by TLC on silica gel plates using benzene-acetone (7:3, v/v) as mobile phase and the final compounds were recrystallized using ethanol-DMF as a solvent. All the solvents and chemicals used were of LR grade and obtained from Merck, CDH (Germany) and S.D. Fine Chemicals (India).

Melting points were determined in open capillary tubes and were uncorrected. The FT-IR spectra were recorded in KBr pellets using a WIN-IR spectrophotometer. ^{1}H-NMR spectra were recorded on a Bruker (300MHz) spectrometer, in DMSO-d_6 using tetramethylsilane (TMS) as internal standard (Table 1).

Synthesis of 3-(3-oxo-3,4-dihydroquinoxalin-2-yl)propionic acid (2)

Equimolar quantities of substituted 1,2-diaminobenzene and a-ketoglutaric acid were dissolved in absolute ethanol in a round bottom flask and refluxed for 30 min. The precipitated crude product was recrystallized to give (**2**).

Synthesis of 3-(3-hydrazone-3,4-dihydro-quinoxalin-2-yl)propionic acid (3)

Equimolar quantities of compound (**2**) and hydrazine hydrate dissolved in absolute ethanol were refluxed for 16 h on water bath. After comple-

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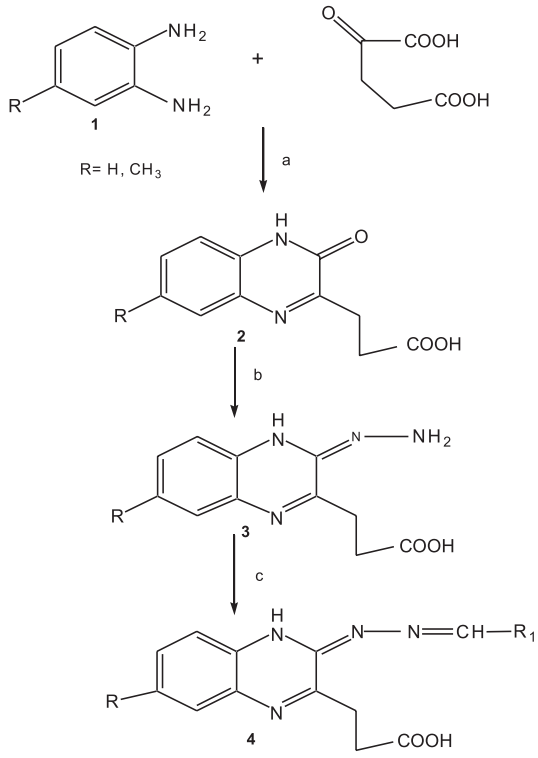
Table 1. Characterization of the synthesized compounds

Compd. no.	R	R_1	M.p. (°C)	% yield	IR (KBr, cm^{-1})	^{1}H-NMR (DMSO-d_6, δ ppm)
4a	H	4F-C_6H_4	212-215	65	3540.39, 3205.06, 1707.59, 1615.37, 1510.32, 1111.51.	12.32 (s, 1H, COOH), 8.05 (s, 1H, NH), 7.21-7.76 (m, 8H, ArH), 5.60 (s, 1H, N=CH), 2.98-3.03 (bs, 2H, CH_2CO), 2.69-2.73 (bs, 2H, CH_2).
4b	H	2Cl-C_6H_4	205-210	70	3513.80, 3271.74, 1717.76, 1653.18, 1492.60, 1014.95.	11.44 (s, 1H, COOH), 8.05 (s, 1H, NH), 7.94-7.95 (d, 2H, ArH), 7.48-7.51(m, 4H, ArH), 7.04-7.41 (d, 2H, ArH), 5.55 (s, 1H, N=CH), 2.98-3.05 (t, 2H, CH_2CO), 2.69-2.73 (t, 2H, CH_2).
4c	H	3-Cl-C_6H_4	216-219	70	3448.95, 1673.71, 1525.85, 1384.34, 1029.85.	12.45 (s, 1H, COOH), 8.68 (s, 1H, NH), 7.91 (s, 1H, ArH), 7.82-7.85 (d, 1H, ArH), 7.50-7.62 (m, 4H, ArH), 7.30-7.45 (m, 2H, ArH), 5.62 (s, 1H, N=CH), 3.00-3.09 (m, 4H, $2CH_2$).
4d	H	4-Cl-C_6H_4	218-221	72	3417.25, 1686.98, 1519.25, 1380.76, 1019.05.	12.60 (s, 1H, COOH), 8.72 (s, 1H, NH), 7.74-7.78 (d, 2H, ArH), 7.27-7.59 (m, 4H, ArH), 5.61 (s, 1H, N=CH), 2.98-3.05 (m, 4H, $2CH_2$).
4e	H	4-OH-C_6H_4	210-212	60	3658.72, 3572.81, 3067.22, 1714.77, 1563.61, 1477.62, 1080.63.	12.48 (s, 1H, COOH), 9.86 (bs, 1H, OH), 8.55 (s, 1H, NH), 7.10-7.76 (m, 8H, ArH), 5.44 (s, 1H, N=CH), 2.71-3.08 (m, 4H, $2CH_2$).
4f	H	4-OCH_3-C_6H_4	212-215	62	3533.55, 3216.53, 1662.83, 1564.61, 1440.97, 1268.44, 1124.18.	12.40 (s, 1H, COOH), 8.58 (s, 1H, NH), 7.48-7.51 (d, 1H, ArH), 7.20-7.36 (m, 4H, ArH), 7.03-7.06 (d, 2H, ArH), 5.52 (s, 1H, N=CH), 3.84 (s, 3H, OCH_3), 2.94-2.97 (m, 4H, $2CH_2$).
4g	H	3,4-OCH_3-C_6H_3	220-223	63	3490.21, 3290.21, 1612.32, 1550.23, 1430.39, 1129.41.	11.23 (s, 1H, COOH), 8.60 (s, 1H, NH), 6.81-7.89 (m, 7H, ArH), 5.51 (s, 1H, N=CH), 3.86 (s, 6H, $2OCH_3$), 2.71-3.11 (m, 4H, $2CH_2$).
4h	H	3,4,5-OCH_3-C_6H_2	235-239	60	3412.58, 3190.39, 1620.91, 1547.79, 1455.16, 1090.34.	12.45 (s, 1H, COOH), 8.60 (s, 1H, NH), 7.78 (bs, 2H, ArH), 7.31-7.37 (m, 7H, ArH), 5.51 (s, 1H, N=CH), 3.86 (bs, 9H, $3OCH_3$), 2.71-3.11(m, 4H, $2CH_2$).
4i	H	2-NO_2-C_6H_4	202-206	58	3490.21, 3220.33, 1621.13, 1540.41, 1461.93, 1103.29.	11.87 (s, 1H, COOH), 8.55 (s,1H,NH), 7.24-7.67 (m, 8H, ArH), 5.62 (s, 1H, N=CH), 2.76-2.83 (m, 4H, $2CH_2$).
4j	H	4-NO_2-C_6H_4	205-209	65	3550.21, 3182.17, 1618.83, 1573.51, 1479.95, 1030.59.	11.63 (s, 1H, COOH), 9.86 (bs, 1H, OH), 8.49 (s, 1H, NH), 7.10-7.76 (m, 8H, ArH), 5.55 (s, 1H, N=CH), 2.73-2.83 (m, 4H, $2CH_2$).
4k	CH_3	4-F-C_6H_4	230-233	59	3510.00, 3130.00, 1663.78, 1605.90, 1435.14, 1125.74.	12.40 (s, 1H, COOH), 8.60 (s, 1H, NH), 7.80-7.85 (m, 7H, ArH), 5.47 (s, 1H, N=CH), 2.71-3.08 (m, 4H, $2CH_2$), 2.39 (s, 3H, CH_3).
4l	CH_3	2-Cl-C_6H_4	242-245	61	3568.95, 3079.57, 1617.54, 1590.12, 1438.33, 1120.82.	12.40 (s, 1H, COOH), 8.36 (s, 1H, NH), 7.80-7.85 (m, 7H, ArH), 6.06 (s, 1H, N=CH), 2.71-3.08 (m, 4H, $2CH_2$), 2.38 (s, 3H, CH_3).
4m	CH_3	3-Cl-C_6H_4	240-242	64	3473.31, 3129.70, 1620.29, 1610.12, 1423.27, 1090.23.	12.40 (s, 1H, COOH), 7.99 (s, 1H, NH), 7.99-7.16 (m, 7H, ArH), 5.60 (s, 1H, N=CH), 2.42 -3.0 (m, 4H, $2CH_2$), 2.40 (s, 3H, CH_3).
4n	CH_3	4-Cl-C_6H_4	248-251	62	3433.31, 3067.62, 1624.15, 1677.54, 1414.69, 1078.57.	12.50 (s, 1H, COOH), 8.72 (s, 1H, NH), 7.89-7.24 (m, 7H, ArH), 5.59 (s, 1H, N=CH), 2.93-3.09 (m, 4H, $2CH_2$), 2.36 (s, 3H, CH_3).
4o	CH_3	4-OH-C_6H_4	219-222	58	3430.31, 3090.70, 1654.29, 1580.12, 1417.64, 111.29.	12.40 (s, 1H, COOH), 9.86 (bs, 1H, OH), 8.44 (s, 1H, NH), 7.79-6.43 (m, 7H, ArH), 5.40 (s, 1H, N=CH), 2.86-3.05 (m, 4H, $2CH_2$), 2.36 (s, 3H, CH_3).
4p	CH_3	4-OCH_3-C_6H_4	265-269	57	3394.35, 3080.87, 1676.72, 1597.57, 1414.79, 1106.84.	12.45 (s, 1H, COOH), 8.63 (s, 1H, NH), 7.80-6.98 (m, 4H, ArH), 5.50 (s, 1H, N=CH), 3.84 (s, 3H, OCH_3), 2.94-2.97 (m, 4H, $2CH_2$), 2.40 (s, 3H, CH_3).
4q	CH_3	2-NO_2-C_6H_4	245-248	59	3403.31, 3089.70, 1660.29, 1602.52, 1413.57, 1113.73.	12.40 (s, 1H, COOH), 8.41 (s, 1H, NH), 8.22-7.14 (m, 7H, ArH), 5.78 (s, 1H, N=CH), 2.86-3.05 (m, 4H, $2CH_2$), 2.36 (s, 3H, CH_3).
4r	CH_3	4-NO_2-C_6H_4	250-254	61	3420.00, 3229.70, 1656.29, 1607.76, 1403.27, 1130.23.	12.55 (s, 1H, COOH), 8.41 (s, 1H, NH), 8.22-7.14 (m, 7H, ArH), 5.78 (s, 1H, N=CH), 2.86-3.05 (m, 4H, $2CH_2$), 2.36 (s, 3H, CH_3).

Table 2. Pharmacological activity of tested compounds

Compound	Antimicrobial activity % zone of inhibition at 100 ppm		Antiinflammatory % inhibition of edema
	S. aureus	*E. coli*	
4a	83.33	72.45	56.30
4b	66.67	68.91	55.84
4c	61.11	60.42	57.76
4d	77.81	70.83	52.90
4e	54.95	63.84	60.85
4f	67.64	67.59	66.04
4g	51.49	58.99	50.41
4h	48.53	56.45	54.23
4i	59.60	49.78	53.12
4j	62.97	46.83	61.07
4k	77.65	70.98	53.28
4l	61.54	67.87	59.86
4m	65.98	54.46	56.95
4n	75.45	69.97	55.87
4o	68.45	57.19	57.66
4p	69.37	62.86	69.85
4q	59.94	58.17	45.74
4r	69.15	67.11	52.08

Ofloxacin (% zone of inhibition at 100 ppm) – 100%
Indomethacin (% inhibition of edema) – 79.5%



Scheme 1 – Preparation of quinoxaline derivatives
a – absol. EtOH/reflux; b – absol. EtOH/NH_2NH_2/reflux;
c – Ar-CHO/absol. EtOH/reflux

tion of reaction, the reaction mixture was cooled and poured onto crushed ice. The solid product so obtained was separated and crystallized to yield compound (**3**).

Synthesis of 3-(3-benzylidene-hydrazono-3,4-dihydroquinoxalin-2-yl)propionic acid (4)

Equimolar quantities of compound (**3**) and appropriate substituted aromatic aldehydes were refluxed on water bath for 10 h using absolute ethanol as a solvent . After completion of reaction the mixture was poured onto crushed ice and the solid product so obtained was recrystallized to give compound (**4**).

RESULTS

Compounds (**4a-r**) were investigated for *in-vitro* antimicrobial activity by cup-plate method and for *in-vivo* antiinflammatory activity by carrageenan induced rat paw edema method using Ugo-Basile plethysmometer (Table 2).

Antimicrobial activity

In-vitro antimicrobial activity was investigated by using cup-plate method (11) on Gram positive microorganism *Staphylococcus aureus* (ATCC 29213) and Gram negative microorganism *Escherichia coli* (ATCC 25922) using ofloxacin as the standard drug.

Anti-inflammatory activity

In-vivo antiinflammatory activity was carried out by Winter et al. method (12), in which paw inflammation in albino rats was induced by carrageenan in hind paw of rat and edema volume was measured using Ugo-Basile plethysmometer. Indomethacin was used as the standard drug.

DISCUSSION AND CONCLUSION

All described quinoxaline derivatives (Table 1) were tested *in-vitro* for antibacterial activity against Gram positive (*S. aureus*) and Gram negative (*E. coli*) microorganisms.. The results obtained indicate that a majority of these compounds show only mod-

erate activity, however, few of them like **4a**, **4d**, **4k**, and **4n** show comparatively good activity against both types of microorganisms. It has been observed that compounds bearing highly electronegative fluoro and chloro substituents at the *para* position of phenyl ring show good activity as compared to compounds bearing these atoms at either *ortho* or *meta* position or the other compounds bearing the less electronegative/electropositive substituents at these positions.

Similarly, all synthesized compounds were evaluated for antiinflammatory activity, and among them only compounds having the methoxy group at the *para* position, i.e. **4f** and **4p,** showed comparatively good percentage of inhibition of edema than the other synthesized compounds. Compounds **4f** and **4p** were further tested for ulcerogenic activity and found to have 'zero' ulcerogenic index.
Quinoxaline derivatives were found to have moderate activity and thus, broaden the scope of its further research.

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