



Received on 13 July 2019; received in revised form, 27 April 2020; accepted, 22 July 2020; published 01 August 2020

SYNTHESIS AND PHARMACOLOGICAL EVALUATION OF SOME NOVEL 1, 2, 3, 4-TETRAHYDROQUINAZOLINONE DERIVATIVES

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Keywords:

1, 2, 3, 4-tetrahydroquinazoline, Isatoic anhydride, Mannich Reaction, Antibacterial, Anti-inflammatory activity

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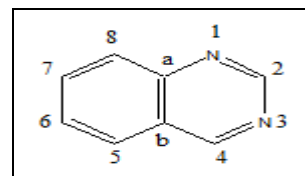
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ABSTRACT: In the study, we have successfully synthesized N-3 substituted tetrahydroquinazolinone by the reaction of isotonic anhydride with aniline derivatives. Thereafter novel derivative would be subjected to synthesize (1, 2, 3, 4-tetrahydroquinazoline) by Mannich reaction with ciprofloxacin and norfloxacin. These synthesized compounds (2a, 2b, 2c, 2d, 2e, 2f, 2g, and 2h) were characterized by FT-IR, ¹H NMR, MASS spectroscopy, and elemental analysis and evaluated for their *in-vitro* antimicrobial activity and anti-inflammatory activity. The antimicrobial activities of synthesized compounds were evaluated against gram-positive and gram-negative bacteria. Amongst these tested compounds 2c, 2d, 2e, 2f exhibited potent antimicrobial activity than the rest of the compounds. All the synthesized compounds were screened for their anti-inflammatory activity using the carrageenan-induced rat paw edema model and exhibited protection against carrageenan-induced edema.

INTRODUCTION: Quinazoline compounds are made up of two fused six-membered simple aromatic rings, *i.e.*, a benzene ring and pyrimidine ring. Quinazoline and their derivatives are an important class of heterocycles with a wide range of pharmacological and biological activities¹. Quinazoline can be divided into two main groups according to their characteristic properties. The first group includes all the quinazoline in which two rings are fully aromatic. These do not behave entirely as pyrimidines. The benzene ring has a profound effect on the properties of pyrimidine ring; it delocalized the π electrons of the 3, 4 double bonds making it reactive. As a consequence of this, quinazoline is reactive toward nucleophilic, which is ready across the 3, 4 double bond².

The second group includes the quinazolines, which lack the full complement of six π electrons in either the pyrimidine or benzene ring. These compounds can be divided into the quinazoline with tautomeric groups in the pyrimidine².



QUINAZOLINE

The derivative in this group shows the characteristic reaction of pyrimidines. 1, 2, 3, 4-tetrahydroquinazoline are reactive and observed in reduced pyrimidines, although fused benzene ring confers on them slightly greater stability, and certainly make them easier to isolate².

Categories of Quinazolinones: Quinazolinones will be classified into the following five categories, based on the substitution patterns of the ring system³.

<p>QUICK RESPONSE CODE</p>	<p>DOI: 10.13040/IJPSR.0975-8232.11(8).3912-22</p> <hr/> <p>This article can be accessed online on www.ijpsr.com</p> <hr/> <p>DOI link: http://dx.doi.org/10.13040/IJPSR.0975-8232.11(8).3912-22</p>
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- 2-Substituted-4(3H)-quinazolinones
- 3-Substituted-4(3H)-quinazolinones
- 4-Substituted-quinazolines
- 2,3-Disubstituted-4(3H)-quinazolinones
- 2,4-Disubstituted-4(3H)-quinazolinones

2-Substituted-4(3H)-quinazolinones:

Amidation and Cyclisation of 2-aminobenzoic Acid Derivatives: The most common approach involves amidation of 2-aminobenzonitrile, 2-aminobenzoic acid, and 2-aminobenzamide. As an example, the reaction of 2-aminobenzonitrile with 3-phenyl-acryloyl chloride followed by oxidative ring closure under basic conditions produced 2-styryl-4(3H) quinazolinone⁴⁻⁵.

3-Substituted-4-quinazolines:

Vilsmeier Reagent in Quinazolinone Synthesis:

A novel dimerisation reaction to furnish 3-substituted-4(3H)-quinazolinones in high yield was developed by Perumal *et al.*, by treating 5-substituted-2-aminobenzoic acid derivatives with the Vilsmeier reagent⁶.

4-Substituted Quinazolines:

Derivatisation of 4(3H)-quinazolinones: 4-Chloroquinazolines, compounds are important synthetic intermediates as they can be derivatised further through the nucleophilic attack at the C-4 position with an intermediate in the synthesis of a quinazoline-derived photoaffinity probe for epithelial growth factor receptor (EGFR)⁷.

2,3-Disubstituted-4(3H)-quinazolines:

Intramolecular Coupling of Azides to Carbonyl Groups: The synthesis of optically active l-vasicinone, a known pyrrolo[2,1-b]-quinazolinone alkaloid, was undertaken by Eguchi *et al.* employing the intramolecular aza-Wittig reaction as the key step in the construction of the quinazolinone ring skeleton⁸.

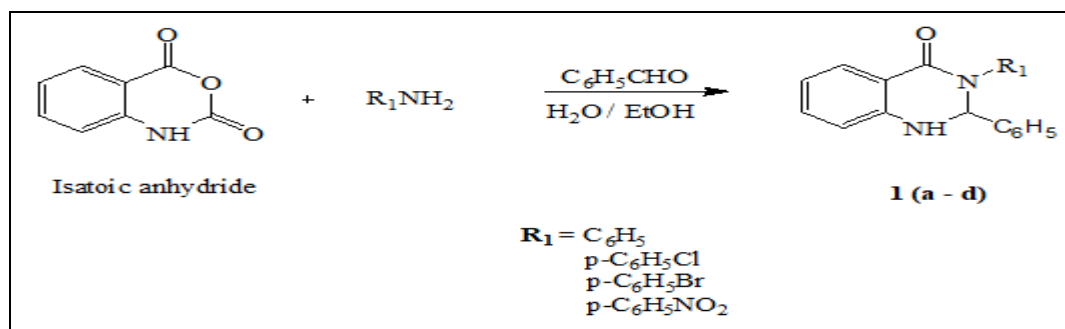
2,4-Disubstituted-4(3H)-quinazolinones and – quinazolines:

2-Substituted-4-aminoquinazolines by Microwave Irradiation: Seijas *et al.*, reported a microwave-promoted synthesis of 4-aminoquinazolines by reacting cyanoaromatic compounds with anthranilonitrile in a domestic microwave oven⁹.

MATERIALS AND METHODS: All chemicals used in the study were of analytical grade purchased from SD Fine-chem limited, LOBA chem., Thomas baker limited, Rankem, CDH, and Qualigens. All the solvents were used after distillation. TLC was run by using silica-coated aluminum sheets (silica gel 60 F254, E Merck, Germany) and visualized in UV light/Iodine chamber. Melting points were determined in open capillary tubes in a Jyoti melting point apparatus and are uncorrected. IR spectra were taken from the FT-IR Affinity-1. NMR spectra were obtained from CDRI, Lucknow, by using a Bruker NMR instrument at 300 MHz. The ¹H chemical shifts are reported as parts per million (ppm) downfield from CDCl₃. MASS spectra were obtained from CDRI, Lucknow. The electrospray mass spectra were recorded on a THERMO finnigan LCQ advantage max ion trap mass spectrometer.

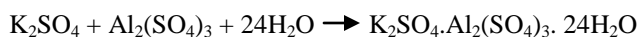
Step 1: General Method for Synthesis of Tetrahydroquinazolinone By Isoatoic Anhydride:

Isoatoic anhydride (1 mmol), primary amine (1.1 mmol) and aldehyde (1 mmol) was dissolved in ethanol (5 mL) and added catalyst alum (0.15 g, 0.4 mmol)¹⁰. The mixture was heated at reflux for a specified period of time. After completion of the reaction as indicated by TLC (eluent: methanol-chloroform), the solid catalyst was separated by filtration. Water (10 mL) was added, and the precipitated product was filtered and recrystallized from EtOH.



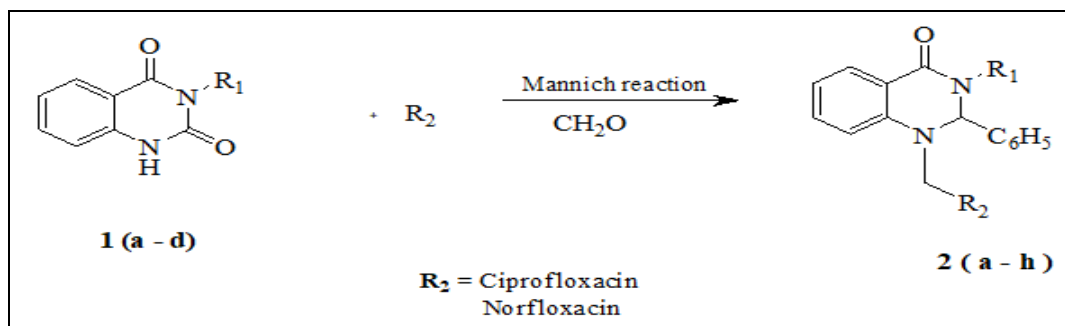
STEP 1: SYNTHESIS OF TETRAHYDROQUINAZOLINONE FROM ISATOIC ANHYDRIDE

Preparation of Catalyst (Alum): Potash alum is prepared by mixing equi-molecular masses of potassium sulphate and aluminum sulphate in water followed by evaporation.



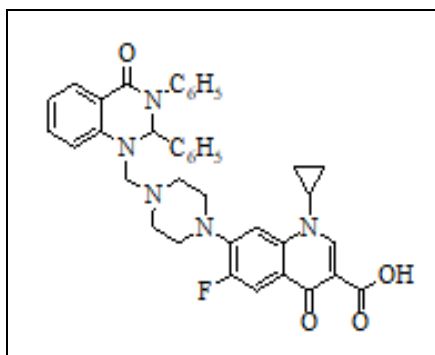
Step 2: General Method for Synthesis of Mannich Base of Tetrahydroquinazolinone Derivatives: A slurry containing tetrahydroquinazolinone with formaldehyde added with

mixture of ciprofloxacin or norfloxacin dissolved in ethanol (10 ml). The reaction mixture was stirred for 4 h at room temperature and refrigerated for 24 h. The products were separated by suction filtration, vacuum dried, and recrystallized from methanol. The synthetic scheme for the preparation of the Mannich base of tetrahydroquinazolinone derivatives is given in **Scheme 2**. The melting point, % yield, and R_f values are given in **Table 1**.

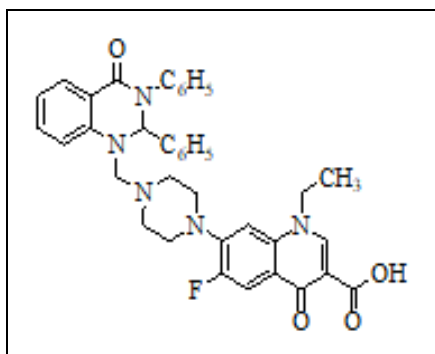


Possible Structures of Mannich Bases of Tetrahydroquinazolinones & Its Derivatives (2a-h):

A. Aniline Derivatives:

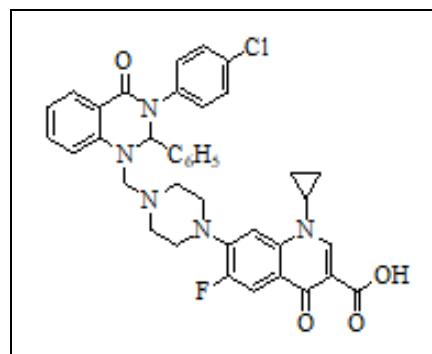


2a. 1-cyclopropyl-6-fluoro-4-oxo-7-(4-((4-oxo-2,3-diphenyl-3,4-dihydroquinazolin-1(2H)-yl)methyl)piperazin-1-yl)-1,4-dihydroquinoline-3-carboxylic acid

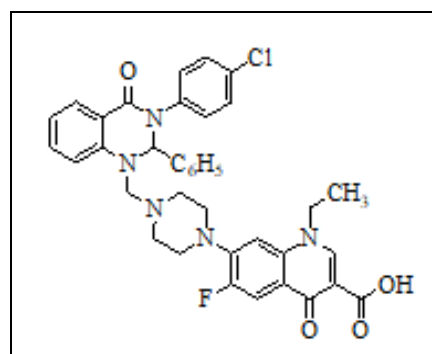


2b. 1-ethyl-6-fluoro-4-oxo-7-(4-((4-oxo-2,3-diphenyl-3,4-dihydroquinazolin-1(2H)-yl)methyl)piperazin-1-yl)-1,4-dihydroquinoline-3-carboxylic acid

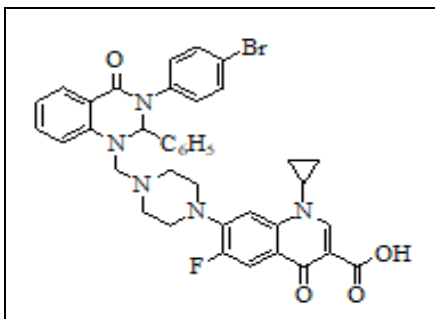
B. p-Chloro Aniline Derivatives:



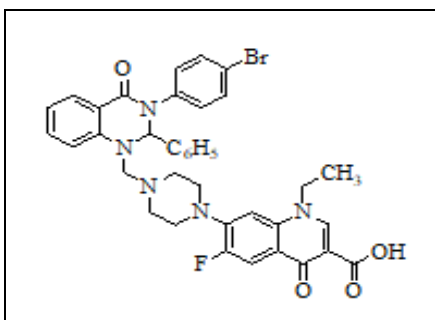
2c. 7-(4-((3-(4-chlorophenyl)-4-oxo-2-phenyl-3,4-dihydroquinazolin-1(2H)-yl)methyl)piperazin-1-yl)-1-cyclopropyl-6-fluoro-4-oxo-1,4-dihydroquinoline-3-carboxylic acid



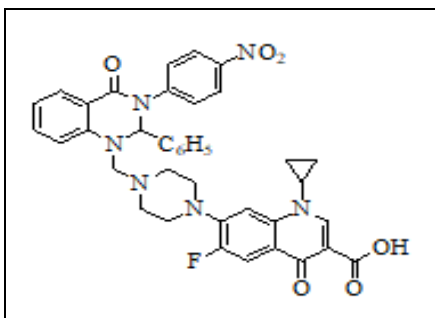
2d. 7-(4-((3-(4-chlorophenyl)-4-oxo-2-phenyl-3,4-dihydroquinazolin-1(2H)-yl)methyl)piperazin-1-yl)-1-ethyl-6-fluoro-4-oxo-1,4-dihydroquinoline-3-carboxylic acid

C. p-Bromo Aniline Derivatives:

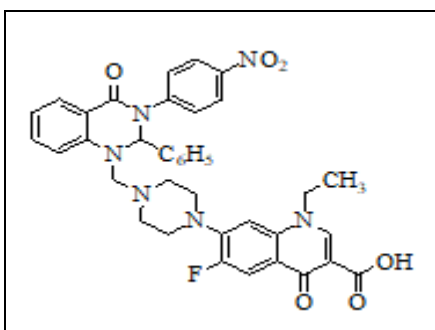
2e. 7-(4-((3-(4-bromophenyl)-4-oxo-2-phenyl-3,4-dihydroquinazolin-1(2H)-yl)methyl)piperazin-1-yl)-1-cyclopropyl-6-fluoro-4-oxo-1,4-dihydroquinoline-3-carboxylic acid



2f. 7-(4-((3-(4-bromophenyl)-4-oxo-2-phenyl-3,4-dihydroquinazolin-1(2H)-yl)methyl)piperazin-1-yl)-1-ethyl-6-fluoro-4-oxo-1,4-dihydroquinoline-3-carboxylic acid

D. p-Nitroaniline Derivatives:

2g. 1-cyclopropyl-6-fluoro-7-(4-((3-(4-nitrophenyl)-4-oxo-2-phenyl-3,4-dihydroquinazolin-1(2H)-yl)methyl)piperazin-1-yl)-4-oxo-1,4-dihydroquinoline-3-carboxylic acid



2h. 1-ethyl-6-fluoro-7-(4-((3-(4-nitrophenyl)-4-oxo-2-phenyl-3,4-dihydroquinazolin-1(2H)-yl)methyl)piperazin-1-yl)-4-oxo-1,4-dihydroquinoline-3-carboxylic acid

Characterization of Compounds:

Chromatographic Analysis: Synthesized compounds were characterized by thin-layer chromatography (TLC) using pre-coated aluminum sheets (10 × 2.5 cm) silica gel G 60 plates F 254, and thickness of plates was 250 μm. The pre-coated plates were activated at 110 °C for 90 minutes in a hot air oven and cooled in a vacuum desiccator on silica gel. The solutions of mannich base of tetrahydroquinazolinones derivatives were prepared by dissolving 1 mg of the pure compound in 5 ml. of acetone and spotted on pre-coated plates with the help of a capillary tube. The plates were run in a pre-saturated TLC chamber using the following solvent systems:

1. Chloroform: Methanol (7%)
2. Chloroform: Methanol (8%)
3. Chloroform: Methanol (9%)

Chromatograms were developed in a TLC chamber saturated with a specific solvent system at 25 °C. The solvent front was allowed to travel approximately 8 cm. Plates were taken out, air-dried, and exposed to iodine vapor by putting in it iodine saturated chamber. The plates were also seen under the UV chamber 254 nm and 366 nm. The R_f values were calculated using the following formula:

The R_f values of the synthesized compound are summarized in **Table 1**.

Melting Point Determination: The measurement of the melting point is a measure of concern in pharmaceutical studies. The most important reason to determine the melting point during synthesis is the identification of synthesized compounds.

Capillary Fusion Method: The melting point of synthesized compounds was determined using the capillary fusion method. Capillary was taken, and one end was sealed by bringing it near the burner flame. The open end of the capillary tube was pushed into a small heap of the compound so that a small plug of the powder was collected in the open end. The tube was tapped gently so that the collected materials settle down. The capillary tube was placed in the melting point determination apparatus. The temperature at which the compound starts to melt was noted with the help of a thermometer. Data came from M.P. determination by capillary fusion method are given in **Table 1**

Infrared Spectroscopy: The samples were previously ground and mixed thoroughly with KBr, using a 1% (w/w) dilution. The KBr disks were prepared by compressing the powders under the force of 10 tons in a hydraulic press. Scans were obtained at a resolution of 1 cm^{-1} from 400-4000 cm^{-1} . The peaks obtained from IR spectroscopy are tabulated in **Table 2**.

$^1\text{H-NMR}$: NMR spectra were obtained from CDRI, Lucknow by using a Bruker NMR instrument at 300 MHz. The ^1H chemical shifts are reported as parts per million (ppm) downfield from CDCl_3 . δ values (in ppm) of the compound are given in **Table 3.3, 3.4, 3.5, 3.6**, respectively.

Mass Spectroscopy: The electrospray mass spectra were recorded on a THERMO finnigan LCQ advantage max ion trap mass spectrometer. The 10 μl samples (dissolve in a solvent such as methanol/ acetonitrile/water) were introduced into ESI source through Finnigan surveyor autosampler. The mobile phase 90:10 Methanol/ACN: H_2O flowed at a rate of 250 $\mu\text{l}/\text{min}$ by MS pump. Ion spray voltage was set at 5.3KV and capillary voltage 34V. The MS scan run up to 2.5 min and spectra's print outs is averaged of over 10 scans at peak top in TLC. This is done by CDRI, Lucknow m/z value of compound is given in **Table 3**.

TABLE 1: PHYSIOCHEMICAL PROPERTIES OF COMPOUND

S. no.	Compound code	Substituent		Molecular formula	Mol. Wt.	Solubility	Melting Point ($^{\circ}\text{C}$)	Yield (%)	R_f value
		R_1	R_2						
1	2a	C_6H_5		$\text{C}_{38}\text{H}_{34}\text{FN}_5\text{O}_4$	643.71	Acetone, Methanol	220 - 240	74.0	0.67
2	2b	C_6H_5		$\text{C}_{37}\text{H}_{34}\text{FN}_5\text{O}_4$	631.70	Acetone, Methanol	220-230	59.5	0.78
3	2c			$\text{C}_{38}\text{H}_{33}\text{ClF}\text{N}_5\text{O}_4$	678.15	Acetone, Methanol	200 - 220	94.0	0.69
4	2d			$\text{C}_{37}\text{H}_{33}\text{ClF}\text{N}_5\text{O}_4$	666.14	HCl, Methanol, Acetone	220-250	80.0	0.88
5	2e			$\text{C}_{38}\text{H}_{33}\text{BrF}\text{N}_5\text{O}_4$	722.60	Acetone, Methanol	210-220	81.96	0.85
6	2f			$\text{C}_{37}\text{H}_{33}\text{BrF}\text{N}_5\text{O}_4$	710.59	HCl, Water, Acetone	220-250	90.90	0.75
7	2g			$\text{C}_{38}\text{H}_{33}\text{FN}_6\text{O}_6$	688.70	Water, Acetone	215 - 240	86.2	0.79
8	2h			$\text{C}_{37}\text{H}_{33}\text{FN}_6\text{O}_6$	676.69	HCl, Methanol, Ethanol	225-240	72.0	0.85

TABLE 2: IR SPECTRAL DATA OF MANNICH BASES OF TETRAHYDROQUINAZOLINONES & ITS DERIVATIVES (2A-H)

S. no.	Compound code	C-N stretching	C-H stretching	C-H (aromatic) bending	-F- stretching	-Cl- Stretching	-Br- stretching	-NO ₂ - stretching	COOH		
									O-H stretching	C=O stretching	C-O stretching
1	2a	1342	2931	933	1118	-	-	-	3078	1627	1265
2	2b	1350	2935	930	1115	-	-	-	3048	1630	1250
3	2c	1342	2931	933	1118	1056	-	-	3448	1735	1273
4	2d	1257	2931	948	1203	1103	-	-	3369	1743	1373
5	2e	1350	2931	933	1249	-	1049	-	3441	1730	1118
6	2f	1373	2931	941	1203	-	1049	-	3441	1620	1257
7	2g	1350	2954	948	1203	-	-	1481	3441	1730	1257
8	2h	1350	2924	941	1273	-	-	1473	3448	1735	1257

TABLE 3: MASS SPECTRAL DATA OF MOST ACTIVE COMPOUNDS

S. no.	Compound	m/z
1	2c	678.4
2	2d	667.1
3	2e	723.4
4	2f	710.5

TABLE 3.1: ¹H NMR SPECTRAL DATA OF COMPOUND 2C

NMR Spectral Data at 300 Mhz (in CDCl ₃) of Compound 2c		
S. no.	δ values (in ppm)	Inference
1	15.12	-OH of carboxylic acid
2	4.12	-CH of cyclopropane 1 δ -N from methine
3	1.33	-CH ₂ cyclopropane 1 δ -N-from methylene
4	6.01	-CH from methine; 1 δ with -N-C=O; 1 δ with -N
5	3.44	-CH ₂ of methylene; 1 δ with -NC; 1δ -N(C)C
6	2.48	-CH ₂ of methylene; 1 δ with -N(C)C; 1 δ with -NC
7	7.47	-CH of 1-benzene; 1 -Cl; 1 -NC(=O)
8	6.04	-CH of 1-benzene; 1 -F; 1 -N-C; 1 -N(C)C; 1 -C=O
9	7.88	-CH of 1-benzene; 1 -Cl; 1 -NC(=O)
10	6.94	-CH of 1-benzene; 1 -N(C)C; 1 -C(=O)N
11	8.01	-CH of 1-benzene; 1 -F; 1 -N-C; 1 -N(C)C; 1 -C=O
12	7.71	-CH of 1-benzene; 1 -N(C)C; 1 -C(=O)N
13	7.43	-CH of 1-benzene; 1 -C
14	7.33	-CH of 1-benzene; 1 -C
15	7.52	-CH of 1-benzene; 1 -N(C)C; 1 -C(=O)N
16	6.97	-CH of 1-benzene; 1 -N(C)C; 1 -C(=O)N
17	7.26	-CH of 1-benzene
18	4.42	-CH ₂ of methylene; 1 δ -NC; 1 δ -N(C)C
19	8.66	-H of 1-ethylene; 1 -N-C; 1 -C(=O)O cis; 1 -C(=O) trans

TABLE 3.2: ¹H NMR SPECTRAL DATA OF COMPOUND 2D

NMR Spectral Data at 300 Mhz (in CDCl ₃) of Compound 2c		
S. no.	δ values (in ppm)	Inference
1	15.12	-OH of carboxylic acid
2	6.01	-CH from methine; 1 δ with -N-C=O; 1 δ with -N
3	3.44	-CH ₂ of methylene; 1 δ with -NC; 1δ -N(C)C
4	2.48	-CH ₂ of methylene; 1 δ with -N(C)C; 1 δ with -NC
5	7.47	-CH of 1-benzene; 1 -Cl; 1 -NC(=O)
6	6.04	-CH of 1-benzene; 1 -F; 1 -N-C; 1 -N(C)C; 1 -C=O
7	7.88	-CH of 1-benzene; 1 -Cl; 1 -NC(=O)
8	6.94	-CH of 1-benzene; 1 -N(C)C; 1 -C(=O)N
9	8.01	-CH of 1-benzene; 1 -F; 1 -N-C; 1 -N(C)C; 1 -C=O
10	7.71	-CH of 1-benzene; 1 -N(C)C; 1 -C(=O)N
11	7.43	-CH of 1-benzene; 1 -C
12	7.33	-CH of 1-benzene; 1 -C
13	7.52	-CH of 1-benzene; 1 -N(C)C; 1 -C(=O)N
14	6.97	-CH of 1-benzene; 1 -N(C)C; 1 -C(=O)N
15	7.26	-CH of 1-benzene
16	4.42	-CH ₂ of methylene; 1 δ -NC; 1 δ -N(C)C
17	4.62	-CH ₂ of methylene; 1 δ -C; 1 δ -N
18	1.13	-CH ₃ of methyl; 1 beta -N
19	9.02	H of 1-ethylene; 1 -N-C; 1 -C(=O)O cis; 1 -C(=O) trans

TABLE 3.3: ¹H NMR SPECTRAL DATA OF COMPOUND 2E

NMR Spectral Data at 300 Mhz (in CDCl ₃) of Compound 2c		
S. no.	δ values (in ppm)	Inference
1	15.12	-OH of carboxylic acid
2	4.12	-CH of cyclopropane; 1 δ -N from methine
3	1.33	-CH ₂ of cyclopropane; 1 beta -N from methylene
4	3.44	-CH ₂ of methylene; 1 δ with -NC; 1δ -N(C)C
5	2.48	-CH ₂ of methylene; 1 δ with -N(C)C; 1 δ with -NC
6	6.04	-CH of 1-benzene; 1 -F; 1 -N-C; 1 -N(C)C ; 1 -C=O
7	6.94	-CH of 1-benzene; 1 -N(C)C; 1 -C(=O)N
8	8.01	-CH of 1-benzene; 1 -F; 1 -N-C; 1 -N(C)C ; 1 -C=O
9	7.71	-CH of 1-benzene; 1 -N(C)C; 1 -C(=O)N
10	7.43	-CH of 1-benzene; 1 -C
11	7.33	-CH of 1-benzene ; 1 -C
12	7.52	-CH of 1-benzene; 1 -N(C)C; 1 -C(=O)N
13	6.97	-CH of 1-benzene; 1 -N(C)C; 1 -C(=O)N
14	7.26	-CH of 1-benzene
15	8.06	-CH of 1-benzene; 1 -Br; 1 -NC(=O)
16	8.74	-CH of 1-benzene; 1 -Br; 1 -NC(=O)
17	4.42	-CH ₂ of methylene; 1 δ -NC; 1 δ -N(C)C
18	6.01	-CH of methine; 1 δ; 1 δ -N-C=O; 1 δ -N
19	8.66	-H of 1-ethylene; 1 -N-C; 1 -C(=O)O cis; 1-C(=O)trans

TABLE 3.4: ¹H NMR SPECTRAL DATA OF COMPOUND 2F

NMR Spectral Data at 300 Mhz (in CDCl ₃) of Compound 2c		
S. no.	δ values (in ppm)	Inference
1	15.12	-OH of carboxylic acid
2	4.62	CH ₂ of methylene; 1 δ-C; 1 δ -N
3	1.31	-CH ₃ of methyl; 1 δ -N
4	3.44	-CH ₂ of methylene; 1 δ with -NC; 1δ -N(C)C
5	2.48	-CH ₂ of methylene; 1 δ with -N(C)C; 1 δ with -NC
6	6.04	-CH of 1-benzene; 1 -F; 1 -N-C; 1 -N(C)C; 1 -C=O
7	6.94	-CH of 1-benzene; 1 -N(C)C; 1 -C(=O)N
8	8.01	-CH of 1-benzene; 1 -F; 1 -N-C; 1 -N(C)C ; 1 -C=O
9	7.71	-CH of 1-benzene; 1 -N(C)C; 1 -C(=O)N
10	7.43	-CH of 1-benzene; 1 -C
11	7.33	-CH of 1-benzene; 1 -C
12	7.52	-CH of 1-benzene; 1 -N(C)C; 1 -C(=O)N
13	6.97	-CH of 1-benzene; 1 -N(C)C; 1 -C(=O)N
14	7.26	-CH of 1-benzene
15	8.06	-CH of 1-benzene; 1 -Br; 1 -NC(=O)
16	8.74	-CH of 1-benzene; 1 -Br; 1 -NC(=O)
17	4.42	-CH ₂ of methylene; 1 δ -NC; 1 δ -N(C)C
18	6.01	-CH of methine; 1 δ; 1 δ -N-C=O; 1 δ -N
19	9.02	-H of 1-ethylene; 1 -N-C; 1 -C(=O)O cis; 1 -C(=O) trans

Pharmacological Screening:

Antimicrobial Screening of Synthesized Compounds by Agar Dilution Method:

Microbiological assay (MBA) is defined as the estimation of potency of a growth-promoting (GPS) or growth-inhibiting substance (GIS) by comparing its quantitative effect on the growth of a specific microorganism with that of a reference standard of defined potency⁵. The agar dilution susceptibility-testing method is utilized for the determination of the minimal inhibitory concentration (MIC) of an antimicrobial agent required to inhibit the growth of a microorganism¹¹.

The agar dilution method is the most well-established method for determining antimicrobial susceptibility. It is commonly used as the standard or reference method for the evaluation of new antimicrobial agents and susceptibility test methods. In addition, it offers the convenience of simultaneously testing a number of isolates, being able to detect mixed cultures or heterogeneous populations, and flexibility in antibiotic selection and concentration range to be tested¹¹.

Methods: Antimicrobial studies of all synthesized compounds were conducted against test

microorganisms, including bacteria (Gram +ve, Gram-ve).

All equipment was cleaned and sterilized by autoclave method at 12 psi for 30 min. Nutrient agar medium was used for the growth of bacteria.

Test Organisms: The pure cultures of the microorganisms were obtained from Awadh laboratory, Kanpur. The purity of culture was checked frequently during the experiment by the staining method. Nutrient Agar media (CDH) was used to isolate pure strains of bacteria.

Preparation of Inoculum: Procedure for preparation of inoculum was adopted as described by Hanlon *et al.*¹²

- Identified pure cultures were kept as stock strains.
- Fresh culture strains were prepared from stock strains, which were used as inoculum.
- The saline dilutions of each culture used were made to appear in turbidity equivalent to 0.5 McFarland standards.
- The fresh subculture of each microorganism was picked up with a sterilized wire loop and introduced to 5 ml saline water.
- It was shaken thoroughly and homogenized to prepare a suspension that was then incubated until its turbidity was visually equivalent to that of 0.5 McFarland standards.
- From this bacterial suspension 1 ml. was taken and mixed with 9 ml. of saline water gave inoculum concentration of 10^7 CFU/ml.

Pouring the Plates:¹²

- Labeled out round Petri plates for each concentration of antibiotics being tested.
- Prepared 1L of media for bacteria the manufacturer instructions (12g in 1000 ml distilled water).
- Cooled the media at water bath at 45 °C.
- Maintained the pH of the medium 7.2 to 7.4 at room temperature.
- Added 15 g of agar on liquid media.

- Added synthesized compound's dilutions on the liquid agar media.
- Swirled the flask to mix thoroughly.
- Poured into round Petri plates on a level surface to a depth of 3-4 mm.
- Plates were allowed to solidify at room temperature.

Preparation of Control Plates:¹² In addition to preparing antibiotic dilution plates, it was also important to prepare control plates. These plates consist of only the agar-based media with no antibiotic added. Test and standard drugs were not soluble in water; therefore, stock solutions were prepared in Ethanol (99%). Ethanol itself showed antibacterial activity; thus, there was a strong need to prepare control plates of ethanol with the same dilutions. The preparation of these plates was carried out by the same procedure as described in section 3.2.3. A control plate with ethanol dilutions was referred to as +ve control while without ethanol was – ve control.

Assay Method: Antimicrobial activity of synthesized compounds was determined using Agar Dilution Method. The apparatus used was sterilized. The procedure was carried out under laminar airflow to observe strictly sterile conditions. All plates were incubated at 37 °C for 48 h in for of bacteria.

Determination of MIC: Effectiveness of a chemotherapeutic agent against a pathogen can be obtained from the minimal inhibitory concentration (MIC). The MIC is the lowest concentration of a drug that prevents the growth of a particular pathogen¹³. Antibiotic plates were read for endpoints on a dark non-reflecting surface. The MIC was read as the first antibiotic concentration that inhibits the growth of the organism completely. The minimum inhibitory concentration (MIC- μ g/ml) was considered to be the lowest concentration that completely inhibited growth on agar plates, disregarding a single colony or a faint haze caused by the inoculum.

The synthesized compounds were evaluated for antibacterial activity by agar dilution method against *Salmonella typhi*, *Vibrio cholera*, *Staphylococcus aureus*, *Klebsiella pneumonia*,

Staphylococcus epidermidis, *Pseudomonas ferundi*. MIC of the compounds tested are shown in *aeruginosa*, *Shigella flexnari*, and *Citrobactor* **Table 4** and **Fig. 1**.

TABLE 4: ANTIMICROBIAL ACTIVITY OF MANNICH BASES OF TETRAHYDROQUINAZOLINONE & ITS DERIVATIVES (2a-h) BY AGAR DILUTION METHOD: MIC IN $\mu\text{g/mL}$ OF THE SYNTHESIZED TEST AND STANDARD COMPOUNDS

Test Organism	MIC ($\mu\text{g/ml}$) of the Test and Standard Compounds					
	2c	2e	2d	2f	Ciprofloxacin	Norfloracin
<i>Salmonella typhi</i>	0.10	0.15	0.25	0.25	0.15	0.50
<i>Vibrio cholera</i>	0.10	0.10	0.19	0.25	0.15	0.50
<i>Klebsiella pneumonia</i>	0.15	0.15	0.50	0.25	0.25	0.50
<i>Shigella flexnari</i>	0.10	0.15	0.25	0.50	0.19	0.75
<i>Citrobactor ferundi</i>	0.10	0.10	0.25	0.25	0.25	0.75
<i>Staphylococcus epidermidis</i>	0.50	0.50	0.50	0.75	0.50	1.00
<i>Staphylococcus aureus</i>	0.25	0.19	0.75	0.75	0.25	1.00

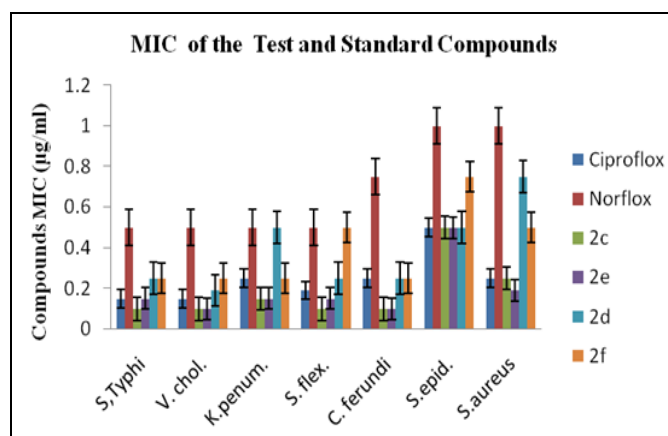


FIG. 1: REPRESENTS THE MIC OF SYNTHESIZED, TEST AND STANDARD COMPOUNDS

Anti-inflammatory Activity:

Procedure:

Materials:

Animals: Wister rats (150-200gm)

Approval: Approved by ethical committee CPCSEA registration no. (1122/ac/07/CPCSEA).

Drug: Indomethacin

Chemical: Carrageenan (1%), Sodium Chloride, CMC

Anti-inflammatory Activity (Carrageenan-Induced Paw Oedema Method in Rats): ¹⁴ Anti-inflammatory activity was performed by carrageenan-induced paw edema method in rats. Indomethacin (10 mg/kg, i.p) was administered as a standard drug for comparison. The synthesized compounds were administered at 10 mg/kg dose level were administered orally 30min prior to the administration of 0.1 ml/kg body weight of carrageenan in saline (1% w/v) into the lateral malleolus of the sub-planter region of the left hind paw. The paw volumes were measured using the mercury displacement technique with the help of a plethysmograph immediately before and 60 min, 120 min, and 180 min after carrageenan injection. The percentage inhibition of paw edema was calculated by using the following formula, and the results are depicted in **Table 5** and **Fig. 2**.

$$\text{Percentage inhibition} = \left[\frac{(\text{Control} - \text{Test})}{\text{Control}} \right] \times 100$$

TABLE 5: THE ANTI INFLAMMATORY ACTIVITY OF COMPOUNDS AT DIFFERENT TIME INTERVALS USING CARRAGEENAN-INDUCED PAW EDEMA IN RATS COMPARED TO INDOMETHACIN

S. no.	Compound code	Volume of Paw Edema (ml)			
		2 h	% inhibition	4 h	% inhibition
1	Control	0.85 \pm 0.011	-	0.8 \pm 0.012	-
2	Standard	0.2 \pm 0.010	76.4	0.23 \pm 0.012	71.25
3	2a	0.45 \pm 0.011	47.0	0.42 \pm 0.023	47.5
4	2b	0.42 \pm 0.016	50.0	0.37 \pm 0.015	53.7
5	2c	0.28 \pm 0.014	67.0	0.25 \pm 0.015	68.7
6	2d	0.36 \pm 0.018	57.6	0.33 \pm 0.017	58.7
7	2e	0.3 \pm 0.018	64.7	0.26 \pm 0.021	67.5
8	2f	0.32 \pm 0.021	62.3	0.29 \pm 0.020	63.7
9	2g	0.56 \pm 0.013	30.0	0.52 \pm 0.011	38.8
10	2h	0.38 \pm 0.019	55.2	0.34 \pm 0.021	57.5

Values are expressed as mean \pm S.E.M (n = 6)

Values in parenthesis (percentage inhibition of edema), Dose =10 mg/kg

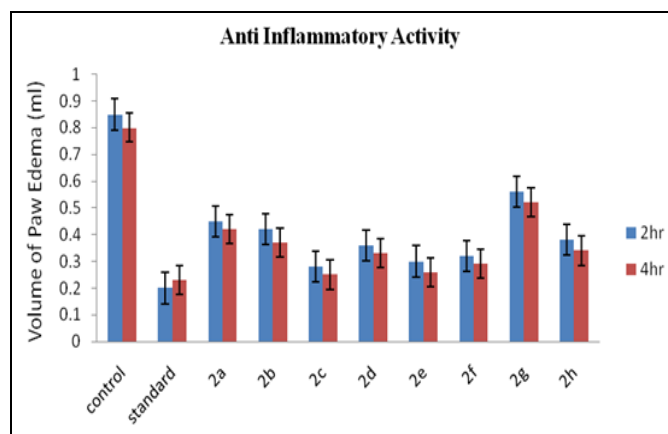


FIG. 2: REPRESENTS ANTI-INFLAMMATORY ACTIVITY OF SYNTHESIZED COMPOUNDS COMPARED TO STANDARD DRUG INDOMETHACIN

RESULTS AND DISCUSSION: The synthesized compounds were evaluated for antibacterial activity by agar dilution method against *Salmonella typhi*, *Vibrio cholera*, *Klebsiella pneumonia*, *Shigella flexnari*, *Citrobactor ferundi*, *Staphylococcus epidermidis* and *Staphylococcus aureus*, in the concentration range of 0.05 - 10 µg/ml. MIC of the compounds tested is shown in **Table 4** and **Fig. 1**.

The compounds 2c and 2e are more potent than ciprofloxacin against *Salmonella typhi*, *Vibrio cholera*, *Shigella flexnari*, *Citrobactor ferundi*, *Klebsiella pneumonia*, and equipotent to that of ciprofloxacin against *Staphylococcus aureus*, *Staphylococcus epidermidis*. Compound 2c is more potent than compound 2e against all these bacteria except *Klebsiella pneumonia*.

Compounds 2d and 2f are more potent than norfloxacin against all these bacteria. Compound 2d is more potent than 2f against all these bacteria except *Klebsiella pneumonia*.

The MIC value of compounds 2c and 2e is lower as compared to the MIC value of compounds 2d and 2f. The potency of ciprofloxacin derivative is higher as compared to norfloxacin derivative against *Salmonella typhi*, *Vibrio cholera*, *Staphylococcus aureus*, *Klebsiella pneumonia*, *Staphylococcus epidermidis*, *Pseudomonas aeruginosa*, *Shigella flexnari* and *Citrobactor ferundi*.

All the synthesized compounds were screened for their *in-vivo* anti-inflammatory activity using the carrageenan-induced rat paw edema model and exhibited protection against carrageenan-induced

edema **Fig. 2a**, and **b**. The protection ranged up to 76.4 percent for reference drug (indomethacin) after 2 hrs and 74.52 after 4 h, while the synthesized compounds (2c & 2e) provided protection up to 68.7 percent and 67.5 percent after 4 h at an equivalent dose. The compounds (2d & 2f) provided protection up to 58.7 percent and 63.7 percent after 4 h.

The result of this study clearly showed that the percentage inhibition for standard drug indomethacin was slightly decreased after 2 h while the percentage inhibition for the synthesized compound was continuously increased after 2 h.

Among all the tested compounds, 2c and 2eshowed remarkable anti-inflammatory activity compared to other derivatives. Compounds (2c & 2e) showed the longer duration of action as compared to standard drug indomethacin

CONCLUSION: Amongst these tested compounds 2c, 2d, 2e, 2f exhibited potent antimicrobial activity than the rest of the compounds against *Salmonella typhi*, *Vibrio cholera*, *Shigella flexnari*, *Citrobactor ferundi*, *Klebsiella pneumonia*. 2c and 2e showed remarkable anti inflammatory activity compared to other derivatives. Compounds (2c & 2e) showed the longer duration of action as compared to standard drug indomethacin.

ACKNOWLEDGEMENT: The author would like to thanks Dr. Abhinav Prasoon Mishra for his support and also thanks to CDRI Lucknow for tested the samples.

CONFLICTS OF INTEREST: No conflict of interest.

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How to cite this article:

Rajput R: Synthesis and pharmacological evaluation of some novel 1, 2, 3, 4-tetrahydroquinazolinone derivatives. *Int J Pharm Sci & Res* 2020; 11(8): 3912-22. doi: 10.13040/IJPSR.0975-8232.11(8).3912-22.

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