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Synthesis and Characterization of 2, 3-Disubstituted Quinazoline-4(3h)-Ones and Their Potential Biological Activity

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Abstract

In the present study, a series of novel 2, 3-disubstituted quinazoline-4(3h)-ones are prepared by condensation of the anthranilic acid with acetic anhydride or benzoyl chloride to get 2-methyl-(4H)-benzo[1,3]oxazin-4-one(3) or 2-phenyl-(4H)- benzo[1,3]oxazin-4-one (5). These are reacting with substituted amines to get title compounds (7a-7g and 8a-8g) and characterized by FTIR, ^{1}H-NMR, Mass spectroscopy. Further, the compounds were screened for the anti-tubercular activity of the synthesized quinazolinones (7a-7g & 8a-8g) was screened against M. tuberculosis H37 RV strain in the Middlebrook 7H9 (MB 7H9 broth) by using Streptomycin and Pyrazinamide as standard drug. The higher anti-tubercular activity of compounds 7b & 8c having thioamido, and guanidino groups exhibited more activity.

Keywords
Quinazolinones, Anti-tubercular activity, Ciprofloxacin, Streptomycin and Pyrazinamide.

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INTRODUCTION:

Heterocyclic compounds containing a ring made up, in addition to carbon atoms, other elements (heteroatom's), most often nitrogen, oxygen, and sulfur, and less frequently phosphorus, boron, and silicon. Benzopyrimidine is usually called Quinazoline **(1)**. It is a bicyclic compound consisting of a pyrimidine system fused at 5th, 6th positions with benzene ring chemical formula is $C_8H_8N_2$ having yellow colored crystalline compound. Its keto derivative quinazolinone ($C_8H_6N_2O$) **(2)** is a building block for approximately 120 naturally occurring alkaloids isolated till date from a number of families of the plant kingdom, microorganisms and from animals.

The first quinazolinone was synthesized in the late 1860's from anthranilic acid and cyanogens to give the 2-cyanoquinazolinone [1, 2]. Interest in the medicinal chemistry of quinazolinone derivatives was stimulated in the early 1950's with the elucidation of a quinazolinone alkaloid 3- [β-keto-γ(3-hydroxy 2-piperdyl)-propyl]-4-quinazolone from an Asian plant *Dichroa febrifuga*, which is an ingredient of a traditional Chinese herbal remedy, effective against malaria. Quinazolinone derivatives are reported to show antibacterial [3] and antifungal

[4] activities. A number of Schiff bases derived from Quinazolinone and its derivatives have been reported with various biological properties such as, antimicrobial [5], Central nervous system (CNS) depressant [6], anti-HIV [7], anti-inflammatory [8], and analgesic [9-14] and as anticancer agents [15].

MATERIALS AND METHODS

The synthesized compounds were screened for anti-tubercular activity, antibacterial activities. Fourier Transform IR spectrometer (model Shimadzu 8700) in the range of 400-4000 cm^{-1} Using KBr pellets and values are reported in cm^{-1} and the spectra were interpreted. ^{1}H-NMR spectra were recorded on DPX-200 MHz NMR spectrometer using DMSO-d_6 and chemical shifts (δ) are reported in parts per million down field from internal reference Tetramethylsilane (TMS) and the Spectra were interpreted. Mass spectra were recorded on Mass spectrophotometer (model Shimadzu) by LC- MS and the spectra were interpreted. Precoated Silica Gel G plates were used to monitor the progress of reaction as well as to check the purity of the compounds: cyclohexane: ethyl acetate (2:1).

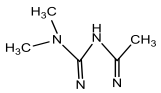
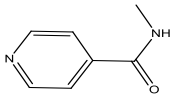
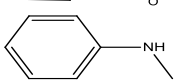
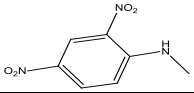
General procedures

Synthesis of 2-methyl-(4H)-benzo[1,3]oxazin-4-one: (3) A mixture of anthranilic acid 1 (0.02 mol, 2.7242 gm) in acetic anhydride 2 (2 ml) was heated for 1hr; the excess solvent was then distilled off under reduced pressure. The reaction mixture was cooled, filtered, washed with petroleum ether, dried and recrystallized with absolute ethanol to get 2-methyl-(4H)-benzo [1,3] oxazin-4-one. Completion of the reaction was determined by thin layer chromatography using cyclohexane: ethyl acetate (2:1) as mobile phase.

Synthesis of 2-phenyl-(4*H*)-benzo [1,3] oxazin-4-one: (5) To a mixture of anthranilic acid (0.1mol) dissolved in pyridine (60 ml) and benzoyl chloride (0.2mol) was added. The mixture was stirred for 30 min followed by treatment with 5% NaHCO3 (15 ml). The solid obtained was recrystallized with ethanol to get 2-phenyl-(4*H*)- benzo [1,3] oxazin-4-one. Completion of the reaction was determined by thin layer chromatography using cyclohexane: ethyl acetate (2:1) as mobile phase.

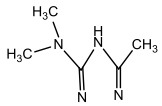
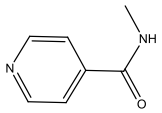
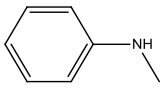
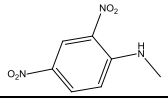
Synthesis of 2-methyl-(4*H*)3-substituted quinazolin-4-one: (7a-7g): 2-methyl-(4*H*)- benzo [1,3] oxazin-4-one(0.01mol) and amino reagent(0.02mol) in ethanol (30ml) was heated under reflux for 3 hrs. Then the reaction mixture was concentrated and solid separated was dried and recrystallized with ethanol to get 2-methyl-4*H*-3-substituted quinazolin-4-one. The homogeneity and purity of the compounds were ascertained by TLC on silica gel G-plates using cyclohexane: ethyl acetate (2:1) and the spots were visualized in iodine chamber.

Table.1: Physical and elemental analysis data of Synthesized compounds (7a-7g)

Compound Code	R	Molecular Formula	Relative Molecular Mass	M.P (^{0}C)	% yield
7a		$C_{10}H_9N_3O_2$	203.0	178-185	79
7b		$C_{10}H_9N_3OS$	219.2	189-196	92
7c		$C_{10}H_{10}N_4O$	202.1	184-189	74
7d		$C_{13}H_{16}N_6O$	274.1	195-202	89
7e		$C_{15}H_{12}N4O_2$	280.2	162-167	72
7f		$C_{15}H_{15}N_3O$	265.3	156-160	88
7g		$C_{16}H_{13}N_5O_5$	355.5	162-165	84

Synthesis of 2-phenyl-(4*H*)3-substituted quinazolin-4-one: (8a-8g): 2-phenyl-(4*H*)-benzo [1,3] oxazin-4-one and amino reagent (0.02 mol) was refluxed for 3-4 hrs in the presence of glacial acetic acid. The reaction mixture was kept at overnight and the product obtained was recrystallized using ethanol to get 2-phenyl-4H-3-substituted quinazolin-4-one. The homogeneity and purity of the compounds were ascertained by TLC on silica gel G- plates using cyclohexane: ethyl acetate (2:1) and the spots were visualized in iodine chamber.

Table.2: Physical and elemental analysis data of Synthesized compounds (8a-8g)

Compound Code	R	Molecular Formula	Relative Molecular Mass	M.P (^{0}C)	% Yield
8a		$C_{15}H_{11}N_3O_2$	265.2	275-280	78
8b		$C_{15}H_{11}N_3OS$	281.3	282-286	92
8c		$C_{15}H_{12}N_4O$	264.2	187-190	64
8d		$C_{18}H_{18}N_6O$	334.3	218-221	88
8e		$C_{20}H_{14}N_4O_2$	342.3	162-167	72
8f		$C_{20}H_{15}N_3O_3$	313.3	198-201	68
8g		$C_{20}H_{13}N_5O_5$	403.3	169-172	74

RESULTS AND DISCUSSION:

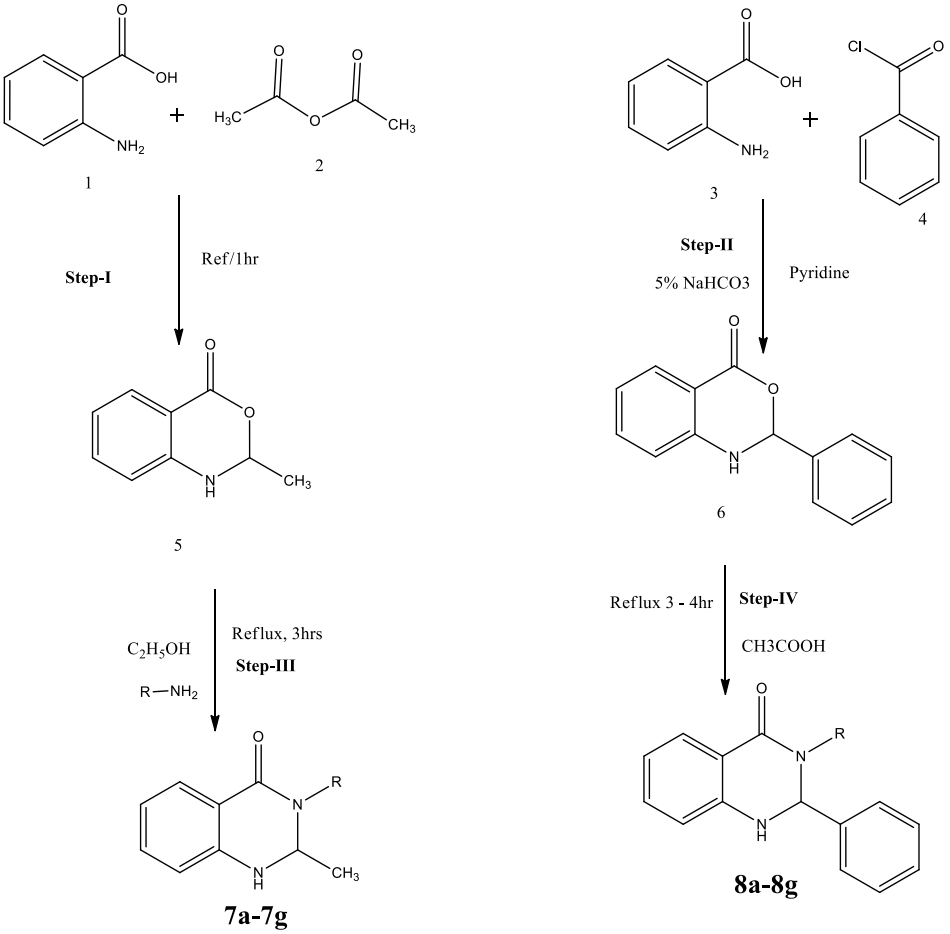
Chemistry: In this study, a series of novel 2, 3-disubstituted quinazoline-4(3h)-ones are prepared by condensation of the anthranilic acid with acetic anhydride and benzoyl chloride to get 2-methyl-(4H)-benzo [1, 3] oxazin-4-one (3) or 2-phenyl-(4H)-benzo [1, 3] oxazin-4-one(5). These are reacting with substituted amines to get title compounds (7a-7g and 8a-8g). All the synthesized compounds have been good %yield and all are characterized by FTIR, ^{1}H-NMR, Mass spectroscopy.

Spectral data: Compound (7a and 7d): 2-methyl-(4*H*) 3-substituted quinazolin-4-one: Mol. formula: $C_{13}H_{16}N_6O$, Yield was 86.76% and m. p. is 199-204 ^{0}C. Elemental analysis: Found C: 57.34% (57.33), N: 30.86% (30.87), O: 5.88% (5.86), H: 5.92% (5.99). The IR (cm-1) spectrum showed the characteristic bands at 2951.4 [C-H stretching (- CH3)], 1768.94, 1861.53 (C-H aromatic out of plane summation bands), 1684.06 [cyclic amide(δ-lactams O=C-NH-)], 1647.4 (C=N stretching [Imines]), 1298.25 [C-N stretching (20Amine)], 1270.7 (C-N Stretching 30 Amine). Well supported by its molecular ion peak at m/z 274.100 in its mass spectrum. The IR (cm-1) spectrum showed the characteristic bands at 2978.4 [C-H stretching (-CH3)]; 1888.54, 1811.38 (C-H aromatic out of plane summation bands); 1689.8 [cyclic amide(δ-lactams O=C- NH_2)]; 1647.4 (C=N stretching [Imines]); 1310 [C-N stretching (30 Amine)]; 1294.3 (C-N Stretching 10 Amine). The 1H NMR (δ ppm) spectrum of compound 7a revealed a singlet at 7.00 – 7.64 (m, 5H, Aromatic), 0.9 (s, 3H, CH_3), 2.00 (s, 2H, NH_2). Well supported by its molecular ion peak at m/z 2073100 in its mass spectrum.

Compound (8c and 8d) 2-phenyl-(4*H*)3-substituted quinazolin-4-one:

Compound (8d): Mol. For: $C_{16}H_{18}N_6O$, Yield was 83% and m. p. is 218-221 ^{o}C, Found C: 64.66% (66.66), N: 25.13% (25.11), O: 4.78% (4.76), H: 5.43% (5.45) The IR (cm^{-1}) spectrum showed the characteristic bands at 3030.44 (C-H stretching), 2964.95 [C-H stretching(-CH3)], 1923.36, 1975.35 (C-H aromatic out of plane

summation bands), 1684.06 [cyclic amide (δ-lactams O=C-NH -)], 1647.4 (C=N stretching [Imines]), 1300.18 [C-N stretching (20 Amine)], 1325.20[(C-N Stretching (30 Amine)]. Well supported by its molecular ion peak at m/z 334.375 in its mass spectrum. The 1H NMR (δ ppm) spectrum of compound 8d revealed a singlet at 2.95 integrating for the six protons of methyl groups, single at 8.26 integrated for the three protons of amines and a multiplet in between 7.45-8.15 accounting for nine aromatic protons. **Compound (8c):** The IR (cm-1) spectrum showed the characteristic bands at 3047.90 (C-H Stretching) 1921.33, 1977.28 (C-H aromatic out of plane summation bands); 1653.20 [cyclic amide(δ-lactams O=C- NH-)]; 1327.19 [C-N stretching (10Amine)]; 1261.60 [C-N Stretching (20 Amine)]; 1344.35 [C-N Stretching (30 Amine)].The 1H NMR (δ ppm) spectrum of compound 7a revealed a singlet at 7.00 – 7.64 (m, 5H, Aromatic), 0.9 (s, 3H, CH3), 2.00 (s, 2H, NH2). Well supported by its molecular ion peak at m/z 264.2 in its mass spectrum.



SCHEME

Pharmacological Evaluation:

➢ **Antitubercular Activities (MABA method):**

The antitubercular activities of the synthesized compounds were determined by Microplate alamar blue assay method (MABA) [Table 3]. [16]

Procedure

Stock solutions of the synthesized compounds and standard drug used were prepared in sterile deionized water and taken in the concentration of 0.1–100 µl/ ml. 200µl of sterile deionized water was added to all outer perimeter wells of sterile 96 wells plate to minimize the evaporation of medium in the test wells during incubation. The 96 wells plate received 100 µl of the Middlebrook 7H9 broth and serial dilutions of compounds were made directly on plate. The final drug concentrations were tested 100–0.2 µg/ml. The plates were covered and sealed with Para film and incubated at 37°C for 5 days. After this, 25 µl of freshly prepared 1:1 mixture of alamar Blue reagent and 10% tween 80 was added to the plate and incubated for 24 h. A blue color in the well was interpreted as no bacterial growth, and pink

color was scored as growth. The MIC was defined as lowest drug concentration which prevented the color change from blue to pink. [15]

➢ **Anti-Inflammatory:**

Anti-inflammatory activity of the newly synthesized compounds was determined by carrageenan induced paw edema assay in rats. Two dose levels (20 mg/kg and 50 mg/kg) of synthesized compounds and Celecoxib (10mg/kg) as standard were administered. [17] The change in the paw volumes were measured before and 1h after carrageenan injection, using the mercury displacement technique with the help of plethysmograph. The percent inhibition of paw edema was calculated from percent inhibition formula.

% inhibition (I) = 100[1 - (a - x) / (b - y)]

Where,

x = mean paw volume of rats before the administration of carrageenan and test compounds or reference compound (test group)

a = mean paw volume of rats after the administration of carrageenan in the test group (drug treated)

b = is the mean paw volume of rats after the administration of carrageenan in the control group

y = mean paw volume of rats before the administration of carrageenan in the control group.

Table 3: Antibacterial activity of synthesized compounds (7a-7g) and (8a-8g) [G –ve Bacteria]

Compounds	Zone of Inhibition									
	E. Coli					Klebsiella				
	75 µg/mL	50 µg/mL	25 µg/mL	10 µg/mL	5 µg/mL	75 µg/mL	50 µg/mL	25 µg/mL	10 µg/mL	5 µg/mL
7a	1	R	R	R	R	1	9	R	R	R
7b	1	R	R	R	R	1	9	R	R	R
7c	R	R	R	R	R	R	R	R	R	R
7d	1	R	R	R	R	1	1	R	R	R
7e	R	R	R	R	R	R	R	R	R	R
7f	1	1	1	R	R	1	R	R	R	R
7g	9	R	R	R	R	9	R	R	R	R
8a	R	R	R	R	R	R	R	R	R	R
8b	9	R	R	R	R	R	R	R	R	R
8c	1	R	R	R	R	1	1	9	R	R
8d	R	R	R	R	R	9	R	R	R	R
8e	1	R	R	R	R	R	R	R	R	R
8f	9	R	R	R	R	R	R	R	R	R
8g	R	R	R	R	R	R	R	R	R	R
Standard	Ciprofloxacin sensitive at 10 µg/mL for E. Coli 32mm and Klebsiella 30mm of zone of inhibition									

Table. 4: Anti-Inflammatory activity of novel 2, 3-disubstituted quinazoline-4(3h)-one's derivatives (% inhibition of paw edema)

% Inhibition of Paw edema	Compounds														
	Diclofenac sodium	7a	7b	7c	7d	7e	7f	7g	8a	8b	8c	8d	8e	8f	8g	8h
10mg/kg	**66.4**	28.6	34.0	43.0	35.8	**57.9**	**59.9**	35.0	36.4	39.3	**58.0**	37.1	**53.3**	36.7	**52.5**	40.1
20mg/kg	**87.3**	44.8	42.1	49.1	46.2	**70.0**	**75.4**	54.2	45.2	63.4	**75.3**	48.1	**83.1**	56.9	**79.2**	55.0

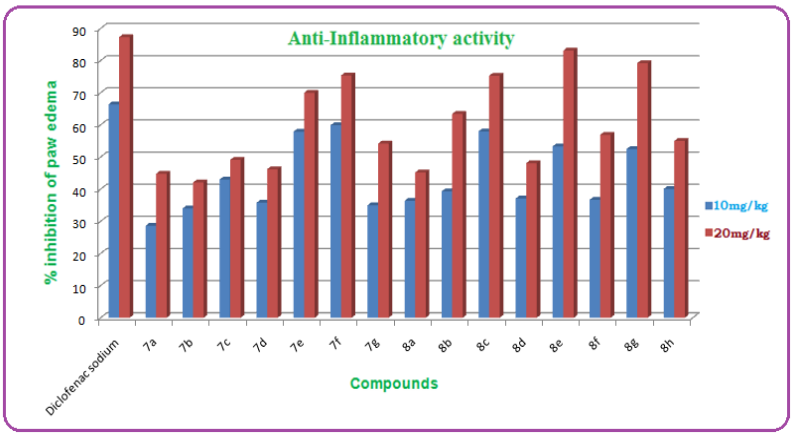


Figure.1: Comparison of Anti-Inflammatory activity of novel 2, 3-disubstituted quinazoline-4(3h)-ones derivatives (% inhibition of paw edema)

CONCLUSION

In summary, a series of novel 2, 3-disubstituted quinazoline-4(3h)-ones was to be synthesized, purify, characterize and evaluate the biological activity. The yield of the synthesized compounds was found to be in the range from 68-86%. In conclusion, the present study highlights the importance of 2, 3-disubstituted quinazoline-4(3h)-ones having various heterocyclic moiety features responsible for the anti-cancer and ant diabetic activities and may serve as a lead molecule for further modification to obtain clinically useful novel entities.

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