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SYNTHESIS OF QUINOZOLINE 2,4 (1H,3H) DIONE AND STUDY OF IT'S ANTI TUBERCULAR ACTIVITY

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ABSTRACT
Synthesis of Quinozoline 2,4 (1H,3H) dione was done and characterized by IR, NMR and mass. The results of anti-tubercular activity revealed that title compounds 7a-e and 8a-c exhibited significant activity. These compounds have amido, thioamido, imidamido, N,Ndimethyl guanidinyl and N-pyridoyl moieties at 3 rd position of quinazolinone ring. The study revealed the necessity of synthesizing many more compounds having these moieties.
Key Words: Quinozoline 2,4 (1H,3H) dione, anti-tubercular activity

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INTRODUCTION
Tuberculosis (TB) was called “phthisis” in ancient Greece, “tabes” in ancient Rome, and “schachepheth” in ancient Hebrew. In the 1700s, TB was called “the white plague” due to the paleness of the patients. TB was commonly called “consumption” in the 1800s even after Schonlein named it tuberculosis. During this time, TB was also called the “Captain of all these men of death.” During the Middle Ages, TB of the neck and lymph nodes was called “scofula.” Scofula was believed to be a different disease from TB in the lungs. Today, our names for TB tell us where TB is located (pulmonary, extrapulmonary) and how to treat it (drug-susceptible, drug-resistant, multidrug resistant, and extensively drug-resistant.). CDC and many organizations around the world are working towards a future where we call TB “history. TB in humans can be traced back to 9,000 years ago in Atlit Yam, a city now under the Mediterranean Sea, off the coast of Israel. Archeologists found TB in the remains of a mother and child buried together. The earliest written mentions of TB were in India (3,300 years ago) and China (2,300 years ago). Throughout the 1600-1800s in Europe, TB caused 25% of all deaths. Similar numbers occurred in the United States. In 1889, Dr. Hermann Biggs convinced the New York City Department of Health and Hygiene that doctors should report TB cases to the health department, leading to the first published report on TB in New York City in 1893. CDC published nationwide TB data for the first time in 1953, reporting 84,304 cases of TB in the United States. CDC publishes TB surveillance data on an annual basis. In 2019, the most recent data available, there were 8,916 reported cases of TB disease in the United States. TB disease is a nationally notifiable disease, however latent tuberculosis infection is not reported to CDC. CDC is researching ways to monitor latent TB infection on a national basis. CDC has a goal of TB elimination in the United States. Ending TB will require a dual approach of maintaining and strengthening current TB

control priorities, while increasing efforts to identify and treat latent TB infection in populations at risk for TB disease. Before the discovery of the bacteria that causes TB, the disease was thought to be hereditary. In the early 1800s, there were "vampire panics" throughout New England. When a TB outbreak occurred in a town, it was suspected that the first family member to die of TB came back as a vampire to infect the rest of the family. To stop the vampires, townspeople would dig up the suspected vampire grave and perform a ritual. On March 24, 1882, Robert Koch announced his discovery that TB was caused by a bacteria in his presentation "Die Aetiologie der Tuberculose" at the Berlin Physiological Society conference. The discovery of the bacteria proved that TB was an infectious disease, not hereditary. In 1905, Koch won the Nobel Prize for Medicine and Physiology. Today, we know TB is an airborne infectious disease, spread when a person with TB disease coughs, speaks, or sings. When a person is diagnosed with TB disease, a contact investigation is done to find and test people (like family members) who may have been exposed to TB. People diagnosed with TB disease or latent TB Infection are then treated. The TB skin test for TB infection measures a person's immune response. The test is performed by injecting a small amount of fluid (called tuberculin) into the skin on the lower part of the arm. A health care worker "reads" the test 48-72 hours later. The TB skin test was developed over time. In 1890, Robert Koch developed tuberculin (an extract of the TB bacilli) as a cure, though it proved to be ineffective. In 1907, Clemens von Pirquet developed a skin test that put a small amount of tuberculin under the skin and measured the body's reaction. Pirquet also invented the term "latent TB infection" in 1909. In 1908, Charles Mantoux updated the skin test method by using a needle and syringe to inject the tuberculin. In the 1930s, American Florence Seibert PhD developed a process to create a purified protein derivative of tuberculin (PPD) for the TB skin test. Prior to this, the tuberculin used in skin tests was not consistent or standardized. Seibert did not patent the technology, but the United States government adopted it in 1940. The TB skin test is still used today and has remained virtually unchanged for almost eighty years. The test and PPD are still listed on the World Health Organization's essential medicines list. A more recent advancement in TB testing has been TB blood tests, or interferon-gamma release assays (IGRAs). Today, we use both TB skin tests and TB blood tests to diagnose TB infection. Additional tests, like x-rays, are needed to diagnose TB disease. When TB was more common in the United States, public health departments often used mobile x-ray vans to test for TB. Mobile clinics are still in use today (1-5).

MATERIALS AND METHODS (6-8)

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 3. Completion of the reaction was determined by thin layer chromatography using cyclohexane: ethyl acetate (2:1) as mobile phase

Synthesis of 2-phenyl-(4H)-benzo[1,3]oxazin-4-one: (5)

To a mixture of anthranilic acid 1 (0.1mol) dissolved in pyridine (60 ml) and benzoyl chloride 4 (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-(4H)- benzo[1,3]oxazin-4-one 5. Completion of the reaction was determined by thin layer chromatography using cyclohexane: ethyl acetate (2:1) as mobile phase.

Synthesis of 2-methyl-(4H)3-substituted quinazolin-4-one: (7a-7g)

2-methyl-(4H)-benzo[1,3]oxazin-4-one 3 (0.01mol) and amino reagent 6 (0.02mol) in ethanol (30ml) was heated under reflux for 3 hrs. Then the reaction mixture was concentrated and solid separated was dried and recrystalized with ethanol to get 2-methyl-4H-3-substituted quinazolin-4-one 7. 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 visualised in iodine chamber.

Synthesis of 2-phenyl-(4H)3-substituted quinazolin-4-one: (8a-8g)

2-phenyl-(4H)-benzo[1,3]oxazin-4-one 5 and amino reagent 6 (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 8. 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 visualised in iodine chamber

Anti-Tubercular Activities

The anti-mycobacterial activities of compounds were assessed against M. tuberculosis using microplate alamar blue assay (MABA). This methodology is non-toxic, uses a thermally stable reagent and shows good correlation with proportional and BACTEC radiometric method. Briefly, 200µL of sterile deionized water was added to all outer perimeter wells of sterile 96 wells plate to minimized evaporation of medium in the test wells during incubation. The 96 wells plate received 100 µL of the Middlebrook 7H9 broth and serial dilution of compounds were made directly on plate. The final drug concentrations tested were 100 to 0.2µg/mL. Plates were covered and sealed with parafilm and incubated at 37ºC for five days. After this time, 25µL of freshly prepared 1:1 mixture of Alamar Blue reagent and 10% tween 80 was added to the plate and incubated for 24 hrs. A blue color in the well was interpreted as no bacterial growth, and pink color was scored as growth. 9) The MIC was defined as lowest drug concentration which prevented the color change from blue to pink101 .

RESULTS AND DISCUSSION

Synthesis was carried out by above procedure.Compound 3 analysed for C9H7NO2, Yield was 89% & m. p. is 195-197 0C. Found C: 66.07%(66.09), N: 6.89%(6.87), O: 19.86%(19.88), H: 4.38%(4.36). The IR (cm -1) spectrum (Fig.1) showed the characteristic bands at 2968.9 (-CH3), 1697.56 (aromatic summation), 1367.70 (C=N-), 1091.84 (C-O Stretching). Compound 7d analysed for C13H16N6O, Yield was 86.76% & m. p. is 199-204 0C. 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 (2 0 Amine)], 1270.7 (C-N Stretching 3 0 Amine). Well supported by its molecular ion peak at m/z 274.100 in its mass spectrum. Compound 5 analysed for C14H19NO2, Yield was 94% & m. p. is 101-1050 C. Found C: 75.33%(75.31), N: 6.27%(6.29) O: 14.33%(14.35), H: 4.06%(4.04). Compound 8d analysed for C16H18N6O, Yield was 83% & m. p. is 218-221 0C. 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 (2 0 Amine)], 1325.20[(C-N Stretching (3 0 Amine)]. Well supported by its molecular ion peak at m/z 334.375 in its mass spectrum (Table-1 and 2).

Table -1 Physical and elemental analysis data of Synthesized compounds (8a-8g)

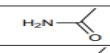
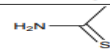
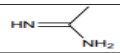
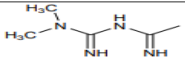
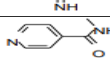
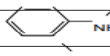
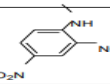
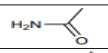
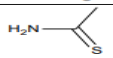
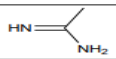
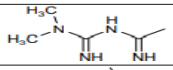
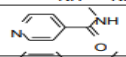
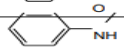
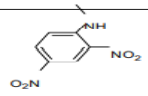
Compound Code	R	Molecular formula	Relative Molecular Mass	M.P (°C)	% yield	Elemental analysis found (calculated) %			
						C	H	N	O
7a		$C_{10}H_9N_3O_2$	203.0162	178-185	79	59.09 (59.11)	4.48 (4.46)	20.66 (20.68)	15.77 (15.75)
7b		$C_{10}H_9N_3OS$	219.262	189-196	92	54.78 (54.76)	14.62 (14.64)	19.16 (19.18)	7.30 (7.32)
7c		$C_{10}H_{10}N_4O$	202.176	184-189	74	59.40 (59.42)	4.98 (4.96)	27.71 (27.70)	7.91 (7.93)
7d		$C_{13}H_{16}N_6O$	274.100	195-202	89	57.34 (57.35)	5.92 (5.94)	30.86 (30.58)	5.88 (5.86)
7e		$C_{15}H_{12}N_4O_2$	280.281	162-167	72	64.28 (64.26)	4.32 (4.34)	19.99 (19.97)	11.42 (11.44)
7f		$C_{15}H_{15}N_3O$	265.375	156-160	88	71.70 (71.71)	5.21 (5.23)	16.72 (16.74)	6.37 (6.35)
7g		$C_{16}H_{13}N_5O_5$	355.508	162-165	84	59.11 (59.13)	4.46 (4.44)	20.68 (20.66)	15.75 (15.77)

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	Elemental analysis found (calculated) %			
						C	H	N	O
8a		$C_{15}H_{11}N_3O_2$	265.266	275-280	78	67.92 (67.90)	4.18 (4.20)	15.84 (15.86)	12.06 (12.08)
8b		$C_{15}H_{11}N_3OS$	281.332	282-286	92	64.04 (64.06)	3.94 (3.92)	14.94 (14.96)	5.69 (5.67)
8c		$C_{15}H_{12}N_4O$	264.281	187-190	64	68.17 (68.19)	4.58 (4.56)	21.20 (21.19)	6.05 (6.07)
8d		$C_{18}H_{18}N_6O$	334.375	218-221	88	64.66 (64.62)	5.43 (5.42)	25.13 (25.15)	4.78 (4.76)
8e		$C_{20}H_{14}N_4O_2$	342.350	162-167	72	70.16 (70.14)	4.12 (4.14)	16.37 (16.35)	9.35 (9.37)
8f		$C_{20}H_{15}N_3O_3$	313.352	198-201	68	76.66 (76.64)	4.82 (4.80)	13.41 (13.43)	5.11 (5.13)
8g		$C_{20}H_{13}N_5O_5$	403.347	169-172	74	59.56 (59.54)	3.25 (3.27)	17.36 (17.38)	19.83 (19.81)

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 drugs. The results of anti-tubercular activity revealed that compounds 7a-7e & 8a-8c exhibited activity at concentrations ranging from 6.25 to 100µg/mL. The remaining compounds showed no activity. It is clear from the present study that the presence of amido, thioamido, and guanidino groups at 3 rd position of quinazolinone nucleus may be necessary for the anti-tubercular activity. In particular, compounds 7b & 8c having thioamido, and guanidino groups exhibited more activity. The study revealed the importance of synthesizing many more compounds with amido, thioamido, and guanidino groups as substituents at 3rd position. Such compounds may emerge as much more potent anti-tubercular agents (table-3)

Table-3 Anti-tubercular Results

Compound	Dose (µg/mL)									
	100	50	25	12.5	6.25	3.125	1.6	0.8	0.4	0.2
7a	S	S	S	R	R	R	R	R	R	R
7b	S	S	S	S	S	R	R	R	R	R
7c	S	S	S	R	R	R	R	R	R	R
7d	S	S	R	R	R	R	R	R	R	R
7e	S	R	R	R	R	R	R	R	R	R
7f	R	R	R	R	R	R	R	R	R	R
7g	R	R	R	R	R	R	R	R	R	R
8a	S	S	R	R	R	R	R	R	R	R
8b	S	S	S	R	R	R	R	R	R	R
8c	S	S	S	S	S	R	R	R	R	R
8d	R	R	R	R	R	R	R	R	R	R
8e	R	R	R	R	R	R	R	R	R	R
8f	R	R	R	R	R	R	R	R	R	R
8g	R	R	R	R	R	R	R	R	R	R
Standard	Sensitive at Pyrazinamide- 3.125µg/mL Sensitive at Streptomycin- 6.25µg/mL									

S – Sensitive, R – Resistant

CONCLUSION

All the title compounds (7a-7g & 8a-8g) were synthesized, characterized and screened for their anti-tubercular. The results of anti-tubercular activity revealed that title compounds 7a-e and 8a-c exhibited significant activity. These compounds have amido, thioamido, imidamido, N,Ndimethyl guanidinyl and N-pyridoyl moieties at 3 rd position of quinazolinone ring. The study revealed the necessity of synthesizing many more compounds having these moieties. Such compounds may emerge as much more potent anti-tubercular agents. The results of anti-bacterial activity revealed that title compounds 7g and 8d exhibited significant activity against Gram positive bacteria. This may be due to the presence of N-phenyl (7g) and N,N-dimethyl guanidinyl moieties at 3 rd position, methyl (7g) and phenyl (8d) at 2 nd positions of quinazolinone ring system. From the results, it was observed that none of the title compounds exhibited significant inhibitory activity on the growth of Gram negative bacteria.

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