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# Synthesis of some 2,3-disubstituted quinazolinone derivatives as antitubercular and antibacterial activity agents

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**ABSTRACT**

*Currently, one third of the world's population is infected with Mycobacterium tuberculosis and 8.9 to 9.9 million new and relapse cases of tuberculosis are reported every year. The emergence of new cases, the increased incidence of multi-drug resistant strains of M. tuberculosis, and the adverse effects of first and second-line antituberculosis drugs have led to renewed research interest in synthetic products in the hope of discovering new antitubercular leads. The reaction of 3-amino-2-(substituted phenyl quinazolin-4-one with 2-acetyl pyridine, 3-acetyl pyridine, 4-acetyl pyridine provided 2-substituted phenyl-(pyridine-4-yl-ethylideneamino) quinazolin-4-(3H)- one. The structure of the compounds has been confirmed by IR, [1]HNMR, Mass spectral data and Elemental analysis. Antitubercular and antibacterial activities were performed by microbroth dilution and cup-plate method respectively. Few compounds have shown good antitubercular activity and better antibacterial activity compared to the standard drug.*

**Key words:** Quinazolin-4(3*H*)-one, antitubercular, antibacterial activity.

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## INTRODUCTION

A number of substituted quinazolin-3(4*H*)-ones were found to exhibit antitubercular [1], antibacterial [2], antimicrobial [3], anti-inflammatory [4] and CNS depressant [5] activities. In continuation of our research work on quinazolinone analogs herein we report the synthesis of title compounds 2-substituted phenyl-(pyridine-4-yl-ethylideneamino) quinazolin-4-(3*H*)- one (2a-i) from 3-amino-2-(substituted phenyl) quinazolin-4-one and evaluation of their antitubercular and antibacterial activities. The intermediate 3-amino-2-(substituted phenyl) quinazolin-4-one (1) was obtained by the reaction of 2-(substituted phenyl) benzoxazin-4-one with hydrazine hydrate, the later compound was prepared by the reaction of substituted aryl acid chloride with anthranilic acid in pyridine (Fig 1, Scheme ).

## MATERIALS AND METHODS

Melting points were measured in open capillary tubes and are uncorrected. IR (KBR) spectra were recorded in film or in potassium bromide disks on a Perkin-Elmer 39 spectrophotometer (υ max in cm-1) and $^1$H NMR spectra on a DPX 300 MHz Bruker FT-NMR spectrophotometer. The chemical shifts were reported as parts per million (δ ppm) using tetramethyl silane (TMS) as internal standard. Mass spectra were obtained on a JEOL-SX-102 instrument using fast atom bombardment (FAB positive). The progress of the reaction was monitored on a readymade silica gel

plates (Merck) using n-hexane: ethyl acetate as a solvent system. Spectral data (UV, IR, 1HNMR and Mass spectra) confirmed the structure of the synthesized compounds.

**PROCEDURE**

**Synthesis of 3-amino-2-(substituted phenyl) quinazolin-4(3*H*)-one (1):**

2-(Substituted phenyl)-1,3-benzoxazin-4-one (0.01 mole) was taken in a round bottomed flask containing absolute alcohol, hydrazine hydrate (0.03 mole) and the contents were refluxed for 5 h. The reaction mixture was cooled to obtain the solid product and was recrystallized from alcohol.

**2-substituted phenyl-(pyridine-4-yl-ethylideneamino) quinazolin-4-(3*H*)- one (2a-i):**

3-Amino-2-(substituted phenyl) quinazoline-4-ones (0.01m) was taken in a round bottomed flask and refluxed with absolute alcohol and acetyl pyridine (0.02m) for 4-5h. The reaction was monitored by TLC. After the completion of reaction contents were cooled to collect the solid crystals and recrystallized from absolute alcohol. All the compounds were prepared by following the similar procedure.

**2-(4-nitro phenyl)-3-(1-pyridin-4-yl-ethylidene-amino) -3*H* quinazolin-4-one (2a):**

$C_{21}H_{15}N_5O_3$, Mol wt: 386, m.p: 233-236, yield: 65.44%, λmax: 223, IR (KBR) $cm^{-1}$: 3223 (Ar C-H str), 1678 (cyc C=O str), 1642 (C=N str), 1348 (Ar-$NO_2$ str).

**2-(4-chloro-phenyl)-3-(1-pyridin-4-ylethylidene-amino)-3*H*-quinazolin-4-one(2b):**

$C_{21}H_{15}N_4OCl$, Mol wt: 375, m.p: 182-185, yield: 91.60%, λmax: 272, IR (KBR) $cm^{-1}$: 1674 (C=0 str), 1453 (Ar C=C), 752 (Ar-Cl str), 740 (Ar C-H out of plane bend).

**2-(2-chloro-phenyl)-3-(1-pyridin-4-ylethylidene-amino)-3*H*-quinazolin-4-one(2c):**

$C_{21}H_{15}N_4OCl$, Mol wt: 375, m.p: 144-148, yield: 39.60%, λmax: 207, IR (KBR) $cm^{-1}$: 3014 (Ar C-H), 1673(C=O), 1442 (Ar C=C), 1338 (CN), 760 (Ar-Cl str).

**2-(4-nitro-phenyl)-3-(1-pyridin-2-yl-ethylidenamino)-3*H* quinazolin-4-one (2d):**

$C_{21}H_{15}N_5O_3$, Mol wt: 386, m.p: 180-185, yield: 82.00%, λmax: 206, IR (KBR) $cm^{-1}$: 2916 (Ali C-H str), 1645 (cyc C=O str), 1450 (Ar-$NO_2$ str),1447 (Ar C=C),1348 (Ar C-N), 853 (Ar C-H out of plane bend); $^1$HNMR-1.24 $CH_3$(3H, s) 7.35-7.83 Ar H(12H, m) 2.5-3.5 Solvent peak(DMSO); m/z = 386 ; CHN Found %= N-18.07, C- 65.36, H-3.96 Calculated %= N-18.18, C-65.45, H- 3.89.

**2-(4-nitro-phenyl)-3-(pyridin-3-yl-ethylidene-amino)-3*H*- quinazolin-4-one (2e):**

$C_{21}H_{15}N_5O_3$, Mol wt: 386, m.p: 185-190, yield: 65.00%, λmax: 223, IR (KBR) $cm^{-1}$: 3304 (N-H str), 1583 (C=Cstr), 1350 (Ar-$NO_2$ str), 1348 (C-N str), 768 (Ar out of plane bend).

**2-(4-chloro-phenyl)-3-(1-pyridin-2-yl-ethylidene-amino)-3*H* quinazolin-4-one (2f)**

$C_{21}H_{15}N_4OCl$, Mol wt: 375, m.p: 200-205, yield: 79.00%, λmax: 206, IR (KBR) $cm^{-1}$: 3011(Ar C-H), 1492 (Ar C=C), 1326 (C-N),908 (Ar C-H out of plane bend), 727 (Ar-Cl str); $^1$HNMR- 1.24 $CH_3$(3H, s) 7.2- 8.8 Ar H(12H, m)2.1-3.3 Solvent peak (DMSO) ; m/z 375; CHN Found % N-14.86 C-67.41, H-3.92 Calculated % N-14.96, C-67.37,H- 4.01.

**2-(4-chloro-phenyl)-3-(pyridin-3-yl-ethylidene-amino)-3*H*- quinazolin-4-one (2g)**

$C_{21}H_{15}N_4OCl$, Mol wt: 375, m.p: 170-175, yield: 82.13%, λmax: 206, IR (KBR) $cm^{-1}$: 1675 (cyc C=O str), 1492 (Ar C=C str), 1317 (C-N), 850 (C-H out of plane bend), 750 (Ar-Cl str).

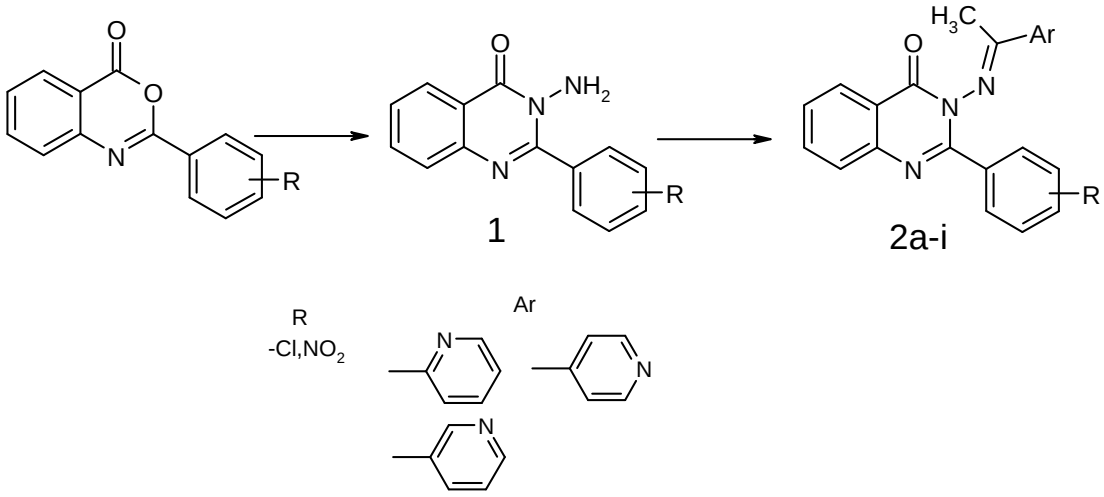
**2-(2-chloro-phenyl)-3-(1-pyridin-2-yl-ethylidene-amino)-3*H* -quinazolin-4-one (2h)**

$C_{21}H_{15}N_4OCl$, Mol wt: 375, m.p: 175-178, yield: 54.60%, λmax: 206, IR (KBR) $cm^{-1}$: 3057(Ar C-H), 1585 (Ar C=C str),757 (Ar Cl str), 740 (C-H out of plane bend); $^1$HNMR- 1.24 $CH_3$(3H, s) 7.25- 8.82 Ar H(12H, m) 2.5-3.3 Solvent peak(DMSO); m/z 375; CHN Found % N-15.07, C- 67.26, H-4.06 Calculated % N-14.97, C- 67.37, H-4.01.

**2-(2-chloro-phenyl)-3-(1-pyridin-3-yl-ethylidene-amino)-3*H*- quinazolin-4-one (2i)**

$C_{21}H_{15}N_4OCl$, Mol wt: 375, m.p: 178-180, yield: 26.60%, λmax: 206, IR (KBR) $cm^{-1}$: 1601(cyc C=O str), 1446 (Ar C=C str), 750 (Ar-Cl str), 672(C-H out of plane bend).

**Fig 1: Scheme**




**Biological Activity**

**Antitubercular Activity:**

All the synthesized compounds were tested for their *invitro* antitubercular activity against *mycobacterium tuberculosis* by agar dilution method [6] with the use of Middlebrook 7H-9 broth and standard strain of *M. tuberculosis* $H_{37}$Rv. The basal medium was prepared according to manufacture's instructions (Hi-Media) and sterilized by autoclaving. 4.5 ml of broth was poured into each one of the sterile bottles. To this, 0.5ml of ADC supplement is added. This supplement contains catalase, dextrose and bovine serum albumin fraction. Then a stock solution of the compound was prepared (10mg / ml). From this appropriate amount of solution is transferred to media bottles to achieve final concentrations of 25, 50, 100ug / ml. Finally 10ul suspension of *M.tuberculosis* strain (100000 organisms/ml, adjusted by Mc Farland's turbidity standard) was transferred to each of the tube and incubated at 37ºC. Along with this one growth control without compound and drug controls were also maintained. The bottles were inspected for growth twice a week for a period of three weeks. The appearance of turbidity was considered as growth and indicates resistance to the compound. The growth was confirmed by making a smear from each bottle and performing a ZN stain. The results are produced in **Table 1.**

**Table 1: Biological activity of the compounds 2a-n**

| Compound Code | **Antitubercular activity (Conc in µg/ml)** | **Antibacterial activity (Zone of inhibition)** | | | |
|---|---|---|---|---|---|
| | Concentration | *E.coli* | *K.pnemoniae* | *B.subtilis* | *S.aureus* |
| 2a | >50 | 14 | 14 | 15 | 11 |
| 2b | >50 | 10 | 11 | 12 | 10 |
| 2c | >50 | 05 | 16 | 10 | 13 |
| 2d | >25 | 12 | 12 | 11 | 12 |
| 2e | >50 | 17 | 16 | 18 | 15 |
| 2f | >50 | 12 | 15 | 14 | 13 |
| 2g | >50 | 16 | 17 | 16 | 12 |
| 2h | >50 | 18 | 16 | 17 | 14 |
| 2i | >50 | 15 | 14 | 17 | 13 |

**Antibacterial activity**

All the synthesized compounds were tested for their antibacterial activity against both gram positive and gram negative organisms viz., *Bacillus subtilis* (NCIM 2697), *Staphylococcus auereus* (NCIM 2079), *Escherichia coli* (NCIM 2065) *and Klebsella pneumonia* (NCIM 5082). The activity was performed by following the procedure of cup plate agar diffusion method [7]. A sterile borer was used to prepare cups of 10 mm diameter in the agar media spread with the microorganisms. 0.1 mL of inoculums (of $10^4$ to $10^6$ CFU / mL population prepared from standardized culture, adjusted with peptone water) was spread on the agar plate by spread plate technique. Accurately measured (0.1 mL) solution of each sample and standard were added to the cups with a micropipette. All the plates were kept in a refrigerator at 2 to 8 ºC for a period of two hours for effective diffusion of test compounds

and standards. Later, they were incubated at 37 ºC for 24 h. The presence of definite zones of inhibition around the cup indicated antibacterial activity. The solvent control was run simultaneously to assess the activity of DMSO, which was used as a solvent for sample. The diameter of the zone of inhibition was measured and recorded in **Table 1.**

## RESULTS AND DISCUSSION

The antitubercular activity of the synthesized compounds revealed that the compound 2-(4-nitro-phenyl)-3-(1-pyridin-2-yl-ethylidenamino)-3*H* quinazolin-4-one **(2d)** showed good activity at a concentration of 25 µg/ml, while all other compounds showed activity at 50 µg/ml against *Mycobacterium tuberculosis*. The antibacterial activity showed that the compounds 2-(4-nitro-phenyl)-3-(pyridin-3-yl-ethylidene-amino)-3*H*- quinazolin-4-one **(2e),** 2-(4-chloro-phenyl)-3-(pyridin-3-yl-ethylidene-amino)-3*H*- quinazolin-4-one **(2g)** and 2-(2-chloro-phenyl)-3-(1-pyridin-2-yl-ethylidene-amino)-3*H* -quinazolin-4-one **(2h)** were active against both the strains of bacterial organism, however the compounds showed least activity against *S aureus*.

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