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Synthesis and antitubercular activity of some new 2, 3-disubstituted quinazolinones

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ABSTRACT

In continuation for the search for newer molecules with minimal side effects, broad spectrum of activity and drug resistant pathogens, especially Mycobacterium tuberculosis a series of 2-(substituted)-N-(4oxo-2-(pyridine-2-yl)quinazolin-3(4H)-yl acetamide and 2 (substituted)-N-(4oxo-2-(pyridine-3-yl)quinazolin-3(4H)-yl acetamide were synthesized. The structure of the compounds has been confirmed by IR, NMR (^{1}H & ^{13}C), mass spectral data and elemental analysis. Antitubercular activity was performed by Microplate Alamar Blue Assay (MABA) method.

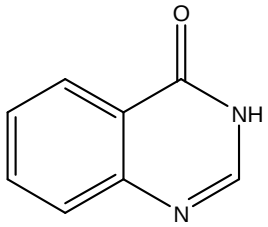
Key words: Quinazolin-4(3H)-acetamide, antitubercular activity.

INTRODUCTION

Medicinal or pharmaceutical chemistry is a scientific discipline at the intersection of chemistry and pharmacology involved with designing, synthesizing and developing pharmaceutical drugs. Medicinal chemistry is a highly interdisciplinary science combining organic with biochemistry, computational chemistry, heterocyclic chemistry, pharmacology, moleculabiology, statistics and physical chemistry [1].

Heterocyclic compounds are abundant in nature and are of great significance to life because their structural subunits exist in many natural products such as vitamins, hormones, antibiotics etc. A practical method for the synthesis of such compounds is of great interest in synthetic organic chemistry [2]. Quinazolinones are an important class of fused heterocycles with a wide range of biological activities like anti-inflammatory [3], antimicrobial [4], antioxidant [5], anticancer [6] and antihypertensive activities [7]. It has been reported that substitution of different heterocyclic moieties at 2 or 3 position of quinazolinone nucleus modulates the biological activity. It is a versatile lead molecule for the design of potential bioactive agents. This characteristic feature of quinazolinones would make a good template for a lead generation library. The quinazolinone moiety is a building block for approximately 150 naturally occurring alkaloids and drugs.

Quinazolinone nucleus is found in many bioactive natural products and also important building blocks in the synthesis of natural and pharmacological compounds. Quinazolinones are the oxidized form of quinazoline and are also part of the quinazoline alkaloids. The structures are defined by the location of the oxygen and the hydrogen on the nitrogen. The quinazolin-4(3H)-one also act as an intermediate or as natural products in many proposed biosynthetic pathways [8].



quinazolin-4(3*H*)-one

Tuberculosis (TB) is one of the most common infectious diseases known by the mankind. About 32% of the world's population is infected by *Mycobacterium tuberculosis*, the main causative agent of TB. Every year, approximately 8 million of the infected people develop active TB, and 2 million individuals die. The World Health Organization estimates that about 30 million people will be infected by *M. tuberculosis* within the next 20 years. The incidence of TB infection has steadily risen in the last decade. The reemergence of TB infection has been further complicated by an increase in the prevalence of drug-resistant TB cases.

Current control efforts are severely hampered due to M. tuberculosis being a leading opportunistic infection in patients with acquired immuno deficiency syndrome and the spreading of multidrug-resistant strains (MDR-MTB). Since no effective vaccine is available, the major strategy to combat the spreading of TB is chemotherapy and the ever-increasing drug resistance, toxicity, side effects of currently used antituberculosis drugs and the absence of their bactericidal activity highlight the need for new, safer, and more effective antimycobacterial compounds. Problems in the chemotherapy of tuberculosis arise when patients develop bacterial resistance to the first-line drugs: isoniazid, rifampicin, ethambutol, streptomycin and pyrazinamide.

Hence based upon the increasing resistance of the pathogen against *M.tuberculosis* the need was felt to synthesize some novel 2,3-disubstituetd quinazolinones and screen for their antitubercular activity. The synthesized compounds were characterized on the basis of IR, ^{1}H and ^{13}C-NMR spectral data, mass spectral data and elemental analysis. Antitubercular activity was performed by Microplate Alamar Blue Assay (MABA) [9] method.

MATERIALS AND METHODS

CHEMISTRY

All the melting points were determined in a Thermonik melting point apparatus and are uncorrected. The IR spectra of the synthesized compounds was recorded on a Fourier Transform IR spectrometer (model Shimadzu 8700) in the range of 400 -4000 using KBr pellets and the value of λmax were reported in cm^{-1}. ^{1}HNMR spectra was recorded on Amx - 400 MHz NMR spectrometer using DMSO and the chemical shifts (δ) reported are in parts per million downfield using tetramethylsilane (TMS) as internal reference. ^{13}C-NMR spectra was recorded on Amx - 400 MHz NMR spectrometer using DMSO and the chemical shifts (δ) reported are in parts per million downfield using tetramethylsilane (TMS) as an internal reference. A mass spectrum was recorded on Mass spectrophotometer (model Shimadzu) by LC-MS 2010A. The purity of the compounds was checked by thin-layer chromatography on silica gel G plates of 0.5mm thickness as stationary phase and combination of n-hexane: ethyl acetate in different ratios as mobile phase. Elemental analysis were analysed by Thermo Finnigan Flash EA 1112 Series.

Synthesis of 2-(pyrindin-2-yl)-4H-benzo[d][1,3]oxazin-4-one)(1) and 2-(pyrindin-3-yl)-4H-benzo[d] [1,3] oxazin-4-one (I)

Nicotinic acid (0.1mol) and thionyl chloride (0.15 mol) were refluxed for 2h. Excess of thionyl chloride was distilled off. The nicotinyl acid chloride was added dropwise into the solution of anthranilic acid in dry pyridine and stirred in cold condition. After complete addition of acid chloride, stirring was continued at room temperature for 6h. The reaction mixture was then poured into crushed ice to obtain the precipitate. It was washed successively with cold water and recrystallized from ethanol .

Similar procedure was followed using isonicotinic acid to obtain the above intermediate **(I).**

Synthesis of 3-amino-2-(pyridine-2-yl) quinazolin-4(3*H*)-one (2) and 3-amino-2-(pyridine-3-yl)quinazolin-4(3*H*)-one (II)

2-(pyrindin-2-yl)-4H-benzo[d][1,3]oxazin-4-one (0.011 mol) obtained from above was reacted with hydrazine hydrate (0.022 mol) using alcohol as the solvent for 5h. The reaction mixture was cooled and the product obtained was recrystallized from alcohol.

Similarly 3-amino-2-(pyridine-3-yl) quinazolin-4(3*H*)-one was carried out using the same procedure to obtain the above intermediate **(II).**

Synthesis of 2-(secondary amino)-acetic acid 3(i-v)
Equimolar ratios of secondary amines and chloroacetic acid were taken and stirred for 3h in dry acetone. The reaction mixture was poured into ice cold water to obtain the precipitate and it was recrystalized using alcohol to obtain the corresponding acetic acid derivatives.

Synthesis of 2-(substituted)-N-(4-oxo-2-pyridin-2-yl-4H-quinazolin-3-yl)-acetamide 4b(i-v) and 2-(substituted) -N-(4-oxo-2-pyridin-3-yl-4H-quinazolin-3-yl)-acetamide IVb(i-v).
The acetic acid derivatives (0.06 mol) **3(i-v)** and thionyl chloride (0.11 mol) were refluxed for 3 h. The reaction mixture was cooled to room temperature and excess of thionyl chloride was distilled off. The residue was cooled and the corresponding acid chloride was added dropwise into the solution of **[2]** in dry pyridine with stirring in cold condition followed by refluxing for 8h. The content were cooled and poured into a beaker containing crushed ice, the solid obtained was filtered, washed with water and recrystallized from ethanol to obtain the titled compounds **4b(i-v).**

Similarly 2-(substituted)-N-(4-oxo-2-pyridin-3-yl-4*H*-quinazolin-3-yl)-acetamide derivatives **IVb(i-v)** were obtained using the above procedure.

Table 1: Physicochemical data of 2-(substituted)-N-(4-oxo-2-pyridin-2-yl-4H-quinazolin-3-yl)-acetamide 4b(i-v)

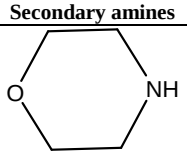
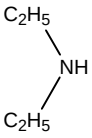
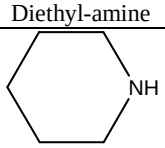
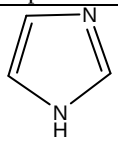
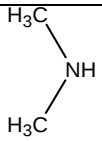
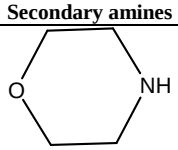
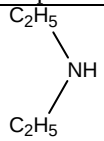
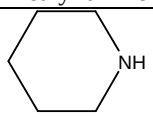
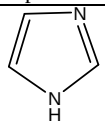
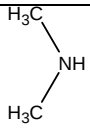
Compound	Secondary amines	Molecular Formula	M.P. (°C)	% Yield
4b(i)	 Morpholine	$C_{19}H_{19}N_5O_3$	210-212	54
4b(ii)	 Diethyl-amine	$C_{19}H_{21}N_5O_2$	220-222	58
4b(iii)	 Piperidine	$C_{20}H_{21}N_5O_2$	240-242	64
4b(iv)	 1*H*-Imidazole	$C_{18}H_{14}N_6O_2$	232-234	70
4b(v)	 Dimethyl-amine	$C_{17}H_{17}N_5O_2$	208-210	72

Table 2: Physicochemical data of 2-(substituted)-N-(4-oxo-2-pyridin-3-yl-4H-quinazolin-3-yl)-acetamide IVb(i-v)

Compound	Secondary amines	Molecular Formula	M.P. (°C)	% Yield
IVb(i)	 Morpholine	$C_{19}H_{19}N_5O_3$	220-222	60
IVb(ii)	 Diethyl-amine	$C_{19}H_{21}N_5O_2$	232-234	64
IVb(iii)	 Piperidine	$C_{20}H_{21}N_5O_2$	248-250	70
IVb(iv)	 1*H*-Imidazole	$C_{18}H_{14}N_6O_2$	238-240	74
IVb(v)	 Dimethyl-amine	$C_{17}H_{17}N_5O_2$	212-214	75

Spectral data:

2-Morpholino-N-(4-oxo-2-(pyridine-2-yl) quinazolin-3(4*H*)-yl)acetamide 4b(i)

IR (KBr, cm^{-1}): 3062 (NH str), 2823 (Ar CH str), 2300 (Al CH str), 1710 (C=O str), 1681 (C=N str), 1601 (C=C str), 1360 (C-N str), 1236 (C-O str).; ^{1}HNMR (DMSO-d_6, δ ppm): 8.61 (s, 1H, NH), 7.59-7.28 (m, 8H, Ar H), 3.30 (s, 2H, CH_2), 2.52 (s, 4H, morpholine), 2.37 (s, 4H, morpholine).; ^{13}CNMR (DMSO, ppm): C(benzene ring)- 128, 127, 134, 123, 146, 127 ; C(cyclic ketone)- 160 ; C(pyrimidine)- 160 ; C(pyridine) -149, 120, 134, 126, 144 ; C(ketone) -170 ; C(morpholine ring) 40, 78.; m/e: 363 and 364 ; CHN: Found C=62.64%, H= 5.07%, N=19.34%. Calculated C=62.46%, H=5.24%, N=19.17%

2-(Diethyl amino)-N-(4-oxo-2-(pyridine-2-yl)quinazolin-3(4*H*)-yl)acetamide 4b(ii)

IR (KBr, cm^{-1}): 3106 (NH str), 2921(Ar CH str), 2861(Al CH str), 1731(C=O str), 1629 (C=N str), 1589 (C=C str), 1393(C-N str).; ^{1}HNMR (DMSO-d_6, δ ppm): 8.375-7.171 (m, 8H, ArH +s, 1H, NH), 2.52 (s, 2H, CH_2), 2.51 (q, 4H, CH_2), 1.33 (t, 6H, CH_3).; ^{13}CNMR (DMSO, ppm): C(benzene ring)- 128, 127, 133, 123, 147, 127 ; C(cyclic ketone)- 168 ; C(pyrimidine)-163 ; C(pyridine)-153, 123, 134, 125, 146 ; C(ketone)- 170 ; C(methyl)-58 ; C(ethyl chain)- 40, 15.; m/e: 348 and 349; CHN: Found C=64.93%, H= 6.99%, N=19.69%. Calculated C=64.94%, H=6.02%, N=19.93%

N-(4-oxo-2-(pyridine-2-yl)quinazolin-3(4*H*)-yl)-2-(piperidin-1-yl)acetamide 4b(iii)

IR (KBr, cm^{-1}):3301 (NH str), 2985 (Ar CH str), 2300 (Al CH str), 1741(C=O str), 1679 (C=N str), 1581(C=C str), 1344 (C-N str).; ^{1}H-NMR (DMSO-d_6, δ ppm): 8.24 (s, 1H, NH), 8.08-7.17 (m, 8H, ArH), 3.34 (s, 2H, CH_2), 2.51 (s, 4H, piperidyl), 1.23 (s, 6H, piperdyl). ; ^{13}C-NMR (DMSO, ppm): C(benzene ring)- 128, 128, 133, 122, 144, 128) ; C(cyclic ketone)-163 ; C(pyrimidine)-163 ; C(pyridine)-155, 116, 134, 119, 149 ; C(ketone)- 170 ; C(methyl)- 57 ; C(piperidyl)- 40, 25.; m/e : 361 and 362; CHN: Found C=66.99%, H= 5.60%, N=19.30%. Calculated C=66.10%, H=5.82%, N=19.27%

2-(1*H*-Imidazole-1-yl)-N-(4-oxo-2-(pyridine-3-yl) quinazolin-3(4*H*)-yl) acetamide IVb(iv)

IR (KBr, cm^{-1}): 3331 (NH str), 3230 (Ar CH str), 2312 (Al CH str), 1743 (C=O str), 1600 (C=N str), 1468 (C=C str), 1323 (C-N str).; ^{1}H-NMR (DMSO-d_6, δ ppm): 8.59 (s, 1H, NH), 8.05- 7.28 (m, 11H, ArH), 4.31 (s, 2H, CH_2); ^{13}C-NMR (DMSO, ppm): C(benzene ring)-128, 127, 130, 123, 148 ; C(cyclic ketone)- 165; C(pyrimidine)- 165 ; C(pyridine)- 128, 148, 154, 123, 136 ; C(ketone)- 170 ; C(methyl)-57 ; C(imidazole ring)- 127, 123, 139.; m/e : 344 and 345; CHN: Found C=62.88%, H= 4.77%, N=24.65%. Calculated C=62.42%, H=4.07%, N=24.27%

2-(Dimethyl amino)-N-(4-oxo-2-(pyridine-3-yl) quinazolin-3(4*H*)-yl) acetamide IVb(v)
IR (KBr, cm^{-1}): 3186 (NH str), 2321 (Ar CH str), 2302 (Al CH str), 1731 (C=O str), 1648 (C=N str), 1541 (C=C str), 1383 (C-N str).; ^{1}HNMR (DMSO-d_6, δ ppm): 8.99 (s, 1H, NH), 8.18-7.26 (m, 8H, ArH), 2.51 (s, 2H, CH_2), 2.40 (s, 6H, CH_3).; ^{13}C-NMR (DMSO, ppm): C(benzene ring)-128, 123, 133, 123, 147, 129 ; C(cyclic ketone)- 164 ; C(pyrimidine)- 164 ; C(pyridine)- 128, 147, 153, 123, 136 ; C(ketone)- 170 ; C(methylene)- 64 ; C(methyl)- 40; m/e : 321 and 322; CHN: Found C=63.16%, H= 5.54%, N=21.19%. Calculated C=63.15%, H=5.30%, N=21.66%

Antitubercular Activity
The antitubercular activity of compounds was assessed against M. tuberculosis using Microplate Alamar Blue Assay (MABA). 200µl of sterile 96 wells plate was taken to minimize evaporation of medium in the test wells during incubation. The 96 wells plate received 100µl of the Middlebrook 7H9 broth and serial dilution of compounds were made directly on plate. The final drug concentrations tested were 100 to 0.2 µg/ml. Plates were covered and sealed with parafilm and incubated at 37°C for five days. After this time, 25µl of freshly prepared 1:1 mixture of Almar Blue reagent and 10% tween 80 was added to the plate and incubated for 24 hrs. A blue colour in the well was interpreted as no bacterial growth, and pink colour was scored as growth.

Table 3: Antitubercular activity of 2-(substituted)-N-(4-oxo-2-pyridin-2-yl-4H-quinazolin-3-yl)-acetamide 4b(i-e) and 2-(substituted)-N-(4-oxo-2-pyridin-3-yl-4H-quinazolin-3-yl)-acetamide IVb(i-e)

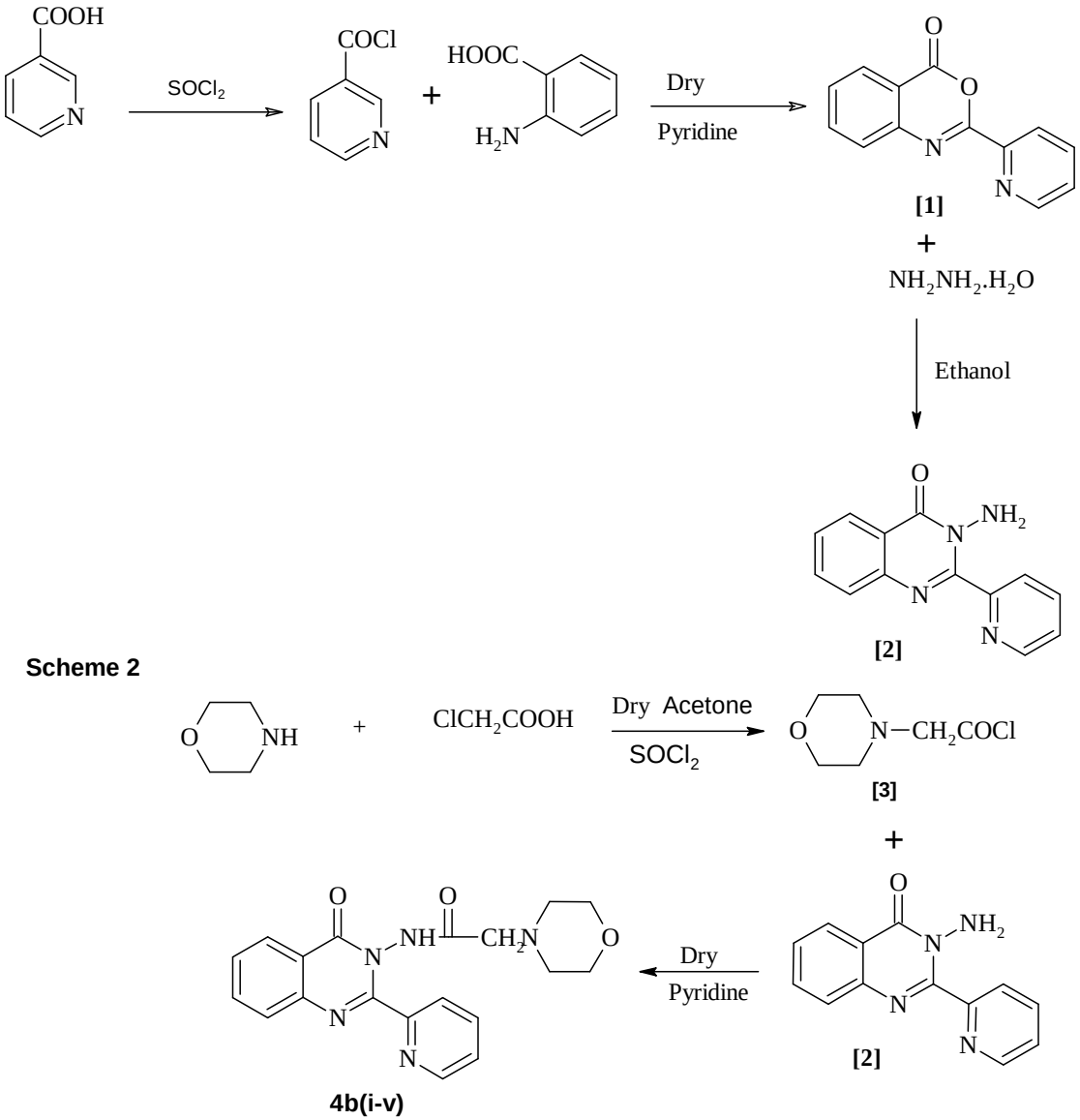
Sl. No.	Compound Code	MIC in µg/ml
1	4b(i)	50
2	4b(ii)	50
3	4b(iii)	50
4	4b(iv)	50
5	4b(v)	50
6	IVb(i)	50
7	IVb(ii)	50
8	IVb(iii)	50
9	IVb(iv)	100
10	IVb(v)	50

Standards : Pyrazinamide- 3.125µg/ml , Streptomycin- 6.25µg/ml
Ciprofloxacin-3.125µg/ml

RESULTS AND DISCUSSION

Chemistry
The synthesis of 2-**(substituted)-N-(4-oxo-2-pyridin-2-yl-4*H*-quinazolin-3-yl)-acetamide 4b(i-v) and 2-(substituted)-N-(4-oxo-2-pyridin-3-yl-4*H*-quinazolin-3-yl)-acetamide IVb (i-v)** were achieved following the steps outlined in the **Scheme 1 and 2.** The intermediates **2-(pyridin-2-yl)-4*H*-benzo[d][1,3]oxazin-4-one)(1) and 2-(pyridin-3-yl)-4*H*-benzo[d][1,3]oxazin-4-one (I)** were obtained by the reaction between nicotinic and isonicotinic acid with thionyl chloride respectively followed by dropwise addition of anthranilic acid in pyridine medium. The second intermediates **3-amino-2-(pyridine-2-yl) quinazolin-4(3*H*)-one (2) and 3-amino-2-(pyridine-3-yl)quinazolin-4(3*H*)-one (II)** were synthesized by reaction between 2-(pyridin-2-yl)-4*H*-benzo[d][1,3]oxazin-4-one)(1) and 2-(pyrindin-3-yl)-4*H*-benzo[d][1,3]oxazin-4-one (I) respectively with hydrazine hydrate. Finally the desired titled compounds were obtained by reaction of the intermediates 3-amino-2-(pyridine-2-yl) quinazolin-4(3*H*)-one **(2)** and 3-amino-2-(pyridine-3-yl)quinazolin-4(3*H*)-one **(II)** respectively with various 2-(secondary amino)-acid chloride derivatives **3(i-e).** All the compounds were obtained in good yield. All the compounds were characterized by spectral analysis. The IR spectra showed the presence of NH stretch from 3062-3331 cm^{-1} and the presence of carbonyl group from 1710-1743 cm^{-1}. In case of ^{1}H-NMR the chemical shift value for NH and methylene protons appeared as singlet in the range of 8.24-8.61 δ ppm and 2.51-4.05 δ ppm respectively. The m/e value also corresponded to the molecular weight of the desired titled compounds. The elemental analysis showed that the percentage of the nitrogen, hydrogen and carbon was found experimentally is equivalent to the calculated values

Scheme 1



Scheme 2

Note :Same procedure as outlined above was followed using isonicotinic acid to obtain titled compounds [IVb(i-e)]

Antitubercular Activity

All the synthesized compounds were screened for in-vitro antitubercular activity by MABA method. It is evident from the table that almost all derivatives have shown moderate antitubercular activity (MIC at 50 µg/ml**)** as compared to the standards which have shown activity at 3.125 µg/ml.

CONCLUSION

The present study describes the synthesis of 2, 3-substituted quinazoline derivatives with various secondary amines. All the compounds have been obtained in good yields and purity. The structure of the compounds was confirmed by IR, NMR, mass and CHN spectral data. All the derivatives were evaluated for antitubercular activity and it was found that the compounds have exhibited moderate activity. In the near future the synthesized compounds shall be considered for QSAR studies and exploited for further structural modifications to obtain more potent molecules

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