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Synthesis, antitubercular, antibacterial and antioxidant activity of some 2-phenyl-3-substituted quinazolin-4(3*H*)-ones

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ABSTRACT

In recent years there is a tremendous increase of drug resistant pathogens, especially mycobacterium tuberculosis leading to the design and development of newer antimycobacterial compounds. The reaction of 2-phenyl-3-chloroacetamido quinazolin-4(3H)-ones with various aromatic amines and thiols gave N-(4-oxo-2-phenylquinazolin-4(3H)-yl)-2-[substituted heteroaryl] acetamide derivatives. 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. The compounds have also been screened for antioxidant activity by DPPH method. All the synthesized compounds have been subjected for physical parameter evaluation. Though the compounds showed moderate antioxidant activity, few compounds have shown good antitubercular activity and better antibacterial activity compared to the standard drug.

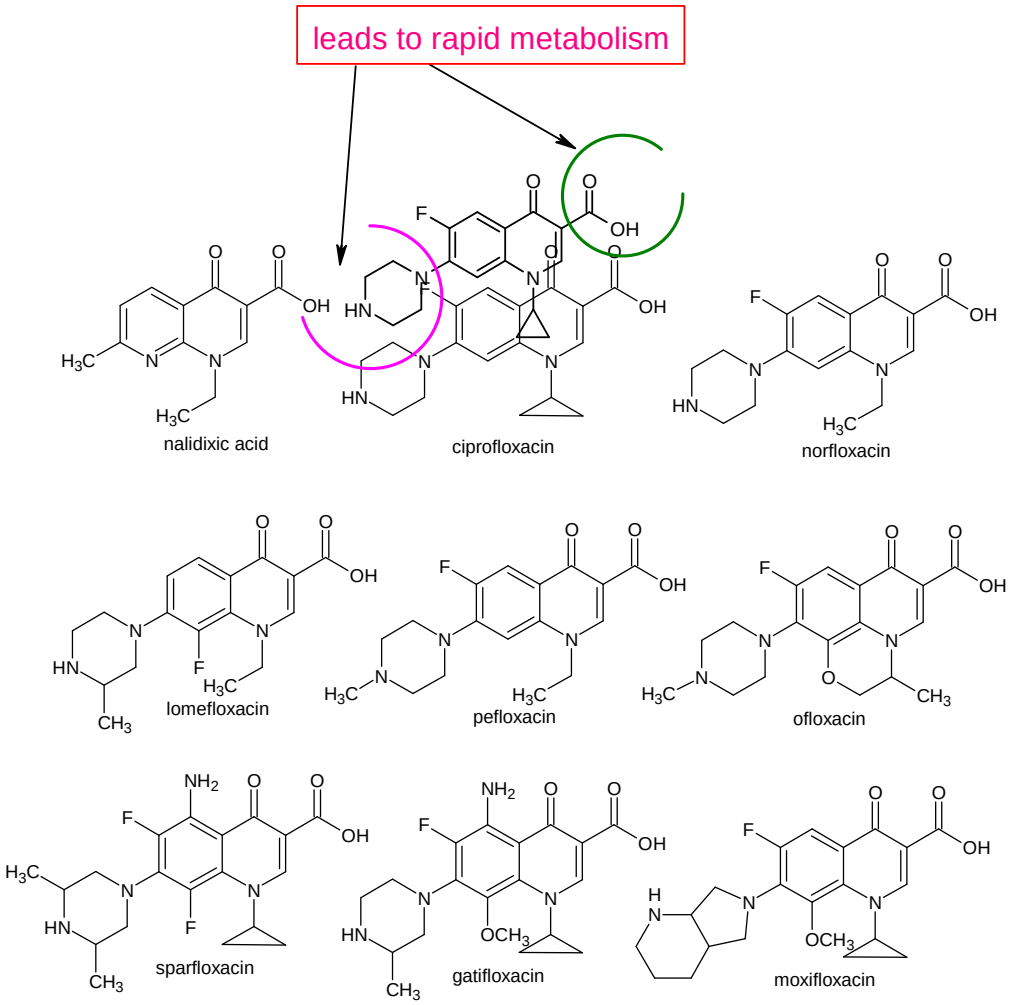
Key words: Quinazolin-4(3H)-one, physical property evaluation, antitubercular, antibacterial activity, antioxidant activity.

INTRODUCTION

The ever growing resistance to antibiotics lead to continuous screening for new biologically effective compounds of either natural or synthetic origin. Quinazolinone derivatives are extensively used in pharmaceutical industry, medicine and in agriculture for their wide scope of

biological activity [1]. Quinazolinone analogs have been reported for various biological activities such as anti-inflammatory [2], antimicrobial [3], antioxidant [4], anticancer [5] and antihypertensive activities [6]. 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.

Fig 1a: Quinolone antibacterials

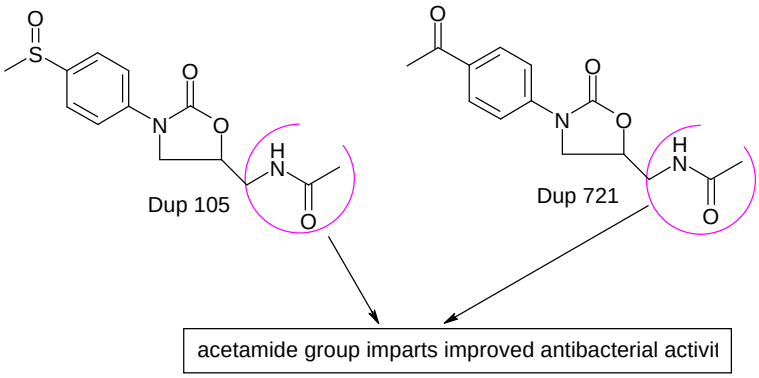


Current control efforts are severely hampered due to M. tuberculosis being a leading opportunistic infection in patients with acquired immuno deficiency syndrome and the spreading of multidrug-resistant strains (MDR-MTB). Since no effective vaccine is available, the major strategy to combat the spreading 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. Moreover it has been well established that substitution of quinazolinone at 2nd and 3rd position lead to molecules with potent biological property. Quinolones are extensively used in the treatment of various bacterial infections (**Fig 1a**). Major metabolic pathway of these drugs is by glucuronide conjugation at the 3-carboxy group producing an inactive metabolite and the piperazine ring on the quinolone drugs are also readily metabolized leading to a reduced antibacterial activity [7].

Dupont have reported DUP 105 and DUP 721 (**Fig 1b**) with an acetamide substitution and have proved that the group imparts improved antibacterial activity. Hence it was thought worthwhile to incorporate a heterocyclic ring at 3rd position of quinazolinone moiety through an acetamide linkage. In the present research work it was aimed to replace the carboxy group at 3rd position with acetamide linkage which would enhance the activity and also limit the metabolic changes and increase the half life of the molecule.

Fig 1b: antibacterials with acetamide group

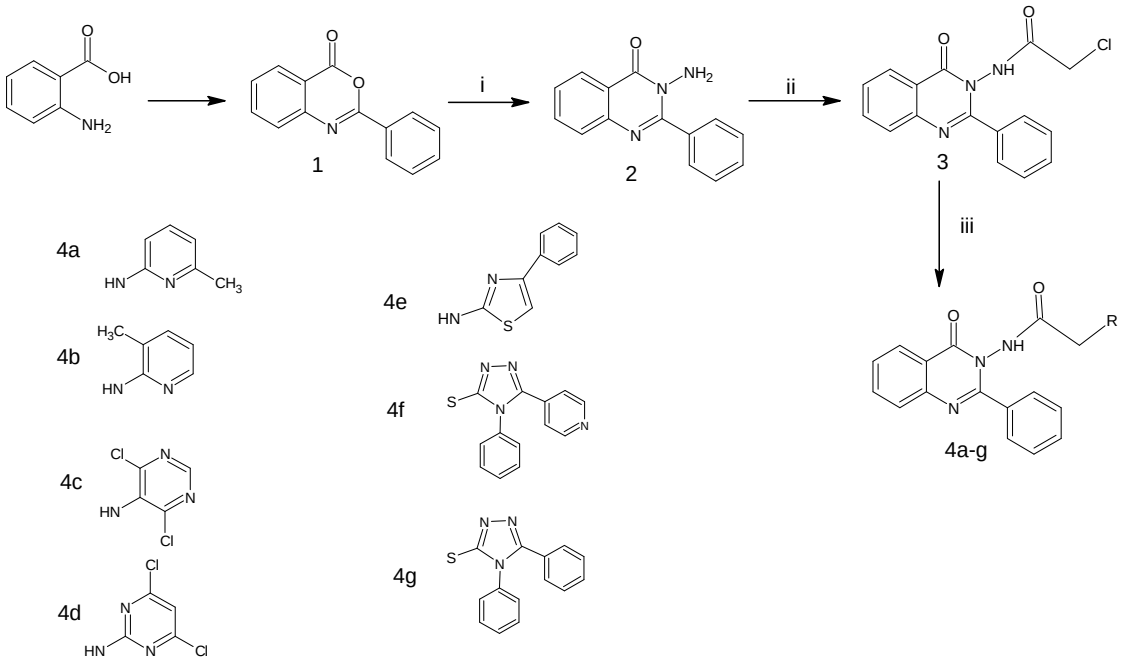


Few of the synthetic antibacterials like nitrofurantoin lead to interstitial pulmonary fibrosis in patients on chronic medication [8], which is due to the generation of oxygen radicals as a result of redox cycling of the drug in the lungs. So to obtain a better knowledge the antioxidant activity of the synthesized molecules was also evaluated.

Chemistry

The compound 2-phenyl benzoxazinone (**1**) and 3-amino-2-phenyl quinazolin-4(3*H*)one (**2**) were prepared according to reported method. Compound **2** was reacted with chloroacetyl chloride in presence of pyridine in dry benzene to obtain 2-chloro-*N*-(4-oxo-2-phenylquinazolin-3(4*H*)-yl)acetamide (**3**). Various substituted pyridyl amine, pyrimidinyl amine and triazolyl thiols were reacted with compound **3** to obtain *N*-(4-oxo-2-phenyl quinazolin-3(4*H*)-yl)-2-[(substituted aryl) amino/mercapto] acetamides **(4a-g) scheme I.**

Scheme 1:



i) NH_2NH_2 , absolute alcohol ii) $ClCOCH_2Cl$,dry benzene,pyridine iii) various heteroaryl amine/thiol,dry DMF

Physical property evaluation

As two of the designed compounds of the present study showed good antibacterial and antitubercular activity when compared with Streptomycin and Pyrazinamide, bioavailability was considered to play an important role for their potency to be bioactive molecules. Hence, a computational study for prediction of ADME properties of the molecules was performed by determination of lipophilicity, TPSA and simple molecular descriptors used by Lipinski in formulating his “rule of five” calculations by using ACD lab software, ChemDraw Ultra and www.molinspiration.com. **Table 1** represents the calculated ClogP, SMV, TPSA and other Lipinski parameters of the synthesized compounds **4a-g.** Polar surface area together with lipophilicity favors for a molecule to cross the biological membranes. Very high TPSA value contributes for a low bioavailability for the molecule. The study of molecular properties of any small molecule can be considered as a unique tool in the field of drug design and also proves that there is a relationship between the physical parameter and the biological activity. Each structure was fully geometry optimized using the ChemDraw Ultra version 8.0 force field with a root mean square deviation (rms) for the least conformation. It was interesting to observe that the Clog P value of the compounds 4e and 4g were 3.6 and 4.1 respectively while all other compounds were found to have less than 3.0.

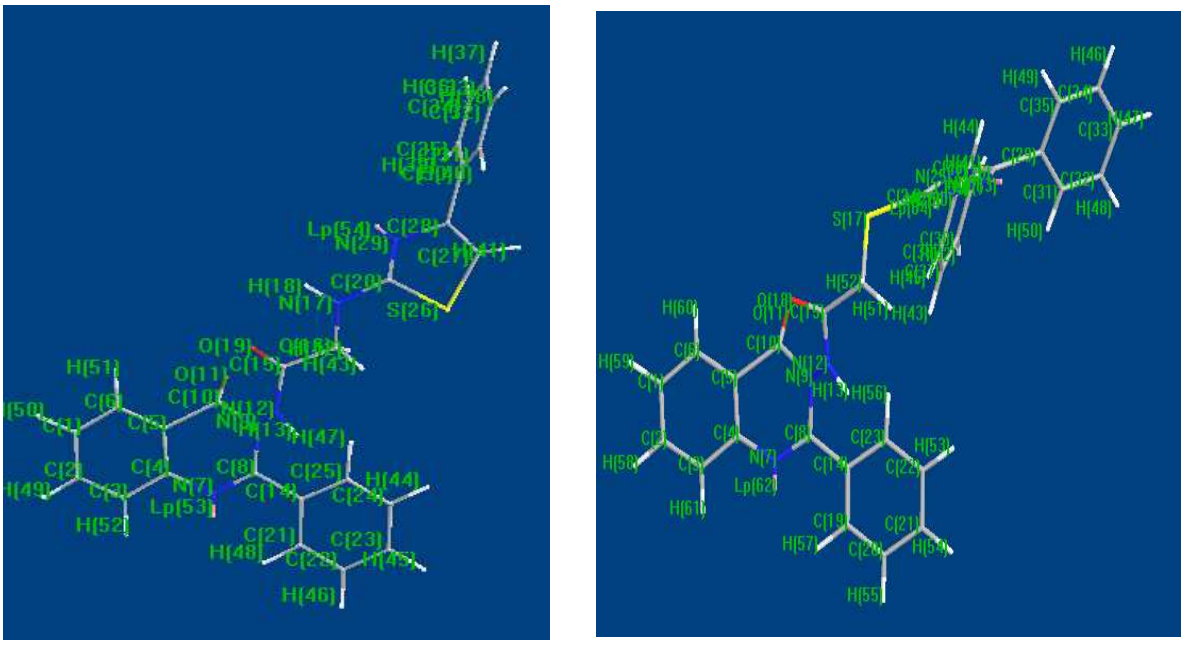
Table 1: Physical Parameters of the compounds 4a-4g.

Comp Code	Mol.Wt	ClogP[a]	SMV[b]	TPSA[c]	HBD[d]	HBA[e]	nrotb[f]	Ref[g]	Steric energy[h]	RMS gradient[i]
Lipinski[j]	≤500	≤5.0			≤5	≤10				
4a	385	2.163	295.8	88.91	2	7	5	111.43	6.73	0.079
4b	385	2.163	295.8	88.91	2	7	5	111.43	6.95	0.090
4c	441	2.538	287.8	101.80	2	8	5	114.65	6.01	0.086
4d	441	2.538	287.8	101.80	2	8	5	114.65	6.87	0.058
4e	453	3.678	330.7	88.91	2	7	6	130.70	13.97	0.095
4f	531	2.730	381.8	107.60	1	9	7	153.60	15.35	0.048
4g	530	4.130	393.1	94.71	1	8	7	155.15	15.19	0.081

a= ClogP value, b= molar volume (A^3), c= topological polar surface area, d=hydrogen bond donor, e= hydrogen bond acceptor, f= number of rotatable bonds, g= refractivity (A^3), h= total steric energy
i= RMS gradient of least energy conformation, j= Lipinski's Rule of 5 for pharmaceuticals [9]

Moreover, we identified that the RMS gradient value of the least steric energy conformation of compound 4e (**Figure 2**) was almost closer to the value of isonicotinic acid hydrazide (INH) which could be one of the factor for the molecule to show good antitubercular activity.

Figure 2: The least energy conformational structure of compound 4e and 4g.



It becomes apparent that ClogP values are not the sole predicting factor for the biological activity. In addition, despite the variation of the molecular shapes of these ligands, measurements of global molecular parameters such as surface area, volume and refractivity also showed their contribution for their biological activity. The biological inefficacy of the inactive compounds

could be attributed to the difficulty to cross the biological membranes due to their physicochemical parameters which prevent their access to their respective binding site.

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 ^1H NMR spectra on a DPX 300 MHz Bruker FT-NMR spectrophotometer. The chemical shifts were reported as parts per million (δ ppm) tetramethyl silane (TMS) as internal standard. Mass spectra were obtained on a JEOL-SX-102 instrument using fast atom bombardment (FAB positive). Elemental analysis was performed on a Perkin–Elmer 2400 C,H,N analyzer. 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 (IR, ^{1}HNMR, Mass spectra and elemental analysis) confirmed the structure of the synthesized compounds and the purity of these compounds were ascertained by microanalysis. Elemental (C,H,N) analysis indicated that the calculated and observed values were within the acceptable limits (±0.4%).

Synthesis of 2-phenyl-4*H*-3,1-benzoxazin-4-one (1)
The compound was synthesized by following the reported procedure [10].

Synthesis of 3-amino-2-phenylquinazolin-4(3*H*)-one (2)
The compound was synthesized by following the reported procedure [11].

Synthesis of 2-chloro-*N*-(4-oxo-2-phenylquinazolin-3(4*H*)-yl)acetamide (3)
The compound was synthesized by following the reported procedure [12].

Synthesis of *N*-(4-oxo-2-phenyl quinazolin-3(4*H*)-yl)-2-[(substituted aryl) amino/mercapto] acetamides (4a-g)
To a solution of 2-chloro-*N*-(4-oxo-2-phenylquinazolin-3(4*H*)-yl)acetamide (**III**) in dry DMF was added substituted heteroaryl amine/thiol slowly with stirring and the reaction mixture was refluxed for 18-24h. The reaction was monitored by TLC, after the completion of reaction, the contents were poured in to a beaker containing crushed ice, neutralized with sodium bicarbonate, the solid obtained was filtered, washed with water and recrystallised form appropriate solvent.

***N*-(4-oxo-2-phenyl quinazolin-3(4*H*)-yl)-2-[(6-methyl-pyridin-2-yl) amino] acetamides (4a)**
Crystallization from ethanol gave cream coloured crystals. mp 259-261ºC, yield 77%. Analysis for $C_{22}H_{19}N_5O_{2.}$. IR (KBr, cm^{-1}): 2927 (CH_2 str), 1689 (cyclic C=O str), 1612 (acyclic C=O str), 1545, 1541 (NH def). ^{1}HNMR (DMSO-d_6, δ ppm): 11.77 (s,1H,NH), 7.15-8.83 (m,12H,ArH), 4.8 (s,1H,NH, exchangeable with D_2O), 3.3 (s,2H, CH_2), 2.5 (s,3H, CH_3), MS (m/z): M^+ 385 (15%) and 296 (100%).

***N*-(4-oxo-2-phenyl quinazolin-3(4*H*)-yl)-2-[(3-methyl-pyridin-2-yl) amino] acetamides(4b)**
Crystallization from ethanol gave buff coloured crystals. mp 142-147ºC, yield 55%. Analysis for $C_{22}H_{19}N_5O_{2.}$ IR (KBr, cm^{-1}): 2923 (CH_2 str), 1691 (cyclic C=O str), 1615 (acyclic C=O str), 1548, 1539 (NH def).

_N_-(4-oxo-2-phenyl quinazolin-3(4*H*)-yl)-2-[(4,6-dichloro-pyrimidin-5-yl) amino] acetamides (4c)

Crystallization from ethanol gave dull coloured crystals. mp 215-218ºC, yield 57%. Analysis for $C_{20}H_{14}Cl_2N_6O_2$. IR (KBr, cm^{-1}): 2923 (CH_2 str), 1670 (cyclic C=O str), 1602 (acyclic C=O str), 1560,1528 (NH def).

_N_-(4-oxo-2-phenyl quinazolin-3(4*H*)-yl)-2-[(4,6-dichloropyrimidin-2-yl) amino] acetamides (4d)

Crystallization from ethanol gave pale brown coloured crystals. mp 232-234ºC, yield 60%. Analysis for $C_{20}H_{14}Cl_2N_6O_2$. IR (KBr, cm^{-1}): 2929 (CH_2 str), 1685 (cyclic C=O str),1604 (acyclic C=O str), 1563, 1552 (NH def).

_N_-(4-oxo-2-phenyl quinazolin-3(4*H*)-yl)-2-[(4-phenylthiazol-2-yl) amino] acetamides (4e)

Crystallization from ethanol gave pale cream coloured crystals. mp 180-183ºC, yield 44%. Analysis for $C_{25}H_{19}N_5O_2S$. IR (KBr, cm^{-1}): 2938 (CH_2 str), 1683 (cyclic C=O str), 1602 (acyclic C=O str), 1562, 1544 (NH def).

_N_-(4-oxo-2-phenyl quinazolin-3(4*H*)-yl)-2-[(4-phenyl-5-pyridin-4-yl) -1,2,4-triazolyl-3-thio] acetamides (4f)

Crystallization from ethanol gave pale cream coloured crystals. mp 117-119ºC, yield 56%. Analysis for $C_{29}H_{21}N_7O_2S$. IR (KBr, cm^{-1}): 2923 (CH_2 str), 1726 (cyclic C=O str), 1681 (acyclic C=O str), 1600, 1548 (NH def).

N-(4-oxo-2-phenyl quinazolin-3(4*H*)-yl)-2-[(4,5-diphenyl-1,2,4-triazolyl)-3-thio] acetamides (4g)

Crystallization from ethanol gave dull coloured crystals. mp 178-180ºC, yield 49%. Analysis for $C_{30}H_{22}N_6O_2S$. IR (KBr, cm^{-1}): 2920 (CH_2 str), 1712 (cyclic C=O str), 1689 (acyclic C=O str), 1600, 1569 (NH def). MS (m/z): M^+ 532 (5%), 339 (78%) and 224 (73%). Calced.:%C, 67.92; H, 4.15; N, 15.84; S, 6.03. Found %C, 67.81; H, 5.37; N, 15.78.

Biological Activity

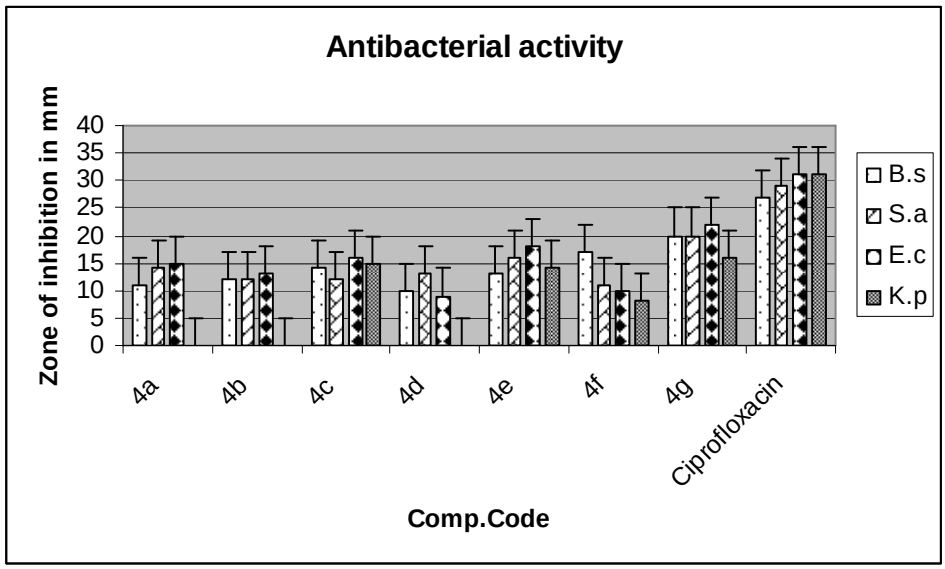
Antibacterial activity

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 [13]. 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 2, Figure 3.**

Table 2: Antibacterial Activity of the compounds 4a-4g.

Sl.No	Comp. Code	Zone of Inhibition in mm			
		Bacillus subtilis **(NCIM 2697)**	***Staphylococcus Aureus*** **(NCIM 2079)**	***Escherichia coli*** **(NCIM 2065)**	***Klebsiella Pneumonia*** **(NCIM 5082)**
1	4a	11	14	15	--
2	4b	12	12	13	--
3	4c	14	12	16	15
4	4d	10	13	09	--
5	4e	13	16	18	14
6	4f	17	11	10	08
7	4g	20	20	22	16
	Ciprofloxacin	27	29	31	31

Figure 3: Antibacterial Activity of the compounds 4a-4g.



Antitubercular Activity:

All the synthesized compounds were tested for their *invitro* antitubercular activity against *mycobacterium tuberculosis* by agar dilution method [14] 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

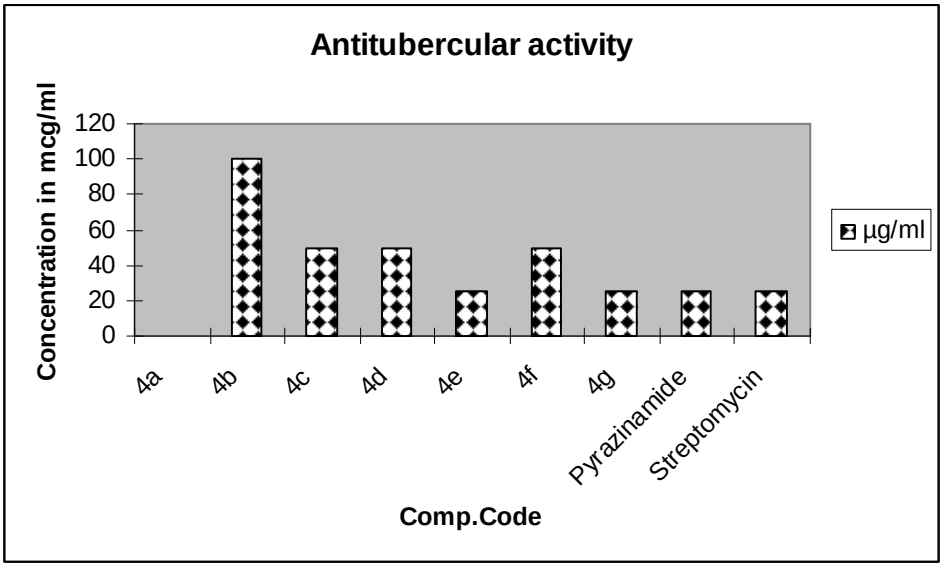
compound and drug controls were also maintained. The bottles were inspected for growth twice a week for a period of three weeks. The appearance of turbidity was considered as growth and indicates resistance to the compound. The growth was confirmed by making a smear from each bottle and performing a ZN stain. The results are produced in **Table 3, Figure 4.**

Table 3: Antitubercular Activity of the compounds 4a-4g.

Sl.No	Compound	25µg/ml	50µg/ml	100µg/ml
1	4a	--	--	--
2	4b	--	--	+
3	4c	--	+	++
4	4d	--	+	++
5	4e	+	++	+++
6	4f	--	+	++
7	4g	+	++	+++
8	Streptomycin	+	++	+++
9	Pyrazinamide	+	++	+++

-- : No activity
+ : Less active
++: Moderately active
+++: Highly active

Figure 4: Antitubercular Activity of the compounds 4a-4g.



Antioxidant Activity

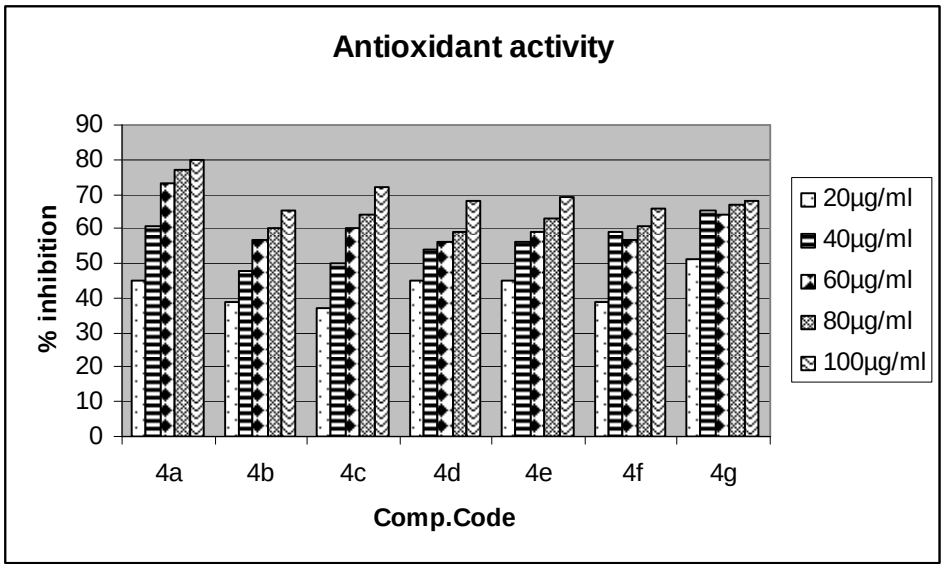
Free radical scavenging activity of the test compounds 9a-j were determined by the 1,1- diphenyl picryl hydrazyl (DPPH) assay method [15]. Drug stock solution (1 mg mL–1) was diluted to final concentrations of 2, 4, 6, 8 and 10 mg mL–1 in methanol. DPPH methanol solution (1 mL, 0.3 mmol) was added to 2.5 mL of drug solutions of different concentrations and allowed to react at room temperature. After 30 min the absorbance values were measured at 518 nm and converted into the percentage antioxidant activity. Methanol was used as the solvent and ascorbic

acid as the standard. The percentage of inhibition was extrapolated against concentration is depicted in **Fig 5**. Results are presented in **Table 4**. The standard drug used was ascorbic acid.

Table 4: Antioxidant Activity at various concentrations of the compounds 4a-4g.

Sl.No	Comp.Code	% Inhibition				
		20 µg/ml	**40 µg/ml**	**60 µg/ml**	**80 µg/ml**	**100 µg/ml**
1	4a	45	61	73	77	80
2	4b	39	48	57	60	65
3	4c	37	50	60	64	72
4	4d	45	54	56	59	68
5	4e	45	56	59	63	69
6	4f	39	59	57	61	66
7	4g	51	65	64	67	68
		2 µg/ml	**4 µg/ml**	**6 µg/ml**	**8 µg/ml**	**10 µg/ml**
	Ascorbic acid	10	15	20	31	54

Figure 5: Antioxidant Activity of the compounds 4a-4g.



RESULTS AND DISCUSSION

This study includes the synthesis of *N*-(4-oxo-2-phenyl quinazolin-3(4*H*)-yl)-2-[(substituted aryl) amino/mercapto] acetamide derivatives, evaluation of antibacterial, antitubercular and antioxidant activity. The compounds were subjected to physical property evaluation. The compounds 4e and 4g containing thiazole and triazole substitution respectively through acetamide linkage from quinazolinone moiety inhibited M*ycobacterium tuberculosis* at a concentration of 25µg/ml while other derivatives of the series inhibited at a higher concentration showing the need of thiazole or triazole moiety for producing the activity and these compounds were also found to be effective on *S.aureus* and *B.subtilis*.

CONCLUSION

The compounds 4e and 4g have shown good antitubercular activity compared with the standard drug Pyrazinamide and Streptomycin. It is obvious that only ClogP is not crucial to produce the desired pharmacological activity, there are almost 100 descriptors that are essential for a molecule to show enhanced biological property. Hence, the compounds synthesized have to be taken as a pharmacophore and exploited further.

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