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Quinazolinone novel derivatives synthesis and their Biological Evaluation as Antimicrobial and Antitubercular agents

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ABSTRACT

At a global level, tuberculosis (TB) is the disease of human values. It suffers from major health, social and economic burden in many countries. There is an inability of an effective vaccine or the use of vaccine were too long and expensive, have increased risk of spread of this disease. A series of quinazolinone derivatives prepared with the help of 2-Amino-3,4,5-trimethoxy benzoic acid and Vilsmeir reagent. Synthesized compounds were characterized by various spectral methods. Different activity like antimicrobial activity of the synthesised compounds were performed, and the most active compounds of the series were further screened for antitubercular activity against bacteria *mycobacterium H37Rv*. Results showed that all the synthesized compounds have antimicrobial activity. Compounds IV c, IV f, IV h and IV o showed maximum activity in the synthesized series. These four compounds further screened for antitubercular activity. Compounds IV c, IV f, IV h and IV o showed significant antitubercular activity.

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INTRODUCTION

Infectious disease like Mycobacterium tuberculosis (Mtb), are the main causative agent of death in human generation. Tuberculosis was first identified by Robert Koch in 1882, since his discovery, till now, the global TB epidemic seems unabeted (Dye and Williams, 2010). Different derivatives of Mtb complex are responsible for human TB, which includes Mtb itself, *Mycobacterium africanum, Mycobacterium bovis, Mycobacterium caprae, Mycobacterium microti, Mycobacterium pinnipedii* and *Mycobacterium canettii*. The major cause of infection and most of the people infected with Mtb have it as asymptomatic latent TB infection (LTBI), which mainly suffers pulmonary TB. (Zumla *et al.*, 2013; Jm, 2009). Most of the people infected with Mtb have it as asymptomatic latent TB infection (LTBI). It was spread via air or from person to person. When people with pulmonary TB different allergy germs into the air. People need to breathing only a few of these germs to become infected.

Last so many years Streptomycin show good activity against Mtb which were obtained from *Streptomyces griseus*, it shows good activity against a TB- specific therapy (Ruiz *et al.*, 2002; Ashforth *et al.*, 2010). Though many studies of streptomycin in humans TB were hopeful, doubts that they consistently cure

patients after realizing the rapid development of drug resistance when a single drug therapy was used for the treatment of TB. There are various Tuberculosis drugs with showed diverse pharmacological action such as para-aminosalicylicc acid, isoniazid, pyrazinamide, cycloserine, kanamycin etc. At that time duration of 18 months or more was used for treatment plan for TB (Zumla *et al.*, 2013; Pym and ., 2008; Shi *et al.*, 2011; Chakraborty *et al.*, 2013; Sirgel *et al.*, 2012b,a; Salian *et al.*, 2012; Pj, 2008; Murillo *et al.*, 2010; STOP, 2011). Its derivatives have been found to show different biological activities such as antimicrobial (Mishra and Jain, 1997; Patel *et al.*, 2006; Al-Salahi *et al.*, 2013), antifungal (Giri *et al.*, 1982), antibacterial (Holla *et al.*, 1998), anti-inflammatory (Srivastav *et al.*, 2009), insecticidal (Singh *et al.*, 2006), CNS depressants (Chaurasia and Sharma, 1985) etc.

Hence, from all these discussions we concluded that there is a requirement to develop anti-TB drugs with improved properties such as increased activity, reduced toxicity, shows the low duration of action, ability to penetrate host cells etc. Due to the above reasons and as a part of our research on the synthetic methods we synthesize compounds (Shaabani *et al.*, 2009, 2011), a series of quinazolinone compounds were synthesized and evaluated for antimicrobial and the compounds which showed potent activity were further performed antitubercular activity.

MATERIALS AND METHODS

Analytical methods- Open capillary tubes method were used to determine the melting point of the synthesized derivatives by Thomas-Hoover melting point apparatus. The purity was checked by TLC (Thin layer chromatography) using Silica gel G coated glass plates taking mobile phase as Ethyl Acetate: N-Hexane (5:5). Spots were visualised by iodine vapours. IR spectras (KBr) were recorded on a Shimadzu FTIR Spectrophotometer. ^{1}H-NMR spectras (DMSO) were taken on a 400 MHz spectrometer and LCMS were entrusted on Shimadzu. All the compounds showed satisfactory analytical results.

Method of preparation of the compounds (IV a-IV o)

The novel reaction occurs by treating 2-amino 3,4,5-trimethoxy benzoic acid (0.05 mmol) with the vilsmeier reagent. This reaction occurred between 80-90^{o}C when two different substituted acids were reacted with a combination of DMF (20ml) and $POCl_3$ (2.5ml). Then, at room temperature, primary amines (0.05 mmol) were made to react with continuous stirring. After amine addition, 90^{o}C temperature were raised, and the reaction were allowed to proceed for 3hrs on a magnetic stirrer. Different substituted anilines derivatives (IV a-o) were prepared.

Spectral data of the synthesized compounds-

6,7,8-trimethoxy 3-o-tolylquinazolin-4(3H)-one (IV a):

Yield - 63%;Creamy solid**; M.P. -** 395-397°C, **IR (KBr): $\bar{\upsilon}(cm^{-1})$; =** 2979 (CH, str, Ali); 1682 (C=O, str, quinazoline); 1628 (C=N, str, quinazoline); 3088 (CH, str, Ar) cm^{-1}, **^{1} H NMR (δ, ppm/ DMSO-*d6*):=**1.88 (t, 3H, -CH_3), 3.88 (m, 9H, 3-OCH_3), 6.35-7.50 (m, 5H, Ar-H), 8.84-9.14 (m, 1H, $Ar_{Quinazoline}$), **MS: m/e** 326.13.

6,7,8-Trimethoxy-3-(naphthalene-2-yl)quinazolin-4(3H)-one (IV b):

Yield- 65%, Fluffy white solid, **M.P. -** 328-331°C, **IR (KBr): $\bar{\upsilon}(cm^{-1})$; =** 2978 (CH, str, Ali); 1681 (C=O, str, quinazoline); 1622 (C=N, str, quinazoline); 3082 (CH, str, Ar) cm^{-1}, **^{1} H NMR (δ, ppm/ DMSO-*d6*):=** 4.09 (m, 9H, 3-OCH_3), 6.08-7.11 (m, 8H, Ar-H), 8.25-8.60 (m, 1H, $Ar_{Quinazoline}$),**MS: m/e** 362.13.

3-(3-Chloro-4-fluorophenyl)-6,7,8-trimethoxyquinazolin-4(3H)-one (IV c):

Yield- 61%,Dark brown solid**; M.P. -** 428-432°C, **IR (KBr): $\bar{\upsilon}(cm^{-1})$; =** 2979 (CH, str, Ali); 1688 (C=O, str, quinazoline); 1628 (C=N, str, quinazoline); 3088 (CH, str, Ar) cm^{-1}, **^{1} H NMR (δ, ppm/ DMSO-*d6*):=** 3.82 (m, 9H, 3-OCH_3), 6.11-7.62 (m, 4H, Ar-H), 8.89-9.12 (m, 1H, $Ar_{Quinazoline}$), **MS: m/e** 364.06.

3-(3-ethoxyphenyl)-6,7,8-trimethoxyquinazolin-4(3H)-one (IV d):

Yield = 54%,Violet solid**; M.P. -** 428-430°C, **IR (KBr): $\bar{\upsilon}(cm^{-1})$; =** 2979 (CH, str, Ali); 1684 (C=O, str, quinazoline); 1626 (C=N, str, quinazoline); 3087 (CH, str, Ar); 1230 (C-O, str) cm^{-1}, **^{1} H NMR (δ, ppm/ DMSO-*d6*):=** 1.68 (t, 3H, -CH_3),1.95-2.05 (m, 2H, -CH_2), 3.86 (m, 9H, 3-OCH_3), 6.21-7.64 (m, 5H, Ar-H), 8.30-8.60 (m, 1H, $Ar_{Quinazoline}$), **MS: m/e** 356.14.

3-(4-Ethylphenyl)-6,7,8-trimethoxyquinazolin-4(3H)-one (IV e):

Yield – 49%**,** Creamy solid; **M.P.** – 407-410°C, **IR (KBr): $\bar{\upsilon}(cm^{-1})$; =** 2972 (CH, str, Ali); 1689 (C=O, str, quinazoline); 1627 (C=N, str, quinazoline); 3087 (CH, str, Ar)cm^{-1}, **^{1} H-NMR (DMSO-*d6*)** δ **=** 1.75 (t, 3H, -CH_3), 1.85-2.12 (m, 2H, -CH_2), 3.79 (m, 9H, 3-OCH_3), 6.45-7.66 (m, 5H, Ar-H), 8.38-8.75 (m, 1H, $Ar_{Quinazoline}$), **MS: m/e** 340.14.

6,7,8-Trimethoxy-3-(4-methoxyphenyl)quinazolin-4(3H)-one (IV

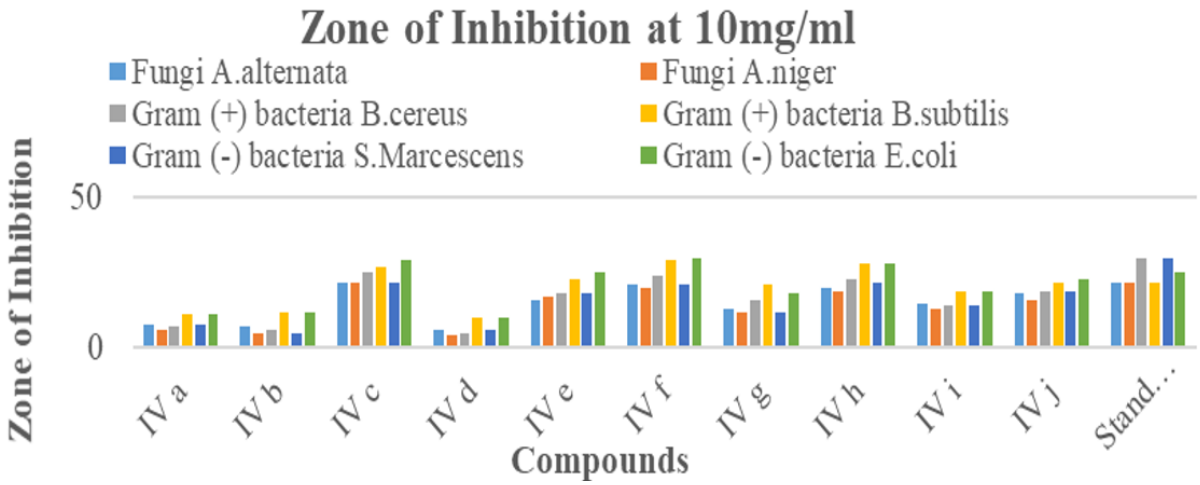


Figure 1: Graphical representation of the zone of Inhibition of the synthesized compounds (IV a-IV o)

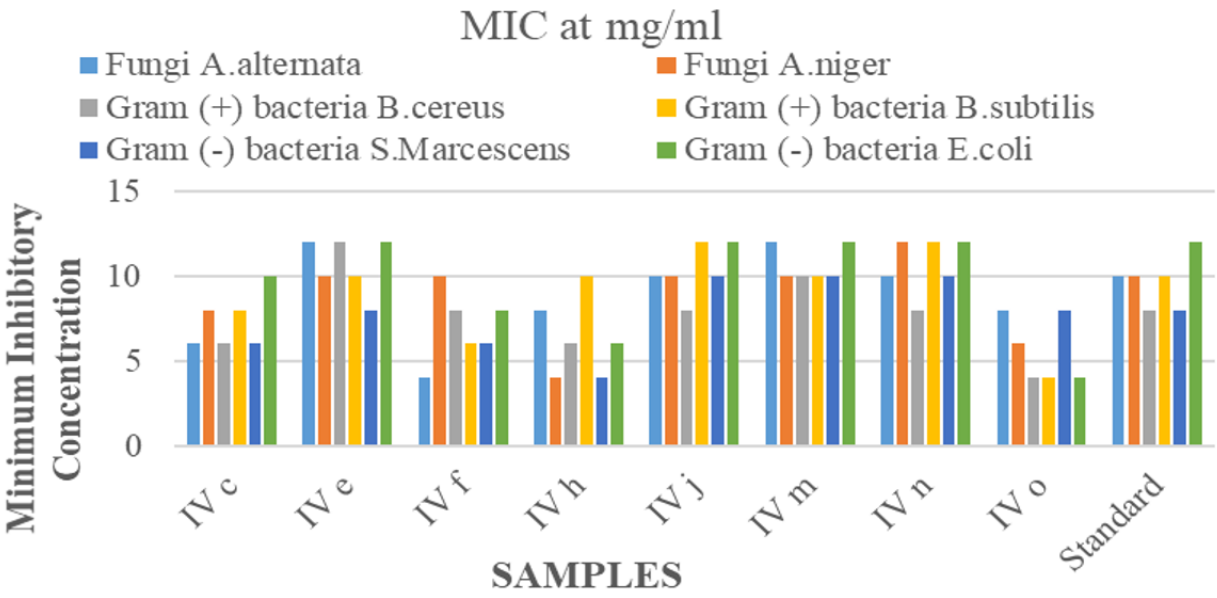


Figure 2: Graphical representation of MIC values of synthesized compounds

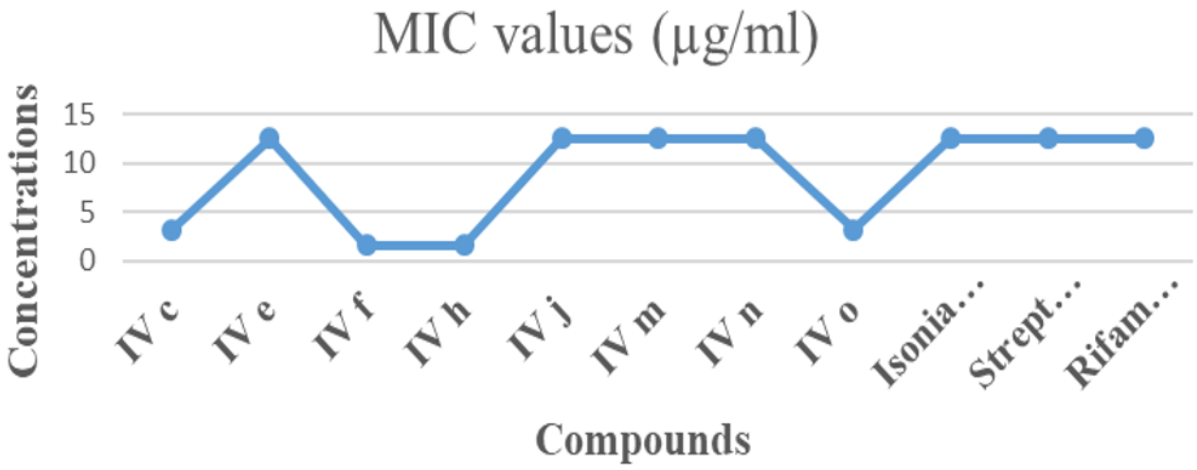
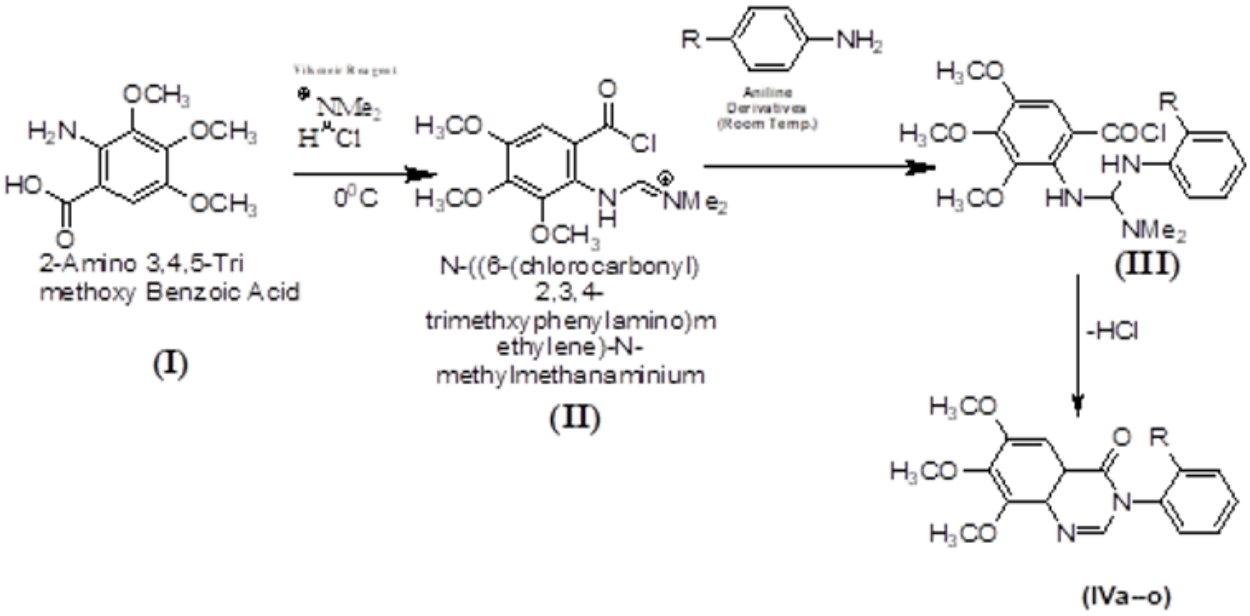


Figure 3: Graphical representation of the MIC value of the synthesized compounds against mycobacterium H37Rv strains



Scheme 1: Synthesis of quinazolin one derivatives

f):

Yield- 53%,Light brown solid**; M.P. –** 417-420°C**, IR (KBr): $\bar{\upsilon}(cm^{-1})$; =** 2982 (CH, str, Ali); 1687 (C=O, str, quinazoline); 1628 (C=N, str, quinazoline); 3085 (CH, str, Ar) cm^{-1}, **^{1} H NMR (δ, ppm/ DMSO-*d6*):=** 3.90 (m, 12H, 3-OCH_3), 6.04-7.39 (m, 5H, Ar-H), 8.92-9.20 (m, 1H, $Ar_{Quinazoline}$), **MS: m/e** 342.12.

6,7,8-Trimethoxy-3-p-tolylquinazolin-4(3H)-one (IV g):

Yield- 61%, Dark black solid; **M.P.** - 395-398°C, **IR (KBr): $\bar{\upsilon}(cm^{-1})$; =** 2978 (CH, str, Ali); 1683 (C=O, str, quinazoline); 1629 (C=N, str, quinazoline); 3087 (CH, str, Ar) cm^{-1}, **^{1} H NMR (δ, ppm/ DMSO-*d6*):=**1.89 (t, 3H, -CH_3), 3.89 (m, 9H, 3-OCH_3), 6.36-7.51 (m, 5H, Ar-H), 8.85-9.15 (m, 1H, $Ar_{Quinazoline}$), **MS: m/e** 326.13.

3-(2,4-Dimethoxyphenyl)-6,7,8-trimethoxyquinazolin-4(3H)-one (IV h):

Yield = 57%, Dark brown solid; **M.P.** - 464-467°C, **IR (KBr): $\bar{\upsilon}(cm^{-1})$; =** 2970 (CH, str, Ali); 1687 (C=O, str, quinazoline); 1629 (C=N, str, quinazoline); 3082 (CH, str, Ar) cm^{-1}, **^{1} H NMR (δ, ppm/ DMSO-*d6*):=** 3.96 (m, 15H, 3-OCH_3), 6.11-6.87 (m, 4H, Ar-H), 8.30-8.89 (m, 1H, $Ar_{Quinazoline}$), **MS: m/e** 372.13.

3-(3-Ethoxyphenyl)-6,7,8-trimethoxyquinazolin-4(3H)-one (IV i):

Yield = 58%, Light black solid; **M.P.** - 428ºC - 430ºC, **IR (KBr): $\bar{\upsilon}(cm^{-1})$; =** 2983 (CH, str, Ali); 1682 (C=O, str, quinazoline); 1630 (C=N, str, quinazoline); 3090 (CH, str, Ar); 1245 (C-O, str) cm^{-1}, **^{1} H NMR (δ, ppm/ DMSO-*d6*):** = 1.70 (t, 3H, -CH_3),1.90-2.05 (m, 2H, -CH_2), 3.90 (m, 9H, 3-OCH_3), 6.25-7.23 (m, 5H, Ar-H), 8.23-8.52 (m, 1H, $Ar_{Quinazoline}$), **MS: m/e** 356.14.

3-(2-Ethylphenyl)-6,7,8-trimethoxyquinazolin-4(3H)-one (IV j):

Yield = 65%, Light cream solid; **M.P.** - 406ºC -409ºC, **IR (KBr): $\bar{\upsilon}(cm^{-1})$; =** 2979 (CH, str, Ali); 1683 (C=O, str, quinazoline); 1625 (C=N, str, quinazoline); 3080 (CH, str, Ar) cm^{-1}, **^{1} H NMR (δ, ppm/ DMSO-*d6*):**= 1.73 (t, 3H, -CH_3),1.92-2.00 (m, 2H, -CH_2), 4.32 (m, 9H, 3-OCH3), 6.60-7.00 (m, 5H, Ar-H), 8.25-8.68 (m, 1H, $Ar_{Quinazoline}$),**MS: m/e** 340.14.

3-(3-Ethylphenyl)-6,7,8-trimethoxyquinazolin-4(3H)-one (IV k):

Yield = 63%, Fluffy white solid; **M.P.** - 406ºC - 408ºC, **IR (KBr): $\bar{\upsilon}(cm^{-1})$; =** 2981 (CH, str, Ali); 1689 (C=O, str, quinazoline); 1622 (C=N, str, quinazoline); 3082 (CH, str, Ar) cm^{-1}, **^{1} H NMR (δ, ppm/ DMSO-*d6*):**= 1.76 (t, 3H, -CH_3),1.85-2.06 (m, 2H, -CH_2), 3.98 (m, 9H, 3-OCH3), 6.98-7.15 (m, 5H, Ar-H), 8.30-8.75 (m, 1H, $Ar_{Quinazoline}$), **MS: m/e** 340.14.

3-(2-Ethoxyphenyl)-6,7,8-trimethoxyquinazolin-4(3H)-one (IV l):

Yield = 48%, Brown solid; **M.P.** - 427ºC -430ºC, **IR (KBr): $\bar{\upsilon}/(cm^{-1})$; =** 2972 (CH, str, Ali); 1683 (C=O, str, quinazoline); 1623 (C=N, str, quinazoline); 3084 (CH, str, Ar); 1230 (C-O, str) cm^{-1}, **^{1} H NMR (δ, ppm/ DMSO-*d6*):**= 1.70 (t, 3H, -CH_3),1.90-2.05 (m, 2H, -CH_2), 3.92 (m, 9H, 3-OCH3), 6.16-6.50 (m, 5H, Ar-H), 8.15-8.25 (m, 1H, $Ar_{Quinazoline}$), **MS: m/e** 356.14.

3-(4-Bromo-4-fluorophenyl)-6,7,8-trimethoxyquinazolin-4(3H)-one (IV m):

Yield = 61%, Dark yellow solid; **M.P.** - 456ºC -459ºC, **IR (KBr):** $\bar{\upsilon}(cm^{-1})$**; =** 2980 (CH, str, Ali); 1689 (C=O, str, quinazoline); 1627 (C=N, str, quinazoline); 3083 (CH, str, Ar) cm^{-1}, **^{1} H NMR (**δ**, ppm/ DMSO-*d6*):**= 3.92 (m, 9H, 3-OCH_3), 6.31-7.49 (m, 4H, Ar-H), 8.30-8.45 (m, 1H, $Ar_{Quinazoline}$), **MS: m/e** 408.01.

3-(3,4-Difluorophenyl)-6,7,8-trimethoxyquinazolin-4(3H)-one (IV n):

Yield = 61%, Black solid; **M.P.** - 397ºC -399ºC, **IR (KBr):** $\bar{\upsilon}(cm^{-1})$**; =** 2982 (CH, str, Ali); 1688 (C=O, str, quinazoline); 1629 (C=N, str, quinazoline); 3083 (CH, str, Ar) cm^{-1}, **^{1} H NMR (**δ**, ppm/ DMSO-*d6*):**= 3.70 (m, 9H, 3-OCH_3), 6.20-6.85 (m, 4H, Ar-H), 8.48-8.90 (m, 1H, $Ar_{Quinazoline}$), **MS: m/e** 348.09.

3-(2-Chloro-4-fluorophenyl)-6,7,8-trimethoxyquinazolin-4(3H)-one (IV o):

Yield = 57%, White solid; **M.P.** - 427ºC -430ºC, **IR (KBr):** $\bar{\upsilon}(cm^{-1})$**; =** 2982 (CH, str, Ali); 1689 (C=O, str, quinazoline); 1621 (C=N, str, quinazoline); 3089 (CH, str, Ar) cm^{-1}, **^{1} H NMR (**δ**, ppm/ DMSO-*d6*):**= 3.85 (m, 9H, 3-OCH_3), 6.30-6.95 (m, 4H, Ar-H), 8.60-8.95 (m, 1H, $Ar_{Quinazoline}$), **MS: m/e** 364.06.

***In-vitro* evaluation of anti-microbial activity**

Antimycobacterial *in-vitro* activity evaluation of the compounds were done by Kriby-Bauer disk diffusion method (Clinical and Laboratory Standards Institute, 2014) against two gram-positive bacterial strains [*Bacillus subtilis* (MTCC 1427), *Bacillus cereus* (MTCC 430)], two gram-negative bacterial strains [*Escherichia coli* (MTCC 405), *Serratia marcescens* (MTCC 4301)] and two fungal strains [*Aspergillus niger* (ITCC 7787), *Alternaria alternate* (ITCC 4522)]. Amoxicillin and Fluconazole were used as standard drugs.

Initial screening of the synthesised compounds and standard drugs carried out at fixed concentration of 10 mg/ml. Results of the screening were recorded for each compound as the average diameter of inhibition zones (IZ) of 24h for bacteria and fungal growth 72h around the discs in mm. Results are shown in Table 2 **.**

Determination of MIC

Minimum Inhibitory Concentration were determined by liquid dilution method against all bacterial and fungal strains (Rohini *et al.*, 2010). Standard stock solution of resultant compounds along with 2mg/ml, 4mg/ml, 6mg/ml, 8mg/ml, 10mg/ml, and 12mg/ml concentrations were prepared with suitable solvent. Bacterial and fungal strains inoculums were also prepared by this concentration using amoxicillin and fluconazole standard solutions. Test tubes in a series containing 1ml derivative solution with varied concentration, 0.2ml of inoculums, and 3.8ml of the sterile water were added. Then, the test tubes were incubated for 24 h to check the turbidity. Same methods were adopted for the rest of the derivatives and with standard drugs. In the test tubes, where there was visually no growth seen called minimum inhibitory concentration of the derivatives. All the resulted values of MIC were shown in Table 3 **.**

Antitubercular activity

Screening for antitubercular activity by Alamar-blue assay Franzblau *et al.* (1998)

Anti- tubercular activity of compounds viz. quinazolinone derivatives were assessed against H37Rv (NCFT/TB/537)- using microplate Alamar blue assay against Mycobacterium sensitive to Isoniazid(H), Streptomycin (S), Rifampicin (R). Deionised water were added to all outer-perimeter wells of 200 μL sterile wells to minimize evaporation of the medium in the test wells during incubation. Middlebrook 7H9 broth (having loopful inoculum of bacteria) were used to fill the wells up to 100 μl at different dilutions of the respective compounds. The maximum concentration of the compound (s) tested was 100 μg/ml. Plates were covered and sealed with parafilm and incubated at 37^{o}C for five days followed by the addition of 25μl of a freshly prepared 1:1 mixture of Alamar blue reagent and 10% Tween 80 to the plates incubated for 24 h. The different colour shows the growth of the bacteria like blue colour in the well was interpreted as no bacterial growth, and a pink colour was scored as growth. MIC values of synthesized compounds along with the MIC of a standard drug were shown in Table 4 **.**

RESULTS AND DISCUSSION

Different derivatives of quinazolinone were prepared by using 2-amino 3,4,5-trimethoxy benzoic acid with vilsmeier reagent. This reaction occurred at 90°C when two different substituted acids were reacted with a combination of DMF and $POCl_3$, followed by the addition of substituted anilines to prepare the final compounds. The analytical data of the synthesised compounds were shown in Table 1 **.**

The 2-Amino 3,4,5-triamino benzoic acid (I) reacted with vilsmeier reagent at 0°C yielded N-((6-(chlorocarbonyl) 2, 3, 4-trimethoxyphenylamino) methylene)-N-methylmethanaminium (II). Then II reacted with primary anilines at room temperature in the presence of DMF to form different substituted

Table 1: Data of the synthesised compounds (IV a-o)

Derivative Code	R	Molecular Formula	Rf value
IV a	2-CH3	C18H18N2O4	0.92
IV b	C4H2	C19H16N2O4	0.75
IV c	3-Cl, 4-F	C17H14ClFN2O4	0.83
IV d	4-OC2H5	C19H20N2O5	0.72
IV e	4-C2H5	C19H20N2O4	0.82
IV f	4-OCH3	C18H18N2O5	0.90
IV g	4-CH3	C18H18N2O4	0.89
IV h	2,4-OCH3	C19H20N2O6	0.76
IV i	3-OC2H5	C19H20N2O5	0.85
IV j	2-C2H5	C19H20N2O4	0.75
IV k	3-C2H5	C19H20N2O4	0.92
IV l	2-OC2H5	C19H20N2O5	0.81
IV m	3-F, 4-Br	C17H14BrFN2O4	0.84
IV n	3,4-F	C17H14F2N2O4	0.89
IV o	2-Cl, 4-F	C17H14ClFN2O4	0.76

Table 2: Different zone of inhibition represents against Gram (+), (-) and fungal strains of synthesised compounds (IV a-o)-

Compd.	Zone of Inhibition at 10 mg/ml					
	Fungi		Gram (+) bacteria		Gram (-) bacteria	
	A.alternata	A.niger	B. cereus	B. subtilis	S.marcescens	E. coli
IV a	08	06	07	11	08	11
IV b	07	05	06	12	05	12
IV c	22	22	25	27	22	29
IV d	06	04	05	10	06	10
IV e	16	17	18	23	18	25
IV f	21	20	24	29	21	30
IV g	13	12	16	21	12	18
IV h	20	19	23	28	22	28
IV i	15	13	14	19	14	19
IV j	18	16	19	22	19	23
IV k	12	14	15	20	11	20
IV l	14	11	13	18	13	21
IV m	18	18	20	24	16	22
IV n	17	15	21	22	17	24
IV o	20	21	22	30	20	27
Standard	22a	22a	30b	22b	30b	25b

[a] Fluconazole[b] Amoxicillin

Table 3: Different MIC values of synthesized and standard compounds

Compd.	Minimum Inhibitory Concentration in mg/ml					
	Fungi		Gram (+) bacteria		Gram (-) bacteria	
	A. alternata	A. niger	B. cereus	B. subtilis	S. Marcescens	E. coli
IV c	06	08	06	08	06	10
IV e	12	10	12	10	08	12
IV f	04	10	08	06	06	08
IV h	08	04	06	10	04	06
IV j	10	10	08	12	10	12
IV m	12	10	10	10	10	12
IV n	10	12	08	12	10	12
IV o	08	06	04	04	08	04
Standard	10a	10a	08b	10b	8b	12b

[a] Fluconazole [b] Amoxicillin

Table 4: Antitubercular activity against mycobacterium H37Rv strains (Alamar blue assay method) of the different synthesized derivatives and the standard compounds

Compounds	MIC values (μg/ml)
IV c	3.12
IV e	12.5
IV f	1.56
IV h	1.56
IV j	12.5
IV m	12.5
IV n	12.5
IV o	3.12
Isoniazid	12.5
Streptomycin	12.5
Rifampicin	12.5

quinazolinones (IV a-IV o). The steps involved in the synthesis were shown in **scheme 1**.

Synthesized compounds were analysed by spectral studies such as Infrared (IR), Proton Nuclear Magnetic Resonance (^{1}H-NMR) and mass spectra. IR spectrum showed absorption band at 2950-2983cm^{-1} (CH, Ali.), 3090-3080 cm^{-1} (CH, Ar), 1690-1680 cm^{-1} (C=O) and 1630-1620cm^{-1} (C=N). Further, ^{1}HNMR showed a multiplet of nine protons at δ (ppm) 3.87-4.50, multiplet for six protons at δ (ppm) 6.16-7.13 and multiplet of one proton of Aryl-quinazoline at δ (ppm) 8.15-8.52 respectively. Mass spectra revealed a molecular ion peak in satisfactory intensity.

Antibacterial and antifungal activity of the synthesized compounds were analysed using *in vitro* Kriby-Bauer disk diffusion method (Clinical and Laboratory Standards Institute, 2014). Gram-positive bacterial strains (*Bacillus subtilis* and *Bacillus cereus*), gram-negative bacterial strains (*E.Coli* and *Serratia marcescens*) and fungal strains (*Aspergillus niger* and *Alternaria alternate*) at 10mg/ml concentration. The zone of inhibition were calculated and compared with amoxicillin and fluconazole. DMSO used as stock solutions of tested derivatives. Synthesized compounds showed good activity against bacterial strains. Compounds IV c, IV f, IV h and IV o showed maximum antibacterial activity in the series. Compounds IV e, IV j, IV m and IV n showed moderate antibacterial activity in the series. Compounds IV g, IV i, IV k and IV l showed least antibacterial activity in the series and the compounds IV a, IV b and IV d showed no antibacterial activity. The synthesised compounds zone of inhibition shown in Table 2 and the graph of the zone of inhibition of all the synthesised compounds were shown in Figure 1.

Among the different synthesized compounds, some of the compounds showed good and moderate activity. Those compounds were further used to determine the MIC value by liquid dilution method. Then we compare the MIC (μg/ml) of most active derivatives and standard drugs against the test strains were shown in Table 3, and the graph of the MIC value of the most potent compounds were shown in Figure 2.

Antitubercular activity

Then furthermost potent and moderate active compounds were undergo antitubercular activity by Alamar-blue assay using Isoniazid (H), Streptomycin (S), Rifampicin (R) as standard drugs and the compounds IV c, IV f, IV h and IV o showed good antitubercular activity. The MIC values of screened derivatives and standard drugs were shown in Table 4**,** and the graph of the standard and the screened compounds of anti-tubercular activity were shown in Figure 3 **.**

CONCLUSIONS

In conclusion, the synthesized compounds of quinazolinone derivatives were prepared from 2-amino 3,4,5-trimethoxy benzoic acid with the help of vilsmeir reagent. All synthesized derivatives of quinazolinone were characterized by spectral analysis. Synthesised derivatives were analysed for in-vitro-antibacterial and antifungal activity using kriby-bauer disk diffusion method using four strains of bacteria and two strains of fungi. Results of the antimicrobial activity showed that synthesised compounds (IV c, IV f, IV h and IV o) were good against gram-positive, gram-negative and fungal strains, whereas synthesised compounds (IV e, IV j, IV m and IV n) were showed moderate against gram-positive, gram-negative and fungal strains. In the 8 derivatives 4 derivatives i.e. (IV c, IV f, IV h and IV o) were highly active against *M. tuberculosis* H37Rv.

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