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Discovery of new coumarin substituted quinazolines as potential bioactive agents

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Abstract In view of the biological importance of some important pharmacophores such as quinazoline, coumarin, and benzothiazole, we frame these moieties in a single molecular scaffold to be screened in vitro for their antimicrobial activity. The newly synthesized analogs have been examined for their in vitro biological efficacy against two Gram-positive bacteria, three Gram-negative bacteria, two fungi, and for antimycobacterial activity against *M. tuberculosis* $H_{37}Rv$. The bioassay results revealed that the majority of final analogs exhibited potential bio efficacies with the remarkable level of MICs comparable to control drugs. The new synthesized compounds were characterized by FT-IR, ^{1}H NMR, ^{13}C NMR, and MS analysis.

Keywords Quinazoline · Coumarin · Benzothiazole · Antimicrobial · Antimycobacterial activity

Introduction

The finding of efficacious new human therapeutic agents is one of humanity's most vital tasks. The great expansion in medicinal research in the past has contributed much to the unparalleled progress of medicine during that period. Improved and more meaningful biological test procedures have provided better guidance in drug discovery by pointing out suggestive observations that could be used in the design

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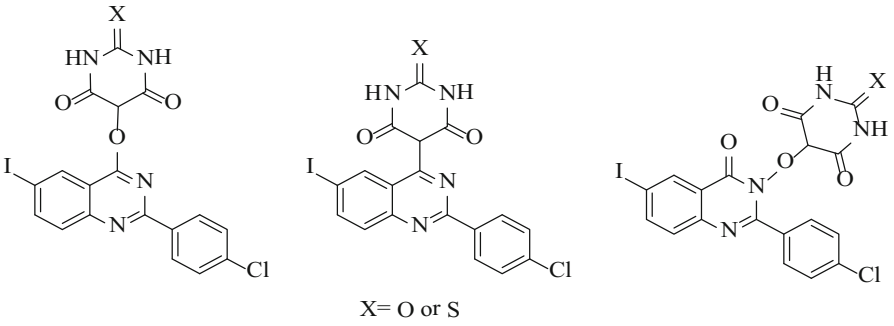
of new therapeutic agents [1]. The elucidation of the structure of many novel molecular leads in recent years has also made possible more rational study of the chemical mode of action of such compounds, and their interaction with drugs. Development of novel classes of drugs with fewer side effects and shorter lengths of treatment are key in continuing the fight against infectious disease. Hence, the field of medicinal chemistry has gained extreme importance for several years with better medical advances [2, 3].

A literature survey revealed that quinazoline, coumarin, and benzothiazole-fused derivatives are important parts of medicinal chemistry in a program of the drug discovery process [4–11]. Mosaad et al. [12] synthesized some quinazoline-based derivatives bearing substitution at the 4th position with ethereal linkage (I), with C–C bond formation at the same position (II) and ethereal linkage at nitrogen atom (III).



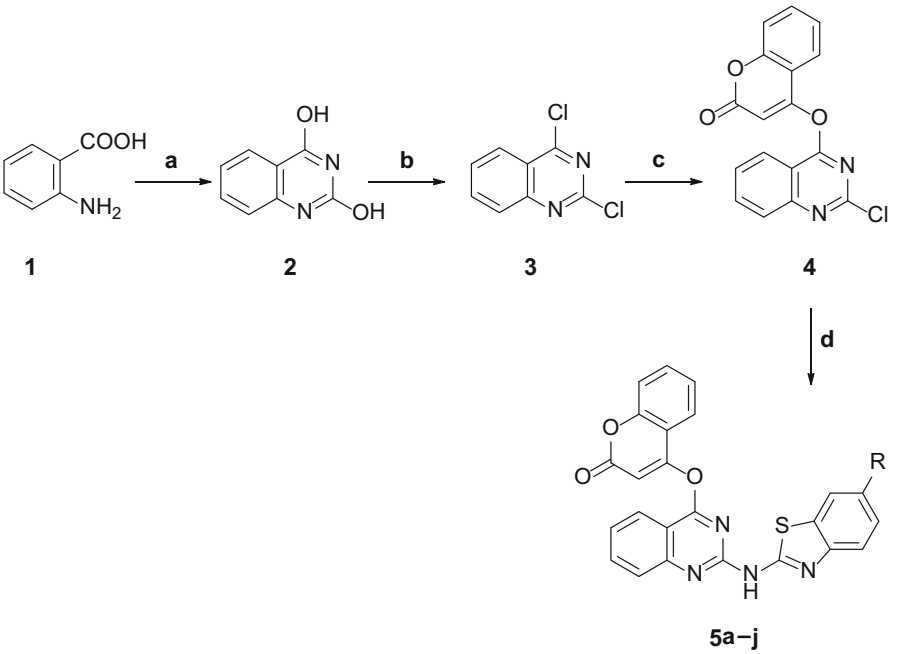
(I) (II) (III)

It was observed that amongst all the three quinazoline derivatives, analog (I) bearing ethereal linkage to the 4th position displayed excellent antimicrobial activities against Gram-positive *S. aureus* and *B. subtilis* bacteria. In an attempt to find new promising pharmacologically active molecules, we report the synthesis and in vitro biological activity of two series of benzothiazole and coumarin incorporated quinazolines. The synthesized analogs contain the ethereal linkage between coumarin and quinazoline rings. The influence of the presence/absence of various electron withdrawing/donating functional groups on the benzothiazole ring for the bioassay is elaborated.

Results and discussion

Chemistry

The synthetic route for the target compounds **5a–j** is outlined in Scheme 1. The initial analog 2,4-dichloroquinazoline (**3**) was synthesized from 2-nitrobenzoic acid according to the reported literature [13–18]. The structure of compound **3** was



Scheme 1 Synthetic protocol for analogs **5a–j**. *Reagents and Conditions* **a** Urea, 180 °C, **b** $POCl_3$, DMA, reflux, **c** 4-hydroxycoumarin, K_2CO_3, Acetone, reflux, **d** 2-aminobenzothiazoles, K_2CO_3, Acetone, reflux

confirmed by ^{1}H NMR and ^{13}C NMR (SI Fig S1–S2) spectrometric analysis. The condensation of 4-hydroxycoumarin with 2,4-dichloroquinzoline (**3**) in the presence of potassium carbonate base yields the intermediate analog **4**. The reaction of this intermediate **4** with various 2-aminobenzothiazole derivatives yields desired analogs **5a–j**. Structure of compound **5a** was confirmed by FT-IR, ^{1}H NMR, and ^{13}C NMR spectrometric analysis. The spectral details of the analog **5a** are discussed here. In the FT-IR spectrum (SI Fig S3), appearance of the characteristic absorption band at 3214 cm^{-1} for N–H group confirms the formation of **5a**. Moreover, the FT-IR spectrum also revealed another two absorption bands at 1691 and 1200 $cm^{-1}$for the C=O and C–O–C groups. The ^{1}H NMR spectrum (SI Fig S4) of compound **5a** also confirmed the presence of two singlets at 10.05 and 6.27 ppm for the NH and coumarin ring proton. Finally, the multiplates were observed between 7.92–7.22 for quinazoline and coumarin protons. The formation of analog **5a** was also supported by ^{13}C NMR spectrum (SI Fig S5), which show peaks at 165.7 and 159.7 ppm due to the presence of C–O–C linkage. Moreover, the appearance of a peak at 154.5 ppm confirms the presence of the C=O group. The mass spectrum (SI Fig S6) further confirmed the assigned structures of **5a** by exhibiting the molecular ion peaks $[M+1]^{+}$ at *m/z* 440.0.

Pharmacology

Antimicrobial activity

Investigation of in vitro antimicrobial activity of the final analogs **5a–j** is summarized in Table 1. The newer analogs were screened against two Gram-positive and three Gram-negative bacteria. From the bioassay results, it can be stated that some of the final analogs displayed excellent antibacterial activity against most of the microorganisms studied. However, the antibacterial property of the each final analog varies by varying structural features of the benzothiazole ring.

Overall, results suggested that compounds have better inhibitory effects against Gram-positive bacteria (MICs 6.25–25 μg/mL) than against Gram-negative ones (MICs 25–50 μg/mL) in terms of MIC. The hydroxyl group that substituted analog **5j** (MIC 6.25 μg/mL) was 50 % more potent than Ampicillin (MIC 12.5 μg/mL) against *S. aureus*, whereas the analog **5h** (OCH_3) was equipotent to the Ampicillin against the same organism. With regard to the activity against *B. cereus*, the best activity was displayed by compounds **5b** and **5j** (MIC 12.5 μg/mL). On the other hand, investigation of antibacterial activity of the synthesized compounds against the three Gram-negative strains revealed that, only a few analogs have been found to be active against the tested strains. Analog **5h** was able to produced good growth inhibitory activity against *E. coli* (MIC 25 μg/mL). However, compounds **5d** and **5h** (MIC 50 μg/mL) exhibited moderate activity against *P. aeruginosa*.

Concerning the antifungal activity of the tested compounds **5b**, **5d**, **5e, 5g**, and **5j** showed sensitivity against the mentioned fungal strains, whereas the rest of the derivatives were reasonably active or insensitive. The methyl group functionalized derivative **5g** displayed exceptional antifungal efficacy 6.25 μg/mL against *A. niger*. Moreover, analog **5e** was also found to be moderately (MIC 12.5 μg/mL) active against the same strain. Interestingly, the chloro group substituted analog **5b** (MIC 6.25 μg/mL) displayed the highest inhibition against *C. albicans*. However, the synthesized analogs showed significant growth inhibition zones in a range of 27–20 mm, when compared with standards of 33–28 mm against both bacterial as well as fungal species.

Antimycobacterial activity

The investigation of in vitro antimycobacterial screening (Table 2) revealed that all the new synthesized compounds showed moderate to good inhibition at 3.12–25 μg/mL against *Mycobacterium tuberculosis* H_{37}Rv.

The primary screening was conducted at a concentration of 6.25 μg/mL using the BACTEC MGIT method only for the first selection of active compounds. The results observed from the BACTEC MGIT method indicated that out of all the tested compounds, two derivatives **5d** and **5j**, exhibited the highest inhibition (99 %) at a constant concentration level (6.25 μg/mL). However, the results of secondary biological screening using the Lowenstein-Jensen MIC method revealed that analog **5d** with a fluoro group to the benzothiazole ring showed the highest inhibition against *M. tuberculosis* H_{37}Rv at 3.12 μg/mL MIC, which is half fold

Table 1 Results of in vitro antimicrobial screening of compounds **5a–j**

Entry	R	Zone of inhibition in mm (MIC in μg/mL)[a]						
		Gram-positive bacteria		Gram-negative bacteria			Fungi	
		S. aureus	*B. cereus*	*E. coli*	*P. aeruginosa*	*K. pneumoniae*	*A. niger*	*C. albicans*
5a	H	14 (200)	<10 (250)	<10 (500)	<10 (200)	<10 (250)	19 (200)	<10 (200)
5b	Cl	**24 (25)**	**25 (12.5)**	13 (100)	17 (100)	15 (100)	24 (50)	**26 (6.25)**
5c	Br	21 (50)	**22 (25)**	19 (100)	20 (62.5)	18 (62.5)	25 (50)	21 (62.5)
5d	F	**22 (25)**	**20 (25)**	**25 (50)**	**25 (50)**	24 (200)	**20 (25)**	18 (62.5)
5e	NO_2	22 (100)	13 (200)	20 (62.5)	23 (100)	21 (100)	**23 (12.5)**	21 (50)
5f	CN	<10 (200)	14 (200)	15 (250)	12 (250)	11 (100)	18 (50)	15 (50)
5g	CH_3	20 (100)	15 (250)	16 (200)	21 (62.5)	16 (250)	**22 (6.25)**	**20 (25)**
5h	OCH_3	**26 (12.5)**	24 (50)	**20 (25)**	**24 (50)**	23 (62.5)	23 (62.5)	20 (50)
5i	OCH_2CH_3	22 (50)	17 (50)	<10 (200)	20 (62.5)	<10 (100)	18 (200)	19 (250)
5j	OH	**27 (6.25)**	**23 (12.5)**	21 (62.5)	**19 (50)**	20 (62.5)	**22 (25)**	**20 (25)**
Ampicillin		30 (12.5)	28 (12.5)	31 (6.25)	28 (25)	33 (25)	–	–
Gentamicina		32 (6.25)	30 (6.25)	29 (12.5)	33 (12.5)	30 (25)	–	–
Fluconazole		–	–	–	–	–	30 (6.25)	32 (6.25)
DMSO		–	–	–	–	–	–	–

[a] Each value is the mean of three independent experiments

Bold values indicate highly active compound against respective microorganisms

Table 2 Results of in vitro antimycobacterial screening of the analogs **5a–j**

Entry	R	BACTEC MGIT method[a]		L. J. MIC method[a]	
		MIC (μg/mL)	% Inhibition	MIC (μg/mL)	% Inhibition
5a	H	>6.25	ND	250	96
5b	Cl	>6.25	ND	25	98
5c	Br	>6.25	ND	12.5	99
5d	F	6.25	99	**3.12**	99
5e	NO_2	>6.25	ND	100	96
5f	CN	>6.25	ND	200	97
5g	CH_3	>6.25	ND	250	97
5h	OCH_3	>6.25	ND	100	96
5i	OCH_2CH_3	>6.25	ND	50	98
5j	OH	6.25	99	**6.25**	99
Ethambutol		3.12	99		
Pyrazinamide		6.25	99		
Rifampicin		0.25	99		
Isoniazid		0.20	99		
DMSO		–	–	–	–

ND Not determine

[a] Each value is the mean of three independent experiments

Bold values indicate highly active compound against respective microorganisms

more active than pyrazinamide. All the remaining derivatives were found to exhibit moderate to poor activity at MIC ranging from 12.5–50 μg/mL.

A structure-activity relationship study revealed that the presence of strong electron donating substitution at the benzothiazole ring improves antibacterial activity. However the presence of electron withdrawing groups is essential for good antifungal activity. Moreover, the presence of halogenated and hydroxyl derivatives have shown high antimycobacterial activity.

Conclusion

In conclusion, we report the synthesis of various benzothiazole and coumarin substituted quinazoline derivatives. The bioassay results reveal that many of the synthesized final analogs have shown remarkable activity against the Gram-positive bacteria and fungal strains as compared with the standards. The hydroxyl group functionalized derivative is found to be half fold more active than ampicillin against *S. aureus*, while chloro and methyl derivatives are equipotent to fluconazole against tested fungal strains. Moreover, the fluoro derivative is half fold more active than pyrazinamide against *M. tuberculosis* $H_{37}Rv$. In short, these compounds represent new structure scaffolds that could be further optimized for future development of more potent and selective bioactive agents.

Experimental

General

All reactions were carried out under a nitrogen atmosphere. Air- and moisture-sensitive solvents and solutions were transferred via syringe or stainless steel cannula. All chemicals were purchased from Sigma Aldrich, Merck, and Fluka. Solvents used were of analytical grade. Anhydrous potassium carbonate was stored in a nitrogen-filled glovebox, ground, and taken out in small quantities and stored in a desiccator. All reactions were routinely checked by TLC. TLC was performed on aluminum-backed silica gel plates (silica gel 60 F254 grade, Merck DC) with spots visualized by UV light. Column chromatography was performed on silica gel LC 60A (70–200 micron). Melting points were determined in open capillaries on a Veego electronic apparatus VMP–D (Veego Instrument Corporation, Mumbai, India) and are uncorrected. ^{1}H NMR and ^{13}C NMR spectra were recorded on a Varian 400 MHz model spectrometer using DMSO-d_6 as a solvent and TMS as internal standard with ^{1}H resonant frequency of 400 MHz and ^{13}C resonant frequency of 100 MHz. The chemical shifts were reported as parts per million (ppm) downfield from TMS (Me_4Si). The splitting patterns are designated as follows; s, singlet; d, doublet; dd, doublet of doublets; t, triplet; m, multiplet. Elemental analyses (C, H, N) were performed using a Heraeus Carlo Erba 1180 CHN analyzer (Hanau, Germany). The mass spectra were measured with a Waters Micromass Q-Tof Micro instrument [time of flight (TOF) mass spectrometer]. Column chromatography was performed on a 1.5 feet (2.5 cm diameter) glass column using silica gel LC 60A (70–200 microns).

*Synthesis of quinazoline-2,4-diol (**2**)*

A flask containing urea (15.0 g, 250 mmol) and 2-aminobenzoic acid (**1**) (25 mmol) was heated at 180 °C. After being stirred for 3 h, the reaction mixture was cooled to 100 °C and an equal volume of water was added. The obtained suspension was left to stir for 10 min, after which it was cooled to room temperature. The precipitate was filtered off and recrystallized from DMF to obtain quinazoline-2,4-diol (**2**) [14]. Light pink solid, Yield: 81 %, M.P. 298–300 °C (lit. [15] 300 °C).

*Synthesis of 2,4-dichloroquinazoline (**3**)*

A mixture of 80 ml $POCl_3$, *N,N*-dimethylaniline (300 mmol) and 2,4-dihydroxyquinazoline (**2**) (150 mmol) was refluxed for 2 h. The cooling mixture was poured into 250 mL ice-water, and the mixture was stirred at room temperature. The resulting precipitate was filtered off and washed with 50 mL water, and dried to give 2,4-dichloroquinazoline (**3**) to be used in the next step without further purification [16]. Yellow solid, Yield: 87 %, M.P. 118–119 °C (lit. [17] 119–120 °C). IR (KBr, cm^{-1}): 3064 (Ar–CH), 1610 (CN), 735(C–Cl). ^{1}H NMR (400 MHz, DMSO–d_6): δ 8.12 (dd, J = 7.2, 1.8 Hz, 1H), 7.97–7.92 (m, 2H, Ar–H), 7.77–7.73 (m, 1H, Ar–H). ^{13}C NMR (100 MHz, DMSO–d_6): δ 162.1, 154.2, 153.9, 148.0, 132.7, 127.5, 126.2, 123.0.

*Synthesis of 4-((2-chloroquinazolin-4-yl)oxy)-2H-chromen-2-one (**4**)*

Potassium carbonate (10 mmol) was added to a solution of 4-hydroxy coumarin (2 mmol) in anhydrous ethanol (20 mL) and was stirred for 5 min. Then, the intermediate **3** (2 mmol) was added into the reaction mixture. The reaction mixture was refluxed for 10 h. After cooling to room temperature, the reaction mixture was treated with 200 mL cold water, and the resulting precipitate was filtered off. The crude product **4** was recrystallized from DMF [18]. White solid, Yield: 72 %, M.P. 192–193 °C.

General synthetic procedure for analogs (5a–j)

A mixture of intermediate **4** (10 mmol), potassium carbonate (12 mmol) and various 2-aminobenzothiazoles (10 mmol) in acetone solvent (30 mL) was refluxed for 8 h. The progress of the reaction was monitored by TLC. After cooling to room temperature, the reaction mixture was treated with 200 mL cold water, and the resulting precipitate was filtered off. The crude products **5a–j** were recrystallized from DMF [19].

*4-((2-(Benzo[d]thiazol-2-ylamino)quinazolin-4-yl)oxy)-2H-chromen-2-one (**5a**)*

White solid, Yield: 74 %, M.P. 197–199 °C. IR (KBr, cm^{-1}): 3214 (N–H), 2948, 2900 (C–H), 1691 (C=O), 1200 (C–O–C). ^{1}H NMR (400 MHz, DMSO-d_6): δ 10.05 (s, 1H, –NH), 8.57 (d, J = 1.7 Hz, 1H), 7.92–7.54 (m, 7H, Ar–H), 7.30–7.22 (m, 4H, Ar–H), 6.27 (s, 1H, coumarin). ^{13}C NMR (100 MHz, DMSO-d_6): δ 165.7 (1C, Cquinazoline–O–C), 160.0 (1C, Cquinazoline–NH–C), 159.7 (1C, Cbenzothiazole–NH–C), 154.5 (1C, Ccoumarin–O–C), 153.2 (1C, C=O), 150.6, 150.4, 140.3, 134.3, 134.0, 131.7, 130.0, 129.6, 129.2, 129.1, 129.0, 127.8, 127.5, 126.4, 121.5, 121.0, 111.9, 109.4, 105.4 (19C, Ar–C). MS, *m/z* 440.0 $[M+1]^+$. Anal calcd for $C_{24}H_{14}N_4O_3S$: C, 65.74; H, 3.22; N, 12.78; S, 7.31 Found C, 65.98; H, 3.07; N, 12.67; S, 7.19.

*4-((2-((6-Chlorobenzo[d]thiazol-2-yl)amino)quinazolin-4-yl)oxy)-2H-chromen-2-one (**5b**)*

Light greenish solid, Yield: 71 %, M.P. 232–234 °C. IR (KBr, cm^{-1}): 3352 (N–H), 3008, 2911 (C–H), 1683 (C=O), 1221 (C–O–C), 748 (C–Cl). ^{1}H NMR (400 MHz, DMSO-d_6): δ 10.28 (s, 1H, –NH), 8.63 (d, J = 1.7 Hz, 1H), 7.79–7.25 (m, 6H, Ar–H), 7.24–6.93 (m, 4H, Ar–H), 5.98 (s, 1H, coumarin). ^{13}C NMR (100 MHz, DMSO-d_6): δ 167.3 (1C, Cquinazoline–O–C), 161.7 (1C, Cquinazoline–NH–C), 160.2 (1C, Cbenzothiazole–NH–C), 158.5 (1C, Ccoumarin–O–C), 155.2 (1C, C=O), 151.1, 149.8, 142.7, 136.2, 135.8, 132.4, 131.9, 130.2, 129.7, 129.5, 129.2, 128.1, 127.1, 127.0, 123.6, 122.4, 115.2, 111.7, 108.0 (19C, Ar–C). MS, *m/z* 474.2 $[M+1]^+$. Anal calcd for $C_{24}H_{13}ClN_4O_3S$: C, 60.95; H, 2.77; N, 11.85; S, 6.78 Found C, 60.87; H, 3.01; N, 11.89; S, 6.73.

*4-((2-((6-Bromobenzo[d]thiazol-2-yl)amino)quinazolin-4-yl)oxy)-2H-chromen-2-one (**5c**)*

White solid, Yield: 69 %, M.P. 216–218 °C. IR (KBr, cm^{-1}): 3283 (N–H), 2931, 2887 (C–H), 1690 (C=O), 1217 (C–O–C). ^{1}H NMR (400 MHz, DMSO-d_6): δ 9.97 (s, 1H, –NH), 8.34 (d, J = 1.7 Hz, 1H), 7.67–7.29 (m, 8H, Ar–H), 7.04–6.73 (m, 4H, Ar–H), 6.03 (s, 1H, coumarin). ^{13}C NMR (100 MHz, DMSO-d_6): δ 165.2 (1C, Cquinazoline–O–C), 161.8 (1C, Cquinazoline–NH–C), 160.1 (1C, Cbenzothiazole–NH–C), 159.7 (1C, Ccoumarin–O–C), 153.2 (1C, C=O), 152.8, 151.0, 141.4, 134.8, 134.5, 131.9, 130.4, 130.1, 129.9, 129.6, 129.2, 128.7, 127.1, 126.5, 123.2, 121.9, 113.4, 112.6, 107.1 (19C, Ar–C). MS, *m/z* 518.0 $[M+1]^+$. Anal calcd for $C_{24}H_{13}BrN_4O_3S$: C, 55.72; H, 2.53; N, 10.83; S, 6.20 Found C, 55.70; H, 2.56; N, 11.05; S, 6.33.

*4-((2-((6-Fluorobenzo[d]thiazol-2-yl)amino)quinazolin-4-yl)oxy)-2H-chromen-2-one (**5d**)*

White solid, Yield: 73 %, M.P. 254–256 °C. IR (KBr, cm^{-1}): 3248 (N–H), 2913, 2901 (C–H), 1687 (C=O), 1228 (C–O–C). ^{1}H NMR (400 MHz, DMSO-d_6): δ 10.09 (s, 1H, –NH), 8.51 (d, J = 1.7 Hz, 1H), 7.83–7.41 (m, 8H, Ar–H), 7.17–7.05 (m, 4H, Ar–H), 6.13 (s, 1H, coumarin). ^{13}C NMR (100 MHz, DMSO-d_6): δ 165.1 (1C, Cquinazoline–O–C), 160.5 (1C, Cquinazoline–NH–C), 159.6 (1C, Cbenzothiazole–NH–C), 154.1 (1C, Ccoumarin–O–C), 153.8 (1C, C=O), 150.7, 150.1, 141.4, 134.8, 134.1, 131.6, 131.0, 130.4, 129.7, 129.2, 129.0, 127.6, 127.0, 126.4, 121.8, 121.5, 117.5, 109.8, 105.3 (19C, Ar–C). MS, *m/z* 458.4 $[M+1]^+$. Anal calcd for $C_{24}H_{13}FN_4O_3S$: C, 63.15; H, 2.87; N, 12.27; S, 7.02 Found C, 63.21; H, 2.69; N, 11.95; S, 6.86.

*4-((2-((6-Nitrobenzo[d]thiazol-2-yl)amino)quinazolin-4-yl)oxy)-2H-chromen-2-one (**5e**)*

Pale yellowish solid, Yield: 64 %, M.P. 203–204 °C. IR (KBr, cm^{-1}): 3303 (N–H), 2960, 2932 (C–H), 1688 (C=O), 1220 (C–O–C). ^{1}H NMR (400 MHz, DMSO-d_6): δ 10.12 (s, 1H, –NH), 8.56 (d, J = 1.7 Hz, 1H), 7.87–7.56 (m, 8H, Ar–H), 7.21–6.94 (m, 4H, Ar–H), 6.04 (s, 1H, coumarin). ^{13}C NMR (100 MHz, DMSO-d_6): δ 167.7 (1C, Cquinazoline–O–C), 163.1 (1C, Cquinazoline–NH–C), 160.4 (1C, Cbenzothiazole–NH–C), 159.9 (1C, Ccoumarin–O–C), 154.2 (1C, C=O), 152.7, 150.5, 140.8, 135.1, 134.5, 131.1, 130.8, 129.6, 129.1, 128.7, 128.2, 127.0, 126.7, 126.4, 122.1, 121.9, 115.2, 111.4, 107.0 (19C, Ar–C). MS, *m/z* 484.1 $[M+1]^+$. Anal calcd for $C_{24}H_{13}N_5O_5S$: C, 59.62; H, 2.71; N, 14.49; S, 6.63 Found C, 59.78; H, 2.55; N, 14.65; S, 6.47.

*2-((4-((2-Oxo-2H-chromen-4-yl)oxy)quinazolin-2-yl)amino)benzo[d]thiazole-6-carbonitrile (**5f**)*

White solid, Yield: 61 %, M.P. 183–184 °C. IR (KBr, cm^{-1}): 3249 (N–H), 2925, 2883 (C–H), 2250 (C≡N), 1681 (C=O), 1237 (C–O–C). ^{1}H NMR (400 MHz,

DMSO-d_6): δ 9.98 (s, 1H, –NH), 8.68 (d, J = 1.7 Hz, 1H), 7.96–7.50 (m, 8H, Ar–H), 7.21–7.02 (m, 4H, Ar–H), 6.25 (s, 1H, coumarin). ^{13}C NMR (100 MHz, DMSO-d_6): δ 165.1 (1C, Cquinazoline–O–C), 159.9 (1C, Cquinazoline–NH–C), 159.5 (1C, Cbenzothiazole–NH–C), 153.8 (1C, Ccoumarin–O–C), 153.2 (1C, C=O), 151.0, 150.4, 140.1, 134.8, 134.2, 131.0, 130.5, 129.1, 129.0, 128.4, 128.1, 127.3, 127.1, 126.5, 121.9, 121.7, 116.0, 113.8, 108.6 (19C, Ar–C), 105.3 (1C, C≡N). MS, *m/z* 463.9 $[M+1]^+$. Anal calcd for $C_{25}H_{13}N_5O_3S$: C, 64.79; H, 2.83; N, 15.11; S, 6.92 Found C, 64.48; H, 2.71; N, 15.23; S, 7.05.

*4-((2-((6-Methylbenzo[d]thiazol-2-yl)amino)quinazolin-4-yl)oxy)-2H-chromen-2-one (**5g**)*

White solid, Yield: 66 %, M.P. 225–227 °C. IR (KBr, cm^{-1}): 3317 (N–H), 2938, 2892 (C–H), 1687 (C=O), 1205 (C–O–C). ^{1}H NMR (400 MHz, DMSO-d_6): δ 10.19 (s, 1H, –NH), 8.61 (d, J = 1.7 Hz, 1H), 8.01–7.50 (m, 8H, Ar–H), 7.23–6.80 (m, 4H, Ar–H), 5.93 (s, 1H, coumarin), 2.42 (s, 3H, –CH_3). ^{13}C NMR (100 MHz, DMSO-d_6): δ 166.2 (1C, Cquinazoline–O–C), 161.8 (1C, Cquinazoline–NH–C), 159.6 (1C, Cbenzothiazole–NH–C), 157.3 (1C, Ccoumarin–O–C), 153.1 (1C, C=O), 150.5, 150.0, 140.7, 137.2, 133.9, 131.5, 130.2, 129.1, 128.7, 128.1, 127.5, 127.0, 126.1, 125.3, 124.5, 122.6, 115.2, 111.7, 109.1 (19C, Ar–C), 22.4 (1C, CH_3). MS, *m/z* 453.0 $[M+1]^+$. Anal calcd for $C_{25}H_{16}N_4O_3S$: C, 66.36; H, 3.56; N, 12.38; S, 7.09 Found C, 66.52; H, 3.60; N, 12.45; S, 7.18.

*4-((2-((6-Methoxybenzo[d]thiazol-2-yl)amino)quinazolin-4-yl)oxy)-2H-chromen-2-one (**5h**)*

Light brownish solid, Yield: 78 %, M.P. 242–244 °C. IR (KBr, cm^{-1}): 3255 (N–H), 2913, 2857 (C–H), 1680 (C=O), 1210 (C–O–C). ^{1}H NMR (400 MHz, DMSO-d_6): δ 10.03 (s, 1H, –NH), 8.51 (d, J = 1.7 Hz, 1H), 7.88–7.64 (m, 8H, Ar–H), 7.25–6.86 (m, 4H, Ar–H), 6.21 (s, 1H, coumarin), 4.22 (s, 3H, –OCH_3),. ^{13}C NMR (100 MHz, DMSO-d_6): δ 165.0 (1C, Cquinazoline–O–C), 160.8 (1C, Cquinazoline–NH–C), 159.4 (1C, Cbenzothiazole–NH–C), 154.5 (1C, Ccoumarin–O–C), 153.1 (1C, C=O), 150.5, 150.0, 141.7, 134.5, 134.3, 131.8, 130.4, 129.8, 129.1, 128.5, 127.4, 126.2, 126.0, 125.4, 121.1, 121.0, 114.5, 108.7, 106.2 (19C, Ar–C), 57.8 (1C, OCH_3). MS, *m/z* 469.2 $[M+1]^+$. Anal calcd for $C_{25}H_{16}N4O4S$: C, 64.09; H, 3.44; N, 11.96; S, 6.84 Found C, 63.88; H, 3.38; N, 11.90; S, 6.99.

*4-((2-((6-Ethoxybenzo[d]thiazol-2-yl)amino)quinazolin-4-yl)oxy)-2H-chromen-2-one (**5i**)*

White solid, Yield: 75 %, M.P. 236–237 °C. IR (KBr, cm^{-1}): 3309 (N–H), 2927, 2895 (C–H), 1690 (C=O), 1210 (C–O–C). ^{1}H NMR (400 MHz, DMSO-d_6): δ 10.11 (s, 1H, –NH), 8.53 (d, J = 1.7 Hz, 1H), 7.78–7.34 (m, 8H, Ar–H), 7.27–6.89 (m, 4H, Ar–H), 6.05 (s, 1H, coumarin), 4.01 (q, J = 6.8 Hz, 2H, –OCH_2CH_3), 1.97 (t, J = 7.2 Hz, 3H, –OCH_2CH_3). ^{13}C NMR (100 MHz, DMSO-d_6): δ 168.3 (1C, Cquinazoline–O–C), 165.7 (1C, Cquinazoline–NH–C), 161.2 (1C, Cbenzothiazole–NH–C),

155.9 (1C, Ccoumarin–O–C), 155.0 (1C, C=O), 153.8, 152.1, 143.5, 133.8, 133.5, 130.1, 130.0, 129.5, 129.1, 129.0, 128.4, 127.5, 127.1, 126.9, 123.7, 121.5, 117.0, 110.4, 109.6 (19C, Ar–C), 58.9 (1C, –O$\underline{C}H_2CH_3$), 15.4 (1C, –OCH$_2\underline{C}H_3$). MS, *m/z* 482.8 $[M+1]^+$. Anal calcd for $C_{26}H_{18}N_4O_4S$: C, 64.72; H, 3.76; N, 11.61; S, 6.65 Found C, 64.58; H, 3.49; N, 11.72; S, 6.80.

*4-((2-((6-Hydroxybenzo[d]thiazol-2-yl)amino)quinazolin-4-yl)oxy)-2H-chromen-2-one (**5j**)*

White solid, Yield: 77 %, M.P. 190–192 °C. IR (KBr, cm^{-1}): 3461 (O–H), 3228 (N–H), 2941, 2905 (C–H), 1684 (C=O), 1235 (C–O–C). ^{1}H NMR (400 MHz, DMSO-d_6): δ 10.23 (s, 1H, –NH), 8.44 (d, J = 1.7 Hz, 1H), 7.85–7.37 (m, 8H, Ar–H), 7.20–6.84 (m, 4H, Ar–H), 6.09 (s, 1H, coumarin), 5.11 (br s, 1H, OH). ^{13}C NMR (100 MHz, DMSO-d_6): δ 164.6 (1C, Cquinazoline–O–C), 161.1 (1C, Cquinazoline–NH–C), 159.8 (1C, Cbenzothiazole–NH–C), 154.3 (1C, Ccoumarin–O–C), 154.0 (1C, C=O), 152.6, 150.3, 147.5, 138.7, 133.4, 132.9, 131.8, 130.3, 129.7, 129.5, 129.2, 128.8, 128.5, 128.0, 124.3, 120.6, 113.2, 111.3, 107.6 (19C, Ar–C). MS, *m/z* 455.1 $[M+1]^+$. Anal calcd for $C_{24}H_{14}N_4O_4S$: C, 63.43; H, 3.11; N, 12.33; S, 7.06 Found C, 63.59; H, 3.04; N, 12.15; S, 6.89.

Microbiology

Methods for in vitro antimicrobial activity evaluation

The synthesized derivatives **5a–j** were examined for their antimicrobial activity against two Gram-positive bacteria (*Staphylococcus aureus* and *Bacillus cereus*), three Gram-negative bacteria (*Escherichia coli*, *Pseudomonas aeruginosa*, and *Klebsiella pneumoniae*) and two fungal species (*Aspergillus niger* and *Candida albicans*) using the paper disc diffusion technique and agar streak dilution method. The Mueller-Hinton agar media were sterilized (autoclaved at 120 °C for 30 min), poured at uniform depth of 5 mm, and allowed to solidify. The microbial suspension (10^5 CFU/mL) (0.5 McFarland Nephelometery Standards) was streaked over the surface of media using a sterile cotton swab to ensure even growth of the organisms. The tested analogs were dissolved in dimethylsulfoxide to give solutions of 3.12–50 μg/mL. Sterile filter paper discs measuring 6.25 mm in diameter (Whatman no. 1 filter paper), previously soaked in a known concentration of the respective test analog in dimethylsulfoxide were placed on the solidified nutrient agar medium that had been inoculated with the respective microorganism, and the plates were incubated for 24 h at (37±1) °C. A control disc impregnated with an equivalent amount of dimethylsulfoxide without any sample was also used and did not produce any inhibition. Ampicillin and gentamicina (100 μg/disc) were used as standard antibacterial drugs while fluconazole (100 μg/disc) was used as an antifungal drug [20].

MIC of the analog was determined by the agar streak dilution method. A stock solution of the synthesized analog (100 μg/mL) in dimethylsulfoxide was prepared and graded quantities of the test analogs were incorporated in a specified quantity of

molten sterile agar, *i.e.*, nutrient agar for evaluation of antibacterial and sabouraud dextrose agar for antifungal activity, respectively. The medium containing the test analog was poured into a Petri dish at a depth of 4–5 mm and allowed to solidify under aseptic conditions. A suspension of the respective microorganism of approximately 10^5 CFU/mL was prepared and applied to plates with serially diluted analogs with concentrations in the range of 3.12–50 μg/mL in dimethylsulfoxide and incubated at (37±1) °C for 24 h (bacteria) or 48 h (fungi). The lowest concentration of the substance that prevents the development of visible growth is considered to be the MIC value [21].

Methods for in vitro evaluation of antimycobacterial activity evaluation

BACTEC MGIT method

The mycobacteria growth indicator tubes (MGIT) containing 4 ml of modified Middle brook 7H9 Broth Base were numbered as per the title compounds to be tested for antituberculosis efficacy by means of various concentrations prepared. The suspension was allowed to stand for 20 min and the tubes were centrifuged at 3000 rpm for 15 min. After that, 10^4–10^7 CFU/mL of prepared *M. tuberculosis H37RV* strain suspension was added in the medium to be incubated and 0.1 mL of an egg-based medium was also added. The MGIT tubes were then tightly recapped, mixed well, and incubated into a BACTEC MGIT instrument at 37 °C until positivity is observed. The readings were measured daily starting from the second day of incubation. Positive cultures were usually detected within 10 days. For reading the actual results, the MGIT tubes were removed from the incubator and placed in the UV light next to a Positive Control tube and an uninoculated tube. Bright fluorescence detected by the corresponding MGIT tube was noticed in the form of a bright orange colour in the bottom of the tube and also an orange reflection on the meniscus [22]. The primary screening was conducted at a concentration of 6.25 μg/mL against *M. tuberculosis* H37Rv in the BACTEC MGIT system. Compounds demonstrating 99 % inhibition in the primary screening were described as most potent compounds. All the other compounds to be tested were re-examined for their actual MIC by adopting the conventional L. J. agar dilution method. The MIC was defined as the lowest concentration inhibiting 99 % of the inoculum.

Lowenstein and Jensen method

The secondary antimycobacterial screening for test compounds was obtained for *M. Tuberculosis H37Rv*, by adopting the L. J. (Lowenstein and Jensen) agar dilution method [23] for the measurement of MIC, and is defined as the lowest concentration of drug which inhibits ≥99 % of bacterial population present at the beginning of the assay. Stock solutions of 250, 200, 100, 62.5, 50, 25, 12.5, 6.25, 3.12, and 1.56 μg/mL dilutions of each test compound in DMSO (dimethylsulfoxide) were added in the liquid L. J. Medium, and then media were sterilized by the inspissation method. A culture of *M. tuberculosis H37Rv* growing on L. J. Medium was harvested in

0.85 % saline in bijou bottles. These tubes were then incubated at 37 °C for 24 h followed by streaking of *M. tuberculosis H37Rv* (5 × 104 bacilli per tube). These tubes were then incubated at 37 °C. Growth of bacilli was seen after 12 days, 22 days and finally 28 days of incubation. Tubes having the compounds were compared with control tubes where medium alone was incubated with *M. tuberculosis H37Rv*. The concentration at which no development of colonies occurred or <20 colonies was taken as the MIC concentration of the test compound. The standard strain *M. tuberculosis H37Rv* was tested with known drugs Isoniazid, Rifampicin, Ethambutol, and Pyrazinamide.

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