DOI 10.1007/s10593-015-1660-2

Chemistry of Heterocyclic Compounds **2015**, *51*(1), 60–66

Synthesis of novel *N*-aryl-4-[6-(2-fluoropyridin-3-yl)quinazolin-2-yl]-piperazine-1-carboxamide or -carbothioamide derivatives and their antimicrobial activity

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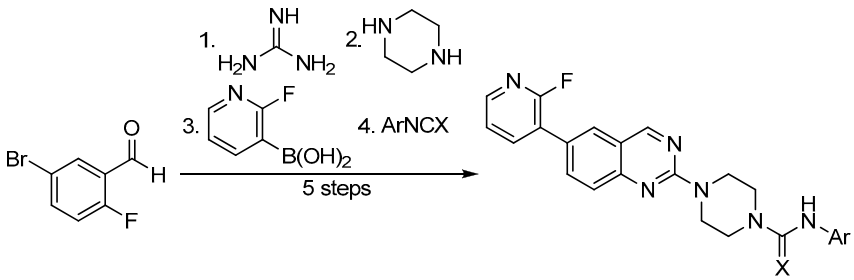
Published in Khimiya Geterotsiklicheskikh Soedinenii, 2015, *51*(1), 60–66

Submitted August 31, 2014
Accepted after revision November 27, 2014



A series of novel *N*-aryl-4-[6-(2-fluoropyridin-3-yl)quinazolin-2-yl]-piperazine-1-carboxamide or -carbothioamide derivatives was synthesized by cyclization of 5-bromo-2-fluorobenzaldehyde and guanidine carbonate in the presence of dimethylacetamide followed by treatment with isoamylnitrite and diiodomethane in the presence of copper iodide to afford 6-bromo-2-iodoquinazoline. The latter was treated with piperazine in the presence of triethylamine followed by (2-fluoropyridin-3-yl)boronic acid in the presence of $Pd(PPh_3)_2Cl_2$ to afford 6-(2-fluoropyridin-3-yl)-2-(piperazin-1-yl)quinazoline which was further treated with various substituted arylisocyanates and arylisothiocyanates to obtain the title compounds. The chemical structures of the synthesized compounds were confirmed by means of LC-MS, ^1H, ^{13}C NMR, IR, and mass spectra and elemental analysis. All the synthesized compounds were evaluated for their *in vitro* antibacterial activity against two Gram-positive and two Gram-negative bacterial species, as well as two different strains of antibiotic-resistant *E. coli* and for antifungal activity against two fungal strains. Some of the compounds have shown potential antimicrobial activity.

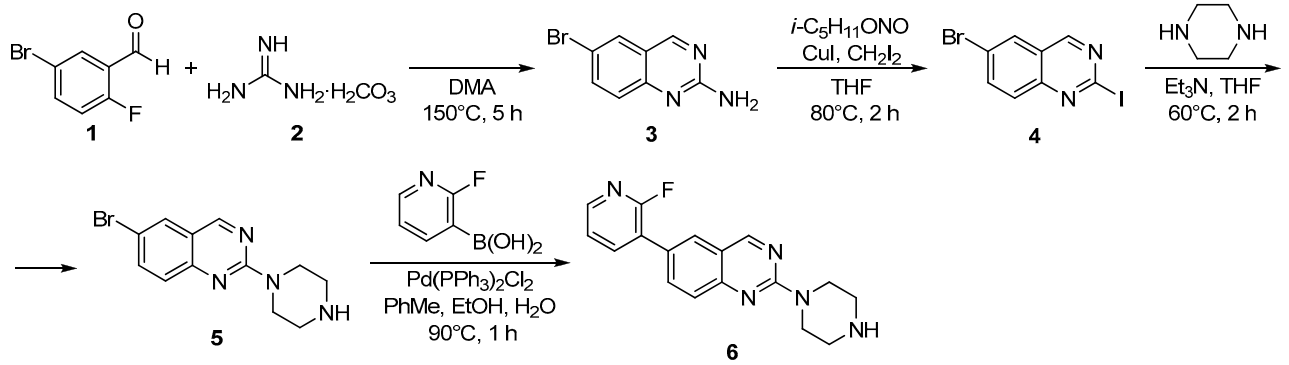
Keywords: arylisocyanates, arylisothiocyanates, piperazine, quinazoline, Suzuki coupling, antimicrobial activity.

Quinazolines are heterocyclic compounds with an important place in the field of medicinal chemistry. The quinazoline ring system is present in the structure of approximately 150 naturally occurring pharmacologically active alkaloids, isolated from a number of families of the plant and animal kingdom, as well as various microorganisms.[1] Quinazoline derivatives possess diverse biological and therapeutic activities such as antibacterial,[2–4] antifungal,[5] anti-inflammatory,[6] anticancer,[2,7] antituberculosis,[8] antiplasmodial,[9] antimicrobial,[10] and antioxidant.[4,11] Hence, quinazoline is a lead moiety for designing potential bioactive agents.

Piperazine and substituted piperazine are important pharmacophores found in many marketed drugs and drugs under clinical trials.[12,13] Pyridine derivatives containing another heterocyclic nucleus also have shown potent pharmacological properties like antifungal[14] and antimicrobial[15] activity. Piperazine moiety, too, was explored for several biological activities including antimicrobial.[16,17] The polarity of the nitrogen atoms of piperazine ring enhances favorable interaction with biomacromolecules and thus confers the biological activity.[18,19] A combination of quinazoline, pyridine, and piperazine heterocyclic rings in one molecule has recently shown to be highly biological active towards several bacterial and fungal species, and these moieties are thought to have a synergistic effect against pathogens causing infectious diseases.[20]

A growing interest and an urgent need for the discovery of new, selective, and promising inhibitors of microbial growth with an improved safety and efficacy profile was the stimulus to present an attractive approach towards design and development of new active compounds. Urea and thiourea are important functional moieties commonly found in natural products, drug intermediates, and their derivatives often display a wide range of biological activities.[21] In particular, urea and thiourea derivatives have exhibited broad spectrum of biological activities such as antimicrobial[22] or antimalarial.[23] Due to the importance of urea and thiourea in pharmacological applications, there is an incentive to develop more

Scheme 1

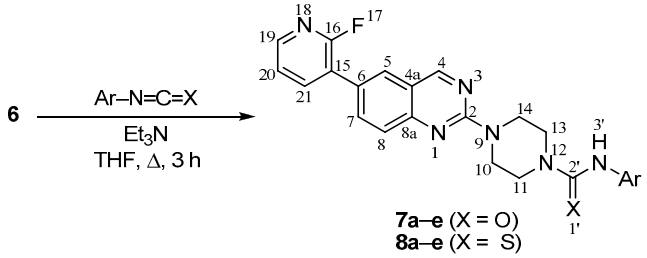


efficient urea and thiourea derivatives as antibacterial and antifungal agents.

Based on the many known applications of quinazoline derivatives, as well as those of urea and thiourea derivatives, we have designed and synthesized a novel series of *N*-aryl-4-[6-(2-fluoropyridin-3-yl)quinazolin-2-yl]-piperazine-1-carboxamide and -carbothioamide derivatives with an objective to screen them for antibacterial and antifungal activities. The synthetic scheme started from 5-bromo-2-fluorobenzaldehyde (**1**) and guanidine carbonate (**2**) in dimethylacetamide (DMA) to form 6-bromoquinazolin-2-amine (**3**) (Scheme 1). Compound **3** was treated with isoamyl nitrite, CuI, and diiodomethane in THF to give 6-bromo-2-iodoquinazoline (**4**). Compound **4** was treated with piperazine in THF to give compound **5** which was used in the Suzuki coupling reaction[24–26] with (2-fluoropyridin-3-yl)boronic acid in toluene–ethanol–water mixture, 1:1:1, to give compound **6** (Scheme 1). Further, compound **6** was treated with various aromatic isocyanates or isothiocyanates to give final products **7a**–**e** and **8a**–**e** in good yields (Table 1).

Compounds **7a**–**e** and **8a**–**e** were characterized by using LC-MS, ^{1}H, ^{13}C NMR, IR spectroscopy and elemental analysis. The IR spectra of all compounds contained absorption bands at 3278–3662, 1625–1672, 1429–1481, and 1313–1365 cm^{-1} corresponding to NH, C=O, C=N, and CN

Table 1. Yields and melting points of the synthesized compounds **7a**–**e** and **8a**–**e**



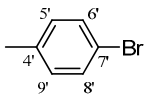
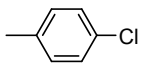
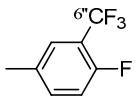
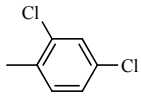
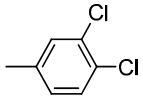
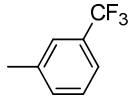
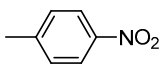
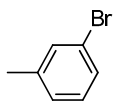
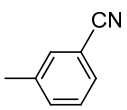
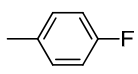
Compound	Ar	Yield, %	Mp, °C	Compound	Ar	Yield, %	Mp, °C
7a		73	262–264	**8a**		74	244–246
7b		78	274–276	**8b**		82	230–232
7c		81	257–259	**8c**		78	214–216
7d		76	246–248	**8d**		78	226–228
7e		79	254–256	**8e**		82	234–236

Table 2. Antibacterial activity of compounds **7a–e** and **8a–e** expressed as DIZ, mm

Compound	Gram-positive bacteria		Gram-negative bacteria			
	S. aureus	*P. aeruginosa*	Donor *E. coli*	Mutant *E. coli*	*K. pneumoniae*	*B. megaterium*
7a	14	13	13	Nd	Nd	13
7b	12	15	12	14	13	12
7c	13	14	12	10	15	14
7d	13	12	10	12	18	14
7e	Nd*	Nd	Nd	Nd	Nd	Nd
8a	9	5	8	9	19	14
8b	14	25	10	14	15	15
8c	17	19	19	14	30	16
8d	16	9	10	13	16	14
8e	16	8	Nd	8	10	19
Amoxiclav	24	24	22	20	22	23

* Nd – Not detected.

groups, respectively. ^{1}H NMR signals at 8.76–9.60 correspond to NH proton and those at 3.50–4.00 – to the piperazine methylene group protons. ^{13}C NMR signals of the C=O, C=S, CH_2N, and C–F group carbon atom were observed around 158.6, 162.5, 43.4, and 160.8 ppm, respectively.

All the synthesized compounds **7a–e** and **8a–e** were evaluated for their antibacterial activity by using agar well diffusion method.[27,28] The synthesized compounds were tested against two Gram-positive (*Staphylococcus aureus* and *Pseudomonas aeruginosa*) and two Gram-negative (*Klebsiella pneumoniae* and *Bacillus megaterium*) bacteria strains. Additionally, two different strains of antibiotic-resistant *E. coli* Gram-negative bacteria were also used in the present study (streptomycin-resistant mutant *E. coli* strain and rifampin-resistant donor *E. coli* strain), and Amoxiclav was used as a standard drug. The antibacterial activity was evaluated based on the diameter of inhibition zone (DIZ), which is the area on an agar plate where the growth of a control organism is prevented by a test compound. The obtained DIZ values are presented in Table 2.

The antibacterial activity was also evaluated in terms of the minimum inhibitory concentration (MIC), which is the lowest drug concentration that prevents visible microorganism growth after overnight incubation.[29] The MIC values are important in diagnostic laboratory to confirm resistance of microorganisms to antimicrobial agents and also to monitor the activity of new antimicrobial agents. MIC measurements were performed using a modified agar well diffusion method.[30]

The obtained MIC values of the tested compounds are presented in Table 3. The results revealed that compound **7b** showed good activity against Gram-positive and Gram-negative bacteria due to the presence of 3-CF_3 and 4-F substituents on the phenylurea benzene ring. Compound **8c** showed potent antibacterial activity against the tested microorganisms due to the presence of 3-CF_3 group on the phenylthiourea ring. Compounds **7d** and **8a** showed good activity against *K. pneumoniae* and *B. megaterium* due to the presence of electron-withdrawing groups NO_2 and Cl. Compound **8b** showed potent activity against *P. aeruginosa* in DIZ measurements and also against both Gram-positive

Table 3. Antibacterial activity of compounds **7a–e** and **8a–e** expressed as MIC, μg/ml

Compound	Gram-positive bacteria		Gram-negative bacteria			
	S. aureus	*P. aeruginosa*	Donor *E. coli*	Mutant *E. coli*	*K. pneumoniae*	*B. megaterium*
7a	75	25	5	Nd	Nd	25
7b	2.5	5	5	5	25	25
7c	50	50	50	50	5	5
7d	25	25	25	25	5	5
7e	Nd*	Nd	Nd	Nd	Nd	Nd
8a	50	100	50	50	5	5
8b	2	2.5	50	25	5	5
8c	5	5	5	25	2.5	5
8d	5	50	50	25	5	25
8e	5	50	Nd	50	50	5
Amoxiclav	–	–	–	–	–	–

* Nd – Not detected.

Table 4. Antifungal activity of compounds **7b**,**c**, and **8a**,**b**,**c**

Compound	*Aspergillus niger* DIZ, mm	*Penicillium spinulosum* DIZ, mm
7b	8	10
7c	5	7
8a	6	5
8b	9	12
8c	10	Nd
Fluconazole	10	12

* Nd – Not detected.

bacteria strains in MIC measurements due to the presence of 2,4-dichlorophenylthiourea functionality. Compound **7c** showed good activity against *K. pneumoniae* and *B. megaterium* in terms of MIC due to the presence of 3,4-dichloro group on the phenylurea ring. Compound **8c** exhibited good antibacterial activity against donor *E. coli* antibiotic-resistant strain due to the presence of 3-CF_3 phenylthiourea functionality, while compound **7b** showed good activity against both donor and mutant *E. coli* antibiotic-resistant strains due to the presence of 3-CF_3 and 4-F substituents on the phenylurea ring. The remaining compounds showed moderate activity against both antibiotic-resistant *E. coli* strains.

Based on the assessment of their antibacterial activity, the compounds **7b**,**c**, **8a–c** were screened for antifungal activity against two fungal strains (*Aspergillus niger* and *Penicillium spinulosum*) using poison plate technique.[31] The results, expressed as DIZ (Table 4), reveal that compounds **7b** and **8b** possess good antifungal activity against *Aspergillus niger* and *Penicillium spinulosum* due to the presence of 3-CF_3 and 4-F or 2-Cl and 4-Cl substituents, respectively, on the phenylurea benzene ring, whereas compound **8c** demonstrates excellent antifungal activity against *Aspergillus niger* due to the presence of 3-CF_3 group on the phenylthiourea benzene ring. The obtained results of the antifungal activity in DIZ are presented in Table 4.

In conclusion, we have synthesized a series of *N*-aryl-4-[6-(2-fluoropyridin-3-yl)quinazolin-2-yl]piperazine-1-carboxamide and *N*-aryl-4-[6-(2-fluoropyridin-3-yl)quinazolin-2-yl]piperazine-1-carbothioamide derivatives in good yields and evaluated the *in vitro* antimicrobial activity of these compounds. The compounds having 3-CF_3 substituent, 3-CF_3 and 4-F, or 2-Cl and 4-Cl substituents on the phenylurea benzene ring have shown potent antimicrobial activity against the tested microorganisms. Based on the above results, some of the compounds may be regarded as potential drug candidates for treatment of different microbial infections caused by multidrug-resistant organisms.

Experimental

IR spectra were recorded on a JASCO FT-IR 5300 spectrometer in KBr pellets. ^{1}H NMR spectra were recorded on Bruker Avance 400 (400 MHz) and Varian Mercury+ 300 (300 MHz) spectrometers in DMSO-d_6. ^{13}C NMR spectra were recorded on a Bruker Avance 400 spectrometer (100 MHz) in DMSO-d_6. TMS was used as internal standard. LC-MS spectra were recorded on an Acquity Ultra Performance Liquid Chromatography instrument coupled with an API 3000 mass spectrometer operating in electrospray positive ionization mode. Elemental analyses were done by using Thermo Scientific Flash 2000 organic elemental analyzer. Melting points were determined in open capillaries on Guna melting point apparatus and are uncorrected. All chemicals were purchased from Sigma Aldrich and Merck, and the solvents were used without further purification. Deionized water was used for all workup procedures, and doubly deionized water was used for biological experiments. All reactions were magnetically stirred and progress of the reactions was monitored by TLC on Merck silica plates.

6-Bromoquinazolin-2-amine (3).[32] Guanidine carbonate (**2**) (4.47 g, 36.9 mmol) was added to a solution of 5-bromo-2-fluorobenzaldehyde (**1**) (5.00 g, 24.6 mmol) in dimethylacetamide (50 ml). The reaction mixture was heated at 150°C for 5 h. The progress of the reaction was monitored by TLC using AcOEt–hexane, 1:1, as eluent. The reaction mixture was cooled and quenched with ice water, and stirred for 30 min. Then the obtained solid was filtered off, and water was removed by azeotropic distillation with PhMe to get 4.00 g (73%) of compound **3**. Mp 270–272°C. ^{1}H NMR spectrum (300 MHz), δ, ppm (*J*, Hz): 7.04 (2H, s, NH_2); 7.37 (1H, d, *J* = 9.0, H Ar); 7.78 (1H, dd, *J* = 9.0, *J* = 2.4, H Ar); 8.05 (1H, d, *J* = 2.4, H Ar); 9.09 (1H, s, H Ar). Mass spectrum, *m/z*: 225 $[M+H]^+$.

6-Bromo-2-iodoquinazoline (4).[33] 6-Bromoquinazolin-2-amine (**3**) (4.00 g, 17.9 mmol) was dissolved in THF (40 ml), and purged with nitrogen gas followed by addition of copper iodide (5.66 g, 17.8 mmol), isoamylnitrite (6.48 g, 55.3 mmol), and diiodomethane (23.91 g, 5 equiv, 89.3 mmol). The reaction mixture was heated at 80°C for 2 h. The progress of the reaction was monitored by TLC using AcOEt–hexane, 1:1, as eluent. The reaction mixture was cooled and quenched with ice water, and extracted with AcOEt (2 × 100 ml). The organic layer was washed with water and brine and dried over sodium sulphate. The solution was filtered and concentrated under reduced pressure. The resultant crude residue was purified by column chromatography using AcOEt–hexane, 1:1, as eluent. Yield 2.60 g (43%). Mp 121–123°C. ^{1}H NMR spectrum (300 MHz), δ, ppm (*J*, Hz): 7.88 (1H, d, *J* = 9.0, H Ar); 8.00 (1H, dd, *J* = 9.0, *J* = 2.1, H Ar); 8.08 (1H, d, *J* = 2.1, H Ar); 9.03 (1H, s, H Ar). Mass spectrum, *m/z*: 335 $[M+H]^+$.

6-Bromo-2-(piperazin-1-yl)quinazoline (5). Piperazine (0.565 g, 6.56 mmol) and triethylamine (1.810 g, 17.9 mmol) were added to a solution of 6-bromo-2-iodoquinazoline (**4**) (2.00 g, 5.97 mmol) in THF (20 ml). The reaction mixture was heated at 60°C for 2 h. The progress of the reaction was monitored by TLC using 10% methanol in chloroform as eluent. The reaction mixture was quenched with water and extracted with 10% methanol in chloroform. The organic layer was dried over sodium sulphate, filtered, and concentrated under reduced pressure. The resultant crude product was purified by column

chromatography using 5–10% methanol in chloroform. Yield 1.200 g (69%). Mp 138–140°C. ^{1}H NMR spectrum (300 MHz), δ, ppm (*J*, Hz): 2.96–2.99 (4H, m, 11,13-CH_2); 3.90–3.94 (4H, m, 10,14-CH_2); 7.47 (1H, d, *J* = 9.0, H Ar); 7.85 (1H, dd, *J* = 9.0, *J* = 2.4, H Ar); 8.13 (1H, d, *J* = 2.1, H Ar); 9.22 (1H, s, H Ar). Mass spectrum (EI, 70 eV), *m/z*: 295 [M(^{81}Br)+H]$^+$. Found, %: C 49.02; H 4.32; N 18.93. $C_{12}H_{13}BrN_4$. Calculated, %: C 49.16; H 4.47; N 19.11.

6-(2-Fluoropyridin-3-yl)-2-(piperazin-1-yl)quinazoline (6). Sodium carbonate (0.90 g, 8.5 mmol) and (2-fluoropyridin-3-yl)boronic acid (0.57 g, 4.1 mmol) were added to a solution of 6-bromo-2-(piperazin-1-yl)quinazoline (**5**) (1.00 g, 3.41 mmol) in PhMe–EtOH–H_2O, 1:1:1. The reaction mixture was degassed with nitrogen, and $Pd(PPh_3)_2Cl_2$ (0.11 g, 0.05 mol %, 1.69 mmol) was added. The reaction mixture was heated at 90°C under nitrogen atmosphere for 1 h. The progress of the reaction was monitored by TLC using $CHCl_3$–MeOH, 9:1, as eluent. The reaction mixture was quenched with water and extracted with $CHCl_3$–MeOH, 9:1 (3 × 100 ml). The organic layer was dried over sodium sulphate, then filtered and concentrated under reduced pressure. The crude product was purified by column chromatography using 5–10% methanol in chloroform. Yield 0.60 g (57%). Mp 158–160°C. ^{1}H NMR spectrum (300 MHz), δ, ppm (*J*, Hz): 2.80–2.83 (4H, m, 10,14-CH_2); 3.83–3.86 (4H, m, 11,13-CH_2); 7.46–7.51 (1H, m, H Ar); 7.58 (1H, d, *J* = 9.0, H Ar); 7.96 (1H, d, *J* = 9.0, H Ar); 8.10 (1H, s, H Ar); 8.17–8.24 (2H, m, H Ar); 9.26 (1H, s, H Ar). Mass spectrum, *m/z*: 310 [M+H]$^+$. Found, %: C 65.91; H 4.99; N 22.37. $C_{17}H_{16}FN_5$. Calculated, %: C 66.01; H 5.21; N 22.64.

Synthesis of *N*-aryl-4-[6-(2-fluoropyridin-3-yl)quinazolin-2-yl)]piperazine-1-carboxamides 7a–e and *N*-aryl-4-[6-(2-fluoropyridin-3-yl)quinazolin-2-yl)]piperazine-1-carbothioamides 8a–e (General Method). Compound **6** (0.10 g, 0.32 mmol) was dissolved in THF (20 ml), than TEA (0.13 ml, 0.96 mmol) was added, followed by the addition a substituted aryl isocyanate (0.48 mmol). The reaction mixture was refluxed for 3 h under nitrogen atmosphere. The progress of the reaction was monitored by TLC using $CHCl_3$–MeOH, 9:1, as eluent. The reaction mixture was cooled, diluted with water, extracted with $CHCl_3$–MeOH, 9:1, and washed with brine. The organic layer was dried over sodium sulphate, filtered, and concentrated under reduced pressure. The obtained crude residue was purified by column chromatography on silica gel (60–120 mesh) using 2–5% methanol in chloroform as the eluent.

***N*-(4-Bromophenyl)-4-[6-(2-fluoropyridin-3-yl)quinazolin-2-yl]piperazine-1-carboxamide (7a)**. Off-white solid. IR spectrum, ν, cm^{-1}: 3448 (N–H), 2922 (C–H), 1654 (C=O), 1475 (C=N), 1313 (C–N), 1238 (C–F). ^{1}H NMR spectrum (400 MHz), δ, ppm (*J*, Hz): 3.58–3.61 (4H, m, 10,14-CH_2); 3.93–3.96 (4H, m, 11,13-CH_2); 7.41–7.51 (4H, m, H Ar); 7.64 (2H, d, *J* = 8.8, H Ar); 7.97–8.00 (1H, m, H Ar); 8.14 (1H, s, H Ar); 8.19–8.25 (2H, m, H Ar); 8.76 (1H, s, NH); 9.31 (1H, s, H Ar). ^{13}C NMR spectrum (100 MHz), δ, ppm: 43.5 (C-2,3,19,20); 113.2 (C-4a); 119.1 (C-15); 120.0 (C-5',9'); 121.4 (C-7'), 122.7 (C-20); 125.4 (C-8); 127.2 (C-6',8'); 128.2 (C-5); 131.0 (C-6); 134.7 (C-7); 139.9 (C-4'); 141.2 (C-21); 146.4 (C-19); 151.1 (C-8a); 154.7 (C-2'); 158.8 (C-4); 160.8 (C-16); 162.5 (C-2). Mass spectrum (EI, 70 eV), *m/z*: 509 [M(^{81}Br)+H]$^+$. Found, %: C 56.63; H 3.78; N 16.29. $C_{24}H_{20}BrFN_6O$. Calculated, %: C 56.82; H 3.97; N 16.56.

4-[6-(2-Fluoropyridin-3-yl)quinazolin-2-yl)-*N*-[4-fluoro-3-(trifluoromethyl)phenyl]piperazine-1-carboxamide (7b). Off-white solid. IR spectrum, ν, cm^{-1}: 3431 (N–H), 2862 (C–H), 1672 (C=O), 1481 (C=N), 1327 (C–N), 1234 (C–F). ^{1}H NMR spectrum (400 MHz), δ, ppm (*J*, Hz): 3.60–3.63 (4H, m, 10,14-CH_2); 3.95–3.97 (4H, m, 11,13-CH_2); 7.48–7.51 (1H, m, H Ar); 7.56 (1H, d, *J* = 8.8, H Ar); 7.64 (1H, d, *J* = 8.8, H Ar); 7.83 (1H, dd, *J* = 8.8, *J* = 2.4, H Ar); 7.97–8.00 (1H, m, H Ar); 8.07 (1H, d, *J* = 2.4, H Ar); 8.14 (1H, s, H Ar); 8.19–8.25 (2H, m, H Ar); 9.07 (1H, s, NH); 9.32 (1H, s, H Ar).^{13}C NMR spectrum (100 MHz), δ, ppm: 43.4 (C-10,11,13,14); 117.8 (C-8'); 119.1 (C-5′); 121.5 (C-6'); 121.9 (C-4a); 122.0 (C-15); 122.7 (C-20); 123.8 (C-6″); 125.4 (C-8); 127.3 (C-9'); 128.2 (C-4'); 131.5 (C-5); 132.5 (C-6); 140.1 (C-7); 141.2 (C-21); 146.4 (C-19); 151.1 (C-8a); 154.4 (C-7'); 158.4 (C-2'); 158.8 (C-4); 160.7 (C-16); 162.5 (C-2). Mass spectrum, *m/z*: 515 [M+H]$^+$. Found, %: C 58.15; H 3.69; N 16.11. Mass spectrum, *m/z*: 515 [M+H]$^+$. Found, %: C 58.15; H 3.69; N 16.11. $C_{25}H_{19}F_5N_6O$. Calculated, %: C 58.37; H 3.72; N 16.34.

***N*-(3,4-Dichlorophenyl)-4-[6-(2-fluoropyridin-3-yl)-quinazolin-2-yl]piperazine-1-carboxamide (7c)**. Off-white solid. IR spectrum, ν, cm^{-1}: 3278 (N–H), 2858 (C–H), 1664 (C=O), 1475 (C=N), 1365 (C–N), 1234 (C–F). ^{1}H NMR spectrum (400 MHz), δ, ppm (*J*, Hz): 3.59–3.61 (4H, m, 10,14-CH_2); 3.94–3.96 (4H, m, 11,13-CH_2); 7.48–7.51 (3H, m, H Ar); 7.62 (1H, d, *J* = 8.8, H Ar); 7.86 (1H, s, H Ar); 7.97 (1H, d, *J* = 9.2, H Ar); 8.14 (1H, s, H Ar); 8.19–8.25 (2H, m, H Ar); 8.91 (1H, s, NH); 9.32 (1H, s, H Ar). ^{13}C NMR spectrum (100 MHz), δ, ppm: 44.0 (C-10, C-11,13,14); 119.7 (C-4a); 120.9 (C-15); 123.2 (C-9'); 123.4 (C-20); 126.0 (C-8); 127.8 (C-5',7'); 128.7 (C-5); 130.6 (C-6); 131.0 (C-7,8'); 135.2 (C-6'), 141.3 (C-4'); 141.7 (C-21); 146.8 (C-19); 146.9 (C-8a); 151.7 (C-4); 155.0 (C-16); 159.3 (C-2'); 163.0 (C-2). Mass spectrum, *m/z*: 497 [M+H]$^+$. Found, %: C 57.79; H 3.67; N 16.72. $C_{24}H_{19}Cl_2FN_6O$. Calculated, %: C 57.96; H 3.85; N 16.90.

4-[6-(2-Fluoropyridin-3-yl)quinazolin-2-yl]-*N*-(4-nitrophenyl)piperazine-1-carboxamide (7d). Off-white solid. IR spectrum, ν, cm^{-1}: 3329 (N–H), 2858 (C–H), 1625 (C=O), 1481 (C=N), 1365 (C–N), 1219 (C–F). ^{1}H NMR spectrum (400 MHz), δ, ppm (*J*, Hz): 3.60–3.95 (8H, m, 10,11,13,14-CH_2); 7.39–7.49 (4H, m, H Ar); 7.62 (2H, d, *J* = 8.8, H Ar); 7.98–8.04 (1H, m, H Ar); 8.00 (1H, s, H Ar); 8.14–8.25 (2H, m, H Ar); 8.77 (1H, s, NH); 9.32 (1H, s, H Ar). ^{13}C NMR spectrum (100 MHz), δ, ppm: 43.5 (C-10,11,13,14); 113.2 (C-4a); 119.1 (C-15); 120.0 (C-5',9'); 121.7 (C-20); 125.2 (C-8); 127.1 (C-6',8'); 128.4 (C-5); 131.1 (C-6); 133.6 (C-7); 139.7 (C-4'); 141.1 (C-7'); 141.3 (C-21); 146.4 (C-19); 151.1 (C-8a); 154.4 (C-2'); 158.6 (C-4); 161.7 (C-16); 181.1 (C-2). Mass spectrum, *m/z*:

474 $[M+H]^+$. Found, %: C 60.66; H 4.18; N 20.52. $C_{24}H_{20}FN_7O_3$. Calculated, %: C 60.88; H 4.26; N 20.71.

***N*-(3-Cyanophenyl)-4-[6-(2-fluoropyridin-3-yl)quinazolin-2-yl]piperazine-1-carboxamide (7e)**. Off-white solid. IR spectrum, ν, cm^{-1}: 3116 (N–H), 2922 (C–H), 1627 (C=O), 1477 (C=N), 1317 (C–N), 1224 (C–F). ^{1}H NMR spectrum (400 MHz), δ, ppm (*J*, Hz): 3.97–4.14 (8H, m, 10,11,13,14-CH_2); 7.33–7.37 (4H, m, H Ar); 7.48–7.52 (1H, m, H Ar); 7.65 (1H, d, *J* = 8.8, H Ar); 8.00 (1H, d, *J* = 8.8, H Ar); 8.15 (1H, s, H Ar); 8.22–8.25 (2H, m, H Ar); 9.33 (1H, s, NH); 9.44 (1H, s, H Ar). ^{13}C NMR spectrum (100 MHz), δ, ppm: 43.1 (C-11,13); 47.5 (C-10,14); 119.1 (C-6'); 121.9 (C-6''); 122.7 (C-15, 20); 125.4 (C-5'); 126.8 (C-8); 127.3 (C-9'); 127.8 (C-7'); 128.2 (C-5,6); 134.7 (C-7,8'); 139.9 (C-4'); 141.1 (C-21); 146.3 (C-19); 151.1 (C-8a); 158.6 (C-2',4); 162.5 (C-16); 181.4 (C-2). Mass spectrum, *m/z*: 454 $[M+H]^+$. Found, %: C 65.98; H 4.39; N 21.36. $C_{25}H_{20}FN_7O$. Calculated, %: C 66.22; H 4.45; N 21.62.

***N*-(4-Chlorophenyl)-4-[6-(2-fluoropyridin-3-yl)quinazolin-2-yl]piperazine-1-carbothioamide (8a)**. Off-white solid. IR spectrum, ν, cm^{-1}: 3421 (N–H), 2862 (C–H), 1632 (C=O), 1473 (C=N), 1346 (C–N), 1223 (C–F). ^{1}H NMR spectrum (400 MHz), δ, ppm (*J*, Hz): 4.00–4.15 (8H, m, 10,11,13,14-CH_2); 7.36 (1H, d, *J* = 8.4, H Ar); 7.41–7.45 (1H, m, H Ar); 7.52 (1H, s, H Ar); 7.65–7.68 (2H, m, H Ar); 8.00 (1H, d, *J* = 8.7, H Ar); 8.18 (1H, s, H Ar); 8.26–8.28 (2H, m, H Ar); 9.36 (1H, s, NH); 9.39 (1H, s, H Ar). ^{13}C NMR spectrum (100 MHz), δ, ppm: 43.2 (C-10,11,13,14); 118.4 (C-5'); 119.1 (C-9'); 121.7 (C-4a); 122.6 (C-15); 123.4 (C-20); 125.1 (C-8); 127.7 (C-6',8'); 128.0 (C-5); 130.1 (C-6, 21); 132.2 (C-7'); 134.1 (C-7); 140.5 (C-4'); 141.7 (C-19); 146.2 (C-8a); 151.0 (C-4); 158.6 (C-16); 162.7 (C-2'); 181.5 (C-2). Mass spectrum, *m/z*: 479 $[M+H]^+$. Found, %: C 60.12; H 4.19; N 17.32; S 6.41 $C_{24}H_{20}ClFN_6S$. Calculated, %: C 60.18; H 4.21; N 17.55; S 6.69.

***N*-(2,4-Dichlorophenyl)-4-[6-(2-fluoropyridin-3-yl)-quinazolin-2-yl]piperazine-1-carbothioamide (8b)**. Off-white solid. IR spectrum, ν, cm^{-1}: 3662 (N–H), 2895 (C–H), 1625 (C=O), 1436 (C=N), 1332 (C–N), 1224 (C–F). ^{1}H NMR spectrum (400 MHz), δ, ppm (*J*, Hz): 3.99–4.07 (4H, m, 10,14-CH_2); 4.08–4.13 (4H, m, 11,13-CH_2); 7.42–7.70 (5H, m, H Ar); 7.74 (1H, s, H Ar); 7.98 (1H, d, *J* = 1.6, H Ar); 8.15 (1H, s, H Ar); 8.19–8.25 (1H, m, H Ar); 9.33 (1H, s, NH); 9.60 (1H, s, H Ar). ^{13}C NMR spectrum (100 MHz), δ, ppm: 43.1 (C-11,13); 47.6 (C-10,14); 119.2 (C-4a); 120.0 (C-15); 121.9 (C-8'); 122.7 (C-20); 127.3 (C-8); 128.2 (C-9'); 128.4 (C-6'); 128.6 (C-5); 129.0 (C-6); 129.4 (C-7'); 134.7 (C-7); 141.8 (C-5'); 146.3 (C-4'); 146.4 (C-21); 151.1 (C-19); 158.4 (C-8a); 158.7 (C-4); 160.8 (C-16); 162.5 (C-2'); 181.2 (C-2). Mass spectrum, *m/z*: 515 $[M(^{37}Cl)+H]^+$. Found, %: C 55.89; H 3.71; N 16.02; S 5.94. $C_{24}H_{19}Cl_2FN_6S$. Calculated, %: C 56.14; H 3.73; N 16.37; S 6.25.

4-[6-(2-Fluoropyridin-3-yl)quinazolin-2-yl]-*N*-[3(trifluoromethyl)phenyl]piperazine-1-carbothiomide (8c). Off-white solid. IR spectrum, ν, cm^{-1}: 3662 (N–H), 2843 (C–H), 1625 (C=O), 1431 (C=N), 1317 (C–N), 1222 (C–F). ^{1}H NMR spectrum (400 MHz), δ, ppm (*J*, Hz): 4.00–4.11 (8H, m, 10,11,12,14-CH_2); 7.23–7.65 (6H, m, H Ar); 7.97–8.00 (1H, m, H Ar); 8.15 (1H, s, H Ar); 8.19–8.25 (2H, m, H Ar); 9.33 (1H, s, NH); 9.48 (1H, s, H Ar). ^{13}C NMR spectrum (100 MHz), δ, ppm: 43.1 (C-11,13); 47.6 (C-10,14); 119.2 (C-4a); 120.4 (C-15); 121.9 (C-5'); 122.7 (C-20); 123.8 (C-6''); 125.5 (C-8); 126.7 (C-8'); 127.3 (C-9'); 128.2 (C-6'); 129.8 (C-7'); 134.7 (C-5); 141.2 (C-6); 141.4 (C-21); 142.6 (C-7); 146.3 (C-4'); 151.1 (C-19); 158.4 (C-8a); 158.7 (C-4); 160.7 (C-16); 162.5 (C-2'); 181.2 (C-2). Mass spectrum, *m/z*: 513 $[M+H]^+$. Found, %: C 58.47; H 3.82; N 16.10; S 5.99. $C_{25}H_{20}F_4N_6S$. Calculated, %: C 58.59; H 3.93; N 16.40; S 6.26.

***N*-(3-Bromophenyl)-4-[6-(2-fluoropyridin-3-yl)quinazolin-2-yl]piperazine-1-carbothioamide (8d)**. Off-white solid. IR spectrum, ν, cm^{-1}: 3398 (N–H), 2862 (C–H), 1627 (C=O), 1429 (C=N), 1332 (C–N), 1220 (C–F). ^{1}H NMR spectrum (400 MHz), δ, ppm (*J*, Hz): 3.99–4.04 (4H, m, 10,14-CH_2); 4.05–4.11 (4H, m, 11,13-CH_2); 7.11–7.51 (5H, m, H Ar); 7.65 (1H, d, *J* = 8.8, H Ar); 8.00 (1H, d, *J* = 8.8, H Ar); 8.15 (1H, s, H Ar); 8.19–8.25 (2H, m, H Ar); 9.33 (1H, s, NH), 9.38 (1H, s, H Ar). ^{13}C NMR spectrum (100 MHz), δ, ppm: 43.1 (C-11,13); 47.4 (C-10,14); 114.6 (C-4a); 119.1 (C-15); 121.9 (C-20); 122.2 (C-7'); 122.7 (C-9'); 125.4 (C-6'); 127.6 (C-5'); 128.2 (C-8); 134.7 (C-8'); 137.2 (C-5); 141.2 (C-6); 146.4 (C-7); 151.1 (C-21); 157.9 (C-4'); 158.4 (C-19); 158.7 (C-8a); 160.3 (C-4); 160.7 (C-16); 162.5 (C-2'); 181.6 (C-2). Mass spectrum, *m/z*: 525 $[M(^{81}Br)+H]^+$. Found, %: C 54.88; H 3.81; N 15.76; S 5.89. $C_{24}H_{20}BrFN_6S$. Calculated, %: C 55.07; H 3.85; N 16.06; S 6.13.

***N*-(4-Fluorophenyl)-4-[6-(2-fluoropyridin-3-yl)quinazolin-2-yl]piperazine-1-carbothioamide (8e)**. Off-white solid. IR spectrum, ν, cm^{-1}: 3367 (N–H), 2860 (C–H), 1674 (C=O), 1429 (C=N), 1323 (C–N), 1234 (C–F). ^{1}H NMR spectrum (400 MHz), δ, ppm (*J*, Hz): 3.59–3.65 (4H, m, 10,14-CH_2); 3.93–3.99 (4H, m, 11,13-CH_2); 7.37–7.67 (4H, m, H Ar); 7.76–7.78 (1H, m, H Ar); 7.95–7.99 (2H, m, H Ar); 8.13 (1H, s, H Ar); 8.19–8.25 (2H, m, H Ar); 8.97 (1H, s, NH); 9.31 (1H, s, H Ar). ^{13}C NMR spectrum (100 MHz), δ, ppm: 43.5 (C-10,11,13,14); 118.9 (C-6'); 119.1 (C-8'); 121.9 (C-4a); 122.7 (C-15); 123.8 (C-20); 125.4 (C-8); 127.3 (C-9', 5'); 128.2 (C-5); 130.1 (C-6); 134.7 (C-7); 140.2 (C-4'); 141.2 (C-19); 146.4 (C-8a); 151.1 (C-4); 154.6 (C-7'); 158.8 (C-16); 160.7 (C-2'); 162.5 (C-2). Mass spectrum, *m/z*: 463 $[M+H]^+$. Found, %: C 62.07; H 4.28; N 17.87; S 6.57. $C_{24}H_{20}F_2N_6S$. Calculated, %: C 62.32; H 4.36; N 18.17; S 6.93.

Antimicrobial activity. Each of the tested compounds (200 μg) was dissolved in DMSO (1 ml). Centrifuged pellets of bacteria from 24 h old culture containing approximately 104–106 colony forming units (CFU) per ml were spread on the surface of nutrient agar plates. Nutrient agar medium was prepared by suspending nutrient agar (28 g) in distilled water (1 l), autoclaved, and cooled to 45°C. Then the agar it was seeded with 15 ml of prepared inocula of the bacteria to obtain concentration 106 CFU/ml. Petri dishes were prepared by pouring 10 ml of seeded nutrient agar. Wells were created in the medium with the help of a sterile metallic borer, and a test solution was added into each of them. The experimental plates were incubated for 24 h at 37°C, and the diameter of the inhibition zone around each well was measured.

Antifungal activity. Test compounds **7b**,**c**, **8a–c** were dissolved in DMSO before mixing with potato dextrose agar (PDA). The final concentration of the compounds in the medium was fixed at 200 μg/ml. Two kinds of fungi were incubated in PDA at 25 ± 1°C for 5 days to get new mycelium for antifungal assay, and then a mycelium disk of approximately 0.45 cm diameter, cut from the culture medium, was picked up with a sterilized inoculation needle and inoculated in the center of the PDA plate. The inoculated plates were incubated at 25 ± 1°C for 5 days. DMSO was added as the negative control to determine possible inhibitory activity of the solvent, while fluconazole Fu 10 (SD 114, Himedia) was used as the positive control. For each treatment, the experiment was repeated three times and the mean value of the diameter of the inhibition zones was calculated.

The authors express their gratitude to IISC, Bangalore, for providing spectral data and to DST-PURSE center, Sri Venkateswara University, Tirupati, for evaluating the antibacterial and antifungal activities.

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