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Synthesis and In Vitro Antimicrobial Evaluation of Piperazine Substituted Quinazoline-Based Thiourea/Thiazolidinone/Chalcone Hybrids[1]

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Abstract—In frames of the search for new biological entities to fight against recent drug-resistant microbial strains, we report a library of quinazoline-based thiourea/4-thiazolidinone/chalcone hybrids. The newly synthesized compounds were studied for efficacy against several bacteria (*Staphylococcus aureus, Bacillus cereus, Pseudomonas aeruginosa*, and *Klebsiella pneumoniae*) and fungi (*Candida albicans* and *Aspergillus clavatus*) using the broth dilution technique. From the biological evaluation, (E)-3-(3,4-dimethoxyphenyl)-1-(4-((4-(4-ethylpiperazin-1-yl)quinazolin-2-yl)amino)phenyl)prop-2-en-1-one was found to be the most active analogue (microbial inhibition concentration 3.12 μg/mL) to inhibit the bacterial growth. The rest of the compounds showed equipotent efficacy (3.12–12.5 μg/mL) as compared to the standard. Final compounds were characterized by FT-IR, ^{1}H NMR, ^{13}C NMR, mass spectroscopy, and elemental analysis.

Keywords: 4-thiazolidinone, antibacterial activity, antifungal activity, chalcone, quinazoline, thiourea

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INTRODUCTION

During the past few decades, the growing population is affected with significant increase in the frequency of severe infectious diseases because of the increasing number of multi-drug-resistant (MDR) microbial pathogens. Increasing number of MDR strains developed by the microbes makes currently used antimicrobial drugs ineffective. Over the past years, alleviation of opportunistic microbial infections became an imperative and challenging problem due to the inactiveness of susceptible microorganism. Rapid multiplication of drug resistant strains poses a severe threat in recent years [1–3]. Such infection readily affects debilitated and immune compromised patients. Hence, there is an urgent need to alleviate drug resistance by providing more effective potential therapeutic agents [4, 5].

The search for novel bioactive agents with higher selectivity and lower toxicity continues to be an area of intensive investigation in synthetic medicinal chemistry. On the path of identifying various chemical substances that may serve as leads for designing novel bioactive agents, nitrogen-containing heterocycles are of particular interest. Quinazoline scaffold has been extensively studied for its many pharmacological properties [6], which include anti-cancer [7], anti-inflammatory [8], anti-bacterial [9], antiviral [10], anti-tubercular [11], anti-malarial [12], and anti-diuretic [13] activities. Quinazoline linked thiourea hybrid derivatives have been identified as impressive antimicrobial agents [14]. Furthermore, the presence of piperazine substituents at C-4 position of quinazoline core has proved to be the active key in various biological effects [15]. Moreover, quinazoline and their condensed products with thiazolidinone derivatives have been reported to possess interesting antimicrobial activity [16, 17].

Encouraged by our previous successful research efforts [10, 18,19] in this regard, we decided to further extend the above methodology to identify more quinazolinyl hybrids. In view of the above-mentioned knowledge of different pharmacophores and in continuation of our research program, we have designed (figure) and synthesized quinazoline-thiourea/thiazolidinone/chalcone hybrids and incorporated piperazine and electronic environment to get single bioactive molecule framework. Compounds were subjected to evaluation of their antibacterial and antifungal potency against various bacterial strains. As a result, some of the derivatives showed excellent activity in the range of 3.12–12.5 μg/mL of MIC.

RESULTS

Chemistry

The scheme outlines the synthetic pathway used to obtain compounds (**Va**–**e**, **VIIIa**–**e**, **Xa**–**e**, and **XIIIa**–**e**). The first step comprises formation of quinazoline-

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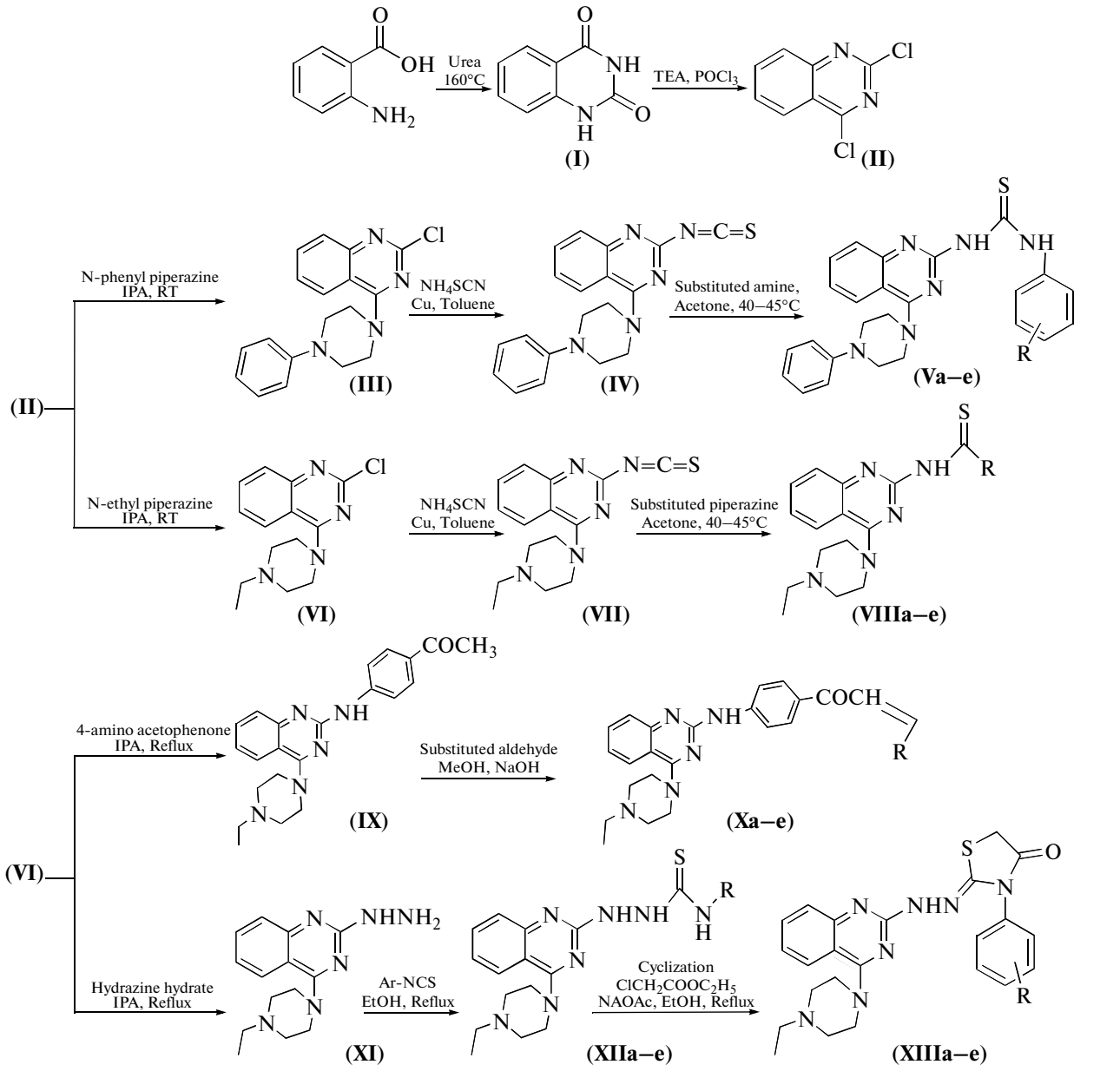
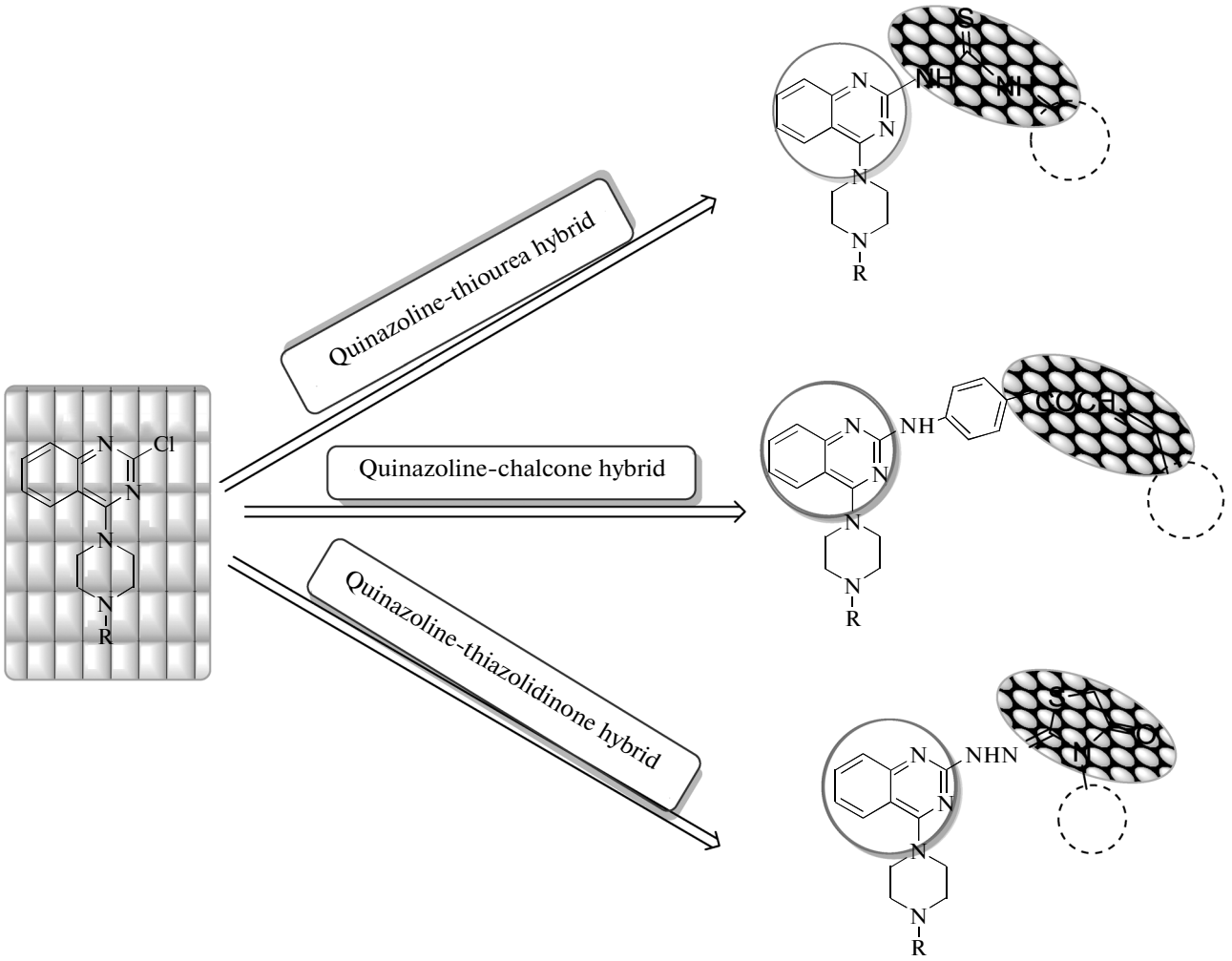
2,4(1*H*,3*H*)-dione (**1**) in very good yield by the reaction of anthranilic acid and urea [20]. Cyclization of intermediate (**I**) with $POCl_3$ forms the 2,4-dichloroquinazoline (**II**). This was further reacted to *N*-phenyl piperazine in isopropyl alcohol to form 2-chloro-4-(4-phenylpiperazin-1-yl)quinazoline(**III**). Compound (**III**) was then reacted to ammonium thiocyanate and various amines to give final thiourea derivatives (**Va–e**).



Scheme. Synthetic route for the synthesis of quinazoline-based final hybrid (**Va–e**, **VIIIa–e**, **Xa–e**, and **XIIIa–e**) derivatives.

Intermediated (**II**) was then further reacted to *N*-ethyl piperazinef-orming 2-chloro-4-(4-ethylpiperazin-1-yl)quinazoline (**VI**) further reacted with ammonium thiocyanate and piperazine to give final (**VIIIa–e**) compounds. Intermediate 2-chloro-4-(4-ethylpiperazin-1-yl)quinazoline (**VI**) was refluxed to replace 2-Cl position with 4-amino acetophenone and hydrazine hydrate that gives 1-(4-((4-(4-ethylpiperazin-1-yl)quinazolin-2-yl)amino)phenyl)ethanone (**IX**) and 4-(4-ethylpiperazin-1-yl)-2-hydrazinylquinazoline (**XI**), respectively. Out of them, (**IX**) was then condensed to various aldehydes to form chalcone (**Xa–e**) analogues, whereas intermediate (**XI**) was reacted with various phenyl isothiocyanate and further cyclized



Design of the title quinazoline-based hybrids.

with chloroethylacetate and sodium acetate to form desired thiazolidinone (**XIIIa**–**e**) derivatives. The accuracy of the synthesis of final compounds was confirmed on the basis of ^{1}H NMR, ^{13}C NMR, and mass spectra and the purity was ascertained by elemental analysis. Physical and analytical data of title compounds are elaborated in Table 1.

Biological Evaluation

All newly synthesized quinazoline derivatives (**Va**–**e**, **VIIIa**–**e**, **Xa**–**e**, and **XIIIa**–**e**) were examined for antimicrobial activity against two gram-positive bacterial strains (*Staphylococcus aureus* MTCC 96, *Bacillus cereus* MTCC 430), two gram-negative bacterial strains (*Pseudomonas aeruginosa* MTCC 741, *Klebsiella pneumoniae* MTCC 109), and two fungal strains (*Aspergillus clavatus* MTCC 1323, *Candida albicans* MTCC 183) using agar dilution method [21]. Ciprofloxacin was used as standard control drug for antibacterial activity, whereas Ketoconazole was used as standard control drug for antifungal activity.

In vitro antibacterial activity. Table 2 shows that all the newly synthesized quinoline scaffolds were found to exhibit good to moderate activity against the specific microbial strain. Bioassay results of the series of (**Va–e**) compounds revealed that final analogue (**Ve**), bearing electron donating methyl group at para position of phenyl ring, was found to be the most active compound that inhibits the gram-positive *S. aureus* bacterial growth at the lowest minimum inhibitory concentration (MIC) value of 3.12 μg/mL. Compound (**Vc**) with chloro group showed lower effectiveness with 12.5 μg/mL MIC against the same bacteria. The presence of an electron snatching substituent, like nitro group (**Vb**), proved to be intrinsic to conduce noteworthy activity at 12.5 μg/mL of MIC against the *B. cereus* strain. Compound (**Vd**) containing chloro group at para position of the phenyl ring showed half-fold (6.25 μg/mL MIC) inhibitory activity against gram-negative *P. aeruginosa* strain as compared to

Table 1. Physical and analytical data of final synthesized quinazolinyl derivatives (**Va**–**e**, **VIIIa**–**e**, **Xa**–**e**, and **XIIIa**–**e**)

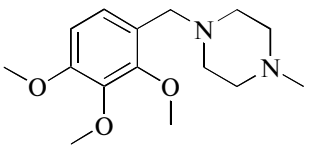
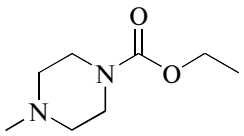
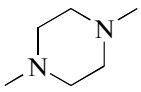
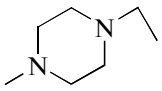
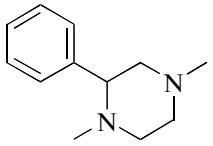
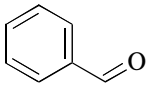
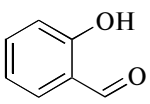
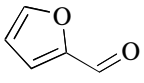
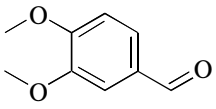
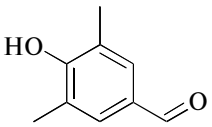
Entry	R	Mol. formula	Mol. weight	Yield, %	mp (°C)	Elemental analysis					
						calcd.			found		
						%C	%H	%N	%C	%H	%N
(Va)	H	$C_{25}H_{24}N_6S$	440.56	72	129–133	68.16	5.49	19.08	68.32	5.47	19.03
(Vb)	2-NO_2	$C_{25}H_{23}N_7O_2S$	485.56	67	162–164	61.84	4.77	20.19	61.67	4.76	20.13
(Vc)	3-Cl	$C_{25}H_{23}ClN_6S$	475.01	65	163–165	63.21	4.88	17.69	63.03	4.87	17.65
(Vd)	4-Cl	$C_{25}H_{23}ClN_6S$	475.01	65	130–131	63.21	4.88	17.69	63.31	4.89	17.64
(Ve)	4-CH_3	$C_{26}H_{26}N_6S$	454.59	67	213–215	68.69	5.76	18.49	68.76	5.77	18.44
(VIIIa)		$C_{29}H_{39}N_7O_3S$	565.73	66	139–141	61.57	6.95	17.33	61.39	6.96	17.29
(VIIIb)		$C_{22}H_{31}N_7O_2S$	457.59	65	162–164	57.74	6.83	21.43	57.86	6.81	21.39
(VIIIc)		$C_{20}H_{29}N_7S$	399.56	64	201–203	60.12	7.32	24.54	59.95	7.31	24.48
(VIIId)		$C_{21}H_{31}N_7S$	413.58	67	110–112	60.99	7.55	23.71	61.09	7.53	23.64
(VIIIe)		$C_{26}H_{33}N_7S$	475.65	67	221–223	65.65	6.99	20.61	65.49	6.97	20.55
(Xa)		$C_{29}H_{29}N_5O$	463.57	66	221–221	75.14	6.31	15.11	74.92	6.32	15.07
(Xb)		$C_{29}H_{29}N_5O_2$	479.57	67	112–114	72.63	6.10	14.60	72.46	6.12	14.59

Table 1. (Contd.)

Entry	R	Mol. formula	Mol. weight	Yield, %	mp (°C)	Elemental analysis					
						calcd.			found		
						%C	%H	%N	%C	%H	%N
(Xc)		$C_{27}H_{27}N_5O_2$	453.54	65	164–166	71.50	6.00	15.44	71.40	6.01	15.48
(Xd)		$C_{31}H_{33}N_5O_3$	523.63	69	207–209	71.11	6.35	13.37	70.91	6.34	13.34
(Xe)		$C_{31}H_{33}N_5O_2$	507.63	67	154–157	73.35	6.55	13.80	73.47	6.54	13.76
(XIIIa)	H	$C_{23}H_{25}N_7OS$	447.56	74	152–154	61.72	5.63	21.91	61.66	5.64	21.86
(XIIIb)	2-CH_3	$C_{24}H_{27}N_7OS$	461.58	66	142–144	62.45	5.90	21.24	62.31	5.88	21.29
(XIIIc)	2-Cl	$C_{23}H_{24}ClN_7OS$	482.00	55	154–158	57.31	5.02	20.34	57.21	5.01	20.31
(XIIId)	2-Cl	$C_{23}H_{24}ClN_7OS$	482.00	61	122–124	57.31	5.02	20.34	57.16	5.01	20.30
(XIIIe)	2-F	$C_{23}H_{24}FN_7OS$	465.55	56	197–200	59.34	5.20	21.06	59.51	5.19	21.00

standard ciprofloxacin drug (3.12 μg/mL MIC), respectively. Analogue (**Va**)manifested excellent inhibition of gram-negative *K. pneumoniae* strain at 12.5 μg/mL of MIC.

For the series (**VIIIa**–**e**) derivatives, compound (**VIIId**) bearing *N*-ethyl piperazine ring showed the strong inhibitory action at 3.12 μg/mL of MIC against gram-positive *S. aureus* strain. Analogues (**VI**–**IIb**) with *N*-ethoxy carbonyl piperazine showed slightly reduced activity of 6.25 μg/mL MIC against the same bacteria. Compound (**VIIIc**) bearing *N*-methyl piperazine moiety appeared with half-fold growth inhibition (6.25 μg/mL MIC) of gram-positive *B. cereus* bacteria as compared to reference ciprofloxacin drug (3.125 μg/mL MIC). Excellent inhibition (3.12 μg/mL of MIC) against gram-negative *P. aeruginosa* strain was noted for trimethoxy phenyl attached piperazine compound (**VIIIa**), which was the most active analogue in the series. Compound (**VIIIb**) exhibited good inhibitory profile at MIC 3.12 and 12.5 μg/mL against gram-negative *K. pneumoniae* and *P. aeruginosa* bacteria, respectively.

In the series of (**Xa**–**e**) derivatives, compound (**Xa**) with unsubstituted phenyl ring showed excellent growth inhibition of gram-positive *S. aureus* bacteria at 3.12 μg/mL of MIC. Two derivatives with 3,4-dimethoxy (**Xd**) and 3,5-dimethoxy,4-hydroxy (**Xe**) substituents on phenyl ring appeared with slightly diminished activity (MIC 12.5 μg/mL) against the same bacteria.

From the bioassay of (**XIIIa**–**e**), compound (**VIIIa**) with unsubstituted phenyl ring displayed remarkable activity (12.5 μg/mL MIC) against *S. aureus* bacteria. The inhibition of *B. cereus* strain's growth at 3.12 μg/mL of MIC was greatly achieved by methyl

Table 2. In vitro antibacterial and antifungal activity in MIC* (μg/mL) of compounds (**Va**–**e**, **VIIIa**–**e**, **Xa**–**e**, and **XIIIa**–**e**)

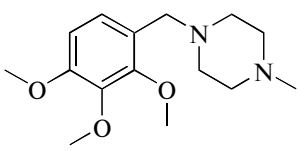
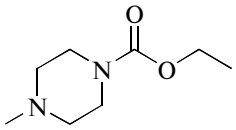
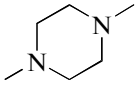
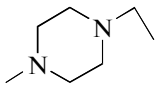
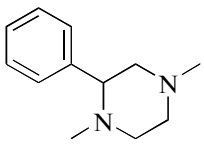
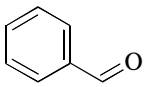
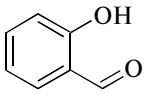
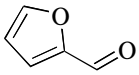
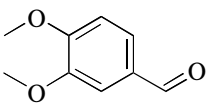
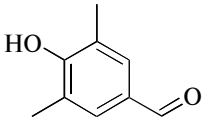
Entry	R	Gram-positive bacteria		Gram-negative bacteria		Fungal strains	
		S. aureus MTCC 96	*B. cereus* MTCC 430	*P. aeruginosa* MTCC 741	*K. pneumoniae* MTCC 109	*A. clavatus* MTCC 1323	*C. albicans* MTCC 183
(Va)	H	25	400	100	6.25	400	3.12
(Vb)	2-NO_2	400	12.5	25	100	6.25	50
(Vc)	3-Cl	12.5	100	400	200	3.12	25
(Vd)	4-Cl	100	50	3.12	25	400	6.25
(Ve)	4-CH_3	3.12	200	25	50	100	12.5
(VIIIa)		25	100	3.12	50	100	400
(VIIIb)		6.25	25	12.5	3.12	200	100
(VIIIc)		100	6.25	25	400	3.12	25
(VIIId)		3.12	200	400	100	200	6.25
(VIIIe)		200	100	50	25	3.12	400
(Xa)		3.12	100	12.5	200	400	100
(Xb)		50	200	100	3.12	50	200

Table 2. (Contd.)

Entry	R	Gram-positive bacteria		Gram-negative bacteria		Fungal strains	
		S. aureus MTCC 96	*B. cereus* MTCC 430	*P. aeruginosa* MTCC 741	*K. pneumoniae* MTCC 109	*A. clavatus* MTCC 1323	*C. albicans* MTCC 183
(Xc)		50	12.5	3.12	400	25	3.12
(Xd)		12.5	100	200	25	3.12	100
(Xe)		12.5	200	100	6.25	200	12.5
(XIIIa)	H	12.5	100	3.12	400	50	12.5
(XIIIb)	2-CH_3	400	3.12	200	100	12.5	200
(XIIIc)	2-Cl	200	25	50	100	3.12	25
(XIIId)	3-Cl	25	100	100	3.12	200	400
(XIIIe)	2-F	100	6.25	50	100	25	3.12
Ciprofloxacin†		3.125	3.125	3.125	3.125	–	–
Ketoconazole†		–	–	–	–	3.125	3.125
DMSO (control)		–	–	–	–	–	–

* MIC, minimum inhibitory concentration. † Standard.

containing substituent at ortho position of phenyl ring (**XIIIb**) whereas (**XIIIe**) analogue bearing fluoro group showed half-fold inhibitory action (6.25 μg/mL MIC) as compared to standard ciprofloxacin drug (3.12 μg/mL MIC). Compound (**XIIIa**) was found potentially active against gram-negative *P. aeruginosa* bacterial strain with MIC 3.12 μg/mL. Compound (**XIIId**) containing orthochloro phenyl ring appeared with significant inhibition of *K. pneumoniae* at lowest MIC value of 3.12 μg/mL. All the remaining analogues were observed with good to promising inhibitory effects at MIC level ranging from 25–400 μg/mL.

In vitro antifungal activity. Antifungal activity data (Table 2) showed that among the (**Va–e**) analogues, (**Vc**) bearing *meta* chloro substituted phenyl ring displayed excellent antigrowth activity at 3.12 μg/mL MIC, whereas (**Vb**) with nitro substituent at *ortho* position of phenyl ring also showed good activity at 6.25 μg/mL MIC against *A. clavatus* fungi. These two derivatives demonstrated equipotent and half-fold antigrowth activity as compared to the control drug ketoconazole (MIC 3.125 μg/mL). Analogue (**Va**) exhibited superior activity against *C. albicans* fungi with the lowest MIC value, i.e. 3.12 μg/mL. Moreover, compounds (**Vd**) exerted potential inhibitory efficiency (6.25 μg/mL) against the same strain. With 12.5 μg/mL MIC, compound (**Ve**) also appeared with good inhibition of *C. albicans* fungal strain's growth.

For (**VIIIa–e**) derivatives, (**VIIIc**) and (**VIIIe**) containing *N*-methyl and *N*-methyl-3-phenyl piperazine ring, respectively, showed equipotent antigrowth activities (3.12 μg/mL MIC) against *A. clavatus* fungi compared to control drug ketoconazole (3.125 μg/mL MIC). Derivative (**VIIId**) appeared with half-fold (6.25 μg/mL) inhibitory action against *C. albicans* fungal strain.

From bioassay data of (**Xa–e**), compound (**Xd**) was found more active analogue for the inhibition of

A. clavatus fungi at 3.12 μg/mL MIC. Compound (**Xc**) displayed strong inhibitory action against *C. albicans* fungi at 3.12 μg/mL MIC. Moreover, analogue (**Xe**) exhibited remarkable antifungal action with 12.5 μg/mL MIC against the same fungi.

In the series of (**XIIIa**–**e**) derivatives, compound (**XIIIc**) showed equal potency (3.12 μg/mL MIC) for the inhibition of *A. clavatus* fungal strain. Moreover, analogue (**XIIIb**) was found remarkably lower active, with 12.5 μg/mL MIC against the same strain. Derivative (**XIIIe**) bearing fluoro substituent appeared with superior antigrowth activity towards *C. albicans* fungi at 3.12 μg/mL MIC. Compound (**XIIIa**) showed diminished inhibitory action at 12.5 μg/mL MIC against the *C. albicans* fungal strain. The activity level of many analogues was found to increase within the scaffolds studied in the research work presented here, whereas all other derivatives appeared with fungal activity varying in the range from 25 to 400 μg/mL MIC.

DISCUSSION

The presented biological evaluation showed novel and innovative results from medicinal point of view. Among the compounds with halogen substituents, mainly chloro substituent showed more remarkable antibacterial and antifungal activity than fluoro group. Methyl group, due to the electron donating tendency, may give good efficacy whereas unsubstitution on phenyl ring lead promising results in antifungal assay. Heterocyclic aldehyde (furfuraldehyde) gave good anticipation in antibacterial and antifungal activity. *N*-ethyl piperazine showed better antibacterial efficacy than *N*-methyl piperazine, therefore one may conclude that ethyl group may improve antibacterial action faster than methyl group. However, for antifungal assay, the conclusion is reverse for the *N*-ethyl and *N*-methyl piperazine. Presumably, the steric hindrance of hydroxyl group between two methyl groups reduces the potential effect in (**Xe**) as compare to other aldehydes.

CONCLUSION

In this article, we have presented the initial efforts made toward the discovery of novel, potentially active quinazoline-linked thiourea/thiazolidinone/chalcone hybrids. Owing to the presence of three pharmacologically active nuclei, viz., quinazoline, piperazines, and thiourea/thiazolidinone/chalcone, in one single molecule, compounds executed potent antimicrobial effect. From the bioassays it is clear that the introduction of appropriate substituent in the quinazoline based ring leads to more active antimicrobial derivatives. It can be stated that the variation of antimicrobial activity may be associated with the nature of tested microorganisms and is due to the chemical structure of the tested compounds. In the present study, higher potency has been observed with the final compounds bearing chalcone bases. Compound (**Xd**) showed the equipotent efficacy towards the bacteria. Most of the compounds appeared with superior and remarkable activity. Therefore, it is concluded that there exists ample scope for further study in this class of compounds in order to discover various biological profiles such as anticancer activity or anti-HIV activity. The study is currently ongoing and the results will be published in due course.

EXPERIMENTAL

Material and Methods

All the chemicals and solvents used for the synthesis work acquired from commercial sources were of analytical grade and were used without further purification. Anthranilic acid, urea, various amines, piperazine, and aldehydes were procured from Sigma Aldrich Chemicals Pvt. Ltd., Mumbai, India. Hydrazine hydrate was purchased from Spectrochem Pvt. Ltd., Mumbai, India. 4-Amino acetophenone and TLC plates were obtained from Merck, Germany. Evaporation of solvents was carried out on a rotary evaporator under reduced pressure or using a high-vacuum pump. Melting points were determined by using open capillary tubes and are uncorrected. TLC was run on E-Merck pre-coated 60 F254 plates and the spots were rendered visible by exposing to UV light or iodine. IR spectra (ν_{max}, cm^{-1}) were recorded on BRUKER TENSOR series FT-IR and SHIMADZU HYPER IR spectrometers using KBr pellets. NMR spectra were recorded by 400 MHz BRUKER AVANCE instrument using TMS as internal standard (chemical shift in δ, ppm) and DMSO-d_6 as a solvent. Spectra were taken with a resonant frequency of 400 MHz for ^1H and 100 MHz for ^{13}C NMR. The splitting patterns are designated as follows; s, singlet; d, doublet; dd, doublet of doublets; and m, multiplet. Elemental analysis was done on "Haraeus Rapid Analyser". The mass spectra were recorded on JEOL SX-102 (EI) instrument with 60 eV ionizing energy.

Quinazoline-2,4(1*H*,3*H*)-dione (I). Anthranilic acid (10 g, 0.07 mol) and urea (43.79 g, 0.73 mol) were stirred at 160°C for 6 h. Reaction was monitored by TLC in hexane–ethyl acetate (6 : 4). After completion of the reaction, the mixture was cooled to 100°C and water was added while stirring for 5 min. The precipitate formed was filtered off and washed with water to yield a solid cake that was suspended in a solution of 0.5 N NaOH and heated to boil for 5–10 min. The mixture was cooled and pH adjusted to 2 with concentrated HCl; the solid was filtered off. After washing with water–methanol (1 : 1), the product was dried, giving white solid of (**I**). Yield 87%; mp 112–113°C [20].

2,4-Dichloroquinazoline (II). Quinazoline-2,4(1*H*,3*H*)-dione (**I**) (5 g, 0.03 mol), triethylamine (6.43 mL, 0.05 mol), and $POCl_3$ (25 mL, 0.27 mol) were refluxed for 7 h (monitored by TLC in toluene–

acetone (5 : 5). After the excess $POCl_3$ was distilled off under vacuum, crushed ice was added to the residue. Reaction mixture was then stirred for 1 h at 0–5°C. The solid product was filtered, washed with water, and dried to give yellow solid of 2,4-dichoro-quinazoline (**II**). Yield 67%; mp 116–117°C.

2-Chloro-4-(4-phenylpiperazin-1-yl)quinazoline (III). To a solution of 2,4-dichloroquinazoline (**II**) (5 g, 0.025 mol) and isopropyl alcohol (40 mL), 1-phenyl piperazine (5.29 g, 0.032 mol) was added and stirred at room temperature for 8–12 h. Reaction progress was observed by TLC in hexane–ethyl acetate (8 : 2). Washing of the crude solid with IPA and drying gives pure solid of (**III**). Yield 69%; mp 124–126°C.

2-Isothiocyanato-4-(4-phenylpiperazin-1-yl)quinazoline (IV). A solution of 2-chloro-4-(4-phenylpiperazin-1-yl)quinazoline (**III**) (2 g, 0.006 mol), ammonium thiocynate (0.93 g, 0.012 mol), and copper powder (0.75 g, 0.012 mol) in anhydrous toluene was refluxed at 90–120°C for 18 h. The completion of the reaction was monitored by TLC in ethanol–acetone (1 : 1). The reaction mixture was filtered and concentrated under reduced pressure. The resulting residue was dissolved in dichloromethane and washed with brine, and dried over Na_2SO_4. The dried solution was concentrated under reduced pressure to obtain the compound (**IV**). Yield 75%; mp 144–146°C.

Synthesis of 1-substituted phenyl-3-(4-(4-phenylpiperazin-1-yl)quinazolin-2-yl)thiourea (Va–e). A solution of 2-isothiocyanato-4-(4-phenylpiperazin-1-yl)quinazoline (**IV**) (0.01 mol) and substituted amine (0.01 mol) in dry acetone was stirred at 40–45°C for 12–18 hrs. The completion of the reaction was monitored by TLC in ethanol–acetone (1 : 1). The reaction mixture was filtered and concentrated under reduced pressure. The resulting residue was dissolved in dichloromethane, washed with brine, and dried over anhydrous Na_2SO_4. The dried solution was concentrated under reduced pressure to obtain the title compounds (**Va–e**).

*Spectral Data of Compounds (**Va–e**)*

1-Phenyl-3-(4-(4-phenylpiperazin-1-yl)quinazolin-2-yl)thiourea (Va). IR: 3234 (N–H, secondary), 3061 (C–H, aromatic), 2935 (C–H, aliphatic), 1666 (C=N), 1327 (C–N), 1234 (C=S); ^{1}H NMR: 9.78 (s, 1H, -NH linked to quinazoline ring), 8.20–7.99 (m, 2H, Ar-H), 7.82 (s, 1H, -NH linked to phenyl ring), 7.75–7.49 (m, 2H, Ar-H), 7.36–7.15 (m, 5H, Ar-H), 7.01–6.58 (m, 5H, Ar-H), 3.59–3.48 (m, 4H, -CH_2piperazine), 3.47–3.01 (m, 4H, –CH_2piperazine); ^{13}C NMR: 180.08, 169.11, 165.86, 156.23, 150.72, 138.56, 132.97, 130.65, 129.77, 128.92, 127.12, 126.89, 123.31, 122.58, 120.11, 115.46, 110.32, 49.85, 47.21; ESI-MS (M + 1): 441.66.

1-(2-Nitrophenyl)-3-(4-(4-phenylpiperazin-1-yl) quinazolin-2-yl)thiourea (Vb). IR: 3275 (N–H, secondary), 3020 (C–H, aromatic), 2944 (C–H, aliphatic), 1667 (C=N), 1445 (-NO_2), 1315 (C–N), 1278 (C=S); ^{1}H NMR: 9.14 (s, 1H), 8.19 (dd, J = 7.5 Hz, 2H), 7.94 (dd, J = 7.1, 1H), 7.78–7.52 (m, 5H), 7.40 (td, J = 7.4 Hz, 1H), 7.09 (t, J = 6.5 Hz, 2H), 6.67–6.57 (m, 3H), 3.66–3.60 (m, 4H), 3.53 (t, J = 4.8 Hz, 2H), 3.43 (t, J = 5.0 Hz, 2H); ^{13}C NMR (100 MHz, DMSO-d_6, δ ppm): 182.24, 163.30, 155.77, 151.30, 150.96, 140.41, 134.99, 134.18, 131.22, 130.18, 129.35, 125.74, 125.26, 124.62, 123.32, 122.82, 120.31, 116.77, 111.60, 49.48, 47.89; ESI-MS (M + 1): 486.24.

1-(3-Chlorophenyl)-3-(4-(4-phenylpiperazin-1-yl) quinazolin-2-yl)thiourea (Vc). IR: 3375 (N–H, secondary), 3045 (C–H, aromatic), 2924 (C–H, aliphatic), 1634 (C=N), 1342 (C–N), 1204 (C=S); ^{1}H NMR: 8.75 (s, 1H), 8.05–7.77 (m, 3H), 7.51 (td, J = 7.2 Hz, 1H), 7.38 (t, J = 4.5 Hz, 1H), 7.11 (t, J = 6.1 Hz, 1H), 7.12–7.01 (m, 4H), 6.65 (dd, J = 6.2 Hz, 3H), 3.72 (dd, J = 4.9 Hz, 3H), 3.57–3.46 (m, 6H); ^{13}C NMR: 178.91, 164.24, 156.15, 150.57, 150.21, 144.35, 132.57, 130.18, 129.64, 128.24, 125.71, 125.01, 124.24, 123.46, 120.64, 120.27, 119.66, 116.34, 111.24, 49.87, 47.24; ESI-MS (M + 1): 476.67.

1-(4-Chlorophenyl)-3-(4-(4-phenylpiperazin-1-yl) quinazolin-2-yl)thiourea (Vd). IR: 3435 (N–H, secondary), 3012 (C–H, aromatic), 2935 (C–H, aliphatic), 1667 (C=N), 1335 (C–N), 1234 (C=S); ^{1}H NMR: 8.10 (dd, J = 7.4 Hz, 1H), 9.02–7.84 (m, 2H), 7.77 (td, J = 6.8, 1H), 7.53 (td, J = 7.0, 1H), 7.35 (d, J = 6.2 Hz, 2H), 7.22 (d, J = 7.1 Hz, 2H), 7.09 (dd, J = 6.3 Hz, 2H), 6.64 (dd, J = 6.1Hz, 4H), 3.72–3.30 (m, 8H); ^{13}C NMR: 178.91, 163.30, 155.77, 151.30, 150.96, 139.93, 130.18, 129.37, 129.35, 128.20, 125.74, 125.26, 123.32, 121.95, 120.31, 116.77, 111.57, 48.54, 45.67; ESI-MS (M + 1): 476.04.

1-(4-(4-Phenylpiperazin-1-yl)quinazolin-2-yl)-3-(*p*-tolyl)thiourea (Ve). IR: 3420 (N–H, secondary), 3035 (C–H, aromatic), 2924 (C–H, aliphatic), 1615 (C=N), 1334 (C–N), 1220 (C=S); ^{1}H NMR: 9.57 (s, 1H), 8.45 (dd, J = 7.2, 1H), 7.95 (dd, J = 7.4, 1H), 7.78 (td, J = 7.3, 1H), 7.54 (t, J = 6.7, 1H), 7.21 (s, 4H), 7.08 (t, J = 7.3 Hz, 2H), 6.62 (dd, J = 7.3 Hz, 3H), 3.66–3.62 (m, 3H), 3.53–3.47 (m, 4H), 3.47–3.43 (m, 2H), 2.32 (s, 3H); ^{13}C NMR: 179.71, 162.00, 155.77, 151.30, 150.96, 137.67, 132.71, 130.30, 130.18, 129.35, 125.74, 125.26, 123.32, 120.97, 120.31, 116.77, 111.60, 50.12, 45.42, 20.24; ESI-MS (M + 1): 455.64.

2-Chloro-4-(4-ethylpiperazin-1-yl)quinazoline (VI). To a solution of 2,4-dichloroquinazoline (**II**) (5 g, 0.025 mol) and isopropyl alcohol (40 mL), 1-ethyl piperazine (3.7 g, 0.032 mol) was added and stirred at room temperature for 8–12 h. Reaction progress was observed by TLC in hexane–ethyl acetate (8 : 2). Washing of the crude solid with IPA and drying gives pure solid of (**VI**). Yield 57%; mp 134–135°C.

4-(4-Ethylpiperazin-1-yl)-2-isothiocyanatoquinazoline (VII). A solution of 2-chloro-4-(4-ethylpiperazin-1-yl)quinazoline (**VI**) (2 g, 0.007 mol), ammonium thiocynate (1.1 g, 0.014 mol), and copper powder (0.91 g, 0.014 mol) in anhydrous toluene was refluxed at 90–120°C for 18 h. The completion of the reaction was monitored by TLC in ethanol–acetone (1 : 1). The reaction mixture was filtered and concentrated under reduced pressure. The resulting residue was dissolved in dichloromethane, washed with brine, and dried over Na_2SO_4. The dried solution was concentrated under reduced pressure to obtain the compound (**VII**). Yield 77%; mp 168–169°C.

Synthesis of *N*-(4-(4-ethylpiperazin-1-yl)quinazolin-2-yl)substituted piperazine-1-carbothioamide (VIIIa–e). A solution of 4-(4-ethylpiperazin-1-yl)-2-isothiocyanatoquinazoline (**VII**) (0.01 mol) and substituted piperazine (0.01 mol) in dry acetone was stirred at 40–45°C for 12–18 h. The completion of the reaction was monitored by TLC in ethanol–acetone (1 : 1). The reaction mixture was filtered and concentrated under reduced pressure. The resulting residue was dissolved in dichloromethane, washed with brine, and dried over anhydrous Na_2SO_4. The dried solution was concentrated under reduced pressure to obtain the titled compounds (**VIIIa–e**).

Spectral Data of Compounds (VIIIa–e)

***N*-(4-(4-Ethylpiperazin-1-yl)quinazolin-2-yl)-4-(2,3,4-trimethoxybenzyl)piperazine-1-carbothioamide (VIIIa).** IR: 3301 (N–H, secondary), 3042 (C–H, aromatic), 2924 (C–H, aliphatic), 1634 (C=N), 1404 (C–N), 1267 (C=S); ^{1}H NMR: 9.45 (s, 1H), 8.12–7.54 (m, 4H), 6.92 (d, J = 7.3 Hz, 1H), 6.62 (d, J = 6.2 Hz, 1H), 4.05 (t, J = 5.4 Hz, 2H), 3.79 (s, 9H), 3.66 (dd, J = 4.9 Hz, 4H), 3.38 (t, J = 5.1 Hz, 2H), 3.33 (t, J = 5.5 Hz, 2H), 2.78 (q, J = 6.2 Hz, 2H), 2.73 (t, J = 5.5 Hz, 2H), 2.68 (t, J = 5.0 Hz, 2H), 2.59 (dd, J = 5.2 Hz, 4H), 1.07 (t, J = 6.4 Hz, 3H); ^{13}C NMR: 180.56, 163.41, 155.24, 154.01, 152.24, 151.72, 140.72, 130.18, 125.78, 125.74, 125.26, 123.84, 123.32, 111.82, 108.12, 60.65, 60.58, 56.79, 51.70, 51.15, 50.06, 48.04, 47.51, 12.34; ESI-MS (M + 1): 566.24.

Ethyl 4-((4-(4-ethylpiperazin-1-yl)quinazolin-2-yl)carbamothioyl)piperazine-1-carboxylate (VIIIb). IR: 3467 (N–H, secondary), 3024 (C–H, aromatic), 2967 (C–H, aliphatic), 1614 (C=N), 1434 (C–N), 1232 (C=S); ^{1}H NMR: 9.43 (s, 1H), 8.11 (dd, J = 7.5 Hz, 1H), 7.91 (dd, J = 7.1 Hz, 1H), 7.77 (td, J = 7.0, 1.5 Hz, 1H), 7.54 (td, J = 7.5, 1.6 Hz, 1H), 4.25–4.14 (m, 4H), 3.62 (dd, J = 5.0 Hz, 4H), 3.39 (t, J = 6.1 Hz, 2H), 3.34 (t, J = 5.3 Hz, 2H), 3.28 (t, J = 4.9 Hz, 2H), 2.75 (t, J = 5.5 Hz, 2H), 2.50 (t, J = 5.0 Hz, 2H), 2.38 (q, J = 6.3 Hz, 2H), 1.37 (t, J = 6.0 Hz, 3H), 1.07 (t, J = 6.3 Hz, 3H); ^{13}C NMR: 182.54, 164.01, 154.41, 153.57, 150.57, 130.37, 126.64, 125.14, 123.24, 113.64, 62.54, 50.56, 49.11, 48.64, 48.04, 44.57, 14.70, 12.34; ESI-MS (M + 1): 458.37.

***N*-(4-(4-Ethylpiperazin-1-yl)quinazolin-2-yl)-4-methylpiperazine-1-carbothioamide (VIIIc).** IR: 3336 (N–H, secondary), 3053 (C–H, aromatic), 2926 (C–H, aliphatic), 1670 (C=N), 1406 (C–N), 1298 (C=S); ^{1}H NMR: 9.57 (s, 1H, -NH linked to quinazoline ring), 8.09–7.51 (m, 4H, Ar-H), 3.98 (m, 4H, -CH_2 N-Me piperazine), 3.34(m, 4H, -CH_2 N-Et piperazine), 2.98 (m, 4H, -CH_2 N-Et piperazine), 2.74 (m, 4H, -CH_2 N-Me piperazine), 2.40 (q, 2H, -CH_2), 2.19 (s, 3H, -CH_3), 1.03 (t, 3H, CH_3); ^{13}C NMR: 180.31, 168.97, 163.12, 155.89, 132.58, 127.44, 126.21, 123.12, 110.63, 53.84, 52.31, 50.58, 49.06, 47.29, 46.12, 13.83; ESI-MS (M + 1): 400.30.

4-Ethyl-*N*-(4-(4-ethylpiperazin-1-yl)quinazolin-2-yl) piperazine-1-carbothioamide (VIIId). IR: 3367 (N–H, secondary), 3015 (C–H, aromatic), 2938 (C–H, aliphatic), 1615 (C=N), 1438 (C–N), 1211 (C=S); ^{1}H NMR: 9.57 (s, 1H), 8.13 (dd, J = 7.4, 1H), 7.93 (dd, J = 7.5 Hz, 1H), 7.77 (td, J = 7.1 Hz, 1H), 7.54 (td, J = 7.3 Hz, 1H), 4.04 (t, J = 5.4 Hz, 2H), 3.65 (t, J = 5.1 Hz, 2H), 3.31 (t, J = 6.0 Hz, 2H), 3.24 (t, J = 6.0 Hz, 2H), 2.75 (dt, J = 5.5 Hz, 4H), 2.53 (dt, J = 5.4 Hz, 4H), 2.39 (dq, J = 6.3 Hz, 4H), 1.07 (td, J = 6.4 Hz, 6H); ^{13}C NMR: 183.56, 162.41, 154.24, 153.24, 132.18, 127.74, 126.26, 124.32, 110.82, 51.47, 51.04, 50.11, 48.24, 47.68, 12.18; ESI-MS (M + 1): 414.25.

***N*-(4-(4-Ethylpiperazin-1-yl)quinazolin-2-yl)-4-methyl-3-phenylpiperazine-1-carbothioamide (VIIIe).** IR: 3424 (N–H, secondary), 3038 (C–H, aromatic), 2924 (C–H, aliphatic), 1634 (C=N), 1426 (C–N), 1228 (C=S); ^{1}H NMR: 9.50 (s, 1H), 8.12 (dd, J = 7.51H), 7.96 (dd, J = 7.2 Hz, 1H), 7.80 (td, J = 7.1 Hz, 1H), 7.57 (d, J = 7.0, 1H), 7.34–7.01 (m, 5H), 4.51 (dd, J = 6.9 Hz, 1H), 4.02 (dt, J = 4.9 Hz, 1H), 3.71–3.57 (m, 2H), 3.52 (dt, J = 5.1 Hz, 1H), 3.39 (t, J = 5.7 Hz, 2H), 3.30 (t, J = 4.9 Hz, 2H), 2.88–2.76 (m, 5H), 2.66–2.54 (m, 3H), 2.29 (s, 3H), 1.09 (t, J = 6.3 Hz, 3H); ^{13}C NMR: 184.12, 166.40, 155.24, 152.24, 141.20, 130.18, 129.63, 128.43, 125.74, 125.51, 125.26, 123.32, 111.82, 66.78, 51.12, 50.89, 50.75, 50.24, 48.10, 47.34, 44.14, 13.5\; ESI-MS (M + 1): 476.14.

1-(4-((4-(4-Ethylpiperazin-1-yl)quinazolin-2-yl)amino)phenyl)ethanone (IX). 2-Chloro-4-(4-ethylpiperazin-1-yl)quinazoline (**VI**) (2 g, 0.007 mol) was refluxed with 4-amino acetophenone (1.05 g, 0.00781 mol) in IPA (20 mL) for 7–8 h. Reaction was monitored by TLC in hexane–ethyl acetate (3 : 2). After completion, crude solid obtained was filtered and

washed with IPA to give solid of desire compound (**IX**). Yield 68%; mp 126–128°C; IR: 3465 (N–H, secondary), 3033 (C–H, aromatic), 2984 (C–H, aliphatic), 1645 (C=O), 1545(–CH=CH–); ^{1}H NMR: 8.11 (dd, J = 7.5, 1.4 Hz, 1H), 7.97 (dd, J = 7.5, 1.4 Hz, 1H), 7.84–7.75 (m, 3H), 7.55 (td, J = 7.4, 1.5 Hz, 1H), 7.11 (d, J = 7.5 Hz, 2H), 5.95 (s, 1H), 3.36 (dt, J = 24.0, 4.9 Hz, 4H), 2.82 (t, J = 4.9 Hz, 2H), 2.65 (t, J = 4.9 Hz, 2H), 2.51 (s, 3H), 2.42 (q, J = 6.3 Hz, 2H), 1.07 (t, J = 6.3 Hz, 3H); ^{13}C NMR: 194.25, 163.86, 156.14, 155.32, 151.62, 143.93, 141.56, 131.63, 130.18, 129.82, 129.04, 128.73, 125.74, 125.26, 124.17, 123.32, 123.15, 117.54, 107.61, 52.82, 50.24, 47.35, 16.22, 13.78; ESI-MS (M + 1): 376.83.

1-(4-((4-(4-Ethylpiperazin-1-yl)quinazolin-2-yl)amino) phenyl)-3-substituted phenylprop-2-en-1-one (Xa–e). 1-(4-((4-(4-Ethylpiperazin-1-yl)quinazolin-2-yl)amino)phenyl) ethanone (**IX**), 0.01 mol, and 0.013 mol of a substituted aldehyde were slowly added in 60 mL of MeOH. 10% 1 N NaOH was then added, and the reaction was stirred for 12–20 h at room temperature. Progress of reaction was followed by TLC in MeOH–acetone (3 : 2). After completion, the pH of the solution was adjusted to 2 with HCl solution. The precipitate thus obtained was filtered off, washed with water, and recrystallized from boiling EtOH to get respective compounds (**Xa–e**).

*Spectral Data of Compounds (**Xa–e**)*

(E)-1-(4-((4-(4-Ethylpiperazin-1-yl)quinazolin-2-yl)amino)phenyl)-3-phenylprop-2-en-1-one (Xa). IR: 3464 (N–H, secondary), 3024 (C–H, aromatic), 2961 (C–H, aliphatic), 1663 (C=O), 1628 (C=N), 1534 (–CH=CH–); ^{1}H NMR: 8.05 (dd, J = 7.51H), 7.94 (dd, J = 7.6 Hz, 1H), 7.79–7.66 (m, 3H), 7.56 (td, J = 7.4 Hz, 1H), 7.28–6.84 (m, 8H), 6.27 (d, J = 6.2 Hz, 1H), 3.28 (dt, J = 5.1 Hz, 4H), 2.77 (q, J = 6.4 Hz, 2H), 2.67 (t, J = 5.1 Hz, 2H), 2.58 (t, J = 5.0 Hz, 2H), 1.07 (t, J = 6.4 Hz, 3H), 1.68 (s, 1H); ^{13}C NMR: 192.28, 163.34, 156.57, 152.84, 144.36, 141.56, 135.88, 131.63, 130.18, 129.46, 129.02, 128.73, 128.06, 125.74, 125.26, 123.32, 122.95, 117.54, 110.11, 51.44, 50.48, 48.21, 12.54; ESI-MS (M + 1): 464.25.

(E)-1-(4-((4-(4-Ethylpiperazin-1-yl)quinazolin-2-yl) amino)phenyl)-3-(2-hydroxyphenyl)prop-2-en-1-one (Xb). IR: 3545 (-OH), 3482 (N–H, secondary), 3022 (C–H, aromatic), 2915 (C–H, aliphatic), 1667 (C=O), 1625 (C=N), 1523 (–CH=CH–); ^{1}H NMR: 8.61 (s, 1H), 8.03 (dd, J = 7.4 Hz, 1H), 7.92–7.82 (m, 2H), 7.80–7.71 (m, 3H), 7.51 (d, J = 7.1 Hz, 1H), 7.29 (d, J = 5.2 Hz, 1H), 7.22–7.06 (m, 3H), 7.22–5.54 (m, 7H), 6.73 (dd, J = 7.4 Hz, 1H), 6.76–5.54 (m, 2H), 5.76 (s, 1H), 3.35 (d, J = 5.0 Hz, 4H), 2.73 (t, J = 5.4 Hz, 2H), 2.50 (t, J = 5.0 Hz, 2H), 2.20 (q, J = 6.3 Hz, 2H), 0.98 (t, J = 6.3 Hz, 3H); ^{13}C NMR: 192.57, 163.84, 158.31, 156.75, 153.35, 141.56, 140.05, 131.63, 131.33, 130.18, 128.73, 128.71, 125.74, 125.26, 123.32, 121.83, 120.64, 117.76, 117.54, 108.34, 52.66, 51.05, 48.28, 12.10; ESI-MS (M + 1): 480.12.

(E)-1-(4-((4-(4-Ethylpiperazin-1-yl)quinazolin-2-yl)amino)phenyl)-3-(furan-2-yl)prop-2-en-1-one (Xc). IR: 3435 (N–H, secondary), 3057 (C–H, aromatic), 2934 (C–H, aliphatic), 1621 (C=O), 1611 (C=N), 1527 (–CH=CH–); ^{1}H NMR: 8.09 (dd, J = 7.5 Hz, 1H), 7.99–7.89 (m, 2H), 7.85 (dd, J = 7.1 Hz, 1H), 7.82–7.72 (m, 3H), 7.61 (d, J = 7.2 Hz, 1H), 7.41 (d, J = 5.0 Hz, 1H), 7.05 (d, J = 7.2 Hz, 2H), 6.99 (dd, J = 7.1 Hz, 1H), 6.63 (t, J = 7.6 Hz, 1H), 3.42–3.36 (m, 2H), 3.32–3.26 (m, 2H), 2.81–2.74 (m, 2H), 2.56–2.50 (m, 2H), 2.40 (q, J = 6.3 Hz, 2H), 1.06 (t, J = 6.3 Hz, 3H); ^{13}C NMR: 193.26, 165.61, 155.82, 151.56, 149.16, 143.04, 141.56, 131.63, 130.18, 128.73, 125.75, 125.26, 123.32, 120.17, 117.54, 112.57, 112.46, 108.25, 52.64, 51.75, 47.51, 13.25; ESI-MS (M + 1): 454.68.

(E)-3-(3,4-Dimethoxyphenyl)-1-(4-((4-(4-ethylpiperazin-1-yl)quinazolin-2-yl)amino)phenyl)prop-2-en-1-one (Xd). IR: 3325 (N–H, secondary), 3055 (C–H, aromatic), 2931 (C–H, aliphatic), 1674 (C=O), 1620 (C=N), 1599 (–CH=CH–), 1300 (C–O–C, aryl), 1041 (O–CH_3); ^{1}H NMR: 8.02 (d, 1H, –CH=CH–), 7.89–7.75 (m, 4H, Ar–H), 7.65–7.48 (m, 2H, Ar-H), 7.42 (d, 1H, –CH=CH–), 7.12–6.87 (m, 5H, Ar-H), 5.19 (s, 1H, –NH), 3.86 (s, 6H, –OCH_3), 3.37 (m, 4H, –CH_2 piperazine), 2.65 (m, 4H, –CH_2 piperazine), 2.39 (q, 2H, –CH_2), 1.11 (t, 3H, –CH_3); ^{13}C NMR: 190.21, 165.76, 162.80, 154.44, 152.32, 150.28, 143.78, 140.55, 135.12, 134.86, 133.64, 132.46, 128.74, 126.88, 125.11, 122.24, 120.68, 115.26, 114.27, 113.34, 112.54, 106.08, 39.99, 39.78, 39.16, 38.74, 13.56; ESI-MS (M + 1): 524.38.

(E)-1-(4-((4-(4-Ethylpiperazin-1-yl)quinazolin-2-yl)amino)phenyl)-3-(4-hydroxy-3,5-dimethylphenyl)prop-2-en-1-one (Xe). IR: 3546 (–OH), 3425 (N–H, secondary), 3085 (C–H, aromatic), 2924 (C–H, aliphatic), 1638 (C=O), 1633 (C=N), 1529 (–CH=CH–); ^{1}H NMR: 8.64 (s, 1H), 8.08 (dd, J = 7.4 Hz, 1H), 8.03–7.92 (m, 2H), 7.80–7.71 (m, 3H), 7.52 (td, J = 7.4 Hz, 1H), 7.38 (d, J = 5.2 Hz, 1H), 7.15 (d, J = 7.5 Hz, 2H), 6.99 (s, 2H), 5.74 (s, 1H), 3.37 (dt, J = 5.1 Hz, 4H), 2.77 (t, J = 5.8 Hz, 2H), 2.56–2.43 (m, 4H), 2.31 (s, 6H), 1.08 (t, J = 6.3 Hz, 3H); ^{13}C NMR: 194.25, 163.86, 156.14, 155.32, 151.62, 143.93, 141.56, 131.63, 130.18, 129.82, 129.04, 128.73, 125.74, 125.26, 124.17, 123.32, 123.15, 117.54, 107.61, 52.82, 50.24, 47.35, 16.22, 13.78; ESI-MS (M + 1): 508.83.

4-(4-Ethylpiperazin-1-yl)-2-hydrazinylquinazoline (XI). 2-Chloro-4-(4-ethylpiperazin-1-yl)quinazoline (**VI**) (2 g, 0.007 mol) was refluxed with hydrazine hydrate (0.39 g, 0.00781 mol) in IPA (20 mL) for 7–8 h. Reaction was monitored by TLC in hexane–ethyl

acetate (3 : 2). After completion, crude solid obtained was filtered and washed with IPA to give solid of desiredcompound (**XI**). Yield 74%; mp 112–113°C; IR: 3318 (N–H, secondary), 3012 (C–H, aromatic), 2929 (C–H, aliphatic), 1644 (C=N); ^{1}H NMR: 8.03 (dd, J = 7.5, 1.6 Hz, 1H), 7.91 (dd, J = 7.4, 1.5 Hz, 1H), 7.76 (td, J = 7.5, 1.4 Hz, 1H), 7.53 (td, J = 7.5, 1.4 Hz, 1H), 3.38 (t, J = 5.1 Hz, 2H), 3.32 (t, J = 5.0 Hz, 2H), 3.06 (s, 1H), 2.96 (s, 1H), 2.73 (t, J = 5.1 Hz, 2H), 2.51 (t, J = 5.1 Hz, 2H), 2.45 (s, 1H), 2.36 (q, J = 6.3 Hz, 2H), 1.06 (t, J = 6.3 Hz, 3H); ^{13}C NMR: 163.04, 150.35, 144.47, 130.18, 125.74, 125.26, 123.32, 114.17, 51.70, 51.70, 50.06, 48.04, 48.04, 12.34; ESI-MS (M + 1): 273.35.

Synthesis of 2-(4-(4-Ethylpiperazin-1-yl)quinazolin-2-yl)-N-Substituted Phenyl Hydrazine Carbothioamide (XIIa–e)

A solution of 0.01 mol of 4-(4-ethylpiperazin-1-yl)-2-hydrazinylquinazoline (**XI**) and equimolar amount of substituted phenyl isothiocyanate in 60 mL of EtOH was heated under reflux for 1–2 h. Reaction was followed by TLC inhexane–ethyl acetate (3 : 2) mobile phase. The precipitate obtained was filtered off, washed with water, and recrystallized from ethanol to get desired compounds (**XIIa–e**).

2-(4-(4-Ethylpiperazin-1-yl)quinazolin-2-yl)-*N*-phenylhydrazinecarbothioamide (XIIa). Yield 68%; mp 134–136°C; IR: 3456 (N–H, secondary), 3027 (C–H, aromatic), 2935 (C–H, aliphatic), 1632 (C=N), 1142 (C=S); ^{1}H NMR: 9.79 (s, 1H), 8.00 (s, 1H), 7.85 (s, 1H), 7.81 (s, 1H), 7.71 (s, 1H), 7.48 (s, 1H), 7.36–7.26 (m, 4H), 7.07 (s, 1H), 3.92 (s, 1H), 3.34–3.26 (m, 4H), 2.70–2.66 (m, 2H), 2.31–2.27 (m, 2H), 2.16–2.04 (m, 2H), 1.08–1.04 (m, 3H); ^{13}C NMR: 179.72, 163.81, 150.37, 149.15, 139.19, 130.18, 128.96, 128.96, 125.74, 125.26, 124.47, 123.32, 121.54, 121.54, 113.98, 51.70, 51.70, 50.06, 48.04, 48.04, 12.34; ESI-MS (M + 1): 408.56.

2-(4-(4-Ethylpiperazin-1-yl)quinazolin-2-yl)-*N*-(o-tolyl)hydrazinecarbothioamide (XIIb). Yield 77%; mp 126–128°C; IR: 3547 (N–H, secondary), 3021 (C–H, aromatic), 2838 (C–H, aliphatic), 1621 (C=N), 1101 (C=S); ^{1}H NMR: 8.02 (dd, J = 7.4, 1.5 Hz, 1H), 7.87 (dd, J = 7.5, 1.4 Hz, 1H), 7.72 (td, J = 7.5, 1.4 Hz, 1H), 7.49 (td, J = 7.5, 1.4 Hz, 1H), 7.31–7.12 (m, 3H), 7.10–6.99 (m, 1H), 5.36 (s, 1H), 3.40 (t, J = 5.2 Hz, 2H), 3.33 (t, J = 5.2 Hz, 2H), 2.85 (s, 1H), 2.73 (t, J = 5.2 Hz, 2H), 2.48 (t, J = 5.1 Hz, 2H), 2.34 (q, J = 6.3 Hz, 2H), 2.24 (s, 3H), 1.06 (t, J = 6.3 Hz, 3H); ^{13}C NMR: 181.16, 163.81, 150.37, 149.15, 138.00, 132.78, 130.18, 129.94, 127.79, 125.74, 125.26, 124.65, 123.32, 123.10, 113.98, 51.70, 51.70, 50.06, 48.04, 48.04, 17.35, 12.34; ESI-MS (M + 1): 422.34.

***N*-(2-Chlorophenyl)-2-(4-(4-ethylpiperazin-1-yl)quinazolin-2-yl)hydrazinecarbothioamide (XIIc).** Yield 69%; mp 144–148°C; IR: 3354 (N–H, secondary), 3011 (C–H, aromatic), 2924 (C–H, aliphatic), 1645 (C=N), 1125 (C=S); ^{1}H NMR: 8.01 (dd, J = 7.5, 1.4 Hz, 1H), 7.93 (ddd, J = 74.5, 7.5, 1.4 Hz, 2H), 8.78–7.51 (m, 3H), 8.78–7.32 (m, 4H), 8.78–7.24 (m, 6H), 8.78–7.06 (m, 7H), 7.02 (td, J = 7.5, 1.5 Hz, 1H), 5.36 (s, 1H), 3.39 (t, J = 5.1 Hz, 2H), 3.32 (t, J = 5.1 Hz, 2H), 2.87 (s, 1H), 2.74 (t, J = 5.1 Hz, 2H), 2.50 (t, J = 5.1 Hz, 2H), 2.37 (q, J = 6.3 Hz, 2H), 1.06 (t, J = 6.3 Hz, 3H); ^{13}C NMR: 181.16, 163.81, 150.37, 149.15, 134.74, 130.18, 129.75, 129.38, 127.75, 125.74, 125.44, 125.26, 125.09, 123.32, 113.98, 51.70, 51.70, 50.06, 48.04, 48.04, 12.34; ESI-MS (M + 1): 442.87.

***N*-(3-Chlorophenyl)-2-(4-(4-ethylpiperazin-1-yl)quinazolin-2-yl)hydrazinecarbothioamide (XIId).** Yield 82%; mp 136–139°C; IR: 3312 (N–H, secondary), 3042 (C–H, aromatic), 2922 (C–H, aliphatic), 1678 (C=N), 1158 (C=S); ^{1}H NMR: 9.28 (s, 1H), 9.14 (s, 1H), 7.99 (dd, J = 7.5, 1.4 Hz, 1H), 7.86 (dd, J = 7.5, 1.4 Hz, 1H), 7.70 (td, J = 7.5, 1.4 Hz, 1H), 7.47 (td, J = 7.5, 1.5 Hz, 1H), 7.27–7.20 (m, 2H), 7.17 (dt, J = 7.3, 1.4 Hz, 1H), 7.10 (dt, J = 7.3, 1.5 Hz, 1H), 5.49 (s, 1H), 3.37 (dt, J = 26.4, 5.0 Hz, 4H), 2.76 (t, J = 5.1 Hz, 2H), 2.51 (t, J = 5.1 Hz, 2H), 2.40 (q, J = 6.3 Hz, 2H), 1.06 (t, J = 6.3 Hz, 3H); ^{13}C NMR: 179.72, 163.81, 150.37, 149.15, 140.58, 133.11, 130.18, 128.83, 125.74, 125.26, 124.05, 123.32, 120.61, 119.66, 113.98, 51.70, 51.70, 50.06, 48.04, 48.04, 12.34; ESI-MS (M + 1): 442.45.

2-(4-(4-Ethylpiperazin-1-yl)quinazolin-2-yl)-*N*-(2-fluorophenyl)hydrazinecarbothioamide (XIIe). Yield 75%; mp 188–190°C; IR: 3524 (N–H, secondary), 3112 (C–H, aromatic), 2850 (C–H, aliphatic), 1645 (C=N), 1164 (C=S); ^{1}H NMR: 9.39 (s, 2H), 7.99 (dd, J = 7.5, 1.4 Hz, 1H), 7.86 (dd, J = 7.5, 1.4 Hz, 1H), 7.71 (td, J = 7.5, 1.4 Hz, 1H), 7.48 (td, J = 7.4, 1.5 Hz, 1H), 7.44–7.21 (m, 1H), 7.16–6.90 (m, 3H), 5.47 (s, 1H), 3.39 (t, J = 5.1 Hz, 2H), 3.33 (t, J = 5.1 Hz, 2H), 2.74 (t, J = 5.0 Hz, 2H), 2.51 (t, J = 5.1 Hz, 2H), 2.36 (q, J = 6.3 Hz, 2H), 1.07 (t, J = 6.3 Hz, 3H); ^{13}C NMR: 181.16, 163.81, 158.99, 150.37, 149.15, 130.18, 127.72, 125.74, 125.72, 125.26, 124.40, 123.94, 123.32, 117.15, 113.98, 51.70, 51.70, 50.06, 48.04, 48.04, 12.34; ESI-MS (M + 1): 426.02.

Synthesis of 2-(2-(4-(4-Ethylpiperazin-1-yl)quinazolin-2-yl)hydrazono)-3-Substituted Phenylthiazolidin-4-one (XIIIa–e)

An aliquot (0.01 mol) of appropriate thiosemicarbazide (**XIIa–e**) and 0.011 mol of chloroethyl acetate were refluxed in 30 mL of absolute EtOH in the presence of 0.04 mol of anhydrous NaOAc for 2 h. Reaction progress was observed by TLC in toluene–acetone (4 : 1). The mixture was then cooled, diluted with water, and allowed to stand overnight. The solid precip-

itated was washed with water, dried, and recrystallized from ethanol to afford final compounds (**XIIIa–e**).

2-(2-(4-(4-Ethylpiperazin-1-yl)quinazolin-2-yl)hydrazono)-3-phenylthiazolidin-4-one (XIIIa). IR: 3298 (N–H, secondary), 3030 (C–H, aromatic), 2931 (C–H, aliphatic), 1730 (C=O), 1672 (C=N), 1305 (C–N), 646 (C–S); ^{1}H NMR: 11.26 (s, 1H, –NH), 7.95 (dd, 1H, Ar-CH),7.68 (d, 2H, Ar-CH), 7.62 (d, 2H, Ar-CH), 7.22 (d, 1H, –CH quinoline), 7.20 (d, 1H, -CH quinoline), 6.99 (dd, 1H, –CH quinoline), 6.95 (d, 1H, -CH quinoline), 4.13 (s, 2H, $-CH_2$ thiazolidinone), 3.52 (t, 4H, $-CH_2$ piperazine), 3.48 (t, 4H, $-CH_2$ piperazine), 1.94 (q, 2H, $-CH_2$ ethyl), 1.22 (t, 3H, $-CH_3$ ethyl); ^{13}C NMR: 172.13, 165.40, 162.82, 155.65, 150.30, 141.16, 140.81, 134.87, 128.92, 126.90, 122.26, 120.96, 116.75, 114.29, 40.01, 39.80, 39.38, 38.76, 12.98; ESI-MS (M + 1): 448.23.

2-(2-(4-(4-Ethylpiperazin-1-yl)quinazolin-2-yl)hydrazono)-3-(*o*-tolyl)thiazolidin-4-one (XIIIb). IR: 3274 (N–H, secondary), 3000 (C–H, aromatic), 2955 (C–H, aliphatic), 1762 (C=O), 1671 (C=N), 1334 (C–N), 647 (C–S); ^{1}H NMR: 9.89 (s, 1H), 7.92 (td, J = 7.7 Hz, 2H), 7.75 (d, J = 7.4 Hz, 1H), 7.53 (d, J = 7.1 Hz, 1H), 7.34–6.47 (m, 4H), 3.89 (s, 2H), 3.32 (t, J = 5.2 Hz, 2H), 3.13 (t, J = 5.1 Hz, 2H), 2.76 (t, J = 5.2 Hz, 2H), 2.55 (t, J = 5.2 Hz, 2H), 2.43 (q, J = 6.3 Hz, 2H), 2.20 (s, 3H), 1.07 (t, J = 6.3 Hz, 3H); ^{13}C NMR: 173.51, 164.21, 150.15, 149.81, 139.55, 136.33, 136.30, 130.96, 130.18, 129.38, 129.37, 128.87, 125.74, 125.26, 123.32, 114.83, 52.54, 50.11, 47.87, 31.04, 17.18, 13.11; ESI-MS (M + 1): 462.34.

3-(2-Chlorophenyl)-2-(2-(4-(4-ethylpiperazin-1-yl)quinazolin-2-yl)hydrazono)thiazolidin-4-one (XIIIc). IR: 3290 (N–H, secondary), 3031 (C–H, aromatic), 2971 (C–H, aliphatic), 1733 (C=O), 1635 (C=N), 1368 (C–N), 640 (C–S); ^{1}H NMR: 8.03 (dd, J = 7.1 Hz, 1H), 7.95 (dd, J = 7.4 Hz, 1H), 7.78 (d, J = 6.5 Hz, 1H), 7.56 (d, J = 7.6 Hz, 1H), 7.46 (dd, J = 7.5 Hz, 1H), 7.39 (dd, J = 7.4 Hz, 1H), 7.22 (d, J = 6.3 Hz, 1H), 7.09 (d, J = 7.5, Hz, 1H), 3.86 (s, 2H), 3.28 (dd, J = 5.2 Hz, 4H), 2.75 (t, J = 5.1 Hz, 2H), 2.50 (t, J = 5.2 Hz, 2H), 2.38 (q, J = 6.3 Hz, 2H), 1.07 (t, J = 6.3 Hz, 3H); ^{13}C NMR: 175.15, 168.64, 152.86, 147.3, 139.55, 136.39, 134.64, 131.26, 130.76, 130.18, 129.79, 129.27, 125.74, 125.26, 123.32, 112.83, 53.75, 50.06, 47.22, 33.57, 14.25; ESI-MS (M + 1): 483.28.

3-(3-Chlorophenyl)-2-(2-(4-(4-ethylpiperazin-1-yl) quinazolin-2-yl)hydrazono)thiazolidin-4-one (XIIId). IR: 3254 (N–H, secondary), 3082 (C–H, aromatic), 2905 (C–H, aliphatic), 1782 (C=O), 1667(C=N), 1373 (C–N), 624 (C–S); ^{1}H NMR: 8.11 (dd, J = 7.5 Hz, 1H), 7.98 (dd, J = 7.4 Hz, 1H), 7.80 (td, J = 6.5 Hz, 1H), 7.59–7.50 (m, 2H), 7.40–7.30 (m, 2H), 7.24–6.98 (m, 1H), 3.90 (s, 2H), 3.37 (d, J = 5.1 Hz, 4H), 2.80 (q, J = 6.4 Hz, 2H), 2.71 (t, J = 5.1 Hz, 2H), 2.60 (t, J = 5.1 Hz, 2H), 1.07 (t, J = 6.4 Hz, 3H); ^{13}C NMR (100 MHz, DMSO-d_6, δ ppm): 174.54, 165.34, 155.27, 144.91, 141.37, 139.18, 132.09, 130.18, 129.29, 127.18, 126.64, 125.74, 125.26, 124.88, 123.32, 116.83, 52.82, 51.67, 49.35, 32.47, 14.35; ESI-MS (M + 1): 483.38.

2-(2-(4-(4-Ethylpiperazin-1-yl)quinazolin-2-yl)hydrazono)-3-(2-fluorophenyl)thiazolidin-4-one (XIIIe). IR: 3354 (N–H, secondary), 3112 (C–H, aromatic), 2950 (C–H, aliphatic), 1710 (C=O), 1661(C=N), 1312 (C–N), 652 (C–S); ^{1}H NMR: 8.14 (dd, J = 7.1 Hz, 1H), 7.97 (dd, J = 7.4 Hz, 1H), 7.71 (d, J = 6.5 Hz, 1H), 7.25 (d, J = 7.6 Hz, 1H), 7.2 (dd, J = 7.5 Hz, 1H), 7.02 (dd, J = 7.4 Hz, 1H), 6.82 (d, J = 6.3 Hz, 1H), 6.79 (d, J = 7.5, Hz, 1H), 3.75 (s, 2H), 3.42 (dd, J = 5.2 Hz, 4H), 2.22 (t, J = 5.1 Hz, 2H), 2.17 (t, J = 5.2 Hz, 2H), 2.11 (q, J = 6.3 Hz, 2H), 1.02 (t, J = 6.3 Hz, 3H); ^{13}C NMR: 175.28, 161.76, 160.34, 156.28, 150.64, 137.35, 131.35, 130.13, 128.94, 126.81, 126.22, 125.74, 125.26, 123.32, 119.49, 111.83, 52.57, 51.38, 49.92, 30.34, 13.55; ESI-MS (M + 1): 466.28.

Biological Screening

Antimicrobial assay. To determine the minimum inhibitory concentration [21], a stock solution of the final synthesized compounds (100 μg/mL) was prepared in dimethyl sulfoxide, and then test compounds were incorporated in a specified quantity of molten sterile agar, i.e., nutrient agar and dextrose agar for antibacterial and for antifungal screening, respectively. Such medium enclosing the test compound 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 10^5 CFU/mL was prepared and added to plates with serially diluted compounds with concentrations in the range of 3.12–100 μg/mL in dimethyl sulfoxide and incubated at (37 ± 1)°C temperature for 24 h (bacteria) or 48 h (fungi). Minimum concentration of the substance that prevents the development of visible growth is considered to be the MIC value.

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