Med Chem Res (2013) 22:225–233
DOI 10.1007/s00044-012-0004-3

MEDICINAL CHEMISTRY RESEARCH

ORIGINAL RESEARCH

Synthesis, characterization, and pharmacological evaluation of 1-[2-(6-nitro-4-oxo-2-phenyl-4*H*-quinazolin-3-yl)-ethyl]-3-phenyl ureas

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Received: 12 December 2011 / Accepted: 24 February 2012 / Published online: 23 March 2012
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Abstract In the present communication, a series based on 1-[2-(6-nitro-4-oxo-2-phenyl-4*H*-quinazolin-3-yl)-ethyl]-3-phenyl-urea have been synthesized by an efficient synthetic protocol. The synthesized compounds were characterized by IR, ^{1}H NMR, ^{13}C NMR spectroscopy, ESI Mass spectrometry, and elemental analysis. The antibacterial and antifungal activity of the compounds were studied against selected strains (*Pseudomonas aeruginosa* ATCC 27853, *Escherichia coli* ATCC 25922, *Staphylococcus aureus* ATCC 25923, *Bacillus subtilis* ATCC 11774, and *Candida albicans* ATCC 66027) by using Kirby Bauer disk diffusion technique and broth dilution technique. All the synthesized compounds showed good antifungal potency against strain *C. albicans*.

Keywords Quinazolin-4(3*H*)-one · Phenyl isocyanates · Antimicrobial activity

Introduction

Design and synthesis of potent antibacterial agents is still the target by many researchers because the treatment of bacterial infection remains a challenging goal due to emerging infectious diseases and increasing number of multidrug resistant microbial pathogens. So, there is an urgent need to develop new antibacterial agents that remains unaffected by existing resistance mechanisms of multidrug resistant bacteria.

In the present communication, synthesis of 6-nitro-2-phenylquinazoline-4(3*H*)-ones and ureido unit (–NH–CO–NH) incorporating structures have been reported on the premises that quinazoline-4-one is a biologically active scaffold bearing diverse bioactivities like antimicrobial, antifungal, anticancer, anti-inflammatory, anticonvulsant, antituberculosis, antihypertensive, antitumor, anti ulcer, proliferative, di hydro folate reductase (DHFR) inhibitor and epidermal growth factor receptor inhibitor (EGFR). (Sharma *et al*., 2011; Pereira *et al*., 2007; Tiwari *et al*., 2006; Raghavendra *et al*., 2007; Kumar *et al*., 2007; Pandey and Bajpai, 2004; Casenghi *et al*., 2004; Al-Rashood *et al*., 2006; Wood *et al*., 2004; Sielecki *et al*., 2001). Moreover, acyclic and cyclic ureas are often biologically active and nontoxic compounds because the ureido (–NH–CO–NH–) unit is a pseudo dipeptide motif, moreover it has been proved that urea linkage (*N*-acetamide derivative) with carbon chain (as a spacer group) represents them better antagonists of a bacterial quorum sensing, and hence they act as potent antibacterial agents (Frezza *et al*., 2006).

Based on above observations and in a view of significant biological properties, a new series of compounds have been synthesized accommodating quinazoline-4(3*H*)-one and ureido linkage in a single molecular frame and screened for antibacterial and antifungal activity.

Results and discussion

Chemistry

Quinazoline-4-one-based derivatives were prepared by a series of reaction as illustrated in Scheme 1. The characteristic cyclization cum condensation reaction of 2-aminobenzoic acid with benzoyl chloride is reported in literature

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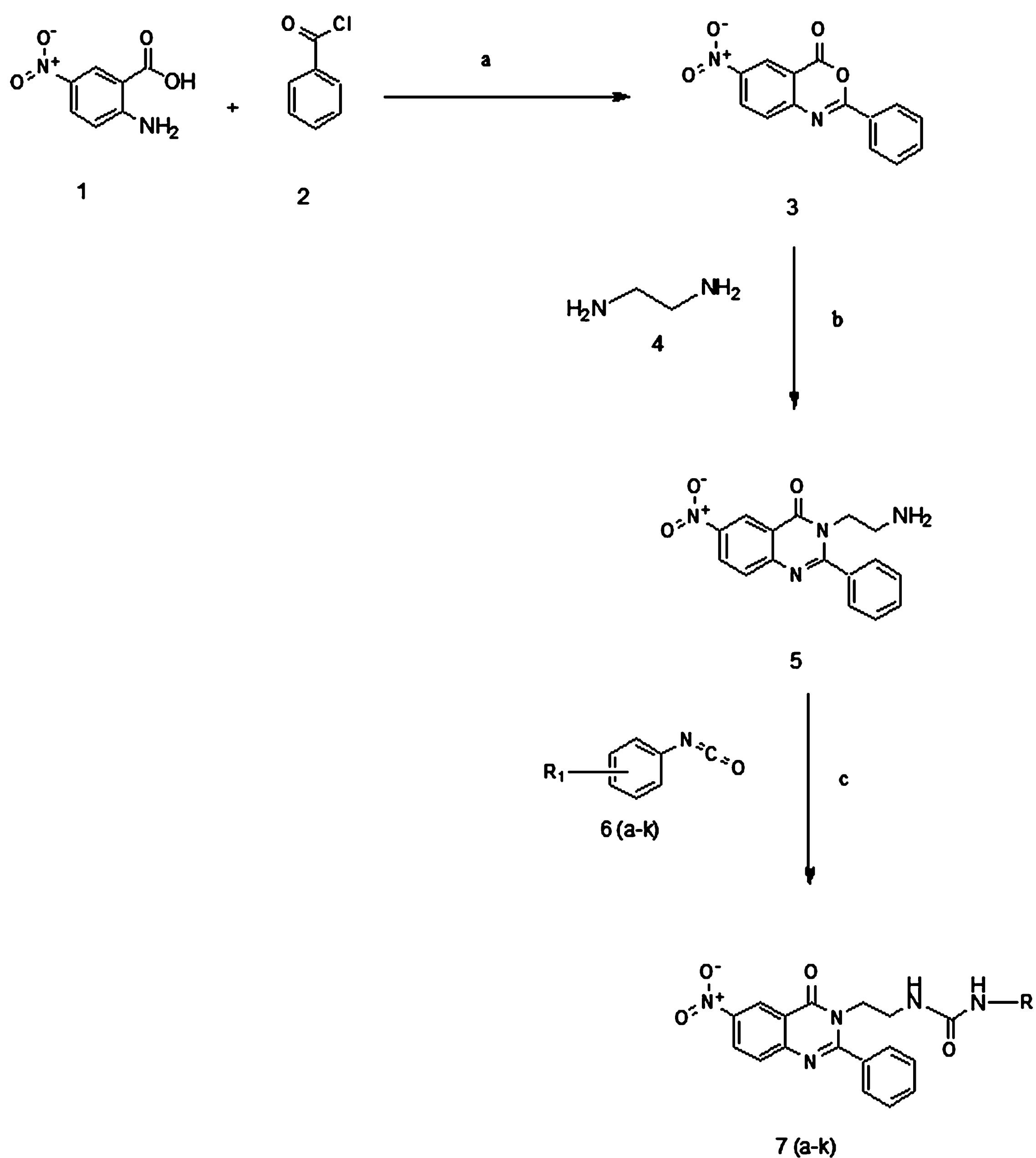


a: pyridine, 0-5 °C, b: EtOH, pyridine, reflux, 7-8 h, C: DMF, Et_3N, reflux, 6-10 h, R: 7a: PhNCO, 7b: Cyclohexyl-NCO, 7c: Transe 4-methyl cyclohexyl-NCO, 7d: O-Cl PhNCO, 7e: P-Cl-PhNCO, 7f: 2-Me-PhNCO, 7g: 4-Me-PhNCO, 7h: P-CF_3-PhNCO, 7i: 3,5-bis(CF_3)-PhNCO, 7j: 4-SCH_3-PhNCO, 7k: 2-Cl-5-CF_3-PhNCO

Scheme 1 Scheme for synthesis of final compounds **7a**–**k**

and this reaction is called as Niementowski reaction (Laddha *et al.*, 2006). The intermediate 6-nitro-2-phenyl-benzo [1,3]oxacin-4-one was synthesized by condensation reaction of 2-amino-5-nitro benzoic acid with equimolar amount of benzoyl chloride using pyridine as a solvent by maintaining temperature 0–5°C. Here, formation of the targeted intermediate was confirmed by C–O stretching of benzoxacine moiety observed at 1178 cm^{-1}. Moreover the C=O stretching was observed at 1772 cm^{-1}, and C=N stretching was observed at 1573 cm^{-1} confirms that cyclization has been carried out successfully. The –O– heteroatom of benzoxacine was converted to –N– hetero atom by nucleophilic substitution reaction resulting into quinazolinone moiety (Al-Obaid *et al.*, 2009; Ammar *et al.*, 2011). Here,

benzoxacine-4-one moiety was converted to quinazoline-4-one moiety by nucleophilic substitution reaction of 6-nitro-2-phenyl-benzo[1,3]oxacin-4-one with equimolar of ethylene diamine by using ethanol as a solvent with catalytic amount of pyridine to give intermediate 3-(2-aminoethyl)-6-nitro-2-phenylquinazolin-4(3*H*)-one. Formation of the resultant intermediate was confirmed by $–NH_2$ stretching frequency observed at 3315 cm^{-1} and disappearance of C–O stretching frequency at 1178 cm^{-1}. Reactions of the intermediate 3-(2-aminoethyl)-6-nitro-2-phenylquinazolin-4(3*H*)-one with different phenyl isocyanate derivatives were carried out by using DMF as a solvent along with equimolar amount of triethyl amine resulted in the synthesis of final compounds (Scheme 1). IR spectra of the compounds showed a characteristic stretching peak of secondary amide at 3303 cm^{-1} that supports successful synthesis of final compounds. The physiochemical characteristics of the synthesized compounds are presented in Table 1.

^{1}H NMR spectra of compound-**7a** revealed signals in the downfield region at 8.9 δppm as a singlet and 6.9 δppm as a triplet indicating the presence of two protons of urea in the linkage at N_3 position of quinazoline-4-one moiety. Two aromatic protons adjacent to the $–NO_2$ group were observed in the downfield region between 8.1 and 8.9 δppm as a singlet and doublet, respectively. Remaining 13 aromatic protons were observed as a multiplet in the aromatic region between 6.9 and 8.0 δppm. A ^{13}C NMR spectrum of compound **7a** was carried out by using DMSO as a solvent. Here, the two most downfield peaks obtained at 166.5 and 165.8 δppm proved the presence of two carbonyl groups in the compound. Carbon attached with Nitrogen heteroatom on both side in the quinazolinone moiety was observed in the downfield region at 145.23 δppm. Further the carbon attached with the nitro group was observed in the downfield region at 141.91 δppm. Peaks at 138.56, 134.92, 134.35, 133.39, 133.30, 129.76, 129.73, 129.46, 128.15, 127.97, 127.92, 127.84, 125.54, 1258.43, 122.25, and 121.77 δppm were for the rest aromatic carbons. Peaks for aliphatic carbon present in the final structure were observed at 40.62 and 39.37 δppm (Silverstein and Webster, 1997).

Biological evaluation

The newly synthesized final compounds (**7a**–**k**) were assayed in vitro antimicrobial activity by using serial broth dilution technique to find out MIC value and were exposed to find out zone of inhibition by using Kirby Bauer disk diffusion technique against various bacterial and fungal strains (*B. subtilis*, *S. aureus*, *E. coli*, *P. aeruginosa*, and *C. albicans*).

Antibacterial evaluation

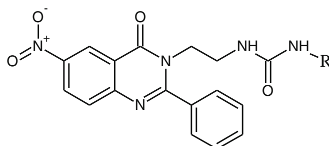
The results of the antibacterial testing of final synthesized compounds against selected gram-positive and gram-negative bacterial strains are reported in Tables 2 (serial broth dilution technique to determine MIC) and 3 (Kirby Bauer technique to determine zone of inhibition), in comparison with the reference drug ciprofloxacin.

Good inhibition was observed for compounds **7c**, **7d**, **7f**, **7g**, and **7j** against strain *E. coli* at 40 µg/ml of MIC, among them compounds **7c** and **7d** showed 11 mm zone of inhibition at concentration 70 µg/ml and compounds **7f**, **7g**, and **7j** showed 11 mm zone of inhibition at 40 µg/ml. Compounds **7b**, **7i**, and **7j** showed inhibition at 40 µg/ml of MIC against gram-negative strain *P. aeruginosa.* All the synthesized compounds showed comparatively poor activity for gram-positive strain *S. aureus*. Compounds **7e**,

Table 1 Physical and analytical data of the synthesized compounds **7a**–**k**

Entry	Mol. formula	% Yield	M.W	M.P. (°C)	Elemental analysis					
					Found (%)			Calculated (%)		
					C	H	N	C	H	N
7a	$C_{23}H_{19}N_5O_4$	73.4	429.42	260–261	64.25	4.42	16.30	64.33	4.46	16.31
7b	$C_{23}H_{25} N_5O_4$	90.47	435.40	230–232	63.32	5.72	16.10	63.4	5.79	16.08
7c	$C_{24}H_{27} N_5O_4$	80.97	449.5	255–256	64.08	6.02	15.52	64.13	6.05	15.58
7d	$C_{23}H_{18}ClN_5O_4$	70.2	463.87	284–285	59.51	3.89	15.12	59.55	3.91	15.10
7e	$C_{23}H_{18}ClN_5O_4$	74.6	463.87	269–270	59.49	3.96	15.11	59.55	3.91	15.10
7f	$C_{24}H_{21}N_5O_4$	75.9	443.45	283–284	64.92	4.79	15.74	65.0	4.77	15.79
7g	$C_{24}H_{21}N_5O_4$	79.4	443.45	289–291	64.94	4.71	15.74	65.0	4.77	15.79
7h	$C_{24}H_{18}F_3N_5O_4$	68.3	497.42	242–244	57.89	3.61	14.06	57.95	3.65	14.08
7i	$C_{25}H_{17}F_6N_5O_4$	60.3	565.42	225–226	53.08	3.01	12.38	53.10	3.03	12.39
7j	$C_{24}H_{21} N_5O_4S$	77.2	475.5	220–221	60.58	4.41	14.72	60.62	4.45	14.73
7k	$C_{24}H_{17}ClF_3 N_5O_4$	80.03	531.87	239–241	54.15	3.19	13.16	54.20	3.22	13.17

Table 2 *In-vitro* antibacterial and antifungal activity (MIC determination by serial broth dilution technique):



Entry	R	MIC in μg/ml				
		Gram-negative strains		Gram-positive strains		Fungal strain
		E.c	*P.a*	*S.a*	*B.s*	*C.a*
7a	Ph	70	100	100	100	20
7b	Cyclohexyl	70	40	200	100	20
7c	4-Me-Cyclohexyl	40	70	100	100	10
7d	2-Cl-Ph	70	70	100	70	20
7e	4-Cl-Ph	40	100	200	70	40
7f	2-Me-Ph	40	40	70	70	20
7g	4-Me-Ph	40	40	70	70	20
7h	4-CF_3-Ph	70	70	70	70	40
7i	3,5-bis (CF_3)-Ph	100	40	200	40	20
7j	4-SCH_3-Ph	40	40	200	70	20
7k	2-Cl-5-CF_3-Ph	70	70	200	100	20
NC	DMSO	–	–	–	–	–
PC	Ciprofloxacin	15	10	5	5	–
PC	Flucanazole	–	–	–	–	5

E.c Escherichi coli, P.a Pseudomonas aeruginosa, S.a Staphylococcus aureus, B.s Bacillus subtilis, C.a Candida albicans

NC negative control, *PC* positive control

7h, and **7i** showed good inhibition potency against gram-positive strain *B. subtilis* at 40 μg/ml of MIC.

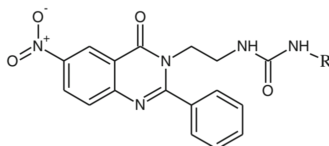
From the results obtained, structure activity relationship studies were carried out and effect of the substituent at the phenyl ring of final compounds have been evaluated. For strain, *E. coli* compounds having electron donating groups (–CH_3 and –SCH_3) at the phenyl or cyclohexyl ring (**7c**, **7f**, **7g**, and **7j**), were found to be more potent in comparison with the parent compound **7a** (unsubstituted phenyl ring) and showed inhibition in terms of MIC at 40 μg/ml, while electron withdrawing groups (–Cl, –CF_3) containing final compounds (**7d**, **7h**, **7i**, and **7k**) were found equipotent (MIC, 70 μg/ml) or less potent (MIC, 100 μg/ml). Final analogs with substituent at phenyl ring showed more effectiveness than unsubstituted compound **7a** against strain *P. aeruginosa*, moreover final compounds with electron donating groups (**7f**, **7g**, and **7j**) were found to be more active (MIC, 40 μg/ml) than electron withdrawing group (**7d**, **7e**, **7h**, **7i**, and **7k**) containing compounds (MIC, 70 or 100 μg/ml). Final compounds with electron withdrawing group (–Cl, –CF_3) at phenyl ring (**7d**, **7e**, **7i**, and **7k**) were found less active (MIC, 200 μg/ml) than compound **7a** against strain *S. aureus*, moreover for the same strain electron donating –CH_3 group at *ortho* and *para* position of phenyl ring enhances its inhibition potency (MIC, 70 μg/ml). Electron withdrawing –CF_3 group at 5th position of phenyl ring (Compounds **7i** and **7k**) decreases inhibition potency against strain *S. aureus* (MIC, 200 μg/ml) in comparison with compound **7a**. Final compounds with substituent at phenyl ring were found to be more effective for inhibition of strain *B. subtilis* (MIC, 40–70 μg/ml) with respect to unsubstituted compound **7a** (Fig. 1).

Antifungal evaluation

The results of the antifungal testing of final synthesized compounds against selected strain *C. albicans* are reported in Table 2 (serial broth dilution technique to determine MIC) and Table 3 (Kirby Bauer technique to determine zone of inhibition), in comparison with the reference drug Flucanazole (Fig. 2).

Synthesized all final compounds (**7a–k**) showed very good anticandida activity (MIC, 10–40 μg/ml). Compound **7c** was found to be the most active against the strain *C. albicans*, showing inhibition potency at 10 μg/ml of MIC and 11 mm of zone of inhibition at 20 μg/ml and

Table 3 In-vitro antibacterial and antifungal activity (determination of zone of inhibition by Kirby bauer disk diffusion technique):



Entry	Zone of Inhibition study (Kirby Bauer disk diffusion technique)														
	E.c			*P.a*			*S.a*			*B.s*			*C.a*		
	IC µg/ml	100 µg/ml ZD (mm)	300 µg/ml	IC µg/ml	100 µg/ml ZD (mm)	300 µg/ml	IC µg/ml	100 µg/ml ZD (mm)	300 µg/ml	IC µg/ml	100 µg/ml ZD (mm)	300 µg/ml	IC µg/ml	100 µg/mll ZD (mm)	300 µg/ml
7a	100	11	16	200	–	14	200	–	14	100	11	15	20	17	20
7b	70	13	18	70	12	17	200	–	12	200	–	13	20	13	21
7c	70	13	17	70	12	21	100	11	14	100	11	14	20	15	20
7d	70	14	18	70	13	19	100	11	14	70	12	15	40	16	20
7e	70	12	14	100	11	16	200	–	12	70	12	16	40	15	21
7f	40	13	16	70	12	18	70	12	14	100	11	14	20	16	19
7g	40	14	17	70	12	17	70	12	14	70	12	15	20	14	17
7h	100	11	15	70	12	18	100	11	13	70	12	14	40	15	20
7i	100	11	14	40	14	19	200	–	12	70	14	19	20	17	24
7j	40	14	18	70	12	19	200	–	12	70	12	16	20	15	20
7k	70	11	18	70	12	19	300	–	12	100	11	14	40	14	20
NC	–	–	–	–	–	–	–	–	–	–	–	–	–	–	—
PC_1	20	NT	NT	10	NT	NT	10	NT	NT	05	NT	NT	–	–	–
PC_2	–	–	–	–	–	–	–	–	–	–	–	–	10	NT	NT

IC Initial concentration (maximum dilution) for appearance of zone of inhibition, *ZD* zone diameter in mm. NC negative control (DMSO effect on microbes), PC_1 positive control Ciprofloxacin, PC_2 positive control Flucanazole, *NT* not tested

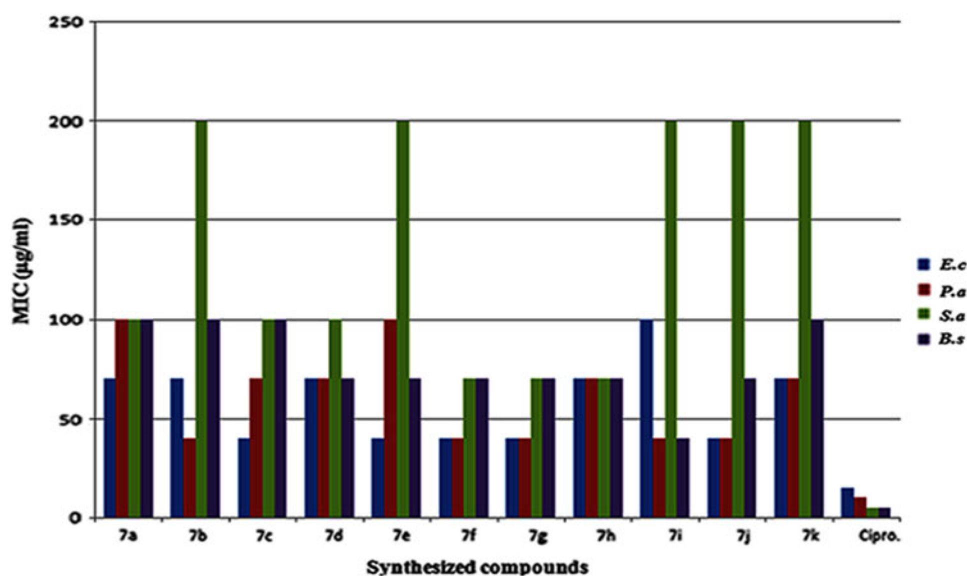


Fig. 1 Graph of MIC against synthesized compounds. Strains used are *E.c.*, *P.a.*, *S.a.*, and *B.s*

20 mm zone of inhibition at 300 µg/ml. Electron withdrawing groups –Cl and $–CF_3$ at *para* position of phenyl ring (compounds **7e**, **7h**) decreases anticandida potency (MIC, 40 µg/ml) in comparison with unsubstituted compound **7a** (MIC, 20 µg/ml). From the results obtained by Kirby Bauer technique, it was concluded that at phenyl or

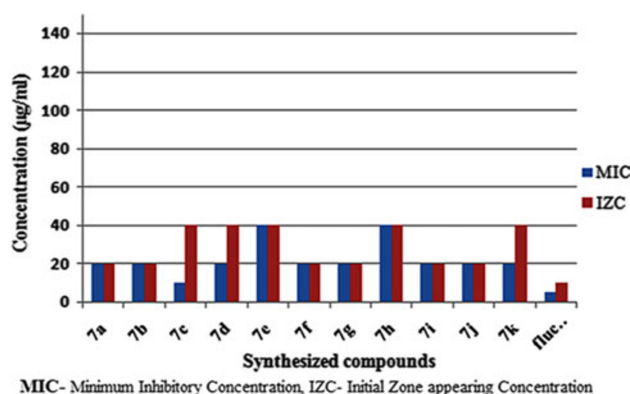


Fig. 2 Graph of dilution concentration against synthesized compounds showing anticandida activity

cyclohexyl ring of final compounds electron donating groups (compounds **7c**, **7f**, **7g**, and **7j**) were more suitable for anticandida activity as for these compounds zone of inhibition starts at concentration 20 µg/ml, while for compounds **7d**, **7e**, **7h**, and **7k** (phenyl ring with electron withdrawing groups) zone of inhibition starts at 40 µg/ml.

Experimental

Chemistry

Materials and instrumentation

All reagents were of analytical reagent grade and were used without further purification. Solvents used were purified by standard procedure before to use. 2-amino-5-nitrobenzoic acid and benzoyl chloride were purchased from ACS Chemicals, Surat. Phenyl isocyanate, Cyclohexyl isocyanate, *trans*-4-methyl cyclohexyl isocyanate, and 4-chloro phenyl isocyanate were gifted by Paushak Pvt Ltd., Vadodara, India and the rest of phenyl isocyanate derivatives were purchased from Sigma Aldrich Chemicals Pvt Ltd., Mumbai, India.

The melting points were determined in open capillaries on a Veego (Model: VMP-D, Veego Instrument Corporation, Mumbai, India) electronic apparatus and are uncorrected. To monitor the reactions as well as to establish the identity and purity of reactants and products, thin layer chromatography was performed on E. Merck Silica gel 0.50-mm plate and spots were visualized under UV radiation. FTIR spectra (4000–400 cm^{-1}) recorded on Shimadzu spectrophotometer (Model: 8400-S, Shimadzu India Pvt Ltd., Mumbai, India) using KBr disk. Nuclear magnetic resonance spectra were recorded on Varian 400 MHz model (Varian India Pvt Ltd., Mumbai, India) using DMSO and or DMF as a solvent and TMS as an internal reference (Chemical shifts are in δppm). ^{1}H NMR and ^{13}C NMR were performed at Center of Excellence, Vapi, India. Elemental analysis was performed using a PerkinElmer, USA 2400-II CHN analyzer.

Preparation of 6-nitro-2-phenyl-4H-3,1-benzoxacine-4-one (3)

To a stirred solution of 2-amino-5-nitrobenzoic acid (**1**) (9.11 g, 0.05 mol) in pyridine (60 ml), benzoyl chloride (**2**) (5.8 ml, 0.05 mol) was added drop wise, maintaining the temperature near 0–5°C for about 2 h. The reaction mixture was stirred for another 2 h at room temperature until a solid product was formed. Progress of the reaction was monitored by TLC using methanol:ethyl acetate (1:9) as an eluent. The resultant reaction mixture was then neutralized with saturated $NaHCO_3$ solution to remove unreacted 2-amino-5-nitrobenzoic acid if any. The resultant reaction mixture was treated with crushed ice followed by filtration and recrystallized with ethanol.

m.p: 182–183°C; yield: 76%; ^{1}H NMR (400 MHz, DMSO, δppm): 8.73 (1H, s, Ar–H, proton adjacent to $–NO_2$ group), 8.64 (1H, d, Ar–H, proton adjacent to $–NO_2$ group), 7.27–7.82 (6H, m, Ar–H); ^{13}C NMR (100 MHz, DMSO, δppm): 169.54 (O–C=O), 158.73 (O–C=N, quinazoline ring), 144.47 ($C–NO_2$), 121.30–128.64 (Ar–C); IR (KBR, cm^{-1}): 1772.62 (O–C=O str. of benzoxacine), 1573.68 (C=N str., benzoxacine), 1507.09 ($–NO_2$ asy.), 1336.36 ($–NO_2$ sym.); Anal calculated for $C_{14}H_8N_2O_4$: C, 62.69; H, 3.01; N, 10.44., Found: C, 62.72; H, 3.12, N, 10.45.

Preparation of 3-(2-aminoethyl)-6-nitro-2-phenylquinazolin-4(3H)-one (5)

A solution of 6-nitro-2-phenyl-4*H*-3,1-benzoxazin-4-one (**3**) (10.7 g, 0.04 mol) in ethanol (65 ml) was taken in dry RBF along with dry pyridine (4 ml) in a catalytic amount. The nucleophile ethylene diamine (**4**) (2.75 ml, 0.005 mol) in ethanol was added in fraction with constant stirring for 10 min. After completion of the addition, the reaction mixture was refluxed for about 10 h. Progress of the reaction was monitored by TLC using toluene:acetone (6:4) as an eluent. The resultant reaction mixture was treated with crushed ice to obtained yellowish product which was filtered, washed, and recrystallized with DMF.

m.p: 264–265°C; yield: 73%; ^{1}H NMR (400 MHz, DMSO, δppm): 8.85 (1H, s, Ar–H, proton adjacent to $–NO_2$ group), 8.69 (1H, d, Ar–H, proton adjacent to $–NO_2$ group), 7.26–7.84 (6H, m, Ar–H), 2.46 (m, 2H, $–CH_2$), 2.92 (t, 2H, $–CH_2$); ^{13}C NMR (100 MHz, DMSO, δppm): 166.58 (C=O), 144.76 (C=N, quinazolinone ring), 144.53

(C–NO_2), 121.38–129.93 (Ar–C), 40.62 (–CH_2), 39.35 (–CH_2); IR (KBR, cm^{-1}): 1692.49 (C=O str., quinazolinone), 1539.68 (C=N str. Of quinazolinone), 1508.09 (–NO_2 asy.), 1335.36 (–NO_2 sym.); Anal calculated for $C_{16}H_{14}N_4O_3$: C, 61.93; H, 4.55; N, 18.06, Found: C, 61.95; H, 4.57, N, 18.07.

*General preparation of final compounds (**7a–k**)*

The synthesized intermediate compound 3-(2-aminoethyl)-6-nitro-2-phenylquinazolin-4(3*H*)-one was dissolved in DMF and equimolar amount of the phenyl isocyanates (**6a–k**) were added drop wise to it and triethylamine was also added in an equimolar amount. After the completion of the addition, the reaction mixture was refluxed for about 6–10 h. The status of the reaction was monitored by TLC using methanol:ethyl acetate (0.5:9.5) as an eluent. After complete conversion, the reaction mixture was poured in crushed ice to separate the product. The obtained product was filtered, washed, and dried.

Characterization of final compounds (**7a–k**)

*1-[2-(6-Nitro-4-oxo-2-phenyl-4H-quinazolin-3-yl)-ethyl]-3-phenyl-urea (**7a**)*

^{1}H NMR (400 MHz, DMSO, δppm): 8.91 (1H, s, –NH), 6.91 (1H, t, –NH), 7.22–8.75 (13H, m, Ar–H), 2.48 (2H, q, –CH_2), 1.20 (2H, t, –CH_2); ^{13}C NMR (100 MHz, DMSO, δppm): 166.51 (C=O), 165.83 (C=O), 145.23 (C=N, quinazolinone ring), 142.37 (C–NO_2), 141.91, 138.56, 134.92, 134.35, 133.39, 133.30, 129.76, 129.73, 129.46, 128.15, 127.97, 127.92, 127.84, 125.54, 1258.43, 122.25, 121.77, 40.62 (–CH_2), 39.37 (–CH_2); IR (KBR, cm^{-1}): 3303.59 (–NH), 1691.0 (C=O str.), 1538.20 (C=N str.), 1507.09 (–NO_2 asy.), 1336.36 (–NO_2 sym.), 1158 (C–N str.); ESI–MS (*m/z*): 430 (M+1).

*1–Cyclohexyl-3-[2-(6-nitro-4-oxo-2-phenyl-4H-quinazolin-3-yl)-ethyl]-urea (**7b**)*

^{1}H NMR (400 MHz, DMSO, δppm): 8.93 (1H, s, –NH), 6.99 (1H, t, –NH), 7.14–8.87 (8H, m, Ar–H), 4.93 (1H, m, –CH), 2.42 (2H, q, –CH_2), 1.84 (4H, q, –CH), 1.37 (6H, m, –CH_2), 1.23 (2H, t, –CH_2); ^{13}C NMR (100 MHz, DMSO, δppm): 166.59 (C=O), 165.73 (C=O), 145.29 (C=N, quinazolinone ring), 142.47 (C–NO_2), 141.95, 134.82, 134.25, 133.59, 133.10, 129.96, 129.27, 128.35, 127.52, 125.53, 122.22, 48.13, 40.68, 39.17, 33.23, 33.63, 25.92, 24.92, 24.32; IR (KBR, cm^{-1}): 3303.59 (–NH), 1691.0 (C=O str.), 1538.20 (C=N str.), 1507.09 (–NO_2 asy.), 1336.36 (–NO_2 sym.), 1158 (C–N str.); ESI–MS (*m/z*): 436 (M+1).

*1-(4-Methyl-cyclohexyl)-3-[2-(6-nitro-4-oxo-2-phenyl-4H-quinazolin-3-yl)-ethyl]-urea (**7c**)*

^{1}H NMR: 8.86 (1H, s, –NH), 6.81 (1H, t, –NH), 7.10 - 8.76 (8H, m, Ar–H), 4.94 (1H, m, –CH), 2.43 (2H, q, –CH_2), 1.84 (4H, q, –CH), 1.35 (5H, m, –CH_2), 1.24 (2H, t, –CH_2), 0.97 (3H, s, –CH_3); ^{13}C NMR (100 MHz, DMSO, δppm): 166.59 (C=O), 165.73 (C=O), 145.29 (C=N, quinazolinone ring), 142.47 (C–NO_2), 141.95, 134.82, 134.25, 133.59, 133.10, 129.96, 129.27, 128.35, 127.52, 125.53, 122.22, 48.13, 40.68, 39.17, 33.23, 33.63, 25.92, 24.92, 24.32, 19.82 (–CH_3); IR (KBR, cm^{-1}): 3303.59 (–NH), 1691.0 (C=O str.), 1538.20 (C=N str.), 1507.09 (–NO_2 asy.), 1336.36 (–NO_2 sym.), 1158 (C–N str.); ESI–MS (*m/z*): 451 (M+1).

*1-(2-Chloro-phenyl)-3-[2-(6-nitro-4-oxo-2-phenyl-4H-quinazolin-3-yl)-ethyl]-urea (**7d**)*

^{1}H NMR (400 MHz, DMSO, δppm): 8.83 (1H, s, –NH), 6.86 (1H, t, –NH), 7.27–8.73 (12H, m, Ar–H), 2.31 (2H, q, –CH_2), 1.35 (2H, t, –CH_2); ^{13}C NMR (100 MHz, DMSO, δppm): 166.41 (C=O), 165.93 (C=O), 145.29 (C=N, quinazolinone ring), 142.31 (C–NO_2), 141.81, 138.66, 134.84, 134.41, 133.49, 133.38 (C–Cl), 129.69, 129.79, 129.47, 128.35, 127.89, 127.99, 127.88, 125.51, 1258.49, 122.19, 121.97, 40.41 (–CH_2), 39.49 (–CH_2); IR (KBR, cm^{-1}): 3303.59 (–NH), 1691.0 (C=O str.), 1538.20 (C=N str.), 1507.09 (–NO_2 asy.), 1336.36 (–NO_2 sym.), 1158 (C–N str.), 850–550 (C–Cl str.); ESI–MS (*m/z*): 465 (M+1).

*1-(4-Chloro-phenyl)-3-[2-(6-nitro-4-oxo-2-phenyl-4H-quinazolin-3-yl)-ethyl]-urea (**7e**)*

^{1}H NMR (400 MHz, DMSO, δppm): 9.07 (1H, s, –NH), 6.92 (1H, t, –NH), 7.25–8.91 (12H, m, Ar–H), 2.54 (2H, q, –CH_2), 1.41 (2H, t, –CH_2); ^{13}C NMR (100 MHz, DMSO, δppm): 166.21 (C=O), 165.33 (C=O), 145.21 (C=N, quinazolinone ring), 142.39 (C–NO_2), 141.91, 138.56, 134.92, 134.35, 133.39, 133.30 (C–Cl), 129.76, 129.73, 129.46, 128.15, 127.97, 127.92, 127.84, 125.54, 1258.43, 122.25, 121.77, 40.62 (–CH_2), 39.37 (–CH_2); IR (KBR, cm^{-1}): 3303.59 (–NH), 1691.0 (C=O str.), 1538.20 (C=N str.), 1507.09 (–NO_2 asy.), 1336.36 (–NO_2 sym.), 1158 (C–N str.), 850–550 (C–Cl str.); ESI–MS (*m/z*): 465 (M+1).

*1-[2-(6-Nitro-4-oxo-2-phenyl-4H-quinazolin-3-yl)-ethyl]-3-o-tolyl-urea (**7f**)*

^{1}H NMR (400 MHz, DMSO, δppm): 8.93 (1H, s, –NH), 6.85 (1H, t, –NH), 7.04–8.63 (12H, m, Ar–H), 2.44 (2H, q, –CH_2), 2.35 (3H, s, –CH_3), 1.22 (2H, t, –CH_2); ^{13}C NMR (100 MHz, DMSO, δppm): 166.11 (C=O), 165.91 (C=O),

145.21 (C=N, quinazolinone ring), 142.44 (C–NO_2), 141.99, 138.65, 134.29, 134.53, 133.32, 133.38, 129.96, 129.73, 129.22, 128.10, 127.82, 127.72, 127.24, 125.92, 125.53, 122.25, 121.77, 40.59 (–CH_2), 39.25 (–CH_2), 11.89 (–CH_3); IR (KBR, cm^{-1}): 3303.59 (–NH), 1691.0 (C=O str.), 1538.20 (C=N str.), 1507.09 (–NO_2 asy.), 1336.36 (–NO_2 sym.), 1158 (C–N str.); ESI–MS (*m/z*): 444 (M+1).

*1-[2-(6-Nitro-4-oxo-2-phenyl-4H-quinazolin-3-yl)-ethyl]-3-p-tolyl-urea (**7g**)*

^{1}H NMR (400 MHz, DMSO, δppm): 8.96 (1H, s, –NH), 6.91 (1H, t, –NH), 7.34–8.89 (12H, m, Ar–H), 2.32 (2H, q, –CH_2), 2.25 (3H, s, –CH_3), 1.29 (2H, t, –CH_2); ^{13}C NMR (100 MHz, DMSO, δppm): 166.51 (C=O), 165.89 (C=O), 145.29 (C=N, quinazolinone ring), 142.54 (C–NO_2), 141.89, 138.55, 134.23, 134.53, 133.52, 133.38, 129.92, 129.63, 129.32, 128.11, 127.93, 12.63, 127.36, 125.83, 125.59, 122.76, 121.77, 40.54 (–CH_2), 39.29 (–CH_2), 11.97 (–CH_3); IR (KBR, cm^{-1}): 3303.59 (–NH), 1691.0 (C=O str.), 1538.20 (C=N str.), 1507.09 (–NO_2 asy.), 1336.36 (–NO_2 sym.), 1158 (C–N str.); ESI–MS (*m/z*): 444 (M+1).

*1-[2-(6-Nitro-4-oxo-2-phenyl-4H-quinazolin-3-yl)-ethyl]-3-(4-trifluoromethyl-phenyl)-urea (**7h**)*

^{1}H NMR (400 MHz, DMSO, δppm): 8.99 (1H, s, –NH), 6.91 (1H, t, –NH), 7.15–8.81 (12H, m, Ar–H), 2.49 (2H, q, –CH_2), 1.26 (2H, t, –CH_2); ^{13}C NMR (100 MHz, DMSO, δppm): 166.35 (C=O), 165.76 (C=O), 145.21 (C=N, quinazolinone ring), 142.35 (C–NO_2), 141.39, 134.31, 133.35, 133.35, 130.32, 129.73, 129.77, 129.41, 128.19, 127.97, 127.82, 127.44, 125.58, 125.43, 123.15, 122.20, 121.71, 119.23 (–CF_3), 40.58 (–CH_2), 39.32 (–CH_2); IR (KBR, cm^{-1}): 3303.59 (–NH), 1691.0 (C=O str.), 1538.20 (C=N str.), 1507.09 (–NO_2 asy.), 1336.36 (–NO_2 sym.), 1283.92 (C–F str.), 1158 (C–N str.); ESI–MS (*m/z*): 498 (M+1).

*1-(3,5-Bis-trifluoromethyl-phenyl)-3-[2-(6-nitro-4-oxo-2-phenyl-4H-quinazolin-3-yl)-ethyl]-urea (**7i**)*

^{1}H NMR (400 MHz, DMSO, δppm): 8.96 (1H, s, –NH), 6.90 (1H, t, –NH), 7.09–8.45 (11H, m, Ar–H), 2.48 (2H, q, –CH_2), 1.27 (2H, t, –CH_2); ^{13}C NMR (100 MHz, DMSO, δppm): 166.47 (C=O), 165.47 (C=O), 145.25 (C=N, quinazolinone ring), 142.39 (C–NO_2), 141.41, 134.33, 133.37, 133.45, 130.49, 129.77, 129.71, 129.41, 128.21, 127.99, 127.78, 127.41, 125.59, 125.49, 123.51, 122.27, 121.79, 119.87 (–CF_3), 119.23 (–CF_3), 40.58 (–CH_2), 39.32 (–CH_2); IR (KBR, cm^{-1}): 3303.59 (–NH), 1691.0 (C=O str.), 1538.20 (C=N str.), 1507.09 (–NO_2 asy.), 1336.36 (–NO_2 sym.), 1283.92 (C–F str.), 1158 (C–N str.); ESI–MS (*m/z*): 566 (M+1).

*1-(4-Methylsulfanyl-phenyl)-3-[2-(6-nitro-4-oxo-2-phenyl-4H-quinazolin-3-yl)-ethyl]-urea (**7j**)*

^{1}H NMR (400 MHz, DMSO, δppm): 8.94 (1H, s, –NH), 6.77 (1H, t, –NH), 6.92–8.71 (12H, m, Ar–H), 2.32 (3H, s, –CH_3), 2.47 (2H, q, –CH_2), 1.20 (2H, t, –CH_2); ^{13}C NMR (100 MHz, DMSO, δppm): 166.51 (C=O), 165.83 (C=O), 145.23 (C=N, quinazolinone ring), 142.37 (C–NO_2), 141.91, 138.56, 134.92, 134.35, 133.39, 133.30, 129.76, 129.73, 129.46, 128.15, 127.97, 127.92, 127.84, 125.54, 1258.43, 122.25, 121.77, 40.62 (–CH_2), 39.37 (–CH_2), 16.70 (–SCH_3); IR (KBR, cm^{-1}): 3303.59 (–NH), 1691.0 (C=O str.), 1538.20 (C=N str.), 1507.09 (–NO_2 asy.), 1336.36 (–NO_2 sym.), 1158 (C–N str.); ESI–MS (*m/z*): 476 (M+1).

*1-(2-Chloro-5-trifluoromethyl-phenyl)-3-[2-(6-nitro-4-oxo-2-phenyl-4H-quinazolin-3-yl)-ethyl]-urea (**7k**)*

^{1}H NMR (400 MHz, DMSO, δppm): 8.95 (1H, s, –NH), 6.84 (1H, t, –NH), 7.04–8.72 (11H, m, Ar–H), 2.44 (2H, q, –CH_2), 1.21 (2H, t, –CH_2); ^{13}C NMR (100 MHz, DMSO, δppm): 166.35 (C=O), 165.76 (C=O), 145.21 (C=N, quinazolinone ring), 142.35 (C–NO_2), 141.39, 134.31, 133.35, 133.35, 130.32 (C–Cl), 129.73, 129.77, 129.41, 128.19, 127.97, 127.82, 127.44, 125.58, 125.43, 123.15, 122.20, 121.71, 119.23 (–CF_3), 40.58 (–CH_2), 39.32 (–CH_2); IR (KBR, cm^{-1}): 3303.59 (–NH), 1691.0 (C=O str.), 1538.20 (C=N str.), 1507.09 (–NO_2 asy.), 1336.36 (–NO_2 sym.), 1158 (C–N str.); ESI–MS (*m/z*): 533 (M+1).

Pharmacology

The newly synthesized compounds **7** (**a**–**k**) were evaluated for their in vitro antibacterial and antifungal activities. The standard strain used for antimicrobial activity was procured from Promotech Life sciences, Bangalore. The in vitro antibacterial activity were performed against gram-positive bacteria *B*. *subtilis* ATCC 11774 and *S. aureus* ATCC 25923 and gram-negative bacteria *E. coli* ATCC 25922 and *P. aeruginosa* ATCC 27853 by using Kirby Bauer technique and serial broth dilution technique. Here, antifungal screening was carried out by using *C. albicans* ATCC 66027 by the same method.

Kirby Bauer is a technique which uses antibiotic impregnated wafers to test whether particular bacteria are susceptible to specific antibiotics. First, the Mueller–Hinton agar media were sterilized (autoclaved at 120°C for half an hour), poured into a Petri dish maintaining 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 agar using a sterile cotton swab for growth of the organisms. Here, all the compounds **7**(**a**–**k**) were dissolved in dimethyl sulfoxide and seven

dilutions (10, 20, 40, 70, 100, 200, and 300 μg/ml) of each compounds were prepared and kept in wafers to measure activity. Sterile filter paper disks measuring 10 mm in diameter, previously soaked in a known concentration of the respective test compound in dimethyl sulfoxide were placed on an agar medium that had been inoculated with the respective microorganism and the resulting petri dishes were incubated for 24 h at 37°C (Murray *et al.*, 1995).

In biological evaluation, DMSO was used as a negative control to check the effect of it on the microbes. A control disk impregnated with a dimethyl sulfoxide without any sample was also used in the same manner and did not show any inhibition. Ciprofloxacin and flucanazole were used as a standard drug for antibacterial and antifungal, respectively.

MICs of all the synthesized compounds were carried out by using serial broth dilution technique (Collins and Lyne, 1970; Mullen *et al.*, 1988). A set of sterilized test tubes with nutrient broth medium capped with cotton plugs were taken. Here, compounds **7**(**a**–**k**) were dissolved in dimethyl sulfoxide and seven dilutions (10, 20, 40, 70, 100, 200, and 300 μg/ml) of each compound were prepared. A fixed volume of culture and diluted drug solution were kept in each test tube and incubated at 37°C for 24 h. The lowest concentration showing no growth of microbes was considered as MIC. The test mixture should contain 10^8 cells/ml. In this evaluation, Ciprofloxacin and Flucanazole were used as a standard for antibacterial and antifungal study, respectively.

Conclusion

The newly synthesized compounds showed remarkable inhibition of the growth of *C. albicans* where as final compounds did not show significant activity towards gram-positive strain *S. aureus*.

The outstanding antifungal properties of this new class of antifungal substances deserve further investigation to clarify mode of action, responsible for the activity observed.

In future, more extensive study can be carried out to determine additional physicochemical and biological parameters, to have a deeper insight into structure activity relationships and to optimize effectiveness of this series of molecules.

Acknowledgments The authors wish to thank the Department of Applied Chemistry, S. V. National Institute of Technology, Surat for providing laboratory facilities and scholarship. The authors wish to offer their deep gratitude to Mr. Paresh Kapopara, Center Diagnosis Laboratory-Surat for carrying out biological screening. The authors are also thankful to Centre of Excellence, Vapi for carrying out spectral analysis.

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