Available online at www.sciencedirect.com
ScienceDirect
European Journal of Medicinal Chemistry 44 (2009) 1188–1197
EUROPEAN JOURNAL OF MEDICINAL CHEMISTRY
http://www.elsevier.com/locate/ejmech

Original article

Antimicrobial studies of some novel quinazolinones fused with [1,2,4]-triazole, [1,2,4]-triazine and [1,2,4,5]-tetrazine rings

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Received 3 April 2008; received in revised form 22 May 2008; accepted 26 May 2008
Available online 3 June 2008

Abstract

Three series of novel and new fused heterocyclic systems, viz. triazolo[4,3-*a*]-quinazolin-7-ones (**4**), [1,2,4,5]-tetrazino[4,3-*a*]-quinazolin-8-ones (**6**) and indolo[2,3-*c*][1,2,4]-triazino[4,3-*a*]-quinazolin-8-ones (**8**) have been synthesized from the key intermediate 3-(substituted-phenyl)-2-hydrazino-quinazolin-4-ones (**3**). Thus, condensation of (**3**) with appropriate aromatic acids in the presence of DCC in dichloromethane afforded the fused system (**4**), while reaction of (**3**) with isatin in methanol gave the corresponding Schiff base (**7**) which on cyclodehydration furnished another fused heterocyclic system (**8**). The intermediate (**3**) on refluxing with substituted-phenylisothiocyanate gave the substituted-thiosemicarbazide (**5**), which on oxidative cyclization with bromine in CCl_4 furnished the novel fused system (**6**). The structures of intermediate and final compounds have been determined by means of IR, ^{1}H NMR, ^{13}C NMR, UV and elemental analysis. All the synthesized compounds have been screened for their antibacterial activity against Gram-negative bacteria, *Escherichia coli*, *Pseudomonas aeruginosa* and Gram-positive bacteria, *Streptococcus pneumoniae, Bacillus subtilis*, as well as demonstrated significant antifungal activity against fungi viz. *Candida albicans*, *Aspergillus fumigatus, Aspergillus flavus*, and *Aspergillus niger*.
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Keywords: Quinazolinone; Antibacterial; Antifungal activity

1. Introduction

The pharmacodynamic versatility of 4-quinazolinone moiety has been documented not only in many of its synthetic derivatives but also in several naturally occurring alkaloids isolated from animals, families of plant kingdoms and from microorganism [1–3]. These isolated quinazolinones derivatives were found to have wide range of biological properties including anti-tumour, sedative, analgesic, antidiabetic, antibacterial, anti-inflammatory, antifungal and anticancer [4–10]. In addition, characterization of potential *N*-methyl-D-aspartate (NMDA) and cholecystokinin antagonists II-lipophilicity studies on quinazolinones are also documented [11].

1,2,4,5-Tetrazines are an important class of heterocycles that find many practical and synthetic applications [12]. In addition, some tetrazoles have been introduced in the design of non-peptide ligands for growth hormone secretagogue (GHS) receptors [13], which shows competitive inhibition potency for the carbapenem and cephamycin-resistant dinuclear zinc mettalo-β-lactamase from *Bacteroides fragilis* [14], and used for treatment of type 2 diabetes [15].

Similarly, derivatives of 1,2,4-triazole and 1,2,4-triazine have been found to possess wide spectrum of pharmacological, medicinal and biological activities [16–19]. The indole nucleus plays an important role as a common denominator for various biocidal activities [20–22].

In light of the above observations and keeping in mind that most of the biologically active quinazolinones are either C-2 or N-3 mono- and/or disubstituted derivatives, we wish to fuse indole, 1,2,4-triazole, 1,2,4-triazine and 1,2,4,5-tetrazine nuclei in between N-1 and C-2 positions of quinazolinone ring to get novel fused systems, triazolo[4,3-*a*]-quinazolin-7-ones,

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doi:10.1016/j.ejmech.2008.05.033

tetrazino[4,3-*a*]-quinazolin-8-ones and indolo[2,3-*c*][1,2,4]-triazino[4,3-*a*]-quinazolin-8-ones, which have not been reported and studied so far. This structural manipulation combines different biodynamic heterocyclic moieties in a parent lead compound quinazolinone, which might have encouraging pharmacological potential.

Moreover, it was considered of interest to substitute various groups on phenyl rings to investigate the influence of such structural variations on the anticipated biological activities.

The above facts and anticipations coupled with our desire to develop efficacious antimicrobial agents and in continuation of our work on fused heterocycles with biological interest [23–26] prompted us to device an efficient and convenient synthetic method of hitherto unknown and novel title compounds triazolo[4,3-*a*]-quinazolin-7-ones, tetrazino[4,3-*a*]-quinazolin-8-ones and indolo[2,3-*c*][1,2,4]-triazino[4,3-*a*]-quinazolin-8-ones. The simple synthesis and antimicrobial results of these newly synthesized compounds are reported in this paper.

2. Chemistry

The required starting 3-(substituted-phenyl)-2-mercapto-quinazolin-4-ones (**1**) were synthesized from anthranilic acid and substituted-phenylisothiocyanates. Methylation of (**1**) with methyl iodide in the presence of fused sodium acetate in ethanol afforded 3-(substituted-phenyl)-2-mercaptomethyl-quinazolin-4-ones (**2**) in good yields. Nucleophilic displacement of mercaptomethyl group of (**2**) with hydrazine hydrate yielded the desired key intermediates (**3**) in excellent yields.

The condensation reaction between key intermediates (**3**) and appropriate aromatic acids in the presence of DCC furnished, triazolo[4,3-*a*]quinazolin-7-ones (**4**), while the reaction of appropriate phenylisothiocyanates with intermediates (**3**) in methanol afforded corresponding thiosemicarbazides (**5**), which on oxidative cyclization with bromine in carbon-tetrachloride furnished tetrazino[4,3-*a*]quinazolin-8-ones (**6**). Schiff bases (**7**) were synthesized by refluxing the intermediates (**3**) and isatin in methanol, which on cyclodehydration with cold concentrated H_2SO_4 yielded indolo[2,3-*c*][1,2,4]triazino[4,3-*a*] quinazolin-8-ones (**8**).

3. Results and discussion

All the final compounds (**4–8**) were prepared in good yields. The physical parameters of these compounds are mentioned in Section 6 and the synthetic route is given in Scheme 1. The structures of the intermediate and final compounds have been elucidated based on their elemental analysis, UV, IR, ^{1}H NMR, ^{13}C NMR and D_2O exchange studies.

3.1. IR studies

IR spectra of compounds (**1–8**) showed useful information about the structure of the compounds.

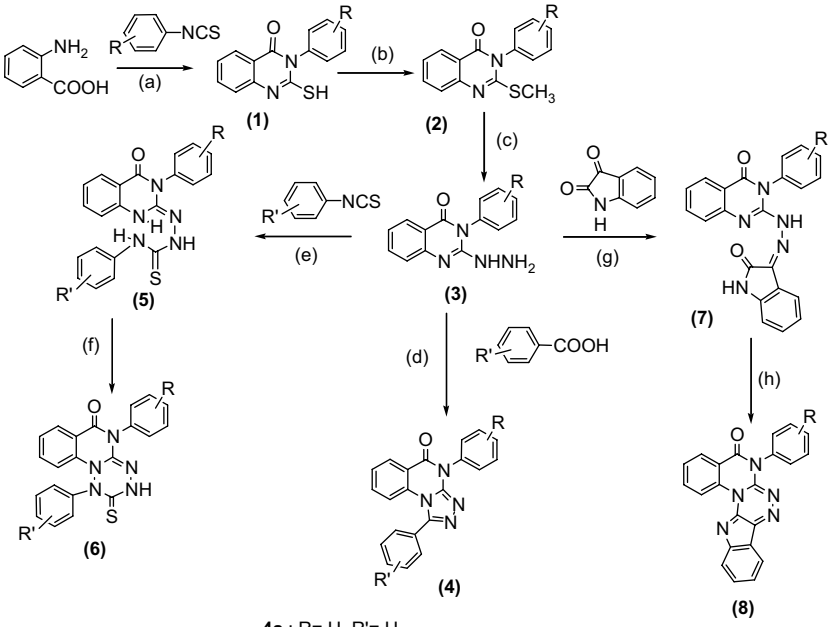
The IR spectra of compounds (**1**) exhibited characteristic bands at 1670–1680 cm^{-1} (>C=O), 1620–1630 cm^{-1} (>C=N) and 2645–2650 cm^{-1} (–SH). The disappearance of peaks at 2645–2650 cm^{-1} and appearance of new peaks at 1217–1221 cm^{-1} of thioether (C–S–C) in (**2**) clearly indicates the methylation at sulfur atom. The band of these thioethers disappeared in the IR spectra of compounds (**3**). The appearance of broad bands at 3425–3433 cm^{-1} (–NH and $-NH_2$) are more evident for the displacement of $-SCH_3$ group of (**2**) by hydrazine hydrate. The IR spectra of compounds (**4**) showed only the significant bands at 1660–1670 cm^{-1} due to the carbonyl group. The oxidative cyclization of compounds (**5**) to compounds (**6**) was confirmed by the presence of peaks at 1220–1235 cm^{-1} (>C=S), 3300–3310 cm^{-1} (–N–H) and weak bands at1440–1450 cm^{-1} (N–N). In the IR spectra of (**7**), peaks at 1660–1675 cm^{-1} and 1650–1660 cm^{-1} indicate the presence of carbonyl group of amide linkages in five and six membered rings, respectively, and broad bands at 3190–3200 cm^{-1} (–N–H). The only prominent peaks at 1655–1665 cm^{-1} due to carbonyl group of six membered amide in the IR spectra of (**8**) indicate the cyclodehydration of (**7**) into (**8**). The other bands of compounds (**7**) disappeared.

3.2. NMR studies

^{1}H NMR spectra of compounds (**1**) showed multiplet at δ 7.2–8.5 ppm for aromatic protons (8H) and singlets at δ 2.9–3.3 ppm for –SH protons. The appearance of new singlets in the ^{1}H NMR spectra of compound (**2**) at δ 2.4–2.8 ppm for mercaptomethyl ($-SCH_3$) protons clearly indicates the methylation at sulfur atom. The ^{1}H NMR spectra of (**3**) displayed a triplet and doublet like signals at δ 8.2–8.4 ppm and δ 4.5–4.8 ppm corresponding to –NH and $-NH_2$ protons of hydrazino functionality, respectively. The disappearance of signals corresponding to the protons of hydrazino functionality ($-NHNH_2$) and appearance of multiplets for aromatic proton in the range of δ 7.1–8.6 ppm clearly indicate the formation of (**4**).

In the ^{1}H NMR spectra of compounds (**5**) a quartet like signal observed at δ 8.5–8.7 ppm is due to three –NH protons, while, aromatic protons resonate at δ 7.1–8.3 ppm. The formation of (**6**) was confirmed by the presence of only a thioamide proton at δ 7.8–8.3 ppm and multiplet at δ 7.0–8.4 ppm. Other signals disappeared. The two singlets at δ 8.2–8.3 and δ 8.7–8.9 ppm in the ^{1}H NMR spectra of compounds (**7**) are due to –N–N–H and amide protons, while multiplet at δ 6.9–8.5 ppm is due to aromatic protons. The disappearance of these signals and appearance of only a multiplet δ 6.8–8.4 ppm in the ^{1}H NMR spectra of (**8**) suggest cyclodehydration of compound (**7**) into (**8**). The signals at δ 8.4 ppm, 8.2 ppm, 8.3 ppm and 8.9 ppm were exchanged with D_2O.

Another strong support for the structures of (**4**), (**6**) and (**8**) is the ^{13}C NMR spectra. The ^{13}C NMR spectra of compounds were taken in DMSO and the signals obtained were all in a good agreement with the proposed structures. In the ^{13}C NMR spectrum of compound **3b** 12 peaks were observed. The carbonyl and imine carbons of quinazolinone ring are resonates at δ 170.0 ppm and 160.1 ppm, respectively. The aromatic carbon atoms resonate at δ 124.2–141.2 ppm. In



Scheme 1. Reagents and conditions: (a) Me_2CO, reflux for 6 h, (b) CH_3COONa, CH_3I, 4 h, stirring, (c) $NH_2NH_2H_2O$, reflux for 6 h, (d) DCC, CH_2Cl_2, 4 h, (e) MeOH, reflux for 6 h, (f) Br_2/CCl_4, 30 min, stirring, (g) MeOH, reflux for 3 h, (h) conc. H_2SO_4, 5–10 °C, 2 h.

the ^{13}C NMR spectrum of compound **4a** 17 peaks were observed. Peak at δ 173.4 ppm is due to carbonyl carbon, δ 149.2–151.3 ppm is of triazole ring carbon atoms and δ 122.4–140.1 ppm for aromatic carbons. In the ^{13}C NMR spectrum of compound **6b** 18 peaks were observed. Peak at δ 190.1 ppm is attributed to thiocarbamide carbon flanked with two nitrogen atoms, δ 170.3 ppm due to carbonyl carbon and other peaks were observed at δ 121.0–157.1 ppm are due to aromatic and imine carbon atoms. The ^{13}C NMR spectrum of compound **8c** showed 21 signals. Carbonyl carbon resonates at δ 175.0 ppm, δ 163.1–166.8 ppm is due to imine carbons, different aromatic carbons were resonate at δ 120.9–152.1 ppm and methoxy carbon atom at δ 51.2 ppm.

4. Biological screening

4.1. Antibacterial screening

Preliminary experiments were carried out to determine the antibacterial activities of titled compounds in vitro against (i) Gram-positive bacteria: *Streptococcus pneumoniae*, *Bacillus subtilis* and (ii) Gram-negative bacteria: *Escherichia coli*, *Pseudomonas aeruginosa* by disc diffusion method [27].

The antibacterial data (Table 1) revealed that all tested compounds of this investigation are moderate to highly active against all the tested pathogenic bacteria. In general, the compounds **4i**, **4l**, **6c** and **8d** having *p*-methoxy, *p*-chloro and *o*-chloro substituent were showed very promising activity with MIC 1.56 μg/mL and 3.12 μg/mL against both Gram-positive *B. subtilis* and Gram-negative *E. coli*, *P. aeruginosa* whereas the rest of the compounds showed moderate activity with MIC 6.25 μg/mL, 12.5 μg/mL, 25 μg/mL and 50 μg/mL as compared to the standard drug Ciprofloxacin (MIC = 1.56 μg/mL, 3.12 μg/mL and 6.25 μg/mL). Compounds **8d** and **6c** were equipotent as Ciprofloxacin against *P. aeruginosa* while, compound **4l** was more potent than standard drug against *S. pneumoniae*. Therefore, it can be inferred that presence of polar substitutent imparts much towards antibacterial power of these compounds. The data also revealed that the activity of compound **4l** > **4i** > **8d** > **6c**. The fusion of different heterocyclic rings in between N-1 and C-2 of parent quinazolinone viz. 1,2,4-triazole, 1,2,4-triazine and 1,2,4,5-tetrazine exerts a significance influence on the antibacterial activity. The presence of triazole ring showed greater activity than that of triazine and tetrazine. The most active compound was **4l**, which contains a triazole ring. This indicates that the presence of triazole moiety is additive towards antibacterial activity in this class of compounds.

Table 1
Antibacterial activities of compounds [**4a**–**8d**]

Compound no.	R	R′	MICs (μg/mL)			
			E. coli	*P. aeruginosa*	*S. pneumoniae*	*B. subtilis*
4a	H	H	50	>50	50	25
4b	H	2-CH_3	25	50	>50	>50
4c	H	2-Cl	12.5	6.25	25	50
4d	4-CH_3	H	25	25	50	50
4e	4-CH_3	2-CH_3	25	>50	25	>50
4f	4-CH_3	2-Cl	12.5	6.25	12.5	25
4g	4-OCH_3	H	12.5	6.25	6.25	12.5
4h	4-OCH_3	2-CH_3	6.25	3.12	25	12.5
4i	4-OCH_3	2-Cl	3.12	1.56	6.25	3.12
4j	4-Cl	H	6.25	12.5	12.5	6.25
4k	4-Cl	2-CH_3	12.5	25	25	12.5
4l	4-Cl	2-Cl	1.56	3.12	3.12	1.56
6a	H	H	>50	50	25	>50
6b	H	4-CH_3	50	50	25	50
6c	H	4-OCH_3	6.25	3.12	25	3.12
6d	4-CH_3	H	25	25	>50	25
6e	4-CH_3	4-CH_3	25	50	50	25
8a	H	–	50	25	>50	50
8b	4-CH_3	–	25	>50	25	25
8c	4-OCH_3	–	12.5	12.5	12.5	6.25
8d	4-Cl	–	3.12	3.12	1.56	6.25
Ciprofloxacin	–	–	1.56	3.12	6.25	1.56

4.2. Antifungal screening

Titled compounds were screened for their antifungal activity against *Candida albicans*, *Aspergillus fumigatus*, *Aspergillus flavus*, and *Aspergillus niger* (recultured) in DMSO by serial plate dilution method [28].

The screening data of antifungal activity of these series of compounds shows moderate to good antifungal activity.

It is of interest that compounds **4i**, **4l** and **8d** showed pronounced antifungal activity against *A. niger* with MIC 1.56 μg/mL, while these compounds showed good activity against *A. fumigatus* and *A. flavus* with MIC 3.12 μg/mL and 6.25 μg/mL which is quite comparable with standard drug Fluconazole (MIC = 1.56 μg/mL, 3.12 μg/mL and 6.25 μg/mL), tested under similar conditions. High activity was demonstrated by **4l** against *A. fumigatus* with MIC 3.12 μg/mL, which is even more than standard drug Fluconazole for same pathogenic fungus. Compounds particularly **4i** and **4l** emerged as very potent compounds of this investigation.

It is interesting to note that a minor alteration in the molecular configuration of investigated compounds may have a pronounced effect on antimicrobial screening, e.g. compounds **4a**, **6a** and **8a** having no substituent in phenyl ring are less active, while compounds having *p*-chloro, *p*-methoxy and *o*-chloro substituent in benzene ring are more active than all other compounds. The compounds **4i** and **4l** have a fused 1,2,4-triazole ring at N-1 and C-2 of parent compound quinazolinone, and were found more potent than other compounds of this investigation, which having triazine and tetrazine ring fused at N-1 and C-2 of parent quinazolinone compound.

5. Conclusion

We have synthesized novel fused quinazolinones (**4**), (**6**) and (**8**) to evaluate them on antimicrobial screen.

From the antimicrobial data it seems that triazolo[4,3-*a*]-quinazolin-7-ones (**4**) seem to be more potent than tetrazino [4,3-*a*]-quinazolin-8-ones (**6**) and indolo[2,3-*c*][1,2,4]-triazino[4,3-*a*]-quinazoline-8-ones (**8**).

The data also revealed that presence of triazole moiety at N-1 and C-2 exerted more influence on the antimicrobial profile than triazine and tetrazine moieties.

Therefore, it can be inferred that presence of triazole nucleus at N-1 and C-2 of quinazolinone ring works better for antimicrobial activity of this series of compounds than others. The importance of such work lies in the possibility that the new compounds might be a more efficacious drug against bacteria and fungi for which a thorough investigation regarding the structure activity relationship, toxicity and its biological effects is essential, which could be helpful in designing more potent antimicrobial agents for therapeutic use.

6. Experimental

6.1. General

Procedure for one typical case for each step has been described. Melting points were taken in open capillaries and are uncorrected. UV spectra were recorded on UV–Visible spectrophotometer (Shimadzu 160). IR spectra were recorded in KBr on a Shimadzu 8201 PC Spectrophotometer (ν_{max} in cm^{-1}), ^1H NMR and ^{13}C NMR spectra in DMSO-d_6 on

a Bruker DRX-300 (300 MHz) spectrometer using TMS as an internal reference (chemical shifts in δ, ppm). Elemental analyses were performed on an elemental Vario EL III Carlo Erba 1108 CHN analyser. Elemental (C, H, N) analysis indicated that calculated and observed values were within the acceptable limits (±0.4%). The purity of compounds was checked by thin layer chromatography on silica gel plate using ether and ethyl acetate as a solvent system. Iodine chamber was used as a developing chamber. All the reagents used were AR grade.

6.2. General procedure for the preparation of 3-(substituted-phenyl)-2-mercapto-quinazolin-4-ones (**1**)

An equimolar mixture of anthranilic acid (0.15 mol) and substituted-phenylisothiocynate (0.15 mol) in acetone (150 ml) was refluxed for 6 h. The solvent was removed and residue was poured into water. The compound thus precipitated was filtered, washed, dried, and recrystallized from aq. ethanol.

6.2.1. 3-(4-Methyl phenyl)-2-mercapto-quinazolin-4-one (**1a**)

Yield 68%; mp 173–175 °C; IR (KBr) cm^{-1}: 2650 (–SH), 1675 (C=O), 1620 (C=N), 1560, 1525, 1440 (C=C); ^1H NMR (DMSO-d_6): δ 7.2–7.6 (m, 4H, ph–H), δ 7.9 (dd, 2H, $J = 8.4$, ph–H), δ 8.2 (dd, 2H, $J = 8.5$, ph–H), 2.4 (s, 3H, $-CH_3$), 3.3 (s, 1H, –SH). Anal. Calcd. for $C_{15}H_{12}N_2OS$: C, 67.14; H, 4.51; N, 10.44. Found: C, 67.01; H, 4.40; N, 10.34.

6.2.2. 3-(Phenyl)-2-mercapto-quinazolin-4-one (**1b**)

Yield 72%; mp 183–185 °C; IR (KBr) cm^{-1}: 2650 (–SH), 1680 (C=O), 1625 (C=N), 1556, 1518, 1432 (C=C); ^1H NMR (DMSO-d_6): δ 7.3–8.4 (m, 9H, ph–H), 2.9 (s, 1H, –SH). Anal. Calcd. for $C_{14}H_{10}N_2OS$: C, 66.12; H, 3.96; N, 11.02. Found: C, 66.01; H, 3.82; N, 11.24.

6.2.3. 3-(4-Meyhoxyphenyl)-2-mercapto-quinazolin-4-one (**1c**)

Yield 70%; mp 192–193 °C; IR (KBr) cm^{-1}: 2645 (–SH), 1672 (C=O),1630 (C=N), 1542, 1526, 1441 (C=C); ^1H NMR (DMSO-d_6): δ 7.3–7.8 (m, 4H, ph–H), δ 8.1 (dd, 2H, $J = 8.5$, ph–H), δ 8.4 (dd, 2H, $J = 8.7$, ph–H), 3.8 (s, 3H, $-OCH_3$), 3.1 (s, 1H, –SH). Anal. Calcd. for $C_{15}H_{12}N_2O_2S$: C, 63.36; H, 4.25; N, 9.85. Found: C, 63.01; H, 4.52; N, 9.62.

6.2.4. 3-(4-Chlorophenyl)-2-mercapto-quinazolin-4-one (**1d**)

Yield 74%; mp 189–191 °C; IR (KBr) cm^{-1}: 2650 (–SH), 1680 (C=O), 1624 (C=N), 1543, 1525, 1412 (C=C); ^1H NMR (DMSO-d_6): δ 7.4–7.8 (m, 4H, ph–H), δ 8.3 (dd, 2H, $J = 8.4$, ph–H), δ 8.5 (dd, 2H, $J = 8.6$, ph–H), 3.0 (s, 1H, –SH). Anal. Calcd. for $C_{14}H_9ClN_2OS$: C, 58.23; H, 3.14; N, 9.70. Found: C, 58.41; H, 3.22; N, 9.64.

6.3. General procedure for the preparation of 3-(substituted-phenyl)-2-mercaptomethyl-quinazolin-4-ones (**2**)

A mixture of 3-(substituted-phenyl)-2-mercapto-quinazolin-4-ones (**1**) (0.1 mol) and anhydrous sodium acetate (0.15 mol) dissolved in dioxane (100 ml) was treated with methyl iodide (0.1 mol) gradually with constant stirring for 4 h. The solvent was removed and the residue was poured into ice-cold water. The compound thus precipitated was filtered, washed, dried, and recrystallized from aq. ethanol.

6.3.1. 3-(4-Methylphenyl)-2-mercaptomethyl-quinazolin-4-one (**2a**)

Yield 71%; mp 167–169 °C; IR (KBr) cm^{-1}: 1670 (C=O), 1625 (C=N), 1590, 1550, 1480 (C=C) 1220 (C–S–C); ^1H NMR (DMSO-d_6): δ 7.2–7.5 (m, 4H, ph–H), δ 7.9 (dd, 2H, $J = 8.3$, ph–H), δ 8.2 (dd, 2H, $J = 8.4$, ph–H), 2.2 (s, 3H, $-CH_3$), 2.5 (s, 3H, $-SCH_3$). Anal. Calcd. for $C_{16}H_{14}N_2OS$: C, 68.06; H, 5.00; N, 9.92. Found: C, 68.21; H, 5.12; N, 9.84.

6.3.2. 3-(Phenyl)-2-mercaptomethyl-quinazolin-4-one (**2b**)

Yield 69%; mp 147–149 °C; IR (KBr) cm^{-1}: 1656 (C=O), 1629 (C=N), 1563, 1545, 1478 (C=C), 1217 (C–S–C); ^1H NMR (DMSO-d_6): δ 7.4–8.2 (m, 9H, ph–H), 2.4 (s, 3H, $-SCH_3$). Anal. Calcd. for $C_{15}H_{12}N_2OS$: C, 67.14; H, 4.51; N, 10.44. Found: C, 67.01; H, 4.32; N, 10.34.

6.3.3. 3-(4-Methoxyphenyl)-2-mercaptomethyl-quinazolin-4-one (**2c**)

Yield 73%; mp 168–171 °C; IR (KBr) cm^{-1}: 1679 (C=O), 1618 (C=N), 1578, 1549, 1470 (C=C), 1221 (C–S–C); ^1H NMR (DMSO-d_6): δ 7.2–7.8 (m, 4H, ph–H), δ 8.2 (dd, 2H, $J = 8.5$, ph–H), δ 8.3 (dd, 2H, $J = 8.6$, ph–H), 3.8 (s, 3H, $-OCH_3$), 2.4 (s, 3H, $-SCH_3$). Anal. Calcd. for $C_{16}H_{14}N_2O_2S$: C, 64.42; H, 4.73; N, 9.39. Found: C, 64.21; H, 4.62; N, 9.44.

6.3.4. 3-(4-Chlorophenyl)-2-mercaptomethyl-quinazolin-4-one (**2d**)

Yield 76%; mp above 200 °C; IR (KBr) cm^{-1}: 1659 (C=O), 1623 (C=N), 1564, 1546, 1476 (C=C), 1218 (C–S–C); ^1H NMR (DMSO-d_6): δ 7.4–7.9 (m, 4H, ph–H), δ 8.3 (dd, 2H, $J = 8.6$, ph–H), δ 8.6 (dd, 2H, $J = 8.7$, ph–H), 2.6 (s, 3H, $-SCH_3$). Anal. Calcd. for $C_{15}H_{11}N_2OSCl$: C, 59.50; H, 3.66; N, 9.25. Found: C, 59.31; H, 3.42; N, 9.34.

6.4. General procedure for the preparation of 3-(substituted-phenyl)-2-hydrazino-quinazolin-4-ones (**3**)

A mixture of 3-(substituted-phenyl)-2-mercaptomethyl-quinazolin-4-ones (**2**) (0.05 mol) and hydrazine hydrate (0.1 mol) in dioxane (50 ml) was refluxed for 6 h. The solvent was removed and residue was poured into ice-cold water. The compound thus precipitated was filtered, washed, dried, and recrystallized from aq. ethanol.

*6.4.1. 3-(4-Methylphenyl)-2-hydrazino-quinazolin-4-one (**3a**)*

Yield 68%; mp 114–116 °C; IR (KBr) cm^{-1}: 3425 (–NH), 1660 (C=O), 1622 (C=N), 1552, 1496, 1415 (C=C); ^1H NMR (DMSO-d_6): δ 7.2–7.7 (m, 4H, ph–H), δ 7.8 (dd, 2H, $J = 8.4$, ph–H), δ 8.1 (dd, 2H, $J = 8.5$, ph–H), 2.3 (s, 3H, $-CH_3$), 4.8 (br s, 2H, $-NH_2$), 8.3 (br s, 1H, NH). Anal. Calcd. for $C_{15}H_{14}N_4O$: C, 67.65; H, 5.30; N, 21.04. Found: C, 67.41; H, 5.42; N, 21.14.

*6.4.2. 3-(Phenyl)-2-hydrazino-quinazolin-4-one (**3b**)*

Yield 73%; mp 111–113 °C; IR (KBr) cm^{-1}: 3430 (–NH), 1656 (C=O), 1631 (C=N), 1536, 1445, 1418 (C=C); ^1H NMR (DMSO-d_6): δ 7.1–8.3 (m, 9H, ph –H), 4.5 (br s, 2H, $-NH_2$), 8.2 (br s, 1H, –NH); ^{13}C NMR (DMSO): δ 170.2, 160.1, 141.2, 140.5, 131.3, 130.9, 130.2, 129.9, 129.1, 127.6, 127.1, 124.2. Anal. Calcd. for $C_{14}H_{12}N_4O$: C, 66.65; H, 4.79; N, 22.21. Found: C, 66.43; H, 4.62; N, 22.34.

*6.4.3. 3-(4-Methoxyphenyl)-2-hydrazino-quinazolin-4-one (**3c**)*

Yield 69%; mp 149–151 °C; IR (KBr) cm^{-1}: 3433 (–NH), 1650 (C=O), 1618 (C=N), 1418, 1450, 1542 (C=C); ^1H NMR (DMSO-d_6): δ 7.2–7.9 (m, 4H, ph–H), δ 8.2 (dd, 2H, $J = 8.4$, ph–H), δ 8.4 (dd, 2H, $J = 8.6$, ph–H), 3.7 (s, 3H, $-OCH_3$), 4.6 (br s, 2H, $-NH_2$), 8.4 (br s, 1H, –NH). Anal. Calcd. for $C_{15}H_{14}N_4O_2$: C, 63.82; H, 5.00; N, 19.85. Found: C, 63.42; H, 5.12; N, 19.74.

*6.4.4. 3-(4-Chlorophenyl)-2-hydrazino-quinazolin-4-one (**3d**)*

Yield 67%; mp 171–173 °C; IR (KBr) cm^{-1}: 3429 (–NH), 1651 (C=O), 1615 (C=N), 1541, 1476, 1412 (C=C); ^1H NMR (DMSO-d_6): δ 7.5–7.9 (m, 4H, ph–H), δ 8.3 (dd, 2H, $J = 8.5$, ph–H), δ 8.6 (dd, 2H, $J = 8.7$, ph–H), 4.7 (br s, 2H, $-NH_2$), 8.2 (br s, 1H, –NH). Anal. Calcd. for $C_{14}H_{11}N_4OCl$: C, 58.65; H, 3.87; N, 12.37. Found: C, 58.49; H, 3.57; N, 12.12.

*6.5. General procedure for the preparation of 5,6-(disubstituted-phenyl)-[1,2,4]-triazolo[4,3-a]-quinazolin-7-ones (**4**)*

A mixture of 3-(substituted-phenyl)-2-hydrazino-quinazolin-4-one (**3**) (0.01 mol), substituted-benzoic acid (0.01 mol) and dicyclohexylcarbodiimide (DCC, 0.01 mol) in dichloromethane (20 ml) was stirred for 3 h. After this solvent was removed and the residue was poured into ice-cold water. The solid mass thus obtained was filtered, washed with water, dried and recrystallized from aq. ethanol.

*6.5.1. 5,6-(Diphenyl)-[1,2,4]-triazolo[4,3-a]-quinazolin-7-one (**4a**)*

Yield 74%; mp 187–189 °C; IR (KBr) cm^{-1}: 1662 (C=O), 1622 (C=N), 1542, 1486, 1400 (C=C), 1112 (C–N–C); ^1H NMR (DMSO-d_6): δ 7.2–8.4 (m, 14H, ph–H), ^{13}C NMR (DMSO): δ 170.2, 151.3, 149.2, 140.1, 137.0, 135.1, 133.1, 132.2, 130.8, 130.0, 129.8, 129.3, 129.0, 128.6, 128.1, 125.2, 122.2. Anal. Calcd. for $C_{21}H_{14}N_4O$: C, 74.55; H, 4.14; N, 16.56. Found: C, 74.41; H, 4.22; N, 16.35; UV (DMF): 289 nm, 223 nm, 206 nm.

*6.5.2. 5-(2-Methylphenyl)-6-(phenyl)-[1,2,4]-triazolo[4,3-a]-quinazolin-7-one (**4b**)*

Yield 73%; mp 176–178 °C; IR (KBr) cm^{-1}: 1660 (C=O), 1618 (C=N), 1531, 1470, 1415 (C=C), 1122 (C–N–C); ^1H NMR (DMSO-d_6): δ 7.1–8.2 (m, 13H, ph–H), 2.3 (s, 3H, $-CH_3$). Anal. Calcd. for $C_{22}H_{16}N_4O$: C, 75.00; H, 4.54; N, 15.90. Found: C, 75.31; H, 4.32; N, 15.75.

*6.5.3. 5-(2-Chlorophenyl)-6-(phenyl)-[1,2,4]-triazolo[4,3-a]-quinazolin-7-one (**4c**)*

Yield 70%; mp 169–171 °C; IR (KBr) cm^{-1}: 1660 (C=O), 1610 (C=N), 1554, 1465, 1405 (C=C), 1130 (C–N–C); ^1H NMR (DMSO-d_6): δ 7.3–8.4 (m, 13H, ph–H). Anal. Calcd. for $C_{21}H_{13}N_4OCl$: C, 67.65; H, 3.48; N, 15.03. Found: C, 67.41; H, 3.32; N, 15.25.

*6.5.4. 5-(Phenyl)-6-(4-methylphenyl)-[1,2,4]-triazolo[4,3-a]-quinazolin-7-one (**4d**)*

Yield 72%; mp 169–171 °C; IR (KBr) cm^{-1}: 1665 (C=O), 1620 (C=N), 1540, 1480, 1400 (C=C), 1110 (C–N–C); ^1H NMR (DMSO-d_6): δ 7.3–7.7 (m, 9H, ph–H), δ 7.9 (dd, 2H, $J = 8.5$, ph–H), δ 8.1 (dd, 2H, $J = 8.5$, ph–H), 2.3 (s, 3H, $-CH_3$). Anal. Calcd. for $C_{22}H_{16}N_4O$: C, 75.00; H, 4.54; N, 15.90. Found: C, 75.31; H, 4.32; N, 15.75.

*6.5.5. 5-(2-Methylphenyl)-6-(4-methylphenyl)-[1,2,4]-triazolo[4,3-a]-quinazolin-7-one (**4e**)*

Yield 62%; mp 177–178 °C; IR (KBr) cm^{-1}: 1666 (C=O), 1630 (C=N), 1525, 1478, 1410 (C=C), 1129 (C–N–C); ^1H NMR (DMSO-d_6): δ 7.2–7.8 (m, 8H, ph–H), δ 7.9 (dd, 2H, $J = 8.3$, ph–H), δ 8.0 (dd, 2H, $J = 8.4$, ph–H), 2.1 (s, 6H, $2 \times -CH_3$). Anal. Calcd. for $C_{23}H_{18}N_4O$: C, 79.31; H, 5.17; N, 16.09. Found: C, 79.11; H, 5.32; N, 16.25.

*6.5.6. 5-(2-Chlorophenyl)-6-(4-methylphenyl)-[1,2,4]-triazolo [4,3-a]-quinazolin-7-one (**4f**)*

Yield 64%; mp 181–183 °C; IR (KBr) cm^{-1}: 1670 (C=O), 1610 (C=N), 1535, 1470, 1419 (C=C), 1135 (C–N–C); ^1H NMR (DMSO-d_6): δ 7.3–7.9 (m, 8H, ph–H), δ 8.0 (dd, 2H, $J = 8.5$, ph–H), δ 8.1 (dd, 2H, $J = 8.6$, ph–H), 2.2 (s, 3H, $-CH_3$). Anal. Calcd. for $C_{22}H_{15}N_4OCl$: C, 68.30; H, 3.88; N, 14.48. Found: C, 68.10; H, 3.66; N, 14.29.

*6.5.7. 5-(Phenyl)-6-(4-methoxylphenyl)-[1,2,4]-triazolo[4,3-a]-quinazolin-7-one (**4g**)*

Yield 70%; mp 180–182 °C; IR (KBr) cm^{-1}: 1660 (C=O), 1635 (C=N), 1540, 1488, 1429 (C=C), 1135 (C–N–C); ^1H NMR (DMSO-d_6): δ 7.2–7.9 (m, 9H, ph–H), δ 8.3 (dd, 2H, $J = 8.5$, ph–H), δ 8.4 (dd, 2H, $J = 8.6$, ph–H), 3.7 (s, 3H, $-OCH_3$). Anal. Calcd. for $C_{22}H_{16}N_4O_2$: C, 71.73; H, 4.34; N, 15.21. Found: C, 71.50; H, 4.20; N, 15.15.

*6.5.8. 5-(2-Methylphenyl)-6-(4-methoxylphenyl)-[1,2,4]-triazolo[4,3-a]-quinazolin-7-one (**4h**)*

Yield 69%; mp 185–187 °C; IR (KBr) cm^{-1}: 1670 (C=O), 1640 (C=N), 1539, 1460, 1420 (C=C), 1126 (C–N–C); ^1H NMR (DMSO-d_6): δ 7.3–7.9 (m, 8H, ph–H), δ 8.2 (dd, 2H, $J = 8.4$, ph–H), δ 8.3 (dd, 2H, $J = 8.5$, ph–H) 3.8 (s, 3H, $-OCH_3$), 2.2 (s, 3H, $-CH_3$). Anal. Calcd. for $C_{23}H_{18}N_4O_2$: C, 72.25; H, 4.71; N, 14.65. Found: C, 72.30; H, 4.50; N, 14.45.

*6.5.9. 5-(2-Chlorolphenyl)-6-(4-methoxylphenyl)-[1,2,4]-triazolo[4,3-a]-quinazolin-7-one (**4i**)*

Yield 73%; mp 188–190 °C; IR (KBr) cm^{-1}: 1664 (C=O), 1629 (C=N), 1530, 1440, 1415 (C=C), 1115 (C–N–C); ^1H NMR (DMSO-d_6): δ 7.3–7.9 (m, 8H, ph–H), δ 8.3 (dd, 2H, $J = 8.4$, ph–H), δ 8.4 (dd, 2H, $J = 8.5$, ph–H) 3.7 (s, 3H, $-OCH_3$). Anal. Calcd. for $C_{21}H_{15}N_4O_2Cl$: C, 65.59; H, 3.72; N, 13.91. Found: C, 65.40; H, 3.50; N, 13.70.

*6.5.10. 5-(Phenyl)-6-(4-chlorophenyl)-[1,2,4]-triazolo[4,3-a]-quinazolin-7-one (**4j**)*

Yield 73%; mp 171–173 °C; IR (KBr) cm^{-1}: 1665 (C=O), 1620 (C=N), 1545, 1425, 1410 (C=C), 1116 (C–N–C); ^1H NMR (DMSO-d_6): δ 7.5–7.9 (m, 9H, ph–H), δ 8.4 (dd, 2H, $J = 8.5$, ph–H), δ 8.6 (dd, 2H, $J = 8.7$, ph–H). Anal. Calcd. for $C_{21}H_{13}N_4OCl$: C, 67.74; H, 3.49; N, 15.05. Found: C, 67.40; H, 3.26; N, 15.34.

*6.5.11. 5-(2-Methylphenyl)-6-(4-chlorophenyl)-[1,2,4]-triazolo[4,3-a]-quinazolin-7-one (**4k**)*

Yield 70%; mp 175–177 °C; IR (KBr) cm^{-1}: 1660 (C=O), 1634 (C=N), 1560, 1440, 1416 (C=C), 1130 (C–N–C); ^1H NMR (DMSO-d_6): δ 7.5–7.9 (m, 8H, ph–H), δ 8.4 (dd, 2H, $J = 8.6$, ph–H), δ 8.5 (dd, 2H, $J = 8.7$, ph–H), 2.3 (s, 3H, $-CH_3$). Anal. Calcd. for $C_{22}H_{15}N_4OCl$: C, 68.30; H, 3.88; N, 14.48. Found: C, 68.15; H, 3.67; N, 14.36.

*6.5.12. 5-(2-Chlorophenyl)-6-(4-chlorophenyl)-[1,2,4]-triazolo[4,3-a]-quinazolin-7-one (**4l**)*

Yield 75%; mp 161–163 °C; IR (KBr) cm^{-1}: 1670 (C=O), 1629 (C=N), 1546, 1419, 1407 (C=C), 1119 (C–N–C); ^1H NMR (DMSO-d_6): δ 7.5–8.0 (m, 8H, ph–H), δ 8.3 (dd, 2H, $J = 8.5$, ph–H), δ 8.5 (dd, 2H, $J = 8.6$, ph–H). Anal. Calcd. for $C_{22}H_{12}N_4OCl_2$: C, 61.91; H, 2.94; N, 13.75. Found: C, 61.80; H, 2.70; N, 13.40.

*6.6. General procedure for the preparation of 3-(substituted-phenyl)-2-[4-substituted-phenyl-thiosemicarbazone]-quinazolin-4-ones (**5**)*

A mixture of 3-(substituted-phenyl)-2-hydrazino-quinazolin-4-one (**3**) (0.01 mol) and substituted-phenylisothiocyanate (0.01 mol) in methanol (25 ml) was refluxed for 4 h. The solvent was removed and water was added to it. The precipitated solid mass was filtered, washed with water, dried and recrystallized from aq. ethanol.

*6.6.1. 3-(Phenyl)-2-[4-(phenyl)-thiosemicarbazone]-quinazolin-4-one (**5a**)*

Yield 71%; mp 161–163 °C; IR (KBr) cm^{-1}: 3325 (–N–H), 1680 (C=O), 1629 (C=N), 1561, 1439, 1427 (C=C), 1235 (C=S), 1119 (C–N–C); ^1H NMR (DMSO-d_6): δ 7.1–8.3 (m, 14H, ph–H), 8.5–8.6 (br s, 3H, –NH). Anal. Calcd. for $C_{21}H_{17}N_5OS$: C, 65.11; H, 4.39; N, 18.08. Found: C, 65.26; H, 4.50; N, 18.24.

*6.6.2. 3-(Phenyl)-2-[4-(4-methylphenyl)-thiosemicarbazone]-quinazolin-4-one (**5b**)*

Yield 70%; mp 169–171 °C; IR (KBr) cm^{-1}: 3331 (–N–H), 1665 (C=O), 1618 (C=N), 1545, 1430, 1415 (C=C), 1221 (C=S),1126 (C–N–C); ^1H NMR (DMSO-d_6): δ 7.2–7.7 (m, 9H, ph–H), δ 7.8 (dd, 2H, $J = 8.4$, ph–H), δ 8.0 (dd, 2H, $J = 8.4$, ph–H), 2.3 (s, 3H, $-CH_3$), 8.5–8.7 (br s, 3H, –NH). Anal. Calcd. for $C_{22}H_{19}N_5OS$: C, 65.83; H, 4.77; N, 17.44. Found: C, 65.66; H, 4.50; N, 17.20.

*6.6.3. 3-(Phenyl)-2-[4-(4-methoxyphenyl)-thiosemicarbazone]-quinazolin-4-one (**5c**)*

Yield 74%; mp 180–181 °C; IR (KBr) cm^{-1}: 3339 (–N–H), 1650 (C=O), 1640 (C=N), 1570, 1441, 1410 (C=C), 1231 (C=S), 1134 (C–N–C); ^1H NMR (DMSO-d_6): δ 7.2–7.8 (m, 9H, ph–H), δ 8.2 (dd, 2H, $J = 8.3$, ph–H), δ 8.3 (dd, 2H, $J = 8.5$, ph–H), 3.8 (s, 3H, $-OCH_3$), 8.5–8.6 (br s, 3H, –NH). Anal. Calcd. for $C_{22}H_{19}N_5O_2S$: C, 65.31; H, 4.56; N, 17.45. Found: C, 65.50; H, 4.36; N, 17.24.

*6.6.4. 3-(4-Methylphenyl)-2-[4-(phenyl)-thiosemicarbazone]-quinazolin-4-one (**5d**)*

Yield 71%; mp 166–167 °C; IR (KBr) cm^{-1}: 3320 (–N–H), 1675 (C=O), 1630 (C=N), 1560 1470, 1420 (C=C), 1230 (C=S),1120 (C–N–C); ^1H NMR (DMSO-d_6): δ 7.2–7.6 (m, 9H, ph–H), δ 7.8 (dd, 2H, $J = 8.4$, ph–H), δ 8.1 (dd, 2H, $J = 8.5$, ph–H), 2.2 (s, 3H, $-CH_3$), 8.5–8.7 (br s, 3H, –NH). Anal. Calcd. for $C_{22}H_{19}N_5OS$: C, 65.83; H, 4.77; N, 17.44. Found: C, 65.60; H, 4.51; N, 17.29.

*6.6.5. 3-(4-Methylphenyl)-2-[4-(4-methylphenyl)-thiosemicarbazone]-quinazolin-4-one (**5e**)*

Yield 71%; mp 185–187 °C; IR (KBr) cm^{-1}: 3331 (–N–H), 1656 (C=O), 1635 (C=N), 1535, 1430,1421 (C=C), 1235 (C=S),1136 (C–N–C); ^1H NMR (DMSO-d_6): δ 7.2–7.6 (m, 4H, ph–H), δ 7.8–7.9 (dd, 4H, $J = 8.3$–8.4, ph–H), δ 8.0–8.1 (dd, 4H, $J = 8.4$–8.6, ph–H), 2.3 (s, 6H, $2 \times -CH_3$), 8.5–8.6 (br s, 3H, –NH). Anal. Calcd. for $C_{23}H_{21}N_5OS$: C, 66.50; H, 5.06; N, 16.86. Found: C, 66.71; H, 5.30; N, 16.53.

*6.7. General procedure for the preparation of 5,7-(disubstituted-phenyl)-6-thioxo-[1,2,4,5]tetrazino[4,3-a]-quinazolin-8-ones (**6**)*

A mixture of 3-(substituted-phenyl)-2-[4-(substituted-phenyl)-thiosemicarbazone]-quinazolin-4-ones (**5**) (0.01 mol) and bromine (0.015 mol) in CCl_4 (10 ml) was stirred at

room temperature for 30 min. The mixture was cooled, when fine crystals separated out which were filtered and recrystallized from aq. ethanol.

*6.7.1. 5,7-(Diphenyl)-6-thioxo-[1,2,4,5]-tetrazino[4,3-a]-quinazolin-8-one (**6a**)*

Yield 71%; mp 180–181 °C; IR (KBr) cm^{-1}: 3300 (–N–H), 1660 (C=O), 1616 (C=N), 1556, 1436, 1415 (C=C), 1220 (C=S),1136 (C–N–C); ^{1}H NMR (DMSO-d_6): δ 7.0–8.3 (m, 14H, ph–H), 8.0 (s, 1H, –N–H). Anal. Calcd. for $C_{21}H_{15}N_5OS$: C, 65.45; H, 3.89; N, 18.18. Found: C, 65.60; H, 3.60; N, 18.33. UV (DMF): 293 nm, 273 nm, 224 nm, 206 nm, 202 nm.

*6.7.2. 5-(4-Methylphenyl)-7-(phenyl)-6-thioxo-[1,2,4,5]-tetrazino[4,3-a]-quinazolin-8-ones (**6b**)*

Yield 73%; mp 172–174 °C; IR (KBr) cm^{-1}: 3305 (–N–H), 1672 (C=O), 1630 (C=N), 1570, 1445, 1400 (C=C), 1235 (C=S), 1140 (C–N–C); ^{1}H NMR (DMSO-d_6): δ 7.2–7.7 (m, 9H, ph–H), δ 7.9 (dd, 2H, $J = 8.5$, ph–H), δ 8.1 (dd, 2H, $J = 8.6$, ph–H), 2.3 (s, 3H, $-CH_3$), 7.8 (s, 1H, –N–H), ^{13}C NMR (DMSO): 190.1, 170.3, 151.1, 148.2, 143.1, 142.1, 141.9, 133.3, 130.1, 129.4, 129.1, 128.1, 126.2, 125.3, 124.1, 121.9, 121.4, 121.0. Anal. Calcd. for $C_{21}H_{17}N_5OS$: C, 66.16; H, 4.26; N, 17.54. Found: C, 66.29; H, 4.40; N, 17.40.

*6.7.3. 5-(4-Methoxyphenyl)-7-(phenyl)-6-thioxo-[1,2,4,5]-tetrazino[4,3-a]-quinazolin-8-ones (**6c**)*

Yield 71%; mp 179–181 °C; IR (KBr) cm^{-1}: 3309 (–N–H), 1650 (C=O), 1615 (C=N), 1565, 1450, 1420 (C=C), 1229 (C=S),1131 (C–N–C); ^{1}H NMR (DMSO-d_6): δ 7.2–7.9 (m, 9H, ph–H), δ 8.2 (dd, 2H, $J = 8.3$, ph–H), δ 8.4 (dd, 2H, $J = 8.6$, ph–H), 3.8 (s, 3H, $-OCH_3$), 8.2 (s, 1H, –N–H). Anal. Calcd. for $C_{21}H_{17}N_5O_2S$: C, 66.61; H, 4.10; N, 16.87. Found: C, 66.20; H, 4.22; N, 16.52.

*6.7.4. 5-(Phenyl)-7-(4-methylphenyl)-6-thioxo-[1,2,4,5]-tetrazino[4,3-a]-quinazolin-8-ones (**6d**)*

Yield 70%; mp 188–189 °C; IR (KBr) cm^{-1}: 3310 (–N–H), 1632 (C=O), 1622 (C=N), 1575, 1459,1436 (C=C), 1220 (C=S), 1121 (C–N–C); ^{1}H NMR (DMSO-d_6): δ 7.3–7.7 (m, 9H, ph–H), δ 7.8 (dd, 2H, $J = 8.4$, ph–H), δ 8.0 (dd, 2H, $J = 8.5$, ph–H), 2.2 (s, 3H, $-CH_3$), 8.3 (s, 1H, –N–H). Anal. Calcd. for $C_{22}H_{17}N_5OS$: C, 66.16; H, 4.26; N, 17.54. Found: C, 66.29; H, 4.31; N, 17.80.

*6.7.5. 5-(4-Methylphenyl)-7-(4-methylphenyl)-6-thioxo-[1,2,4,5]-tetrazino-[4,3-a]-quinazolin-8-ones (**6e**)*

Yield 69%; mp 193–194 °C; IR (KBr) cm^{-1}: 3301 (–N–H), 1670 (C=O), 1610 (C=N), 1564, 1444, 1430 (C=C), 1235 (C=S),1131 (C–N–C); ^{1}H NMR (DMSO-d_6): δ 7.2–7.7 (m, 4H, ph–H), δ 7.8–8.0 (dd, 4H, $J = 8.3$–8.4, ph–H), δ 8.0–8.2 (dd, 4H, $J = 8.4$–8.6, ph–H), 2.1 (s, 6H, $2 \times -CH_3$), 8.2 (s, 1H, –N–H). Anal. Calcd. for $C_{23}H_{19}N_5OS$: C, 66.82; H, 4.60; N, 16.94. Found: C, 66.61; H, 4.40; N, 16.50.

*6.8. General procedure for the preparation of 3-(substituted-phenyl)-2-(isatinhydrazone-3′-yl)-quinazolin-4-ones (**7**)*

A mixture of 3-(substituted-phenyl)-2-hydrazino-quinazolin-4-one (**3**) (0.01 mol) and isatin (0.01 mol) in methanol (20 ml) was refluxed for 2 h. The solvent was removed and the residue was poured into water. The solid mass thus obtained was filtered washed with water, dried and recrystallized from aq. ethanol.

*6.8.1. 3-(Phenyl)-2-(isatinhydrazone-3′-yl)-quinazolin-4-ones (**7a**)*

Yield 69%; mp 174–176 °C; IR (KBr) cm^{-1}: 3190 (–N–H), 1660 (C=O, five membered ring), 1660 (C=O, six membered ring), 1630 (exo C=N), 1610 (endo C=N), 1510, 1444, 1400 (C=C); ^{1}H NMR (DMSO-d_6): δ 6.9–8.0 (m, 13H, ph–H), 8.2 (br s, 1H, –N–H), 8.8 (br s, 1H, –N–H). Anal. Calcd. for $C_{22}H_{15}N_5O_2$: C, 69.29; H, 3.93; N, 18.37. Found: C, 69.50; H, 3.32; N, 18.51.

*6.8.2. 3-(4-Methylphenyl)-2-(isatinhydrazone-3′-yl)-quinazolin-4-ones (**7b**)*

Yield 71%; mp 181–183 °C; IR (KBr) cm^{-1}: 3200 (–N–H), 1675 (C=O, five membered ring), 1655 (C=O, six membered ring), 1620 (exo C=N), 1600 (endo C=N), 1530, 1460, 1420 (C=C); ^{1}H NMR (DMSO-d_6): δ 7.2–7.8 (m, 8H, ph–H), δ 7.9 (dd, 2H, $J = 8.3$, ph–H), δ 8.2 (dd, 2H, $J = 8.4$, ph–H), 2.2 (s, 3H, $-CH_3$) 8.3 (br s, 1H, –N–H), 8.9 (br s, 1H, –N–H). Anal. Calcd. for $C_{23}H_{17}N_5O_2$: C, 69.87; H, 4.30; N, 17.72. Found: C, 69.60; H, 4.49; N, 17.80.

*6.8.3. 3-(4-Methoxyphenyl)-2-(isatinhydrazone-3′-yl)-quinazolin-4-ones (**7c**)*

Yield 69%; mp 185–186 °C; IR (KBr) cm^{-1}: 3200 (–N–H), 1660 (C=O, five membered ring), 1650 (C=O, six membered ring), 1625 (exo C=N), 1616 (endo C=N), 1545, 1440, 1415 (C=C); ^{1}H NMR (DMSO-d_6): δ 7.2–8.0 (m, 8H, ph–H), δ 8.3 (dd, 2H, $J = 8.5$, ph–H), δ 8.4 (dd, 2H, $J = 8.6$, ph–H), 3.8 (s, 3H, $-OCH_3$) 8.3 (br s, 1H, –N–H), 8.7 (br s, 1H, –N–H). Anal. Calcd. for $C_{23}H_{17}N_5O_3$: C, 67.15; H, 4.13; N, 17.03. Found: C, 67.35; H, 4.30; N, 17.10.

*6.8.4. 3-(4-Chlorophenyl)-2-(isatinhydrazone-3′-yl)-quinazolin-4-ones (**7d**)*

Yield 69%; mp 171–173 °C; IR (KBr) cm^{-1}: 3195 (–N–H), 1673 (C=O, five membered ring), 1660 (C=O, six membered ring), 1622 (exo C=N), 1620 (endo C=N), 1540, 1432, 1419 (C=C); ^{1}H NMR (DMSO-d_6): δ 7.5–7.9 (m, 8H, ph–H), δ 8.3 (dd, 2H, $J = 8.4$, ph–H), δ 8.5 (dd, 2H, $J = 8.6$, ph–H), 8.2 (br s, 1H, –N–H), 8.8 (br s, 1H, –N–H). Anal. Calcd. for $C_{22}H_{14}N_5O_2Cl$: C, 63.13; H, 3.36; N, 16.84. Found: C, 63.25; H, 3.49; N, 16.98.

6.9. General procedure for the preparation of 3-(substituted-phenyl)-indolo-[2,3-c] [1,2,4]-triazino[4,3-a]-quinazolin-8-ones (**8**)

Slurry of 3-(substituted-phenyl)-2-(isatinhydrazone-3′-yl)-quinazolin-4-ones (**7**) (0.01 mol) in conc. H_2SO_4 (5.0 ml) was made below 15 °C and left for 2 h. Sufficient amount of ice-cold water was added to the reaction mixture and the precipitated mass was filtered and neutralized with ammonia solution. The compound thus obtained was filtered, dried, and recrystallized from aq. ethanol.

6.9.1. 3-(Phenyl)-indolo[2,3-c][1,2,4]-triazino[4,3-a]-quinazolin-8-ones (**8a**)

Yield 70%; mp 178–180 °C; IR (KBr) cm^{-1}: 1655 (C═O), 1630 (C═N), 1510, 1440, 1400 (C═C); ^{1}H NMR (DMSO-d_6): δ 6.8–8.0 (m, 13H, ph–H). Anal. Calcd. for $C_{22}H_{13}N_5O$: C, 72.72; H, 3.58; N, 19.28. Found: C, 72.51; H, 3.70; N, 19.52; UV (DMF): 301 nm, 253 nm, 240 nm, 220 nm, 212 nm.

6.9.2. 3-(4-Methylphenyl)-indolo[2,3-c][1,2,4]-triazino[4,3-a]-quinazolin-8-ones (**8b**)

Yield 71%; mp 187–189 °C; IR (KBr) cm^{-1}: 1660 (C═O), 1630 (C═N), 1510, 1440, 1400 (C═C); ^{1}H NMR (DMSO-d_6): δ 7.2–7.8 (m, 8H, ph–H), δ 7.9 (dd, 2H, $J = 8.2$, ph–H), δ 8.2 (dd, 2H, $J = 8.3$, ph–H), 2.3 (s, 3H, $-CH_3$). Anal. Calcd. for $C_{23}H_{15}N_5O$: C, 73.20; H, 3.97; N, 18.56. Found: C, 73.46; H, 3.80; N, 18.29.

6.9.3. 3-(4-Methoxyphenyl)-indolo[2,3-c][1,2,4]-triazino[4,3-a]-quinazolin-8-ones (**8c**)

Yield 69%; mp above 200; IR (KBr) cm^{-1}: 1662 (C═O), 1625 (C═N), 1519, 1450, 1410 (C═C); ^{1}H NMR (DMSO-d_6): δ 7.3–8.0 (m, 8H, ph–H), δ 8.3 (dd, 2H, $J = 8.4$, ph–H), δ 8.4 (dd, 2H, $J = 8.5$, ph–H), 3.7 (s, 3H, $-OCH_3$). Anal. Calcd. for $C_{23}H_{15}N_5O_2$: C, 70.22; H, 3.81; N, 17.81. Found: C, 70.03; H, 3.50; N, 17.43.

6.9.4. 3-(4-Chlorophenyl)indolo[2,3-c][1,2,4]-triazino[4,3-a]-quinazolin-8-ones (**8d**)

Yield 68%; mp 176–178 °C; IR (KBr) cm^{-1}: 1665 (C═O), 1630 (C═N), 1530, 1462, 1421 (C═C); ^{1}H NMR (DMSO-d_6): δ 7.5–8.0 (m, 8H, ph–H), δ 8.3 (dd, 2H, $J = 8.5$, ph–H), δ 8.4 (dd, 2H, $J = 8.5$, ph–H). Anal. Calcd. for $C_{22}H_{12}N_5OCl$: C, 66.41; H, 3.01; N, 17.61. Found: C, 66.30; H, 3.19; N, 17.78.

6.10. Antibacterial screening

Preliminary experiments were carried out to determine the antibacterial activities of titled compounds (**4a–4l**, **6a–6e** and **8a–8d**) in vitro against (i) Gram-positive bacteria: *S. pneumoniae*, *B. subtilis* and (ii) Gram-negative bacteria: *E. coli*, *P. aeruginosa* by disc diffusion method [27]. The bacterial strains were subcultured in broth agar and incubated for 18 h at 37 °C and then freshly prepared bacterial cells were spread onto nutrient agar plate in a laminar flow cabinet. Sterilized paper disks (6.0 mm in diameter) were placed on the nutrient agar plates. Five milligrams of each test compounds were dissolved in 1 mL of dimethylsulfoxide (DMSO) separately to prepare stock solution. From stock solution different concentrations 50 μg/mL, 25 μg/mL, 12.5 μg/mL, 6.25 μg/mL, 3.12 μg/mL and 1.56 μg/mL of each compound were prepared. Thus, proper amounts of the different concentrations of compounds were pipetted on the blank disks, which were placed on the plates. The plates were incubated at 37 °C for 24 h. The minimum inhibitory concentrations (MICs), the lowest concentration (μg/mL) of the test compound that resulted no visible growth on the plates were recorded in Table 1. DMSO was used as a solvent control to ensure that solvent had no effect on bacterial growth. Ciprofloxacin was designated in our experiment as a control drug.

6.11. Antifungal screening

Titled compounds were screened for their antifungal activity against *C. albicans*, *A. fumigatus*, *A. flavus*, and *A. niger* (recultured) in DMSO by serial plate dilution method [28]. Test compound (5 mg) were dissolved in 1 mL of dimethylsulfoxide (DMSO) and solution was diluted with water (9 mL). Further progressive dilutions with melted Muller-Hinton agar were performed to obtain required concentrations of 50 μg/mL, 25 μg/mL, 12.5 μg/mL, 6.25 μg/mL, 3.12 μg/mL and 1.56 μg/mL. Petridishes were inoculated with 1.5 x 10^{-4} colony forming units (cfu) and incubated at 37 °C for 26 h. The minimum inhibitory concentrations (MICs) in μg/mL were noted. To ensure that solvent had no effect on fungal growth a control test was performed with test medium

Table 2
Antifungal activities of compounds [**4a–8d**]

Compound no.	R	R′	MICs (μg/mL)			
			C. albicans	*A. fumigatus*	*A. flavus*	*A. niger*
4a	H	H	25	>50	>50	25
4b	H	2-CH_3	50	25	25	>50
4c	H	2-Cl	25	50	12.5	6.25
4d	4-CH_3	H	>50	25	50	50
4e	4-CH_3	2-CH_3	25	12.5	50	12.5
4f	4-CH_3	2-Cl	12.5	25	50	6.25
4g	4-OCH_3	H	6.25	12.5	25	3.12
4h	4-OCH_3	2-CH_3	12.5	6.25	25	6.25
4i	4-OCH_3	2-Cl	6.25	3.12	3.12	1.56
4j	4-Cl	H	12.5	6.25	12.5	25
4k	4-Cl	2-CH_3	6.25	12.5	3.12	12.5
4l	4-Cl	2-Cl	1.56	3.12	6.25	1.56
6a	H	H	25	>50	>50	25
6b	H	4-CH_3	>50	25	12.5	25
6c	H	4-OCH_3	6.25	12.5	25	3.12
6d	4-CH_3	H	25	12.5	12.5	25
6e	4-CH_3	4-CH_3	25	50	12.5	25
8a	H	–	>50	25	25	>50
8b	4-CH_3	–	25	12.5	25	50
8c	4-OCH_3	–	6.25	6.25	3.12	12.5
8d	4-Cl	–	3.12	6.25	3.12	1.56
Fluconazole	–	–	1.56	6.25	3.12	1.56

supplemented with DMSO at the same dilutions as used in the experiment. Fluconazole was used as a standard drug. The results are incorporated in Table 2.

Acknowledgement

The authors are thankful to the Head, Sophisticated Analytical Instrument Facility, Central Drug Research Institute, Lucknow, for providing IR, ^{1}H NMR, & ^{13}C NMR spectral data. Authors are grateful to CSIR, New Delhi for financial assistance. The authors are thankful to Prof. S. K. Sengupta & Dr. O. P. Pandey, Department of Chemistry, D.D.U. Gorakhpur University, Gorakhpur, respectively, for providing laboratory facilities for antifungal activities. Authors are also thankful to Department of Biotechnology, D.D.U. Gorakhpur University, Gorakhpur, for help in evaluating antibacterial activities.

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