Available online at www.sciencedirect.com
ScienceDirect
European Journal of Medicinal Chemistry 44 (2009) 2328–2333
EUROPEAN JOURNAL OF MEDICINAL CHEMISTRY
http://www.elsevier.com/locate/ejmech

Laboratory note

Synthesis and anti-microbial screening of some Schiff bases of 3-amino-6,8-dibromo-2-phenylquinazolin-4(3*H*)-ones

Perumal Panneerselvam*, Bilal Ahmad Rather, Dontireddy Ravi Sankar Reddy, Natesh Ramesh Kumar

Department of Pharmaceutical Chemistry, C.L. Baid Metha College of Pharmacy, Jyothi Nagar, Rajiv Gandhi Salai, Thorapakkam, Chennai 600 097, Tamil Nadu, India

Received 27 February 2008; received in revised form 20 March 2008; accepted 8 April 2008
Available online 29 April 2008

Abstract

In the present study, a series of novel Schiff bases were synthesized by condensation of 3-amino-6,8-dibromo-2-phenylquinazolin-4(3*H*)-ones with different aromatic aldehydes via cyclized intermediate 6,8-dibromo-2-phenyl benzoxazin-4-one. The chemical structures were confirmed by means of IR, ^{1}H NMR, ^{13}C NMR, Mass spectral and Elemental analysis. These compounds were screened for anti-bacterial (*Staphylococcus aureus* ATCC-9144, *Staphylococcus epidermidis* ATCC-155, *Micrococcus luteus* ATCC-4698, *Bacillus cereus* ATCC-11778, *Escherichia coli* ATCC-25922, *Pseudomonas aeruginosa* ATCC-2853, and *Klebsiella pneumoniae* ATCC-11298) and anti-fungal (*Aspergillus niger* ATCC-9029 and *Aspergillus fumigatus* ATCC-46645) activities by paper disc diffusion technique. The minimum inhibitory concentrations (MICs) of the compounds were also determined by agar streak dilution method. Among the synthesized compounds 3-(3,4,5-trimethoxybenzylideneamino)-6,8-dibromo-2-phenylquinazolin-4(3*H*)-one **10** was found to be the most potent anti-microbial activity with MICs of 18.9, 19.1, 18.8, 21.7, 18.2, 19.3, 16.7, 8.6 and 10.1 μg/ml against above mentioned respective strains. Compounds were found to exhibit more anti-fungal than anti-bacterial activity.

Keywords: Quinazolin-4(3*H*)-one; Schiff base; Anti-bacterial; Anti-fungal

1. Introduction

The search of new anti-microbial agents with reduced toxicity and lower side effects is of continuous process. One of the most frequently encountered heterocyclic in medicinal chemistry is quinazolin-4(3*H*)-one and its derivatives were reported to possess diverse biological applications including anti-microbial [1–5], analgesic and anti-inflammatory [6,7], anti-convulsant [8], anti-cancer [9], anti-tubercular [10], anti-malarial [11], anti-viral [12] and anti-helmintic [13] activities. The literature survey revealed that the presence of substituted aromatic ring at position 3 as well as substituent like methyl and phenyl groups at position 2 is necessary requirement for its medicinal properties. Quinazolin-4(3*H*)-ones with substitution at 3rd position has been reported to be associated with anti-microbial properties [14,15]. The various substitutions at 3rd position of the quinazolin-4(3*H*)-ones which were reported are substituted phenyl ring moieties [16,17], bridged phenyl rings [18,19], heterocyclic rings [20,21] and aliphatic system [22,23]. In general Schiff bases and presence of pharmacophores like $-NO_2$, −Br, phenolic −OH, and −Cl are reported to possess anti-microbial activities [5,24,25]. These observations led to the conception that a series of novel Schiff bases of 3-amino-6,8-dibromo-2-phenylquinazolin-4(3*H*)-ones **1**–**12** were synthesized using different aromatic aldehydes by condensation and their chemical structures were confirmed by IR, ^{1}H NMR, ^{13}C NMR, Mass spectral and Elemental analysis. These compounds were screened for their anti-bacterial activity against four Gram-positive bacteria (*Staphylococcus aureus* ATCC-9144, *Staphylococcus*

* Corresponding author. Tel.: +91 44 24960151/24960425, +91 9442712951 (mobile).
E-mail address: pps2k2000@yahoo.co.in (P. Panneerselvam).

0223-5234/$ - see front matter © 2008 Elsevier Masson SAS. All rights reserved.
doi:10.1016/j.ejmech.2008.04.010

epidermidis ATCC-155, *Micrococcus luteus* ATCC-4698 and *Bacillus cereus* ATCC-11778), three Gram-negative bacteria (*Escherichia coli* ATCC-25922, *Pseudomonas aeruginosa* ATCC-2853 and *Klebsiella pneumoniae* ATCC-11298) and anti-fungal (*Aspergillus niger* ATCC-9029 and *Aspergillus fumigatus* ATCC-46645) activities by paper disc diffusion technique. The minimum inhibitory concentrations (MICs) of the compounds were also determined by agar streak dilution method.

2. Chemistry

In the present study 3,5-dibromoanthranilic acid **1** was reacted with benzoyl chloride in the presence of pyridine to form cyclized intermediate 6,8-dibromo-2-phenyl benzoxazin-4-one **2** which further treated with hydrazine hydrate resulted in 3-amino-6,8-dibromo-2-phenylquinazolin-4(3*H*)-one **3**. The compound **3** was subjected to react with various aromatic aldehydes in the presence of ethanol as a solvent to form a series of novel Schiff bases **1**–**12**.

3. Biology investigation

3.1. Anti-microbial activity

All the synthesized compounds were screened for anti-microbial activities by paper disc diffusion technique. The tested micro-organism strains were: *S. aureus* (ATCC-9144), *S. epidermidis* (ATCC-155), *M. luteus* (ATCC-4698), *B. cereus* (ATCC-11778), *E. coli* (ATCC-25922), *P. aeruginosa* (ATCC-2853), *K. pneumoniae* (ATCC-11298), *A. niger* (ATCC-9029) and *A. fumigatus* (ATCC-46645). The observed data on the anti-microbial activity of the synthesized compounds and standard drugs are given in Table 1.

4. Results and discussion

In this present work a novel series of quinazolin-4(3*H*)-ones compounds were synthesized. Synthetic scheme illustrates the way used for the synthesis of target compounds. As a starting material 3,5-subsituted anthranilic acid and benzoyl chloride were used to produce Schiff bases of substituted quinazolin-4(3*H*)-one via the intermediate substituted benzoxazin-4-one and substituted 3-amino-2-phenylquinazolin-4(3*H*)-one. The structures of the compounds were characterized by IR, ^{1}H NMR, ^{13}C NMR, Mass spectral data and Elemental analysis. All the synthesized compounds were active against all tested micro-organisms with the range of MIC values for *S. aureus* (17.3–35.4 μg/ml), *S. epidermidis* (19.1–33.6 μg/ml), *M. luteus* (17.2–34.2 μg/ml), *B. cereus* (19.3–36.1 μg/ml), *E. coli* (18.2–33.4 μg/ml), *P. aeruginosa* (19.3–37.2 μg/ml), *K. pneumoniae* (16.7–35.7 μg/ml), *A. niger* (8.6–15.2 μg/ml) and *A. fumigatus* (10.1–15.9 μg/ml). 3-(3,4,5-Trimethoybenzylideneamino)-6,8-dibromo-2-phenylquinazolin-4(3*H*)-one **10** was found to exhibit the most potent in vitro anti-microbial activity with the MICs of 8.6, 10.1, 16.7, 18.2, 18.8, 18.9, 19.1, 19.3 and 21.7 μg/ml against *A. niger*, *A. fumigatus*, *K. pneumoniae*, *E. coli*, *M. luteus*, *S. aureus*, *S. epidermidis*, *P. aeruginosa* and *B. cereus*, respectively. Compound **7** exhibited significant anti-microbial activity when compared to standard drugs Ciprofloxin and Ketokonazole. Other compounds **1**–**6**, **8**–**9**, **11**–**12** showed mild to moderate anti-bacterial and anti-fungal activity. The results revealed that most of the synthesized compounds exhibited significant anti-fungal activity. The most potent anti-bacterial and anti-fungal activity exhibited by

Table 1
Anti-microbial activity of the synthesized compounds

Compound	In vitro activity-zone of inhibition in mm (MIC in μg/ml)								
	S. aureus, ATCC-9144	*S. epidermidis*, ATCC-155	*M. luteus*, ATCC-4698	*B. cereus*, ATCC-11778	*E. coli*, ATCC-25922	*P. aeruginosa*, ATCC-2853	*K. pneumoniae*, ATCC-11298	*A. niger*, ATCC-9092	*A. fumigatus*, ATCC-46645
1	17 (35.4)	18 (33.6)	19 (31.8)	20 (29.4)	17 (33.4)	19 (28.7)	21 (27.7)	24 (9.7)	22 (11.1)
2	19 (32.1)	20 (22.2)	18 (34.2)	19 (31.9)	19 (29.9)	16 (36.2)	20 (33.4)	23 (10.1)	24 (12.3)
3	20 (21.8)	24 (21.3)	18 (28.2)	21 (19.9)	22 (20.4)	17 (37.2)	22 (24.3)	22 (12.2)	23 (13.6)
4	19 (27.9)	25 (19.8)	21 (26.2)	19 (30.1)	20 (29.1)	18 (32.1)	23 (22.7)	19 (14.7)	22 (15.9)
5	21 (22.6)	22 (21.8)	23 (30.1)	22 (23.6)	26 (19.8)	19 (36.1)	21 (34.6)	25 (10.7)	23 (11.6)
6	18 (31.1)	20 (22.6)	23 (25.1)	21 (21.9)	24 (19.8)	22 (21.2)	19 (29.1)	22 (15.2)	24 (14.6)
7	24 (17.3)	22 (20.9)	26 (17.2)	21 (19.3)	23 (21.8)	25 (22.6)	27 (19.0)	24 (9.1)	21 (10.7)
8	19 (28.4)	20 (25.6)	23 (33.8)	19 (36.1)	27 (20.2)	26 (22.8)	25 (21.8)	25 (10.2)	22 (12.6)
9	22 (22.4)	22 (21.8)	23 (27.4)	20 (32.8)	23 (30.2)	21 (34.2)	20 (35.7)	24 (14.4)	25 (10.9)
10	26 (18.9)	25 (19.1)	24 (18.8)	23 (21.7)	27 (18.2)	24 (19.3)	28 (16.7)	26 (8.6)	24 (10.1)
11	23 (21.6)	23 (24.2)	24 (28.1)	17 (35.1)	24 (31.2)	19 (33.4)	23 (28.2)	24 (12.7)	23 (15.2)
12	20 (23.8)	24 (21.4)	21 (22.8)	23 (21.9)	25 (20.1)	19 (25.1)	25 (19.8)	24 (11.8)	22 (14.6)
Ciprofloxacin (100 μg/disc)	29 (0.2)	31 (0.39)	30 (0.1)	29 (0.3)	32 (0.2)	33 (0.25)	33 (0.1)	–	–
Ketoconazole (100 μg/disc)	–	–	–	–	–	–	–	30 (6.1)	29 (0.23)
Dimethyl formamide	–	–	–	–	–	–	–	–	–

compound **10** might be due to the presence of electron donating substituent three methoxy groups on the benzylideneamino moiety of the 2-phenyl substituted quinazolin-4(3*H*)-one. Similarly 3-(4-hydroxybenzylideneamino)-6,8-dibromo-2-phenylquinazolin-4(3*H*)-one **7** also exhibited significant anti-microbial activity due to the presence of phenolic –OH group on the benzylideneamino group of the quinazoline-4(3*H*)-one moiety. While other compounds, though they contain both electron withdrawing groups like nitro, chloro and electron donating group like methoxy group do not exhibit significant in vitro anti-microbial activity (Scheme 1).

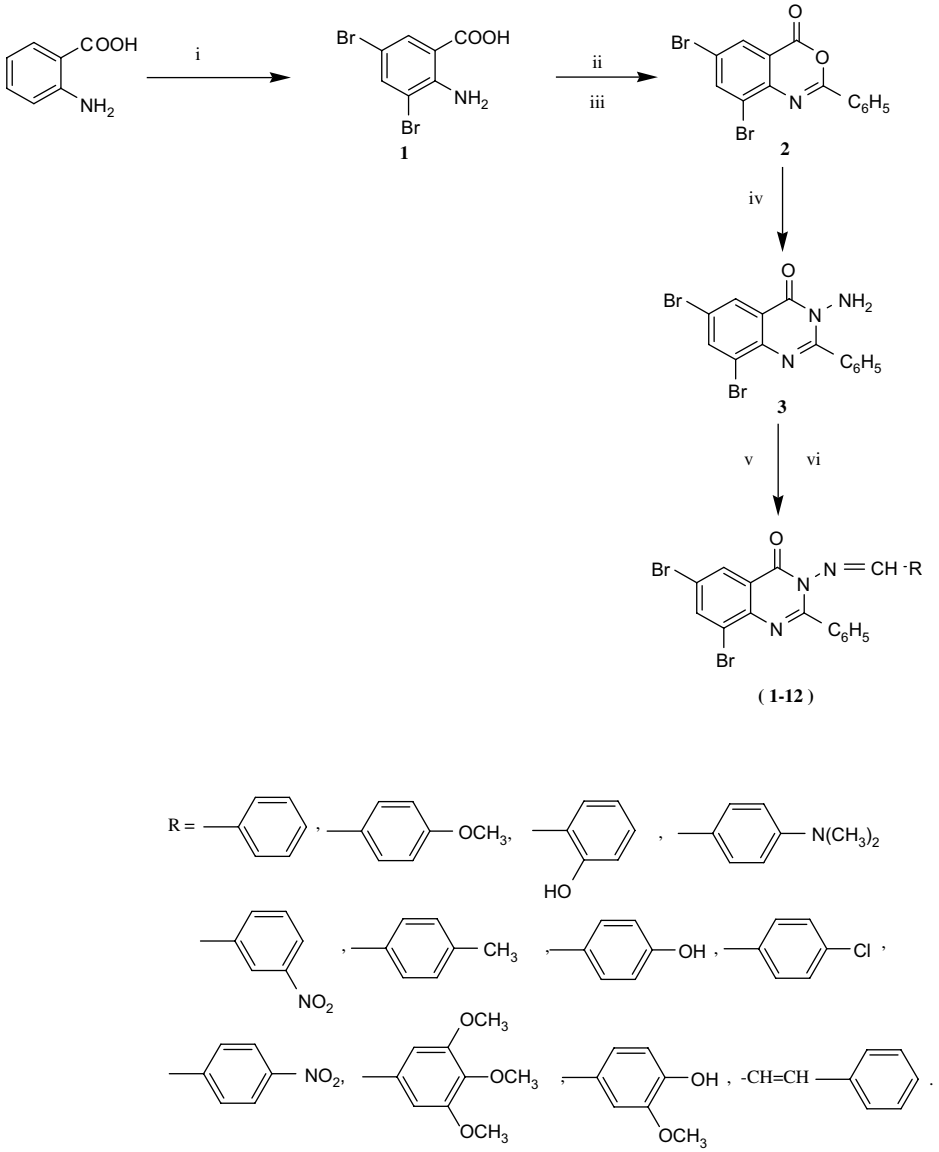
5. Conclusion

The anti-microbial activity of the synthesized compounds may be due the presence of the versatile pharmacophores and bromine which might increase the lipophilic character of the molecule, which facilitate the crossing through the biological membrane of the micro-organism and thereby inhibit their growth.

6. Experimental protocols

6.1. Chemistry

The melting points were taken in open capillary tube and are uncorrected. The IR spectra of the compounds were recorded on ABB Bomem FT-IR spectrometer MB 104 with KBr pellets. The ^{1}H NMR (300 MHz) and ^{13}C NMR (300 MHz) spectra were recorded on a Bruker 300 NMR spectrometer. Mass spectra were recorded on Shimadzu GC–MS QP 5000. Microanalyses were obtained with an



Scheme 1. Synthesis of Schiff bases of 3-amino-6,8-dibromo-2-phenylquinazolin-4(3*H*)-ones **1**–**12**. Reagents: (i) Br_2 in boiling glacial acetic acid; (ii) 0 °C in dry pyridine; (iii) C_6H_5COCl; (iv) $NH_2NH_2 \cdot H_2O$; (v) C_2H_5OH; and (vi) R-CHO, reflux, 3 h.

Elemental analyses system GmbH VarioEL V300 element analyzer. The purity of the compounds was checked by TLC on pre-coated SiO_2 gel (HF_{254}, 200 mesh) aluminium plates (E Merk) using ethyl acetate:*n*-hexane (20:80) and visualized in UV chamber. IR, ^{1}H NMR, ^{13}C NMR, Mass spectra and Elemental analysis were consistent with the assigned structures.

*6.1.1. General method of synthesis **1–12***

The 3-amino-6,8-dibromo-2-phenylquinazolin-4(3*H*)-one was prepared according to reported literatures [2,26,27]. Equimolar quantities (0.01 mol) of 3-amino-6,8-dibromo 2-phenylquinazolin-4(3*H*)-one and substituted aromatic aldehydes were dissolved in sufficient quantity of ethanol and refluxed for 6–8 h then kept aside for 3 days, the product which separated out was filtered, dried and recrystallised from absolute ethanol.

*6.1.1.1. 3(Benzylideneamino)-6,8-dibromo-2-phenylquinazolin-4(3H)-one **1***. Yield 82%; mp 231–233 °C; IR: 1771 (C=O), 1557 (C=C), 1607 (C=N), 1201 (C–O–C), 549 (C–Br), 3124 (C–H), 1258 (C–N) cm^{-1}; ^{1}H NMR ($CDCl_3$): δ 8.09 (s, 1H, H-5, Ar-H), 8.12 (s, –N=CH–), 7.82 (s, 1H, H-7, Ar-H), 7.25–7.60 (m, 10H, Ar-H); ^{13}C NMR ($CDCl_3$): δ 164.1 (C-2), 160.2 (C-4), 154.3 (C-9), 143.6 (–N=*C*H–), 139.6 (C-7), 133.7 (C-1′), 131.2 (C-4′), 129.3 (C-6′ and C-2′), 128.8 (C-3′ and C-5′), 128.7 (C-1‴), 128.6 (C-3″ and C-5″), 130.4 (C-4″), 126.2 (C-2″ and C-6″), 131.6 (C-5), 125.4 (C-10), 123.9 (C-6), 113.5 (C-8); EI-MS (*m*/*z*): M$^+$ 483.6 (M + 2, 28%); Anal. Calcd. for $C_{21}H_{13}Br_2N_3O$: C, 52.20; H, 2.71; N, 8.70; Found: C, 52.21; H, 2.70; N, 8.71.

*6.1.1.2. 3(4-Methoxybenzylideneamino)-6,8-dibromo-2-phenylquinazolin-4(3H)-one **2***. Yield 68%; mp 235–237 °C; IR: 1771 (C=O), 1557 (C=C), 1605 (C=N), C–N (1376), 1010 (C–O–C), 853 (C–H), 3125 (C–H, Ar), 549 (C–Br), cm^{-1}; ^{1}H NMR ($CDCl_3$): δ 8.07 (s, 1H, H-5, Ar-H), 8.12 (s, –N=CH–), 3.72 (s, 1H, H-7, –OCH_3), 7.27–7.62 (m, 9H, Ar-H); ^{13}C NMR ($CDCl_3$): δ 163.9 (C-2), 163.0 (C-4′), 160.3 (C-4), 153.9 (C-9), 142.9 (N=*C*H–), 139.6 (C-7), 132.3 (C-5), 130.4 (C-2′), 130.3 (C-4″), 130.2 (C-2′ and C-4″), 128.7 (C-1″), 128.9 (C-3″ and C-5″), 126.1 (C-1′), 126.3 (C-2″ and C-6′), 125.6 (C-10), 123.8 (C-6), 114.2 (C-3′ and C-5′), 113.4 (C-8), 4.01 (–*O*CH_3); EI-MS MS (*m*/*z*): M$^+$ 513.1 (M + 2, 30%); Anal. Calcd. for $C_{22}H_{15}Br_2N_3O_2$: C, 51.49; H, 2.95; N, 8.19; Found: C, 51.48; H, 2.96; N, 8.20.

*6.1.1.3. 3(2-Hydroxybenzylideneamino)-6,8-dibromo-2-phenylquinazolin-4(3H)-one **3***. Yield 65%; mp 226–228 °C; IR: 1771 (C=O), 1576 (C=C), 1607 (C=N), 1377 (C–N), 1431 (C–H), 3125 (C–H, Ar), 508 (C–Br), 3565 (O–H, s), 1201 (O–H, b) cm^{-1}; ^{1}H NMR ($CDCl_3$): δ 8.0 (s, 1H, H-5, Ar-H), 8.13 (s, –N=CH–), 7.83 (s, 1H, H-7, Ar-H), 7.29–7.63 (m, 8H, Ar-H), 5.29 (s, 1H, Ar-H); ^{13}C NMR ($CDCl_3$): δ 163.9 (C-2), 160.3 (C-4′), 161.2 (C-2′), 153.6 (C-9), 143.7 (–N=*C*H–), 139.8 (C-7), 131.6 (C-5), 130.6 (C-6′), 132.6 (C-4′), 130.4 (C-4″), 128.9 (C-3″ and C-5″), 126.2 (C-2″ and C-6″), 128.7 (C-1″), 125.3 (C-10), 123.9 (C-5′), 118.6 (C-1′), 121.5 (C-3′), 116.0 (C-5″), 43.6 (C-8); EI-MS (*m*/*z*): M$^+$ 499.9 (M + 2, 25%); Anal. Calcd. for $C_{21}H_{13}Br_2N_3O_2$: C, 50.53; H, 2.63; N, 8.42; Found: C, 50.52; H, 2.64; N, 8.43.

*6.1.1.4. 3(4-(Dimethylamino)benzylideneamino)-6,8-dibromo-2-phenylquinazolin-4(3H)-one **4***. Yield 71%; mp 225–227 °C; IR: 1793 (C=O), 1557 (C=C), 1375 (C–N) 1603 (C=N), 1445 (C–H), 3082 (C–H, Ar), 559 (C–Br) cm^{-1}; ^{1}H NMR ($CDCl_3$): δ 8.02 (s, 1H, H–5, Ar-H), 8.13 (s, –N=CH–), 7.82 (s, 1H, H–7, Ar-H), 7.23–7.64 (m, 9H, Ar-H), 2.80 (s, 6H, –$N(CH_3)_2$); ^{13}C NMR ($CDCl_3$): δ 163.9 (C-2), 160.2 (C-4), 153.4 (C-9), 151.9 (C-4′), 143.9 (–N=*C*H–), 139.9 (C-7), 131.4 (C-5), 130.1 (C-2′ and C-6′), 130.2 (C-4″), 128.9 (C-3″ and C-5″), 126.2 (C-2″ and C-6″), 125.4 (C-10), 123.9 (C-6), 114.6 (C-3′ and C-5′), 113.6 (C-8), 40.4 (–$N(CH_3)_2$); EI-MS (*m*/*z*): M$^+$ 526.2 (M + 2, 30%); Anal. Calcd. for $C_{23}H_{18}Br_2N_4O$: C, 52.50; H, 3.45; N, 10.65; Found: C, 52.51; H, 3.46; N, 10.64.

*6.1.1.5. 3(3-Nitrobenzylideneamino)-6,8-dibromo-2-phenylquinazolin-4(3H)-one **5***. Yield 64%; mp 222–224 °C; IR: 1703 (C=O), 1561 (C=C), 1348 (C–N), 1667 (C=N), 1432 (C–H), 3125 (C–H, Ar), 529 (C–Br), 1561 (C–NO_2) cm^{-1}; ^{1}H NMR ($CDCl_3$): δ 8.03 (s, 1H, H-5, Ar-H), 8.12 (s, –N=*C*H–), 7.80 (s, 1H, H-7, Ar-H), 7.20–7.60 (m, 8H, Ar-H); ^{13}C NMR ($CDCl_3$): δ 163.8 (C-2), 160.1 (C-4), 154.4 (C-9), 143.2 (–N=CH–), 139.3 (C-7), 148.4 (C-3′), 135.4 (C-6′), 134.7 (C-1′), 131.2 (C-5), 130.4 (C-4″), 128.9 (C-3″ and C-5′), 129.7 (C-3′), 125.3 (C-10), 123.9 (C-6), 123.4 (C-4′), 128.7 (C-1′), 126.3 (C-2″ and C6″), 124.2 (C-2′), 113.4 (C-8); EI-MS (*m*/*z*): M$^+$ 528.1 (M + 2); Anal. Calcd. for $C_{21}H_{12}Br_2N_4O_3$: C, 47.76; H, 2.29; N, 10.61; Found: C, 47.77; H, 2.26; N, 10.60.

*6.1.1.6. 3(4-Methylbenzylideneamino)-6,8-dibromo-2-phenylquinazolin-4(3H)-one **6***. Yield 78%; mp 219–221 °C; IR: 1702 (C=O), 1669 (C=C), 1398 (C–N), 1684 (C=N), 1448 (C–H), 3096 (C–H, Ar), 550 (C–Br) cm^{-1}; ^{1}H NMR ($CDCl_3$): δ 8.09 (s, 1H, H-5, Ar-H), 8.12 (s, –N=CH–), 7.82 (s, 1H, H-7, Ar-H), 7.25–7.61 (m, 9H, Ar-H), 2.30 (s, 3H, –CH_3); ^{13}C NMR ($CDCl_3$): δ 164.3 (C-2), 160.2 (C-2), 154.9 (C-9), 143.4 (–N=*C*H–), 139.6 (C-7), 129.6 (C-5′), 140.1 (C-4′), 130.9 (C-1′), 131.2 (C-5), 130.2 (C-4″), 129.2 (C-3′ and C-5′), 129.1 (C-2′ and C-6′), 128.7 (C-1′), 128.6 (C-3′ and C-5′), 126.1 (C-2″ and C-6′), 125.3 (C-10), 123.9 (C-6), 113.4 (C-8), 24.2 (–*C*H_3); EI-MS (*m*/*z*): M$^+$ 497.1 (M + 2, 35%); Anal. Calcd. for $C_{22}H_{15}Br_2N_3O$: C, 53.15; H, 3.04; N, 8.45; Found: C, 53.14; H, 3.05; N, 8.44.

*6.1.1.7. 3(4-Hydroxybenzylideneamino)-6,8-dibromo-2-phenylquinazolin-4(3H)-one **7***. Yield 75%; mp 238–240 °C; IR: 1771 (C=O), 1665 (C=C), 1377 (C–N), 1665 (C=N), 1447 (C–H), 3132 (C–H, Ar), 550 (C–Br), 3525 (O–H, s), 1202 (O–H, b) cm^{-1}; ^{1}H NMR ($CDCl_3$): δ 8.08 (s, 1H, ;H-5, Ar-H), 8.12 (s, –N=*C*H–), 5.91 (s, Ar–O*H*), 7.31–7.62 (m, 9H, Ar-H); ^{13}C NMR ($CDCl_3$): δ 164.4 (C-2), 160.6 (C-4), 154.7

(C-9), 160.9 (C-4′), 143.3 (−N=*C*H−), 139.5 (C-7), 131.4 (C-5), 128.7 (C-1′), 126.3 (C-2′ and C-6′), 116.9 (C-3′ and C-5′), 125.3 (C-10), 123.8 (C-6), 128.7 (C-1′), 128.6 (C-3′ and C-5′), 126.3 (C-2″ and C-6′), 113.6 (C-8); EI-MS (*m/z*): M^+ 499.15 (M + 2, 30%); Anal. Calcd. for $C_{21}H_{13}Br_2N_3O_2$: C, 50.53; H, 2.63; N, 8.42; Found: C, 50.54; H, 2.62; N, 8.43.

6.1.1.8. 3(4-Chlorobenzylideneamino)-6,8-dibromo-2-phenylquinazolin-4(3H)-one ***8***. Yield 70%; mp 244–246 °C; IR: 1703 (C=O), 1683 (C=C), 1377 (C−N), 1670 (C=N), 1447 (C−H), 3125 (C−H, Ar), 529 (C−Br), 784 (C−Cl) cm^{-1}; ^1H NMR ($CDCl_3$): δ 8.07 (s, 1H, H-5, Ar-H), 8.13 (s, −N=C*H*−), 7.80 (s, H-7, Ar-H), 7.29–7.64 (m, 9H, Ar-H); ^{13}C NMR ($CDCl_3$): δ 164.8 (C-2), 160.4 (C-4), 154.4 (C-9), 136.4 (C-4′), 143.9 (−N=*C*H−), 139.5 (C-7), 131.6 (C-5), 131.9 (C-1′), 130.6 (C-2′ and C-6′), 130.2 (C-4′), 128.9 (C-3′ and C-5′), 128.7 (C-1′), 125.4 (C-10), 129.2 (C-3′ and C-5′), 126.7 (C-2″ and C-6′), 123.7 (C-6), 113.6 (C-8); EI-MS (*m/z*): M^+ 517.6 (M + 2, 32%); Anal. Calcd. for $C_{21}H_{12}Br_2ClN_3O$: C, 48.72; H, 2.34; N, 8.12; Found: C, 48.71; H, 2.35; N, 8.11.

6.1.1.9. 3(4-Nitrobenzylideneamino)-6,8-dibromo-2-phenylquinazolin-4(3H)-one ***9***. Yield 78%; mp 228–230 °C; IR: 1703 (C=O), 1667 (C=C), 1378 (C−N), 1607 (C=N), 1433 (C−H), 3115 (C−H, Ar), 529 (C−Br), 1523 (C−NO_2) cm^{-1}; ^1H NMR ($CDCl_3$): δ 8.09 (s, 1H, H-5, Ar-H), 8.13 (s, −N=C*H*−), 7.84 (s, H-7, Ar-H), 7.31–7.80 (m, 9H, Ar-H); ^{13}C NMR ($CDCl_3$): δ 164.3 (C-2), 160.7 (C-4), 54.3 (C-9), 150.7 (C-4′), 143.8 (−N=*C*H−), 139.2 (C-1′), 139.5 (C-7), 131.4 (C-5), 130.3 (C-4″), 130.2 (C-2′ and C-6′), 128.9 (C-3′ and C-5′), 128.7 (C-1′), 125.4 (C-10), 121.3 (C-3′ and C-5′), 126.2 (C-2″ and C-6′), 123.6 (C-6), 113.7 (C-8); EI-MS (*m/z*): M^+ 528.9 (M + 2, 30%); Anal. Calcd. for $C_{21}H_{12}Br_2N_4O_3$; C, 47.76; H, 2.29; N, 10.61; Found: C, 47.75; H, 2.28; N, 10.62.

6.1.1.10. 3(3,4,5-Trimethoxybenzylideneamino)-6,8-dibromo-2-phenylquinazolin-4(3H)-one ***10***. Yield 72%; mp 236–238 °C; IR: 1703 (C=O), 1670 (C=C), 1377 (C−N), 1607 (C=N), 1449 (C−H), 3126 (C−H, Ar), 518 (C−Br), 1202 (C−OCH_3) cm^{-1}; ^1H NMR ($CDCl_3$): δ 8.10 (s, 1H, H-5, Ar-H), 8.14 (s, −N
ce:glyph name="dbnd"/>C*H*−), 7.83 (s, H-7, Ar-H), 6.76–7.62 (m, 5H, Ar-H), 7.71 (H-7, Ar-H), 6.99 (s, H6′, Ar-H), 3.70 (s, 9H, $(OCH_3)_3$); ^{13}C NMR ($CDCl_3$): δ 164.2 (C-2), 160.3 (C-4), 154.2 (C-9), 150.9 (C-3′ and C-5′), 143.4 (−N=*C*H−), 141.6 (C-4′), 139.6 (C-7), 131.3 (C-5), 130.2 (C-4″), 128.7 (C-1′), 128.9 (C-3′ and C-5′), 125.6 (C-10), 128.1 (C-1′), 126.2 (C-2″ and C-6″), 123.7 (C-6), 113.7 (C-8), 106.7 (C-2′ and C-6′), 53.3 ($OCH_3)_3$; EI-MS (*m/z*): M^+ 570.3 (M + 2, 40%); Anal. Calcd. for $C_{24}H_{19}Br_2N_3O_4$: C, 50.29; H, 3.34; N, 7.33; Found: C, 50.28; H, 3.35; N, 7.34.

6.1.1.11. 3(4-Hydroxy-3-methoxybenzylideneamino)-6,8-dibromo-2-phenylquinazolin-4(3H)-one ***11***. Yield 82%; mp 246–248 °C; IR: 1704 (C=O), 1686 (C=C), 1377 (C−N), 1667 (C=N), 1447 (C−H), 3125 (C−H, Ar), 529 (C−Br), 1200 (C−OCH_3), 3489 (O−H, s), 1301 (O−H, b) cm^{-1}; ^1H NMR ($CDCl_3$): δ 8.10 (s, 1H, H-5, Ar-H), 8.16 (s, −N=C*H*−), 7.80 (s, 1H-7, Ar-H), 7.29–7.62 (m, 7H, H-2′, H-3′, H-4′, H-5′, H-6′, H-2″, H-3″, Ar-H), 7.09 (s, 1H, H-6′, Ar-H); ^{13}C NMR ($CDCl_3$): δ 164.2 (C-2), 160.2 (C-4), 154.3 (C-9), 151.6 (C-5′), 143.6 (−N=*C*H−), 138.7 (C-4′), 139.9 (C-7), 131.4 (C-5), 130.5 (C-4″), 126.3 (C-2″ and C-6″), 128.7 (C-3′ and C-5′), 128.7 (C-1′), 128.5 (C-1′), 125.6 (C-10), 123.9 (C-6), 127.4 (C-1′), 122.8 (C-2′), 119.7 (C-6′), 117.2 (C-3′), 113.4 (C-8), 56.70 (−OCH_3); EI-MS (*m/z*): M^+ 529.1 (M + 2, 30%); Anal. Calcd. for $C_{22}H_{15}Br_2N_3O_3$: C, 49.93; H, 2.86; N, 7.94; Found: C, 49.94; H, 2.85; N, 7.95.

6.1.1.12. 3(3-Phenylallylideneamino)-6,8-dibromo-2-phenylquinazolin-4(3H)-one ***12***. Yield 79%; mp 228–231 °C; IR: 1704 (C=O), 1667 (C=C), 1378 (C−N), 1667 (C=N), 1401 (C−H), 3126 (C−H, Ar), 550 (C−Br) cm^{-1}; ^1H NMR ($CDCl_3$): δ 8.10 (s, 1H, H-5, Ar-H), 7.89 (s, −N=C*H*−), 7.82 (s, H-7, Ar-H), 6.62–6.63 (d, 5.8H-2, −N=CH−CH=C*H*−), 7.31–7.65 (m, 10H, Ar-H), 5.61 (s, −N=CHC*H*=CH); ^{13}C NMR ($CDCl_3$): δ 164.9 (C-2), 160.3 (C-4), 154.6 (C-9), 139.01 (N=CHCH=*C*H), 135.3 (C-1′), 139.6 (C-7), 131.4 (C-5), 137.6 (N=*C*H), 130.2 (C-4″), 128.5 (C-3′ and C-5′), 128.9 (C-3′ and C-5′), 126.4 (−N=CH−CH=CH−), 125.5 (C-10), 126.5 (C-2′ and C-6′), 126.1 (C-2″ and C-6′), 123.4 (C-6), 113.6 (C-8); EI-MS (*m/z*): M^+ 509.58 (M + 2, 25%); Anal. Calcd. for $C_{23}H_{15}Br_2N_3O$: C, 54.25; H, 2.97; N, 8.25; Found: C, 49.92; H, 2.87; N, 8.24.

6.2. Anti-microbial screening

The anti-bacterial activity of the synthesized compounds was tested against four Gram-positive bacteria (*S. aureus* ATCC-9144, *S. epidermidis* ATCC-155, *M. luteus* ATCC-4698 and *B. cereus* ATCC-11778) and three Gram-negative bacteria (*E. coli* ATCC-25922, *P. aeruginosa* ATCC-2853 and *K. pneumoniae* ATCC-11298) using nutrient agar medium (Hi-Media Laboratories, India). The anti-fungal activities of the compounds were tested against two fungi namely *A. niger* ATCC-9029 and *A. fumigatus* ATCC-46645 using sabouraud dextrose agar medium (Hi-Media Laboratories, India).

6.2.1. Paper disc diffusion technique

The sterilized [28] (autoclaved at 120 °C for 30 min) medium (40–50 °C) was inoculated (1 ml/100 ml of medium) with the suspension (10^5 cfu ml^{-1}) of the micro-organism (matched to McFarland barium sulphate standard) and poured into a petridish to give a depth of 3–4 mm. The paper impregnated with the test compounds (μg ml^{-1} in dimethyl formamide) was placed on the solidified medium. The plates were pre-incubated for 1 h at room temperature and incubated at 37 °C for 24 and 48 h for anti-bacterial and anti-fungal activities, respectively. Ciprofloxacin (100 μg/disc) and Ketoconazole (100 μg/disc) were used as standard for anti-bacterial and anti-fungal activity, respectively. The observed zone of inhibition is presented in Table 1.

6.2.2. Minimum inhibitory concentration (MIC)

MIC [29] of the compound was determined by agar streak dilution method. A stock solution of the synthesized compound (100 μg ml^{-1}) in dimethyl formamide was prepared and graded quantities of the test compounds were incorporated in specified quantity of molten sterile agar (nutrient agar for anti-bacterial activity and sabouraud dextrose agar medium for anti-fungal activity). A specified quantity of the medium (40–50 °C) containing the compound was poured into a petridish to give a depth of 3–4 mm and allowed to solidify. Suspension of the micro-organism was prepared to contain approximately 10^5 cfu ml^{-1} and applied to plates with serially diluted compounds in dimethyl formamide to be tested and incubated at 37 °C for 24 and 48 h for bacteria and fungi, respectively. The MIC was considered to be the lowest concentration of the test substance exhibiting no visible growth of bacteria or fungi on the plate. The observed MIC is presented in Table 1.

References

[1] V. Alagarsamy, V. Rajasolomon, R. Meena, K.V. Ramseshe, Biol. Pharm. Bull. 28 (2005) 1091–1094.
[2] V. Alagarsamy, G. Muruganantham, R. Venkateshaperumal, Biol. Pharm. Bull. 26 (12) (2003) 1711–1714.
[3] P. Kant, Indian J. Heterocycl. Chem. 15 (2006) 221–224.
[4] M.K. Srivastava, B. Mishra, Nizamuddin, Indian J. Chem. 40B (2001) 342–344.
[5] P. Mishra, P. Pannerselvam, S. Jain, J. Indian Chem. Soc. 72 (1995) 559–560.
[6] V. Alagarsamy, V. Rajasolomon, G. Vanikavitha, V. Pallchamy, S. Amuthalakshmi, M. Ravi Chandran, A. Arnold Sujin, A. Thangathirupathi, R. Revathi, Biol. Pharm. Bull. 25 (2002) 1432–1435.
[7] P. Pannerselvam, R.V. Pradeepchandran, S.K. Sridhar, Indian J. Pharm. Sci. 65 (2003) 268–273.
[8] V. Alagarsamy, A. Thangathirupathy, S.C. Mandal, S. Rajasekaran, S. Vijayakumar, R. Revathi, J. Anburaj, S. Arunkumar, S. Rajesh, Indian J. Pharm. Sci. 68 (2006) 108–111.
[9] V. Murgan, Caroline C. Thomas, G.V.S. Rama Sarma, E.P. Kumar, Indian J. Pharm. Sci. 65 (2003) 386–389.
[10] P. Nandy, M.T. Vishalakshi, A.R. Bhat, Indian J. Heterocycl. Chem. 15 (2006) 293–294.
[11] R. Lakhan, O.P. Singh, R.L. Singh, J. Indian Chem. Soc. 64 (1987) 316–318.
[12] V.K. Pandey, M. MukeshTandon, Indian J. Heterocycl. Chem. 15 (2006) 399–400.
[13] R. Rastogi, S. Sharma, Indian J. Chem. 21B (1982) 744.
[14] S.K.V. Seshavataram, N.V.S. Rao, Proc. Indian Acad. Sci. 85A (1997) 81.
[15] M.M. Said, M.M.M. Hussein, Bull. Fac. Pharm. 32 (1994) 341.
[16] P.N. Bhargava, S. Prakash, Indian J. Chem. 39B (1997) 18.
[17] T.M. Abdel-Rahman, Heterocycl. Commun. 3 (1997) 535.
[18] P. Kumar, C. Nath, K.P. Bhargava, K. Shanker, Pharmazie 37 (1982) 802.
[19] T. Aono, S. Marui, F. Itoh, M. Yamooka, M. Nakao, Eur. Patent No. 735035, 1996.
[20] F. Roubinek, J. Vavrina, Z. Bundesinsky, Collect. Czech. Chem. Commun. 47 (1982) 630.
[21] M.L. Elliot, W.M. Welch, Pct. Int. Appl. WO 97-43276 (1997).
[22] M. Ishikawa, H. Azuma, Y. Eguchi, S. Ito, T. Naito, H. Ebisawa, I. Kotoku, J. Pharmacobio-Dyn. 5 (1982) 8.
[23] M.K. Ibrahim, J. Al-Azhar, Pharm. Sci. 22 (1998) 9.
[24] Y.D. Kulkarni, A. Rowhani, J. Indian Chem. Soc. 67 (1990) 46–47.
[25] P.N. P.MishraGupta, A.K. Shakya, J. Indian Chem. Soc. 68 (1991) 618–619.
[26] S.Alvin Wheeler, Oates, J. Am. Chem. Soc. 32 (1910) 770–773.
[27] D.T. Zentmyer, E.C. Wagner, J. Org. Chem. 14 (1946) 967–981.
[28] S.H. Gillespie, Medical Microbiology-Illustrated, Butterworth Heinemann Ltd., United Kingdom, 1994, pp. 234–247.
[29] P.M. Hawkey, D.A. Lewis, Medical Bacteriology – a Practical Approach, Oxford University Press, United Kingdom, 1994, pp. 181–194.