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Design, synthesis and biological evaluation of new quinazoline derivatives as antimicrobial and anti-fungal agents

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ABSTRACT

A new series of 6- bromo-2-(4-chlorophenyl)quinazolin-4(3H)-one derivatives 2-7 were synthesized. Their chemical structures were confirmed by spectral and elemental analysis. The synthesized compounds were evaluated for their anti-microbial activity using the agar diffusion technique. The tested compounds were evaluated against, Gram-positive bacteria (Bacillus subtilis, Staphylococcus aureus), Gram- negative bacteria (Escherichia coli and Pseudomonas aeruginosa), Yeast (Candida albicans) and Filamentous Fungus (Aspergillus niger). Minimum inhibitory concentration (MIC) of the compounds which showed antimicrobial activity was determined in vitro. Compound ***3b*** *has broad spectrum activity against Gram-positive bacteria, Gram-negative bacteria, Yeast and Filamentous Fungus with MICs 150, 150, 75, 312, 625 and 625 µg/mL against (Bacillus subtilis ATCC 6633, Staphylococcus aureus ATCC 3555, Escherichia coli ATCC 23282 Pseudomonas aeruginosa ATCC 10145, Candida albicans IMRU 3669 and Aspergillus niger ATCC 16404) respectively.*

Key words: Quinazolinone; Chalcones; Antimicrobial; Anti-fungal.

INTRODUCTION

The increasing incidence of resistant and multi-resistant bacterial strains has become a serious problem all over the world [1]. The reasons for antimicrobial resistance increase are due to the excessive use of antibiotic and inaccurate diagnosis [2, 3]. This encourage searching for new antimicrobial agents. Quinazolinones are classes of fused heterocycles that are of considerable interest due to their wide range of biological activities such as anticancer [4-8], anti-psychotic [9], anti-inflammatory [10,12], anti-fungal [12-14] and antimicrobial activities[15-18].

Literature survey revealed that 2,3 disubstituted quinazolinone derivatives show promising antimicrobial and anti-fungal activities [19,20]. On the other hand bromo quinazolinone derivatives substituted at 3rd position with phenyl ring show potential antimicrobial and anti-fungal activities [21,22]. Chacones, pyrazole, isoxazole and thiopyrimidine have diverse biological activities including antibacterial and anti-fungal activities [23-26].

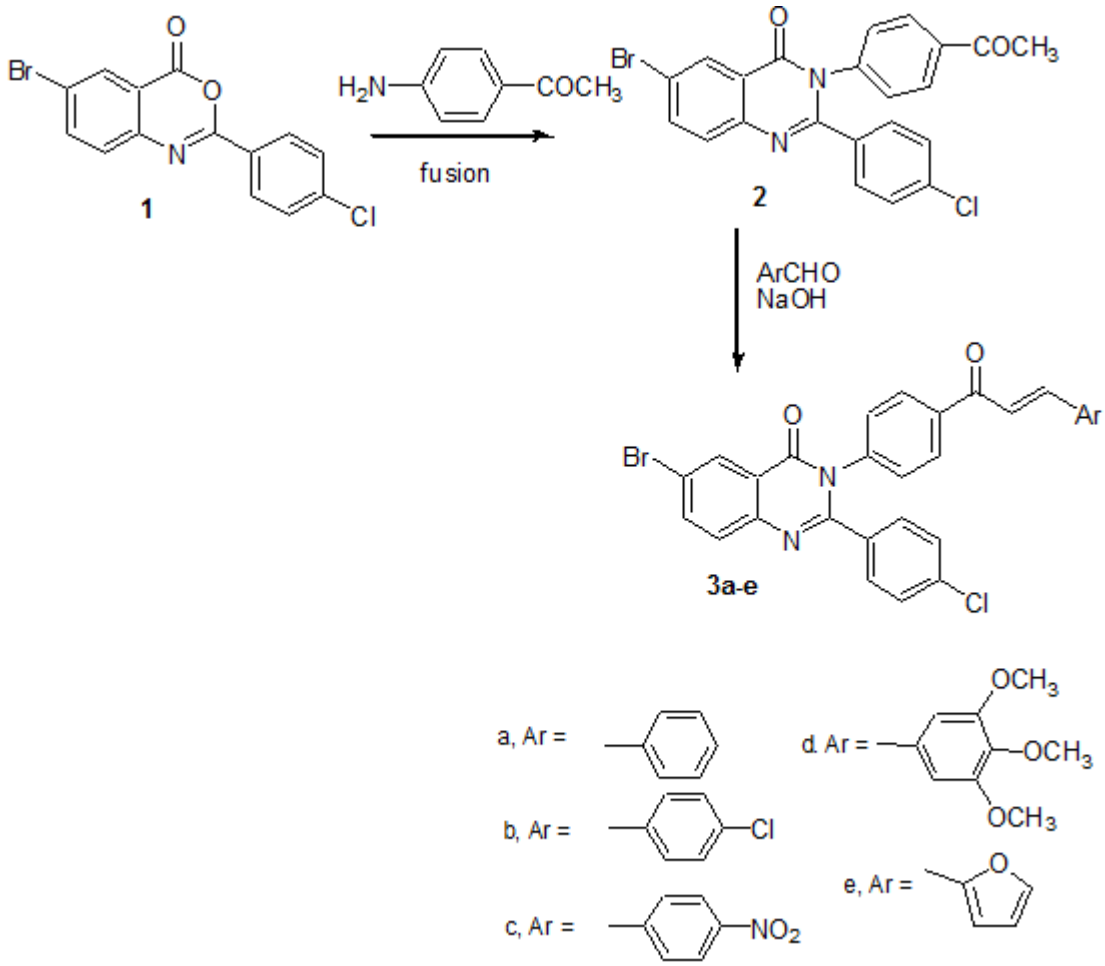
Based on this finding and continuation of our drug research program concerning with synthesis of new safer and more biologically active quinazolin-4-one derivatives [4-8, 22], we synthesized new bromo quinazolinone derivatives substituted at 3rd position with chalcones, pyrazole, isoxazole or thio pyrimidine moiety bearing para chloro phenyl moiety at position 2.

EXPERIMENTAL SECTION

2.1. Chemistry

All melting points are uncorrected, elemental analyses were carried out in the microanalytical unit of National Research Centre and Cairo University, Egypt. IR spectra were recorded on FT-IR spectrophotometer- Nexus 670-

Nicolet (USA) and Perkin Elmer-9712 spectrophotometer. ^{1}H NMR spectra were determined on a Varian-Gemmini-300 MHz.. ^{13}C NMR (DMSO-d_6) spectra were recorded at 100.62 MHz at the aforementioned research center in Cairo university. Mass spectra were determined on Finnigan Mat SSQ 7000, mode EI 70 eV (Thermo Inst. Sys. Inc. USA). Thin layer chromatography was carried out on silica gel 60 F254 (Merck) plates using chloroform/ petroleum ether/ methanol (7:4:1) as an eluent system.



Scheme 1: Synthesis of compounds 3a-e

***2.1.1. 3-(4-acetylphenyl)-6-bromo-2-(4-chlorophenyl)quinazolin-4(3H)-one* (2)**

A mixture of the benzoxazine **1** (0.01 mol) and p-aminoacetophenone (0.01 mol) was heated together upon fusion at 160 ^{o}C on sand bath for 2 h. After cooling, the crude mass was crystallized from methanol twice to give redish brown crystals of **2**, m.p. 210^{o}C in 80% yield. Analysis for $C_{22}H_{14}BrClN_2O_2$, Calcd.: % C, 58.24; H, 3.11; N, 6.17, Found: % C, 58.31; H, 3.17; N, 6.20, IR spectrum (KBr, cm^{-1}) showed characteristic absorption bands at 3380 (C-H aromatic), 1710 (C=O of acetyl), at 1690 (C=O of quinazolinone), 1630 (C=N) and at 1590 (C=C). ^{1}HNMR spectrum (DMSO-d_6, ppm) showed signals at 2.6 (3H, s, $COCH_3$) and 7.5-8.1 (m, 11H, Ar-H). ^{13}C NMR (DMSO-d_6) d ppm: 198, 165, 160.5, 147.5, 143, 137.5, 136.4, 136.3, 135.7, 129.2, 129, 128.5, 126.5, 124.6, 124, 123, 121.7, 26.5. MS (m/z, R.I.): 453 (100.0%), 452 (70.3%), 456 (30.6%).

***2.1.2. General method for the preparation of* 3a-e:**

A mixture of the ketone **2** (0.002 mol) and the appropriate aromatic aldehyde, (0.002 mol) in ethanol (10 mL). 5 % NaOH in ethyl alcohol (10 mL) was added drop wise within 15 minutes. The reaction mixture was refluxed for 6 h then cooled and the crude precipitated material was filtered off, air dried and then crystallized from the proper solvent to give the chalcones **3a-e** respectively.

2.1.2.1. ***(E)-6-bromo-2-(4-chlorophenyl)-3-(4-cinnamoylphenyl)quinazolin-4(3H)-one (3a)***

Crystallized from ethanol to give brown crystals, m.p. 170^{o}C in 70% yield. Analysis for $C_{29}H_{18}BrClN_2O_2$; Calcd %C, 64.28; H, 3.35; N, 5.17; Found: %C, 64.30; H, 3.30; N, 5.19; IR: $\upsilon_{max.}$/cm^{-1} 1685 (C=O quinazolinone), 1660 (C=O of the α,β - unsaturated ketone) and at 1590 (C=N).^{1}H-NMR (DMSO-d_6, ppm): 6.8-7.0 (2H, d, d, CH=CH) and 7.2-8.1 (m, 16H, Ar-H). ^{13}C NMR (DMSO-d_6): 189,167, 160,148, 145, 139, 137, 136, 135.2, 132.3, 134, 131,

130, 129.8, 129.5, 129.3, 128, 126.7, 124.9, 124.6, 123. 121.7, 121.3.MS (m/z, R.I.): 542 (100.0%), 540.02 (50.2%), 541.03 (22.4%)

2.1.2.2. ***(E)-6-bromo-2-(4-chlorophenyl)-3-(4-(3-(4-chlorophenyl)acryloyl)phenyl)quinazolin-4(3H)-one (3b)***
Crystallized from ethanol to give yellow crystals, m.p. 205^{o}C in 75% yield. Analysis for $C_{29}H_{17}BrCl_2N_2O_2$; Calcd %C, 74.99; H, 4.50; N, 6.25; Found: %C, 74.97; H, 4.49; N, 6.24; IR: $\upsilon_{max.}$/cm^{-1} 1690 (C=O quinazolinone), 1660 (C=O of the α,β - unsaturated ketone) and at 1610 (C=N).^{1}H-NMR spectrum (DMSO-d_6, ppm): 7.0-7.2 (2H, d, d, CH=CH) and 7.3-8.24(m, 15H, Ar-H). ^{13}C NMR (DMSO-d_6) d ppm: 190,168, 161,148, 146.2, 139.5, 138, 136.6, 135.2, 133.3, 135, 1342, 131, 130, 129.6, 129.3, 128, 126.9, 125.9, 124.8, 123.1.121.9, 121.5. MS (m/z, R.I.): 575 (100.0%), 573 (42.0%), 577 (50.1%).

2.1.2.3. ***(E)-6-bromo-2-(4-chlorophenyl)-3-(4-(3-(4-nitrophenyl)acryloyl)phenyl)quinazolin-4(3H)-one (3c)***
Crystallized from glacial acetic acid to give white crystals, m.p. 210^{o}C in 75% yield. Analysis for $C_{29}H_{17}BrClN_3O_4$; Calcd %C, 77.76; H, 4.66; N, 6.48; Found: %C, 77.72; H, 4.64; N, 6.46; IR: $\upsilon_{max.}$/cm^{-1} 1700 (C=O quinazolinone), 1670 (C=O of the α,β - unsaturated ketone) and at 1600 (C=N).^{1}HNMR (DMSO-d_6, ppm) : 6.9-7.1 (2H, d, d, CH=CH) and 7.4-8.5(m, 15H, Ar-H). ^{13}C NMR (DMSO-d_6): 191, 169, 164, 149, 147, 145, 142, 137, 136.5, 134, 132.3, 132, 130,130.5, 129.9, 129, 128.5, 125, 124.5, 123.8, 123, 122.2,122. MS (m/z, R.I.): 585 (100%), 587 (40.0%), 588 (31.5%).

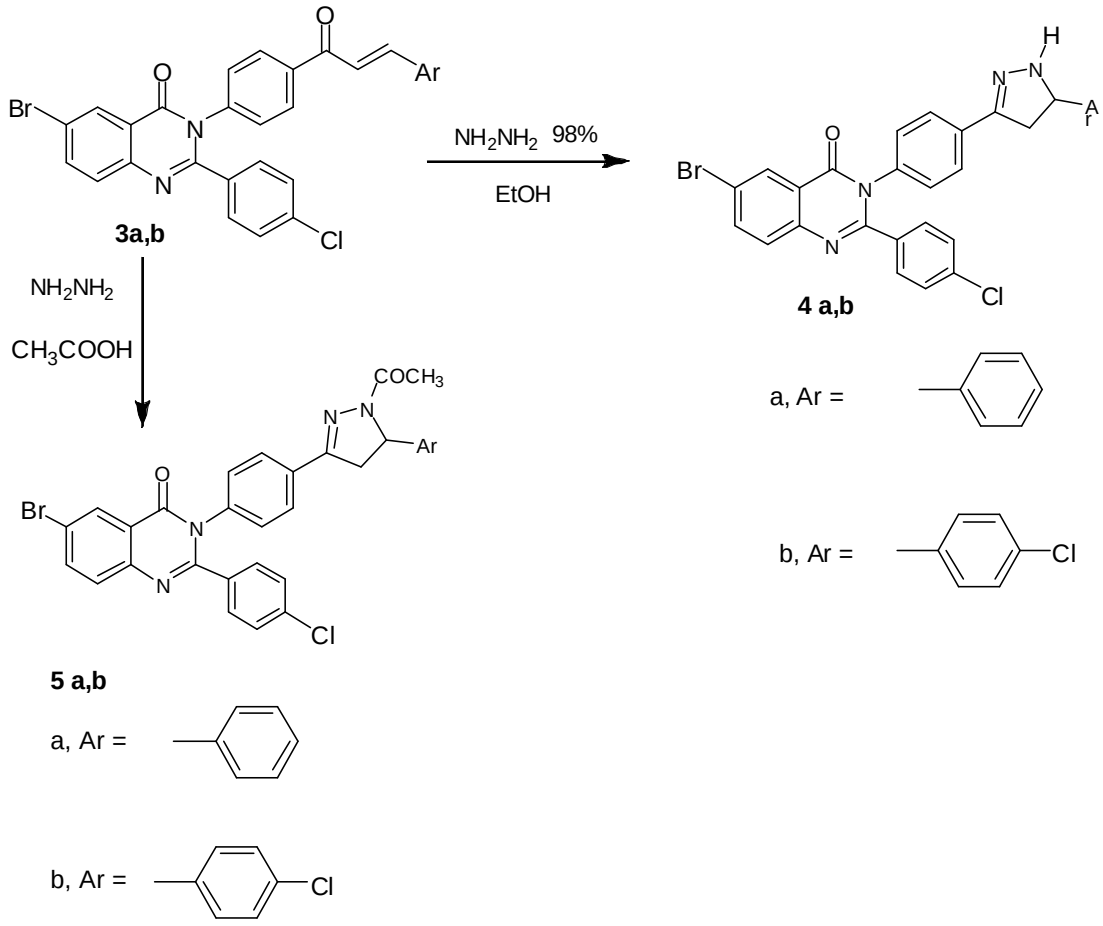
*2.1.2.4.****(E)-6-bromo-2-(4-chlorophenyl)-3-(4-(3-(3,4,5-trimethoxyphenyl)acryloyl)phenyl)quinazolin-4(3H)-one (3d)***
Crystallized from ethanol to give yellow crystals, m.p. 260^{o}C in 70% yield. Analysis for $C_{32}H_{24}BrClN_2O_5$; Calcd %C, 60.82; H, 3.83; N, 4.43, Found: %C, 60.90; H, 3.89; N, 4.48. IR: $\upsilon_{max.}$/cm^{-1} 1700 (C=O quinazolinone), 1680 (C=O of the α,β - unsaturated ketone) and at 1590 (C=N).^{1}H-NMR (DMSO-d_6, ppm): 3.9 (9H, s, OCH3) , 6.8-7.0 (2H, d, d, CH=CH) and 7.1 -8.2 (m, 13H, Ar-H). ^{13}C NMR (DMSO-d_6) d ppm: 189.5, 163, 160.7, 152, 148, 146, 138.5, 136.3, 136, 133, 132, 131.3, 129.1, 128.7, 126.5, 126.3, 124.9, 124.6, 123, 122, 103.8, 61, 56.2. MS (m/z, R.I.): 633 (100%), 632 (70.0%), 630 (33%).

2.1.2.5. ***(E)-6-bromo-2-(4-chlorophenyl)-3-(4-(3-(furan-2-yl)acryloyl)phenyl)quinazolin-4(3H)-one (3e)***
Crystallized from ethanol to give reddish brown crystals, m.p. 220^{o}C in 70% yield. Analysis for $C_{27}H_{16}BrClN_2O_3$; Calcd %C, 60.98; H, 3.03; N, 5.27; Found: %C, 60.98; H, 3.03; N,5.25; IR: $\upsilon_{max.}$/cm^{-1} 1720 (C=O quinazolinone), 1685 (C=O of the α,β - unsaturated ketone) and at 1600 (C=N).^{1}H-NMR (DMSO-d_6, ppm): 6.7-6.9 (2H, d, d, CH=CH) and at 7.0-8.2 (m, 14H, Ar-H). ^{13}C NMR (DMSO-d_6): 190, 167, 161, 151.5, 147, 144, 139, 136.3, 135.7, 133.5, 132.5, 132, 131.5, 129.2, 129.1, 127.3, 126.5, 124.9, 124.5, 121, 120.9, 113.8, 112.9.MS (m/z, R.I.): 533 (100.0%), 530 (76.2%), 532 (20.9%).

2.1.3. ***General method for the preparation of 4a,b****:*
A mixture of the chalcone **3a**,**b** (0.005 mol) and hydrazine hydrate (2.5 mL, 0.005 mol, 98%) in abs. ethanol (25 mL) was heated under reflux for 6 h. After cooling, the separated material was filtered off, air dried and then crystallized from the proper solvent to give **4a,b** respectively.

*2.1.3.1.****6-bromo-2-(4-chlorophenyl)-3-(4-(5-phenyl-4,5-dihydro-1H-pyrazol-3-yl)phenyl)quinazolin-4(3H)-one (4a)***
Crystallized from ethanol to give red crystals, m.p. 110^{o}C in 70% yield. Analysis for $C_{29}H_{20}BrClN_4O$; Calcd %C, 62.66; H, 3.63; N, 10.08, Found: %C, 62.62; H, 3.59; N, 10.10; IR: $\upsilon_{max.}$/cm^{-1} 3380 (NH), 3020(CH, aromatic), 1660 (C=O) and 1630(C=N). (DMSO-d_6, ppm): 3.6 (d,d, 2H, CH_2, pyrazoline ring), 3.95 (t, 1H, CH of pyrazoline, 7.0 (s, NH, exchangeable with D_2O), 7.3 -8.2 (m, 16H, Ar-H). ^{13}C NMR (DMSO-d_6) d ppm: 166, 161, 151.8, 147.7, 143.9, 136.3, 135.9, 135.1, 132.4, 132.1, 129.4, 129.2, 129, 128.5, 126.9, 126.5, 124.5, 123, 121.7, 49.2, 42.6. MS (m/z, R.I.): 558 (100%), 555 (35%), 559 (25%).

*2.1.3.2.****6-bromo-2-(4-chlorophenyl)-3-(4-(5-(4-chlorophenyl)-4,5-dihydro-1H-pyrazol-3-yl)phenyl)quinazolin-4(3H)-one (4b)***
Crystallized from ethanol to give white crystals, m.p. 290^{o}C in 70% yield. Analysis for $C_{29}H_{19}BrCl_2N_4O$; Calcd %C, 59.01; H, 3.24; N,9.49; Found: %C, 59.12; H,3.29; N,9.51; IR: $\upsilon_{max.}$/cm^{-1} 3395 (NH), 3035(CH, aromatic), 1675 (C=O) and 1640(C=N). ^{1}H-NMR (DMSO-d_6, ppm) 3.6 (d,d, 2H, CH_2, pyrazoline ring), 3.95 (t, 1H, CH of pyrazoline), 7.2 (s, NH, exchangeable with D_2O), 7.4 -8.3 (m, 15H, Ar-H). ^{13}C NMR (DMSO-d_6): 169, 162, 152.9, 151.7, 129.7, 137.8, 137.2, 136.5, 135.5, 134, 133, 132, 130, 129.8, 129, 126.9, 125, 124, 122, 121.7, 49.9, 43.4. MS (m/z, R.I.): 592 (100%), 591 (50%), 589 (20%).



Scheme 2: Synthesis of the target compounds 4a, b, 5a,b

2.1.4. ***General method for the preparation of (5a,b):***

A mixture of the chalcone **3a**,**b** (0.005 mol) and hydrazine hydrate (2.5 mL, 0.005 mol, 98%) in presence of (10 mL) of glacial acetic acid was heated under reflux for 10 h. After cooling, the mixture was poured into ice/water and filtered off, air dried and then crystallized from the proper solvent to give **5a,b** respectively.

*2.1.4.1.**3-(4-(1-acetyl-5-phenyl-4,5-dihydro-1H-pyrazol-3-yl)phenyl)-6-bromo-2-(4-chlorophenyl)quinazolin-4(3H)-one (5a)***

Crystallized from methanol to give reddish brown crystals, m.p. 180^{o}C in 70% yield. Analysis for $C_{31}H_{22}BrClN_4O_2$; Calcd %C, 62.27; H, 3.71; N, 9.37; Found: %C, 62.29; H, 3.80; N, 9.30; IR: $\upsilon_{max.}$/cm^{-1} 3040(CH, aromatic), 1690 (C=O quinazolinone, 1680 (C=O, acetyl) and 1635(C=N). ^{1}H-NMR (DMSO-d_6, ppm) 2.2 (3H, s , $COCH_3$), 3.6 (d,d, 2H, CH_2, pyrazoline ring), 3.90 (t, 1H, CH of pyrazoline, 7.0 -8.3 (m, 16H, Ar-H). ^{13}C NMR (DMSO-d_6): 168.5, 165, 161, 151.7, 148, 141.7, 137, 135.9, 135, 129.5, 129.3, 129, 128.5, 126.7, 124.9, 124.5, 123, 122, 58.9, 40, 23.5. MS (m/z, R.I.): 598 (100.0%), 596 (80%), 599 (35.0%).

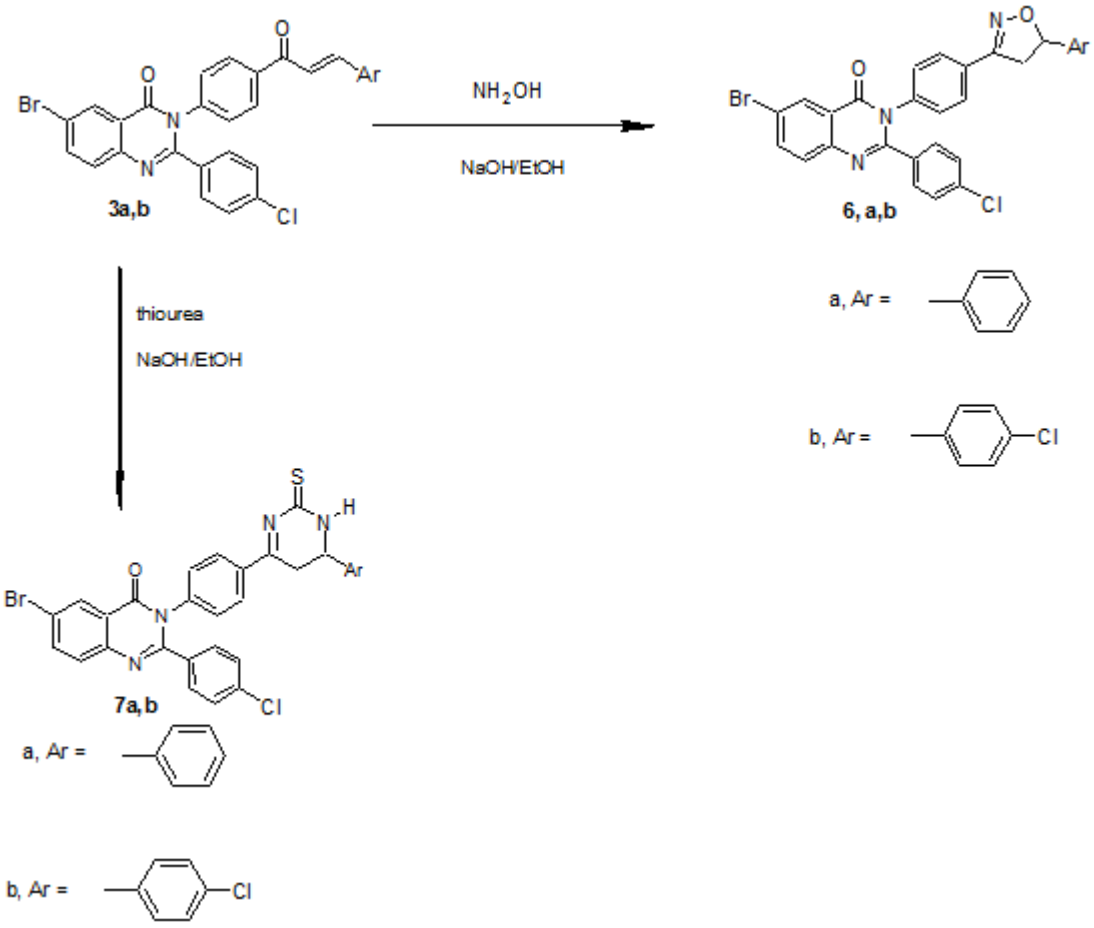
*2.1.4.2.**3-(4-(1-acetyl-5-(4-chlorophenyl)-4,5-dihydro-1H-pyrazol-3-yl)phenyl)-6-bromo-2-(4-chlorophenyl) quinazolin-4(3H)-one (5b)***

Crystallized from ethanol to give brown crystals, m.p. 122^{o}C in 65% yield. Analysis for $C_{31}H_{21}BrCl_2N_4O_2$; Calcd %C, 58.88; H, 3.35; N, 8.86; Found: %C,58.80; H,3.29; N, 8.79. IR: $\upsilon_{max.}$/cm^{-1} 3040(CH, aromatic), 1700 (C=O quinazolinone, 1685 (C=O, acetyl) and 1645(C=N). ^{1}H-NMR (DMSO-d_6, ppm) 2.2 (3H, s , $COCH_3$), 3.6 (d,d, 2H, CH_2, pyrazoline ring), 3.95 (t, 1H, CH of pyrazoline, 7.4 -8.5 (m, 15H, Ar-H). ^{13}C NMR (DMSO-d_6): 170, 166, 161.4, 153, 151.9, 148, 137.8, 137.3, 136.3, 135.9, 135.5, 132.3, 132, 129.5, 129.1, 128.8, 126.7, 124.9, 124.5, 123.3, 121.7, 59.5, 40.1, 23.5. MS (m/z, R.I.): 634 (100%), 633 (40%), 631 (20%).

2.1.5. ***General method for the preparation of (6a,b)***

A mixture of chalcone **3a**,**b** (3 mmol) and hydroxylamine hydrochloride (5 mmol) in sodium hydroxide solution (0.5g NaOH in 0.5mL water) in ethanol (60 mL) was refluxed for 10 h. The product obtained upon cooling was

filtered off, washed with water and recrystalized from the proper solvents to obtain the desired compounds **6a,b** respectively.



Scheme 3: Synthesis of the target compounds 6a, b, 7a,b

4.1.5.1. ***6-bromo-2-(4-chlorophenyl)-3-(4-(5-phenyl-4,5-dihydroisoxazol-3-yl)phenyl)quinazolin-4(3H)-one (6a)***
Crystallized from ethanol to give yellow crystals, m.p. 155^{o}C in 65% yield. Analysis for $C_{29}H_{19}BrClN_3O_2$; Calcd %C, 62.55; H, 3.44; N, 7.55; Found: %C, 62.50; H, 3.39; N, 7.50.IR: $\upsilon_{max.}$/cm^{-1} 3025(CH, aromatic), 1665 (C=O) and 1635 (C=N). ^{1}H-NMR (DMSOd6, δ ppm): 3.6-3.75 (d, d, 2H, CH_2, isoxazoline ring), 5.9 (t, 1H, CH, isoxazoline ring), 7.2 -8.2 (m, 16H, Ar-H). ^{13}C NMR (DMSO-d_6) d ppm: 164, 160.6, 157, 147.9, 143.5, 136.3, 135.9, 135.1, 133.4, 130, 129.5, 129.2, 128, 127.5, 126.7, 126, 124.6, 124.3, 121.9, 124, 82.5, 43.MS (m/z, R.I.): 557 (100.0%), 556 (64%), 558 (30%).

*2.1.5.2.****6-bromo-2-(4-chlorophenyl)-3-(4-(5-(4-chlorophenyl)-4,5-dihydroisoxazol-3-yl)phenyl)quinazolin-4(3H)-one (6b)***
Crystallized from glacial acetic acid to give white crystals, m.p. 104^{o}C in 65% yield. Analysis for $C_{29}H_{18}BrCl_2N_3O_2$; Calcd %C, 58.91; H, 3.07; N,7.11; Found: %C, 58.95; H, 3.10; N,7.19. IR: $\upsilon_{max.}$/cm^{-1} 3045 (CH, aromatic), 1690 (C=O) and 1640 (C=N). ^{1}H-NMR (DMSOd6, δ ppm): 3.65-3.8 (d, d, 2H, CH_2, isoxazoline ring), 6.0 (t, 1H, CH, isoxazoline ring and 7.35 -8.40 (m, 15H, Ar-H). ^{13}C NMR (DMSO-d_6) d ppm: 167, 162.3, 156.4, 153.1, 147.9, 138.1, 136.5, 135.7, 135, 134.7, 132.5, 130.2, 130, 128.7, 127.7, 127, 125.2, 125, 124, 121.7, 84.5, 42.MS (m/z, R.I.): 593 (100%), 592 (40%), 590 (39%).

2.1.6. ***General method for the preparation of (7a,b)***
A mixture of the chalcone **3a**,**b** (0.005 mol) and thiourea (0.005 mol) in presence of 0.5 gram of NaOH in 5mL of water. The mixture was refluxed in (25mL) of ethanol for 6 h then concentrated under vaccum and neutralized with dilute HCl. The precipitated material was filtered off, washed with water, dried and crystallized from ethanol to give **7a,b**.

2.1.6.1. ***6-bromo-2-(4-chlorophenyl)-3-(4-(6-phenyl-2-thioxo-1,2,5,6-tetrahydropyrimidin-4-yl)phenyl)quinazolin-4(3H)-one (7a)***

Crystallized from ethanol to give yellow crystals, m.p. 240^{o}C in 60% yield. Analysis for $C_{30}H_{20}BrClN_4OS$; Calcd %C, 60.06; H, 3.36; N, 9.35;Found: %C, 60.06; H, 3.36; N, 9.35; IR: $\upsilon_{max.}$/cm^{-1} 3480-3220(OH enolic of pyrimidine), 3025(CH, aromatic), 1690 (C=O), 1620 (C=N) and 1270(C=S). ^{1}H-NMR (DMSOd6, δ ppm): at 3.4(2H, d, CH_2 0f pyrimidinone), 5.8(1H, t, CH of pyrimidinone), and 7.2 -8.3 (m, 17H, Ar-H and pyrimidone). ^{13}C NMR (DMSO-d_6): 187, 165, 164.4, 161.6, 147.7, 145.5, 136.5, 136.2, 134.7, 134, 132.5, 130.4, 130.1, 129.7, 129.5, 126.9, 124.6, 124.3, 123.5, 122, 52.2, 42.4.MS (m/z, R.I.): 601 (100%), 602 (50%), 599 (44%).

*2.1.6.2.****6-bromo-2-(4-chlorophenyl)-3-(4-(6-(4-chlorophenyl)-2-thioxo-1,2,5,6-tetrahydropyrimidin-4-yl)phenyl) quinazolin-4(3H)-one (7b)***

Crystallized from glacial acetic acid to give white crystals, m.p. 110^{o}C in 60% yield. Analysis for $C_{30}H_{19}BrCl_2N_4OS$; Calcd %C, 56.80; H, 3.02; N, 8.83; Found: %C, 56.84; H, 3.09; N,8.89; IR: $\upsilon_{max.}$/cm^{-1} 3485-3230 (OH enolic of pyrimidine), 3040(CH, aromatic), 1700 (C=O), 1635 (C=N) and 1280(C=S). ^{1}H-NMR (DMSOd6, δ ppm): at 3.55(2H, d, CH_2 0f pyrimidinone), 5.7(1H, t, CH of pyrimidinone), 7.35 -8.4 (m, 16H, Ar-H and pyrimidone). ^{13}C NMR (DMSO-d_6): 189, 165.6, 163, 161.4, 153.9, 149.2, 137.8, 137.1, 136.5, 136, 134.7, 134, 133.4, 131.4, 131, 129.3, 127, 125.6, 125.3, 124, 121.7, 56.4, 44.6. MS (m/z, R.I.): 634 (100.0%), 632 (30%), 636 (48%).

2.2. *Antimicrobial screening*

The anti-bacterial activity of the synthesized compounds was tested against strains isolated from animal by products and were accused of being a direct cause of food intoxication in human. The tested compounds were evaluated against, Gram-positive bacteria (*Bacillus subtilis* ATCC 6633, *Staphylococcus aureus* ATCC 35556), Gram-negative bacteria (*Escherichia coli* ATCC 23282 and *Pseudomonas aeruginosa* ATCC 10145), Yeast (*Candida albicans* IMRU 3669) and *Filamentous Fungus* (*Aspergillus niger* ATCC 16404). The bacteria and yeast were grown on nutrient agar while the fungus was grown on Czapek`s Dox agar medium.

2.2.1 *Paper disc diffusion technique*

The sterilized medium [27] (autoclaved at 120 ^{o}C for 30 min) (40-50 ^{o}C) was inoculated (1 ml/100 ml of medium) with the suspension (105 cfu/ml) of the micro-organism (matched to 0.9 McFarland barium sulphate standard) and poured into a Petri dish to give a depth of 3-4 mm. The paper impregnated with the test chemicals (14 compounds) each dissolved in conc. (100 mg/ml in DMF) was placed on the solidified medium. The plates were preincubated for 1 h at room temperature and incubated at 37-28 ^{o}C for 24-48 h for anti-bacterial and anti-fungal activities, respectively.

The negative control was DMF showed no biological activity against the tested microorganisms, and a standard antibiotics; Tetracycline (30 mcg) as antibacterial, Nalidixic acid (30 mcg) as anti-yeast and Fluconazole (100 ppm) as anti-fungal were used as a positive control.The observed inhibition zone is presented in Table 1.

2.2.2. *Minimum inhibitory concentration (MIC)*

MIC [28] of the compound was determined by agar streak dilution method. A stock solution of the synthesized compound (1000 µg /ml) in DMF was prepared and graded quantities of the test compounds were incorporated in specified quantity of molten sterile agar. A specified quantity of the medium (40-50 ^{o}C) containing the compound was poured into a Petri dish to give a depth of 3-4 mm and allowed to solidify. Suspension of the micro-organism was prepared to contain approximately 105 cfu/ml and applied to plates with serially diluted compounds in DMF to be tested and incubated at 37 ^{o}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 2.

RESULTS AND DISCUSSION

3.1. Chemistry

Fusion between compound **1** and p-amino acetophenone at 160°C afforded 3-(4-acetylphenyl)-6-bromo-2-(4-chlorophenyl)quinazolin-4(3H)-one **2** (scheme 1). Claisen-Schmidt condensation of the acetyl derivative **2** with different aldehydes namely, benzaldehyde, p-chlorobenzaldehyde, p- nitobenzaldehyde , 3,4,5 trimethoxy benzaldehyde and/or furan-2-carboxaldehyde in ethanolic sodium hydroxide solution afforded the corresponding α,β-unsaturated ketones (chalcones) **3a-e** respectively (scheme 1).

Cyclocondensation of the unsaturated ketone **3a,b** with hydrazine hydrate in absolute ethanol afforded the corresponding pyrazoline derivatives **4a,b.** While cyclocondensation of the unsaturated ketone **3a,b** with hydrazine hydrate in glacial acetic acid afforded the corresponding acetyl pyrazoline derivatives **5a,b** (Scheme 2).

When α,β-unsaturated ketones **3a,b** were allowed to react with hydroxylamine hydrochloride in ethanolic sodium hydroxide solution, afforded the corresponding isoxazolines **6a,b** respectively. Cyclocondensation of the chalcones **3a,b** with thiourea in presence of NaOH afforded the corresponding tetrahydropyrimidine-thione derivatives **7a,b** respectively (Scheme 3).

Table, (1): Antimicrobial activity of the tested compounds measured in vitro activity-zone of inhibition in mm

Test organism / Compuond ID	*Bacillus subtilis*	*Staph. aureus*	*Escherichia coli*	*Pseud. aeruginosa*	*Candida albicans*	*Aspergillus niger*
2	-ve	-ve	-ve	-ve	-ve	-ve
3a	-ve	-ve	-ve	-ve	18	17
3b	22	22	31	21	15	15
3c	-ve	-ve	-ve	-ve	-ve	-ve
3d	-ve	-ve	19	18	-ve	-ve
3e	-ve	-ve	-ve	-ve	17	19
4a	-ve	-ve	-ve	-ve	17	19
4b	-ve	-ve	-ve	-ve	-ve	-ve
5a	-ve	-ve	-ve	-ve	19	21
5b	19	21	-ve	-ve	-ve	-ve
6a	-ve	-ve	-ve	-ve	-ve	-ve
6b	-ve	-ve	-ve	-ve	-ve	-ve
7a	-ve	-ve	-ve	-ve	-ve	-ve
7b	-ve	-ve	-ve	-ve	-ve	-ve
Reference	28	28	31	34	25	27

Table (2): Minimum Inhibitory Concentrations (MIC) of the compounds shows antimicrobial activity measured by (µg/mL)

Test organism / Compuond ID	*Bacillus subtilis*	*Staph. aureus*	*Escherichia coli*	*Pseud. aeruginosa*	*Candida albicans*	*Aspergillus niger*
3a	-----	-----	-----	-----	625	625
3b	150	150	75	312	625	625
3d	-----	-----	625	625	-----	-----
3e	-----	-----	-----	-----	625	625
4a	-----	-----	-----	-----	625	625
5a	-----	-----	-----	-----	625	625
5b	625	312	-----	-----	-----	-----

3.2. Antimicrobial activity

In this present work novel series of 6-bromo quinazolin-4(3H)-ones compounds were synthesized. The synthesized compounds were evaluated for their anti-microbial activity using the agar diffusion technique. The tested compounds were evaluated against, Gram-positive bacteria (*Bacillus subtilis* ATCC 6633, *Staphylococcus aureus* ATCC 35556), Gram-negative bacteria (*Escherichia coli* ATCC 23282 and *Pseudomonas aeruginosa* ATCC 10145), Yeast (*Candida albicans* IMRU 3669) and Filamentous Fungus (*Aspergillus niger* ATCC 16404). (Table 1)

Minimum inhibitory concentration (MIC) of the compounds which showed antimicrobial activity was determined in vitro using a broth micro-dilution assay. The MIC values showed 100 % inhibition of microbial growth (Table 2).

The anti-bacterial data (Table 1) revealed that compound **3b** has broad spectrum activity against Gram-positive bacteria, Gram-negative bacteria, Yeast and Filamentous Fungus with MICs 150, 150, 75, 312, 625 and 625 µg/mL against (*Bacillus subtilis* ATCC 6633, *Staphylococcus aureus* ATCC 3555, *Escherichia coli* ATCC 23282 *Pseudomonas aeruginosa* ATCC 10145, *Candida albicans* IMRU 3669 and *Aspergillus niger* ATCC 16404) respectively. Compound **3d** has promising activity against Gram-negative bacteria *Escherichia coli* ATCC 23282 *Pseudomonas aeruginosa* ATCC 10145 with MICs 625 µg/mL. On the other hand compound **5b** show potent activity against Gram-positive bacteria *Bacillus subtilis* ATCC 6633, *Staphylococcus aureus* ATCC 3555 with MICs 625, 312 µg/mL respectively. Compounds **3a, 3e, 4a** and **5a** exert potent anti-yeast and anti-fungal activity against *Candida albicans* IMRU 3669 and *Aspergillus niger* ATCC 16404 with MICs 625 µg/mL.

CONCLUSION

We have synthesized novel series of quinazolin-4(3H)-ones compounds to evaluate them on anti-microbial screen. 6-bromo quinazolinone derivatives bearing para chloro phenyl moiety at position 2 and substituted at 3rd position with 4-chalcone moiety bearing 4-choro phenyl **3b** show remarkable antimicrobial, anti-yeast and anti fungal

activity. 4-chalcone moiety bearing 3,4,5-trimethoxyphenyl **3d** show potent activity against Gram-negative bacteria. On the other hand 4-chalcone moiety having phenyl or furan-2-yl abolish antimicrobial activity but have potent anti-yeast and anti- fungal activity. While substitution with 4-nitrophenyl at the same position afford compound **3c** with no antimicrobial or anti-fungal activity.

Substitution at 3rd position with 5-phenyl- pyrazol or 1-acetyl-5-phenyl moiety give compound **4a**, **5a** respectively with promising anti-yeast and anti fungal activity. While substitution at the same position with 1-acetyl-(4-chlorophenyl) moiety give **5b** which have potent activity against Gram-positive bacteria. Compounds **6a,b** and **7a,b** with dihydro isoxazol or thioxo-tetrahydro pyrimidinyl moiety repectively at 3rd position of quinazolinone nucleus has neither antimicrobial nor anti fungal activity.

REFERENCES

[1] L. D. Högberg, A. Heddini ,O. Cars, *Trends Pharmacol Sci*. **2010**, 31(11), 509-515.
[2] B. Huttner , H. Goossens , T. Verheij,S. Harbarth , CHAMP consortium, *Lancet Infect Dis*. **2010**, 10(1), 17-31.
[3] R.E. Jsturiz, *Int. J. Antimicrob. Agents* **2010**, 36, 519-522.
[4] M. F. Ahmed, A. Belal, M. Youns, *Med. Chem. Res*. **2015**, DOI 10.1007/s00044-015-1357-1.
[5] M. F. Ahmed, M. Youns, *Arch. Pharm. Chem. Life Sci*. **2013**, 346, 610-617.
[6] M. F. Ahmed, M. Youns, A. Belal *Acta Pol. Pharm*. **2016**, 73(1).
[7] M. F. Ahmed, A. Belal, *Arch. Pharm. Chem. Life Sci*. **2015**, 348, 1–11
[8] M. F. Ahmed, A. Belal, *Res. Chem. Intermed*. **2015**, DOI. 10.1007/s11164-015-2048-8
[9] M. Alvarado, M. Barceló, L. Carro, C. F. Masaguer, E. Raviña, *Chem. Biodivers* **2006**, 3, 106–117.
[10] Mohamed F. Zayed, Memy H. Hassan, *Saudi Pharm. J*. **2014**, 22, 157–162
[11] S. M. Mosaad, M.K. Mohsen , M.M. Emad, N. Abotaleb, , M. KHEDR, F. A. Marwa, *Acta Pol. Pharm. Drug* Res. **2011**, 68, 665-675.
[12] M. A. El-Hashash, D. B. Guirguisand, Y. A. El-Badry. *Der Pharma Chemica*, **2011,** 3 (6), 147-159.
[13] C. P. Mani, T. Yakaiah, G. Gayatri, K. K. Pranay K, B. Narsaiah,U. S. N. Murthy, A. R. R. Raghu., *Eur. J. Med. Chem.*, **2011**, 45(1), 78-84.
[14] M. B. Deshmuhk, S. Patil, S. S. Patil , S. D. Jadhav, *Indian J Pharm. Sci.*, **2010**, 72 (4), 500-504.
[15] S. K. Pandey , A. Singh, A. Singh A, Nizamuddin.. *Eur. J. Med. Chem*., **2009**, 44, 1188-1197.
[16] D. J. Connolly, D. Cusack, T. P. O. Sullivan,P. J. Guiry, *Tetrahedron*., **2005**, 61, 10153-10202.
[17] S.B. Mhaske, N. P. Argade, *Tetrahedron*, **2006**,62, 9787–9826
[18] Kadi A.A. *J. Saudi Chem. Soc*., **2011**, 15, 95–100
[19] M. M. Aly, Y. A. Mohamed, K. M. El-Ba youki, W. M. Basyouni , S. Y. Abbas, *Eur. J. Med. Chem*., **2010**, 45, 3365-3373.
[20] G. P. Suresha, R. Suhas, W. Kapfo, D. C. Gowda, *Eur. J. Med. Chem*., **2011**, 46, 2530-2540.
[21] P. Panneerselvam, B.A. Rather, D.R.S. Reddy, N.R. Kumar, *Eur. J. Med. Chem*., **2009**, 44 (5), 2328-2333.
[22] M. S. Mohamed, M. M. Kamel, E. M. M. Kassem, N. botaleb, S. AbdEl-moez, M. F. Ahmed, *Eur. J. of Med*. Chem. **2010**, 45, 3311-3319.
[23] X. Fang, B. Yang, Z. Cheng, P. Zhang, M. Yang, *Res Chem Intermed*. **2014,** 40, 1715–1725
[24] V.J. Hushare, P. R. Rajput, *RASAYAN J. Chem*. **2012**, 5, 121-126
[25] E. M. Sharshira, N. M. M. Hamada, *Molecules* **2012**, 17, 4962-4971.
[26] T. I. El-Emary, Sh. A. Abdel-Mohsen, Phosphorus, Sulfur, and Silicon and the Related Elements, **2006,** 181:11, 2459-2474.
[27] S.H. Gillespie, Medical Microbiology-Illustrated. Butterworth Heinemann Ltd., United Kingdom, **1994**, 234-247.
[28] P.M. Hawkey, D.A. Lewis, Medical Bacteriology - a Practical Approach. Oxford University Press, United Kingdom, **1994**, 181-194.