SYNTHESES OF NEW HETEROCYCLES DERIVED FROM 2-PHENYL-3,1-BENZOXAZIN-4-ONE AND THEIR ANTIBACTERIAL AND ANTIFUNGAL ACTIVITY

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ABSTRACT

A series of novel substituted N-(6-substituted-4H-benzo[e][1,3]oxazin-3-yl)- 2-[4-(4-oxo-2-phenyl-4H-quinazolin-3-yl)-phenoxy]-acetamides (9a-b) and N-(6-substituted-2-thione-4H-benzo[e][1,3]oxazin-3-yl)-2-[4-(4-oxo-2-phenyl-4H-quinazolin-3-yl)-phenoxy]-acetamides (10a-b) were designed and synthesized. The structures of the newly synthesized compounds have been confirmed by IR, ^{1}H NMR and mass spectra. The compounds have also been screened for their biological activity.

KEY WORDS

Quinazolinone derivatives, IR, NMR, mass spectral data and biological activity.

INTRODUCTION

Quinazoline and quinazolinone possess interesting pharmacological activities[1], including anticonvulsant, antibacterial and diabetic activity[2,3]. Many compounds containing quinazolinone moiety are known as drugs e.g. methaquinalon[4] as a sedative and piriqualone[5,6] as an anticonvulsant.

Several 1,3-benzoxazine derivatives reported in the literature were found to possess analgesic, anti-inflammatory, tranquillizing, sedative, antibacterial, bacteriostatic[7-9], smooth muscle relaxant and spermicidal activities[10,11]. These observations together with our interest in synthetic potential of 4-(3H)-quinazolin-4-one prompted us to synthesize some new derivatives 4-(3H)-quinazolin-4-one with the objective of screening them for their biological activity.

RESULTS AND DISCUSSION

2-Amino benzoic acid **1** was reacted with benzoyl chloride **2** in pyridine to form 2-phenyl-3,1-benzoxazin-4-one[12,13] **3** which on condensation with 4-amino phenol gave 3-(4-hydroxy-phenyl)-2-phenyl-3H-quinazolin-4-one[14] **4.** Esterification of **4** at 50°-55°C with ethyl chloroacetate in presence of potassium carbonate in DMF afforded [4-(4-oxo-2-phenyl-4H-quinazolin-3-yl)-phenoxy]-acetic acid ethyl ester **5.** The same reaction was found to be incomplete in acetone even after heating for 48 hours. The reaction in acetone reached completion in 4 minutes with total conversion of compound **4** to compound **5** under microwave irradiation and the compound **5** obtained was finally crystallised from acetone.

The compound **5** can also be prepared in high yields by the following alternative method:

The condensation of 2-phenyl-3,1-benzoxazin-4-one **3** with 4-(amino-phenoxy)- acetic acid ethyl ester[15] gave [4-(4-oxo-2-phenyl-4H-quinazolin-3-yl)-phenoxy]-acetic acid ethyl ester **5**. The structure of compound **5** obtained by both the methods was established by analytical and spectral data. The compound **5** on condensation with hydrazine hydrate in ethanol afforded [4-(4-oxo-2-phenyl-4H-quinazolin-3-yl)-phenoxy]-acetic acid hydrazide **6** in good yields. The hydrazide **6** on reaction with substituted salicylaldehyde afforded Schiff's base [4-(4-oxo-2-phenyl-4H-quinazolin-3-yl)-phenoxy]-acetic acid (2-hydroxy-substituted benzilidene)-hydrazides **7a** and **7b** respectively. The selective reduction of Schiff's base **7a** and **7b** with sodium borohydride in tetrahydrofuran gave [4-(4-oxo-2-phenyl-4H-quinazolin-3-yl)-phenoxy]-acetic acid-N-(2-hydroxy-substituted benzyl)-hydrazides **8a** and **8b**. The reaction of compounds **8a** and **8b** with formaldehyde in isopropyl alcohol furnished N-(6-substituted-4H-benzo[e][1,3]oxazin-3-yl)-2-[4-(4-oxo-2-phenyl-4H-quinazolin-3-yl)-phenoxy]-acetamides **(9a-b)** whereas the reaction of compounds **8a** and **8b** with carbon disulphide in ethanol in presence of potassium carbonate gave N-(6-substituted-2-thione-4H-benzo[e][1,3]oxazin-3-yl)-2-[4-(4-oxo-2-phenyl-4H-quinazolin-3-yl)-phenoxy]-acetamides **(10a-b) [SCHEME 1].**

BIOLOGICAL ACTIVITY

Antibacterial activity

All the newly synthesized compounds **(9a-b)** and **(10a-b)** were screened in **vitro** for their antibacterial activity against **Staphylococcus aureus, Escherichia coli, Bacillus subtilis and Salmonella typhosa** by the ditch-plate technique[16] using concentrations of 2 mg/mL. Nutrient agar was employed as culture media and DMF was used as solvent control for antibacterial activity.

Antifungal activity

The compounds **(9a-b)** and **(10a-b)** synthesized were screened for their antifungal activity against **Aspergillus niger, Candida albicans, Cryptococcus neoformans and Thielaviopsis paradoxa** by paper-disc diffusion method[17] at concentrations of 2 mg/mL. Nutrient agar was employed as culture media and DMF was used as solvent control for antifungal activity.

The known compounds such as ampicillin, amoxicillin, norfloxacin, penicillin and griseofulvin were used for comparison purpose. The diameter of zone of inhibition was measured in mm. The antibacterial and antifungal screening data are recorded in **Table-1.**

From **Table-1,** it can be seen that the compounds **9b, 10a** and **10b** exhibited remarkable activity against **Staphylococcus aureus,** compound **10b** showed notable activity against **Bacillus subtilis** and **Salmonella typhosa** and compounds **9b** and **10b** displayed a high degree of activity against **Escherichia coli.**

From **Table-2,** it can be seen that the compounds **9b** and **10b** exhibited good activity against **Aspergillus niger** and the compounds **9a, 10a** and **10b** showed maximum activity against **Cryptococcus neoformans.** The compounds **9b** and **10b** showed good activity against **Candida albicans and Thielaviopsis paradoxa.**

TABLE-1

Biological Activity Data

Compounds	Zone of Inhibition in mm Antibacterial activity			
	S. aureus	E. coli	B. subtilis	S.typhosa
9a	11	10	11	8
9b	17	16	12	13
10a	16	12	8	11
10b	17	18	17	16

TABLE-2

Biological Activity Data

Compounds	Zone of Inhibition in mm			
	Antifungal activity			
	A. niger	C. albicans	C.neoformans	T. paradoxa
9a	12	9	16	12
9b	15	17	8	16
10a	14	11	15	9
10b	17	16	17	16

EXPERIMENTAL

Melting points were taken in open capillaries and are uncorrected. IR spectra (KBr in cm^{-1}) were recorded on Jasco 410 plus FTIR spectrophotometer. ^1H NMR spectra were recorded on a Bruker 500 MHz NMR spectrophotometer using DMSO-d_6 as solvent and TMS as internal standard (chemical shifts in δ ppm). The mass spectra of compounds were determined on Schimadzu model No. QP 2010. The elemental analysis was carried out on a Perkin Elmer elemental analyzer and sulphur analysis was obtained by oxygen-flask method. The purity of the compounds was monitored by thin layer chromatography. The TLC was carried out on precoated 0.2 mm silica gel $_{60}F^{254}$ plates.

2-Phenyl-3,1-benzoxazin-4-one (3)

To a solution of 2-amino benzoic acid **1** (13.7 g, 0.1 mole) in 100 mL dry pyridine, benzoyl chloride **2** (14.0 mL, 0.11 mole) was added dropwise with constant stirring at 25°-30°C and stirred further for about 1 hour. The reaction mixture was then poured into water. The solid obtained was filtered, washed with 5% sodium bicarbonate solution and recrystallised from methanol, yield 82%, m.p. 122-123°C; IR (KBr) 1762 (C=O), 1598 (C=N), 1571, 1495, 1473 (C=C, aromatic).

3-(4-Hydroxy-phenyl)-2-phenyl-3H-quinazolin-4-one (4)

A mixture of compound **3** (22.3 g, 0.1 mole) and 4-aminophenol (11.88 g, 0.11 mole) in 200 mL pyridine was refluxed for about 7 hours. The reaction mixture was then allowed to cool to room temperature and left overnight. The separated solid was filtered, washed with methanol and recrystallized from ethanol, yield 50%, m.p. 240-242°C; IR (KBr) 3292 (O-H), 1650 (CO·N), 1604 (C=N), 1577, 1541, 1512, 1485 (C=C, aromatic).

[4-(4-Oxo-2-phenyl-4H-quinazolin-3-yl)-phenoxy]-acetic acid ethyl ester (5)

Method I

A solution of compound **4** (3.14 g, 0.01 mole) in 50 mL dry acetone was heated in presence of anhydrous potassium carbonate (1.38 g, 0.01 mole) and ethyl chloroacetate (1.47 g, 0.012 mole) under microwave irradiation for 4 minutes. The reaction mixture was cooled and filtered to separate out potassium chloride and unreacted potassium carbonate. Acetone was removed from the filtrate under reduced pressure to one-third of the initial volume and the product obtained on cooling was filtered, washed with water and recrystallised from acetone, yield 50%, m.p. 179-180°C; IR (KBr) 1735 (C=O ester), 1650 (CO·N), 1606 (C=N), 1577, 1544, 1508, 1448 (C=C, aromatic); NMR (DMSO-d_6) δ 1.24 (t, 3H, CH_3), 4.1 (q, 2H, CH_2), 4.7 (s, 2H, O·CH_2·C), 6.9-8.6 (m, 13H, ArH); Anal. Calcd. for $C_{24}H_{20}N_2O_4$: C, 72.00; H, 5.00; N, 7.00. Found : C, 71.96, H, 5.02, N, 6.94.

The same reaction was carried out in N,N-dimethyl formamide as solvent instead of acetone. The reaction mixture was heated for about 4 hours and the compound **5** obtained was filtered, washed with water and crystallized from acetone, yield 40%, m.p. 180°C.

The analytical and spectral data were in accordance with the data obtained above for compound **5** synthesized in acetone as solvent.

[4-(4-Oxo-2-phenyl-4H-quinazolin-3-yl)-phenoxy]-acetic acid ethyl ester (5)

Method II

2-Phenyl-3,1-benzoxazin-4-one (**3**; 4.46 g, 0.02 mole) and (4-amino-phenoxy)-acetic acid ethyl ester (4.29 g, 0.022 mole) in 100 mL pyridine was refluxed for about 12 hours. The reaction mixture was then allowed to cool to room temperature and left overnight. The crystalline solid obtained was filtered, washed with ethanol and recrystallized from ethanol, yield 82%, m.p. 179-180°C; IR (KBr) 1735 (C=O ester), 1650 (CO·N), 1606 (C=N), 1577, 1544, 1508, 1448 (C=C, aromatic); ^{1}H NMR (DMSO-d_6) δ 1.24 (t, 3H, CH_3), 4.1 (q, 2H, CH_2), 4.7 (s, 2H, O·CH_2·C), 6.9-8.6 (m, 13H, ArH); Anal. Calcd. for $C_{24}H_{20}N_2O_4$: C, 72.00; H, 5.00; N, 7.00. Found : C, 72.05; H, 5.05; N, 6.97.

[4-(4-Oxo-2-phenyl-4H-quinazolin-3-yl)-phenoxy]-acetic acid hydrazide (6)

A mixture of compound **5** (4.0 g, 0.01 mole) and 99% hydrazine hydrate (0.5 mL, 0.015 mole) in 500 mL of ethanol was refluxed for about 8 hours. The reaction mixture was then allowed to cool to room temperature. The separated white coloured crystalline solid was filtered, washed with ethanol and recrystallised from ethanol, yield 90%, m.p. 218-220°C; IR (KBr) 3315, 3272 (NH·NH_2), 1674 (C=O amide), 1652 (CO·N), 1603 (C=N), 1585, 1508, 1450 (C=C, aromatic); ^{1}H NMR (DMSO-d_6) δ 4.3 (s, 2H, NH_2), 4.4 (s, 2H, OCH_2C), 6.9-8.6 (m, 13H, ArH), 9.3 (s, 1H, NH); Anal. Calcd. for $C_{22}H_{18}N_4O_3$: C, 68.39; H, 4.66; N, 14.50. Found : C, 68.41; H, 4.65; N, 14.52.

[4-(4-Oxo-2-phenyl-4H-quinazolin-3-yl)-phenoxy]-acetic acid (2-hydroxy-benzilidene)-hydrazide (7a)

Compound **6** (3.86 g, 0.01 mole) was condensed with salicylaldehyde (1.22 g, 0.01 mole) in 99% ethanol (40 mL) in presence of 1-2 drops of glacial acetic acid with continuous stirring over a period of 12 hours. The white coloured solid obtained was filtered, washed with ethanol and recrystallized from DMF : methanol (3 : 1), yield 82%, m.p. 225-226°C; IR (KBr) 3257 (NH), 1678 (CO·N), 1624 (C=N), 1593, 1508, 1489 (C=C, aromatic), 1087 (C-O-C); ^{1}H NMR (DMSO-d_6) δ 4.8 (s, 2H, O·CH_2·C), 5.1 (s, 1H, CH), 6.9-8.6 (m, 17H, ArH), 10.0 (s, 1H, NH-C=O), 11.0 (s, 1H, OH); Anal. Calcd. for $C_{29}H_{22}N_4O_4$: C, 71.02; H, 4.49; N, 11.43. Found : C, 71.00; H, 4.43; N, 11.52.

[4-(4-Oxo-2-phenyl-4H-quinazolin-3-yl)-phenoxy]-acetic acid (5-bromo-2-hydroxy-benzilidene)-hydrazide (7b)

Similarly compound **7b** was synthesized from compound **6** and bromosalicylaldehyde as per procedure given in **7a.**

7b: White-coloured solid, yield 77%, m.p. 234-236°C; IR (KBr) 3246 (NH), 1685 (CO·N), 1602 (C=N), 1558, 1508, 1489 (C=C, aromatic), 1074 (O·C·O); ^{1}H NMR (DMSO-d_6) δ 4.7 (s, 2H, OCH_2C), 5.1 (s, 1H, CH), 6.9-8.6 (m, 16H, ArH), 10.4 (s, 1H, NH·C=O), 11.6 (s, 1H, OH); Anal. Calcd. for $C_{29}H_{21}BrN_4O_4$: C, 61.16; H, 3.69; N, 9.84. Found : C, 61.21; H, 3.75; N, 9.92.

[4-(4-Oxo-2-phenyl-4H-quinazolin-3-yl)-phenoxy]-acetic acid N-(2-hydroxy-benzyl)-hydrazide (8a)

[4-(4-Oxo-2-phenyl-4H-quinazolin-3-yl)-phenoxy]-acetic acid -2-hydroxy-benzilidene)-hydrazide (**7a**; 2.45 g, 0.005 mole) was suspended in 25 mL terahydrofuran. Sodium borohydride (0.19 g, 0.006 mole) was then added pinch wise at 20°-25°C with continuous stirring for 2 hours. The reaction mixture was then warmed up to 35°C and stirred for another hour. It was allowed to cool to room temperature, 200 mL of water was added followed by neutralization with hydrochloric acid at pH 6.5 under cold condition. The white-coloured product obtained was filtered, washed with water and recystallized from terahydrofuran, yield 76%, m.p. 176-178°C; IR (KBr) 3317 (NH·NH), 1689 (CO·N), 1602 (C=N), 1585, 1508, 1489 (C=C, aromatic), 1098 (C·O·C); ^{1}H NMR (DMSO-d_6) δ 3.8 (s, 2H, N·CH_2·C), 4.4 (s, 2H, O·CH_2·C), 4.6 (s, 1H, NH), 6.8-8.6 (m, 17H, ArH), 9.6 (s, 1H, NH·C=O), 9.7 (s, **1H, OH**); Anal. Calcd. for $C_{29}H_{24}N_4O_4$: C, 70.73; H, 4.88; N, 11.38. Found : C, 70.69; H, 4.85; N, 11.42.

[4-(4-Oxo-2-phenyl-4H-quinazolin-3-yl)-phenoxy]-acetic acid N-(5-bromo-2-hydroxy-benzyl)-hydrazide (8b)

Similarly compound **8b** was synthesized from compound **7b** as per procedure given in **8a**.
8b: White-coloured solid, yield 71%, m.p. 151-152°C; IR (KBr) 3257 (NH), 1653 (CO·N), 1606 (C=N), 1541, 1508, 1489 (C=C, aromatic), 1072 (C·O·C); ^{1}H NMR (DMSO-d_6) δ 3.8 (s, 2H, N·CH_2·C), 4.5 (s, 2H, O·CH_2·C), 4.6 (s, 1H, NH), 6.8-8.6 (m, 17H, ArH), 9.6 (s, 1H, NH·C=O), 9.8 (s, **1H, OH**); Anal. Calcd. for $C_{29}H_{23}BrN_4O_4$: C, 60.94; H, 4.02; N, 9.81. Found : C, 61.01; H, 4.08; N, 9.85.

N-(4H-benzo[e][1,3]oxazin-3-yl)-2-[4-(4-oxo-2-phenyl-4H-quinazolin-3-yl)-phenoxy]-acetamide (9a)

To the solution of compound **8a** (0.246 g, 0.0005 mole) in 25 mL isopropyl alcohol was added 37% formaldehyde solution (0.05 mL, 0.0006 mole) and the reaction mixture was heated for about 6 hours. The hot solution was charcoalised, filtered and allowed to stand overnight at room temperature. The white-coloured product **9a** obtained was filtered, washed with water and recrystallised from isopropyl alcohol, yield 66%, m.p. 143-144°C; IR (KBr) 3290 (NH), 1683 (CO·N), 1602 (C=N), 1541, 1508, 1489 (C=C, aromatic), 1076 (C·O·C); 1H NMR (DMSO-d_6) δ 4.1 (s, 2H, N·CH_2·O), 4.4 (s, 2H, O·CH_2·C), 4.8 (s, 2H, N·CH_2·C), 6.8-8.6 (m, 17H, ArH), 9.7 (s, 1H, NH·C=O); MS (m/z): 504 [M^+], 313, 224, 205, 193, 179, 166, 153, 146, 120, 109, 105, 92, 877, 78, 65, 51; Anal. Calcd for $C_{30}H_{24}N_4O_4$: C, 71.42; H, 4.76; N, 11.11. Found : C, 71.45; H, 4.73; N, 11.12.

N-(6-bromo-4H-benzo[e][1,3]oxazin-3-yl)-2-[4-(4-oxo-2-phenyl-4H-quinazolin-3-yl)-phenoxy]-acetamide (9b)

Similarly compound **9b** was synthesized from compound **8b** as per procedure given in **9a**.

9b: White-coloured solid, yield 61%, m.p. 166-167°C; IR (KBr) 3290 (NH), 1651 (CO·N), 1604 (C=N), 1541, 1508, 1487 (C=C, aromatic), 1072 (C·O·C); ^{1}H NMR (DMSO-d_6) δ 4.1 (s, 2H, N·CH_2·O), 4.5 (s, 2H, O·CH_2·C), 4.9 (s, 2H, N·CH_2·C), 6.8-8.6 (m, 16H, ArH), 9.7 (s, 1H, NH·C=O); MS (m/z): 583 [M^+], 313, 223, 205, 195, 179, 166, 153, 146, 120, 109, 105, 90, 78, 77, 63, 51; Anal. Calcd. for $C_{30}H_{23}BrN_4O_4$: C, 61.74; H, 3.95; N, 9.60. Found : C, 61.84; H, 3.91; N, 9.66.

N-(2-thione-4H-benzo[e][1,3]oxazin-3-yl)-2-[4-(4-oxo-2-phenyl-4H-quinazolin-3-yl)-phenoxy]-acetamide (10a)

[4-(4-Oxo-2-phenyl-4H-quinazolin-3-yl)-phenoxy]-acetic acid N-(2-hydroxy-benzyl)-hydrazide (**8a;** 0.246 g, 0.0005 mole) was dissolved in 25 mL ethanol. Carbon disulphide (0.6 mL, 0.0009 mole) was added and the reaction mixture was heated in presence of potassium carbonate (0.92 g, 0.0066 mole) for 4 hours. The excess ethanol was distilled under

reduced pressure till 2-3 mL of ethanol was left behind. The viscous liquid obtained on cooling was poured into 20 mL of cold water followed by neutralization with 1:1 hydrochloric acid under cold condition. The white-coloured product obtained was filtered, washed with water and recrystallised from ethanol, yield 55%, m.p. 164-165°C; IR (KBr) 3282 (NH), 1653 (CO N), 1604 (C=N), 1541, 1508, 1489 (C=C, aromatic), 1417 (C=S), 1087 (C O C); ^{1}H NMR (DMSO-d_6) δ 4.5 (s, 2H, O·CH_2·C), 5.5 (s, 2H, N·CH_2·C), 6.8-8.6 (m, 17H, ArH), 10.2 (s, 1H, NH·C=O); MS (m/z): 534 [M^+], 313, 237, 224, 205, 206, 179, 166, 152, 146, 120, 109, 105, 92, 78, 77, 65, 51; Anal. Calcd. for $C_{30}H_{22}N_4O_4S$: C, 67.42; H, 4.12; N, 10.49; S, 6.00. Found : C, 67.38; H, 4.06; N, 10.51; S, 5.95.

N-(6-bromo-2-thione-4H-benzo[e][1,3]oxazin-3-yl)-2-[4-(4-oxo-2-phenyl-4H-quinazolin-3-yl)-phenoxy]-acetamide (10b)

Similarly compound **10b** was synthesized from compound **8b** as per procedure given in **10a**.

10b: White-coloured solid, yield 52%, m.p. 169-170°C; IR (KBr) 3257 (NH), 1697 (CO·N), 1593, 1508, 1487 (C=C, aromatic), 1373 (C=S), 1078 (C-O-C); ^{1}H NMR (DMSO-d_6) δ 4.6 (s, 2H, O·CH_2·C), 5.5 (s, 2H, N·CH_2·C), 6.8-8.6 (m, 16H, ArH), 10.3 (s, 1H, NH·C=O); MS (m/z): 613 [M^+], 313, 237, 224, 205, 206, 179, 166, 152, 146, 120, 105, 109, 92, 78, 77, 65, 51; Anal. Calcd. for $C_{30}H_{21}BrN_4O_4S$: C, 58.73; H, 3.43; N, 9.14; S, 5.22. Found : C, 58.80; H, 3.40; N, 9.20; S, 5.26.

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SCHEME 1