SYNTHESES OF SOME NOVEL [4-(4-OXO-2-PHENYL-4H-QUINAZOLIN-3-YL)-PHENOXY]-ACETIC ACID [1-SUBSTITUTED AMINOMETHYL-2-OXO-1,2-DIHYDRO-INDOL-3-YLIDENE]-HYDRAZIDE DERIVATIVES AND THEIR POTENTIAL BIOLOGICAL ACTIVITY

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Abstract : [4-(4-Oxo-2-phenyl-4H-quinazolin-3-yl)-phenoxy]-acetic acid [2-oxo-1,2-dihydro-indol-3-ylidene]-hydrazide (7) on reaction with formaldehyde and various secondary amines in N,N-dimethyl formamide afforded Mannich bases [4-(4-oxo-2-phenyl-4H-quinazolin-3-yl)-phenoxy]-acetic acid [1-substituted aminomethyl-2-oxo-1,2-dihydro-indol-3-ylidene]-hydrazides (8a-d). 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.
Keywords : [4-(4-oxo-2-phenyl-4H-quinazolin-3-yl)-phenoxy]-acetic acid ethyl ester, 1H-indole-2,3-dione, IR, spectral studies and biological activity.

Introduction

Quinazolinones exhibit wide spectrum of biological activities including anthelmintic[1], anti-inflammatory[2], anticonvulsant[3], hypoglycemic[4], antitubercular[5], antibacterial[6], anticancer[7] and CNS depressant[8] activities. Indole and its derivatives represent one of the most active class of compounds possessing a wide spectrum of pharmacological activities. Indole-2,3-dione and its derivatives have been found to posses antimicrobial, anticonvulsant, antileukemic, antifertility, antiviral and CNS depressant activities[9-11]. Indole-2,3-dionhydrazones and indole-2,3-dione Mannich bases are known to have potential antiviral[12] and antimicrobial activity[13]. Also quinazolinone such as tryptanthrin have been found to have remarkable antimalarial activity[14]. In view of the above observations, we have synthesized some novel Mannich bases with the objective of screening them for their biological activity.

Results and Discussion

2-Amino benzoic acid **1** was allowed to react with benzoyl chloride **2** in pyridine to form 2-phenyl-3,1-benzoxazin-4-one[15,16] **3** which on condensation with 4-amino phenol gave 3-(4-hydroxy-phenyl)-2-phenyl-3H-quinazolin-4-one[17] **4** which on esterification at 50°-55°C with ethyl chloroacetate in presence of potassium carbonate in DMF formed [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. But 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 method:
The condensation of 2-phenyl-3,1-benzoxazin-4-one **3** with (4-amino-phenoxy)- acetic acid ethyl ester[18] 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 compound **6** on reaction with 1H-indole-2,3-dione gave [4-(4-oxo-2-phenyl-4H-quinazolin-3-yl)-phenoxy]-acetic acid [2-oxo-1,2-dihydro-indol-3-ylidene]-hydrazide **7.** The compound **7** on further reaction with formaldehyde and various secondary amines in N,N-dimethyl formamide gave Mannich bases [4-(4-oxo-2-phenyl-4H-quinazolin-3-yl)-phenoxy]-acetic acid [1-substituted aminomethyl-2-oxo-1,2-dihydro-indol-3-ylidene]-hydrazides (**8a-d**) **[Scheme-1].**

Biological Activity
Antibacterial Activity

All the newly synthesized compounds (**8a-d**) were screened in **vitro** for their antibacterial activity against Staphylococcus aureus, Escherichia coli, **B**acillus subtilis and Salmonella typhosa by the ditch-plate technique[19] using concentrations of 2 and 5 mg/ml. Nutrient agar was employed as culture media and DMF was used as solvent control for antibacterial activity.

The compounds **8a** & **8b** showed high activity against Escherichia coli and Staphylococcus aureus at the concentration level of 2 mg/ml. The compounds **8c** & **8d** exhibited moderate activity against both Escherichia coli and Staphylococcus aureus at the concentration level of 5 mg/ml.

Antifungal Activity

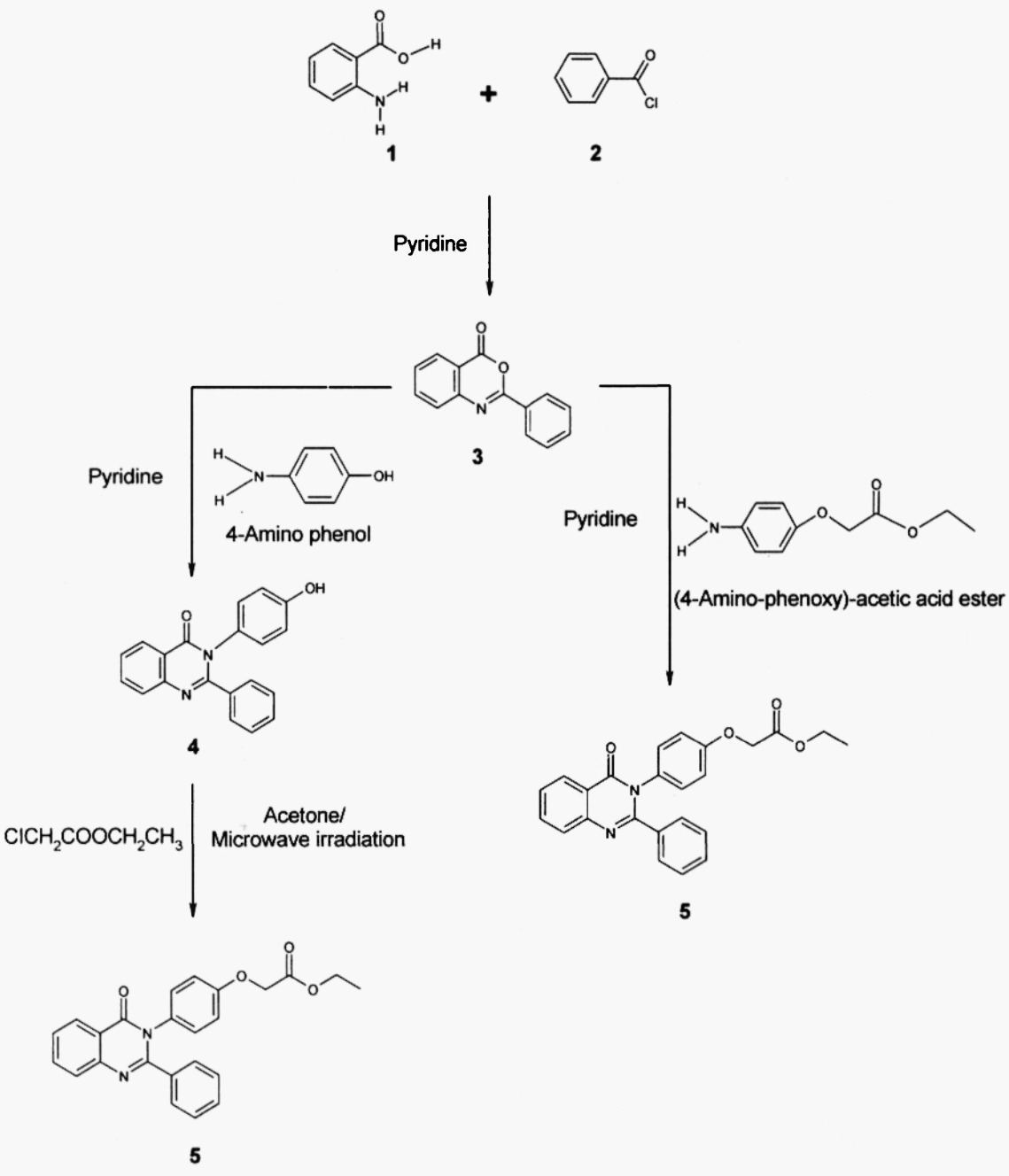
The compounds **(8a-d)** synthesized were screened for their antifungal activity against Aspergillus niger, Candida albicans, Cryptococcus neoformans and Thielaviopsis paradoxa by paper-disc diffusion method[20] at concentrations of 2 and 5 mg/ml. Nutrient agar was employed as culture media and DMF was used as solvent control for antifungal activity.

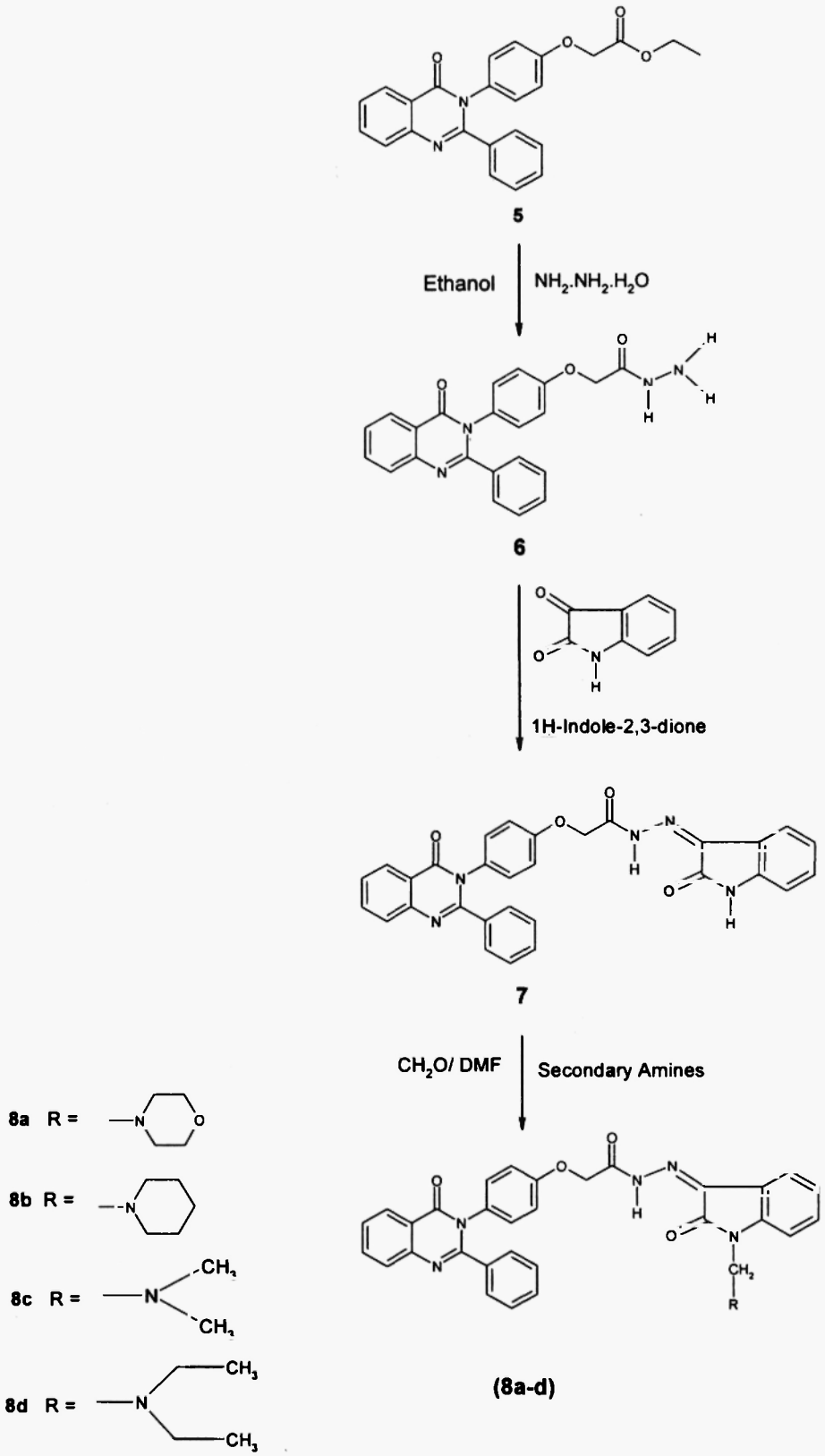
The compounds **8a, 8b and 8c** showed marked activity against Aspergillus niger, Candida albicans and Cryptococcus neoformans at the concentration level of 2 mg/ml and moderate activity against Thielaviopsis paradoxa at the concentration level of 5 mg/ml. The compound **8d** showed moderate activity against Aspergillus niger and Cryptococcus neoformans at the concentration level of 5 mg/ml and no activity against Candida albicans and Thielaviopsis paradoxa at the concentration level of 5 mg/ml. Known compounds, such as ampicillin, amoxcillin, norfloxacin and penicillin were used for comparison purpose. The diameter of zone of inhibition was measured in mm

Inhibition zone diameter in mm :	High activity	15-18 mm
	Moderate activity	11-14 mm
	Weak Activity	< 11 mm

Antimalarial Activity

The effort to find new antimalarial activity is still a high priority given to the increasing malarial emergency due to chloroquine resistant Plasmodium falciparum strains. The chloroquine-resistant Plasmodium falciparum malarial parasite was cultured in vitro and the sensitivity of parasite to the newly synthesized compounds was evaluated using the tritiated Hypoxanthine incorporation assay[21]. The compounds **(8a-d)** were tested for antimalarial activity and the only compound [4-(4-oxo-2-phenyl-4H-quinazolin-3-yl)-phenoxy]-acetic acid [1-(1-methyl-1-morpholin-4-yl-ethyl)-2-oxo-1,2-dihydro-indol-3-ylidene]-hydrazide **8a** was found to be most active against Plasmodium falciparum strains and its 50% inhibitor concentration (IC50) values were 17.89μM.


1
2
Pyridine
3
Pyridine
4-Amino phenol
Pyridine
(4-Amino-phenoxy)-acetic acid ester
4
5
ClCH2COOCH2CH3
Acetone/
Microwave irradiation
5


5
Ethanol
$NH_2.NH_2.H_2O$
6
1H-Indole-2,3-dione
7
CH_2O/ DMF
Secondary Amines
8a R =
8b R =
8c R =
8d R =
(8a-d)

Scheme-1

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. ^{1}H 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 with Schimadzu model No.QP 2010. The elemental analysis was carried out on a Perkin Elmer CHN analyzer. The purity of the compounds was monitored by thin layer chromatography. 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 cm^3 dry pyridine, benzoyl chloride 2 (14.0 cm^3, 0.11 mole) was added drop wise 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. 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 cm^3 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. 242°C; IR (KBr) 3292 (OH), 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 100 cm^3 dry acetone was heated in presence of anhydrous potassium carbonate (1.38 g, 0.01 mole) with ethyl chloroacetate (1.47 cm^3, 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 total volume and the product obtained on cooling was filtered, washed with water and recrystallised from acetone, yield 50%, m.p. 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, OCH_2C), 6.9-8.6 (m, 13H, ArH); Anal.Calcd. for $C_{24}H_{20}N_2O_4$ (400): C, 72.00; H, 5.00; N, 7.00 % Found : C, 71.96, , 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

A mixture of compound **3** (4.46 g, 0.02 mole) and (4-amino-phenoxy)-acetic acid ethyl ester (4.29 g, 0.055 mole) in 100 cm^3 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. 180°C; IR (KBr) 1735 (C=O ester), 1650 (CON), 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, OCH_2C), 6.9-8.6 (m, 13H, ArH); Anal.Calcd. for $C_{24}H_{20}N_2O_4$ (400): 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 2 cm^3 of 99% hydrazine hydrate in 500 cm^3 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. 220°C; IR (KBr) 3315, 3272 (NHNH_2), 1674 (C=O amide), 1652 (CON), 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 $C_{22}H_{18}N_4O_3$ (386): 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-oxo-1,2-dihydro-indol-3-ylidene]-hydrazide (7)

Compound **6** (3.86 g, 0.01 mole) was dissolved in 100 cm^3 N,N-dimethyl formamide to which 1H-indole-2,3-dione (1.44 g, 0.011 mole) was added and the mixture heated at 70°-75°C in presence of 1-2 drops of glacial acetic acid for about 16 hours. The solid obtained was filtered, washed with ethanol and recrystallized from DMF: ethanol (1:1), yield 85%, m.p. 230°C; IR (KBr) 3284 (NH), 1716, 1701, 1653 (CON), 1602 (C=N), 1541, 1521, 1508, 1489 (C=C, aromatic); ^{1}H NMR (DMSO-d_6) δ 4.9 (s, 2H, OCH_2C), 6.9-8.6 (m, 17H, ArH), 10.8 (s, 1H, NH, Indole), 13.6 (s, 1H, NH); Anal.Calcd. for $C_{30}H_{21}N_5O_4$ (515): C, 69.90; H, 4.07; N, 13.59. Found: C, 69.95; H, 4.13; N, 13.66.

[4-(4-Oxo-2-phenyl-4H-quinazolin-3-yl)-phenoxy]-acetic acid (1-substituted aminomethyl-2-oxo-1,2-dihydro-indol-3-ylidene)-hydrazides (8a-d) : The compound (7) (0.257 g, 0.0005 mole) was dissolved in 1 cm^3 N,N-dimethylformamide. A slight excess of 40% formaldehyde (0.05 cm^3, 0.00062 mole) and appropriate secondary amine (0.006 mole) was added with vigorous stirring. The reaction mixture was stirred at room temperature (30°C) for 24 hours. The crystalline solid separated out was filtered, washed with water and finally recrystallised from DMF : ethanol (1:2) to give compounds (8a-d).

[4-(4-Oxo-2-phenyl-4H-quinazolin-3-yl)-phenoxy]-acetic acid [1-morpholin-1-ylmethyl-2-oxo-1,2-dihydro-indol-3-ylidene]-hydrazide (8a): Pale yellow-coloured solid, yield 68%, m.p. 232°C; IR (KBr) 3293 (NH), 1721, 1707, 1653 (CON), 1602 (C=N), 1541, 1521, 1508, 1489 (C=C, aromatic); ^{1}H NMR (DMSO-d_6) δ 2.5 (t, 4H, CH_2NCH_2), 3.5 (t, 4H, CH_2OCH_2), 4.5 (s, 2H, NCH_2N), 4.8 (s, 2H, OCH_2C), 6.9-8.6 (m, 17H, ArH), 13.6 (s, 1H, NH); MS (m/z): 614 [M^+], 371, 327, 313, 299, 223, 205, 179,

166, 146, 132, 118, 109, 105, 90, 77; Anal.Calcd. for $C_{35}H_{30}N_6O_5$ (614) : C, 68.40; H, 4.88; N, 13.68. Found: C, 68.48; H, 4.95; N, 13.71.

[4-(4-Oxo-2-phenyl-4H-quinazolin-3-yl)-phenoxy]-acetic acid (1-piperidine-1-ylmethyl-2-oxo-1,2-dihydro-indol-3-ylidene)-hydrazide (8b): Pale yellow- coloured solid, yield 66%, m.p. 234°C; IR (KBr) 3311 (NH), 1705, 1683,1635 (CON), 1600 (C=N), 1585, 1521, 1508 (C=C, aromatic); ^{1}H NMR (DMSO-d_6) δ 1.3-1.5 (m, 6H, $CH_2CH_2CH_2$ of piperidine), 2.6 (t, 4H, CH_2NCH_2 of piperidine), 4.5 (s, 2H, NCH_2N), 4.9 (s, 2H, OCH_2C), 7.0-8.6 (m, 13H, ArH) 13.6 (s, 1H, NH); MS (m/z): 613 [M^+], 371, 327, 313, 297, 234, 224, 205, 206, 179, 166,161, 146, 132, 118, 109, 105, 90, 77; Anal.Calcd. for $C_{36}H_{32}N_6O_4$ (612): C, 70.58; H, 5.22; N, 13.72. Found: C, 70.53; H, 5.26; N, 13.79.

[4-(4-Oxo-2-phenyl-4H-quinazolin-3-yl)-phenoxy]-acetic acid (1-dimethyl amino-1-ylmethyl-2-oxo-1,2-dihydro-indol-3-ylidene)-hydrazide (8c): Pale yellow-coloured solid, yield 76%, m.p. 209°C; IR (KBr) 3348 (NH), 1708, 1695, 1685 (CON), 1600 (C=N), 1541, 1521, 1508 (C=C, aromatic); ^{1}H NMR (DMSO-d_6) δ 2.2 (s, 6H, CH_3NCH_3), 4.5 (s, 2H, NCH_2N), 4.9 (s, 2H, OCH_2C), 6.9-8.6 (m, 17H, ArH), 13.6 (s, 1H, NH); MS (m/z): 572 [M^+], 371, 353, 327, 313, 314, 297, 268, 237, 224, 205, 194, 179, 166, 146, 118, 109, 105, 90, 77; Anal.Calcd. for $C_{33}H_{28}N_6O_4$ (572): C, 69.23; H, 4.89; N, 13.68. Found: C, 69.29; H, 4.83; N, 13.65.

[4-(4-Oxo-2-phenyl-4H-quinazolin-3-yl)-phenoxy]-acetic acid (1-diethyl amino-1-ylmethyl-2-oxo-1,2-dihydro-indol-3-ylidene)-hydrazide (8d): Pale yellow- coloured solid, yield 72%, m.p. 202°C; IR (KBr) 3329 (NH), 1705, 1685, 1637 (CON), 1600 (C=N), 1575, 1521, 1508 (C=C, aromatic); ^{1}H NMR (DMSO-d_6) δ 1.0 (t, 3H, CH_3), 2.6 (q, 2H, CH_2), 4.5 (s, 2H, NCH_2N), 4.9 (s, 2H, OCH_2C), 6.9-8.6 (m, 17H, ArH), 13.6 (s, 1H, NH); MS (m/z): 601 [M^+], 371, 353, 327, 313, 314, 297, 268, 237, 224, 205, 179, 166, 146, 118, 109, 105, 90, 77; Anal.Calcd. for $C_{35}H_{32}N_6O_4$ (600): C, 70.00; H, 5.33; N, 14.00. Found: C, 70.12; H, 5.36; N, 14.08.

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