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Synthesis of thiazolyl quinazolones for studying their antiviral activity against *Japanese encephalitis virus* (JEV), a RNA virus of high pathogenicity

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Abstract- The synthesis of 2-Phenyl-3,1-benzoxazine-4-one 1 have been obtained from Antharanilic acid by stirring in dry pyridine at room temperature. The compound 1 are then converted to their respective quinazolone 2 by treatment with hydrazine hydrate and Compound 3 by treatment with 1,1-biphenyl-4,4-diamine. The compound 4&5 on heating with aromatic aldehyde to give 3-(p-Arylidenoamino diphenyl)/2-Phenyl-3-arylidene-amino-4-(3H) quinazolone. Interaction of compound 4&5 with thioglycollic acid undergoes cyclization to give3-(p-Arylidenoamino diphenyl)/2-Phenyl-3-{3'-(2-aryl-4-oxo-1',3'-l)]quinazolones. The in vitro anti-JEV activity of these compounds has also been evaluated.
Keywords- quinazolone, 1,1-biphenyl-4,4-diamine, dimethylformamide (DMF), thioglycollic acid, Antharanilic acid

Introduction

The pharmacological properties exhibited by thiazolyl quinazolones1 derivatives have been of much significance in recent years. These compounds have been demonstrated to be associated with varying degree of antiviral activity in vitro and in vivo both [1-6]. The antiviral activity has been attributed to a delay of penetration of virus into the cells [7]. The extent of pharmacological effects of quinazolone derivatives depends on the active group to which it is attached. Thus, some quinazolone derivative have been demonstrated to exhibit varying degree of virus inhibitory properties against Semilike forest virus (SFV), Ranikhet disease virus (RDV) and Encephalomyocarditis virus in experimental animal [8,9].

Pharmacological Activity

Compound 6a-a & 7a-e were bioevaluated for their antiviral activity against an animal virus viz. *Japanese encephalitis virus* (JEV) (P20778), an RNA virus of greater pathogeicity in vitro. The mean lethal dose (LD50) of the virus in mice was calculated before each experiment following the procedure of Read and Muench [10]. the slandered method of Sidwell and Huffman [11] was followed for performing cytotoxity test and antiviral assay in vitro with slight modification [12]. The physical and spectral characterization data of evaluated compounds are presented in Table-II.

Experimental Procedure

1H NMR spectra was obtained with a DRX-3000 Bruker FT-NMR spectrophotometer taking (CH3)4Si (TMS) as an internal standard. The chemical shifts are reported as parts per million (ppm). IR spectra were obtained with a FT-IR Perkin-Elmer (model). Mass spectra were taken from JEOL-SX-102 instrument using fast atom bombardment (FAB-positive) technique. All melting points were determined in open capillary tube and are uncorrected. The nitrogen analyses carried out on CARLO-ERBA EA1108 elemental analyses. Thin-layer chromatography (TLC) was performed on readymade silica gel plates [13,14].

2-Phenyl-3,1-benzoxazine-4-one 1

Antharanilic acid (0.2 mole) was dissolved in dry pyridine (100 ml) by stirring at room temperature. The solution was cooled to 0oC and acid chloride (0.4 mole) was added slowly with constant stirring. When the addition was complete, the resultant reaction mixture was stirred for half an hour at room temperature. It was treated with 10% NaHCO3 solution in order to dissolve any unreacted acid. The solid thus obtained, was washed repeatedly with water and air dried. The crude benzoxazine thus obtained, was recrystallized from diluted ethanol as white crystalline mass m.p. 124oC13, Yield 85%.

3-(p-Amino diphenyl/2-Phenyl-3-amino-4-(3H)-quinazolone 2 (2&3)

Compound 2 was synthesized in the following manner. A mixture of 2-Phenyl-3,1-benzoxazine-4-one (0.05 mole) and 1,1-biphenyl-4,4-diamine (0.05 mole) in anhydrous pyridine (50 ml) was heated under reflux on a sand bath for 6 hours under anhydrous conditions. Subsequently, the reaction mixture was poured into ice cold water (100 ml) containing conc. HCl (10 ml). A solid started to separate out, which was allowed to settle down for 1 hour. It was filtered off and washed sucessessively with water. After drying in vacuum, it was recrystallized from ethanol. m.p. 185oC, yield 75%. Analysis for C25H19N3 N: Calculated: 18.66, Found: 18.59. IR(KBr): 3442 (N – H str), 3055 (Ar-H), 1733 (C=O), 1648 (C=N), 1611-1421 (C=C skeletal);

Compound 3 was synthesized in the following manner. A mixture of 2-phenyl-3,1-benzoxazine-4-one (0.2 mole) and hydrazine hydrate in anhydrous pyridine (30 ml) was heated under reflux for 6 hours under anhydrous reaction

condition. Subsequently, the reaction mixture was poured into ice-cold water (100 ml) containing concentrated HCl (10 ml). A solid started to separate out, which was allowed to settle down for 1 hour. It was filtered off and washed successively with water. After drying under vacuum it was recrystallized from diluted ethanol. It melted at 166oC5, yield 78%. Analysis for C13H11N3O N: Calculated:11.14, Found: 11.11. . IR(KBr): 3451 (N – H str), 3045 (Ar-H), 1723 (C=O), 1655 (C=N), 1610-1420 (C=C skeletal);

3-(p-Arylidenoamino diphenyl)/2-Phenyl-3-arylidene-amino-4-(3H) quinazolone (4a-e & 5a-e)

A mixture of 3-(p-Arylidenoamino diphenyl)/2-Phenyl-3-arylidene-amino-4-(3H) quinazolone (0.02 mole) and (0.02 mole) of an appropriate aryaldehyde in absolute EtOH (30 ml) in presence of glacial acetic acid (1 ml) was refluxed for 8-10 hours. Excess of solvent was removed under reduced pressure. The solid obtained, was washed with cold water. Several times and recrystallized from methanol. Characterization data of the compounds thus synthesized, are given in Table-I.

4a: IR(KBr): 2911 (Ar-H), 2340 (N-N), 1631(ter. amide C=O), 1610 (C=N), 1600-1415 (C=C skeletal), 784 (Ar-Cl);

4b: 3391 (Ar-OH), 3042 (Ar-H), 1630-1423 (C=C skeletal), 1614 (C=N), 1611 (ter.amide C=O);

4c: 1625 (C=N), 3363 (Ar-OH), 1650 (tert. amide C=O), 1645 (C=C skeletal), 2360 (N-N), 2933 (Ar-C-H-str.); 1H NMR (CDCl3): d: 1.22-2.78(m, 10H, CH2 in ring), 4.72 (s, 1H, N-CH-R), 6.76-7.91(m, 17H, Ar-H), 5.10 (s, 1H, Ar-OH);

4d: 3015 (Ar-H), 2985 (C-H str), 2332(N-N), 1665 (C=N), 1638 (ter. amide);

4e: 3038 (Ar-H), 2350(N-N), 1642-1433 (C=C skeletal), 1652 (ter.amide C=O)

5a: IR(KBr): 2355 (N-N), 1631 (ter. Amide C=O), 1612 (C=N), 1600-1435 (C=C skeletal), 779 (Ar-Cl);

5b: 3415 (Ar-OH), 2343 (N-N), 1637 (ter. Amide C=O), 1620 (C=N), 1590-1445 (C=C skeletal);

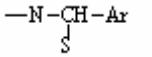
5c: 1632 (C=N), 3340 (Ar-OH), 2365 (N-N), 2947 (Ar-CH-str.), 1650(tert. amide C=O), 1652 (C=C skeletal), 1H NMR (CDCl3): d 4.48 (s, 1H, -N-CH-R), 6.40-7.86 (m, 13H, Ar-H), 5.22 (s, 1H, Ar-OH);

5d: 1653 (C=N), 1635 (ter. Amide C=O), 1624-1469 (C=C skeletal)

3-(p-Arylidenoamino diphenyl)/2-Phenyl-3-{3'-(2-aryl-4-oxo-1',3'-l)]quinazolone (6a-e& 7a-e)

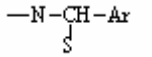
The target compounds were synthesized in the following manner. Thus, a mixture of 3-(p-Arylidenoamino diphenyl)/2-Phenyl-3-arylidene-amino-4-(3H) quinazolone (0.01mole) and thioglycollic acid (0.01mole) containing in trace ZnCl2 (0.1 gm) in dimethylformamide (DMF) was heated under reflux for 4 hours. It was poured into crushed ice and stirred vigorously. Solidification occurred after fifteen minutes. It was filtered off and washed with cold water. Recrystallization from ethanol gave analytically pure sample. The compounds of this category are presented in Table –I along with their characterization data.

6a: : IR (KBr): 1632 (ter. amide C=O), 1612 (C=N), 1615 (C=C skeletal), 2926(Ar-H), 754 (Ar-Cl) :^{1}H NMR ($CDCl_3$): δ 6.61-7.76 (m, 21H, Ar-H), 4.83 (brs, 1H, s, replaceable-OH),



3.27 (s, 1H,), 3.36 (s, 2H, O=C-CH_2-S)

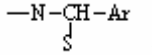
6b: : IR (KBr): 1164 (C-N), 690 (Ar-Cl), 1634 (ter. amide C=O), 3016(Ar-H), 1636 (C=C, skeletal) ^{1}H NMR ($CDCl_3$): δ 6.63-7.85 (m, 21H, Ar-H), 4.86 (brs, 1H, s, replaceable-OH),



3.29 (s, 1H,), 3.39 (s, 2H, O=C-CH_2-S)

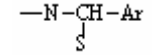
6c: IR (KBr): 1623 (C=N), 2855 (C–H str.), 3061 (Ar-H str.), 1682 (tert. amide, C=O), 3665 (Ar-OH), 2345 (N–N);

^{1}H NMR ($CDCl_3$): δ 6.68-7.81 (m, 21H, Ar-H), 4.89 (brs, 1H, s, replaceable-OH),



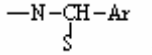
3.21 (s, 1H,), 3.35 (s, 2H, O=C-CH_2-S)

6d: IR (KBr): 3033 (Ar-H), 3445(Ar-OH), 2355 (N-N), 1626-1463 (C=C skeletal), 1625 (ter. amide C=O), ^{1}H NMR ($CDCl_3$): δ ^{1}H NMR ($CDCl_3$): δ 6.63-7.75 (m, 21H, Ar-H), 4.81 (brs, 1H, s, replaceable-OH),



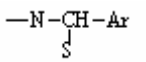
3.27 (s, 1H,), 3.30(s, 2H, O=C-CH_2-S)

6e: IR (KBr): 3053 (Ar-H), 3465 (Ar-OH), 2373 (N-N), 1618-1475 (C=C skeletal), 1632 (ter. amide C=O), ^{1}H NMR ($CDCl_3$): δ ^{1}H NMR ($CDCl_3$): δ 6.71-7.85 (m, 21H, Ar-H), 4.860 (brs, 1H, s, replaceable-OH),



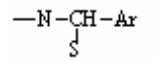
3.22 (s, 1H,), 3.31 (s, 2H, O=C-CH_2-S)

7a: IR (KBr): 1633 (C=N), 1665 (tert. amide C=O), 735 (C-Cl str.), 2325 (-N-N-), 2960 (Ar-CH-str.), 1645-1665 –(C=C skeletal). ^{1}H NMR ($CDCl_3$): δ ^{1}H NMR ($CDCl_3$): δ 6.63-7.73 (m, 13H, Ar-H), 4.79 (brs, 1H, s, replaceable-OH),



3.31 (s, 1H,), 3.40 (s, 2H, O=C-CH_2-S)

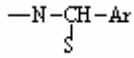
7b: IR (KBr): 3030 (Ar-H), 3410 (Ar-OH), 2355 (N-N), 1640-1450 (C=C skeletal), 1646 (ter. amide C=O). ^{1}H NMR ($CDCl_3$): δ ^{1}H NMR ($CDCl_3$): δ 6.65-7.81 (m, 13H, Ar-H), 4.89 (brs, 1H, s, replaceable-OH),



3.29 (s, 1H,), 3.38 (s, 2H, O=C-CH_2-S)

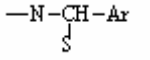
7b : Mass (FAB): 415 (M^+), 398, 389, 338, 322, 245, 220, 332145, 119, 105 (Base peak);

7c: IR (KBr): 3045 (Ar-H), 3455(Ar-OH), 2385 (N-N), 1618-1465 (C=C skeletal), 1640 (ter. amide C=O), ^{1}H NMR ($CDCl_3$): δ ^{1}H NMR ($CDCl_3$): δ 6.69-7.79 (m, 13H, Ar-H), 4.82 (brs, 1H, s, replaceable-OH),



3.31(s, 1H,), 3.28 (s, 2H, O=C-CH_2-S)

7d: IR(KBr): 3065 (Ar-H), 3485(Ar-OH), 2365 (N-N), 1610-1469 (C=C skeletal), 1635 (ter. amide C=O),1634(C=N), ^{1}H NMR ($CDCl_3$): δ ^{1}H NMR ($CDCl_3$): δ 6.70-7.88 (m, 13H, Ar-H), 4.83 (brs, 1H, s, replaceable-OH),



3.32 (s, 1H,), 3.41 (s, 2H, O=C-CH_2-S)

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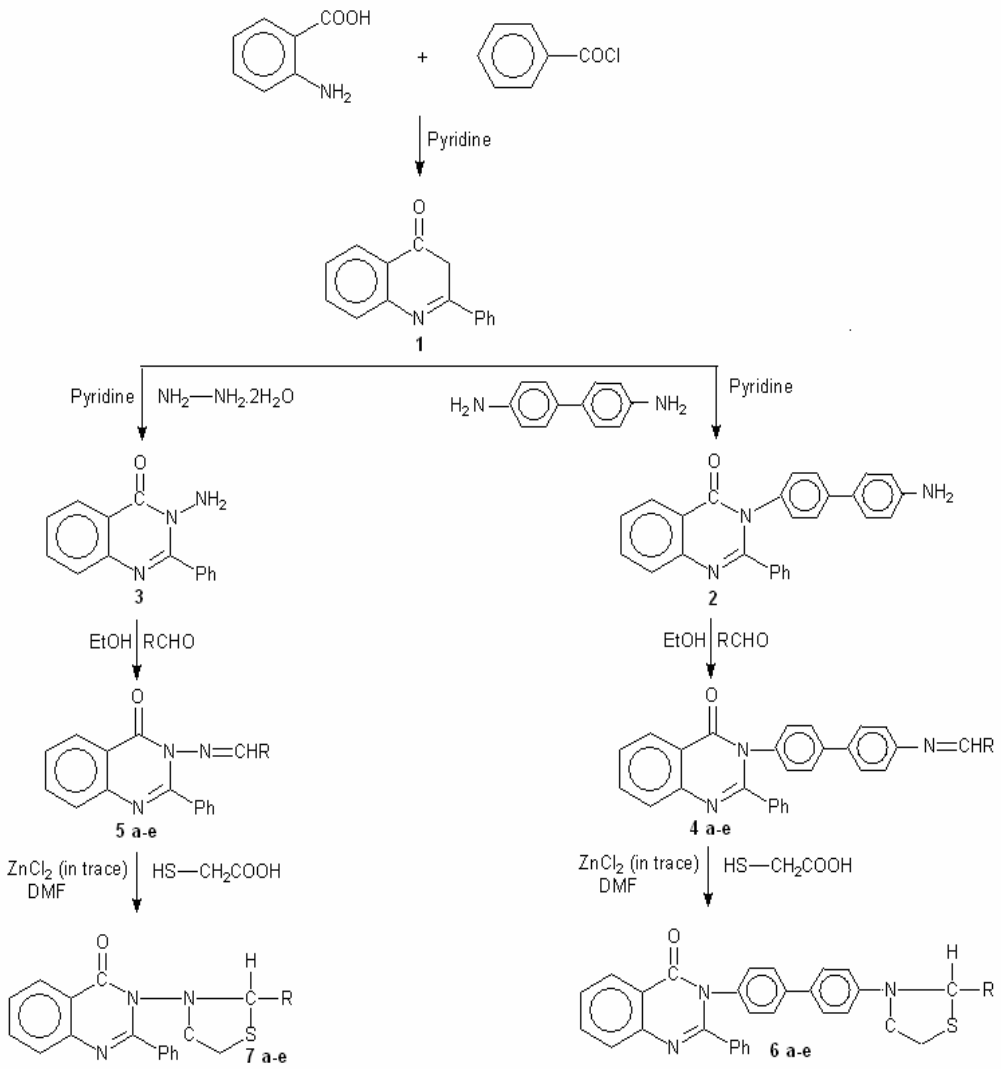
Table I-Characterization data for compound 4a-e,5a-e,6a-e and 7a-e

Compound No.	**R**	**m.p. (°C)**	**Yield (%)**	**Molecular formula**	**Analysis (%) nitrogen (Calcd) Found**
4a	p-Chlorophenyl	153	40	$C_{33}H_{22}N_3O_2Cl$	(7.96) 7.93
4b	p-Hydroxyphenyl	102	60	$C_{33}H_{23}N_3O_2$	(8.51) 8.45
4c	o-Hydroxyphenyl	102	48	$C_{33}H_{23}N_3O_2$	(8.51) 8.43
4d	4-OH, 3-OCH_3, -phenyl	142	55	$C_{34}H_{23}N_3O_3$	(8.03) 7.98
4e	3-OH, 4-OCH_3, -phenyl	142	45	$C_{34}H_{23}N_3O_3$	(8.03) 7.95
5a	p-Chlorophenyl	147	68	$C_{21}H_{14}N_3OCl$	(11.68) 11.61
5b	p-Hydroxyphenyl	142	70	$C_{21}H_{15}N_3O_2$	(12.31) 12.36
5c	o-Hydroxyphenyl	215	55	$C_{21}H_{15}N_3O_2$	(12.31) 12.38
5d	4-OH, 3-OCH_3-phenyl	90	63	$C_{22}N_{17}N_3O_3$	(11.32) 11.27
5e	3-OH, 4-OCH_3-phenyl	90	58	$C_{22}N_{17}N_3O_3$	(11.32) 11.28
6a	p-Chlorophenyl	153	55	$C_{35}H_{26}N_3O_2SCl$	(7.34) 7.28
6b	p-Hydroxyphenyl	102	45	$C_{35}H_{27}N_3O_2S$	(7.59) 7.53
6c	o-Hydroxyphenyl	102	65	$C_{35}H_{27}N_3O_2S$	(7.59) 7.62
6d	4-OH, 3-OCH_3, -phenyl	142	45	$C_{36}H_{29}N_3O_3S$	(7.20) 7.16
6e	3-OH, 4-OCH_3, -phenyl	142	45	$C_{36}H_{23}N_3O_3S$	(7.20) 8.17
7a	p-Chlorophenyl	153	60	$C_{23}H_{18}N_3OSCl$	(10.01) 10.07
7b	p-Hydroxyphenyl	102	70	$C_{23}H_{19}N_3O_2S$	(10.47) 10.41
7c	o-Hydroxyphenyl	102	50	$C_{23}H_{19}N_3O_2S$	(10.47) 10.39
7d	4-OH, 3-OCH_3, -phenyl	142	45	$C_{23}H_{21}N_3O_3S$	(9.74) 9.71
7e	3-OH, 4-OCH_3, -phenyl	142	45	$C_{24}H_{21}N_3O_3S$	(9.74) 9.76

Table II- Anti-Japanese encephalitis virus (JEV) in vitro, activity data of compound: 6 (a-e) & 7 (a-e)

Compound No.	R	Dose µg/ml	% cytopathic effect (CPE inhibition)
6a	p-OH-phenyl	250	–
6b	p-Cl-phenyl	250	75
6c	o-OH-phenyl	250	25
6d	3-OH, 4-OCH_3, -phenyl	250	50
6e	4-OH, 3-OCH_3, -phenyl	250	-
7a	p-OH-phenyl	250	25
7b	p-Cl-phenyl	250	12.5
7c	o-OH-phenyl	250	25
7d	3-OH, 4-OCH_3, -phenyl	250	50
7e	4-OH, 3-OCH_3, -phenyl	250	12.5



Scheme - 1