Academic Sciences International Journal of Pharmacy and Pharmaceutical Sciences

ISSN- 0975-1491 Vol 4, Issue 4, 2012

Research Article

ONE-POT SYNTHESIS OF NOVEL QUINAZOLINE DERIVATIVES AND THEIR ANTIMICROBIAL ACTIVITY

SUBARNA GANGULI[1], MK PANIGRAHI[2], PRATIKSHA SINGH[3] AND P.K.SHUKLA[3#]

[1]Calcutta Institute of Pharmaceutical Technology and Allied Health Sciences, Banitabla, Uluberia, Howrah-711316, W.B, [2]Hi-Tech college of Pharmacy, Bhubaneshwar-751025,[3,3#]Central Drug Research Institute, Lucknow-226001 India

Received: 16 Jun 2012, Revised and Accepted: 27 July 2012

ABSTRACT

One-pot synthetic methodology was adopted to synthesise final compounds. The synthesis of final compounds was done from starting compounds 2-(4-substituted)Phenyl benzoxazin-4-one and 2-Phenyl-benzoxazin-4-one. They were prepared from Anthranilic acid and 4-substituted and unsubstituted benzoyl chloride derivatives in presence of pyridine. A set of six Schiff bases were synthesized by reacting 2-(4-substituted)Phenyl-3-amino Quinazoline -4-3(H)one and 2-Phenyl-3-amino Quinazoline -4-3(H)one with various substituted aromatic aldehydes in glass vials and placed in an oil bath at 80 degree celcius. Structural elucidation was done by spectroscopic method. The final compounds were screened for their antimicrobial activity

INTRODUCTION

Quinazolines are the derivatives of benzopyrimidine ring system. These compounds are used in medicine because of their wide spectrum of biological activities. It is known and well documented in the literature that there exists a connection between wide spectrums of biological activities with the molecules having quinazoline moiety [1]. Hence quinazoline-4(3H)-one is a lead compound for designing potential bioactive agents. Quinazoline derivatives possess a variety of activities like antibacterial, antifungal, anti-HIV[2,3] anthelmintic [4], CNS depressant [5] and antitubercular [6]. Antitumor activities of 2,3-dihydro-2-aryl-4-quinazolinones are also reported [7,8]. Some reports suggest that 2-styryl quinazolin-4-ones (SQZ) [9,10] are effective inhibitors of tubulin polymerization. The 2,3-disubstituted quinazolones have been demonstrated to be associated with potent antiviral and antihypertensive acitivities [11]. Synthesis of naturally occurring bioactive alkaloid having quinazolone system, vascinone is reported recently [12]. Qunazolines possess antimalarial activity[13] and hyperlipidemic activity[14]

Due to various types of biological activity found in Quinazoline derivatives, objective has been taken to synthesise Quinazoline derivatives in order to evaluate their antimicrobial activity against pathogenic bacteria and fungi.

MATERIALS AND METHODS

Experimental

Melting points were determined in open capillary tubes and were uncorrected. Purity of the compounds was checked by TLC on silica gel G plates using benzene and Petroleum ether solvent system in 7:3 ratio. Iodine chamber was used as a visualizing agent. IR-spectra were recorded using KBr pellets on a SHEMADZU 8000 series spectro-photometer. NMR spectra on BRUKER 400 MHz Spectrophotometer using DMSO as solvent and TMS as internal standard (chemical shift values expressed in ppm). ESI-MS was also done for structural elucidation of the compounds.

Procedure

Step 1: Preparation of 2-Phenyl benzoxazin-4-one (aa1a):

Anthranilic Acid (0.01mole) was dissolved in Pyridine. Benzoyl Chloride in 0.01 mole was added to it and was cooled. The reaction mixture was stirred at room temperature. A little quantity of the sample was taken and dissolved in water to check the formation of benzoxazin. As Anthranilic acid, benzoyl chloride and Pyridine are water soluble so they will dissolve in water but Benzoxazin being insoluble it forms a precipitate. When Benzoxazin was formed, then the entire reaction mixture was poured into a beaker containing 250 ml of water containing 10%sodium bicarbonate. The benzoxazin so formed was filtered, dried and recrystallised from ethanol. A pale brown coloured crystalline compound was obtained. Melting point is 121 degree celcius. Purity of the compound was checked by TLC using benzene and Petroleum ether solvent system in 7:3 ratio.

Preparation of 2-(4-substituted phenyl)-benzoxazin-4-one (aa2a):

Anthranilic Acid (0.01mole) was dissolved in Pyridine. p-substituted Benzoyl Chloride in 0.01 mole was added to it and was cooled. The reaction mixture was stirred at room temperature. A little quantity of the sample was taken and dissolved in water to check the formation of benzoxazin. As Anthranilic acid, p-substituted benzoyl chloride and Pyridine are water soluble so they will dissolve in water but Benzoxazin being insoluble, it forms a precipitate. When Benzoxazin was formed, then the entire reaction mixture was poured into a beaker containing 250 ml of water containing 10%sodium bicarbonate. The benzoxazin so formed was filtered, dried and recrystallised from ethanol. A pale brown coloured crystalline compound was obtained. Melting points were checked. Purity of the compounds were checked by TLC using benzene and Petroleum ether solvent system in 7:3 ratio.

Step 2: Preparation of 2-Phenyl -3-amino Quinazoline-4(3H)-one (aa1b):

0.01 mole of aa1a sample was taken in ethanol. To this equimolar quantity of hydrazine hydrate was added and refluxed for 2 hours. This was cooled. Product so formed was filtered, dried and recrystallised using ethanol. Purity of the compound was checked by TLC using benzene and Petroleum ether solvent system in 7:3 ratio. Melting point was found to be 181 degree celcius.

Preparation of 2-(4-substituted phenyl)-3-amino Quinazoline -4(3H)-one(aa2b):

0.01 mole of aa2a sample was taken in ethanol. To this equimolar quantity of hydrazine hydrate was added and refluxed for 2 hours. This was cooled. Product so formed was filtered, dried and recrystallised using ethanol. Purity of the compound was checked by TLC using benzene and Petroleum ether solvent system in 7:3 ratio. Melting points were checked.

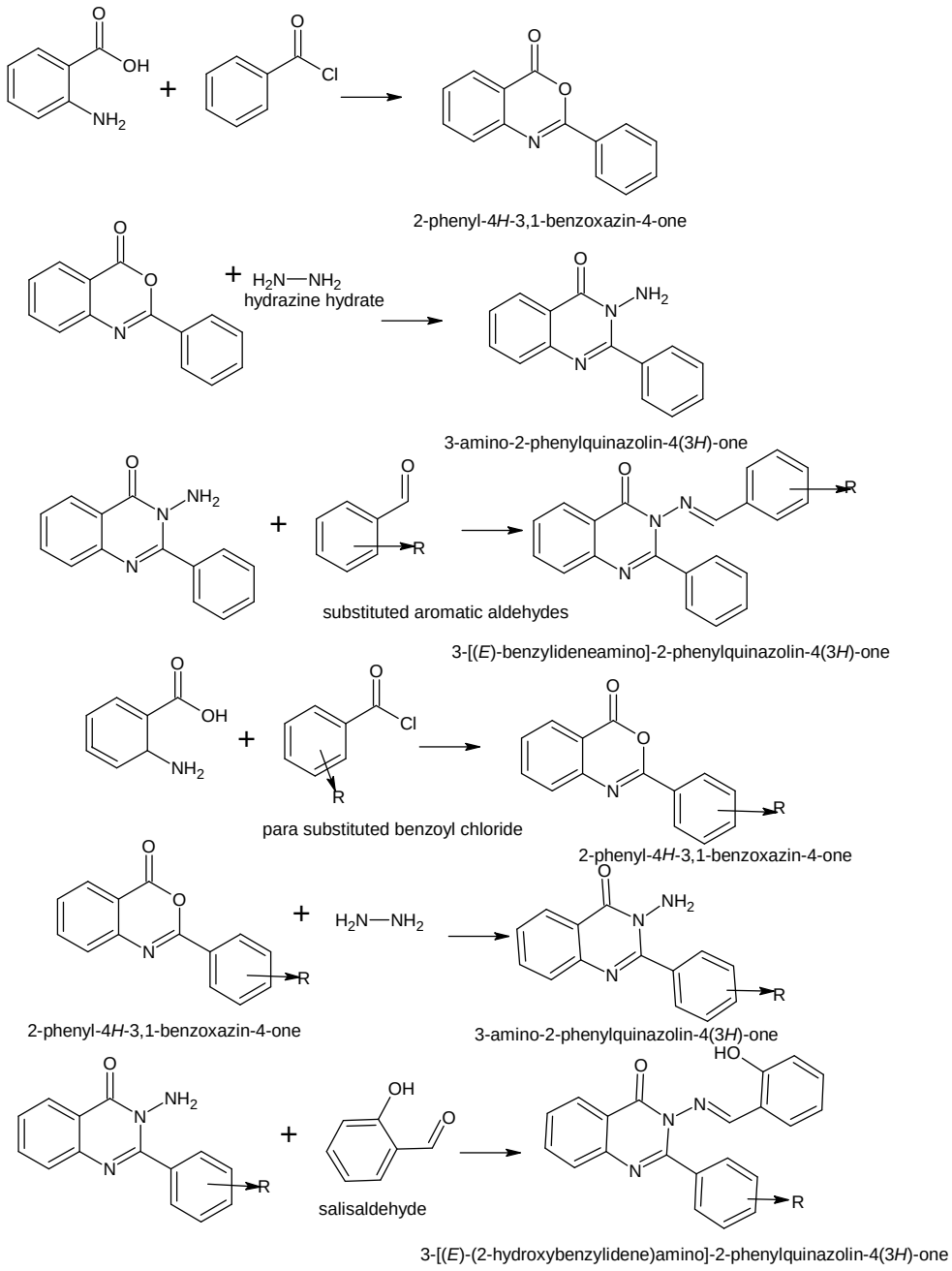
Step 3: Preparation of 2-Phenyl-3-[(substituted benzylidene)amino] quinazoline-4(3H)-one (aa1c):

Compound aa1b was taken in a watch glass. To this equimolar quantity of substituted aromatic aldehydes were added and mixed well with a glass rod. This mixture was transferred into a glass vial and was then placed in an oil bath and heated at 80 degree celcius. Heating was done for 0.5 to 1 hour. Reaction was monitored by TLC using benzene and ethyl acetate in 7:3 ratio. Melting points were checked.

Preparation of 2-(4-substitutedPhenyl)-3-amino quinazoline-4(3H)-one (aa2c):

Compound aa2b was taken in a watch glass. To this equimolar quantity of substituted aromatic aldehydes were added and mixed well with a glass rod. This mixture was transferred into a glass vial and was then placed in an oil bath and heated at 80 degree celcius. Heating was done for 0.5 to 1 hour. Reaction was monitored by TLC using benzene and ethyl acetate in 7:3 ratio. Melting points were checked.

The Reaction is shown below:



Study of Antibacterial Activity by Turbidometric method (for compounds aa1c1-3)

Minimum Inhibitory Concentration (MIC) [15]values for all the synthesized compounds against Gram-positive bacteria (*S. aureus* 6571 and *B. subtilis*) and Gram-negative bacteria (*E. coli* K12 and *S. dysenteriae* 6) were obtained by a turbidometric method. The test compound was dissolved in DMF to prepare the stock solution and aseptically filtered through bacterial membrane. The required volume of filtrate was transferred to tubes containing a defined volume of nutrient broth to achieve a desired concentration of the compound. The concentrations of the tested compound were 800, 700,600, 500, 400, 300, 200, 100, 50, and 25 µg/mL, in comparison with the standard drug ampicillin. The tubes containing nutrient broth were inoculated with 12 hrs old liquid culture (0.1 mL) in duplicate. The tubes were incubated at 37degree Celcius for 18-24 hrs and the relative growths in the tubes were determined turbidometrically in a photoelectric colorimeter. The O.D. values, recorded at 530 nm were plotted against concentrations of the test compounds to get the standard curve and the MIC values of the compounds against the test organism were determined.

Microorganisms

The Microorganisms used were Gram-positive bacteria (*S. aureus* 6571 and *B. subtilis*) and two Gram-negative bacteria (*E. coli* K12 and *S. dysenteriae* 6).

Study of antibacterial and Antifungal Activity (for compounds aa2c1-3)

The *in vitro* antbacterial and antifungal activity of synthesized compounds were evaluated against 4 pathogenic bacteria viz, *E. coli* (ATCC 9637), Pseudomonas aeruginosa (ATCC BAA-427), Staphyloccus aureus (ATCC 25923), Klebsiella pneumoniae (ATCC 27736) and 6 viz. pathogenic fungi, *Candida albicans* (Ca), *Cryptococcus neoformans* (Cn), *Sporothrix schenckii* (Ss), *Trichophyton mentagrophytes (Tm), Aspergillus fumigatus* (Af), *Candida parapsilosis* (Cp, ATCC22019), by broth micro-dilution technique as per guidelines of Clinical and laboratory Standard Institute (CLSI)[1-2] Mueller Hinton broth for bacteria and RPMI 1640 Medium buffered with MOPS [3-(N-morpholino) propanesulphonic acid] for fungi in microtitre plates. The starting concentration of compound in first well was 50µg/ml and its 2 fold dilutions as follows 25, 12.5, 6.25, 3.12, 1.56, 0.78, 0.39 and so on. Inocula of test culture were maintained using by McFarland standard and $1\text{-}5\times10^3$ cells were inoculated in each well. Microtitre plates were incubated for 24 h (for bacteria), 24-48 h (yeasts) and 72-96 h (mycelial fungi) at 35ºC. After incubation minimal inhibitory concentrations (MIC) were determined by visual observation as well as on a spectrophotometer (Molecular Devices, USA) at 492nm. Gentamycin and fluconazole were used as reference antibacterial and antifungal agents respectively.

RESULTS AND DISCUSSIONS

From the literature survey it reveals that Quinazoline derivatives have been reported for number of pharmacological activities and some molecules have shown significant activities and some compounds show moderate and good activities. Compound Aa1c1 showed 100 200 mcg/ml MIC against *S. aureus* 6571 and *B. subtilis*.It showed MIC of 100 and 200 against *E. coli* K12 and *S. dysenteriae* 6.

Compound Aa1c2 showed 200 and 200 mcg/ml MIC against *S. aureus* 6571 and *B. subtilis*. It showed MIC of 300 and 400 against *E. coli* K12 and *S. dysenteriae* 6.

Compound Aa1c3 showed 200 and 300 mcg/ml MIC against *S. aureus* 6571 and *B. subtilis*. It showed MIC of 400 and 300 against *E. coli* K12 and *S. dysenteriae* 6.Here we have synthesized some novel Quinazoline-4(3H)-one Schiff bases and screened them for their anti-bacterial and anti-fungal activities.The results are as follows:

Table1: Minimum Inhibitory conc.in mcg/ml for compounds Aa2c1-3.

MM No.	Code No.	Date of testing	Minimum inhibitory conc. (MIC) in µg/ml against									
			BACTERIA				FUNGI					
			1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**
S11-606	Aa2c1	Dec	>50	>50	>50	>50	>50	>50	>50	>50	>50	>50
S11-607	Aa2c2	2011	>50	>50	>50	>50	>50	>50	>50	>50	>50	>50
S11-608	Aa2c3		>50	>50	**12.5**	>50	**6.25**	**12.5**	**50**	**50**	**25**	6.25
	Gentamicin		0.78	0.78	0.39	0.78						
	Fluconazole						0.5	1.0	**2.0**	1.0	2.0	1.0

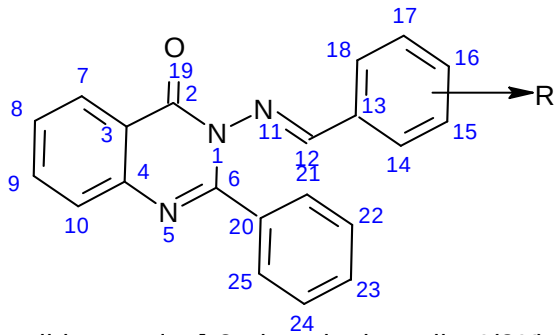
1. E. coli (ATCC 9637) *2*. Pseudomonas aeruginosa (ATCC BAA-427), 3. Staphyloccus aureus (ATCC 25923), 4. Klebsiella pneumoniae (ATCC 27736). 5*. Candida albicans* 6. *Cryptococcus neoformans* 7. *Sporothrix schenckii, 8. Trichophyton mentagrophytes, 9. Aspergillus fumigatus* 10. *Candida parapsilosis* (ATCC-22019)

Fungi were tested by NCCLS method in RPMI 1640 medium and bacteria in Mueller Hinton Broth

1. National Committee for clinical laboratory standards, 2003. Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically. Approved standard M7–A6. NCCLS, Wayne, Pa.

2. National Committee for Clinical Laboratory Standards, Reference Method for Broth Dilution Antifungal Susceptibility Testing of Yeasts; Approved Standard, NCCLS Document M27-A. National Committee for Clinical Laboratory Standards, Wayne, PA, USA, 1997

Numbering of 3-[(*E*)-benzylideneamino]-2-phenylquinazolin-4(3*H*)-one:



3-[(*E*)-benzylideneamino]-2-phenylquinazolin-4(3*H*)-one

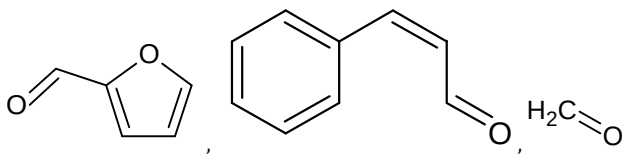
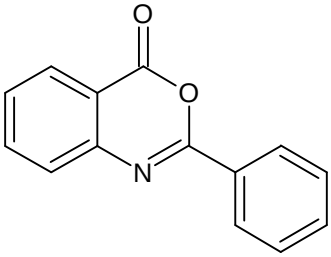
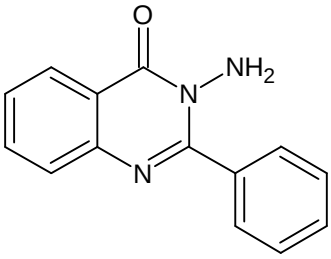
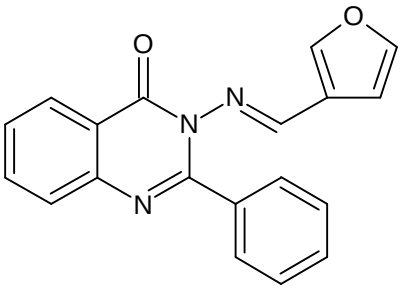
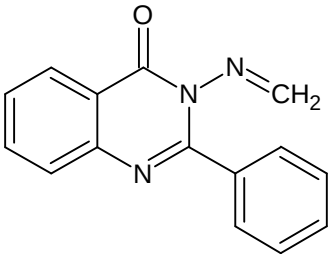
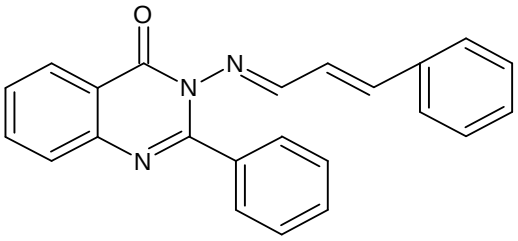
R= 

Table 2: The physicochemical parameters of the compounds aa1 series

S. No.	Compd. No.	Structure	Chemical name	M.W.	M.P Degree celcius	% yield
1	Aa1a		2-phenyl-4*H*-3,1-benzoxazin-4-one	223.23	119-121	93
2	Aa1b		3-amino-2-phenylquinazolin-4(3*H*)-one	237.26	180-182	89
3	Aa1c1		3-[(*E*)-(furan-3-ylmethylidene)amino]-2-phenylquinazolin-4(3*H*)-one	315.35	196-198	89
4	Aa1c2		3-(methylideneamino)-2-phenylquinazolin-4(3*H*)-one	249.27	176-178	78
5	Aa1c3		2-phenyl-3-{(*E*)-[(2*E*)-3-phenylprop-2-en-1-ylidene]amino}quinazolin-4(3*H*)-one	315.41	206-208	88

Spectral data of 2-Phenyl-3-[(substituted benzylidene)amino] quinazoline-4(3H)-one (aa1c)

Aa1c1: 1HNMR (DMSO,δppm): 7.88(1H,s,imine);7.58-7.66(4H fused aromatic); 7.88-8.60(5H,m,aromatic);7.26-7.31(3H,t,furan).

ESI-MS: 315

IR(KBr): 3200 cm^{-1}(aromatic C-H, str.),3342 cm^{-1}(furan-H,str.),1688 cm-1(quinazolone C=O), 1525 cm^{-1}(C=N group),643 cm^{-1}(aromatic C-H, bending).

Aa1c2: 1HNMR (DMSO,δppm): 7.88(1H,s,imine);7.58-7.66(4H fused aromatic); 7.88-8.60(5H,m,aromatic);6.9-7.0(CH_2).

ESI-MS: 249.27

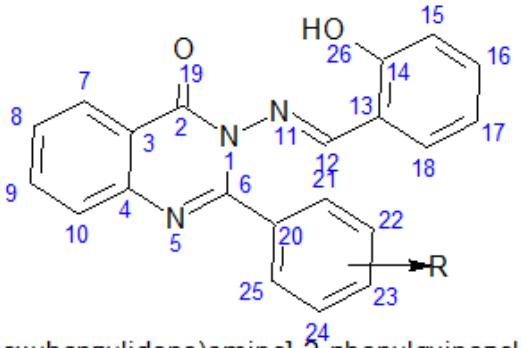
IR(KBr): 3200 cm^{-1}(aromatic C-H, str.), 1688 cm-1(quinazolone C=O), 1525 cm^{-1}(C=N group),643 cm^{-1}(aromatic C-H, bending).

Aa1c3: 1HNMR (DMSO,δppm): **8**.24(1H,s,imine);8.57 and 8.60(2H,m,alkenyl);7.88-7.98(4H,m,fused aromatic),7.22-7.66(10H,m,aromatic).

ESI-MS: 315

IR(KBr): 3257cm^{-1}(aromatic C-H,str.),1631 cm^{-1}(quinazolone C=O),1448 cm^{-1}(C=N group), 752cm^{-1}(aromatic C-H, bending), 3083cm^{-1}(C=C-H)

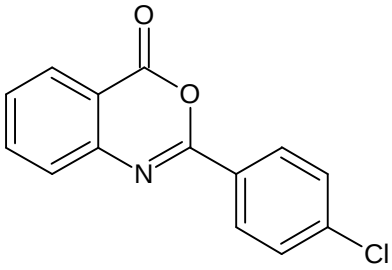
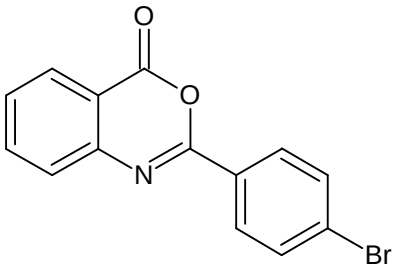
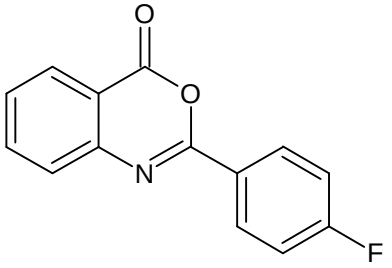
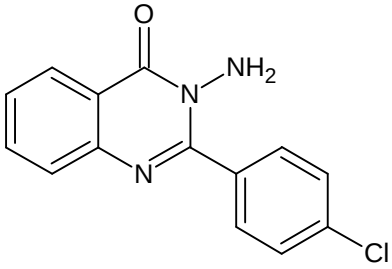
Numbering of 3-[(*E*)-(2-hydroxybenzylidene)amino]-2-phenylquinazolin-4(3*H*)-one (aa2c):

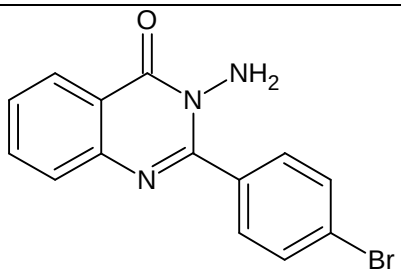
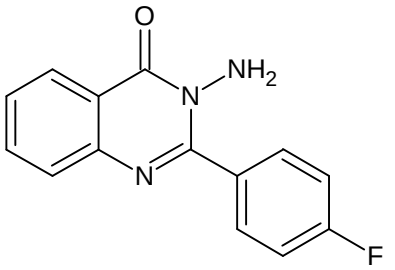
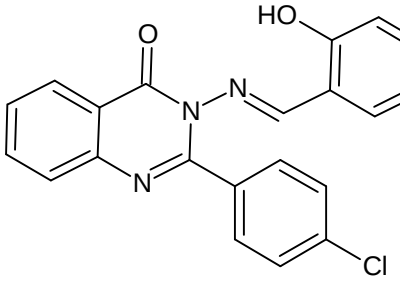
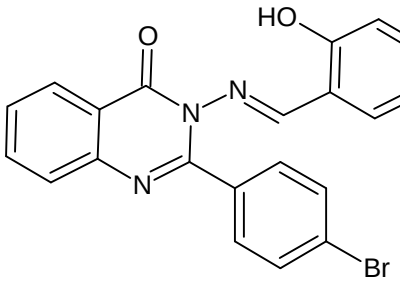
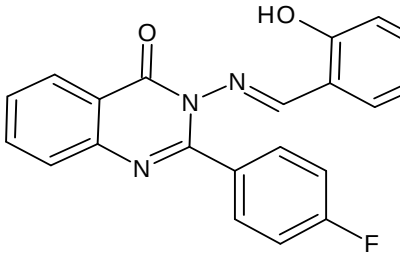


3-[(*E*)-(2-hydroxybenzylidene)amino]-2-phenylquinazolin-4(3*H*)-one

R= Cl,Br,F

Table 3: The physicochemical parameters of the compounds aa2 series.

S. No.	Comp. No.	Structure	Chemical name	M.W.	M.P. (in deg ree celcius)	% yield	Percentage yield
1	Aa2a1		2-(4-chlorophenyl)-4*H*-3,1-benzoxazin-4-one	257.68	74-76	95	
2	Aa2a2		2-(4-bromophenyl)-4*H*-3,1-benzoxazin-4-one	302.13	168-170	93	
3	Aa2a3		2-(4-fluorophenyl)-4*H*-3,1-benzoxazin-4-one	241.21	164-166	94	
4	Aa2b1		3-amino-2-(4-chlorophenyl)quinazolin-4(3*H*)-one	271.71	166-168	88	

5	Aa2b2		3-amino-2-(4-bromophenyl)quinazolin-4(3*H*)-one	316.16	184-186	87
6	Aa2b3		3-amino-2-(4-fluorophenyl)quinazolin-4(3*H*)-one	255.25	176-178	88
7	Aa2c1		2-(4-chlorophenyl)-3-[(*E*)-(2-hydroxybenzylidene)amino]quinazolin-4(3*H*)-one	375.8	194-196	89
8	Aa2c2		2-(4-bromophenyl)-3-[(*E*)-(2-hydroxybenzylidene)amino]quinazolin-4(3*H*)-one	420.2588	190-192	91
9	Aa2c3		2-(4-fluorophenyl)-3-[(*E*)-(2-hydroxybenzylidene)amino]quinazolin-4(3*H*)-one	359.3532	208-210	94

Spectral data of 2-(4-substitutedPhenyl)-3-amino quinazoline-4(3H)-one (aa2c):

Aa2c1: 2-(4-chlorophenyl)-3-[(*E*)-(2-hydroxybenzylidene)amino] quinazolin-4(3*H*)-one.

1HNMR (DMSO,δppm): H-18 (7.60), H-17 (7.59), H-16 (7.58), H-15 (7.57). OH(10.1),s,1H,H-C=N (9.2), H-7(6.94),H-8 (6.97), H-9(6.98), H-10 (6.97)H-25(7.39), H-24(7.45).

ESI-MS: 376.26

IR(KBr): 3200 cm^{-1}(aromatic C-H, str.), 1688 cm-1(quinazolone C=O), 1525 cm^{-1}(C=N group),643 cm^{-1}(aromatic C-H, bending),750-700cm^{-1}(C-Cl stretching)

Aa2c2:: 2-(4-bromophenyl)-3-[(*E*)-(2-hydroxybenzylidene) amino] quinazolin-4(3*H*)-one.

1HNMR (DMSO,δppm): H-18 (7.26), H-17 (7.35), H-16 (7.37), H-15 (7.38). OH(10.059),s,1H,H-C=N (9.152), H-7(7.79),H-8 (7.81), H-9(7.83), H-10 (7.85)H-25(8.37), H-24(8.34).

ESI-MS: 420.0852

IR(KBr): 1688 cm-1(quinazolone C=O), 1525 cm^{-1}(C=N group),643 cm^{-1}(aromatic C-H, bending),600-500cm^{-1}(C-Br stretching).

Aa2c3: 2-(4-Fluorophenyl)-3-[(*E*)-(2-hydroxybenzylidene) amino] quinazolin-4(3*H*)-one.

1HNMR (DMSO,δppm): H-18 (7.46), H-17 (7.45), H-16 (7.57), H-15 (7.48). OH(9.5),s,1H,H-C=N (9.2), H-7(7.9),H-8 (7.82), H-9(7.84), H-10 (7.86)H-25(8.47), H-24(8.54).

ESI-MS:359.7315

IR(KBr): 1688 cm-1(quinazolone C=O), 1525 cm^{-1}(C=N group),643 cm^{-1}(aromatic C-H, bending), 1100-1000 cm^{-1} (C-F stretching).

ACKNOWLEDGEMENT

The authors are thankful to The Cultivation of Science,Kolkata for ESI-MS report.

REFERENCE

1. Spirkova, K., Stankovsky, S., Mrvova, A., Cipak, L. Synthesis and Biological Activity of Some 2-substituted Quinazolin-4-ones. Chem. Pap. **1999;** 53: 272-275.
2. Alagarsamy, V., Giridhar, R., Yadav, H. R., Revathi, R., Rukmani, K.; De Clercq. E. Anti HIV,antibacterial and antifungal activities of some novel1,4-disubstituted-1,2,4-triazolo[4,3a]quinazolin-5(4h)-ones. Indian J. Pharm. Sci. **2006**, *68:* 532-535.
3. Srivastava, V. K.; Singh, A., Gucati, A., Shankar, K. Antiparkinsonian agent from qunazonyl thioazolidinones and azetidinones. Indian J. Chem. **1987**; *26:* 652-656.
4. Gupta, D. P., Ahmed, S., Kumar, A., Shankar, K. Newer Quinazolinone Derivatives as Anthelmintic Agents. Indian J. Chem. **1988;** 27: 1060-1062.
5. Wang HJ, Wei CX, Deng XQ, Li FL, Quan ZS. Synthesis and evaluation on anticonvulsant and antidepressant activities of 5-Alkoxy-tetrazolo[1,5-a]quinazolines. Arch Pharm (Weinheim). **2009**; 342:671-5.
6. Joshi, V., Chaurasia, R. P. Synthesis of Some New 4-Quinazolinone-2-carboxylhydrazides and their Tosyl Derivatives Having Potential Biological Activity. Indian J. Chem. **1987;** *26*: 602-604.
7. Yale, H. J., Kalkstein, M. Substituted 2,3-dihydro-4(1H)-quinazolinones, a new class of inhibitors of cell multiplication. J. Med. Chem. **1967;** 10: 334-336.
8. Neil, G. L., Li, L. H., Buskirk, H. H., Moxley, T. E. Antitumor effects of the antispermatogenicagent, 2,3-dihydro-2-(1-naphthyl)-4(1H)-quinazolinone (NSC-145669*)*.Cancer Chemother. Rep. **1972;** 56: 163-173.
9. Jiang, J. B, Hesson, D. P., Dusak, B. A. Dexter, D. L., Kang, G. J., Harmel E. Synthesis and Biological Evaluation of 2-Styrylquinazolin-4(3H)-ones, a New Class of Antimitotic Anticancer Agents which Inhibit Tubulin Polymerization. J. Med. Chem. **1990;** 33:1721-1728.
10. Lin, C. M., Kang, G. J., Roach, M. C., Jiang, J. B., Hesson, D. P., Luduena, R. F., Hamel, E.Investigation of the mechanism of the interaction of tubulin with derivatives of 2-styrylquinazolin-4(3H)-one**.** Mol. Pharmacol. **1991;** 40: 827-832.
11. Pandey, V. K., Tusi,S., Tusi, Z., Raghubir, R., Dixit, M., Joshi, M. N. Heterocyclic Compounds Thiazolyl quinazolones as Potential Antiviral and Antihypertensive Agents. IndianJ. Chem. **2004;** 43:180-184.
12. Kamal, A., Devaiah, V., Sankaraiah, N., Reddy, K. L. A Polymer-assisted Solution-Phase Strategy for the Synthesis of Fused [2,1-b] quinazolones and the Preparation of Optically Active Vasicinone. Synlett **2006;** 2609-2612.
13. Jian Guan, Quan Zhang, Michael O'Neil, Nicanor Obaldia III, Arba Ager,Lucia Gerena, and Ai J. Lin. Antimalarial Activities of New Pyrrolo[3,2-f]Quinazoline-1,3-Diamine Derivatives. Antimicrobial Agents And Chemotherapy,**2005;**49:12; 4928–4933.
14. Subarna Ganguli,M.Firdous, Tara Sankar Maity, Raj Kumar Bera, Mahendra Panigrahi. The study of antihyperlipidemic activities of schiff bases of 4(3H)quinazolinone derivatives in rats.International Journal of Pharmacy and Pharmaceutical Sciences,2012;4:175-178.
15. Srikanth Kagithoju, Vikram Godishala, Shiva Krishna Pabba, Hima Bindhu Kurra and N. Rama Swamy. Anti bacterial activity of flower extract of Pongamia pinnata linn. - an elite medicinal plant. International Journal of Pharmacy and Pharmaceutical Sciences,2012;4:130-132.