Academic Sciences International Journal of Pharmacy and Pharmaceutical Sciences

ISSN- 0975-1491 Vol 4, Suppl 3, 2012

Research Article

SYNTHESIS, CHARACTERIZATION, PRELIMINARY QSAR STUDIES AND INVITRO ANTIOXIDANT ACTIVITY OF SOME NOVEL 2, 3-DISUBSTITUTED QUINAZOLINONE DERIVATIVES

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Received: 18 Oct 2011, Revised and Accepted: 25 Sep 2011

ABSTRACT

A series of some novel 2, 3-disubstituted quinazolinone derivatives were synthesized by condensing 2-methyl/ 2-phenyl/6-bromo-2-methyl/6-bromo-2-phenyl/6, 8-dibromo-2-methyl/ 6,8-dibromo-2-phenyl benzoxazines with compounds containing amino group. The chemical structures of the newly synthesized compounds were confirmed by IR, ^{1}H-NMR, ^{13}C-NMR, Mass spectral data and elemental analysis. Title compounds were subjected to preliminary QSAR study using Mol inspiration software. All the synthesized compounds were screened for their invitro anti-oxidant activity by 1, 1-diphenyl-2, 2-picryl hydrazyl free radical (DPPH) method.

Keywords: Quinazolinone, Benzoxazine, Preliminary QSAR study, Invitro antioxidant (DPPH).

INTRODUCTION

Quinazolinone ring system was rewarded as a promising molecule because of its broad spectrum of biological activities like anti-histaminic[1], anti-cancer[2-3], anti-HIV[4], anti-inflammatory[5], analgesic[6], anti-diabetic[7], anti-bacterial[8], anti-fungal[9], anti-oxidant[10], anti-tubercular[11], anti-convulsant[12] etc. Anti-oxidant compounds like phenolic acids, polyphenols and flavanoids scavenge free radicals such as peroxide, hyperoxide or lipid peroxyl and thus inhibit the oxidative mechanisms that lead to degenerative diseases. Reactive oxygen species (ROS) such as superoxide anions, hydrogen peroxide, hydroxyl and nitric oxide radicals play an important role in oxidative stress related to the pathogenesis of various important diseases[13]. Antioxidants act as a major defence against radical mediated toxicity by protecting the damages caused by free radicals. Anti-oxidant agents are effective in the prevention and treatment of complex diseases, like Atherosclerosis, Stroke, Diabetes, Alzheimer's disease and Cancer[14]. The various analytical methods used to measure the radical scavenging activity of antioxidants against free radicals are 1,1-diphenyl-2,2-picryl hydrazyl free radical (DPPH) radical, the superoxide anion radical (O_2), the hydroxyl radical (OH), peroxyl radical (ROO), the malondialdehyde (MDA) or thiobarbituric acid reactive substances (TBARS) assay.

Keeping in view the diverse therapeutic activities of quinazolinones, the present study involves the synthesis of 2, 3-disubstituted quinazolinone derivatives and to study them for their preliminary QSAR using Mol inspiration software and invitro anti-oxidant activity by DPPH method. Preliminary QSAR studies include log P value, Molar Volume, number of rotatable bonds Topological Polar surface area.

MATERIALS AND METHODS

Melting points were determined using an open ended capillary tube method and are uncorrected. The completion of the reaction was checked by TLC using a silica gel G as stationary phase and the spot is visualized by UV-chamber. FT-IR Spectra were recorded on a Perkin-Elmer 1800 FT-IR in KBr disc. ^{1}H-NMR spectra were recorded at 400 MHZ on a Brucker FT-NMR spectrophotometer using TMS as internal standard. Mass spectra were recorded using a Thermo Finnigan LCQ Advantage MAX 6000 ESI Mass spectrometer. Elemental analysis was undertaken with Perkin Elmer-2400 instrument and the measured values agreed within 0.4% with the calculated. The synthetic strategy to synthesize the target compounds is depicted in scheme 1 & 2 and the scheme details are given in Table 1.

Synthesis of 2-methyl-4*H*-Benzoxazine-4-one[15]

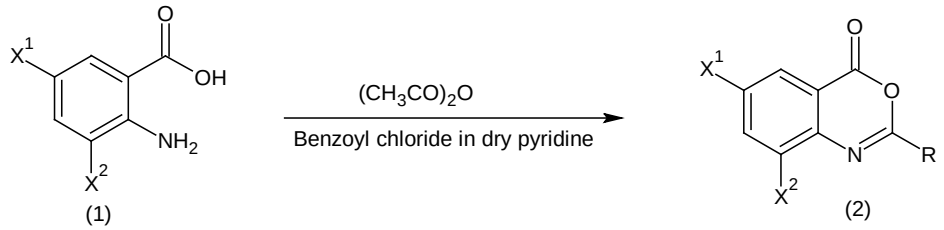
A mixture of disubstitued anthranilic acid, (1) (R^1, R^2= H, Br) (0.12 mol) in Acetic anhydride (0.2 mol) was refluxed for 4 Hrs. The excess solvents were then distilled off under reduced pressure. The reaction mixture was filtered, washed, dried and recrystallized with absolute ethanol.

Synthesis of 2-phenyl-4*H*-Benzoxazine-4-one[16]

Disubstitued anthranilic acid, (1) (X^1, X^2= H, Br) (0.1 mol) was dissolved in 50 ml of dry pyridine. To this solution, Benzoyl chloride (0.2 mol) was added drop wise with constant stirring at low temperature. The reaction mixture was cooled. When the addition of Benzoyl chloride was completed, the resultant mixture was treated with 10% sodium bicarbonate. The reaction mixture was filtered and washed repeatedly with water to remove inorganic materials. The crude product obtained was recrystallized from ethanol.

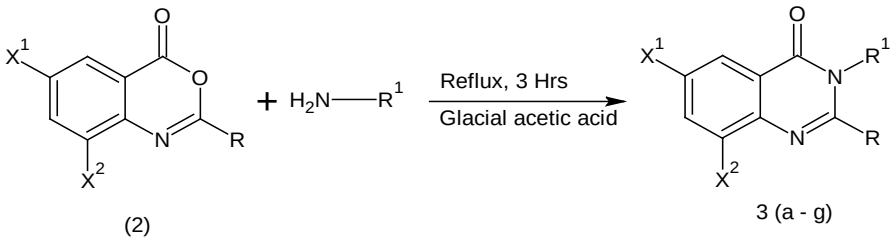
General Procedure for the synthesis of title compounds

Various substituted benzoxazine-4-ones (2) (0.01 mol) and o-Toludine/ pyridine-4-carbohydrazide/ Hydrazine Hydrate (0.01 mol) were refluxed for 3 hours in presence of glacial acetic acid. The reaction mixture was allowed to cool at room temperature. The crude product was recrystallized using absolute alcohol. The pyhsico chemical parameters of the synthesized compounds were represented in table 2 and the preliminary QSAR studies were shown in table 3.



4,6-disubstituted anthranilic acid

6,8-disubstituted methyl/phenyl benzoxazine-4-one

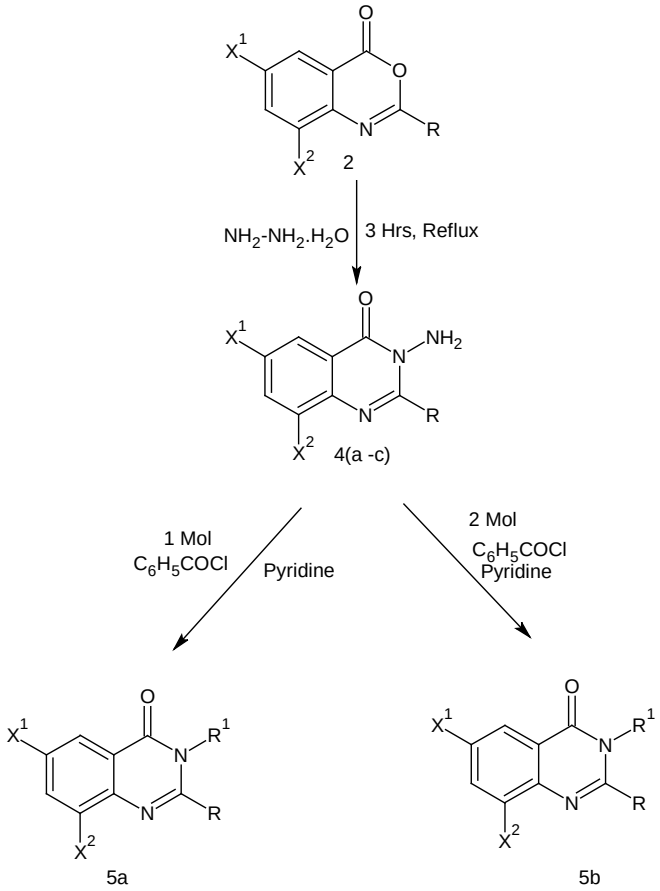


Scheme 1

X^1, X^2 = H, Br

R= CH_3, C_6H_5

R^1 = -$C_6H_4CH_3$, -$NHCOC_6H_4N$



Scheme 2

X^1, X^2 = H, Br

R= CH_3, C_6H_5

R^1= -$NHCOC_6H_5$, -N $(COC_6H_5)_2$

Table 1: Scheme details

S. No.	Compound Code	X^1	X^2	R	R^1
1	3a	H	H	CH_3	-$C_6H_4CH_3$
2	3b	H	H	C_6H_5	-$C_6H_4CH_3$
3	3c	Br	H	C_6H_5	-$C_6H_4CH_3$
4	3d	Br	Br	CH_3	-$C_6H_4CH_3$
5	3e	Br	Br	C_6H_5	-$C_6H_4CH_3$
6	3f	H	H	CH_3	$NHCOC_6H_4N$
7	3g	H	H	C_6H_5	$NHCOC_6H_4N$
8	4a	H	H	CH_3	-NH_2
9	4b	H	H	C_6H_5	-NH_2
10	4c	Br	Br	C_6H_5	-NH_2
11	5a	H	H	C_6H_5	$NHCOC_6H_5$
12	5b	H	H	C_6H_5	-$N(COC_6H_5)_2$

Table 2: Physico chemical parameter of the synthesized compounds

S. No.	Compound code	Molecular formula	Molecular Weight (in gms)	Percentage yield	Rf value	Melting point (^{0}C)
1	3a	$C_{16}H_{14}N_2O$	250.29	62	0.54	84
2	3b	$C_{21}H_{16}N_2O$	312.36	68	0.64	78
3	3c	$C_{21}H_{15}BrN_2O$	391.26	72	0.61	94
4	3d	$C_{16}H_{12}Br_2N_2O$	408.08	70	0.64	98
5	3e	$C_{21}H_{14}Br_2N_2O$	470.15	71	0.53	90
6	3f	$C_{15}H_{12}N_4O_2$	280.28	58	0.58	170
7	3g	$C_{20}H_{14}N_4O_2$	342.35	68	0.68	142
8	4a	$C_9H_9N_3O$	175.18	62	0.62	138
9	4b	$C_{14}H_{11}N_3O$	237.25	60	0.72	144
10	4c	$C_{14}H_9Br_2N_3O$	395.04	64	0.71	132
11	5a	$C_{21}H_{15}N_3O_2$	341.36	49	0.65	130
12	5b	$C_{28}H_{19}N_3O_3$	445.46	50	0.75	80

Table 3: Preliminary QSAR Study of synthesized compounds

S. No.	Compounds	M.W	Log P	TPSA	Nrotb	M.V
1	3a	250.301	3.026	34.897	1	232.76
2	3b	312.372	5.054	34.897	2	287.60
3	3c	329.197	3.811	34.897	1	250.64
4	3d	408.09	4.54	34.897	1	268.53
5	3e	470.164	6.576	34.89	2	323.379
6	3f	280.287	0.644	76.887	2	243.429
7	3g	341.37	3.961	63.995	3	302.433
8	4a	175.191	0.525	60.92	0	156.079
9	4b	237.26	2.553	60.92	1	210.92
10	4c	395.054	4.075	60.92	1	246.698
11	5a	341.37	3.961	63.995	3	302.433
12	5b	445.478	5.239	72.277	4	393.206

M.W – Molecular Weight; TPSA – Topological Polar Surface Area; n rotb – Number of Rotatable bonds; M.V – Molar Volume

Determintion of Anti-Oxidant Activity

Free radical scavenging activity[17] of the title compounds were determined by 1, 1-diphenyl-2, 2-picryl hydrazyl free radical (DPPH) assay method. Test compound stock solutions (1mg/ml) were diluted to final concentration of 10, 20, 30, 40 and 50 µg/ml in methanol. DPPH in methanol solution (1 ml, 0.1 mmol) was added to 2.5 ml of test solution of different concentrations and allowed to react at room temperature. After 30 min the absorbance was measured at 517 nm. Methanol was used as the solvent and Ascorbic acid as standard. The percentage antioxidant activity (% inhibition) against concentration was calculated by using the following formula and the results were showed in Table 4.

$$\% \text{ Anti-oxidant activity} = \frac{(A_{Control} - A_{Test})}{(A_{Control})} X\ 100$$

Where, $A_{Control}$ - Absorbance of control sample

A_{Test} - Absorbance of Test sample

RESULTS AND DISCUSSION

The compounds were synthesized as per scheme 1 &2, where various 2,3- disubstituted benzoxazine 4-ones was reacted with o-Toludine/ Pyridine-4-carbohydrazide/ Hydrazine Hydrate under reflux for three hours in the presence of glacial acetic acid. The structures of the synthesized compounds were confirmed by IR, ^{1}H-NMR, ^{13}C-NMR, Mass spectra and CHN analysis. The results of all the characterization techniques including melting point determination, thin layer chromatography, IR, ^{1}H-NMR, ^{13}C-NMR, Mass spectra and elemental analysis positively confirm the formation of the title compounds. Further all the synthesized analogs presented significant values in preliminary QSAR studies and all compounds obey Lipinski rule of five violations. The anti-oxidant activity of the synthesized compounds may be due to the presence of methyl and unsubstituted phenyl group at 2 nd position of quinazolinone ring. When compared to standard drug, compound 3a, 3d, 3f and 4a (possessing methyl group at 2 nd position) showed 84.54 %, 81.96%, 79.89%, and 79.2% of antioxidant activity respectively at a concentration of 100 µg/ml.

Table 4: Invitro anti-oxidant activity of the synthesized compounds

S. No.	Compound code	% Inhibition at a concentration (mcg/ml)				
		10	20	30	40	50
1	Standard	73.52	76	77.59	79.52	85.2
2	3a	76.32	79.34	81.35	82.27	84.54
3	3b	71.21	71.84	74.77	75.14	77.24
4	3c	65.11	67.3	71.48	73.13	75.58
5	3d	66.24	72.31	75.28	77.82	81.96
6	3e	68.85	71.13	73.13	75.31	76.51
7	3f	72.97	74.24	75.51	77.97	79.89
8	3g	66.5	67.71	67.94	68.35	69.02
9	4a	72.64	73.3	75.54	76.8	79.2
10	4b	58.27	61.23	63.5	64.48	65.28
11	4c	64.14	66.32	69.18	72.24	73.12
12	5a	54.16	58.88	63.98	65.15	66.81

Spectral data of the Synthesized Compounds

Compound 3a: 2-Methyl-3-(2-methylphenyl) quinazolin-4(3*H*)-one

Yield 62%; TLC R_f= 0.54; mp 84; log P 2.5; IR(KBr): 1672 (C=O str), 1152.7 (C=C), 1604.5(C=N), 1448.8 (CH_3), 3397.5 (C-H Ar); ^{1}H-NMR ($CDCl_3$): δ 6.64 (s, 1H, Ar-H), 6.6 (s, 1H, Ar-H), 7.4- 8(m,6H, Ar- H), 2.8 (s, 3H, CH_3), 2.23(s, 3H, CH_3); ^{13}C-NMR: δ 24.19, 25.52, 29.75, 44.63, 57.96, 120.3, 122.6, 123.9, 125.7, 126.8, 127, 128, 130.1, 131.66, 134.8, 139.9; EI-MS (M/Z): 250 (M+1). Anal. Calcd for $C_{16}H_{14}N_2O$: C, 76.78; H, 5.64; N, 11.19; O, 6.39; Found C, 76.74; H, 5.62; N, 11.15; O, 6.33.

Compound 3b: 3-(2-Methylphenyl)-2-phenylquinazolin-4(3*H*)-one

Yield 68%; TLC R_f= 0.64; mp 78; log P 4.4; IR(KBr): 1666 (C=O str), 1517.3(C=C), 1595.5(C=N), 1026 (C-N), 1448.1 (C-H), 2923 (C-H, Ar); ^{1}H-NMR ($CDCl_3$): δ 7.0 (s, 1H, Ar-H), 7.1 (s, 1H, Ar-H), 7.2-7.8 (m, 11H, Ar-H), 2.3 (s, 3H, CH_3); ^{13}C-NMR: δ 87.5, 93.5, 98.2, 120.4, 122.7, 125.8, 126.5, 126.5, 126.8, 127.0, 127.2, 127.4, 128.8, 131.9; EI-MS (M/Z): 312 (M+1). Anal. Calcd for $C_{21}H_{16}N_2O$: C, 80.75; H, 5.16; N, 8.97; O, 5.12; Found C, 80.71; H, 5.11; N, 8.62; O, 5.10.

Compound 3c: 6-Bromo-3-(2-methylphenyl)-2-phenylquinazolin-4(3*H*)-one

Yield 72%; TLC R_f= 0.61; mp 94; log P 5.21; IR (KBr): 1666.6 (C=O str), 1594, 1515 (C=C), 1447.3(C- H), 3047.1, 3568.4 (C-H, Ar), 531 (C-Br). Anal. Calcd for $C_{21}H_{15}BrN_2O$: C, 64.46; H, 3.86; Br, 20.42; N, 7.16; O, 4.09; Found C, 64.24; H, 3.63; Br, 20.02; N, 7.13; O, 4.01.

Compound 3d: 6, 8-Bibromo-2-methyl-3-(2-methylphenyl) quinazolin-4(3*H*)-one

Yield 70%; TLC R_f= 0.64; mp 98; log P 4; IR (KBr): 3373.4 (C-H, Ar), 1672(C=O str), 1493(C=C), 2535 (C- H), 1617(C=N), 530,656 (C-Br). Anal. Calcd for $C_{16}H_{12}Br_2N_2O$: C, 47.09; H, 2.96; Br, 39.16; N, 6.86; O, 3.92; Found C, 46.9; H, 2.73; Br, 39.11; N, 6.76; O, 3.85.

Compound 3e: 6, 8-Bibromo-3-(2-methylphenyl)-2-phenylquinazolin-4(3*H*)-one

Yield 71%; TLC R_f= 0.53; mp 90; log P 5.94; IR (KBr): 3403.3 (C-H, Ar), 1671(C=O str), 1512(C=C), 1447(C-H), 1603(C=N), 548, 699(Br). Anal. Calcd for $C_{21}H_{14}Br_2N_2O$: C, 53.65; H, 3.00; Br, 33.99; N, 5.96; O, 3.40; Found C, 53.21; H, 2.93; Br, 33.41; N, 5.76; O, 3.28.

Compound 3f: *N*-(2-Methyl-4-oxoquinazolin-3(4*H*)-yl) pyridine-4-carboxamide

Yield 58%; TLC R_f= 0.58; mp 170; log P 1.2; IR (KBr): 3428.8 (C-H, Ar), 1669(C=O str), 1543(C=C), 1605(C=N), 3428 (N-H str); ^{1}H-NMR ($CDCl_3$): δ 7.4-7.5 (m, 4H, Ar-H), 7.28-8.6 (4H, Ar-H in pyridine), 2.37 (s, 3H, CH_3), 2(s, 1H, NH). Anal. Calcd for $C_{15}H_{12}N_4O_2$: C, 64.28; H, 4.32; N, 19.99; O, 11.42; Found C, 64.18; H, 4.31; N, 19.74; O, 11.25.

Compound 3g: *N*-(4-Oxo-2-phenylquinazolin-3(4*H*)-yl) pyridine-4-carboxamide

Yield 68%; TLC R_f= 0.68; mp 142; log P 2.16; IR (KBr): 3057.7 (C-H, Ar), 1685(C=O str), 1526(C=C), 1452(C-H), 1599(C=N), 1239(C-N), 3502(N-H str); ^{1}H-NMR ($CDCl_3$): δ 7.31-7.38 (m, 5H, Ar-H), 7.41 (s, 1H, Ar-H), 7.53-7.57 (m, 3H, Ar-H), 7.6-7.72 (m, 5H, Ar-H); EI-MS (M/Z): 342 (M+1). Anal. Calcd for $C_{20}H_{14}N_4O_2$: C, 70.17; H, 4.12; N, 16.37; O, 9.35; Found C, 70.15; H, 4.10; N, 16.32; O, 9.32.

Compound 4a: 3-Amino-2-methylquinazolin-4(3*H*)-one

Yield 62%; TLC R_f= 0.62; mp 138; log P 0.73; IR (KBr): 3540.2(C-H, Ar), 1658.2 (C=O str), 1598.4 (C=C), 1427.8 (C-H), 3302 (N-H str), 1475.9 (C=N). Anal. Calcd for $C_9H_9N_3O$: C, 61.70; H, 5.18; N, 23.99; O, 9.13; Found C, 61.68; H, 5.16; N, 23.36; O, 9.11.

Compound 4b: 3-Amino-2-phenylquinazolin-4(3*H*)-one

Yield 60%; TLC R_f= 0.72; mp 144; log P 1.71; IR (KBr): 3307.1 (C-H, Ar), 1661.2 (C=O str), 1566.9(C=C), 1472.1 (C- H), 3216.8 (N-H str); ^{1}H-NMR ($CDCl_3$): δ 5.02 (s, 2H, NH_2), 7.47-7.48 (d, 2H, Ar-H), 7.73-7.77 (m, 5H, Ar-H), 8.25-8.27 (d, 2H, Ar-H); ^{13}C-NMR: δ 120.19, 126.6, 127.06, 127.9, 128.2, 129.3, 130.2, 134.1, 134.49. Anal. Calcd for $C_{14}H_{11}N_3O$: C, 70.87; H, 4.67; N, 17.71; O, 6.74; Found C, 70.84; H, 4.62; N, 17.52; O, 6.71.

Compound 5a: *N*-(4-Oxo-2-phenylquinazolin-3(4*H*)-yl) benzamide

Yield 49%; TLC R_f= 0.65; mp 130; log P 3.42; IR (KBr): 3307.7(C-H,Ar), 1660.7 (C=O str), 1566.4 (C=C), 3034.1 (C- H), 1252.1 (C-N), 3216.8 (N-H str); ^{1}H-NMR ($CDCl_3$): δ 7.47-7.51 (m, 5H, Ar-H), 7.73-7.78 (m, 6H, Ar-H), 7.01(s, 1H, Ar-H), 7.05 (s, 1H, Ar-H), 8.27 (s, 1H, N-H), 8.29 (s, 1H, Ar-H); ^{13}C-NMR: δ 120.2, 126.6, 127, 127.9, 128.2, 129.3, 130.2, 134.1, 134.5, 147, 154.6, 131.6. Anal. Calcd for $C_{21}H_{15}N_3O_2$: C, 73.89; H, 4.43; N, 12.31; O, 9.37; Found C, 73.86; H, 4.41; N, 12.30; O, 9.32.

Compound 5b: *N*-(4-Oxo-2-phenylquinazolin-3(4*H*)-yl) dibenzamide

Yield 50%; TLC R_f= 0.75; mp 80; log P 4.75; IR (KBr): 3307.7 (C-H, Ar), 1660.7 (C=O str), 1566.4 (C=C), 3034.1 (C- H), 1252.1 (C-N), 3216.8 (N-H str). Anal. Calcd for $C_{28}H_{19}N_3O_3$: C, 75.49; H, 4.30; N, 9.43; O, 10.77; Found C, 75.46; H, 4.29; N, 9.41; O, 10.75.

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