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Synthesis, antioxidant and anticancer activity of quinazoline derivatives.

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Abstract
Sodium hydroxide on treatment with bromine and pthalamide followed by reaction with hydrochloric acid gave anthranillic acid **1**. Compound **1** on further reaction with sodium hydroxide and benzoyl chloride yields 2-phenyl benzoxizanone **2**, Compound **2** further reactions with substituted anilines yielded the corresponding **2a** to **2f** respectively. All the synthesized compounds have been screened for their antioxidant and anticancer activity.

Key Words
Quinazoline substituted aniline, antioxidant, anticancer activity.

Introduction
Cancer is continuing to be a major health problem in developing as well as undeveloped countries[1-9]. Surpassing heart diseases, it is taking the position number one killer due to various worldwide factors. Although major advances have been made in the chemotherapeutic management of some patients, the continued commitment to the laborious task of discovering new anticancer agents remains critically important. In the course of identifying various chemical substances which may serve as leads for designing novel antitumor agents, we are particularly interested in the present work with quinazoline derivatives which have been identified as a new class of cancer chemotherapeutic agents with significant therapeutic efficacy against solid tumors[7-9]. It is well known that quinazoline derivatives are potent inhibitors of epidermal growth factor receptor (EGFR)[10-18]. The epidermal growth factor receptor (EGFR) is cellular trans-membrane tyrosine kinases that is over-expressed in a significant number of human tumors (e.g., breast, ovarian, colon, and prostate), their expression levels often correlate with vascularity, and is associated with poor prognosis in patients.

Heterocycles containing quinazoline rings are associated with a wide range of biological properties such as analgesic[19,20], antioxidant[21-23], anticancer[24-28], antiinflammatory[29,30],anticonvulsant[31],antibacterial[32], antifungal[33] and antimycobacterial[34,35] activity. The known methods for the preparation of quinqzolines are based on reaction of anthranillic acid with benzoyl chloride or acetic anhydride. In view of the previous rationale and in continuation of an ongoing program aiming at finding new structure leads with potential chemotherapeutic activities , new series of 2,3 disubsituted quinazolines have been synthesized and screened for antioxidant and anticancer activity.

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Materials and Methods
The starting material, anthranillic acid **1** was prepared in laboratory. Melting points were determined in open capillary tubes and are uncorrected. IR spectra were recorded in KBr discs (V_{max} in cm^{-1}) on Perkin-Elmer FT-IR (Spectrum ONE) spectrometer and ^1H NMR spectra on bruker AMX (400 MHz) spectrometer using DMSO-d_6 as solvent unless otherwise stated, using TMS as an internal standard (chemical shifts in δ,ppm) and mass spectra on a Jeol SX-102 (FAB) mass spectrometer.

1. Synthesis of anthranillic acid

Prepared a solution of 30gm of sodium hydroxide in 120ml of water in a 350ml conical flask and cool to 0^oC (or) below in a bath of ice and salt. Added 26.2g (8.4ml) of bromine in one portion and shake until all bromine has reacted. The temperature will rise somewhat, cool again to 0^oC or below. Meanwhile prepared a solution of 22gms of sodium hydroxide in 80ml of water. Added 24gms of finely powdered pthalimide in one portion to cold solution of sodium hypobromite solution in the form of smooth paste with water rapidly with stirring. Removed the flask from the cooling bath and shaked vigorously until a clear yellow solution is obtained (for 5 min). Added the prepared NaOH solution rapidly in one portion, heat the solution to 80^oC for about 2 min and filter if

necessary. Cool in ice and add concentrated HCl slowly with stirring until the solution is just neutral (about 60ml). Precipitated the Anthranillic acid completely by the gradual addition of glacial acetic acid (20-25ml). Recrystallized from hot water with addition of little decolorizing carbon. Collect the acid on a buchner funnel and dry at 100^{o}C.

2. Synthesis of 2-phenyl benzoxizanone

The hydroxyl/amino compound is either suspended or dissolved in an excess of freshly prepared 10% sodium hydroxide solution together with small excess of benzoyl chloride, and the resulting mixture is shaken vigorously in ambient conditions. Under these conditions, benzoylation proceeds smoothly. Thus solid benzoylated product which is insoluble in aqueous medium gets separated.

3. Synthesis of 2a, 2b, 2c, 2d, 2e and 2f

An equimolar mixture of **2** and substituted anilines was refluxed for 6hrs with glacial acetic acid and progress of reaction was monitored by TLC. After completion of reaction, contents were poured onto crushed eyes to form solid mass which was collected and recrystallized from ethanol.

2a: 2, 3 diphenylquinazoline-4(3H)-one 62.44%, m.p. 98^{o}C. ^{1}H NMR (DMSO-d_6): δ 7.0-7.9 (m, 14H, Ar-H); IR (KBr): 1435 (C=C), 1179 (C-N), 1618 (C=N), 1664 (C=O); *m/z* (%) 298 (7), 210 (19), 160 (100), 120 (22).

2b: 3-(4-chlorophenyl)-2-phenylquinazoline-4(3H)-one 68.56%, m.p. 158^{o}C. ^{1}H NMR (DMSO-d_6): δ 7.2-8.3 (m, 13H, Ar-H); IR (KBr): 822 (C-C), 1447 (C=C), 1179 (C-N), 1607 (C=N), 1665 (C=O), 606 (C-Cl); *m/z* (%) 334 (M+2, 4), 332 (11), 236 (25), 160 (100), 108 (35).

2c: 3-(4-nitrophenyl)-2-phenylquinazoline-4(3H)-one 85.34%, m.p. 170^{o}C. ^{1}H NMR (DMSO-d_6): δ 7.2-8.1 (m, 13H, Ar-H); IR (KBr): 824 (C-C), 1458 (C=C), 1179 (C-N), 1633 (C=N), 1679 (C=O), 1505 (C-NO_2); *m/z* (%) 343 (6), 312 (10), 250 (41), 160 (100), 134 (40).

2d: 3-(4-bromophenyl)-2-phenylquinazoline-4(3H)-one 43.25%, m.p. 110^{o}C. ^{1}H NMR (DMSO-d_6): δ 7.2-7.9 (m, 13H, Ar-H); IR (KBr): 819 (C-C), 1447 (C=C), 1176 (C-N), 1647 (C=N), 1668 (C=O), 504 (C-Br); *m/z* (%) 378 (M+2, 5), 376 (7), 262 (32), 160 (100), 108 (25).

2e: 3-(3-chlorophenyl)-2-phenylquinazoline-4(3H)-one 35.72%, m.p. 158^{o}C. ^{1}H NMR (DMSO-d_6): δ 7.0-7.9 (m, 13H, Ar-H); IR (KBr): 830 (C-C), 1488 (C=C), 1169 (C-N), 16308 (C=N), 1668 (C=O), 606 (C-Cl); *m/z* (%) 334 (M+2, 4), 332 (12), 236 (25), 160 (100), 108 (35).

2f: 2-phenyl-3-p-tolyquinazoline-4(3H)-one 52.16%, m.p. 90^{o}C. ^{1}H NMR (DMSO-d_6): δ 2.25 (s, 3H, CH_3), 7.0-8.1 (m, 13H, Ar-H); IR (KBr): 819 (C-C), 1454 (C=C), 1180 (C-N), 1603 (C=N), 1662 (C=O), 2732 (C-CH_3); *m/z* (%) 312 (8), 262 (16), 160 (100), 120 (20).

Anticancer activity by using MTT assay[36-38] against MCF-7 breast cancer cell line.

MTT [(3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyl tetrasodium bromide)] is a pale yellow substrate that is cleaved by living cells to yield a dark blue formazan product. This process requires active mitochondria, and even freshly dead cells do not cleave significant amount of MTT. Thus the amount of MTT cleaved is directly proportional to the number of viable cells present, which is quantified by colorimetric methods. This assay was performed at Deshpande Laboratories, Bhopal using the standard operating procedures. Briefly the compounds were dissolved in DMSO and serially diluted with complete medium to get the concentrations a range of test concentration. DMSO concentration was kept < 0.1% in all the samples. Cell lines maintained in appropriate conditions were seeded in 96 well plates and treated with different concentrations of the test samples and incubated at 37 °C, 5% CO2 for 96 hours. MTT reagent was added to the wells and incubated for 4 hours; the dark blue formazan product formed by the cells was dissolved in DMSO under a safety cabinet and read at 550nm. Percentage inhibitions were calculated and plotted with the concentrations used to calculate the IC50 values.

Antioxidant activity by p-NDA (p-nitroso dimethyl aniline)[21-23] radical scavenging method.

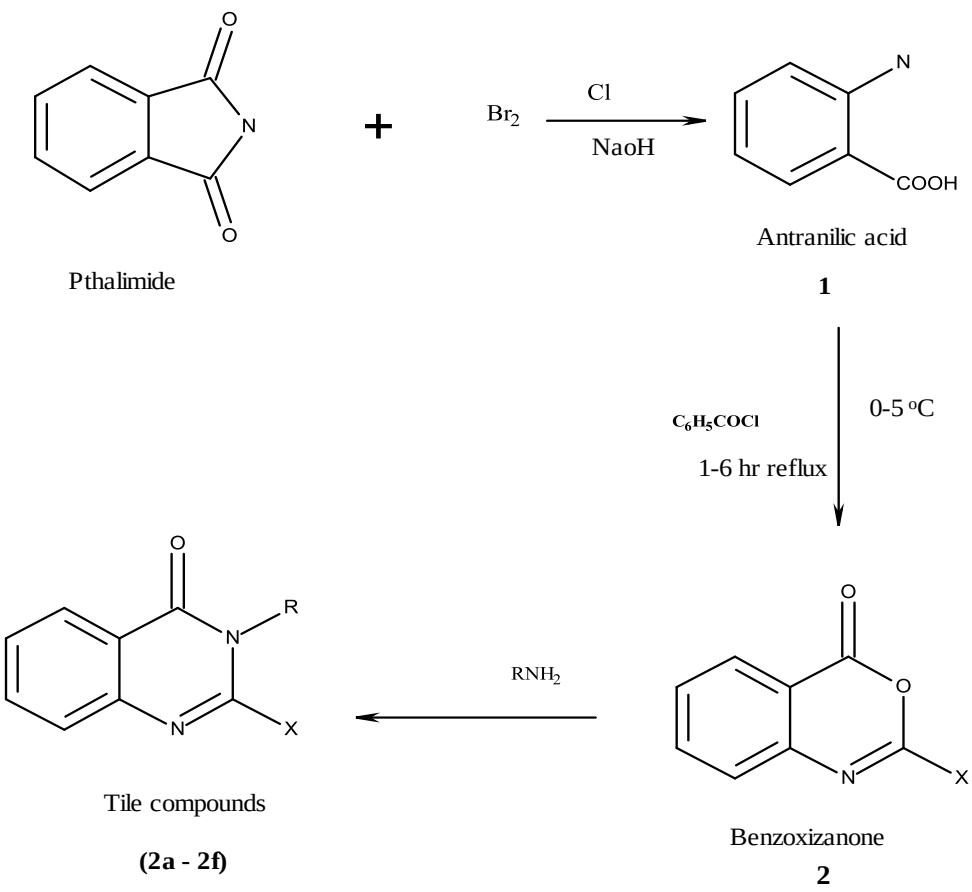
To a solution containing ferric chloride (0.1mM, 0.5ml), EDTA(0.1mM, 0.5ml), ascorbic acid(0.1mM, 0.5ml), hydrogen peroxide(2mM, 0.5ml) and p-nitroso dimethyl aniline (0.01mM, 0.5ml) in phosphate buffer (P^{H} 7.4, 20Mm) were added various concentrations of the test compounds

in distilled DMSO or dissolving solvent or alcohol to produce a final volume of 3ml. Absorbance was measured at 440nm (Elizabeth and Rao, 1990).

$$\text{p-NDA radical scavenging activity(\%)} = \frac{[\text{Abs(control)}-\text{Abs(standard)}}{[\text{Abs(control)}]\times 100}$$

Results and Discussion

The synthesized compounds were evaluated for their antioxidant as well as anticancer activities, in comparison with the standards, namely, ascorbic acid, penicillin G, streptomycin sulfate and amphotericin B respectively. In overall bioassay **(Table 2)** in general the compounds **2b** and **2f** exhibited good antioxidant activity. In **(Table 3)** the compounds **2b, 2e** and **6f** exhibited good anticancer activity against MCF-7 breast cancer cell lines.



Pthalimide

Antranilic acid

1

Tile compounds

(2a - 2f)

Benzoxizanone

2

Scheme 1

Table 1: Number of substituents at the position X and R

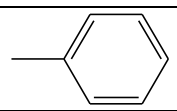
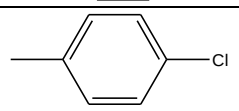
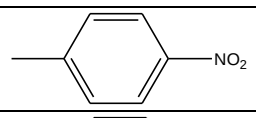
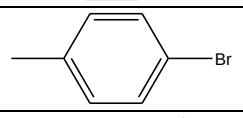
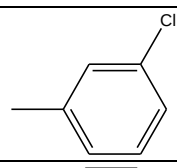
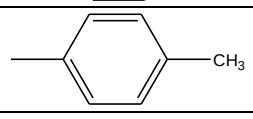
X	R
C_6H_5	
C_6H_5	
C_6H_5	
C_6H_5	
C_6H_5	
C_6H_5	

Table 2: Antioxidant activity by p-NDA radical scavenging method.

Compounds	% Radical scavenging method concentrations (µg/ml)				
	10 µg/ml (%)	20 µg/ml (%)	40 µg/ml (%)	80 µg/ml (%)	IC_{50} µg/ml
2a	25.41	28.38	33.37	36.65	161.49
2b	66.00	67.69	69.63	73.26	<50
2c	17.82	22.11	27.06	30.69	186.57
2d	20.46	23.43	24.75	34.53	183.45
2e	34.98	39.93	40.92	46.57	101.58
2f	60.72	64.35	66.65	68.64	<50
Ascorbic acid	74.30	76.40	78.20	79.01	<50

Table 3: Anticancer activity, Percentage growth inhibition of synthesized compounds.

Compounds	IC_{50} values in (µM)	% Growth inhibition at different concentrations (µM)				
		0.01	0.1	1	10	100
2a	9	0	3.2	14.7	20.6	21.1
2b	6	0	7.2	30.0	54.5	60.5
2c	8	0	2.1	11.3	20.3	22.8
2d	7	0	3.3	12.7	25.9	28.4
2e	6	0	7.5	29.4	54.1	60.7
2f	4	0	12.3	31.5	61.3	67.9

Conclusion

This study reports the successful synthesis of some new quinazoline derivatives. The antioxidant and anticancer screening studies were also performed in the study. The substituted anilines are the active components present in many standard drugs and it is known to increase the pharmacological activity of the molecules. The oxidant screening suggests that among the newly synthesized compounds, **2b** and **2f** exhibited good activity and the anticancer screening suggest that **2b, 2e** and **2f** exhibited good anticancer activity against MCF-7 breast cancer cell line.

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