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Synthesis and Evaluation of Cytotoxic Activity of 3-((1*H*-Benzo[d]imidazol-2-yl)methyl)-2-phenylquinazolin-4*(3H)*-one Derivatives on MCF-7 Cell Line

Baljeet Singh[1]*, Shailesh Sharma[2], Manish Sinha[3], S. Sagar[4]

Abstract

Cancers, particularly, breast cancer is one of the deadliest disease in women and requires to be treated with lowest side-effects and toxicity. Quinazolinone and Benzimidazole nucleus are privileged nucleus with reported anti-cancer activity. Hence, combined derivatives of 3-((1H-Benzo[d]imidazol-2-yl)methyl)-2-phenylquinazolin-4(3*H*)-one E(1-8) were prepared and tested for cytotoxicity on MCF-7 cell line. Compound E6 showed 70.74% inhibition of cell growth as compared to 86.96% inhibition by standard doxorubicin.

Keywords: Breast cancer, Cytotoxic, MCF-7, Quinazolinone-4(3*H*)-one

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Introduction

Cancer is the phase in which body cells start to grow uncontrollably due to some factors arising from physical and chemical changes in the body. Normally, cells are controlled by certain signals that govern events of cell life cycle such as division, differentiation, and death of cells, but due to some variations, cell growth goes out of control which is known as cancer.[1] Nearly 10 million deaths were caused by cancer in year 2020. "In the terms of new cases in 2020, the most common cases of cancer aroused due to breast cancer (2.26 million), lung cancer (2.21 million), and colon and rectum cancer (1.93 million). Similarly, there were 1.41 million cases of prostate cancer, 1.20 million cases of skin (non-melanoma) cancer, and 1.09 million cases of stomach cancer. Breast cancer is the most common malignant tumor in women and the second most deadly cancer in women, which accounts for approximately 23% of all cancers in females."[2-4] It can occur in breast tissue of either sex but females are more prone to it. The majority of breast cancers appears in the ducts hence called ductal cancer where as a fewer cases that appear in sacs or lobules are known as lobular cancers.[5] Cancer of breast can reach out to other regions of body such as lymph glands, bones, and liver.[1] "Several factors associated with risk of breast cancer are age, sex, race, lack of childbearing or breast-feeding, higher hormone levels, economic status, and dietary iodine deficiency."[6]

Quinazolinone and its derivatives have gained attention due to their antitumor properties. There are several prominent anticancer drugs such as Idelalisib, Erlotinib, Lapatinib, Canertinib, Raltitrexed, and Thymitaq which possess quinazolinone nucleus.[7-9] Similarly Rajitha Gali *et al.* have reported preparation of Indolylmethylene benzo[h]thiazolo[2,3-b]quinazolinones derivative 8c ($IC_{50=}$2.59) which had shown better inhibition of MCF7 cell line as compared to Doxorubicin (IC_{50} = 3)[3]. Kumar *et al.* reported synthesized of imidazolone fused quinazolinone derivatives among which 4e shows markedly good activity in inhibition of MCF7 cell line with IC_{50} value of 8.9 as compared to 26.2 of standard Cisplastin.[7] Methoxylated 2-benzylthio-quinazoline-4(3H)-ones derivatives series was prepared by El-Messery *et al.* which were evaluated against several cell lines including cell line for breast cancer. Derivative 40, 64, and 66 have GI_{50} value of 2.2, 2.4, and 2.6 μM, respectively, as compared to standard 5-Flourouracil which produced GI_{50} value of 22.6 μM.[10]

In five-membered aromatic nitrogen heterocycles, benzimidazole is among top five most commonly used moiety among pharmaceuticals approved by U.S. FDA. It is a heterocyclic motif of importance present in many natural products, in material science fuel cells, in ionic liquids, and in pharmaceutical industry. Being an important scaffold in number of bio-active molecules and isoster of purine nucleosides, it is most commonly explored for anti-cancer drug development.[11,12] Some of potent benzimidazole containing antitumor derivatives is bendamustine,[13] selumetinib,[14] Hoechst-33258,[15] and PPTMB (2-Phenyl-5-(pyrrolidin-1-yl)-1-(3,4,5-trimethoxybenzyl)-1H-benzimidazole).[16] Paul *et al.* have reported preparation of Quinazolinone-benzimidazole conjugates among which Compound 14 shows superior inhibitory activity against methotrexate (IC_{50}=0.011 μM against 0.02 μM of doxorubicin).[17] Hence, considering anti-cancer activities of Quinazolinone and Benzimidazole, novel derivatives E(1-8) were synthesized by combining both the nucleus and were tested on MCF-7 cell line with hope to get anti-cancer agents with better activity.

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Materials and Methods

Chemicals procured for the synthesis from Spectrochem Pvt. Ltd. and Loba Chemicals Pvt. Ltd were of LR grade. Sigma-Aldrich provided the anthranilamide. Solvents used in the reaction had been purified before use. Reaction monitoring was done with Thin Layer Chromatographic technique (TLC) using Silica gel pre-coated plates having F254 indicator. The eluent used for chromatogram development was combination of chloroform: methanol (9:1). ^{1}H NMR (400 MHz) and ^{13}C NMR (100 MHz) spectra were developed using Bruker 400 using TMS (Tetramethylsilane) as internal standards ($\delta = 0.0$ ppm) and solvent DMSO-d_6 at Indian Institute of Technology, Ropar, India. The ^{1}H NMR spectra were reported in following pattern: δ (multiplicity, coupling constant *J*, and number of protons). Multiplicities are written as by s (singlet), d (doublet), dd (doublet of doublets), ddd (doublet of doublets of doublets), t (triplet), and m (multiplet). IR spectrum was recorded on FTIR-ATR Alpha-E made of Bruker at ASBASJSM College of Pharmacy, Bela, India.

Experimental

The strategy to synthesize target compounds is depicted in Scheme 1.

Where R=Br, Cl and R_1 = H, Cl, CH_3, NO_2

Reagents and reaction conditions: (i) DMSO, stirring at 100 °C, (ii) 4N HCl, reflux for 3 h (iii) DMF, K_2CO_3

Synthesis of 2-phenylquinazolin-4*(3H)*-one derivatives B(1-2) In 20 ml DMSO, 0.05 mol (1.0 equivalent) Anthranilamide and 0.06 mol (1.2 equivalent) substituted aldehyde A(1-2) was dissolved in a flat bottom flask. After that, the solution was agitated at 100°C for 12–18 h. Completion of reaction was tracked using TLC technique and after completion, it was cooled off to room temperature. To resulting solution, mixed 250 ml of cold water and precipitates produced were collected by filtration which were then recrystallized in ethanol.[18]

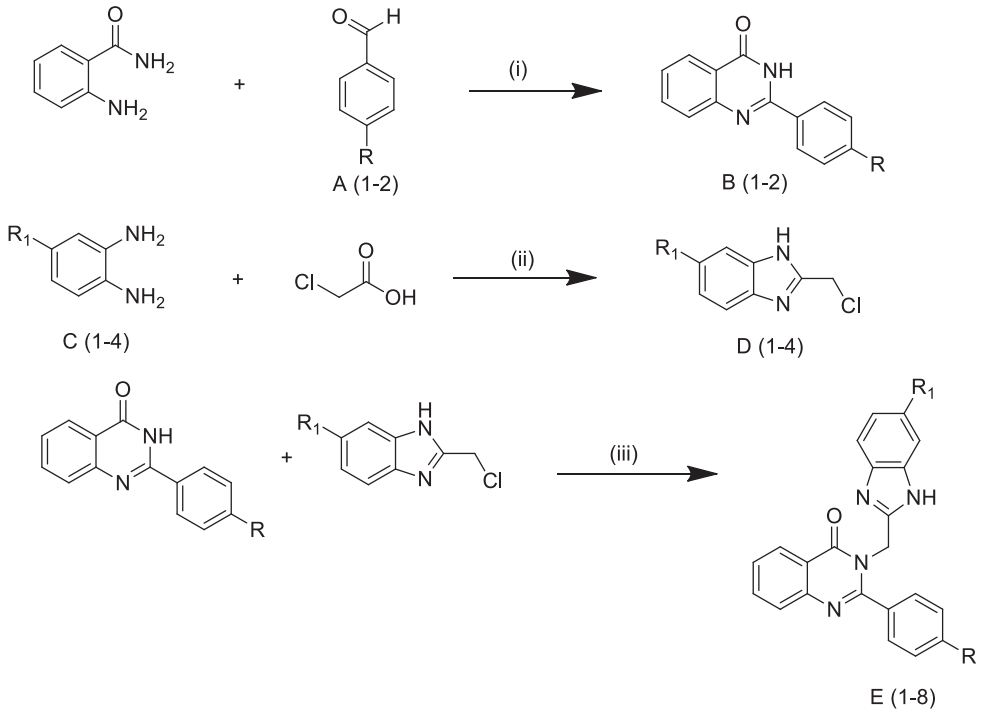
Synthesis of 2-(chloromethyl)-1*H*-benzo[d]imidazole derivatives D(1-4) The compounds D(1-4) were prepared using method adopted by Kaur H. and Singh B. 0.1mol of C(1-4) derivatives, 0.1 mol of chloroacetic acid and 50 ml of 4N HCl was mixed in a flask and was refluxed for 3 h. Then, it was kept to attain room temperature and then basified using ammonium hydroxide solution to get crude derivatives which were separated and then recrystallized from methanol to get D(1-4).[19]

General Procedure Synthesis of Compounds E(1-8)

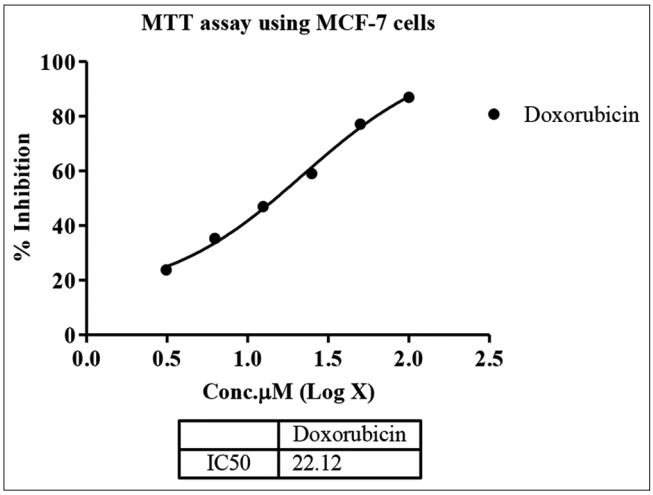
2 mmol of derivative B(1-2) and 2 mmol of derivative D(1-4), 6 mmol K_2CO_3, and 20 ml DMF (Dimethylformamide) were transferred to a flask with flat bottom and shaken on magnetic stirrer for 18 h at RT (room temperature). After that, added water to separate solid precipitates from the liquid. To obtain the final product, the solids were filtered, then recrystallized in hot ethanol.

Evaluation of Cytotoxic Activity

Skanda Life Sciences Pvt Ltd. at Banglore, India, tested cytotoxic activity. The MTT (3-[4, 5- dimethylthiazol-2-yl]-2, 5-diphenyl tetrazolium bromide) system uses mitochondrial dehydrogenases to measure the behavior of living cells. The MTT approach is simplistic, precise, and repeatable. MTT, a tetrazolium salt which is aqueous soluble, when prepared in salt solution or media without phenol red, yields a yellowish solution. The tetrazolium ring of dissolved MTT is in viable cells cleaved by mitochondrial dehydrogenase enzymes, resulting in an insoluble purple formazan. DMSO, Pure propanol, acidified isopropanol, or ethanol may be used to solubilize this water-insoluble formazan. The purple solution that results is spectrophotometrically calculated. A difference in the amount of formazan produced in response to rise or reduction in cell number indicates the extent of the effects generated by the test material.

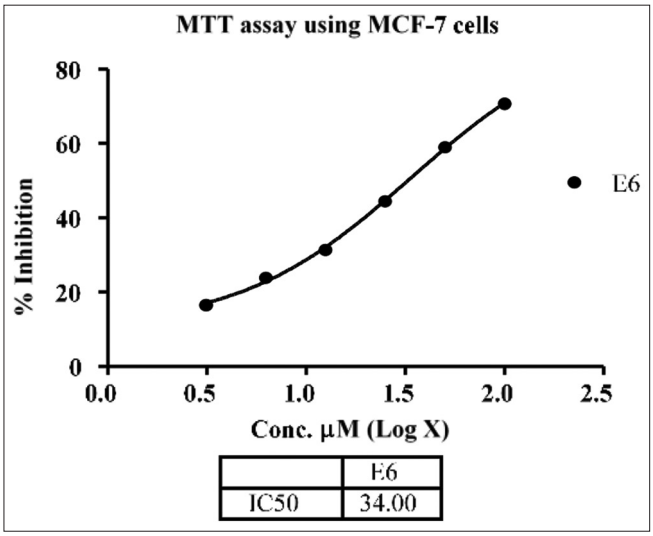


Scheme 1: Scheme for Synthesis of compounds (E1-E8)



	Doxorubicin
IC50	22.12

Graph 1: Graph representing the % inhibition and IC_{50} value of doxorubicin



	E6
IC50	34.00

Graph 2: Graph representing the % inhibition and IC_{50} value of E6

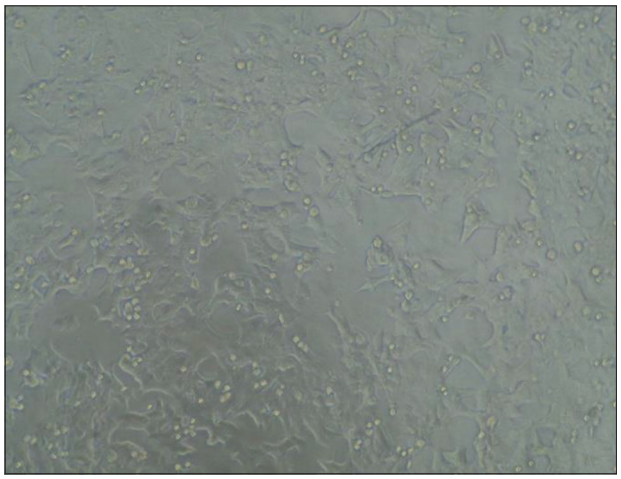


Figure 1: MCF-7 cells on treatment with Standard Doxorubicin at 3.125 µM concentration (23.75% inhibition)

Preparation of Standard and Test Solution

The drug doxorubicin was chosen as the standard. Doxorubicin 10 mM stock was taken and dulbecco's modified eagle medium (DMEM) plain media was used to prepare serial two fold dilutions ranging from 100 µM –3.125 µM. Similarly, for test solution, 10 mM stock of Samples was prepared for cytotoxicity tests and again DMEM plain media was used to prepare serial twice fold dilutions ranging 100 µM –3.125 µM.

Cell Lines and Culture Medium

MCF-7 cell lines were acquired from American type culture collection and cultivated in DMEM added with inactivated fetal bovine serum (FBS) 10%, penicillin (100 IU/ml), and streptomycin (100 g/l) until confluent at 37°C in a humidified environment of 5% CO_2. The cells were separated using a cell dissociating solution combining trypsin (0.2%), EDTA (0.02%), and glucose in phosphate buffer saline (PBS) (0.05%). The viability of the cells is tested before centrifugation. A 96-well plate was seeded with 50,000 cells per well and cultured for 24 h at 37° Celsius in a 5% CO_2 incubator.

Procedure

"Using particular media having 10% FBS, monolayer cell culture was first trypsinized first and then cell count was adjusted to 5.0×10^5 cells/ml. Then, added 100 µl of diluted cell suspension (50,000 cells/well) to every well of the 96 well microtiter plate. The microtiter plate was kept for 24 h after which a partial monolayer was formed. Supernatant was then discarded and monolayer was rinsed with medium once, and 100 µl of various test drug doses were mixed in partial monolayer in microtiter plates. Then, plates were kept in 5% CO_2 environment for 24 h at temperature of 37°C. After 24 h of incubation, the test solutions (100 µM–3.125 µM) added in two-fold dilution in the microtiter plate wells was cast-off and to each well on microtiter plate, 100 µl of MTT (5 mg/10 ml MTT in PBS) was poured. The plates were further stored in 5% CO_2 environment at 37°C for further 4 h. After fulfillment of above mentioned conditions, the supernatant was removed, and 100 µl of DMSO was poured into wells of microtiter plates. The plates were then shaken gently to dissolve formed formazan. At a wavelength of 590 nm, the absorbance was measured using a microplate reader. The percentage growth inhibition was determined using the formula below, and the dose-response curves for each cell line were used to produce the concentration of test drug needed to inhibit cell growth by 50% (IC_{50}) value."[20,21]

$$\% \text{ Inhibition} = \frac{(\text{Optical density of control} - \text{Optical density of sample})}{\text{Optical density of control}} \times 100$$

Result and Discussion

Synthesis of Derivatives

Eight compounds were synthesized and various techniques involving Melting point (m. pt.), IR, ^{1}H NMR, and ^{13}C NMR spectroscopy were used to characterize them. The data reported here shows physicochemical properties such as chemical formula, melting point, % yield [Table 1] followed by IR, ^{1}H NMR, and ^{13}C NMR data.

Table 1: Physicochemical properties of compounds E (1-8)

Compound	*R*	R_1	*Chemical formula*	*Melting point (°C)*	*% Yield*	*Color*
E1	Br	H	$C_{22}H_{15}BrN_4O$	204–06	60%	Light yellow
E2	Br	Cl	$C_{22}H_{14}BrClN_4O$	260–62	92%	Dark brown
E3	Br	CH_3	$C_{23}H_{17}BrN_4O$	196–98	52%	Brown yellow
E4	Br	NO_2	$C_{22}H_{14}BrN_5O_3$	224–26	56%	Light orange
E5	Cl	H	$C_{22}H_{15}ClN_4O$	220–22	59%	Light yellow
E6	Cl	Cl	$C_{22}H_{14}Cl_2N_4O$	271–73	89%	Brown
E7	Cl	CH_3	$C_{23}H_{17}ClN_4O$	208–10	66%	Light brown
E8	Cl	NO_2	$C_{22}H_{14}ClN_5O_3$	228–30	52%	Orange

3-((1H-benzo[d]imidazol-2-yl)methyl)-2-(4-bromophenyl) quinazolin-4(3H)-one (E1)

IR (in cm^{-1}) C-Br 630, C-N 1310, C=C 1615,1475, C=O 1715, C-H 2930, C-H Ar 3075, N-H 3510 and ^{1}H NMR 11.01 (s, 1H, NH), 8.21–8.13 (m, 3H), 7.78 (ddd, J=8.3, 7.0, 1.2 Hz, 1H), 7.74–7.66 (m, 2H), 7.64 (dd, J=8.5, 1.2 Hz, 1H), 7.60–7.52 (m, 1H), 7.51–7.42 (m, 2H), 7.22–7.13 (m, 2H), 5.03 (s, 2H, CH_2). ^{13}C NMR δ 162.57, 154.83, 151.10, 148.05, 138.88, 136.27, 135.68, 134.50, 133.09, 130.68, 126.66, 126.43, 124.95, 123.99, 122.30 (d, J=6.9 Hz), 121.41, 116.63, 114.89 (ArC), 42.34(CH_2).

2-(4-Bromophenyl)-3-((6-chloro-1H-benzo[d]imidazol-2-yl) methyl)quinazolin-4(3H)-one (E2)

IR (in cm^{-1}) C-Br 670, C-Cl 680, C-N 1280, C=C 1625,1480, C=O 1720, C-H 2950, C-H Ar 3100, N-H 3250 and ^{1}H NMR 11.17 (s, 1H, NH), 8.18–8.15 (m, 3H), 7.78 (ddd, J=8.25, 7.1, 1.2 Hz, 1H), 7.76–7.65 (m, 3H), 7.64–7.52 (m, 2H), 7.50 (ddd, J=8.2, 7.1, 1.3 Hz, 1H), 7.38 (dd, J=8.2, 2.2 Hz, 1H), 5.06 (s, 2H, CH_2). ^{13}C NMR δ 161.75, 155.88, 150.71, 149.55, 138.64, 137.43, 136.66, 133.95, 132.99, 131.86, 128.46, 126.76, 125.93, 124.05, 123.99, 122.65, 120.91, 119.57, 113.90(ArC), 42.41(CH_2).

2-(4-Bromophenyl)-3-((6-methyl-1H-benzo[d]imidazol-2-yl) methyl)quinazolin-4(3H)-one (E3)

IR (in cm^{-1}) C-Br 615, C-N 1260, C=C 1610,1475, C=O 1710, C-H 2840, C-H Ar 3140, N-H 3480 and ^{1}H NMR 11.21 (s, 1H, NH), 8.31–8.23 (m, 3H), 7.80 (ddd, J=8.4, 7.0, 1.2 Hz, 1H), 7.75–7.60 (m, 3H), 7.50 (ddd, J=8.2, 7.1, 1.3 Hz, 1H), 7.40–7.30 (m, 2H), 7.24 (ddt, J=8.6, 2.8, 0.7 Hz, 1H), 5.03 (s, 2H, CH_2), 2.41 (d, J=1.1 Hz, 3H, CH_3). ^{13}C NMR δ 163.7, 156.33, 152.33, 148.05, 138.56, 135.68, 135.17, 134.44 (d, J=12.6 Hz), 133.09, 130.68, 126.66, 126.43, 124.95, 123.99, 123.63, 121.41, 115.93, 113.55(ArC), 43.45 (CH_2), 21.36 (CH_3).

2-(4-Bromophenyl)-3-((6-nitro-1H-benzo[d]imidazol-2-yl) methyl)quinazolin-4(3H)-one (E4)

IR (in cm^{-1}) C-Br 625, C-N 1320, N=O 1540, C=C 1600,1480, C=O 1710, C-H 2960, C-H Ar 3150, N-H 3400 and ^{1}H NMR (400 MHz, DMSO-d6)) δ 11.70 (s, 1H, NH), 8.35 (d, J=2.1 Hz, 1H), 8.25–8.17 (m, 3H), 8.10 (dd, J=9.0, 2.1 Hz, 1H), 7.83–7.74 (m, 2H), 7.72–7.64 (m, 2H), 7.62 (dd, J=8.5, 1.2 Hz, 1H), 7.50 (ddd, J=8.2, 7.1, 1.3 Hz, 1H), 5.03 (s, 2H, CH_2). ^{13}C NMR δ 162.57, 154.83, 150.79, 149.65, 144.27, 142.46, 136.13, 135.68, 134.50, 134.09, 131.88, 127.66, 127.13, 125.05, 124.59, 123.41, 118.51, 117.83, 109.55(ArC), 42.44 (CH_2).

3-((1H-Benzo[d]imidazol-2-yl)methyl)-2-(4-chlorophenyl) quinazolin-4(3H)-one (E5)

IR (in cm^{-1}) C-Cl 770, C-N 1130, C=C 1605, 1460, C=O 1700, C-H 2850, C-H Ar 3010, N-H 3430 and ^{1}H NMR 11.07 (s, 1H), 8.39–8.31 (m, 2H),

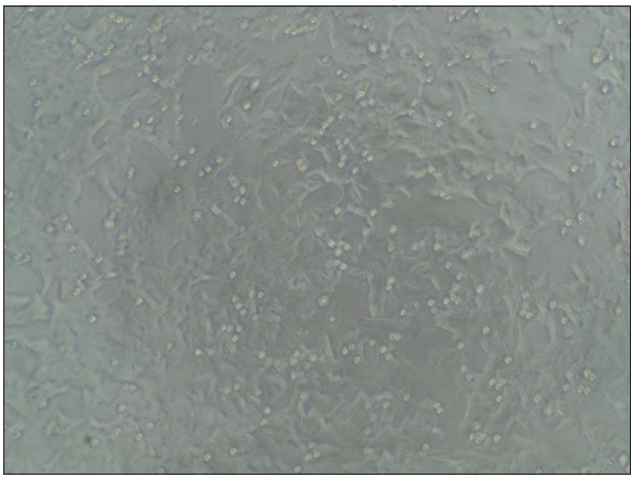


Figure 2: MCF-7 cells on treatment with Compound E6 at 3.125 μM concentration (16.56% inhibition)

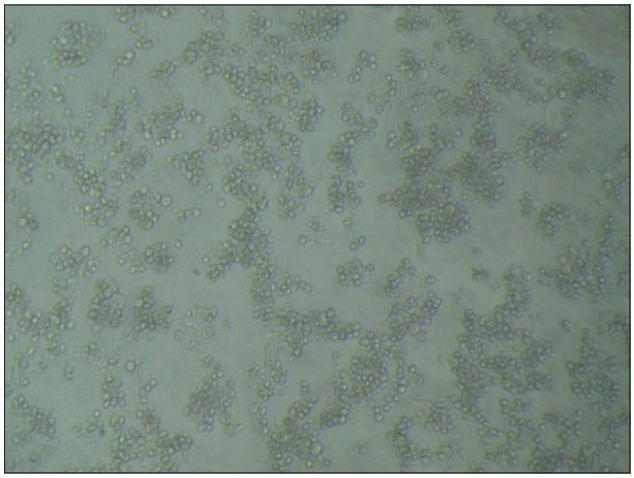


Figure 3: MCF-7 cells on treatment with Standard Doxorubicin at 100 μM concentration (86.96% inhibition)

8.16 (dd, J=8.0, 1.3 Hz, 1H), 7.75 (ddd, J=8.35, 7.0, 1.2 Hz, 1H), 7.68 (dd, J=8.45, 1.2 Hz, 1H), 7.59–7.42 (m, 5H), 7.25–7.12 (m, 2H), 5.08 (s, 2H). ^{13}C NMR δ 164.79, 154.72, 151.10, 148.05, 138.88, 136.33 (d, J=12.0 Hz), 135.19, 134.50, 130.39 (d, J=18.1 Hz), 126.66, 125.63, 123.95, 122.30 (d, J=6.9 Hz), 121.41, 116.63, 114.89(ArC), 42.34(CH_2).

3-((6-chloro-1H-benzo[d]imidazol-2-yl)methyl)-2-(4-chlorophenyl) quinazolin-4(3H)-one (E6): IR (in cm^{-1}) C-Cl 730, C-N 1150, C=C 1610, 1470, C=O 1724, C-H 2955, C-H Ar 3100, N-H 3380 and ^{1}H NMR (400 MHz, DMSO-d6)) δ 11.22 (s, 1H, NH), 8.49–8.41 (m, 2H), 8.16 (dd, J=8.0, 1.3 Hz, 1H), 7.80 (ddd, J=8.3, 7.0, 1.2 Hz, 1H), 7.70–7.56 (m, 3H), 7.55–7.44 (m, 3H), 7.38 (dd, J=8.2, 2.2 Hz, 1H), 5.04 (s,

Table 2: Percent inhibition of MCF-7 cell lines by compounds E (1-8) and doxorubicin

		MCF-7		
Sample	*Conc. in μM*	*Optical density at 590 nm*	*% inhibition*	*IC_{50} in μM*
Control	0	0.598	0.00	
E1	3.125	0.588	1.67	–
	6.25	0.547	8.53	
	12.5	0.521	12.88	
	25	0.447	25.25	
	50	0.403	32.61	
	100	0.354	40.80	
E2	3.125	0.590	1.34	–
	6.25	0.558	6.69	
	12.5	0.540	9.70	
	25	0.532	11.04	
	50	0.483	19.23	
	100	0.454	24.08	
E3	3.125	0.566	5.35	–
	6.25	0.532	11.04	
	12.5	0.487	18.56	
	25	0.421	29.60	
	50	0.389	34.95	
	100	0.336	43.81	
E4	3.125	0.574	4.01	–
	6.25	0.534	10.70	
	12.5	0.477	20.23	
	25	0.412	31.10	
	50	0.388	35.12	
	100	0.358	40.13	
E5	3.125	0.568	5.02	–
	6.25	0.514	14.05	
	12.5	0.452	24.41	
	25	0.400	33.11	
	50	0.388	35.12	
	100	0.326	45.48	
E6	3.125	0.499	16.56	34
	6.25	0.455	23.91	
	12.5	0.410	31.44	
	25	0.332	44.48	
	50	0.245	59.03	
	100	0.175	70.74	
E7	3.125	0.588	1.67	–
	6.25	0.561	6.19	
	12.5	0.487	18.56	
	25	0.423	29.26	
	50	0.389	34.95	
	100	0.354	40.80	
E8	3.125	0.577	3.51	–
	6.25	0.552	7.69	
	12.5	0.462	22.74	
	25	0.412	31.10	
	50	0.384	35.79	
	100	0.359	39.97	
Doxorubicin	3.125	0.456	23.75	22.17
	6.25	0.387	35.28	
	12.5	0.317	46.99	
	25	0.245	59.03	
	50	0.137	77.12	
	100	0.078	86.96	

2H, CH_2). ^{13}C NMR δ 166.54, 156.79, 150.71, 148.05, 138.64, 136.41 (d, J=3.8 Hz), 135.19, 133.46, 131.23 (d, J=18.1 Hz), 128.46, 126.66, 126.43, 124.95, 122.65, 121.41, 119.57, 113.90(ArC), 42.41(CH_2).

2-(4-Chlorophenyl)-3-((6-methyl-1H-benzo[d]imidazol-2-yl) methyl)quinazolin-4(3H)-one (E7)

IR (in cm^{-1}) C-Cl 685, C-N 1230, C=C 1605, 1475, C=O 1720, C-H 2840, C-H Ar 3050, N-H 3400 and ^{1}H NMR 11.25 (s, 1H, NH), 8.38–8.29 (m, 2H), 8.16 (dd, J=8.0, 1.3 Hz, 1H), 7.79 (ddd, J=8.41, 7.01, 1.2 Hz, 1H), 7.66 (dd, J=8.48, 1.2 Hz, 1H), 7.59–7.47 (m, 3H), 7.45–7.35 (m, 2H), 7.27 (ddt, J=8.6, 2.8, 0.8 Hz, 1H), 5.09 (s, 2H, CH_2), 2.43 (d, J=1.12 Hz, 3H, CH_3). ^{13}C NMR δ 163.93, 154.54, 150.33, 148.05, 136.48 (d, J=17.0 Hz), 135.18 (d, J=1.4 Hz), 134.44 (d, J=12.6 Hz), 130.39 (d, J=18.1 Hz), 126.66, 125.43, 124.95, 123.63, 121.41, 115.93, 113.55(ArC), 42.41(CH_2), 21.36(CH_3).

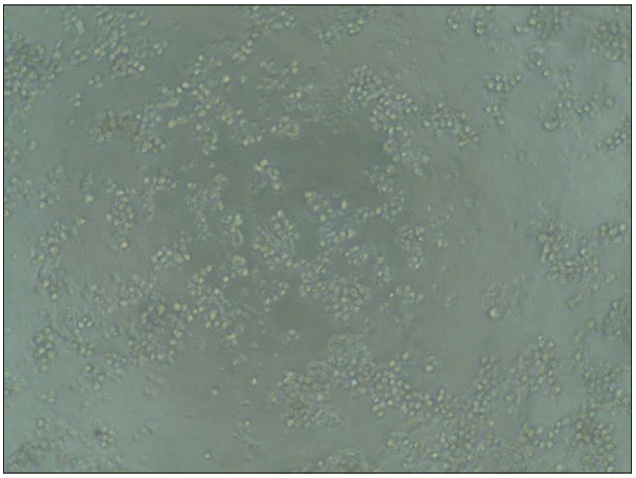


Figure 4: MCF-7 cells on treatment with compound E6 at 100 μM concentration (70.74% inhibition)

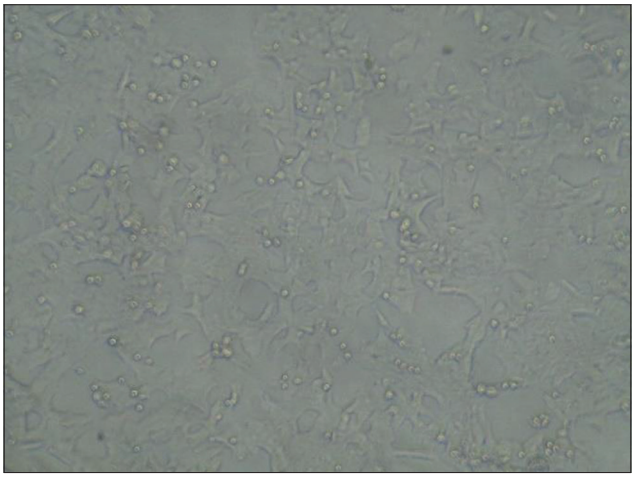


Figure 5: MCF-7 control cells (0 % inhibition)

2-(4-Chlorophenyl)-3-((6-nitro-1H-benzo[d]imidazol-2-yl) methyl)quinazolin-4(3H)-one (E8)

IR (in cm^{-1}) C-Cl 790, C-N 1205, N=O 1520, C=C 1605,1475, C=O 1717, C-H 3010, C-H Ar 3135, N-H 3390 and ^{1}H NMR 11.72 (s, 1H, NH), 8.39–8.31 (m, 3H), 8.20–8.07 (m, 2H), 7.85–7.76 (m, 2H), 7.64 (dd, J=8.5, 1.2 Hz, 1H), 7.55–7.44 (m, 3H), 5.02 (s, 2H, CH_2). ^{13}C NMR δ 165.52, 156.87, 151.97, 150.25, 144.27, 142.46, 136.39, 136.13, 135.19, 134.50, 130.39 (d, J=18.1 Hz), 128.68, 126.43, 126.05, 122.41, 118.51, 117.83, 109.55(ArC), 42.42(CH_2).

Cytotoxic Activity

Using the MTT assay system, compounds E(1-8) were tested for cytotoxicity on MCF7 cell lines using doxorubicin as a reference

drug. All of the synthesized compounds had weak activity, but compound E6 had IC_{50} value of 34 compared to doxorubicin's IC_{50} value of 22.17. Table 2 and Figures 1-5 shows the percent inhibition of MCF-7 cell lines by the identified compounds in detail.

Conclusion

A series of eight compounds with a quinazolinone nucleus were synthesized and characterized using a variety of techniques including melting point, IR, and ^{1}H NMR and ^{13}C NMR. Cytotoxicity of compounds E(1-8) was measured against MCF-7 cell lines and one of them, E6, demonstrated comparable cytotoxic activity when compared to standard doxorubicin, with IC_{50} values of 34 μM for E6 and 22.17 μM for doxorubicin, respectively [Graphs 1 and 2].

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