SYNTHESIS OF SOME NEW PHTHALIZINE DIONE DERIVATIVES WITH THEIR ANTI-MICROBIAL AND ANTI-CANCER ACTIVITY

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Abstract

Starting from methyl-1,4-Dioxo-3-phenyl-3,4-dihydro-1H-phthalazin-2-yl-acetate (**2**) eighteen newly Phthalazine dione derivatives were synthesized. The starting material **2** was prepared by N-alkylation of 2-phenyl-2,3-dihydrophthalazine-1,4-dione **(1)** with ethyl chloro acetate under reflux over night. 2-(1,4-dioxo-3-phenyl-3,4-dihydrophthalazin-2(1H)-yl)acetohydrazide **(3)** was prepared by hydrazinolysis of ester **2** with hydrazine hydrate under reflux. Monopeptide methyl-3-[2-(1,4-dioxo-3-phenyl-3,4-dihydro-1H-phthalazin-2-yl)-acetylamino]-alkanoate **4a-c** were prepared *via* azide coupling method by coupling of hydrazide **3** with different methyl ester of glycine, β-alanine and L-leucine respectively. The hydrazides 2-(1,4-Dioxo-3-phenyl-3,4-dihydro-1H-phthalazin-2-yl)-N-(2-hydrazinocarbonyl-ethyl)-amides **5a-c** were prepared by hydrazinolysis of esters **4a-c** with hydrazine hydrate respectively. Similarly; dipeptides methyl-3-[2-(1,4-Dioxo-3-phenyl-3,4-dihydro-1H-phthalazin-2-yl)-acetylamino]-propionylamino-alkanoates **6a-i** were prepared form coupling of methyl esters of glycine, β-alanine and L-leucine with hydrazides **5a-c** *via* azide coupling method. Schiff's base hydrazones N-[2-(Arylidene-hydrazinocarbonyl)-ethyl]-2-(1,4-dioxo-3-phenyl-3,4-dihydro-1H-phthalazin-2-yl)-amides **7a-i** were prepared by condensation of hydrazides **5a-c** with different aldehydes such as 4-chlorobenzaldehyde, 4-methoxybenzaldehyde and 4-nitrobenzaldehyde.

The anti-bacterial activities of the synthesized compound were screened *in vitro* against *E.coli, Salmonella* and *Staphylococcus aureus* with comparison to 2-phenyl-2,3-dihydrophthalazine-1,4-dione **(1).** The results showed that most compounds have activities against *E.coli* and a little compounds were sensitive to *Salmonella* but there

no significance response to *Staphylococcus aureus*. Also the anti-cancer activities were assayed and some synthesized phthalazinedione has high activity in inhibition of HEPG2 and MCF-7 cancer cell lines.

Keywords: Chemoselective, phthalazinone, azide coupling, monopeptide and dipeptide, Schiff`s basses, anticancer, anti-microbial

INTRODUCTION

Recently, Our research group focused their efforts on searching for new anticancer drugs[1,2] where the anticancer drugs research is never ending to obtain lower toxicity and more selectivity products towards tumor cells.

Phthalazine dione and its derivatives have attracted much attention to chemists and pharmacologists because of their broad spectrum biological activities and applications where it possessing versatile chemical, industrial and biological properties such as antitumor[3], cytotoxic[4], anticonvulsant, cardiotonic, vasorelaxant, antimicrobial and anti-inflammatory properties[5]

It was reported that many Phthalazine nucleus has emerged as a promising and attractive one in the development of novel anticancer agents[6-9]
such as 5 amino-2,3-dihydro-1,4-phthalazinedione **A** figure 1 has therapeutic use to identification of poly(ADP-ribose) polymerase, an enzyme that responds to DNA damage and to application in treating skin aging, Alzheimer`s, atherosclerosis, osteoarthritis, osteoporosis, age-related macular degeneration, muscular dystrophy, immune senescence, viral infections and cancer as diseases involving the function of poly (ADP-ribose) polymerase. Also 3-Amino-2-chloro-1-[3-(5-methyl-1-phenyl-1H-[1,2,3]triazol-4-yl(-1-phenyl-1H-pyrazol-4-yl]-7-nitro-1H-pyrazolo[1,2-b]phthalazine-5,10-dione **B** figure 1 has antiproliferative efficacy on human hepatic cancer cell lines. More over 6-Amino-1-methyl-3-phenylamino-2,3-dihydro-1H-pyrazolo[1,2-b]phthalazine-5,10-dione **C** figure 1 which used as anti-hypoxic and antipyretic

agent. 4-[3-(4-Cyclopropanecarbonyl-piperazine-1-carbonyl)-4-fluoro-benzyl]-3,4-dihydro-2H-phthalazin-1-one. Olaparib (Lynparza®) **D** figure 1 is an oral small molecule phthalazine based poly ADP-ribose polymerase (PARP) inhibitor being developed for the treatment of solid tumors.

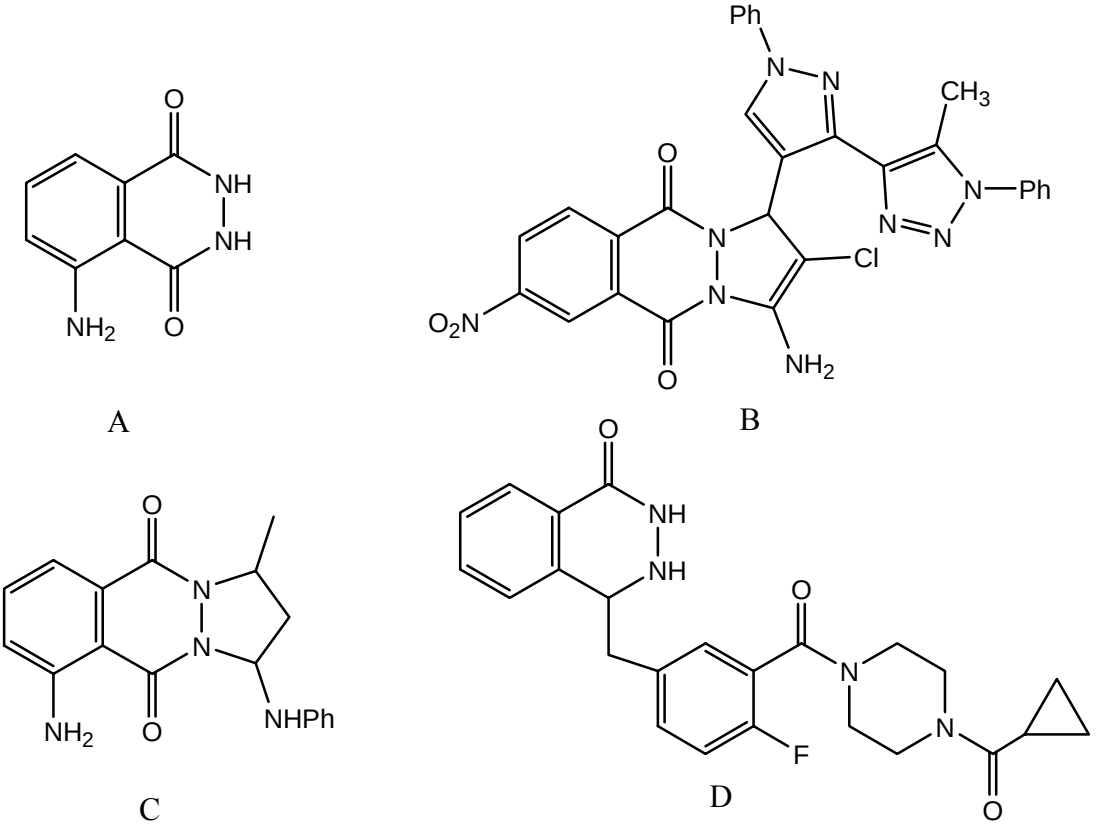


Figure 1 Some common phthalizine compounds used as anti cancer drugs

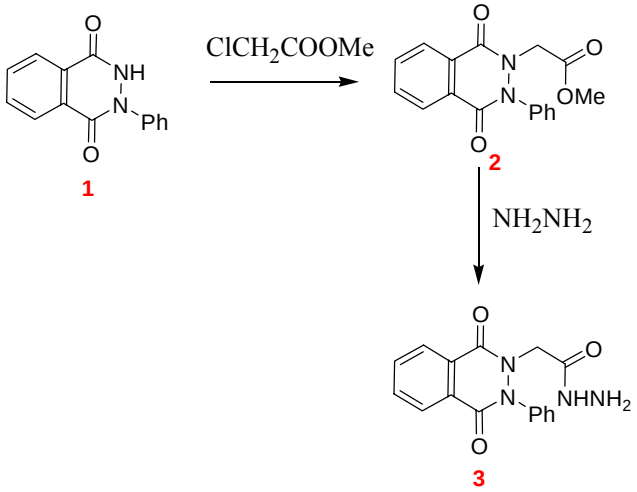
Therefore, many methods have been reported for synthesis of phthalazine derivatives[10]. A series of differently substituted 1,4-phthalazinedione derivatives were synthesized in good yield starting from phthalic anhydride or 4-chlorophthalic anhydride[11].

RESULTS & DISCUSSION:

Our research group reported early that[12-15], how we can control on chemoselective alkylation in both amides and thioamides. As extension of this studies, we achieve N-Alkylation of 2-phenyl-2,3-dihydrophthalazine-1,4-dione **(1)** with ethyl

choloracetate in acetone which proceed selectively on N atom not at O atom or even in competition reaction at both atoms. We can explain that depending on their behavior towards electrophiles according to reaction control points as basisty and neucleophilicity of both N and O atoms. The product was methyl-1,4-Dioxo-3-phenyl-3,4-dihydro-1H-phthalazin-2-yl-acetate (**2**) prove that the N atom in present system is stronger neucleophile more than Oxygen i.e this reaction new evidence for basis of chemoselective reactivity of heterocyclic amides towards electrophiles scheme1.

The ester **2** underwent hydrazinolysis under reflux with hydrazine hydrate to produce hydrazide 2-(1,4-dioxo-3-phenyl-3,4-dihydrophthalazin-2(1H)-yl)acetohydrazide **(3)** in excellent yield. Scheme 1



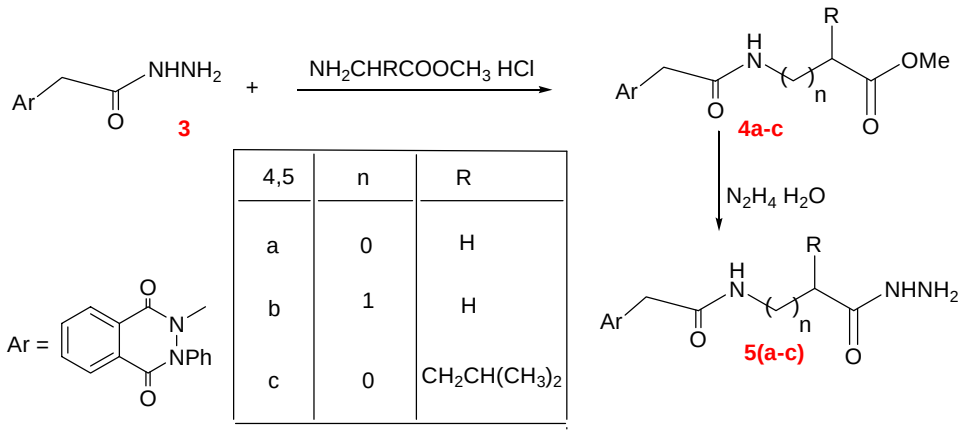
Scheme 1 Synthesis of ester **2** and corresponding hydrazide **3** from phthalizine dione

The charachterstic ^{1}H- NMR spectral peaks for hydrazide **3** :^{1}H- NMR of compound **3** ^{1}HNMR (DMSO-*d6*): δ 4.32 (s, 2H, NCH_2), 6.89-8.32 (m, 9H, Ar-H), 11.50 (s, 1H, NH, D_2O exchangeable), NH_2 protons seemed to be exchanged by the solvent.

The hydrazide **3** coupled with amino acid methyl ester *via* azide coupling method which converted to the mono peptide methyl-3-[2-(1,4-dioxo-3-phenyl-3,4-dihydro-1H-phthalazin-2-yl)-acetylamino]-alkanoate **4a-c**. On hydrazinolysis of esters **4a-c** with hydrazine hydrate corresponding hydrazides **5a-c** were obtained in very good yield**.** Scheme 2

General procedures for racemization-free azide coupling method[16,17], for synthesis of compounds **4(a-c)**

To cold solution (0°C) of hydrazide **3** (3.67gm, 0.01mol) in acetic acid (15ml), 1N HCl (15ml) and water (25ml) was added a solution of $NaNO_2$ (0.7g, 0.01mol) in cold water (15ml). The reaction mixture was stirred at (0°C) for 15 minute. The yellow syrup formed was extracted with cold Ethyl acetate (30ml), washed with cold 5%Na_2CO_3 (30ml) and finally dried over Na_2SO_4. To this solution amino acid ester $NH_2(CHR)COOMe.HCl$ (0.01mol) in ethyl acetate 20ml containing 2ml of Et_3N were added. The reaction mixture was kept at (0°C) for 24hrs then at room temp for another 24 hrs. The solution was evaporated to dryness. The residue was crystallized from petroleum ether /ethyl acetate to give the desired product.



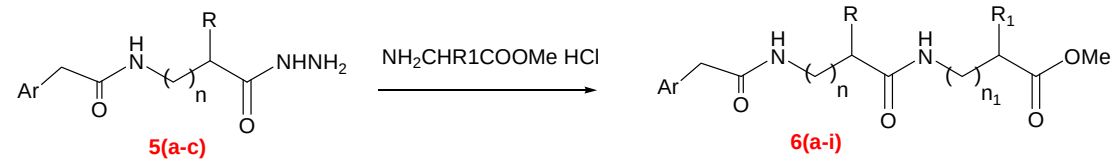
4,5	n	R
a	0	H
b	1	H
c	0	$CH_2CH(CH_3)_2$

Scheme 2 Synthesis of monopeptides **4a-c** and their corresponding hydrazides **5a-c**

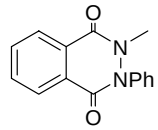
The chemical structure of the synthesized methyl 2-(2-(1,4-dioxo-3-phenyl-3,4-dihydrophthalazin-2(1H)-yl)acetamido)acetate **(4a)** was confirmed by ^{1}HNMR which showed the following signals: a broad signal of $NHCH_2$ at 6.91ppm, duplet signal of $-NHCH_2$ at 4.07ppm and singlet signal of OCH_3 at 3.69ppm

Similarly; The hydrazides **5a-c** which could be re-coupled with another amino acids *via* azide coupling method to give di-peptied derivatives methyl-3-[2-(1,4-Dioxo-3-

phenyl-3,4-dihydro-1H-phthalazin-2-yl)-acetylamino]-propionylamino-alkanoates **6a-i** (Scheme 3)**.**



Ar =



5&6	n	R	n_1	R_1
a	0	H	0	H
b	0	H	1	H
c	0	H	0	$CH_2CH(CH_3)_2$
d	1	H	0	H
e	1	H	1	H
f	1	H	0	$CH_2CH(CH_3)_2$
g	0	$CH_2CH(CH_3)_2$	0	H
h	0	$CH_2CH(CH_3)_2$	1	H
i	0	$CH_2CH(CH_3)_2$	0	$CH_2CH(CH_3)_2$

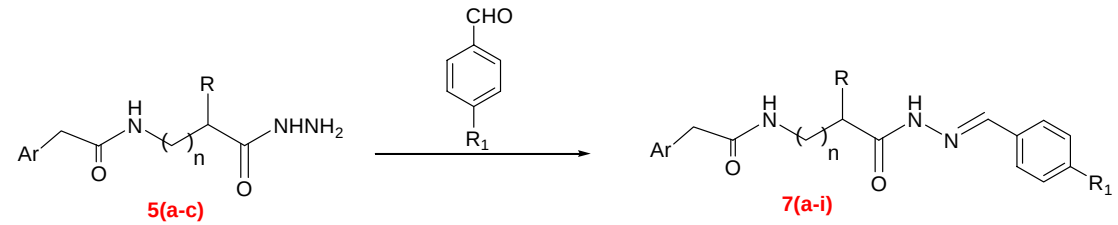
Scheme 3 Synthesis of dipeptides **6a-i** from corresponding hydrazides **5a-c**

The structure of **6a** was elucidated *via* different analysis methods for example the ^{1}HNMR of methyl 2-(2-(2-(1,4-dioxo-3-phenyl-3,4-dihydrophthalazin-2(1H)-yl)acetamido)acetamido)acetate (**6a**) showed the following characteristic signals three singlet signal of three (-CH_2) at 4.83, 4.00 and 3.87 ppm and singlet signal of OCH_3 at 3.65ppm.

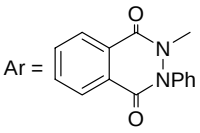
More over the hydrazides **5a-c** were condensed with carbonyl group of different aldehydes to give the corresponding Schiff`s bases **7a-i**.

General procedures[18] for preparation of Schiff's base derivatives **7a-i**

A mixture of hydrazides **5a-c** (0.01mol) and appropriate aromatic aldehydes (0.01mol) was refluxed in ethanol (25ml) for 12hrs, after cooling the collected solid crystallized from the proper solvent scheme 4.



5&7	n	R	R_1
a	0	H	Cl
b	0	H	OMe
c	0	H	NO_2
d	1	H	Cl
e	1	H	OMe
f	1	H	NO_2
g	0	$CH_2CH(CH_3)_2$	Cl
h	0	$CH_2CH(CH_3)_2$	OMe
i	0	$CH_2CH(CH_3)_2$	NO_2



Scheme 4 Synthesis of Schiff`s bases **7a-i** from corresponding hydrazides **5a-c**

The structure of **7a** was elucidated *via* different analysis methods for example the ^{1}HNMR of N-[2-(4-Chloro-benzylidene-hydrazinocarbonyl)-ethyl]-2-(1,4-dioxo-3-phenyl-3,4-dihydro-1H-phthalazin-2-yl)-acetamide **7a** showed the following signals: a broad signal of NH at 11.47ppm, multiplet signal of 4H of aromatic ring of Chlorobenzaldhyde at 7.37-7.52ppm and singlet signal of CH at 10.11ppm.

Biological activity of the synthesized compounds.

Molecular Docking Methodology:

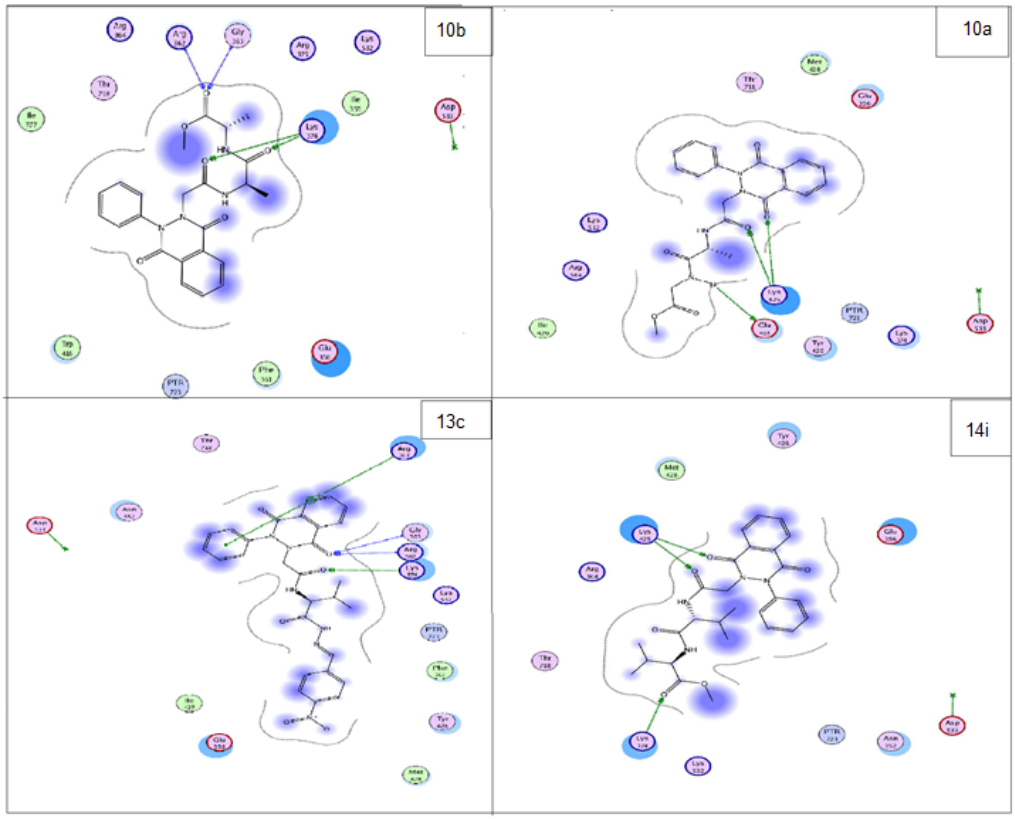
Bioinformatics including molecular modeling studies are very valuable at the present time in the field of drug discovery, saving money and effort needed for the screening of new compounds by guiding and confining the investigation to possible target/ targets. The use of docking simulation studies in our project is quite important to help in predicting the possible mode of action and structure activity relationship of the active derivatives and guiding the research future directions in compounds

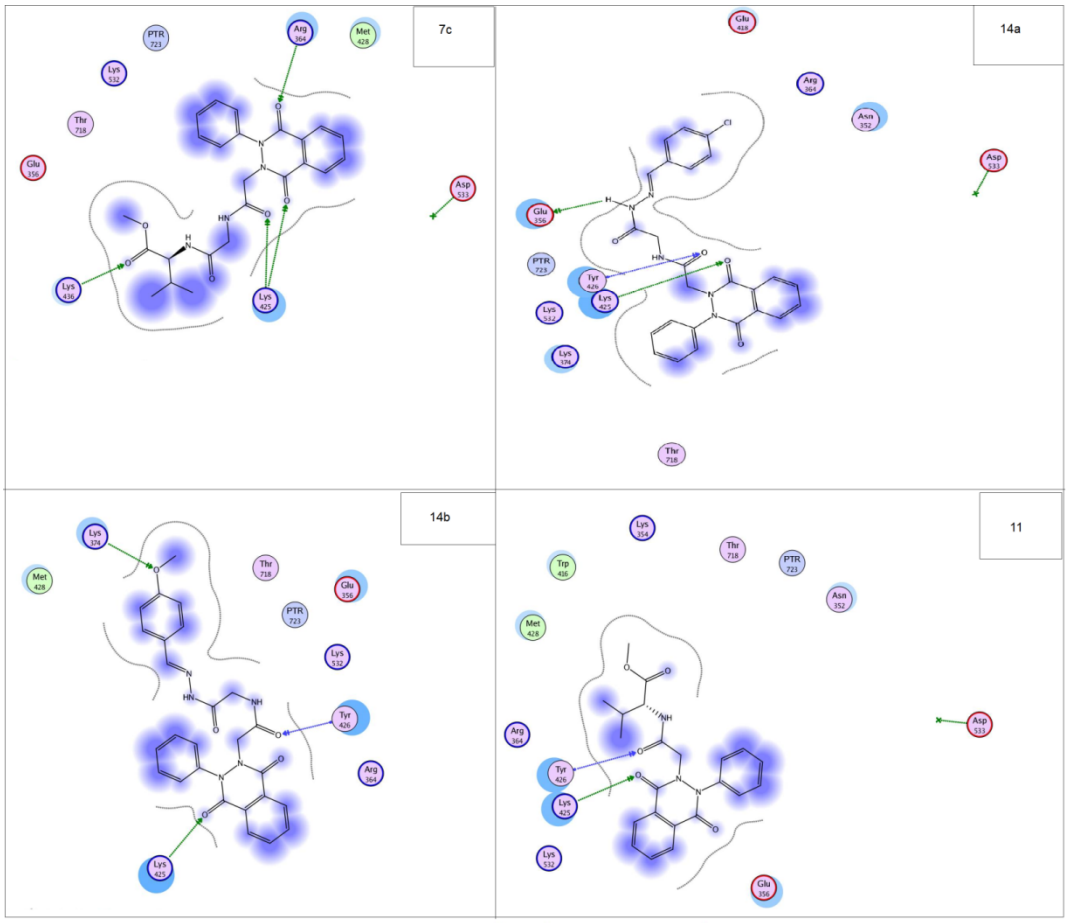
optimization and biochemical enzyme assay for the possible target enzymes. Key interactions at protein–protein interfaces constitute important targets for small molecule inhibition because of their specific arrangements and biological importance[19].

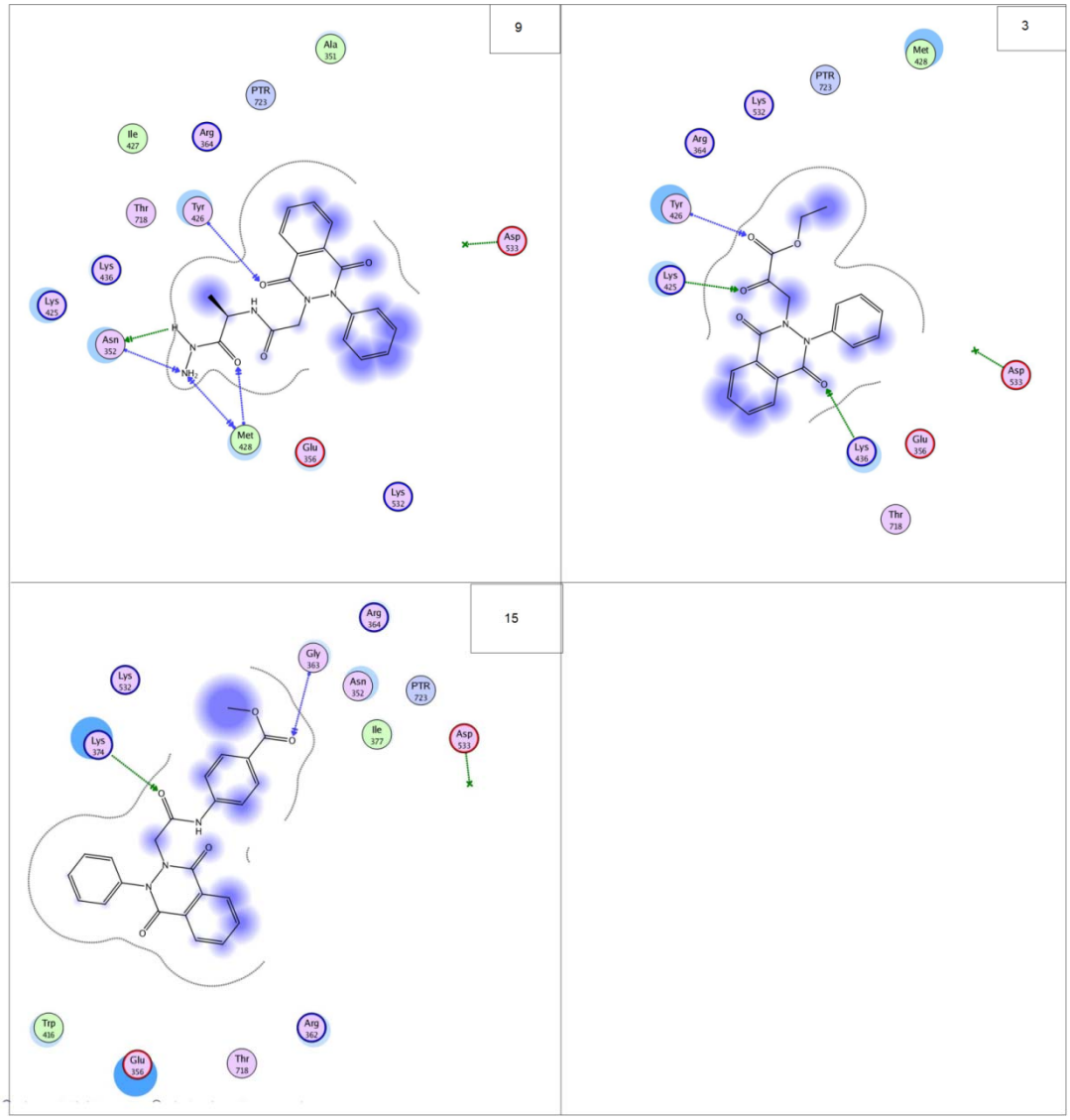
All the molecular modeling studies were carried out on Intel® Core™ i3 CPU, 2.40 GHZ processor, and 3 GB memory with Windows 7 operating system using Molecular Operating Environment (MOE 2008-10 Chemical Computing Group, Canada) as the computational software[20, 21]. Anti-bacterial activities of the synthesized benzotriazinone derivatives were investigated through correlation with E. coli Fab-H inhibitory activities, and the anti-cancer activity of compounds were screened through detection of their ability to act as Vitamin D receptor. The crystal structure of E. coli FabH-CoA complex structure (PDB code: 1HNJ), and the crystal structure of the nuclear receptor for vitamin D bound to its natural ligand (PDB code: 1DB1) were obtained from the freely accessible Protein data bank. The docking studies were performed after the verification process which was performed by redocking of the co crystallized ligand into the active site using the default settings. The synthesized derivatives were docked within the active site of the crystallized structures using the MOE dock tool in MOE, performed with the default values. Different conformers for each compound are imported by systematic conformational of the MOE and saved in an mdb data base file to be docked into the active site of the receptor. Each complex was analyzed for interaction, 2D and 3D images were taken by using MOE visualizing tool.

The results were evaluated based on binding affinity calculation together with cluster size determination and visually through possible interaction with key residues at the active site.

Anti-cancer activity:




7c
14a
14b
11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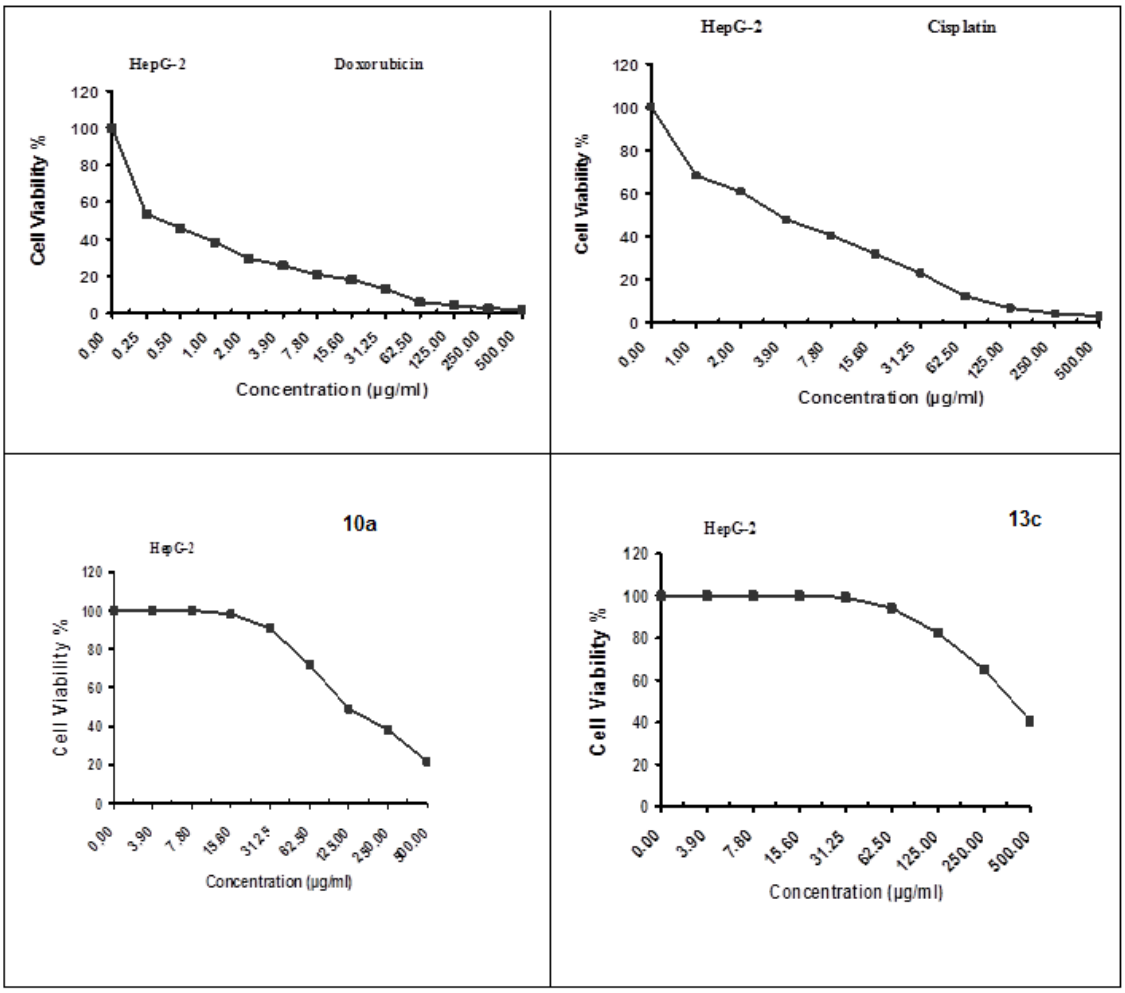
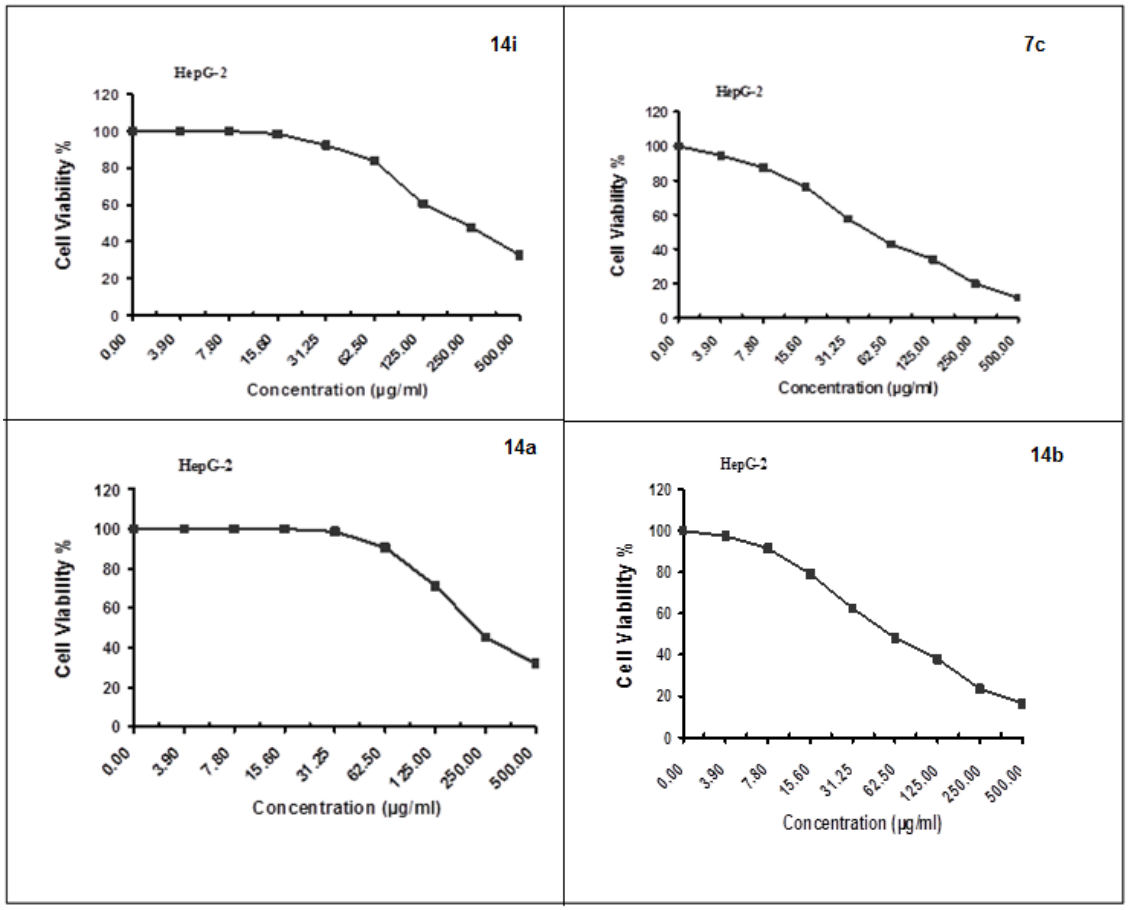
PTR 723
Ile 427
Arg 364
Thr 718
Tyr 426
Asp 533
Lys 436
Lys 425
Asn 352
Met 428
Glu 356
Lys 532
3
Met 428
PTR 723
Lys 532
Arg 364
Tyr 426
Lys 425
Asp 533
Lys 436
Glu 356
Thr 718
15
Arg 364
Gly 363
Lys 532
Asn 352
PTR 723
Lys 374
Ile 377
Asp 533
Trp 436
Arg 362
Glu 358
Thr 718

Anti-cancer activity (HEPG -2 & MCF-7 Cell Lines)

Evaluation of cytotoxicity against HepG-2& MCF-7 cell lines




14i
HepG-2
Cell Viability %
120
100
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60
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20
0
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3.90
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62.50
125.00
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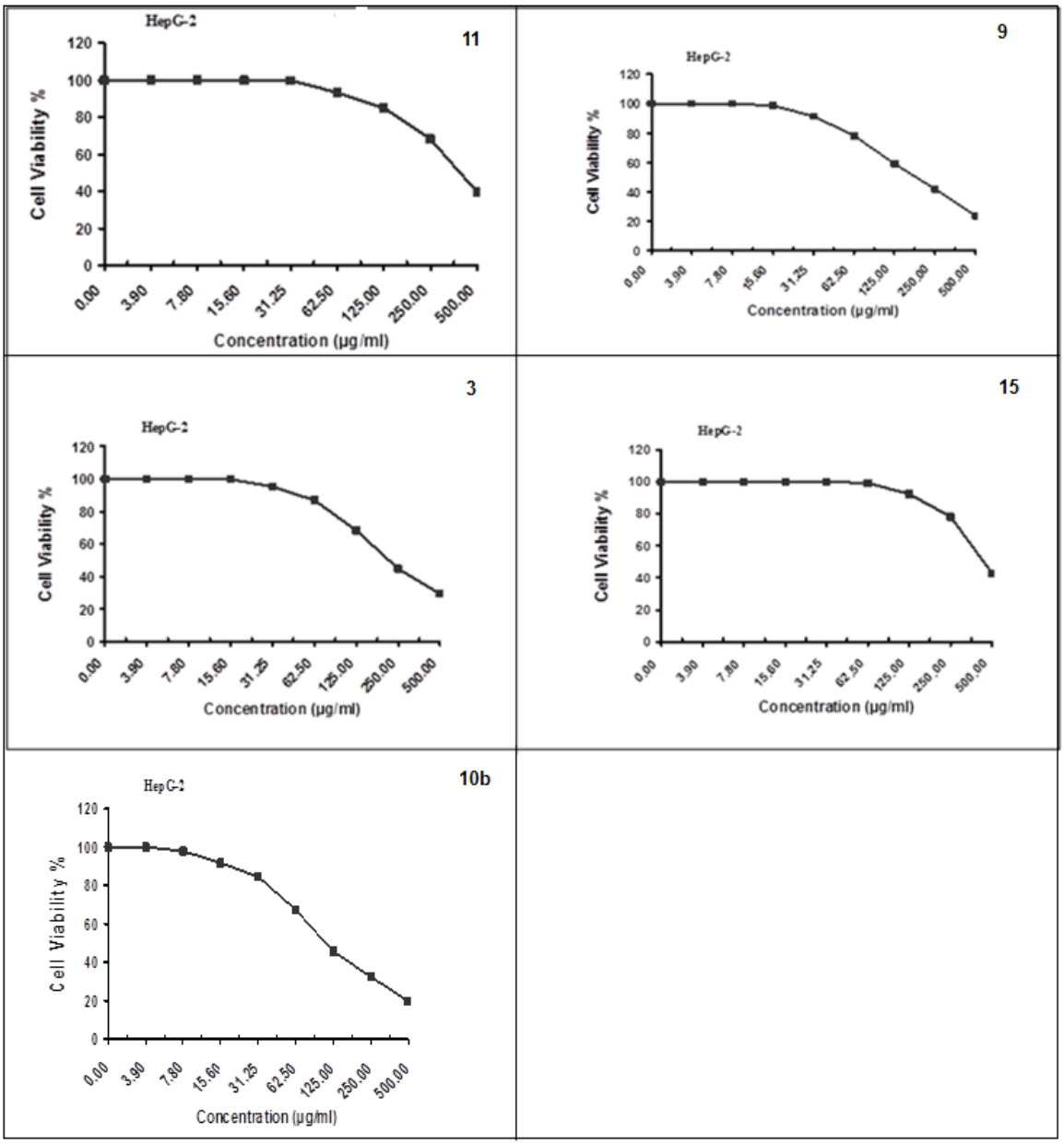
500.00
Concentration (µg/ml)
7c
HepG-2
Cell Viability %
Concentration (µg/ml)
14a
HepG-2
Cell Viability %
Concentration (µg/ml)
14b
HepG-2
Cell Viability %
Concentration (µg/ml)


HepG-2
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Concentration (µg/ml)
HepG-2
9
Cell Viability %
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HepG-2
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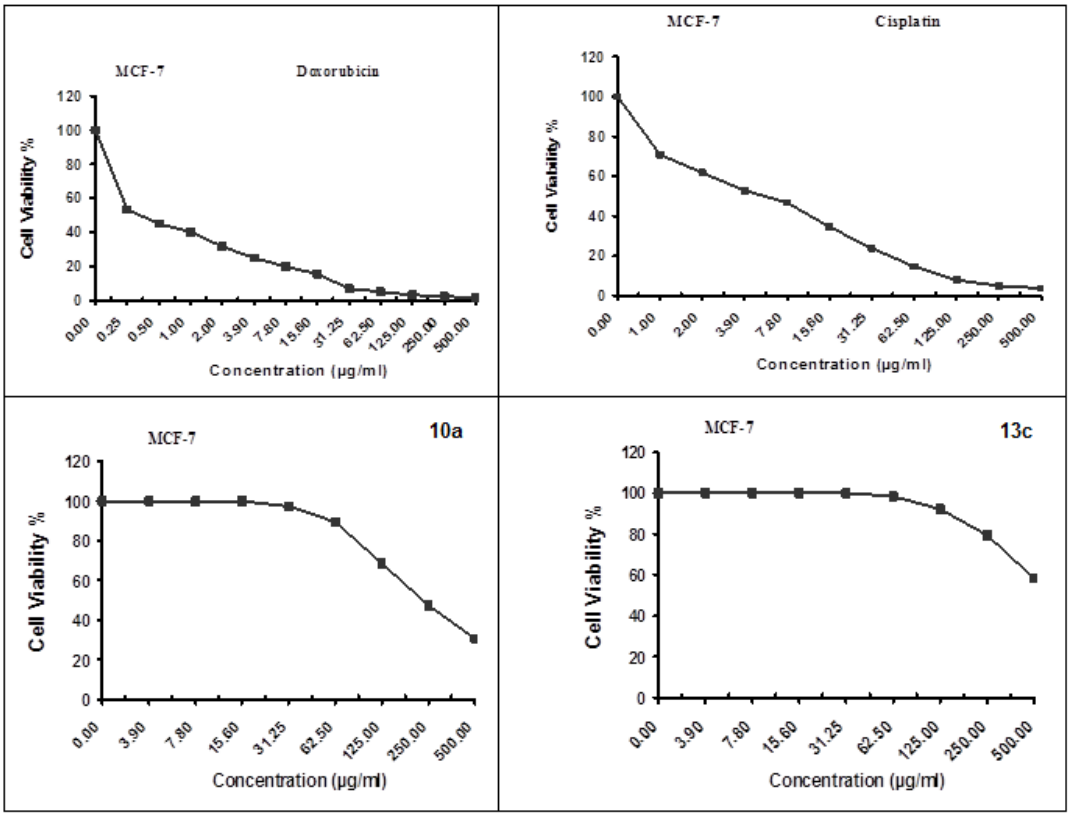
Concentration (µg/ml)
HepG-2
10b
Cell Viability %
Concentration (µg/ml)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Concentration (µg/ml)
MCF-7
Cisplatin
Cell Viability %
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Concentration (µg/ml)
MCF-7
10a
Cell Viability %
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31.25
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Concentration (µg/ml)
MCF-7
13c
Cell Viability %
120
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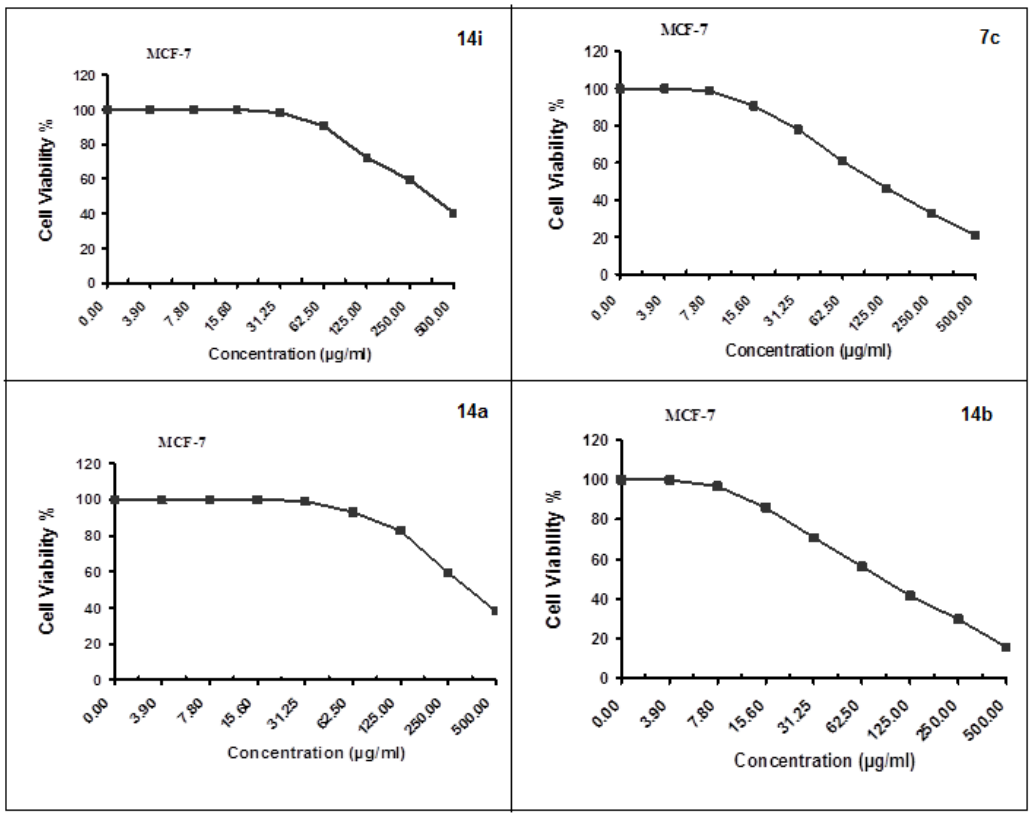
Concentration (µg/ml)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MCF-7
Cell Viability %
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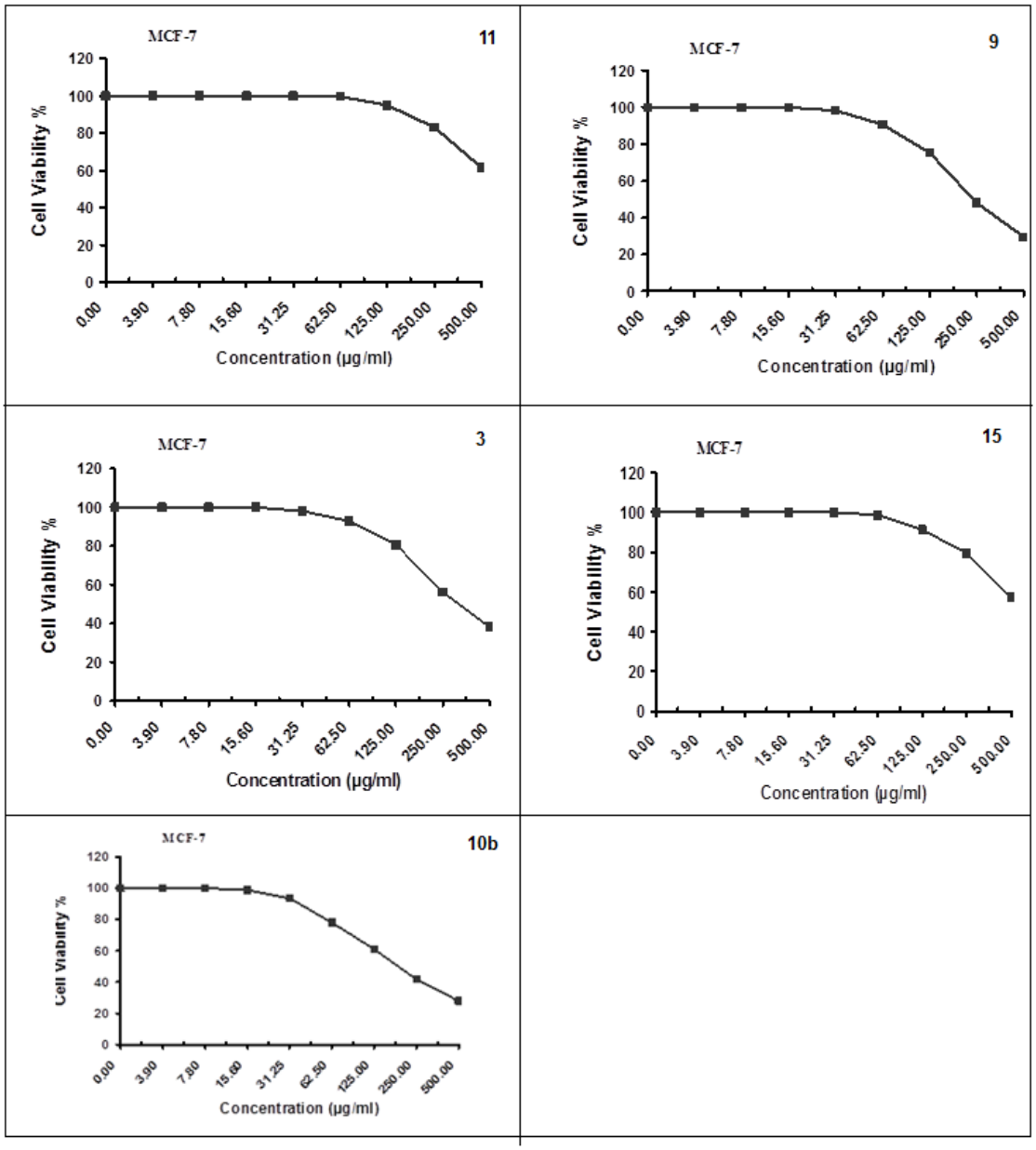
Concentration (µg/ml)
7c
MCF-7
Cell Viability %
Concentration (µg/ml)
14a
MCF-7
Cell Viability %
Concentration (µg/ml)
14b
MCF-7
Cell Viability %
Concentration (µg/ml)


MCF-7
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Cell Viability %
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Concentration (µg/ml)
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Cell Viability %
Concentration (µg/ml)
MCF-7
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Cell Viability %
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MCF-7
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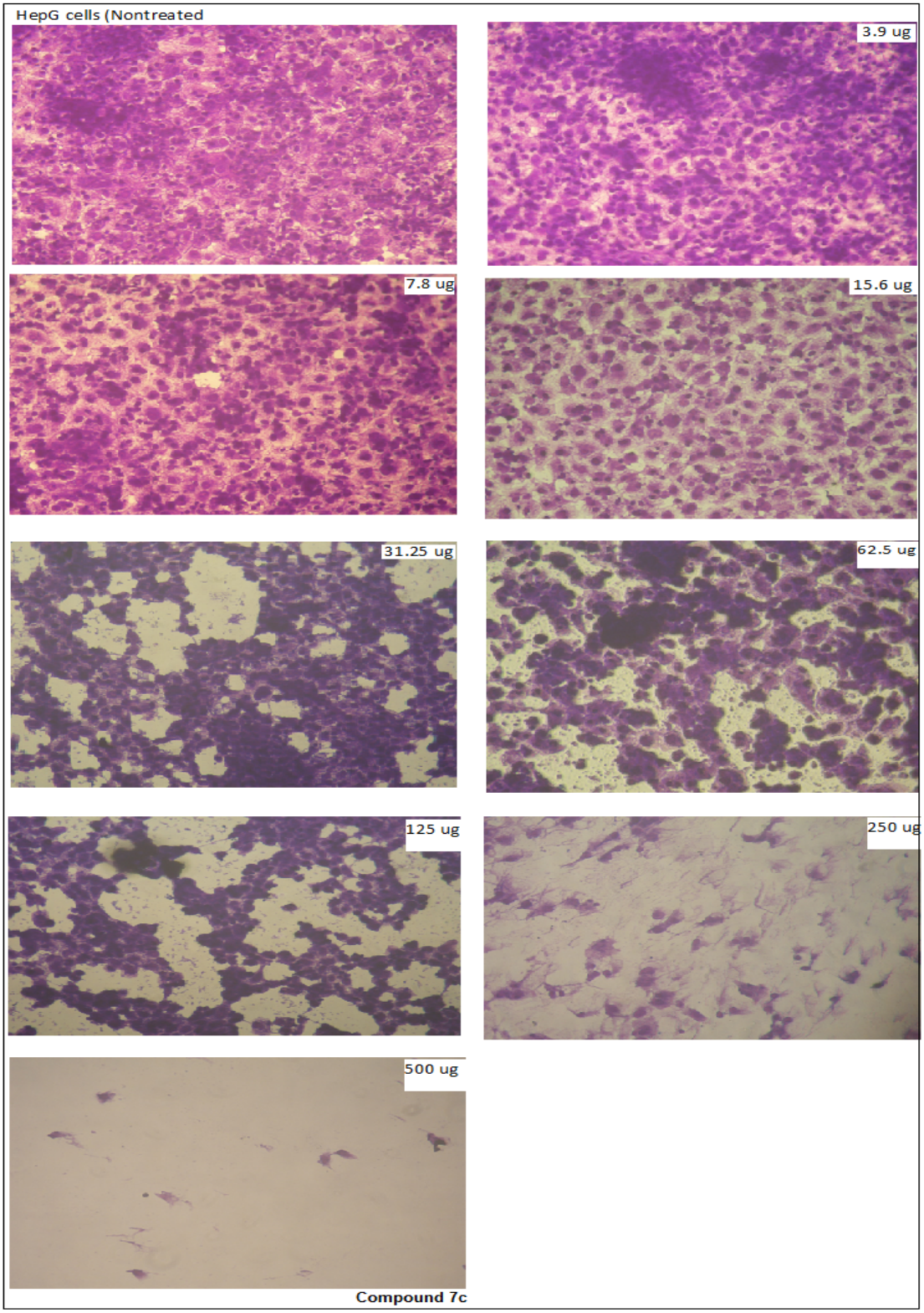
Cell Viability %
Concentration (µg/ml)
MCF-7
10b
Cell Viability %
Concentration (µg/ml)



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