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SYNTHESIS, CHARACTERISTICS AND ANTICANCER ACTIVITY OF NOVEL 2-THIOHYDANTOIN ANALOGUES

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Abstract
Breast cancer is the most commonly diagnosed cancer in female, hence there is an urgent demand for the discovery of novel anticancer drugs with potent activity but also safe for long-term application. Toward this aim, the current study described the synthesis and characterization of novel analogues of thiohydantoins. The results revealed that the synthesized compounds possess a cytotoxic activity against MCF-7 cell line in best to moderate level. Among synthesized compounds, compound 1d exhibited the most potent cytotoxic activity with IC50 of 0.74 µg/ml, compared to Doxorubicin drug (IC50 value 0.68 µg/ml). This review highlights that, synthesized compounds endowed with promising anti-breast cancer properties.

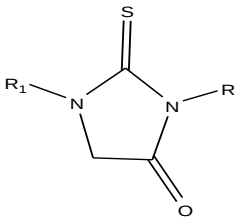
Key word: Cancer, Thiohydantoin, MCF-7 cell line etc

1. Introduction

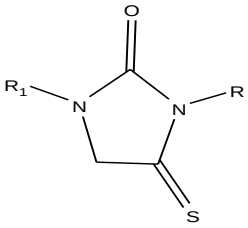
Cancer is a universal disease, which effect on the particular organs as well as disturb to whole body system[i]. The cancer occurs due to uncontrolled progression of normal cells. The numbers of factors influenced on the normal cell due to which the transformation of a normal cell to a cancerous cell[ii]. In universe a different types of cancer are present out of which, the breast cancer is very common to the entire world and normally diagnosed in female candidates. In worldwide an estimated value for the breast cancer grasp more than one million women's. The periodical statistical data will be observed for this disease varied widely such as in 2008, nearly about 421,000 cases were recovered for breast cancer, where as in 2009–2010, more than 49,500 women were diagnosed with breast cancer in Europe. From the breast cancer about 11,600 women's and 75 men were died in 2010. The estimated data caused from this cancer is more than 458,000 women in 2008 worldwide. In 2008, the new cases 184,450 were appeared in persistent stages and this number varies to 230,480 in 2011 in USA. This estimated value is increases day by day and new cases (~268,600) for breast cancer was identified in women, the breast cancer also detected in men in 2019[iii].Environmental factors and dietary habits are the primary source of breast cancer induction with some secondary factors like virus-mediated genetic disturbances [iv]. In an average about 42,170 women in the U.S. predictable to die in 2020[v]. Now a day number of therapies are present to cure this devastating disease such as

radiotherapy, hormone therapy, chemotherapy, proton beam therapy, targeted drug therapy, clinical trials, immunotherapy, cryoablation etc[vi-viii] .Including this, the another way also available to treat the cancer and remove carcinogenic organ from body by surgery, preventive diagnostic, staging, palliative, supportive, cryosurgery, laser, electro surgery and various others [ix-x].

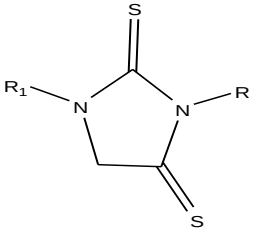
Thiohydantoin containing a nonaromatic ring along with sulfur anolog. In thiohydantoin one or more oxygen atoms were replaced by sulfur[xi-xv]. The number of thiohydantoins are present like 2-thiohydantoin[xvi] (Figer-1), 4-thiohydantoin[xvii] (Figer-2), and 2, 4-thiohydantoin[xviii] (Figer-3).



2-Thiohydantoin
Figer-1



4-Thiohydantoin
Figer-2



2,4-Thiohydantoin
Figer-3

The well-known analogue is 2-thiohydantoins because it shows wide range of applications as anticonvulsant[xix], antiepileptic[xx], antimicrobial[xxi], antiviral[xxii], antineoplastic[xxiii], hypolipidemic[xxiv], antithrombiotic[xxv] and potential antitumor activities[xxvi-xxvii].

Recently Abdellatif *et al*. reported the synthesis of hybrid (3,5-disubstituted)-2-thiohydantoin-pyrazole compounds with considerable anticancer activity toward MCF-7, A-549, and HCT-116 cell lines[xxviii]. Elhady *et al*. reported the synthesis of novel 2‑thioxoimidazolidin‑4‑one derivatives bearing pyrazole, triazole and benzoxazole moieties and explore its anticancer properties based on the group present in 2-thiohydantoin moiety [xxix]. The current study aims to design and develop novel 2-thiohydantoin derivatives and explore anticancer applications.

2. Experiment

The starting material were commercially available in analytical grade. The purity of resultant compound was check by using TLC. The IR spectra were recorded in KBr by using FT-(IR Perkin Elmer -Spectrum RX-IFTIR).Mass spectra were recorded on mass spectrometer while 1HNMR were recorded on FT NMR Spectrometer (Bruker Avance Neo 500 MHz).

2.1 General Procedure for synthesis of 2-Thiohydantoin:- A mixture of aurone (0.01 M) and N-substituted thiourea (0.01 M) were taking in round bottom flask along with 10% KOH and Ethanol as a solvent. A reaction mixture was reflux about 3 hr. The mixture had been cooled, poured into ice cold water and filtered using a suction pump. The final product recrystallized with Ethanol.

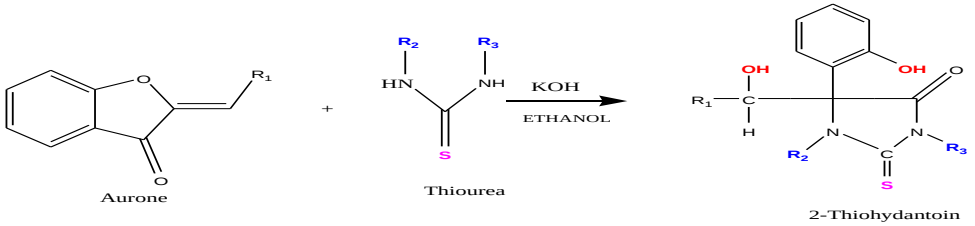


Table-01

Sr. no.	Compounds	R_1	R_2	R_3
1.	1a	C_4H_3O	C_6H_5	C_6H_5
2.	1b	C_6H_4Cl	C_6H_5	H
3.	1c	C_6H_5	CH_3	CH_3
4.	1d	C_7H_7	CH_3	CH_3

2.2 Preparation of 5-(hydroxyl(4-methoxyphenyl)methyl)-5-(2-hydroxyphenyl)-1,3-diphenyl-2-thioxoimidazolidin-4-one(1a)

2-(4-methoxybenzylidene) benzofuran-3(2H)-one (0.01M) was refluxed with N,N-diphenyl thiourea (0.01M) in presence of 10% KOH and appropriate ethanol solvent up to 3 hours. After completion of reaction, cooled the mixture and poured in to ice cold water. After filtering and washing with diluted HCl and water, a solid product was produced. The product was crystallized by using ethanol.

Mol. Formula $C_{29}H_{24}O_4N_2S$: yellowish Crystalline solid, m.p 258^oC , yield 70%, Elemental analysis (%):C,70.14; H,4.87; N,5.64; O,12.89; S,6.46; IR (KBr cm-1) 3617.5 (O-H), 3016 (=CH), 1614 (C=N), 1438 (Ar C=C),ESI-MS[M+H]+ Calculated for $C_{29}H_{24}O_4N_2S$: *m/z* 496.15, 497.15,498.15 ; ^{1}H-NMR (500 MHz, DMSO) δ3.76 (s, 3H), 5.68 (s,1H), 6.86-7.38 (m, *J* =8.4,1.1 Hz, 11H), 7.43 7.70 (m, 6H),

2.3 Preparation of 5-((4-chlorophenyl)(hydroxy)methyl)-5-(2-hydroxyphenyl)-3-phenyl-2-thioxoimidazolidin-4-one (1b)

2-(4-chlorobenzylidene)benzofuran-3(2H)-one(0.01M) was refluxed with N-phenyl thiourea (0.01M) in presence of 10% KOH and appropriate ethanol solvent up to 3 hours. After completion of reaction, cooled the mixture and poured in to ice cold water. After filtering and washing with diluted HCl and water, a solid product was produced. The product was crystallized by using ethanol.

Mol. Formula $C_{22}H_{17}O_3N_2SCl$: faint yellowish Crystalline solid, m.p 228^oC, yield 74%, Elemental analysis (%):C,62.19; H,4.03; N,6.59; O,11.30; S,7.55;Cl,8.34. IR (KBr cm-1) 3616.5 (O-H), 3268.1 (N-H), 1682(Amide C=O), 1436 (Ar C=C), 755.2 (C-Cl); ESI-MS[M+H]+ Calculated for $C_{22}H_{17}O_3N_2SCl$: *m/z* 424.06, 426.06, 425.07, 427.07. ^{1}H-NMR (500 MHz, DMSO) δ5.58 (s, 1H), 7.04 (m, *J* = 8.0,7.8 Hz, 1H), 7.48 (m, *J* = 8.3,1.6,0.5 Hz, 8H), 8.02(m, *J* = 8.0,1.4 Hz, 1H).

2.4 Preparation of 5-(hydroxyl (phenyl) methyl)-5-(2-hydroxyphenyl)-1,3-dimethyl-2-thioxoimidazolidin-4-one(1c)

2-benzylidenebenzofuran-3(2H)-one (0.01M) was refluxed with N,N-dimethyl thiourea (0.01M) in presence of 10% KOH and appropriate ethanol solvent up to 3 hours. After completion of reaction, cooled the mixture and poured in to ice cold water. After filtering and washing with diluted HCl and water, a solid product was produced. The product was crystallized by using ethanol.

Mol. Formula $C_{18}H_{18}O_3N_2S$: faint red crystalline solid, m.p 210^oC, yield 76%, Elemental analysis (%): C, 63.14; H, 5.30; N, 8.88; O, 14.02; S, 9.36.

IR (KBr cm-1) 3520.5 (O-H), 3268.1 , 1712(Amide C=O), 1436 (Ar C=C), 755.2 ; ESI-MS M+H]+ Calculated for $C_{18}H_{18}O_3N_2S$: *m/z* 342.10, 343.11, 344.10,. ^{1}H-NMR (500 MHz, DMSO) δ 3.08 (3H, s), 3.40 (3H, s), 5.64 (1H, s), 6.64 (1H, m, *J* = 8.3, 1.3, 0.5 Hz), 7.04 (1H, m, *J* = 8.0, 7.7, 1.3 Hz).

2.5 Preparation of 5-(hydroxy(p-tolyl)methyl)-5-(2-hydroxyphenyl)-1,3-dimethyl-2-thioxoimidazolidin-4-one(1d)

2-(4-methylbenzylidene)benzofuran-3(2H)-one (0.01M) was refluxed with N,N-dimethyl thiourea (0.01M) in presence of 10% KOH and appropriate ethanol solvent up to 3 hours. After completion of reaction, cooled the mixture and poured in to ice cold water. The solid product obtained which was filtered and washed with dilute HCl and water. The product was crystallized by using ethanol.

Mol. Formula $C_{19}H_{20}O_3N_2S$: faint orange crystalline solid, m.p 224^{o}C, yield 73%, Elemental analysis (%): C, 64.02; H, 5.66; N, 7.86; O, 13.47; S, 9.01. IR (KBr cm-1) 3416.5 (O-H), 1720(Amide C=O), 1436 (Ar C=C), 755.2; ESI-MS[M+H]+ Calculated for $C_{19}H_{20}O_3N_2S$: *m/z* 356.12,357.12,358.12. ^{1}H-NMR (500 MHz, DMSO) δ 2.27 (3H, s), 3.08 (3H, s), 3.40 (3H, s), 5.62 (1H, s), 6.64 (1H, m, *J* = 8.3, 1.3, 0.5 Hz), 7.04 (1H, m, *J* = 8.0, 7.7, 1.3 Hz), 7.15-7.35 (5H, 7.21 (m, *J* = 8.0, 1.2, 0.5 Hz).

3. Result and discussion

Biological assessment

The anti-cancer activity of all synthesized compounds has been evaluated against cell lines MCF-7 (breast carcinoma cell line). Cytotoxicity of the 2-thiohydantoin derivatives on MCF-7 cell line (Procured from NCCS Pune) was determined by NRU (Neutral Red Uptake) Assay. The cells (5000-8000 cells/well) were cultured in 96 well plates for 24 h in DMEM medium (Dulbecco's Modified Eagle Medium-AT149-1L) supplemented with 10% FBS (Fetal Bovine Serum - HIMEDIA-RM 10432) and 1% antibiotic solution at 37°C with 5% CO2. Next day, medium was removed and fresh culture medium was added to each well of the plate. 5 µl of Treatment dilutions (of different concentrations) were added to the defined wells and treated plates were incubated for 24 h. 100 µl of NRU (SRL Chem-36248) (40 µg/ml in PBS - phosphate buffered saline) was added to the defined wells and incubated (Heal Force-Smartcell CO2 Incubator-Hf-90) for 1 h. After that medium was removed, NRU was dissolved in 100 µl of NRU Destain solution. Finally plates were read at 550/660 nm using Elisa Plate Reader (iMark BioRad-USA). IC-50 Was calculated.

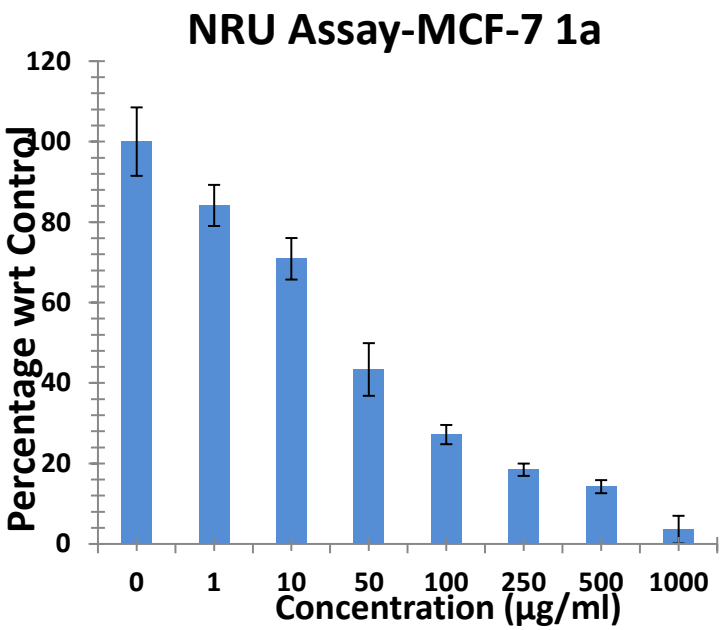
Table 2 show the IC 50 value of the synthesized compounds as well as reference compound Doxorubicin against breast carcinoma cell line MCF-7. Tables 3, 4, 5 and 6, show percentage viability in vitro cytotoxicity against breast carcinoma cell line MCF-7.

Table 2

Sample	IC50 value (µg/ml)
1a	**36.06**
1b	**27.31**
1c	**2.19**
1d	**0.74**
Doxorubicin (Reference)	**0.68**

Table 3. Cytotoxicity of compounds 1a against breast carcinoma cell line MCF-7

Concentration µg/mL	Viability (%)
0	100
1	84.14239
10	70.87379
50	43.3657
100	27.18447
250	18.4466
500	14.23948
1000	3.559871



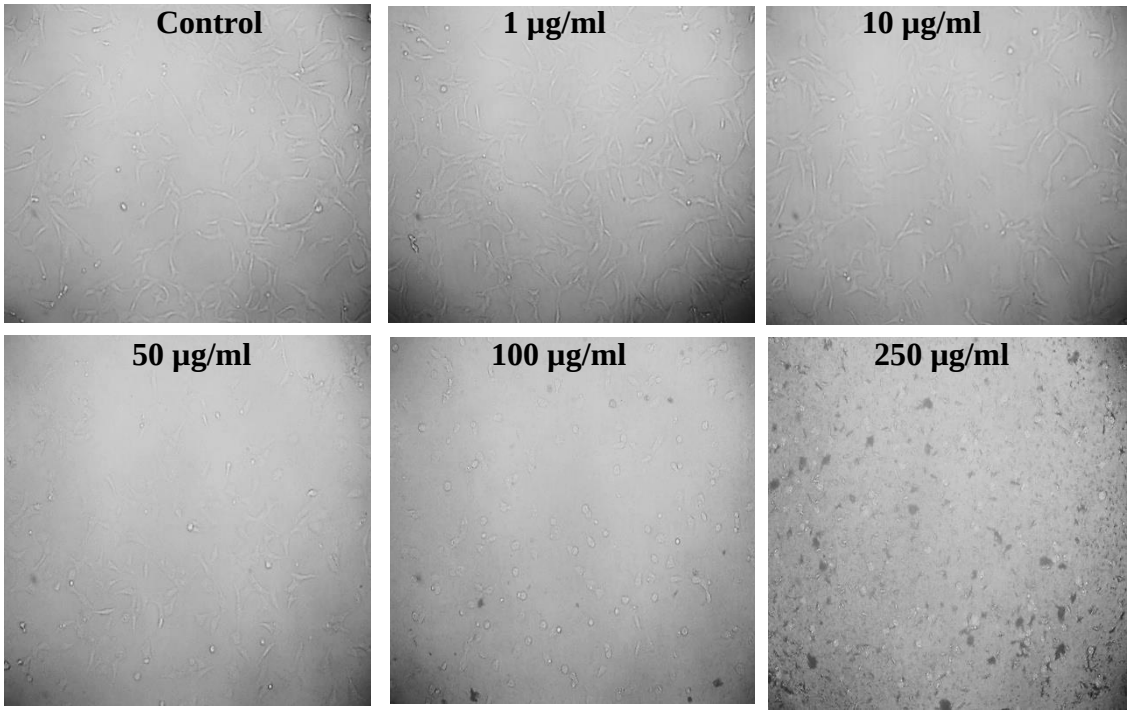
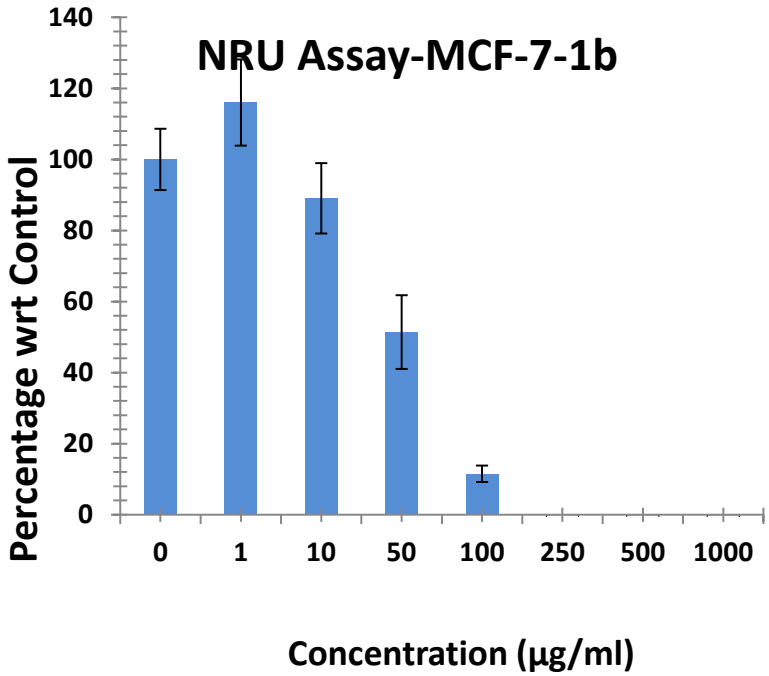


Table 4. Cytotoxicity of compounds 1b against breast carcinoma cell line MCF-7

Concentration µg/mL	Viability (%)
0	100
1	116.0112
10	89.04494
50	51.40449
100	11.51685
250	-9.55056
500	-51.1236
1000	-46.9109



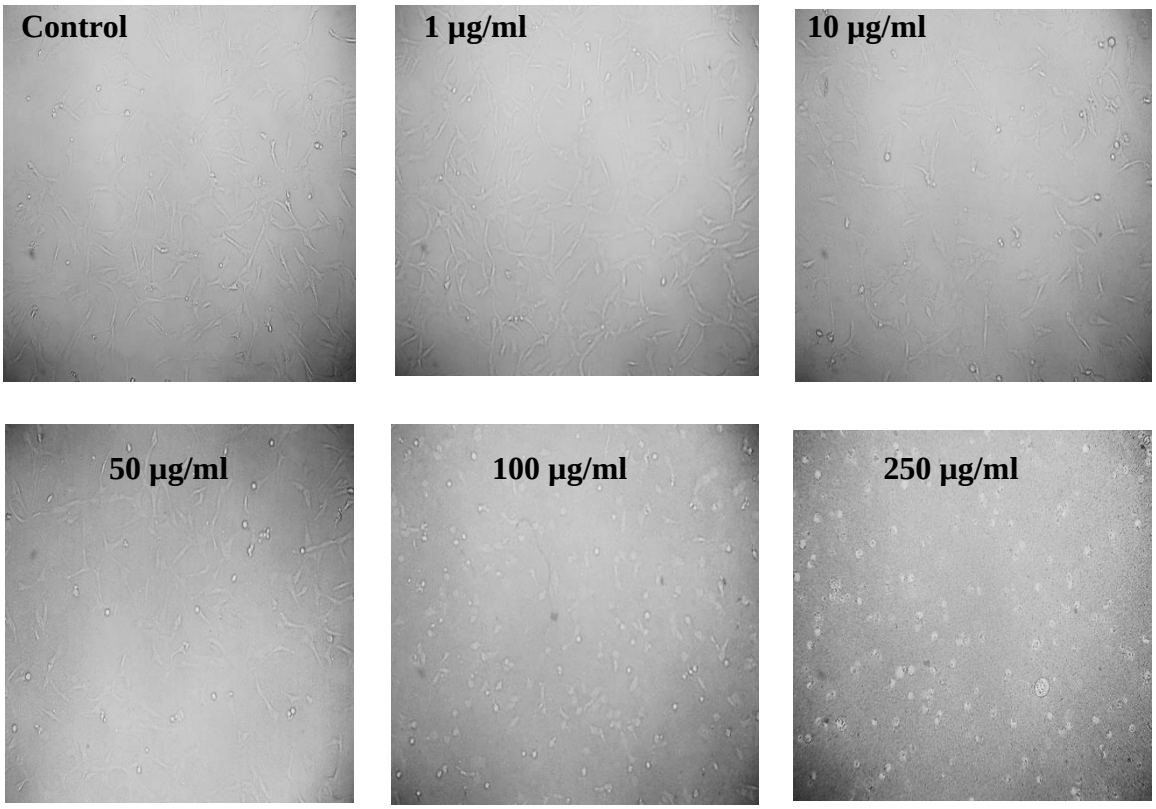
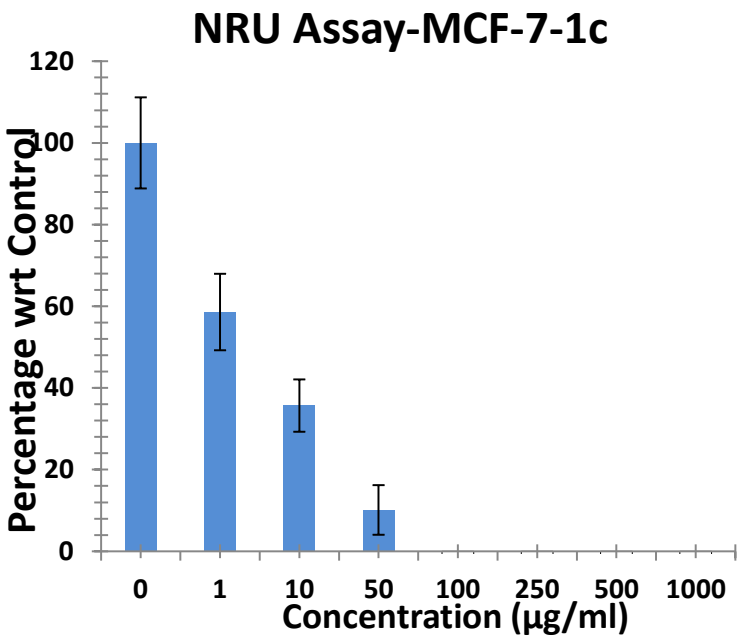


Table 5. Cytotoxicity of compounds 1c against breast carcinoma cell line MCF-7

Concentration µg/mL	Viability (%)
0	100
1	58.59031
10	35.68282
50	10.13216
100	-12.3348
250	-7.04846
500	-23.348
1000	-89.4273



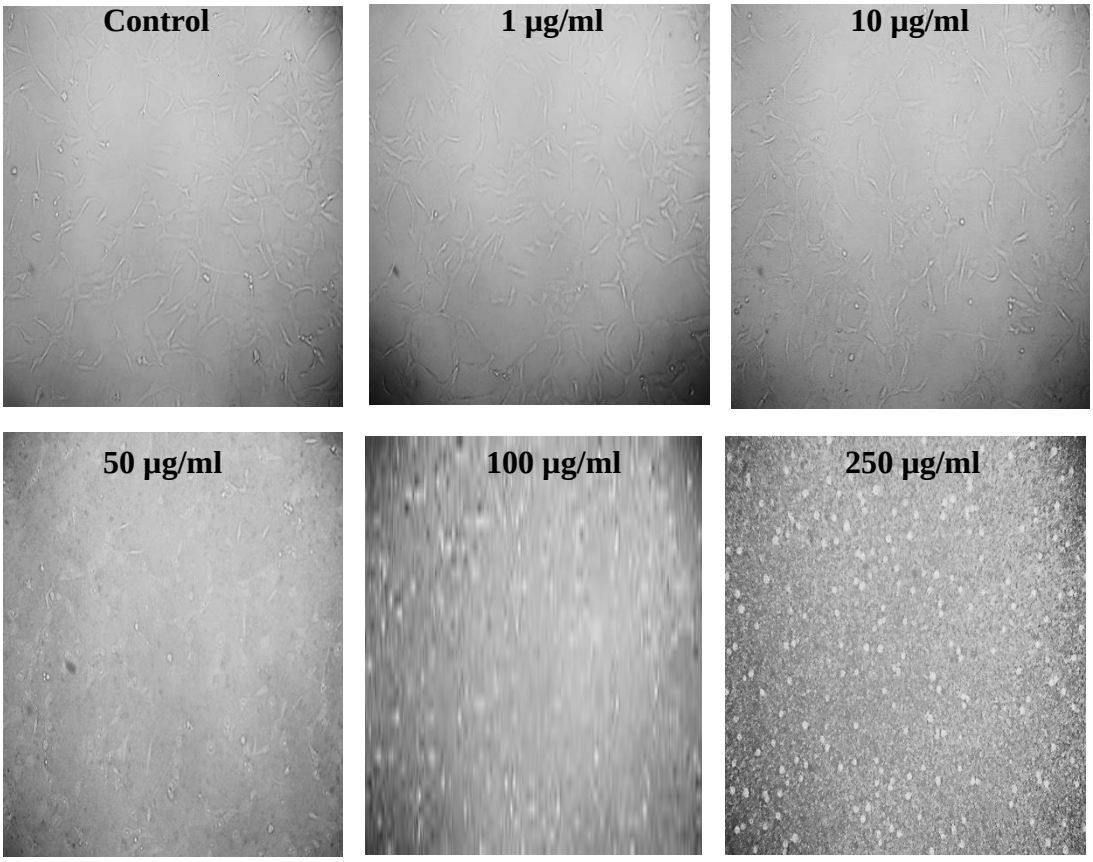
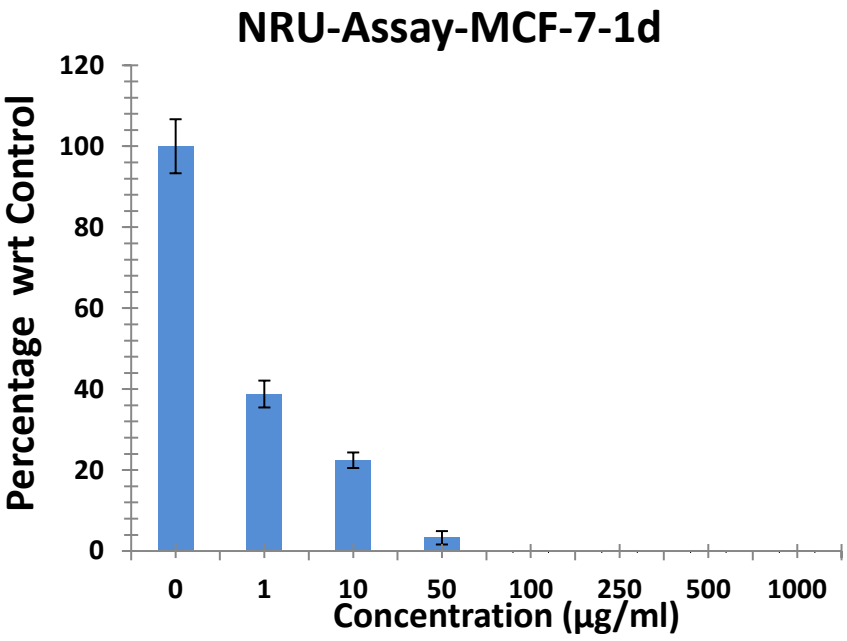
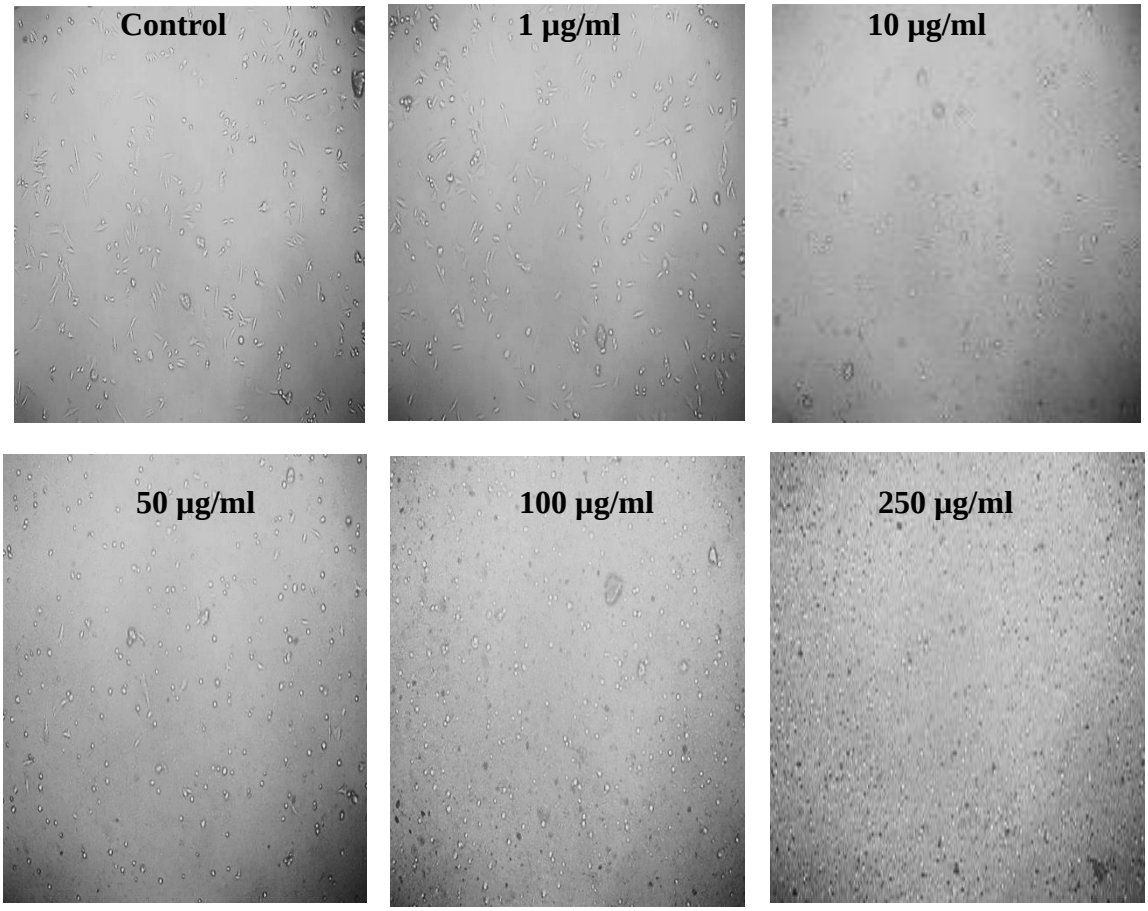


Table 6. Cytotoxicity of compounds 1d against breast carcinoma cell line MCF-7

Concentration µg/mL	Viability (%)
0	100
1	38.78505
10	22.42991
50	3.271028
100	-2.02492
250	-2.49221
500	-22.5857
1000	-11.0592





The resulting data of the 50% inhibition concentration (IC50) summarized in Table 2 showed that, the synthesized 2-Thiohydantoin compounds have different activity against breast cancer cell line MCF-7. Data examination revealed that, the tested compounds showed good to moderate activity. The compound 1c and 1d shows best activity against MCF-7 cell line. Doxorubicin is a anticancer drugs used since 1960. If synthesized compound compare to Doxorubicin then compound 1c and 1d shows best result while compound 1a and 1b shows moderate result again MCF-7 cell line

Generally the structure and biological activity of the compound is responsible for anticancer activity. In 2-Thiohydantoin number of groups like phenyl, benzyl, hydroxyl etc. present at N1 and N3 increases the reactivity against cancerous cell.

4. Conclusion

In this work, we report the effective synthesis of 2-thiohydantoin derivatives and evaluated anticancer property. In present study the compound 1d is very effective against MCF-7 cell line due to methyl group attached to N1 and N3 position. The other compounds like 1a, 1b and 1c shows moderate activity. If different groups are attached at different positions which are responsible to change the effectiveness against MCF-7 line. These results highlight the significance of these compounds as promising new prospects for the discovery and development of innovative chemotherapeutic drugs in the treatment of breast cancer.

Conflicts of interest

The authors declare there is no conflict of interest.

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References

i Wahab R.; Dwivedi S.; Umar A.; Singh S.; Hwang I.H.; Shin H.S.; Musarrat J.; Al- Khedhairy A. A.; Kim Y. S.; ZnO Nanoparticles Induce Oxidative Stress in Cloudman S91 Melanoma Cancer Cells; J. Biomed Nanotech; 2013, **9** (3), 441–449.

ii Bray F.; Jemal A.; Grey N.; Ferlay J.; Forman D.; Global cancer transitions according to the Human Development Index : a population-based study. Lancet Oncol; 2012, **13** (8), 790–801.

iii Nardin S.; Mora E.; Varughese F.M.; Avanzo F.D.; Vachanaram A.R.; Rossi V.; Saggia C.; Rubinelli S.; Gennari A.; Breast Cancer Survivorship, Quality of Life, and Late Toxicities; Front Oncol.; 2020, **10**, 864.

iv Su N.N.; Wu Q.; and Cui J.; Applications and prospects of light environment control technology for vegetable seedling cultivation in factory; China Vegetables; 2013, **4**, 006.

v American Cancer Society. Breast Cancer Facts & Figures 2019-2020. Atlanta:American Cancer Society, Inc. 2019. https://www.cancer. org/content/dam/

cancer-org/research/cancer- facts-and-statistics/breastcancer-facts-and-figures/breast-cancer-facts-and- figures-2019-.pdf.

vi Chen K.; Wei1 J.; Ge C.; Xia W.; Shi Y.; Wang H.; Jiang X.;.Application of autoplanning in radiotherapy for breast cancer after breast conserving surger; Scientific Reports; 2020,**10**, 10927.

vii Cammarata F.P.; Forte G.I.; Broggi G.; Bravatà V.; Minafra L.; Pisciotta P.; Calvaruso M.;Tringali R.; Tomasello B.; Torrisi F.; Petringa G.; Cirrone G.; Cuttone G.; Acquaviva R.; Caltabiano R.; Russo G.; Molecular Investigation on a Triple Negative Breast Cancer Xenograft Model Exposed to Proton Beams; Int. J. Mol. Sci.; 2020, **21** (17), 6337.

viii Li J.; Li M.; Tian L.; Qiu Y.; Yu Q.; Wang X.; Guo R.; He Q.;. Facile strategy by hyaluronic acid functional carbon dot-doxorubicin nanoparticles for CD44 targeted drug delivery and enhanced breast cancer therapy. Inter. J. Pharmaceutics; 2020, **578**, 119122.

ix He X.; Zhang Q.; Feng Y.; Li Z.; Pan Q.; Zhao Y.; Zhu W.; Zhang N.; Zhou J.; Wang
L.; Wang M.; Liu Z.; Zhu H.; Shao Z., Wang L.; Resection of liver metastases from breast cancer: a multicentre analysis; Clin. Transl. Oncol.; 2020, **22** (4), 512–521.

x Wahab R.; Siddiqui M.A.; Saquib Q.; Dwivedi S.; Ahmad J.; Musarrat J.; Al-Khedhairy A.A.; Shin H.S.; ZnO nanoparticles induced oxidative stress and apoptosis in HepG2 and MCF-7 cancer cells and their antibacterial activity; Colloids Surf. BBiointerfaces; 2014, **117**, 267–276.

xi Johnson T.B.; Scott W.M.; J. Am. Chem. Soc.; 1913**, 35**, 1136–1143.

xii Johnson T.B.; Bengis R.; J. Am. Chem. Soc.; 1913, **35**, 1605-1606.

xiii Johnson T.B.; Hemingway E.H.; J. Am. Chem. Soc.; 1916, **38**, 1550-1557.

xiv Cromwell L.D.; Stark G.R.; Biochemistry; 1969, **8**, 4735–4740.

xv Wyzlic I.M.; Tjarks W.; Soloway A.H.; Perkins D.J.; Burgos M.; O'Reilly K.P.; Inorganic Chemistry; 1996, 35, 4541–4547.

xvi Johnson T.B.; Scott W.M.; J. Am. Chem. Soc.; 1913, **35**, 1130–1136.

xvii El-Sharif A.; Hussein A.; El-gaby M.; Atalla A.; Ahmed A.; Phosphorus, Sulfur, and
Silicon; 2001, **170**, 47-63.

xviii Ahmedova A.; Marinova P.; Paradowska K.; Marinov M.; Wawer I.; Mitewa M.; Polyhedron; 2010**, 29**, 1639-1645.

xix Davis R.A.; Aalbersberg W.; Meo S.; Moreira da Rocha R.; Ireland C. M.; Tetrahedron; 2002, **58**, 3263.

xx Kandra L.; Remenyik J.; Batta G,; Somsâk L.; Gyêmânt G.; Park K. H.; Carbohydr Res.; 2005, **340**, 1311.

xxi Roue N.; Bergman J.; Tetrahedron; 1999, **55**, 14729.

xxii Chang W. J.; Kulkarni M. V.; San C. M.; J Comb Chem.; 2006, **8**, 141.

xxiii Fuentes J.; Salameh B. A. B.; Pradera M. A.; Córdoba F. J. F.; Gasch C.; Tetrahedron; 2006, **62**, 97.

xxiv Beloglazkina E.K.; Majouga A. G.; Romashkina R. B.; Zyk N.V.;Tetrahedron Lett.; 2006, **47**. 2957.

xxv Lin M.J.; Sun C. M.; Tetrahedron Lett. ; 2003, **44**, 8739.

xxvi Chazeau V.; Cussac M.; Boucherle A.; Eur J Med Chem.; 1992, **27**, 615.

xxvii Kononowicz K. K.; Wojciechowska J. K.; Phosphorus SulfurSilicon Relat Elem.; 1992, **73**, 235.

xxviii Abdellatif K.R.A.; Fadaly W.A.A.; Mostafa Y.A.; Zaher D.M.; Omar H.A.;

Thiohydantoin derivatives incorporating a pyrazole core: Design, synthesis and biological evaluation as dual inhibitors of topoisomerase-I and cycloxygenase-2 with anti-cancer and anti-inflammatory activities;Bioorg. Chem.; 2019, **91**, 103132.

xxix Elhady H.A.; El‑Sayed R.; Al‑Nathali H.S.; Design, synthesis and evaluation of anticancer activity of novel 2‑thioxoimidazolidin‑4‑one derivatives bearing pyrazole, triazole and benzoxazole moieties; Chemistry Central Journal; 2018, **12**(1),51.

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