Open Access **Baghdad Science Journal** **P-ISSN: 2078-8665**
Published Online First: May, 2023 **2024, 21(1): 133-145** **E-ISSN: 2411-7986**

DOI: https://doi.org/10.21123/bsj.2023.7978

Study of the Anticancer and Antimicrobial Biological Activity of a New Series of Thiohydantoin Derivatives

Qusay Hussein Hays* *Dakhil Zughayir Mutlaq*[2]*

Department of Chemistry, College of Education for Pure Sciences, University of Basrah, Basrah, Iraq
*Corresponding author: www.qasm44@gmail.com
E-mails address: dakhil.mutlaq@uobasrah.edu.iq

Received 18/10/2022, Revised 4/2/2023, Accepted 5/2/2023, Published Online First 20/5/2023, Published 01/1/2024

Abstract:

Recently, some prostate cancer patients have acquired resistance to the second -generation drugs (anzalutamide and apalutamide) prescribed for the treatment of this disease due to the emergence of the F876L mutation, which represents a challenge to modern medicine. In this study, a new series of 2-thiohydantoin derivatives were prepared through the reaction of different derivatives of maleimide (1c-4c) with isothiocyanate derivatives. The prepared compounds were diagnosed using FT-IR,^{1}H-NMR ,^{13}C-NMR, Mass spectra. The prepared series compounds has been studied against prostate cancer cells. The MTT assay was used to determine the activity of the prepared compounds against prostate cancer cells. The data indicated, depending on the IC50 values, that some of the prepared compounds have anti-prostate cancer activity. The results indicate that compounds 1d and 2d have good anti-prostate cancer activity compared to the rest of the compounds. The prepared series of compounds were also studied against selected types of bacteria and fungi, as the results showed that some of the compounds in the series had anti-bacterial and anti-fungal activity.

Keywords: Anti-bacterial activity, Antifungal activity, Isothiocyanate, Prostate cancer, Thiohydantoin.

Introduction:

Hydantoin was first isolated by Bayer in 1861 in the course of her research on uric acid [1]. 2-thiohydantoin was first reported in 1913, and by evaluating the evidence, it was found that the first compound prepared for this type of compound was by Peter Klason in 1890 [2]. 2-thiohydantoin is one of the biologically active molecules and has diverse activities to supply the medical and pharmaceutical industries in facing the challenges faced by modern medicine after the spread of drug-resistant diseases, which are increasingly spreading all over the world, where it has been used as anti- cancer [3-8], anti-leishmaniasis [9], an antiviral [10], protect PC12 cells [11], antibacterial [12-14], also showed anti-TMV activity [15], anti-neuroinflammatory [16]. Perhaps one of the most important things that drew the attention of researchers to the thiohydantoin nucleus in the last decade is that it is the main nucleus in the second-generation of drugs (enzalutamide and apalutamide) intended for the treatment of prostate cancer. Enzalutamide and apalutamide were discovered in a series of 2-thiohydantoin derivatives prepared in 2006 by Sawyers and Jung in a series of UCLA patent applications and subsequently published in a journal in 2010[17-18]. In 2012, enzalutamide was officially approved as a drug for the treatment of prostate cancer by the US Food and Drug Administration, and it was approved in the European Union in 2013 and in Japan in 2014. As for apalutamide, it was approved in the United States of America as a drug for the treatment of prostate cancer in 2018 and in the European Union in 2019 [19]. Prostate cancer is one of the most common diseases in men. In 1989, flutamide was approved as a drug for the treatment of prostate cancer by the US Food and Drug Administration, while bicalutamide was approved in 1995, and these drugs became ineffective as cancer progressed to the stage of hormone resistance. The ineffectiveness of the first-generation anti-prostate cancer drugs led to the design of the second-generation drugs (enzalutamide and apalutamide), but unfortunately, as the disease progressed to the stage of resistance to this generation of drugs, the second-generation drugs have become ineffective at the present time .On the contrary, in some cases, their use leads to

strengthening The disease instead of being cured, and the reason is due to the emergence of the F876L mutation[20-21]. In this study, we prepared a new series of 2-thiohydantoin derivatives and they were studied against prostate cancer using the MTT assay [22]. In comparison to the other compounds, the data show that compound 1d has good anti-prostate cancer activity. The compounds were also studied against selected types of bacteria and fungi, and it was clear from the results that some compounds had anti-bacterial and anti-fungal activity under study.

Materials and Methods:

Infrared spectra of the prepared compounds were recorded using a Japanese-made Shimadzu 8400 FT.IR device and in the form of potassium bromide tablets in the (400–4000) region at room temperature. At room temperature, ^{1}H and ^{13}C-NMR spectra were recorded using BRUCKER-500MHz and 125MHz instruments, DMSO-d6 as a solvent, and TMS as an internal reference. All chemical displacements were measured in ppm. Mass spectra were recorded using a quadruple device analyzer spectrometer 5975 Agilent with electron collision technology at electronic energy of 70 eV.1-(4-bromophenyl)-1H-pyrrole-2,5-dione(1a), 1-(p-tolyl) -1H-pyrrole-2,5-dione(2a),1-(4-methyl-3-nitrophenyl) -1H-pyrrole-2,5-dione(3a), 1-(4-acetylphenyl) -1H-pyrrole-2,5-dione(4a), acetone, hexane, absolute ethanol, acetonitrile from(Sigma-Aldrich). Phenyl isothiocyanate, glacial acetic acid, Isoniazid, TLC, cyclohexyl isothiocyanate from (Merck).

Synthesis of compounds (1c-4) [23, 24]

The first step: 0.01mol of different maleimides (1a–4) was dissolved with 0.01mol of Isoniazid in 30ml of absolute ethanol and the mixture was left under reflux and stirred for 6–12 hrs. The reaction was monitored by TLC (thin layer chromatography). After the reaction was completed, it was cooled, filtrated, and washed in absolute ethanol. Recorded spectra data of the prepared compounds:

N'-(1-(4-bromophenyl)-2,5-dioxopyrrolidin-3-yl)isonicotinohydrazide(1c):

White solid powder; yield 63 %, mp= 245-247 ºC

N'-(1-(4-methyl-3-nitrophenyl)-2,5-dioxopyrrolidin-3-yl)isonicotinohydrazide (3c):

White solid powder; yield 53 %, mp= 236-238 ºC

N'-(1-(4-acetylphenyl)-2,5-dioxopyrrolidin-3-yl)isonicotinohydrazide(4c):

White solid powder; yield 65%; mp= 229-231 ºC , FT-IR and NMR spectra data are listed in tables (1),(2)and (3).

Table 1. FT-IR spectra data of (1c-4) compounds

Comp. Symbol	N-H amide cm^{-1}	N-H cm^{-1}	C-HAr. cm^{-1}	C-H aliph. cm^{-1}	C=O Imidazolidine-2,4-dione cm^{-1}	C=O imidazolidine-2,4-dione cm^{-1}	C=O Amid cm^{-1}	C=C cm^{-1}	C-N cm^{-1}
1c	3317	3217	2995	2883	1784	1712	1637	1546,1494, 1469	**1406**
3c	3415	3284	3080	2983 ,2929	1788	1718	1658	1537,1496	**1344**
4c	3415	3311	3001	2997	1786	1716	1681	1604,1544	**1404**

Table 2. ^{1}HNMR spectra data of (1c-4) compounds

Com. Symbol	H_n	Ar-H	H_m	H_r	H_k	Hi	CH_3
1c	10.47 (d,J=6.3 , 1H)	8.77–8.70 (m , 2H) 7.80-7.65 (m , 4H) 7.29-7.23 (m, 2H) 8.74 (d, J=5.0 , 2H) 8.02 (s , 1H) 7.74 (d , J=8.2 , 1H) 7.65 (d , J=8.3 , 1H) 7.59 (d ,J=8.3 , 1H)	6.15 t ,J=5.8, (1H)	4.24 (dt,J=8.8,4.4,1H)	3.09 (dd ,J=18.0 ,8.5 ,1H)	2.80 (dd ,J=18.0 , 3.8 , 1H)	–
3c	10.51 (s , 1H)		6.20 (s, 1H)	4.32-4.27 (m,1H)	3.12 (dd , 18.1 , 8.4 ,1H)	2.83 (dd , 17.8 , 8.4 ,1H)	2.57 (s , 3H)

4c	10.51 (d ,J= 5.8 , 1H)	8.76-8.70 (m , 2H) 8.09-8.07 (m , 2H) 7.76-7.73 (m, 2H) 7.49-7.45 (m ,2H)	6.19 (d ,J=5.9, 1H)	4.28 (dd,J=8.7,4.2,1H)	3.13 (dd , 18.0 , 8.5 ,1H)	2.83 (dd ,J=17.9, 3.9 , 1H)	2.61 (s , 3H)

Table 3. ^{13}C-NMR spectra data of (1c-4) compounds

Comp.symbol	C=O imidazolidine-2,4-dione	C=O imidazolidine-2,4-dione	C=O Isonicotinohydrazide	C-Ar	CHr	$CH_i H_k$	CH_3
1c	174.98	174.50	164.39	150.29, 139.90, 131.90, 131.58, 128.93 121.21, 121.18	57.53	34.52	_
3c	174.89	174.41	164.37	150.27, 148.57, 139.89, 133.33, 133.12, 131.61, 130.96, 122.69, 121.18	57.65	34.58	19.45
4c	174.98	174.48	164.41	150.30, 139.91, 136.28 136.23 128.80, 126.86, 121.20	57.59	34.60	26.81

Synthesis of compounds (1d-4) and (1e-4) [23, 24]

In this experiment, 0.01mol of maleimide derivatives (1c–4) was dissolved in 30ml of acetonitrile as a solvent and few drops of glacial acetic acid as a catalyst.. Then was added 0.01mol of cyclohexyl isothiocyanate or phenyl isothiocyanate. The mixture was left under reflux with stirring for 16–70 hrs. The reaction was monitored by TLC (thin layer chromatography). After the reaction was completed, the solvent was evaporated at room temperature and then recrystallized by a mixture of acetone and hexane.

Recorded spectra data of the prepared compounds:

N-(5-(2-((4-bromophenyl)amino)-2-oxoethyl)-3-cyclohexyl-4-oxo-2-thioxoimidazolidin-1-yl) isonicotinamide (1d):

White solid powder; yield 55 %; mp= 159-162 ºC . MS *m/z* (%): 530 (M^+, 9.2), 129 (28), 106 (72), 71 (52), 43 (100).

N-(3-cyclohexyl-4-oxo-5-(2-oxo-2-(p-tolylamino)ethyl)-2-thioxoimidazolidin-1-yl)isonicotinamide(2d):

White solid powder; yield 25%, mp = 247-250. MS *m/z* (%): 465 (M^+ , 47), 344 (30), 211 (29), 129 (64), 106 (100) ,78 (52), 55 (42).

N-(3-cyclohexyl-5-(2-((4-methyl-3-nitrophenyl)amino)-2-oxoethyl)-4-oxo-2-thioxoimidazolidin-1-yl)isonicotinamide(3d):

White solid powder; yield 68 %; mp= 140-142 ºC. MS *m/z* (%): 510 (M^+, 12), 216 (55), 178 (35), 106 (100), 78 (48), 55 (38).

N-(5-(2-((4-acetylphenyl)amino)-2-oxoethyl)-3-cyclohexyl-4-oxo-2-thioxoimidazolidin-1-yl)isonicotinamide(4d):

Onion solid powder; yield 40 %; mp= 178-181 ºC. MS *m/z* (%): 493 (M^+, 12), 371 (28), 215 (57), 106 (100), 78 (78), 55 (91).

N-(5-(2-((4-bromophenyl)amino)-2-oxoethyl)-4-oxo-3-phenyl-2-thioxoimidazolidin-1-yl)isonicotinamide(1e):

White solid powder; yield 25%; mp= 272-275 ºC. MS *m/z* (%): 525 (M^+, 5.5), 135 (33), 106 (100), 78 (81), 51 (47) .

N-(4-oxo-5-(2-oxo-2-(p-tolylamino)ethyl)-3-phenyl-2-thioxoimidazolidin-1-yl)isonicotinamide(2e):

White solid powder; yield 44%; mp= 246- 248ºC. MS *m/z* (%): 459 (M^+, 5.7), 135(25), 106 (100), 78 (63) , 51 (38).

N-(5-(2-((4-methyl-3-nitrophenyl)amino)-2-oxoethyl)-4-oxo-3-phenyl-2-thioxoimidazolidin-1-yl)isonicotinamide (3e):

White solid powder; yield 25%; mp= 170-172 ºC. MS *m/z* (%): 135 (100), 77 (181), 106 (68), 51(52).

N-(5-(2-((4-acetylphenyl)amino)-2-oxoethyl)-4-oxo-3-phenyl-2-thioxoimidazolidin-1-yl)isonicotinamide (4e):

White solid powder; yield 45 %; mp=241-243 ºC. MS *m/z* (%): 487 (M^+, 0.2), 215 (78), 135 (66), 106 (100), 78 (78), 51 (47). FT-IR and NMR spectra data are listed in tables 4, 5 and 6.

Table 4. FT-IR spectra data of (1d-4) and (1e-4) compounds

Comp. Symbol	N-H amide cm^{-1}	N-H cm^{-1}	C-HAr. cm^{-1}	C-H aliph. cm^{-1}	C=O cm^{-1}	C=O cm^{-1}	C=O Amid cm^{-1}	C=C cm^{-1}	C=S cm^{-1}	C-N cm^{-1}
1d	3277	3248	3051	2933,2856	1739	1689	1662	1602,1537	1489	**1359**
2d	3412	3292	3041	2935,2854	1751	1705	1631	1543, 1514	1429	**1361**
3d	3238	3414	3045	2933,2858	1751	1695	1614	1531	1413	**1357**
4d	3257	3234	3099	2929,2856	1751	1687		1597, 1533	1411	**1361**
1e	3394	3180	3122	2906	1761	1701	1593	1521,1489	1448	**1338**
2e	3483	3400	3045	2912	1761	1703	1681	1614,1523	1444	**1303**
3e	3261	3498	3105, 3053	2978,2931	1766	1689	1598	1527,1500	1425	**1334**
4e	3396	3483	3062	2910	1759	1747	1699	1595,1535	1408	**1303**

Table. 5: ^{1}HNMR spectra data of (1d-4) and (1e-4) compounds

Com. Symbol	Hd	He	Ar-H	H_c	H_f	Ha ,Hb	CH_3	H-cyclohexyl
1d	11.65 (s , 1H)	10.24 (s , 1H)	8.79-8.77 (m, 4H) 7.51-7.46 (m, 4H)	4.63 (t , J=4.4 ,1H)	4.50-4.44 (m ,1H)	3.03 (d ,J=4.3 ,2H)	-	2.99 (dd ,J=22.6 ,12.2 ,2H) 1.83 (d , J=13.1 ,2H) 1.78-1.71 (m ,2H) 1.65 (d ,J=13.0 ,1H) 1.26 (d ,J=18.0 , 3H)
2d	11.66 (s , 1H)	9.98 (s , 1H)	8.77 (d J=5.1 ,2H) 7.79-7.78 (m ,2H) 7.41-7.40 (m , 2H) 7.09 (d ,J=8.1 , 2H)	4.63 (t , J=4.3 ,1H)	4.50-4.45 (m ,1H)	3.01-2.99 (m ,2H)	2.27 (s,3H)	2.23-2.16 (m ,2H) 1.83 (d , J=13.3 ,2H) 1.74 (t, J=15.1 ,2H) 1.65 (d ,J=13.1 ,1H) 1.29-1.14 (m ,3H)
3d	11.63 (s , 1H)	10.49 (s , 1H)	8.77 (d ,J=5.2,2H) 8.26 (d ,J=2.3 ,2H) 8.18 (d,J=5.2 , 2H) 7.68 (dd ,J=8.5,2.4 , 1H) 7.42 (d ,J=8.3,1H)	4.68 (t, J=4.3,1H)	4.52-4.45 (m, 1H, H_f)	3.06 (d ,J=4.5,2H)	2.45 (s,3H)	2.20-2.17 (m ,2H) 1.83 (d , J=12.3 ,2H) 1.78-1.72 (m ,2H) 1.64 (d ,J=12.9 ,1H) 1.32-1.14 (m ,3H)

4d	11.64 (s , 1H)	10.45 (s , 1H)	8.79-8.78 (m ,2H) 7.92-7.91 (m ,2H) 7.80-7.79 (m , 2H) 7.16 (t ,J=7.8 , 1H) 7.66 (t ,J=8.5 ,1H)	4.66 (t , J=4.4 ,1H)	4.52-4.46 (m, 1H, H_f)	3.09–3.08 (m ,2H)	2.51 (s,3H)	2.25-2.17 (m ,2H) 1.85-1.64 (m, 5H) 1.32-1.15 (m, 3H)
1e	11.80 (s , 1H)	10.34 (s , 1H)	8.81-8.80 (m,2H) 7.84-7.82 (m ,2H) 7.59-7.42 (m, 9H)	4.94 (d , J=4.2 ,1H)	-	3.24-3.16 (m ,2H)	-	-
2e	11.83 (s , 1H)	10.11 (s , 1H)	8.18-8.80 (m,2H) 7.84-7.83 (m ,2H) 7.57 (t, J=7.6 , 2H) 7.48 (dd, J= 13.4,7.3Hz, 3H) 7.42 (d, J= 7.3Hz, 2H) 7.12 (d, J=8.0 , 2H)	4.92 (t , J=4.0 ,1H)	-	3.17 (d ,J=4.3 ,2H)	2.25 (s,3H)	-
3e	11.76 (s , 1H)	10.57 (s , 1H)	8.80 (d, J=4.5 , 2H) 8.32 (t, J=2.2 , 1H) 7.83 (dt, J=4.2,1.8 , 2H) 7.75 (dd, J=8.4 , 2.4,1H) 7.60-7.53 (m , 2H) 7.51-7.47 (m , 1H) 7.45-7.42 (m , 3H)	4.99 (t d, J=4.3,2.2 ,1H)	-	3.24 (q,J=3.3,2.9,2H)	2.47 (s,3H)	-
4e	11.79 (s , 1H)	10.56 (s , 1H)	8.80 (d, J=5.0 , 2H) 8 7.95 (d, J=8.3 , 2H) 7.83 (d, J=5.0 , 2H) 7.73 (d, J=5.0 , 2H) 7.58 (t, J=7.7 , 2H 7.50 (t, J=7.3 , 1H) 7.44 (d, J=7.7 , 2H)	4.97 (t , J=3.9 ,1H)	-	3.25 (d, J=4.1 , 2H)	2.53 (s,3H)	-

Table 6. ^{13}C-NMR spectra data of (1d-4) and (1e-4) compounds

Comp.symbol	C=S	C=O Thioimidazole	ArNHC=O	NNH C=O	C-Ar	CHc	CH_f	CH_aH_b	C-cyclo-hexyl	CH_3
1d	184	171.88	166.68	164	150.53, 138.56, 138.28, 131.58, 121.41, 120.99, 114.85	59.02	55.74	34.86	28.60, 27.88, 25.60, 25.46, 24.93	-
2d	184	171.94	166.14	164.27	150.49, 138.58, 136.41, 132.14, 129.06, 121.40, 119.07	59.07	55.69	34.79	28.57, 27.87, 25.58, 25.45, 24.91	20.43
3d	184	171.75	167.08	164.22	150.34, 148.52, 138.67, 137.73, 133.12, 127.23, 123.67, 121.44, 114.30	59.02	55.73	34.91	28.56, 27.89, 25.56, 25.45, 24.90	19.14
4d	184	171.83	167.14	164.22	150.37, 143.16, 138.69, 131.78, 129.46, 121.46, 118.34	58.99	55.73	34.99	28.57, 27.87, 25.58, 25.45, 24.91	26.39
1e	183	171.43	166.96	164.39	150.58, 138.56, 138.26, 133.83, 131.64, 129.07, 128.98, 128.52, 121.47, 121.10, 114.98	59.68	-	35.07	-	-
2e	183	171.53	166.45	164.37	150.58, 138.57, 136.42, 133.86, 132.29, 129.17, 129.07, 128.95, 128.54, 121.48, 119.15	59.73		34.99	-	20.49
3e	183	171.29	167.39	164.39	150.52, 148.59, 138.55, 137.71, 133.76, 133.18, 129.03, 128.96, 128.50, 127.33, 123.77, 121.43, 114.40	59.69	-	35.07	-	19.14

4e	183	171.38	167.42	164.37	150.55, 143.14, 138.57, 133.81, 131.87, 129.51, 129.05, 128.96, 128.51, 121.44, 118.46	59.66	-	35.20	-	26.42

Results and Discussion:

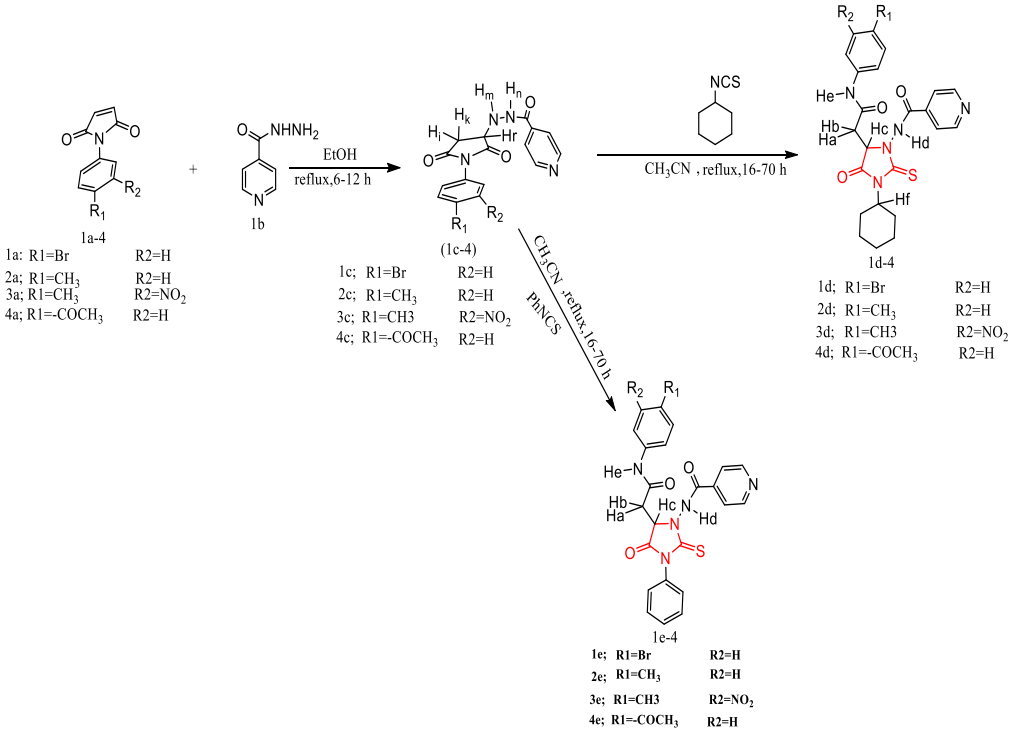
In this study, the authors prepared a new series of thiohydantoin derivatives. In the first step, a new series of succinimide derivatives 1c–4 were prepared by reacting different maleimides 1a–4 with isoniazid in absolute ethanol, and the time required for the reaction to occur was 6–12 hrs. In the second step, and to obtain the final product, different succinimide derivatives (1c-4) were reacted with isothiocyanate derivatives in acetonitrile as a solvent and few drops of glacial acetic acid as a catalyst. The period required for the reaction to occur was 16-70 hrs. Scheme 1. The reaction mechanism for the synthesis of compounds 1d-4 and 1e-4 is illustrated in Scheme. 2, as the synthesis process goes through an intermediate compound that suffers a rearrangement process to give the final product. The compounds of the prepared series were diagnosed by FT-IR, ^{1}HNMR, ^{13}C-NMR and mass spectra. The chemical structures of all the resulting succinimide derivatives 1c, 3c and 4c were confirmed by FT-IR, ^{1}H-NMR, and ^{13}C-NMR. The IR spectrum was used to identify the functional groups of these compounds. The NH amide and NH groups were observed in the range of 3564–3317 and 3483–3217 cm^{-1}, respectively. The absorption bands in the 1788–1637 cm^{-1} area are linked to C=O. The band in the range 1546-1409 cm^{-1} was assigned to the C=C aromatic stretching.

The succinimide derivatives 1c, 3c and 4c were used to generate ^{1}H-NMR spectra. The compounds were also distinguished by the appearance of signal doublet of doublets at around δ 3.13-3.09 and 2.83-2.80, which belong to H_i and H_k protons, respectively. Because the hydrogen atoms of the methylene group are adjacent to the chiral center. The H_r signal is at δ 4.32-4.27. The signals at around δ 6.20–6.15 were assigned to the protons of H_m. Signals of singlet at around δ 10.57–10.11 were due to H_e. signals (doublet and signal) for protons H_n in the arrange at δ 10.51-10.47. Aromatic protons were given the signals (multiplet, doublet and singlet) at roughly δ 8.77–7.23. The methyl groups responsible for the singlet are at δ 2.57.

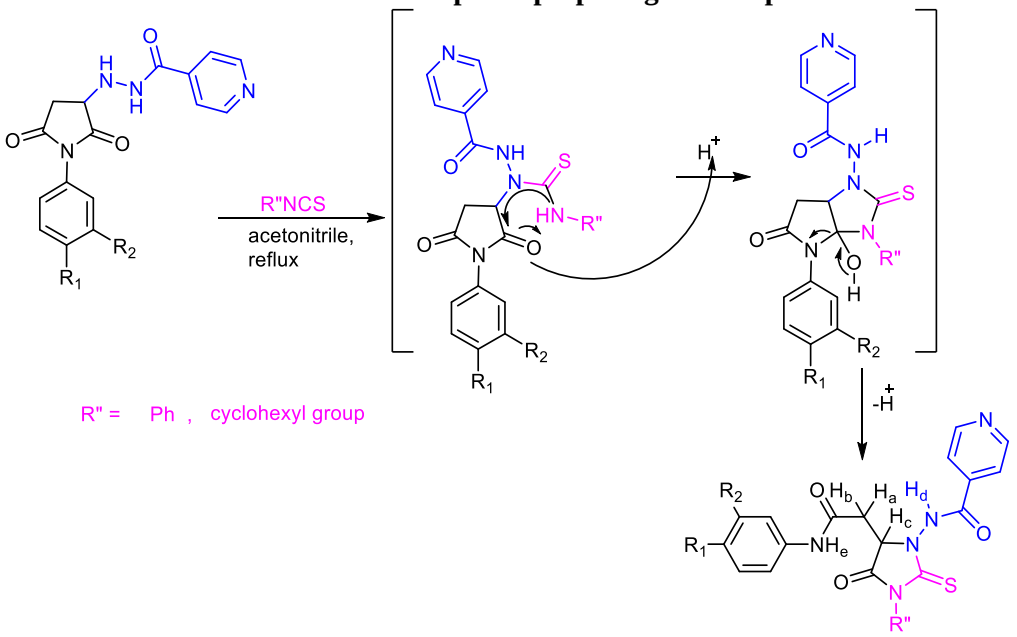
The ^{13}C- NMR of the compounds 1c, 3c and 4c that showed signals at around δ 197-164 were attributed to carbonyl groups. The signals of the carbon aromatic ring appeared in the range of δ 150-121. The aliphatic carbons are present in the range δ 58.27–21.45.

The obtained spectra data indicates the correctness of the structures of the prepared compounds 1d–4d and 1e–4e. The most important bands recorded by the FT-IR spectra, 3479-3300 (NH amide group), 3300-3200 (NH), 3186-3000 (CH - *aromatic ring*), 1449-1400 (thiocarbonyl group), 1350-1300 (C-N). The recorded NMR spectra of the prepared compounds were characterized by the fact that the signals appeared in almost identical chemical shifts. The ^{1}HNMR spectrum revealed a triple signal for Hc at 4.99 to 4.63 and a multiple for Ha and Hb at 3.2 to 2.7. The protons of aromatic rings showed multiple signals in the region of 8.83–7.09. The two singlets at δ 11.83 to 11.63 and δ 10.57 to 9.98 correspond to protons H_d and H_e, respectively. Signals of a multiplet were observed at δ 4.52-4-44 attributed to the H_f, while protons of the cyclohexyl group showed a multiple signals in the region at δ 2.25-1.14. ^{13}CNMR spectra confirmed the presence of all carbon atoms in compounds 1d-4 and 1e-4. Signals appearing in carbon's aliphatic range 59.07–14 were observed. The carbonyl carbons of ketone, thiocarbonyl carbons, and the carbonyl carbons appeared in the regions 206–196, 184.46–183, and 171.94–164, respectively. The recorded mass spectra of the compounds 1d-4 and 1e-4 showed the following molecular ions (*m/z*): 530 $[M]^+$, 465 $[M]^+$, 510 $[M]^+$, 493 $[M]^+$, 524 $[M]^+$, 459 $[M]^+$ 487 $[M]^+$, 513 $[M]^+$.

Baghdad Science Journal
2024, 21(1): 133-145
P-ISSN: 2078-8665
E-ISSN: 2411-7986



Scheme 1. shows the steps for preparing the compounds

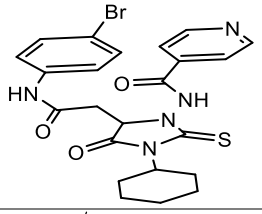
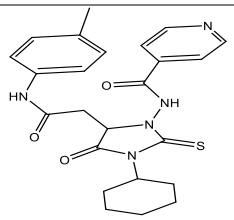
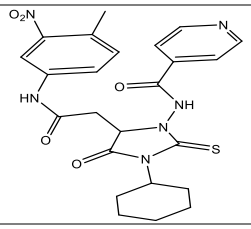
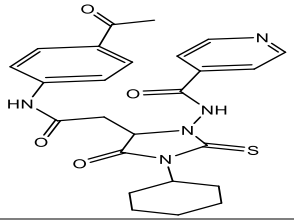
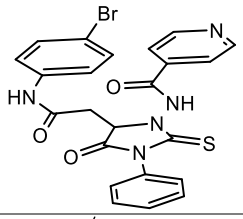
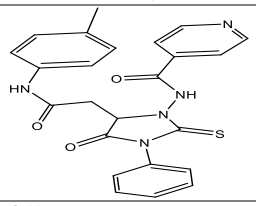
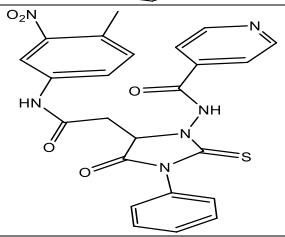
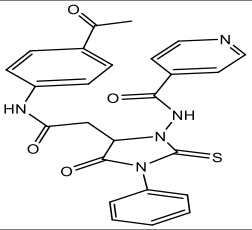


Scheme 2. shows the mechanism of preparation of compounds (1d-4) and (1e-4).

The anti-prostate cancer activity: [22]

The discovery of anti-prostate cancer drugs is one of the most important challenges facing modern medicine, and the reason is the large number of mutations that occur in AR (androgen receptor). In this study, we examined a new series of thiohydantoin derivatives as anti-prostate cancer agents. Depending on the IC50 value of the studied compounds, we note that there is a noticeable variation in the activity of the compounds against prostate cancer cells Fig.1.The difference in activity is attributed to the different Ar-substitutions in the prepared series. Among the compounds studied, compounds 1d, 2d, and 3d showed anti-prostate cancer activity Fig.2. The results showed that compound 1d has good activity compared to the rest of the series compounds, and the reason is due to the presence of the bromine atom. Also, the good activity of compound 2d compared to compound 3d is due to the presence of the methyl group, as shown in Table. 7.

Table 7. shows the values of IC50 for the compounds under study against prostate cancer cells.

Compound symbol	Compound structure	PC_3 cells IC_{50} in µg/mL	
1d		46.873	active
2d		51.836	active
3d		93.588	active
4d		234.851	Inactive
1e		393.917	Inactive
2e		202.865	Inactive
3e		221.503	Inactive
4e		265.008	Inactive

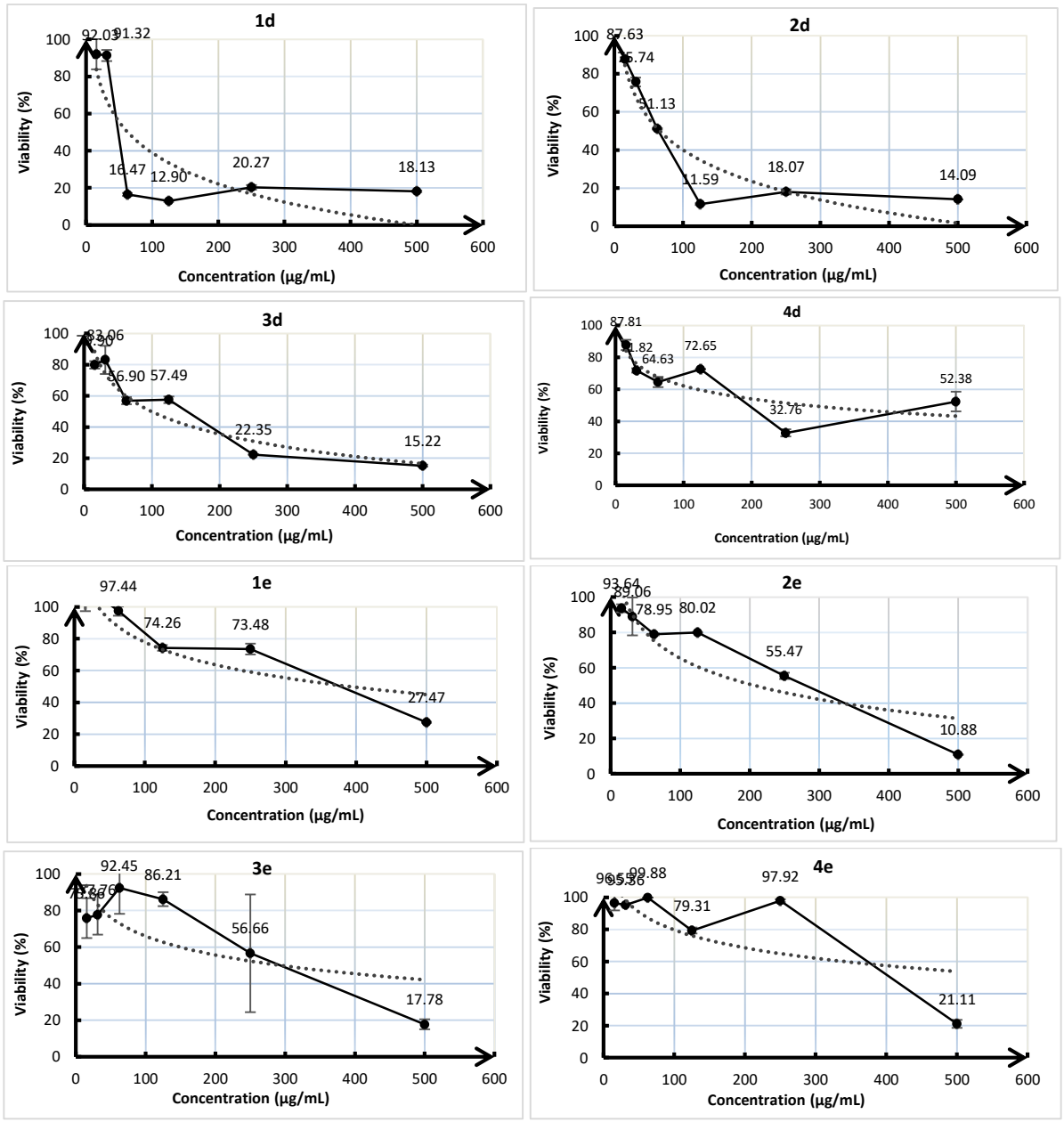
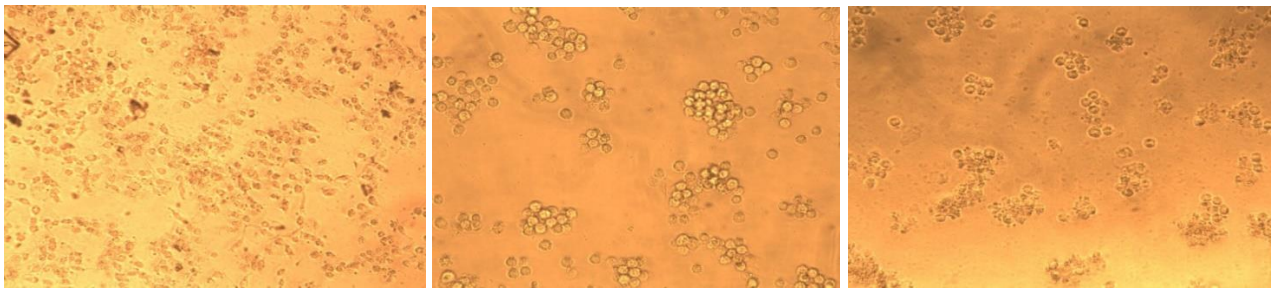


Figure 1. Graph of the response of the prepared compounds against prostate cancer cell lines.



1d 2d 3d

Figure 2. The effect of compounds (1d, 2d and 3d) on prostate cancer cells.

Evaluation of prepared compounds against bacteria and fungi: [25,26]

Most of the heterocyclic compounds are characterized by their anti-bacterial and anti-fungal activity[27,28]. The biological activity of thiohydantoin derivatives is found in a wide range of heterocyclic compounds, and the biological activity of this type of compound usually depends on the nature of the substituent groups on the thiohydantoin ring.

Previous studies confirm that compounds containing in their structures the thiohydantoin nucleus have anti-bacterial and anti-fungal properties [29-33]. In this study, we evaluated the prepared thiohydantoin series against selected strains of bacteria and fungi.The prepared compounds were studied as anti-bacterial E. coli and Staph compared with Spiromycin as a positive control. Also, the prepared series of compounds were evaluated as anti-fungal Candida albicans and Aspargilus. The growth inhibition of bacteria and fungi under study was measured in mm, as one concentration of 100 mg/ml was prepared for each compound.50 µL of each concentration was taken and added to each well (7 mm diameter holes cut in the agar gel, 20 mm apart from one another). Plates were incubated for 24 hours at 36ºC ± 1ºC, under aerobic conditions. The obtained data indicate that some compounds of the prepared series have anti-bacterial and anti-fungal activity. The two compounds 1c and 3c showed anti-fungal activity against Aspargilus, while the rest of the compounds under study did not show any activity. The data showed that all the compounds under study had anti-Candida albicans activity. All the compounds under study showed antibacterial activity against staph except the compounds 2d, 3e and 4e, which showed no activity against this particular type of bacteria. The compounds 1c, 3c, and 1d showed anti-bacterial activity against *E. coli*, while the rest of the compounds under study did not have anti-bacterial activity against this type of bacteria, Table 8.

Table 8. Shows the data for studying the prepared compounds against bacteria and fungi.

Compound symbol	Aspargilus	Candida albican	Staph	**E.Coli**
1c	17	25	16	**12**
3c	17	21	18	**13**
1d	-	16	16	**12**
2d	-	13	-	**-**
3d	-	23	15	**-**
4d	-	22	14	-
1e	-	40	15	**-**
2e	-	31	12	**-**
3e	-	26	-	**-**
4e		30	-	
Spiromycin	No tested	No tested	23	**23**

Negative -; No activity

Conclusion:

The aim of this study was to prepare compounds that have anti-prostate cancer activity. The relevant dose-response curve data confirm that the substituent groups in the aromatic rings of the prepared series of compounds have a significant effect on their anti-prostate cancer activity. The two compounds 1d and 2d showed moderate activity against prostate cancer cells, and further study is needed to increase their activity. When replacing the phenyl group in the compounds 1e, 2e and 3e with a cyclohexyl group 1d, 2d, 3d, it is noted that the activity of the compounds against prostate cancer increases. Hence, we recommend keeping the cyclohexyl group in the event that further study is conducted on this type of compound for its role in increasing activity.

Acknowledgment:

We appreciate the cooperation of the Deanship of the College of Education for Pure Sciences at the University of Basra. We also appreciate the assistance of the Head of the Chemistry Department at the College of Education for Pure Sciences at the University of Basra for facilitating many of the obstacles that accompanied this work.

Authors' declaration:

- Conflicts of Interest: None.
- We hereby confirm that all the Figures and Tables in the manuscript are mine ours. Besides, the Figures and images, which are not mine ours, have been given the permission for republication attached with the manuscript
- Ethical Clearance: The project was approved by the local ethical committee in University of Basrah.

Authors' Contributions Statement:

Q. H. H. participated in the following roles: conducting and following up all reactions, measuring spectra of the prepared compounds, follow-up of the anti-cancer activity measurements of the prepared compounds. D. Z. M. participated in the following roles: Interpretation of the spectra data of the prepared compounds, following up on the antibacterial activity measurements of the prepared compounds, and the interpretation of their results, Manuscript review and proofreading.

Authors' Declaration:

- Conflicts of Interest: None.
- We hereby confirm that all the Figures and Tables in the manuscript are mine ours. Besides, the Figures and images, which are not mine ours, have been given the permission for re-publication attached with the manuscript.
- Ethical Clearance: The project was approved by the local ethical committee in University of Basrah.

References:

1. Baeyer A. Vorläufige Notiz über das Hydantoïn. Liebigs Ann Chem. 1861;117(2):178–180. https://doi.org/10.1002/jlac.18611170204.
2. Klason P. An apparatus for preparing chloride. P Chem Ztg. 1890; 14: 543.
3. Han X , Wang C, Qin C, Xiang W, Fernandez-Salas E, Yang, C Y, et al. Discovery of ARD-69 as a highly potent proteolysis targeting chimera (PROTAC) degrader of androgen receptor (AR) for the treatment of prostate cancer. J. Med. Chem. 2019; 62(2): 941-964.https://doi.org/10.1021/acs.jmedchem.8b01631 .
4. Khodair A I, Bakare S B, Awad M K, Nafie M S. Design, synthesis, DFT, molecular modelling studies and biological evaluation of novel 3-substituted (E)-5-(arylidene)-1-methyl-2-thioxoimidazolidin-4-ones with potent cytotoxic activities against breast MCF-7, liver HepG2, and lung A549. J Mol Struct. 2021; 1229: 129805.https://doi.org/10.1016/j.molstruc.2020.129805.
5. Xu X , Ge R , Li L, Wang J , Lu X, Xue S. Exploring the tetrahydroisoquinoline thiohydantoin scaffold blockade the androgen receptor as potent anti-prostate cancer agents. Eur J Med Chem. 2018; 143: 1325-1344. https://doi.org/10.1016/j.ejmech.2017.10.031.
6. AbdulJabar L A, Al-Shawi A A A , Mutlaq D Z. Anti-liver and anti-breast cancer activities of 2-thioxo-4- imidazolidinone derivatives. Med Chem Res. 2021; 30: 1943–1953. https://doi.org/10.1007/s00044-021-02769-8.
7. Wang A, Wang Y, Meng X , Yang Y. Design, synthesis and biological evaluation of novel thiohydantoin derivatives as potent androgen receptor antagonists for the treatment of prostate cancer. Bioorg Med Chem. 2021; 31: 115953. doi: https://doi.org/10.1016/j.bmc.2020.115953.
8. Elbadawi M M, Khodair A I, Awad M K, Kassab S E, Elsaady M T, Abdellatif K R. Design, synthesis and biological evaluation of novel thiohydantoin derivatives as antiproliferative agents: A combined experimental and theoretical assessments. J Mol Struct. 2022; 1249: 131574. https://doi.org/10.1016/j.molstruc.2021.131574.
9. Camargo P G, Bortoleti B T D S , Fabris M, Gonçalves M D , Tomiotto-Pellissier F , Costa I N, et al. Thiohydantoins as anti-leishmanial agents: n vitro biological evaluation and multi-target investigation by molecular docking studies. J Biomol Struct Dyn. 2022; 40(7): 3213-3222. https://doi.org/10.1080/07391102.2020.1845979.
10. Chen L, Hao Y, Song H, Liu Y, Li Y, Zhang J, Wang Q. Design, synthesis, characterization, and biological activities of novel spirooxindole analogues containing hydantoin, thiohydantoin, urea, and thiourea moieties. J Agric Food Chem. 2020; 68(39): 10618-10625. https://doi.org/10.1021/acs.jafc.0c04488 .
11. Peng X R, Zhang R R, Liu J H, Li Z R, Zhou L, Qiu M H. Lepithiohydimerins A—D: Four Pairs of Neuroprotective Thiohydantoin Dimers Bearing a Disulfide Bond from Maca (Lepidium meyenii Walp.). Chin J Chem. 2021; 39(10): 2738-2744. https://doi.org/10.1002/cjoc.202100353 .
12. Tejchman W, Orwat B, Korona-Głowniak I, Barbasz A, Kownacki I, Latacz G, et al. Highly efficient microwave synthesis of rhodanine and 2-thiohydantoin derivatives and determination of relationships between their chemical structures and antibacterial activity. RSC Adv. 2019;9(67): 39367-39380. https://doi.org/10.1039/C9RA08690K .
13. De Carvalho P G , Ribeiro J M , Garbin R P B , Nakazato G , Yamada Ogatta S F , de Fátima. Synthesis and antimicrobial activity of thiohydantoins obtained from L-amino acids. Lett Drug Des Discov. 2020; 17(1): 94-102. https://doi.org/10.2174/1570180816666181212153011.
14. Mutlaq D Z, Al-Shawi A A, AbdulJabar L A. Antioxidant and antimicrobial activities of some novel 2-thiohydantoin derivatives. Egypt. J. Chem. 2021; *64*(3): 1315-1321. https://doi.org/10.21608/EJCHEM.2020.47419.2963 .
15. Huang Y, Guo Z, Song H, Liu Y, Wang L, Wang Q. Design, synthesis, and biological activity of β-carboline analogues containing hydantoin, thiohydantoin, and urea moieties. J Agric Food Chem. 2018; 66(31): 8253-8261. https://doi.org/10.1021/acs.jafc.8b03087.
16. Lee T H, Khan Z, Kim S Y , Lee K R. Thiohydantoin and hydantoin derivatives from the roots of Armoracia rusticana and their neurotrophic and anti-neuroinflammatory activities. J Nat Prod. 2019; 82(11): 3020-3024. https://doi.org/10.1021/acs.jnatprod.9b00527.
17. Sawyers C L , Jung M E, Chen C D , Ouk S, Welsbie D, Tran C, et al. Diarylhydantoin Compounds. PCT Int. Patent Appl. WO2006/124118, November 23, (2006).
18. Jung M E , Ouk S , Yoo D, Sawyers C L, Chen C, Tran C, et al. StructureActivity Relationship for Thiohydantoin Androgen Receptor Antagonists for CastrationResistant Prostate Cancer (CRPC). J Med. Chem. 2010; 53: 2779-2796. https://doi.org/10.1021/jm901488g .
19. Brave M, Weinstock C, Brewer J R, Chi D C, Suzman D L, Cheng J, et al. An FDA Review of Drug Development in Nonmetastatic Castration-resistant Prostate CancerFDA Review: Drug Development in nmCRPC. Clin Cancer Res. 2020; 26(18): 4717-4722. https://doi.org/10.1158/1078-0432.CCR-19-3835.
20. Korpal M, Korn J M, Gao X, Rakiec D P, Ruddy D A, Doshi S, et al. An F876L mutation in androgen

receptor confers genetic and phenotypic resistance to MDV3100 (enzalutamide). Cancer Discov. 2013; 3(9): 1030-1043. https://doi.org/10.1158/2159-8290.CD-13-0142
21. Joseph J D, Lu N, Qian J , Sensintaffar J , Shao G , Brigham D , et al. A Clinically Relevant Androgen Receptor Mutation Confers Resistance to Second-Generation Antiandrogens Enzalutamide and ARN-509AR F876L Confers Enzalutamide and ARN-509 Resistance. Cancer Discov. 2013; 3(9): 1020-1029.; https://doi.org/10.1158/2159-8290.CD-13-0226.
22. Meerloo J V, Kaspers G J, Cloos J. Cell sensitivity assays: the MTT assay. Cancer cell culture. 2011; 731:237. https://doi.org/10.1007/978-1-61779-080-5_20 .
23. Bouzroura S, Hammal L, Nedjar- Kolli B, Balegroune F, Hamadene M, Poulain S. Synth. Commun. 2008; 38: 448-455. https://doi.org/10.1080/00397910701316987 .
24. Troin Y, Bentarzi Y, Nedjar-Kolli B, Plas A, Chalard P. Arkivoc. 2010; 328-337. http://dx.doi.org/10.3998/ark.5550190.0011.a27.
25. Smania Jr A , Monache F D , Smania E D F A , Cuneo R S. Antibacterial activity of steroidal compounds isolated from Ganoderma applanatum (Pers.) Pat.(Aphyllophoromycetideae) fruit body. Int J Med Mushrooms. 1999; 1(4):325-3330. https://doi.org/10.1615/IntJMedMushr.v1.i4.40 .
26. Thangavelu R, Devi P G, Gopi M, Mustaffa M M .Management of Eumusae leaf spot disease of banana caused by Mycosphaerella eumusae with Zimmu (Allium sativum× Allium cepa) leaf extract. Crop Prot. 2013;46: 100-105. https://doi.org/10.1016/j.cropro.2012.12.022.
27. Ali Z Z M, Abdulla A M. Synthesis and Antimicrobial Schreening of New 4, 5, 6, 7-Tatra Hydro Benzo Thiophene Derivatives. Baghdad Sci J. 2019; 16(1): 68-77. http://dx.doi.org/10.21123/bsj.2019.16.1.0068.
28. Alsahib S A. Characterization and Biological Activity of Some New Derivatives Derived from Sulfamethoxazole Compound. Baghdad Sci J. 2020; 17(2): 471-480. DOI: http://dx.doi.org/10.21123/bsj.2020.17.2.0471.
29. Wu Y, Ding X , Xu S , Yang Y , Zhang X , Wang C , et al. Design and synthesis of biaryloxazolidinone derivatives containing a rhodanine or thiohydantoin moiety as novel antibacterial agents against Gram-positive bacteria. Bioorg Med Chem Lett. 2019; 29(3): 496-502. https://doi.org/10.1016/j.bmcl.2018.12.012 .
30. AbdulJabar L A, Mutlaqa D Z, Al-Shawia A A A. Sythesis of Novel 2-Thioxo-4-Imidazolidinone Derivatives and Evaluate Their Antibacterial, And Antifungal Activities. Egypt J Chem. 2021:64; 3059 - 3067. https://doi.org/10.21608/EJCHEM.2021.66960.3442 .
31. Kania A , Tejchman W, Pawlak A M , Mokrzyński K , Różanowski B , Musielak B M , et al. Preliminary Studies of Antimicrobial Activity of New Synthesized Hybrids of 2-Thiohydantoin and 2-Quinolone Derivatives Activated with Blue Light. Molecules. 2022; 27(3): 1069. https://doi.org/10.3390/molecules27031069.
32. Ghasempour L, Asghari S, Tajbakhsh M, Mohseni M. One-pot synthesis of new hydantoin (thiohydantoin) derivatives and evaluation of their antibacterial and antioxidant activities. J Heterocycl Chem. 2020; 57(12): 4136-4148. https://doi.org/10.1002/jhet.4120 .
33. Mollanejad K, Asghari S, Jadidi K. Diastereoselective synthesis of pyrrolo [1, 2-c] imidazoles using chiral thiohydantoins, malononitrile, and aldehydes and evaluation of their antioxidant and antibacterial activities. J Heterocycl Chem. 2020; 57(2): 556-564. https://doi.org/10.1002/jhet.3762.

دراسة الفعالية البايولوجية المضادة للسرطان والميكروبات لسلسلة جديدة من مشتقات الثيوهيدانتوين

قصي حسين هايس **داخل زغير مطلق**

قسم الكيمياء، كلية التربية للعلوم الصرفة، جامعة البصرة، البصرة، العراق

الخلاصة :

في الأونة الأخيرة اكتسب بعض مرضى سرطان البروستات مقاومة أدوية الجيل الثاني (أنزالوتاميد وابالوتاميد) المقررة لعلاج هذا المرض والسبب يعود الى ظهور الطفرة F876L والتي تمثل تحديا للطب الحديث .في هذه الدراسة تم تحضير سلسلة جديدة من مشتقات 2-ثايوهيدانتوين وذلك بوساطة تفاعل مشتقات الماليميايد (1c-4c) مع مشتقات الايزوثايوسيانات. (FT-IR ,^{1}H-NMR ,^{13}C-NMR, Mass spectra). تم تشخيص المركبات المحضرة باستخدام تم دراسة سلسلة المركبات المحضرة ضد خطوط خلايا سرطان البروستات . تم استخدام فحص MTT لتحديد نشاط المركبات المحضرة ضد خطوط خلايا سرطان البروستات أذ أشارت البيانات وبالأعتماد على قيم IC50 أن بعض المركبات المحضرة لها نشاط مضاد لخطوط خلايا سرطان البروستات . تشير النتائج إلى أن المركبين 1d و 2d لهما نشاط جيد ضد خطوط خلايا سرطان البروستات مقارنة مع بقية مركبات السلسلة قيد الدراسة . كما تمت دراسة سلسلة المركبات المحضرة ضد أنواع مختارة من البكتيريا والفطريات، حيث أظهرت النتائج أن بعض مركبات السلسلة المحضرة لها نشاط مضاد للبكتيريا والفطريات قيد الدراسة .

الكلمات المفتاحية : الفعالية المضادة للبكتريا، الفعالية المضادة للفطريات، أيزوثايوسيانات، سرطان البروستات، ثيوهيدانتوين