Anti-Liver and Anti-Breast Cancer Activities of Novel 2-Thioxo-4-Imidazolidinone Derivatives

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Abstract

MTT assay and flowcytometry analysis were used to examine the anti-liver (HepG2) and anti-breast cancer (MCF-7) activities of twelve compounds derived from 2-thioxo-4-imidazolidine. The compounds 5a-h and 7f demonstrated significant anticancer activity against breast cancer cells, while the compounds 5a, ab, and 5d-h demonstrated significant anticancer activity against liver cancer cells, with varying IC50 values as compared to Cisplatin as the positive control. Among these compounds, we chose 5a, 5d, and 5h to detect cell cycle phases and late apoptosis. Compound 5a arrested MCF-7 cells in the S phase, while compound 5d arrested cells in the G1 phase. Compound 5a arrested S phase HepG2 cells, compound 5d arrested S phase cells, and compound 5h arrested G2 phase HepG2 cells. Compound 5a had a higher ratio of late apoptosis than compounds 5d and 5h on both cancer cells. Finally, the development of these compounds as new anti-breast and anti-liver agents warrants further research to understand the mechanism of action, especially against breast cancer.

1. Introduction

Liver metastases occur in approximately 50% of all patients with metastatic breast cancer, with 5–12% of patients developing liver metastases as the primary site of breast cancer recurrence. Metastatic liver disease can affect liver function and endanger patients' lives. Breast cancer liver metastases are associated with a poor prognosis [1]. Chemotherapy for breast cancer and liver cancer kills cancer cells by using anticancer medicines that have negative side effects [2]. As a result, research and production of new anti-liver and anti-breast cancer agents with less side effects is still ongoing. Many heterocyclic families have been studied for their anti-tumor activity [3]. Heterocyclic compounds improve drug design, pharmacokinetics, and pharmacodynamics properties by increasing lipophilicity, polarity, precise targeting, and other physicochemical properties [4]. The five-member heterocyclic family 2-thiohydantoin (2-thioxo-4-imidazolidinone) is one of these heterocyclic families [5, 6]. Klason developed the first thiohydantoin or 2-thioxo-4-imidazolidinone ring in 1890 [7]. The thiohydantoin moiety is used in a large number of biologically active compounds [8]. In the preparation of biologically active compounds, heterocyclic moieties or functions in the skeleton of 2-thioxoimidazolidine-4-one or thiohydantoin are widely used [9–11]. The studies revealed that 2-thioxo-4-imidazolidinone has anti-cancer activity and that these groups may be used to discover new anticancer agents [12, 13]. In our study, we examined the function of new 2-thioxo-4-imidazolidinone derivatives against liver and cancer cells using the MTT assay, morphology changes, cell cycle, and apoptosis.

2. Result And Discussion

AbdulJabar et al. [14] characterized eight 2-thioxo-4-imidazolidinone compounds, while the four new compounds 7e-h were synthesized by reacting malimide derivatives 3e-h with phenylisothiocyanate 6 in the presence of acetonitrile and three drops of glacial acetic acid. Reflux for a duration ranging from 16 to 53 hours. TLC confirmed the reaction, and re-crystallization of the compounds in ethanol yielded a strong yield, as shown in Scheme 1.

These four compounds were diagnosed by proton and carbon NMR spectrum, mass spectrometry, and infrared spectrum, and the results were identical to the prepared compounds. The ^1H -NMR spectra of compounds 7e-h displayed the characteristic patterns of the ABC system belonging to the COCH_2CH fragment, with the inclusion of two signals as a triplet at 4.93–4.85 ppm for H_c and a multiplet at 3.22–3.08 ppm due to the H_a and H_b protons. The H_d and H_e protons showed two singlets signals at 11.41–11.37 ppm and 10.32–10.09 ppm, respectively. The two signals of the aromatic ring protons showed a doublet in the region of 7.86–7.84 ppm and a multiplet at 7.64–7.03 ppm. The presence of all carbon atoms for compounds 7e-h was confirmed by ^{13}C -NMR spectra. Signals appearing in carbon's aliphatic range at 60.24–20.93 ppm were observed for the CH_c , CH_aH_b and CH_3 . The signal of C_1 showed in the regions of 184.57-184.48 ppm, while signals of C_2 , C_3 and C_4 appeared in the regions of 172.11-165.94 ppm.

The FT-IR spectra of 2-thioxo-4-imidazolidinones 7e-h show characteristic absorptions at 3319 – 3182 cm^{-1} for the NH and NHPH, 1755 – 1600 cm^{-1} for the carbonyl ($\text{C} = \text{O}$) and 1438 – 1433 cm^{-1} for the thiocarbonyl ($\text{C} = \text{S}$) groups. The mass spectra of 7e-h showed a molecular ion (m/z): 492 $[\text{M}]^+$, 538 $[\text{M} + \text{H}]^+$, 472 $[\text{M}]^+$ and 458 $[\text{M}]^+$, respectively. The mass spectra confirmed the correctness of the prepared structures.

2.1 Anticancer activity of 2-thioxo-4-imidazolidinone derivatives

The discovery of new anticancer agents is a major challenge for researchers due to the high risk of cancer disease and the adverse side effects of chemotherapy. Pharmaceuticals, Agrochemicals, pet products, sanitizers, developers, enzymes, corrosion inhibitors, copolymers, and dyestuff are all examples of heterocyclic compounds in use. Furthermore, the heterocyclic structures are the precursor to many anticancer agents on the market. Some studies [15–17] demonstrated the importance of 2-thioxo-4-imidazolidinone in cancer treatment.

2.1.1 Anti-liver cancer activity

Using the MTT test, we investigated the cytotoxicity of 12 compounds derived from 2-thioxo-4-imidazolidinone against liver cancer cells (HepG2). 7 compounds (5a, ab, and 5d-h) showed potential activity and their IC_{50} values were calculated. The IC_{50} values were (1.312, 2.345, 1.350, 1.669, 1.983, 1.860, 1.597) μM . **Table 1** shows the IC_{50} values of the compounds in comparison to the IC_{50} value of Cisplatin as a positive control, which was 3.994 μM . Compound 5b demonstrated substantial activity and outperformed the other compounds. We chose the compounds 5a, 5d, and 5h based on their availability in the laboratory to investigate cell morphology changes, cell cycle, and apoptosis. Using the IC_{50} value for each compound, Fig. 1 shows that compound 5a caused more morphological changes in HepG2 cells than compounds 5d and 5h (HepG2). Furthermore, a cell cycle flowcytometry experiment revealed that compounds 5a and 5d arrested cells in the S phase, while compound 5h arrested cells in the G2 phase, as

shown in Figs. 2, 3, and 4. This suggests that the composition of compounds influences the cell cycle of liver cancer cells. Furthermore, late apoptosis (Q2) of HepG2 cells affected by IC50 values of compounds 5a, 5d, and 5h revealed that late apoptosis of compound 5a (27.6 percent) is higher than compound 5d (5.63 percent) and compound 5h (5.47 percent), and compared to the control late apoptosis (Q4).

2.1.2 Anti-breast cancer activity

Similarly, twelve 2-thioxo-4-imidazolidinone derivatives were tested against breast cancer cells (MCF-7). Among these compounds, nine (5a-h and 7f) demonstrated good behavior, and IC50 values were calculated. The IC50 values were (3.476, 2.063, 1.577, 2.709, 2.360, 2.489, 2.101, 2.140, 1.593) μM , respectively, as compared to the 3.653 μM IC50 value of Cisplatin as a positive control, **Table 1**. We chose two compounds (5a and 5d) based on their abundance to investigate morphology changes, cell cycle, and apoptosis. Compound 5a was found to be more toxic than compound 5d based on the IC50 value used to observe the morphology changes of MCF-7 cells (Fig. 1). Compound 5a inhibited cell growth in the S phase, while compound 5d inhibited cell growth in the G1 phase. Late apoptosis (Q2) of compound 5a was 24.6 percent, while compound 5d was 13.3 percent, compared to the control's late apoptosis (Q4), as shown in Figs. 5 and 6. Compound 5a has a high effect on morphology cells, arrested cells in the same S step, and reduced late apoptosis in both cancer cells (HepG2 and MCF-7). Compound 5d had a lower effect than compound 5a and arrested cells in various stages of HepG2 and MCF-7 cells.

The different locations and types of functional groups in compounds can explain their anticancer activity against liver and breast cancer cells. According to the MTT assay findings, compound 5a had higher activity on MCF-7 than HepG2, which had a chlorine group in R1 and a hydrogen atom in R2, while compound 5b had a chlorine group in R1 and a hydrogen atom in R2, and had medium activity on both MCF-7 and HepG2 cells. The compound 5c, which contains a bromide group in R1 and a hydrogen atom in R2, demonstrated low activity against MCF-7 and no activity against HepG2. Compound 5(d-h) demonstrated medium activity toward MCF-7 and low activity toward HepG2. Compound 7f had low activity on MCF-7 and no activity on HepG2, while compounds 7e, 7g, and 7h had no activity on any cancer cell. For both cancer cells, the activity levels of both compounds were compared to Cisplatin, which served as a supportive influence. These anti-liver and anti-breast cancer activities can occur as a result of the donate and withdraw groups' role in increasing/decreasing the potential toxicity of compounds.

3. Materials And Methods

3.1 Chemistry

AbdulJabar et al. [14] prepared and characterized eight compounds (5a-h). The four new compounds (7e-h) were characterized using FT-IR spectra (Perkin-Elmer equipment), nuclear magnetic resonance (^1H and ^{13}C -NMR) spectra (recorded in DMSO- d_6 on a BRUCKER-500 spectrometer operating at 500 MHz and 125 MHz, respectively), and mass spectra (MS) (recorded on a Probe Agilent (HP) MSD5973 As a result, the

purification of compounds was investigated using thin-layer chromatography (TLC) and melting point. All chemical shifts are provided in parts per million (ppm) from the internal tetramethylsilane (TMS) norm.

3.1.1 Synthesis of compounds (7e-h):

Step 1

Phenylhydrazide (2a) or 4-methylphenyl hydrazide (2b) (0.01 mol) was applied to a 30 ml ethanol solution of maleimide (1a-d) (0.01 mol). The mixture that resulted was refluxed for 6–12 hours. The white precipitate was collected by filtration and recrystallized from ethanol after cooling to room temperature, yielding compounds 3a-h.

Step 2

In 30 ml of acetonitrile supplemented with 3 drops of glacial acetic acid, a mixture of compounds prepared in step 1 (0.01 mol) and phenyl isothiocyanate 6 (0.011 mol) was heated to reflux with magnetic stirring for 16 to 53 hours. After allowing the solution to cool to room temperature, the white precipitate was collected, washed with diethyl ether, and re-crystallized from acetonitrile to yield products 7e-h.

3.1.2 The characterization of compounds (7e-h):

3.1.2.1 *N*-(5-(2-((4-chlorophenyl)amino)-2-oxoethyl)-4-oxo-3-phenyl-2-thioxoimid-azolidin-1-yl)-4-methylbenzamide (7e)

Yield 55 %; white solid; mp.= 269–270 °C; ^1H NMR (500 MHz, DMSO- d_6) δ 11.37 (s, 1H, H_d), 10.31 (s, 1H, H_e), 7.84 (d, J = 8.2 Hz, 2H, H-Ar), 7.64–7.30 (m, 12H, H-Ar), 4.89 (t, J = 3.8 Hz, 1H, H_c), 3.22–3.08 (m, 2H, H_a , H_b), 2.37 (s, 3H, CH_3); ^{13}C NMR (125 MHz, DMSO- d_6) δ 184.49 (C_1 = S), 172.00 (C_4 = O), 167.35 (C_2 = O), 165.95 (C_3 = O), 143.13, 138.32, 134.35, 129.55, 129.46, 129.32, 129.15, 128.96, 128.24, 127.32, 121.10 (C-Ar), 60.17 (CH_c), 35.43 (CH_aH_b), 21.55 (CH_3); IR (KBr, cm^{-1}): 3305 (NH *amide*), 3219 (Ph-NH), 3064 (CH-*arom.*), 2995 (CH-*aliph.*), 1753 (C = O *asym.*), 1664 (C = O *sym.*), 1602 (C = O *amide*), 1539, 1494 (C = C *arom.*), 1433 (C = S), 1330 (C-N); MS (m/z): 492 [M] $^+$.

3.1.2.2 *N*-(5-(2-((4-bromophenyl)amino)-2-oxoethyl)-4-oxo-3-phenyl-2-thioxo imi d-azolidin-1-yl)-4-methylbenzamide (7f)

Yield 90 %; white solid; mp.= 262–263 °C; ^1H NMR (500 MHz, DMSO- d_6) δ 11.37 (s, 1H, H_d), 10.32 (s, 1H, H_e), 7.84 (d, J = 7.9 Hz, 2H, H-Ar), 7.58–7.54 (m, 4H), 7.53–7.48 (m, 3H, H-Ar), 7.41 (d, J = 7.8 Hz, 2H, H-Ar), 7.33 (d, J = 7.9 Hz, 2H, H-Ar), 4.90 (s, 1H, H_c), 3.19–3.15 (m, 2H, H_a , H_b), 2.38 (s, 3H, CH_3); ^{13}C NMR (125 MHz, DMSO- d_6) δ 184.48 (C_1 = S), 171.99 (C_4 = O), 167.37 (C_2 = O), 165.94 (C_3 = O), 143.12, 138.72,

134.33, 132.05, 129.55, 129.46, 129.32, 129.11, 128.95, 128.23, 121.48, 115.34 (C-Ar), 60.16 (CH_C), 35.45 (CH_aH_b), 21.55 (CH₃); IR (KBr, cm⁻¹): 3273 (NH *amide*), 3182 (Ph-NH), 3047 (CH-*arom.*), 2993 (CH-*aliph.*), 1753 (C = O *asym.*), 1666 (C = O *sym.*), 1610 (C = O *amide*), 1531, 1489 (C = C *arom.*), 1436 (C = S), 1330 (C-N); MS (m/z): 538 [M + H]⁺.

3.1.2.3 4-Methyl-N-(4-oxo-5-(2-oxo-2-(*p*-tolylamino)ethyl)-3-phenyl-2-thioxoimid azoli din-1-yl)benzamide (7g)

Yield 73 %, white solid; mp.= 250–251 °C; ¹H NMR (500 MHz, DMSO-*d*₆) δ 11.41 (s, 1H, Hd), 10.09 (s, 1H, He), 7.86 (d, *J* = 7.9 Hz, 2H, H-Ar), 7.57–7.29 (m, 9H, H-Ar), 7.12 (d, *J* = 8.1 Hz, 2H, H-Ar), 4.89 (t, *J* = 3.9 Hz, 1H, H_C), 3.2–3.08 (m, 2H, H_a, H_b), 2.37 (s, 3H, CH₃), 2.25 (s, 3H, CH₃); ¹³C NMR (125 MHz, DMSO-*d*₆) δ 184.57 (C₁ = S), 172.11 (C₄ = O), 166.88 (C₂ = O), 165.96 (C₃ = O), 143.11, 136.91, 134.41, 132.65, 129.58, 129.55, 129.44, 129.29, 129.16, 128.99, 128.26, 119.56 (C-Ar), 60.24 (CH_C), 35.38 (CH_aH_b), 21.56 (CH₃), 20.93 (CH₃); IR (KBr, cm⁻¹): 3305 (NH *amide*), 3221 (Ph-NH), 3035 (CH-*arom.*), 2997 (CH-*aliph.*), 1755 (C = O *asym.*), 1666 (C = O *sym.*), 1610 (C = O *amide*), 1539, 1498 (C = C *arom.*), 1433 (C = S), 1330 (C-N); MS (m/z): 472 [M]⁺.

3.1.2.4 4-Methyl-N-(4-oxo-5-(2-oxo-2-(phenylamino)ethyl)-3-phenyl-2-thioxoimid azolid in-1-yl) benzamide (7h)

Yield 90 %; white solid; mp.= 268–269 °C; ¹H NMR (500 MHz, DMSO-*d*₆) δ 11.40 (s, 1H, H_d), 10.17 (s, 1H, H_e), 7.85 (d, *J* = 7.8 Hz, 2H, H-Ar), 7.60–7.03 (m, 12H, H-Ar), 4.93–4.85 (m, 1H, H_C), 3.21–3.13 (m, 2H, H_a, H_b), 2.37 (s, 3H, CH₃); ¹³C NMR (125 MHz, DMSO-*d*₆) δ 184.52 (C₁ = S), 172.08 (C₄ = O), 167.14 (C₂ = O), 165.96 (C₃ = O), 143.11, 139.39, 134.39, 129.55, 129.45, 129.30, 129.21, 128.98, 128.25, 123.76, 119.54 (C-Ar), 60.21 (CH_C), 35.43 (CH_aH_b), 21.55 (CH₃); IR (KBr, cm⁻¹): 3319 (NH *amide*), 3221 (Ph-NH), 3059 (CH-*arom.*), 2912 (CH-*aliph.*), 1753 (C = O *asym.*), 1668 (C = O *sym.*), 1600 (C = O *amide*), 1546, 1498 (C = C *arom.*), 1438 (C = S), 1327 (C-N); MS (m/z): 458 [M]⁺.

3.2 MTT assay

The MTT assay was performed on 96-well plates using the Mohammed et al. system with some modifications [18] to assess the IC₅₀ of new compounds. Cancer cell lines were seeded at a density of 1x10⁴ cells per well. Following a 24 hours incubation period, the cells were treated with the compounds dissolved in DMSO by taking 1 µl of each compound and diluting it using the normal dilution procedure to obtain five concentrations, and the effect was observed for 72 hours. After removing the growth medium, 28 µl of MTT solution was added and incubated for 2 hours at 37°C. After removing the MTT solution, 100 µl of DMSO (Dimethyl Sulfoxide) was applied to the cells and incubated for 15 minutes at 37°C. The absorbance at 620 nm was measured with a micro-plate reader to estimate cell viability. As a positive regulation, cisplatin was used.

3.3 Flowcytometry analysis

3.3.1 Apoptosis

Flowcytometry was used to identify late apoptosis using the process of Al-Shawi et al. with some modifications [19]. In brief, cancer cells were treated for 48 hours with the IC₅₀ values of compounds 5a, 5d, and 5h. The cells were harvested, rinsed twice with PBS, and labeled with 5 µl FITC-conjugated annexin V, as directed by the manufacturer. The samples were immediately analyzed on a flowcytometer after being incubated in the dark for 10 minutes and then labelled with PI (Beckman Coulter, Epics XL).

3.3.2 Cell Cycle

Flowcytometry research has established the G₁, S, and G₂ stages. Mossa and AlShawi et al., with some modifications [20]. For 48 hours, cancer cells were treated with IC₅₀ values of compounds 5a, 5d, and 5h. The cells were then washed with PBS and fixed in 70% ice-cold ethanol overnight at 4°C. After washing twice with PBS, cells were stained for 30 minutes in the dark at room temperature with a solution containing 50 µg/ml PI and 100 g/ml RNase A. Flow cytometry was used to examine the stained cells (Beckman Coulter, Epics XL).

Statistical analysis

The IC₅₀ values of the compounds were estimated using GraphPad Prism 8.1 for Windows. The experiments were carried out three times.

4. Conclusion

For the first time, twelve novel 2-thioxo-4-imidazolidinone derivatives were tested for anti-liver and anti-breast cancer activity. Seven compounds exhibited medium to low anti-liver activity, with compound 5b exhibiting higher activity than the others. Nine compounds were found to have anti-breast cancer efficacy, with compound 5a having the highest toxicity as compared to Cisplatin, the positive control. Compound 5a cell morphology and flowcytometry findings against both cancer cells suggested that compounds 5a, 5d, and 5h could be developed as anti-breast cancer agents and warrant further research to improve its role in fighting breast cancer. Furthermore, recommend that the compound 5b be studied in relation to liver cancer and even breast cancer. Future research may employ molecular docking studies to better understand the interactions of possible compounds with liver and breast cancer proteins.

Declarations

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Compliance with Ethical Standards:

- Conflicts of interest: The authors declare there is no conflict of interest
- Research involving human participants and/or animals: Not applicable
- Informed consent: Not applicable

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Tables

Table 1: GraphPad Prism software for Windows was used to calculate the IC₅₀ values of compounds 5a-h and 7f against MCF-7 cells and compounds 5a, ab, and 5d-h against HepG2 cells.

Compound Symbole	R1	R2	MCF-7 cells IC ₅₀ in µM	HepG2 cells IC ₅₀ in µM
**Cisplatin	-	-	3.653	3.994
5a	H	H	3.476	1.312
5b	Cl	H	2.063	2.345
5c	Br	H	1.577	*NA
5d	CH ₃	H	2.709	1.350
5e	H	CH ₃	2.360	1.669
5f	Cl	CH ₃	2.489	1.983
5g	Br	CH ₃	2.101	1.860
5h	CH ₃	CH ₃	2.140	1.597
7e	H	CH ₃	*NA	*NA
7f	Cl	CH ₃	1.593	*NA
7g	Br	CH ₃	*NA	*NA
7h	CH ₃	CH ₃	*NA	*NA

NA**= No activity; *Cisplatin**= Standard anticancer drug as positive control

Figures

Figure 1

The morphology of MCF-7 and HepG2 cells treated with the IC₅₀ values of compounds 5a, 5d, and 5h was shown.

Figure 2

Compound 5a cell cycle and apoptosis were demonstrated using IC50 against the HepG2 cell.

Figure 3

Compound 5d cell cycle and apoptosis were demonstrated using IC50 against the HepG2 cell.

Figure 4

Compound 5h cell cycle and apoptosis were demonstrated using IC50 against the HepG2 cell.

Figure 5

Compound 5a cell cycle and apoptosis were demonstrated using IC50 against the MCF-7 cell.

Figure 6

Compound 5d cell cycle and apoptosis were demonstrated using IC50 against the MCF-7 cell.

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