Research Article

Synthesis and Biological Evaluation of Novel Isoxazole-Amide Analogues as Anticancer and Antioxidant Agents

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Cancer now is one of the leading causes of mortality in the world. There has been a lot of effort to discover new anticarcinogenic agents that allow treatment with fewer side effects. A series of isoxazole-carboxamide derivatives (2a–2g) were synthesised and evaluated for their cytotoxic activity against breast (MCF-7), cervical (HeLa), and liver (Hep3B) cancer cell lines and their antioxidant activity in the 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay. The results showed that 2d and 2e were the most active compounds against Hep3B cells, with a half-maximal inhibitory concentration (IC_{50}) of around 23 μg/ml; 2d showed the highest activity against HeLa cells, with an IC_{50} 15.48 μg/ml. However, 2a had the lowest IC_{50} (39.80 μg/ml) against MCF-7 cells. By contrast, compound 2g was inactive against all cancer cell lines, with IC_{50} values >400 μg/ml. Both 2d and 2e reduced Hep3B secretion of alpha-fetoprotein (to 1829:33 ± 65.91 ng/ml and 1758:66 ± 54.04 ng/ml, respectively). Furthermore, in cell cycle analysis, 2d and 2e induced a delay in the G2/M phase of 18.07%, which is similar to the doxorubicin positive control. Moreover, 2d and 2e reduced the necrosis rate of Hep3B threefold and instead shifted the cells to apoptosis. Our results indicate that 2d and 2e have potent and promising anticancer activity. However, compound 2a was the most active as antioxidant agent (IC_{50} = 7.8 ± 1.21 μg/ml) compared with Trolox as a positive control (IC_{50} 2.75 μg/ml).

1. Introduction

Cancer is the leading cause of death throughout the world [1–3]. Indeed, 25% of the deaths in developing countries are due to cancer [4–6]. In 2018, cancer was responsible for 9.6 million deaths. During a person’s life, 1 out of 5 men and 1 out of 6 women will develop cancer [7]. Environmental factors such as diet, obesity, alcohol consumption, physical inactivity, radiation, sunlight, and viral infection as well as genetic factors lead to the development of cancer [8]. Over the years, scientists have explored a myriad of treatments for each type of cancer, including chemotherapy, hormonal treatment, radiation, and surgery [9].

Chemotherapy has been widely used, particularly against inoperable cancer [10], as the primary therapy or as an adjunct therapy before and/or after another treatment [11]. However, chemotherapy use is restricted because it has weak effectiveness, minimal selectivity against target cells, and undesirable side effects such as alopecia, nausea, and vomiting [12, 13]. Many natural extracts with anticancer activity have been evaluated in recent decades [14]. One of these compounds, combretastatin (Figure 1), was isolated...
from the African plant *Combretum caffrum* and has been modified to find new derivatives with antitumour activity, such as combretastatin A-4 phosphate (fosbretabulin) (Figure 1). That compound has been approved by the Food and Drug Administration (FDA) for thyroid cancer [15].

Different types of heterocyclic derivatives such as isoxazole are used extensively as agrochemicals in medicine; indeed, they are efficient in anticancer chemotherapy [15, 18]. Researchers have found that the isoxazole ring imparts it with anticancer [19, 20], hypoglycemic [21], pain killing, bactericidal [20, 22], antiviral (for HIV) [23], and anti-inflammatory [24] activities. The isoxazole ring has been used as a core structure for many anticancer medications [25]. For example, resorcylic 4,5-diarylisoxazole amides have shown a potent inhibitory effect on heat shock protein (HSP90) [26]. A derivative of diarylisoxazole (Figure 2, st. 1) was discovered for its activity against androgen receptor (AR-) expressing breast cancer cells [27]. Leflunomide, an immunosuppressant used in chemotherapy, is another isoxazole derivative. Other researchers reported that the 3,5-dimethylisoxazole (Figure 2, st. 2) functional group mimics acetylated lysine (KAc); they utilised this functional group to improve bromodomain inhibitors, with positive effects on cancer cells [28]. In another work, researchers tested a hybrid molecule, with a trimethoxyphenyl moiety combined with the isoxazole ring (arylamino-isoxazolyl-2-propenone; Figure 2, st. 3). The compound showed cytotoxic activity (low half-maximal inhibitory concentration (IC<sub>50</sub>) values) against several cancer cell lines: cervical adenocarcinoma (Hela), lung adenocarcinoma (A549), breast carcinoma (MCF7), and hepatocellular carcinoma (HCT116) [29].

Oxidants are formed as a normal product of aerobic metabolism, but they can be produced at elevated rates under pathophysiological conditions. Thus, an imbalance between oxidants and antioxidants in favour of oxidants potentially leads to damage that forms the core of oxidative stress. The biologically active agents that work to slow or prevent the cell damage caused by those free radicals are called antioxidants [30, 31]. Environmental stress is usually the primary impetus for the formation of these unstable free radicals. While the human body produces endogenous antioxidants, other agents are found in natural plants and foods; examples include β-carotene, R-tocopherol (vitamin E), and ascorbic acid (vitamin C). Other antioxidants can be chemically synthesised [30–33]. In the last few decades, researchers have synthesised many agents that have marked antioxidant activity, such as quinolinone-3-aminooamide [31], thienopyrimidine, thienopyrazole [34], and N-aryl-1,4-dihydropyridine derivatives [35]. These substances, like Trolox, a water-soluble vitamin E analogue, and rebamipide (Figure 2), exhibit effective antioxidant properties by scavenging unstable free radicals [31, 36, 37].

The current project is aimed at synthesising novel isoxazole-carboxamide derivatives (2a–2g) with or without a methoxyphenyl moiety and at evaluating some of their biological activity such as antioxidant and anticancer activities on HeLa, MCF-7, HepG2, and HepB3 cancer cell lines.

2. Materials and Methods

2.1. Chemistry. All chemicals were obtained from Alfa Aesar and Sigma-Aldrich. SMP-II Digital Melting Point Appliances are used to determined melting points and are uncorrected. <sup>13</sup>C-NMR and <sup>1</sup>H-NMR spectra were recorded in DMSO-d<sub>6</sub> and were performed on two NMR instruments. The first was a Bruker 500 MHz-Avance III High-Performance Digital FT-NMR spectrometer at the Faculty of Science, Department of Chemistry, the University of Jordan, Jordan. The second was a Bruker 300 MHz-Avance III High-Performance Digital FT-NMR spectrometer at the NMR facility at the Doping and Narcotics Analysis Laboratory of the Faculty of Pharmacy, Anadolu University, Turkey. Tetramethylsilane was used as the internal standard. All chemical shifts were recorded as δ (ppm). High-resolution mass spectra data (HRMS) were collected using a Waters LCT Premier XE Mass Spectrometer (high sensitivity orthogonal acceleration time-of-flight instrument) using the ESI (+) method (the instrument was coupled to an AQUITY Ultra Performance Liquid Chromatography system (Waters Corporation, Milford, MA, USA)) at Pharmacy Faculty, Gazi University, Ankara, Turkey.

2.1.1. General Procedure of Isoxazole-Carboxamide Synthesis (2a–2g). 3-(2-Chlorophenyl)-5-methylisoxazole-4-carboxylic acid (1) (1.5 mmol) was dissolved in dichloromethane (12 ml). To this mixture DMAP (0.3 mmol), EDC (1.8 mmol) were added and was allowed to stir under nitrogen gas at room temperature for 30 min. After that, the appropriate aniline derivative (1.8 mmol) was added and the mixture was allowed to stir for 24–48 h. The reaction was monitored by TLC, and at the end of the reaction, the solvent was removed under reduced pressure and dissolved again in

![Figure 1: Combretastatin, CA-4P (fosbretabulin), and CA-4 structures.](image-url)
dichloromethane, then extracted with 1% NaHCO₃ and brine. The organic layer was dried by drying agent Na₂SO₄ and evaporated under reduced pressure. The obtained product was purified by flash chromatography using the appropriate solvent system or by the crystallization utilising appropriate solvent system [38].

2.1.2. N-(4-(tert-butyl)phenyl)-3-(2-chlorophenyl)-5-methylisoxazole-4-carboxamide (2a). Silica gel column was purified by chromatography using n-hexane: ethyl acetate solvent system (70:30); solid product, M.P. 175-176°C, yield 77%; ¹H NMR (DMSO-d₆) δ: 10.02 (1H, s), 7.43-7.58 (6H, m), 7.31 (2H, d, J = 8.6 Hz), 2.65 (3H, s), and 1.24 (9H, s); ¹³C NMR (DMSO-d₆) δ ppm: 169.98, 160.40, 159.71, 146.74, 136.57, 132.84, 132.24, 131.97, 130.09, 127.98, 127.79, 125.78, 120.04, 115.13, 34.50, 31.54, 12.73. HRMS (m/z): [M+H]⁺ calcd for C₂₁H₂₂ClN₂O₂ 369.1356, found 369.1364.

2.1.3. N-(4-chloro-2,5-dimethoxyphenyl)-3-(2-chlorophenyl)-5-methylisoxazole-4-carboxamide (2b). Purified by silica gel column chromatography using n-hexane: ethyl acetate solvent system (70:30); solid product, M.P. 115-117°C, yield 67%; ¹H NMR (DMSO-d₆) δ: 8.83 (1H, s), 8.02 (1H, s), 7.55-7.74 (4H, m), 7.07 (1H, s), 3.75 (3H, s), 3.52 (3H, s), and 2.75 (3H, s); HRMS (m/z): [M+H]⁺ calcd for C₁₉H₁₇Cl₂N₂O₄ 407.0565, found 407.0558.

2.1.4. 3-(2-chlorophenyl)-N-(3,5-dimethoxyphenyl)-5-methylisoxazole-4-carboxamide (2c). Purified by silica gel column chromatography using n-hexane: ethyl acetate solvent system (65:35); solid product, M.P. 255-257°C, yield 82%; ¹H NMR (DMSO-d₆) δ: 9.99 (1H, s), 7.45-7.59 (4H, m), 7.24 (1H, d, J = 2.1 Hz), 7.02 (1H, dd, J = 8.7, 2.1 Hz), 6.87 (1H, d, J = 8.7 Hz), 3.70 (3H, s), 3.69 (3H, s), and 2.65 (3H, s); ¹³C NMR (DMSO-d₆) δ ppm: 169.89, 160.44, 159.45, 148.94, 145.76, 132.83, 132.70, 132.23, 131.99, 130.11, 127.99, 127.82, 115.13, 114.56, 105.26, 56.19, 55.81, and 12.79; HRMS (m/z): [M+H]⁺ calcd for C₁₉H₁₈ClN₂O₄ 373.0955, found 373.0951.

2.1.5. 3-(2-Chlorophenyl)-N-(3,4-dimethoxyphenyl)-5-methylisoxazole-4-carboxamide (2d). Purified by silica gel column chromatography using n-hexane: ethyl acetate solvent system (75:25); solid product, M.P. 199-200.5°C, yield 90%; ¹H NMR (DMSO-d₆) δ: 9.99 (1H, s), 7.47-7.59 (4H, m), 7.23 (1H, d, J = 2.1 Hz), 7.02 (1H, dd, J = 8.4, 2.1 Hz), 6.87 (1H, d, J = 8.7 Hz), 3.70 (3H, s), 3.68 (3H, s), and 2.65 (3H, s); HRMS (m/z): [M+H]⁺ calcd for C₁₉H₁₈ClN₂O₄ 373.0955, found 373.0948.

2.1.6. 3-(2-Chlorophenyl)-N-(2,5-dimethoxyphenyl)-5-methylisoxazole-4-carboxamide (2e). Purified by silica gel column chromatography using n-hexane: ethyl acetate solvent system (65:35); solid product, M.P. 188-190°C, yield 78%; ¹H NMR (DMSO-d₆) δ: 8.27 (1H, s), 7.79 (1H, s), 7.58-7.68 (4H, m), 6.86 (1H, d, J = 9 Hz), 6.06 (1H, dd, J = 9.3 Hz), 3.67 (3H, s), 3.48 (3H, s), and 2.75 (3H, s); ¹³C NMR (DMSO-d₆) δ ppm: 173.86, 158.94, 153.35, 143.18, 135.35, 133.35, 132.79, 132.52, 130.51, 128.30, 127.77,
127.22, 111.94, 108.76, 107.43, 56.55, 55.85, and 13.17; HRMS (m/z): [M+H]+ calcd for C_{11}H_{18}ClN_{2}O_{4} 373.0955, found 373.0954.

2.1.7. 3-(2-Chlorophenyl)-5-methyl-N-phenylisoxazole-4-carboxamide (2f). Purified by silica gel column chromatography using n-hexane: ethyl acetate solvent system (70:30); solid product, M.P. 201-202°C, yield 69%. 1H NMR (DMSO-d_{6}) δ: 10.14 (1H, s), 7.47-7.58 (6H, m), 7.30 (2H, t, J = 7.5 Hz), 7.07 (1H, t, J = 7.2 Hz), and 2.66 (3H, s). 13C NMR (DMSO-d_{6}) δ ppm: 170.10, 160.45, 159.86, 139.13, 132.79, 132.25, 132.03, 130.10, 129.19, 127.90, 127.83, 124.35, 120.27, 115.06, and 12.77. HRMS (m/z): [M+H]+ calcd for C_{11}H_{18}ClN_{2}O_{4} 373.0948, found 373.0948.

2.1.8. 3-(2-Chlorophenyl)-5-methyl-N-(3,4,5-trimethoxyphenyl) isoxazole-4-carboxamide (2g). Purified by silica gel column chromatography using n-hexane: ethyl acetate solvent system (70:30); solid product, M.P. 214-215.5°C, yield 90%; 1H NMR (DMSO-d_{6}) δ: 10.06 (1H, s), 7.47-7.60 (4H, m), 6.93 (2H, s), 3.71 (6H, s), 3.61 (3H, s), and 2.66 (3H, s). 13C NMR (DMSO-d_{6}) δ ppm: 170.03, 160.42, 159.68, 153.19, 135.33, 134.31, 132.85, 132.19, 131.99, 130.12, 127.98, 127.83, 115.07, 97.93, 60.57, 56.21, and 12.80. HRMS (m/z): [M+H]+ calcd for C_{20}H_{20}ClN_{2}O_{5} 403.1048, found 403.1055.

2.2. Biological Methods

2.2.1. Cell Culture and Cytotoxicity Assay. Hep3B, HeLa, and MCF7 cancer cell lines were cultured in Roswell Park Memorial Institute- (RPMI-) 1640 medium and supplemented with 10% foetal bovine serum, 1% L-glutamine, and 1% penicillin-streptomycin in a humidified atmosphere with 5% CO₂ at 37°C. The cells were seeded at 2×10⁴ cells/well in a 96-well plate. After 72 h, the cells were confluent; the medium was changed and cells were incubated with various concentrations (500, 100, 50, 10, and 1 µg/ml) of the synthesised compounds for 24 h [14]. Cell viability was assessed with the Cell Titer 96® Aqueous One Solution Cell Proliferation (MTS) assay according to the manufacturer’s instructions (Promega Corporation, Madison, WI). Briefly, at the end of the treatment, 20 µl of MTS solution per 100 µl of medium was added to each well and incubated at 37°C for 2h. The absorbance was measured at 490 nm [39, 40].

2.2.2. Alpha-Fetoprotein (aFP) Analysis. Hep3B cells were cultured in Dulbecco’s Modified Eagle Medium (DMEM) with 10% foetal bovine to examine the level of aFP as a marker of tumour activity. We marked Hep3B cells by labeling their surface with HBsAg (Water et al., 1998). Hep3B cells were incubated with DDW and silica in 10 µl/ml for 24 h. A commercially available enzyme-linked immunosorbent assay (ELISA) kit (R&D Systems, Inc., Minneapolis, MN, USA) was used to assess the level of aFP in the medium.

2.2.3. Apoptosis and Cell Cycle Analysis. Hep3B cells were trypsinised (0.05% trypsin/0.53 mM EDTA), washed, and adjusted to 1×10⁶ cells/ml (in saline containing 1% albumin; Biological Industries, Israel) for 10 min to determine their purity. The cells were fixed with 4% paraformaldehyde and permeabilised in 0.1% saponin in phosphate-buffered saline (PBS) for 20 min at room temperature. They were then stained with an anti-human HBsAg monoclonal antibody (R&D System) for 30 min at room temperature. The cells were then incubated with propidium iodide (PI) to stain fragmented DNA and Annexin V conjugated to fluorescein isothiocyanate (FITC) (R&D systems) to stain phosphatidylserine (PS) according to the manufacturer’s instructions. The cells were analysed by flow cytometry (Becton-Dickinson LSR 11, Immunofluorometry Systems, Mountain View, CA, USA). Apoptotic cells were defined as Annexin V (+)/PI (-). Viable cells were defined as Annexin V (-)/PI (-). In each experimental setting, unstained controls, immunoglobulin G (IgG) isotype controls, and FMO controls were used [41].

To analyse the cell cycle, Hep3B was fixed in cold 70% ethanol for at least 30 min at 4°C. After washing two time in PBS, the cells were treated with 50 µl RNase (100 µg/ml), stained with 5 µl propidium iodide (PI) (50 µg/PI/100 ml solution), and analysed with flow cytometry [41].

2.3. Antioxidant Activity. The antioxidant activity was measured for synthesised compounds 2a–2g. A 1 mg/ml solution of each compound was prepared by dissolving 1 mg in 1 ml of methanol; this stock was diluted with methanol to obtain several concentrations (2, 5, 10, 20, 50, and 100 µg/ml). One millilitre of each concentration was mixed with 1 ml of methanol and 1 ml of 2,2-diphenyl-1-picrylhydrazyl (DPPH) solution. The solution was incubated for 30 min in the dark at room temperature. A blank solution was prepared by replacing the plant fraction with methanol [42]. Trolox was used as a positive control. The absorbance was measured by a UV-Vis spectrophotometer at 517 nm then compared with the control. The antioxidant activity was calculated with the following equation:

\[ I(\%) = \left( \frac{\text{ABS}_{\text{test}} - \text{ABS}_{\text{blank}}}{\text{ABS}_{\text{blank}}} \right) \times 100\% \]  

where I (%) is the percent antioxidant activity and ABS is the absorbance at 517 nm.

The antioxidant IC₅₀ for each synthesised compounds was calculated using BioDataFit 1.02 (data fit for biologists) [43].

2.4. Statistical Analyses. The antioxidant activity was measured in triplicate for each sample, while the cytotoxic test was measured in duplicate for each sample. The obtained results are presented as the mean ± standard deviation. Statistical analysis was performed using the GraphPad Prism software version 6.01 (GraphPad Software, San Diego, CA, USA). Three or more groups were compared with one-way analysis of variance (ANOVA) followed by Bonferroni’s post hoc test.

3. Results and Discussion

3.1. Chemistry. The isoxazole-carboxamide derivatives (2a–2g) were synthesised as outlined in Figure 3. The coupling to form the isoxazole-carboxamide compounds 2a–2g was
afforded by using EDC as an activating agent and DMAP as a covalent nucleophilic catalyst; the active intermediates were reacted with the aniline derivatives [44]. High-resolution mass spectrometry (HRMS) was used to confirm the synthesis of these seven compounds. They were purified by using column chromatography (a 70:30 mixture of n-hexane:ethyl acetate solvent). The proton nuclear magnetic resonance (1H-NMR) peaks confirmed the synthesis of these compounds. There was one proton in the range of 8.27–10.14 ppm as a singlet peak for the N-H amide of each compound; there were 6–9 protons (depending on whether or not the phenyl was substituted) in the aromatic area and another

\[ \text{Table 1: IC}_{50} \text{ of isoxazole compounds and doxorubicin on different cancer cells (Hep3B, HeLa, and MCF-7).} \]

| Code | R Group | IC\(_50\) (µg/ml) |
|------|---------|------------------|
| 2a   | ![Image](image1.png) | 79.43 ± 2.34, 18.62 ± 0.79, 39.80 ± 1.63 |
| 2b   | ![Image](image2.png) | Inactive, 45.70 ± 1.67, 85.10 ± 2.72 |
| 2c   | ![Image](image3.png) | Inactive, Inactive, 75.80 ± 2.36 |
| 2d   | ![Image](image4.png) | 23.98 ± 1.83, 15.48 ± 0.89, Inactive |
| 2e   | ![Image](image5.png) | 23.44 ± 1.99, 32.35 ± 3.05, 63.10 ± 2.14 |
| 2f   | ![Image](image6.png) | 81.28 ± 2.23, Inactive, 98.5 ± 2.57 |
| 2g   | ![Image](image7.png) | Inactive, Inactive, Inactive |
| DOX  | ![Image](image8.png) | 1.25 ± 0.19, 2.13 ± 0.07, 1.55 ± 0.23 |

Note: \( P \) value ≤ 0.05.

Figure 3: 1+ aniline derivatives dissolved in 15 ml DCM, then DMAP and EDC added under nitrogen gas stir for 24 h.
3 protons around 2.66 ppm as a singlet peak, which refers to the methyl group on the isoxazole ring. The $^{13}$C-NMR spectrum showed a C signal of a carbonyl group around 170 ppm; a signal at 12–13 ppm indicated an aliphatic carbon methyl.

3.2. Biological Evaluations

3.2.1. Cytotoxic Evaluation of the Compounds 2a–2g. Table 1 shows the cytotoxic effect of compounds 2a–2g on MCF-7, HeLa, and Hep3B cancer cell lines. Compounds 2d and 2e showed the greatest anticancer activity against Hep3B cells, while the rest of the compounds had a high IC$_{50}$; a compound with an IC$_{50}$ > 100 μg/ml is listed as "inactive" in Table 1. Compound 2d was the most potent against HeLa cells (IC$_{50}$ = 18.62 μg/ml), and compound 2a was also active against HeLa cells (IC$_{50}$ = 39.80 μg/ml). However, the other compounds showed weak anticancer activity against MCF-7 cells (IC$_{50}$ values from 63.10 to 588.80 μg/ml).

3.2.2. Alpha-Fetoprotein Results. According to cytotoxicity results and because 2d and 2e were the most active against Hep3B cells, we evaluated the levels of aFP secreted into the medium to examine the inhibitory effects of the synthesised compound on cell proliferation. Compounds 2d and 2e reduced Hep3B secretion of aFP to $2383.33 \pm 2407.33$ ng/ml, respectively, compared with $2519.2 \pm 198.05$ ng/ml in untreated cells. Compounds 2c and 2f did not reduce aFP secretion (2383.33 and 2407.33 ng/ml, respectively) compared with 2d and 2e (Figure 4). Overall, 2d and 2e have anticancer activity against Hep3B cells (Figure 4).

3.2.3. 2d and 2e Inhibited DNA Cell Cycle of Hep3B Cells. Because 2d and 2e were cytotoxic to Hep3B cells, they were assessed to determine whether they disturbed the cell cycle. Doxorubicin (DOX) was used to a positive control to induce cell cycle progression. The data in Figure 5 show a similar proportion of cells in the G1 phase following treatment with compounds 2d or 2e or DOX. Compounds 2d and 2e also showed similar behaviour to DOX in reducing the percent of cells in the S as well as the G2/M phase ($P < 0.05$). Indeed, 2d and 2e reduced the proportion of cells in the G2/M phase up to 3.6-fold. These data indicate that 2d and 2e markedly delay mitosis, suggesting they have potential anticancer characteristics.
3.3. Antioxidant Evaluation. We estimated the antioxidant activity of hepatocellular cancer cells and thus accelerating their death. Antioxidants were evaluated as Annexin V (+)/PI (-); this population was distinguished from late apoptotic and necrotic cells, which were considered Annexin V (+)/PI (-). Untreated Hep3B cells showed 57.33% ±7.5% apoptotic cells; treatment with 2d and 2e reduced apoptosis to 14.53% ±3.4% and 18.26% ±2.1%, respectively (Figure 6). Treatment with 2d and 2e increased the Annexin V (+)/PI (+) fraction (apoptotic/necrotic cells) to 67.66% ±4.9% and 69.67% ±3.3%, respectively, compared with 41.66% ±1.52% in untreated cells. Our data strongly suggest that compounds 2d and 2e have anticancer properties through increasing the necrotic activity of hepatocellular cancer cells and thus accelerating their death.

3.4. Apoptosis vs. Necrosis Test. We next determined whether 2d and 2e induced apoptosis (programmed cell death) and necrosis. Cells undergoing apoptosis have their PS phospholipids translocated from the inner face of the plasma membrane to the cell surface. Therefore, apoptotic cells can be identified by the presence of PS on the cell surface using Annexin V–FITC staining and flow cytometric analysis. Cells were also stained with PI, which can enter the cell only when the plasma membrane is damaged. Apoptosis was evaluated as Annexin V (+)/PI (-); this population was distinguished from late apoptotic and necrotic cells, which were considered Annexin V (+)/PI (-). Untreated Hep3B cells showed 57.33% ±7.5% apoptotic cells; treatment with 2d and 2e reduced apoptosis to 14.53% ±3.4% and 18.26% ±2.1%, respectively (Figure 6).

Data Availability
The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

Conflicts of Interest
The authors declare that they have no competing interests.

Authors’ Contributions
A.E. and M.H. conceived and designed the current study. A.E., M.H., J.A., A.J., S.Q., I.A., A.S., R.Z., O.H., S.H., and A.M. analyzed the data obtained. This paper was written by A.E., M.H., and J.A. and drafted by all authors. All authors read and approved the final manuscript.

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