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Original Article

Evaluation of 2(3*H*)-Benzoxazolone Derivatives Containing Piperidine Substituents as Cytotoxic and Apoptotic Agents: An *in vitro* and *in silico* Study

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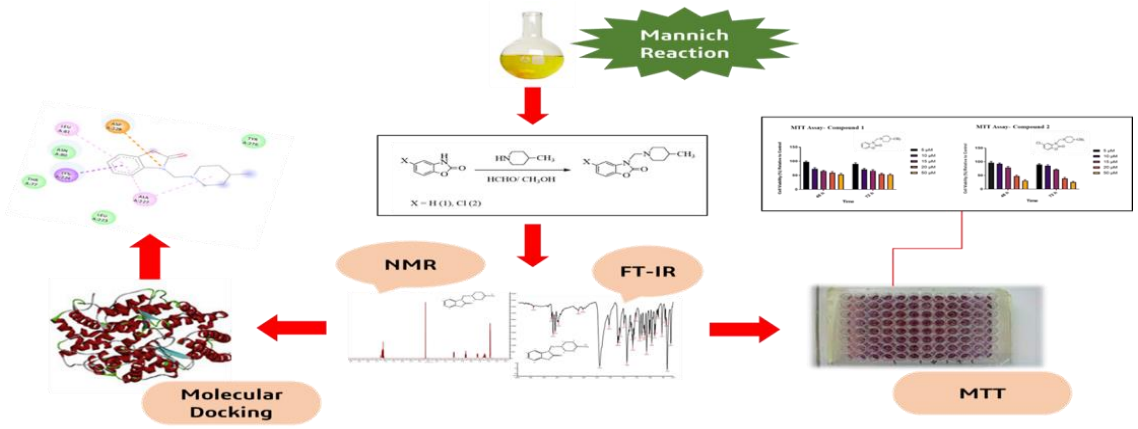
ABSTRACT

To create chemical structures with various pharmacological actions, heterocyclic compounds play a key role as pharmacophores. The pharmacological effects of derivatives of 2(3*H*)-benzoxazolone include analgesic, anti-inflammatory, antibacterial, anti-nociceptive, and anticancer activities. The aim of this study was to examine the cytotoxic and apoptotic effects of newly synthesized 2(3*H*)-benzoxazolone derivatives against metastatic MDA-MB-231 breast cancer cell lines *in vitro* and *in silico*. The structural verifications of target compounds were performed by elemental analysis, FT-IR, and NMR spectra. MTT assay was used to assess the cytotoxic effects of the compounds in terms of the decreased cell viability. TUNEL assay was used to confirm the apoptotic activities of the compounds. The MTT results revealed that compound **2**, which has a chlorine substituent at the 5th position of the core structure, had the maximum activity at a concentration of 50 µM during a 72-hour incubation period. The results demonstrated that 2(3*H*)-benzoxazolone derivatives with piperidine substituents efficiently reduced the cell survival in the target cell line. The molecular docking results also supported the experimental data. Furthermore, the *in-silico* investigation demonstrated that the synthesized compounds have desirable drug-like characteristics for the oral drug-delivery system. In addition, the findings showed that chlorination of the benzoxazolone ring influences the apoptotic activity, suggesting that these derivatives might be promising innovative anticancer medications in the future.

GRAPHICAL ABSTRACT



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Introduction

Cancer is the second largest cause of mortality worldwide and a potentially fatal condition [1]. Nearly 10 million cancer-related deaths were recorded by the World Health Organization (WHO) in 2020 [1]. The most frequent malignancy in women is breast cancer [2]. Anticancer research is crucial for the development of novel lead compounds, particularly in women because of the rise in breast cancer cases globally. Breast cancer research and novel drug synthesis models are being reported with an increase in breast incidence and mortality [3-9]. Given that there are several active sites on its basic structure, the 2(3*H*)-benzoxazolone scaffold plays a significant role in medicinal chemistry. The pharmacological effects of 2(3*H*)-benzoxazolone derivatives include analgesic [10], anti-inflammatory [10], antiviral [11], antifungal [12], anti-nociceptive [13], anticancer [14], and anticonvulsant [15] effects. Nevertheless, there are very few studies available that examine the cytotoxic and apoptotic effects of compounds based on benzoxazolone. Bilginer *et al.* have investigated 6-(3-aryl-2-propenoyl)-2(3*H*)-benzoxazolone derivatives for their antiproliferative efficacy against the colon cancer cell line, HCT116 [16]. In the same study, caspase-3 activation and DNA measurements were utilized to assess apoptosis in the treated cells, while lactate dehydrogenase (LDH) activity was evaluated to analyse necrosis [16]. In another study, similar chalcone-like benzoxazolone derivatives were synthesized, examined for cytotoxicity against the different human leukemia cell lines, and were found to be effective [17].

Disubstituted piperidines were also reported to demonstrate various biological activities in the literature [18]. In recent years, there have been several publications on studies evaluating the cytotoxicity of piperidine derivatives [19, 20]. Due to their high reactivity, Mannich bases, including piperidine derivatives, are crucial in the discovery of synthetic pathways in pharmaceutical chemistry [21]. Ognyan *et al*. investigated the potential anticancer properties of benzoxazolone Mannich bases containing trimethoxyphenyl propenoyl groups at positions 6 in the primary structure [22]. This study examined the target compounds for their cytotoxicity and discovered mild to moderate activity against a number of leukemia and breast cancer cell lines, including SKW-3, MDA-MB-231, HL-60, and BV-173. BV-173 had the highest chemosensitivity to the investigated compounds, followed by SKW-3 and HL-60 cell lines [22].

Apoptosis has been defined as programmed cell death or cell suicide. Apoptosis is an energy dependent and physiological event [23]. Numerous illnesses and diseases can be brought on by the apoptotic program being dysfunctional or out of balance [23]. In cancer cells, it is a targeted process. One of the most important and well-known indicators of apoptosis is caspase-3 [24]. It is a member of the cysteine-aspartate protease family, one of the six protease families with significant roles in both neuropathology and normal neuronal development. Caspases-3 eventually causes apoptosis, which involves DNA strand damage and cleavage, for both mechanistic routes [24]. Caspase-3 activity suggests that the downstream portion of apoptotic pathway has been reached. Therefore, caspase-3 is an important target in cancer research. Consequently, regardless of the intrinsic or extrinsic mechanism, apoptosis is a desired state in cancer cells. Among breast cancer cell lines, MDA-MB-231 is a triple-negative breast cancer (TNBC) cell line that is extremely aggressive, invasive, and poorly differentiated because it lacks the expression of the oestrogen receptor (ER), the progesterone receptor (PR), and HER2 (human epidermal growth factor receptor 2) [25]. MDA-MB-231 is one of the most commonly used cell lines in breast cancer research. The TUNEL assay may be used to analyse the DNA fragmentation during apoptosis by using fluorescence microscopy or flow cytometry techniques [26, 27]. Furthermore, the quantitative real-time polymerase chain reaction approach may detect the quantity of growth arrest-specific 2 (GAS2) in tissues [28].

All of these prior findings and a comprehensive literature review showed that compounds with benzoxazolone and piperidine scaffolds have

considerable cytotoxic effects on several cancer cell types including MDA-MB-231. Further investigations incorporating cytotoxicity assessments of piperidine containing benzoxazolone derivatives indicate the potential in the creation of effective chemotherapeutic drugs. Based on these previous findings in the literature, the current work was launched with the goal of screening synthesized derivatives for the cytotoxic and apoptotic effects of benzoxazolone derivatives against MDA-MB-231 cell line. The experimental data were further supported by in silico molecular docking and ADME analysis for drug likeness of synthesized compounds. *In silico* molecular docking and ADME study for drug likeness of synthesized compounds supported the experimental findings.

Materials and methods

All chemicals were purchased from Merck (formerly Sigma-Aldrich, Darmstadt, Germany). Mettler Toledo FP900 Thermosystem (Mettler-Toledo, Greifensee, Switzerland) was used to measure the melting points and the data were uncorrected. FT-IR spectra were recorded as films on a Spectrum Two FT-IR Spectrometer (PerkinElmer, Inc., Waltham, MA, USA). The NMR spectra of substances synthesized in this work were obtained by using a Jeol 400 MHz spectrometer (Peabody, MA, USA). The chemical shifts were recorded in parts per million (ppm) units and tetramethylsilane (TMS) was used an an internal reference. The splitting patterns are denoted by the letters s (singlet), d (doublet), t (triplet), q (quartet), and m (multiplet). Elemental analyses were examined for C, H, and N on Leco CHNS 932 analyser (Leco-932, St. Joseph, MI, USA) and the analyses were within ± 0.4% of the theoretical values.

General synthesis method

Mannich reaction was used for the synthesis, according to a previously described method [29]. 20 mmol 2(3H)-benzoxazolone derivatives and 20 mmol of 4-methylpiperidine were dissolved separately in the appropriate amount of methanol and mixed. Following that, 20 mmol of 37% w/v formaldehyde solution was added. The mixture was heated under reflux for 2 hours. After dumping the liquid into the ice bath, the significant precipitation occurred with a light yellow colour. The precipitate was filtered, rinsed with cold methanol, vacuum dried, and recrystallized in ethanol.

*3-{[4-methylpiperidin-1-yl]methyl}-2-benzoxazolone (Compound **1**)*

Yellow powder, Yield: 81.50%; mp 92-94 °C, IR (KBr) (ν_{max}/ cm^{-1}): 2759-2949 (C-H), 1760 (lactam, C=O) cm^{-1}; ^{1}H-NMR (400 MHz, $CDCl_3$): δ 7.1-6.7 (m, 4H; Aromatic), 4.6 (s, 2H, methylene), 3.1 and 1.6 (dd, 4H, piperidine H^6-H^8), 2.3 (t, 4H, piperidine H^5-H^9), 1.2-1.4 (m, 1H, piperidine H^7), 0.8 (d, 3H, H^{10}), ^{13}C-NMR (100 MHz, $CDCl_3$): δ 155.4, 143.3, 131.2, 124.6, 121.5, 108.7 (Aromatic-C), 65.2 (methylene-CH_2), 51.2, 34.0, 30.1 (piperidine-C), 21.7 (methyl-CH_3). Anal. Calc. for $C_{14}H_{18}N_2O_2$ C, 68.27; H, 7.37; N, 11.37; Found C, 68.24; H, 7.34; N, 11.35.

*5-chloro-3-{[4-methylpiperidin-1-yl]methyl}-2-benzoxazolone (Compound **2**)*

Yellow powder, Yield: 74.5%; mp 108-110 °C; IR (KBr) (ν_{max}/ cm^{-1}): 2759-2949 (C-H), 1760 (lactam, C=O) cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.1-7.3 (m, 3H, Aromatic), 4.6 (s, 2H, H^4), 3.1 and 1.6 (dd, 4H, piperidine H^6-H^8), 2.3 (t, 4H, piperidine H^5-H^9), 1.2-1.4 (m, 1H, piperidine H^7), 0.8 (d, 3H, H^{10}). ^{13}C-NMR (100 MHz, $CDCl_3$): δ 158.5, 143.3, 131.2, 124.6, 121.5, 108.7 (Aromatic-C), 65.2 (methylene-CH_2), 51.2, 34.0, 30.1 (piperidine-C), 21.7 (methyl-CH_3). Anal. Calc. for $C_{14}H_{17}ClN_2O_2$ C, 59.89; H, 6.10; N, 9.98; Found C, 59.81; H, 6.08; N, 9.96.

Cell culture and MTT assay procedure

Dulbecco's modified eagle medium (DMEM) (Gibco) was used to grow the human breast cancer cell lines MDA-MB-231 (HTB-26, ATCC), which were supplemented with 10% fetal bovine serum (FBS), human insulin of 4 mg/mL, and 1% penicillin/streptomycin. The cells were kept at a constant temperature of 37 °C in a humidified incubator with 5% CO_2. By using the MTT assay, the reduction in cell viability was examined. All stock solutions of compounds were dissolved in

dimethyl sulfoxide (DMSO, Sigma-Aldrich) and diluted in growth media at various concentrations. In 96-well plates (2×10^3 cells/well), cells were plated. Cells were serum deprived for 24 hours in culture media with 0.1% FBS after they had reached around 80% confluence. 500 ng/mL to 2.5 g/mL of RANKL (Sigma-Aldrich) was used to stimulate cells for 24 hours. The media was changed for 200 µl of new medium, and each well received 50 µl of MTT reagent (5 mg/mL; Sigma-Aldrich). After 4 hours incubation period at 37 °C, the medium was changed for 200 µl of DMSO and 25 µl of glycine buffer. An ELISA reader (Labsystem Multiskan Ms) operating at 570 nm was used to measure the absorbance. Untreated cells were assumed to have a 100% absorbance rate. The studies and chemical dilutions were carried out three times.

TUNEL assay and apoptosis

By using a commercial in situ apoptosis detection kit (*In Situ* Cell Death Detection Kit AP, Roche), DNA fragmentation was discovered by labelling the apoptotic cells with a particular staining. The DNA fragmentation results from the activation of Ca/Mg-dependent endonuclease enzymes in apoptotic cells. On 6-well plates with coverslips, 5×10^4 MDA-MB-231 cells were cultured for 24 hours. The cells were fixed in a 4% paraformaldehyde solution after being rinsed with PBS. To improve permeability, the cells were treated in 0.1% Triton X-100 in 0.1% sodium citrate for 1 hour at 4 °C. The cells were then exposed to compound **1** and compound **2**, respectively for 1 hour at 37 °C in the dark. Cells were further exposed to the medium alone. A light microscope was used for analysis (Olympus BX40, Tokyo, Japan).

Molecular docking and ADME analysis

The synthesized compounds and X-ray crystal structure of human caspase-3 (PDB ID: 5I9B) in complex with PRD_000238, native ligand [30-32] were prepared by using LigPrep and Protein Preparation Wizard in Maestro of Schrödinger-2021 software package, respectively [33-35]. The resolution of the crystal structure of human caspase-3 was determined as 1.80 Å. The standard precision module of the Schrödinger Suite (Glide SP) was utilized to perform the molecular docking calculations [36]. The native ligand's docking pose and crystal conformation were discovered to be 1.291 Å. The 2D interactions were investigated by using Discovery Studio Visualizer software. The pharmacokinetic characteristics and drug-likeness of the synthesized compounds were assessed by using the preADMET prediction service depending on the 2D molecular structure of the studied derivatives.

Statistical analysis

Data were expressed as mean ± standard deviation (SD). GraphPad Prism 9 software was used to evaluate the results. Where relevant, differences between groups were analysed statistically by using the Mann-Whitney test, with a p-value <0.05 indicating statistical significance.

Results and Discussion

Synthesis and molecular docking

Through the Mannich reaction, benzoxazolone derivatives, and 4-methylpiperidine were used to create Mannich bases, as displayed in Figure 1. Condensation in the formaldehyde presence resulted in the formation of a methylene bridge between these two structures. Indicating that the Mannich reaction was successful, the synthesized compounds had IR stretching bands of the lactam ring at 1760 cm^{-1} (C=O) and lacked the N-H bands of the piperidine and benzoxazolone rings, which typically are presented between 3100 and 3400 cm^{-1}. At 4.6 ppm, a singlet signal was detected, demonstrating the presence of a methylene bridge. In addition, the aromatic protons in ^{1}H-NMR spectra are visible as a multiplet between 6.7 and 7.3 ppm. The integral values match to the proposed structures of the synthesized compounds.

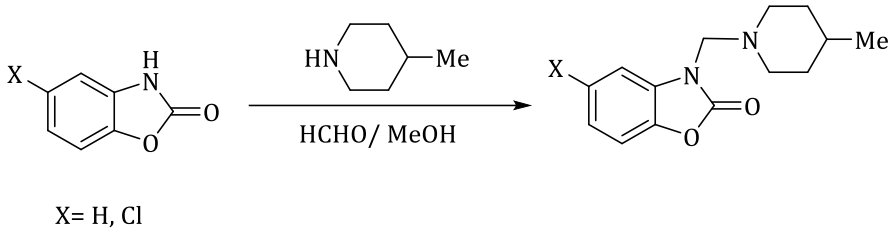

Figure 1: General synthesis method of compounds **1** and **2**

Furthermore, a docking study was performed on synthesized compounds against caspase-3 enzyme. The docking scores and their common interacting amino acids with the active site of caspase-3 are listed in Table 1 for each derivative and the native ligand (PRD_000238). The 2D ligand-receptor interactions and electrostatic investigation of compound **1** and compound **2** in the active site of caspase-3 are depicted in Figures 2 and 3, respectively. Compared the synthesized compounds with the native ligand, the two compounds had similar docking scores and shared interaction residues. Moreover, both compounds produced an H-bond with THR77 and TYR276, which is thought to be one of the essential amino acids for caspase-3 activation. On the other hand, a hydrophobic pocket including the amino acid residues LEU81, LEU223, and LYS224 was created when a chlorine substituent was presented at position 5 of the benzoxazolone ring. This hydrophobic pocket appears to affect docking scores, implying that in future studies, any additional hydrophobic groups, such as small-chain alkyl groups like methyl or ethyl substituents could be replaced with chlorine at position 5 and tested for their effects on how similar derivatives interact with caspase-3.

Table 1: The docking scores of the synthesized derivatives and their common amino acid interactions with the native ligand in the binding pocket of caspase-3

Compound's Name	Docking Score (kcal/mol)	Interacting Amino Acids
Native Ligand (PRD_000238)	-8.582	THR77, ARG207, SER209, LEU223, LYS224, TYR276.
Compound **1**	-7.245	THR77, LEU223, LYS224, TYR276
Compound **2**	-7.868	THR77, LEU223, LYS224, TYR276

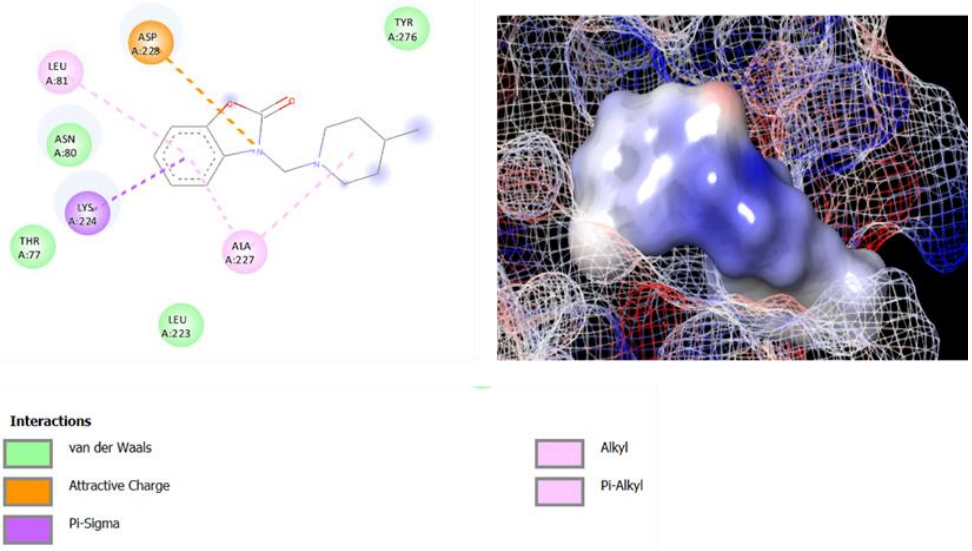

Figure 2: The 2D ligand-receptor interactions (on the left) and electrostatic investigation (on the right) of compound **1** in the caspase-3 active site

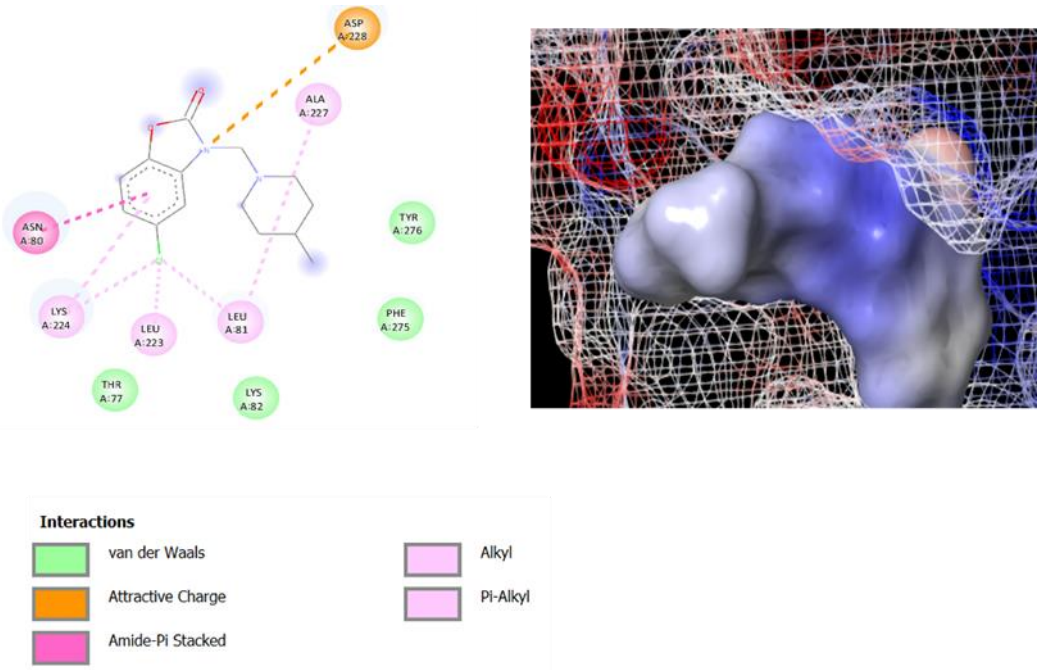

Figure 3: The 2D ligand-receptor interactions (on the left) and electrostatic investigation (on the right) of compound 2 in the caspase-3 active site

ADME properties and drug-likeness assessment

The analysis of the ADME characteristics of bioactive compounds is a key restriction throughout drug development that influences compound selection [37]. Around half of the applicants failed throughout the early stages of development due to unsatisfactory ADME profiles [38]. To avoid this failure, in-silico approaches for predicting pharmacokinetic features of the bioactive compounds and guiding the early phases of drug development have been efficiently employed. Furthermore, investigating pharmacokinetic features using in silico techniques prior to do the experimental studies assists in the identification of potential lead molecules. The evaluation of ADME properties were summarized in Table 2. The synthesized compounds initially displayed a moderate Caco-2 cell line permeability and an outstanding human intestinal absorption (HIA) value of 99%.

These results suggest that the other processes besides a passive diffusion may be involved in the absorption via the gut. This is due to the lack of carrier proteins, cells responsible for the secretion of mucus, and extracellular elements, which can similarly affect absorption in Caco-2 cell models [39]. In addition, the Caco-2 system's tight connections reduce the amount of substances that may pass through to be absorbed paracellularly [39]. Furthermore, the compounds were investigated to block the CYP2C9 enzyme, which might result in drug interactions with medications depending on CYP2C9 for their metabolism. Since just the free portion of the drug is responsible for pharmacological effect [40], the high plasma protein binding capacity might impact the drugs' efficacy. Both compounds' plasma protein binding capacities were not particularly high. Therefore, they could exhibit the optimal plasma half-life, distribution volume, and clearance rates. Moreover, the synthesized compounds were impermeable across blood-brain barrier. When an attempt to prevent or address the brain functions throughout the drug design and synthesis stage, brain penetration is a crucial issue to take into account. The danger of harmful CNS side effects and toxicity is decreased or eliminated when compounds are not permeable to the blood-brain barrier [41].

Besides, Lipinski's rule of five consists of four physicochemical parameter criteria [42], which include the H-bond donors and acceptors, molecular mass, and logP value that define the

drug-likeness for the oral administration system. In general, oral medications need these criteria to exhibit excellent intestinal permeability and water solubility profiles. Poor bioavailability and absorption are probably going to occur. The *in-silico* study revealed that all synthesized compounds followed Lipinski's rule. Thus, these compounds have a greater chance of commercial success due to the decreased attrition rates throughout drug design and clinical investigations.

Cell viability

Target compounds were administered to MDA-MB-231 cells at several doses (5, 10, 15, 20, and 50 µM) for 48 and 72 hours, respectively. The acquired data demonstrated a dosage and time-dependent substantial reduction in cell viability for all doses of both compounds compared with the control group. The MTT results of compound 1 are demonstrated in Figure 4. According to the results, compound 2 with a chlorine substituent in place of hydrogen at position 5 of the primary structure was generally more effective at lowering the percentage of cell viability. In addition, both compounds were most efficient in inhibiting cell growth for a 72-hour incubation period at 50 µM concentrations.

cells

The MDA-MB-231 cells that had been treated with 50 µM doses of both compounds for 72 hours, the apoptotic effects of the investigated compounds were assessed by using the TUNEL assay. The findings show that both compounds are efficient in preventing DNA fragmentation in MDA-MB-231 cells during apoptosis. The results are summarized in Table 3. TUNEL analysis of DNA fragmentation in MDA-MB-231 cells treated with synthesized compounds under light microscope is indicated in Figure 5.

Table 2: Some important *in silico* based pharmacokinetic properties of synthesized compounds

Compound	Lipinski's rule of five	BBB	HIA%	PPB %	Caco2-P (nm/sec)	CYP2C9 blockage
Compound **1**	Fit	0.21	99.39	47.18	40.43	Nil
Compound **2**	Fit	0.44	99.09	64.61	51.80	Nil

BBB: Blood-brain barrier penetration of the compound, HIA: Human intestinal absorption, PPB: Plasma protein binding ability, Caco2-P: Caco2 (human colorectal carcinoma) cell permeability, and CYP2C9: Cytochrome-P450 2C9.

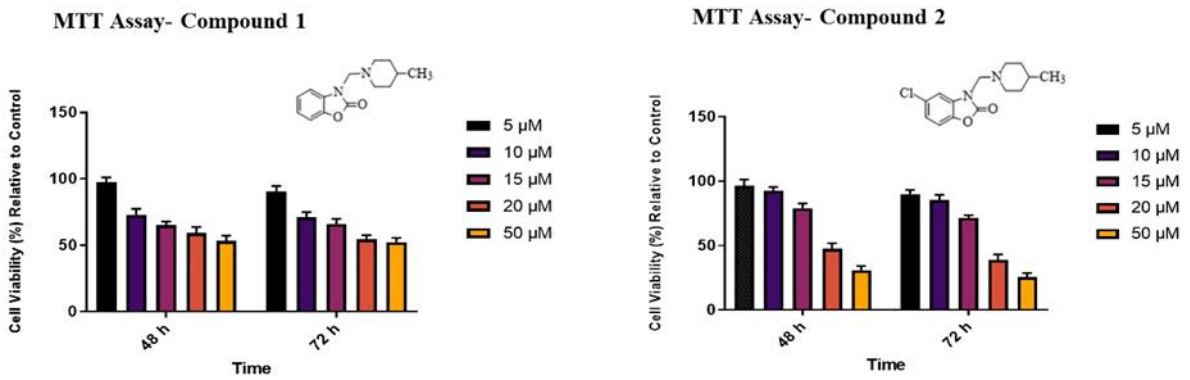

Figure 4: The effects of compound **1** and compound **2** on cell viability of MDA-MB-231 cells

Table 3: The results were reported as means ± SD and were significant compared with the control group for compound 1 ($p < 0.01$) and compound 2 ($p < 0.001$). Data were compared by using the Mann-Whitney test

Compound 1	Compound 2	Control Group
71.63 ± 10.98	83 ± 6	34.84 ± 4.02

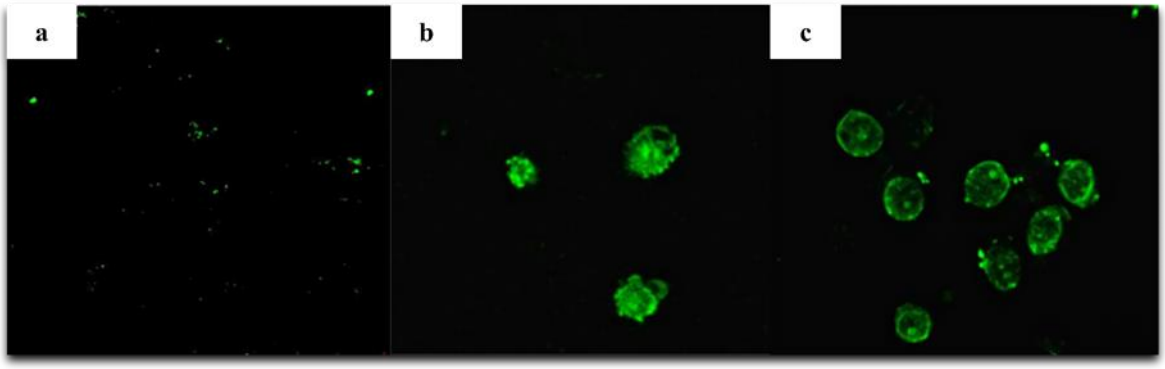

Figure 5: Evaluation of DNA fragmentation by TUNEL assay in MDA-MB-231 cells exposed to synthesized compounds. a) Cells without treatment b) Cells treated with 50 µM of compound **1** for 72 hours c) Cells treated with 50 µM of compound **2** for 72 hours

Conclusion

The cytotoxic and proapoptotic characteristics of these newly synthesized 2(3*H*)-benzoxazolone derivatives against MDA-MB-231 cell line were screened for the first time. Inhibiting the growth, proliferation, and induction of apoptosis in these metastatic breast cancer cells was more effectively accomplished by compound **1** than by compound **2**. Furthermore, a TUNEL assay investigation was used to confirm these findings. The TUNEL assay data revealed that both compounds were capable of inducing DNA fragmentation during apoptosis in MDA-MB-231 cells. These results clearly demonstrated that the substitution of 5th position of the main ring affected the apoptotic process. The presence of a chlorine substituent (compound **2**), on the other hand, altered docking scores in molecular docking analysis, providing a much more desired result. A comprehensive investigation of how different types of substituents affect all possible cancer pathways through connected derivatives of these substances would be valuable. Using immunohistochemical analyses, the additional evaluation of these drug candidates' possible apoptotic pathways could be carried out.

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Authors' contributions

All authors contributed to data analysis, drafting, and revising of the paper and agreed to be responsible for all the aspects of this work.

Conflict of Interest

The author declared that they have no conflict of interest.

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References

[1]. Sung H., Ferlay J., Siegel R.L., Laversanne M., Soerjomataram I., Jemal A., Bray F., Global cancer statistics 2020: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries, *CA: a Cancer Journal for Clinicians*, 2021, **71**:209 [Crossref], [Google Scholar], [Publisher]

[2]. Zhang R., Yang Y., Dong W., Lin M., He J., Zhang X., Tian T., Yang Y., Chen K., Lei Q.Y., Zhang S., Xu Y., Lv L., D-mannose facilitates immunotherapy and radiotherapy of triple-negative breast cancer via degradation of PD-L1, *Proceedings of the National Academy of Sciences*,

2022, **119**:e2114851119 [Crossref], [Google Scholar], [Publisher]

[3]. Fadhil H.A., Samir A.H., Mohammed Y.A., Al. Rubaei Z.M.M., 'Synthesis, characterization, and in vitro study of novel modified reduced graphene oxide (RGO) containing heterocyclic compounds as anti-breast cancer', *Eurasian Chemical Communications*, 2022, **4**:1156 [Crossref], [Google Scholar], [Publisher]

[4]. Al Abdeen S.H.Z., Mustafa Y.F., Mutlag S.H., 'Synthesis of disubstituted anisolodipyrone-derived ester compounds: The search for new bioactive candidates', *Eurasian Chemical Communications*, 2022, **4**:1171 [Crossref], [Google Scholar], [Publisher]

[5]. Nashaan F.A., Al-Rawi M.S., Alhammer A., Rabie A.M., Tomma J.H., 'Synthesis, characterization, and cytotoxic activity of some imides from galloyl hydrazide', *Eurasian Chemical Communications*, 2022, **4**:966 [Crossref], [Google Scholar], [Publisher]

[6]. Lauro F., Francisco D., Marcela R., Virginia M., Elizabeth M., Lenin H., Maria L., Elodia G., Eduardo P., Regina C., Alondra A., Jhaira C., Design and synthesis of two steroid derivatives from 2-nitroestrone and theoretical evaluation of their interaction with BRCA-1, *Asian Journal of Green Chemistry*, 2019, **3**:216 [Crossref], [Google Scholar], [Publisher]

[7]. Alimoradzadeh R., Moosavi N., Karimkoshteh A., Sadeghi Z., Milanifard M., Ismaili A., Investigation of the Chemistry of Metformin by Targeting the Nrf2 Signaling Pathway (A response Surface Methodology Approach), *Chemical Methodologies*, 2022, **6**:166 [Crossref], [Google Scholar], [Publisher]

[8]. Abdulghani S., Al-Rawi M., Tomma J., Synthesis of New 1, 2, 4-triazole Derivatives with Expected Biological Activities, *Chemical Methodologies*, 2022, **6**:59 [Crossref], [Google Scholar], [Publisher]

[9]. Moghaddam A., Zamani H., Karimi-Maleh H., A new sensing strategy for determination of tamoxifen using Fe3O4/graphene-ionic liquid nanocomposite amplified paste electrode, *Chemical Methodologies*, 2021, **5**:373 [Crossref], [Google Scholar], [Publisher]

[10]. Tugcu G., Koksal M., A QSAR Study for Analgesic and Anti-inflammatory Activities of 5-/6-Acyl-3-alkyl-2-Benzoxazolinone Derivatives, *Molecular Informatics*, 2019, **38**:e1800090 [Crossref], [Google Scholar], [Publisher]

[11]. Moitra P., A combinatorial approach of structure-based virtual screening and molecular dynamics simulation towards the discovery of a highly selective inhibitor for VP9 coat protein of Banna virus, *Bioorganic Chemistry*, 2019, **86**:15 [Crossref], [Google Scholar], [Publisher]

[12]. Petrov O., Gerova M., Petrova K., Ivanova Y., New imidazole derivatives of 2 (3H)-benzazolones as potential antifungal agents, *Journal of Heterocyclic Chemistry*, 2009, **46**:44 [Crossref], [Google Scholar], [Publisher]

[13]. Chanchal S., Rajnish K., Avijit M., Ajay K., Rakesh S., Shivali M., Mohd A.M., Benzothiazole: Synthetic Strategies, Biological Potential, and Interactions with Targets, *Mini-Reviews in Organic Chemistry*, 2022, **19**:242 [Crossref], [Google Scholar], [Publisher]

[14]. Erdag E., "Synthesis of Novel 2(3H)-Benzoxazolone Mannich Bases as Potential Agents for Future Studies of Cancer Treatment", *Journal of Pharmaceutical Research International*, 2021, **32**:23 [Crossref], [Google Scholar], [Publisher]

[15]. Gambacorta G., Baxendale I.R., Continuous-Flow Hofmann Rearrangement Using Trichloroisocyanuric Acid for the Preparation of 2-Benzoxazolinone, *Organic Process Research & Development*, 2022, **26**:422 [Crossref], [Google Scholar], [Publisher]

[16]. Bilginer S., Bardaweel S.K., Sabbah D.A., Gul H.I., Docking studies and antiproliferative activities of 6-(3-aryl-2-propenoyl)-2 (3h)-benzoxazolone derivatives as novel inhibitors of phosphatidylinositol 3-kinase (PI3Kα), *Anti-Cancer Agents in Medicinal Chemistry (Formerly Current Medicinal Chemistry-Anti-Cancer Agents)*, 2021, **21**:716 [Crossref], [Google Scholar], [Publisher]

[17]. Ivanova Y., Momekov G., Petrov O., Karaivanova M., Kalcheva V., Cytotoxic Mannich bases of 6-(3-aryl-2-propenoyl)-2 (3H)-benzoxazolones, *European Journal of Medicinal Chemistry*, 2007, **42**:1382 [Crossref], [Google Scholar], [Publisher]

[18]. Seck R., Gassama A., Cojean S., Cavé C. Synthesis and antimalarial activity of 1, 4-disubstituted piperidine derivatives, *Molecules*, 2020, **25**:299 [Crossref], [Google Scholar], [Publisher]

[19]. Syame S.M., Mohamed S.M., Elgabry E.A., Darwish Y.A.A., Mansour A.S., Chemical characterization, antimicrobial, antioxidant, and cytotoxic potentials of Swietenia mahagoni, *AMB Express*, 2022, **12**:77 [Crossref], [Google Scholar], [Publisher]

[20]. Mitra S., Anand U., Jha N.K., Shekhawat M.S., Saha S.C., Nongdam P., Rengasamy K.R.R., Proćków J., Dey A., Anticancer applications and pharmacological properties of piperidine and piperine: a comprehensive review on molecular mechanisms and therapeutic perspectives, *Frontiers in Pharmacology*, 2022, **12**:772418 [Crossref], [Google Scholar], [Publisher]

[21]. Roman G., Mannich bases in medicinal chemistry and drug design, *European Journal of Medicinal Chemistry*, 2015, **89**:743 [Crossref], [Google Scholar], [Publisher]

[22]. Ognyan P.I., Yordanka I.B., Mariana G.S., Georgi M.T., Synthesis and cytotoxicity of new Mannich bases of 6-[3-(3, 4, 5-Trimetoxyphenyl)-2-propenoyl]-2 (3H)-benzoxazolone, *Letters in Drug Design & Discovery*, 2020, **17**:515 [Crossref], [Google Scholar], [Publisher]

[23]. Elmore S., Apoptosis: a review of programmed cell death, *Toxicologic pathology*, 2007, **35**:495 [Crossref], [Google Scholar], [Publisher]

[24]. Julien O., Wells J.A., Caspases and their substrates, *Cell Death & Differentiation*, 2017, **4**:1380 [Crossref], [Google Scholar], [Publisher]

[25]. Simu S., Marcovici I., Dobrescu A., Malita D., Dehelean C.A., Coricovac D., Olaru F., Draghici G.A., Navolan D., Insights into the behavior of triple-negative mda-mb-231 breast carcinoma cells following the treatment with 17β-ethinylestradiol and levonorgestrel, *Molecules*, 2021, **26**:2776 [Crossref], [Google Scholar], [Publisher]

[26]. Jamalvandi M., Khanahmad H., Irani S., Bastaminejad S., *Journal of Isfahan Medical School*, 2020, **38**:694 [Crossref], [Publisher]

[27]. Jamalvandi M., Khanahmad H., Irani S., Bastaminezhad S., *Selection and Characterization of single-stranded DNA aptamers against interleukin-5*, 2019, **14**:515 [Crossref], [Google Scholar], [Publisher]

[28]. Izadi-Ajeerlo B., Bastaminejad S., Basati G., Upregulated expression of the growth arrest-specific-2 (gas2) gene in colorectal cancer, and its relation to cancer progression and prognosis, *Journal of Isfahan Medical School,* 2019, **37**:93 [Crossref], [Google Scholar], [Publisher]

[29]. Gökhan N., Köksal M., Küpeli E., Yeşilada E., Erdoğan H., Some new Mannich bases of 5-methyl-2-benzoxazolinones with analgesis and anti-inflammatory activities, *Turkish Journal of Chemistry,* 2005. **29**:445 [Crossref], [Google Scholar], [Publisher]

[30]. Kirubhanand C., Selvaraj J., Rekha U.V., Vishnupriya V., Nalini D., Mohan S.K., Vijayalakshmi P., Rajalakshmi M., Ponnulakshmi R., Bioinformation, Molecular docking data of piperine with Bax, Caspase 3, Cox 2 and Caspase 9, *Bioinformation*, 2020, **16**:458 [Crossref], [Google Scholar], [Publisher]

[31]. Hossain S.L., Mathews M., Nagarajappa V.S.B., Kumar B.K., Yelamaggad C.V.V., Singh C.R., Antiproliferative, apoptosis-inducing activity and molecular docking studies of sydnones compounds, *Journal of Cancer Research and Therapeutics*, 2021, **18**:681 [Crossref], [Google Scholar], [Publisher]

[32]. Al-Jumaili M.H.A., Siddique F., Abul Qais F., Hashem H.E., Chtita S., Rani A., Uzair M., Almzaien K.A. Analysis and prediction pathways of natural products and their cytotoxicity against HeLa cell line protein using docking, molecular dynamics and ADMET, *Journal of Biomolecular Structure*

and Dynamics, 2020, 1-13 [Crossref], [Google Scholar], [Publisher]

[33]. Greenwood J.R., Calkins D., Sullivan A.P., Shelley J.C., Towards the comprehensive, rapid, and accurate prediction of the favorable tautomeric states of drug-like molecules in aqueous solution, *Journal of Computer-aided Molecular Design*, 2010, **24**:591 [Crossref], [Google Scholar], [Publisher]

[34]. Shelley J.C., Chollet A., Frye L., Greenwood J.R., Timlin M.R., Uchimaya M., Epik: a software program for pK a prediction and protonation state generation for drug-like molecules, *Journal of Computer-aided Molecular Design*, 2007, **21**:681 [Crossref], [Google Scholar], [Publisher]

[35]. Sastry G.M., Adzhigirey M., Day T., Annabhimoju R., Sherman W., Protein and ligand preparation: parameters, protocols, and influence on virtual screening enrichments, *Journal of Computer-aided Molecular Design*, 2013, **27**:221 [Crossref], [Google Scholar], [Publisher]

[36]. Friesner R.A., Banks J.L., Murphy R.B., Halgren T.A., Klicic J.J., Mainz D.T., Repasky M.P., Knoll E.H., Shelley M., Perry J.K., Shaw D.E., Francis P., Shenkin P.S., Glide: a new approach for rapid, accurate docking and scoring. 1. Method and assessment of docking accuracy, *Journal of Medicinal Chemistry*, 2004, **47**:1739 [Crossref], [Google Scholar], [Publisher]

[37]. Hughes J.P., Rees S., Kalindjian S.B., Philpott K.L., Principles of early drug discovery, *British Journal of Pharmacology*, 2011, **162**:1239 [Crossref], [Google Scholar], [Publisher]

[38]. Alavijeh M.S., Palmer A.M., The pivotal role of drug metabolism and pharmacokinetics in the discovery and development of new medicines, *Curr. Opin. Investig. Drugs*, 2004, **7**:755 [Google Scholar], [Publisher]

[39]. Lemmens G., Van Camp A., Kourula S., Vanuytsel T., Augustijns P., Drug Disposition in the Lower Gastrointestinal Tract: Targeting and Monitoring, *Pharmaceutics*, 2021, **13**:161 [Crossref], [Google Scholar], [Publisher]

[40]. Chaudhry S.R., Muhammad S., Eidens M., Klemm M., Khan D., Efferth T., Weisshaar M.P., Pharmacogenetic prediction of individual variability in drug response based on CYP2D6, CYP2C9 and CYP2C19 genetic polymorphisms, *Current Drug Metabolism*, 2014, **15**:711 [Crossref], [Google Scholar], [Publisher].

[41]. Pardridge W.M., Drug transport across the blood–brain barrier, *Journal of Cerebral Blood Flow & Metabolism*, 2012, **32**:1959 [Crossref], [Google Scholar], [Publisher]

[42]. Benet L.Z., Hosey C.M., Ursu O., Oprea T.I. BDDCS, the Rule of 5 and drugability, *Advanced Drug Delivery Reviews*, 2016, **101**:89 [Crossref], [Google Scholar], [Publisher]

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