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Investigation of some 3-substituted-2(3H)-benzoxazolone derivatives against caspase-3 : A molecular docking study

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Abstract

In the development of pharmacological probes, 2(3H)-benzoxazolones are viewed as privileged scaffolds. The functionalization of the nitrogen atom at the third position of the 2(3H)-benzoxazolone moiety is of importance because the electrical properties of this atom can be crucial for biological activity. The purpose of this study was to use in silico techniques to examine the affinities of 3-substituted-2(3H)-benzoxazolone derivatives against the caspase-3 enzyme. These compounds are predicted to have strong anticancer action on several cancer cell lines. In order to determine the potential binding modes of compounds with similar structures synthesized in this study and earlier studies with caspase-3, molecular docking studies were conducted. It was found that the majority of the compounds formed hydrogen bonds with the Arg207 amino acid residue, which is thought to be crucial in activities. The design and manufacture of future therapeutic compounds with anticancer properties may therefore benefit from understanding provided by this research.

Keywords: 2(3H)-Benzoxazolone; Mannich reaction; Piperazine; Caspase-3; Molecular docking

1. Introduction

A report from the World Health Organization (WHO) estimates that by 2030, 13.1 million people will die from cancer [1]. Chemotherapeutics are alkylating anticancer medications that are commonly utilized in clinics for cancer therapy [2]. In general, common side effects associated with chemotherapeutic drugs are nausea, vomiting and dose-related myelosuppression, although more specific toxicities may be present [3]. From a clinical point of view, drug toxicity appears to be an additional cause of morbidity and mortality in patients [4]. The drug resistance problems and low selectivity of such drugs encourage researchers to develop anticancer drug candidates that will be safer, more selective, and will have fewer side effects.

2(3*H*)-Benzoxazolone derivatives are essential compounds in pharmacological probe development because different chemical alterations can be done at various places in the core structure [5]. The derivatives synthesized over the nitrogen atom in the third position of the benzoxazolone ring have a wide range of biological activity [6]. Compounds with benzoxazolone in their structure have been shown to have analgesic [7], anti-inflammatory [8], antibacterial [9], and cytotoxic [10] properties. However, there are just a few cytotoxic activity investigations of these molecules in the literature. According to *Ivanova et al*., chalcone compounds with benzoxazolone in their structure have antileukemic effects through triggering apoptotic cell death [11]. Furthermore, *Erdag et al.* discovered that Mannich bases of benzoxazolone produced on the third position via Mannich reaction exhibit cytotoxic effects on the MCF-7 breast cancer cell line [12]. In the same study, it was reported that the chemical characteristics of the substituents at the fifth position of the benzoxazolone ring can affect the apoptotic cell pathways extrinsically or intrinsically.

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Apoptosis is the process by which environmental variables cause DNA-damaged cells to undergo programmed cell death in order to shield the organism from additional harm [13]. It suggests a cell death mechanism that is fundamentally different from necrosis, which is known as the traditional cell death mechanism [14]. The activation of a group of cysteine-aspartic proteases known as caspases is necessary for the beginning of apoptosis [14]. The mitochondrial apoptosis pathway is another name for intrinsic apoptosis [15]. This type of apoptosis is brought on by either a positive or negative pathway and is dependent on factors released from mitochondria. On the other hand, extrinsic apoptosis is a controlled cell death mechanism linked to changes in the microenvironment, where extracellular agents are active. It is mostly regulated by two types of cell membrane receptors [16]. The first of them are death receptors, which are triggered when the appropriate ligands bind to them. The second are receptors, which are activated when the concentration of some ligands drops below a predetermined threshold. For this reason, extrinsic apoptosis is also known as the death receptor pathway of apoptosis.

Caspase-3 is one of the best known and most critical biomarkers of apoptosis [17]. It belongs to the cysteine-aspartate protease family, which is one of the six protease families that have important functions in normal neuronal development and neuropathology [17]. It is one of the important effectors of apoptosis as it can lyse the cell in case of activation. Eventually, for both mechanism pathways, caspases-3 triggers apoptosis which includes damage and cleavage of the DNA strand [18]. The activation of caspase-3 indicates that downstream of apoptotic pathway had been reached. Consequently, apoptosis is a desirable condition in cancer cells, regardless of intrinsic or extrinsic pathway.

The usage of the benzoxazolone ring in the molecular design of therapeutic candidates with anticancer capabilities, as well as the influence of various substituents on the benzoxazolone ring on apoptotic pathways, have become more important as a result of these previous findings. Caspase-3 is an essential target for both experimental and *in silico* anticancer investigations since both the extrinsic and intrinsic routes result in caspase-3 activation and cell death. The purpose of this study was *in silico* investigation of the affinities of some 3-substituted-2(3*H*)-benzoxazolone derivatives that were predicted to exhibit considerable anticancer activity on different cancer cell lines in a previous study and against caspase-3 enzyme.

2. Material and methods

2.1. Chemical methods

All reagents and solvents were purchased from Merck (formerly Sigma-Aldrich, Darmstadt, Germany) and were purified by standard procedures. Melting points are uncorrected. FT-IR spectra were recorded as films or KBr pellets on a Spectrum Two FT-IR Spectrometer (PerkinElmer, Inc., Waltham, MA, USA). NMR spectra of compounds prepared for the first time in this study were acquired on a Jeol 400 MHz spectrometer (JEOL USA, Inc., Peabody, MA, USA) at 400 and 100 MHz respectively. Fully decoupled ^{13}C NMR spectra were reported. Chemical shifts were reported in ppm (parts per million) units relative to the internal standard tetramethylsilane (TMS = 0.00 ppm), and the splitting patterns were described as follows: s (singlet), d (doublet), t (triplet), q (quartet), and m (multiplet). Elemental analyses (C, H, N) were performed with on Leco CHNS 932 analyzer and the analyses were within ± 0.4% of the theoretical values.

2.2. General synthesis method

Synthesis was carried out via Mannich reaction with a previously published procedure [19]. 15 mmol 2(3*H*)-benzoxazolone derivatives and 15 mmol of 1-(3-Methylphenyl)piperazine derivative derivative were dissolved separately in 10 ml of methanol and mixed. The procedure was followed by addition of 20 mmol of 37% w/v formaldehyde solution. The mixture was heated under reflux for 2 hours and significant precipitation occured after pouring the mixture onto ice bath. The reaction was controlled by TLC in benzene:methanol (9:1). The mixture was filtered off, washed with cold methanol, dried in *vacuo* and recrystallized in an appropriate solvent.

2.2.1. 5-Chloro-3-{[4-(3-methylphenyl)] piperazino-1-yl}-2-benzoxazolone (1)

White solid (70.3, yield %); mp 171.4 ºC. FT-IR (cm^{-1}) 2800-3065 (C-H), 1765 (C=O). ^1H NMR (400 MHz, $CDCl_3$), δ (ppm): 7.3-6.9 (m, 7H, Ar-CH), 4.7 (s, 2H, CH_2), 2.9 (t, 4H, pip-CH_2 H^2, H^6), 2.8 (t, 4H, pip-CH_2 H^3, H^5), 2.3 (s, 3H, CH_3);^{13}C NMR (100 MHz, $CDCl_3$) δ156.3, 151.9, 145.6, 137.2, 132.9, 131.2, 128.6, 123.6, 121.3, 118.1, 110.7, 108.3, (Ar-C), 65.1 (CH_2), 51.4, 51.3 (pip-C), 22.6 (CH_3). Anal. Calc. for $C_{19}H_{20}ClN_3O_2$ C, 63.77; H, 5.63; N, 11.74; Found C, 63.69; H, 5.61; N, 11.65.

2.2.2. 3-{[4-(3-methylphenyl)] piperazino-1-yl}-2-benzoxazolone (2)

Brown solid (81.5, yield %); mp 154.3 ºC. FT-IR (cm^{-1}) 2800-3065 (C-H), 1765 (C=O). ^1H NMR (400 MHz, $CDCl_3$), δ (ppm): 7.3-6.9 (m, 8H, Ar-CH), 4.7 (s, 2H, CH_2), 2.9 (t, 4H, pip-CH_2 H^2, H^6), 2.8 (t, 4H, pip-CH_2 H^3, H^5), 2.3 (s, 3H, CH_3);^{13}C NMR

(100 MHz, $CDCl_3$) δ156.3, 151.9, 145.6, 137.2, 132.9, 131.2, 128.6, 123.6, 121.3, 118.1, 110.7, 108.3, (Ar-C), 65.1 (CH_2), 51.4, 51.3 (pip-C), 22.6 (CH_3). Anal. Calc. for $C_{19}H_{21}N_3O_2$ C, 70.57; H, 6.55; N, 12.99; Found C, 70.51; H, 6.52; N, 12.91.

2.3. Data Set

In addition to the new compounds synthesized in this study (compound **1** and compound **2**), other compounds with similar structures, whose synthesis and spectroscopic analyzes were reported before [20], were also used in the molecular docking study. These compounds were; 5-Chloro-3-{[4-(2-ethylbenzyl)]piperazino-1-yl}-2-benzoxazolone (**3**), 3-{[4-(2-ethylbenzyl)]piperazino-1-yl}-2-benzoxazolone (**4**), 5-Chloro-3-{[4-cyclopropyl]piperazino-1-yl}-2-benzoxazolone (**5**), 3-{[4-cyclopropyl]piperazino-1-yl}-2-benzoxazolone (**6**), 5-Chloro-3-{[4-(2-bromophenyl)]piperazino-1-yl}-2-benzoxazolone (**7**), and 3-{[4-(2-bromophenyl)]piperazino-1-yl}-2-benzoxazolone (**8**), respectively.

2.4. Molecular docking

In this study, some newly synthesized and some previously reported 3-substituted-2(3H)-benzoxazolone derivatives were investigated using Maestro Schrödinger 2021-4 package program. The selected derivatives and a high resolution (1.80 Å) X-ray crystal structure of human caspase-3 (PDB ID: 5I9B) in complex with PRD_000238, native ligand [21-23] were prepared using LigPrep and Protein Preparation Wizard in Maestro of Schrödinger-2021 software package, respectively [24-26]. The docking calculations were performed using the Glide SP (standard precision) module of Schrödinger Suite [27]. The docked pose and the crystal conformation of the native ligand (PRD_000238) was found as 1.291 Å.

3. Results and discussion

The traditional Mannich reaction is a condensation reaction between a carbonyl molecule containing at least one alpha hydrogen and a primary or secondary amine, or in rare cases, ammonia, in the presence of formaldehyde [28]. Mannich bases are preferred when creating prodrugs because of their high water solubility in chemical structures [28]. Additionally, a wide range of biological functions are associated with Mannich bases [29]. By using the synthesis technique described in the literature [19], 3-methylphenylpiperazine was reacted with 2(3*H*)-benzoxazolone derivatives through Mannich reaction, in order to produce 3-substituted-2(3*H*)-benzoxazolone derivatives as indicated in Figure 1.

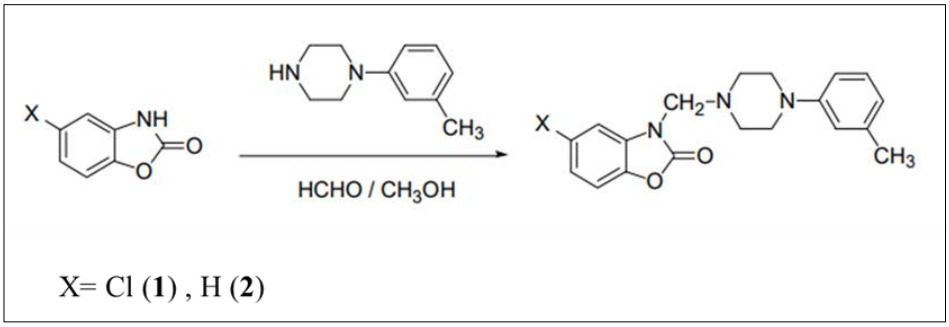

Figure 1 Synthesis of compound 1 and compound 2

The structural characterizations were carried out using FT-IR, ^{1}H NMR, ^{13}C NMR, and elemental analysis methods. During the Mannich reaction, a methylene bridge was formed between the N-H group in the 3rd position of the benzoxazolone ring and the N-H group of the piperazine ring in the presence of formaldehyde. In the FT-IR spectra of all Mannich bases, the expected N–H bands of the benzoxazolone and piperazine rings at 3100–3400 cm^{-1} has disappeared, indicating that a reaction took place between these heterocycles. In addition, the carbonyl (C=O) stretching band of benzoxazolone lactam group has appeared at 1765 cm^{-1} as expected. In the ^{1}H NMR spectra of all derivatives, the methylene protons were observed as a singlet at 4.7 ppm which was another evidence of Mannich reaction between these structures. Regarding the evaluation of chemical shifts in the ^{1}H NMR spectrum, it was clear that aromatic and aliphatic protons were observed in the regions expected for the respective compounds. The integral values of the aromatic protons match the proposed structures.

In this article, a docking study was performed on eight 3-substituted-2(3*H*)-benzoxazolone derivatives against caspase-3 enzyme. The structures of previously published compounds were given in **Figure 2**. The docking scores and interacting amino acid residues were listed in **Table 1** for each compound and the native ligand (PRD_000238). The 2D/3D ligand-receptor interactions of novel derivatives (compound 1 and compound 2) were shown in **Figure 3.**

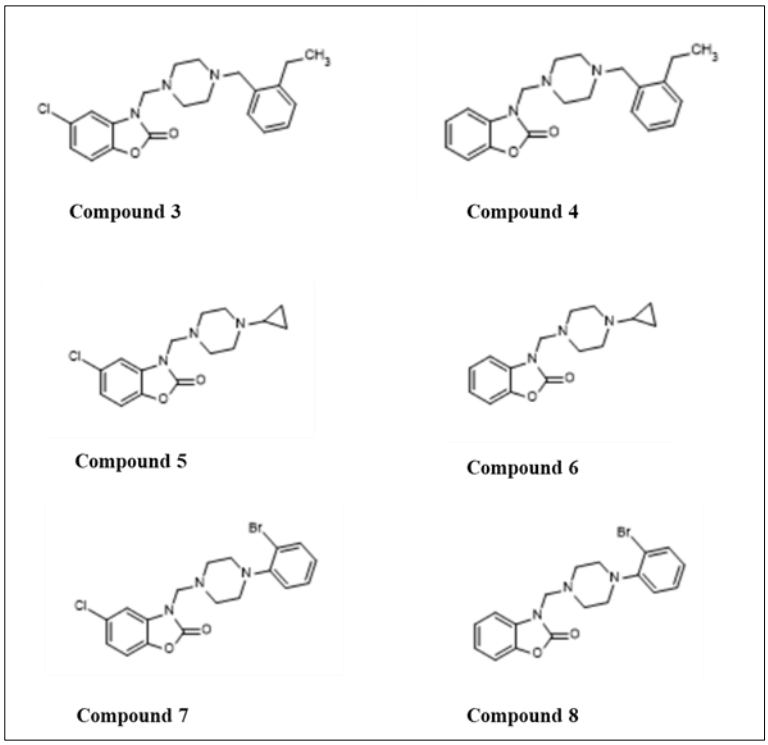

Figure 2 The structures of previously published 3-substituted-2(3H)-benzoxazolone derivatives [20]

Table 1 The docking scores of tested compounds and their interacting amino acid residues in the active site of caspase-3

Compound	Docking Score	Interacting Amino Acids
Native Ligand (PRD_000238)	-8.982	MET61, SER205,ARG207
Compound 1	-7.103	ARG207
Compound 2	-8.105	TRP206, ARG207
Compound 3	-4.858	GLY122
Compound 4	-5.089	HIE121, GLY122
Compound 5	-7.583	THR166, ARG207
Compound 6	-8.428	CYS163, THR166, ARG207
Compound 7	-6.893	PHE256, ARG207
Compound 8	-7.018	MET61, PHE256, ARG207

Given the results of earlier in *silico* studies that have been published in the literature, it was clear that certain amino acid residues, including SER65, SER205, ARG207, and SER209 may have a significant impact on the activation of caspase-3 [23]. Compounds 1, 2, 5 and 6 showed better docking scores compared to other derivatives. Newly synthesized derivatives (compounds **1** and **2**) both generated H-bond with ARG207 which is among one of the crucial amino acid residues for caspase-3 activity. In general, the results showed that carbonyl group of benzoxazolone core structure is significant for the formation of hydrogen bonds with ARG207 residue. However, compounds 3 and 4 contain ethylbenzylpiperazine group which is bulky enough to create a possible steric hindrance, creating a different position within the active site. Therefore, no hydrogen bonds were created with the critical amino acid residues. This might be

the reason of their lowest docking scores among the other derivatives. As seen clearly in **Figure 3** and **Figure 4** , compound **2** showed π–π stacking type of interaction with TRP206 but there was no interaction with TRP206 in compound **1**. On the other hand, the presence of a chlorine substituent at position 5 of the benzoxazolone ring resulted in the creation of a hydrophobic pocket containing the amino acid residues CYS163, LEU168, and PHE256. The creation of this hydrophobic pocket indicates that any additional hydrophobic groups, such as small-chain alkyl substituents like the methyl group or other halogens, may be replaced with chlorine and examined for their impact on how similar drugs interact with caspase-3 in future studies.

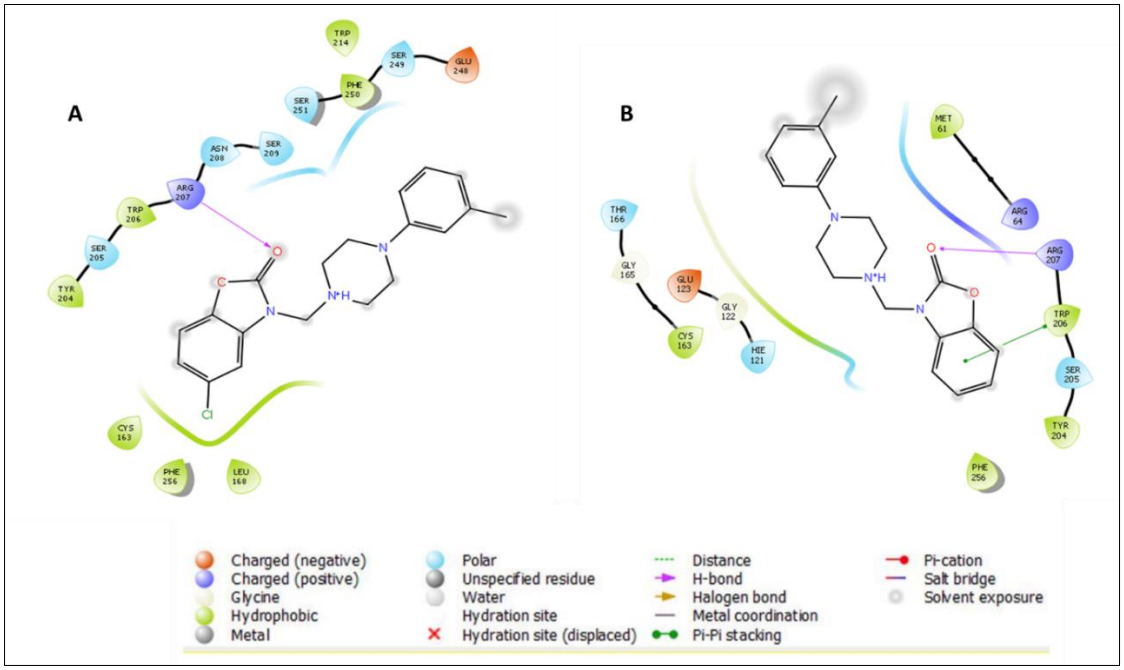

Figure 3 The 2D/3D ligand-receptor interactions of novel derivatives (compound 1 and compound 2)

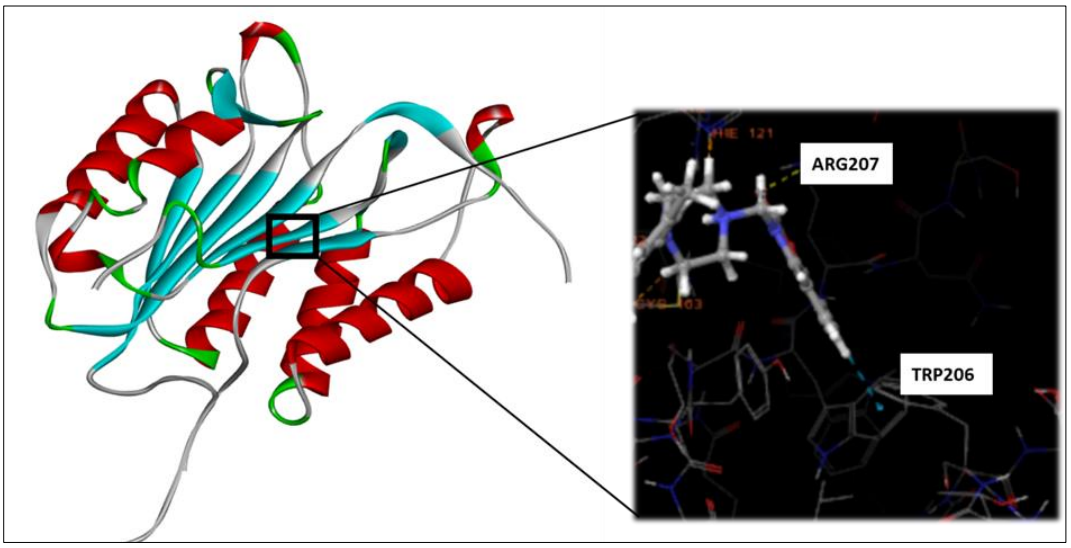

Figure 4 The 3D structure and ligand-receptor interactions of compound 2 against caspase-3

4. Conclusion

In conclusion, a molecular docking investigation revealed that the primary amino acid residue through which 3-substituted-2(3H)-benzoxazolone derivatives interact with the caspase-3 active site is ARG207. In addition to in *silico*

investigations, it is anticipated that these compounds will eventually shed light on the synthesis of related derivatives as well as cytotoxicity studies on various cancer cell lines employing immunohistochemical assessments or MTT assay methods.

Compliance with ethical standards

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Disclosure of conflict of interest

The author declares no conflict of interest.

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