Original Article

Synthesis, Docking And Biological Evaluation Of Novel Isoxazole Analogues As Anticancer And Antioxidant Agents

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Abstract

Novel isoxazole were designed and subjected for molecular docking study, which revealed that the designed compounds possess significant to moderate interaction with the targeted enzyme topoisomerase II. Among them compound OX1, OX3, OX5 and OX 6 (-9 kcal/mol) showed similar docking compared to Adriamycin (-12.76 kcal/mol). The invitro anti-oxidant activity studies were carried out for synthesised molecules. Based on the experimental results, among all the compounds synthesized, compound OX5 substituted with acetaldehyde shows good inhibition (-78.241 µg/mL) compared to all the tested compounds. The in-vitro cytotoxic evaluation was carried out for the synthesised compounds and it is compared with well-known anticancer drug Adriamycin. The results were found that the synthesized compounds are relatively non-toxic at 100 µg concentration in tested cell line. Among the tested compounds, compound OX5 substituted with chloro benzene group was found to have IC_{50} values -26.32 µg/mL against MCF – 7 cancer cell lines. Compound OX6 substituted with hydroxy-benzene was found to have IC_{50} values -29.57 µg/mL against MCF-7 cancer cell line. Based on the study the compound OX1, OX3, OX5 and OX6 which is substituted with a bulky group like Methyl, Methylbenzene, Chlorobenzene, and Hydroxy benzene have emerged to be the most active compounds.

KEY WORD: Adriyamycin, Anticancer Drugs, DPPH, MCF-7 Breast Cancer, Molecular Docking.

1. INTRODUCTION

Cancer is the leading cause of death throughout the world [1, 2]. Indeed, 25% of the deaths in developing countries are due to cancer [3]. In 2020, cancer was responsible for 9.6 million deaths [4]. 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 [5]. Over the years, scientists have explored a myriad of treatments for each type of cancer, including chemotherapy, hormonal treatment, radiation, and surgery [6]. Chemotherapy has been widely used, particularly against inoperable cancer as the primary therapy or as an adjunct therapy before and/or after another treatment [7, 8]. However, chemotherapy use is restricted because it has weak effectiveness, minimal selectivity against target cells, and undesirable side effects such as alopecia, queasiness, and vomiting [9, 10]. Many natural extracts with anticancer activity have been evaluated in recent decades [11].

Different types of heterocyclic derivatives such as isoxazole are used extensively as agrochemicals in medicine; indeed, they are efficient in anticancer chemotherapy [12]. Researchers have found that the isoxazole ring imparts it with anticancer [13], hypoglycemic [14], analgesic, bactericidal [12], antiviral [13], and anti-inflammatory [15] activities. The isoxazole ring has been used as a core structure for many anticancer medications [15]. For example, resorcinylic 4,5-diarylisoxazole amides have shown a potent inhibitory effect on heat shock protein (HSP90) [16]. A derivative of diarylisoxazole was discovered for its activity against androgen receptor- (AR-) expressing breast cancer cells. Leflunomide, an immunosuppressant used in chemotherapy, is another isoxazole derivative. Other researchers reported that the 3,5- dimethylisoxazole functional group mimics acetylated lysine (KAc); they utilised this functional group to improve bromodomain inhibitors, with positive effects on cancer cells [17].

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 [18]. The biologically active agents that work to slow or prevent the cell damage caused by those free radicals are called antioxidants. 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 [19]. In the last few decades, researchers have synthesised many agents that have marked antioxidant activity, such as quinolinone-3-aminoamide [16], thienopyrimidine, thienopyrazole [18], and N-aryl-1,4-dihydropyridine derivatives [19]. These substances, like Trolox, a water soluble vitamin-E analogue, and rebamipide, exhibit effective antioxidant properties by scavenging unstable free radicals [20-22]. The current project is aimed at synthesising novel isoxazole derivatives (**OX1**–**OX6**) with or without a methoxyphenyl moiety and at evaluating some of their biological activity such as antioxidant and anticancer activities on MCF-7 cancer cell line.

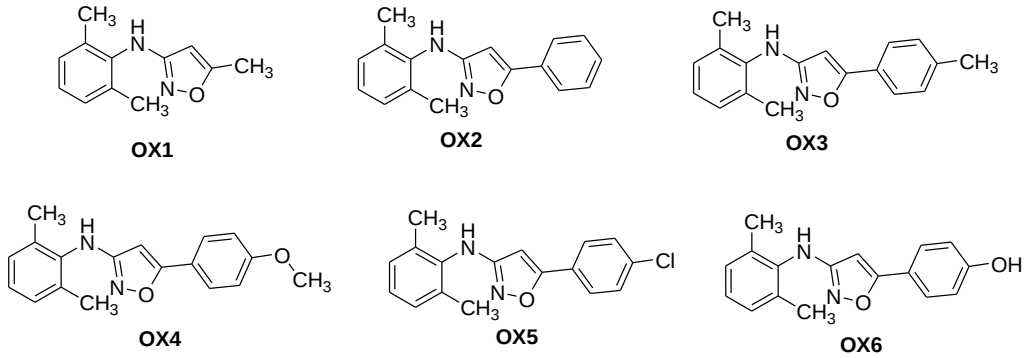

Figure 1 : Newly designed isoxazole scaffold

2. MATERIALS AND METHODS

2.1. Materials

All the solvents (Analytical grade) and reagents were dried and purified. The reactions were performed on sterile glassware. All reagents and solvents were obtained from the supplier or recrystallized/redistilled unless otherwise noted. Purity of the synthesized compounds was carried out by thin layer chromatography (TLC). The reaction progress was monitored by TLC using precoated silica 60 F254, 0.25 mm aluminium plates (Merck) and n-hexane: ethyl acetate (5:5) as the mobile phase. The developed chromatogram was visualized by UV chamber and melting points were determined in open capillary tubes using Veego VMP-1 melting point apparatus and were uncorrected. Infrared (IR) spectra were recorded on a Perkin-Elmer Fourier Transform-Infrared Spectrometer using KBr pellets. Elemental analyses (C, H, and N) were done with a Shimadzu analyzer (Mumbai, Maharashtra, India) and all analyses were consistent (within 0.4%) with theoretical values. The ^{1}H NMR and ^{13}C NMR spectra of synthesized compounds were recorded on a Bruker NMR Spectrometer (Billerica, MA, USA) at 400 MHz frequency in deuterated DMSO and $CDCl_3$ and using TMS as an internal standard (chemical shift in ppm). The mass spectra of compounds were scanned on Shimadzu ESI-MS.

2.2. Experimental section

2.2.1. Step 1: synthesis of N-(2, 6-dimethylphenyl) acetamide (A)

A mixture contains xylidine (0.01mol) and 40 ml DMF was added to the mixture of acetyl chloride (0.012 mol) in triethylamine (0.012mol) and then refluxed for 1h at 150-155^{O}C until the starting material disappeared, tested by TLC using ethyl acetate and n hexane (5:5) as mobile phase. After completion of the reaction, the precipitate formed upon cooling and it was filtered and recrystallized from ethanol to achieve the N-(2,6-dimethylphenyl) acetamide **(A)**.

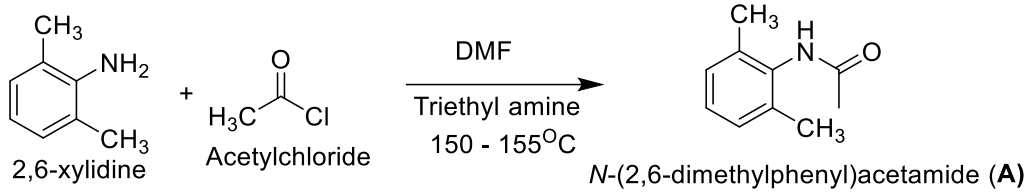

Figure 2 : Synthesised N-(2, 6-dimethylphenyl)acetamide (A)

2.2.2. Step 2: synthesis of Substituted (Z)-N-(2,6-dimethylphenyl)-2-enamide (B1 to B6)

Various substituted aldehyde, N-(2,6-dimethylphenyl)acetamide **(A)** and pyridine (1:1: 0.15 mol.) were mixed in a flask, heated on an oil-bath at 130-140^{O}C ~ for 4 hours, and when the contents were cold, methyl alcohol was added and the whole was left overnight. The

product of Substituted (Z)-N-(2,6-dimethylphenyl)-2-enamide was taken out as before by partial concentration of the filtrate and subsequent addition of acidulated water.

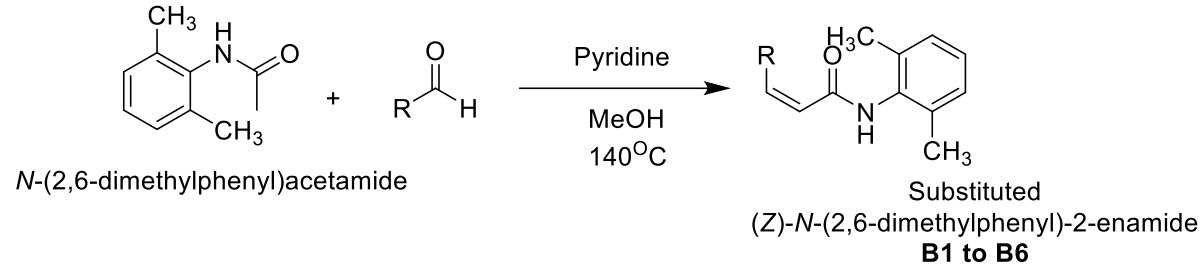

Figure 3 : Substituted (Z)-N-(2,6-dimethylphenyl)-2-enamide (B1 to B6)

2.2.3. Step 3: Synthesis of N-(2, 6-dimethylphenyl)-2-substituted-2,3-dihydrobenzo [b][1,4]thiazepin-3-amine.

The mixture of N-(2,6-dimethylphenyl)but-2-enamide derivative and hydroxylamine hydrochloride(1:1) was stirred at 50°C in aqueous media, 5-arylisoxazole derivatives were obtained in good yields. Completion of reaction was checked by TLC using ethyl acetate and n hexane (5:5) as mobile phase.

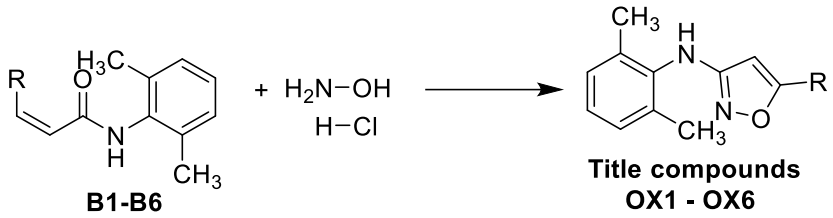

Figure 4 : Substituted N-(2, 6-dimethylphenyl)-2-substituted-2,3-dihydrobenzo [b][1,4]thiazepin-3-amine

2.3. Characterization

2.3.1. Characterization of 4-(3-((2, 6-dimethylphenyl) amino) isoxazol-5-yl) phenol (OX1)

$C_{12}H_{14}N_2O$; Colour: White solid; % yield: 71%; MP: 171 – 173^{O}C; IR (KBr) cm^{-1}: 3074 (NH stretching, Amine); 2985 (CH stretching, Aromatic); 2732 (CH stretching, Alkane); 1452 (CN bending); 850 Aromatic ring; ^{1}H NMR (500 MHz, DMSO) δ 8.33 (s, 1H), 6.99 (d, J = 7.5 Hz, 2H), 6.87 (dd, J = 7.8, 7.2 Hz, 1H), 5.89 (s, 1H), 3.32 (s, 1H), 2.32 (d, J = 13.2 Hz, 9H). ^{13}C NMR (126 MHz, DMSO) δ 170.31, 134.70, 134.50, 129.99, 129.36, 129.30, 128.79, 128.02, 127.88, 127.26, 127.02, 126.63, 18.89, 17.77. Mass: 202 m/z; found 202 m/z.

2.3.2. Characterization of N-(2,6-dimethylphenyl)-5-phenylisoxazol-3-amine (OX2)

$C_{17}H_{16}N_2O$; Colour: White solid; % yield: 76%; MP: 183 – 185^{O}C; 3074 (NH stretching, Amine); 2985 (CH stretching, Aromatic); 2732 (CH stretching, Alkane); 1452 (CN bending); 850 Aromatic ring; ^{1}H NMR (500 MHz, DMSO) δ 9.17 (s, 1H), 7.70 – 7.58 (m, 2H), 7.46 – 7.40 (m, 2H), 7.35 (s, 1H), 7.05 – 6.89 (m, 2H), 6.85 (s, 1H), 6.71 (s, 1H), 3.35 (s, 1H), 2.33 – 2.29 (m, 6H); ^{13}C NMR (126 MHz, DMSO) δ 170.30, 155.81, 154.37, 130.00, 129.31, 128.03, 127.26, 127.03, 119.01, 64.86, 23.44. Mass: actual 264 m/z; found 265 (M+1).

2.3.3. Characterization of N-(2,6-dimethylphenyl)-5-(p-tolyl)isoxazol-3-amine (OX3)

$C_{18}H_{18}N_2O$; Colour: yellow solid; % yield: 78%; MP: 193 – 195^{O}C; IR (KBr) cm^{-1}: 3074 (NH stretching, Amine); 2985 (CH stretching, Aromatic); 2732 (CH stretching, Alkane); 1452 (CN bending); 850 Aromatic ring; ^{1}H NMR (500 MHz, DMSO) δ 8.97 (s, 1H), 7.67 – 7.53 (m, 2H), 7.37 – 7.23 (m, 2H), 7.04 – 6.89 (m, 2H), 6.83 (d, J = 7.0 Hz, 1H), 6.39 (s, 1H), 4.79 (s, 1H), 2.37 – 2.33 (m, 3H), 2.33 – 2.29 (m, 6H); ^{13}C NMR (126 MHz, DMSO) δ 170.30, 155.81, 130.00, 129.31, 128.03, 127.26, 127.03, 119.01, 108.17, 64.86, 23.44, 20.90. Mass: actual 278 m/z; found 279 m/z (M+1).

2.3.4. Characterization of N-(2, 6-dimethylphenyl)-5-(4-methoxyphenyl) isoxazol-3-amine (OX4)

$C_{18}H_{18}N_2O_2$; colour: White solid; % yield: 73%; MP: 185 – 188^{O}C; IR (KBr) cm^{-1}: 3363 (NH stretching, Amine); 2985 (CH stretching, Aromatic); 2600 (CH stretching, Alkane); 1455 (CN bending); 851 Aromatic ring; ^{1}H NMR (500 MHz, DMSO) δ 7.70 – 7.55 (m, 2H), 7.12 – 6.97 (m, 3H), 6.97 – 6.89 (m, 1H), 6.82 (s, 1H), 6.25 (s, 1H), 4.78 (s, 1H), 3.83 – 3.79 (m, 3H), 2.33 – 2.29 (m, 6H). ^{13}C NMR (126 MHz, DMSO) δ 170.30, 155.81, 154.86, 154.37, 129.99, 129.31, 128.03, 127.26, 127.03, 119.01, 108.41, 64.86, 18.89. Mass: actual 294 m/z; found 295 (M+1) m/z.

2.3.5. Characterization of 5-(4-chlorophenyl)-N-(2, 6-dimethylphenyl) isoxazol-3-amine (OX5)

$C_{17}H_{15}ClN_2O$; Colour: White solid; % yield: 73%; MP: 185 – 188^{O}C; IR (KBr) cm^{-1}: 3074 (NH stretching, Amine); 2985 (CH stretching, Aromatic); 2732 (CH stretching, Alkane); 1452 (CN bending); 850 Aromatic ring; 751 (C-Cl stretching); ^{1}H NMR (500 MHz, DMSO)

δ 7.65 – 7.52 (m, 2H), 7.52 – 7.38 (m, 2H), 7.05 – 6.90 (m, 2H), 6.85 (s, 1H), 6.64 (s, 1H), 3.33 (s, 1H), 2.33 – 2.29 (m, 6H); ^{13}C NMR (126 MHz, DMSO) δ 149.52, 148.31, 130.74, 130.46, 124.56, 124.30, 124.18, 122.98, 121.93, 121.86, 108.86, 108.59, 106.99, 106.84, 101.84, 101.66, 18.38. Mass: actual 298 m/z; found 297 (M-1) m/z.

2.3.6. Characterization of 4-(3-((2, 6-dimethylphenyl) amino) isoxazol-5-yl) phenol (OX6)

$C_{17}H_{16}N_2O_2$; colour: White solid; % yield: 72%; MP: 186 – 189^{O}C; IR (KBr) cm^{-1}: 3074 (NH stretching, Amine); 2985 (CH stretching, Aromatic); 2732 (CH stretching, Alkane); 1452 (CN bending); 850 Aromatic ring; ^{1}H NMR (500 MHz, DMSO) δ 7.56 – 7.42 (m, 2H), 7.05 – 6.85 (m, 4H), 6.82 (s, 1H), 6.24 (s, 1H), 4.78 (s, 1H), 4.07 (s, 1H), 2.33 – 2.29 (m, 6H). ^{13}C NMR (126 MHz, DMSO) δ 170.31, 155.82, 154.37, 134.50, 130.00, 129.31, 128.03, 127.26, 127.03, 124.46, 119.01, 108.40, 64.86, 20.60. Mass: actual 280 m/z; found 280 m/z.

2.4. In-silico molecular docking studies

2.4.1. Devices and materials

The three dimensional structures produced on a computer may be manipulated on the screen to show different views of the structures. With more complex molecular mechanics programs it is possible to superimpose one structure on top of another. In other words, it is possible to superimpose the three dimensional structure of a potential drug on its possible target site. This process, which is often automated, is known as docking. It enables to evaluate the fit of potential drugs (ligand) to their target site. If the structure of a ligand is complementary to that of its target site the ligand is more likely to be biologically active. The research work was done in-silico by utilizing bioinformatics tools. Also, we utilize some of the offline programming's like protein data bank (PDB) www.rcsb.org/pdb, PubChem database, Marvin sketch. The molecular docking studies were carried out through PyRx 0.9 [23].

2.4.2. Preparation of protein

By utilizing protein data bank, we took the topoisomerase II **(PDB ID: 1A35)** with a resolution of 1.90A^{o}. From the protein we removed the crystal water, followed by the addition of missing hydrogens, protonation, and ionization. The SPDBV (swiss protein data bank viewer) force field was applied for energy minimization. Prepared protein is validated by utilizing the Ramachandran plot [24].

2.4.3. Identification of active sites

Identification of active amino acid present in the protein is detected by using Protein-ligand interaction profile (PLIP) https://plip-tool.biotec.tu-dresden.de/plipweb/plip/index offline tool in google [25].

2.4.4. Preparation of ligands

The molecules are designed in two and three-dimensional structures using Marvin sketch tool and the structure was optimized in 3D optimization in Marvin sketch and saved as pdb format [26].

2.5. DPPH radical assay

The free radical scavenging activity of the synthesized compounds was determined by 1, 1- diphenyl-2-picrylhydrazyl (DPPH). Briefly, 1ml of sample was added to 3ml of 0.1mM ethanolic DPPH solution at different concentration (25, 50, 100, 250, 500µg/ml). Then, the mixture was vigorously shaken and left to stand for 30 minutes under subdued light. The absorbance was measured at 517 nm in a UV spectrophotometer. Lower absorbance of the reaction mixture indicates higher free radical scavenging activity. Ascorbic acid, which is a good antioxidant, was taken as a standard in this study. For control, DPPH solution (3 ml) was mixed with ethanol and used for measuring the absorbance value. The decrease in absorbance of DPPH solution on addition of test samples in relation to the control was used to calculate the antioxidant activity. All tests were performed in triplicate [27].

2.6. In-vitro anticancer activity

The in - vitro cytotoxicity of the synthesized compounds was assessed against MCF-7 cancer cell line using SRB assay. The monolayer culture of the cell line was trypsinized, followed by adjusting the cell count to 1.0 × 105 cells/ mL by means of DMEM medium containing 10% FBS. The diluted cell suspension (0.1 mL) was added to each well of the 96-well microtiter plate. The test wells were added with various concentrations (100 µL) of test samples, and the control wells received media (100 µL). The plates were then incubated at 37 °C for 72 h in 5% CO2 atmosphere. After this duration, the cultures were fixed with trichloroacetic acid (25 µL, 10% w/v) and stained for 30 min with sulforhodamine B (0.4% w/v) in acetic acid (1% v/v). Unbound dye was cleared by 4 washes with acetic acid (1% v/v), and protein-bound dye was extracted with 10 mM unbuffered Trisbase [tris (hydroxymethyl) aminomethane]. The optical density of the protein-bound dye was recorded at 540 nm [28]. The percentage cell viability (CV) was calculated using the following formula:

$$\text{Cell viability} = \frac{\text{Mean OD of individual test group}}{\text{Mean OD of control group}} \times 100$$

The concentration of test samples required to inhibit cell growth by 50% was tabulated from the dose–response for each cell line.

3. RESULTS AND DISCUSSION

3.1. Synthetic work

The compound N-(2,6-dimethylphenyl)acetamide (A) was prepared by the reaction with A mixture of xylidine (0.01mol) in 40 ml DMF was added to the mixture of acetyl chloride in triethylamine. Reaction mixtures were refluxed for 1h at 150-155^{O}C until the starting material disappeared by TLC using ethyl acetate and n hexane (5:5) as mobile phase. Further the compound A react with various substituted aldehyde, and pyridine at the temperature of 130-140^{O}C ~ for 4 hours, and when the contents were cold, methyl alcohol was added and the whole was left overnight. The product of Substituted (Z)-N-(2,6-dimethylphenyl)-2-enamide **(B1 – B6)** was formed. Further the compound B1 – B6 was reacted with hydroxylamine hydrochloride was stirred at 50°C in aqueous media, 5-arylisoxazole derivatives were obtained in good yields. Completion of reaction was checked by TLC using ethyl acetate and n hexane (5:5) as mobile phase. The IR spectrum of the final synthesized compounds showed absorption bands around 3300–3200 cm^{-1} for amine NH, while the distinguishing broad absorption peaks at 3030 were observed for CH aromatic, peaks at 1464 - 1400 cm^{-1} for CN bending, and 800–700 cm^{-1} for aromatic rings. These compounds also exhibited appropriate peaks at corresponding ppm in their ^{1}H NMR spectra. The ^{1}H NMR spectra of the synthesized compounds revealed a singlet signal at 8.7 for H of NH, doublet signal at 3 - 4 shows the CH_3 group and a signal at 7.5–6.5 for H of aromatic ring. The ^{13}C NMR spectra of synthesized compounds revealed a signal at 170 for carbonyl carbon, a signal at 155–160 for ethene carbon, signal at 20 for CH_3 group and a signal at 120–145 for aromatic carbon. The corresponding molecular ion peaks in the ESI–MS spectra were in conformity with the assigned structures. All the synthesized compounds were subjected to short-term in vitro cytotoxicity studies using MCF-7 cell line and in-vitro anti-oxidant screening using DPPH scavenging method.

3.2. Molecular docking studies

Based on literature studies of isoxazole derivatives, the 6 compounds were designed and subjected to molecular docking studies. Molecular docking was carried out through PyRx 0.9 to predict the interactions model of the protein to its inhibitors, which was performed to elucidate the binding mode competence of topoisomerase II and 6 isoxazole analogues. The designed molecules were docked along with the reference standard, adriyamycin (ADR). The docking energy of our designed compounds ranged from -7.8 to -9 kcal/mol indicated good binding affinities to the target receptor, and the results are depicted in **Table 1**. Among the docked compounds, derivatives OX1, OX3, OX5 and OX6 (-9 kcal/mol) showed a significant binding energy towards the targeted enzyme. The compounds OX1 possess pi-pi stacking bond with amino acid Tyr231 and it also possess vendar-waals interaction with amino acids such as Pro220, Ala 228, Tyr231, Glu255, Thr258, Pro357 and Pro358. The roles of certain crucial amino acids in the ligand-binding domain of the human topoisomerase II inhibitors were also established. Major non-covalent interactions between the studied ligands and the ligand-binding domain of the topoisomerase II inhibitors were investigated. These amino acids have been repeatedly implicated during ligand interaction with the topoisomerase II inhibitors and also play important role in the inhibition of the ligand-binding domain of topoisomerase II inhibitors. It was further observed in the docking studies of the compounds that OX1, OX3, OX5, and OX6 demonstrated favorable hydrophobic binding interactions with the proteins. These non-covalent interactions, van der Waals, columbic interaction, π–π interaction, and hydrogen interaction, are shown in **Figure 5 to 10**.

Table 1 : Docking Results

Ligand	Binding Affinity
OX1	-9
OX2	-7.8
OX3	-9
OX4	-8.8
OX5	-9
OX6	-9
ADR	-12.76

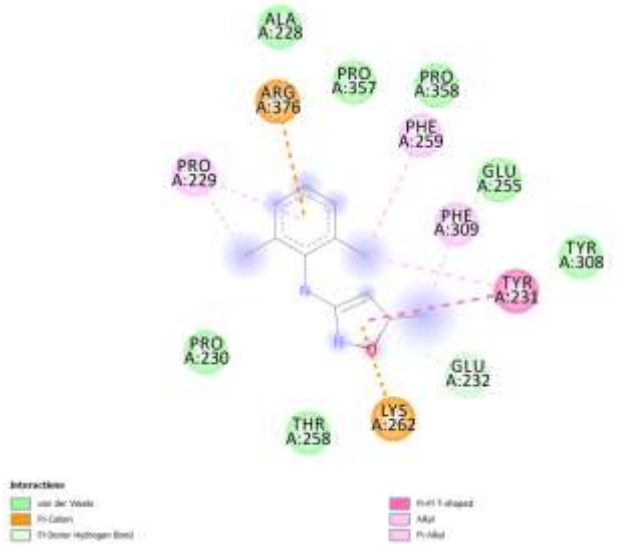

Figure 5 : Fitting pose with interactions of compound **OX1** in the pocket of **1A35** in 2D view.

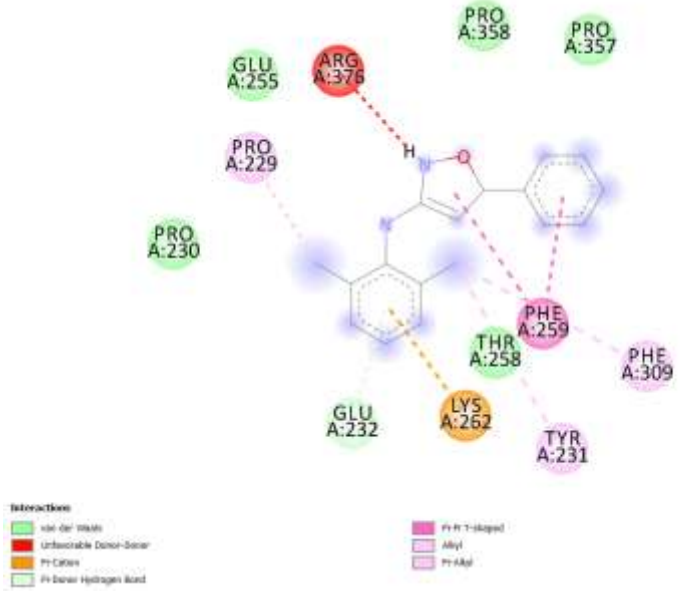

Figure 6 : Fitting pose with interactions of compound **OX2** in the pocket of **1A35** in 2D view.

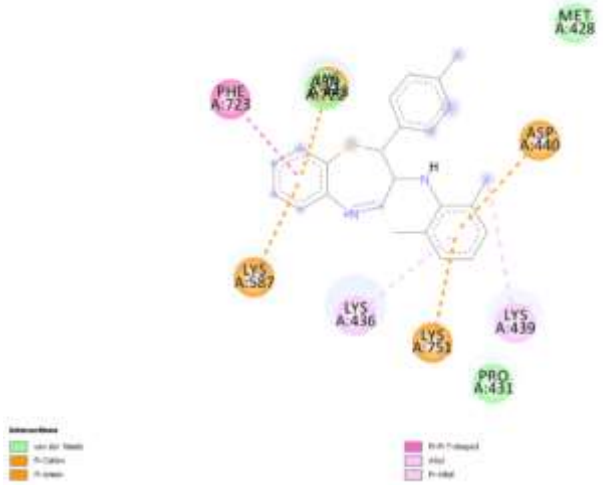

Figure 7 : Fitting pose with interactions of compound **OX3** in the pocket of **1A35** in 2D view.

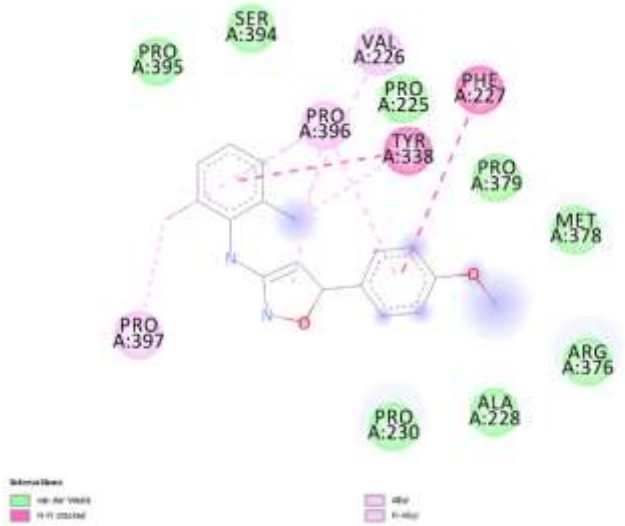

Figure 8 : Fitting pose with interactions of compound **OX4** in the pocket of **1A35** in 2D view

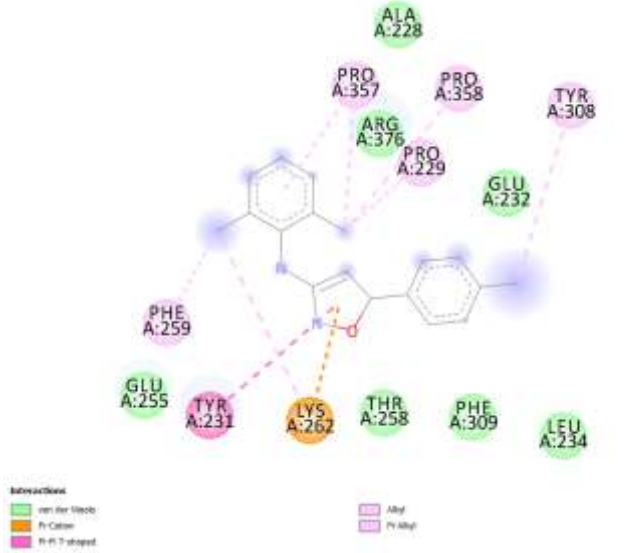

Figure 9 : Fitting pose with interactions of compound **OX5** in the pocket of **1A35** in 2D view

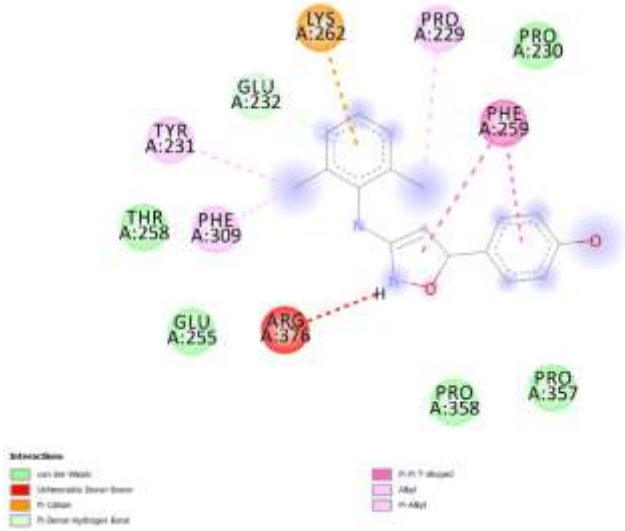

Figure 10 : Fitting pose with interactions of compound **OX6** in the pocket of **1A35** in 2D view

3.3. Antioxidant activity

The free radical scavenging activity of all compounds were carried out in the presence of the stable free radical (1,1-diphenyl-2-picrylhydrazyl) DPPH using ascorbic acid antioxidant agents as positive control. Although a number of methods are available for determination of the antioxidant activity, the DPPH method is very common, rapid and has been shown to be one of the most appropriate methods. The inhibitory effects of different concentrations of synthesized compounds on DPPH radical are presented in **Table 2.** The antioxidant activity is expressed in terms of IC_{50}. Based on the experimental results, among all the compounds synthesized compound OX5 substituted with chloro-benzene shows good inhibition (-78.241 µg/mL) compared to all the tested compounds. Remaining all the tested compounds shows -91.439 µg/mL to -167.615 µg/mL.

Table 2 : Antioxidant activity

Name of the compound	**(IC_{50}µg/mL)**
OX1	-98.589
OX2	-167.615
OX3	-108.589
OX4	-158.241
OX5	-78.241
OX6	-91.439

3.4. In-vitro cytotoxic evaluation

The targets compounds **(OX1 – OX6)** were evaluated for their anticancer activity against MCF – 7 cancer cell lines. The IC_{50} values (concentration required to growth inhibition of 50%) for the synthesised compounds were determined using SRB assay. The cytotoxic evaluation results and IC_{50} values were listed in **Table 3** and well-known anticancer drug Adriamycin was used as positive control. The results indicated that the all the tested compounds exhibited significant cell growth inhibition compared to reference standard against MCF – 7 cancer cell lines. The results were found that the synthesized compounds are relatively non-toxic at 100 µg concentration in tested cell line. Among the tested compounds, compound OX5 substituted with chloro-benzene group were found to have IC_{50} values -26.32 µg/mL against MCF – 7 cell lines cancer cell lines. Compound OX6 substituted with hydroxy-benzene were found to have IC_{50} values -29.57 µg/mL against MCF-7 cancer cell line. It was observed that most of the tested compounds demonstrated considerable anti-proliferative activity against tested cell lines. The compounds possessing halogen substitution and hydroxy substitution of the 2-phenyl ring on the isoxazole showed promising anticancer activities in the tested cell lines. The observed activity can be attributed to the activation of the ring by the electron-withdrawing nature of the halogen atoms.

Table 3 : Invitro cytotoxic evaluation of synthesised compounds against MCF - 7 cell line

Name of the compound	**MCF 7 (IC_{50}µg/mL)**
OX1	-76.32
OX2	-97.88
OX3	-59.42
OX4	-99.72
OX5	-26.32
OX6	-29.57
Adriyamycin	-23.54

4. CONCLUSION

The physicochemical and spectroscopic data confirmed the structural integrity of the newly synthesized compounds. The investigated molecules displayed a similar manner to protein binding to the active site of Topoisomerase II protein **(PDB ID:1A35)** in molecular docking studies. The calculated docking energies indicated that its interaction with Topoisomerase II is favorable, but only to a limited extent. Based on the literature all the synthesised compounds are subjected to antioxidant activity by DPPH method. All the tested compounds exhibited maximum activity compared to the standard drug Ascorbic acid. Based on the study the compound OX5 and

OX6 which is substituted with a electronegative groups like Chlorine and hydroxy group produce a significant activity compared with standard drugs. All the synthesized compounds were screened for their in vitro viability test against MCF – 7, cancer cell lines. Compounds OX5 and OX6 emerged to be the most active compounds against tested cell lines. In addition, ADMET prediction results indicated that these compounds might possess less toxicity and pharmacokinetic properties. The study thus serves as an attempt to progress toward the discovery of novel drugs. Additional derivatives may be prepared and further extended in-depth investigations into in-vivo activity would be implemented to establish a SAR (Structural activity relationship) for rational study.

REFERENCES

1. R. Sekhon and N. Bhatla, "Gynecological cancer update," Asian Journal of Oncology, 2016, 2, 61.
2. J. Didkowska, U. Wojciechowska, M. Mańczuk, and J. Łobaszewski, "Lung cancer epidemiology: contemporary and future challenges worldwide," Annals of Translational Medicine, 2016, 4(8), 150.
3. M. Hawash, "Highlights on specific biological targets; cyclindependent kinases, epidermal growth factor receptors, Ras protein, and cancer stem cells in anticancer drug development," Drug Research, 2019, 69(9), 471–478.
4. K. O. M. Almohammadawi, H. S. Q. Alhilfi, and A. S. H. Alshewered, "Epidemiological data of 1418 cancer cases of inpatient in Al-Sadder Teaching Hospital, Misan Province from 2011-2018 (Surveillance study)," Medical Science, 2018, 22, 455–461.
5. J. E. Bennett, G. A. Stevens, C. D. Mathers et al., "2030: Worldwide trends in non-communicable disease mortality and progress towards sustainable development goal target 3.4," The Lancet, 2018, 392, 1072–1088.
6. S. M. Abu Bakr, S. S. Abd El-Karim, M. M. Said, and M. M. Youns, "Synthesis and anticancer evaluation of novel isoxazole/pyrazole derivatives," Research on Chemical Intermediates, 2016, 42(2), 1387–1399.
7. J. Ferlay, M. Colombet, I. Soerjomataram et al., "Estimating the global cancer incidence and mortality in 2018: GLOBOCAN sources and methods," International Journal of Cancer, 2019, 144 (8), 1941–1953.
8. Y.-H. Chu, S.-L. Tzeng, C.-W. Lin, M.-H. Chien, M.-K. Chen, and S.-F. Yang, "Impacts of microRNA gene polymorphisms on the susceptibility of environmental factors leading to carcinogenesis in oral cancer," PLoS One, 2012, 7(6), 39777.
9. M. T. E. Puts, B. Tapscott, M. Fitch et al., "A systematic review of factors influencing older adults' decision to accept or decline cancer treatment," Cancer Treatment Reviews, 2016, 41(2), 197–215.
10. C.-Y. Huang, D.-T. Ju, C.-F. Chang, P. M. Reddy, and B. K. Velmurugan, "A review on the effects of current chemotherapy drugs and natural agents in treating non–small cell lung cancer," Bio Medicine, 2017, 7 (4), 23–23.
11. M. Arruebo, N. Vilaboa, B. Sáez-Gutierrez et al., "Assessment of the evolution of cancer treatment therapies," Cancers, 2011, 3 (3), 3279–3330.
12. R. Oun, Y. E. Moussa, and N. J. Wheate, "The side effects of platinum-based chemotherapy drugs: a review for chemists," Dalton Transactions, 2018, 47(19), 6645–6653.
13. M. S. Aslam, S. Naveed, A. Ahmed, Z. Abbas, I. Gull, and M. A. Athar, "Side effects of chemotherapy in cancer patients and evaluation of patients opinion about starvation based differential chemotherapy," Journal of Cancer Therapy, 2014, 5 (8), 817–822.
14. N. A. Jaradat, S. Al-lahham, A. N. Zaid et al., "Carlina curetum plant phytoconstituents, enzymes inhibitory and cytotoxic activity on cervical epithelial carcinoma and colon cancer cell lines," European Journal of Integrative Medicine, 2019, 30, 100933.
15. M. M. Hawash and S. N. Baytas, "Antiproliferative activities of some biologically important scaffolds," FABAD Journal of Pharmaceutical Sciences, 2018, 43,9–77.
16. P. Nathan, M. Zweifel, A. R. Padhani et al., "Phase I trial of combretastatin A4 phosphate (CA4P) in combination with bevacizumab in patients with advanced cancer," Clinical Cancer Research, 2012, 18(12), 3428–3439.
17. E. Abma, S. Daminet, P. Smets, Y. Ni, and H. De Rooster, "Combretastatin A4-phosphate and its potential in veterinary oncology: a review," Veterinary and Comparative Oncology, 2017, 15 (1), 184–193.
18. S. Shahinshavali, R. Sreenivasulu, V. Guttikonda, D. Kolli, and M. Rao, "Synthesis and anticancer activity of amide derivatives of 1, 2-isoxazole combined 1, 2, 4-thiadiazole," Russian Journal of General Chemistry, 2019, 89 (2), 324–329.
19. J.-P. Yong, C.-Z. Lu, and X. Wu, "Potential anticancer agents. I. Synthesis of isoxazole moiety containing quinazoline derivatives and preliminarily in vitro anticancer activity," Anti-Cancer Agents in Medicinal Chemistry, 2014, 15 (1), 131–136.
20. R. N. Kumar, G. J. Dev, N. Ravikumar et al., "Synthesis of novel triazole/isoxazole functionalized 7-(trifluoromethyl)pyrido[2,3- _d_]pyrimidine derivatives as promising anticancer and antibacterial agents," Bioorganic & Medicinal Chemistry Letters, 2016, 26 (12), 2927–2930.
21. C. A. Kumar, B. Veeresh, K. Ramesha et al., "Antidiabetic studies of 1-benzhydryl-piperazine sulfonamide and carboxamide derivatives," Journal of Applied Chemistry, 2017, 6, 240.
22. V. K. Govindappa, J. Prabhashankar, B. B. A. Khatoon, M. B. Ningappa, and A. K. Kariyappa, "Synthesis of 3, 5-diaryl-isoxazole-4-carbonitriles and their efficacy as antimicrobial agents," Pharmacy Chemica, 2012, 4, 2283–2287.
23. A. Sysak and B. Obmińska-Mrukowicz, "Isoxazole ring as a useful scaffold in a search for new therapeutic agents," European Journal of Medicinal Chemistry, 2017, 137, 292–309.
24. Kaviarasan L, Gowramma B, Identification of Benzimidazole Containing 4H-Chromen- 4-One Derivative as Potential MAP Kinase Inhibitors by In-Silico Approaches, Journal of Receptor and Signal Transduction Research. 2021, 41, 153–8.
25. de Oliveira CS, Lira B, Barbosa-Filho JM, Lorenzo JG and de Athayde-Filho PF. Synthetic approaches and pharmacological activity of 1, 3, 4-oxadiazoles: a review of the literature from 2000–2012. Molecules, 2012, 17(9), 10192-10231.
26. Kaviarasan L, Gowramma B, Kalirajan R, Mevithra M, Chandralekha S, Molecular Docking Studies and Synthesis of a New Class of Chroman 4 One Fused 1,3,4 Thiadiazole Derivatives and Evaluation for Their Anticancer Potential, Journal of the Iranian Chemical Society, 2020, 17(8), 2083–94.
27. Onkol T, Cakir B, Sahin FM. Synthesis and antinociceptive activity of 2-[(2-Oxobenzothiazolin-3-yl) methyl]-5- aminoalkyl/aryl-1,3,4-thiadiazole. Turk. J. Chem., 2002, 28, 461.
28. Kaviarasan L, Elizabeth E, Praveen TK, Kalirajan R, Manal M, Pulla Prudviraj, Gowramma B. 1,3,4-Thiadiazolo (3,2-A) Pyrimidine-6-Carbonitrile Scaffold as PARP1 Inhibitor, Anti-Cancer Agents in Medicinal Chemistry 2021, 21(15), 2050–65.