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SYNTHESIS AND DESIGN OF A NOVEL SUBSTITUTED MORPHOLINE DERIVATIVES AND EVALUATION FORTHEIR ANTI-CANCER ACTIVITY

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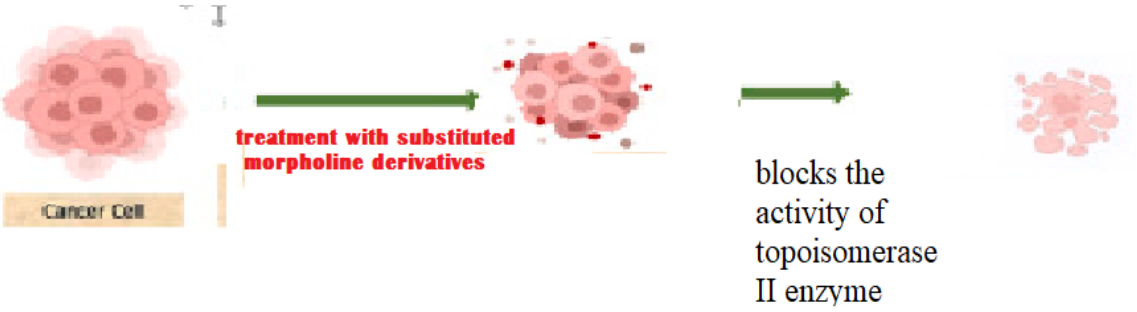
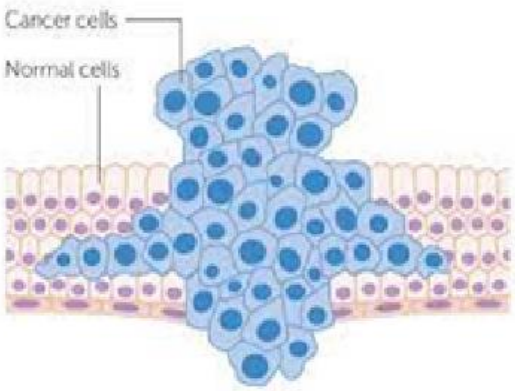
ABSTRACT

Synthesis and design of a novel substituted morpholine derivatives and evaluation for their anti-cancer activity. The docking study of the designed compound was studied against topoisomerase and the result of docking studies revealed that all compoundspossess significant to moderate interaction with the target enzyme. Amongthese compounds are subjected to *in-vitro* cytotoxicity study by SRB assay method with cell lines MDA-MB-231 cell lines. All the tested compounds displayed an IC_{50}> 115 µg/mL at a concentration range of 30–250 µg/mL. Among the tested compounds, derivative M5 substituted with methoxy shows a significant IC_{50} value (81.92 µg/ml) and followed by compound M2 substituted with methyl derivative (88.27 µg/ml) shows good inhibition in the breast cancer cell line.Remaining all other tested compounds shows well to moderate cytotoxic activity on the tested cell line.





KEYWORDS: Docking study, topoisomerase II, Docking study, in-vitro studies, MDA-MB-231, cytotoxic activity.

1. INTRODUCTION

Heterocyclic agents are used for the synthesis of many drugs since they exhibit a specific form of chemical reactivity. During the period of 1940-1970, there was an urgent requirement for the discovery of newer drugs for their treatment against numerous ailments. Proving the proverb "Necessity is the mother of invention", during the decade of the 40 to 70s, a large number of drugs were introduced.[1,2] Therefore, this period is regarded as the "Golden Period" for new drug discovery. Morpholine was first synthesized by dehydration of diethanolamine in the year 1898.[3] The compound of morpholine tends to exhibit a characteristic amine odour. The compound is miscible in water and serves as an organic solvent for dyes, resins, shellac, waxes and casein. Morpholine remains an extremely versatile chemical with several applications. Over 25,000 tonnes of morpholine are produced throughout the world annually.[4] The major preparation process involves the reaction of diethylene glycol with ammonia in the presence of catalysts and hydrogen. Also, the compound serves as an intermediate for manufacturing rubber chemicals as well as optical brighteners. The compound has also been used extensively as a corrosion inhibitor for several steam boiler systems and serves as a catalyst in several organic reactions. The compound has also been used in synthesizing organic molecules.[5,6]

The derivatives of morpholine are being regarded as a significant class composed of heterocyclic compounds that have gained considerable interest in recent years as an anti-inflammatory local anaesthetic, anticancer, appetite suppressant, neuroprotective, antituberculosis, antidepressant, antitumor, antimalarial, antiparasitic and with Hypolipidemicactivities.[7] Due to its improved pharmacokinetic properties, such compounds

are widely utilized by the pharmaceutical industry for drug design. Biological activity exhibited by molecules comprising of morpholine moiety is immense and especially, nitrogen-substituted morpholines serve as drugs exhibiting a wide spectrum of numerous biological activities. The commercially synthesized antibiotic linezolid with morpholine moiety has an excellent antimicrobial agent[8] and Gefitinibis clinically utilized for treating chemo-resistant non-small cell lung cancer patients.[9] Moclobemideis a drug used for depression. Phendimetrazineis widely prescribed as an appetite suppressant and is similar to amphetamine.[10] Furthermore, fenpropimorphis reported as a fungicide and is widely used for controlling diseases that affect cereal productivity.[11]

Process chemistry acts as an interplay for several underlined factors like the freedom to operate, polymorphism and the efficient analytical methods for determining the quality and green chemistry.

In the current scenario, the main purpose of R&D with regard to generic API players is to establish a non-infringing process that meets up with the above-defined issues over tremendous restrictions on patents.[12] The cost of API depends on the following parameters such as raw materials (reactants, catalysts, solvents and reagents), the robustness of the process (concise process and high yields), cycle time and recovery.

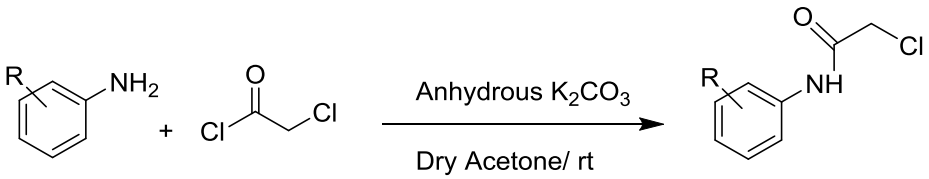
2. MATERIALS AND METHODS

All the chemicals were purchased from Nice Chemicals. Analytical TLC was performed on Pre-coated sheets of silica gel G/UV-254 of 0.2mm thickness (Macherey-Nagel, Germany) using analytical grade solvent and visualized with iodine spray (10% w/w I2 in silica gel) or UV light. The melting point was determined in capillary tubes and is uncorrected. IR spectra were taken as KBr pellets for Perkin Elmer Spectrum FT-IR solids. 1H NMR (400MHz) and 13C NMR (100 MHz) spectra were recorded in DMSO-d6 solution with TMS as an internal standard on Bruker instrument. Spin multiplicities are given as s (singlet), d (doublet), t (triplet) and m (multiplet). The coupling constant (J) is given in hertz. Mass spectra were recorded on an LC-MS spectrometer.In the molecular scenario in the modern drug design, docking is commonly used to understand the interaction between the target ligand-receptor and the target lead molecule's binding orientation with its protein receptor and is quite frequently used to detect the associations between the target components. The research work was done in-silico by utilizing bioinformatics tools. Also, we utilize some of the offline

programmings like protein data bank (PDB) www.rcsb.org/pdb, PubChem database, Marvin sketch. The molecular docking studies were carried out through Discovery studio.

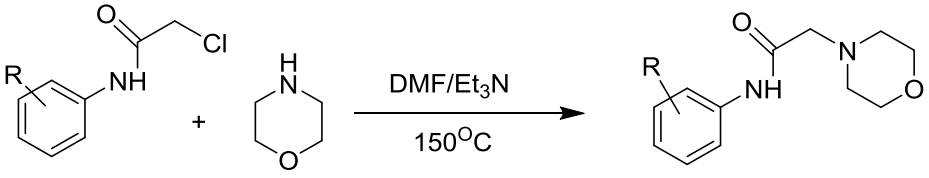
2.1.General procedure for the synthesis of substituted 2-chloro-N-phenylacetamide derivatives

Substituted amino benzene 1.0 gm (0.0011 mol, 1.0 equiv) in acetone was added potassium carbonate 0.24 gm (0.00179mol, 1.5 equiv). After stirring for 15 min at room temperature, 2 chloroacetylchloride (174-184) 0.242 gm (0.00143 mol, 1.2 equivalent) was added, and the reaction mixture was stirred at room temperature for 4−12 hrs. Progress of the reaction was monitored with TLC using n-hexane: ethylacetate (1:2 to 1:6) as eluent, sports are detected by using UV spectroscopy. The mixture was filtered, the filtrate was concentrated, and the residue thus obtained was purified.



2.2.procedure for the synthesis of N-MorpholineDerivatives (1-10))

A mixture of substituted 2-chloro-N-phenylacetamide (0.01mol) in 40 ml DMF was added to the mixture of morpholine (0.012 mol) in triethylamine (0.012mol). Reaction mixtures were refluxed for 1h at 150-1550C until the starting material disappeared by TLC. After the reaction, the precipitate formed upon cooling and was filtered and recrystallized from ethanol to achieve the final compounds.



2.2.1. Synthesis of compound 2-morpholino-N-(p-tolyl)acetamide (M1)

$C_{13}H_{18}N_2O_2$; White crystalline solid; MP 199-201°C; Rf: 0.95; % yield: 89%; IR (cm^{-1}): (NH Stretching amine), (CH Stretching aromatic), (CH Stretching alkane), (C=O Stretching amide), (aromatic ring);^{1}H NMR (500 MHz, DMSO) δ 7.45 (d, J = 7.5 Hz, 2H), 7.23 (d, J = 7.4 Hz, 2H), 6.93 (s, 1H), 3.79 (t, J = 4.7 Hz, 4H), 3.26 (s, 2H), 2.69 (t, J = 4.7 Hz, 2H), 2.57 (t, J = 4.8 Hz, 2H), 2.34 (s, 3H). ^{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, 124.45, 123.71, 119.40, 119.01, 40.54, 20.89, 17.16. Mass: found 236 m/z; actual: 234 m/z.

2.2.2. Synthesis of compound 2-morpholino-N-(o-tolyl)acetamide (M2)

$C_{13}H_{18}N_2O_2$; White crystalline solid; MP 228-231°C; Rf: 0.95; % yield: 89%; IR (cm^{-1}): (NH Stretching amine), (CH Stretching aromatic), (CH Stretching alkane), (C=O Stretching amide), (aromatic ring); ^{1}H NMR (500 MHz, DMSO) δ 7.45 (d, *J* = 7.5 Hz, 2H), 7.23 (d, *J* = 7.4 Hz, 2H), 6.93 (s, 1H), 3.79 (t, *J* = 4.7 Hz, 4H), 3.26 (s, 2H), 2.69 (t, *J* = 4.7 Hz, 2H), 2.57 (t, *J* = 4.8 Hz, 2H), 2.34 (s, 3H). ^{13}C NMR (126 MHz, DMSO) δ 155.82, 134.70, 134.50, 129.99, 129.36, 129.30, 128.79, 128.02, 127.88, 127.26, 127.02, 126.63, 124.45, 123.71, 119.40, 119.01, 40.54, 20.89, 17.16. Mass: found 236 m/z; actual: 234 m/z.

2.2.3. Synthesis of compound N-(4-chlorophenyl)-2-morpholinoacetamide (M3)

$C_{12}H_{15}ClN_2O_2$; White crystalline solid, MP 223-225°C; Rf: 0.98; % yield: 91%; IR (cm^{-1}): (NH Stretching amine), (CH Stretching aromatic), (CH Stretching alkane), (C=O Stretching amide), (aromatic ring); ^{1}H NMR (500 MHz, DMSO) δ 7.44 (d, J = 7.5 Hz, 2H), 7.38 (d, J = 7.5 Hz, 2H), 6.93 (s, 1H), 3.79 (t, J = 4.7 Hz, 4H), 3.26 (s, 2H), 2.69 (t, J = 4.7 Hz, 2H), 2.57 (t, J = 4.7 Hz, 2H). ^{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, 124.45, 123.71, 119.40, 119.01, 67.61, 64.87. Mass: found 256 m/z; actual: 254 m/z.

2.2.3.Synthesis of compound N-(4-methoxyphenyl)-2-morpholinoacetamide (M5)

$C_{13}H_{18}N_2O_3$; White crystalline solid, MP: 211-214 °C; Rf: 0.98; % yield: 90%; IR (cm^{-1}): (NH Stretching amine), (CH Stretching aromatic), (CH Stretching alkane), (C=O Stretching amide), (aromatic ring); ^{1}H NMR (500 MHz, DMSO) δ 9.82 (s, 1H), 7.47 (d, *J* = 7.5 Hz, 2H), 6.97 (d, *J* = 7.5 Hz, 2H), 6.87 (s, 1H), 3.79 (dd, *J* = 12.0, 7.3 Hz, 7H), 3.26 (s, 2H), 2.69 (t, *J* = 4.8 Hz, 2H), 2.57 (t, *J* = 4.7 Hz, 2H). ^{13}C NMR (126 MHz, DMSO) δ 170.31, 134.50, 129.99, 129.36, 129.30, 128.79, 128.02, 127.88, 127.26, 127.02, 126.63, 124.45, 67.61, 64.87, 40.54. Mass: 251 found m/z; actual: 250 m/z.

2.2.4. Synthesis of compound N-(2-methoxyphenyl)-2-morpholinoacetamide (M6)

$C_{13}H_{18}N_2O_3$; White crystalline solid, MP 187-191°C; Rf: 0.97; % yield: 90%; IR (cm^{-1}): (NH Stretching amine), (CH Stretching aromatic), (CH Stretching alkane), (C=O Stretching amide), (aromatic ring); ^{1}H NMR (500 MHz, DMSO) δ 9.82 (s, 1H), 7.47 (d, *J* = 7.5 Hz, 2H), 6.97 (d, *J* = 7.5 Hz, 2H), 6.87 (s, 1H), 3.79 (dd, *J* = 12.0, 7.3 Hz, 7H), 3.26 (s, 2H), 2.69 (t, *J*=

4.8 Hz, 2H), 2.57 (t, *J* = 4.7 Hz, 2H). ^{13}C NMR (126 MHz, DMSO) δ 170.31, 134.50, 129.99, 129.36, 129.30, 128.79, 128.02, 127.88, 127.26, 127.02, 126.63, 124.45, 67.61, 64.87, 40.54. Mass: found 250 m/z; actual: 250 m/z.

2.2.5. Synthesis of compound (N-(4-hydroxyphenyl)-2-morpholinoacetamide (M11)

$C_{12}H_{16}N_2O_3$; White crystalline solid, MP 122-125°C; Rf: 0.98; % yield: 90%; IR (cm^{-1}): (NH Stretching amine), (CH Stretching aromatic), (CH Stretching alkane), (C=O Stretching amide), (aromatic ring); ^{1}H NMR (500 MHz, DMSO) δ 9 .79 (s, 1H), 7.33 (d, *J* = 7.5 Hz, 2H), 6.88 – 6.81 (m, 3H), 3.79 (t, *J* = 4.7 Hz, 4H), 3.61 (s, 1H), 3.26 (s, 2H), 2.69 (t, *J* = 4.8 Hz, 2H), 2.57 (t, *J* = 4.8 Hz, 2H). ^{13}C NMR (126 MHz, DMSO) δ 170.31, 129.99, 129.36, 129.30, 128.79, 128.02, 127.88, 127.26, 127.02, 126.63, 67.61, 64.87. Mass: found 237 m/z; actual: 236 m/z.

2.2.6. Synthesis of compound N-(3-chloro-4-hydroxyphenyl)-2-morpholinoacetamide (M13)

$C_{12}H_{15}ClN_2O_3$; White crystalline solid, MP: 112-116 °C; Rf: 0.97; % yield: 91%; IR (cm^{-1}): (NH Stretching amine), (CH Stretching aromatic), (CH Stretching alkane), (C=O Stretching amide), (aromatic ring); ^{1}H NMR (500 MHz, DMSO) δ 9.79 (s, 1H), 9.26 (s, 1H), 7.61 (d, *J* = 1.4 Hz, 1H), 7.05 (dd, *J* = 7.5, 1.4 Hz, 1H), 6.88 (s, 1H), 6.81 (d, *J* = 7.5 Hz, 1H), 4.34 (s, 1H), 3.79 (t, *J* = 4.7 Hz, 4H), 3.26 (s, 2H), 2.69 (t, *J* = 4.7 Hz, 2H), 2.57 (t, *J* = 4.8 Hz, 2H). ^{13}C NMR (126 MHz, DMSO) δ 170.31, 129.99, 129.36, 129.30, 128.79, 128.02, 127.88, 127.26, 127.02, 126.63, 67.61, 64.87. Mass: found 272 m/z; actual: 270 m/z.

2.2.7.. Synthesis of compound N-(2-formyl-5-hydroxyphenyl)-2-morpholinoacetamide (M14)

$C_{12}H_{16}N_2O_4$; White crystalline solid, MP 100-103 °C; Rf: 0.98; % yield: 90%; IR (cm^{-1}): (NH Stretching amine), (CH Stretching aromatic), (CH Stretching alkane), (C=O Stretching amide), (aromatic ring); ^{1}H NMR (500 MHz, DMSO) δ 9.79 (s, 1H), 9.26 (s, 1H), 7.61 (d, *J*= 1.4 Hz, 1H), 7.05 (dd, *J* = 7.5, 1.4 Hz, 1H), 6.88 (s, 1H), 6.81 (d, *J* = 7.5 Hz, 1H), 4.34 (s, 1H), 3.79 (t, *J* = 4.7 Hz, 4H), 3.26 (s, 2H), 2.69 (t, *J* = 4.7 Hz, 2H), 2.57 (t, *J* = 4.8 Hz, 2H). ^{13}C NMR (126 MHz, DMSO) δ 170.31, 129.99, 129.36, 129.30, 128.79, 128.02, 127.88, 127.26, 127.02, 126.63, 67.61, 64.87. Mass: found 265 m/z; actual: 264 m/z.

2.2.8. Synthesis of compound N-methyl-2-morpholino-N-phenylacetamide (M18)

$C_{13}H_{18}N_2O_2$; White crystalline solid, MP 240-241°C; Rf: 0.99; % yield: 91%; IR (cm^{-1}): (NH Stretching amine), (CH Stretching aromatic), (CH Stretching alkane), (C=O Stretching amide), (aromatic ring); ^{1}H NMR (500 MHz, DMSO) δ 9.79 (s, 1H), 9.26 (s, 1H), 7.61 (d, *J* = 1.4 Hz, 1H), 7.05 (dd, *J* = 7.5, 1.4 Hz, 1H), 6.88 (s, 1H), 6.81 (d, *J* = 7.5 Hz, 1H), 4.34 (s, 1H), 3.79 (t, *J* = 4.7 Hz, 4H), 3.26 (s, 2H), 2.69 (t, *J* = 4.7 Hz, 2H), 2.57 (t, *J* = 4.8 Hz, 2H). ^{13}C NMR (126 MHz, DMSO) δ 168.50, 156.63, 137.77, 135.17, 128.98, 128.09, 126.06, 125.64, 124.94, 123.54, 123.24, 121.60, 65.51, 62.44, 19.18. Mass: found 233 m/z; actual: 234 m/z.

2.2.9. Synthesis of compound N-(4-chlorophenyl)-N-ethyl-2-morpholinoacetamide (M20)

$C_{14}H_{19}ClN_2O_2$; White crystalline solid, MP 243-245°C; Rf: 0.98; % yield: 90%; IR (cm^{-1}): (NH Stretching amine), (CH Stretching aromatic), (CH Stretching alkane), (C=O Stretching amide), (aromatic ring); ^{1}H NMR (500 MHz, DMSO) δ 7.37 (d, *J* = 7.5 Hz, 2H), 7.00 (d, *J* = 7.5 Hz, 2H), 3.85 (q, *J* = 6.3 Hz, 1H), 3.75 (t, *J* = 4.7 Hz, 4H), 3.56 (q, *J* = 6.3 Hz, 1H), 3.25 (s, 2H), 2.64 (t, *J* = 4.7 Hz, 2H), 2.50 (t, *J* = 4.7 Hz, 2H), 1.30 (t, *J* = 6.2 Hz, 3H). ^{13}C NMR (126 MHz, DMSO) δ 170.30, 155.98, 153.95, 135.46, 134.50, 131.51, 130.00, 129.31, 128.03, 127.26, 127.03, 124.45, 119.01, 108.17, 64.86, 56.41, 18.07. Mass: found 282 m/z; actual: 282 m/z.

2.3. Molecular Dockingstudies

Devices and Materials

In the molecular scenario in the modern drug design, docking is commonly used to understand the interaction between the target ligand-receptor and the target lead molecule's binding orientation with its protein receptor and is quite frequently used to detect the associations between the target components. The research work was done in-silico by utilizing bioinformatics tools. Also, we utilize some of the offline programmings like protein data bank (PDB) www.rcsb.org/pdb, PubChem database, and Marvin sketch. The molecular docking studies were carried out through Discovery Studio.

5.5.2. Preparation of protein

By utilizing the offline program protein data bank (PDB), we take the tyrosinase II (PDB ID:3NM8) was obtained from the PDB website. From the protein, we removed the crystal water, followed by the addition of missing hydrogens, protonation, ionization, and energy minimization. The SPDBV (swiss protein data bank viewer) force field was applied for

energy minimization. The prepared protein is validated by utilizing the Ramachandran plot 43.

5.5.3. Identification of active sites

Identification of active amino acid present in the protein is detected by using the Protein-ligand interaction profile (PLIP) https://plip-tool.biotec.tu-dresden.de/plipweb/plip/index offline tool in Google. From this, I found the active amino acid present in the protein44.

5.5.4. Preparation of Ligands

By utilizing the Marvin sketch tool, the molecules are designed in two and three-dimensional structures. After the designed molecule, the structure was optimized in 3D optimization in Marvin sketch and saved as a pdf format.

5.6. *In vitro* anticancer activity

The *in vitro* cytotoxicity of the synthesized compounds was assessed against the MDA-MB-231 cancer cell line using an 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% CO_2 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 four washes with acetic acid (1% v/v), and protein-bound dye was extracted with 10 mMunbufferedTris base [tris (hydroxymethyl) aminomethane]. The optical density of the protein-bound dye was recorded at 540 nm. 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. .

6. RESULTS AND DISCUSSION

6.1. Molecular docking studies

Based on literature studies of morpholine derivatives, the 20 compounds were designed for our study and these compounds were subjected to molecular docking studies. Molecular docking was carried out through discovery studio to predict the interactions model of the protein to its inhibitors. The molecular docking was performed to elucidate the binding mode competence of topoisomerase II (PDB ID:1A35) and morpholine analogues. The designed molecules were docked along with the native ligand and a reference standard, doxorubicin. The docking energy of our designed compounds ranged from 8 to 10 kcal/mol indicated good binding affinities to the target receptor, and the results are depicted in **Table 1**. Among the docked compounds, derivative M5 (-9.7 kcal/mol)showed a significant binding energy towards the targeted enzyme. The compounds M5 possess3 hydrogen bond with following amino acids Gly 490, Asn 491, Thr 501. The roles of certain crucial amino acids in the ligand-binding domain of the human topoisomerase inhibitors were also established. Major non-covalent interactions between the studied ligands and the ligand-binding domain of the Acetylcholinesterase inhibitors were investigated. These amino acids have been repeatedly implicated during ligand interaction with the tyrosinase inhibitors and also play important role in the inhibition of the ligand-binding domain of tyrosinase inhibitors. These non-covalent interactions, van der Waals, columbic interaction, π–π interaction, and hydrogen interaction, are shown in **Figure 1 to 7**.

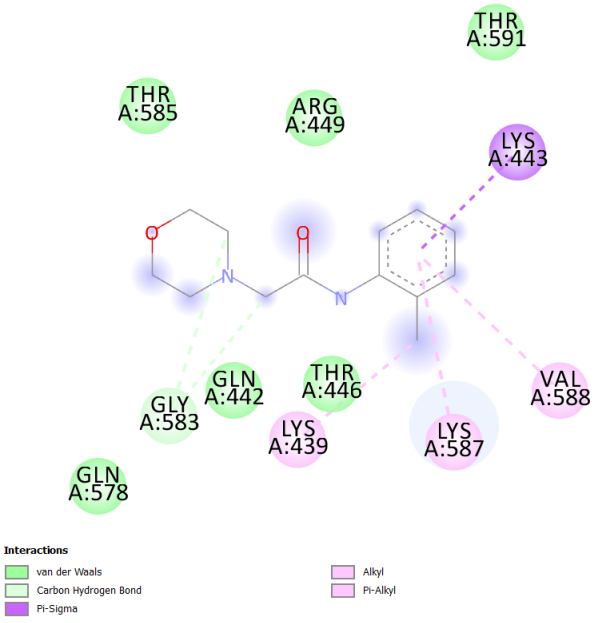


Figure 1: Fitting pose with interactions of M2 in the pocket of 1A35 in 2D view.

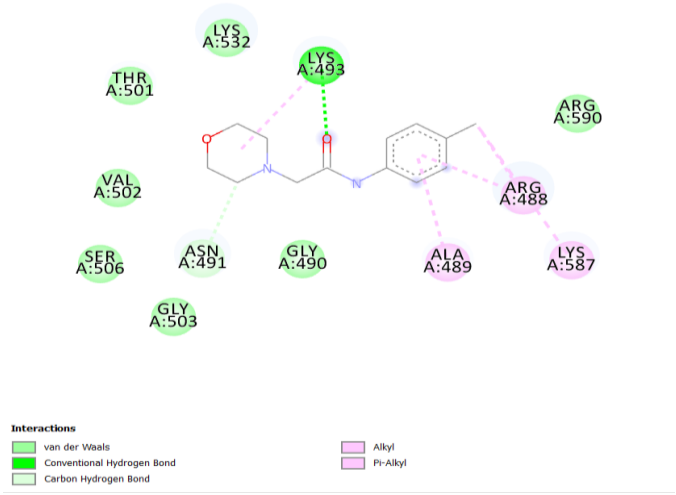


Figure 2. Fitting pose with interactions of M3 in the pocket of 1A35 in 2D view.

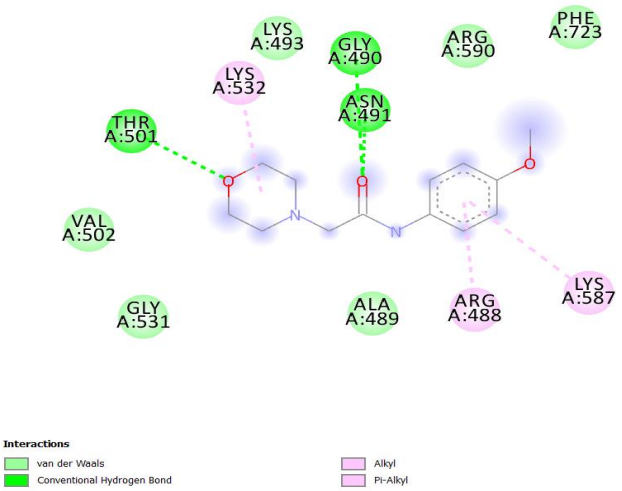


Figure 3. Fitting pose with interactions of M5 in the pocket of 1A35 in 2D view.

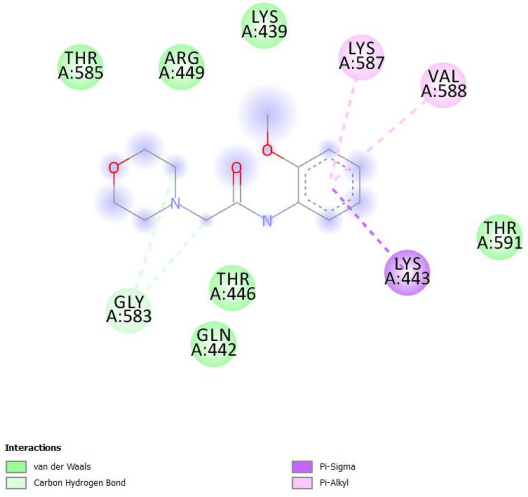


Figure 4. Fitting pose with interactions of M6 in the pocket of 1A35 in 2D view.

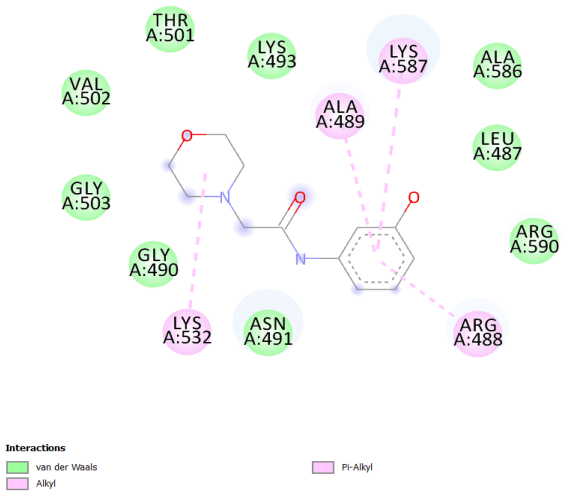


Figure 5. Fitting pose with interactions of M11 in the pocket of 1A35 in 2D view.

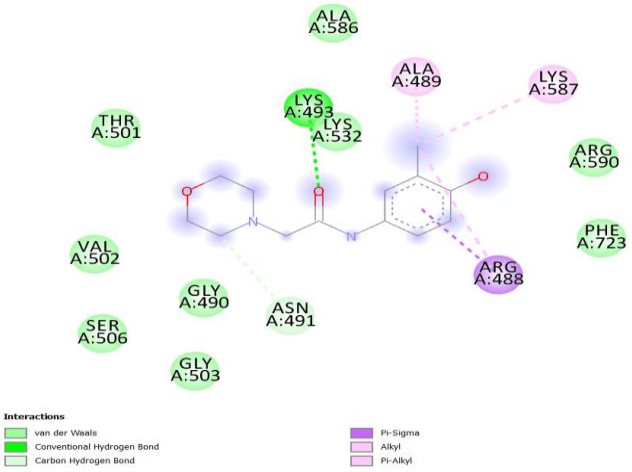


Figure 6. Fitting pose with interactions of M13 in the pocket of 1A35 in 2D view.

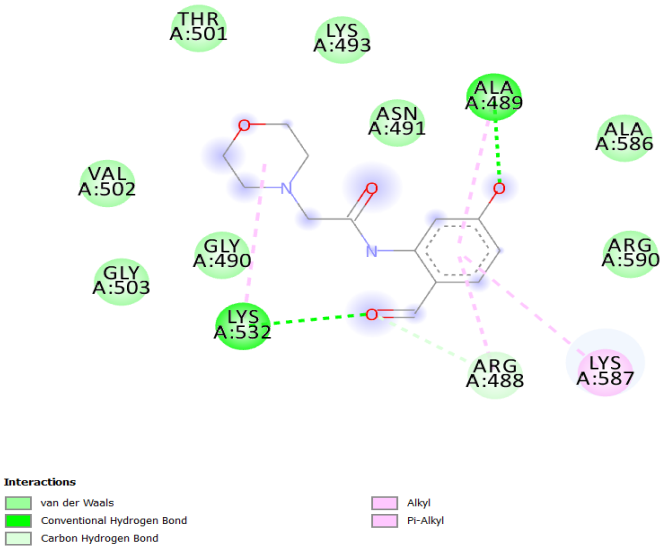


Figure 7. Fitting pose with interactions of M14 in the pocket of 1A35 in 2D view.

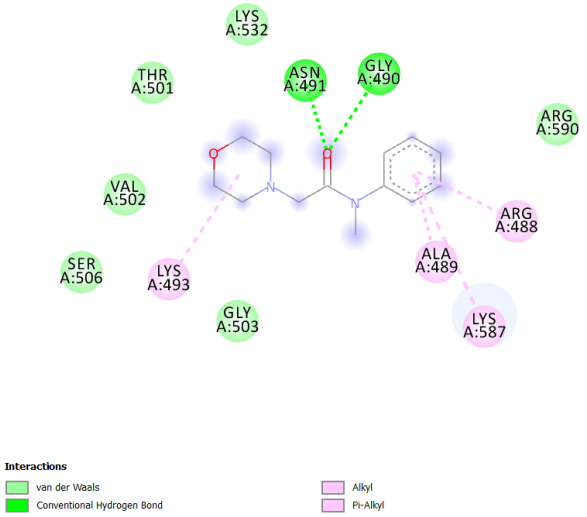


Figure 8. Fitting pose with interactions of M18 in the pocket of 1A35 in 2D view.

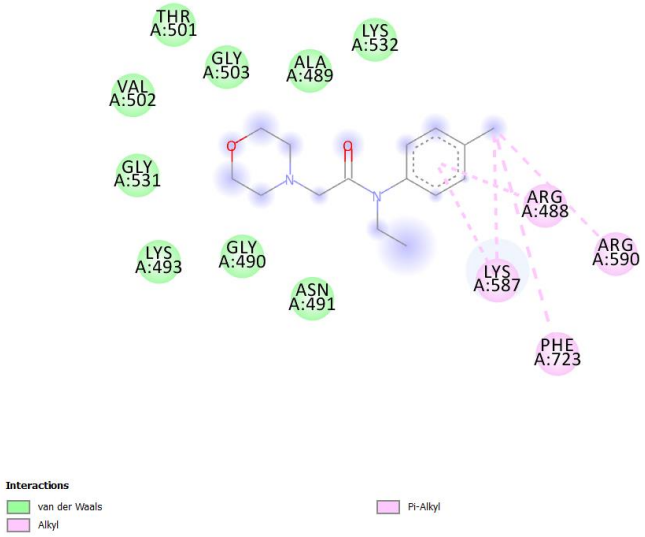


Figure 9. Fitting pose with interactions of M20 in the pocket of 1A35 in 2D view.

Table 1: Docking score.

Ligand	Binding energy
M1	-9.0
M2	-9.6
M3	-9.1
M4	-8.2
M5	-9.7
M6	-9.2
M7	-8.9
M8	-9.1
M9	-9.1
M10	-9.0
M11	-9.1

M12	-8.1
M13	-8.8
M14	-8.8
M15	-7.9
M16	-7.8
M17	-7.7
M18	-8.9
M19	-7.8
M20	-9.1

Table 2: docking results.

Ligand	Binding Affinity
1	87.02
2	33.37
3	65.83
4	89.84
5	64.98
6	85.97
7	91.35
8	86.24
9	87.21
10	99.57
STD	15.23

7.1. *In-vitro* anticancer activity

Results of anticancer activity of the compounds were expressed as IC_{50} values which were determined by plotting the percentage cell viability versus concentration of the sample on a logarithmic graph and reading of the control. The experiments were performed in triplicates, and then, the final IC_{50} values were calculated by taking an average of triplicate experimental results. The results of in-vitro anti-cancer activity expressed in IC_{50} (µg/mL) are expressed in **Table 2** and were compared to Doxorubicin. There are 10 compounds subjected to *in-vitro* cytotoxicity study by SRB assay method with cell lines MDA-MB-231 cell lines. All the tested compounds displayed an IC_{50}> 115 µg/mL at a concentration range of 30–250 µg/mL. Among the tested compounds, derivative M5 substituted with methoxy shows a significant IC_{50} value (81.92 µg/ml) and followed by compound M2 substituted with methyl derivative (88.27 µg/ml) shows good inhibition in the breast cancer cell line. Remaining all other tested compounds shows good to moderate cytotoxic activity on the tested cell line.

Table 2: Date for *in vitro* cell line study.

Sl No	Compound code	MDA-MB-231 (IC_{50}µg/ml)
1	M1	114.16
2	M2	88.27
3	M3	96.54
4	M5	81.92
5	M6	123.39
6	M11	98.34
7	M13	106.23
8	M14	91.35
9	M18	130.35
10	M20	100.32
8	DOX	23.14

CONCLUSION

- In the present work we designed the novel set of morpholine molecule as GyraseD inhibitors.
- All the designed compounds were subjected to molecular docking studies using discovery studio software.
- Based on docking score all the compounds show significant docking score among them compound M1, M2, M3, M5, M6, M11, M13, M14, M18 and M20 shows top ten compound. Further we selected these compounds for synthesis in conventional method.
- Based on the scheme presented in material method section, the designed compounds were synthesized and purified by the recrystallization techniques.
- All our synthesized compounds were purified by column chromatography and characterized by FT-IR, ^{1}H-NMR, ^{13}C-NMR and LC-MS spectral data.
- In the ^{1}H-NMR spectra splitting patterns for the aromatic protons were observed to be in agreement with the substitution pattern of respective compounds.
- In the ^{13}C-NMR spectrum of synthesized compounds, the carbonyl carbon of benzamide and aromatic SP^2 hybridized carbon signals appeared in the expected region.
- These 10 compounds are subjected to *in-vitro* cytotoxicity study by SRB assay method with cell lines MDA-MB-231 cell lines. All the tested compounds displayed an IC_{50}> 115 µg/mL at a concentration range of 30–250 µg/mL. Among the tested compounds, derivative M5 substituted with methoxy shows a significant IC_{50} value (81.92 µg/ml) and followed by compound M2 substituted with methyl derivative (88.27 µg/ml) shows good inhibition in the breast cancer cell line.
- Based on the above binding this study may be concluded as the substitution in the morpholine molecules blocks the activity of topoisomerase II enzyme in cancer cells.

Ethical approval

This article does not contain any human participants and animal work.

Disclosure statement

This author declares that there is no conflict of interest.

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