

DESIGN AND SYNTHESIS OF DENSELY FUNCTIONALIZED NOVEL SPIROOXINDOLES AS ANTICANCER AGENTS

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ABSTRACT

A series of new Spirooxindole derivatives were designed and synthesized to evaluate their antiproliferative activity by MTT assay. The result revealed most of the compounds exhibited strong antiproliferative properties in comparison with positive control Doxorubicin. Particularly compound 8 showed significant inhibitory activity for both MCF-7 and HeLa cancer cell lines. A molecular docking study was performed to evaluate the potential mechanism of antiproliferative activity. It revealed that designed Spirooxindole derivatives strongly interact with hydrophobic amino acids of the p53 binding site of MDM2. Inhibition of p53-MDM2 interaction results in the reactivation of p53 which is corroborated by the significant antiproliferative activity of the compounds against cancer cell lines. These data signify the potential of designed spirooxindole derivatives as anticancer agents.

Keywords: Spirooxindole, Curcumin Analogs, 1,3 Dipolar Cycloaddition, Azomethine Ylides, Anti-Proliferative Activity

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INTRODUCTION

The number of patients suffering from cancer is increasing and is the second worldwide cause of death after cardiovascular diseases.¹ The carcinogenesis mirrors the evolutionary process, where uncontrollable cell proliferation is caused by mutation and selection acting on cells in a tissue.² For metastatic cancers, chemotherapy is the preferred choice of treatment in which antineoplastic agents inhibit the rapidly dividing cancer cells, however, it also affects normal cells with fast-growing rates.³ The toxicity and side-effects such as bone marrow depression, alopecia, nephrotoxicity, and hepatotoxicity of conventional anticancer agents are thus attributed to the inability to discriminate and destruction of normal cells such as hair follicles, bone marrow, and gastrointestinal tract cells.⁴ One way to enhance the specificity of small molecular inhibitors towards cancer cells is to use agents with natural scaffolds. Natural scaffolds such as coumarins⁵, pyrans⁶, and spirooxindoles⁷ provide promising approaches to constructing chemical architectures with improved selectivity towards cancer cells and are highly effective for killing existing tumor cells. Using a combinatorial strategy, the natural scaffolds can be incorporated with effective pharmacophores for targeted therapy. Spirocyclic oxindoles are prevalent in a variety of natural products. Moreover, they are widely explored for their bio-active properties such as antibacterial^{8,9}, antifungal¹⁰, antimalarial¹¹, and anticancer¹² properties. Currently, one of the spirooxindole-based compounds MI-888¹² is in preclinical evaluation for the treatment of cancer. The structural characteristics of spirooxindoles consist of an oxindole core with spiro ring fused and varied heterocyclic motifs. The mechanism of action of this class of compounds deals with the inhibition of p53-MDM2 interaction.¹³ The effective inhibition of this heteromeric interaction causes the reactivation of tumor suppressor gene p53 and regression of tumor progression. Here, we report the design and synthesis of 8 novel spirooxindoles prepared via 1,3 Dipolar Cycloaddition^{14,15} of azomethine ylides. Dipoles were prepared separately in situ from the reaction between Isatin and four α -amino acids such as L-Proline, Thioproline, Sarcosine, and Pipecolic acid. Our design strategy consists of 1) a core group that comprises of spiro oxindole scaffold, R1, and R2 heterocycles, and

2) a side chain pharmacophore containing N-Boc protected piperidiny thiazole moieties. The rationale for selecting a piperidiny thiazole-based sidechain is inspired by our previous article Shanmugasundaram P *et al.* (2019)¹⁶ in which we demonstrated the significant bioactivity of this moiety. We designed and synthesized compounds 1 to 8 by varying R1 and R2 heterocycles (Fig.-1) for structure-activity relation.

EXPERIMENTAL

Chemistry

Spirooxindoles 1-8 were prepared by the multicomponent reaction of chalcones, isatin, and amino acids without any catalyst. According to the literature, *in situ*, the generation of azomethine ylides (1,3-dipole) was achieved by the reaction between isatin and α -amino acids. Further, this ylide was reacted with activated alkene which acts as a dipolarophile, giving rise to pyrrolidine-containing spirooxindole. The detailed synthetic procedure, ¹H NMR, Mass spectra, IR, and purity data are provided in the supplementary information.

Anti-Proliferative Activity by MTT Assay

Growth inhibitory properties of compounds 1-8 were assessed against MCF-7 and HeLa cell lines Fig.-1. IC₅₀ values of the compounds at 48 hrs. were measured by incubating cells with Dimethyl sulfoxide solutions of the selected compounds, washed, and then cell proliferation was evaluated by MTT test; Concentration needed to inhibit cell proliferation by 50% as determined from dose-response curves by exponential decay fitting ($r^2 > 0.9$). The data represent the average of three independent studies. The detailed methodology of culture and maintenance of mammalian cell lines, cell line revival protocol, preparation of cell line testing, cell morphology changes by staining and imaging, quantification of cell death, and protocol of MTT assay are provided in the supplementary data. We used Doxorubicin as a control for our study. Detailed procedures and photomicrographs are provided in the supplementary information.

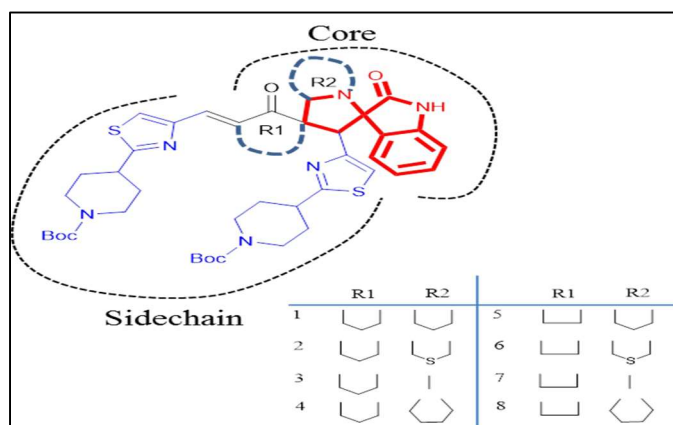


Fig.-1: Design Strategy of Spirooxindole Derivatives Containing Pharmacophoric Scaffolds for Structure-Activity Correlation

Docking Study

The inactivation of tumor suppressor protein p53 is one of the common mechanisms for cancer progression.¹⁷ In silico studies of the synthesized spiro pipecolic (SP) molecules were carried out to study molecular level insight and screen the compound activity. The docking studies for the synthesized compounds were carried out to study atomistic level details and to screen the compound activity. To perform docking studies, we used crystal structure PDB ID: 5LAW¹⁸, obtained from a protein data bank. We used Autodock Vina^{19,20} for docking studies. Protein with PDB format was chosen as a receptor (macromolecule) and was saved as a PDBQT file. The binding pocket of the receptor was chosen according to the co-crystallized ligand binding site. The size of the grid box was set to 37.5 Å, 30 Å, and 30 Å in the x, y, and z directions, respectively, centered on the docking site with 0.375 nm spacing for each dimension. The exhaustiveness of global search and maximum number of binding modes were set to 8 and 20 respectively. Default setting was used as scoring function weights and terms and 3 kcal/mol was set as the maximum energy difference. The results were analyzed by Discovery Studio Visualizer.²¹

RESULTS AND DISCUSSION

The antiproliferative activity of the test compounds 1-8 was assessed against MCF-7 and HeLa cell lines using MTT assay using positive control Doxorubicin. For mechanistic investigation molecular docking study was conducted to assess the inhibiting potential of the compounds for p53-MDM2 interactions. To establish the structure-activity relation and identify the differential role of the various functional groups we divided the structural skeleton of the compounds 1-8 into two regions namely core and side-chain. The core region comprises three functional units namely 1) Pyrrolidine containing spiro oxindole scaffold 2) R1 heterocycle which is contributed by starting material and 3) R2 heterocycle which is provided by respective α - amino acids Fig.-1. Furthermore, the Growth inhibition study revealed that the bulkiness of R1 and R2 heterocyclic groups has a significant effect on bioactivity. This observation was corroborated by the lower IC₅₀ values observed for a series of compounds 1, 2, 3, 4, 5, 6, and 8 having bulkier R2 scaffolds in comparison with compound 7. Particularly, compound 8 showed higher anti-proliferative activity for both MCF-7 and HeLa cancer cell lines compared to other candidates. Moreover, based on the docking scores, we find the top 5 candidates are compound 8, 5, 4, 3, and 2. Interestingly, there is significant correlation exists between the antiproliferative activity of compounds 8 and 5 with their respective docking score. It is very well established that the p53 binding site of the MDM2 is lined by the hydrophobic amino acids, as can be seen in Fig.-5.

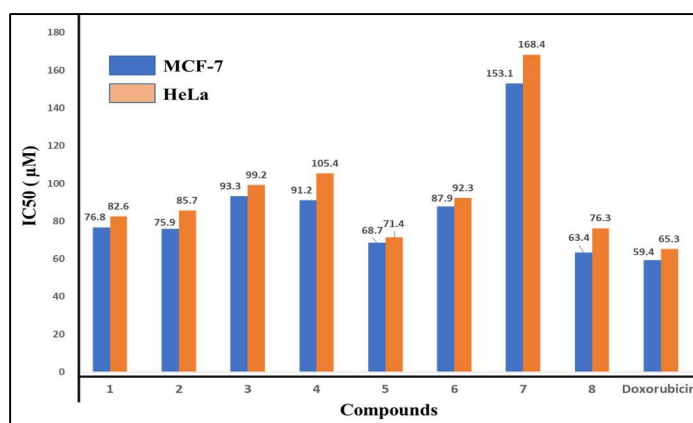


Fig.-2: Graphical Representation of Anti-Proliferative Activity of Compound 1-8 and Doxorubicin Against MCF-7 and HeLa Cancer Cell Lines

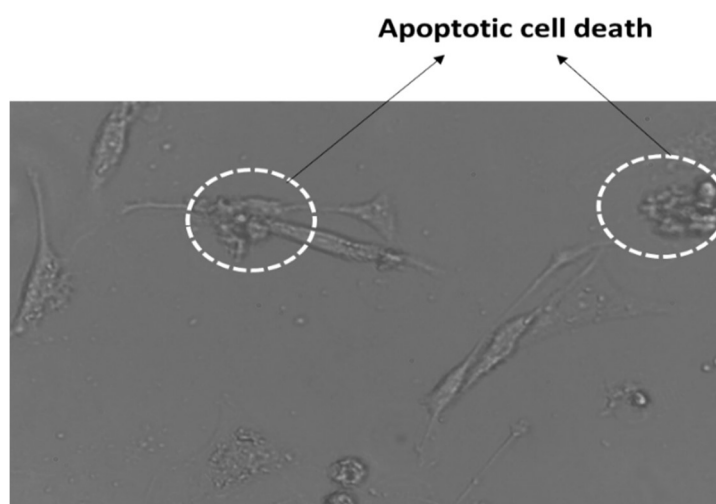


Fig.-3: Photomicrograph of Cellular Death of MCF-7 Cell Line Caused by Compound 8 Showing Similarity in Apoptotic Cell Death

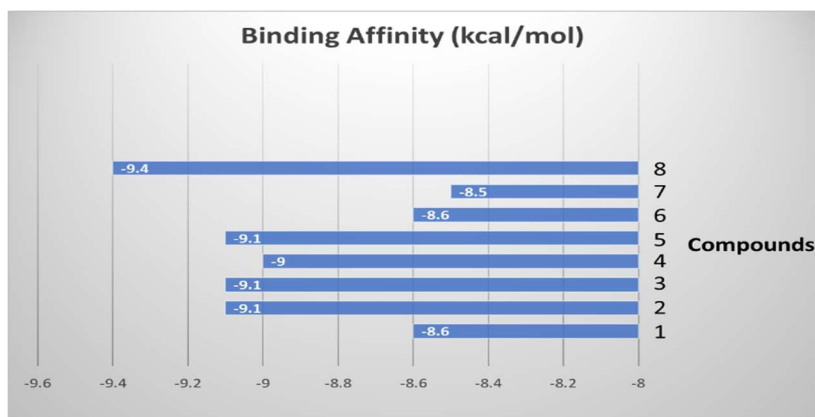


Fig.-4: Binding Affinity Score of Compounds 1-8 for Docking Study

It is very well established that the p53 binding site of the MDM2 is lined by the hydrophobic amino acids, as can be seen from the snapshot below. The interaction profile between compound 8 and MDM2, suggests favorable hydrophobic interactions between the ligand tail and key amino acid (LEU:57, GLY:58, ILE:61, and HIS:96) in the p53 binding cleft. His96 offers hydrogen-bonded and π -sulfur interaction to the thiazole ring. This compound forms multiple π -alkyl interactions with Ala, Lys, and Met amino acids. The π -sigma interaction between Leu54 and ligands and van der Waals contributions from other residues corroborate activity against the cancer cells. The second active compound 5, also interacts with the p53 binding site via hydrophobic interactions, especially with the cleft lining residues such as Ile19, Pro20, Ala21, Glu23, Gln24, Leu54, Leu57, Gly58, Gln59, Phe91, and Ile103. The Phe55 provides π - π interactions to the benzyl ring. The alkyl- π , and alkyl-alkyl interactions from Isoleucine residues and Lysine residues make the overall complex energetically stable.

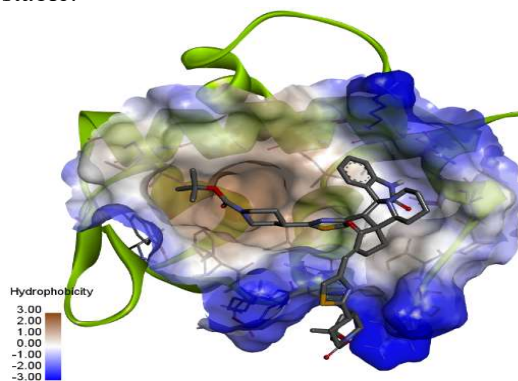


Fig.-5: Interaction Profile of Compound 8 in the MDM-2 Binding Site of p53

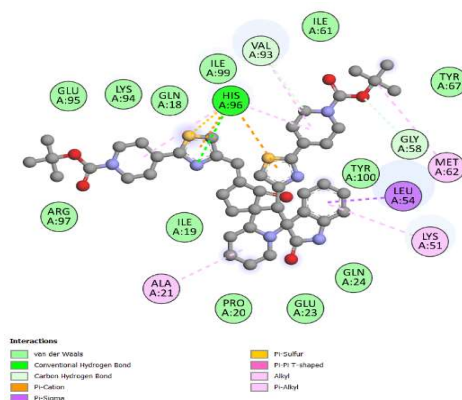


Fig.-6: Interaction Profile of Compound 5 in the MDM-2 Binding Site of p53

CONCLUSION

A good correlation of the docking score and anti-proliferative activity of designed spirooxindole derivatives i.e., compounds 1-8 provides more insight into the mechanism of their anti-proliferative activity against cancer cell lines. Furthermore, bulky scaffolds containing heterocyclic motifs and their relatively strong non-covalent association with the MDM-2 binding site of p53 confers them as potential candidates as anticancer agents for future studies.

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CONFLICT OF INTERESTS

The authors declare that there is no conflict of interest.

AUTHOR CONTRIBUTIONS

All the authors contributed significantly to this manuscript, participated in reviewing/editing, and approved the final draft for publication. The research profile of the authors can be verified from their ORCID ids, given below:

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