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Investigation of In-vitro Anticancer Activity of Letrozole Derivatives

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Abstract

The increasing prevalence of cancer has driven the demand for novel therapeutic agents. This study focuses on the synthesis, characterization, and evaluation of anticancer activity of Letrozole derivatives against HeLa cell lines and other cancer types. The compounds were evaluated through NCI's tumor cell culture screening program and MTT assays. Compounds Sch.3 (58) and Sch.4 (69) demonstrated notable inhibition on HL-60 (TB) leukemia and HeLa cell lines, respectively, with Sch.4 (69) exhibiting up to 68.4% growth inhibition. This study highlights the potential of Letrozole derivatives as anticancer agents with promising efficacy across various concentrations.

Keywords:

Letrozole derivatives, anticancer activity, HeLa cell lines, NCI screening, MTT assay, leukemia.

Introduction

Cancer remains a leading cause of mortality globally, with its incidence projected to increase due to population growth, aging, and lifestyle changes [1,2]. Cervical cancer, caused primarily by persistent infection with high-risk human papillomavirus (HPV) strains, is a significant global health challenge, especially in low- and middle-income countries [3]. Despite advances in prevention and treatment, the five-year survival rate for advanced cervical cancer remains low, underscoring the need for novel therapeutic agents [4,5].

Letrozole, a non-steroidal aromatase inhibitor, is widely used to treat hormone-dependent cancers, particularly postmenopausal breast cancer [6,7]. It acts by inhibiting the aromatase enzyme, which converts androgens to estrogens, thereby reducing estrogen-dependent tumor growth [8]. Recent research has focused on modifying Letrozole's structure to enhance its efficacy and expand its applicability to other cancer types [9].

The Developmental Therapeutics Program (DTP) at the National Cancer Institute (NCI), USA, provides a robust platform for screening compounds against cancer cell lines. The NCI-60 human tumor cell line panel is a valuable resource for evaluating potential anticancer agents across a spectrum of cancers, including leukemia, melanoma, and cervical cancer [10,11]. This program emphasizes precise endpoints, such as net cell killing and tumor regression, rather than mere growth inhibition, offering a comprehensive assessment of compound activity [12,13].

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In vitro assays, such as the MTT assay, are indispensable for preliminary evaluation of anticancer activity. The MTT assay measures cellular metabolic activity as an indicator of cell viability and proliferation, offering insights into a compound's cytotoxic potential [14,15]. HeLa cells, derived from cervical cancer, serve as an ideal model for studying the efficacy of anticancer agents due to their robust growth characteristics and clinical relevance [5].

This study explores the synthesis, characterization, and evaluation of Letrozole derivatives for anticancer activity using the NCI screening program and MTT assay. By assessing their effects on HeLa cells and other cancer types, this research aims to identify promising candidates for further development as anticancer therapeutics.

Material and Method

Compound Synthesis and Characterization: Letrozole derivatives were synthesized and characterized through NMR and IR spectroscopy to confirm structural modifications.

In-vitro NCI Screening Protocol: All synthesized compounds were submitted to NCI for evaluation against their 60-cell line panel. Compounds were tested at five concentrations starting from 10^{-4} M. Growth inhibition was measured as described in the NCI-DTP protocol [5].

MTT Assay on HeLa Cell Lines: HeLa cells were cultured in DMEM with 10% fetal bovine serum. Cells were seeded into 96-well plates and treated with test compounds (1-1000 nM concentrations). Letrozole served as the positive control. MTT was added post-treatment, and absorbance at 370 nm was recorded using a UV-Visible spectrophotometer [6,7].

Results and Discussion

NCI Screening Results:

The interpretation of one dose compare graph gives the cancer cell line sensitivity over the screening compound. It is a characteristic fingerprint for the screened compounds. The value of 100 growth percent means no growth inhibition. A value of 40 growth percent refers to 60 percent growth inhibition. A value of 0 means no net growth over the course of the experiment. A value of -40 growth percent refers to 40 percentlethality. A value of -100 growth percent means all cells are killed. The compounds **Sch.3 (58)** and **Sch.4 (69)** exhibited exceptional anti-leukemia activity. The compound **Sch.3 (58)** showed 32 percent lethality on HL-60 (TB) cell line. It also showed growth inhibition on K-562 and SE cell lines of leukemia. In terms of selectivity ratio, **Sch.3 (58)** and **Sch.4 (69)** compounds were interestingly more selective for HL-60(TB) and CCRF-CEM leukemia cell lines respectively. Compound **Sch.3 (38), Sch.3 (40)** and **Sch.4 (69)** showed growth inhibition on LOX IMVI melanoma cell line, HOP-92 nonsmall cell lung cancer cell line, CCRF-CEM Leukemia cell line respectively. Compound **Sch.3 (58)** interestingly exhibited **32 percent lethality** against HL-60 (TB) cell lines of leukemia where as**Sch.4 (69)** showed **68.4% growth inhibition** against CCRF-CEM Leukemia cell line.

The mean graph representation of the NCI screening results highlighted Sch.3 (58) and Sch.4 (69) as highly active against leukemia cell lines, specifically HL-60 (TB) and CCRF-CEM. Sch.3 (58) demonstrated 32% lethality on HL-60 (TB) cells, while Sch.4 (69) exhibited 68.4% growth inhibition on CCRF-CEM cell lines.

MTT Assay on HeLa Cells:

Anticancer screening on HeLa cell line results were depicted in Figure 8.13 to 8.16. The bars marked **Letrozole** in all the histograms depicts positive control in all the 96 well plates. The other bars with respective compound codes in histogram are the cell viability percentages of HeLa cell line against the respective compounds. All the compounds including Letrozole were tested on four concentrations (1000 nM, 100 nM, 10 nM, 1 nM) to test the effective concentration window. 1000 nM concentration of all the derivatives has shown significant inhibition on HeLa cells equivalent to Letrozole. However, 1 nM concentrations of all the derivatives showed an insignificant effect on HeLa cells in 48 hours and 72 hours incubation. Interestingly compound **Sch.3 (58)** and **Sch.4 (69)** have decreased the cell viability of HeLa cells better than the remaining derivatives in 42-hour incubation of 96 well plate. A similar effect was observed on 1000 nM concentrations of **Sch.3 (58)** and **Sch.4 (69)** derivatives inhibition effect on HeLa cells on 72

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hour incubation period. Compounds **Sch.1 (10), Sch.1 (12)** and **Sch.3 (54)** also showed significant inhibition equivalent to letrozole in all the four concentrations. Especially these compounds showed significant percentage growth inhibition of **57.8 %, 53.1 %, 58.9 %** respectively against HeLa cell line at 1000nm concentration. These preliminary biological screening studies have given positive anticancer activity for these new classes of derivatives.

Letrozole derivatives, particularly Sch.3 (58) and Sch.4 (69), showed significant inhibition of HeLa cell growth at 1000 nM, comparable to Letrozole. Derivatives Sch.1 (10), Sch.1 (12), and Sch.3 (54) also displayed notable activity, with growth inhibition percentages of 57.8%, 53.1%, and 58.9%, respectively. These findings underscore their potential as effective anticancer agents for cervical cancer [8,9].

Figures 1-4 illustrate the percentage viability of HeLa cells after 48 and 72 hours of treatment with various derivatives, confirming their efficacy compared to Letrozole.

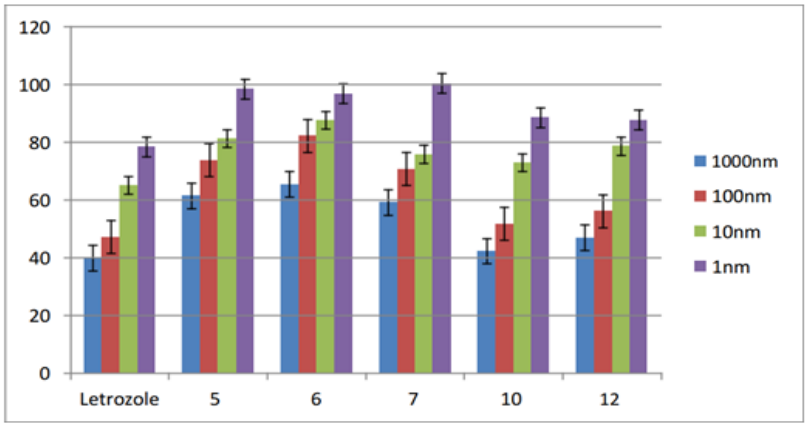

Figure 1: Percentage viability of HeLa cells after 48 hours incubation; Letrozole-positivecontrol, 5=Sch.1 (5); 6=Sch.1 (6); 7=Sch.1(7);10= Sch.1 (10); 12=Sch.1(12);

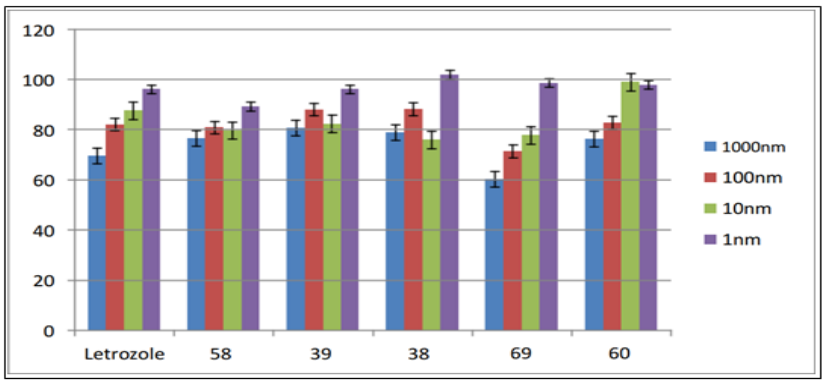

Figure 2: Percentage viability of HeLa cells after 48 hours incubation; Letrozole-positive control, 58= Sch. 3(58); 39=Sch.3 (39); 38= Sch.3 (39); 69= Sch.4 (69); 60=Sch.4 (60);

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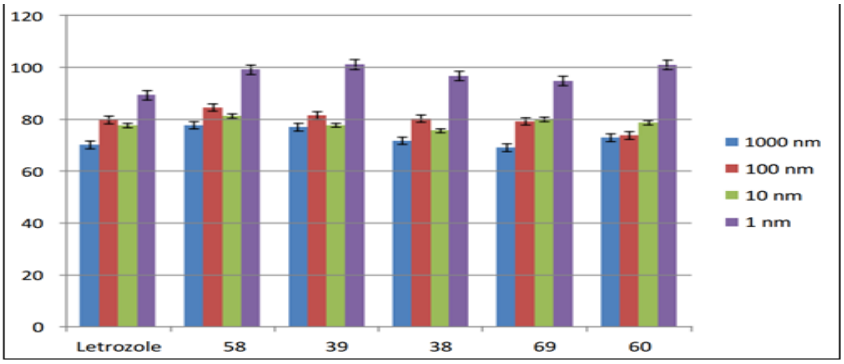

Figure 3: Percentage viability of HeLa cells after 72 hours incubation;Letrozole-positivecontrol, 58=Sch.3(58);39=Sch.3(39);38=Sch.3(39);69= Sch.4(69);60=Sch.4(60);

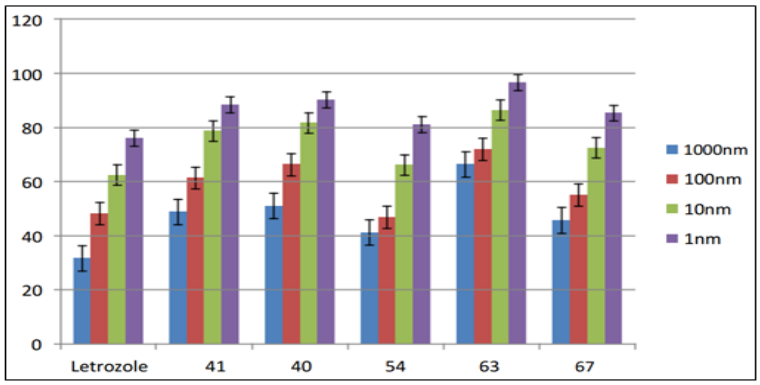

Figure 4: Percentage viability of He La cells after 48 hours incubation; Letrozole-positive control, **41=Sch. 3(41)**; 40=**Sch.3 (40)**; **54=Sch.3 (54)**; **63=Sch.4 (63)**; **67= Sch.4 (67)**;

Conclusion

This study highlights the promising anticancer potential of Letrozole derivatives, particularly against leukemia and HeLa cell lines. Compounds Sch.3 (58) and Sch.4 (69) emerged as potent candidates, demonstrating \superior growth inhibition at higher concentrations. Future research will focus on optimizing these derivatives for enhanced efficacy and reduced toxicity.

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