Pharmacological Evaluation of New 4, 5-dihydro-1H- Pyrazole-1-yl acetate Derivatives as anti-cancer agents

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Abstract:

A series of nine novel 4, 5-dihydro-1H-pyrazole-1-yl acetate derivatives (IVa-i) by Shahlaa et al. was investigated in vitro for their antiproliferative activity against two cancer cell lines, breast cancer cell line (MCF-7) and lung cancer cell lines (A549), According to the cytotoxicity effect of these compounds, IVa, IVc and IVi compounds have antiproliferative effect with percentage (81.30%, 87.4% & 54.66%) respectively at 72h treatment on MCF-7 cell line compared to other compounds, these results indicate that the new compound IVc have the higher antiproliferative percent comparable to tamoxifen as a standard anti-tumour for oestrogen receptor positive breast cancer cell line after 72h followed by IVa after 72h (83.31%). cytotoxicity effect of compound IVb was highest among tested compounds on lung cancer cell line (A549) with antiproliferative percentage (58.49% & 75.04%) at 48 & 72h respectively, but it is less than erlotinib as a standard anti-tumour for lung cancer cell line cytotoxicity effect (77.10% & 82.46%) at these times. three compounds (IVa, IVc & IVi) have antiproliferative effect on breast cancerous cell line (MCF-7) and compound (IVc) have inhibition percentage comparable to that of the authorized medication Tamoxifen. One compound (IVb) had antiproliferative activity, but less than that of erlotinib on lung cancerous cell line (A549) and there is good agreement between our docking results and the experimental results.

Key words: Pyrazole, anticancer, 4, 5-dihydro-1H- Pyrazole, MCF-7, A549

التقييم الدوائي لمشتقات البيرازول الجديدة تحتوي على dihydro-4,5 اسيتات كعوامل مضادة للسرطان

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الخلاصة:

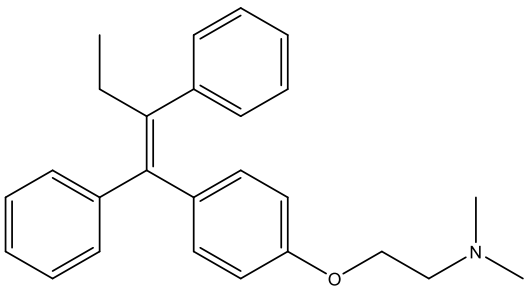
سلسلة من تسعة مشتقات جديدة dihydro-1H- pyrazole-1-yl acetate (IVa-i)-4,5 بواسطة Shahlaa et al. تم فحصها في المختبر لنشاطها المضاد للتكاثر ضد سطرين من الخلايا السرطانية ، خطوط خلايا سرطان الثدي (MCF-7) والرئة (A549) ، وفقًا لتأثير السمية الخلوية لهذه المركبات ، فإن مركبات IVa و IVc و IVi لها تأثير مضاد للتكاثر بنسبة مئوية (81.30٪ ، 87.4٪ ، 54.66٪) على التوالي عند 72 ساعة من العلاج على خط خلايا MCF-7 مقارنة

بالمركبات الأخرى ، تشير هذه النتائج إلى أن المركب الجديد IVc يحتوي على نسبة عالية من مضادات التكاثر مقارنة بالتاموكسيفين الذي يعد الدواء القياسي للمقارنة بعد 72 ساعة يليه IVa بعد 72 ساعة (83.31٪). كان تأثير السمية الخلوية لمركب IVb هو الأعلى بين المركبات المختبرة على خط خلايا سرطان الرئة (A549) مع نسبة مضاد للتكاثر (58.49٪ و 75.04٪) عند 48 و 72 ساعة على التوالي ، ولكنه أقل من تأثير السمية الخلوية للإيرلوتينيب الذي يعد الدواء القياسي للمقارنة (77.10٪ و 82.46٪) عند هذه الأوقات. ثلاثة مركبات (IVa و IVc و IVi) لها تأثير مضاد للتكاثر على خط الخلايا السرطانية للثدي (MCF-7) والمركب (IVc) له نسبة تثبيط مماثلة للتاموكسيفين المصرح به. مركب واحد (IVb) له مضادات التكاثر ، ولكن أقل من الإيرلوتينيب على خط الخلايا السرطانية في الرئة (A549) وهناك اتفاق جيد بين نتائج الالتحام والنتائج التجريبية.

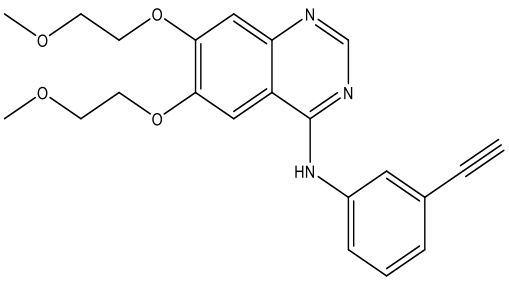
الكلمات المفتاحية: بيرازول، مضاد للسرطان، 4،5-ثنائي هيدرو-1H- البيرازول، خطوط سرطان الثدي، خطوط سرطان الرئة

Introduction

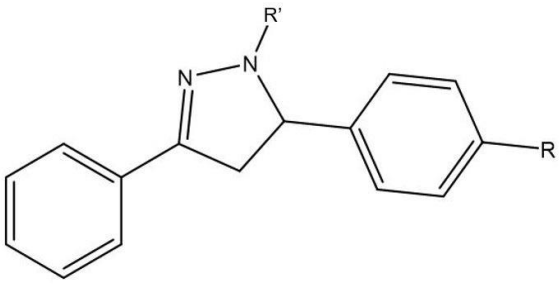
Cancer is characterized by abnormal multiplying and spreading of the body's own cells. Both malignant and benign tumors exhibit uncontrolled proliferation, but malignancies are distinguished by the ability to de-differentiate, become invasive, and spread [1]. The genesis of cancer involves more than one genetic change. Other factors may involve the actions of promoters, co-carcinogens, hormones etc. which, while not themselves are carcinogenic, but increase the likelihood that genetic mutation(s) would result in cancer [2]. Hormone antagonists can be effective in the treatment of several types of hormone-sensitive tumors. They can be classified as antiestrogens and anti-androgens. Estrogen and progesterone are the primary regulators of breast tissue growth and differentiation. Estrogen receptor alpha (ERα) and ERα antagonists (fulvestant, tamoxifen, letrozole, and anastrozole) play a role in the treatment of breast cancer [3]. Tyrosine kinases are largely deregulated in lung cancer and specifically in non-small cell lung cancer (NSCLC). Therefore, the inhibition of pathogenic kinases is a breakthrough development in cancer research, treatment and care, which clinically improve the quality of life [4]. In our study, we evaluated a series of 4, 5-dihydro-1H- Pyrazole-1-yl acetate derivatives with a structural likeness to tamoxifen and erlotinib, anti-cancer agents (Figure 1) against MCF-7 and A549 cell lines for breast and lung cancer respectively. Useful and great therapeutic value of the pyrazole nucleus have been recognized for a long time and the widest range of activities of this nucleus has been evaluated. However, as the first synthetic organic compound with the pyrazoline-5-one nucleus, it has found widespread use as a drug. Later on, many modifications to the pyrazole nucleus were attempted and several compounds were synthesized that are now used to treat a variety of diseases including pain, cancer, inflammation, tuberculosis, and bacterial diseases [5,6]. Literatures have been reported that disubstituted and trisubstituted pyrazole derivatives have good anticancer activity against different cell lines including MCF-7 and A549 [7-13].



Tamoxifen



Erlotinib



General structure of (IV_{a-i})

Figure (1): Structural demonstration of anticancer Tamoxifen, Erlotinib and IV_{a-i}.

Materals & Methods

In vitro Determination of Anti-proliferative Effect of the tested compound on human cell line

The ability of the previously 9 synthesized compounds (IVa-i) by Shahla et al. (2021 unpublished data) to prevent the prevent the growth of cancer and work as antiproliferative two human cancer cell lines were used. Lung cancer cell line, human non-small cell lung cancer cell line (A549) and human breast cancer cell line (MCF-7).

In vitro cytotoxicity

Cell Culture

Lung cancer cell line (A549) and breast cancer cell line (MCF-7) were purchased from American Type Culture Collection ATCC and stored at University of Mustansiriyah in the Biomedical Research Centre Cell Bank. MCF-7 and A549cell lines in this study were used as model cancer cells.

Maintenance, storage and resuscitation of cell line

A549 cells in Roswell Park Memorial Institute-1640 (RPMI-1640) medium were maintained and supplemented with 1 percent Penicillin-Streptomycin-Amphotericin B 100X with 1 percent L-Glutamine and 0.5 percent fetal bovine serum (FBS) as antiseptic. MCF-7 cells were cultured in Dulbecco's Modified Eagle's Medium (DMEM) liquid medium, with 1 percent penicillin–streptomycin and 10 percent fetal bovine serum (FBS) as antiseptic. Obtained cells pellet stored at minus eighty °C for twenty-four hours and for long time were stored under liquid nitrogen. All steps for maintenance, storage and resuscitation done according to Marin V et al and Qusay A et al. 1[4, 15].

Cell Viability and Inhibitory Concentration (IC50) by MTT Assay Colorimetric Assay

The MTT assay was used to assess the effects of compounds IVa-i well as tamoxifen and erlotinib as a control of

breast cancer and lung cancer respectively on (A549) and (MCF-7) cancer cell lines viability. A hundred microliters from all cells suspensions (A549) and (MCF-7) were dispensed into nighty six well flat-bottom tissue culture plates at concentrations of 5 x 103 cells/well and incubated for 24h under standard conditions, 4 x 103 cells/well for 48 hours incubation and 3 x 103 cells/well for 72 hours incubation. After 24h, the cells were treated with (0.15, 0.32, 0.75, 1.5, 3.12, 6.25, 12.5, 25, 50 and 100 µM) of the compounds IVa-i. The cell culture medium was removed and cultures were incubated with medium containing 30 microliters of MTT solution (3 mg/ml MTT in PBS) (3-(4,5-Dimethylthiazol-2-yl)-2,5 Diphenyltetrazolium Bromide) for four hours. at 37° C After a recovery period 24h, 48h and 72h. After the incubation period 4hrs. By gentle inversion and tapping onto paper this medium was removed. Only 100 µl growth media were used in the control wells. In each well 100µl of dimethyl sulfoxide was added, the plates were then kept in the dark for about 15 to 20 minutes at room temperature. The assay was done in a triplicate, the results were evaluated after the multiscan reader measured the absorbance of each well at a wavelength of 540 nm and corrected for background absorbance at a wavelength of 650 nm. The optical density (OD) of the wells devoid of compounds was used to gauge the viability of the cells. The minimal extract concentration that reduced the viability of the cultured cells to 50% after 72 hours was known as the inhibitory concentration 50% (IC50). (16-18)

Data Analysis

All statistical analysis of compounds kinetic and characterization were performed using the nonlinear curve fitting software Origin 9.1 software. IC50 was done by using the nonlinear curve fitting software prism pad software. Values of $p < 0.05$ were considered statistically significant.

Results & Discussion

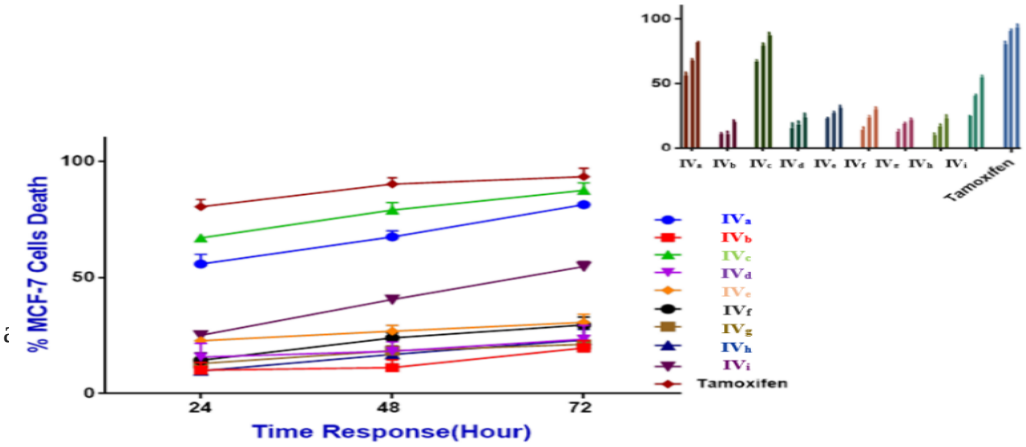
Assessment of tumor cell growth inhibitory ability of nine synthesized compounds (IVa-IVi) in vitro

Cell Death percentage of synthesized compounds in two cell line

Breast cancer cell line (MCF-7) and lung cancer cell lines (A549) were treated with 60µM synthesized compounds (IVa-IVi) as well as tamoxifen and erlotinib as a control of breast cancer and lung cancer respectively, at different time 24, 48, 72h. According to the cytotoxicity effect of these compounds, IVa, IVc and IVi compounds have antiproliferative effect with percentage (81.30%, 87.4% & 54.66%) respectively at 72h treatment on MCF-7 cell line compared to other compounds, these results indicate that the new compound IVc have the higher antiproliferative percent comparable to tamoxifen after 72h followed by IVa after 72h (83.31%) as seen in figure 2 and table 1. However, cytotoxicity effect of compound IVb was highest among the tested compounds on lung cancer cell line (A549) with antiproliferative percentage (58.49% & 75.04%) at 48 & 72h respectively, but it is less than erlotinib cytotoxicity effect (77.10% & 82.46%) at these times, as seen in figure 3 and table 2.

Table (1): Cell death percentage against MCF-7 cell line of compound IV_a-IV_i and tamoxifen as control

Compounds	% Cell Death		
	24h	48h	72h
IV_a	55.78	67.40	81.30
IV_b	10.05	11.19	19.63
IV_c	66.97	78.96	87.40
IV_d	15.70	18.25	23.43
IV_e	22.77	26.83	30.60
IV_f	14.32	23.93	29.51
IV_g	12.97	18.43	21.21
IV_h	9.77	16.84	23.06
IV_i	25.09	40.50	54.66
Tamoxifen	80.38	83.31	90.15



d by MTT
compounds

Table (2): Cell death percentage against A549 cell line of compound IVa-IVi and Erlotinib as control

Compounds	% Cell Death		
	24h	48h	72h
IV_a	25.05	36.11	39.63
IV_b	42.33	58.49	75.04
IV_c	17.09	18.20	23.93
IV_d	14.00	15.04	17.23
IV_e	9.77	16.84	23.06
IV_f	24.31	35.90	42.81
IV_g	25.15	28.95	32.64
IV_h	12.45	15.14	27.03
IV_i	9.76	12.65	15.67
Erlotinib	68.60	77.10	82.40

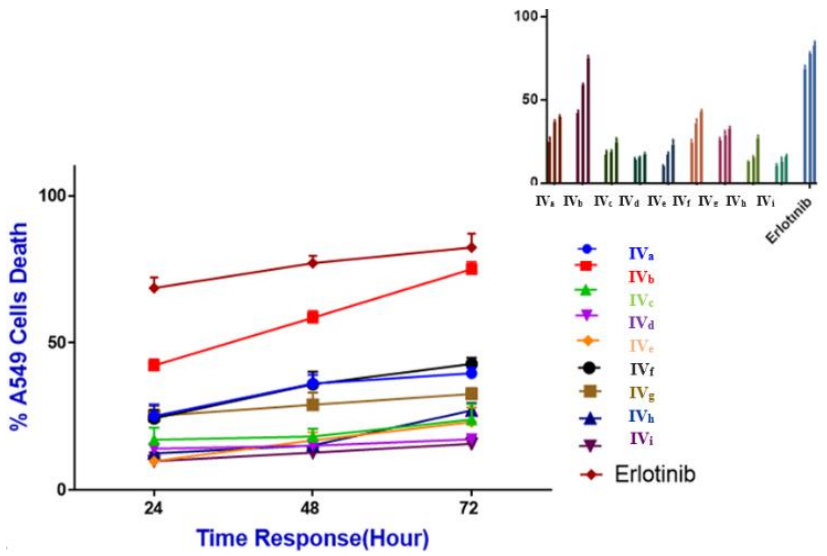


Figure (3): ***In vitro*** **% cell death of the lung cancer (A549) cells was detected by MTT assay. The results of A549 cells post 24, 48 and 72h treatment of 60 µM of all compounds compared with Erlotinib as control.**

Comparison of IC_{50} values between compounds (IV_a, IV_c, IV_i) and tamoxifen in breast cancer cell line (MCF7)

Dose-response curve was generated by Prism Pad 8.1 using nonlinear regression analysis for compounds in MCF-7 cells are shown in figures 4. By using the MTT test, the IC50 values were determined for a range of compound concentrations (100-1.56 µM). Thus IC_{50} (minimum concentration of compounds can kill 50% of the cells) for compounds IV_a, IV_c and IV_i compared to the positive control tamoxifen were determined.

Tested compound had good activity (IC_{50} values range from 27.53-60µM), but higher than that of tamoxifen (IC_{50}=18.02).

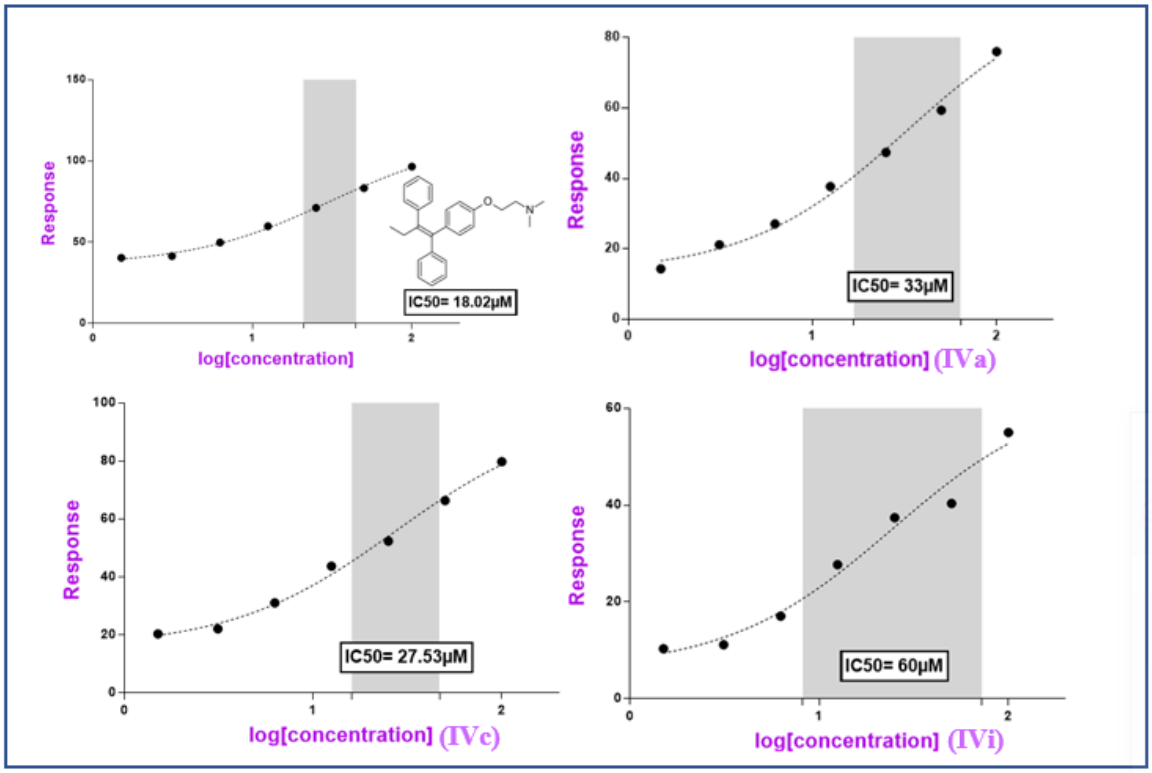


Figure (4): Dose-response curves for compounds (IV_a, IV_c, IV_i) and Tamoxifen (Control). MCF-7 cells were treated for 72h with 1.56, 3.12, 6.25, 12.5, 25, 50 and 100 µM doses of (IV_a, IV_c, IV_i) and Tamoxifen.

According to these results, the new compounds (IVa, IVc& IVi) had antiproliferative effect against breast cancer cells line specially IVc which appeared more potent compound between the nine synthesized compounds as anti-cancer with IC50 =27.53µM. However, compound IVa have an approximate comparable activity to that of IVc while compound IVi have lower activity than them. Compound IVc may had blocking effect against ER-α receptor which gave promising antitumor activity as this study results fits with docking study results inside the active site of ER-α that showed compound IVc bind by hydrophobic interactions with the amino acids that surrounded it and had good docking score very close to the tamoxifen as demonstrated earlier.

Comparison of IC50 values between compound IVb and Erlotinib in lung cancer cell line (A549)

Prism Pad 8.1 generated a dose-response curve using nonlinear regression analysis on A549 cells line were demonstrated. IC50 values were determined from a range of concentrations for compound IVb (100-1.56 µM) by MTT assay to the positive control erlotinib. Compound IVb have good activity (IC50 =35.02µM), but significantly lower than Erlotinib (IC50 = =25.23µM as shown in figure 5.

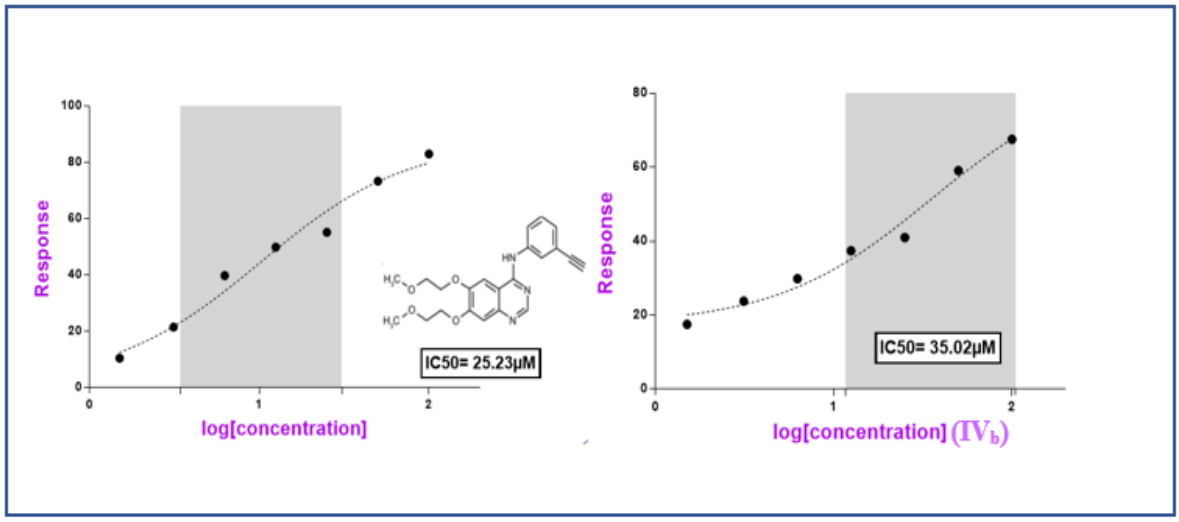


Figure (5): Dose-response curve for compound IVb & erlotinib. A549 cells were treated for 72h with 1.56, 3.12, 6.25, 12.5, 25, 50 and 100 µM doses of compound IVb & erlotinib.

Compound IVb considered the most potent compound between the nine tested compounds against the A549 cell line in comparison with erlotinib. Compound IVb may had blocking effect against EGFR receptor which gave promising antitumor activity as this study results fits with docking study results inside the active site of EGFR that showed compound IVb bind by hydrophobic interactions and one pi-cation interaction with the amino acids that surrounded it and docking score accepted in comparison to Erlotinib as demonstrated earlier.

Conclusion

A series of nine novel synthesized 4,5-dihydro-1H-pyrazole-1yl-acetate derivatives were tested in vitro to evaluate their cytotoxicity to breast and lung cancerous cell line (MCF-7 and A549) respectively by the MTT assay in comparison with tamoxifen and erlotinib respectively. Data results of cytotoxicity study showed that three compounds (IVa, IVc & IVi) have antiproliferative effect on breast cancerous cell line (MCF-7) and compound (IVc) have inhibition percentage comparable to that of the authorized medication tamoxifen. One compound (IVb) had antiproliferative, but less than that of erlotinib on lung cancerous cell line (A549) and there is good agreement between our docking results and the experimental results.

Conflict of interest:

The authors have no conflicts of interest regarding this investigation.

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