RESEARCH ARTICLE

The Evaluation of Antiproliferative Effect of Imatinib Derivatives against Breast and Colon Cell-Lines

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ABSTRACT

Background: Cancer is considered as one of the major leading causes of death. Tyrosine kinase inhibitors are recognized for their potential antiproliferative effects.

Materials and methods: In the previous study, the authors designed, synthesized, and characterized two imatinib derivatives. These derivatives were biologically evaluated with the utilization of MCF-7, HCT116, and MDCK cell lines.

Results: In respect to the imatinib standard, compound 2b has superior activity against HCT116 cell line (IC50; 15.88 µg/mL against 18.52 µg/mL for imatinib) and an improved cytotoxic activity on MDCK cell line (IC50; 0.654 mg/mL against 0.272 mg/mL for imatinib).

Conclusion: The two synthesized compounds showed biological activity against cancerous cell lines and improved cytotoxic activity against normal non-cancerous cell line with respect to the imatinib standard.

Keywords: Antiproliferative, Breast cancer, Cell lines, Colon cancer, HCT116, IC50, Imatinib analogs, MCF-7, MDCK, Tyrosine kinase inhibitor.

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INTRODUCTION

On a global scale, cancer is a formidable health problem, and its incidence is high, and it ranks a close second only to heart disease as the top cause of death in America and worldwide. One-quarter of all deaths in the USA are caused by cancer.[1-3] Accordingly, there is a necessity to invent and develop drug candidates to encounter this undefeatable disease. However, the treatment of cancer with chemotherapeutic agents is associated with many side effects.[4] In the past several years, we have seen dramatic changes in anticancer drug development. Even though numerous kinase inhibitors have been discovered recently, and several have been successfully developed for the treatment of cancer, including imitanib (Gleevec), gefitinib (Iressa) and erlotinib (Terceva), and still there is strong demand for the discovery of improved anticancer drugs.[5] One of the approaches in the development of the new anticancer drugs is the targeting of enzymes those involved in the pathways of signal transduction. These are the oncogene protein kinases which regulates cellular growth and proliferation.[6-9]

In the same context, researchers considered tyrosine kinases as a potential target to inhibit or cure breast cancers.[10] Consequently, a reasonable number of tyrosine kinase inhibitors (TKI) have been synthesized and biologically evaluated.[11-13] In previous work, our team has designed, synthesized, and characterized three potential imatinib derivatives.[14] In this work, the authors aimed to evaluate these derivatives biologically.

MATERIALS AND METHODS

Materials

Table 1: Utilized materials with their manufacturers and countries of origin.

#	*Material*	*Manufacturer*	*Country*
1	Imatinib (99.8%)	BLDpharm	CHINA
2	N-(4-methyl-3-((4-(pyridine-3-yl)pyrimidin-2-yl)amino) phenyl)benzamide (2a) (99.65%)	Medicinal Chemistry Lab*	IRAQ

Contd.

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#	*Material*	*Manufacturer*	*Country*
3	N-(4-methyl-3-((4-(pyridine-3-yl)pyrimidin-2-yl)amino) phenyl)pivamide (2b) (99.69%)	Medicinal Chemistry Lab*	IRAQ
4	MTT	Roth	GERMANY
5	Cellulose acetate membrane filter pore size 0.2 µm diameter 25 mm.	chm	SPAIN
6	Dimethyl sulphoxide (99%)	CDH	INDIA
7	Celltreat® 96 Well Cell Culture Plates	CELLTREAT Scientific Products	USA

*These compounds were synthesized and characterized in previously published work.[14]

Table 2: The used instruments with their originators and manufacturers.

#	*Instrument*	*Country*	*Manufacturer*
1	Microplate reader 800 TS	USA	BioTek
2	Inverted Microscope	GERMANY	Zeiss
3	Incubator UN 55	GERMANY	Memmert
4	Clean Bench	KOREA	LabTech

The materials utilized in this study are listed in Table 1.

Cell Lines

The cell lines utilized in this work are; MCF-7 Breast cancer, MDCK kidney normal cells, and HCT116 colorectal cancer cells.

Instruments

The instruments utilized in this study are tabulated in Table 2 .

Methods:

Obeying a restricted protocol that was revealed extensively in previous work,[14] the cell lines were prepared, and the time of the drug candidate application was optimized to be 24 hours. After that, the MTT stock and working solutions were prepared to get homogenized solutions of the cell-medium with 10% MTT to be utilized in the cell-lines. Then these solutions with concentrations of (50, 25, 12.5, 6.25, and 3.125) µg/mL were applied on the mentioned cell-lines and the results were recorded with plate reader at 630 nm.

RESULTS

In previously published work,14 two imatinib derivatives were designed, synthesized, and characterized. These derivatives are tabulated in Table 3.

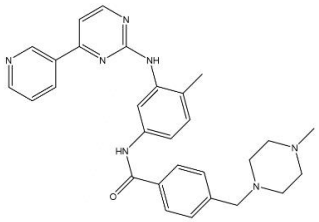
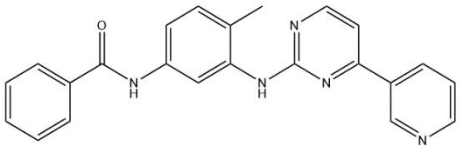
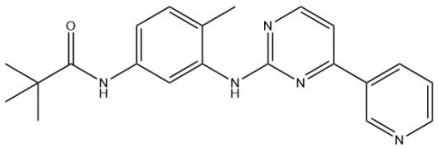
The biological effect of imatinib and the synthesized compounds on the cancerous and normal cell-lines are shown in Figures 1, 2, and 3:

The IC50 for imatinib and the synthesized compounds for the HCT116, MCF-7, and the MDCK cell lines are calculated in Table 4.

DISCUSSION

The cell lines were chosen and selected carefully. For the HCT116 cell lines, except for skin cancers, colorectal cancer

Table 3: Some chemical parameters of the imatinib and the synthesized derivatives.

Chemical formula (Code)	*Structure Chemical name*
$C_{29}H_{31}N_7O$ Imatinib	 *N*-(4-methyl-3-((4-(pyridin-3-yl)pyrimidin-2-yl)amino)phenyl)-4-((4-methylpiperazin-1-yl)methyl)benzamide
$C_{23}H_{19}N_5O$ (2a)	 N-(4-methyl-3-((4-(pyridine-3-yl)pyrimidin-2-yl)amino)phenyl)benzamide
$C_{21}H_{23}N_5O$ (2b)	 *N*-(4-methyl-3-((4-(pyridin-3-yl)pyrimidin-2-yl)amino)phenyl)pivalamide

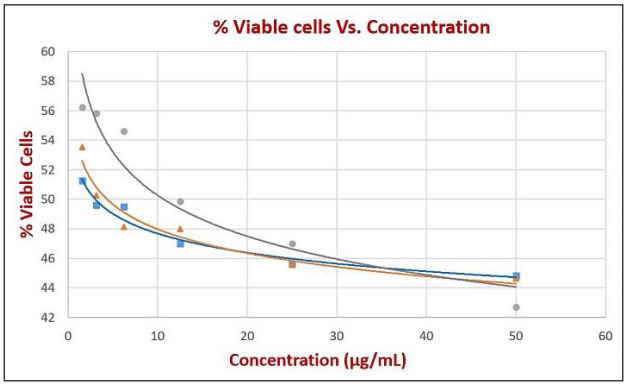


Figure 1: The percent viable cells of MCF-7 breast cancer cell line versus concentration of imatinib standard (■), compound 2a (▲), and compound 2b (●) after 24-hours' incubation.

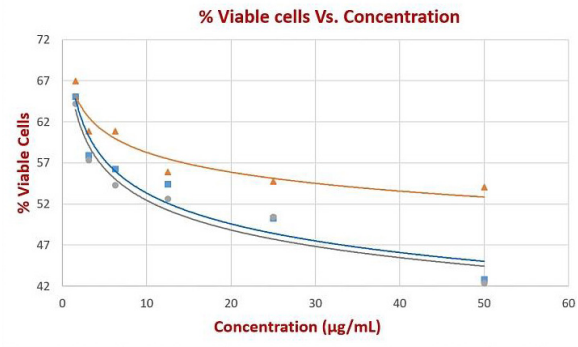


Figure 2: The percent viable cells of HCT116 colon cancer cell line versus concentration of imatinib standard (■), compound 2a (▲), and compound 2b (●) after 24-hours' incubation.

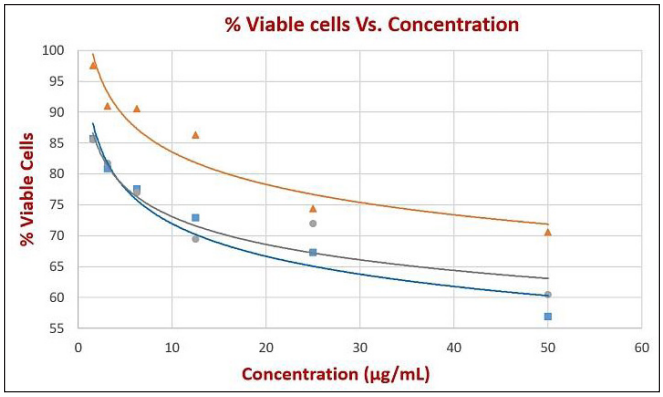


Figure 3: The percent viable cells of MDCK kidney normal cell line versus concentration of imatinib standard (■), compound 2a (▲), and compound 2b (●) after 24-hours' incubation

Table 4: A summary for IC50 for the cell lines and chemical compounds specified.

Cell line	*IC50 Imatinib (µg/mL)*	*IC50 2a (µg/mL)*	*IC50 2b (µg/mL)*
MCF-7	3.03	4.31	10.6
HCT116	18.52	122.29	15.88
MDCK	0.272	2.329	0.654

is considered as the third proposed cancer in the United States for both genders. Moreover, the American Cancer Society has estimated the number of colorectal cancer cases for 2019 as 44,180 new cases of rectal cancer and 101,420 new cases of colon cancer in the United States of America.[15] For American women, breast cancer (with the exception of skin cancers) is considered as the number one in occurrence in the United States. Statistically, the American Cancer Society has proposed the cases number of breast cancer as 268,600 new cases of invasive breast cancer and 62,930 new cases of non-invasive breast cancer in the United States for 2019.[16]

Reasonably, to estimate the selectivity of the candidate drugs towards the cancerous cell-lines, their effect against the non-cancerous cell-lines has to be estimated through.[17-19] Huge number of studies conducted the MDCK cell-line for the cell viability studies.[20-28] Alongside their availability, the non-cancerous MDCK cell-lines were chosen.

Notably, Table 4 reveals the ultimate results of this study as it shows the activity of the chemicals that were synthesized on both cancer and non-cancer cell lines. The obtained data is classified below for each cell-line as revealed below:

1. For the MCF-7 cell line, the imatinib standard has the lowest IC50 value. The half-maximal inhibitory concentration (IC50) is a measure of the effectiveness of the synthesized chemical compound as an antiproliferative agent against a given cell-line. On the other hand, compound 2a has the middle value for IC50 (1.42-times of that of imatinib). Finally, compound 2b has the highest IC50 value (3.5-times of that of imatinib).
2. For HCT116 cell line, the IC50 for compound 2b has the lowest value, followed by the imatinib standard, then lastly compound 2a. Whereas compound 2b has 0.86-times the IC50 value with respect to imatinib standard, compound 2a has 6.6-times the IC50 value for the imatinib standard against the cancer cell-line.
3. For the MDCK cell line, the imatinib standard possesses the lowest IC50 value, which means the most toxic in respect to compounds 2a and compound 2b. In other words, both compound 2a and compound 2b have lower cytotoxicity profiles than the imatinib standard.

CONCLUSIONS

In this work, two of imatinib analogs possess antiproliferative activity. The biological effects are concluded for compound 2b, which possesses better activity and lower cytotoxicity than the imatinib standard in HCT116 and MDCK cell-lines, respectively.

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ABBREVIATIONS

HCT116, Human Colorectal Carcinoma; MCF-7, Michigan Cancer Foundation -7; MDCK, Madin-Darby Canine Kidney; TK, Tyrosine Kinase; TKI, Tyrosine Kinase Inhibitor.

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