How to Cite:
Gani, I. H., & Al-Obaidi, Z. (2022). Synthesis, characterization, and pharmacological evaluation of new imatinib analogues as antiproliferative agents. *International Journal of Health Sciences*, *6*(S6), 9152–9159. https://doi.org/10.53730/ijhs.v6nS6.12416

Synthesis, characterization, and pharmacological evaluation of new imatinib analogues as antiproliferative agents

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***Abstract*---**Cancer is the second cause of death after the cardiovascular disease. There are many target receptors for a drug that acts as an anticancer. One of these targets is the tyrosine kinase which is considered one of the most platforms for cancer therapeutics in the recent years. For instance, imatinib is known as a prototype that acts by inhibiting tyrosine kinase receptors. Nevertheless, after one year of receiving imatinib, many patients developed resistance to treatment. Hence, there is an unmet necessity to develop new compounds in an attempt to tackle the resistance issues. In the current work, new imatinib analogues were synthesized with approved attributes. The structures were characterized and confirmed by spectral tools (FTIR,1HNMR, and 13CNMR). Furthermore, the physicochemical properties were acquired with variant reliable techniques (melting point by DSC, Retention factor by TLC, and material description alongside the physical appearance). Moreover, the cytotoxicity of the compound against human lung cancer cell line (A549) was investigated by viability test with an MTT reagent. As a result, compound IIX possesses IC50 =0.623 μM which is three-fold more potent than the reference drug, imatinib (IC50=2.479 μM).

***Keywords*---**tyrosine kinase, HNMR, MTT, A549, imatinib.

Introduction

Cancer has emerged in the last decades as one of the most critical public health challenges(Siegel et al., 2022), and the second largest cause of death after heart disease Cancer is defined by uncontrolled cell growth leading to tumour development(Yahya & Alqadhi, 2021). Receptors of tyrosine kinase (RTKs), are important regulatory signalling proteins that control cancer cell proliferation and metastasis(*Protein Tyrosine Kinases: From Inhibitors to Useful Drugs (Cancer Drug Discovery and Development) - PDF Drive*, n.d.). Several compounds targeting RTKs have been employed in oncology as first or second-line treatment in various forms of cancer throughout the last two decades(Pottier et al., 2020). TKIs are effective in targetting a range of tumour types(Rimassa et al., 2019), In clinical practice, there are five TKIss (imatinib, nilotinib, bosutinib, dasatinib and ponatinib) with diverse safety profiles that are readily available in various conditions(García-Gutiérrez & Hernández-Boluda, 2019).

Imatinib is an antitumor drug that inhibition of many tyrosine kinases(Manley et al., 2002), most notably the BCR-ABL1 (whose over activation can cause CML (chronic myeloid leukaemia),c-kit (contributed to the development of tumours in gastrointestinal), platelet-derived growth factor receptor (PDGFR), and the native ABL1 kinase, which has widespread expression([Hochhaus et al., 2017),(Bernal-Bello et al., 2020)]). At 12 months, the estimated percentage of complete cytogenetic response (CCyR) with imatinib was 69% only. After five and ten years of follow-up, 31 and 52% of patients treated with imatinib, respectively, stopped therapy. The most common reason for therapy cessation was a poor therapeutic response (11%), with just 4% of patients discontinuing treatment owing to adverse effects(Gugliotta et al., 2015),(Viganò et al., 2014). There is a need to develop new derivatives because of the challenges of resistance due to the mutation and adverse effects of anticancer drugs. This work aims to synthesise new compounds as Imatinib derivatives and then check their cytotoxicity of them against the A549 cell line.

Materials and Methods

All material with reagents are supplied by commercial (BLD, Sigma Aldrich, Spain, India), 6-methyl-N1-(4-(pyridin-3-yl)pyrimidin-2-yl)benzene-1,3-diamine was supplied by BLD company China. For cytotoxicity assay, RPMI1640 Gibco, Belgium), Trypsin (Capricorn, German), FBS(Fisher Scientific, USA), and PBS(Capricorn, German) as shown in table (1).

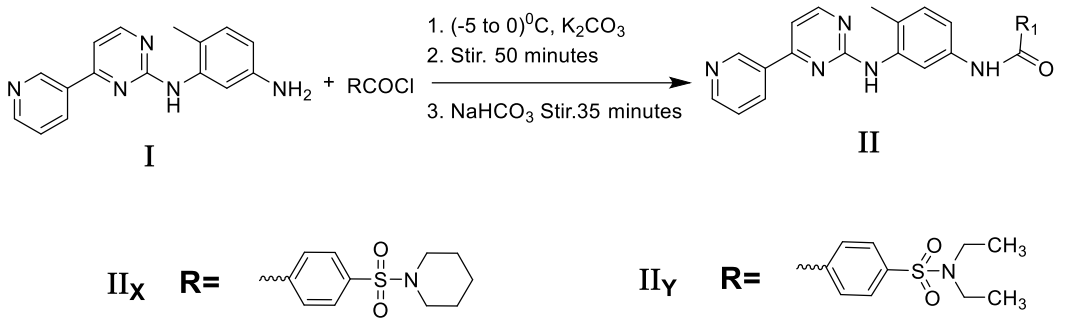
Table 1
All instruments with company supplier

Instrument	Company	Country of origin
IHNMR	Bruker	USA
13CNMR	Broker	USA
DSC	Shimadzu	Japan
FT-IR	shimadzu	Japan
Biosafety cabinet	Telstar BioII	Germany

Microplate Reader GloMax	Promega	Germany
Inverted Microscope	Optica	Korea
Instrument	Company	Country of origin
^{I}HNMR	Bruker	USA
^{13}CNMR	Broker	USA

Chemical synthesis

The scheme of reaction for synthesized compounds is illustrated below.



Scheme 1. Reveals the pathway for the syntheses of the chemical compounds

Synthesis of compound IIX

Approximate 385.39 mg of Acyl chloride (2.2 mmol) was added to the stirred mixture solution of 499.19 mg of the compound 1[6-methyl-N1-(4-(pyridin-3-yl)pyrimidin-2-yl)benzene-1,3-diamine](1.8 mmol) and 1.24 g of K_2CO_3 (9 mmol) in 14 mL of solvent tetrahydrofuran at -5 to 0C^0 then, the mixture continuous string for 50 minutes at -5°C. After this, the mixture of reaction has been transferred into the cold solution of 13 mL of 10% Sodium bicarbonate and stirred for 35 minutes at a temperature below 0°C. A solid yellow pale precipitation will form, and the precipitate was collected for washing with (hexane, and water) finally, drying the precipitate to obtain a powder.

N-(4-methyl-3-((4-(pyridin-3-yl)pyrimidin-2-yl)amino)phenyl)-4-(piperidin-1-ylsulfonyl)benzamide(C28H28N6O3S)

Pale Yellow powder(27.52% yield);MP 217.14C^0; **IR** (KBr) v (cm-1): 3390(N-H of amide linkage),3035(C-H of Ar),1670 (C=O) ,1577 (C=N aromatic ring). **^{1}H-NMR**: δ1.38(m,2H, CH2 of piperidine ring of Para position), δ1.52(m,4H, CH2 of piperidine ring of meta position), δ2.95(m,4H, CH2 of piperidine ring of Ortho position), δ 2.10(s,3H, CH3 on benzene ring),δ 8.73 (s, 1H, NH of Sec. amine), δ 7.24 to 9.28(11H, Ar H), δ 10.54 (s, 1H, NH of amide linkage).**^{13}CNMR**: δ17.72 (CH3 on a benzene ring), δ23.31(CH2 of piperidine ring at Para position), δ25.60(CH2 of piperidine ring at meta position), δ47.07(CH2 of piperidine ring at meta position),δ108.06 to 162.11(Ar C), δ164.73(C=O).

Synthesis of compound IIY

Approximate 608.4 mg of Acyl chloride (2.2 mmol) was added to the stirred mixture solution of 499.19 mg from the compound 1 [6-methyl-N1-(4-(pyridin-3-yl)pyrimidin-2-yl)benzene-1,3-diamine] (1.8 mmol) and 1.24 g of K_2CO_3 (9 mmol) in 14 mL of solvent tetrahydrofuran at -5 to 0C0 and the mixture continuous stirred for 50 minutes at -5C^0, Orange precipitate has occurred, After this, the mixture of reaction has been transferred to the cold solution of 13 mL of 10% Sodium bicarbonate and stirred for 35 min at a temperature below 0°C. A solid yellow precipitate will form, and the precipitate was collected for washing with(hexane, and water), finally, dring to obtain a powder. The filtrate was separated into two layers by using a separatory funnel with a mixture of ethanol and ether, after this, take the ethanol layer and then filtrated then dried to obtain a white powder of amide.

4-(N,N-diethylsulfamoyl)-N-(4-methyl-3-((4-(pyridin-3-yl)pyrimidin-2-yl)amino)phenyl)benzamide(C27H28N6O3S)

White soft powder(75.03% yield);MP 251C^0; **IR** (KBr) v (cm-1): 3383(N-H of amide linkage),3105(C-H of Ar),1670 (C=O) ,1585 (C=N aromatic ring). **^{1}H-NMR**: δ1.05(Quintet,6H,$(CH3)_2$), δ 2.26(s,3H, CH3 on benzene ring), δ 3.23(septet,4H, CH2),δ 8.72 (s, 1H, NH of Sec . amine), δ 7.24 to 9.24((11H, Ar H), δ 10.50 (s, 1H, NH of amide linkage).**^{13}CNMR**: δ14.61 (di-methyl of aliphatic chain), δ 18.18(CH3 on a benzene ring), δ42.38(CH2 Ortho to sulfonamide), δ108.06 to 162.10(Ar C), δ164.72(C=O).

ADME-Tox study

A drug must have an appropriate physicochemical characteristic to be effective in individuals (in vivo). A novel compound should study the ADME assay to determine if these compounds are safe for more development. (Muhsin et al., 2021),(Ismaeel et al., 2020),(Salih & Salih, 2020). Physicochemical properties for two compounds were determined by using software Swiss site(Daina et al., 2017) with ADMET lab 2(Xiong et al., 2021) servers, an important parameter illustrated below in table2.

Table 2
ADME-Tox properties of new compounds

Compound	M. Wt. (g/mol)	TPSA A^2	Rule of 5	Log p
IIX ($C_{28}H_{28}N_6O_3S$)	528.63	83	Coincide	3.53
IIY ($C_{27}H_{28}N_6O_3S$)	516.62	120	Coincide	3.39

Results and Discussions

Cytotoxicity assay
IC 50 determination against A549 cell line by MTT reagent

MTT is the test that requires the activity of mitochondrial reductase enzyme to convert the yellow dye MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide] to an insoluble purple colour formazan. The formazan can be dissolved by DMSO solvent, and the concentration is evaluated using optical density at 570 nm((Kumar et al., 2018),(Tolosa et al., 2015)). The viability test was evaluated against a human lung cancer cell line (A549) which was supplied by ATCC and cultured at the pharmacology lab/pharmacy college/Mustansiriyah University. The two compounds were incubated for 48 hours with different concentrations: (20, 10, 5, 2.5, 1.25, 0.625, 0.313, 0.156, 0.078, and 0.039)µM, and used imatinib drug as reference. Compound IIY gives high potency threefold time than imatinib where IC50 =0.623, While imatinib drug IC50=2.479. as shown in table (3) and figures (1-3).

Table 3
Shows the IC50 of the compounds with the reference imatinib

compound	IC50 µM
Imatinib	2.479
IIX	2.489
IIY	0.623

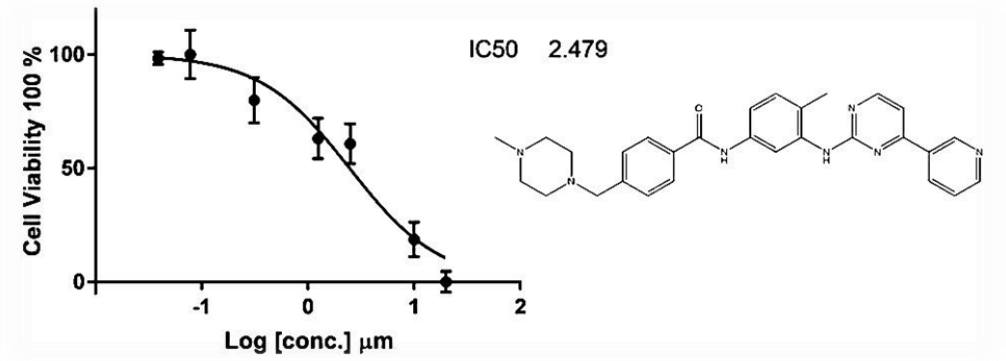


Figure 1. Cell viability curve of Imatinib against A549 cell line which gives IC50=2.479

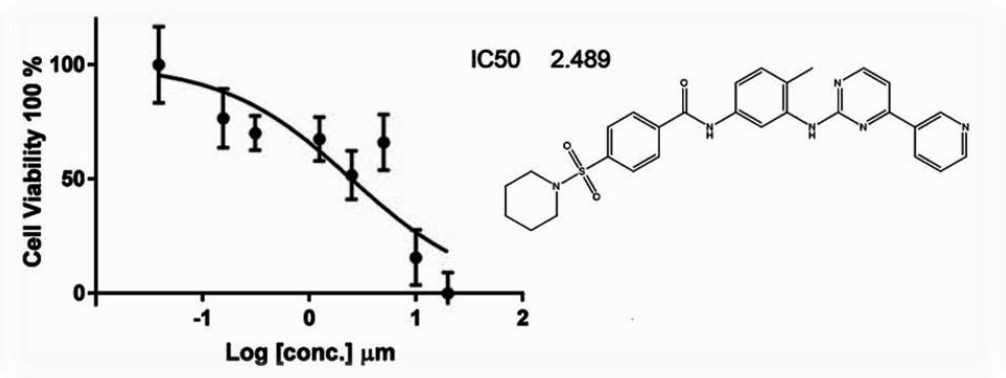


Figure 2. Cell viability curve of compound 1IX against A549 cell line which gives IC50=2.489

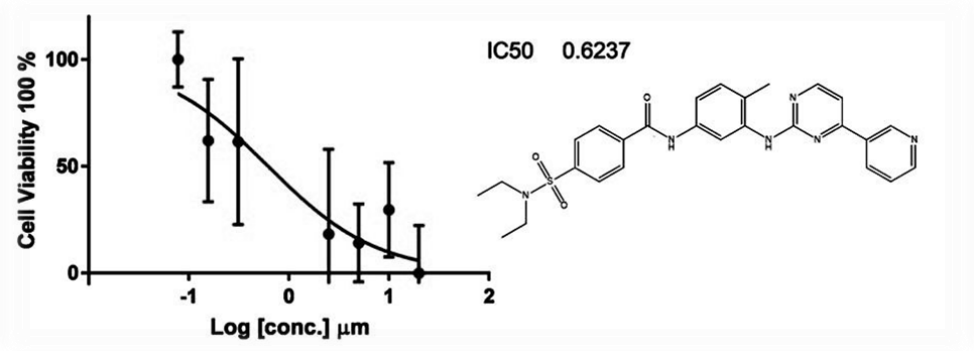


Figure 3. Cell viability curve of compound IIY against A549 cell line which gives IC50=0.6237

Conclusion

New compounds were synthesized and proven structures by using spectra (IR,^{1}HNMR,^{13}CNMR), the cytotoxicity was determined against the A549 cell line using imatinib as a reference, and the IC50 of newly compounds high potent compared to the reference.

Acknowledgments

The researchers take this opportunity to thank Mustansiriyah University in Baghdad, Iraq, for their support and advice with this project (www.uomustansiriyah.edu.iq).

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