RESEARCH ARTICLE

Synthesis of thiophene hydrazone derivatives by Japp Klinegmann reaction method for anticancer activity against MCF-7 cell line

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ABSTRACT

The creation of novel anticancer drugs is the aim of this research. Our decision about which compounds to synthesis was aided by docking studies. Using data from the protein databank and refinement from BIOVIA discovery, the protein structure of AGF183 (PDB5IZQ). It was subjected to molecular docking investigations using PyRx 0.8 Autodock Vina software. following the creation of a novel sequence of thiophene-2,5-carbohydrazide. Final chemicals such as thiophene-2,5-carbohydrazide derivatives (G1 and G2) are produced via the Japp Klinegmann process. This reaction makes it possible to produce Schiff bases without any problems. Diethylthiophene-2,5-dicarboxylate was reacted with an aromatic diazonium salt to create the final products. When compared to the reference drug imatinib, the synthesized compounds exhibited the strongest anticancer effect. The study conducted on MCF-7 cell line demonstrated that compounds G1 and G2 demonstrated the most potent anticancer effect. Compared to the reference value of imatinib, which is 52.77 µg/ml and has G1 and G2 docking scores of -8.8, -8.6, and the standard medication methotrexate (-11.87 kcal/mol), their IC50 values were 46.52 µg/ml and 50 µg/ml. On base of characterization results above show that it Japp Klinegmann Synthesis method is useful for synthesis of thiophene hydrazone derivatives and its findings imply that the MCF-7 cells, which are utilized to potential treatment for human breast cancer..

Keywords: Diethyl thiophene -2,5- dicarboxylate, MCF-7 Cell, methotrexate, Autodock Vina.

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INTRODUCTION

Cancer it is the most dengerous diseases, it is constantly vital to develop anti-cancer technologies. In order to meet the pressing need for the development of effective chemotherapeutic agents as well as to mitigate the issues related to anticancer medications currently on the market, such as drug resistance and toxicity, numerous researchers are assiduously striving to employ significant heterocyclic motifs. Many researchers are constantly working to make new anticancer drugs using thiophene basic scaffolds[1]. For a long time, mono-, di-, or trisubstituted thiophene derivatives have been documented as anticancer agents. Numerous commercially available anticancer drugs that contain thiophene cores work by affecting various cancer-related pathways. Worldwide, a lot of research has been done recently to find new drugs with anti-cancer properties. These investigations are now more important than ever due to several issues, including rapidly growing patient populations, toxicity, serious side effects of prescription drugs, and cancers that have evolved to respond to treatment. Chemotherapy is a commonly used anti-cancer treatment because of its effect on tumor cells[2].Therefore, it is essential to develop new anticancer drugs that either eradicate or inhibit the proliferation of cancer cells without endangering healthy cells. Promising candidates for the development of new biologically active therapeutic molecules for their significant pharmacological properties, which have attracted the attention of researchers in recent years[3]. Based on our literature review, we are interested in hydrazone derivatives and sulfur-containing heterocycles. One type of receptor that is widely present in epithelial cancer cells is the folate receptor (FR). In recent clinical trials, against antifolate such as methotrexate (MTX) have been shown to bind to FRα and destroy cancer cells[4]. These investigations also revealed that while certain folate receptor ihibitor, such as MTX, RTX, and PDX, have similar binding affinities for the FA receptor, others, such as PTX, had higher affinities for FRα than FA[5].

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The current work addressed this issue by creating new analogs of FAs with heterocyclic rings recently introduced to anticancer medications and examining how they alter the binding affinity of FRα[5]. As a result, novel molecules that interact with FRα could be produced substances with the highest affinity for the FRα interaction. Present-day drugs such as vintafolide and methotrexate act on folate receptors to function as folate transporters; however, these drugs are quite cytotoxic[6]. One of the worst diseases is cancer, so the development of anti-cancer technology is always necessary. Many researchers are working seriously to use important heterocyclic motifs to address the urgent need for the development of potent chemotherapeutic agents and to alleviate the problems associated with anticancer drugs currently on the market, such as drug resistance and toxicity[7]. Many researchers are constantly working to make new anticancer drugs using thiophene, one of the basic scaffolds. For a long time, mono-, di-, or trisubstituted thiophene derivatives have been documented as anticancer agents. Numerous commercially available anticancer drugs that contain thiophene cores work by affecting various cancer-related pathways. Worldwide, a lot of research has been done recently to find new drugs with anti-cancer properties. These investigations are now more important than ever due to several challenges, including rapidly growing patient populations, toxicity, serious side effects of prescription drugs, and cancer developing resistance to treatment. Chemotherapy is a commonly used anti-cancer treatment because of its effect on tumor cells. However, several anticancer drugs are well known to be toxic and have major side effects. Therefore, it is crucial to develop new anticancer drugs that either eradicate or inhibit the proliferation of cancer cells without endangering healthy cells[8]. The bioactive scaffolds that are necessary for the creation of drugs are small chemical molecules[9]. Prospective prospects for the synthesis of new physiologically active anti-cancer therapy compounds are hydrazones. By creating two new molecules, the requirement for novel compounds that address the FRα issue has been addressed. Old research that has been published shows that all the molecules that have been proposed have a similar structure to folic acid and our study sets them apart because our molecules have a thiophene-2,5-carbohydrazide ring different from folic acid, which makes them very new and this compound is very potent and has a high affinity for FRα[10]. The most efficient technique for the production of thiophene-2,5-carbohydrazide is the nucleophilic substitution reaction on the carbonyl group, which is used in the synthesis of the thiophene-hydrazone derivative. This reaction involves the interaction of diethylthiophene-2,5-dicarboxylate with an ary diazonium salt[11-13]. The synthesis of thiophene-2,5-hydrazone derivatives with aromatic rings proceeds without problems. Preparation of the organometallic derivative is not necessary for this type of reaction. Moreover, this reaction is very interesting from the point of view and non-toxic. However, in most reported results of this reaction so far, diethylthiophene-2,5-dicarboxylate was used to obtain thiophene-2,5-carbohydrazide. Here we describe the synthetic steps involved in the formation of thiophene-2,5-carbohydrazide derivatives using the ary diazonium salt. We also investigate structure-activity relationships regarding the anticancer properties of these derivatives.

MATERIAL AND METHOD

All of the compounds have been used in pure grade without purification and are readily available in the market. Using the KBr disc approach, IR spectra were recorded on a Jasco/FT/IR-4100 type A. Using DMSO as a solvent, 1H and 13C NMR spectra were obtained on a Bruker Avance Neo 500 MHz. Using an LC-MSD-Trap-SL device, mass spectra were captured in the electrospray ionization (ESI) mode. All the reactions were monitored by HPTLC on Merck, TLC Al plates silica gel 60 F 254. The organic extracts were dried over anhydrous Na_2SO_4.

Process for the synthesis

Scheme(1) G1–G2 compound synthesis.

Dimethyl thiophene -2,5-dicarboxylate added with Aromatic diazonium salt with methanol as a solvent to produce Thiophene-2,5-dicarbohydrazide . The mixture refluxing for 12 hours. Before being recrystallized from ethanol, cooling the reaction mixture and the yellow-colored crystalline product filtered and cleaned with methanol.

RESULTS AND DISCUSSION

N'2-[(1E)-ethylidene]-N'5-[(1Z)-ethylidene] thiophene-2,5-dicarbohydrazide derivatives was prepared by using starting

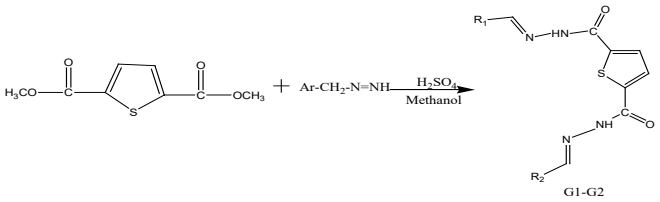


Figure 1:

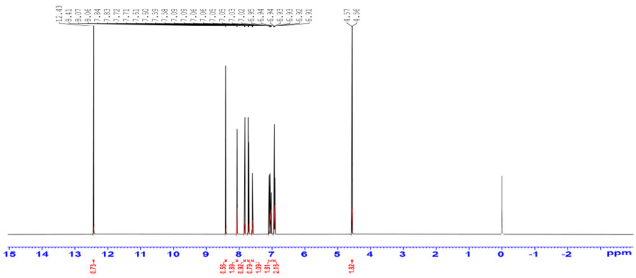


Figure 2:FTIR spectra of G1

Table 1: Synthetic Derivative (G1-G2) from the scheme.

S. No	*Derivatives*	*R1*	*R2*
1	G1		
2	G2		

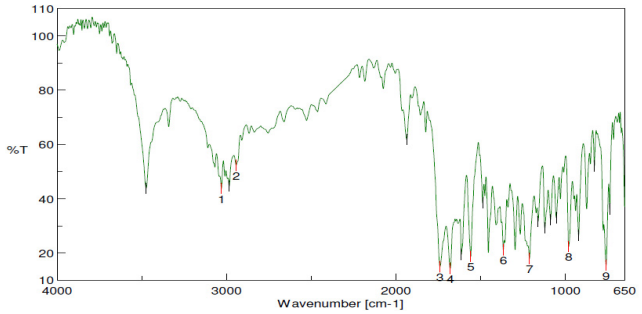


Figure 3:NMR of G1

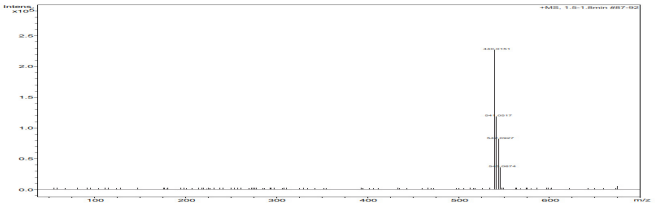


Figure 4: Mass of G1

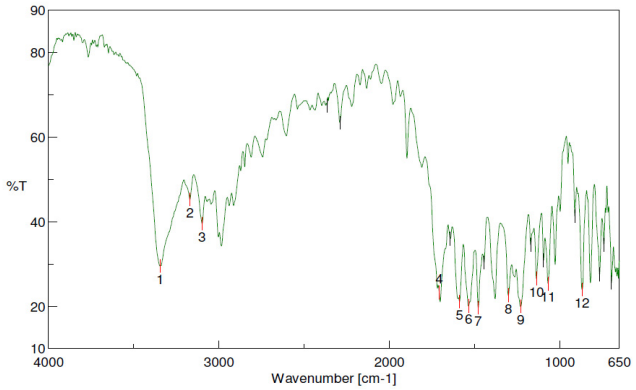


Figure 5: FTIR of G2 Compound

material dimethylthiophene-2,5-dicarboxylate. Figures 2-4 shows the FTIR, NMR and mass of G1.

N'2-[(1E)-(4-fluorothiophen-2-yl)methylidene]-N'5-[(1Z)-(4-fluorothiophen-2-yl)methylidene]thiophene-2,5-dicarbohydrazide)(4):"Yellow powder, IR (KBr,cm^{-1}):(3029.62) C=N-H,(2942)-C=C,(758.852)C-S,(1678.73)C=N,1739.48 (C=O) ;MS: *m/z* 423.20;^{1}H NMR(500 MHz, Chloroform-d) δ 12.43 (s, 1H), 8.41 (s, 1H), 8.07 (d, J = 4.2 Hz, 2H), 7.83 (d, J = 6.8 Hz, 1H), 7.72 (d, J = 6.8 Hz, 1H), 7.60 (dd, J = 6.0, 5.1 Hz, 1H), 7.11 – 7.01 (m, 2H), 6.97 – 6.89 (m, 2H), 4.57 (d, J = 6.1 Hz, 2H).exact mass calcd. For $C_{16}H_{10}F_2N_4O_2S_3$: 423.20; found: 423.20. Figures 5-7 shows the FTIR, NMR and mass of G2.

N'2-[(1E)-(4-chlorothiophen-2-yl)methylidene]-N'5-[(1Z)-(4-chlorothiophen-2-yl)methylidene]thiophene-2,5-dicarbohydrazide(5):"Yellow powder, IR (KBr,cm^{-1}):(3345.89) C=N-H,(3099.05)-C=C,(1069)C-S ,(1707.66) C=N,1588.0 (C=O) ;MS: *m/z* 456.20;^{1}H NMR((500 MHz, Chloroform-d) δ 12.32 (s, 1H), 8.37 (s, 1H), 8.03 (s, 2H), 7.83 (d, J = 6.8 Hz, 1H), 7.72 (d, J = 6.8 Hz, 1H), 7.48 – 7.43 (m, 2H), 7.37 (d, J =

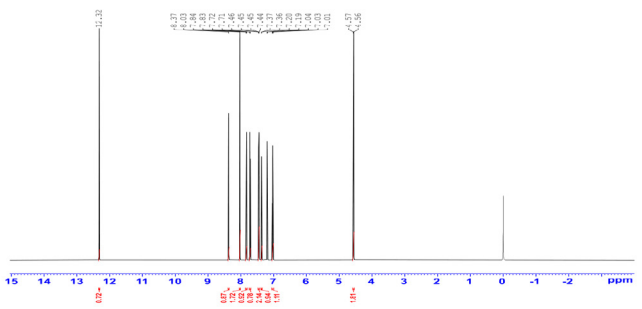


Figure 6:NMR of G2 Compound

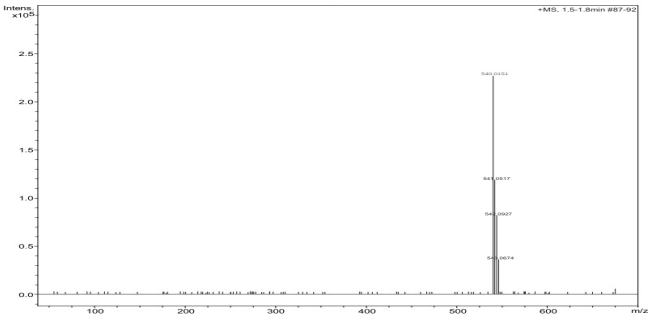


Figure 7: Mass of G2 Compound

1.6 Hz, 1H), 7.03 (t, J = 6.3 Hz, 1H), 4.57 (d, J = 6.1 Hz, 2H). exact mass calcd. For $C_{16}H_{10}C_{l2}N_4O_2S_3$: 456.20; found: 456.20.

Biological evaluation

Anticancer Activity

Thiophene-hydrazone, which become evolved and produced, is examined in vitro for antiproliferative activity against the Human MCF-7 cellular line using the MTT assay (three,4,5-dimethylthiazol-2-yl)-2,five-diphenyltetrazolium bromide, with Imatinib, a verified anticancer medication, as a reference chemical. In a ninety six-well plate, 1×10^5 cells/ml might be delivered. The mobile be counted became measured the usage of Neubauer's chamber. The plate will subsequent be incubated at 370 ^{0}C in a CO_2 incubator for one day. After one day of incubation, plate may be tested beneath an inverted microscope. 100 µl of scaffold extract media can be introduced

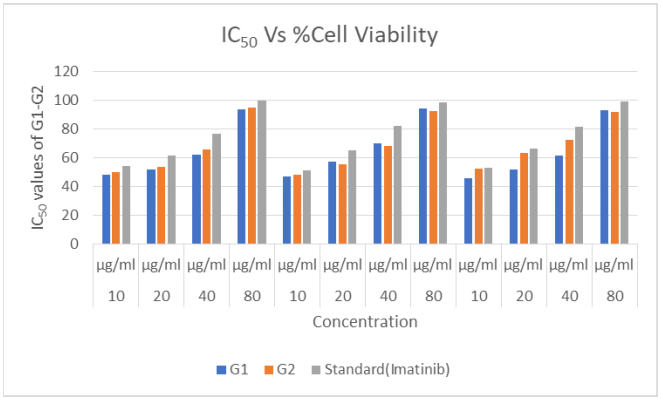


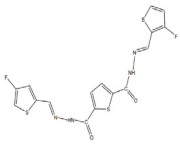
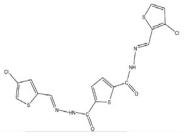
Figure 8: Anticancer activity of G1-G2

Table 2: Anticancer activity of the synthesized compound G1 and G2

Human Breast Cancer Cell Line (MCF-7)												
Percentage of inhibitions on MCF-7 at concentration of µg/ml (IC_{50})												
Sample Concentrations (µg/ml)												
	Experiment 1				*Experiment 2*				*Experiment 3*			
	10 µg/ml	*20 µg/ml*	*40 µg/ml*	*80 µg/ml*	*10 µg/ml*	*20 µg/ml*	*40 µg/ml*	*80 µg/ml*	*10 µg/ml*	*20 µg/ml*	*40 µg/ml*	*80 µg/ml*
G1	47.67	51.65	62.06	93.56	46.53	56.77	69.51	93.87	45.37	51.68	61.04	92.67
G2	49.69	53.23	65.57	94.59	48.05	55.31	68.12	92.19	52.26	63.36	72.29	91.63
Control	38.67	49.28	61.28	82.38	37.35	47.27	59.27	88.28	35.21	43.18	54.31	79.15
Standard (Imatinib)	54.16	61.12	76.28	99.33	51.13	65.24	81.69	98.27	53.03	66.07	81.05	98.86

in triplicate. After that, this plate will be incubated in a CO_2 incubator at 370 ^{0}C for 24 hours. After 24 hours of incubation, the plate can be examined under an inverted microscope. Scaffold check samples could be withdrawn and changed with a hundred µl of new DMEM media. Then 10µl of 5mD/ml MTT reagent can be implemented to each properly.After 4 hours of incubation,plate can be eliminated and studied underneath an inverted microscope, with photographs taken. The medium might be eliminated with the aid of flipping the plate, and every nicely will receive 200µl of acidic isopropanol. After 1 hour, absorbance may be measured at 492nm on a 96-nicely plate reader. Table 2 and Figure 8 shows anticancer activity of the synthesized compound G1 and G2.

Table 3: Docking of the AGF(183) 5IZQ protein with Compound (G1-G2) score values

Derivative Structure	*Docking Interaction*	*Docking Energy (kcal/mol)*
G1		-8.8
G2		-8.6

Docking

Chemsketch was used to draw the intended structures. The ionization and tautomeric states of the structures were adjusted using BIOVIA Discovery Studio. Using the steepest descent algorithm and the MMFF94 force field, the optimized structures were subjected to energy minimization. The RCSB Protein data repository was accessed to obtain the 3.60 Å resolution structure of FRα that has been previously reported. With BIOVIA Discovery Studio, the protocol for improving protein structure was carried out. A docking investigation was conducted against the FRα for designed structures. PyRx 0.8 was used to carry out the docking procedure. In the PyRx 0.8 GiRD Auto-Dock Vina wizard unit, the prepared protein and ligand structure were imported and chosen. Sizes were X(-5.9649), Y(20.3364), Z(-8.5258), Dimensions: X: 23.8028, y: 25.9727, Z: 25.0006 coordinates. The standard value for exhaustiveness was 8. The docked pose with the highest negative binding affinity of each chemical was stored in pdb format, and further binding interactions were examined using BIOVIA Discovery Studio. Table 3 shows the docking of the AGF(183) 5IZQ protein with Compound (G1-G2) score values

The potential binding mechanism of the recommended inhibitors with the human folate receptor alpha crystal structure was examined using molecular docking analysis. Docking analysis was utilized as a first step of assessment to identify viable candidates from the generated inhibitors that may be further examined for the development of anticancer drugs targeting human folate receptor alpha. Three constructed structures in all were examined in comparison to human folate receptor alpha. The binding affinities of all designed inhibitors ranged from -8.2 kcal/mol to -10.2 kcal/mol. The compound G1 was found to have of -8.8 kcal/mol with formation of interaction like hydrogen bond interaction with THR82,TRP102, carbon hydrogen bond interaction with SER174,ASP81,LYS136,TRP171, Pi cation interaction with TYR85,HIS135,TYR60, Pi sulphur interaction with TYR85,HIS135,TYR60, and vander waal interactions with TRP64,TRP134 ,TRP140 ,PHE62 ,GLN100 ,GLUY86 ,ARG103 ,ARG106. G2 showed binding energy of -8.6 kcal/mol and was found to be interacting with via formation of hydrogen bond interaction with HIS135,TRP140 Carbon hydrogen bond with TRP171,SER174 , pi sulphur interaction with PHE62,TYR85, pi-pi T shaped interaction with TYR60,TRP102, Alkyl interaction with TYR175 and vander waal interactions with ASP81,TRP64,TRP134, TRP138,GLY137, LYS136,ARG106 , ARG103, GLU86

CONCLUSION

Two thiophene-2,5-dicarbohydrazide compounds containing aromatic rings were synthesized and coupled to FRα. Higher binding energies were demonstrated for all chemicals. The results showed that the internal FRα active site interacts with region and ASP81, Arg106, Arg103, Trp64, Phe62, Tyr60,

Trp134, and Trp138. These results indicate that enhancement of binding affinity to FRa requires electron-donating and electron-withdrawing groups on aromatic, polycyclic, and heterocyclic rings. However, due to time and resource constraints, the study conducted here provided only a theoretical prediction of the characteristics required for potential lead candidates. Much work needs to be done and many aspects of reaching the clinical level need to be looked at to ensure that current efforts are not abandoned. Only two drugs are effective in the treatment of FRα: methotrexate and vintafolide. It is suggested that compounds could be synthesized with anticancer properties. G1 and G2 have docking scores of -8.8, -8.6 kcal/mol. Synthesis of compounds will allow confirmation of the activity to be determined. Diethylthiophene-2,5-dicarboxylate is a starting material that can be used in a new way to synthesize thiophene-2,5-carbohydrazide. This approach enables the synthesis of the necessary intermediates for the final step of the thiophene-2,5-dicarbohydrazide process. New methodology can now be incorporated, many surrogate patterns not available from previously published approaches of this kind. Many of the selected candidates demonstrated improved antitumor activity compared to the reference medication, imatinib. Compared to the reference drug Imantib, which has an IC50 value of 52.77 µg/ML, the new derivatives G1 to G2 showed the strongest growth inhibitory effects. Half maximal inhibitory concentration or IC50 and percent cell death were measured at different values. Among the series tested, G2 showed the greatest antitumor activity, with an IC50 value of 50 µg/ml, compared to the IC50 value of the reference drug Imantib of 52.77 µg/ml. The IC50 values of compounds G1 and G2 show that IC50 values (46.52 and 50.00 µg/ml). Promising results current research have prompted further investigation of this family of compounds as novel chemotherapeutic agents against cancer.

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