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Novel Synthesis and Anticancer Activity of Thiophene Hydrazone Derivatives.

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ABSTRACT:

Developing new anticancer agents aim in this investigation. Docking studies have helped us choose which chemicals to synthesize. PyRx 0.8 Autodock Vina software was used to conduct molecular docking studies on protein structure AGF183(PDB5IZQ), which was obtained from a protein data bank and improved using the BIOVIA discovery studio. following the synthesis of thiophene-2,5-carbohydrazide in a new sequence. To create the final compounds, such as thiophene-2,5-carbohydrazide derivatives (F1-F3), a nucleophilic substitution process on the carbonyl group was required. The seamless production of Schiff bases is made possible by this reaction. Final products were formed by treating diethyl thiophene -2,5-dicarboxylate to condensation with hydrazine hydrate and adding aromatic aldehyde. Synthesized derivatives exhibited the strongest antitumor efficacy when measured against the reference substance imatinib.MCF-7 Cell Line test revealed that Compounds F1 and F3 exhibited the strongest antitumor activity. Their IC50 values were 48.2 µg/ml, 51.15 µg/ml, and 49.00 µg/ml, respectively, compared to the reference value of Imatinib is 52.77 µg/ml and docking score of F1-F3 (-8.2,-10.2,-8.5 kcal/mol) and the standard drug methotrexate (-11.87 kcal/mol). These results suggest that the new chemicals may be harmful to MCF-7 cells used for human breast cancer.

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

1.INTRODUCTION:

One of the worst diseases is cancer, so the development of anti-cancer technology is always necessary. Many researchers are earnestly working on the use of important heterocyclic motifs to address the urgent need for the development of potent chemotherapeutic agents as well as to alleviate the problems associated with anticancer drugs currently on the market, such as drug resistance and toxicity. Many researchers are constantly working to make new anticancer drugs using thiophene, one of the basic scaffolds (Metwally M et al.,2024).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 cancer developing resistance to treatment (Hasdemir B et al.,2023). 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. Promising candidates for the development of new biologically active therapeutic molecules due to their significant pharmacological properties, which have attracted the attention of researchers in recent years(Chawla P et al.,2020). 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). Antifolates such as edatrexate (EDX), pralatrexate (PDX), raltitrexed (RTX), pemetrexed (PTX), methotrexate (MTX), and pralatrexed (PDX) have been shown to bind to FRα and destroy cancer cells in recent clinical trials. These investigations also revealed that while certain antifolates, such as MTX, RTX, and PDX, have similar FA receptor binding affinities, others, such as PTX, had higher FRα affinities than FA(Elgubb A et al.,2024). In this case, multitargeting appears as a potential strategy for new antifolate drugs. need new molecules that act on FRα, this problem was overcome by the current work, which produced new analogues of FAs with heterocyclic rings recently added to anticancer drugs and investigated how they affect the binding affinity of FRα.(Saeed Met al.,2023)Analogs showing the

highest affinity for the FRα interaction were selected for molecular dynamics analysis aimed at a deeper insight into the binding mechanism. Existing drugs such as methotrexate and vintafolide act on folate receptors as a folate transporter, they are highly cytotoxic drugs (Noor U et al.,2019). The Data and Safety Monitoring Board (DSMB) recommended that the study be stopped after a planned data analysis revealed that vintafolide unable to increase progression-free survival (PFS) in patients with platinum-resistant ovaries. One of the worst diseases is cancer, so the development anti-cancer technology always necessary. Many researchers are earnestly working on the use of 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. Many researchers are constantly working to make new anticancer drugs using thiophene, one of the basic scaffolds(Matela G.,2020).. 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 cancer developing resistance to treatment. Chemotherapy is a commonly used anti-cancer treatment because of its effect on tumor cells (Saini D et al.,2018). 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. The bioactive scaffolds that are essential for the creation of drugs are small chemical molecules. Hydrazones are small organic molecules in which R1 and R2 represent different functional groups. They are an important class of organic compounds that are given by the general formula R1−NHN=CH−R2. Hydrazones are a promising candidate for the development of new biologically active therapeutic molecules for cancer. The need for new molecules that act on the FRα, problem was overcome by proposing three new molecules. 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. The most efficient technique for the production of

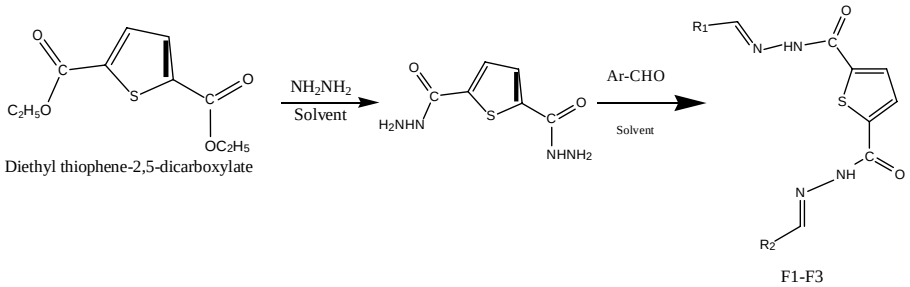
thiophene-2,5-carbohydrazide is the nucleophilic substitution reaction on the carbonyl group, which is used in the synthesis of the thiophene-hydrazone derivative. This reaction involves the interaction of diethyl thiophene-2,5-dicarboxylatewith an aromatic aldehyde in the presence of hydrazine hydtrate. The synthesis of several thiophene-2,5-hydrazone derivatives with aromatic rings proceeds without problems. The preparation of the organometallic derivative is not necessary for this type of reaction. In addition, this reaction is very interesting from the point of view of atom economy and non-toxic. However, in most of the results of this reaction reported so far, diethyl thiophene-2,5-dicarboxylate was used to give thiophene-2,5-carbohydrazide. Here we describe the synthetic steps involved in the formation of thiophene-2,5-carbohydrazide derivatives using aromatic hydrazine condense with the aromatic Aldehyde group. We also investigate structure-activity relationships regarding the anticancer properties of these derivatives.

2.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.

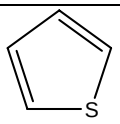
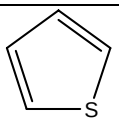
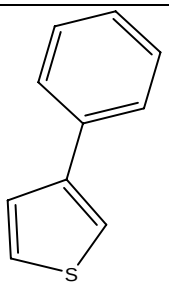
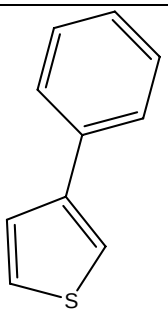
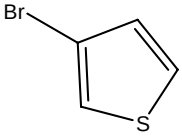
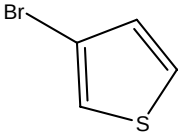
General procedure for the synthesis-

Scheme(1) Synthesis of the compounds F1-F3.



diethyl thiophene -2,5-dicarboxylate added with hydrazine hydrate with methanol as a solvent and sulphuric acid was added dropwise with aromatic-aldehyde soution. The mixture refluxing in an oil bath at 80°C for 18hours. The reaction mixture was cooled, and the yellow-colored crystalline product was filtered and washed with methanol before being recrystallized from ethanol.

Table (1) Synthetic Derivative(F1-F3) from the scheme.

Sr. No	Derivatives	R1	R2
1	F1		
2	F2		
3	F3		

3.RESULTS AND DISCUSSION:

N'2-[(1E)-ethylidene]-N'5-[(1Z)-ethylidene] thiophene-2,5-dicarbohydrazidederivatives wasprepared by using starting material diethylthiophene-2,5-dicarboxylate.

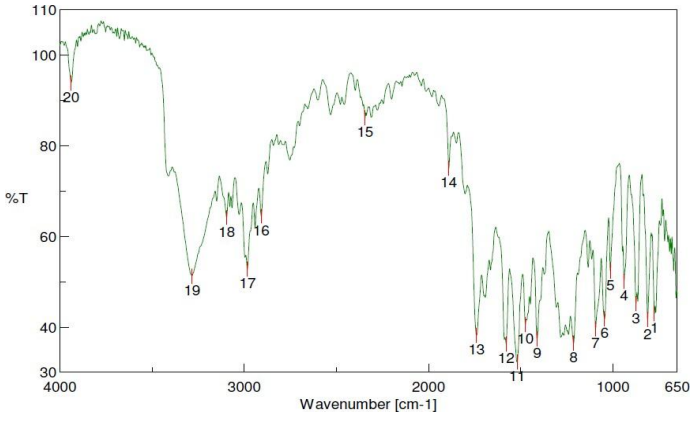
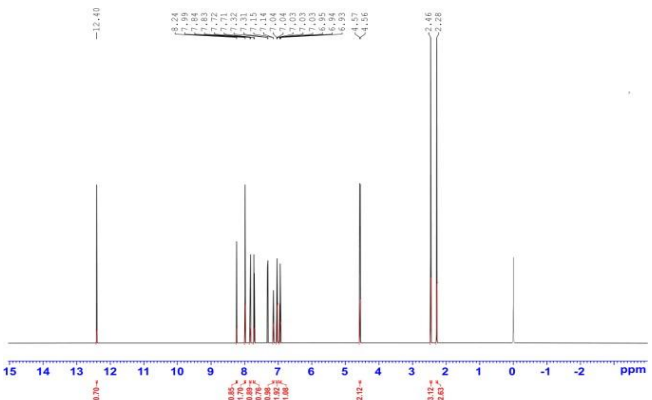
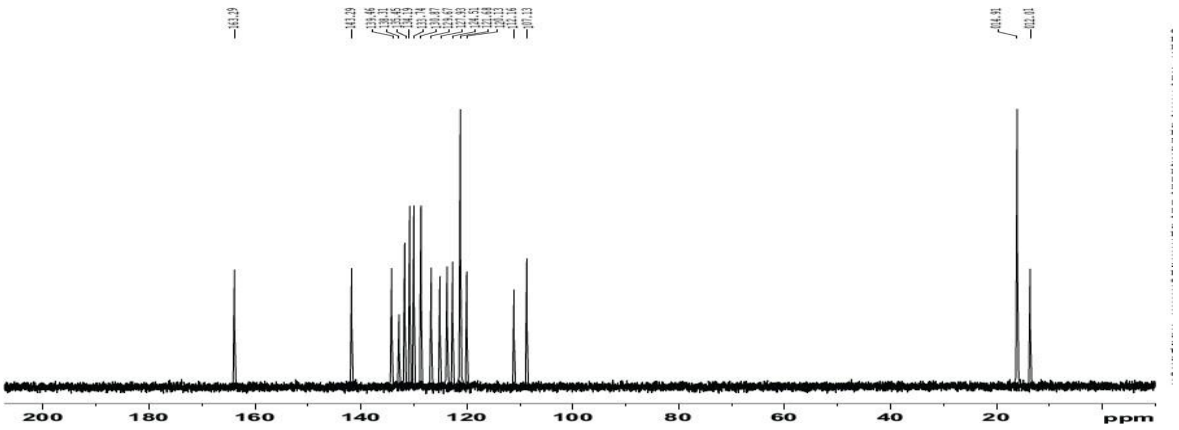


Figure (1) FTIR spectra of F1 compund

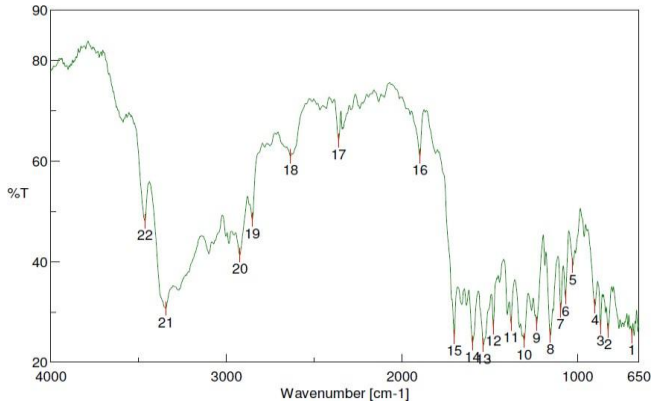


Figure(2) NMR of F1 compund

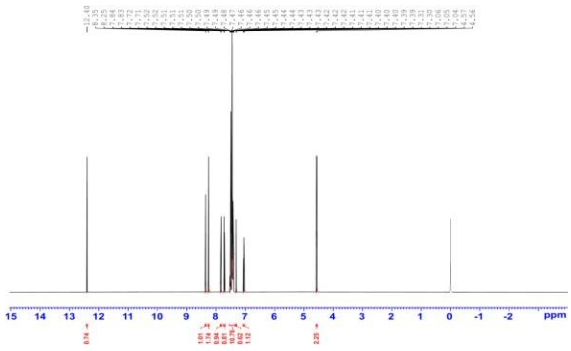


Figure(3)Mass of F1 compound

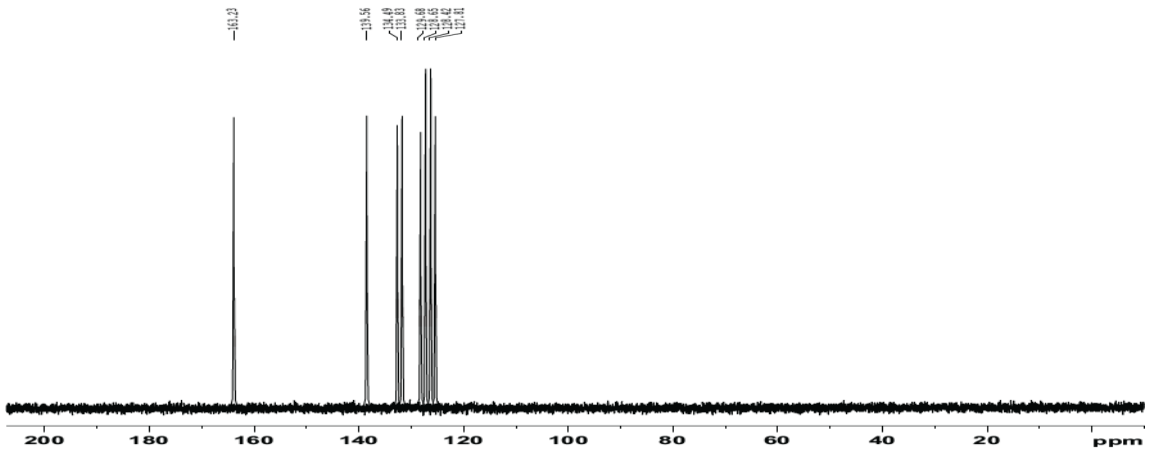
1. N'2,N'5-bis[(1E)-(thiophen-2-yl)methylidene]thiophene-2,5-dicarbohydrazide (1): **"**Yellow powder, IR (KBr,cm^{-1}) :(3283.21) C=N-H, ,(2344.05)-C=C,(774.279)C-S,(1739.48) C=N , 1889.9 (C=O); MS: *m/z* 388 .12;^{1}H NMR (500 MHz, Chloroform-d) δ 12.40 (s, 1H), 8.24 (s, 1H), 7.99 (s, 2H), 7.83 (d, J = 6.8 Hz, 1H), 7.72 (d, J = 6.8 Hz, 1H), 7.14 (d, J = 1.8 Hz, 1H), 7.06 – 7.01 (m, 2H), 6.94 (t, J = 6.3 Hz, 1H), 4.56 (d, J = 6.3 Hz, 2H), 2.46 (s, 3H), 2.28 (s, 3H). exact mass calcd. For $C_{16}H_{12}N_4O_2S_3$: 388; found: 388.49.Shown in figure(1,2,3)



Figure(4)FTIR of F2 Compound

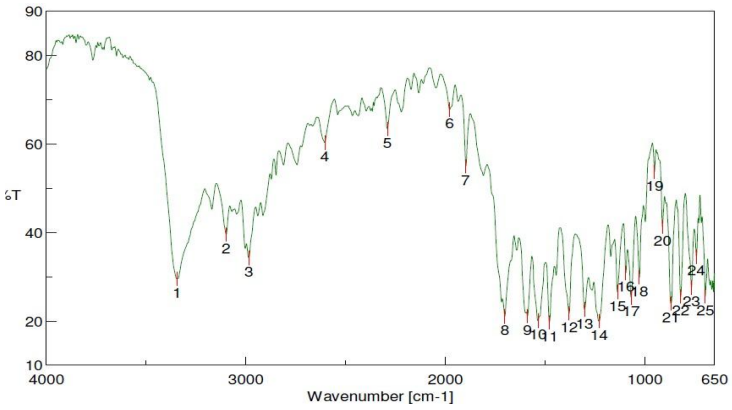


Figure(5)NMR of F2 Compound

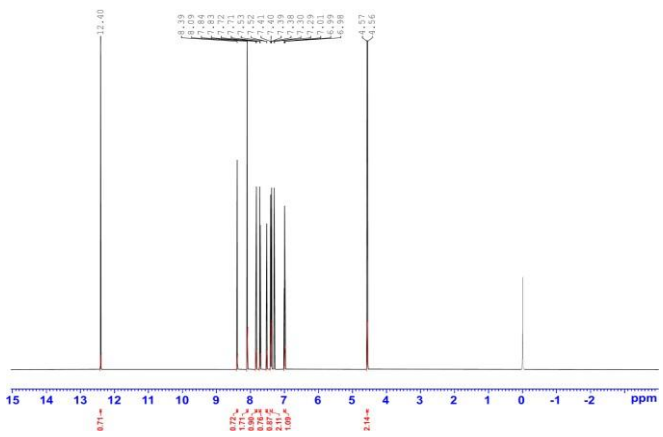


Figure(6)Mass of F2 Compound

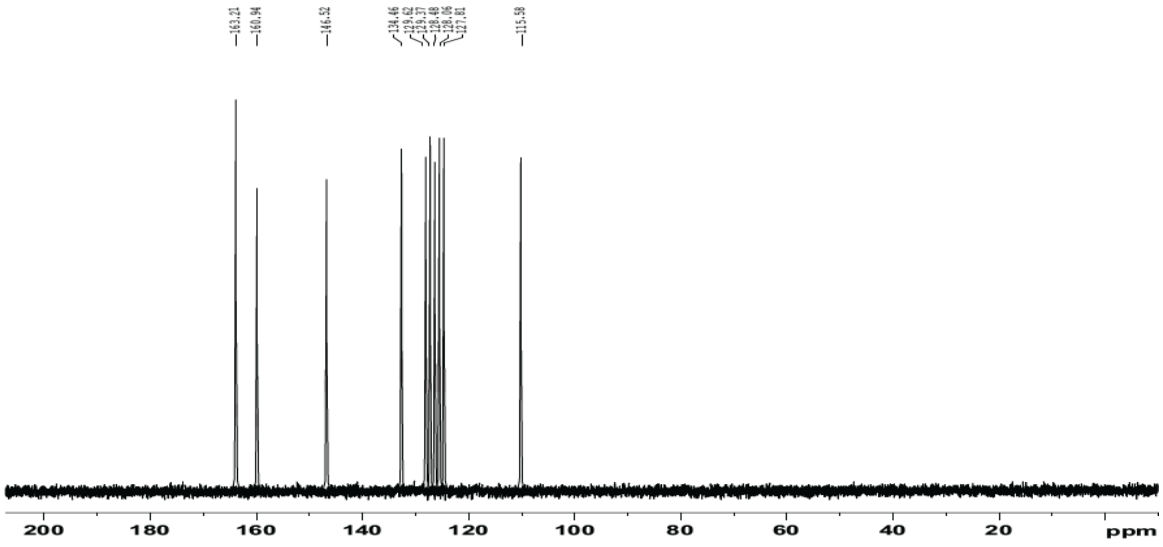
2. N'2-[(1E)-(4-phenylthiophen-2-yl)methylidene]-N'5-[(1Z)-(4-phenylthiophen-2-yl)methylidene]thiophene-2,5-dicarbohydrazide(2):**"**Yellow powder, IR (KBr,cm^{-1}) :(3345.89) C=N-H,(2635)-C=C,(690.391)C-S,(1702.84) C=N ,1897.61 (C=O); MS: *m/z* 440;^{1}H NMR (500 MHz, Chloroform-d) δ 12.40 (s, 1H), 8.35 (s, 1H), 8.25 (s, 2H), 7.83 (d, J = 6.8 Hz, 1H), 7.72 (d, J = 6.8 Hz, 1H), 7.54 – 7.37 (m, 11H), 7.31 (s, 1H), 7.05 (t, J = 6.1 Hz, 1H), 4.56 (d, J = 6.3 Hz, 2H). exact mass calcd. For $C_{28}H_{20}N_4O_2S_3$: 440; found: 440shown in figure(1,2,3)



Figure(7)FTIR spectra of Compound F3



Figure(8)NMR of Compound F3

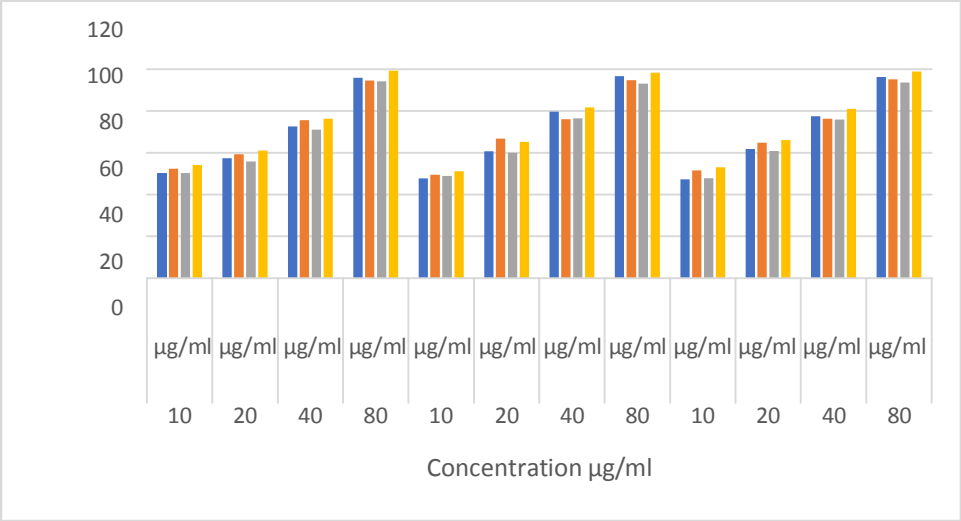


Figure(9)Mass of Compund F3

3. N'2-[(1E)-(4-bromothiophen-2-yl)methylidene]-N'5-[(1Z)-(4-bromothiophen-2-yl)methylidene]thiophene-2,5-dicarbohydrazide(3):"Yellow powder, IR (KBr,cm^{-1}) :(3345.89)C=N-H,(2604.39)-C=C,(741.496)C-S,(1701.87)C=N, 1896.65(C=O) ;MS: *m/z* 545.20;^{1}H NMR(500 MHz, Chloroform-d) δ 12.40 (s, 1H), 8.39 (s, 1H), 8.09 (s, 2H), 7.83 (d, J = 6.8 Hz, 1H), 7.72 (d, J = 6.8 Hz, 1H), 7.52 (d, J = 1.7 Hz, 1H), 7.43 – 7.36 (m, 2H), 6.99 (t, J = 6.3 Hz, 1H), 4.56 (d, J = 6.3 Hz, 2H).exact mass calcd. For $C_{16}H_{10}Br_2N_4O_2S_3$: 545.20; found: 545.20.Shown in figure(7,8,9)

Table(2)Anticancer activity of the synthesized compound

HumanBreastCancerCellLine (MCF-7)												
PercentageofinhibitionsonMCF-7atconcentrationofμg/ml(IC_{50})												
SampleConcentrations(μg/ml)												
	Experiment1				Experiment2				Experiment3			
	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
F1	50.37	57.32	72.56	95.76	47.64	60.65	79.61	96.67	47.28	61.75	77.44	96.25
F2	52.45	59.32	75.58	94.54	49.45	66.76	75.99	94.76	51.57	64.86	76.26	95.18
F3	50.26	55.92	71.08	94.13	48.93	60.04	76.49	93.07	47.83	60.87	75.85	93.66
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



Figure(10) Anticancer activity of F1-F3

Biological evaluation

Anticancer Activity

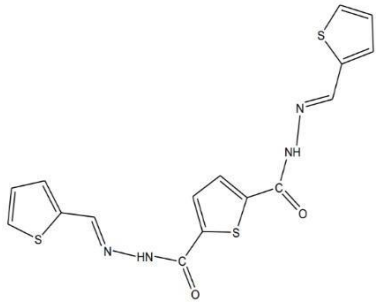
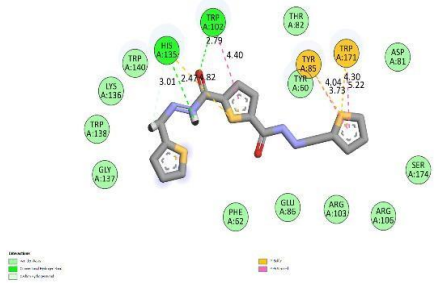
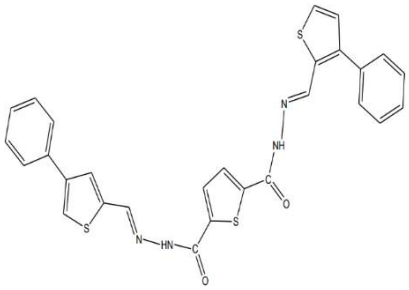
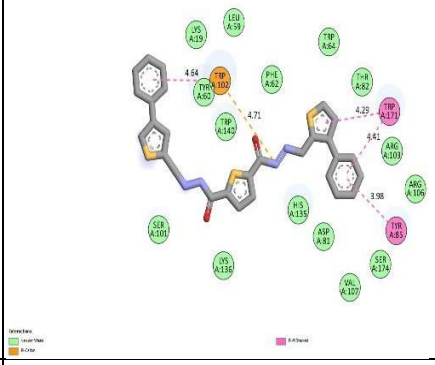
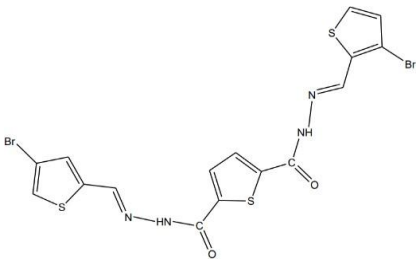
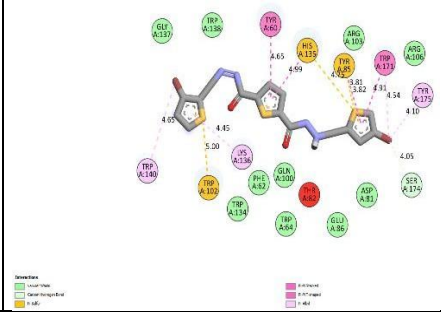
Thiophene-hydrazone, which become evolved and produced, is examined in vitro for antiproliferative activity against the Human MCF-7 cellular line using the MTT assay (three,4,5-dimethylthiazol-2-yl)-2,five-diphenyltetrazolium bromide, with Imatinib, a verified anticancer medication, as a reference chemical. 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}Cin a CO_2 incubator for 24 hours. After 24 hours of incubation, the plate may be tested beneath an inverted microscope. Sterile scaffold take a look at samples can be added in triplicate.100 µl of scaffold extract media can be introduced 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.The plate might be wrapped in aluminum foil and incubated for 4 hours in a CO_2 incubator. After 4 hours of incubation, the 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.

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)Docking of the AGF(183) 5IZQ protein with Compound (D1-D25) score values

Derivative	Structure	Docking Interaction	**Docking Energy (kcal/mol)**
F1			**-8.2**
F2			**-10.2**
F3			**-8.5**

Molecular docking analysis was used to investigate the possible binding mechanism of the suggested inhibitors with the human folate receptor alpha crystal structure. 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. F1 was found to be interacting with human folate receptor alpha through the formation of hydrogen bonds with TYR85, TRP171, pi sulphur interactions with TYR85, TRP171, pi-pi T shaped interactions with TRP102 and PHE62, and vander waal interactions with SER174, GLY137, TRP138, LYS136, TRP140, TYR60, ASP81, ARG106, ARG103, GLU86, and PHE62. F1's binding energy was found to be -8.2 kcal/mol..With a binding affinity

of -10.2 kcal/mol, F2 interacted with the target by forming vander waal interactions with SER101, LUS136,HIS135,ASP81, VAL107,SER174, ARG106, ARG103,THR82, TRP64,PHE62,LEU59,LYS19,TYR60, and TRP140, as well as pi-cation interactions with TRP102, TYR85, and TRP171.With the formation of interactions such as the carbon hydrogen bond with SER174, the Pi-Sulfur interaction with TRP102,HIS135,TYR85, the Alkyl interaction with TRP140,LYS136,TYR175, the Pi-Pi t SHAPED interaction with TRP171,TYR60, and the vander waal interaction with GLY137,TRP138,ARG103,ARG106,ASP81,GLU86,TRP64,TRP134,PHE62, the compound F3 demonstrated a binding affinity of -8.5 kcal/mol. Shown in table(3).

4.CONCLUSION:

Three thiophene-2,5-dicarbohydrazide compounds containing aromatic rings were synthesized and aligned with FRα.Higher binding energies have been demonstrated for all chemicals. The findings demonstrated interactions between the inner region of the FRα active site and ASP81, Arg106, Arg103, Trp64, Phe62, Tyr60, Trp134, and Trp138. These results imply that boosting the binding affinity towards FRα requires electron-donating and electron-withdrawing groups on aromatic, polycyclic, and heterocyclic rings.However, due to time and resource constraints, the study done here only yielded a theoretical prediction of the characteristics required for potential lead candidates. There is a lot of work to be done and many aspects of reaching the clinical level to be looked at in order to make sure that current efforts are not abandoned. Only two drugs are effective in treating FRα: methotrexate and vintafolide. It is suggested that the compounds could be synthesized having anticancer characteristics.F1, F2, and F3 have a docking score of -8.2,-10.2, and -8.5 kcal/mol. The synthesis of the compounds will allow the confirmation of the activity to be determined.Diethylthiophene-2,5-dicarboxylate is a starting material that can be used in a novel way to synthesize thiophene-2,5-carbohydrazide. This approach allows for the synthesis of the necessary intermediates for the final step of the thiophene-2,5-dicarbohydrazide process.New methodology, many replacement patterns that were not available from previously published approaches of this kind can now be incorporated. The robustness of this process was demonstrated by producing a large number of compounds. Spectrum data is used to assess each synthesized compound. Many of the selected candidates have shown improved antitumor activity when compared to the reference medication, imatinib. When compared to the reference drug Imantib, which has an IC50 value of 52.77 µg/ML, the novel derivatives F1 to F3 showed the most potent growth inhibitory effects. The half maximum inhibitory concentration, or IC50, and the percentage of cell death were measured at various values.Among the tested series, F2 showed the most anti-tumor activity, with an IC50 value of 51.15 µg/mL, compared to the reference medication doxorubicin's IC50 value of 51.57 µg/mL. The

IC50 values of compounds F1 and F2 indicate that they may have cytotoxic effects on MCF-7 human breast cancer cells (48.13 µg/mL and 49.00 µg/mL, respectively). The promising outcomes of the present investigation have made it necessary to carry out further research into this family of compounds as novel cancer chemotherapeutic agents, as indicated in Table (2).

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