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DOCKING AND BIOLOGICAL EVALUATION OF A NOVEL DERIVATIVE OF METFORMIN AND ITS SYNERGISTIC ACTIVITY WITH 5-FU AS ANTICANCER AGENTS.

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ABSTRACT:

Background: This current study was focused on the design and synthesis of a novel derivative of metformin and uracil to study the efficacy of both moieties as anticancer agents. **Methods:** The target compound was successfully designed and prepared by condensation of uracil-5-methyl chloride with metformin in anhydrous pyridine as an acid scavenger. The prepared compound could be considered as a chemical isoster of both 5-FU and metformin in an attempt to augment the anticancer activity of 5-FU. Both target compound and 5-FU were subjected to anticancer activity in a comparative manner against a human A-2780 ovarian, HT-29 colon, MCF-7 breast, and HEPG-2 liver carcinoma cells. **Results:** The compound showed a promising activity relative to 5-Fluorouracil which was used as a reference standard. **Conclusion:** the synergistic activity of the compound and 5-FU was studied that showed an amazing results.

KEYWORDS: *Metformin; 5-FU; Metformin derivative; Synergistic anticancer activity.*

INTRODUCTION:

Cancer is a deadly disease that defeats a person in many cases and causes his end, but there is no despair with life and no life with despair. The term denotes to any one of a large number of diseases characterized by the development of abnormal cells that divide uncontrollably and have the ability to infiltrate and destroy normal body tissues. It has the ability to spread

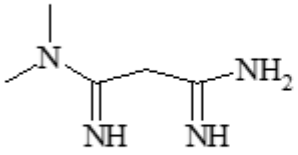
throughout the body thus, constituting the second cause of death in the world[1-3]. From this stand point and for many decades, millions of scientists and researchers around the world have been rushing to make an effort to conduct and develop research in order to develop solutions and strategies to confront this disease that is destroying people 'lives and draining government resources. Positive results are few in this field, and until now there is no ideal solution to this disease, but hope exists and attempts are continuing. Treatment and control methods vary from surgery to the use of chemotherapeutic agents .Surgery plays a crucial role in cancer treatment also, it allows the examination of tissue samples as a diagnostic agent .[4,5] Many types of surgery are involved including cryosurgery, laser surgery, mohs surgery and robotic surgery[6]. More over in many cases surgery is often used in combination with radiation therapy using high energy particles or waves such as X-rays, gamma rays, electron beams or protons to destroy or damage cancer cells [7]. Both methods could be combined with the use of chemotherapeutic agents that kill or slow the growth of cancer cells. There are many common types such as alkylating agents as cyclophosphamide that treats various cancers including breast, ovarian and lymphoma. Also , Busulfan that used for chronic myeloid leukemia. In addition to Anti-metabolites such as Methotrexate that treats breast, lung and ovarian cancers, as well as leukemia and lymphoma. Also[8], 5-Fluorouracil that is effective in colorectal ,breast and stomach cancers[9]. Furthermore, some anthracyclines such as Doxorubicin and Daunorubicin are involved in leukemia treatment[10]. Other methods involve hormonal therapy[11] , bone marrow and blood transplants. Also biological therapy is a choice in which substances which are produced naturally in patient 'own body to block the cancer cell growth[12]. 5-Fluorouracil is one of the oldest and classical antimetabolic agent for cancer treatment specially, colorectal, esophageal , stomach, pancreatic, breast and cervical cancer. As a cream it is used for actinic keratosis, basal cell carcinoma and skin warts[13]. It was patented in 1956 and prescribed in early 1662, in 2022,it was the 270th most commonly recommended drug in the united states with more than 900,000 prescriptions[14]. It acts as antimetabolic agent by thymidylate synthase inhibition thus interrupting the pyrimidine thymidylase which is a nucleotide required for DNA replication . Thymidylate synthase methylate deoxyuridine monophosphate (Dump) to form thymidine monophosphate (dTMP) , Administration of 5-FU causes a scarcity in dTMP so rapidly dividing cancerous cells undergo cell death via thymineless death. Unexpectedly, and despite the relative success[14], 5- FU resistance is a significant challenge in cancer treatment, particularly in colorectal cancer[15]. One of the recommended strategies to overcome 5-FU resistance is the combination therapy by combining the drug with other chemotherapeutic agents or targeted therapies that can enhance its efficacy.

These combinations include the incorporation with Leucovorin, Oxaliplatin, Irinotecan to acts as topoisomerase inhibitor in addition to 5-FU effects[16]. It is also combined with Bevacizumab, vascular endothelial growth factor (VEGF) inhibitor enhancing 5-FU efficacy by inhibiting angiogenesis. More over it could be combined with Cetuximab, an epidermal growth factor receptor inhibitor that enhances 5-FU efficacy by inhibiting signaling[17]. A promising combination therapy was recently researched was 5-FU combination with Metformin but needs further future researches[18-22].

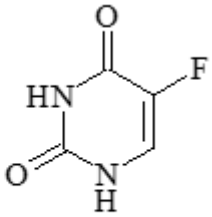
Metformin is a popular famous widely prescribed drug for treatment of type 2 diabetus , but recently it showed promising anticancer activity against pancreatic, colorectal, ovarian and other cancers[23]. Because , it has less adverse effects and being an inexpensive drug, it could be used as a chemotherapeutic agent against cancer as it inhibits cell proliferation, inducing apoptosis and reducing angiogenesis. Several mechanisms have been proposed to explain the cytotoxic effect of metformin[23] It may act by activation of adenosine monophosphate – activated protein kinase (AMPK) pathway, which regulates energy metabolism and has been implicated in cancer prevention. . Also, metformin decreases insulin and insulin-like growth factor-1 (IGF-1) signaling which can promote cancer cell growth. In addition metformin may increase oxidative stress in cancer cells, leading to cell death.

Studies have shown that the combination of metformin and 5-FU can enhance the anticancer efficacy in various cancer cell lines and animal models. Also, the combination of metformin and 5-FU has been shown to increase apoptosis (programmed cell death) in cancer cells. This combination may offer a promising therapeutic strategy for cancer treatment. However further research is needed to fully understand the synergistic effects of these two medications and to determine their optimal dosing and administration regimens. Here, we developed a synthetic and biological plan to use a metformin and uracil analog hoping, in the future to get more investigations in this field. The started by the preparation of target compound using uracil as a block unit, Thus a classical reaction of uracil is its chloromethylation which is a complex reaction that can lead to various products depending on the conditions. For example, if uracil is reacted with formaldehyde and HCl gas with a temperature control (0-5^{o}C),5-chloromethyluracil is obtained in a fair yield .This compound was best prepared indirectly from chlorination of 5-hydroxymethyl uracil in a reasonable yield. This compound was used in synthesis of nucleoside analogs and as a cross-linking agent and was reacted in many literatures with huge numbers of amines giving uracil derivatives[25-34]. In our research we use metformine as an amine to prepare our target compound. This uracil-metformin derivative

had been exhibited promising anticancer activities as a chemical isoster of 5-FU ands F metformin.



Metformin



5-Fluorouracil

MATERIALS AND METHODS:

Chemistry:

All melting points are uncorrected and were measured using an Electrothermal IA 9100 apparatus (Shimadzu, Japan). IR spectra were recorded as potassium bromide pellets on a Perkin-Elmer 1650 spectrophotometer (USA). ^{1}HNMR spectra (300MHz) were recorded in dimethyl sulfoxide (DMSO) by employing tetramethyl silane (TMS) as an internal standard on Varian Mercury 300 MHz NMR Spectrometer (Varian, UK) and the chemical shifts (δ) were expressed as ppm against TMS as internal standard. Mass spectra were recorded on a 70 ev EI Ms-QP 1000 (Shimadzu,Japan). Microanalyses were operated using vario, Elementar apparatus (Shimadzu) and carried out in Microanalytical unit, Central Services Laboratory, National Research Centre, Dokky , Giza, Egypt. The progress of all the reactions was monitored by TLC on silica gel 60 for TLC (Merck) using chloroform-methanol (3:1) as mobile phase and spots were visualized by iodine vapors or by irradiation with UV-light (254nm).

Biology:

All cell lines were purchased from national research centre, Dokky, Giza, Pharmacology unit. They were imported and derived from various cell banks and repositories from American Type Culture Collection (ATCC), European Collection of Authenticated Cell Cultures (ECACC),German Collection of Microorganisms and Cell Culture (DSMZ) and Addex. Dyes were purchased from sigma chemical Co. Preliminary studies were conducted with each of many dyes to determine whether each stained cells more intensively at acidic, neutral, or basic pH. One of these anionic dyes was sulforhodamine B (SRB) which was dissolved in 1% acetic acid for cell staining and extracted from cells with un buffered Tris base. The absorption maximum of the dye was determined with a DU-70 scanning spectrophotometer (Beckman

Instruments, Inc, Fullerton, CA).

Bio.RPMI-1640 medium was purchased from sigma chemical company.

Cells:

We performed preliminary experiments with the human A-2780 ovarian, HT-29 colon, MCF-7 breast, and HEPG -2 liver carcinoma cells. The method applied is similar to that reported by Skehan, P. Stock cultures were grown in T-75 containing 50 MI of RPMI-1640 medium with glutamine, bicarbonate and 5% fetal calf serum. Medium was changed at 48-hours intervals. Cells were dissociated with 0.25% trypsin and 3Mm 1,2-cyclohexanediaminetetracetic acid in NKT buffer (137Mm NaCl, 5.4Mm KCl, and 10 Mm tris; pH 7.4) Experimental cultures were plated in microtiter plates (Costar and Cambridge, Ma) containing 0.2 mL of growth medium per well at densities of 1,000-200, 000 cells per well.

Dyes:

Dyes were purchased from Sigma Chemical Co. Preliminary studies were conducted with each of these 31 dyes to determine whether each stained cells more intensely at acidic, neutral, or basic pH. The anionic dyes bromophenol blue, chromotrope 2R, Coomassie brilliant blue, naphthol yellow S, Orange G, and sulforhodamine B (SRB) were dissolved in 1% acetic acid for cell staining and extracted from cells with 10 mM unbuffered Tris base [tris(hydroxymethyl) aminomethane]. The cationic dyes acridine orange, azure A, azure B, phenosafranin, safranin O, thionin, and toluidine blue O were dissolved in unbuffered 10mM tris base to stain cells and were resolubilized for measurement of optical density with either 1% or 10% acetic acid . The cationic dyes ethidium bromide, propidium iodide, and pyronin B were dissolved in water for staining Although these are excellent fluorescent dyes, their staining intensity was poor at visible wavelengths. Crystal violet was dissolved in 10% ethanol and 90% water at a neutral pH; its staining intensity varied considerably from one cell line to another. The absorption maximum of each dye in each solubilizing solution was determined with a DU-70 scanning spectrophotometer (Beckman Instruments, Inc., Fullerton, CA).

1. EXPERIMENTAL:

5-Hydroxymethyl uracil: 2 It was prepared as in literature by treating uracil (14.2 g, 0.1mol) and paraformaldehyde (4g, 0.13 mol) in 170 ml aq. solution of KOH(0.42N) . The mixture is allowed to stand for 72h at 50^{o}C,then it was monitored by T.L.C, diluted with distilled water (450ml), the mixture was stirred with 40 g. of freshly washed Dowex 50 | H^{+} form, and then filtered. The slightly acidic filtrate was concentrated under reduced pressure to 30 ml, and

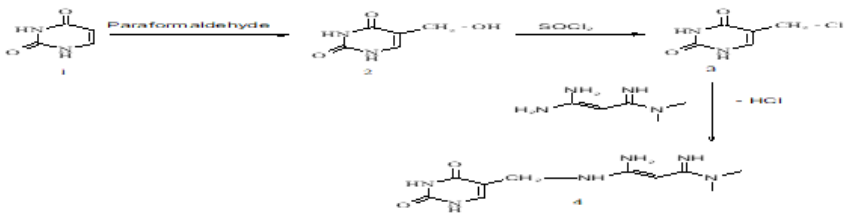
refrigerated. The product was recrystallized from water, yield 13.76 (80%),m.p 289ºC.

5-Chloromethyl uracil: 3 A solution of thionyl chloride (3.3 ml, 0.04 mol) in dry chloroform (20ml) was added dropwise into a solution of (2) (6.02g., 0.03 mol) in dry chloroform (60ml) containing 3ml of pyridine, then the mixture was refluxed for 1 hr.in anhydrous conditions. The product was separated by extraction with diethyl ether and the residue was crystallized from DMF|water to give 6g.,80% of compound (3), m.p 350ºC. **5-[N-(N-(N,N-Dimethylcarbamimidoyl)carbamimidoylmethyl)-2,4-dioxo-1,2,3,4-tetrahydro pyrimidine 4.**

A mixture of compound 3 (1.13 mole) ,Metformin (1.13 mole) and pyridine (0.016 mole) in absolute ethanol (50 ml) was refluxed for 10 hours, then cooled, filtered and re-crystallized from DMF/water.

Yield: 75%: m.p: 215-217 ºC: IR (KBr cm^{-1}): 3446, 3359,3230 (NH), 3163 (CH, aromatic),2987 (CH,aliphatic), 1683,1665 (2C= O). 1HNMR (DMSO-d6), δ:3.86 (s,6H,2CH_3,4.93 (s,2H,CH2), 3.5,3.8,3.9,9.8(s,4NH of biguanide, D_2O exchangeable), 8.10 (s,1H,pyrimidine-H6), 10.5,11.00 (s,2NH,D_2O exchangeable of pyrimidine), ^{13}CNMR (DMSO-d6) δ: 33.01,56.11, 118.32,130.54,147.67,153.98,169.09,177.23. MS: m/z (%), 253 (M^+,53.3%), Anal. Calcd, for $C_9H_{15}N_7O_2$: C, 35.76; H, 4.67; N, 27.80. Found: C,35.66; H, 4.58; N,27.69.

Scheme: 1



BIOLOGY

Cytotoxic Activity:

Cell Fixation:

Washing cultures with buffer prior to fixation to remove serum protein commonly caused detachment and loss. To avoid this potential problem, cultured were fixed with TCA before washing. Cells attached to the plastic substratum were fixed by gently layering 50µL of cold50%TCA (4^OC) on top of the growth medium in each well to produce a final TCA

concentration of 10% The cultures were incubated at 4°C for 1 hour and then washed five times with tap water to remove TCA, growth medium and law-molecular weight metabolites and serum plates were air dried and then stored until use. Background optical densities were measured in wells incubated with growth medium without cells. Cells in suspension were allowed to settle out of solution. When these cells were physically resting on the bottom of the wells,50 µL of cold 80%TC.

A (4^{O}c) was gently layered on top of the overlying growth medium. The cultures were left undisturbed for 5 minutes and then refrigerated at 4^{0}C for an additional hour of fixation. This procedure led to the attachment of single cell suspensions to the plastic substratum provided that cells were in contact with it when the fixative was applied. This method was as effective in promoting cell attachment as were cytospinning and using the macromolecular adhesive Cell-Talk (Biopolymers, Famington, CT). However, it did not adequately attach cells that grew as floating aggregates rather than as single cell suspensions. Small cell lung carcinoma lines were particularly unsuited to this method of fixation.

Procedure:[35]

Preparation of the sample: Target compound 3 was dissolved in DMSO .Cells were plated in 96-multiwell plate (104 cells | well) for 24 h before treatment with the test compound to allow attachment of cell to the wall of the plate different concentrations of compound 3 (0, 2.5, 5, and 10 µg|ml) were added to the cell monolayer in triplicate wells individual dose, monolayer cells were incubated with the compounds for 48h at 37°C and in atmosphere of 5% CO_2, after 48h, cells were fixed, washed and stained with SRB stain, excess stain was washed with acetic acid and attached stain was recovered with Tris-EDTA buffer, color intensity was measured in an ELISA reader, the relation between surviving fraction and drug concentration is plotted to get the survival curve of each tumor cell line after the specified compound and the IC 50 was calculated.

Table 1. In vitro cytotoxic activity (IC_{50}) of the newly prepared compound 4 and 5-Fluorouracil against a human A-2780 ovarian, HT-29 colon, MCF-7 breast, and HEPG-2 liver carcinoma cells.

	A-2780 (µg m / L)	**HT-29 (µg m / L)**	**MCF-7 (µg m / L)**	**HEPG-2 (µg m / L)**
Compound 4	0.73	0.83	0.55	-
Metformin +Compound 3	0.52	0.60	0.53	-
5-FU + Metformin	0.50	0.55	0.57	5.00

5-FU + Compound 4	0.50	0.54	0.52	1.04
5-Flurouracil	0.52	0.61	0.67	5.00

IC_{50} is defined as the concentration which results in 50% decrease in cell number as compared with that of the control structures in the absence of an inhibitor. The value of IC_{50}% for the reference drug 5-Fluorouracil against HEPG2 was maintained by the National Institute of Cancer, Cairo University, Cairo, Egypt (The liver carcinoma cells were found to be more resistant to 5-FU as a reference drug).

RESULTS:

-Compound **4** showed promising activity against ovarian, colon, breast cell lines compared to 5-Fluorouracil but was inactive against liver cell line. Thus 5-UF is still somewhat more reactive than compound 3,this is because the atomic size of fluorine atom is approximately near to that of hydrogen in the five position of uracil, thus 5-FU is the proto type of anti- metabolites as anticancer agents.

-Combination of compound 4 with metformin gave a synergistic action where it was relatively more active than 5-FU but still resistant liver carcinoma cells

-Combination of 5-FU and metformin increases the anticancer activity but to a smaller degree.

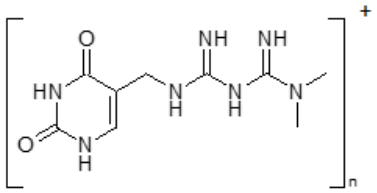
-Combination of 5-FU and compound 4 gave a more potent synergistic activity and in unexpected manner give positive activity against liver carcinoma cells.

DISCUSSION: Anti- metabolites are a class of anticancer agents that interfere with DNA and RNA synthesis, thereby inhibiting cancer cell growth and proliferation. They work by mimicking the structure of essential nutrients or molecules, thereby disrupting cellular metabolism, they include folic acid antagonists such as methotrexate and pemetrexed, also purine analogs such as mercaptopurine and thioguanine. Moreover, adenosine deaminase inhibitors such as pentostatin are considered. A classical group is pyrimidine analogs such as fluorouracil and cytarabine. They have shown various efficacy in treating various types of cancer for example, methotrexate and mercaptopurine are involved in leukemia, lymphoma and breast cancer also, 5-Flurouracil for colorectal cancer. Thus, Anti- metabolites are a crucial class of anticancer agents that have shown significant efficacy in treating various types of cancer. However , resistance and side effects remain major challenges. Ongoing research aims to develop new anti-metabolites with improved efficacy and reduced toxicity. This research represents a close cooperation link between individuals in the pharmaceutical field,

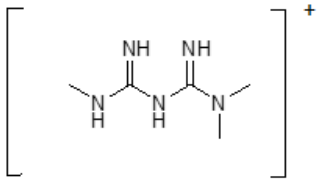
especially pharmaceutical chemistry, which aims to synthesize new effective drugs, as well as pharmacology, which aims to track these drugs to know their biological effects with the aim of solving some of the problems of this field in order to reach new effective and safe drugs. The beginning was to look at an effective compound such as 5-FU, a classical proto type of anti-metabolites but resistance to 5-FU is a significant clinical problem. Resistance is acquired due to reduced expression of the nucleoside transporter ENT1, which is responsible for 5-FU uptake into cancer cells. In addition, resistance is attributed to elevated expression of the enzyme dihydropyrimidine dehydrogenase (DPD) which breaks down 5-FU into its inactive metabolites. In some caces, mutations or over expression of the target enzyme TS, which can reduce the binding affinity of 5-FU. Various strategies were recommended to over come 5-FU resistance. Firstly, using 5-FU in combination with other agents, also by increasing the dose but this may also increase toxicity. . In addition pro-drugs was a tool.

In this research, we try to prepare a compound similar to it but with the specific aim of replacing the fluorine atom with a sulfonamide of metformin. Firstly uracil was indirectly chloromethylated by hydroxyl methylation of uracil using paraformaldehyde then chlorination of the product by refluxing with thionyl chloride. The chloromethyl derivative was condensed with metformin as an amine in presence of pyridine as an acid binder to give our target compound 4. Thus compound 4 was designed and synthesized to have both uracil and metformin nuclei in one compound.

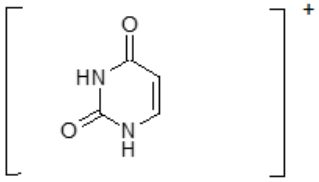
In IR-spectrum compound 4 should intensely peaks at 3446,3359,3230(6NH), 3163 (CH aromatic), 2987 (CH aliphatic), 1683,1665 (2C = O). The mass spectrum reveals a peak at m/z 253(53.3%) corresponding to $[M]^+$.The base peak (100%R.A) appeared at 111 corresponding to uracil cation, a peak at m/z 142 (38%) corresponding to metformin $+CH_2$-cation and others as shown.



M/Z 253



M/Z 142



M/Z 111

1HNMR spectrum showed peaks at δ 3.86 ppm corresponding to two methyl groups. Also, peak δ 4.93 ppm corresponding to methylene group, in addition NH 's protons at δ 3.5,3.8,3.9,9.8 of metformin . Furthermore, a peak at δ 8.10 ppm of pyrimidine. Also, peaks at δ10.5,11.00 corresponding to 2NH of pyrimidine.13CNMR-spectrum showed different carbons at δ .01,56.11, 118.32,130.54,147.67,153.98,169.09,177.23.

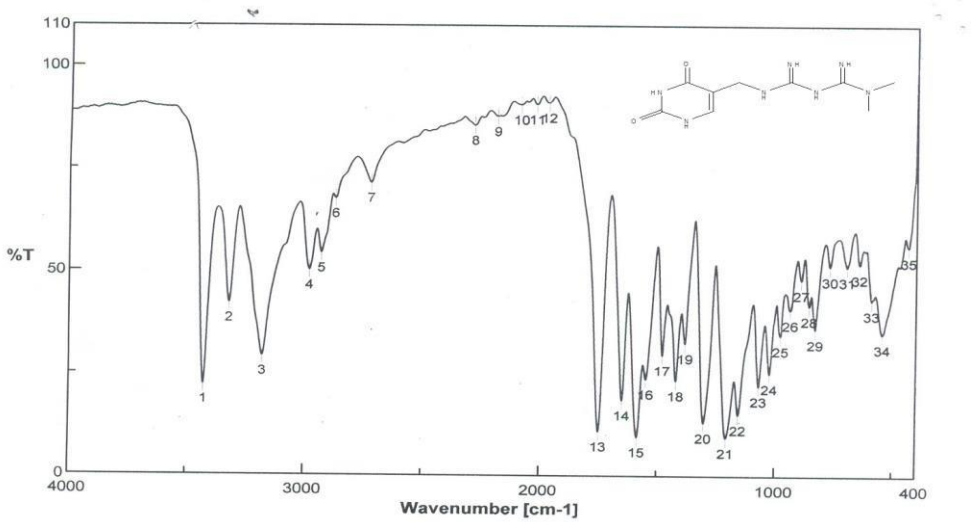


Fig.1 IR-Spectrum of target uracil-metformin compound 4.

Recent literatures have acknowledged that these types of compounds might have promising anticancer activity as antimetabolites by inhibition of nucleic acid synthesis.

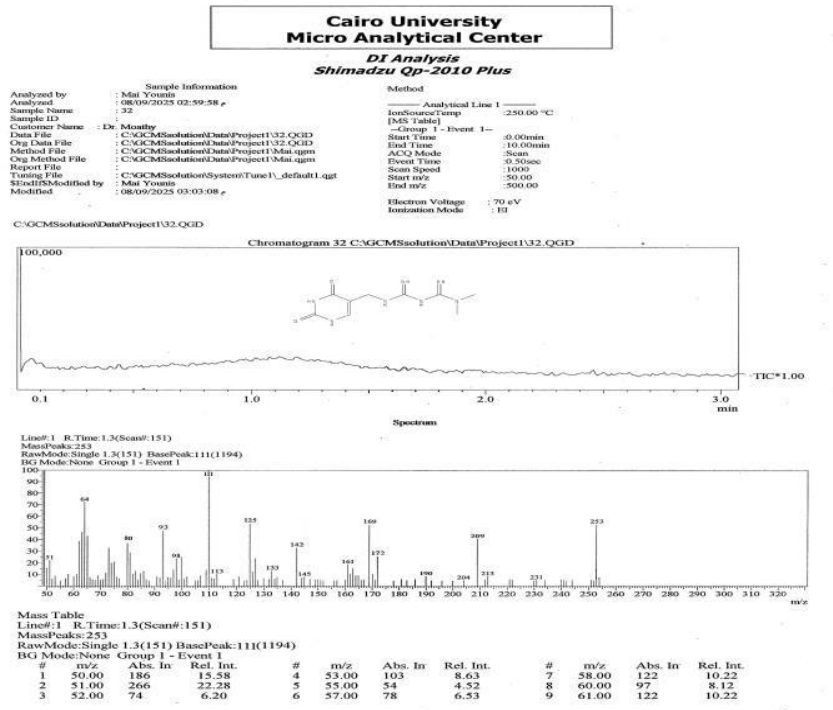


Fig.2 Ms-Spectrum of target uracil-metformin compound 4.

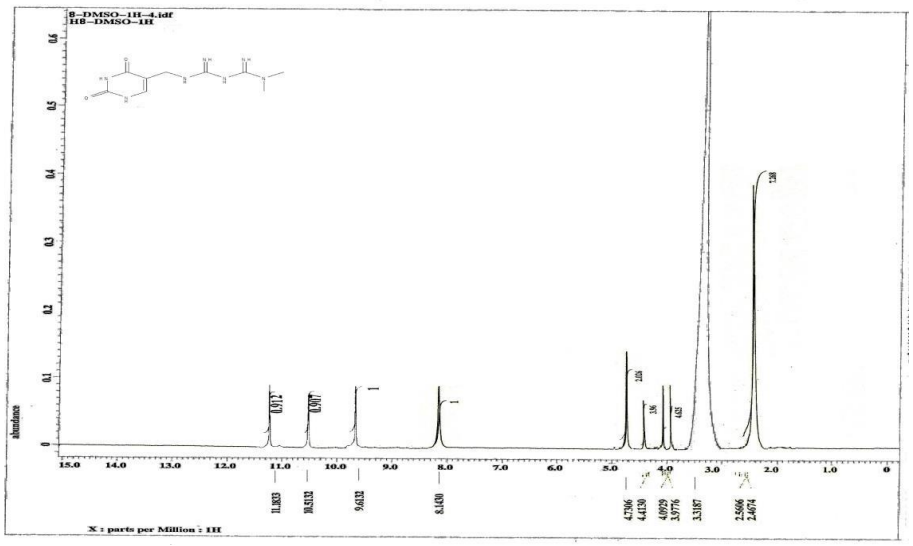
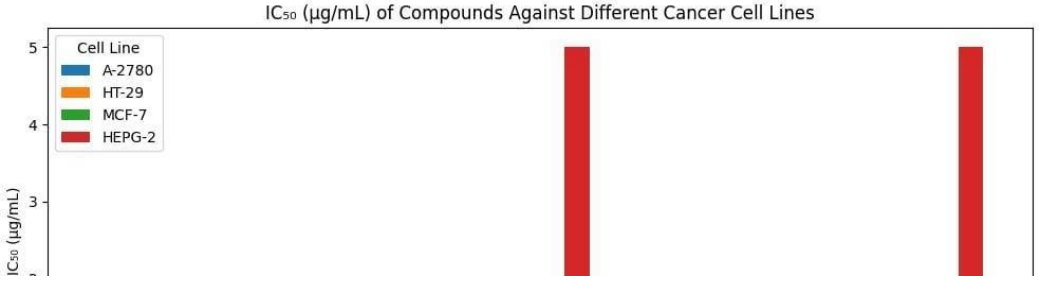


Fig.3 [1]HNMR-Spectrum of target uracil-metformin compound 3.



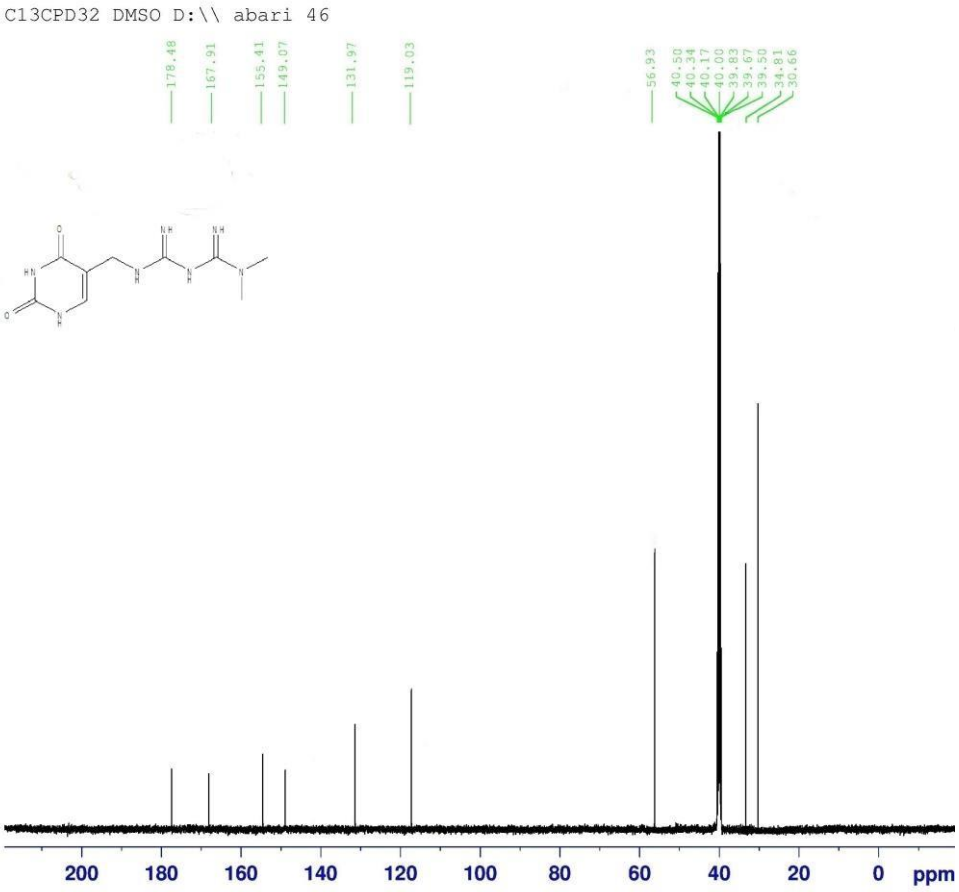


Fig.4 ^{13}CNMR-Spectrum of target uracil-metformin compound 4.

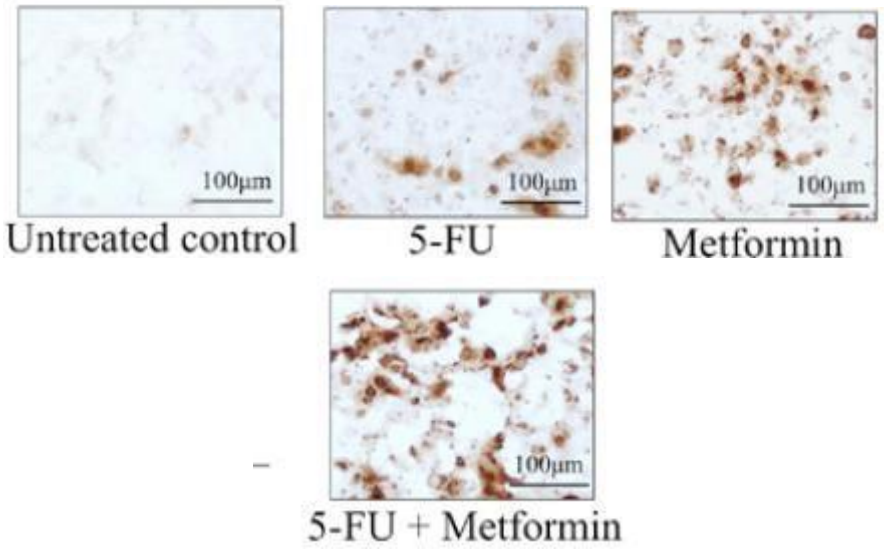


Fig.5 Cell apoptosis in colon cell lines for 5-FU,Metformin and its combination.

	mol	rseq	mseq	S	rmsd_refine	E_conf	E_place	E_score1	E_refine	E_score2	E_orig	Rec_index	PLIF_raw	FP↓PLIF	PLIF_ligidx
1	metformin-UMP	1	1	-7.9812	1.8781	-631.4351	-84.8104	-14.2784	-54.3180	-7.9812	0.7350	1	[[2,2,2,2,3,24,	1 2 3 4 5	[[3,53],[3,53],
2	metformin-UMP	1	1	-7.9789	2.9339	-630.4966	-77.8839	-11.8496	-57.0578	-7.9789	1.0321	1	[[3,53,2,2,52,5	1 2 3 4 5	[[2,2],[2,2],[2
3	metformin-UMP	1	1	-7.8376	2.6281	-619.8818	-100.4321	-12.4512	-49.3341	-7.8376	1.7990	1	[[52,2,3,24,24,	1 2 3 4 5	[[2,3],[2,3],[2
4	metformin-UMP	1	1	-7.8109	1.4205	-625.5499	-99.2817	-10.2929	-55.1825	-7.8109	1.0521	1	[[3,20,21,52,2,	1 2 3 4 5	[[2,2],[2,2],[2
5	metformin-UMP	1	1	-7.7736	1.3791	-618.0255	-77.4230	-10.3279	-45.8903	-7.7736	2.7990	1	[[2,11,49,2,2,3	1 2 3 4 5	[[3,53],[3,53],
6	metformin-UMP	1	1	-7.6492	2.3026	-634.6335	-86.0082	-11.3087	-55.9077	-7.6492	2.4116	1	[[3,21,29,2,52,	1 2 3 4 20	[[53,53],[53,53
7	metformin-UMP	1	1	-7.6322	1.2356	-611.4481	-80.5137	-10.4133	-40.3388	-7.6322	1.4019	1	[[11,21,2,2,3,2	25 26 27 2	[53,53,53,53,11
8	metformin-UMP	1	1	-7.5734	3.1168	-630.3683	-82.1514	-10.2367	-46.1174	-7.5734	1.4261	1	[[13,21,27,2,3,	25 26 27 2	[[2,11],[2,11],
9	metformin-UMP	1	1	-7.5711	1.5528	-624.7560	-84.3945	-10.3245	-51.4213	-7.5711	2.4190	1	[[27,29,52,2,2,	1 2 3 4 5	[[2,2,3],[2,3],
10	metformin-UMP	1	1	-7.4660	3.0222	-621.0485	-67.9369	-10.6099	-40.3835	-7.4660	0.7619	1	[[3,35,2,2,2,20	27 28 46 2	[[52,53],[52,53
11	metformin-UMP	1	1	-7.4290	2.8162	-613.2201	-82.5379	-10.1438	-46.5619	-7.4290	1.9314	1	[[2,24,52,2,2,2	1 2 3 4 23	[[53,53],[53,53
12	metformin-UMP	1	1	-7.3821	1.7542	-627.5092	-107.4970	-10.1239	-44.6596	-7.3821	1.3990	1	[[52,11,24,52,5	49 50 51 5	[53,53,[2,53],[
13	metformin-UMP	1	1	-7.3135	2.8159	-623.9042	-69.0518	-10.4851	-48.7045	-7.3135	1.3992	1	[[52,2,3,11,52,	1 2 3 4 5	[[2,3],[2,3],[2
14	metformin-UMP	1	1	-7.2868	2.2570	-633.9640	-70.6531	-11.8729	-49.6163	-7.2868	2.9990	1	[[3,2,2,52,52,5	1 2 3 4 5	[[2,2],[2,2],[2
15	metformin-UMP	1	1	-7.2542	2.2065	-627.7953	-93.4394	-10.0865	-33.0361	-7.2542	2.9990	1	[[21,2,2,3,3,20	25 26 27 2	[52,52,[2,52],5
16	metformin-UMP	1	1	-7.1209	2.9301	-628.1960	-69.0745	-10.4796	-53.0801	-7.1209	0.0200	1	[[2,27,2,3,52,5	1 2 3 4 21	[[3,52],[3,52],
17	metformin-UMP	1	1	-7.0460	2.3094	-627.1677	-89.1644	-11.1920	-48.3064	-7.0460	0.4142	1	[[53,2,2,3,52,5	66 67 68 6	[[3,52],[3,52],
18	metformin-UMP	1	1	-7.0319	3.6476	-636.2789	-87.3327	-10.7780	-49.0904	-7.0319	3.3990	1	[[3,27,29,2,52,	66 67 68 6	[[2,53],[2,53],
19	metformin-UMP	1	1	-7.0035	2.3777	-625.5574	-83.1999	-12.2570	-38.0666	-7.0035	2.0190	1	[[51,2,3,3,52,5	1 2 3 5 6	[[3,3,52],[3,3,
20	metformin-UMP	1	1	-6.9768	1.7765	-629.7673	-123.4033	-10.5701	-44.1965	-6.9768	0.0330	1	[[3,21,52,20,35	80 81 82 8	[35,35,[52,53],

Molecular docking table.

The molecular docking results of the newly synthesized compound (compound 4) revealed promising binding affinities toward the target enzyme active sites, comparable to or even exceeding those reported for 5-Fluorouracil, the docking energies ranged between -6,97 and -7.98 kcal/mol, indicating strong and thermodynamically favorable interactions between the ligand and the receptor pocket. Among the generated conformations, the top-ranked pose (E_orig = -7.98 kcal/mol;RMSD_refine = 1.87AO)showed optimal spatial accommodation within the enzyme' catalytic cavity, suggesting a stable ligand-receptor complex. The low RMSD_refine value reflects a good alignment and reproducibility of the docking pose, emphasizing the reliability of binding prediction. Analysis of the E_refine and E_place parameters indicates that compound 4 achieves efficient placement and favorable refinement energy, supporting the hypothesis that the conjugation of metformin with compound 4 moiety enhances molecular interactions. This modification likely contributes additional hydrogen bond donors/acceptors and π-π stacking capability, which improve the binding efficiency compared to 5-FU.Furthermore, the Protein-Ligand Interaction Fingerprint (PLIF) results demonstrate consistent contacts with key active site residues responsible for substrate stabilization and catalytic activity. The presence of multiple hydrogen bonds and hydrophobic interactions suggests that metformin-compound 4 can effectively mimic the binding behavior of 5-FU while possibly exhibiting altered pharmacokinetic or toxicity profiles.

Collectively, these results indicate that metformin-compound 4 may serve as a promising structural scaffold for developing new anticancer agents with potential advantages in metabolic stability and reduced cytotoxicity. Further in vitro and in vivo studies are required to confirm the compounds biological activity and to compare its inhibitory potency selectively and pharmacological behavior against 5-FU.

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CONCLUSION:

Combination of 5-FU and metformin showed a promising synergistic activity compared to5-FU or metformin single treatment using different cell lines. The design of uracil and metformin in one structure augment the cytotoxic activity to a noticeable manner.

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