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ANTI-CANCER ACTIVITIES OF 6-ARYL -5-CYANO-2-THIOURACIL DERIVATIVES

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ABSTRACT

In this study, the anticancer activities of ten 6-aryl -5-cyano-2-thiouracil derivatives were evaluated using three human cell lines of Breast (**MCF7**), Colon (**HCT116**) and Liver (**HEPG2**) cancers. All the tested compounds are active against three cell lines. Compound **5** was highly selective to inhibit three cell lines in comparison with the antitumor agent 5-Flurouracil as a control.

Keywords: 6-aryl-5-cyano-2-thiouracils, Breast (**MCF7**), Colon (**HCT116**) and Liver (**HEPG2**) cancers.

INTRODUCTION

Pyrimidine derivatives are well known for their pharmacological activities. Various drugs containing pyrimidine nucleus were synthesized and used as anticancer agents like 5-Fluorouracil (5-FU), Tegafur and Thioguanine^[1] (**fig.1**). An interest in pyrimidine derivatives as anticancer agents has led to the preparation and anticancer evaluation of hundreds of such molecules. For example, 2-cyanopyrimidines^[2], hydrazino pyrimidine-5-carbonitriles^[3], 1,3-dialkylated-pyrimidin-2,4-diones^[4] and 4-

anilino-2-(2-pyridyl)pyrimidines were evaluated as a new class of potent anticancer agents^[5]. Some series of N-(2-(trifluoromethyl)pyridine-yl) anthranilic acid derivatives show potent in vitro antiproliferative activity against human tumor cells^[6]. 2-Thiouracils and 6-aryl-2-thiouracils are well known for their antifungal, antibacterial^[7], anticancer^[8] and antiviral activity^[9]. In view of these facts, we aimed to investigate these thiouracil derivatives for their anti-cancer activities. Ten compounds were

subjected to 3 human tumor cell lines and all of these compounds are active against three cell lines.

Materials and Methods

Chemistry

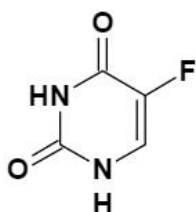
From a series of recently synthesized pyrimidine derivatives ^[10], ten compounds (**1-10, fig. 2**) have been selected as representative examples of the various classes for evaluation for their antitumor activity.

Anti-cancer screening

Experimental

Measurement of Potential Cytotoxicity by (SRB) assay :

The ten thiouracil derivatives (**1-10**) were subjected to a screening system for evaluation of their antitumor activity against 3 cell lines of human cancer, namely Breast (MCF7), Colon (HCT116) and Liver (HEPG2) cancer obtained from pharmacology screening unit of the National Cancer Institute (NCI), Cairo University, Egypt, following the standard procedure (Skehan et al., 1990) ^[11] in comparison to the known anticancer drugs: 5-Fluorouracil



5 - Fluorouracil

Potential cytotoxicity of the selected Thiouracil derivatives was tested using the method as follows ¹¹:

- Cells were plated in 96-multiwell plate (10^4 cells/well) for 24 hr before treatment with the compound (s) to allow attachment of cells to the wall of the plate.
- Different concentrations of the compound under test (0, 1, 2.5, 5, 10 μ g/ml) were added to the cell monolayer. Triplicate wells were prepared for each individual dose.
- Monolayer cells were incubated with the compound(s) for 48 hrs at 37°C and in an atmosphere of 5% CO₂.
- After 48 hr, cells were fixed, washed and stained with Sulfo-Rhodamine-B stain.
- Excess stain was washed with acetic acid and attached stain was recovered with Tris EDTA buffer.
- Color intensity is measured in an ELISA reader.
- The relation between surviving fraction and drug conc. is plotted to get the survival curve of each tumor cell line after the specified compound.

RESULTS

The results are expressed as growth inhibition of 50 % (IC₅₀) which is the concentration of the compound causing 50 % reduction in the net protein increase (as measured by a sulforhodamine B (SRB) protein Staining) in control cells during compound incubation. The results have been given in (Table 1, fig. 3).

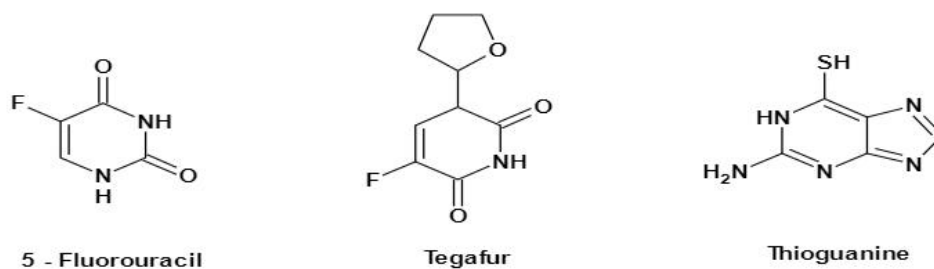
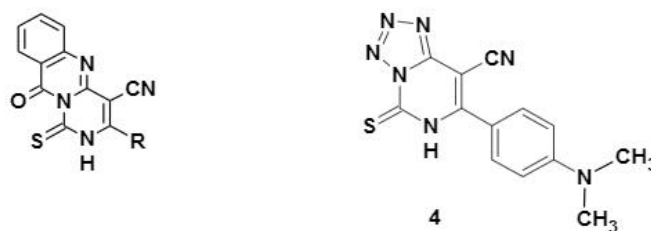
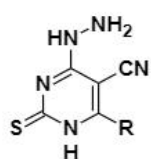


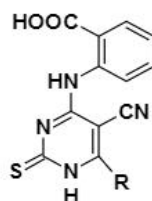
Fig . 1. Pyrimidine derivatives as anticancer agents.



- 1, R = P - F C₆H₄
 2, R = P - N(CH₃)₂ C₆H₄
 3, R = 3,4,5 - (OCH₃)₃ C₆ H₂ .



- 5, R = P - FC₆H₄
 6, R = P - N(CH₃)₂ C₆H₄
 7, R = 3,4,5 - (OCH₃)₃ C₆ H₂ .



- 8, R = P - FC₆H₄
 9, R = P - N(CH₃)₂ C₆H₄
 10, R = 3,4,5 - (OCH₃)₃ C₆ H₂ .

Fig .2.Synthesized compounds 1-10 .

Table I. IC₅₀ values, in µg/ml concentrations, of tested compounds.

Compounds	Cell Lines		
	MCF7	HCT116	HEPG2
1	2.35	1.21	1.41
2	5.17	1.14	0.87
3	4.03	3.56	0.54
4	3.36	0.94	2.35
5	2.35	0.94	0.50
6	3.96	1.68	1.14
7	4.03	4.09	0.47
8	2.95	2.82	0.40
9	2.72	1.01	0.81
10	3.56	3.96	0.60
5-FU	2.6	2.8	-

MCF7: Breast carcinoma cell line. HCT116: colon carcinoma cell line. HEPG2: Liver carcinoma cell line.

5-FU: 5- Fluorouracil.

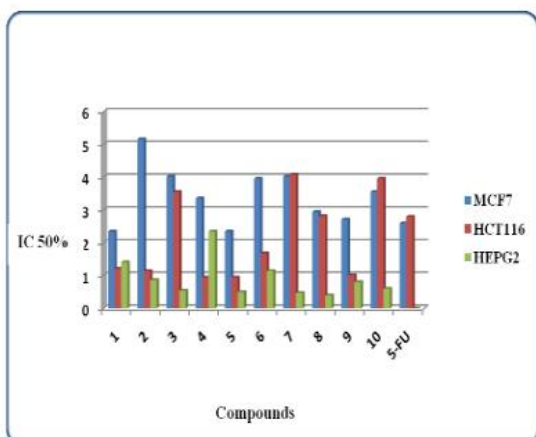


Fig 3. anticancer activity of compounds 1-10.

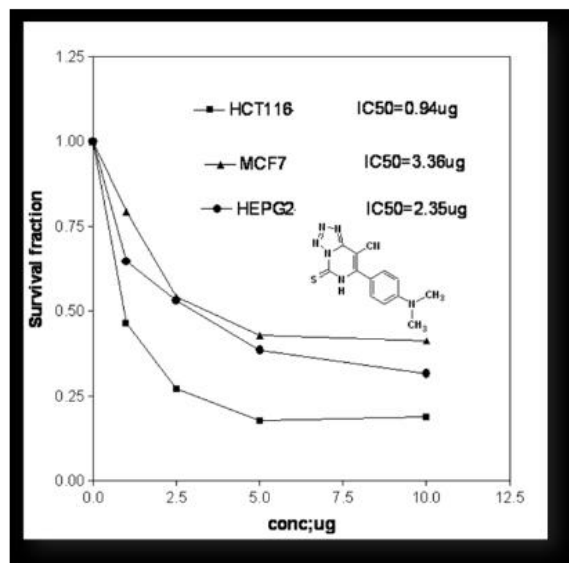


Fig.4: The relation between surviving fraction and drug conc for compound 4 .

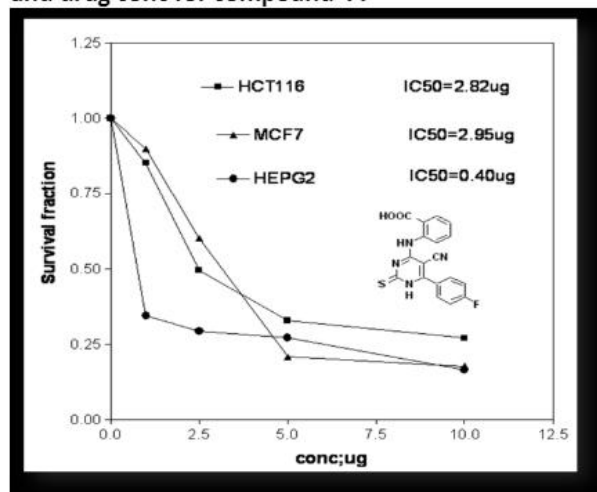


Fig.5: The relation between surviving fraction and drug conc for compound 8 .

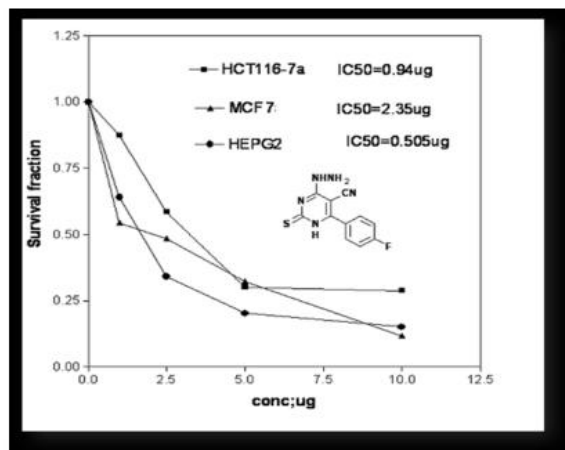


Fig.6: The relation between surviving fraction and drug conc for compound 5 .

DISCUSSION

The antitumor activity results indicated that all the tested compounds are active against three cell lines in comparison to the known anticancer drugs: 5 - Flurouracil.

Table 1 showed that the compounds **2** and **3** (with nitrogen heterocyclic ring in the molecule) showed moderate anticancer activity against MCF7 cell line while **1** and **2** showed high selectivity for HCT116 and HEPG2 cell lines. The tetrazolo pyrimidine **4** showed high anticancer activity against HCT116 cell line than MCF7 and HCT116 cell lines. Among compounds **5**, **6** and **7** (with hydrazine hydrate group in the molecule), compound **5** exhibited high anticancer activity against HCT116, HEPG2 and MCF7 cell lines. Moreover the 4 - amino pyrimidines **8**, **9** and **10** (with anthranilic acid moiety) showed high selectivity for HEPG2 cell line.

Structural-activity relationship (SAR).

From the above obtained results (table 1), we can conclude that the anticancer activity is due to:

- (i) The presence of nitrogen heterocyclic rings.
- (ii) The presence of nitrile generally enhancing the activity as well as hydrazine hydrate moiety.
- (iii) The presence of 2-thiouracil moiety is essential for activity.

Conclusions

The most active compounds being **1** and **5** against Breast cancer MCF7 cell line; **4,5**, **9** and **2** against colon cancer HCT116 cell line (fig.4) and **8,7**, **5** and **3** against liver cancer HEPG2 cell line (fig.5). Compound **5** found to be the prominent cytotoxic and selective ones toward liver cancer HEPG2 cell line, colon cancer HCT116 cell line and Breast cancer MCF7 cell line in comparison with the anti tumor agent 5-Flurouracil as a control (fig.6).

Acknowledgements

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