

EVALUATION OF ANTICANCER ACTIVITY OF SOME THIOURACIL DERIVATIVES

Mosaad Sayed Mohamed¹, Mahmoud Moustafa Youns² and Naglaa Mohamed Ahmed^{*1}

^{1,1*} Pharmaceutical Organic Chemistry Department, Faculty of Pharmacy, Helwan University,
 Ein Helwan, Post Code No.11795, Cairo, Egypt

²Biochemistry Department, Faculty of Pharmacy, Helwan University, Ein Helwan, Post Code No.11795, Cairo, Egypt

*Corresponding Author Email: nogamoon2005@yahoo.com

ABSTRACT

Some 2-thiouracils and fused 2-thiouracils were evaluated for their cytotoxicity study. The synthesized compounds were tested for their cytotoxicity effects towards (CaCo-2) cancer cell line using SRB assay. The results showed that compound **1b** displayed promising cytotoxic activity and found to be more potent than standard, doxorubicin.

KEY WORDS

2-thiouracil, fused 2-thiouracil, CaCo-2, SRB assay.

INTRODUCTION

Cancer is the second leading cause of human death [1]. Although there are many therapeutic strategies, including chemotherapy and radiotherapy, high systemic toxicity and drug resistance are the main problems in the treatment of cancer. Therefore, scientists are focused on finding new therapeutic targets for cancer and discovering novel antineoplastic drugs [2]. Pyrimidine ring is the building unit of DNA and RNA which explains the fact that pyrimidine derivatives exhibit diverse pharmacological activities. Indeed, many pyrimidine-bearing skeletons showed potent anticancer activity. For example, 2-cyanopyrimidines [3], hydrazine pyrimidine - 5 - carbonitriles [4], 1, 3-dialkylated-

pyrimidine-2, 4- diones[5], aniline- 2 - (2-pyridyl) pyrimidines, 2-hdrazinyl- 4 -morpholinotheino [3,2-d] pyrimidine [6] and N-trisubstituted (at C2,C4 and C6) pyrimidines [7]. Different drugs such as purvalanol B, olomoucine, and its analogue roscovitine that contain pyrimidine ring are potent CDK2 inhibitors [8-10]. Additionally; 2-thiouracils are potential therapeutics as antiviral, anticancer, antibacterial and antifungal agents [11-17]. In particular, 6-n-propyl-2-thiouracil (6-PTU, 1) is antithyroid drug [18] where its S-alkylation (2) and N3-alkylation (3) products (Fig.1) have been recently reported as novel antibacterial and cytotoxic agents [19, 20].

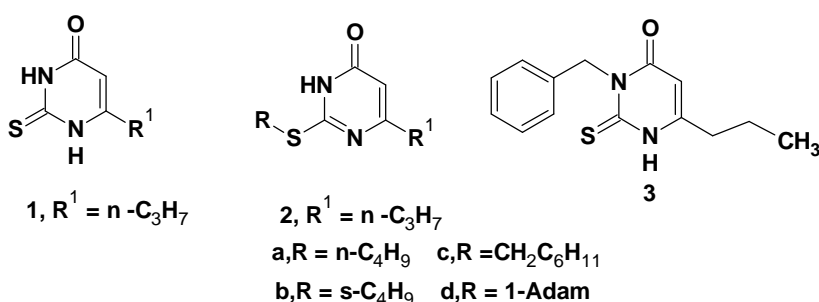


Fig.1. Chemical structures of 2-thiouracil derivatives

A series of trifluoromethylated hexahydro-2-thiopyrimidines have been recently reported as novel cytotoxic agents against colon cancer cell line

(COLO320HSR)^[21]. From literature survey, it was found that 5-cyano-2-thiouracil derivatives and their condensed heterocycles exerted promising anticancer

activity against most human cancers e.g. Breast (MCF-7), colon (HCT-116), liver (HEPG-2), leukemia (MOLT-4), cervical cancer (HELa) and renal cancer^[13,22-27].

In continued quest of new anticancer agents we herein report the anticancer activity of certain thiouracil derivatives.

MATERIALS AND METHODS

Chemistry

From a series of recently synthesized thiouracil derivatives [27], ten compounds (1a, b-5a, b, Fig. 2) have been selected as representative examples of the various classes for evaluation for their antitumor activity.

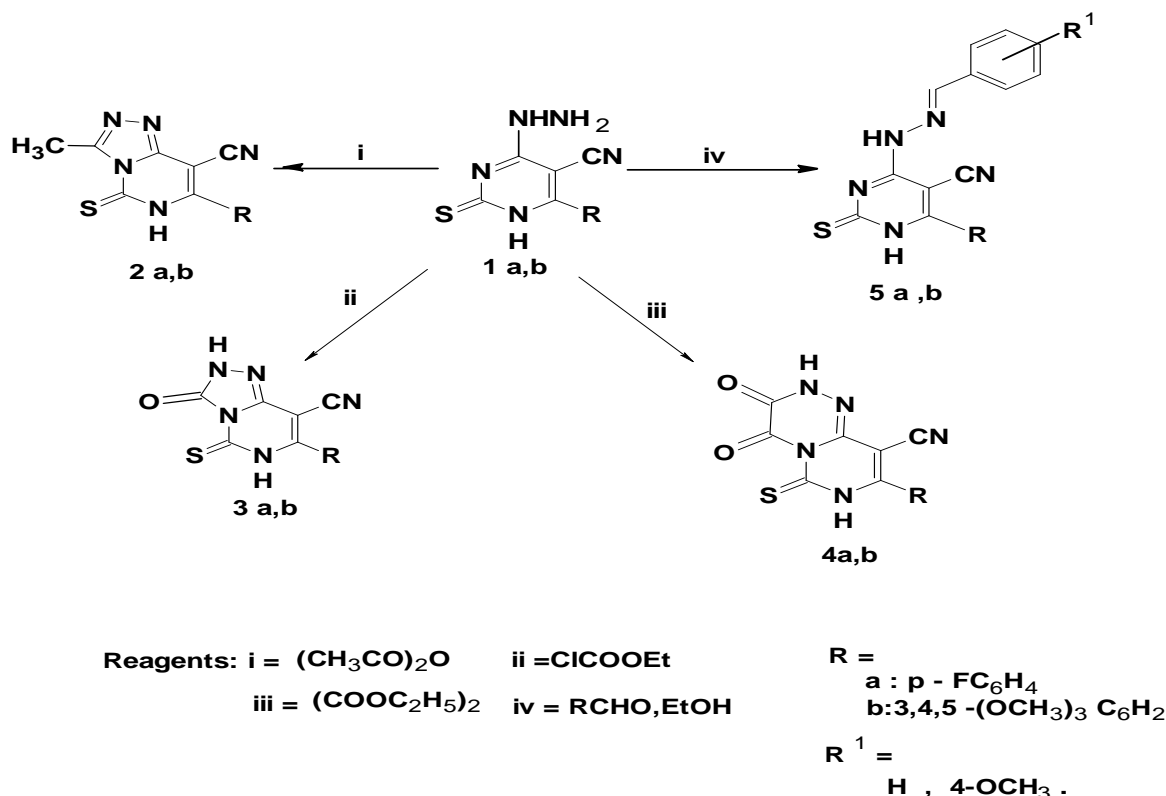


Fig.2. Synthetic pathway for the preparation of compounds 1-5 (a, b).

In vitro cytotoxicity activity

Thiouracils were subjected to a screening system for evaluation of their anticancer activity against cell line of human cancer, namely colon (CaCo-2)cancer obtained from pharmacology screening unit of the National Cancer Institute (NCI), Cairo University, Egypt, following the Sulfo Rhod-amine-B-stain (SRB) assay method^[28] in comparison to the known anticancer drugs: Doxorubicin. The SRB assay, which was developed in 1990, is one of the most widely used methods where it relies on the ability of SRB to bind to protein components of the cells that have been fixed to tissue-culture plates by trichloroacetic acid (TCA). As the binding of SRB is stoichiometric, the amount of dye extracted from stained cells is directly proportional to the cell mass.

Materials, methods, and reagents

Fetal calf serum (FCS) was from Invitrogen Co. (Carlsbad,CA). DMEM medium was from Cambrex (New Jersey,USA). DMSO, doxorubicin, penicillin, streptomycin, and sulforhodamine B (SRB) were from Sigma Chemical Co. (St. Louis, USA). Samples: Stock solutions of compounds were prepared in DMSO and kept at 20 °C. Appropriate dilutions of the compounds were freshly prepared just prior to the assays. Final concentrations of DMSO did not interfere with the cell growth.

SRB cytotoxic assay

The cultured colon carcinoma cell CaCo-2 from the National Cancer Institute (NCI, Cairo, Egypt) is routinely maintained in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10 % fetal calf

serum (FCS), antibiotics (100 U/mL penicillin, 100 µg/mL streptomycin). Cells were plated in 96-multiwell plate (10^4 cells/well) for 24 h before treatment with the compounds to allow attachment of cells to the wall of the plate. Test compounds were dissolved in DMSO and diluted with saline to the appropriate volume. Different concentrations of the compound under test (0, 1, 2.5, 5, 10 µg/mL) were added to the cell monolayer. Triplicate wells were prepared for each individual dose. Monolayer cells were incubated with the compounds for 48 h at 37°C

and in an atmosphere of 5 % CO₂. After 48 h, 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 at a wavelength of 570 nm. Results are expressed as means of at least three independent experiments performed in duplicate. The results are expressed as growth inhibition of 50 % (IC₅₀) of cells (**Table 1**).

Table 1. IC₅₀ values^a (in µg/mL) for cytotoxic activity of the compounds against CaCo-2 cell by SRB assay

Compounds	IC ₅₀ (µg/mL)
1a	44.47±7.05
1b	10.42 ± 0.65
2a	16.73± 2.12
2b	48.3 ± 5.33
3a	15.16 ± 0.64
3b	29.05±1.911
4a	19.27±1.65
4b	13.104±1.095
5a	16.232±1.53
5b	34.18±3.39
Doxorubicin	12 ± 0. 043

IC₅₀ values a (in µg/mL), which the concentration required for a 50 % of cell growth inhibition. Results are presented as a mean ± SEM of three independent experiments performed in duplicate.

RESULTS AND DISCUSSION

Cytotoxic activity

Many thiouracil derivatives have been synthesized to evaluate their antitumor activities as trial to get more effective and less toxic agents. The antitumor activity results indicated that all the tested compounds are active against CaCo-2 cell line (**Table 1**). Compound **1a** (with hydrazine hydrate group in molecule) showed moderate cytotoxic activity against CaCo-2 cell line (IC₅₀= 44.47µg/mL), compound **1b** (with three

methoxy groups on phenyl ring) exhibited high anticancer activity (IC₅₀=10.42 µg/mL). Compound **2a** (with triazole moiety) and **3a** (with 3-methyl triazole moiety) showed significant cytotoxic activity (IC₅₀ = 16.73 and 15.16 µg/mL, respectively), while compounds **2b** and **3b** showed moderate cytotoxic activity (IC₅₀ = 48.3 and 29.05 µg/mL, respectively). Compounds **4a** and **b** (with triazine moiety) showed significant cytotoxic activity (IC₅₀ = 19.27 and 13.10 µg/mL, respectively) against CaCo-2 cell line. Compound **5a** (with phenyl hydrazono moiety) showed significant activity (IC₅₀ = 16.232 µg/mL) and compounds **5b** showed moderate activity (IC₅₀ = 34.18 µg/mL) (**Fig.3**).

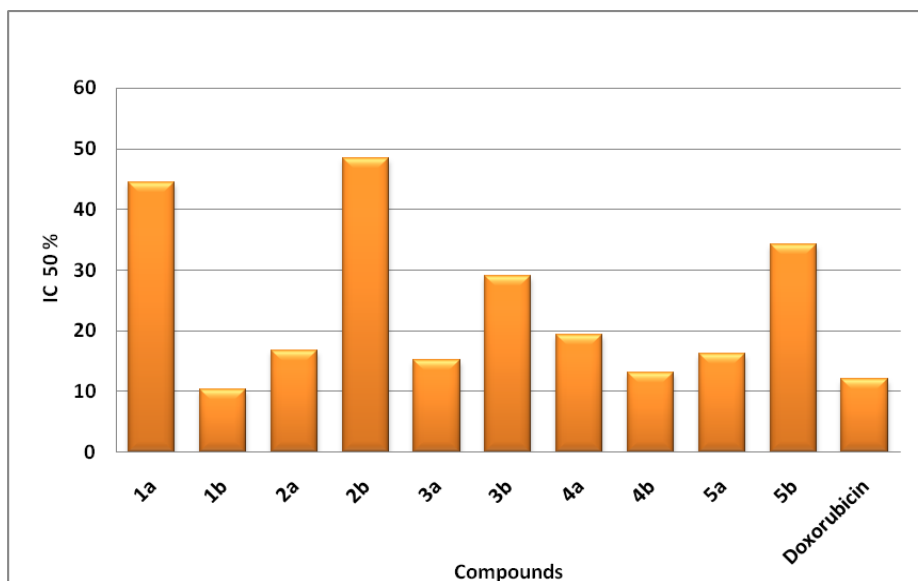


Fig. 3 .Screening of anticancer activity by the SRB assay shows that 1bhas highest activity (more potent than the reference drug (RF). Each value represents a mean \pm SEM (n = 3).

CONCLUSION

The most active compound being 1b and found to be the prominent cytotoxic toward colon cancer CaCo-2 cell line in comparison with the anti tumor agent Doxorubicin as a control. In conclusion, the preliminary biological studies lead to the identification of novel cytotoxic agents. The findings demonstrate thiouracils and fused thiouracils as novel leads for further development as medicinal agents.

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***Corresponding Author:**

Naglaa Mohamed Ahmed*

Email: nogamoon2005@yahoo.com