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SYNTHESIS OF A NEW DERIVATIVE OF 5-FLUOROURACIL BASED ON 2-CHLORO-N-(4-IODOPHENYL)ACETAMIDE AND STUDY OF ITS BIOLOGICAL ACTIVITY AGAINST CANCER CELLS

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Abstract

In this work, a new compound was synthesized by the reaction of 2-chloro-N-(4-iodophenyl) acetamide with 5-fluorouracil. The process was carried out in two stages, first, the chloroacetylation reaction of 4-iodoaniline was carried out and in the second stage, the new product was synthesized based on the reaction of the obtained compound with 5-fluorouracil. The structure of the product was confirmed by ^{1}H,^{13}C NMR, IR and Mass spectrometry. The liquefaction temperature, reaction yield, solubility of the obtained product were determined and the biological activity was studied in 3 types of cancer cells: Hela (cervical cancer cell), HT-29 (colon cancer cell) and MCF-7 (breast cancer cell).

Keywords: *5-Fluorouracil (5-Fu), chloroacetyl chloride, 4-iodoaniline, 2-chloro-N-(4-iodophenyl) acetamide*

Introduction

Today, the synthesis of new drugs for the treatment of cancer is of great importance in the pharmaceutical fields. Although many anticancer drugs have been developed and are used in the treatment of cancer, most of them have adverse effects. 5-Fluorouracil and its derivatives have been used for many years in the treatment of tumor diseases: gastrointestinal, colon, head, neck and breast cancer. 5-Fluorouracil is a strong anticancer agent, but it also has many negative side effects. Patients who used 5-Fluorouracil experienced adverse effects such as inflammation of the mouth and intestines, hair loss, and central nervous system damage. However, many derivatives of 5-fluorouracil have been shown to have much better biological activity than 5-fluorouracil.

5-Fluorouracil (5-Fu) was first synthesized in 1957 and is one of the anticancer agents routinely used to treat tumors such as colon, stomach, and breast cancer (Cunning-

ham D., James R. D., 2001). Due to the lack of tumor selectivity of 5-Fu, its therapeutic use causes serious damage to the bone marrow, gastrointestinal tract, and central nervous system. To overcome these problems, many modifications of the structure of 5-Fu have been made, and a number of drugs have been synthesized with its amino acids, peptides, phospholipids, and polymers (Zhang F. M. et al., 2006). 5-Fu is a cytotoxic agent that inhibits rapidly proliferating cancer cells in conventional chemotherapy.Researchers have tried various methods to reduce the toxicity of 5-Fu, including strategies to modify its chemical structure and to deliver the drug to a specific site.

Several low molecular weight derivatives of 5-fluorouracil have been developed, including 5-fluoro-2'-deoxyuridine, 1-(2-tetrahydrofuryl)-5-Fu, and 3,5-dioctanoyl 5-fluoro-2-deoxyuridine (Malet-Martino M., Martino R. 2002; Wang J. X., Sun X., Zhang Z. R., 2002). Many compounds of 5-Fu containing a nitro group in the aromatic ring have been obtained, and their cytotoxicity and radiosensitizing activity have been studied.

The coupling of 2,4-dinitrophenylamine with 5-fluorouracil through three carbon atoms resulted in the formation of 3[3-(2,4-dinitro-phenylamino)-propyl]-5-fluoro-1-H-pyrimidine-2,4-dione, which has radiosensitizing properties, but not cytotoxicity, under aerobic conditions (Khalaj A. et al., 2006). In addition, 2,4-dinitrobenzoyl, 2,4- and 3,5-dinitrobenzoyl, 2,4-dinitrophenylacetyl derivatives of 5-Fu were reacted with di-T-butoxycarbonate $(Boc)_2O$, 4-Dimethylaminopyridine (4-DMAP), K_2CO_3, KI salts and DMFA, acetonitrile solvents at 78 °C for 4 hours. The structure of the obtained products was confirmed by IR, NMR and Mass spectrometry methods[6]. New sulfonyl derivatives of 5-Fu (5-fluoro-1 (arylsulfonyl) pyrimidine-2,4 (1H, 3H)-diones) were synthesized by the reaction of 5-fluorouracil with sulfonyl chloride (Yan X. et al., 2009).

Polymeric conjugates of 5-Fu play an important role in the pharmaceutical industry. In particular, the conjugate of polyethylene glycol with 5-fluorouracil, which is a smooth, spatially uniform 44.3 ± 5.8 nm nanoparticles with the participation of recombinant human serum albumin, has advanced the pharmaceutical and therapeutic fields (Sharma A., Kaur A., Jain U. K., Chandra R., Madan J., 2017). Scientists have synthesized a new derivative of 5-Fu based on pectin (PT), a high-molecular polysaccharide. In the process of obtaining this derivative, the monochloroacetic acid derivative of 5-Fu was first synthesized, then a solution of pectin in DMSO was added to the resulting product and stirred at 50° C for 96 hours, then ethanol was added to the reaction mixture and filtered.

The obtained 5-Fu-PT derivative was dried at 60 ° C for 24 hours and then subjected to HPLC analysis, and the analysis showed that 5-Fu-PT did not contain free intermediate 5-Fu-acetic acid (Wang Q. W., Liu X. Y., Liu L., Feng J., Li Y. H., Guo Z. J. and Mei Q. B. 2007; Liu L. et al., 2008). Nowadays, obtaining metal complexes and developing drugs based on them is one of the urgent tasks of the chemistry field. Therefore, a new ruthenium-based 5-Fu complex [Ru(5-FU)(PPh3)2(bipyr)]PF6, which has a high cytotoxic effect on cancer cells, was synthesized in analytical purity and high yield. Its apoptosis induction effect in human colon carcinoma HCT116 cells was evaluated (Silva V. R. et al., 2018).

In recent years, Uzbek scientists have synthesized the paraaminoazobenzene derivative of 5-Fu in high yield. In the reaction process, an intermediate product based on chloroacetyl chloride of paraaminobenzene was initially obtained, and 5-Fu was reacted with it in DMFA solvent to obtain the target product. The biological activity of the product was studied in cancer cells (Ochilov Sh. E. U. et al., 2024). Based on the above data, we also synthesized a new 4-iodoaniline-based compound of 5-Fu to reduce the negative effects of 5-Fu, improve its properties such as selective action, high biological activity and easy solubility in water. Its structure was confirmed using Mass,^{1}H,^{13}C NMR and IR spectroscopy. The biological activity of the resulting product was studied in 3 types of cancer cells: Hela (cervical cancer cell), HT-29 (colon cancer cell) and MCF-7 (breast cancer cell).

Experimental part

The M-560 device was used to measure the liquidus temperature of the obtained product.^{1}H and^{13}C NMR spectra were obtained in DMSO solvent on VARIAN MR

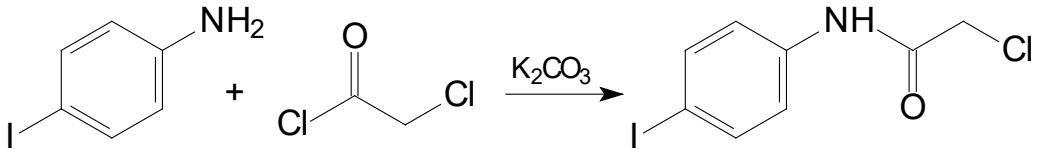
400 MHz spectrometers. Mass analysis was studied using high-resolution mass spectrometry (HRMS) AB SCIEX QSTAR Elite. IR spectra (KBr pellet) were studied on Perkin Elmer FT-IR/NIR Spectrometr Spectrum 3, 4000–400 cm^{-1}.

Results and discussions

Synthesis method

The synthesis of a new derivative of 5-fluorouracil based on 4-iodoaniline was carried out in two stages. First, 0.01 mol (2.955 g) of 4-iodoaniline was placed in a 100 ml round-bottomed flask and dissolved in 50 ml of acetonitrile. 0.01 mol (1.38 g) of K_2CO_3 was weighed and added to the mixture on an analytical balance. The solution was stirred with a magnetic stirrer at a temperature of –1–3° C for 30 min in a cold condition, and 0.01 mol (0.8 ml) of chloroacyl chloride was added dropwise. Then, the reaction was carried out for 2 h, and then the mixture was checked by TLC (hexane: acetone, 2–3). The intermediate 2-chloro-N-(4-iodophenyl)acetamide was obtained in 80% yield.

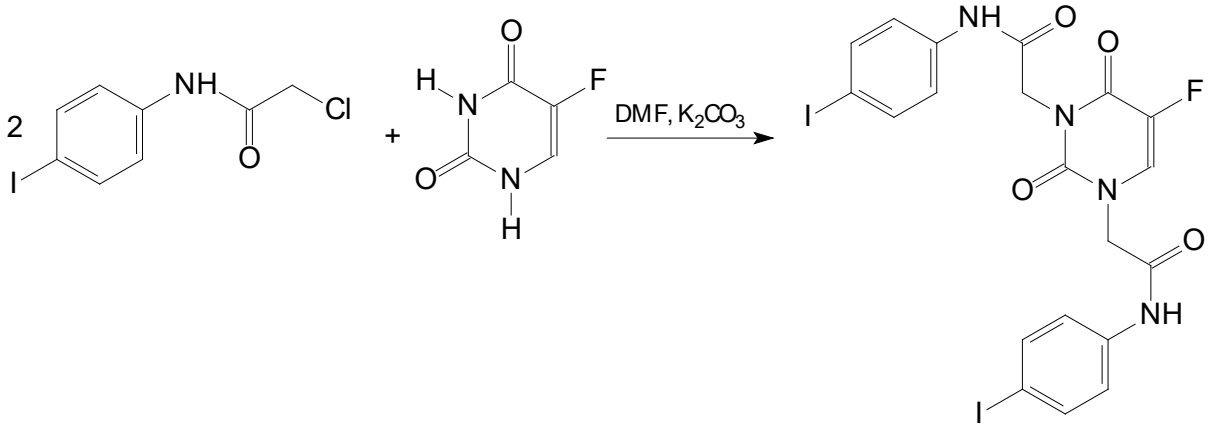
Scheme 1. *The first stage of the reaction*



Name: 2-chloro-N-(4-iodophenyl)acetamide

In the second step of the reaction, 0.625 mmol (0.081 g) of 5-fluorouracil was dissolved in 10 ml of DMFA at room temperature and K_2CO_3 (1.25 mmol, 0.1725 g) was added. Then, 1.25 mmol (0.37 g) of 2-chloro-N-(4-iodophenyl)acetamide was added under stirring and the reaction was carried out for 5 h at room temperature. The reaction mixture was checked by TLC (hexane: acetone, 2: 3) and cleaned.

Scheme 2. *Synthesis of the target compound*



Product name: 2,2'-(5-fluoro-2,4-dioxopyrimidine-1,3(2H,4H)-diyl) bis(N-(4-iodophenyl)acetamide)

Yield: 74%; m.p.: 275–277 °C. R_f = = 0.71.^{1}H NMR (400 MHz, DMSO-D6) δ 8.28 (s, 1H), 8.26 (s, 1H), 7.65 (dd, J = 8.9, 6.5 Hz, 3H), 7.41 (dd, J = 11.2, 8.9 Hz, 3H), 4.64 (s, 3H), 4.60 (s, 3H), 3.33 (s, 1H).^{13}C NMR ((101 MHz, DMSO-D_6) δ 165.22, 164.77, 156.67, 156.41, 149.70, 138.48, 138.36, 137.51, 137.45, 137.15, 130.56, 130.22, 121.87, 121.27, 121.22, 109.57, 87.16, 86.94, 51.11, 43.95. HR-ESI–MS: m/z 648.92 $[M - H]^+$ calcd for $C_{20}H_{15}FN_4O_4I_2$. IR (KBr pellet): ν=3281.51 (NH, 1736.15 (–C=O, amid), 1645.13, 1596.95 (–C=O, 5-Fu), 1382.97 (–CH_2-C=O), 1197.72 (C-F) $см^{-1}$.

Figure 1. *IR spectrum of 2,2'-(5-fluoro-2,4-dioxopyrimidine-1,3(2H,4H)-diyl) bis(N-(4-iodophenyl)acetamide)*

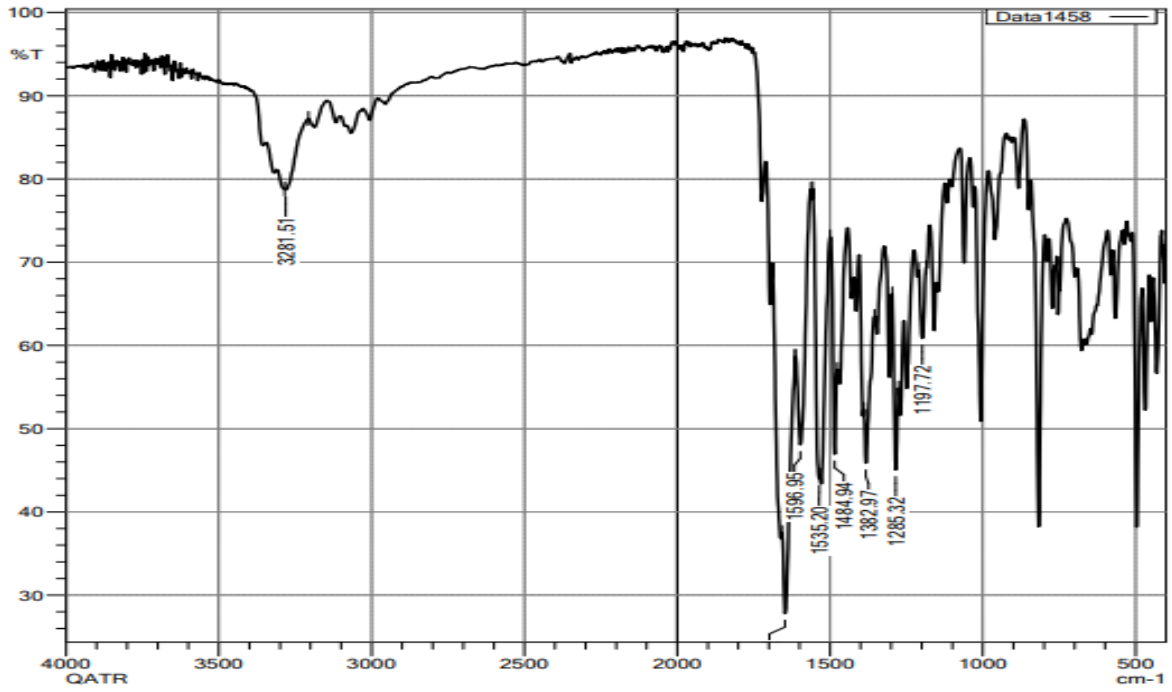
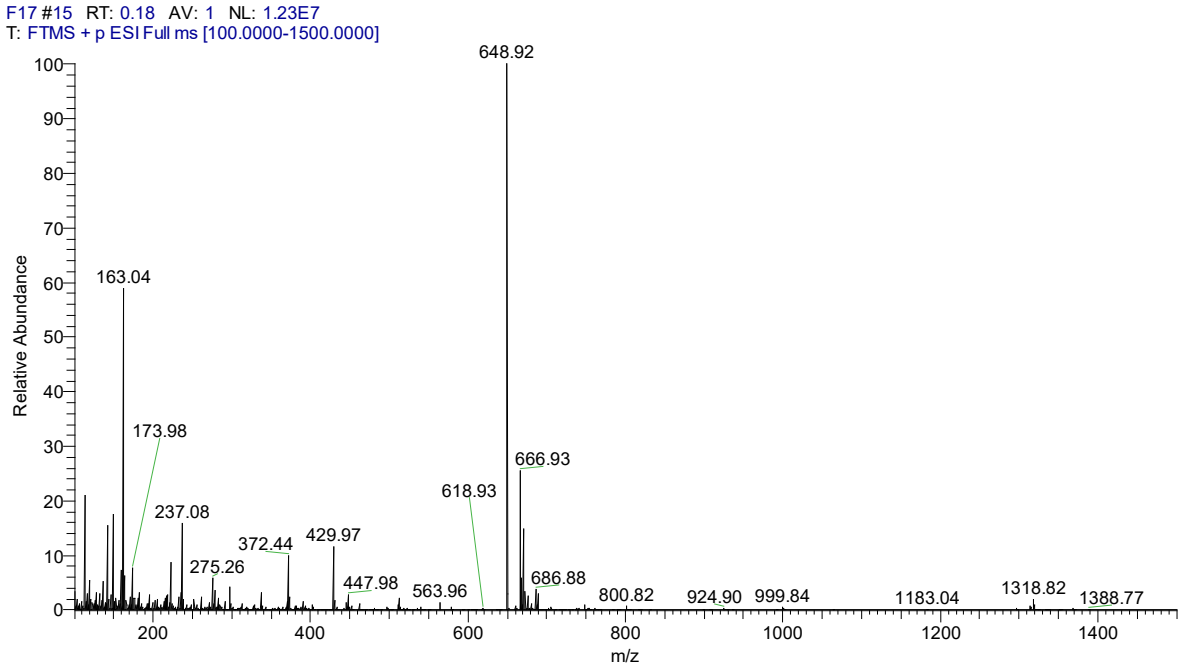


Figure 2. *Mass spectrum of 2,2'-(5-fluoro-2,4-dioxopyrimidine-1,3(2H,4H)-diyl) bis(N-(4-iodophenyl)acetamide)*



Biological activity

The biological activity of 2,2'-(5-fluoro-2,4-dioxopyrimidine-1,3(2H,4H)-diyl)bis(N-(4-iodophenyl)acetamide) was studied in 3 different types of cancer cells (50 mmol/l). The product showed an inhibition rate of 87.63% (5-Fu=27.08%) for Hela (cervical cancer cell), 82.90% (5-Fu = = 40.75%) for HT-29 (colon cancer cell) and 64.20% (5-Fu = 41.49%) for MCF-7 (breast cancer cell). The IC_{50} values of the product were Hela=10.73±0.54 μM and HT-29 = = 5.19 ± 0.26 μM, respectively.

Conclusion

2,2'-(5-Fluoro-2,4-dioxopyrimidine-1,3(2H,4H)-diyl)bis(N-(4-iodophenyl) acetamide) was synthesized for the first time and its chemical structure was confirmed using IR,^{1}H,^{13}C NMR and Mass spectrometry methods. When the biological activity of the obtained compound was studied in terms of its inhibitory properties against Hela (cervical cancer cell), HT-29 (colon cancer cell) and MCF-7 (breast cancer cell), it was observed that the inhibitory activity of the obtained substance on Hela cell was 3-fold stronger than 5-Fu, 2- fold more active than 5-Fu against HT-29 and 1.5- fold more active than 5-Fu against MCF-7 cells.

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