RESEARCH
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SONOCRYSTALLIZATION OF A NOVEL SYNTHESIZED ANTICANCER DERIVATIVE OF 2-THIOURACIL-6-SULPHONAMIDE TO ENHANCE ITS WATER SOLUBILITY.

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Abstract
A potent and promising anticancer agent, 2-thiouracil-5-(2,4,6-trimethylphenyl) sulphonamide 3, was synthesized by reacting 2-thiouracil-5-sulphonyl chloride 2 with 2,4,6-trimethylphenyl amine in the existence of pyridine as an acid scavenger. Its poor water solubility, however, limits the in vivo screening, which led us to employ a non-conventional and secure ultra-sonic-assisted process to solubilize it to improve its water solubility and bioavailability for use in future studies. Target compound 3 melt was added to deionized water that was kept at 60 degrees Celsius and simultaneously exposed to ultrasonic energy in a process known as melt sonocrystallization. After the distributed droplets solidified, the resulting agglomerates were filtered out, allowed to dry at room temperature, and then tested for saturated solubility. The target molecule was tested as an anticancer agent against human A-2780 ovarian, HT-29 colon, MCF-7 breast, and HEPG-2 liver carcinoma cells both before and after sonocrystallization. In comparison to 5-Fluorouracil, which was employed as a benchmark, it demonstrated an encouraging action.
Keywords: 2-Thiouracil; 2-Thiouracil-5-sulphonamide; Aqueous Solubility; 5-Fluorouracil; anticancer agent; sonocrystallization.

INTRODUCTION

2. Thiouracil is a significant bioactive chemical that has drawn the interest of numerous researchers, particularly those in the field of pharmaceutical chemistry. The compound exhibits multiple electrophilic substitution processes at the fifth position. A frequently employed reaction is the chlorosulphonation reaction, which involves refluxing chlorosulphonic acid at 120 Celsius degree to produce 2-thiouracil-5-sulphonyl chloride derivative [1]. This derivative is utilized in current studies to prepare 2-thiouracil-5-sulphonamides through condensation with multiple aromatic amines while pyridine is present as an acid scavenger. Promising properties included antibacterial, antifungal [2], antiviral [3], anticancer [4-6], antihypertensive [7], and thrombolytic [8] properties in a few of these compounds. Most of these compounds underwent in vitro screening; however, because of their low water solubility and thus low bioavailability, in vivo screening proved challenging.

To address these issues, numerous attempts have been made. Recently, these sulphonamides' inclusion complexation with cyclodextrins improved their aqueous solubility. In order to address issues with aqueous solubility and bioavailability, sonocrystallization—a prominent technique in the field of solubility enhancement—involves using ultrasonic energy to create fine drug particles [9].

It provides a more secure approach of enhancing solubility by dissolving drugs without the need for a solvent or carrier. The crystal characteristics of the solution to be crystallized can be altered by subjecting it to fluctuating sound pressure within the frequency range of 15 KHz to 10 MHz. Additionally, smaller crystals with smaller sizes will be created because ultrasonic irradiation shortens the induction time of crystals and boosts the nucleation rate. Furthermore, collisions brought on by this irradiation may cause existing crystals to fragment. The novel crystals have a rapid rate of dissolution and are water soluble, which may enhance the pharmacokinetics and pharmacodynamics of bioactive new medications [10–13].

2. Material and Methods:

The Electrothermal IA 9100 apparatus (Shimadzu, Japan) was used to measure all melting points. The Perkin-Elmer 1650 spectrophotometer (USA) was used to record IR spectra. The Varian Mercury 300 MHz NMR Spectrometer (Varian, UK) was used to record 1HNMR spectra (300 MHz) in dimethyl sulfoxide (DMSO). Tetramethyl silane (TMS) was used as an internal benchmark for 1HNMR spectra (300 MHz), and the chemical shifts (δ) were expressed as ppm against TMS. The Vario, Elementar apparatus (Shimadzu) was employed to capture mass spectra. The Vario, Elementar apparatus (Shimadzu) was used to operate microanalyses. Using a mobile phase of chloroform-methanol (3:1), TLC was used to track the progression of all operations using silica gel 60 for TLC (Merck). Patterns were seen by iodine vapors or by exposure to ultraviolet (UV) rays (254 nm).

3. Experimental:

4-Oxo-2-thioxo-1,2,3,4-tetrahydropyrimidine-5-sulphonyl chloride: 2

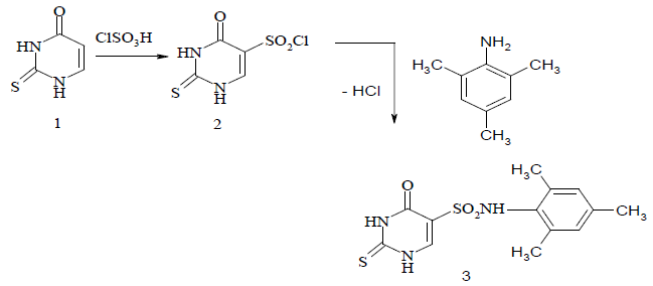
It was prepared as in literature by Fathalla 2002[1].

4-Oxo-2-thioxo-1,2,3,4-tetrahydropyrimidine-5-sulphonic acid-N-(2,4,6-trimethylphenyl) amide derivative 3, or N-Mesityl-4-oxo-2-thioxo-1,2,3,4-tetrahydropyrimidine-5-sulphonamide.

After refluxing for eight hours in 100% ethanol (50 ml), compound 2 (1.13 mole), 2,4,6-trimethyl phenyl amine (1.13 mole), and pyridine (0.016 mole) were combined. The combination was then cooled, filtered, and re-crystallized from DMF/water.

Yield: 74%: m.p: 193-195 ºC: IR (KBr cm^{-1}): 3358 (NH), 3163 (CH, aromatic), 2959 (CH, aliphatic), 1680 (C= O), 1228 (C=S),1116,1346 (SO_2). 1HNMR (DMSO-d_6), δ: 2.0 (3H,s,1CH_3), 2.2 (6H,s,2CH_3), 7.9,8.0 (3H,aromatic), benzene), 8.1 (s,1H,pyrimidine), 10.1,10.7,11.7, (s,3NH,D_2O exchangeable,^{13}CNMR:δ17.7 (2C,s,2CH_3),25.7(1C,s,CH_3), 121.1(1C,s,pyrimidine), 128.1,128.2,159.3(6C,s,benzene),165.1(1C,s,C = O), 177.3(1C,s,C= S), MS: m/z (%), 325.41 (M+,12.3%), Anal. Calcd, for $C_{13}H_{15}N_3O_3S_2$: C, 12.91; H, 4.65; N, 12.91. Found: C,12.81; H, 4.73; N,12.95.

Scheme 1



Sonocrystallization procedure:

In a container kept at 195°C with a paraffin oil bath, compound 3 was melted. The molten sample was placed into a vessel holding 50 milliliters of deionized water, which was kept at 60 degrees Celsius using a thermostatic water bath. The sample was then sonicated for 15 minutes at an amplitude of 80% and a cycle rate of 0.8 per second utilizing a probe ultrasonicator (IKasonic U 200 s control). Filtration was used to separate and dry the product that was left over after the distributed droplets solidified at room temperature. A saturated solubility research was conducted using the proportion of MSC compound 3 agglomerates.

Solubility determination:

Saturated solubility measurement was performed to determine the product's solubility following sonocrystallization. Ten milliliters of distilled water kept at 37 degrees Celsius were mixed with an excess of MSC sample, and the mixture was agitated for over 34 hours. After that, the mixture was centrifuged for 10 minutes at 7000 rpm. After being properly diluted, utilizing a UV spectrophotometer, the supernatant's wavelength was determined at 247 nm.

The saturated Compound 3 solubility was recorded at 12.35 µg/ml, while that of MSC-Compound 3 reached 18.27 µg/ml. The enhanced solubility is attributed to the reduced size of the individual crystals comprising the agglomerates.

4.Biology:

Cytotoxic Activity:

Cells:

Using human A-2780 ovarian, HT-29 colon, MCF-7 breast, and HEPG -2 liver cancer cells, we conducted early investigations. The technique used is comparable to what P. Skehan reported. In T-75, which had 50 MI of RPMI-1640 medium enriched with bicarbonate, glutamine, and five percent fetal calf serum, stock cultures were cultivated. The medium was switched every 48 hours. 3 Mm 1,2-cyclohexanediaminetetracetic acid and 0.25% trypsin were used to dissociate the cells in NKT buffer (137 Mm NaCl, 5.4 Mm KCl, and 10 Mm tris; pH 7.4). Microtiter plates (Costar and Cambridge, Ma) were used to plate experimental cultures, with 0.2 mL growth media in each well and densities ranging from 1,000 to 200 000 cells/well.

Dyes:

We bought them from Sigma Chemical Co. Initial investigations were carried out using various dyes to see whether they cells were stained more strongly at pH values that were basic, neutral, or acidic. Sulforhodamine B (SRB), one of these anionic dyes, was removed from cells using unbuffered Tris base and diluted in 1% acetic acid for cell labeling. Using a DU-70 scanning spectrophotometer (Beckman Instruments, Inc., Fullerton, CA), the dye's maximum absorption was ascertained.

Cell Fixation:

Rinsing colonies with buffer prior to fixing to eliminate serum protein frequently resulted in loss and detachment. Before being washed, the cultures were treated with TCA to prevent this possible issue. To fix cells adhered to the plastic substratum, 50µL of cold 50% TCA (4ºC) was meticulously sprinkled over each well's growing media, yielding a final TCA concentration of 10%. After an hour of incubation at 4ºC. After five rounds of tap water cleaning, the cultures were cleared of low molecular weight byproducts, growth media, and TCA. Once they were air drying, the serum plates were stored until they were required again. The growth medium-free wells were used to measure the background optical densities. The suspension of cells was permitted to settle out of the solution. Upon the well bottoms, these cells were submerged in 50 µL of cold, 80% TC.

The growth medium was covered with a thin layer of A (4º C). After five minutes of inactivity, the cultures were fixed for an additional hour in a refrigerator set at 4º C. This procedure allowed single-cell suspensions to stick to the plastic substratum as long as the cells had been in contact with it when the solution of fixative was used. The application of the macromolecular adhesive Cell-Talk (Biopolymers, Famington, CT) as well as cytospinning were equally successful in encouraging cell attachment as this technique. It did not, however, sufficiently adhere to cells that instead of multiplying as single-cell suspensions, accomplished so as suspended clusters. Particularly unsuitable for this fixation technique were small cell lung carcinoma lines.

Procedure:
The sample was prepared by dissolving target chemical 3 in DMSO and another sample in water following its sonocrystallization.
Before being medicated with the study drug, the cells had been placed for 24 hours in 96 multiwell plates, with 104 cells per well. Various concentrations of compound 3 (both before and after sonocrystallization) (0, 2.5, 5, and 10 µg|ml) were individually dosed and injected into the cell monolayer in triplicate wells. Next, the chemical compounds had been incubated with the cells for 48 hours at 37 degrees Celsius in an environment with five percent carbon dioxide (CO2). The cells were left aside, washed, and stained with SRB stain after these 48 hours. Tris-EDTA buffer was used to extract the bound stain after the remaining stain was eliminated with acetic vinegar. Next, an ELISA reader was used to determine the intensity of coloring. The correlation between the dosage amount and the percentage of cells that survive has been plotted to determine the survival curve of every kind of tumor cell after the IC 50 and the particular drug have been determined.

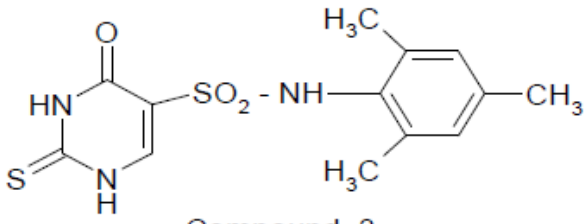
Table 3. In vitro cytotoxic activity (IC_{50}) of the newly prepared compound 3 before and after sonocrystallization and 5-Fluorouracil against a human A-2780 ovarian, HT-29 colon, MCF-7 breast, and HEPG-2 liver carcinoma cells.

Compound 3	A-2780 (µg m/L)	HT-29 (µg m/L)	MCF-7 (µg m/L)	HEPG-2 (µg m/L)
Before Sonocrystallization	0.72	0.84	0.54	-
After Sonocrystallization	0.54	0.64	0.51	-
5-Flurouracil	0.51	0.61	0.67	5.00

The IC50 index was validated by Cairo University's National Institute of Cancer in Cairo, Egypt. for the reference drug 5-Fluorouracil versus HEPG2. (The hepatic cancerous cells were found to be more resistant to 5-FU as a reference medication).

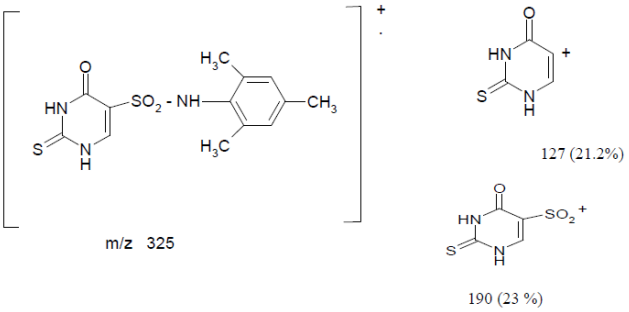
Results:
Compound 3 was inert versus a liver cell line, but it had encouraging efficacy against ovarian, colon, and breast cell lines when compared to 5-fluorouracil. It is observed that the studied compound's sonocrystallization increased its antitumor efficacy.

Discussion:
To address several issues about pharmaceutical studies, this work is an example of the serious and kind collaboration between members of pharmaceutical chemistry and pharmaceutical departments. Preparing our target compound 3, beginning with 2-thiouracil, has been the main focus from the moment we began. The +M-effects of both OH and SH-groups chemically activated 2-thiouracil at position 5 for electrophilic substitution reactions. Chlorosulphonation is a versatile reaction that has recently gained significant attention. The synthesis of our target sulphonamide derivative 3 involved refluxing of 2-thiouracil and chlorosulphonic acid for 8 hours, generating a fair yield of active sulphonyl derivative 2. This was then condensed with 2,4,6-trmethylphenyl amine (mesityl amine) in the presence of the catalytic quantity of pyridine as an acid binder. Elements studies and various spectrum data[14] were used to confirm the structure of compound 3.



Compound 3

Compound 3 should have strong peaks in the IR spectrum at 3358 (NH), 3163 (CH aromatic), 2959 (CH aliphatic), 1680 (C = O), 1228 (C=S), 1116, and 1346 (SO2). A peak at m/z 325 (27%) in the mass spectrum is found to correspond to [M]+. The mesityl cation's base peak (100% R.A.) and the 2-thiouracil cation's peak (m/z 127) were apparent among other peaks as indicated.



Along with aromatic protons at7.0,8.0, and 8.1 as well as peaks at10.1,10.7,11.7 corresponding to three NH and D2O exchangeable protons, the 1HNMR spectra revealed peaks at 2.0,2.2 ppm for three methyl protons. Furthermore, the 13CNMR spectra revealed distinct carbons at 19.4 (2CH3), 25.7 (CH3), 121.1,128.1,128.2,130.0, 159.3 (aromatic carbons) 165.1 (C=O), and 177.3 (C=S) .
Recent research has recognized that by inhibiting the manufacturing of nucleic acids, these kinds of chemicals may have promised anticancer activity as antimetabolites. However, their aqueous solubility issue still has an impact on their bioavailability, which in turn influences how they are formulated in pharmaceuticals and restricts their therapeutic efficacy. A new solvent-free method for producing small particles with high water solubility characteristics is sonocrystallization. Human A-2780 ovarian, HT-29 colon, MCF-7 breast, and HEPG-2 liver cancer cells were used to test the antitumor potential of our selected target medications in vitro. Although the compound's melted mass was sonicated for 15 minutes to disperse it into small particles with high aqueous solubility, it has shown encouraging activity to boost the compound's water solubility[15].

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Conclusion:

In this work, a novel particle engineering methodology called melt sonocrystallization was implemented. It involved using of ultrasonic sound energy directed towards the molten mass of 4-Oxo-2-thioxo-1,2,3,4-tetrahydropyrimidine-5-sulphonic acid-N-(2,4,6-trimethylphenyl) amide derivative to generate fine particles that could potentially enhance the compound's aqueous solubility and bioavailability, making it a viable anticancer agent for future studies using animal models. The target chemical was not potent against HEPG-2 liver carcinoma cells, while it was effective against human A-2780 ovarian, HT-29 colon, and MCF-7 breast cancer cells.

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