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Enhancement of The Solubility of *N*-(3-acetylphenyl)-4-Oxo-2-Thioxo-1,2,3,4-Tetrahydropyrimidine-5-Sulfonamide Through Inclusion Complexation with Cyclodextrins

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ABSTRACT

A new thiouracil-5-sulfonamide derivative (1) was prepared from nucleophilic condensation of 2-thiouracil-5-sulfonyl chloride with m-aminoacetophenone using pyridine as an acid binder. The bad solubility of this sulfonamide is enhanced through its inclusion complexation with cyclodextrins (CDs). The solubility of the sulfonamide in water and in 0.1N HCl was determined at gradient temperatures. The stability constants of the complexes were determined using ultrafilteration and UV-spectrophotometric methods. The results showed that the sulfonamide has low solubility in water (<5µg / ml) and in 0.1NHCl (<7µg / ml). The highest solubility was obtained with HP-ß-CD while the least one was with ß-CD. We arranged the apparent stability constants of the formed complexes as follow, ß-CD > HE-ß-CD > HP-ß-CD and their values were decreased as the temperature increased. The stability constants were attained using ultrafilteration method at room temperature.

Keywords: Thiouracil, 5-sulfonamide cyclodextrin complexes; dissolution; stability constants, ultrafilteration.

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INTRODUCTION

A newly *N*-(3-acetylphenyl)-4-oxo-2-thioxo-1,2,3,4-tetrahydropyrimidine-5-sulfonamide belongs 2-thiouracil-5-sulfonamides series were prepared in 2002 by Fathalla et al[1], then the preparation of a large number of them in the past several years and proved a great diversity in its biological activities as antibacterial, antifungal, [2]antiviral, [3] anti-inflammatory[4] and anticancer agents.[5-7] But a major problem of these compounds is the water solubility. In early 2020, an attempt to enhance the aqueous solubility of them is performed by salt formation as disodium salt.[7] Here, we developed a technique to improve the solubility with a rapid dissolution rate depending on inclusion complexation technique.[8] Cyclodextrins and their derivatives are able to form inclusion complexes with various compounds improving their solubility, stability and bioavailability.[9]They are safe and nontoxic as they are nearly not absorbed from the intestinal tract.[10] Our aim of this research is to discover and illustrate the effect of the type and concentration of different CDs, namely ß-cyclodextrin (ß-CD), hydroxyethyl-ß-CD (HE-ß-CD) and hydroxypropyl-ß-CD(HP-ß-CD) on the solubility of the 2-thiouracil-5-sulfonamide (2-T-5-S).We also determined and studied the stability constants of the complexes using ultrafilteration and UV-spectrophotometric methods.

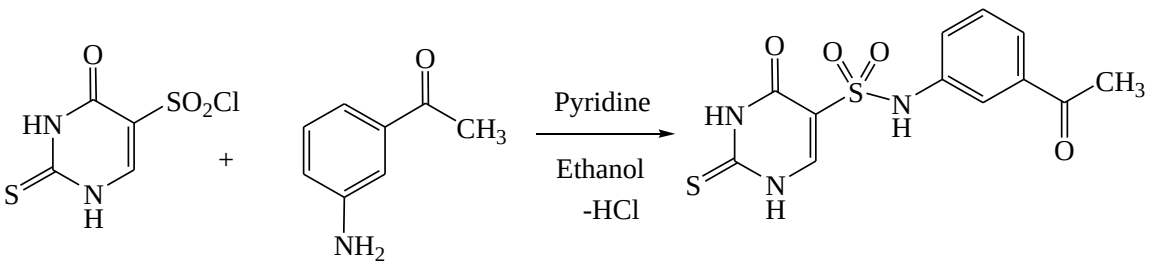
MATERIALS AND METHODS

Melting point was uncorrected and was determined in capillary tube on a Boetius melting point microscope. Microanalyses were performed by the micro analytical unit at Cairo University. IR spectrum was recorded as KBr pellets on a Beckmann infra spectrophotometer PU9712 using KBr disc. ^{1}HNMR spectra were determined on a Joel EX 270 MHz spectrometer using tetramethylsilane as an internal standard. Mass spectra (MS) were recorded on a Finigan SSQ 7000 Mass spectrometer at 70 ev. The reaction was followed and checked by TLC using Chloroform/Methanol (3:1) and spots were examined under a UV-lamp.

Experimental:

Synthesis of *N*-(3-acetylphenyl)-4-oxo-2-thioxo-1,2,3,4-tetrahydropyrimidine-5-sulfonamide:

A mixture of 2-thiouracil-5-sulfonyl chloride (2.0 g,0.01 mol),*m*-aminoacetophenone (1.2 g, 0.01 mol) in(50 ml) absolute ethanol and (0.4 ml) of anhydrous pyridine was refluxed for 8 h. The reaction mixture was cooled and neutralized with 1N HCl, poured on crushed ice, the formed precipitate was filtered off, washed several times with water, dried, and crystallized from DMF/water.
Yield: 75%; m.p.: 256-258°C: IR (KBr cm^{-1}): 3227 (NH), 3168 (CH, aromatic), 2980(CH,aliphatic), 1686 (C=O),1279 (C=S),1130,1320 (SO_2).^{1}HNMR (DMSO-d_6), δ: 2.5(s,3H,CH_3-CO), 7.0-7.2 (m,4H,aromatic-H),8.1(s,1H,pyrimidine-H_6), 9.3,10.0,10.2 (s,3NH`s, D_2O exchangeable), MS: m/z (%), 325.36 (M^+,12.3%), Anal.Calcd. for $C_{12}H_{11}N_3O_4S_2$: C, 44.30; H, 3.41; N, 12.91; Found: C,44.29; H,3.51; N,12.85.



a) Materials:

Cyclodextrins were purchased from sigma Aldrich Co, Germany;Polyvinylpyrrolidine (PVP) with average molecular weight of 40000 was obtained from Winlab, UK. All other chemicals and solvents were of analytical grade.

b) Methods:

I. Solubility studies of 2-T-5-S in water and 0.1N HCl at different temperatures:

Solution of 2-T-5-S (16µg/ml) in 0.1N HCl was prepared with the help of 1ml of dimethylformamide (DMF).Different concentrations (8-30 µg/ml) of 2-T-5-S in 0.1NHCl were prepared and assayed spectrophotometrically at 256 nm using spectro UV-VIS RS single beam spectrophotometer Labomed, Inc. (USA). Solubility determinations in water and in 0.1N HCl at different temperatures were carried out according to the method described byBrewster et al.[11] Excess amounts (10 mg) of 2-T-5-S were added to 10 ml of distilled water or 0.1N HCl separately. The tubes were sonicated for 1 hr using an ultrasonic bath (RaypanR.Espinar.s.l, Spain) , then equilibrium was completed in a water bath at 25 and 37 ^{o}C for distilled water and at 25,37,45 and 50^{O}C for 0.1NHCl for further 24 hrs (Table 1). Aliquots were withdrawn using plastic syringe, filtered through Millipore filter (0.45µm, HA-type) and assayed spectrophotometrically for substance content.

II- Characterization of 2-T-5-S – CDs complexes:

II.A-Ultraviolet spectrophotometric method:

The concentration of 2-T-5-S was kept constant at 2.765 x 10^{-5}M (equivalent to 20 mg/ml and dissolved with the help of DMF) while the ß-CD, HE-CD and HP-ß-CD concentrations were varied from 8.656 x $10^{-4}$to 3.436 x 10^{-3} M. Spectral shift method was used[12]. Spectral shift method was used[12] by allowing the mixturesto stand for 24hrs before recording the UV absorbance spectra with UV-Visible spectrophotometer 1601, PC,Shimadzu-Japan. The changes in absorbance of 2-T-5-S by the addition of CDs were recorded at 254 nm. The stability constant can be calculated using Benesi-Hildebrand equation:

$$\frac{b}{\Delta A} = \frac{1}{S_T K_{1:1} \Delta\varepsilon[L]} + \frac{1}{S_T \Delta\varepsilon}$$

$$K_{1:1} = \frac{y - intercept}{\text{slope}}$$

Where, b is the cell thickness, ΔA is the change in absorbance, S_T is molar concentration of absorbing solute, $K_{1:1}$ is stability constant, Δε is the difference in molar absorptive between the complexed and uncomplexed forms of the substrate and L is the ligand concentration.

II.B-Ultrafiltration:

Ultrafiltration is a useful method to assess complex formation in aqueous solution[13] and can be used to confirm the stoichiometry of the crystalline complexes.[14] It is similar to the equilibrium dialysis, at which two compartments are separated by a semipermeable membrane, the membrane being permeable to one interact ant (2-T-5-S) and impermeable to the macromolecule. The binding equilibrium between macromolecule and the substance is established, and then pressure (by the force of the compressed nitrogen) is applied to force the solution through the ultrafiltration membrane. Since the macromolecule cannot penetrate the membrane. 2-T-5-S concentration in the filtrate is equal to the free (unbound) substance concentration in the presence of the macromolecule. The total substance concentration in the presence of macromolecule is also measured, and the molar ratio of bound substance to macromolecule is calculated.Ultrfiltration was chosen as a fast and simple method for studying the interaction between 2-T-5-S and CD, and the complex stability constants were determined.

Determination of stability constant and stoichiometry using ultrafiltration:

2-T-5-S is dissolved in 50 ml of 0.1N HCl with the aid of DMF (1.70 X 10^{-5} M). The prepared solutions either alone or in the presence of different concentrations of ß-CD, HE- ß-CD and HP- ß-CD (8.5 x 10^{-5}, 1.7 x 10^{-4}, 2.55 x 10^{-4}, 3.4 x 10^{-4}, and 8.5 x 10^{-4} M for each CD) were filled into the stirred filtration cell (Amicon, model 8050-USA) . The stirrer was adjusted at a revolution rate of 8 rpm and the solution was forced at pressure of 20 pound per square inch (P.S.I) using nitrogen compressor through AmiconDiaflo ultrafiltration membrane YM2 (Molecular weight cut-off 1000) and collected in fractions (4 ml each) in succession. After collecting seven fractions, the pressure was released, and the residual content of the cell was also collected and analyzed. The fractions of 2-T-5-S were analyzed spectrophotometrically, and the results were used for calculating the stability constants (Table 2). Filtrates were collected over a constant time period of 0-105 min. and were assayed for total 2-T-5-S filtered and expressed as the percentage of 2-T-5-S in the filtrate per hr. The stability constant of 2-T-5-S-CDs complexes could be calculated[13], were [2-T-5-S-CD] and $[2\text{-T-5-S}]_f$ are the concentrations of the complexes and free 2-T-5-S , respectively.

$$K_{1:1} = \frac{[2-\mathrm{T}-5-\mathrm{S}-\mathrm{CD}]}{[CD][2-T-5-S]_f}$$

Assuming that the total concentration, $[2\text{-T-5-S}]_f$ of the substance is given by the sum of the complexed and free substance, and CD is present in excess, then the equation may be written as:

$$K_{1:1} = \frac{[2-\mathrm{T}-5-\mathrm{S}]t}{[CD][2-T-5-S]_f}$$

The value of $[2\text{-T-5-S}]_f$ could be directly obtained from the mean value of the effluent concentrations after reaching equilibrium. Since $[2\text{-T-5-S}]_f$ and CD concentrations were arbitrarily chosen, the value of $K_{1:1}$ could readily be calculated.

RESULTS AND DISCUSSION

Due to the presence of hydroxyl groups placed on the surface of cyclodextrins andan internal non-polar hole, the inclusion of hydrophobic compounds such as sulfonamide derivatives takes place mainly by hydrophobic interactions with the walls of cyclodextrin cavity(host).[15]Otherwise, van der Walls and other forces such as dipole–dipole interactions, may be contributingin the binding of the guest sulfonamide molecules. Complexation with cyclodextrins and formation of complexes is a rather simple processdespite the presence number of factors and different forces involved in.

Characterization of 2-T-5-S-CDs complexes:

I-UV-Spectrophotometric method:

Ultraviolet spectroscopy is a useful tool to determine inclusion complexation in a solution if it causes a change in the absorption spectrum of a guest molecule[16] although frequently only small shifts are observed on the UV spectra of included guests. So, the putative formation of an inclusion complex of 2-T-5-S in aqueous solution has been studied by UV spectroscopy. The absorption spectrum of 2-T-5-S varied slightly by adding ß-HE and HP-ß-CDs to its solution. No bathochromic shift was observed for 2-T-5-S absorption in the presence of CDs, but the intensity of the absorption is slightly decreased with increasing ß-CD and HE-ß-CD concentrations. This decrease may be due to partial shielding of chromophore electrons within the CD cavity.[17] In contrast, the

intensity of the absorption maximum peak of 2-T-5-S is increased with increasing HP-ß-CD concentration. These data indicate that an interaction between 2-T-5-S and CDs occurred in solution. Similar results have been reported for ß-CD and HP-ß-CD with ibuprofen, dimethyl-ß-CD with hydrocortisone butyrate[18] and α-CD with allopurinol.[19]Assuming 1:1 stoichiometry, Benese-Hildebrand equation was applied to calculate the stability complexes constant.[20] The K_{11} values determined for ß-CD, HE-ß-CD and HP-ß-CD complexes with 2-T-5-S were 2168.7, 1657.8 and 453.7 M^{-1} respectively. (Table 4)

II-Ultrafiltration method:

The ultrafiltration profiles of 2-T-5-S in the presence of different concentrations of ß-CD, HE-ß-CD and HP-ß-CD are shown in table 4. During the early stages of the effluence, the concentration of the drug is low, but the filtrate concentration reaches a constant level after 16 ml filtration. At this level, a state of equilibrium was attained, and the concentration of 2-T-5-S filtered represented the amount of the free substance molecules at the respective CD added. A distinct fall in the free amount of 2-T-5-S filtered with increasing of CD concentration was observed. Undoubtedly, this indicates the formation of 2-T-5-S-CD complex having a higher combined molecular weight than the free drug molecule alone.

CONCLUSION

The UV spectrophotometry and ultrafiltration of 2-T-5-S with ß-CD and HE-ß-CD in 0.1N HCl at 25,37, 45 and 50 ^{0}C indicated the formation of soluble complexes of 1:1 stoichiometry. HP-ß-CD showed the formation of higher order complexes. The apparent stability constant, which reflects the affinity of CDs to the drug determined by the solubility method was arranged in the following order, ß-CD > HE-ß-CD > HP-ß-CD for all methods. The estimated enthalpies and entropies for the interaction of 2-T-5-S with CDs indicated that the interaction is exothermic.

Table I: Solubilizing efficiency of ß-CD, HE-ß-CD and HP-ß-CD and HP-CD on 2-T-5-S in 0.1 N HCl at different temperatures:

System	T (^{0}C)	CD conc. (x10^{-3})	Solubility in 0.1N HCl (S_0)(x10^{-6}M)± SD	Solubility in CD (S)(x10^{-5} M)± SD	Solubilizing power of CDs(S/So) ± SD
ß-CD	25	1.8	2.16 ± 0.01	19. 7 ± 2.08	91.76
	37		3.25 ± 0.01	24.2 ± 3.15	82.12
	45		4.44 ± 0.03	32.0 ± 3.52	67.68
	50		5.53 ± 0.09	33.0 ± 3.00	56.38
HE-ß-CD	25	5	2.17 ± 0.01	28. 1 ± 3.51	129.49
	37		3.22 ± 0.01	39.4 ± 2.08	126.36
	45		4.44 ± 0.03	47.4 ± 1.52	106.75
	50		5.55 ± 0.09	52.4 ± 11.12	92.40
HP-ß-CD	25	5	2.17 ± 0.01	55. 0 ± 3.10	251.15
	37		3.22 ± 0.01	77.0 ± 1.52	245.98
	45		4.44 ± 0.03	86.4 ± 3.21	193.69
	50		5.55 ± 0.09	94.1 ± 5.50	166.08

Table 2: Stability constants and thermodynamic parameters for complexation of 2-T-5-S with CDs:

System	T (^{0}C)	K1:1 (M^{-1})	K1:2 (M^{-1})	ΔH^o (K.cal/mol)	ΔF^o (K.cal/mol)	ΔS^o (cal /mol /^{o}K)
ß-CD-2-T-5-S	25	5934.2	-	-3.97	-5.13	3.91
	37	4937.0			-5.23	4.16
	45	4156.0			-5.26	4.05
	50	3531.6			-5.24	3.93
HE-ß-CD-2-T-5-S	25	3375.8	-	-2.95	-4.81	6.24
	37	3207.5			-4.97	6.51
	45	2668.8			-4.98	6.35
	50	2297.1			-4.96	6.22
HP-ß-CD-2-T-5-S	25	2953.6	63.1	-2.51	-4.73	7.45
	37	2704.0	52.0		-4.86	7.58
	45	2357.0	37.0		-4.90	7.51
	50	2127.4	26.9		-4.92	7.46

ΔH^o=standard enthalpy change, ΔF^o= Gibbs free energy changes, ΔS^o = standard entropy change, K1:1 and K1:2 = stability constants of complexes with stoichometry 1:1 and 1:2, respectively.

Table 3: Ultraviolet spectral data for 5-T-5-S in 0.1 N HCl with ß-CD, HE-ß-CD and HE-ß-CD recorded at 254 nm (Mean ± SD)

CD Concentration x 10^{-4} (M)	Absorbance (A) ± SD in presence of					
	ß-CD		HE-ß-CD		HP-ß-CD	
	(A) ± SD X (10^{-4})	(Δ A)	(A) SD X(10^{-4})	(Δ A)	(A) SD X(10^{-4})	(Δ A)

0.000	0.5898 ± 3.0	-	0.5696 ± 3.0	-	0.5797 ± 3.0	-
8.566	0.5756 ± 5.2	0.0142	0.5771 ± 5.1	0.0127	0.5902 ± 1.0	0.0004
14.278	0.57468 ± 1.1	0.0152	0.5756 ± 3.1	0.0142	0.5957 ± 1.1	0.0059
22.844	0.5734 ± 9.0	0.0164	0.5742 ± 5.2	0.0156	0.5968 ± 3.1	0.0070
28.556	0.5712 ± 1.5	0.0185	0.5729 ± 8.0	0.0169	-	-
34.260	0.5696 ± 7.0	0.0202	0.5698 ± 3.7	0.0200	0.5981 ± 5.0	0.0093

Table 4: Apparent stability constants determined by different methods at 25°C.

System	Solubility method	Ultrafilteration method	UV spectrophotometric method
ß-CD-2-T-5-S	5821.2	5937.3	2178.6
HE-ß-CD-2-T-5-S	3375.8	3354.7	1697.2
HP-ß-CD-2-T-5-S	2953.6	3137.6	423.5

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