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Supramolecular assembly with hydrogen bonded network solid formed by reaction of cyanuric chloride with thiourea: Spectral and anitmicrobial studies

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Interaction of thiourea (Tu) with cyanuric chloride (CC) in aqueous solution yields a compound, $(Tu)_2(CA)_2(NCS)(H_2O)_3$, (CA-TU) (CA = cyanuric acid). The elemental composition, TG-DTA and mass spectrum has confirmed the proposed composition. Infrared bands at 2026 cm^{-1} and 1725 cm^{-1} indicates –NCS and >C=O groups respectively along with the –OH and –NH stretching bands. ^{1}H NMR indicates protonated H_2O (δ 13.35) and N-H (δ 6.05 - 7.01). The ^{13}C NMR reveals –NCS carbon resonances at δ 116.99 and at δ 178.83 due to thiourea carbon. The signals at δ 150.39, 148.41 and 145.34 are due to CA carbonyls. Thermal studies indicate that step-wise decomposition leading to NH_4NO_3 takes place and thereafter, to nil residue. The compound is a supramolecular assembly of extensively H-bonded networked solid. The compound has been found to be more effective towards Gram positive bacteria than Gram negative bacteria. The action is superior towards fungi compared to that of the standard Amphotericin-B. The presence of sulphur and nitrogen moieties make it a good antifungal agent.

Keywords: Supramolecular H-bonded solid, Cyanuric acid (CA) – Tu (thiourea) - NCS, Spectral characterization, H-bonded framework solid, Antimicrobial activity

1,3,5-Triazine derivatives have fascinated chemists and have been a key research area of interest[1-4]. These molecules have gained prominence as it finds applications in pharmaceutical, textile, plastics, rubber industries and as pesticides, dyestuffs, optical bleaches, explosives and surface active agents[5].

Heterocyclic scaffolds are profitably exploited in the realms of medicinal chemistry. A molecule from hybridisation of different heterocyclic moiety helps in tackling resistance and efficacy problems[6]. 1,3,5-triazines (s-triazine) owes its superiority as a building block in heterocyclic scaffolding compared to isomeric 1,2,3-triazine and 1,2,4-triazine. The broad spectrum of biological activities displayed by s-triazine are well documented[7].

The structurally symmetric s-triazine with its multifarious bio-activity have been comprehensively reviewed[8]. For the convenient and cost effective synthesis[9] of s-triazine derivatives, 2,4,6-trichloro-1,3,5-triazine or commercially known as cyanuric chloride (CC) is an attractive starting material. The three chlorine atoms in the CC molecule could be easily subjected to nucleophilic substitution reactions with almost all types of nucleophiles (S, O and N)[10]. Substitution reduces the reactivity of s-triazine nucleus, this opens the scope of temperature controlled condition, to achieve the required degree of substitution[11].

Cyanuric chloride (CC) has gained prominence owing to its mild nature, environmental friendliness, commercial availability and low cost and as a green source of HCl *in situ*. These features make cyanuric chloride as a potential starting material in organic syntheses. As an oxophilic reagent CC is employed for the conversion of carboxylic acids into acid chlorides, esters, amides, peptides, macrolactones and strategically as catalyst[12].

Derivatives of s-triazine were screened for their antimicrobial activity[13-15]. The chemistry of cyanuric acid and cyanurates are comprehensively reviewed in the literature[16]. Thiourea derivatives were known to evince biological activity like: antibacterial, antioxidant, anticancer, anti-inflammatory, anti-Alzheimer, anti tuberculosis and antimalarial properties[17]. In this pursuit, we intend to study the interaction of thiourea (Tu) with the CC under aqueous conditions and screen the resulting compound for its antimicrobial activity.

Experimental Section

General Information

Cyanuric chloride, AR grade (Loba) and thiourea, LR grade (Loba) were employed for the synthesis as such with distilled water.

Elemental analyses were performed on Thermos Fischer instrument. Nietzsche STA 449F3 instrument was utilized to record the thermal analysis. ESI mode of ionization method was applied to record the mass spectrum using Agilent mass spectrometer. NMR experiments were performed on Bruker Avance 500MHz instrument in DMSO-d_6. IR spectra were recorded employing solid state (KBr) sampling method on Thermo Scientific Nicolet iS50 spectrometer. UV-Visible spectrum was obtained through Perkin Elmer LAMBDA 950 spectrometer.

Reaction of cyanuric acid with thiourea

Cyanuric chloride (1.84 g, 0.01 mole) and thiourea (2.28 g, 0.03 mole) were mixed and heated in water (80 cm^3) at 60°C with constant stirring (Scheme 1). A gum like mass was formed at first which on stirring for 1.5 h got disintegrated. The compound was filtered and washed with water and dried in the desiccator using anhyd. $CaCl_2$ to yield 2.60 g (35%) of CA-TU.

Anti bacterial studies

Stock cultures of *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Bacillus subtilis and Salmonella typhi* were maintained at 4°C on nutrient agar slant. Active cultures for experiments were prepared by transferring a loop full of culture from the stock cultures into the test tubes containing nutrient broth, that were incubated for 24 h at 37ºC.

Antibacterial activity of the extracts were determined by disc diffusion method on Mueller Hinton agar (MHA) medium. Mueller Hinton Agar (MHA) medium was poured into the petri plate. After the medium solidified, the inoculum were spread on the solid plates with sterile swab moistened with the bacterial suspension. The disc were placed in MHA plates and 20 µL of sample (concentration: 1000µg, 750µg and 500 µg) were dispensed on the disc. The antibacterial activity of test were compared with Ampicillin (20µL/disc). The plates were incubated at 37ºC for 24 h, the antibacterial activity was determined by measuring the diameter of zone of inhibition[18]. All the experiments were conducted in triplicates.

Anti fungal studies

Stock cultures of *Aspergillus niger* and, *Rhizopus* were maintained at 4°C on Sabouraud Dextrose agar slant. Active cultures for experiments were prepared by transferring the stock cultures into the test tubes containing Sabouraud Dextrose broth that were incubated at 48 h at RT.

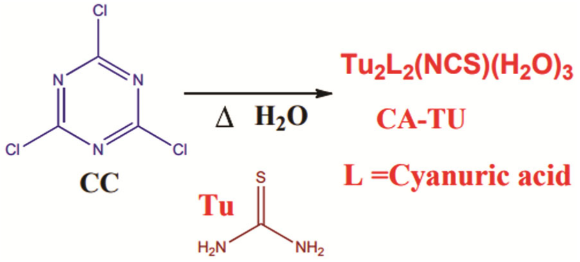
Sabouraud Dextrose agar (SDA) medium is poured into the petri plate. After the medium has solidified, the inoculums were spread on the solid plates with sterile swab moistened with the fungal suspension. Amphotericin-B is taken as standard. Samples and standard of 20 µL each were added in sterile discs and placed in SDA plates. The plates were incubated at 28ºC for 24 h. Then antifungal activity was determined by measuring the diameter of zone of inhibition.

Results and Discussion

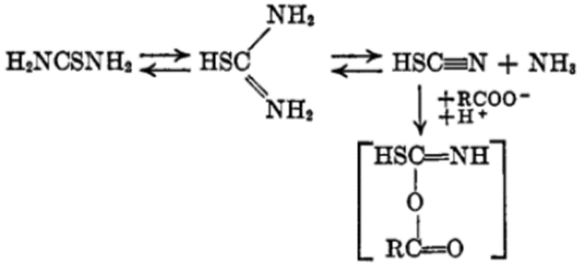
The elemental analyses indicated that the composition is $(Tu)_2$ $L_2(NCS)(H_2O)_3$(L = Cyanuric acid). Expt (calcd): C: 19.52(20.68), H: 3.40(3.45),N : 28.78(29.50). S: 18.46(18.39).

Synthesis

Formation of amides by heating thiourea and carboxylic acids were reported (Scheme 2)[19] through the formation of thiocyanic acid as the reaction intermediate . The dissociated thiocyanic acid, protonates the cyanuric acid and sandwiches NCS^- ion between them. The thiocyanate has resulted from the thiourea.



Scheme 1 — Preparation of CA-TU compound



Scheme 2 — Mechanism of formation of thiocyanate from thiourea[19]

Infrared Spectrum

The IR spectrum(Fig. 1 and Table 1) indicated a broad band at 3508 cm^{-1} and 3434 cm^{-1} due to ν(O-H) and ν(N-H). A band at 2026 cm^{-1}confirmed ν(C-N) of NCS moiety[20,21]. A prominent band at 1725 cm^{-1} indicated ν(C=O) from carbonyl group. The bands appearing at 1294 cm^{-1} and 1143 cm^{-1} were attributed to ν(C=N) of cyanuric acid and ν(C-O), which arises due to tautomerism[22]. A band at 454 cm^{-1} is due to δ(NCS) of the thiocyanate group. The appearance of bands 772 (Ref. 23) and 892 cm^{-1} account for ν(CS) of thiourea and that of -NCS group.

NMR Spectra

^{1}H NMR: The signals appearing from δ 6.05 to 7.37 (Fig. 2) are due to N-H protons in different environment and are H-bonded to N-H group. Similarly, the broad signals spanning from δ 6.05 to 13.35 could be attributed to highly deshielded protons. The ^{1}H NMR spectrum indicated a three dimensional network with H-bonding. The peak at δ 13.35 is due to the protanated -OH and H+ in between the two cyanuric acid moieties with NCS− as the anion in between these moieties as well. The NH$_2$ group of thiourea, the - NH, -C=O and -C-OH groups interact extensively through H-bonding leading to a supramolecular assembly. The present observations therefore reveal continuous signature of peaks between δ 6-7 which can be taken as a characteristic of NH and those falling in the region δ 9.0 to 10.5-13.0, due to OH protons of different strength of extended and extensive H-bonding. The broad peak at δ 4.77 indicated three dimensional H-bond with the involvement of NH$_2$ of thiourea. It is well documented in the literature that the peaks from δ 6.05 to 6.64 are from the NH of the cyanuric acid[24]. A three dimensional supramolecular H-bonded network has been proposed.

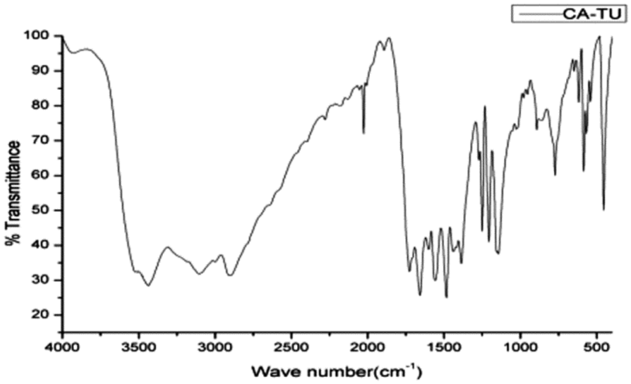


Fig. 1 — IR spectrum of CA-TU

Table 1 — IR assignments for L$_2$ (Tu)$_2$(NCS).(H$_2$O)$_3$ (CA-TU)

IR band frequencies (cm^{-1})	Assignment
3508.31	ν(O-H)
3434.78	ν(N-H)
2026.08	ν(C≡N)
1725.87	ν(C=O)
1657.46	δ(N-H)
1484.08	In-plane ring stretching or ν(N-C-N)
1143.37	ν(C-O)
892.45	ν(C=S) NCS
772.80	ν(CS) Tu
567.02	δ(C=O)
454.01	δ(NCS)

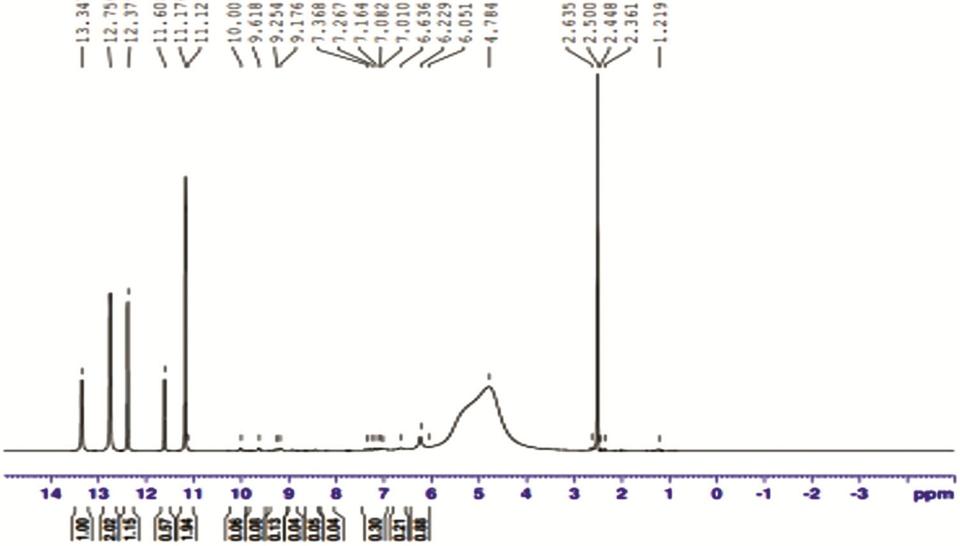


Fig. 2 — ^{1}H NMR spectrum of CA-TU in DMSO-d_6

^{13}C NMR: The ^{13}C NMR study (Fig. 3) confirmed the presence of -NCS functionality with resonance at δ 116.99[25]. The resonances at δ 178.83 and δ 176.34 account for thiourea in its different conformation, which is influenced by the supramolecular assembly through H-bonding network[26]. The signals at δ 150.39, 148.41 and 145.34 are due to the carbons of the cyanuric acid experiencing different chemical environment due to tautomerism and resonance effect. An extensive three dimensional hydrogen bonding network has been proposed through a protonated CA molecule and Tu units with NCS^{-1} being sandwiched between the CA units. This extensive H-bonding observation is in agreement with the literature[27-29] as reported by Nagaraja and co-workers.

Mass spectral investigation

The mass spectral study presented the molecular ion information, capturing [MH]$^+$ ion (Fig. 4). The isotopic abundance of ^{13}C, ^{34}S and ^{15}N give rise to cluster of peaks spanning 523-525 Da. This is in agreement with the elemental analyses and thermal studies. An

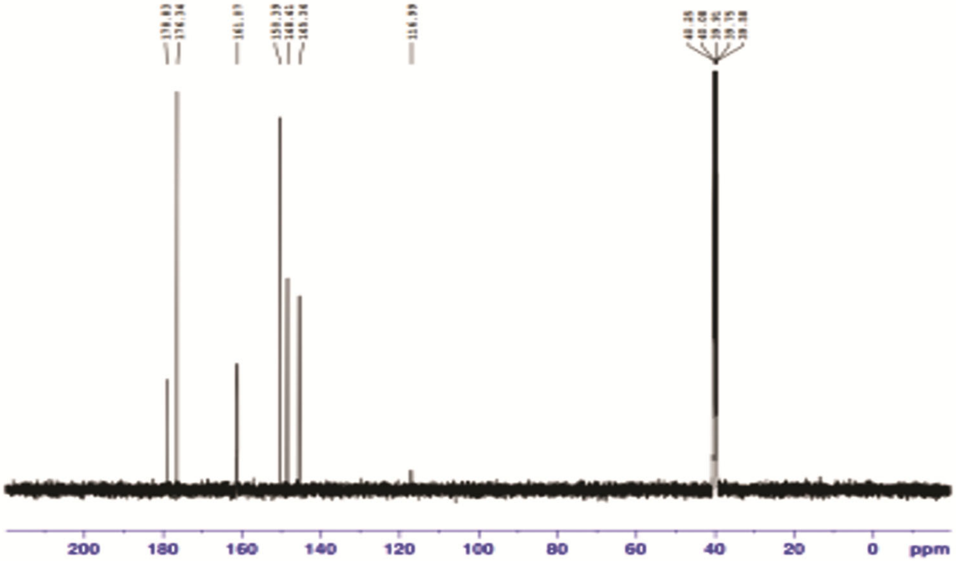


Fig. 3 — ^{13}C NMR spectrum of CA-TU in DMSO-d_6

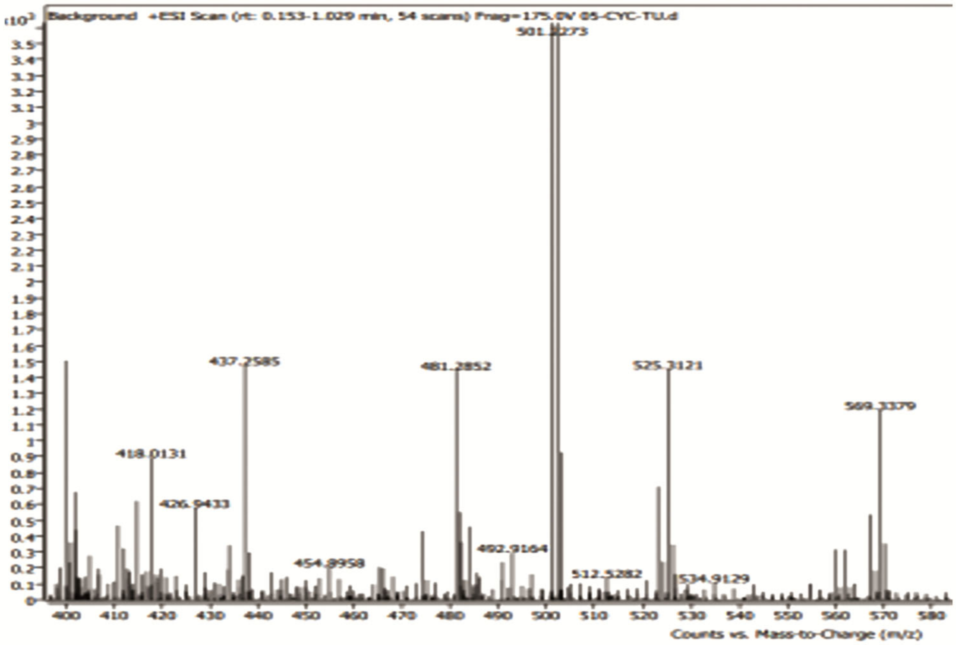


Fig. 4 — Mass spectral pattern of CA-TU with base peak and M$^+$ ion

elaborate fragmentation pattern with successive loss of -NCS or H_2O or Tu were observed (Fig. 4 and Fig. 5).

Themal studies

The first step is due to the loss of three water molecules corresponding to 10.00% (Calcd: 10.34%), second step involves the loss of -NCS group corresponding to 12.80% (Calcd: 13.01%). The compound decomposed with the loss of one thiourea molecule in each step and the loss of two cyanuric acid moieties together yielding NH_4NO_3 as the residue (Fig. 6 and Fig. 7). Above 650°C, the NH_4NO_3 decomposes to N_2O and H_2O leaving nil residue. The heat involved in each stage has been depicted in Table 2.

UV-Visible spectrum

It absorbs at 380 nm due to n→π* transistion and shows a strong absorption at 320 nm due to π→π* transistion.

Anti microbial activity

The CA-TU has been tested against Gram positive, Gram negative bacteria and fungus. The compound exhibited satisfactory activity against *Staphylococcus aureus*, which is Gram positive than *Pseudomonas aeruginosa*, a Gram negative bacteria (Fig. 8, Table 3). The effect is pronounced on *Bacillus subtilis* and *Salmonella typhi* and comparable to the standard, Ampicillin. This sulphur containing moieties are expected to be good anti microbial agents and can be

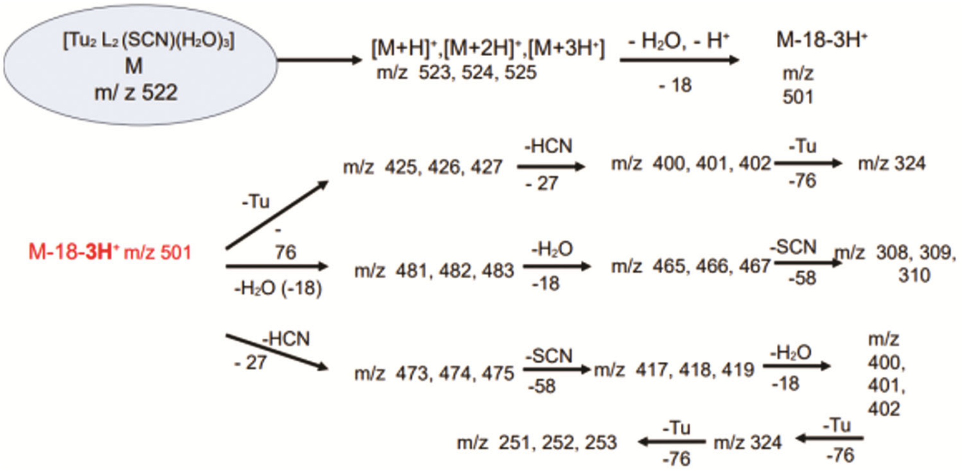


Fig. 5 — Mass spectral fragmentation pattern of CA-TU

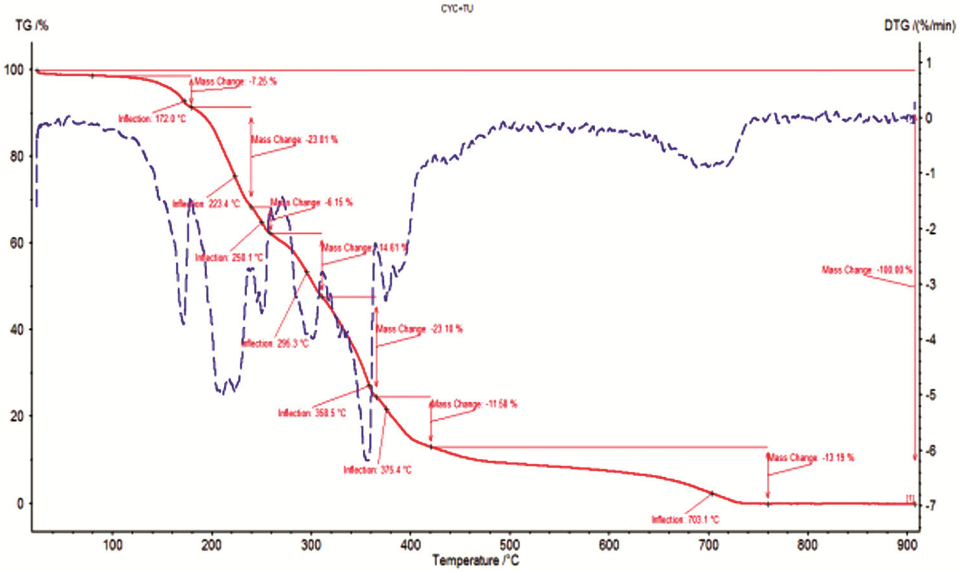


Fig. 6 — TG-DTG pattern of CA-TU in nitrogen atmosphere at a heating rate of 10°C min^{-1}

Table 2 — TG-DSC studies of L_2 $(Tu)_2(NCS)(H_2O)_3$ (CA-TU)

Temperature range (°C)	Wt. Loss (%) Expt	Wt. Loss (%) Theor	Loss or phase formed	Temperature (°C)	Heat J/g
110-180	10.10	10.34	Loss of three H_2O molecule	175	75.19
190-220	20.90	21.45	Loss of NCS group	201	70.22
230-260	35.92	36.01	Loss of one $(NH_2)_2CS$	260	51.89
260-300	51.23	50.57	Loss of one $(NH_2)_2CS$	282	22.63
310-370	80.93	80.84	Loss of one cyanuric acid	320	178.60
380-400	85.32	84.67	Decomposition of another cyanuric acid yielding NH_4NO_3 residue	380	
640-730	100	100	Decomposition of NH_4NO_3 leaving nil residue	723	193.25

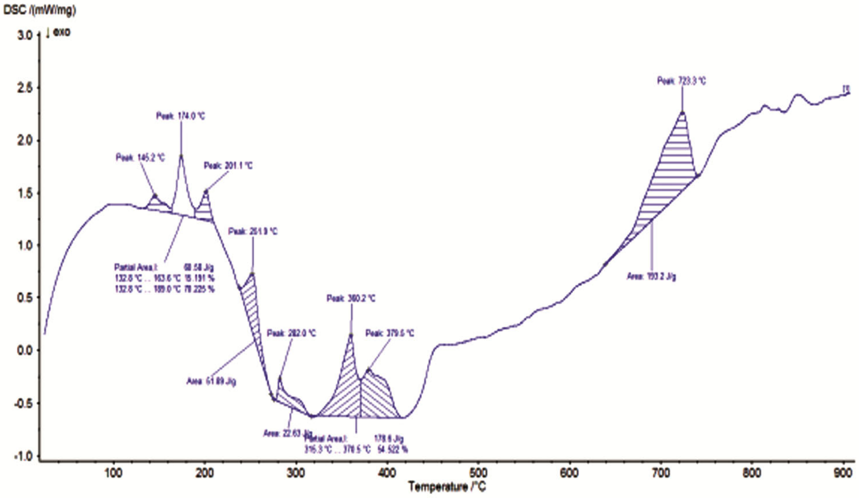


Fig. 7 — DSC pattern of CA-TU in nitrogen atmosphere at a heating rate of 10°C min^{-1}

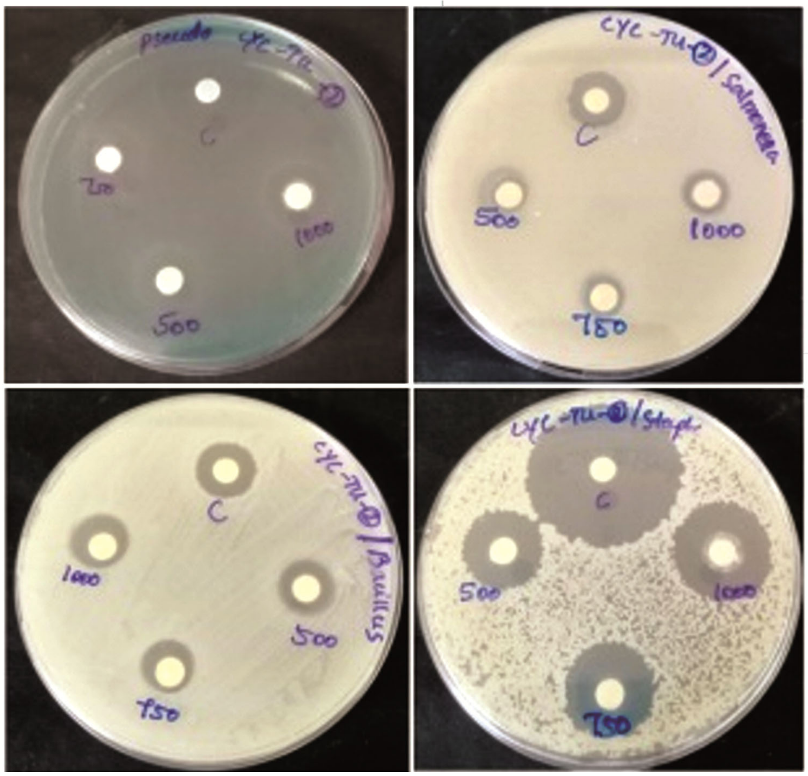


Fig. 8 — Anti bacterial studies on CA-TU

Table 3 — Antimicrobial activity results of CA-TU

Organisms	Zone of Inhibition (mm)			
	Sample (µg/mL)			
	1000	750	500	Standard
Staphylococcus aureus	23 mm	18 mm	18 mm	37 mm
Pseudomonas aeruginosa	15 mm	11 mm	9 mm	15 mm
Bacillus subtilis	13 mm	11 mm	11 mm	15 mm
Salmonella typhi	11 mm	9 mm	8 mm	14 mm
Aspergillus niger	27 mm	17 mm	15 mm	14 mm
Rhizopus	30 mm	18 mm	15 mm	15 mm

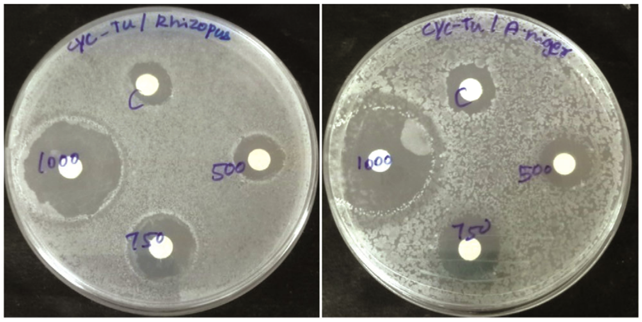


Fig. 9 — Antifungal activity of 1. *Rhizopus* 2. *Aspergillus niger*

tested against several other microorganisms and pathogens. Such screening is essential for dermatology and its use in pre and post operative surgeries for disinfecting tools and surroundings. Our studies indicated that generally, Gram positive bacteria are usually susceptible to, heterocyclic and sulphur containing compounds than Gram negative bacteria. In the case of fungi (Fig. 9) *Aspergillus niger* and *Rhizopus*, the CA-TU showed a significant enhancement in the activity compared to the standard (Amphotericin-B).

Conclusion

Interaction of thiourea with cyanuric chloride yielded a H-bonded network solid in aqueous solution. This involved -NH$_2$ groups of thiourea, cyanuric acid -C-OH, C=O and -N-H groups along with water molecules, including the protonated species. The bands at 2026 cm^{-1}, 1725 and 772.8 cm^{-1} arise due to ν(C≡N) of thiocyanate, ν(C=O) of CA and Tu ν(C=S). The ^{1}H NMR indicated protonated H$_2$O (δ 13.35) and N-H at δ 6.05-7.01. ^{13}C NMR indicated the presence of -NCS, Tu and CA carbons. Elemental analyses, thermal studies and mass spectra suggested the composition. Mass spectrum studies revealed several steps with a different ionised species due to loss of NCS, Tu, and CA. TG-DTA studies indicated that stepwise decomposition leading to NH$_4$NO$_3$ and further to nil residue. The microbiological examination indicated that the compound is more active against Gram positive than Gram negative bacteria. Its action against fungi is quite impressive and is attributed to the presence of -NCS, Tu, CA and the H-bonded network. The coexistence of a single compound with -NCS, Tu, CA groups due to H-bonded framework in the solid state is quite interesting.

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References

1 Smolin E M & Rapoport L, *S-Triazines and Derivatives in the Chemistry of Heterocyclic Compounds*, (NY: Interscience Publishers, New York), 1959.
2 Quirke M E, *Comprehensive Heterocyclic Chemistry*, (NY: Pergamon Press, New York), 1984, p. 457.
3 Bartholomew D, *Comprehensive Heterocyclic Chemistry II / 6, Six-membered rings with two or more heteroatoms and fused carbocyclic derivatives / vol. ed.: A.J. Boulton*, (Oxford: Pergamon), 1996, p. 575.
4 Comins D & O'Connor S, *Regioselective Substitution in Aromatic Six- Membered Nitrogen Heterocycles. Advances in Heterocyclic Chemistry*, (NY: Academic Press, New York), 1988, p. 199. (https://doi.org/10.1016/s0065-2725(08)60263-9).
5 Huthmacher K & Most D, *Cyanuric Acid and Cyanuric Chloride*. Ullmann's Encyclopedia of Industrial Chemistry, 2000. (https://doi.org/10.1002/14356007.a08_191).
6 Claudio Viegas-Junior Eliezer J Barreiro & Carlos Alberto Manssour Fraga, *Curr Med Chem*,14 (2007) 1829. doi:https://doi.org/10.2174/092986707781058805.
7 Singh S, Mandal M K, Masih A, Saha A, Ghosh S K, Bhat H R & Singh U P, *Arch Pharm*, 354 (2021) 1. doi:https://doi.org/10.1002/ardp.202000363.
8 Ali M I & Naseer, M M, *RSC Adv*, 13 (2023) 30462. doi:https://doi.org/10.1039/d3ra05953g.
9 Blotny G, *Tetrahedron*, 62 (2006) 9507. doi:https://doi.org/10.1016/j.tet.2006.07.039.
10 Sharma A, Sheyi R, de la Torre B G, El-Faham A & Albericio F, *Molecules*, 26 (2021) 864. doi:https://doi.org/10.3390/molecules26040864.
11 Sharma A, El-Faham A, de la Torre B G & Albericio F, *Front Chem*, 6, (2018) 516. https://doi.org/10.3389/fchem.2018.00516.
12 Banerjee B, Priya A, Kaur J, Kaur M, Singh A & Sharma A, *Synth Commun*, 53 (2023) 855. https://doi.org/10.1080/00397911.2023.2201889.
13 Dinari M, Gharahi F & Asadi P, *J Mol Struct*, 1156 (2018) 43. https://doi.org/10.1016/j.molstruc.2017.11.087.
14 Liu H, Long S, Rakesh K P & Zha G -F, *Eur J Med Chem*, 185 (2020) 111804. https://doi.org/10.1016/j.ejmech.2019.111804.
15 Abd Alhameed R, Almarhoon Z N, Sholkamy E, Ali Khan S, Ul-Haq Z, Sharma A, G. de la Torre B, Albericio F & El-Faham A, *J Fungi*, 6 (2020) 237. https://doi.org/10.3390/jof6040237.

16 Seifer G B, *Russ J Coord Chem*, 28 (2002) 301. https://doi.org/10.1023/a:1015531315785.

17 Agili F A, *Chemistry*, 6 (2024) 435. https://doi.org/10.3390/chemistry6030025.

18 Mangaiyarkkarasi P & Arulantony S, *Int J Curr Pharm Res*, 8 (2016) 43. (https://www.journals.innovareacademics.in/index.php/ijcpr/article/view/13879).

19 Rahman A -U, Medrano M A & Jeanneret B E, *J Org Chem*, 27 (1962) 3315. https://doi.org/10.1021/jo01056a509.

20 Jones L H, *J Chem Phys*, 25 (1956) 1069. https://doi.org/10.1063/1.1743101.

21 Lieber E, Rao R & Ramachandran J, *Spectrochim. Acta*, 13 (1959) 296. https://doi.org/10.1016/0371-1951(59)80030-8.

22 Newman R & Badger R M, *J Ame Chem Soc*, 74 (1952) 3545.

23 Kumar V S & Sundararajan R S, *JMMCE*, 03 (2015) 118.

24 Cheng H, Cheng Y-J, Bhasin S, Zhu, J-Y, Xu, X-D, Zhuo, R-X. & Zhang, X-Z, *Chem Comm*, 51 (2015) 6936. https://doi.org/10.1039/c5cc00501a.

25 Kargol J A, Crecely R W & Burmeister J L, *Inorg Chem*, 18 (1979) 2532. https://doi.org/10.1021/ic50199a040.

26 Sullivan R H, Nix P, Summers E L & Parker S L, *Org Magn Reson*, 21 (1983) 293. https://doi.org/10.1002/omr.1270210502.

27 Ponnuvel A, Kala, A P, Nagaraja K S & Karnan C, . *Acta Cryst E*, 77 (2021) 1019. https://doi.org/10.1107/s2056989021009324.

28 Ponnuvel A, Nivithaa S, Kala A, Ramkumaar G R, Nagaraja K S & Karnan C, *J Chem Crystallogr*, 53 (2023) 507. https://doi.org/10.1007/s10870-023-00989-x.

29 Elango M, Subramanian V & Sathyamurthy N, *J Chem Sci*, 129 (2017) 873. https://doi.org/10.1007/s12039-017-1259-5.