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Supporting information for article:

A new bioactive cocrystal of coumarin-3-carboxylic acid and thiourea: detailed structural features and biological activity studies

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Biological activities evaluations

a) Cytotoxicity assay Protocol

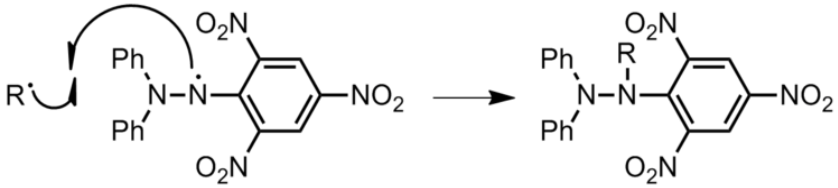
Cytotoxic activity of compounds was evaluated in 96-well flat-bottomed micro plates by using the standard MTT (3-[4, 5-dimethylthiazole-2-yl]-2, 5-diphenyl-tetrazolium bromide) colorimetric assay 40 . For this purpose, 3T3 (mouse fibroblast) cells were cultured in Dulbecco's Modified Eagle Medium, supplemented with 5% of foetal bovine serum (FBS), 100 IU/mL of penicillin and 100 µg/mL of streptomycin in 75 cm^2 flasks, and kept in 5% CO_2 incubator at 37 °C. Exponentially growing cells were harvested, counted with haemocytometer and diluted with a particular medium. Cell culture with the concentration of 5×10^4 cells/mL was prepared and introduced (100 µL/well) into 96-well plates. After overnight incubation, medium was removed and 200 µL of fresh medium was added with different concentrations of compounds (1-30 µM). After 48 hrs, 200 µL MTT (0.5 mg/mL) was added to each well and incubated further for 4 hrs. Subsequently, 100 µL of DMSO was added to each well. The extent of MTT reduction to formazan within cells was calculated by measuring the absorbance at 540 nm, using a micro plate reader (Spectra Max plus, Molecular Devices, CA, USA). The cytotoxicity was recorded as concentration causing 50% growth inhibition (IC_{50}) for 3T3 cells. The percent inhibition was calculated by using the following formula:

% inhibition = 100-((mean of O.D of test compound – mean of O.D of negative control)/ (mean of O.D of positive control – mean of O.D of negative control)*100).

The results (% inhibition) were processed by using Soft- Max Pro software (Molecular Device, USA).

b) DPPH Radical Scavenging Assay

DPPH (2, 2-diphenyl-1-picrylhydrazyl radical), is a dark-colored crystalline powder composed of stable free-radical molecules. DPPH has major application in laboratory research most notably in antioxidant assays. At radical state, the ethanol solution of this compound is appeared to be deep violet and has strong absorbance at 517 nm, which when reacts with an antioxidant (e.g., vitamin C) get reduced to the molecular form (DPPHH) which appeared as pale yellow with decrease in absorbance. This change in absorbance can be a measure to the radical scavenging power of the test sample.



Ant-oxidant assay protocol

Pure sample: 0.5 mM in DMSO
Crude Sample: 0.5 mg/mL in DMSO
DPPH (Wako Chemicals USA, Inc.) Solution: 0.3 mM in Ethanol

The DPPH assay is typically run by the following procedure:

DPPH solution (95 µL, 300 µM) in Ethanol is mixed with test solution (5 µL, 500 µM). The reaction is allowed to progress for 30 min at 37 °C and absorbance is monitored by multiplate reader, SpectraMax340 at 517 nm. Upon reduction, the colour of the solution fades (violet to pale yellow). Percent Radical Scavenging Activity (%RSA) is determined by comparison with a DMSO containing control. The concentration that causes a decrease in the initial DPPH concentration by 50% is defined as IC 50 value. The IC_{50} values of compounds were calculated by using the EZ-Fit Enzyme kinetics software program (Perrella Scientific Inc. Amherst, MA, USA). *N*-acetylcysteine, and ascorbic acid are used as the reference compounds.

c) Leishmanicidal activity

Leishmaniasis is a common disease endemic to the tropical areas of the Mediterranean region. Several species of the leishmania protozoa such as *L. Major*, *L. Donovani*, *L. Tropica*, etc. are responsible for causing this disease. Only a few drugs such as *N*-methyglucamine antimonite, amphotericin, and pentamidine are used to treat this disease. They are expensive and exhibit some toxic effects to man and animals. The discovery of anti-leishmanicidal drugs is therefore highly desirable. Promastigotes are the extracellular flagellated form of the parasite, and they can be used for *in vitro* determination of leishmanicidal activity of natural compounds.

MATERIALS

- Tissue culture

- 96 wells plate
- Roswell Park Memorial Institute (RPMI) -1640
- Fetal bovine serum(FBS)
- Distilled water
- Di-methyl sulfoxide (DMSO)
- Absolute methanol
- Incubator
- Light microscope
- Leishmania promastigotes
- Neubauer chamber
- Amphotericin –B, pentamidine
- Test samples

96 MICROTITRE PLATE BIO ASSAY:

Stock Solution of Test (Experimental Compounds) For Assay:

Take 1 mg of experimental compound/crude extract, dissolve in 50 µL of DMSO, and dilute with 950 µL of RPMI-1640 media.

PROTOCOL:

- Leishmania promastigotes are grown in bulk, early, in liquid medium RPMI-1640 Supplemented with 10% foetal bovine serum.
- Parasites at log phase are centrifuges at 2000 rpm for 10 minutes.
- Discard supernatant.
- Add fresh media to dilute the pallet material, till final density of 10^6 cells/mL are found.
- 100 µL of media is added in all wells except in first column which receives 180 µL media.
- Last two rows are for –ve and +ve control.
- Add 20 µL of stock of test compound in to first well and mix it well. Following this make serial dilution.
- Plate incubates at dark at 25 °C for 72 hours.
- After 72 hours activity of drugs/test compounds assessed microscopically using improved Neubauer chamber.

The cultures is,

L. Major. 50155 (ATCC)

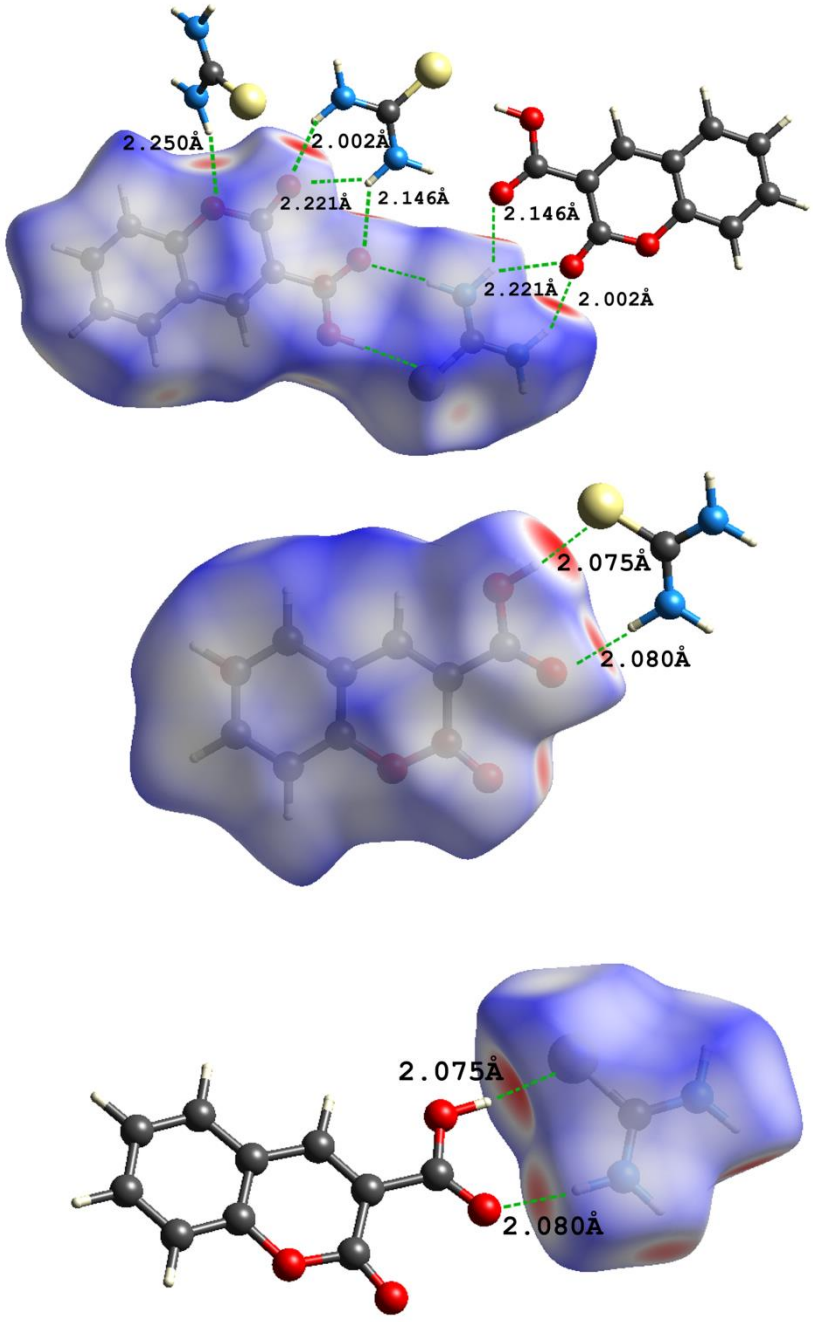


Figure S1 The intermolecular interactions of the **CU:TH** co-crystal with the neighbouring moieties are shown in figure. The S···H–O and O···H–N is important interactions responsible for strong hydrogen bonds as indicated by the red spots on the Hirshfeld surface.

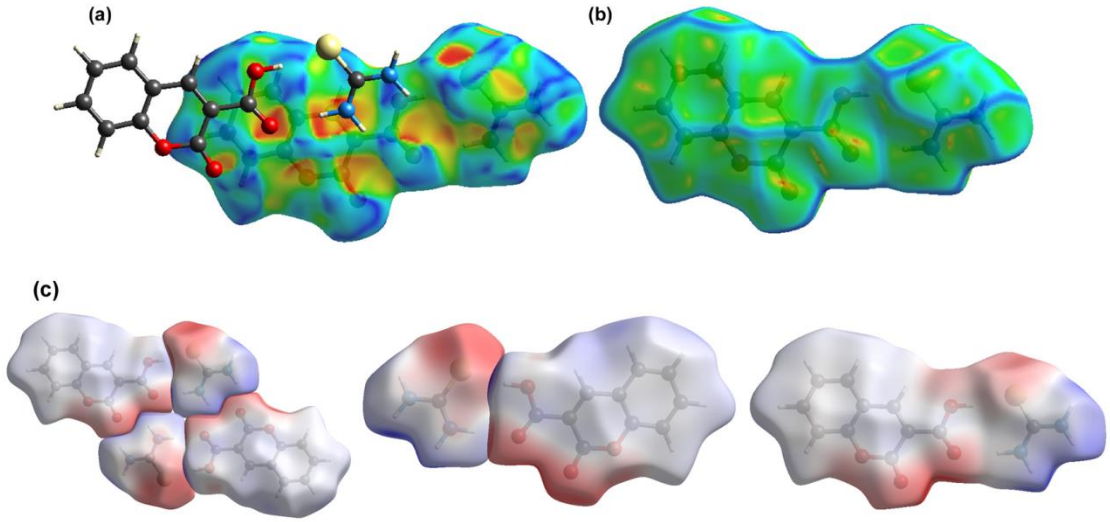


Figure S2 Hirshfeld surface of the co-crystal plotted over (a) shape-index, (b) curvedness, and (c) calculated electrostatic potential (the red and blue regions represent negative and positive electrostatic potentials, respectively).

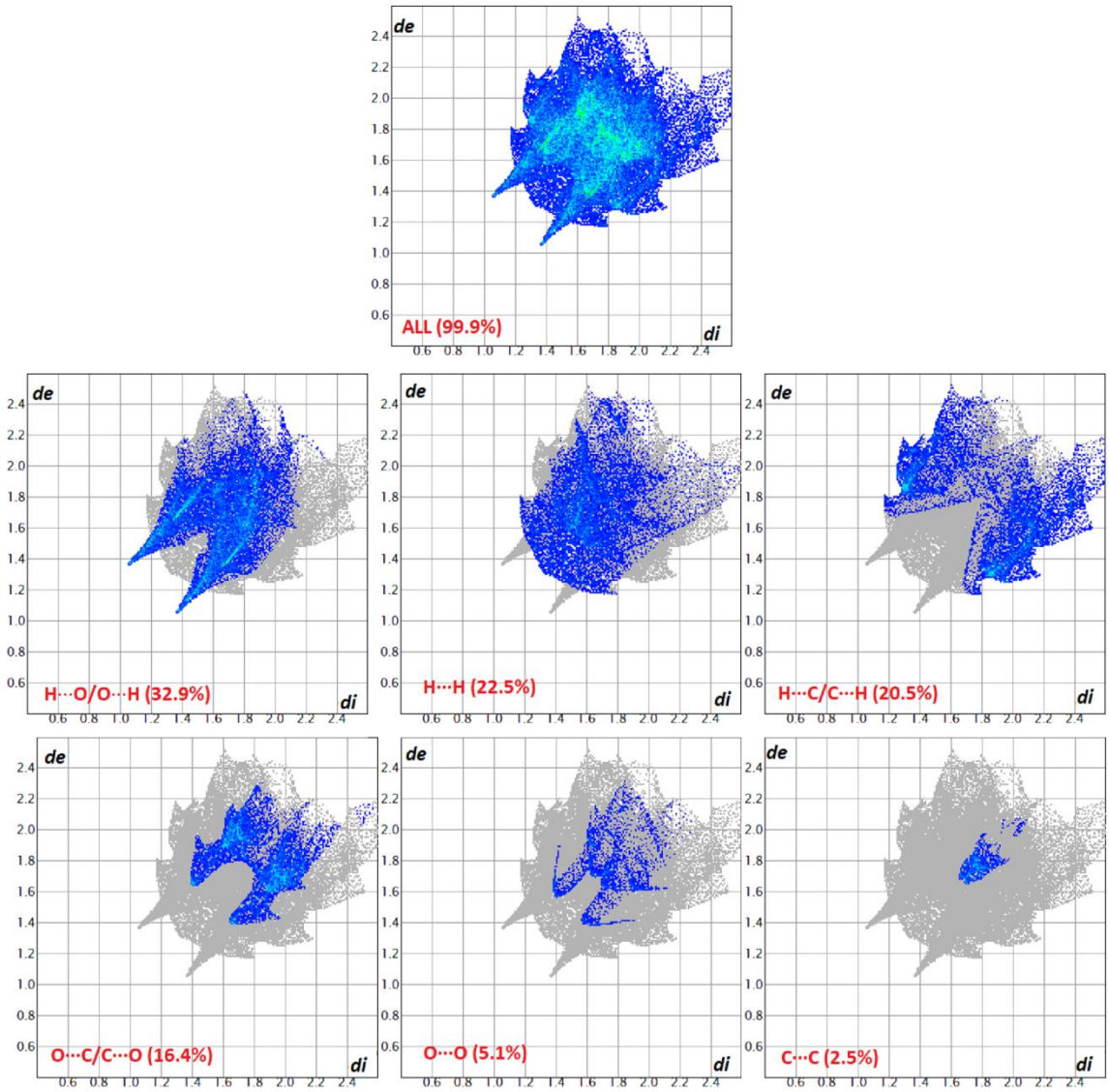


Figure S3 The two-dimensional fingerprint plots for coumarin-3-carboxylic acid (**CU**), with the percentage contribution of the intermolecular contacts.

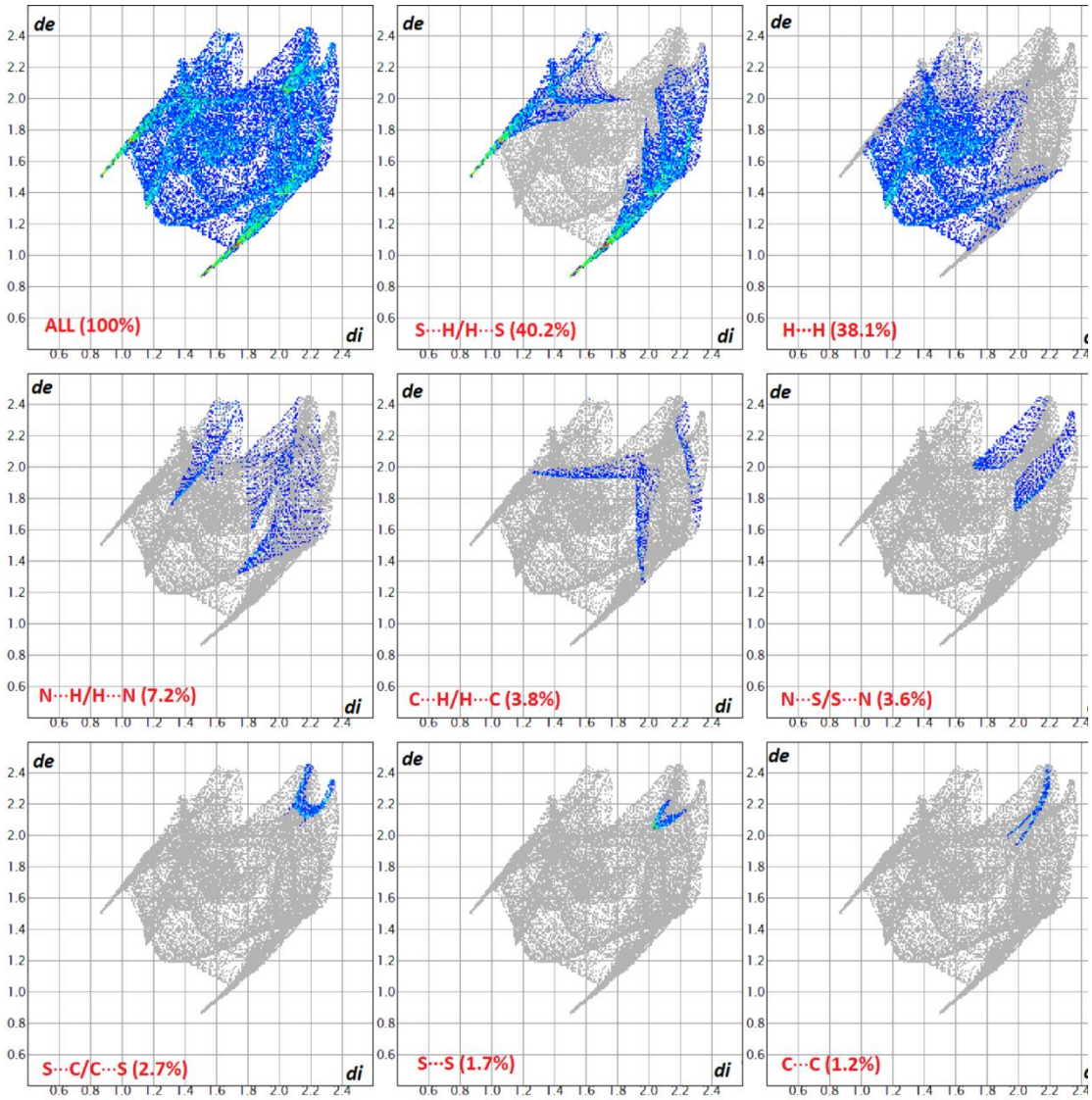


Figure S4 The two-dimensional fingerprint plots for thiourea (**TH)** with the percentage contribution of the intermolecular contacts.

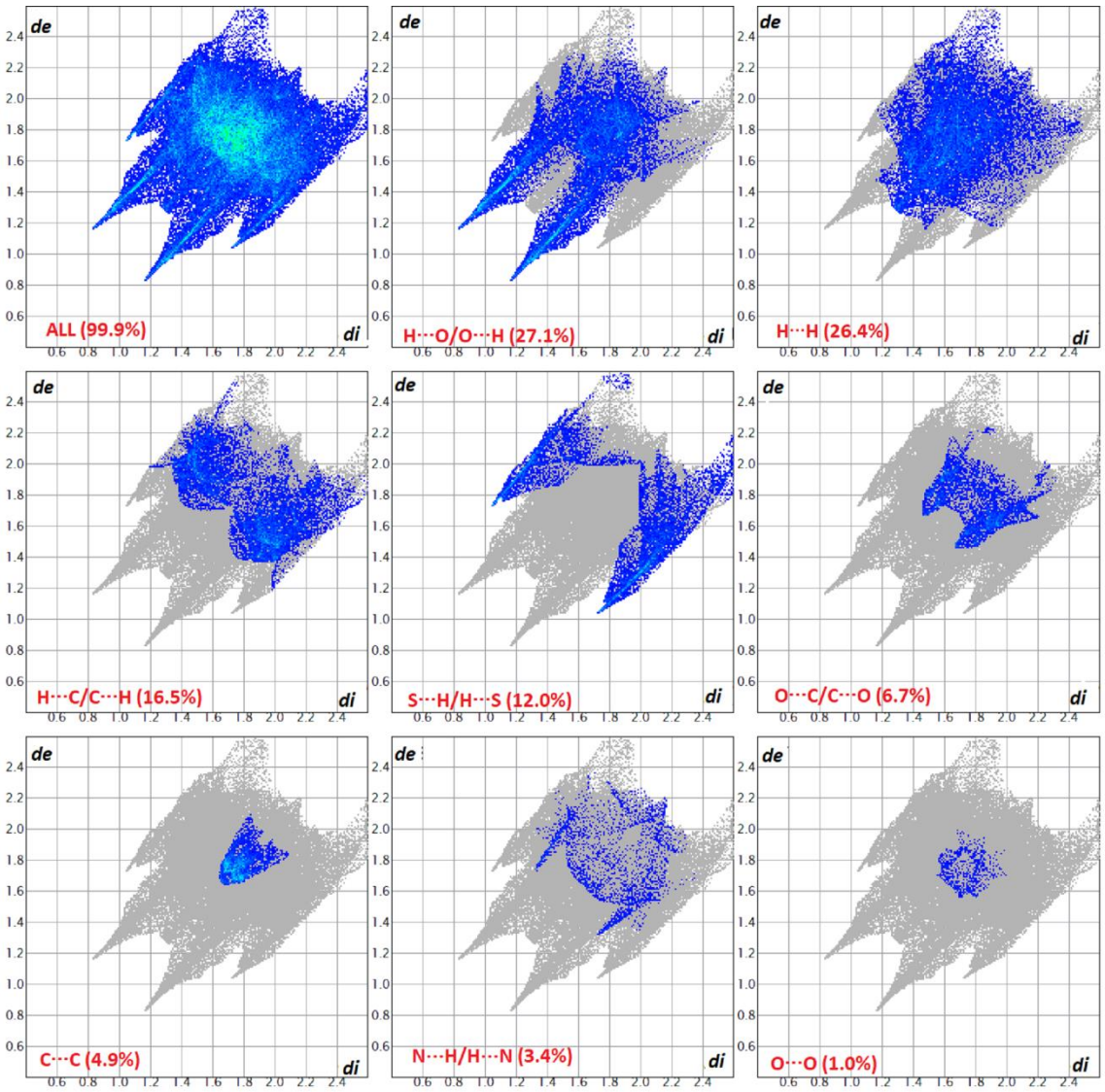


Figure S5 The two-dimensional fingerprint plots for **CU:TH** co-crystal with the percentage contribution of the intermolecular contacts of crystal packing.

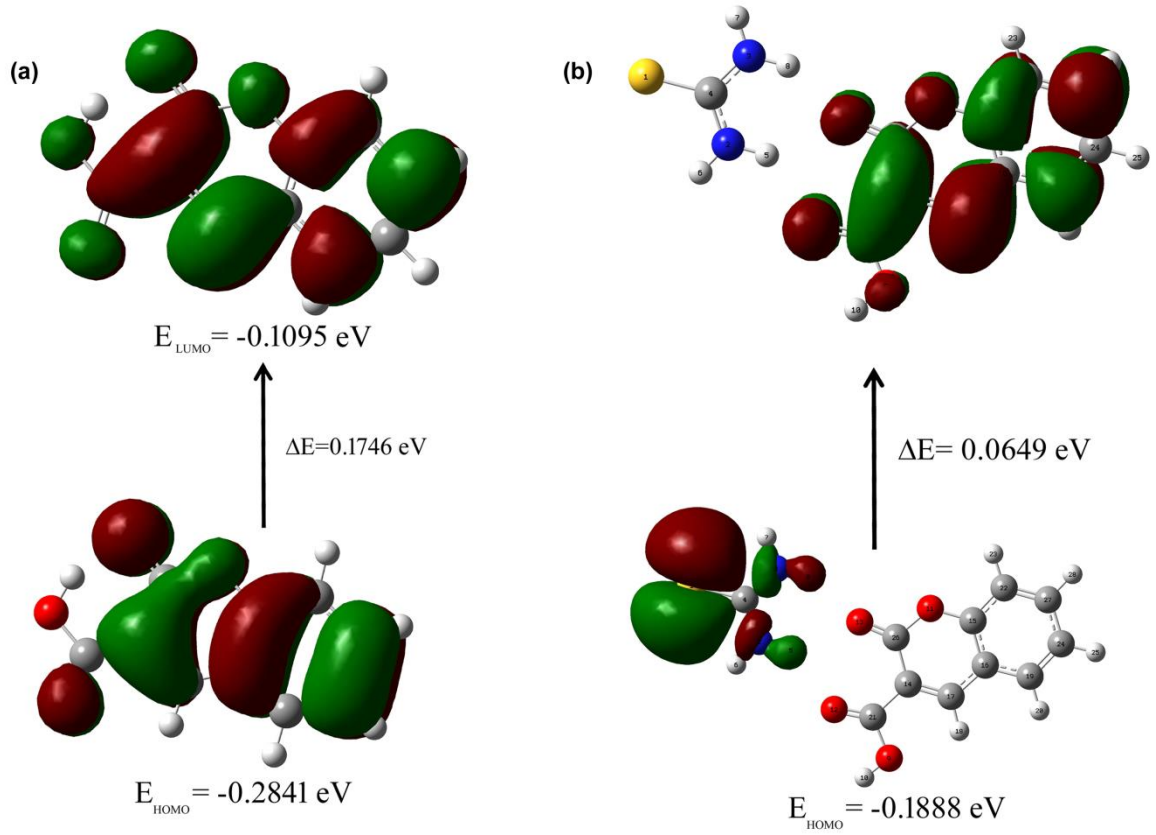


Figure S6 The HOMO-LUMO energy gap of **CU** and **CU:TH** calculated at the PBE1PBE theory level.

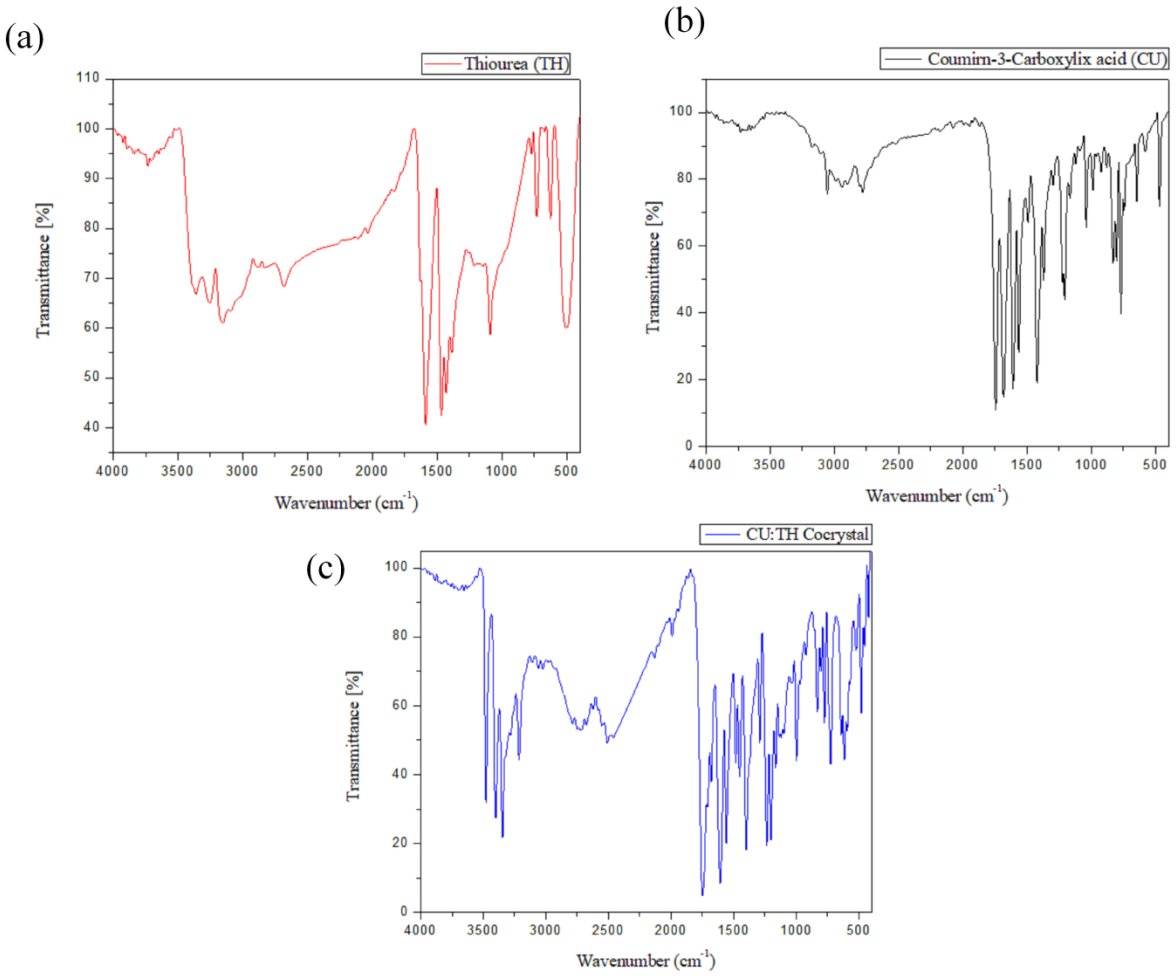


Figure S7 FT-IR Spectra of (a) thiourea (**TH**), (b) coumarin-3-carboxylic acid (**CU**), and (c) (**CU:TH**) co-crystal.

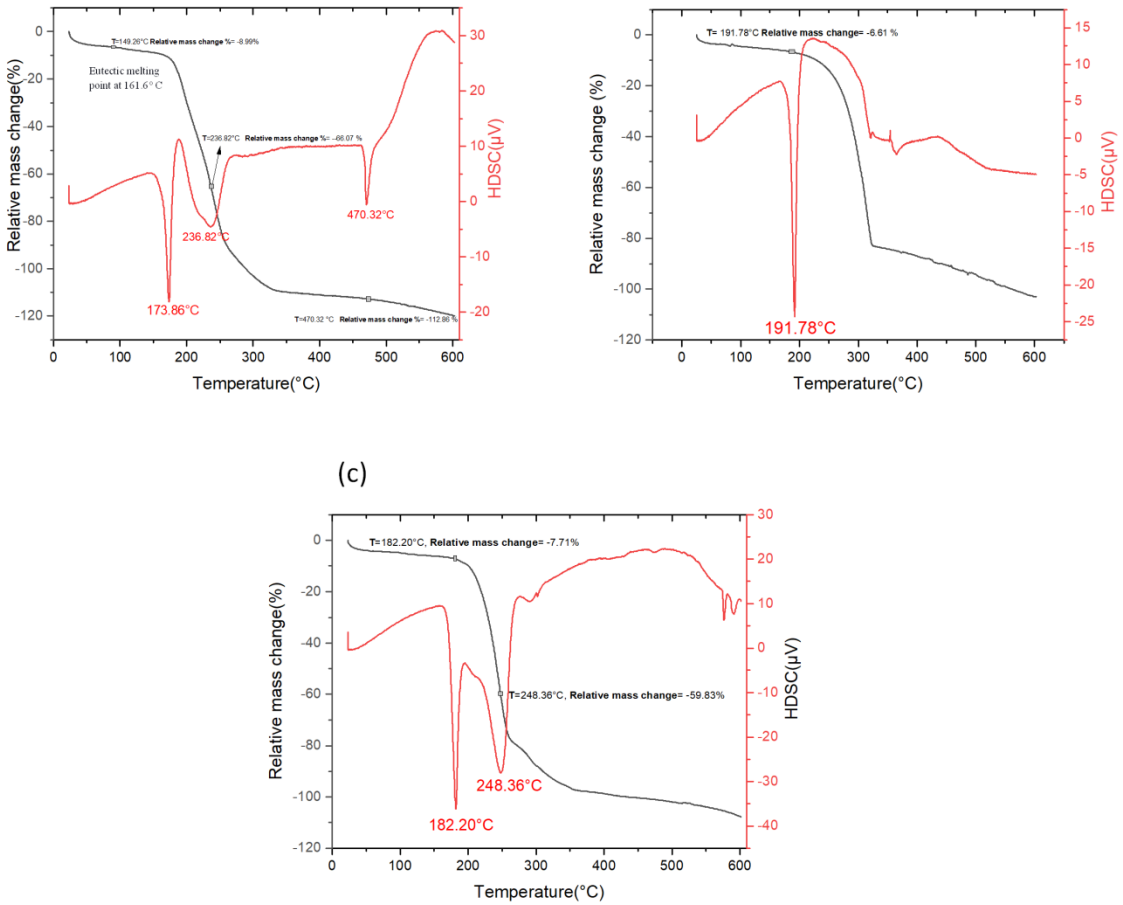


Figure S8 DSC/TGA spectra of (**a**) **CU:TH** co-crystal, (**b**) Pure **CU** and **(C)** pure **TH.**