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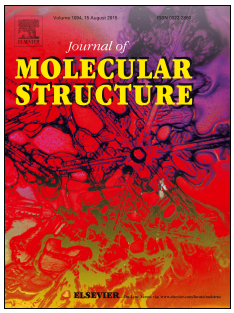

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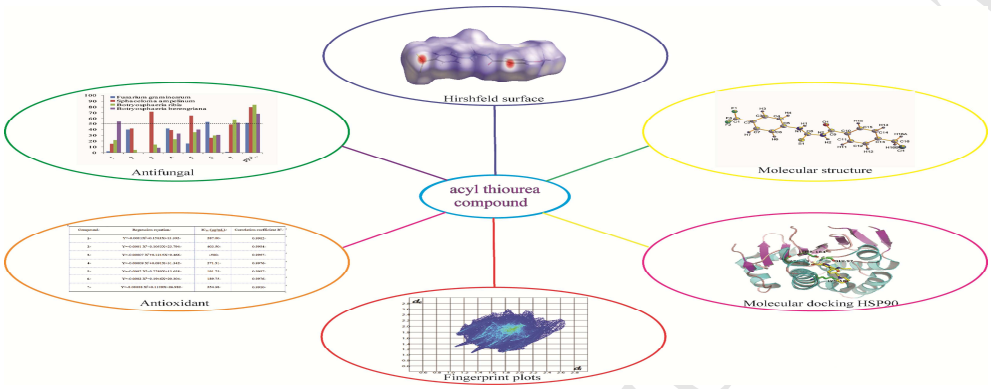
Graphical abstract: In the present study, a series of acyl thiourea derivatives (7 compounds) has been synthesized and characterized by some spectroscopic techniques. The molecular structure of five compounds was determined by a single crystal X-ray analysis. The intermolecular contacts of five crystal structures have been preformed based on the Hirshfeld surface and their associated 2D fingerprint plots. All the synthesized compounds were preliminarily screened for their *in vitro* anti-fungal and antioxidant activities. *In silico* molecular docking studies were performed to screen against heat shock protein HSP90.



Synthesis, characterization, and *in vitro* evaluation and *in silico* molecular docking of thiourea derivatives incorporating 4-(trifluoromethyl)phenyl moiety

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Abstract: A series of acyl thiourea derivatives bearing 4-(trifluoromethyl)phenyl moiety (7 compounds) has been synthesized and characterized by FT-IR, ^{1}H and ^{13}C NMR spectroscopy and elemental analyses. The molecular structure of five compounds (**2**, **4**, **5**, **6** and **7**) was determined by single crystal X-ray diffraction analysis. The crystal structures revealed that the carbonyl thiourea units in all determined compounds are mostly planar due in part to the formation of intramolecular N-H···O=C and C-H···S=C hydrogen bonds that form two S (6) rings. The intermolecular contacts of five crystal structures have been preformed based on the Hirshfeld surface and their associated 2D fingerprint plots. All the synthesized compounds were preliminarily screened for their *in vitro* anti-fungal activity. Especially, compounds **4**, **5** and **6** showed a good anti-fungal activity for four different kinds of fungi. Furthermore, all prepared thiourea derivatives were screened for antioxidant potential activity by DPPH free radical scavenging and the excellent activity were found compounds **5** and **6** with the IC_{50} value of 191.75 μg/mL and 189.75 μg/mL, respectively. *In silico* molecular docking studies were performed to screen the thiourea derivatives against heat shock protein HSP90.

Keywords: 4-(trifluoromethyl)phenyl moiety; antioxidant; thiourea derivatives; molecular docking; Hirshfeld surface; supermolecular chemistry

1. Introduction

Due to unique properties of fluorinated compounds, the incorporation of the trifluoromethyl group into such prototype molecules in place of a methyl group and a chlorine atom is now a well-accepted strategy in medicinal and structural chemistry [1]. First, chemicals with trifluoromethyl group have wide applications in medicine, agrochemicals and organic electronics. A. Bielenica and coworker reported fluoromethyl substituents on the benzene ring can improve antimicrobial potency [2]. And secondly, trifluoromethyl group

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of aromatic rings always increases the lipophilicity of the molecule [3-4]. More importantly, the compounds incorporation of the trifluoromethyl group can form, from the molecular structure point of view, the X-H···F hydrogen bonds that provide driving forces for self-assembly in the compounds.

Compounds possessing acyl thiourea as a structural motif (-CONHCSNH-) have significant importance in medicinal chemistry as many of them are known to exhibit interesting pharmacological properties such as antimicrobial [5-7], anticancer [8-10], antinociceptive [11-13], anticonvulsant [14], urease inhibitory [15, 16], antioxidant [17], anti-HIV [18], anti-inflammatory [19]. In addition, acyl thiourea compounds also possessed others applications such as heterocyclic synthesis intermediate, non-ionic surfactants [20], organocatalysts [20-25], metal coordination [26-30], and anion receptors [31-37]. And thiourea derivatives have attracted great attention due to the potential in antioxidant and anti-fungal activity in recently.

Free radical, an atom or molecule with an unpaired electron possessing an ability to plunder electron from stable surrounding compounds, leading to the change of cell structure, the destruction of the cellmembrane system and the variation of DNA, is considered as an important pathogenesis of various diseases. Less than 14 units free radical belong to the normal range for human. But free radical over the standard value will result in diseases such as cancers, hepatitis, diabetes and uremia. To counteract those negative effects, scavenging free radical is an attractive strategy. In previous studies, a diverse variety of antioxidants had been researched, including thiourea compounds [16, 38, 39]. However, the antioxidant activity of those compounds is far from meeting people's expectation, so the synthesis of acyl thiourea derivatives which possess a good antioxidant activity is highly needed.

Similarly, plant fungicidal disease is one of the most serious plant diseases which can lead to agricultural loss and reductions, and it is also closely relevant to food security in the world. Therefore, anti-fungal agents play a significant role in agricultural production. Unfortunately, some of them (arsenic, mercurial and organophosphate pesticides) are prohibited to use because they can be harmful to the atmosphere and soil. In the past decades, thiourea derivatives were widely applied to agriculture for their low toxicity, low residue and environmental friendly. The only drawback is that some thiourea compounds have not sufficient lipophilicity to enhance the rate of cell penetration and transport of a drug to an active site [40]. Hence, the continued efforts to design new anti-fungal agents with good biopotency, bioavailability and lipophilicity are the subjects of current medicinal chemistry research.

In this paper, we synthesized novel thiourea derivatives incorporating 4-(trifluoromethyl)phenyl moiety and evaluated them for *in vitro* anti-fungal and antioxidant activities. This study focuses on characterizing the structure of acyl thioureas incorporating trifluoromethyl group by the FTIR, NMR, X-ray analysis, Hirshfeld surface and 2D fingerprint plots technology and considers the anti-fungal and antioxidant activities of these compounds. Besides, the possible anticancer mechanism of thiourea derivatives by

molecular docking was studied in detail.

2. Experimental

2.1. Materials and methods

All the chemicals were commercially available from Sigma Aldrich (St Louis, MO, USA). The solvents were of laboratory reagent grade, and were used without further purification. Microwave syntheses were carried out using a BILON-CW-1000 microwave synthesizer with the appropriate absorption power settings. Elemental analyses were on an ELEMENTAR Vario EL □ elemental analyzer. The melting points were determined on a Cossim KER3100-08S apparatus. The FT-IR (KBr pellets) spectra were recorded in the 400-4000 cm^{-1} range using a Bruker EQUINOX 55 FT-IR spectrometer. ^{1}H NMR spectra were obtained in Dimethylsulfoxide-d_6 by using a Bruker 400 MHz spectrometer. ^{1}H NMR (400 MHz): internal standard solvent DMSO-d_6 (2.50 ppm from TMS): internal standard TMS; the splitting of proton resonances in the reported ^{1}H NMR spectra were remarked as s=singlet, d=double, t=triplet, dt=doublet triplet and m=multiple. Single crystal X-ray experiments were performed with Mo *Kα* radiation (λ=0.071073nm) with Bruker D-QUEST diffractometer.

2.2. Synthesis of thiourea derivatives

A solution of prepared substituted benzoyl chloride (1.4057-1.8904 g, 10 mmol) in dry tetrahydrofuran (40 mL) was added drop wise to a three-necked round-bottomed flask containing potassium thiocyanate (1.4577 g, 15 mmol). The mixtures were refluxed for approximately 2 hours at 60 □. A solution of 4-Aminotrifluorotoluene (1.5307 g, 9.5 mmol) in tetrahydrofuran (20 mL) was added and reacted in microwave heating 60-65 □ for about 90 seconds. The resulting mixture was pushed into 500 mL water and filtered off, washed with ethanol and dried in vacuo. The forming compounds were grown at room temperature from organic solvents for single crystals of compounds **2**, **4**, **5**, **6** and **7**.

2.2.1. Synthesis of 1-(benzoyl)-3-(4-trifluoromethylphenyl)thiourea, 1

White soild. Yield: 86%. mp. 137.8-138.5 □. Anal. Calc. for $C_{15}H_{11}F_3N_2OS$ (%): C, 55.55; H, 3.42; N, 8.64. Found: C, 55.50; H, 3.29; N, 8.65. FT-IR (KBr): v, cm^{-1} 3395 (N-H), 3208 (H-N), 2981(C-H, Ph), 1670 (C=O), 1522(C=C, Ph), 1267 (C-F), 780 (C=S). ^{1}H NMR (400 MHz, DMSO-d_6): δ, ppm 12.75 (s, 1H, NH), 11.73 (s, 1H, NH), 7.97-8.00 (m, 4H, Ar-H), 7.80 (d, J=8.44 Hz, 2H, Ar-H), 7.68 (t, J=7.41 Hz, 1H, Ar-H), 7.55 (t, J=7.70 Hz, 2H, Ar-H). ^{13}C NMR (101 MHz): δ, ppm 179.9 (C=S), 168.7 (C=O), 142.2 (Ar-C), 133.7 (Ar-C), 132.5 (Ar-C), 129.2 (2C), 128.9(2C), 126.5 (Ar-C), 126.3 (m, 2C), 125.1 (2C), 123.2 (CF_3).

2.2.2. Synthesis of 1-(4-methoxybenzoyl)-3-(4-trifluoromethylphenyl)thiourea, 2

White solid. Yield: 84%. mp. 154.4-156.1 □. Anal. Calc. for $C_{16}H_{13}F_3N_2O_2S$ (%): C, 54.23; H, 3.70; N, 7.91. Found: C, 54.21; H, 3.58; N, 7.93. FT-IR (KBr): v, cm^{-1} 3330 (N-H), 3208 (H-N), 2972 (C-H, Ph), 1668 (C=O), 1535 (C=C, Ph), 1259 (C-F), 780 (C=S). ^{1}H NMR (400 MHz, DMSO-d_6): δ, ppm 12.86 (s, 1H, NH), 11.54 (s, 1H, NH), 7.96-8.04 (m, 4H, Ar-H), 7.79 (d, J=8.49 Hz, 2H, Ar-H), 7.08 (d, J=8.91 Hz, 2H, Ar-H), 3.86 (s, 3H, OCH_3). The forming compound was grown at room temperature from diethyl ether for single crystal.

2.2.3. Synthesis of 1-(4-methylphenyl)-3-(4-trifluoromethylphenyl)thiourea, 3

White solid. Yield: 79%. mp. 154.9-156.5 □. Anal. Calc. for $C_{16}H_{13}F_3N_2OS$ (%): C, 56.80; H, 3.87; N, 8.28. Found: C, 56.78; H, 3.78; N, 8.28. FT-IR (KBr): v, cm^{-1} 3351 (N-H), 3208 (H-N), 2931 (C-H, Ph), 1679 (C=O), 1525 (C=C, Ph), 1254 (C-F), 772 (C=S). ^{1}H NMR (400 MHz, DMSO-d_6): δ, ppm 12.81 (s, 1H, NH), 11.63 (s, 1H, NH), 7.91-7.99 (m, 4H, Ar-H), 7.79 (d, J=8.59 Hz, 2H, Ar-H), 7.36 (d, J=8.06 Hz, 2H, Ar-H), 2.40 (s, 3H, CH_3). ^{13}C NMR (101 MHz, DMSO): δ, ppm 180.0 (C=S), 168.5 (C=O), 144.2 (Ar-C), 142.2 (Ar-C), 129.6 (Ar-C), 129.5(2C), 129.3 (2C), 126.8 (Ar-C), 126.2 (m, 2C), 125.0 (2C), 123.2 (CF_3), 21.6 (CH_3).

2.2.4. Synthesis of 1-(4-benzylchloride)-3-(4-trifluoromethylphenyl)thiourea, 4

White solid. Yield: 82%. mp. 167-169 □. Anal. Calc. for $C_{16}H_{12}ClF_3N_2OS$ (%): C, 51.55; H, 3.24; N, 7.51. Found: C, 51.53; H, 3.17; N, 7.49. FT-IR (KBr): v, cm^{-1} 3301 (N-H), 3198 (H-N), 2986 (C-H, Ph), 1674 (C=O), 1528 (C=C, Ph), 1264 (C-F), 774 (C=S). ^{1}H NMR (400 MHz, DMSO-d_6): δ, ppm 12.71 (s, 1H, NH), 11.75 (s, 1H, NH), 7.96-8.01 (m, 4H, Ar-H), 7.80 (d, J=8.40 Hz, 2H, Ar-H), 7.61(d, J=8.10 Hz, 2H, Ar-H), 4.86 (s, 2H, CH_2Cl). The precipitated thiourea was recrystallized from methanol-methylene chloride (1:2).

2.2.5. Synthesis of 1-(4-fluorobenzene)-3-(4-trifluoromethylphenyl)thiourea, 5

White solid. Yield: 83%. mp. 129.9-131.1 □. Anal. Calc. for $C_{15}H_{10}F_4N_2OS$ (%): C, 52.63; H, 2.94; N, 8.18. Found: C, 52.65; H, 2.78; N, 8.28. FT-IR (KBr): v, cm^{-1} 3292 (N-H), 3023 (H-N), 3000 (C-H, Ph), 1674 (C=O), 1526 (C=C, Ph), 1264 (C-F), 773 (C=S). ^{1}H NMR (400 MHz，DMSO-d_6): δ, ppm 12.68 (s, 1H, NH), 11.77 (s, 1H, NH), 8.06-8.10 (m, 2H, Ar-H), 7.97 (d, J=8.04 Hz, 2H, Ar-H), 7.80 (d, J=8.55 Hz, 2H, Ar-H), 7.39 (t, J=8.83 Hz, 2H, Ar-H). Colourless crystal suitable for X-ray diffraction study was got from ethyl acetate.

2.2.6. Synthesis of 1-(4-chlorobenzene)-3-(4-trifluoromethylphenyl)thiourea, 6

White solid. Yield: 81%. mp. 135.8-136.5 □. Anal. Calc. for $C_{15}H_{10}F_3N_2ClOS·0.5$ (H_2O) (%): C, 48.99; H, 3.01; N, 7.62. Found: C, 48.98; H, 2.90; N, 7.64. FT-IR (KBr): v, cm^{-1} 3246 (N-H), 3195 (H-N), 2940 (C-H, Ph), 1677 (C=O), 1266 (C-F), 782 (C=S). ^{1}H NMR (400 MHz，DMSO-d_6): δ, ppm 12.63 (s, 1H, NH), 11.79 (s, 1H, NH), 7.95-8.01 (m, 4H, Ar-H), 7.79 (d, J=8.59 Hz, 2H, Ar-H), 7.61-7.64 (m, 2H, Ar-H). This compound was recrystallized from ethyl acetate in two weeks.

2.2.7. Synthesis of 1-(4-nitrobenzene)-3-(4-trifluoromethylphenyl)thiourea, 7

Cyan solid. Yield: 56%. mp. 154.8-155.2 ℃. Anal. Calc. for $C_{15}H_{10}F_3N_3O_3S·0.5$ (C_2H_5OH) (%): C, 48.98; H, 3.34; N, 10.71. Found: C, 48.94; H, 3.19; N, 10.65. FT-IR (KBr): v, cm^{-1} 3293 (N-H), 3034 (H-N), 2942 (C-H, Ph), 1699 (C=O), 1265 (C-F), 783 (C=S). ^{1}H NMR (400 MHz，DMSO-d_6): δ, ppm 12.51 (s, 1H, NH), 12.09 (s, 1H, NH), 8.36 (d, J=8.70 Hz, 2H, Ar-H), 8.18 (d, J=8.75 Hz, 2H, Ar-H), 7.97 (d, J=8.39 Hz, 2H, Ar-H), 7.81 (d, J=8.61 Hz, 2H, Ar-H), 4.36 (t, J=5.00 Hz, 1H, OH), 3.44 (td, J=6.96, 11.85 Hz, 2H, CH_2), 1.06 (t, J=6.99 Hz, 3H, CH_3). The solid product obtained was recrystallized from ethanol to afford cyan diamond crystalline solid.

2.3. X-ray crystallography

Crystals were obtained by slow evaporation method and subjected to X-ray diffraction. SHELXS 97 was used for structure solution and refinement using the direct methods. All the non-hydrogen atoms were obtained by the full-matrix-block least-squares method on F^2 with anisotropic thermal parameters [41]. The hydrogen atoms were added according to the theoretical models. All molecular plots and packing diagrams were drawn using Diamond 3.0 and additional metrical data were calculated using PLATON [42]. In addition, the analysis of molecular crystal structures (compounds **2**, **4**, **5**, **6** and **7**) using CrystalExplorer 3.1 program has gained the Hirshfeld surfaces their 2D fingerprint plots [43]. This work made the intermolecular interactions to visualize clearly.

2.4. Evaluation of anti-fungal activity

Those thiourea derivatives (**1-7**) were screened for their in vitro anti-fungal activity by disk diffusion method. Anti-fungal activity of the synthesized thiourea derivatives was determined by the mycelial growth rate method. Each thiourea derivative was dissolved in dimethyl sulfoxide (DMSO), which was added into the sterile PDA equably. The same volume of mixed PDA was poured into 90 mm petri dishes in the same volume quintuplicate. The concentration of tested thiourea compounds were 100 mg/L. And then, the phytopathogen was inoculated in each plate of 8 mm diameter. The diameter of the *Fusarium*

graminearum and *Sphaceloma ampelinum* on the PDA plate was surveyed after 72 h, while they needed 120 h for *Botryosphaeria ribis* and *Botryosphaeria berengriana.* Pyrimethanil was used as negative control (as standard drug). 100 μL of DMSO was added to 900 μL of PDA medium to reveal the effect of DMSO. The inhibition rate of synthesized thiourea compounds was measured using the formula.

Inhibition rate(%)= [(diameter of control-diameter of test compound)/(diameter of control-8)]×100.

2.5. DPPH free radical scavenging assay

The antioxidant activity of those thiourea derivatives (**1-7**) was measured by DPPH scavenging assay according to these papers [44-46]. There was 10 mg of thiourea derivative that it was dissolved in 2 mL DMSO as a stock solution. From the stock, 20 μL, 40 μL, 60 μL, 80 μL and 100 μL were taken before adding in 880 μL, 860 μL, 840 μL, 820 μL and 800 μL of methanol, respectively. 100 μL of DPPH was added into the mentioned solutions and the mixed solutions were incubated 30 min at 37 ℃. For this step, 100 μg/mL, 200 μg/mL, 300 μg/mL, 400 μg/mL and 500 μg/mL were formed as the final concentration of the thiourea derivative. Different volumes of DMSO were added corresponding methanol to form 900 mL solution as blank control groups, which was to reveal the scavenging effect of DMSO. After incubation, the absorption was measured by spectrophotometer at 517 nm. Ascorbic acid (Vitamin C) was used as the standard compound. Results were expressed as percent inhibition, calculated from the formula [47].

Inhibition(%) =[100-(Abs of test compound/Abs of control)×100]

2.6. Molecular docking

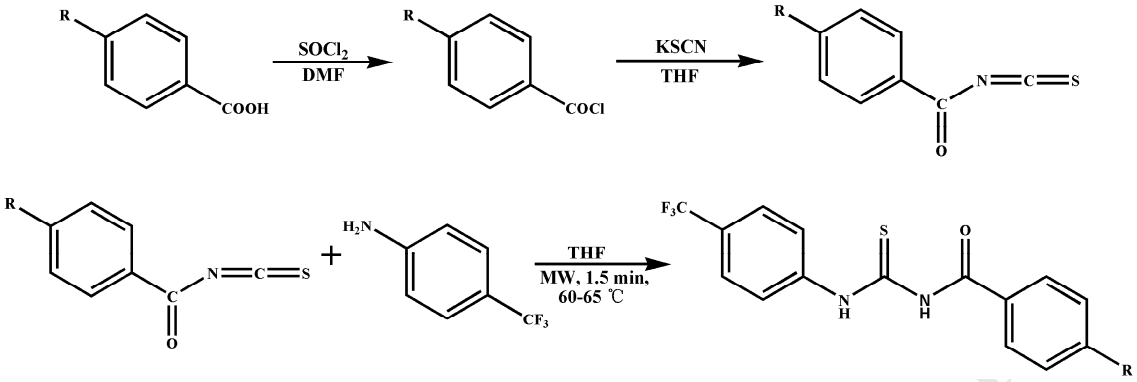
In molecular docking studies were carried out to screen against HSP90 by Autodock 4.2.6. The structure of the protein was downloaded from RSCB Protein Data Bank. The 2 dimension structure of thiourea derivatives were obtained from Chem Office 2004 and ZINC Website. The 2D structure was turned into 3D format, and saved as PDB file. In autodock program, 7 acyl thiourea derivatives were treated with energy minimization. The protein was prepared by extracting ligand substructures and generating some active pockets. The prepared protein was saved as the formation of PDBQT. Then, the molecular docking will be carried out by the program. After docking, the binding energy and the binding site will be analyzed by the program of PyMOL 1.7.

3. Results and discussion

3.1. Synthesis

An robust and efficient microwave assisted solution phase parallel synthesis protocol [48] was performed because the synthesized time was drastically reduced. Acyl thiourea derivatives incorporating

4-(trifluoromethyl)phenyl moiety (**1-7**) were obtained from the corresponding carboxylic acid, potassium thiocyanate and aminotrifluorotoluene in tetrahydrofuran (**Scheme 1**).



R=H(1), OCH_3(2), CH_3(3), CH_2Cl(4), F(5), Cl(6), NO_2(7)

Scheme 1. Synthesis of acyl thiourea derivatives.

3.2. Spectroscopy

The FT-IR spectrum of the synthesized thiourea compounds (**1-7**) exhibited a broad band around 3300 cm^{-1} (-NH) due to the strong electron-withdrawing group which is at the para position of N-benzene ring ($-CF_3$) and the formation of the C=O···H-N intramolecularhydrogen bond. The C=O···H-N intramolecular hydrogen bond generated a red-shift of the υ(N-H) mode compared with the others υ(N-H) stretching. A very strong (2991-3208 cm^{-1}) band suggested the formation of the group of H-N(CS). A strong (1667-1692 cm^{-1}) and a medium intensity (772-783 cm^{-1}) bands have been observed in the FT-IR spectrum, which can be ascribed to the stretching vibration of C=O and C=S in all the compounds, respectively. It is interesting that the υ(C=S) of the thiourea compound is assigned to the absorption appeared at 1325 cm^{-1} in a reported paper [49]. This phenomenon having such a low frequency suggested that the formation of C=S···H-X hydrogen bonds make to generate a red-shift in this paper, as determined from the X-ray analysis in the title species [50-53]. Meanwhile, the strong (1667-1692 cm^{-1}) bands corresponds to the υ(C=O) mode in all synthesized compounds, which is similar in frequency to the C=O···H-X hydrogen bonding found in many small-molecular studies [54]. Strong bonds could be observed in the 1602-1611 cm^{-1} in the synthesized compounds, which were mainly originated in the C-C stretching vibrations of the benzene rings. The amide and thiourea groups present a characteristic bond in the 1500-1600 cm^{-1} in the IR spectrum, which originated by the N-H deformation mode. For the synthesized compounds, 1525-1552 cm^{-1} are assigned to this mode. Medium intensity bands expected to appear in the 1400-1300 cm^{-1} region in the vibrational spectra of thiourea compounds are assigned to the C-N stretching vibration. However, the situation becomes complicated because C-N stretching modes are usually coupled in symmetric and antisymmetric motions [55, 56]. Therefore, the NCN antisymmetric and symmetric stretching vibrations of the title compounds are

assigned to 1350-1342 cm^{-1} and 1171-1156 cm^{-1} in the infrared spectra of compounds **1-7**, respectively. The C-F stretching modes originate medium intensity absorptions in the 1123-1149 cm^{-1}.

In the ^{1}H NMR spectrum of all synthesized thiourea compounds, the protons of the two -NH groups were obtained as single peak at 12.03-12.88 and 11.54-12.09 ppm, respectively. The protons of benzene rings were observed at 7.01-8.37 ppm. In the ^{13}C NMR spectrum of compounds **1** and **3**, the chemical shifts of carbons pertaining to CONH and CSNH moieties of the substituted thiourea ligand resonate at 168.66 ppm and 179.94 ppm for **1**, 168.48 ppm and 179.97 ppm for **3**. Duo to the impact of F element, the carbon atoms of the benzene rings will generate the coupling phenomenon. The chemical shifts of carbons of 4-amino-trifluoro cresyl are mostly identical in the compounds **1** and **3** (see **Fig. S8 and Fig. S9** in the Electronic Supplementary Material of this paper).

3.3. Three-dimensional structures

Compounds **2**, **4**, **5**, **6** and **7** crystallize in the P-1, P-1, P-1, $P2_12_12$ and C 1 2/c 1 spatial group with an only nonequivalent molecule adopting the S-shaped conformation, respectively, as is shown in Fig. 1.

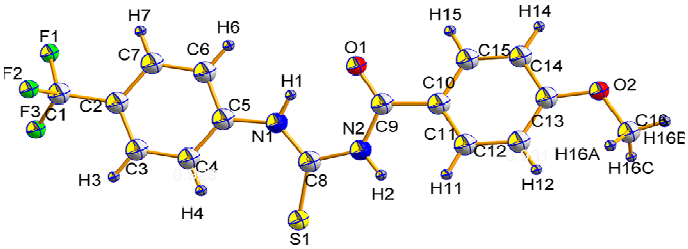
The C-S and C-O bond lengths both show the expected double-bond character in all the five molecular structures (**2**, **4**, **5**, **6** and **7**). The C(O)-N1, C(S)-N1 and C(S)-N2 bond lengths (see **Tables S1-S5**) indicate the partial double-bond character typical for this type of compounds which are in good agreement with the report related trifluoromethyl substituted benzene species [57-62]. Compared to the unsubstituted [63] 1-benzoyl-3-phenylthioureas, the trifluoromethyl substitution at C4 does not result in any significant effect on these bond lengths which are consistent with the paper reported by M.K. Rauf. [57].

The five molecular structures (**2**, **4**, **5**, **6** and **7**) consist of similar thiourea cores (-CONHCSNH-) with different substitution groups. The cores are mostly planar with O-C-N-C and C-N-C-S torsion angles of 1.90° and 172.89° for **2**, -5.50° and -171.26° for **4**, -4.01° (molecular □), 4.79° (molecular □) and -166.65° (molecular □), 173.94° (molecular Ⅱ) for **5**, 0.28° and 175.06° for **6**, 4.97° and 175.06° for **7** as well. The crystal structure of compounds **2**, **4**, **5**, **6** and **7** along with their crystal packing diagrams are depicted in **Fig. 1**. Data collections and structure refinement details are summarized in **Table 1**. Intra- and intermolecular hydrogen bond parameters are listed in **Table 2**. All the selected torsion angles and lengths of all acyl thiourea compounds are shown **Tables S1-S5**.

The dihedral angle between the benzene ring of p-trifluoromethylaniline (Ring A) and the benzene ring of substituted benzoyl chloride (Ring B) are 10.45°, 40.06°, 10.91°, 2.63° and 8.55° for compounds **2**, **4**, **5**, **6** and **7**, respectively. The compounds **2**, **4**, **5**, **6** and **7** of the corresponding angles with respect to the (N/C/S/N/C/O) plane are 11.98°, 28.09°, 20.66°, 30.75° and 29.89° for the Ring A and 21.92°, 67.13°, 31.23°,

28.82° and 32.39° for the Ring B, respectively.

It can be clearly seen that both intra- and intermolecular hydrogen bonds are presented in all of those five acyl thiourea derivatives. Intramolecular N-H···O and C-H···S hydrogen bonds form two S (6) rings and contribute to the relative planarity of the acyl thiourea moieties [64, 65], which are quite different from the others trifluoromethyl substitution at the C2 or C3 [57-62]. In addition, the two molecules of compound **2** have two different orientations and a pair of intramolecular C-H···F (-x, 1-y, 1-z) interactions form a second form of dimer in the cell unit. The crystal packing of compound **4** is stabilized by intermolecular N-H···S (-x, -y, -z) hydrogen bonds, wherein atom S act as acceptor forming R_2^2 (8) ring. The packing diagrams of compound **5** shows the alternate N-H···S(1-x, 1-y, -z) action is bolstered by a C-H···S (1-x, 1-y, -z) contact generating an R_2^1 (7) ring. The crystal packing of compound **6** demonstrates intermolecular N-H···O (x, y, 1+z) hydrogen bonds form D motif. An intramolecular C-H···F and an intermolecular C-H···F (-1+x, y, 2+z) interactions form an S (5) ring and a second form of dimer, respectively. Intramolecular N-H···O actions form C (4) motif, and the alternate N-H···O (x, -1+y, z) actions are bolstered by a C-H···O (x, y, -1+z) contact generating an R_2^1 (7) ring in the packing diagrams of compound **7**. The mode of the crystal packing of compounds **6** and **7** belong to a part of supermolecular chemistry. Compared with the reported trifluoromethyl substitution thiourea derivatives [57-62], compounds 5 and 6 were stabilized by forming intermolecular hydrogen bonds with water and ethanol molecular, respectively. The C-H···F hydrogen bonds to provide driving forces for self-assembly were formed in compounds **2** and **6** due to the incorporation of the trifluoromethyl group. Geometrical parameters of the C-H···π interactions for compounds **2** and **4** were calculated by PLATON and the result was shown in **Table S7**.



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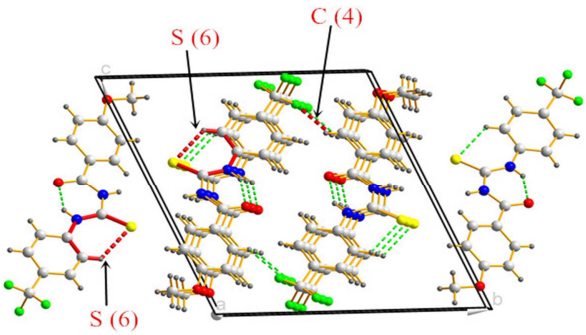
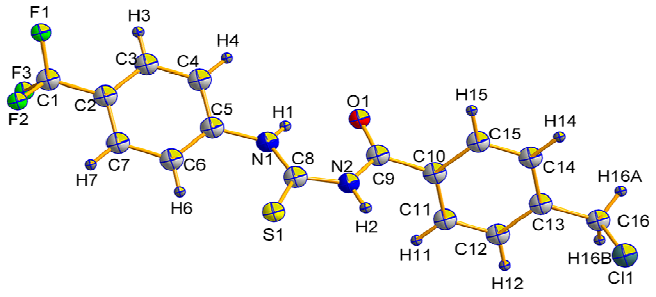


The crystal packing of **2**



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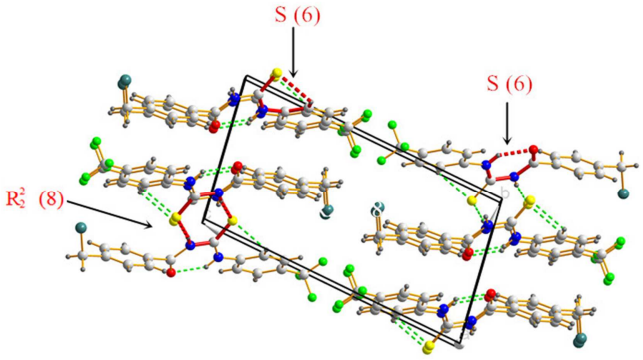
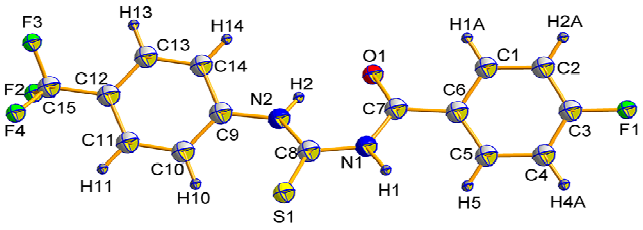


The crystal packing of **4**



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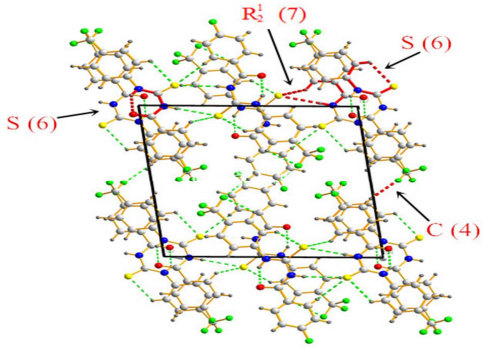
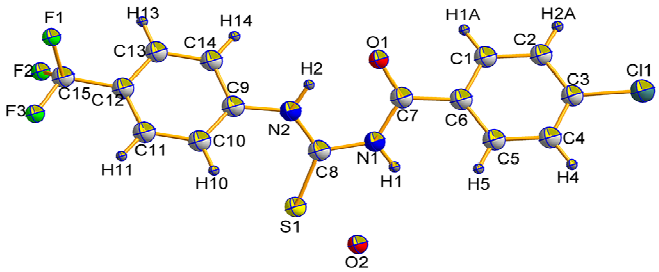


The crystal packing of **5**



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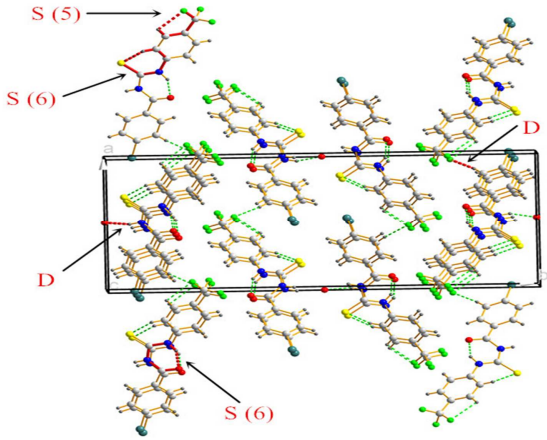
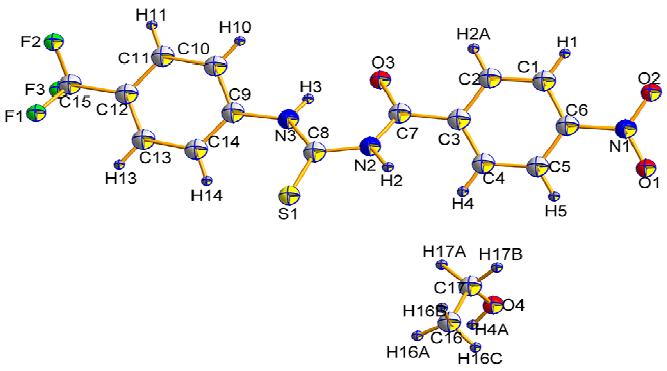


The crystal packing of **6**



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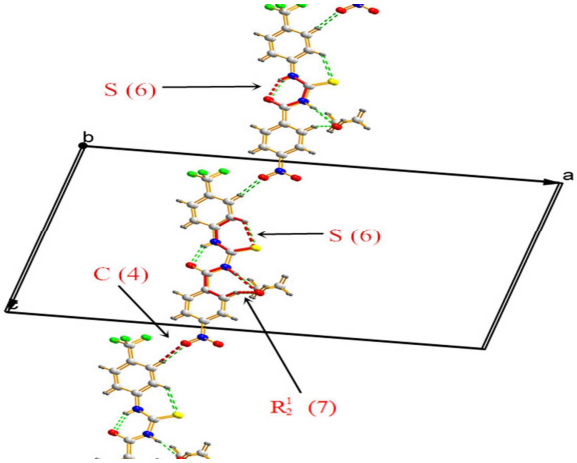


The crystal packing of **7**

Fig. 1. The crystal structures of compounds **2**, **4**, **5**, **6** and **7**. Displacement ellipsoids are drawn at the 50% probability level.

Table 1

Crystal data and structure refinement parameters for compounds **2** and **4**-**7**.

Compound	Compound **2**	Compound **4**	Compound **5**	Compound **6**	Compound **7**
Chemical formula	$C_{16}H_{13}F_3N_2O_2S$	$C_{16}H_{12}ClF_3N_2OS$	$C_{15}H_{10}F_4N_2OS$	$C_{15}H_{10}F_3N_2ClOS$ ·0.5 (H_2O)	$C_{15}H_{10}F_3N_3O_3S$· 0.5 (C_2H_5OH)
Formula weight	354.35	372.79	342.31	366.76	392.35
Temperature (K)	296(2)	296(2)	296(2)	296(2)	296(2)
Wavelength (Å)	0.71073	0.71073	0.71073	0.71073	0.71073
a (Å)	4.8291(18)	6.663(3)	7.807(2)	11.776(4)	30.380(7)
b (Å)	13.259(5)	8.250(4)	13.593(4)	28.887(9)	7.5658(17)
c (Å)	13.880(5)	15.438(6)	14.312(4)	4.6167(14)	15.421(3)
α (degree)	114.099(6)	78.748(8)	81.965(4)	90.00	90.00
β (degree)	95.442(7)	80.811(7)	85.382(4)	90.00	102.474(4)
γ (degree)	90.250(7)	80.398(8)	81.585(5)	90.00	90.00
Volume ($Å^3$)	806.7(5)	813.6(6)	1484.9(7)	1570.5(8)	3460.9(13)
Z	2	2	4	4	8
Dc (Mg/m^3)	1.459	1.522	1.531	1.551	1.506
Crystal system	Triclinic	Triclinic	Triclinic	Orthorhombic	Monoclinic
Space group	P-1	P-1	P-1	P2(1)2(1)2	C 1 2/c 1
Index ranges	$-5 \le h \le 5$, $-15 \le k \le 14$, $-12 \le l \le 16$	$-7 \le h \le 7$, $-8 \le k \le 9$, $-18 \le l \le 17$	$-9 \le h \le 8$, $-16 \le k \le 13$, $-17 \le l \le 17$	$-14 \le h \le 10$, $-33 \le k \le 34$, $-5 \le l \le 5$	$-5 \le h \le 5$, $-15 \le k \le 14$, $-12 \le l \le 16$
Absorption Coefficient (μ)	0.244	0.400	0.266	0.415	0.243
F (000)	364	380	696	744	1608
Theta range for data collection (^{o})	1.68 to 25.10	1.36 to 25.10	1.44 to 25.10	1.41 to 25.09	1.37 to 27.20
Reflections collected	4004	4020	7416	7782	9515
Independent reflections	2840	2860	5237	2801	3749
R (int)	0.0366	0.0390	0.0215	0.0819	0.0452

Goodness-of-fit on F^2 (S)	1.072	1.025	1.029	1.053	1.000
R factor (%); R_1, R_2	0.1358, 0.0967	0.1284, 0.0717	0.1318, 0.0718	0.1024, 0.0741	0.1464, 0.0710

Table 2

The intermolecular and intramolecular hydrogen bonds for compounds **2**, **4**, **5**, **6** and **7**. Hydrogen-bond geometry: Distance, Å; angle, °.

Compound	Donor-Hydrogen··· Acceptor	D-H (Å)	H···A (Å)	D··· A (Å)	D-H···A (°)
2	N1-H1···O1	0.8600	1.9000	2.637(7)	142.00
	C4-H4···S1	0.9300	2.5400	3.202(7)	128.00
	C15-H15···F1[a]	0.9300	2.4900	3.262(8)	140.00
4	N1-H1···O1	0.8600	1.9600	2.672(6)	140.00
	N2-H2···S1[b]	0.8600	2.6000	3.451(5)	172.00
	C6-H6···S1	0.9300	2.6400	3.200(6)	120.00
5	N1-H1···S2[d]	0.8600	2.8700	3.711(5)	167.00
	N2-H2···O1	0.8600	1.8900	2.616(6)	141.00
	N3-H3···S1[d]	0.8600	2.8500	3.705(5)	173.00
	N4-H4···O2	0.8600	1.8600	2.588(6)	142.00
	C2-H2A···F4[f]	0.9300	2.4800	3.390(9)	167.00
	C5-H5···S2[d]	0.9300	2.8600	3.509(6)	128.00
	C10-H10···S1	0.9300	2.7100	3.234(6)	117.00
	C17-H17···F8[c]	0.9300	2.4500	3.364(8)	166.00
	C20-H20···S1[d]	0.9300	2.7800	3.539(6)	140.00
	C25-H25···S2	0.9300	2.6300	3.216(5)	121.00
	C26-H26···F8	0.9300	2.4100	2.729(7)	100.00
6	N1-H1···O2[f]	0.8600	2.2200	3.008(6)	152.00
	N2-H2···O1	0.8600	1.8900	2.600(7)	139.00
	C2-H2A···F3[g]	0.9300	2.5200	3.443(8)	172.00

	C10-H10···S1	0.9300	2.7300	3.222(7)	114.00
	C11-H11···F3	0.9300	2.4200	2.741(8)	100.00
7	N3-H3···O3	0.8600	1.9000	2.638(4)	142.00
	N2-H2···O4	0.8600	2.3900	3.217(14)	161.00
	N2-H2···O4^{h}	0.8600	2.3200	3.174(13)	170.00
	C4-H4···O4^{h}	0.9300	2.5300	2.972(13)	110.00
	C5-H5···O1	0.9300	2.4500	3.244(6)	144.00
	C13-H13···O2^{g}	0.9300	2.4400	3.326(6)	160.00
	C14-H14···S1	0.9300	2.6800	3.239(5)	119.00

Symmetry equivalent position: (a) -x, 1-y, 1-z; (b) -x, -y, -z; (c) 1+x, -1+y, z; (d) 1-x, 1-y, -z; (e) -1+x, y, 2+z; (f) x, y, 1+z; (g) x, y, -1+z; (h) x, -1+y, z.

3.4. Hirshfeld Surface Analysis

Hirshfeld surface analysis and fingerprint plots were carried out for the purpose of studying the nature of the intermolecular contacts and their quantitative contributions to the supramolecular assembly of the crystal structure of the synthesized compounds [66-68]. The 3D d_{norm} Hirshfeld Surface for compounds **2**, **4**, **5**, **6** and **7** are represented in the **Fig. 2**, where the value of d_{norm} is defined for the equtation [69]:

$$d_{norm}=(d_i- r_i^{vdW})/ r_i^{vdW} +(d_e+ r_e^{vdW})/ r_e^{vdW}.$$

The color of blue, white and red on Hirshfeld Surface is represented the d_{norm} value of positive, zero and negative, and those colors implied the intermolecular contacts are longer, equal or shorter than van der Waals radii (r^{vdW}), respectively. As the **Fig. 2** shows, the most intense red regions occur near to the F atom and the C-H group where the F atom can be served as acceptor and the C atom can be served as donor in the C-H···F hydrogen bond of compound **2,** such as illustrated in the **Fig.1** (The crystal packing of 2) and discussed in the three-dimensional structures analysis. The red regions of the C=S group and the N-H group are assigned to strong C-H···S hydrogen bonds, theother two red regions represent C···S contacts. TheC=S group appear to two red regions that is due to C-H···S=C and N-H···S=C hydrogen bonds in the compound **5** (**Fig.1.** the crystal packing of **5** and **Table 2**). It is observed that hydrogen atom of the amide group and fluorine atom are involved in interaction contacts, resulting in red regions in the Hirshfeld surface of compound **6** (**Fig.1.** the crystal packing of **6** and **Table 2**). As can be seen in the Hirshfeld surface of the compound **7,** the red region appear near to the O atom of the nitro group which can be assigned to C-H···O hydrogen bonds (**Fig.1.** the crystal packing of **7** and **Table 2**).

The fingerprint points of the main intermolecular contacts for all five molecules are shown in the **Fig. S10A**. The S···H, F···H, O···H and H···H contacts are presented in the **Fig. S10B, C, D** and **E**, respectively. For compound **2**, the F···H contacts, with sharp pairs of spikes centered near a (d_e+d_i) sum of 2.4 Å, correspond to C-H···F hydrogen bond (**Fig. S10C**). The S···H and O···H contacts have pairs of spikes that centered near a (d_e+d_i) sum of 2.8 and 2.7 Å, respectively. The shortest contacts correspond to the very close H···H contacts, showing a spike centered near a (d_e+d_i) sum of 2.6 Å. The compounds **6** and **7** are quite different from the others compounds because there are some contacts with an asymmetric pairs of spikes. For compound **6**, the O···H contacts have an asymmetric pairs of spikes, indicating O···H and H···O contacts with different (d_e+d_i) distances near 2.1 and 2.5 Å, respectively. From the Hirshfeld surface analysis and fingerprint plots point of view, the hydrophobic interactions together with the hydrogen bonds provide driving forces for self-assembly in the five compounds.

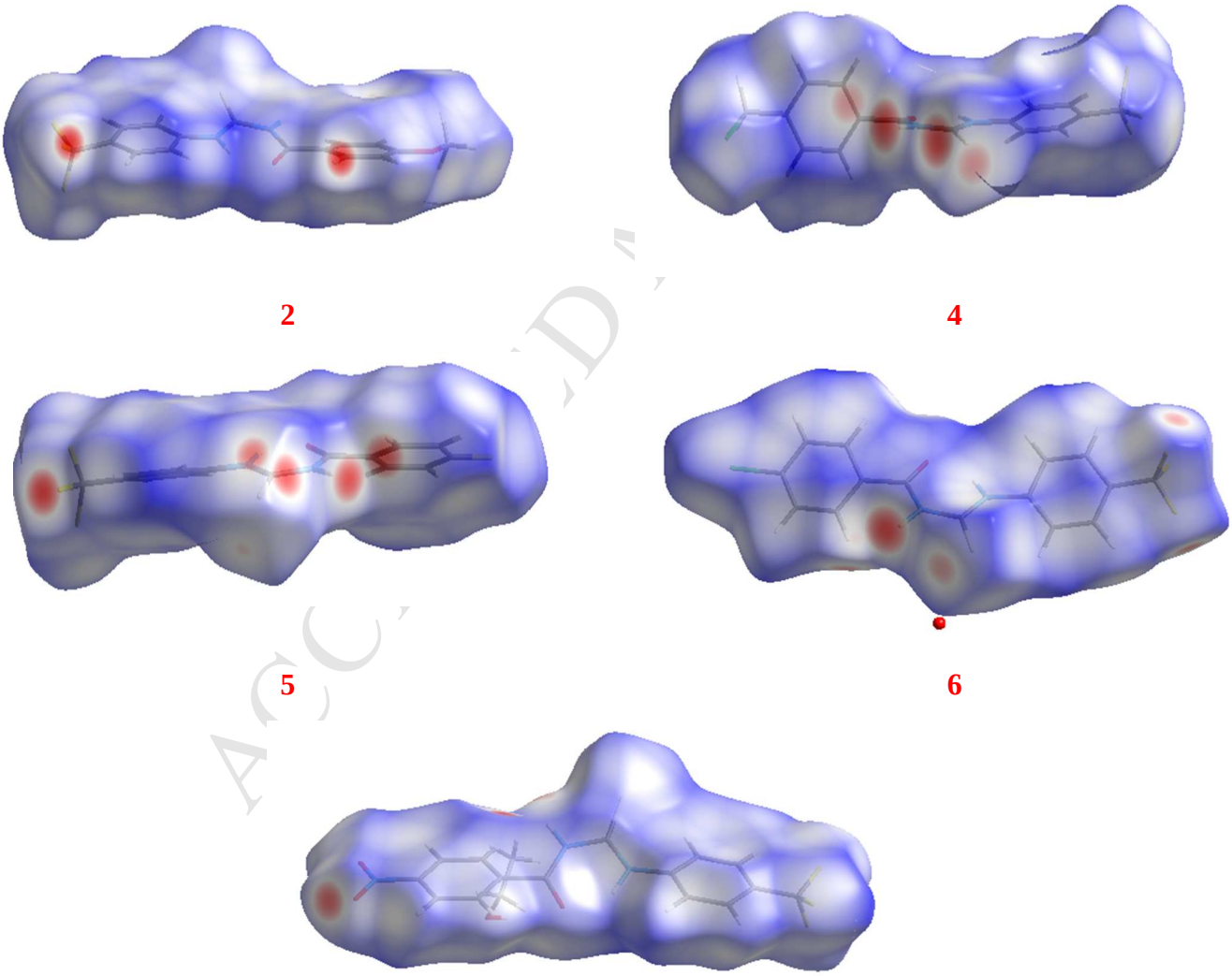
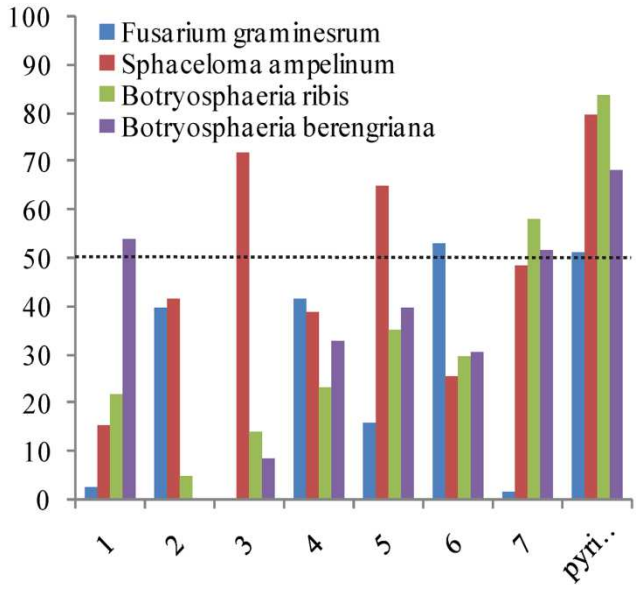


Fig. 2 Views of the Hirshfeld surface for 5 crystal compounds with thermal ellipsoids plotted at 50% probability level.

3.5. Anti-fungal

Anti-fungal activity of thiourea derivatives incorporating 4-(trifluoromethyl)phenyl moiety was measured by the disk diffusion method against four strains which include *Fusarium graminearum*, *Sphaceloma ampelinum*, *Botryosphaeria ribis* and *Botryosphaeria berengriana*. The result of anti-fungal experiment is listed in **Fig. 3**. Compounds **1** (53.90%), **3** (71.70%) and **7** (58.23%) exhibited a better inhibition effect than other candidates for *Botryosphaeria berengriana*, *Sphaceloma ampelinum* and *Botryosphaeria ribis*, respectively. Note that compound 6 (53.22%) against *Fusarium graminearum* had a better inhibition effect than the standard drug (51.1% for pyrimethanil). The inhibition rate of compound **2** (-0.61%) against *Botryosphaeria berengriana* indicated the compound does not have effect on the fungal, as well as compound **3** (-0.87%) against *Fusarium graminearum*. Those negative values may be the operating errors. Compounds **4**, **5** and **6** possess a better inhibition activity for four different kinds of fungi than compounds **1** [17], **2**, **3** and **7**, comprehensively. That indicates incorporation of halogen atom(s) within the thiourea derivatives is able to enhance the lipophilicity of the medicines which is helpful for the efficacy of anti-fungal.



--- 50% inhibition

Fig. 3. Inhibition rate of 7 thiourea derivatives. Each value represents a mean (n=5).

3.6. DPPH assay

The result of scavenging free radical of 7 compounds using DPPH method is shown in **Table 3**. DPPH in its radical from has an absorbance at 517 nm which eventually disappear when it is reduced by the antioxidant compounds. In those regression equations, X represents the concentration of synthesized thiourea derivatives which range from 100 μg/mL to 500 μg/mL. However, Y exhibits the rate of scavenging free radical. The excellent activities are found for compounds **5** and **6** with the IC_{50} value of 191.75 μg/mL

and 189.75 μg/mL, respectively, which are lower than the value of 250 μg/mL in the report [70]. Ascorbic acid exhibited a strong antioxidant activity (much lower 100 μg/mL) as a positive control.

Table 3

The antioxidant activity of synthesized thiourea derivatives.

Compound	Regression equation	IC_{50} (μg/mL)	Correlation coefficient R^2
1	$Y=-0.0001X^2+0.1563X+13.392$	287.00	0.9962
2	$Y=-0.0001\ X^2+0.1053X+23.794$	403.50	0.9994
3	$Y=-0.00007\ X^2+0.1215X+0.466$	›500	0.9967
4	$Y=-0.00009\ X^2+0.091X+31.342$	271.51	0.9976
5	$Y=-0.0002\ X^2+0.2280X+13.658$	191.75	0.9997
6	$Y=-0.0002\ X^2+0.1946X+20.304$	189.75	0.9976
7	$Y=-0.00008\ X^2+0.1109X+26.982$	254.38	0.9910

3.7. Molecular docking

It was reported that acyl thiourea derivatives possess excellent pharmaceutical activities like antibacterial [5-7], anti-fungal [8-10], antioxidant [17] and anti-HIV [18] etc. In recent years, acyl thiourea compounds have developed a new and important apply--anticancer. Therefore, docking was implemented to screen the acyl thiourea compounds against HSP90 enzyme.

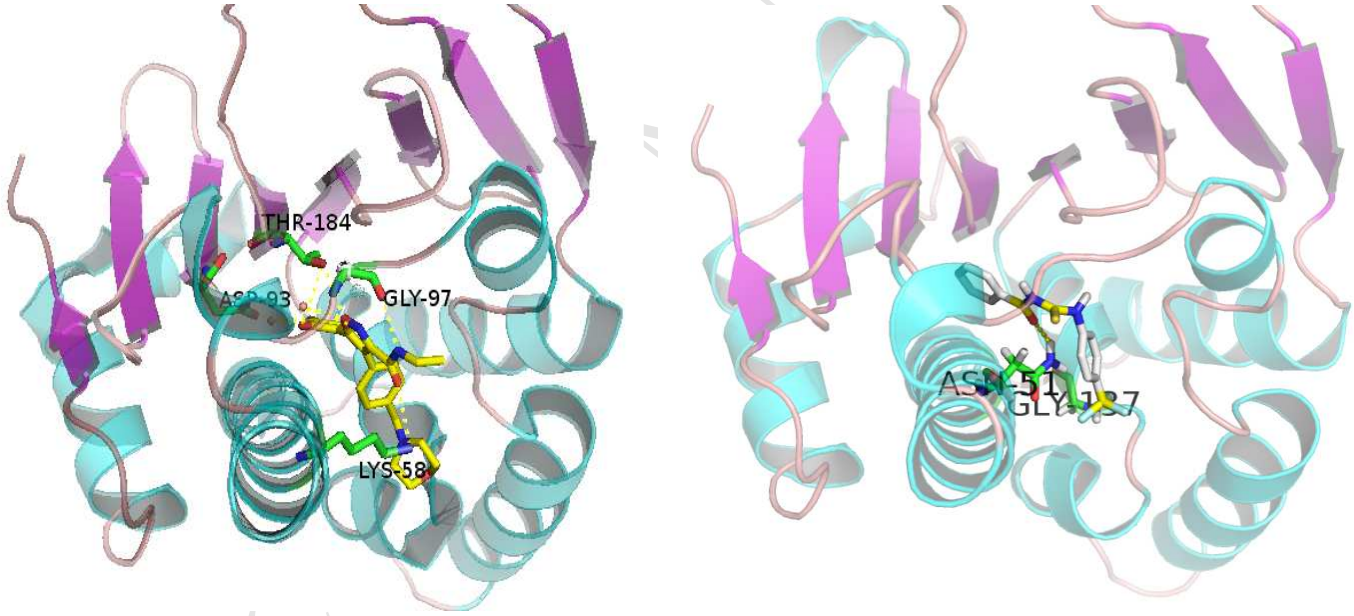
HSP90

Heat shock protein (HSP90) is a master regulator of many kinds of cancer-associated proteins. Inhibitors of HSP90 play an essential and important role in disrupting oncogenic signaling network at multiple levels [71]. So the inhibitors of HSP90 are a promise and broad spectrum anticancer agents. In this paper, docking studies of thiourea compounds against HSP90 were done by AUTODOCK which was installed on the window operation system. The PDB file of HSP90 was obtained from RSCB Protein Data Bank (PDB ID: 2VCJ). **Fig. 4(a)** and **(b)** show the model bonding of the co-crystal ligand and compound **1** with the active pocket of HSP90. It was observed that molecule preferred to interact with the HSP90 protein and made several polar and non-polar interactions. All of acyl thiourea derivatives bind at the residues of HSP90 as shown in **Fig. 5**. Asn51 and Gly137 are important amino acid residues of HSP90, the two residues can form hydrogen bond with thiourea compounds to enhance the stability of the complex. The docking

energy, hydrogen bond, the inhibition constant K_i and the residue of HSP90 are listed in **Table S6**. The active pocket of HSP90 is mainly made of Ser50, Asn51, Ser52, Lys58, Asp93, Thr94, Gly97, Met98, Asp102, Leu107, Gly137, Phe138, Val148, Lys153, His154, Thr184 and Val186. The docking energy ($\triangle G_{binding}$) produced by AutoDock is sum of two factor as [72]:

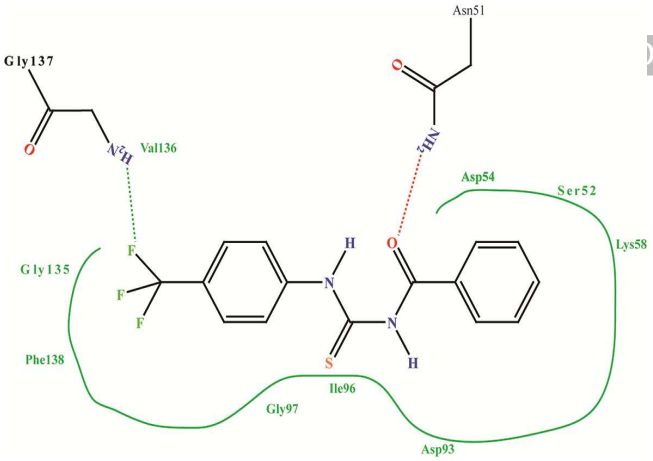
$$\triangle G_{binding} = \triangle G_{Intermolecular\ Energy} + \triangle G_{Torsional\ Energy}$$

The intermolecular energy of compound **3** is -8.02 kcal/mol, the torsional energy is 1.49 kcal/mol. Compound **3** bound at the active site of HSP90 with the best binding energy (-6.53 kcal/mol) among 7 thiourea derivatives. Binding free energy, i.e., $\triangle G_{binding}$ was determined for each. Molecules with lowest $\triangle G_{binding}$ values were considered as the most stable molecule with highest affinity for interaction with the receptor. In addition, the inhibition constant K_i is defined as the concentration of competing ligand in a competition assay which would occupy 50% of the receptors if no radio ligand were present. The inhibition constant of the compound 3 have a best value (16.29 μmol/L) which demonstrated this compound with highest affinity for receptor among 7 thiourea derivates. Hence, the relative ability of compound **3** to dock with the HSP90 protein is the best in all synthesized thiourea derivatives.

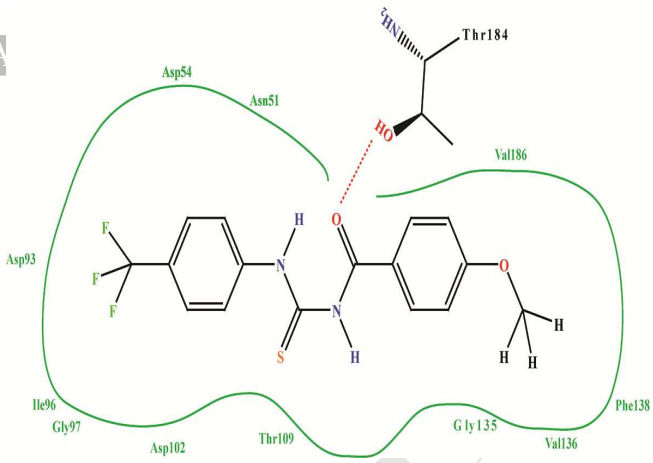


(a) (b)

Fig. 4. Cartoon view of HSP90 exhibiting the binding pocket of its co-crystal ligand (a) and the synthesized thiourea compound **1** (b). The formation of two polar (hydrogen) bonds shown as yellow dashed lines .



1



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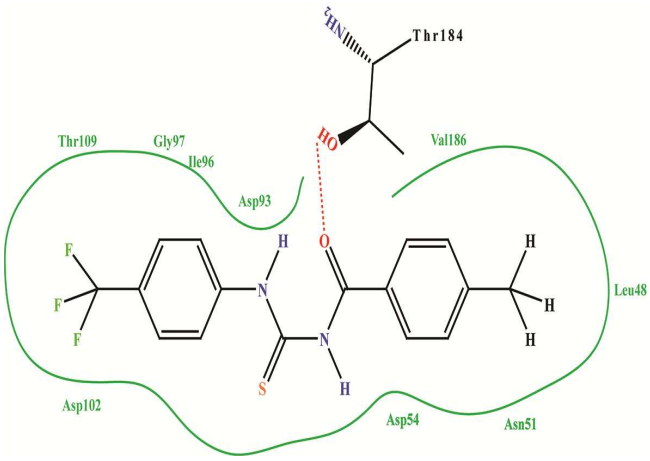

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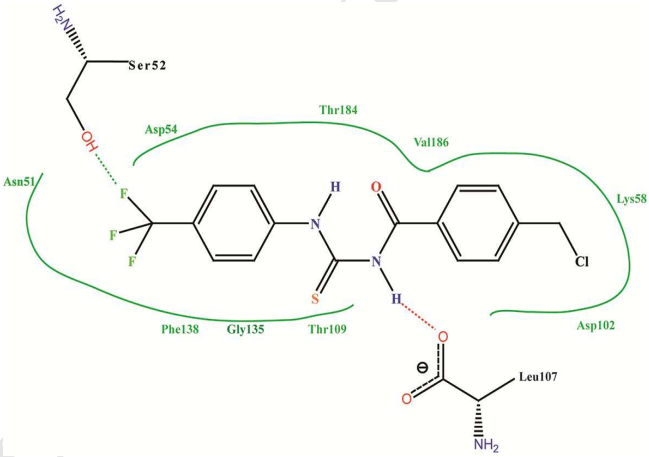

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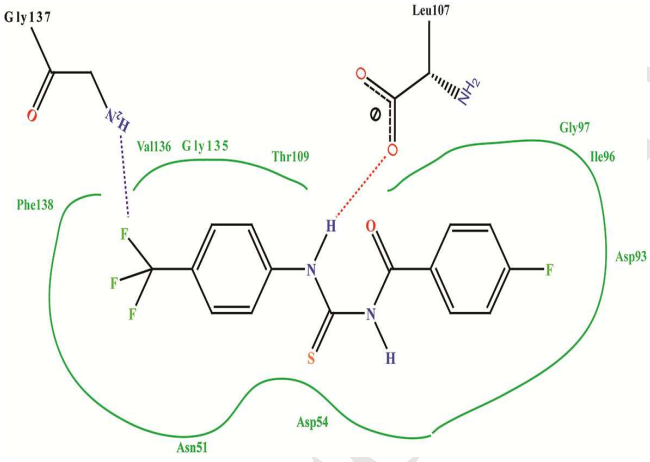

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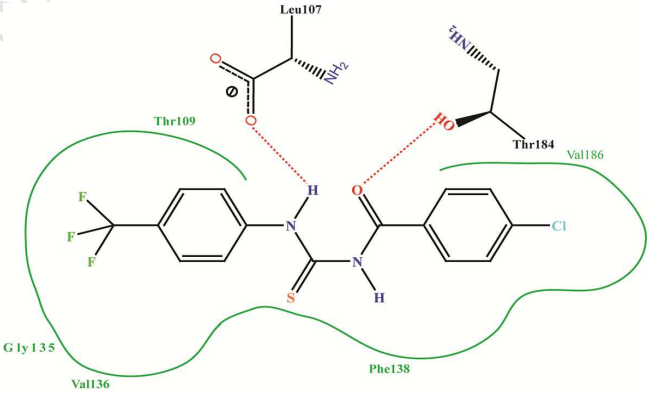

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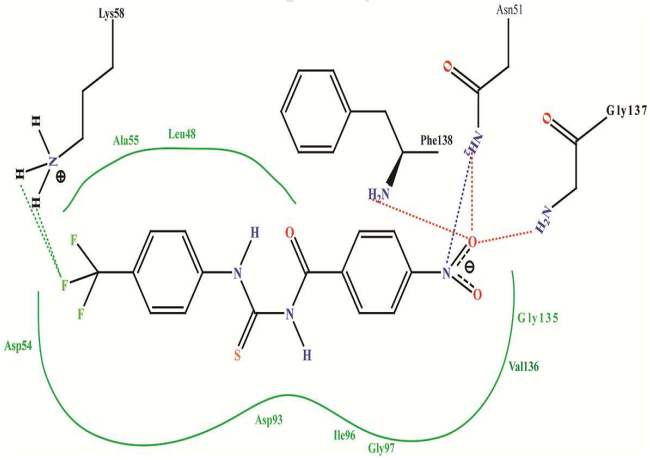

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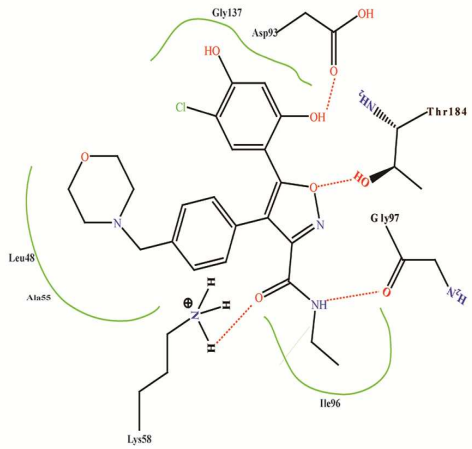

Isoxazole derivatives (Co-crystal ligand)

Fig. 5. All of thiourea compounds and co-crystal ligand showing hydrogen bonds at the active site of HSP90.

4. Conclusion

In our study, 7 acyl thiourea derivatives were prepared using the mentioned method in this paper and all of them were characterized by some spectroscopic techniques. The crystal structures of the 5 compounds (**2**, **4**, **5**, **6** and **7**) were firstly elucidated and uploaded at the Cambridge Structure Data Center. The crystal structures revealed that the carbonyl thiourea units in all determined compounds are mostly planar due in part to the formation of intramolecular N-H···O=C and C-H···S=C hydrogen bonds that form two S (6) rings. The C-H···F hydrogen bonds to provide driving forces for self-assembly were formed in compounds 2 and 6 due to the incorporation of the trifluoromethyl group. The anti-fungal activity of 7 synthesized compounds were initially screened by the disk diffusion method and the result showed compounds **4**, **5** and **6** had a better inhibition rate for four different kinds of fungi, comprehensively. Compared with compounds **1**, **2**, **3** and **7**, halogen atom(s) are able to enhance its lipophilicity that may be helpful for the anti-fungal activity for compounds **4**, **5** and **6**. *In vitro* DPPH anti-oxidant assay of the synthesized thiourea compounds, compounds **5** and **6** had great antioxidant activities which IC_{50} value are up to 191.75 μg/mL and 189.75 μg/mL, respectively. Molecular docking study of 7 thiourea derivatives against HSP90 exhibited at the active residues similar to the co-crystal ligand, which can suggest the 7 compounds may have the anti-cancer activity. To our study, the further research on in vitro the inhibition activity of 7 compounds against HSP90 is in progress and the docking can really to help to promote the development of medicinal chemistry.

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Appendix A. Supplementary material

Crystallographic data for the structures reported in this paper have been deposited with Cambridge Crystallographic Data Center as supplementary publication nos. CCDC 1470485 for **2**, CCDC 1470493 for **4**, CCDC 1472915 for **5**, CCDC 1485740 for **6** and CCDC 1485741 for **7**. Copies of the data can be obtained, free of charge, on application to CCDC, 12 Union Road, Cambridge CB2 1EZ, UK, (fax: +44 1223 336033 or e-mail: deposit@ccdc.cam.ac.uk)." Other supporting materials can be found in the Electronic Supplementary Material of this paper.

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Highlights:

1. Six acyl thiourea compounds were firstly prepared and characterized.
2. It is firstly reported intramolecular N-H···O=C and C-H···S=C hydrogen bonds form two S (6) rings in all the determined compounds.
3. Intermolecular contacts were explored using both the Hirshfeld surface and their 2D fingerprint plots.
4. All synthesized acyl thiourea derivatives showed anti-fungal and antioxidant activities.
5. Molecular docking against heat shock protein was implemented for biological evaluation.