

Synthesis, spectroscopic characterization, biological activities, X-ray diffraction and molecular docking studies of 2-methyl-3-(thiazol-2-ylcarbamoyl)phenylacetate

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ABSTRACT

We performed a different methodology for amide bond formation, the 2-methyl-3-(thiazol-2-ylcarbamoyl)phenylacetate (MTP) compound, which was prepared from the reaction of 3-acetoxy-2-methylbenzoic anhydride with thiazol-2-amine. The MTP compound was characterized with the assistance of various spectral techniques including IR, ¹H NMR, ¹³C NMR, XRD and elemental analysis. The MTP has been crystallized in the monoclinic space group *P*₂₁/*n*. The ground state molecular structure (GSMS) of the optimized MTP was obtained using the DFT/B3LYP/6–31G(d,p) method. Then, intermolecular interactions for the MTP crystal were conducted by the 2D and 3D Hirshfeld analyses. Next, the DFT-optimized structure of MTP compound was used to perform molecular docking studies with the proteins of bacterial and fungal organisms in order to find the most preferred binding mode of ligand within the protein cavity. Druglikeness assay, ADME and Toxicology studies have been carried out to predict whether the MTP has an effective drug characteristics or not. The antimicrobial activity of the MTP was tested in terms of antibacterial and antifungal activities. The results revealed that this MTP showed the best activity against *B. licheniformis* among four bacterial species and *A. flavus* and *C. utilis* among five fungal species. These findings indicate that this and similar compounds with thiazole ring can be used as antibacterial agents in the future.

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1. Introduction

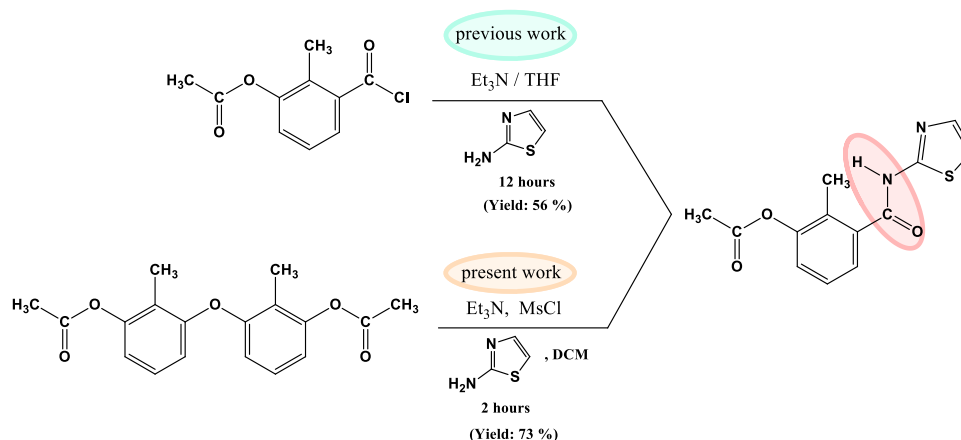
Amide bonds hold a special place in the chemistry field since amide bonds exist in the structures of pharmaceuticals, agrochemicals and many other biological active molecules [1–3]. Besides, these bonds are crucially important to every form of life because these bonds connect amino acids with each other to form proteins and peptides, which are the core constituents of the human body [4,5]. Compounds carrying these bonds have been found to have a wide range of biological activities such as *in vivo* nematocidal activity [6], anticancer activity [7,8], antibacterial [9–10], antiviral [11,12], anticonvulsant [13], antitubercular [14], antimicrobial [15], anti-inflammatory [16], antioxidant [17], antidiabetic [18] and anti-HIV [19]. As a consequence of the ubiquity of amide bonds, methods for the synthesis of amides have a major impact on every branch of chemical science.

Accordingly, we performed a different methodology for the amide bond formation. Based on the model reaction methodology, the amide compound was effectively synthesized from the treatment of acid anhydride derivatives with a secondary amine under the minimally optimized reaction conditions. In the previous study, we utilized a well-known, common method to synthesize the MTP, in which both the reaction time was long (like 12 hours) and the yield was low (56%) [20]. Therefore, we employed a different method, which allows us to obtain the MTP compound in a shorter time (4 hour) and with a higher yield (73%). Perhaps the most important result of this model reaction was that it did not form a racemate product. In order to confirm the amide bond formation and structure of the MTP, we utilized various spectral techniques such as IR, ¹H NMR and ¹³C NMR.

A Hirshfeld surface analysis was performed in which visual representations and fingerprint drawings based on Hirshfeld surfaces were interpreted. DFT calculation was made in order to compare the experimental values with the theoretical values. Using the data obtained by theoretical calculation, HOMO LUMO energy values provided information about their biological and chemical

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Scheme 1. Synthesis of the MTP compound.

activities. In this way, information about their biological activities can be obtained for compounds for which biological analyzes cannot be performed. The compound was optimized using the DFT/B3LYP/6–31G(d,p) basis sets, and its theoretical NMR values were analyzed using the B3LYP/6–31G(d,p) method. In addition, the optimized structure was also used in molecular docking calculations. Molecular docking study was carried out especially to predict residues affecting functional groups of ligands and to understand Ligand-Receptor interaction. The compound was docked in proteins from bacterial or fungal organisms the *Bacillus licheniformis* (PDB ID:6B7K), *Micrococcus luteus* (PDB ID:4EWP), *Enterobacter aerogenes* (PDB ID:1GMU), *Proteus vulgaris* (PDB ID:4MCX) and *Aspergillus flavus* (PDB ID:1R56), *Aspergillus niger* (PDB ID:3K4P), *Aspergillus parasiticus* (PDB ID:3HRQ), *Candida albicans* (PDB ID:1ZAP) and *Candida utilis* (PDB ID:3VGB) to gain information about the drug's efficacy and predict the most likely mode of interaction.

2. Experimental procedures

2.1. Measurement and reagents

Reagents, starting materials and solvents were purchased from commercial sources (Carlo Erba, Merck, ABCR, or Sigma-Aldrich) and were used without further purification. The solvents were of analytical grade. The melting points were measured with a Stuart SMP 30. IR spectra were recorded on a Bruker Vertex 80V. The ¹H and ¹³C NMR spectra were recorded either using a Bruker/ULtraschilt operating at 300 MHz spectrometer using TMS as the internal standard. Elemental analyses were performed on a Elementar Vario Micro Cube (Germany) elemental analyzer at ODUMARAL in Ordu University.

2.2. Synthesis of 2-methyl-3-(thiazol-2-ylcarbonyl) phenylacetate (MTP)

The starting compound, 3-acetoxy-2-methylbenzoic anhydride, was prepared from the reaction of 3-acetoxy-2-methylbenzoic acid with 3-acetoxy-2-methylbenzoyl chloride according to the procedure as described in the literature [21]. 3-acetoxy-2-methylbenzoic anhydride (0.5 mmol, 1.0 equiv) was dissolved in CH₂Cl₂ (DCM) (12 mL) and methanesulfonyl chloride (MsCl) (0.55 mmol, 1.1 equiv) at 0 °C was added. Then, Et₃N (2.2 mmol, 2.2 eq) was added. The reaction mixture was stirred for 45 minutes without changing its temperature and thiazol-2-amine (1.1 mmol, 2.2 eq) was added. Progress of the reaction was monitored using TLC. After 4 h, the reaction mixture was diluted with DCM (35 mL) and washed with 1N aq HCl (10 mL), NaHCO₃ (10 mL) and brine (10 mL), respectively.

The organic layer was dried over anhydrous sodium sulfate, filtered and concentrated under reduced pressure to give the desired product. The crude product was crystallized from ethanol. The synthesis reaction is given in Scheme 1 [22]. Physical data: 73%; m. p. 208–210°C; Anal. calc. for C₁₃H₁₂N₂O₃S: C, 56.51; H, 4.38; N, 10.14; S, 11.60 Found C, 56.57; H, 4.39; N, 10.16; S, 11.59.

2.3. X-ray crystallography

XRD measurement of MTP compound was registered at 296 K on a Bruker D8 QUEST diffractometer (MoKα radiation, λ=0.71073 Å). Data reduction: Bruker SAINT [23], Cell refinement: Bruker SAINT [23], Data collection: Bruker APEX2 [24]. The single crystal structure was solved by direct methods using SHELXS-97 [25] and refined using SHELXL-97 [26] software. The figures of the MTP compound were drawn by using WINGX-2014 [27], ORTEP-2014 [27] and MERCURY-2021 [28] software. All other H atoms were positioned geometrically and were refined with a riding model with U_{iso} 1.2 times that of attached atoms. Details of the crystal data, data collection, and structure refinement parameters are described in Table 1.

2.4. Hirshfeld surface analysis

Electron distribution is calculated by Hirshfeld surface analysis [29], which is an important analysis to determine the spherical atomic electron densities [30,31] of a given crystal structure [32–36].

The equation expressing the contact distance (d_{norm}) depending on the d_i, d_e and vdW radius has been given in our previous study [37]. In this paper, the Hirshfeld surfaces are charted with d_{norm} and 2D fingerprint plots given were determined using Crystal Explorer 17.5 [38].

2.5. Theoretical aspects

2.5.1. Structural simulation

Becke 3-Lee-Yang-Parr (B3LYP) and 6-31G(d,p) basis set were selected for the calculations of the complex and Gaussian 09W package program and Gauss View 5.0 interface program were used for all calculations [39]. The initial data for the molecular optimization of the MTP compound were used to determine its atomic coordinates in the crystallographic study. The ¹H and ¹³C NMR chemical shifts were obtained by the Gauge Independent Atomic Orbital (GIAO) approach [40,41] and converted to the TMS scale. The ¹H and ¹³C NMR chemical shifts were converted to the TMS scale by subtracting the calculated absolute chemical shielding of TMS

Table 1
Crystallographic data for MTP compound.

Formula	C ₁₃ H ₁₂ N ₂ O ₃ S
Formula weight	276.31
Temperature(K)	293
Radiation, λ(MoK _α)	0.71073
Crystal system	Monoclinic
Space group	P2 ₁ /n
Unit cell dimensions	
a, b, c(Å)	16.482 (14), 11.022 (6), 7.422 (4)
α, β, γ (°)	90, 101.60(3), 90
Volume (Å ³)	1320.7(15)
Z	4
Calculated density (Mg.m ⁻³)	1.390
μ(mm ⁻¹)	0.25
F(000)	576
Crystal size (mm)	0.05 × 0.12 × 0.14
θ range (°)	1.26–28.33
Index	–22 ≤ h ≤ 22
ranges	–14 ≤ k ≤ 14
	–9 ≤ l ≤ 9
Measured Reflections	30997
Independent reflections	3284
Reflections observed [I ≥ 2σ(I)]	2008
Refinement method	Full matrix least-squares on F ²
Data/restraints/parameters	3284/0/176
Goodness-of-fit on F ²	1.067
Final R indices [I ≥ 2σ(I)]	R ₁ = 0.0499; wR ₂ = 0.1286
R indices (all data)	0.1013
Δρ _{max} ; Δρ _{min} (eÅ ⁻³)	0.226; –0.381

($\delta = \Sigma O - \Sigma$, where δ is the chemical shift, Σ is the absolute shielding and ΣO is the absolute shielding of TMS), whose value are 31.8821 and 182.466 ppm, respectively. The GIAO ¹H and ¹³C NMR chemical shift values were obtained by using the B3LYP /6–31G(d,p) model.

2.5.2. Molecular docking simulation

Molecular docking was used to predict how a molecule interacts with a protein, DNA or RNA [42]. For this purpose, Crystal structures of proteins were downloaded from RCSB-PDB (protein data bank, <https://www.rcsb.org>) and used for docking. We performed the docking studies by using AutoDock 4.2.6 [43]. Non-polar hydrogen atoms were joined, and rotatable bonds were removed. Kollman charges were added by AutoDock tools [44]. The grid maps with different points and 1Å° grid-points spacing were generated using AutoGrid.exe application [45]. Docking simulation was achieved by Lamarckian genetic algorithm (LGA) [46]. For twenty-three variables, genetic Algorithm parameters were set maximum of 250,000,000 energy estimation and 300-population size. The each docking experiment was set to close after maximum of 250,000,000-energy estimation [46]. Most favorable docked conformations were selected based on the free binding energy of protein–compound and analyzed to investigate the interaction.

2.5.3. Druglikeness and toxicology

Druglikeness of the MTP compound was carried out by SwissADME web server to predict ADME (Adsorption (A), distribution (D), metabolism (M), excretion (E)) parameters, pharmacokinetic properties, druglike nature and medicinal chemistry friendliness of one or multiple small molecules to support drug discovery [47].

ProTox-II (https://tox-new.charite.de/protox_II/) is a free web server used to estimate various toxicological endpoints of various chemical compounds [48]. While this calculation software developed for drug toxicity studies provide great advantages in terms of accessibility, accuracy and speed, they will also be able to be compared with in vitro and in vivo experimental studies. To assess the toxicity of the MTP compound, the acute toxicity, bioaccumulation factors, developmental toxicity and mutagenicity of these organic

Table 2
Dihedral angles (°) between planes.

Planes	Dihedral angles (°)
P1–P2	56.92
P2–P3	6.61
P1–P3	61.08

compounds were estimated by the quantitative structure-activity relationship (QSAR) method using the Toxicity Estimation Software Tool (T.E.S.T.) [49].

2.6. Measurements of antimicrobial activity

2.6.1. Minimal inhibitory concentration (MIC) method

The antimicrobial activities of the MTP compound was determined using *Bacillus licheniformis* NRRL-B 1001; *Micrococcus luteus* NRRL-B 1013 as Gram-staining-positive bacteria, *Enterobacter aerogenes* NRRL B-3567; *Proteus vulgaris* NRRL-B 123 as Gram-staining-negative and *Aspergillus flavus* NRRL-1957; *Aspergillus niger* ATCC 16404; *Aspergillus parasiticus* NRRL-465; *Candida albicans* ATCC 1023 and *Candida utilis* NRRL-Y900 as fungi.

Minimal inhibitory concentration (MIC) of the MTP compound was determined using the broth microdilution method [50] carried out in 96-well microplates. The MTP compound used in this study was dissolved in dimethyl sulfoxide (DMSO) at a suitable concentration. The cultures were obtained from nutrient broth for all the bacterial strains after 24 h of incubation at 37°C. Fungi were maintained in nutrient broth after incubation for 24 h at 28°C. Bacterial and fungi cells were homogenized in nutrient broth. The turbidity of bacterial and fungi suspensions was set at a concentration of approximately 10⁶ cells/ml. Only inoculated broth was used as controls. 100 µL suspension of each microorganism and 100 µL suspension of compound tested were added into the wells. The microplate with no growth of microorganism was recorded to represent the MIC enounced in µg/mL. Amoxicillin and Tetracycline were used as the reference standard for antibacterial activity, while Ketoconazole was used as the reference standard for antifungal activity.

3. Results and discussions

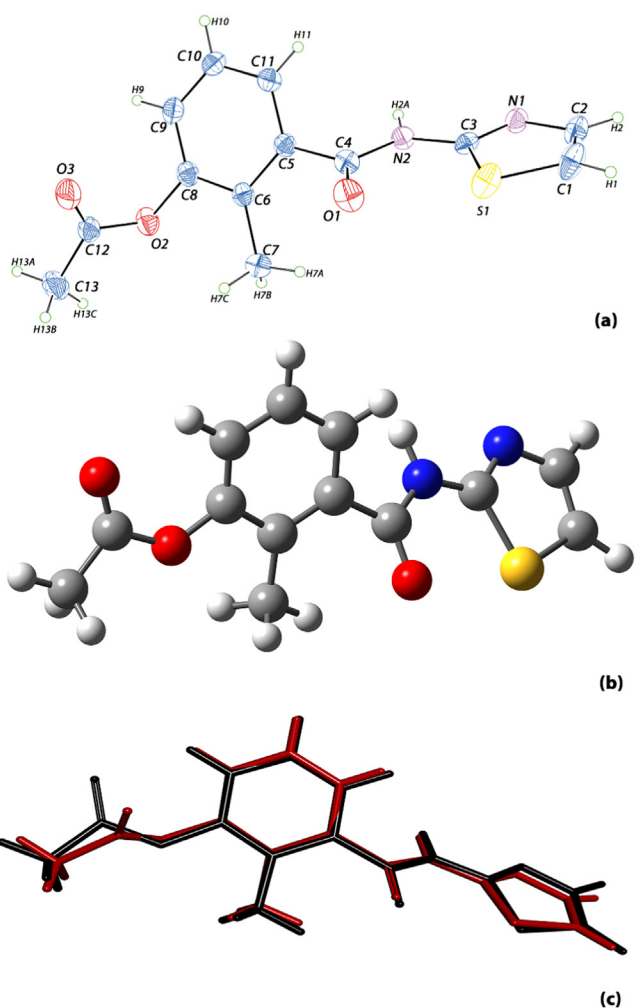
3.1. Description of crystal structure

The MTP compound crystallized in the P2₁/n monoclinic space group. The asymmetric unit contains one molecule. The scheme of the labelled atoms plotted ORTEP-2014 [27] is shown in Fig. 1a. The molecular geometry of the MTP compound is non-planar. The dihedral angles between the P1, P2 and P3 planes seen in Fig. 2 are listed in Table 2. The P1 plane is more twist than the other planes. It is likely because the C5–C4 bond length is longer than the C3–N2 bond length. It may be due to the steric effects of the groups attached to the benzene ring. Bond lengths and bond angles related to X-ray diffraction and theoretical calculations with literature are given in Table 3. The compound is compatible with it when compared to the literature [51]. The experimental value of the C12–O2–C8 bond angle is 116.62° and the theoretical value is 127.14°. The reason for this difference is the inclusion of the O3 atom in the hydrogen bond (Table 4), causing the COCH₃ group to twist. Such changes can be observed because theoretical calculations ignore interactions such as hydrogen bonding due to vacuum environment. While the dihedral angle between C9–C8–O2–C12 is –72.34 according to the experimental results, its theoretical value is 2.74 (Fig. 1b). The deviation seen in Fig. 1c is due to this dihedral angle and its RMSD value is 0.0918.

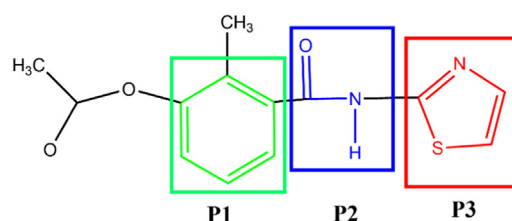
Table 3

Bond lengths (Å) and bond angles (°) related to X-ray diffraction and theoretical calculations with literature.

Bond lengths (Å)	XRD	DFT	[51]	Bond angles (°)	XRD	DFT	[51]
S1-C1	1.716(3)	1.811	1.721(3)	C1-S1-C3	88.24(12)	86.09	88.5(10)
S1-C3	1.722(2)	1.825	1.731(2)	C12-O2-C8	116.62(19)	127.14	-
O2-C12	1.354(3)	1.391	-	C4-N2-C3	124.4(2)	126.55	123.13(17)
O2-C8	1.414(3)	1.418	-	C3-N1-C2	109.6(2)	111.75	110.11(18)
O1-C4	1.221(3)	1.251	1.224(2)	C8-C6-C5	116.1(2)	116.98	-
N2-C4	1.359(3)	1.388	1.372(3)	C8-C6-C7	121.2(2)	118.99	-
N2-C3	1.378(3)	1.385	1.377(3)	C9-C8-O2	119.1(2)	123.91	-
O3-C12	1.188(3)	1.231	-	C6-C8-O2	116.9(2)	112.99	-
N1-C3	1.297(3)	1.308	1.306(3)	O1-C4-N2	121.4(2)	120.90	121.9(2)
N1-C2	1.384(3)	1.396	1.381(3)	O1-C4-C5	123.4(2)	124.29	121.62(19)
C6-C7	1.499(3)	1.511	-	N2-C4-C5	115.21(19)	114.81	116.43(17)
C5-C4	1.488(3)	1.494	1.454(3)	N1-C3-S1	115.50(17)	115.06	115.13(15)
C12-C13	1.492(2)	1.502	-	N2-C3-S1	123.08(18)	123.56	123.28(14)
				O3-C12-O2	122.8(2)	124.55	-
				O3-C12-C13	126.5(3)	125.96	-
				O2-C12-C13	110.7(3)	109.49	-

**Fig. 1.** (a) ORTEP, (b) the optimized and (c) the superimposition (red; XRD and black; theoretical) view of the MTP compound.

Another factor affecting molecular conformation is non-covalent interactions. These interactions affect it due to the repulsive and attractive forces that occur. In addition, as it is known, these interactions provide the formation of the three-dimensional structure of a crystal. The crystal structure of compound displays an intermolecular hydrogen bond, three C—H...O interaction and $\pi \cdots \pi$

**Fig. 2.** The P1, P2 and P3 planes of compound.**Table 4**

The geometric data of non-covalent interactions (Cg1: C1-C2-N1-C3-S1).

D-H...A	D-H (Å)	H...A(Å)	D...A(Å)	D-H...A(°)
N2—H2A...N1 ⁱ	0.871	2.067	2.932(2)	172
C7—H7A...O1	0.960	2.460	3.114(3)	125
C7—H7C...O3 ⁱⁱ	0.960	2.530	3.460(3)	163
C9—H9...O3 ⁱⁱⁱ	0.930	2.590	3.449(3)	153
Cg...Cg	Cg—Cg	$\alpha(^{\circ})$	$\beta(^{\circ})$	$\gamma(^{\circ})$
Cg1...Cg1 ^{iv}	3.810(3)	0	20.7	20.7

Symmetry codes: (i) $-x+1, -y+1, -z+1$; (ii) $-x, -y, -z$; (iii) $-x, -y, -z-1$; (iv) $-x+1, -y, -z+1$

interactions in 3D network. The geometric data of these interactions are listed in Table 4. By linking two independent identical molecules with the intermolecular N—H...N hydrogen bond, $R_2^2(8)$ synthon forms (Fig. 3) [52]. A, B and C molecules connected by N2—H2A...N1ⁱ hydrogen bonds are connected to each other by C7—H7C...O3ⁱⁱ interactions (Fig. 4) and form a 1D polymeric structure in the a -direction and form an $R_2^2(14)$ motifs according to graph-set notation. 1D chains are connected by C9—H9...O3ⁱⁱⁱ. These chains in [100] direction are resulted in 2D polymeric sheets, formed in the (101) plane (Fig. 5). 2D layers are connected with $\pi \cdots \pi$ (Cg1...Cg1; Cg1: C1-C2-N1-C3-S1) interactions and formed a three-dimensional supramolecular structure.

3.2. Molecular properties

3.2.1. Mulliken population analysis (MPA)

The effective atomic charges calculated by Mulliken analysis, the most popular population analysis, give information about the charge distribution on the chemical bond. Although mulliken charge analysis cannot give an accurate result, it provides comments about nucleophilic (rich electron) or electrophilic (poor electron) indexes and determination of regions sensitive to molecular interactions. Mulliken population analysis provides preliminary in-

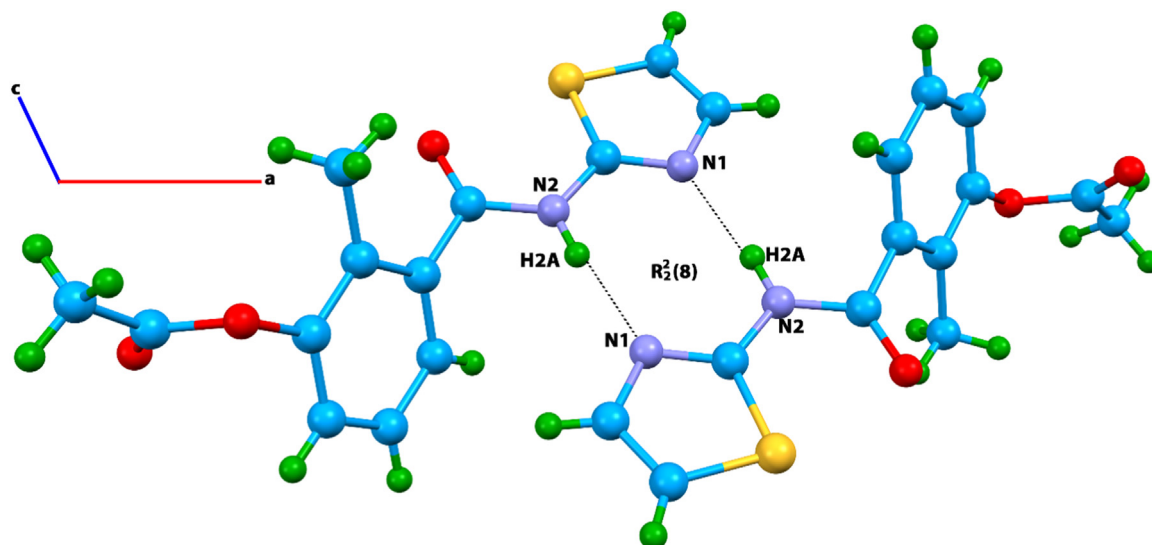


Fig. 3. $R_2^2(8)$ synthon formed by linking two independent identical molecules with the intermolecular N—H...N hydrogen bond.

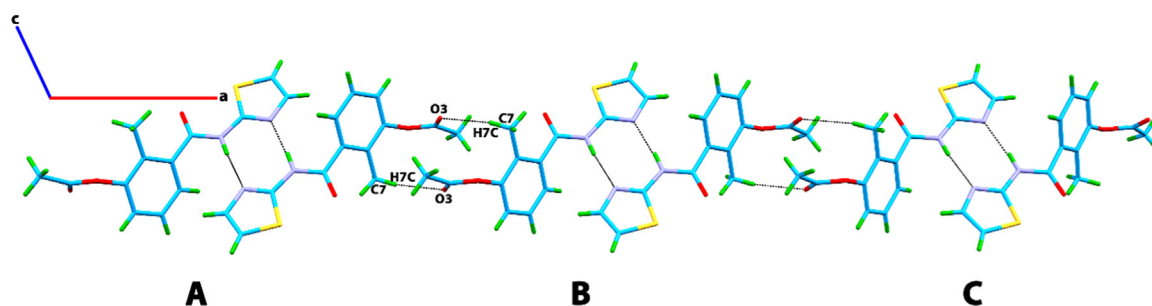


Fig. 4. 1D chain formed by C7—H7C...O3ⁱⁱ interactions between „A, B and C„ molecules in the [100] direction.

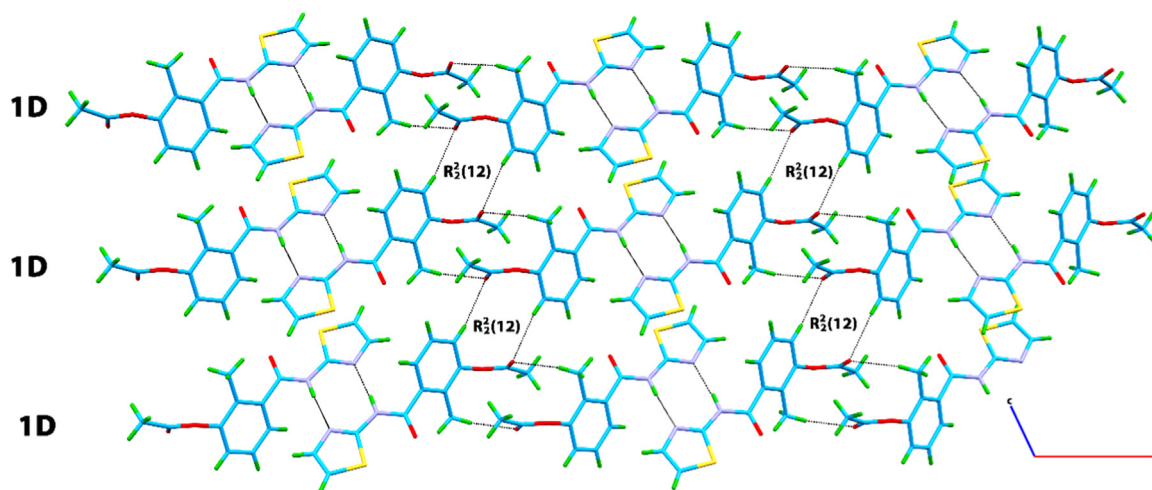


Fig. 5. 2D polymeric sheets connecting by same H-bonds forming between 1D chains in the (101) plane.

formation about chemical reaction-prone regions, electron density distribution and molecular electrostatic potential. Atomic charges affect the molecular polarization, dipole moment, electronic structure and more often the behavior of a molecular system. Atomic charges obtained by Mulliken population analysis of the complex are listed in Table 5. As can be seen in the results, all hydrogen atoms have clear positive charges. It appears that the H2A atom has a greater positive atomic charge (0.355491e) than other hydrogen atoms. In addition, the results in Table 5 show that O2, O1, N2, O3 and N1 atoms have higher negative value than other atoms.

Especially, N2 atom (-0.678474e) has a maximum negative charge. This may be due to the presence of intra-molecular hydrogen bond (Table 3). Also, the carbon atoms (C6, C8, C4, C3, C2) that are connected to the O2, O1, N2, O3 and N1 atoms have positive charges than other carbon atoms due to steric effect.

3.2.2. Chemical reactivity

FMOs also play an important role in molecular interactions. While E_{HOMO} represents the ability to give an electron, E_{LUMO} represents the ability to receive an electron. Since the energy gap be-

Table 5
MPA of MTP compound.

Atom	MPA	Atom	MPA	Atom	MPA	Atom	MPA
S1	0.501229	C8	0.212320	C7	-0.437976	C1	-0.476102
O2	-0.566938	C4	0.455591	H7A	0.174886	H1	0.171330
O1	-0.437871	C3	0.133249	H7C	0.151111	C13	-0.455959
N2	-0.678474	C12	0.528376	H7B	0.165555	H13C	0.184855
O3	-0.405068	C11	-0.131385	C2	0.086305	H13A	0.180580
N1	-0.396746	H11	0.147004	H2	0.159753	H13B	0.186612
C6	0.090744	C9	-0.078173	C10	-0.156599	H2A	0.355491
C5	-0.006323	H9	0.199442	H10	0.143189		

Table 6

Quantum chemical descriptors of the MTP compound with literature.

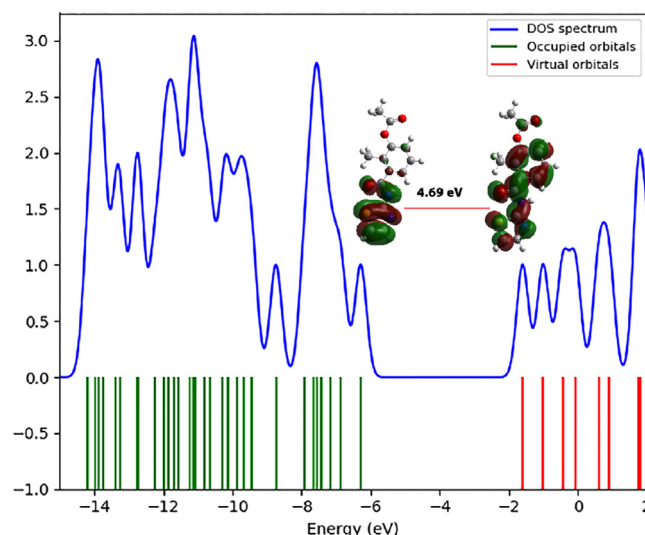
	MTPDFT-B3LYP/631-G	[57]
E_{HOMO} (eV)	-6.29	-5.44
E_{LUMO} (eV)	-1.60	-0.98
E_{GAP} (eV) ($ E_{\text{HOMO}} - E_{\text{LUMO}} $)	4.69	4.46
I (eV) ($-E_{\text{HOMO}}$)	6.29	5.44
A (eV) ($-E_{\text{LUMO}}$)	1.60	0.98
η (eV) ($\frac{I-A}{2}$)	2.34	2.23
σ (eV ⁻¹) ($\frac{1}{\eta}$)	0.43	0.45
χ (eV) ($\frac{I+A}{2}$)	3.14	3.21
μ (eV) ($-\frac{I+A}{2}$)	-3.14	-3.21
ω (eV) ($\frac{\mu^2}{2\eta}$)	2.11	2.31
N (eV ⁻¹) ($\frac{1}{\omega}$)	0.47	0.43
ΔN_{MAX} ($-\frac{\omega}{\eta}$)	1.34	1.44
S (eV ⁻¹) ($\frac{1}{2\eta}$)	0.21	0.22

E_{GAP} : Energy band gap I : Ionization potential; A : Electron affinity; η : Chemical hardness; σ : Chemical softness; χ : Electronegativity; μ : Chemical potential; ω : Electrophilic index; N : Nucleophilic index; ΔN_{MAX} : Maximum charge transfer; S : Global Softness

tween these orbitals is closely related to electron conductivity, it is a critical parameter defining the molecular electron transition [53]. The smaller the HOMO-LUMO energy difference, the easier the interaction will be [54]. Thus, it will be easier for the reactants to interact and react. Energy gaps are also responsible for the chemical and spectroscopic properties of molecules. While $I = -E_{\text{HOMO}}$ is the lowest energy required to remove an electron from a molecule, $A = -E_{\text{LUMO}}$ is the amount of energy required to add a electron to a molecule. Quantum chemical definitions of the compound are listed in Table 6.

Hardness and softness are a decisive criterion for defining the chemical reactivity or kinetic stability of a molecule. Soft molecules have small energy gaps, while hard molecules have large energy gaps [55]. Therefore, it can be said that hard molecules are more stable than soft molecules. Electronegativity refers to electrons or electron density in an atom or a functional group the ability to attract to itself. The electrophilicity index factor is used to evaluate energy reduction that is the maximum electron flow between the HOMO and LUMO orbitals [56]. Compounds with small electrophilic index or high nucleophilic index have a small biological activity. As the ΔN_{MAX} and global softness (S) values of the compounds increase, their biological activities increase. $E_{\text{HOMO}} = -6.29$ eV, $E_{\text{LUMO}} = -1.60$ eV belonging to compound, ΔE values were 4.69 eV. Results given in the Table 6 can be interpreted as follows:

- (1) These parameters and the ΔE value show that the MTP compound has a higher kinetic stability, lower chemical reactivity and lower intramolecular charge transfer.
- (2) The compound has a electrophilic index and nucleophilic index close to similar compounds [57]. That is, it shows average biological activity.

**Fig. 6.** The total density of states (TDOS) curves for the MTP compound.

- (3) Similarly, since the ΔN_{MAX} and S values of the compound are slightly lower, its biological activity is slightly decreased compared to the literature [57].

Density of states (DOS) gives information about the state distribution of the system. That is, the DOS results are important for estimating the occupancy of states, calculating optical transition probabilities, and/or calculating transition rates upon absorption and emission of light. TDOS spectra was generated by GaussSum software [58]. With the TDOS analysis results given in Fig. 6, it presents the maximum state density on the occupied orbitals as a curve.

3.2.3. Molecular Electrostatic Potential (MEP)

MEP is a useful descriptor to visualize electrophilic or nucleophilic reactive regions of molecules and to show electrostatic potential regions for color grading [59]. 3D graphs of the molecular electrostatic potential of the compound are shown in Fig. 7a. In the MEP map, different colors are represented by different values of the electrostatic potential: green represents the zero potential region, red represents the most negative region and blue represents the most positive electrostatic potential region. Potential increases in order; red < orange < yellow < green < blue. The color code of this map ranges from -0.0735 au to +0.0735 au, where blue indicates strongest attraction and red indicates repulsion. Positive sites are located around hydrogen atoms, which are associated with nucleophilic reactivity [60,61]. Negative regions are found around the oxygen atoms (O1, N1 and O3). As shown in Fig. 7a, the most positive potential region is N2-H2A. Negative potential fields are found around electronegative atoms, while the remaining species are surrounded by orange, yellow, and green. Consequently, the MTP compound is suitable to both electrophilic and nucleophilic reactions.

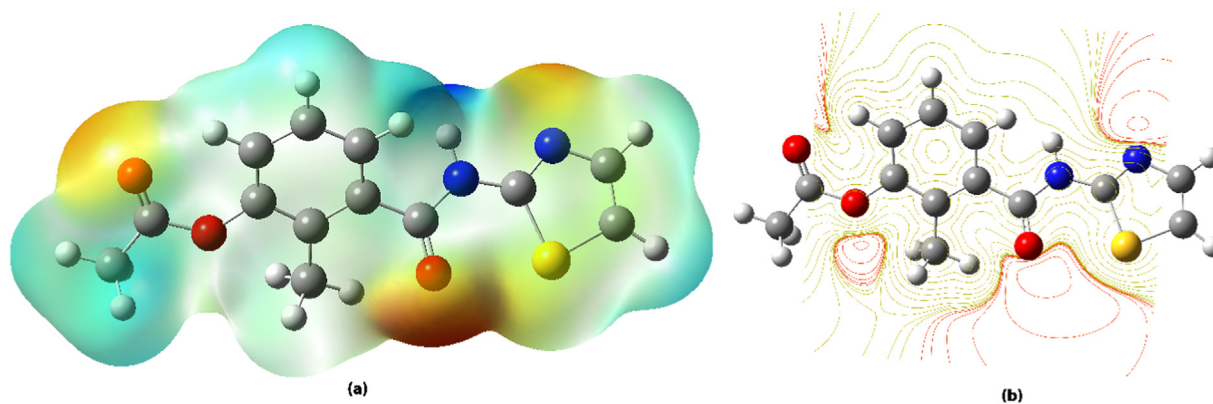


Fig. 7. MEP (a) and MEP contour (b) image of compound.

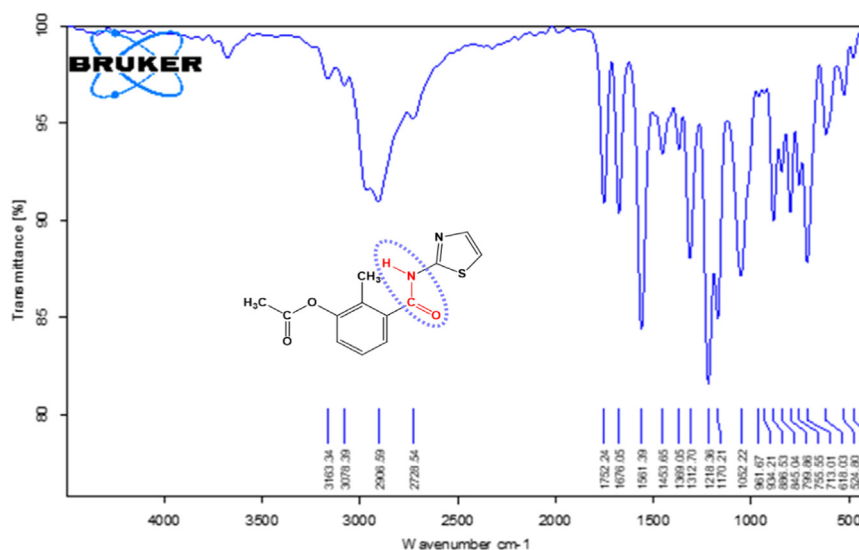


Fig. 8. IR spectrum of the MTP compound.

MEP top-up maps are drawn in two dimensions and used to show lines of constant intensity or luminosity. Negative and positive potential lines are seen on this map (Fig. 7b). Electron-rich lines around oxygen and nitrogen on thiazole ring are theoretically potential active sites of the molecule.

3.3. Vibrational analysis

Experimental IR spectrum of the MTP compound is shown in Fig. 8. The important characteristic absorption band for amides appeared at 3164 cm^{-1} corresponding to the N–H stretching vibration. The other characteristic strong absorption band appeared at 1676 cm^{-1} was described as C=O stretching vibration while the other strong band 1752 cm^{-1} was attributed to C=O stretching vibration of ester carbonyl. The reason why the strong C=O stretching vibration of the ester carbonyl group is higher than the normal stretching vibration of the ester can be explained by the resonance of the phenyl group with oxygen. Another group stretching vibration called as amide II, the C–N stretching vibration with the N–H bending vibration bands, this mode was observed at 1453 cm^{-1} . These frequency values were closely in agreement with similar compounds in the literature [62,63].

3.4. NMR analysis

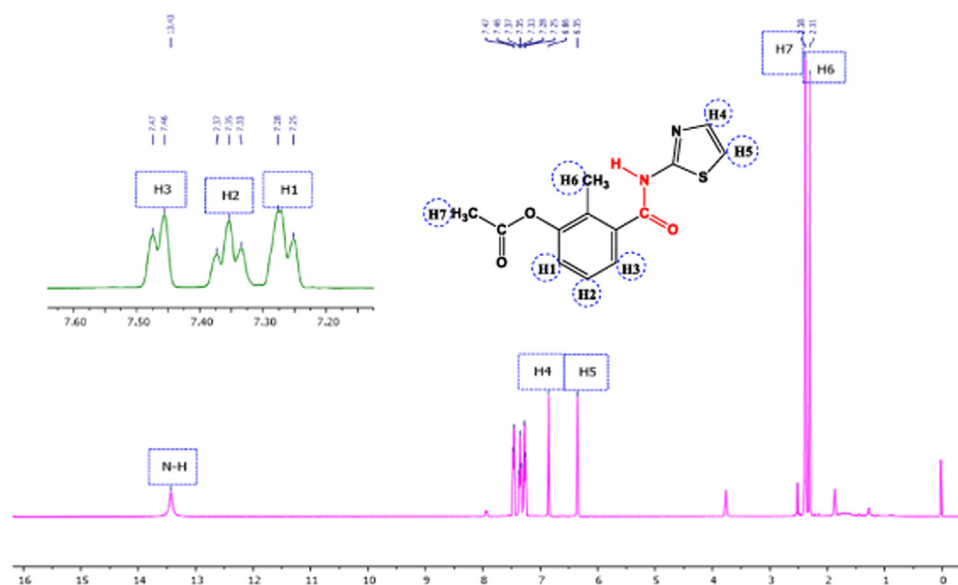
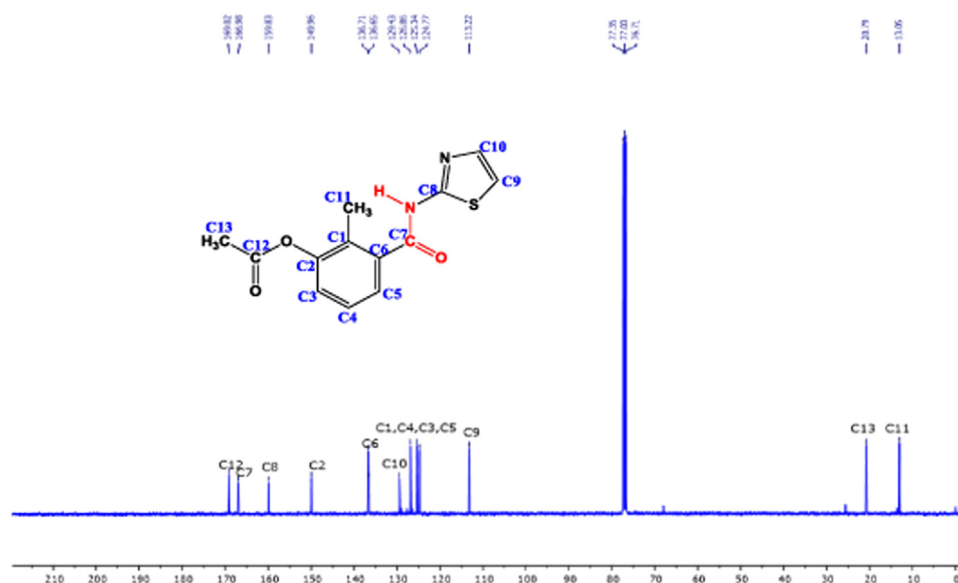
The ^1H NMR spectrum of the MTP compound was recorded in CDCl_3 (Fig. 9). On the other hand, the experimental and theoret-

Table 7

Experimental and theoretical ^1H and ^{13}C NMR chemical shifts the MTP compound.

Atom	Experimental	Calculated	Atom	Experimental	Calculated
C1	129.43	117.84	H1	7.26	8.02
C2	149.96	141.24	H2	7.35	6.51
C3	125.34	107.05	H3	7.46	6.49
C4	126.86	111.05	H (NH)	13.43	7.52
C5	124.71	107.46	H4	6.86	6.35
C6	136.71	119.77	H5	6.35	6.60
C7	166.98	154.02	H6a	2.38	1.64
C8	159.83	151.10	H6b		1.51
C9	113.22	116.05	H6c		1.92
C10	136.65	118.29	H7a	2.31	1.99
C11	13.05	3.13	H7b		1.96
C12	169.02	163.54	H7c		1.11
C13	20.79	11.28			

ical ^1H and ^{13}C NMR chemical shift values of the compound in CDCl_3 are listed Table 7. The NH proton of amide function have resonated at 13.43 ppm as a singlet. The experimental ^1H NMR signal for proton NH was observed at 13.43 ppm, while the same was calculated at 7.52 ppm [64]. Three methyl protons have resonated at 2.31 ppm as a singlet and the three methyl protons attached to ester carbonyl have resonated at 2.38 ppm as a singlet. The aromatic protons of the phenyl and thiazole rings appeared in the region of $\delta = 6.35\text{--}7.47\text{ ppm}$ (Fig. 9). The H3 proton coupled to the H2 proton showed a doublet at 7.46 ppm and the H2

Fig. 9. ^1H NMR spectrum of the MTP compound.Fig. 10. ^{13}C NMR spectrum of the MTP compound.

proton gave a triplet by coupling H1 and H3 as being 7.35 ppm. The H1 proton coupled to the H2 proton and showed a doublet peak at 7.26 ppm. The protons of the thiazole ring, H4 and H5, interacted with each other to give as doublets at 6.86 ppm and 6.35 ppm, respectively (Fig. 9). These data were in agreement with those previously reported for similar compounds in the literature [57,63].

This MTP compound was further characterized by ^{13}C NMR (Fig. 10). The chemical structure was confirmed by the presence of 13 different peaks as expected. The C12, carbon atom of the ester carbonyl group observed at 169.02 ppm, whereas, the C7, amide functional group carbon was observed at 166.98 ppm. The aromatic C8 and C2 carbons were the most downfield in comparison with the other carbons of the aromatic rings and so these carbons gave the signal at 159.83 ppm and 149.96 ppm, respectively. The C1, C3, C4, C5 and C6, the other carbons of the aromatic rings, were at 129.43 ppm, 125.34 ppm, 126.86 ppm, 124.71 ppm, 136.71 ppm, respectively. The C9 and C10, the thiazole ring carbons resonated at 113.22 ppm and 136.65 ppm, respectively. The methyl carbon

attached to ester carbonyl group ($-\text{OCOCH}_3$) gave a signal at 20.79 ppm, while the signal at 13.05 ppm belonged to methyl group carbon on the phenyl ring. The results were in agreement with the reported values in the literature [57,63].

3.5. Hirshfeld surface analysis (HS)

The Hirshfeld surfaces of the MTP compound are illustrated in Fig. 11, showing surfaces that have been mapped over d_{norm} , d_i , d_e , shape indices, curvedness are -0.5445 to 1.1060 , 0.7722 to 2.5716 , 0.7733 to 2.5366 , -1 to 1 and -4 to 4 $^\circ\text{A}$, respectively. What the color scale of the d_{norm} means was explained in our previous study [37]. The Hirshfeld surface is the graphical representation of which d_i represents two distances. One is the distance from a given point on HS to the nearest nucleus inside the surface, and the other one is distance from a point on HS to the nearest nucleus outside the surface. The distances between the nuclei are Van der Waals separations between atoms, the red region represents shorter interactions, the blue colored region represents longer interactions, and

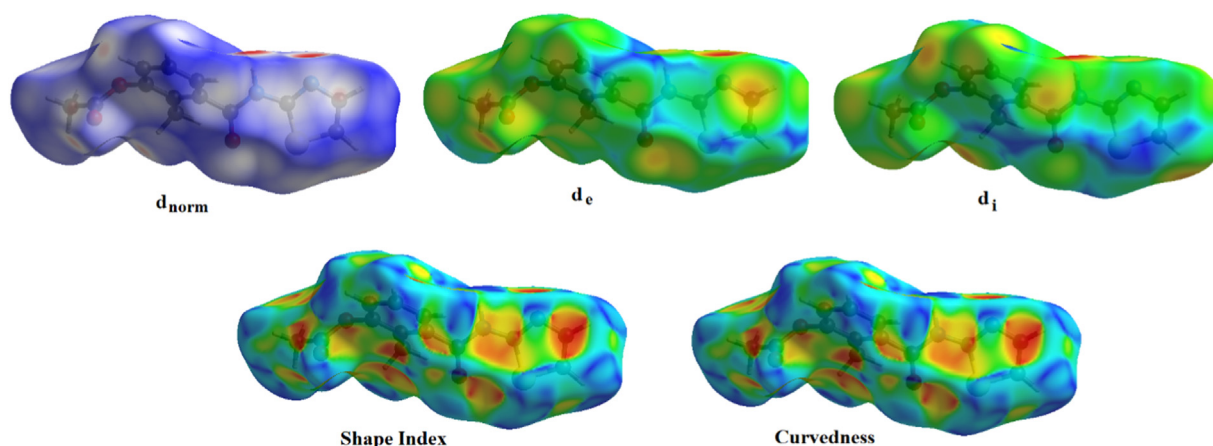


Fig. 11. Hirshfeld surfaces analysis of complex by d_{norm} , d_i , d_e , shape index (top) and curvedness mapping.

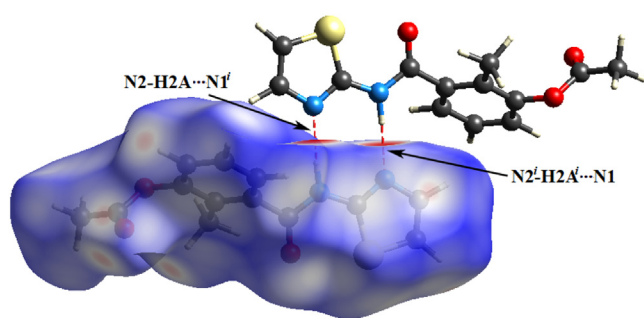


Fig. 12. Hirshfeld surface mapped with d_{norm} function with N-H...N hydrogen bonds.

Table 8

Intermolecular interactions in the complex according to XRD and Hirshfeld results.

Interaction	H...O (XRD) Å	H...O (Hirshfeld) Å
N2-H2A...N1	2.932	2.930

the white region represents equal interactions [65,66]. Light red spots are due to C-H...O interactions. The shape-index and curvedness surfaces have been shown to convey the information about each donor-acceptor pair and to measure how much shape effectively divides the surfaces into set of patches respectively (Fig. 11). This is used in crystal stacking (C7-H7C...O3ⁱⁱ)

The 3D package structure of the molecule was formed by the interaction of N-H...N intermolecular hydrogen bonds that contribute to the crystal packaging, shown on the d_{norm} surface in Fig. 12. In Table 8, short intermolecular interactions of the MTP compound according to XRD and Hirshfeld results are given. The representation of the 2-dimensional fingerprint is given in Fig. 13. The largest contributions of the total Hirshfeld surface are H...H/H...H (37.8%), C...H/H...C (15.5%), O...H/H...O (24.4 %) and N...H/H...N (8.7%) belongs to their interactions (Fig. 13). Hirshfeld surface analysis is one of the best ways to understand the intermolecular interactions with the crystalline environment of the molecule.

3.6. Druglikeness and ADME studies

A perfect drug is not only the one having the best binding interaction with the target, but also the activity of this drug in the human system must be looked at. Therefore, it is necessary to examine the absorption, distribution, metabolism and excre-

Table 9

The physicochemical properties, druglikeness and the pharmacological properties with SwissADME prediction of the compounds.

Physicochemical Properties		Druglikeness		Pharmacokinetic properties	
MW	286.39	WLogP	0.71	GI	High
H-A	4	SA	3.97	BBB	No
TPSA	92.73	LogS	-	CYP1A2 inhibitor	No
Rotatable bonds	5		2.14	CYP2C6 inhibitor	No
MR	78.97			LogK _p	-7.08
N atoms	19				

MW=Molecular Weight (g/mol); **H-A**=no. of H-bond acceptors; **H-D** = no. of H-bond donor; **LogS**=Solubility; **TPSA**=Total polar surface area; **MR**=Molar refractivity; **Natoms**=number of atoms; **WLogP**=Lipophilicity; **SA**=Synthetic Accessibility; **GI**=Gastro intestinal; **BBB**= Blood brain barrier; **P-gp**=P-glycoprotein; **CYP1A2**= cytochrome P450 family 1 subfamily A member 2 (PDB: 2H14); **CYP2D6**= cytochrome P450 family 2 subfamily D member 6 (PDB:5TFT); **Log K_p**=Skin permeation (cm/s)

tion (ADME) properties of the MTP compound. Orally administered compounds must pass through the gastrointestinal tract rapidly and be fully *absorbed*, properly *distributed*, *metabolized* slowly, and *excreted* harmlessly and accurately. If a drug fails, it may be due to poor ADME properties [67]. The physicochemical data of MTP compound was calculated using OpenBabelGui [68] and SwissADME software [69]. We may distinguish the non-drug like and drug like molecules by Lipinski's five rules [70]. It can be said that the druglikeness to the molecules that comply with two or more of the Lipinski's rules given in the following showed high success or non-druglikeness properties for less.

- MW < 500 g/mol
- LogP < 5
- H-A (no. of H-bond acceptors) < 5
- H-D (no. of H-bond donor) < 10
- 40 < MR (Molar refractivity) < 130

Investigation of these properties can help in early preclinical developments and prevent costly late pre-clinical and clinical failures. To find out the drug-like properties, the MTP compound was evaluated using Lipinski's rule of five. The results were given in Table 9 and the radar graphs were figured in Fig. S1. Molar refraction (MR) is the total polarization capacity of one mole of a compound. It depends on temperature, pressure and refractive index [71]. The octanol/water partition coefficient (logP) is related to the polar or hydrophobic character of a compound. The polar surface area (PSA) or topological polar surface area (TPSA) of a molecule is the surface sum over all polar atoms or molecules, including bonded hydrogen atoms. LogP, MW (molecular weight) and TPSA

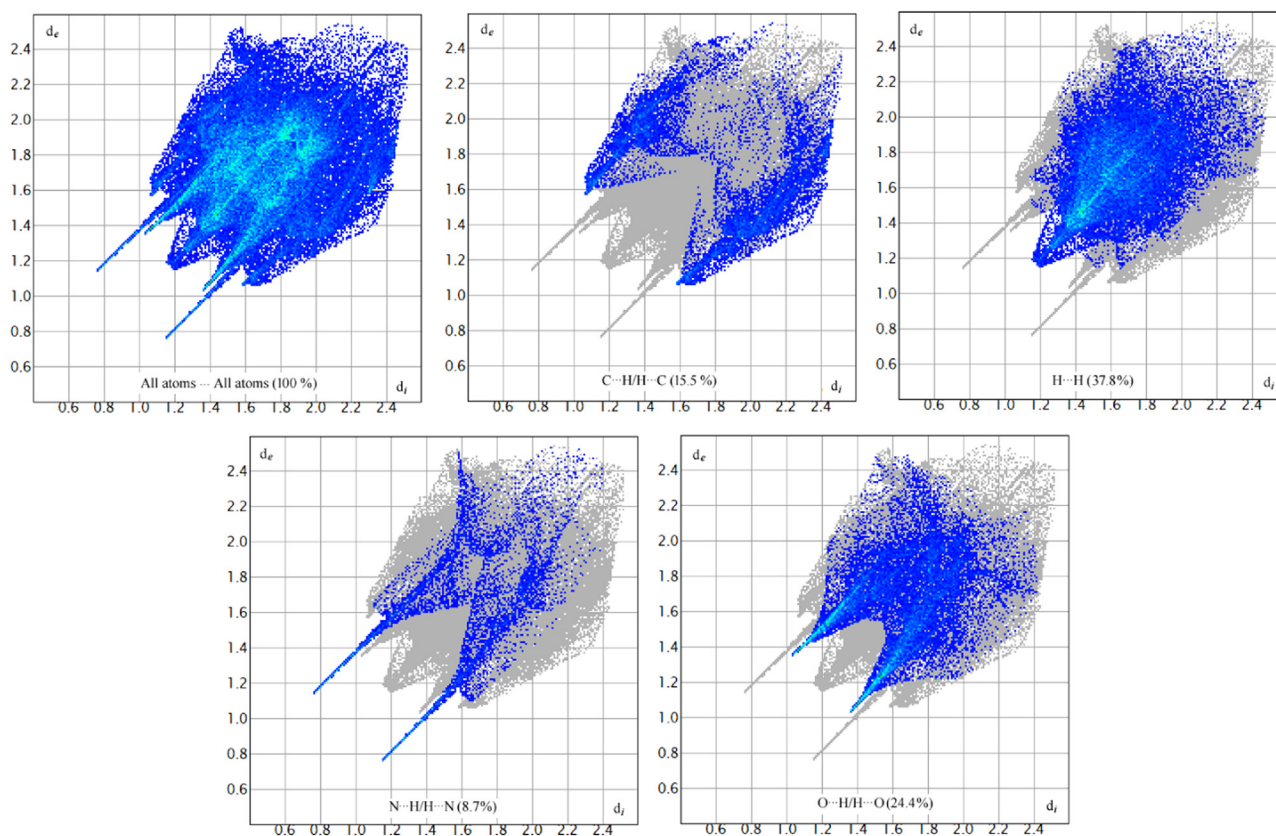


Fig. 13. Finger-print plot of percentages of contacts on the Hirshfeld surface in the compound.

values are all related to oral bioavailability, membrane permeability. In addition, HBA (Hydrogen bond acceptor) and HBD (hydrogen bond donor) also play an important role in identifying drug candidates. Results showed that new synthesized MTP compound owned the drug-likeness properties. The synthesized MTP compound is found to be no Lipinski violations. Hence, it implies that this compound has the drug ability properties/druglikeness properties.

In addition, the skin permeability coefficient ($\log K_p$) is determined by the molecular size and lipophilicity that appear in the correlation. The $\log K_p$ value of our compound is found to be -7.08. Boiled-Egg is correlated between WLOGP and TPSA to evaluate HIA and BBB as indicators of passive gastrointestinal absorption and brain penetration barrier, respectively. The white area indicates the area with a high probability of passive absorption through the gastrointestinal tract, while the yellow area (yolk) indicates a high probability of brain penetration. Compound, red dot appearing in white area indicates its off-substrate role against P-gp (PGP-).

The study of toxicity is an important drug development process and frequently used in preclinical and clinical trial phases. Thus, this study provides savings in terms of workload, cost and time for the future experimental studies [72].

The LD_{50} of the compound in the ProTox-II server was found below 5000 mg/kg (LD_{50} =3000 mg/kg). The lower the LD_{50} value, the higher the lethal value. Accordingly, the MTP compound is in class 5 ($2000 < LD_{50} \leq 5000$) which is harmful if swallowed. The mutagenicity, acute toxicity, immunotoxicity, hepatotoxicity, carcinogenicity and cytotoxicity of the compound are found to be inactive. Oral rat LD_{50} , 96h D. magna LC_{50} , 96h fat-headed minnow LC_{50} value means that the chemical compound in the water will kill 50% of the fat-headed minnow after 96 hours. The number of chemical compounds (mg/kg body weight) that caused 50% of the

rats to die after oral ingestion was calculated using T.E.S.T software and listed in Table 10.

3.7. Molecular docking studies

The binding sites of the protein are mainly its big and small cavities. The studies related to protein-small molecule interactions can help understand the molecular mechanisms of drug and protein-drug interactions in drug design. Molecular docking is a useful computational technique to predict these interactions and to find binding sites and best orientation of the small molecules. Therefore, crystal structures of proteins from bacterial and fungal organisms were downloaded from the Protein Data Bank (PDB). These are the *Bacillus licheniformis* (PDB ID:6B7K), *Micrococcus luteus* (PDB ID:4EWP), *Enterobacter aerogenes* (PDB ID:1GMU), *Proteus vulgaris* (PDB ID:4MCX) and *Aspergillus flavus* (PDB ID:1R56), *Aspergillus niger* (PDB ID:3K4P), *Aspergillus parasiticus* (PDB ID:3HRQ), *Candida albicans* (PDB ID:1ZAP) and *Candida utilis* (PDB ID:3VGB). The DFT calculation has been used to get energy minimized geometry optimized structure of the MTP compound. The docking studies have been performed to predict the most probable mode of binding of the MTP compound with proteins by Autodock 4.2.6, Autodock Tool 1.5.6 [73,74] and BIOVIA Discovery studio Visualizer software 2019 [75]. The most favored binding modes of MTP compound with proteins are presented in Table 11.

As shown in Fig. 14, there are various types of interactions of MTP compound with *B. licheniformis*. The HD group of ASN257 residue in *B. licheniformis* interacts with O atom of the Acethyl group in the compound via H-bond interaction. Moreover, LYS18 residue in *B. licheniformis* interacts with thiazole ring of the MTP compound via H-bond interaction. The O atoms of the carbonyl groups moiety in the MTP compound interact with ARG47 and

Table 10

Toxicity prediction values and results of the MTP compound and its intermediates calculated by T.E.S.T and Pro-TOX II.

Acute toxicity		Developmental toxicity		Mutagenicity	
Oral rat LD ₅₀ (mg/Kg)	Daphnia magna LC ₅₀ (96 h)(mg/L)	Predicted value	Predicted result	Predicted value	Predicted result
963.43	11,33	0.23	Developmental non-toxicant	0.39	Negative
Organ toxicity	Toxicity Endpoints	Immunotoxicity	Mutagenicity	Cytotoxicity	LD ₅₀ (mg/kg)
Hepatotoxicity	Carcinogenicity	Inactive	Inactive	Inactive	3000
Inactive	Inactive				

Table 11

Molecular docking energy values for ligand–protein interaction.

Protein ID	Affinity (kcal.mol ⁻¹)	Est. free energy of binding (kcal.mol ⁻¹)	Est. inhibition constant, K _i (µM)	vdW+ Hbond+ desolve energy (kcal.mol ⁻¹)	Electrostatic energy (kcal.mol ⁻¹)	Final Total Internal Energy (kcal.mol ⁻¹)	Torsional Free Energy (kcal.mol ⁻¹)	Unbound System's Energy (kcal.mol ⁻¹)	Total internal energy (kcal.mol ⁻¹)
6B7K	-7.7	-5.85	51.92	-6.96	-0.08	-0.28	+1.19	-0.28	-7.04
4EWP	-7.2	-4.84	282.37	-6.11	+0.08	-0.44	+1.19	-0.44	-6.04
1GMU	-7.0	-4.33	672.13	-5.51	-0.01	-0.58	+1.19	-0.58	-5.52
4MCX	-7.5	-4.95	236.45	-5.86	-0.28	-0.57	+1.19	-0.57	-6.14
1R56	-7.9	-5.55	85.99	-6.59	-0.15	-0.58	+1.19	-0.58	-6.74
3K4P	-6.9	-5.14	171.09	-6.24	-0.09	-0.48	+1.19	-0.48	-6.33
3HRQ	-6.9	-4.76	323.25	-5.87	-0.08	-0.56	+1.19	-0.56	-5.96
1ZAP	-6.3	-3.96	1250.00	-5.02	-0.14	-0.50	+1.19	-0.50	-5.16
3VGB	-7.0	-5.61	77.28	-6.54	-0.26	-0.56	+1.19	-0.56	-6.80

Table 12

The minimum inhibition concentrations (MIC's) of the MTP compound.

Sample	Minimum inhibition concentration (µg/mL)								
	Gram-staining-positive			Gram-staining-negative		Fungi			
	B. licheniformis	M. luteus	E. aerogenes	P. vulgaris	A. flavus	A. niger	A. parasiticus	C. albicans	C. utilis
MTP Compound	256	512	256	512	256	512	512	1024	1024
Amoxicillin	<2	>1000	>1000	>1000	-	-	-	-	-
Tetracycline	<2	8	8	8	-	-	-	-	-
Ketoconazole	-	-	-	-	1	1	1	2	2

GLN238 residues in *M. luteus* via H-bond interaction. There are non-covalent interactions such as the π -alkyl and the π -stacked between the MTP compound and *E. aerogenes*. ARG29 and HIS-1 residues in *P. vulgaris* interact with carbonyl groups of the MTP compound via H-bond interaction. As well, THR57 and ASP58 residues in *A. flavus* interact with N1, O1 and O1 atoms of in the MTP compound, respectively, via hydrogen bond interactions. In Fig. 14, the MTP compound interacts with *A. flavus* via non-covalent interactions such as the π -anion, π -cation and the π -sulfur. In particular, ASP58, HIS256, PHE159 of *A. flavus* interact with the thiazole and benzene rings of the MTP compound via the π -anion, π -cation and the π -sulfur interactions. The TRP376 residue in *A. niger* interacts with N atom of the thiazole ring via H-bond interaction. There are only H-bond and π -sulfur interactions between *A. parasiticus* and the MTP compound. The ASN323 and ASN153 residue of *C. albicans* interact with carbonyl group via H-bond interactions. Finally, one of the important interactions in the *C. utilis* is hydrogen bond interaction and occurs between the NH group of the GLN86 residue and the N1 atom of the thiazole ring in the MTP compound (Table S1).

The free binding energies of proteins and MTP compound are calculated and results are given in Table 11. It has been observed that the estimated free energy of binding between *B. licheniformis* and the MTP compound as the largest. However, it has found that the estimated free binding energy is the smallest between the *C. albicans* protein and the MTP compound. The inhibition constant was calculated as the lowest in the *B. licheniformis* protein (51.92 µM). The inhibition constant is very important as it predicts how a compound will inhibit an enzyme and how it might interact with

a substrate for the enzyme. The lower the inhibition constant, the higher the probability of a chemical reaction between the ion and the receptor antigen. Therefore less drug is needed to inhibit enzyme activity [76].

3.8. Evaluation of antimicrobial activity

The antimicrobial activity of the MTP compound was determined according to the minimum inhibitory concentration (MIC). Amoxicillin and tetracycline were used for bacteria and ketoconazole was utilized for fungi as the standard control. The results are demonstrated in Table 12.

In our previous study (Yakan et al. 2020), the activity of the MTP molecule against six bacterial species including three gram-positive (*S. aureus*, *E. faecalis* and *B. cereus*) and gram-negative bacteria (*E. coli*, *P. aeruginosa* and *K. pneumoniae*) was investigated. The MTP compound exhibited the highest activity against *K. pneumoniae* among these bacterial strains compared to the reference amoxicillin. In this study, the activities of the MTP compound against four bacterial species including *B. licheniformis*, *M. luteus*, *E. aerogenes* and *P. vulgaris* were analyzed. In addition, activity studies were conducted for five different fungi species including *A. flavus*, *A. niger*, *A. parasiticus*, *C. albicans* and *C. utilis*.

As shown in Table 12, the MTP compound exhibited better activity against the Gram-staining-positive *M. luteus*, and the Gram-staining-negative *E. aerogenes* and *P. vulgaris* than Amoxicillin standard. Also, this MTP compound showed a lower effect on *B. licheniformis* compared to the Amoxicillin and Tetracycline standards. When compared to the Ketoconazole standard, it exhibited lower

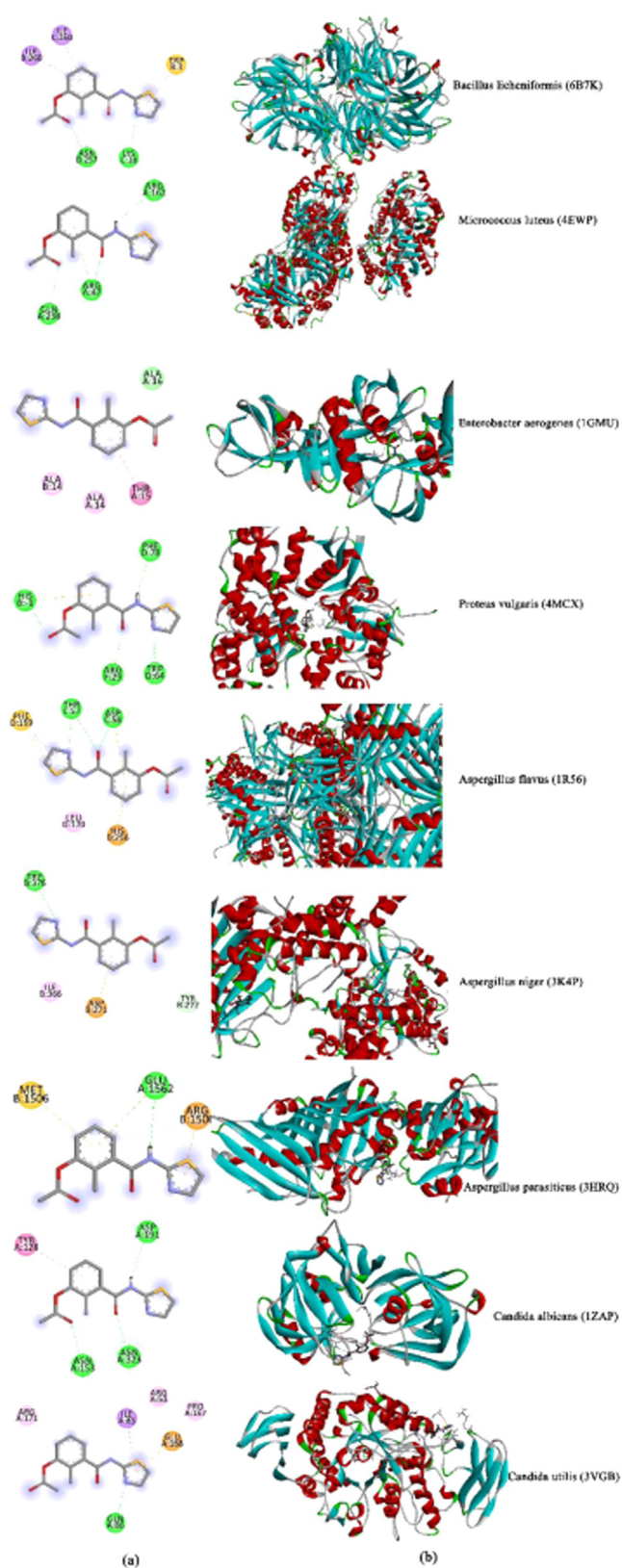


Fig. 14. Best fit binding of MTP compound in the proteins cavity (a) 2D; (b) 3D.

antifungal activity for fungi *A. flavus*, *A. niger*, *A. parasiticus*, *C. albicans*, and *C. utilis*.

Thiazole and its derivatives have shown potential antimicrobial activity against various pathogen microorganisms. Cakmak et al. (2022) found that *N*-(thiazol-2-yl)furan-2-carboxamide have a good antibacterial effect against *S. aureus*, *E. faecalis*, *K. pneumoniae* and *P. aeruginosa* [77]. Pawar, et al. (2016) synthesized a series of acetamide substituted thiazole derivatives (13a-e) and all the derivatives displayed potent antibacterial activity against *E. coli*, *S. aureus*, and *B. subtilis* [78]. A similar study was published in 2010 by Gouda and his colleagues. They examined the antibacterial activities of a number of thiazole and pyrazole derivatives (20-21) against various bacteria, and found that all the synthesized derivatives showed better antibacterial activity than the reference drug molecules [79].

4. Conclusions

In this study, we employed a different methodology for amide bond formation, where the MTP compound was synthesized in a high yield from the reaction of 3-acetoxy-2-methylbenzoic anhydride with thiazol-2-amine. In this model reaction study, methanesulfonyl chloride (MsCl) was used to activate the anhydride. Yet, in our previous study, we pre-activated carboxylic acids using thionyl chloride, which was a traditional method. The MTP compound that we obtained in this study has higher yield compared to the previous study. The structure of this MTP compound confirmed through X-ray diffraction, IR, ^{13}C and ^1H NMR spectroscopies and elemental analysis. This MTP compound was crystallized in the $P2_1/n$ monoclinic space group and the asymmetric unit contains one molecule. Considering the experimental antimicrobial values, the MTP compound exhibits the best activity against Gram-staining-positive *B. licheniformis*, Gram-staining-negative *E. aerogenes* and fungi *A. flavus*. However, according to molecular docking results, the MTP compound showed the best activity against Gram-staining-positive *B. licheniformis*, fungi *A. flavus* and *C. utilis*. This activity is due to the presence of S and N heteroatoms in the thiazole ring in the structure of the MTP compound. These findings show that this and similar compounds with thiazole ring can be assessed as promising antibacterial agents.

5. Author contribution statement

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Synthesis, Spectroscopic Characterization, Writing-Review, Visualization & Editing, Supervision.

Tuğba Aycan: Department of Physics, Faculty of Arts and Sciences, Sinop University, Sinop, Turkey

X-Ray Studies, Molecular Docking Studies, Druglikeness and toxicology, Theoretical calculations, Writing-Review.

Filiz Öztürk: Karadeniz Advanced Technology Research and Application Center, Ondokuz Mayıs University, Samsun, Turkey

Hirshfeld analysis, Theoretical calculations, Writing-Review.

Aysel Veyisoğlu: Department of Medical Services and Techniques, Vocational School of Health Services, Sinop University, Sinop, Turkey

Biological Studies, Writing-Review.

Supplementary

CCDC Deposition Number 2169704 contains the supplementary crystallographic data for this study (<https://www.ccdc.cam.ac.uk/>). Radar graph and BOILED-Egg radar of compound (Fig. S1 and Table S1) was provided.

Declaration of Competing Interest

The author declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:[10.1016/j.molstruc.2022.133937](https://doi.org/10.1016/j.molstruc.2022.133937).

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