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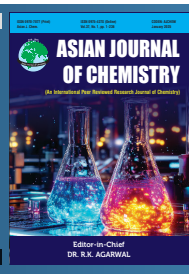


# Synthesis and Biological Evaluation of New Chalcone based Thiazole Derivatives: Antimicrobial, Antifungal Activity, ADME and Molecular Docking Studies

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The Claisen-Schmidt condensation reaction of 2-acetyl thiophene with heterocyclic carboxaldehyde or substituted benzaldehyde in the presence of alkaline medium was used to synthesize novel thiophene chalcones. Several chalcone heterocycle hybrids appear to have more activity than the standards. These chalcones can be hydroaminated to provide good yields of amino thiazole derivatives. The newly synthesized compounds were characterized with spectroscopic techniques including FT-IR, $^{1}$H NMR, $^{13}$C NMR and HRMS. In addition to a seed germination inhibition test and an evaluation of the synthesized compounds *in vitro* antibacterial activity against a variety of bacteria and fungi, the synthesized aminothiazole derivatives underwent molecular docking and *in silico* ADMET studies.

**Keywords: Chalcones, Thiazoles, Antibacterial activity, Antifungal activity, Seed germination.**

## INTRODUCTION

The structure of chalcones, α,β-unsaturated carbonyl compounds, allows them to interact with a variety of biological targets by nucleophilic attack, which is crucial to their pharmacological characteristics [1]. The incorporation of heterocyclic moiety into the chalcone has further enhanced their medicinal relevance [2]. Thiazoles, exhibit a wide range of biological activities and serve as valuable components in medicinal chemistry for several biological applications [1]. 2-Amino thiazoles are heterocyclic compounds with extensive applications in drug design. These scaffolds have been identified as key pharmacophores due to their antiviral, anticancer, antimicrobial, antidiabetic and anti-inflammatory activities [3-5]. The biological activity of thiazoles is closely related to their substitution patterns at positions 2 and 4, which allow for versatile interactions with biological targets.

Heterocyclic chalcones, incorporating bioactive heterocycles such as pyridine, furan, thiophene and thiazole, have shown significant promise in medicinal chemistry [6,7]. The pharmacological potential of these compounds can be attributed to their ability to undergo nucleophilic addition reactions, such as the aza-Michael reaction, facilitating the formation of C-N bonds essential for drug development [8]. The incorporation of 2-amino thiazole moieties into chalcone derivatives has resulted in the development of novel compounds with enhanced anticancer activities, such as antiestrogenic effects, apoptosis induction, tubulin polymerization inhibition and suppression of angiogenesis [9]. Structural modifications, particularly in the aryl moieties or the enone linker of chalcones, have led to the identification of potent anticancer agents [9]. Bioisosteric substitution of the chalcone aryl groups with heterocyclic moieties has further improved their specificity and potency [10].

Thus, motivated by the numerous pharmacological applications linked to heterocycle based chalcones, the synthesis of novel 3-(2-amino thiazole)-1-(2-thienyl)-3-heteroaryl-1-oxo propane derivatives in moderate to good yields using the Claisen-Schmidt condensation of aromatic aldehydes and thiophene ketone were accomplished. Moreover, the modified agar well diffusion assay method is used to screen the compounds for antibacterial and antifungal activity and seed germination inhibition test. Furthermore, the ADME and molecular docking studies were carried out to understand their biological activity.

## EXPERIMENTAL

The melting points were determined using an open capillary tube method and are uncorrected. The progress of the reaction was examined using a TLC method using benzene-ethyl acetate mixture in a 7.0:0.5 v/v ratio, when exposed towards UV light. Using deuterated chloroform ($CDCl_3$) as a solvent and tetramethyl silane (TMS) as an internal standard respectively, 400 M Hz Bruker-NMR spectrometer was employed to record the $^1H$ NMR and $^{13}C$ NMR. The Perkin-Elmer spectra was analyzed to obtain an infrared spectrum in the 4000-400 $cm^{-1}$ region. Mass spectra were acquired employing with the Lynx SCN781 spectrometer TOF mode. The compounds were purified using column chromatography utilizing Merck silica gel (60-120 mesh).

**Synthesis of chalcones (3a-f):** Stirred a solution of different benzaldehyde (**2a-e**, 5 mmol) in aqueous NaOH solution (5 mmol) for 4 h at room temperature and 2-acetyl thiophene (**1a**, 5 mmol) was dissolved in 95% ethyl alcohol (12 mL). For 4 h, a solution comprising acetophenone (**1f**, 5 mmol), benzaldehyde (**2f**, 5 mmol) and aqueous NaOH solution (5 mmol) in 12 mL of 95% ethyl alcohol was shaken at room temperature. Thin layer chromatography (TLC) plates were used to track the progress of the reaction. Once the reaction complete, the resultant product was placed in an ice-cold water overnight. It was washed in cold water after the solid was separated and recrystallize with ethyl alcohol.

**1,3-Di(thiophen-2-yl)prop-2-en-1-one (3a):** Pale yellow solid, yield: 90%, m.p.: 95-97 ºC; IR (KBr, $\nu_{max}$, $cm^{-1}$): 1631 (C=O), 1562 (C=C), 721 ( C-S-C); $^1H$ NMR ($CDCl_3$) δ ppm: 7.807 (d, 1H, *J* = 15.3 Hz, HC=C), 7.670 (d, 1H, *J* = 15.5 Hz, C=CH), 7.173-7.994 (m, 6H, *J* = 6.5-7.5 Hz, Th-H); $^{13}C$ NMR ($CDCl_3$) δ ppm: 120.4, 128.2, 128.4, 128.9, 131.7, 132.1, 133.8, 136.4, 140.1, 145.5, 181.5; MS *m/z*: 220.9 ($M^+$).

**3-(Furan-2-yl)-1-(thiophen-2-yl)prop-2-en-1-one (3b):** Brown solid, yield: 84%, m.p.: 69-71 ºC; IR (KBr, $\nu_{max}$, $cm^{-1}$): 1640 (C=O), 1586 (C=C), 1230 (C-O-C), 722 (C-S-C); $^1H$ NMR ($CDCl_3$) δ ppm: 6.711 (d, 1H, *J* = 15.3 Hz, HC=C), 6.720-7.855 (m, 3H, *J* = 7.6 Hz, Fu-H), 7.584 (d, 1H, *J* = 15.5 Hz, C=CH), 7.154-7.853 (m, 3H, *J* = 6.5 Hz, Th-H); $^{13}C$ NMR ($CDCl_3$) δ ppm: 112.7, 116.4, 119.1, 128.2, 129.9, 130.1, 133.8, 145.0, 145.6, 151.4, 181.7; MS *m/z*: 205.0 (M+1).

**3-(Pyridin-3-yl)-1-(thiophen-2-yl)prop-2-en-1-one (3c):** Pale brown solid, yield: 89%, m.p.: 92-94 ºC; IR (KBr, $\nu_{max}$, $cm^{-1}$): 1651 (C=O), 1414 (C=C), 714 (C-S-C), 1229 (C=N); $^1H$ NMR ($CDCl_3$) δ ppm: 7.100 (d, 1H, *J* = 15.3 Hz, HC=C), 7.741 (d, 1H, *J* = 15.5 Hz, C=CH), 7.203-7.897 (m, 3H, *J* = 6.5 Hz, Th-H), 7.615-8.862 (m, 4H, *J* = 7.6 Hz, Py-H); $^{13}C$ NMR ($CDCl_3$) δ ppm: 123.8, 128.0, 130.5, 132.2, 134.0, 135.4, 135.8, 140.2, 143.9, 148.2, 149.9, 190.6; MS *m/z*: 216.0 (M+1).

**3-(4-Methoxyphenyl)-1-(thiophen-2-yl)prop-2-en-1-one (3d):** Pale yellow solid, yield: 88%, m.p.: 66-68 ºC; IR (KBr, $\nu_{max}$, $cm^{-1}$): 1641 (C=O), 1510 (C=C), 728 (C-S-C); $^1H$ NMR ($CDCl_3$) δ ppm: 3.883 (s, 3H, Me-H), 6.955 (d, 1H, *J* = 15.3 Hz, HC=C), 7.201-7.692 (m, 4H, *J* = 7.6 Hz, Ar-H), 7.694 (d, 1H, *J* = 15.5 Hz, C=CH), 7.213-7.884 (m, 3H, *J* = 6.5 Hz, Th-H); $^{13}C$ NMR ($CDCl_3$) δ ppm: 55.4, 114.2, 114.2, 119.3, 127.4, 128.1, 130.2, 131.4, 131.2, 133.5, 143.9, 145.8, 161.7, 182.0; MS *m/z*: 245.0 (M+1).

**3-(3,4-Dimethoxyphenyl)-1-(thiophen-2-yl)prop-2-en-1-one (3e):** Pale yellow solid, yield: 89%, m.p.: 65-67 ºC; IR (KBr, $\nu_{max}$, $cm^{-1}$): 1643 (C=O), 1579 (C=C), 728 (C-S-C); $^1H$ NMR ($CDCl_3$) δ ppm: 3.942-3.952 (s, 6H, Me-H), 6.980 (d, 1H, *J* = 15.3 Hz, HC=C), 7.143-7.236 (m, 3H, *J* = 7.6 Hz, Ar-H), 7.774 (d, 1H, *J* = 15.5 Hz, C=CH), 7.148-7.868 (m, 3H, *J* = 6.5 Hz, Th-H); $^{13}C$ NMR ($CDCl_3$) δ ppm: 55.9, 55.9, 111.1, 111.1, 119.4, 123.1, 127.6, 128.1, 130.1, 133.6, 144.1, 145.7, 149.2, 151.4, 190.0; MS *m/z*: 275.0 (M+1).

**1,3-Diphenylprop-2-en-1-one (3f):** Pale yellow solid, yield: 96%, m.p.: 56-58 ºC; IR (KBr, $\nu_{max}$, $cm^{-1}$): 1640 (C=O), 1564 (C=C); $^1H$ NMR ($CDCl_3$) δ ppm: 7.498 (d, 1H, *J* = 15.3 Hz, HC=C), 7.330-7.891 (m, 10H, *J* = 7.6 Hz, Ar-H), 7.943 (d, 1H, *J* = 15.5 Hz, C=CH); $^{13}C$ NMR ($CDCl_3$) δ ppm:120.1, 127.5, 128.1, 128.1, 128.5, 128.5, 128.7, 128.7, 129.1, 129.1, 134.6, 136.5, 138, 144.6, 184.8; MS *m/z*: 209 (M+1).

**General procedure for synthesis of chalcone amino thiazole derivatives (5a-f):** 2-Amino thiazole (**4**, 0.01 mol) and chalcones (**3a-f**, 0.01 mol) in ethyl alcohol were mixed and stirred to obtain a homogeneous solution and then left to stand for one night. The progress of the reaction was monitored with TLC and once it was completed, the reaction mixture was cooled in an ice bath. The solid products were separated and then dried. The products **5a-f** were purified by column chromatography using silica gel (60-120 mesh) and benzene:ethyl acetate (7.0:0.5 v/v) as an eluent.

**3-(Thiazol-2-ylamino)-1,3-di(thiophen-2-yl)propan-1-one (5a):** Light brown solid, yield: 82%, m.p.: 59-60 ºC; IR (KBr, $\nu_{max}$, $cm^{-1}$): 3119 (N-H), 1634 (C=O), 1567 (C=N), 1064 (C-N), 737 (C-S-C); $^1H$ NMR ($CDCl_3$) δ ppm: 3.765-3.798 (d, 2H, *J* = 7.0 Hz, $H_2C$-), 6.204 (t, 1H, *J* = 7.1 Hz, -CH), 6.855 (d, 1H, *J* = 7.3 Hz, thiazole-S-CH), 6.871 (s, 1H, NH), 7.226 (d, 1H, *J* = 7.3 Hz, thiazole-N-CH), 6.891-7.880 (m, 6H, *J* = 6.6 Hz, Th-H); $^{13}C$ NMR ($CDCl_3$) δ ppm: 71.3, 75.5, 110.1, 126.0, 127.0, 127.5, 128.2, 129.9, 133.8, 135.8, 138.6, 143.8, 162.1, 183.6; MS *m/z*: 321.0 (M+1).

**3-(Furan-2-yl)-3-(thiazol-2-ylamino)-1-(thiophen-2-yl)-propan-1-one (5b):** Dark brown solid, yield: 80%, m.p.: 43-45 ºC; IR (KBr, $\nu_{max}$, $cm^{-1}$): 2930 (N-H), 1631 (C=O), 1564 (C=N), 1244 (C-O-C), 1034 (C-N), 735 (C-S-C); $^1H$ NMR ($CDCl_3$) δ ppm: 3.982-3.991 (d, 2H, *J* = 7.0 Hz, $H_2C$-), 6.411 (t, 1H, *J* = 7.1 Hz, -CH), 6.799 (d, 1H, *J* = 7.3 Hz, thiazole-S-CH), 6.802 (s, 1H, NH), 7.324 (d, 1H, *J* = 7.3 Hz, thiazole-N-CH), 6.531-7.711 (m, 3H, *J* = 7.5 Hz, Fu-H), 6.819-7.880 (m, 3H, *J* = 6.6 Hz, Th-H); $^{13}C$ NMR ($CDCl_3$) δ ppm: 71.1, 73.5, 108, 109, 111.2, 127.6, 132.4, 133.9, 136, 140.1, 141.1, 149, 162.1, 182.9; MS *m/z*: 300.0 (M-4).

**3-(Pyridin-3-yl)-3-(thiazol-2-ylamino)-1-(thiophen-2-yl)propan-1-one (5c):** Dark brown semi-solid, yield: 76% m.p.: 51-53 ºC; IR (KBr, $\nu_{max}$, $cm^{-1}$): 2955 (N-H), 1613 (C=O), 1513 (C=N), 1180 (C-N), 722 (C-S-C); $^1H$ NMR ($CDCl_3$); δ 3.818-3.900 (d, 2H, *J* = 7.0 Hz, $H_2C$-), 6.321 (t, 1H, *J* = 7.1 Hz, -CH), 6.784 (d, 1H, *J* = 7.3 Hz, thiazole-S-CH), 6.813 (s, 1H, NH), 7.111 (d, 1H, *J* = 7.3 Hz, thiazole-N-CH), 7.321-7.811 (m, 3H, *J* = 6.6 Hz, Th-H), 7.392-8.716 (m, 4H, *J* = 7.5

Hz, Py-H); $^{13}$C NMR ($CDCl_3$): δ 70.6, 73.2, 111, 122, 128.2, 131, 134.4, 135, 136, 140, 142.7, 145, 149.3, 161.7, 183; MS *m/z*: 311.1 (M-4).

**3-(4-Methoxyphenyl)-3-(thiazol-2-ylamino)-1-(thiophen-2-yl)propan-1-one (5d):** Light brown solid, yield: 83% m.p.: 49-50 ºC; IR (KBr, $\nu_{max}$, $cm^{-1}$): 3118 (N-H), 1633 (C=O), 1566 (C=N), 1064 (C-N), 722 (C-S-C); $^1$H NMR ($CDCl_3$) δ ppm: 3.784-3.876 (d, 2H, *J* = 7.0 Hz, $H_2C$-), 3.902 (s, 3H, Me-H), 6.540 (t, 1H, *J* = 7.1 Hz, -CH), 6.856 (d, 1H, *J* = 7.3 Hz, thiazole-S-CH), 6.877 (s, 1H, NH), 7.286 (d, 1H, *J* = 7.3 Hz, thiazole-N-CH), 6.972-7.357 (m, 4H, *J* = 7.6 Hz, Ar-H), 7.379-7.822 (m, 3H, *J* = 6.6 Hz, Th-H); $^{13}$C NMR ($CDCl_3$) δ ppm: 55.4, 76.7, 77.3, 108.7, 114.1, 114.1, 124.8, 127.4, 128.2, 130.3, 131.5, 133.5, 138.8, 143.9, 145.7, 161.7, 182.1; MS *m/z*: 341.0 (M-3).

**3-(3,4-Dimethoxyphenyl)-3-(thiazol-2-ylamino)-1-(thiophen-2-yl)propan-1-one (5e):** Dark brown solid, yield: 81%, m.p.: 52-54 ºC; IR (KBr, $\nu_{max}$, $cm^{-1}$): 3116 (N-H), 1636 (C=O), 1565 (C=N), 1062 (C-N), 723 (C-S-C); $^1$H NMR ($CDCl_3$) δ ppm: 3.795-3.824 (d, 2H, *J* = 7.0 Hz, $H_2C$-), 3.841-3.863 (s, 6H, Me-H), 5.245 (t, 1H, *J* = 7.1 Hz, -CH), 6.796 (d, 1H, *J* = 7.3 Hz, thiazole-S-CH), 6.817 (s, 1H, NH), 7.201 (d, 1H, *J* = 7.3 Hz, Th-N-CH), 6.905-6.971 (m, 3H, *J* = 7.6 Hz, Ar-H), 7.246-7.889 (m, 3H, *J* = 6.6 Hz, thiazole-H); $^{13}$C NMR ($CDCl_3$) δ ppm: 56.6, 56.6, 76.7, 77.4, 109.8, 111.1, 118.8, 123.2, 128.3, 133.6, 134.5, 138.6, 138.7, 141.2, 145.7, 148.5, 149.2, 151.5; MS *m/z*: 375.0 (M+1).

**1,3-Diphenyl-3-(thiazol-2-ylamino)propan-1-one (5f):** Brown solid, yield: 85%, m.p.: 51-53 ºC; IR (KBr, $\nu_{max}$, $cm^{-1}$): 2999 (N-H), 1629 (C=O), 1564 (C=N), 1055 (C-N), 724 (C-S-C); $^1$H NMR ($CDCl_3$) δ ppm: 3.137-3.687 (d, 2H, *J* = 7.0 Hz, $H_2C$-), 4.809 (t, 1H, *J* = 7.1 Hz, -CH), 7.189 (d, 1H, *J* = 7.3 Hz, thiazole-S-CH), 7.199 (s, 1H, NH), 7.211 (d, 1H, *J* = 7.3 Hz, thiazole-N-CH), 7.286-7.887 (m, 10H, *J* = 7.6 Hz, Ar-H); $^{13}$C NMR ($CDCl_3$) δ ppm: 72, 75, 112.1, 126.1, 126.8, 126.8, 128.1, 128.1, 128.4, 128.4, 128.9, 128.9, 134.2, 136.1, 137, 141, 162.1, 192.0; MS *m/z*: 309.0 (M+3).

**Antibacterial activity:** The antibacterial activity of the synthesized compounds **5a-f** was evaluated using a modified agar well diffusion assay [11]. The 18-24 h old bacterial cultures and newly subcultured bacteria were used for the assay. The Mueller-Hinton agar (MHA) as medium and the bacterial pathogens *viz*. *Escherichia coli* [MTCC-1599], *Staphylococcus aureus* [MTCC-4734] and *Pseudomonas aeruginosa* [MTCC-1934] were employed. Wells with a diameter of 6 mm were developed in the agar using a sterile borer and different concentrations (5 mg/mL, 2.5 mg/mL and 1.25 mg/mL) of compounds **5a-f** were added to the wells. Streptomycin, ciprofloxacin and chloramphenicol at a concentration of 1 mg/mL were used as positive control, while DMSO served as the negative control. After incubation for 24 h at 37 ºC, the zone of inhibition was measured.

**Antifungal activity:** The antifungal efficacy of synthesized compounds **5a-f** was investigated using the agar well diffusion approach using *Candida albicans* [MTCC-1637], *Aspergillus brasiliensis* [MTCC-1344] and *Aspergillus flavus* [MTCC-9606] as test fungal pathogens. The spore suspension from the test fungus culture was swabbed onto sterile (SDA) sabouraud dextrose agar (Himedia Laboratories Pvt. Ltd., India) medium. The wells were developed with a sterile borer on sabouraud dextrose agar medium plates and loaded with different concentrations (5 mg/mL and 2.5 mg/mL). Standard antibiotic (fluconazole, 1 mg/mL) were used as a positive control and DMSO was used as a negative control. After being incubated for 2-3 days at 27 ºC, a zone of inhibition was measured [12].

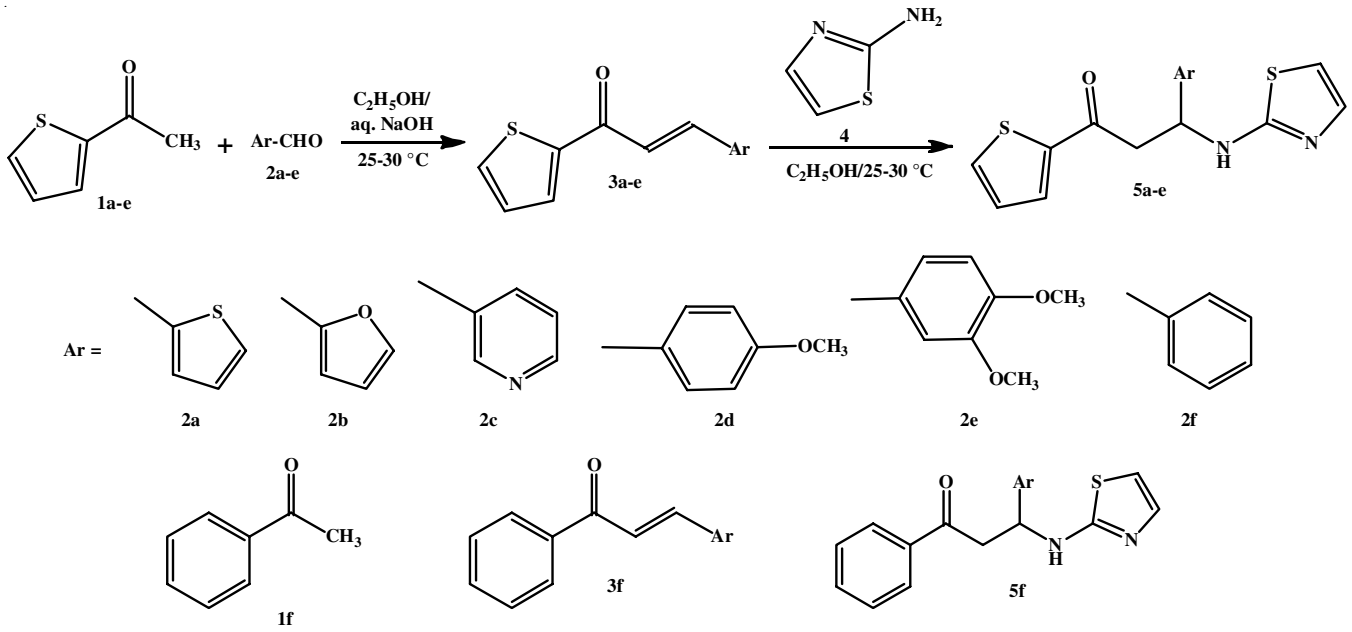
**ADMET studies:** ADMET studies of the synthesized molecules has been done using an online tool pkCSM sofware. The smiles version of the molecules was drawn from ChemDraw professional 16.0 and used to obtain the ADMET data [13].

**Molecular docking:** Docking studies were carried out against proteins of PDB IDs 1MWT, 2UV0, 4DUH. The optimal orientation of a ligand (drug) and a target protein to form a stable complex is predicted by molecular docking. Vina, Biovia Discovery Studio 2021 Client and AutoDock Tools-1.5.7 were used to download and prepare 3D protein structures from the RCSB Protein Data Bank by eliminating unnecessary components. Following the addition of polar hydrogens, proteins were stored in PDBQT format. Chem3D 16.0 was used to draw the ligands, AutoDockTools 1.5.7 was used to prepare them and PDBQT format was saved. Proteins, ligands and configuration files were placed in a folder containing Vina executables and the docking command was then executed. Biovia Discovery Studio was used to assess and illustrate binding affinities (kcal/mol) [14].

**Seed germination inhibition test:** The assessment of seed germination is one of simplest methods of environmental biomonitoring. This method was formulated to evaluate the toxicological impact of polluted or contaminated liquid samples on seed germination [15]. The inhibitory effect on seed germination is being used to screen the cytotoxic and anti-proliferative compounds [16] and also used to study anti-proliferative effect of calcium channel blockers [17]. Healthy greengram seeds were chosen, thoroughly washed 3-4 times with distilled water and sterilized by immersing them in 10% sodium hypochlorite for 20 min to inhibit fungal proliferation. The seeds were repeatedly washed in distilled water. The experiment was performed in duplicate for compounds **5a-f** and the control. Three layers of Whatman No. 1 filter paper were placed, followed by the introduction of 10 mL of 10% alcoholic solution of compounds **5a-f** and finally 10 mL of distilled water were added to the control plate. Ten prepared seeds were placed in Petri dishes and sealed with parafilm. All the Petri dishes were kept at 24 ºC for 72 h in dark.

## RESULTS AND DISCUSSION

The synthetic method involved by the reaction of 2-acetyl thiophene (**1a**) with various benzaldehyde (**2a-e**) and acetophenone (**1f**) with benzaldehyde (**2f**) in alkaline medium, a series of chalcones (**3a-f**) was prepared. Chalcone based amino thiazole derivatives (**5a-f**) were synthesized in substantial yield through the amino addition reaction of chalcones (**3a-f**) in the presence of alcohol with 2-amino thiazole (**Scheme-I**) yielding




**Scheme-I:** Synthetic pathway of amino thiazole derivatives **(5a-f)**

the products in the range of 76-85%. The IR, $^1$H NMR,$^{13}$C NMR and MS studies were used to confirm the structural assignments, with compound **5d** serving as the typical compound from the series. The N-H stretching, C=N group, carbonyl group, C-N group and thiophene C-S-C group are responsible for significant peaks in the IR at 3118, 1566, 1633,1064 and 722 cm$^{-1}$, respectively. Compound **5d** showed an $M^+$ ion peak at *m/z* (M-3) value that corresponded to its molecular mass. The methylene protons were detected at a triplet in the spectrum on δ 6.540 (*J* = 7.1 Hz) ppm and a doublet on δ 3.784-3.876 (*J* = 7.0 Hz) ppm for the $^1$H NMR containing $H_2C$-C and C-CH, respectively. At δ 6.856 (*J* = 7.3 Hz) ppm, the thiazole rings S-CH proton signal became visible as a doublet, whereas the N-CH proton signal manifested as a doublet at δ 7.286 (*J* = 7.3 Hz) ppm. With each of the hydrogen within the methoxy group attached through the aromatic rings *p*-position on δ 3.902 ppm the signal was showed through a singlet. The signal for one NH hydrogen of the linked 2-amino group of thiazoles appeared as a singlet at δ 6.877 ppm. The thiophene ring was responsible for all of the several signals from all the three protons and δ 7.379-7.822 (*J* = 6.6 Hz) ppm, while the aromatic protons were responsible for all the signals with all four protons in δ 6.972-7.357 (*J* = 7.6 Hz) ppm. In the $^{13}$C NMR spectra, molecule **5d** displayed signals at δ 182.1, 77.3 and 76.7 ppm. The carbonyl propane's C-1, C-2 and C-3 carbons are identified through this indication. A methoxy carbon signature was found at δ 55.4 ppm. At δ 108.7, 138.8 and 16 1.7 ppm, three carbons displayed a signal associated with the thiazole ring. A variety indicates show up at δ 128.2, 131.5, 133.5 and 143.9 ppm, which were identified as carbons in the thiophene ring surface. The other indications at δ 114.1, 124.8, 127.4, 130.3 and 145.7 ppm that were clearly associated with aromatic carbons. The structure of the synthesized compounds **5a-f** is further confirmed by the identical and consistent pattern signals found in their mass spectra, $^1$H NMR, IR and $^{13}$C NMR spectra.

**Antibacterial activity:** A modified agar well prepetition experiment was utilized to evaluate the antibacterial activity of the synthesized compounds **5a-f**. When compared to positive control, the antibacterial activity is minimal. Among six compounds with *E. coli*, compound **5c** has high antibacterial activity. The degree of antibacterial activity is **5b > 5a > 5e > 5d** in decreasing sequence. In compound **5f**, the activity is almost negligible. The order of degree of activity for *S. aureus* is **5d > 5b**, **5c > 5a > 5e > 5f** in that order whereas the degree of activity for *P. aeruginosa* is **5b > 5c**, **5a > 5e > 5d > 5f**. This indicates that among the synthesized chalcone amino thiazole derivatives, compound **5f** showed the minimum antibacterial activity since, it did not contain either heterocyclic ring or any substituent group (Table-1).

**Antifungal activity:** When compared to standard antibiotics, the antifungal activity of the synthesized compounds is far less. The results obtained among six compounds, for *Candida albicans*, compound **5b** has some antifungal activity, all other compounds almost have similar activity of order **5b > 5b**, **5c**, **5a**, **5e** and nil for **5f**. For *A. brasiliense*, the activity is in the order **5e > 5b > 5a > 5c**, **5d** and nil for **5f**, whereas *A. flavus* have not shown any antifungal activity. Since compound **5f** lacked a heterocyclic ring and other substituent groups, it exhibited no antifungal activity among the chalcone amino thiazole derivatives (Table-2).

### *In silico* studies

**ADMET studies:** High solubility often correlates with better absorption. Compounds **5d**, **5e**, **5f** have better solubility compared to **5a**, **5b**, **5c**. In order to determine a compound's absorption capability in the human digestive system, studies on Caco-2 permeability are necessary. Compound **5a** has very high permeability, which suggests that this compound has excellent potential for intestinal absorption, likely due to favourable lipophilicity and size. Compound **5b** has moderate permeability,

TABLE-1
ZONE OF INHIBITION VALUES OF THE ANTIBACTERIAL ACTIVITY OF COMPOUNDS **5a-f** AT DIFFERENT CONCENTRATIONS AGAINST PATHOGENIC BACTERIA

| Test pathogenic bacteria | Zone of inhibition values in diameter (mm) | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | **5a** (mg/mL) | | | **5b** (mg/mL) | | | **5c** (mg/mL) | | | **5d** (mg/mL) | | |
| | 5.0 | 2.5 | 1.25 | 5.0 | 2.5 | 1.25 | 5.0 | 2.5 | 1.25 | 5.0 | 2.5 | 1.25 |
| *E. coli* | 15 | 14 | 13 | 16.5 | 15.5 | 14.5 | 18 | 17 | 16 | 12 | 11 | 10 |
| *S. aureus* | 17 | 15 | 14 | 17 | 16 | 15 | 17 | 16.5 | 15 | 18 | 17 | 16 |
| *P. aeruginosa* | 16 | 15.5 | 15 | 18.5 | 17.5 | 16.5 | 16 | 15 | 14 | 15 | 14 | 13 |
| | **5e** (mg/mL) | | | **5f** (mg/mL) | | | Streptomycin (STM) | | Ciprofloxacin (CPFX) | | Chloramphenicol (CHL) | |
| | 5.0 | 2.5 | 1.25 | 5.0 | 2.5 | 1.25 | | | | | | |
| *E. coli* | 14 | 13 | 12 | 3.0 | 2.5 | 2.0 | 19.10 | | 28.13 | | 23.06 | |
| *S. aureus* | 15 | 14 | 13 | 4.0 | 3.8 | 3.5 | 10.16 | | 33.93 | | 26.03 | |
| *P. aeruginosa* | 16 | 15 | 14 | 4.5 | 4.0 | 3.8 | 22.06 | | 23.96 | | 24.10 | |

TABLE-2
ZONE OF INHIBITION VALUES OF THE ANTIFUNGAL ACTIVITY OF COMPOUNDS **5a-f** AT DIFFERENT CONCENTRATIONS AGAINST PATHOGENIC FUNGI

| Test pathogenic fungi | Zone of inhibition values in diameter (mm) | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | **5a** (mg/mL) | | **5b** (mg/mL) | | **5c** (mg/mL) | | **5d** (mg/mL) | | **5e** (mg/mL) | | **5f** (mg/mL) | | Fluconazole |
| | 5.0 | 2.5 | 5.0 | 2.5 | 5.0 | 2.5 | 5.0 | 2.5 | 5.0 | 2.5 | 5.0 | 2.5 | |
| *Candida albicans* | 10.16 | 10.00 | 13.05 | 12.05 | 10.16 | 10.00 | 10.05 | 10.00 | 11.20 | 10.15 | 0.00 | 0.00 | 16.5 |
| *Aspergillus brasiliensis* | 13.10 | 12.10 | 14.10 | 13.00 | 11.05 | 10.05 | 13.10 | 10.20 | 15.05 | 14.05 | 0.00 | 0.00 | 15.0 |
| *Aspergillus flavous* | 11.15 | 10.15 | 12.00 | 10.00 | 11.00 | 10.00 | 10.05 | 10.15 | 11.10 | 10.15 | 0.00 | 0.00 | 19.0 |

this may have a reasonable absorption profile, but there might be some factors (such as size or functional groups) limiting its absorption compared to compound **5a**. Compound **5d** has moderate permeability, similar to compound **5b**, it indicates a decent absorption potential, but improvements might be necessary for optimal bioavailability. Compound **5e** has low permeability and compound **5f** has moderate permeability (Table-3).

All the six compounds have a high permeability as the percentage of Intestinal absorption is greater than 80%. The log $K_p$ values foe skin permeability is slightly less than -2, which indicates moderate skin permeability in the synthesized compounds. P-glycoprotein does not bind to compounds **5a**-**e** or significantly interact with it, indicating that they are neither carried by it nor interact with it. P-glycoprotein can transport compound **5f**, which could impact its absorption, distribution and possible medication interactions. Compound **5f** is a substrate for P-glycoprotein. Compounds **5a**, **5b**, **5c** and **5f** do not inhibit P-glycoprotein, meaning they are unlikely to affect the transport of other substrates through this protein. Compounds **5d** and **5e** are identified as inhibitors of P-glycoprotein.

Table-4 has been showed volume of distribution at steady state (VDss) is an important pharmacokinetic parameter that helps assess how a drug distributes throughout the body. Compounds **5a**, **5b**, **5c** show low volume of distribution whereas compounds **5d**, **5e**, **5f** show high volume of distribution. Fraction unbound (Fu) refers to the portion of a drug which is not bound to plasma proteins in the bloodstream. Compound **5a** has low value indicates that only about 2.9% of the drug is unbound in the plasma. Compound **5b** compared to compound **5a** has a comparatively larger proportion unbound at 12.1%. Given that a greater amount can interact with target areas, it suggests a better possibility for therapeutic efficacy. With 9.9% unbound, compound **5c** falls between **5a** and **5b**. Compound **5d** may limit the efficacy of the drug due to high plasma protein binding. Compound **5e** is the lowest fraction unbound among the compounds. This suggests substantial binding and could mean reduced therapeutic activity, making it essential to consider dosing adjustments or alternative formulations. Blood-brain barrier (BBB) permeability refers to the ability of substances to cross the BBB, a selective barrier that protects the brain from harmful substances while allowing essential nutrients to pass. Negative blood–brain barrier (BBB) permeability is a term that describes when a compound is unlikely to cross the BBB, except compounds **5d** and **5e** others can cross the BBB as observed (Table-4).

TABLE-3
PREDICTION OF ABSORPTION IN GASTROINTESTINAL OF SYNTHESIZED ANALOGUES BY pkCSM TOOL

| Compound | Water solubility (log mol/L) | Caco2 permeability (log Papp in $10^{-6}$ cm/s) | Intestinal absorption (human) (% Absorbed) | Skin permeability (log Kp) | P-glycoprotein substrate | P-glycoprotein I inhibitor |
|---|---|---|---|---|---|---|
| **5a** | -4.163 | 2.169 | 88.37 | -2.752 | No | No |
| **5b** | -3.934 | 1.447 | 91.487 | -2.753 | No | No |
| **5c** | -3.745 | - | 92.595 | -2.821 | No | No |
| **5d** | 4.386 | 1.59 | 91.741 | -2.861 | No | Yes |
| **5e** | 4.628 | 1.342 | 92.75 | -2.909 | No | Yes |
| **5f** | 4.8 18 | 1.87 | 92.105 | -2.64 | Yes | No |

TABLE-4
PREDICTION OF DISTRIBUTION IN GASTROINTESTINAL OF SYNTHESIZED ANALOGUES

| Compound | VDss (human) (log L/kg) | Fraction unbound (human) (Fu) | BBB permeability (log BB) | CNS permeability (log PS) |
|---|---|---|---|---|
| **5a** | -0.013 | 0.029 | 0.161 | -1.192 |
| **5b** | -0.176 | 0.121 | 0.203 | -2.804 |
| **5c** | -0.222 | 0.099 | 0.067 | -2.817 |
| **5d** | 0.219 | 0.033 | -0.119 | -2.132 |
| **5e** | 0.168 | 0.017 | -0.257 | -2.319 |
| **5f** | 0.478 | 0.03 | 0.31 | -1.728 |

TABLE-5
PHARMACOKINETIC PROPERTY (METABOLISM AND EXCRETION) BY pkCSM TOOL

| Compound | Metabolism | | | | | | | Excretion | |
|---|---|---|---|---|---|---|---|---|---|
| | CYP2D6 substrate | CYP3A4 substrate | CYP1A2 inhibitior | CYP2C19 inhibitior | CYP2C9 inhibitior | CYP2D6 inhibitior | CYP3A4 inhibitior | Total clearance | Renal OCT2 substrate |
| **5a** | No | Yes | Yes | Yes | Yes | No | No | 0.185 | No |
| **5b** | No | Yes | Yes | Yes | Yes | No | No | 0.272 | No |
| **5c** | No | Yes | Yes | Yes | Yes | No | No | 0.164 | No |
| **5d** | No | Yes | Yes | Yes | Yes | No | No | 0.28 | No |
| **5e** | No | Yes | Yes | Yes | Yes | No | Yes | 0.426 | Yes |
| **5f** | No | Yes | Yes | Yes | Yes | No | Yes | 0.297 | No |

Individual variations in the CYP2D6 enzyme can affect drug metabolism, leading to differences in drug efficacy and risk of side effects. Thus, the synthesized compounds are not prone to CYP2D6 substrate. Similarly, CYP3A4 metabolizes several drugs and the interactions can occur when multiple drugs are taken that affect the enzyme's activity, either increasing or decreasing the metabolism of these substrates. Only compound **5e** and **5f** are prone to CYP3A4 inhibitor (Table-5).

Total clearance is a pharmacokinetic parameter that represents the volume of plasma from which a drug is completely removed per unit of time. Compound **5e** has the highest clearance, suggesting it is eliminated more efficiently. This may allow for a more frequent dosing schedule or a lower risk of accumulation. Whereas compound **5c** has the lowest clearance value among the group, suggesting it is eliminated more slowly compared to the others. This could indicate a higher potential for accumulation in the body, necessitating careful dosing. Renal OCT2 (organic cation transporter 2) is a crucial transporter in the kidneys that mediates the uptake of organic cations from the renal tubular fluid into renal epithelial cells. The majority of the synthesized compounds (**5a**, **5b**, **5c**, **5d** and **5f**) do not interact with OCT2, suggesting that their renal clearance may occur through other mechanisms or transporters (Table-5).

**Molecular docking studies:** Molecular docking studies were carried out for the synthesized compounds **5a-f** with the various amino acid residues in the binding pocket of the active site of protein 1MWT, 2UV0 and 4DUH were exhibits a good interaction with binding scores as shown in Table-6. Among these, compound **5f** showed good docking score of -5.7 kcal/mol for 1MWT and compound **5f** showed good docking score of -7.5 kcal/mol for 2UV0 and **5e** showed good docking score of -7.1 kcal/mol for 1MWT. The 2D and 3D representation of protein-ligand interactions is shown in Figs. 1-3. Hence, the

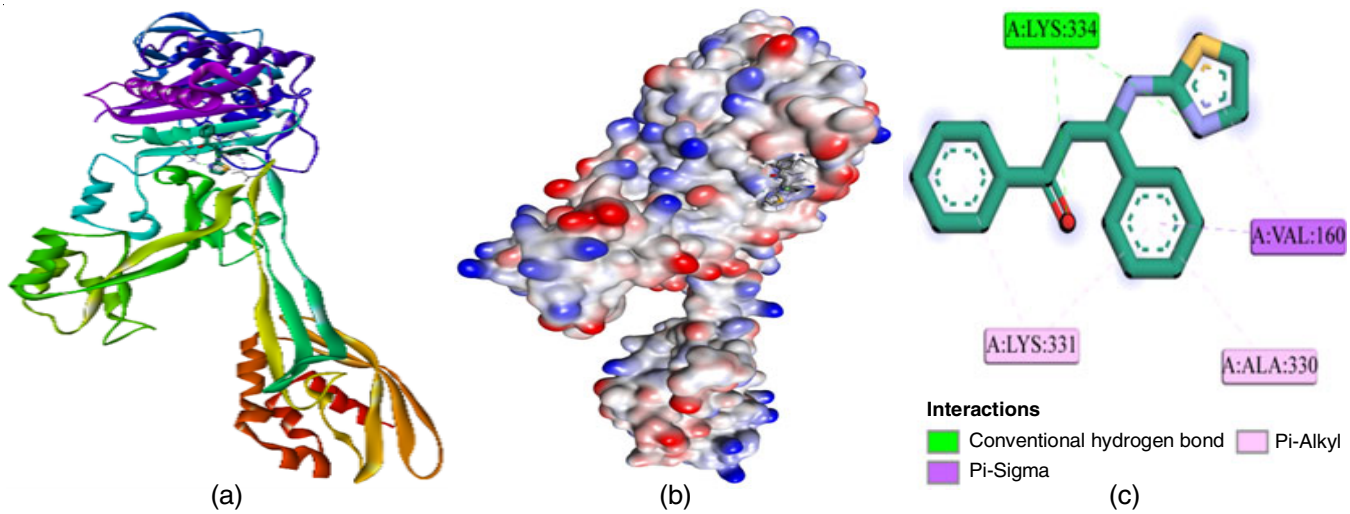



Fig. 1. (a) Protein ligand complex of compound **5f** with active site of 2UV0 (b) Docking interaction at surface as interpolated charge (c) 2-D representation of protein -ligand interactions

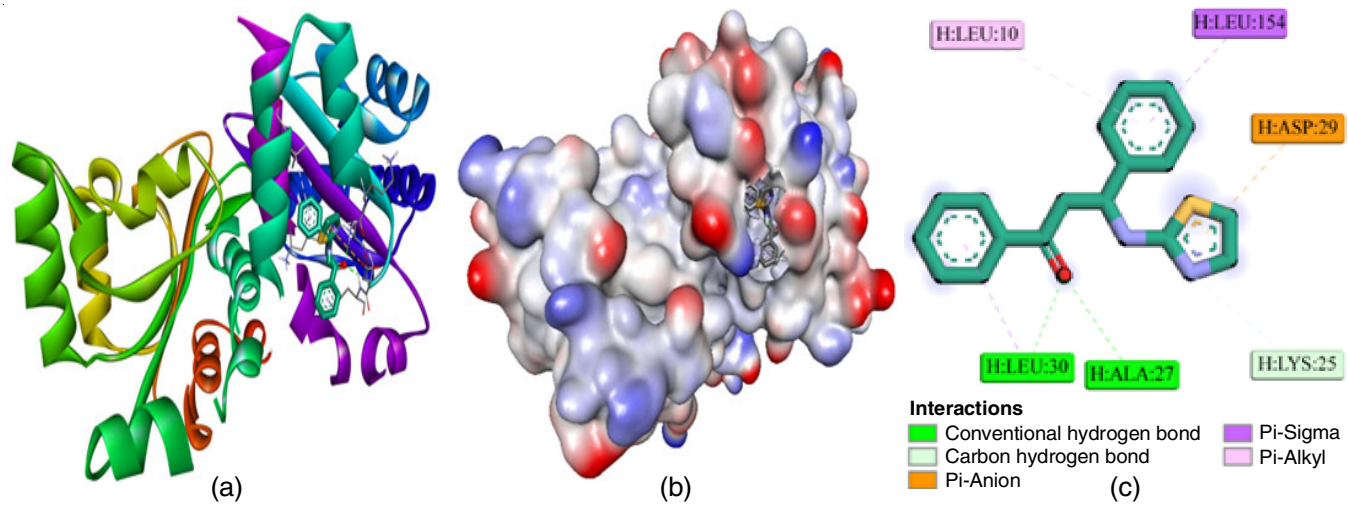



Fig. 2. (a) Protein ligand complex of compound **5f** with active site of 1MWT (b) Docking interaction at surface as interpolated charge (c) 2-D representation of protein-ligand interactions

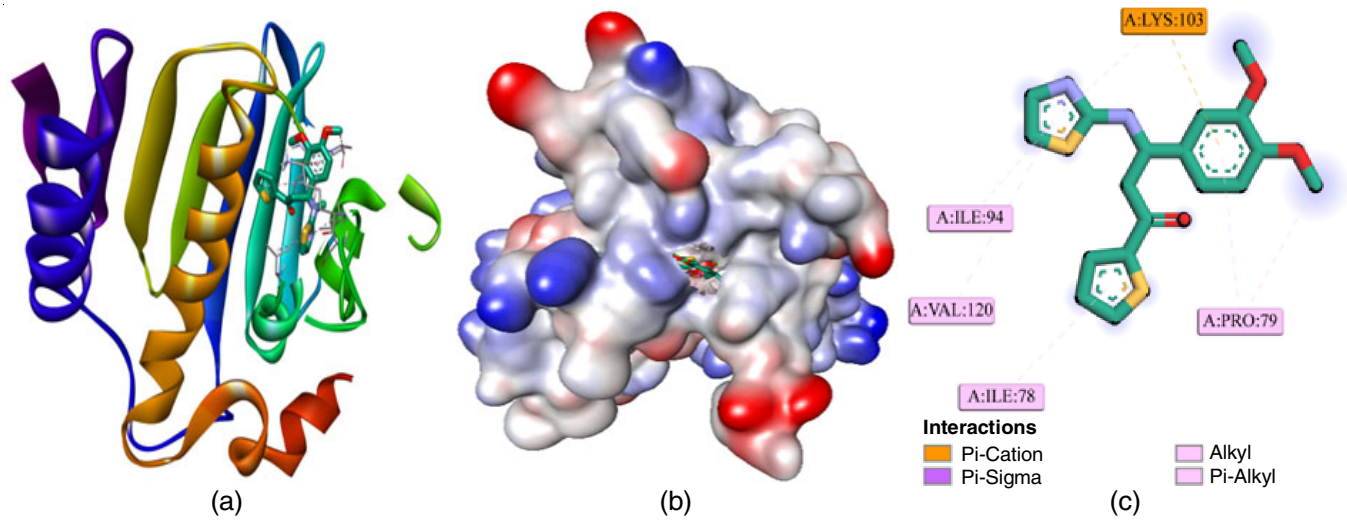



Fig. 3. (a) Protein ligand complex of compound **5e** with active site of 4DUH (b) Docking interaction at surface as interpolated charge (c) 2-D representation of protein-ligand interactions

TABLE-6
BINDING AFFINITY OF COMPOUNDS **5a-f** AND PROTEIN 1MWT, 2UV0 AND 4DUH

| Compound | Docking score | | |
|---|---|---|---|
| | 1MWT | 2UV0 | 4DUH |
| **5a** | -4.9 | -6.3 | -6.7 |
| **5b** | -4.9 | -6.4 | -4.8 |
| **5c** | -4.9 | -6.6 | -5.7 |
| **5d** | -5.1 | -6.1 | -5.3 |
| **5e** | -5.1 | -6.2 | -7.1 |
| **5f** | -5.7 | -7.5 | -5.5 |

current work highlights the bactericidal activity and antifungal activity of chalcones that might outcome as a promising drug component in the future pharmaceuticals.

**Seed germination inhibition test:** The assay was conducted on the green gram (*Vigna radiata*) by using Petri dishes. The assay was conducted for the synthesized compounds **5a-f** and control in triplicates. Compound **5a** has high antiproliferative activity when compared to all other compounds (Fig. 4).

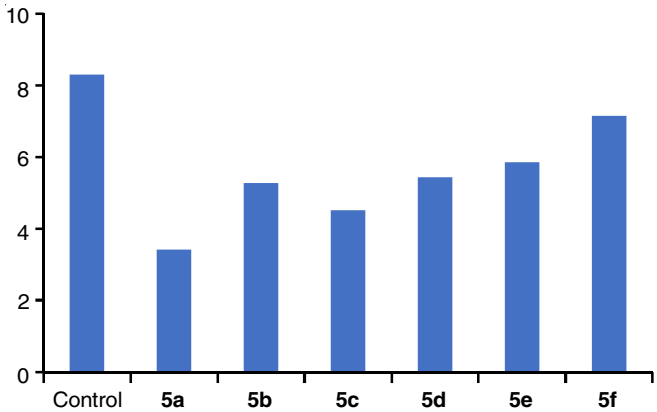



Fig. 4. Effect of compounds on seed germination

The compounds in decreasing sequence of antiproliferative activity are **5a** > **5c** > **5b**, **5d** > **5e**. Compound **5f** has the least antiproliferative activity since it did not contain either heterocyclic ring or any substituent group.

## Conclusion

The synthesis of hybrid chalcone-amino thiazoles by hydroamination followed by Claisen-Schmidt condensation and the *in vitro* antibacterial and antifungal activity has illustrated the significance of the work. The newly synthesized compounds demonstrated medium to acceptable antibacterial and antifungal activity against the microorganisms studied, making the modified agar well diffusion assay examination of these compounds still a topic of interest. These molecules in drug development was further supported by the molecular docking experiments. Understanding the binding affinities and interaction methods of target proteins like 1MWT, 2UV0 and 4DUH was made possible using docking simulations. Strong interactions were shown by compounds **5e** and **5f** in particular, suggesting that they are the suitable candidates for additional pharmacological investigation. By forecasting positive pharmacokinetic characteristics, such as excellent absorption, distribution and metabolic profiles for the majority of derivatives, the computational ADMET study provided an additional layer of validation. While compounds with low toxicity criteria show their potential safety, those with high permeability and moderate clearance rates show promise for oral bioavailability. Although the antibacterial and antifungal activity results were not found to be expected and different, but seed germination inhibition test study had a very good result. This information will be useful for further and future innovation.

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## CONFLICT OF INTEREST

The authors declare that there is no conflict of interests regarding the publication of this article.

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