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To cite this article: Demokrat Nuha, Asaf Evrim Evren, Meral Yılmaz Cankılıç & Leyla Yurttaş (2021): Design and synthesis of novel 2,4,5-thiazole derivatives as 6-APA mimics and antimicrobial activity evaluation, Phosphorus, Sulfur, and Silicon and the Related Elements, DOI: [10.1080/10426507.2021.1946537](https://doi.org/10.1080/10426507.2021.1946537)


To link to this article: <https://doi.org/10.1080/10426507.2021.1946537>

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## Design and synthesis of novel 2,4,5-thiazole derivatives as 6-APA mimics and antimicrobial activity evaluation

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### ABSTRACT

Nine new thiazole derivatives were synthesized considering 6-acetyl penicillanic acid (6-APA) and investigated for their antimicrobial activity. Ethyl 2-(2-mercaptoacetamido)-4-methylthiazole-5-carboxylate derivatives (**3a–3i**) were gained with a two-step synthetic method using conventional Hantzsch thiazole synthesis. The structural elucidation of the compounds was performed by <sup>1</sup>H-NMR, <sup>13</sup>C-NMR spectral data and HRMS. All compounds were tested on eleven bacteria and sixteen fungi species and minimum inhibitory concentration (MIC) was determined for each. Compounds **3d** (4-methyl-2-(2-((5-methyl-1,3,4-thiadiazol-2-yl)), **3f** (4-methyl-2-(2-((5-nitro-1*H*-benzimidazol-2-yl) and **3g** (4-methyl-2-(2-((5-methyl-4*H*-1,2,4-triazol-3-yl) bearing thiazole, 5-nitrobenzimidazole and triazole rings respectively exhibited high antimicrobial activity against most of the strains. *In silico* physicochemical properties were calculated for the compounds and it was detected that they comply with the rules of drug availability.

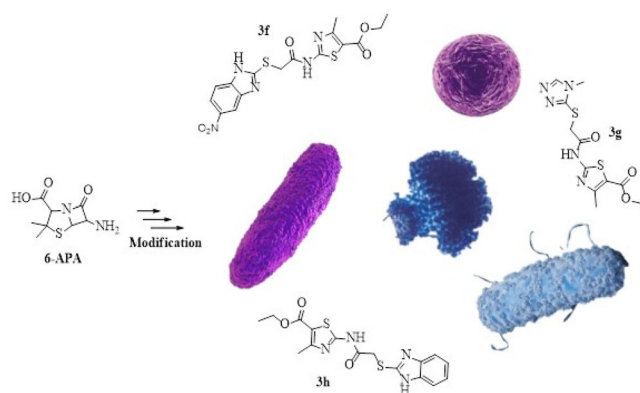
### ARTICLE HISTORY

Received 5 February 2021  
Accepted 18 June 2021

### KEYWORDS

Thiazole; 6-APA; antibacterial activity; antifungal activity; physicochemical property prediction

### GRAPHICAL ABSTRACT

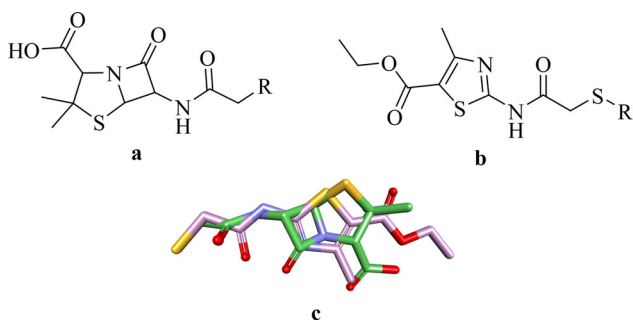


### Introduction

There is a great concern in the clinical setting due to developing resistance against antimicrobial agents which occurs in several phenotypes such as multi-drug, extended-drug, and pan-drug resistance [1–3]. As the development of antimicrobial resistance (AMR) interrupts not only the treatment of infections but also the treatment of any kind of disease [4], antimicrobial studies have been prioritized. Hence, many kinds of antimicrobial studies such as developing new (potential) drugs and searching for new pathways were published in the literature [5–9]. In the past decades depending on antibiotics misuse, scientists did not approach the future optimistically [10–12]. Unfortunately, a list of

new resistant bacteria was published recently [13]. These cautions denote that we will need new antimicrobial agents to heal patients or to prevent them from infections very soon.

Since chemotherapeutics including the thiazole moiety are commonly used in several chronic diseases including cancer [14–16], hypertension [17], diabetes [18], Parkinson's and/or Alzheimer's diseases [19], analgesic [20], anxiety [21], and also various infections [22–26]. Thus, we examined the antimicrobial properties of this ring. For this purpose, our research group aimed to design new thiazole derivatives that may show cytotoxicity against pathogenic microbes. Inspired by the core structures of penicillin derivatives, since



**Figure 1.** The core structure of penicillin derivatives (a) (6-APA), the core structure of the designed molecules (b), and overlay of 6-acetyl penicillanic acid (pink carbons) and designed compounds (green carbons) without R groups (c) [overlay module was performed via Discovery Studio Visualizer (DSV, 2017.R2) [27]].

the thiazole side is substituted with dimethyl and carboxylic acid groups (Figure 1), our core structure is formed of carboxylic acid ester and methyl substitutions. Also, the acetamide structure was preserved but it linked to the other ring system with a sulfur atom. Although  $\beta$ -lactam moiety is defined as a pharmacophore, we thought that the acetamide linkage may mimic it, since the carbonyl oxygen is close to thiazole in our design.

According to the above information and based on the need to develop new and effective antimicrobial agents, novel 2-acetamidethiazole derivatives were synthesized and evaluated for their antibacterial and antifungal activity.

## Results and discussion

### Chemistry

The compounds **3a–3i** were synthesized as summarized in Scheme 1.

In this study, we synthesized nine new compounds which included the ethyl 2-(2-mercaptoacetamido)-4-methylthiazole-5-carboxylate nucleus in their core structures. The structures of the synthesized compounds (**3a–3i**) were confirmed by  $^1\text{H-NMR}$ ,  $^{13}\text{C-NMR}$ , and high-resolution mass spectroscopy (HRMS). In the first step, ethyl 2-chloro-3-oxobutanoate and thiourea were reacted at room temperature for ring closure to obtain ethyl 2-amino-4-methylthiazole-5-carboxylate (**1**) [28]. Then compound **1** was acetylated with chloroacetyl chloride according to a method reported [29]. Finally, the obtained product ethyl 2-(2-chloroacetamido)-4-methylthiazole-5-carboxylate (**2**) was reacted with 2-mercaptoazole derivatives to gain the final products ethyl 2-(2-mercaptoacetamido)-4-methylthiazole-5-carboxylate derivatives (**3a–3i**) as shown in Scheme 1. All the synthesized compounds were characterized by analytical and spectral data. The  $^1\text{H-NMR}$  spectra of compounds showed that ethoxy protons were observed at  $\delta$  1.24–1.27 ppm ( $\text{CH}_3$ ) as a triplet and at  $\delta$  4.15–4.23 ppm ( $\text{CH}_2$ ) as a quartet, at  $\delta$  2.46–2.55 ppm ( $\text{CH}_3$ ) for 4-methyl thiazole protons which were singlet peaks. Acetamide protons were observed at  $\delta$  3.96–4.44 ppm ( $\text{CH}_2$ ) as a singlet. A broad singlet peak seen at  $\delta$  12.78–12.86 ppm indicated the acetamide N–H proton. The appearance of a pair of singlet, doublets, triplets, and/or multiplets at  $\delta$  6.73–8.56 ppm was due to the

aromatic protons of the aromatic rings. The  $^{13}\text{C-NMR}$  spectra of compounds showed signals at  $\delta$  17.47–18.05 ppm for 4-methyl thiazole carbon ( $\text{CH}_3$ ), at  $\delta$  14.64–14.87 ppm for ethoxy carbon ( $\text{CH}_3$ ), and at  $\delta$  59.84–61.04 ppm for ethoxy carbon ( $\text{CH}_2$ ), at  $\delta$  35.52–40.48 ppm for acetamide ( $\text{CH}_2$ ), at  $\delta$  97.44–165.42 ppm for aromatic carbon and at 167.22–172.96 for carbonyl ( $\text{C}=\text{O}$ ) carbon. HRMS data confirmed the molecular formulae as expected.

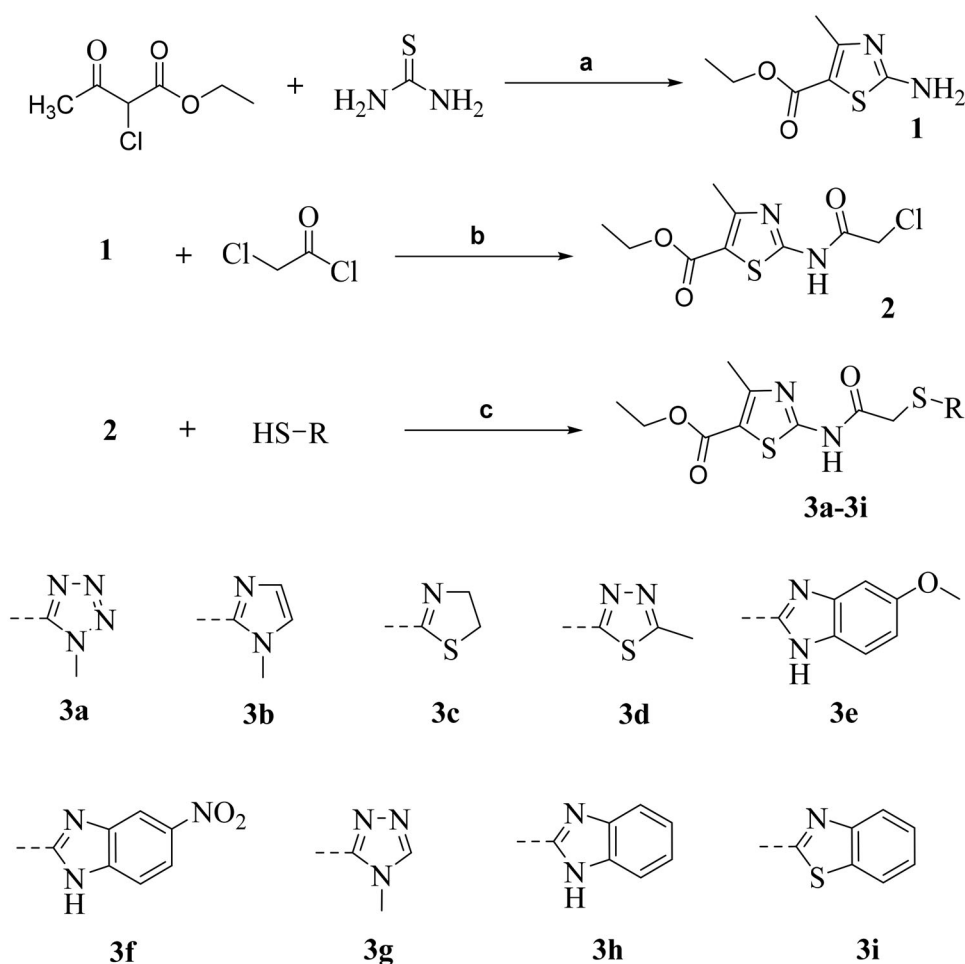
### ADME parameters

The computational results were shown in Table 1. According to these results, there was no violation of Lipinski's rule of five [30]. These scores are in line with the activity potential of those compounds. It is thought that the synthesized compounds might have a good pharmacokinetic profile. Thus, the drug-likeness of the compounds was dedicated to positive.

### Antimicrobial activity evaluation

The antimicrobial activity of the compounds was tested on various types of microorganisms. The tested bacteria strains are *B. cereus*, *B. subtilis*, *M. luteus*, and *S. aureus* as Gram-positive species, *E. faecalis*, *P. vulgaris*, *K. pneumoniae*, *E. coli*, *S. typhimurium*, *Y. enterocolitica*, and *E. aerogenes* as Gram-negative species, and the results were given as minimum inhibitory concentration (MIC) as shown in Table S1 (Supplemental Materials). In general, the tested compounds were more active against Gram-negative than Gram-positive strains. None of the compounds showed activity against *B. subtilis* and *B. cereus*. Compound **3g** exhibited the same potency as that of the standard drug chloramphenicol whereas compound **3f** showed half of the potency of the standard drug against *M. luteus*. Likewise, compound **3f** exhibited half the activity against *S. aureus*. Remarkably, compounds **3d** and **3g** exhibited fourfold antibacterial activity (MIC: 31.25  $\mu\text{g/mL}$ ), whereas **3f** (MIC: 62.50  $\mu\text{g/mL}$ ) showed twofold activity compared to chloramphenicol (MIC: 125  $\mu\text{g/mL}$ ). Among Gram-negative bacterial strains, *E. coli* and *S. typhimurium* were the most resistant types. Compounds **3d** and **3g** inhibited *P. vulgaris* and *E. aerogenes* at 31.25  $\mu\text{g/mL}$  and compound **3f** inhibited the same bacteria at 62.50  $\mu\text{g/mL}$  concentration while chloramphenicol had a MIC value of 125  $\mu\text{g/mL}$ . *K. pneumoniae* was the most susceptible bacteria to which compounds **3d** and **3f** exhibited twofold activity, whereas compounds **3c** and **3g** showed the same potency as that of the standard drug. Against *Y. enterocolitica*, MIC value was determined 31.25  $\mu\text{g/mL}$  for compound **3f**, whereas compounds **3d**, **3e**, and **3g** had MIC values of 62.50  $\mu\text{g/mL}$  which were similar to that of chloramphenicol.

The antifungal activity of the compounds was tested against four *Candida*, five *Aspergillus*, four *Penicillium*, two *Fusarium*, and one *Alternaria* species and the findings were represented in Table S2 (Supplemental Materials). Among *Candida* strains, *C. parapsilosis* was the most sensitive type that compounds **3c** and **3d** showed twofold activity of that of the standard drug whereas **3g** exhibited similar potency to ketoconazole. Compounds **3d** and **3g** inhibited *C.*



**Scheme 1.** The synthesis diagram of the compounds **3a–3i**. Reagents and conditions: (a) EtOH, r.t., 15 h; (b) TEA, THF, ClCOCH<sub>2</sub>Cl, 0–5 °C, then r.t. 3 h; (c) Acetone, Potassium carbonate, r.t., 6 h

**Table 1.** Physicochemical, pharmacokinetic, and medicinal chemistry properties of the final compounds (by SwissAdme) **3a–3i**.

	Physicochemical properties					Pharmacokinetics		Medicinal chemistry	
	HBA	HBD	TPSA	Log P <sub>o/w</sub>	Log S	GIA	Log K <sub>p</sub>	RoF (V)	SA
<b>3a</b>	7	1	165.43	1.30	−4.72	Low	−7.23	Yes (0)	3.24
<b>3b</b>	5	1	139.65	1.82	−4.64	High	−6.90	Yes (0)	3.18
<b>3c</b>	5	1	159.49	2.17	−5.49	Low	−6.63	Yes (0)	3.49
<b>3d</b>	6	1	175.85	2.46	−6.18	Low	−6.48	Yes (0)	3.32
<b>3e</b>	6	2	159.74	2.75	−6.56	Low	−6.28	Yes (0)	3.32
<b>3f</b>	7	2	196.33	2.00	−7.18	Low	−6.47	Yes (0)	3.37
<b>3g</b>	6	1	152.54	1.44	−4.30	Low	−7.32	Yes (0)	3.25
<b>3h</b>	5	2	150.51	2.79	−6.40	Low	−6.08	Yes (0)	3.22
<b>3i</b>	5	1	162.96	3.59	−7.58	Low	−5.55	Yes (0)	3.29
RF-1	5	3	115.38	0.53	−3.16	High	−7.46	Yes (0)	2.78
RF-2	5	0	69.06	3.55	−5.51	High	−6.46	Yes (1)	4.45

**HBA:** H-bond acceptor, **HBD:** H-bond donor, **TPSA:** Topologic polar surface area (Å<sup>2</sup>) **Log P<sub>o/w</sub>:** Lipophilicity, *Consensus* Log P<sub>o/w</sub> (Average of all five predictions), **Log S:** Water Solubility, **GIA:** Gastrointestinal absorption, **Log K<sub>p</sub>:** skin permeation (cm/s) **RoF (V):** Lipinski's Rule of Five (violation number), **SA:** Synthetic accessibility from 1 (very easy) to 10 (very difficult). **RF- 1:** Chloramphenicol, **RF-2:** Ketoconazole.

*albicans* at the same MIC value of 62.50 µg/mL while compound **3d** inhibited *C. glabrata* at half the potency of the standard drug. Compounds **3d** and **3g** attracted attention due to their inhibition potential as in other mold species. Among *Aspergillus* strains, *A. niger* was the most resistant mold, while *A. fumigatus* was the most susceptible kind. Compounds **3d** and **3h** displayed antifungal activity at two-fold activity whereas compounds **3f** and **3g** showed the same potency as that of ketoconazole against *A. fumigatus*.

Compounds **3d** and **3g** showed equipotent activity to the standard drug against *A. ochraceus* which was a clinic isolate. Among *Penicillium* species, *P. chrysogenum* was the toughest kind whereas *P. notatum* was the most sensitive one to which compounds **3c**, **3d**, **3e**, **3f**, **3h**, and **3i** exhibited equipotent and **3g** twofold antifungal activity compared to the standard drug. Against *P. citrinum*, compounds **3d** and **3e** showed significant activity while compound **3g** inhibited *P. expansum*, distinctively. All compounds except **3a**, **3b**,

and **3i** executed satisfying antifungal activity against both *Fusarium* strains that against *F. moniliforme* compounds **3c**, **3d**, and **3g** and against *F. solani* compound **3g** exhibited twofold antifungal activity. None of the compounds showed activity against *A. alternata*.

Nine ethyl 4-methyl-2-(2-((heteroaryl)thio)acetamido)-thiazole-5-carboxylate (**3a–3i**) derivatives were tested for their antimicrobial activity potential. Thiazole derivatives were widely studied molecules focusing on many pharmacological aspects and they were known with antimicrobial effect profile, particularly. The thiazole ring exists in the structure of many active compounds in the market and the literature [31]. When the compounds were evaluated structurally, it was seen that the compounds **3d**, **3f**, and **3g** were active on both Gram-positive and Gram-negative bacteria in terms of antibacterial activity. These compounds carry 5-methyl-1,3,4-thiadiazole, 5-nitrobenimidazole and 4-methyl-triazole, respectively. When the compounds were evaluated in terms of antifungal activity, similarly it was observed that the compounds **3d** and **3g** were prominent with higher efficiency, but it was also determined that the compounds **3c** bearing 2-thiazoline and **3e** bearing 5-methoxybenzimidazole had widespread antifungal effects. Additionally, compounds **3f** and **3h** exhibited activity comparable to the standard drug, especially prominent in some genres. No significant activity was observed for compounds **3a**, **3b**, and **3i**, except for exceptions. No clear correlation was detected when the virtual physicochemical parameters and biological findings were considered together.

Also, it was concluded that all compounds have a good pharmacokinetic profile considering values of log P and violations of Ro5.

For future studies, the design of molecules should be improved according to the following suggested points. The thiazole ring system may be reduced to a non-aromatic form. The sulfur atom at the chain may be changed to oxygen or nitrogen atoms. The end rings [(benz)azoles] may be changed with the phenyl derivatives. The ester may be converted to a carboxylic acid or its derivatives. As a pharmacophore residue, the  $\beta$ -lactam ring should be added to molecules. The positions of the carboxyl side and hydrophobic side may be changed to each other as in penicillin derivatives.

## Materials and methods

### Chemistry

All chemicals used in the syntheses were purchased either from Merck Chemicals (Merck KGaA, Darmstadt, Germany) or Sigma-Aldrich Chemicals (Sigma-Aldrich Corp., St. Louis, MO, USA). The reactions and the purities of the compounds were observed by thin-layer chromatography (TLC) on silica gel 60 F254 aluminum sheets obtained from Merck (Darmstadt, Germany). Melting points of the synthesized compounds were recorded by MP90 digital melting point apparatus (Mettler Toledo, Ohio, USA) and were presented as uncorrected. <sup>1</sup>H-NMR and <sup>13</sup>C-NMR spectra were recorded by a Bruker 300 MHz and 75 MHz digital FT-NMR

spectrometer (Bruker Bioscience, Billerica, MA, USA) in DMSO-*d*<sub>6</sub>, respectively. In the NMR spectra, splitting patterns were designated as follows: s: singlet; d: doublet; t: triplet; m: multiplet. Coupling constants (*J*) were reported as Hertz. High-resolution mass spectrometric (HRMS) studies were performed using an LC/MS-IT-TOF system (Shimadzu, Kyoto, Japan). The Supplemental Materials file contains antimicrobial activity assay, the antibacterial and antifungal activity of the compounds (Tables S1–S2), and sample HRMS, <sup>1</sup>H and <sup>13</sup>C-NMR spectra for products **3** (Figures S2–S28).

### General synthesis of ethyl 2-amino-4-methylthiazole-5-carboxylate (**1**)

Ethyl 2-chloro-3-oxobutanoate (2.38 g, 14.45 mmol) was added to a solution of thiourea (1 g, 13.14 mmol) in ethanol (40 mL) at room temperature. The mixture was stirred for 15 h at 80 °C. The reaction was monitored with TLC (EtOAc:PET = 1:2). After the reaction was completed, ethanol was removed, and the crude product was washed with cold ethanol and crystallized from ethanol. m. p. 173–175 °C [28], yield 82%.

### The synthesis of ethyl 2-(2-chloroacetamido)-4-methylthiazole-5-carboxylate (**2**)

Triethylamine (2.45 g, 24.19 mmol) was added to a solution of ethyl 2-amino-4-methylthiazole-5-carboxylate (**1**) (3 g, 16.12 mmol) in THF (80 mL) in an ice bath. 2-chloroacetyl chloride (1.52 g, 19.35 mmol) mixed with THF (10 mL) and added dropwise to the mixture. The mixture was stirred for 3 h in an ice bath. The reaction was monitored with TLC (EtOAc:PET = 1:1). After the reaction was completed, THF was removed, and the crude product was washed with water. Yield 85% [29].

### General synthesis of ethyl 2-(2-mercaptoacetamido)-4-methylthiazole-5-carboxylate derivatives (**3a–3i**)

Mercapto derivatives (1.14 mmol) were added to a solution of ethyl 2-(2-chloroacetamido)-4-methylthiazole-5-carboxylate (**2**) (0.3 g, 1.14 mmol) in acetone (25 mL). K<sub>2</sub>CO<sub>3</sub> (0.23 g, 1.71 mmol) was added to the mixture and the mixture was stirred for 6 h at room temperature. The reaction was monitored with TLC (EtOAc:PET = 1:3). After the reaction was completed, acetone was removed, and the crude product was washed with water and recrystallized from ethanol.

### Ethyl 4-methyl-2-(2-((1-methyl-1H-tetrazol-5-yl)thio)acetamido)thiazole-5-carboxylate (**3a**)

m. p. 177–178 °C, yield 78%, <sup>1</sup>H-NMR (300 MHz, DMSO-*d*<sub>6</sub>, ppm)  $\delta$  1.24 (t, *J* = 7.11 Hz, 3H, O-CH<sub>2</sub>-CH<sub>3</sub>), 2.46 (s, 3H, thiazole-CH<sub>3</sub>), 3.97 (s, 3H, tetrazole-CH<sub>3</sub>), 4.14 (s, 2H, S-CH<sub>2</sub>), 4.15–4.19 (m, 2H, O-CH<sub>2</sub>-CH<sub>3</sub>). <sup>13</sup>C-NMR (75 MHz, DMSO-*d*<sub>6</sub>, ppm)  $\delta$  14.86 (O-CH<sub>2</sub>-CH<sub>3</sub>), 17.99 (thiazole-CH<sub>3</sub>), 34.02 (tetrazole-CH<sub>3</sub>), 40.48 (S-CH<sub>2</sub>), 59.86

(O-CH<sub>2</sub>-CH<sub>3</sub>), 110.60, 154.66, 157.25, 163.43, 169.62, 170.94. HRMS (m/z): [M + H]<sup>+</sup> calculated 343.0642; found 343.0646.

**Ethyl 4-methyl-2-(2-((1-methyl-1H-imidazol-2-yl)thio)acetamido)thiazole-5-carboxylate (3b)**

m. p. 165–166 °C, yield 81%, <sup>1</sup>H-NMR (300 MHz, DMSO-d<sub>6</sub>, ppm) δ 1.27 (t, *J* = 7.11 Hz, 3H, O-CH<sub>2</sub>-CH<sub>3</sub>), 2.53 (s, 3H, thiazole-CH<sub>3</sub>), 3.59 (s, 3H, imidazole-CH<sub>3</sub>), 3.96 (s, 2H, S-CH<sub>2</sub>), 4.23 (q, *J* = 7.12 Hz, 2H, O-CH<sub>2</sub>-CH<sub>3</sub>), 6.94 (d, *J* = 1.20 Hz, 1H, Ar-H), 7.26 (d, *J* = 1.18 Hz, 1H, Ar-H). <sup>13</sup>C-NMR (75 MHz, DMSO-d<sub>6</sub>, ppm) δ 14.66 (O-CH<sub>2</sub>-CH<sub>3</sub>), 17.48 (thiazole-CH<sub>3</sub>), 33.46 (imidazole-CH<sub>3</sub>), 37.52 (S-CH<sub>2</sub>), 60.96 (O-CH<sub>2</sub>-CH<sub>3</sub>), 114.52, 124.16, 129.11, 139.51, 156.73, 160.13, 162.53, 168.49. HRMS (m/z): [M + H]<sup>+</sup> calculated 341.0737; found 341.0743.

**Ethyl 2-(2-((4,5-dihydrothiazol-2-yl)thio)acetamido)-4-methylthiazole-5-carboxylate (3c)**

m. p. 133–134 °C, yield 85%, <sup>1</sup>H-NMR (300 MHz, DMSO-d<sub>6</sub>, ppm) δ 1.27 (t, *J* = 7.11 Hz, 3H, O-CH<sub>2</sub>-CH<sub>3</sub>), 2.53 (s, 3H, thiazole-CH<sub>3</sub>), 3.46 (t, *J* = 7.92 Hz, 2H, dihydrothiazole), 4.09 (t, *J* = 8.07 Hz, 2H, dihydrothiazole), 4.17 (s, 2H, S-CH<sub>2</sub>), 4.23 (q, *J* = 7.08 Hz, 2H, O-CH<sub>2</sub>-CH<sub>3</sub>). <sup>13</sup>C-NMR (75 MHz, DMSO-d<sub>6</sub>, ppm) δ 14.66 (O-CH<sub>2</sub>-CH<sub>3</sub>), 17.50 (thiazole-CH<sub>3</sub>), 36.17 (dihydrothiazole), 36.30 (S-CH<sub>2</sub>), 60.92 (O-CH<sub>2</sub>-CH<sub>3</sub>), 64.36 (dihydrothiazole), 114.39, 156.71, 160.46, 162.55, 162.94, 167.62. HRMS (m/z): [M + H]<sup>+</sup> calculated 346.0348; found 346.0349.

**Ethyl 4-methyl-2-(2-((5-methyl-1,3,4-thiadiazol-2-yl)thio)acetamido)thiazole-5-carboxylate (3d)**

m. p. 254–255 °C, yield 87%, <sup>1</sup>H-NMR (300 MHz, DMSO-d<sub>6</sub>, ppm) δ 1.27 (t, *J* = 7.11 Hz, 3H, O-CH<sub>2</sub>-CH<sub>3</sub>), 2.55 (s, 3H, thiazole-CH<sub>3</sub>), 2.67 (s, 3H, thiadiazole-CH<sub>3</sub>), 4.23 (q, *J* = 7.08 Hz, 2H, O-CH<sub>2</sub>-CH<sub>3</sub>), 4.37 (s, 2H, S-CH<sub>2</sub>), 12.86 (brs, 1H, -NH). <sup>13</sup>C-NMR (75 MHz, DMSO-d<sub>6</sub>, ppm) δ 14.64 (O-CH<sub>2</sub>-CH<sub>3</sub>), 15.66 (thiazole-CH<sub>3</sub>), 17.48 (thiadiazole-CH<sub>3</sub>), 37.04 (S-CH<sub>2</sub>), 61.04 (O-CH<sub>2</sub>-CH<sub>3</sub>), 114.76, 156.74, 159.77, 162.46, 164.16, 166.45, 167.22. HRMS (m/z): [M + H]<sup>+</sup> calculated 359.0301; found 359.0306.

**Ethyl 2-(2-((5-methoxy-1H-benzo[d]imidazol-2-yl)thio)acetamido)-4-methylthiazole-5-carboxylate (3e)**

m. p. 178–179 °C, yield 89%, <sup>1</sup>H-NMR (300 MHz, DMSO-d<sub>6</sub>, ppm) δ 1.25 (t, *J* = 7.11 Hz, 3H, O-CH<sub>2</sub>-CH<sub>3</sub>), 2.51 (s, 3H, thiazole-CH<sub>3</sub>), 3.76 (s, 3H, O-CH<sub>3</sub>), 4.14 (s, 2H, S-CH<sub>2</sub>), 4.18 (q, *J* = 7.08 Hz, 2H, O-CH<sub>2</sub>-CH<sub>3</sub>), 6.73 (dd, *J*<sub>1</sub> = 2.43 Hz, *J*<sub>2</sub> = 6.27 Hz, 1H, Ar-H), 6.96 (d, *J* = 2.34 Hz, 1H, Ar-H), 7.32 (d, *J* = 8.70 Hz, 1H, Ar-H). <sup>13</sup>C-NMR (75 MHz, DMSO-d<sub>6</sub>, ppm) δ 14.76 (O-CH<sub>2</sub>-CH<sub>3</sub>), 17.81 (thiazole-CH<sub>3</sub>), 37.37 (S-CH<sub>2</sub>), 55.90 (O-CH<sub>3</sub>), 60.34 (O-CH<sub>2</sub>-CH<sub>3</sub>), 97.44, 110.76, 112.36, 115.10, 135.01, 140.16, 149.88, 155.66,

157.09, 163.04, 165.42, 170.88. HRMS (m/z): [M + H]<sup>+</sup> calculated 407.0842; found 407.0851.

**Ethyl 4-methyl-2-(2-((5-nitro-1H-benzo[d]imidazol-2-yl)thio)acetamido)thiazole-5-carboxylate (3f)**

m. p. 216–217 °C, yield 85%, <sup>1</sup>H-NMR (300 MHz, DMSO-d<sub>6</sub>, ppm) δ 1.26 (t, *J* = 7.08 Hz, 3H, O-CH<sub>2</sub>-CH<sub>3</sub>), 2.52 (s, 3H, thiazole-CH<sub>3</sub>), 4.14 (s, 2H, S-CH<sub>2</sub>), 4.21 (q, *J* = 7.08 Hz, 2H, O-CH<sub>2</sub>-CH<sub>3</sub>), 6.16 (s, 1H, benzoimidazole-NH), 7.41 (d, *J* = 8.82 Hz, 1H, Ar-H), 7.88 (dd, *J*<sub>1</sub> = 2.31 Hz, *J*<sub>2</sub> = 6.51 Hz, 1H, Ar-H), 8.21 (d, *J* = 2.25 Hz, 1H, Ar-H). <sup>13</sup>C-NMR (75 MHz, DMSO-d<sub>6</sub>, ppm) δ 14.68 (O-CH<sub>2</sub>-CH<sub>3</sub>), 17.62 (thiazole-CH<sub>3</sub>), 35.52 (S-CH<sub>2</sub>), 60.80 (O-CH<sub>2</sub>-CH<sub>3</sub>), 110.72, 113.92, 115.65, 140.14, 144.34, 150.61, 156.96, 161.36, 162.70, 170.10. HRMS (m/z): [M + H]<sup>+</sup> calculated 422.0587; found 422.0588.

**Ethyl 4-methyl-2-(2-((4-methyl-4H-1,2,4-triazol-3-yl)thio)acetamido)thiazole-5-carboxylate (3g)**

m. p. 256–257 °C, yield 79%, <sup>1</sup>H-NMR (300 MHz, DMSO-d<sub>6</sub>, ppm) δ 1.27 (t, *J* = 7.11 Hz, 3H, O-CH<sub>2</sub>-CH<sub>3</sub>), 2.54 (s, 3H, thiazole-CH<sub>3</sub>), 3.59 (s, 3H, triazole-CH<sub>3</sub>), 4.15 (s, 2H, S-CH<sub>2</sub>), 4.23 (q, *J* = 7.08 Hz, 2H, O-CH<sub>2</sub>-CH<sub>3</sub>), 8.56 (s, 1H, Ar-H), 12.78 (brs, 1H, -NH). <sup>13</sup>C-NMR (75 MHz, DMSO-d<sub>6</sub>, ppm) δ 14.65 (O-CH<sub>2</sub>-CH<sub>3</sub>), 17.47 (thiazole-CH<sub>3</sub>), 31.31 (triazol-CH<sub>3</sub>), 36.64 (S-CH<sub>2</sub>), 61.04 (O-CH<sub>2</sub>-CH<sub>3</sub>), 114.72, 146.84, 148.70, 156.72, 159.73, 162.47, 167.67. HRMS (m/z): [M + H]<sup>+</sup> calculated 342.0689; found 342.0694.

**Ethyl 2-(2-((1H-benzo[d]imidazol-2-yl)thio)acetamido)-4-methylthiazole-5-carboxylate (3h)**

m. p. 223–224 °C, yield 81%, <sup>1</sup>H-NMR (300 MHz, DMSO-d<sub>6</sub>, ppm) δ 1.25 (t, *J* = 7.08 Hz, 3H, O-CH<sub>2</sub>-CH<sub>3</sub>), 2.48 (s, 3H, thiazole-CH<sub>3</sub>), 4.03 (s, 2H, S-CH<sub>2</sub>), 4.15 (q, *J* = 7.11 Hz, 2H, O-CH<sub>2</sub>-CH<sub>3</sub>), 7.08–7.12 (m, 2H, Ar-H), 7.42–7.45 (m, 2H, Ar-H). <sup>13</sup>C-NMR (75 MHz, DMSO-d<sub>6</sub>, ppm) δ 14.87 (O-CH<sub>2</sub>-CH<sub>3</sub>), 18.05 (thiazole-CH<sub>3</sub>), 38.76 (S-CH<sub>2</sub>), 59.84 (O-CH<sub>2</sub>-CH<sub>3</sub>), 110.59, 114.27, 121.57, 140.12, 152.02, 157.38, 163.47, 169.75, 172.96. HRMS (m/z): [M + H]<sup>+</sup> calculated 377.0737; found 377.0738.

**Ethyl 2-(2-(benzo[d]thiazol-2-ylthio)acetamido)-4-methylthiazole-5-carboxylate (3i)**

m. p. 155–156 °C, yield 88%, <sup>1</sup>H-NMR (300 MHz, DMSO-d<sub>6</sub>, ppm) δ 1.24 (t, *J* = 7.11 Hz, 3H, O-CH<sub>2</sub>-CH<sub>3</sub>), 2.52 (s, 3H, thiazole-CH<sub>3</sub>), 4.19 (q, *J* = 7.08 Hz, 2H, O-CH<sub>2</sub>-CH<sub>3</sub>), 4.44 (s, 2H, S-CH<sub>2</sub>), 7.32–7.37 (m, 1H, Ar-H), 7.41–7.47 (m, 1H, Ar-H), 7.79 (d, *J* = 8.22 Hz, 1H, Ar-H), 8.01 (d, *J* = 7.89 Hz, 1H, Ar-H). <sup>13</sup>C-NMR (75 MHz, DMSO-d<sub>6</sub>, ppm) δ 14.68 (O-CH<sub>2</sub>-CH<sub>3</sub>), 17.62 (thiazole-CH<sub>3</sub>), 37.85 (S-CH<sub>2</sub>), 60.66 (O-CH<sub>2</sub>-CH<sub>3</sub>), 113.51, 121.52, 122.31, 124.94, 126.84, 135.23, 152.98, 156.88, 162.67, 162.73, 166.56, 168.39. HRMS (m/z): [M + H]<sup>+</sup> calculated 394.0348; found 394.0348.

## Prediction of the physicochemical properties

The physicochemical, pharmacokinetic, and medicinal chemistry properties of the compounds were calculated, virtually by using SwissAdme software [32]. HBA as H-bond acceptor, HBD as H-bond donor, TPSA as Topologic polar surface area ( $\text{\AA}^2$ ), Log Po/w as consensus Log Po/w (Average of all five predictions), Log S as water solubility, GIA as Gastrointestinal absorption, Log Kp as skin permeation (cm/s), RoF (V) as Rule of Five (violation number), SA as Synthetic accessibility from 1 (very easy) to 10 (very difficult) were predicted for final molecules and standard drugs chloramphenicol and ketoconazole.

## Conclusions

In this study, nine new ethyl 2-(2-mercaptoacetamido)-4-methylthiazole-5-carboxylate derivatives (**3a–3i**) were obtained using thiourea and ethyl 2-chloro-3-oxobutanoate as starting materials. After acetylation reaction, the final compounds were gained. Ethyl 2-(2-mercaptoacetamido)-4-methylthiazole-5-carboxylate was the core structure that overlaid the 6-APA molecule. All final compounds were tested on twenty-seven microorganism strains. Among the compounds, **3f** (5-nitrobenzimidazole), **3g** (*N*-methyltriazole), and **3h** (benzimidazole) exhibited high antibacterial activity whereas **3g** and **3h** showed prominent antifungal activity compared to the standard drugs. *K. pneumoniae*, *A. fumigatus* and *F. moniliforme* were the most susceptible genres.

## Acknowledgment

We gratefully thank Anadolu University DOPNA Laboratory for analyzing the synthesized compounds.

## Disclosure statement

The author confirms that this article's content has no conflict of interest.

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