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Synthesis of Some Heterofunctionalized Penicillanic Acid Derivatives and Investigation of Their Biological Activities

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6-Substituted amino-penicillanic acid esters were synthesized starting with 6-apa. The compounds containing a 1,3-thiazole- or 1,3-thiazolidinone nucleus linked to the penicillanic acid skeleton via a hydrazino linkage were obtained from 6-apa. The treatment of carbonylamino and carbonothioylamino compounds with 4-chlorophenacyl bromide or ethyl bromoacetate gave 6-bis{4-[1,3-thiazol(idinone)amino]benzoyl}amino derivatives of 6-apa. Benzyl derivatives were synthesized in several steps, starting with 4-aminobenzoyl chloride. The treatment of 4-{[3-benzyl-4-oxo-1,3-thia(oxa)-zolidin-2-ylidene]amino}benzoyl chlorides with 6-apa in ethanolic solution produced the 6-[bis(4-{[3-benzyl-4-oxo-1,3-thiazolidin-2-ylidene]amino}benzoyl)amino] derivative of penicillanic acid, while the reaction of the same intermediates in DMF gave the mono-substituted amino derivative of 6-apa. The synthesized compounds were screened for their biological activities, and some of them were found to possess good to moderate antimicrobial activity. Moreover, some of the compounds displayed antiurease, anti-β-lactamase, and/or antilipase activities.

Keywords: 6-Amino penicillanic acid / Anti β-lactamase activity / Antilipase activity / Antimicrobial activity / Anti-urease activity / 1,3-Oxa(thia)zole

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Introduction

As a result of enhanced antimicrobial resistance, infectious diseases account for a substantial part of deaths worldwide. Although World Health Organization has defined the antimicrobial agents as miracle drugs and leading weapons for the treatment of infectious diseases, a number of clinically efficacious antimicrobial agents, which are actively used, have become less effective due to antimicrobial resistance [1–5]. Furthermore, treatment of infectious diseases is more difficult in immunodeficient patients such as those infected with human immunodeficiency virus (HIV) [1]. The rising prevalence of multi-drug-resistant bacteria has served the medicinal chemists to design and synthesize novel antimicrobial agents, which are active against these resistant strains in current treatment. Besides, the newly synthesized agents should have fewer side effects on the human body because the use of antimicrobial drugs to treat with infections causes several problems in some cases, especially patients with impaired liver or kidney functions [6, 7]. Recent studies have shown that administration of highly potent antimicrobial agents at appropriate dosage will not only eradicate bacterial growth, but also minimize the probability of resistance formation [1].

Design and synthesis of new combinational chemotherapeutics constitute a major challenge of medicinal chemistry, which aims to overcome the antimicrobial resistance [8]. Besides the development of completely new agents possessing different chemical properties than those of the existing ones, another approach is to combine two or more pharmacophores into a single molecule. Therefore, a single molecule containing more than one pharmacophore, each with a different mode of action, could be beneficial for the treatment of microbial infectious [9–13]. These synergistic antimicrobial combinations have several major advantages, including the potential to slow down the development of drug resistance, a broader antimicrobial spectrum, and a potential reduction in the dose and toxicity of each drug [14].

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β-Lactam derivatives constitute a class of the most important antibacterial agents in the current clinical regimen, although the threat of resistance is getting stronger. Besides their applications as antibacterials, β-lactams are increasingly used as inhibitor of other medicinally important targets [15]. Nowadays, the industrial production of semi-synthetic β-lactam antibiotics is mostly carried out by chemical syntheses containing complex routes in which extreme temperatures (*i.e.*, −30°C) and organochloride solvents are involved. But, these methods are being criticized since they are potential sources of environmental damages [16]. Accordingly, enzymatic synthesis of β-lactam antibiotics received great attention from industry and academy as a green chemistry alternative. However, low yield and selectivity restricted the industrial application of the enzymatic methods [17].

Among several privileged structures, thiazolidinone ring is one of the most attractive targets for combinatorial synthesis, since its structure–activity relationship belongs to an important class of N- and S-containing heterocycles, which are widely used in drug design and synthesis studies [18, 19].

Oxazolidinones are reported as a unique class of antibacterials interacting with the bacterial ribosome to inhibit bacterial protein synthesis by preventing formation of 70S initiation complex [20–22]. This mechanism of action allows organisms, which are resistant to other antimicrobials, to remain susceptible to oxazolidinone inhibition [23].

Substituted piperazines were reported as important pharmacophores, which are involved in a number of therapeutically important drugs such as crixivan, an HIV protease inhibitor, norfloxacine, ciprofloxacine, levofloxacine, second-generation fluoroquinolones, eperezolid, ranbezolid, which are oxazolidinone class antibacterials, and an antifungal drug, itraconazole [24].

It is known that the production of enzymes such as the serine-β-lactamase (SBLs) and metallo-β-lactamase (MBLs) in bacteria increasingly causes the resistance against a broader range of common β-lactam antibiotics such as penams, carbapenems, and cephalosporins. Thus, the development of an inhibitor for SBLs and MBLs is an attractive approach to maintain the usefulness of existing antibiotics. Due to this reason, β-lactamase inhibitors have gained importance to overcome the antibacterial resistance [25, 26]. In this context, a number of natural and synthetic compounds have been reported to possess anti β-lactamase activity, which catalyzes the hydrolysis of the CO–N bond in the molecules of penicillins and cephalosporins. However, only a few of them have found a field of use at clinical settings.

Bacterial urease enzymes, which accelerate hydrolysis of urea to ammonia gas with the reaction rate at least 10^{14} over the spontaneous reaction, have been reported as important virulent factors including several important pathogeneses such as pyelonephritis, hepatic coma, peptic ulceration, injection-induced urinary stones, and stomach cancer [27, 28]. Moreover, the mixing of NH_3 to the atmosphere from urea is subsequently deposited on land or water. The result of this is eutrophication and acidification of natural ecosystems on a regional scale [29].

Obesity can lead to a series of serious diseases including but not limited to hypertension, hyperlipidemia, arteriosclerosis, and type II diabetes [30]. Pancreatic lipase plays an important role in digestion of fat. Pancreatic lipase inhibitors, such as orlistat, have been used as therapeutic agents for curing obesity [31]. However, some side effects including fecal incontinence, flatulence, and steatorrhea have been reported for orlistat [32, 33]. The compounds possessing anti-lipase activity can be the alternatives of orlistat.

In the present study, as a part of our ongoing study on the synthesis of bioactive hybrid molecules, we aimed to obtain new penicillanic acid derivatives containing azole moieties in a more environment-friendly way.

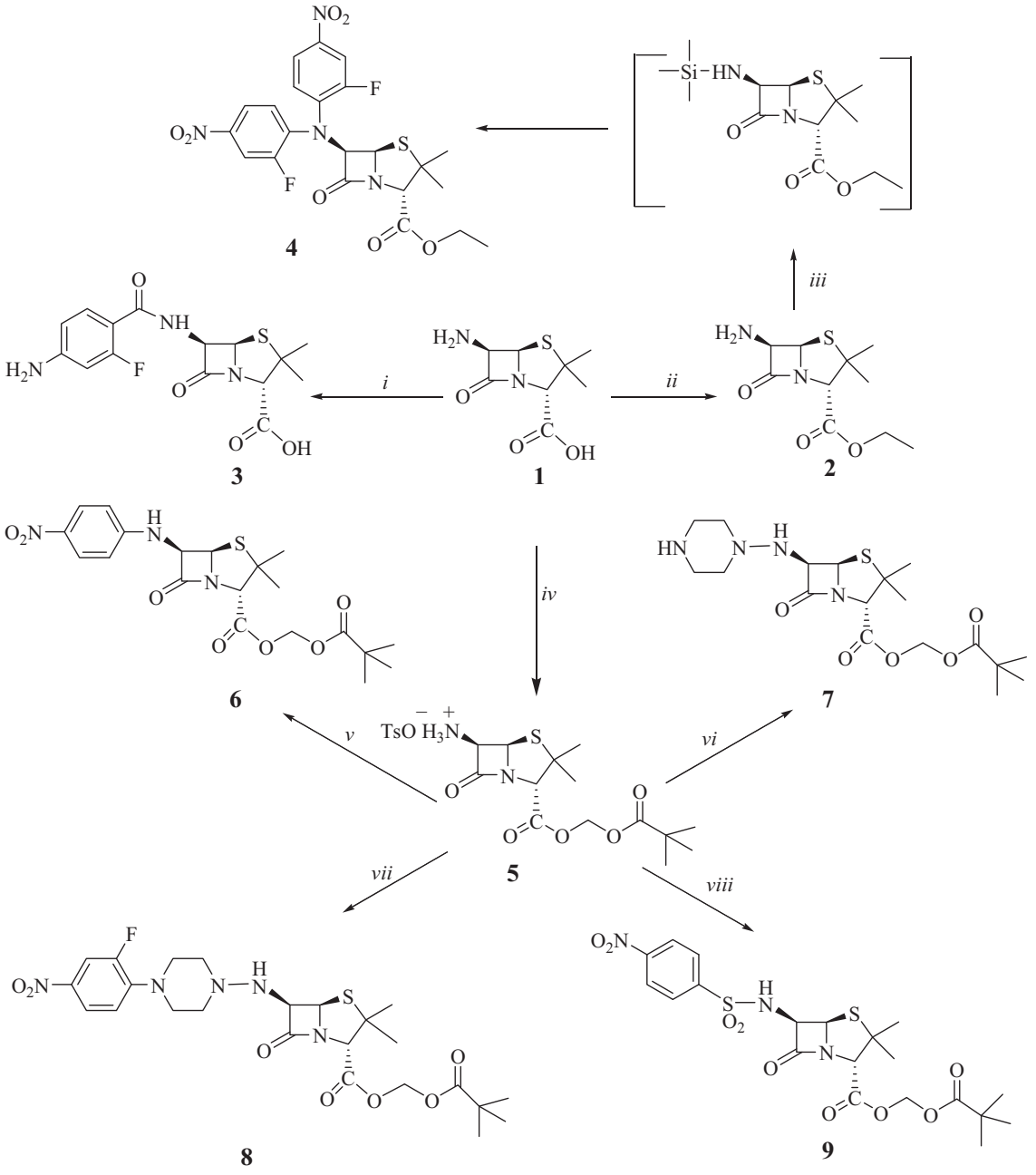
Results and discussion

Chemistry

The main aim of the present study is to design and synthesize new penicillanic acid derivatives incorporating piperazine, 1,3-thiazol(idinone), or 1,3-oxazol(idinone) ring within a single structure, and to investigate their biological activities. The core structure of the present study, penicillanic acid, belongs to the β-lactam class compounds, which constitute one of the most important antibacterial agents, besides their other applications including β-lactamase inhibitory effect, etc. [15–17]. Moreover, the pharmacological importance of other groups, namely 1,3-oxazole, 1,3-thiazole, and piperazine, which were combined with penicillanic acid skeleton in the present study, was well documented [18–24]. In addition, in one of our previous studies [34] the discovery of some azole compounds as antiurease agents served to merge the azole groups, namely 1,3-oxazol(idin)e and 1,3-thiazol(idin)e, with the penicillanic acid skeleton. Another reason for choosing these azole groups for hybridization is that some hybrid compounds carrying 1,3-oxazol(idin)e or 1,3-thiazol(idin)e nucleus were obtained as antilipase agents in our laboratory [35, 36].

The intermediate and target compounds were synthesized according to the reactions outlined in Schemes 1–5. The starting compound (+)-6-aminopenicillanic acid (6-apa, **1**) was provided commercially.

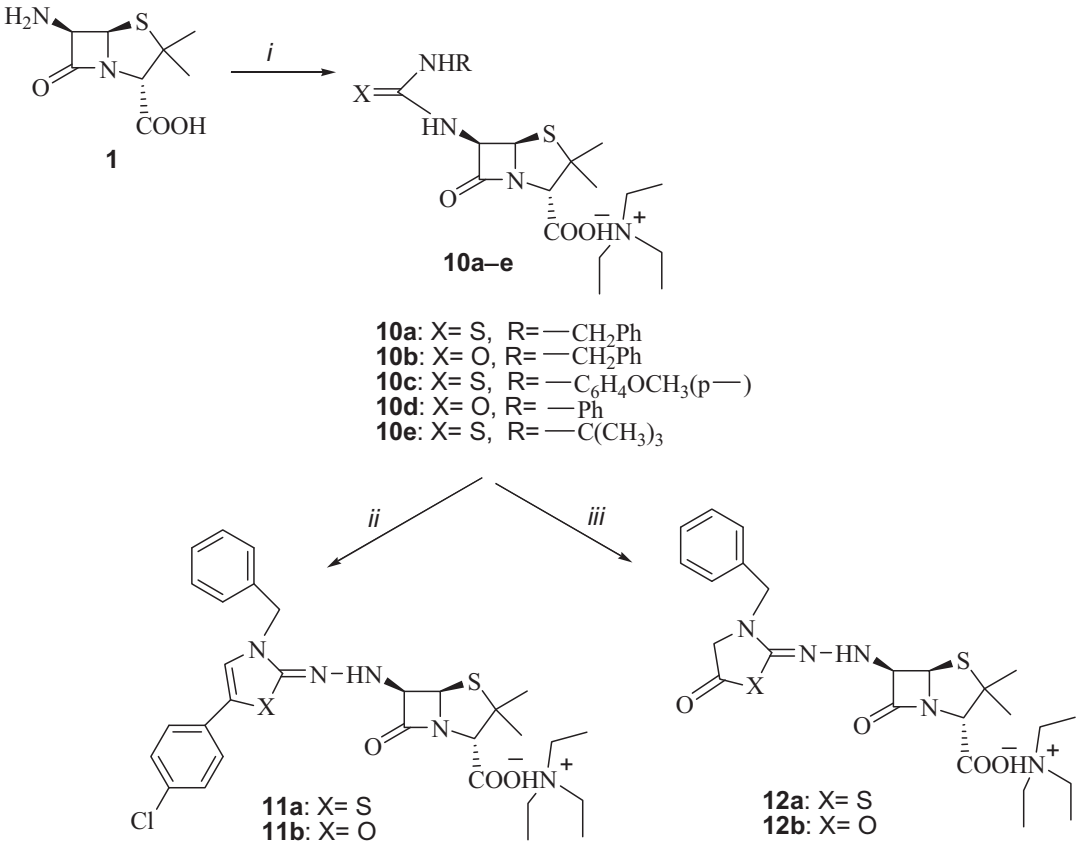
Compound **3** was synthesized via the reaction of (+)-6-amino penicillanic acid (6-apa, **1**) with 2-fluoro-4-aminobenzoic acid in the presence of ethyl chloroformate and triethylamine at low temperature. This idea originated from the need to incorporate an amide function to molecule,



Scheme 1. *i*: Ethylchloroformate, 2-fluoro-4-aminobenzoic acid, Et_3N, 6-apa, stirring in THF at room temperature; *ii*: catalytic amount of H_2SO_4, reflux in ethanol; *iii*: Et_3N, 6-apa, H_2NCONH_2 in dry DCM, trimethyl chlorosilane, *n*-BuLi at N_2 atm, 4-bromo-2-fluoronitrobenzene; *iv*: Et_3N, 6-apa, chloromethylpivalate and then *p*-TSA; *v*: Et_3N, 4-bromonitrobenzene in DCM; *vi*: piperazine in DCM at room temperature; *vii*: Et_3N and 1-(2-fluoro-4-nitrophenyl)piperazine, stirring in DCM at room temperature; *viii*: Et_3N, 4-nitrobenzene sulfonyl chloride, stirring in DCM at room temperature.

because amide bond is essential for antibacterial activity, especially in β-lactam antibiotics. For instance, ampicillin, penicillin G, penicillin V, nafcillin, meticillin, oxacillin, pivampicillin, and talampicillin incorporate an amide function instead of amine [24].

The treatment of 6-aminopenicillanic acid ethyl ester (**2**) with 3,4-difluoro nitrobenzene resulted in disubstitution of NH_2 protons and generated compound **4**, when 6-apa itself was used in this reaction; no change could be observed due to the low reactivity of compound **1** toward nucleophilic



Scheme 2. *i*: RNCX, Et_3N, DMF, room temperature; *ii*: (*4*-)$ClC_6H_4COCH_2Br$, CH_3COONa, reflux in ethanol; *iii*: $BrCH_2CO_2Et$, CH_3COONa, reflux in ethanol.

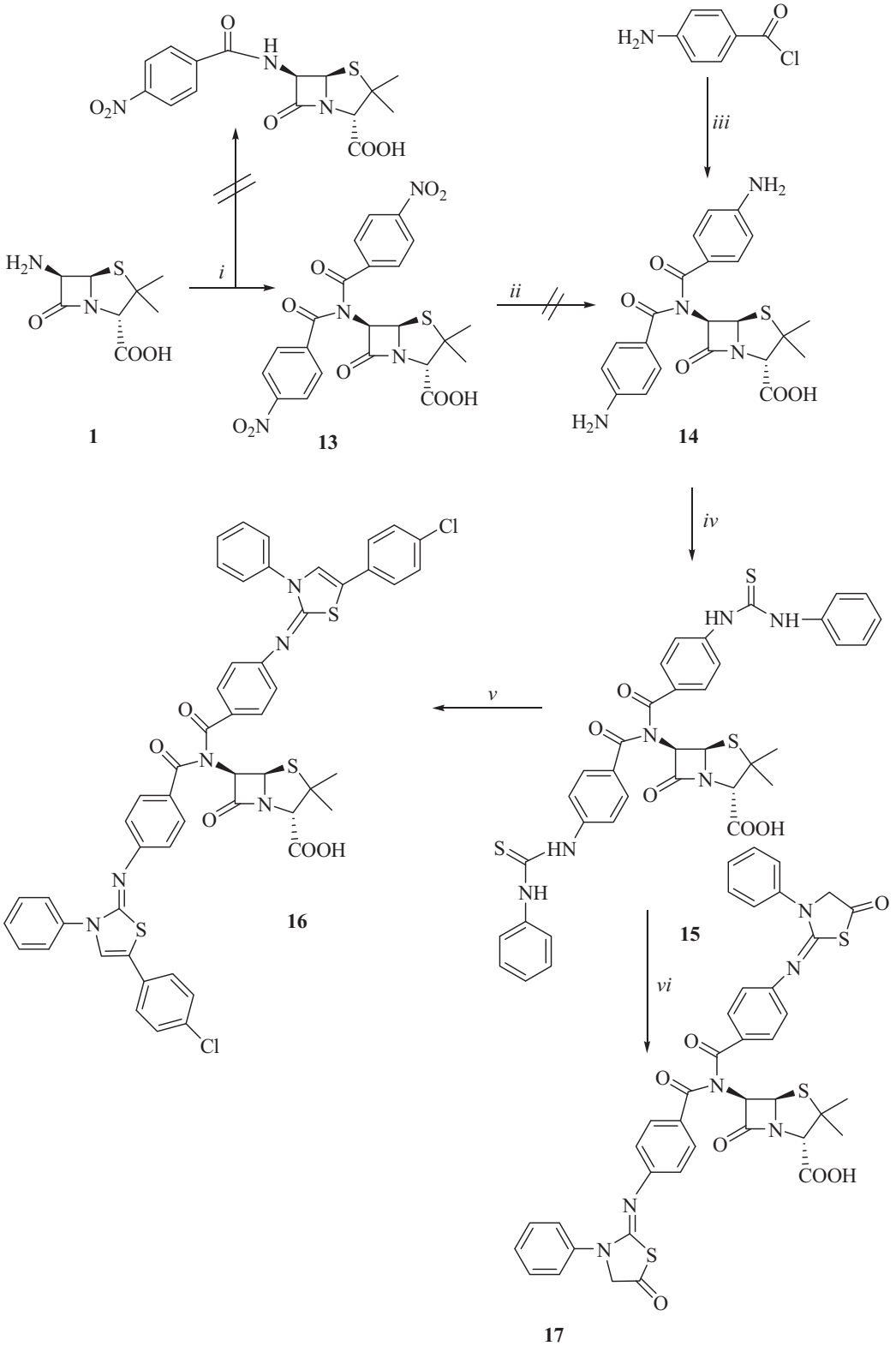
aromatic substitution. It was reported that the conversion of 6-apa to compound **5** increases the reactivity of the amine-nitrogen of 6-apa toward nucleophilic substitution [37]. The substitution of one of two amine protons by 4-nitrophenyl nucleus was carried out starting from compound **5**, and thus, compound **6** was obtained.

The signals derived from phenyl moiety were present at the aromatic region in the ^1H and ^{13}C NMR spectra of compounds **3**, **4**, and **6**. The mass spectra and elemental analysis were consistent with mono-substitution of amine protons of 6-apa, leading to the formation of compounds **3** and **6**. On the other hand, the mass spectrum and the elemental analysis supported the disubstitution for compound **4**.

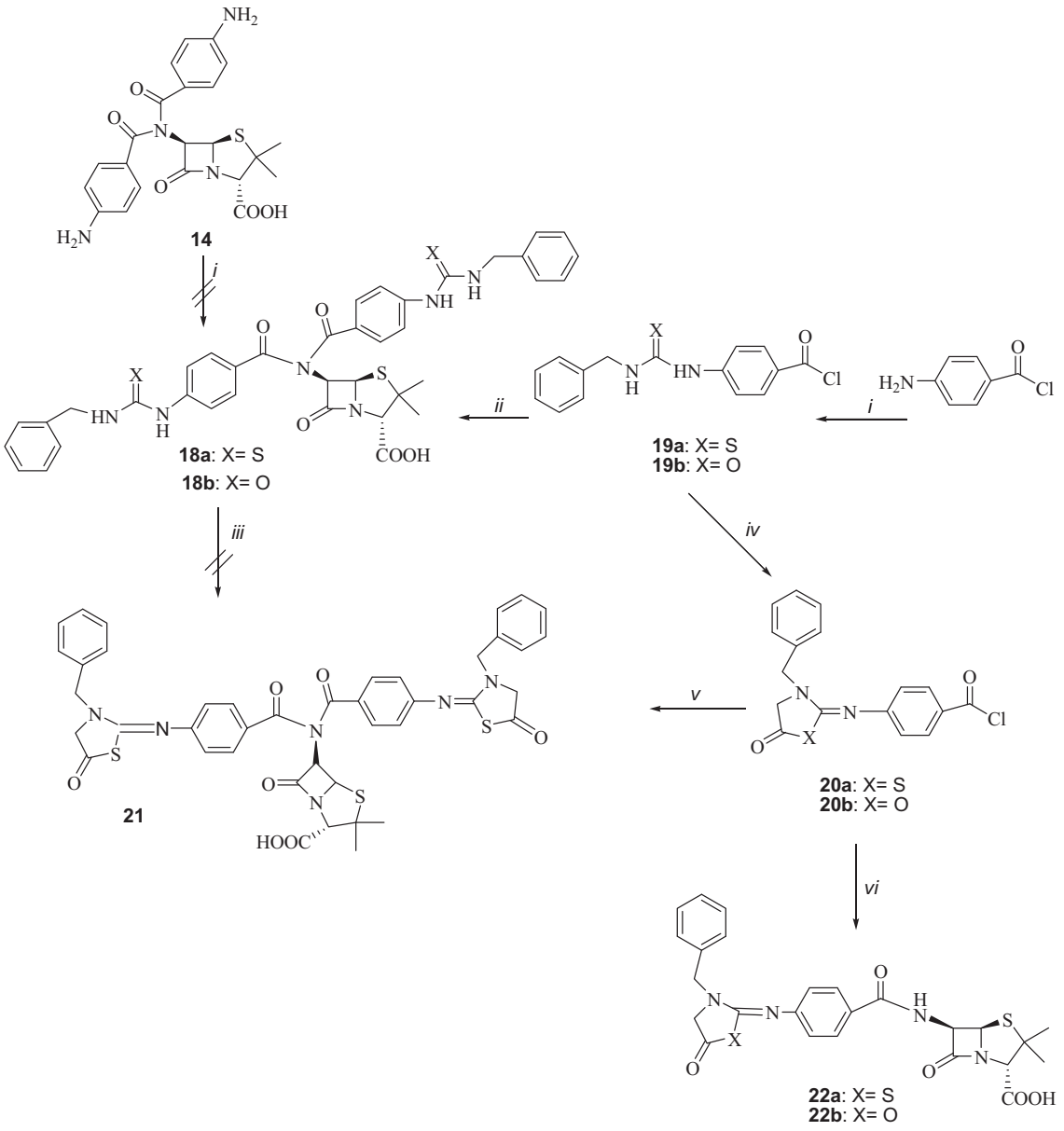
With the aim to bring a sulfonamide function to penicillanic acid core structure, compound **9** was obtained by the reaction of compound **5** with 4-nitrobenzene sulfonyl-chloride in anhydrous DMF; this idea originated from the fact that the antimicrobial activities of sulfonamides were well documented [24]. Compounds **7** and **8**, which were obtained by the reaction of compound **5** with the corresponding amines in dichloromethane, contain a piperazine nucleus as an additional pharmacophore linked to the penicillanic acid skeleton. In the ^1H and ^{13}C NMR spectra of compounds **7** and **8**, the signals originating from the piperazine moiety were observed at the related chemical shift value. In addition, ^1H and ^{13}C NMR spectra of compound **8** displayed additional signals appearing in the aromatic region due to 4-nitrophenyl moiety. The stretching bands due to nitro group were present in the FT-IR spectra of compounds **6** and **8**.

Compounds **10a–e** were obtained from the reaction of compound **1** with several isocyanates and isothiocyanates as important intermediates leading to the formation of 1,3-oxazol(idinone) and 1,3-thiazol(idinone) derivatives.

As different from compound **1**, ^1H and ^{13}C NMR spectra of compounds **10a–e** exhibited additional signals due to (thio)-urea and triethylammonium moieties at the related chemical shift values. In addition, these compounds gave relatively stable $[M]^+$, $[M+1]^+$, $[M-1]^+$, or $[M+2]^+$ ion peaks and elemental analysis data. The intermolecular cyclization of compounds **10a**,**b** with ethyl bromoacetate in boiling absolute



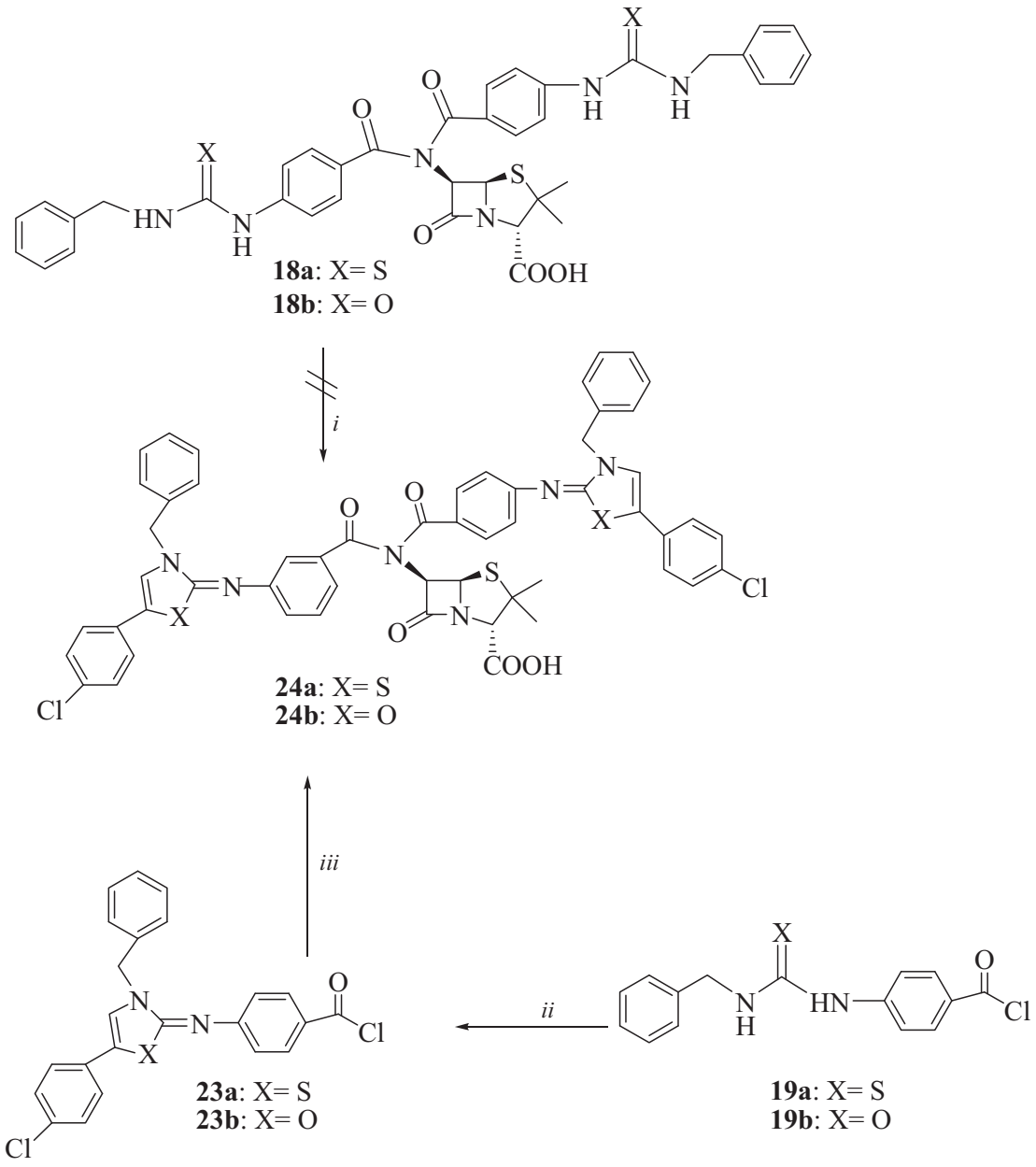
Scheme 3. *i*: K_2CO_3, (*4*-)$NO_2C_6H_4COCl$, acetone–water, rt; *ii*: Pd-C, H_2NNH_2, reflux in *n*-butanol; *iii*: 6-apa, K_2CO_3, acetone–water, rt; *iv*: PhCNS, reflux in ethanol; *v*: (*4*-)$ClC_6H_4COCH_2Br$, CH_3COONa, reflux in ethanol; *vi*: $BrCH_2CO_2Et$, CH_3COONa, MW 200 W.



Scheme 4. *i*: $C_6H_5CH_2CNS(O)$ MW at 150 W or reflux in ethanol; *ii*: 6-apa, K_2CO_3, acetone–water, stirring at rt; *iii* and *iv*: $BrCH_2CO_2Et$, CH_3COONa, reflux in ethanol; *v*: 6-apa, K_2CO_3, in DMF, rt; *vi*: 6-apa, K_2CO_3, acetone–water, rt or MW (for **22b**) 150 W.

ethanol afforded the corresponding 5-oxo-1,3-thiazole (**12a**) or 5-oxo-1,3-oxazole (**12b**) derivatives, while the cyclocondensation of the same intermediates (**10a**,**b**) with 4-chloropenacylbromide produced 1,3-thiazole (**11a**) or 1,3-oxazole (**11b**) compounds. With the conversion of compounds **10a**,**b** into **11a**,**b**, one of two NH signals disappeared in the ^{1}H NMR spectra, while additional signals originating from chlorophenyl nucleus were recorded in the aromatic region in the ^{1}H and ^{13}C NMR spectra of compounds **11a**,**b**. Moreover, compounds **11** and **12** displayed reasonable elemental analysis data consistent with the assigned structures.

With the aim to obtain a useful intermediate for further cyclizations and also to bring an amide function, the NH_2 position of 6-apa (**1**) was functionalized to two 4-nitrophenylbenzoyl groups in acetone–water at cold-room temperature; thus, compound **13** was prepared. In this reaction, monoacyl derivative of 6-apa could not be obtained.



Scheme 5. *i*: (*4*-)$ClC_6H_4COCH_2Br$, CH_3COONa, reflux in ethanol; *ii*: (*4*-)$ClC_6H_4COCH_2Br$, CH_3COONa, MW (150 W for **23a**) or reflux (for **23b**); *iii*: 6-apa, K_2CO_3 in DMF, rt.

Our efforts on the reduction of nitro groups to amino groups in different reduction conditions resulted in failure due to decomposition of β-lactam ring. The synthesis of the amine **14** was achieved by the reaction of 4-nitrobezoyl chloride with 6-apa at cold-room temperature. The FT-IR spectrum of **13** demonstrated two sharp signals pointing the presence of nitro groups, whereas the FT-IR spectrum of **14** displayed absorption bands derived from amine functions. The signals belonging to two phenyl nuclei were recorded at aromatic region in the ^1H and ^{13}C NMR spectra of compounds **13** and **14**. In addition, these compounds displayed reasonable mass fragmentation; and their elemental analyses are consistent with the assigned structures.

The treatment of compound **14** with phenylisothiocyanate yielded the corresponding bithiourea derivative, **15**. However our attempt to transform the amino groups of **14** into

benzylthiourea and benzylurea function was unsuccessful because obtained viscous reaction mixture was very complex and the components could not be separated and identified. However, the synthesis of (thio)urea derivatives, **18a** and **18b**, could be achieved by the treatment of 6-apa with **19a**,**b**, which were obtained starting from 4-aminobenzoyl chloride.

In the FT-IR spectra of compounds **19a**,**b**, the absence of any signal due to $-NH_2$ group and the presence of additional C=O (or C=S) stretching bands constitute an evidence for condensation between 4-aminobenzoyl chloride and benzylisocyanate or benzylisothiocyanate. In the ^{1}H NMR spectra of compounds **19a**,**b**, two D_2O exchangeable peaks at 6.77–8.44 and 8.98–9.93 ppm attributed to two NH protons. Moreover, in the ^{1}H and ^{13}C NMR spectra of these compounds, the crowding in the aromatic region that originated from additional phenyl ring supported the condensation. When compounds **19a**,**b** were converted to **18a**,**b**, additional signals were observed at the related chemical shift values in the ^{1}H NMR spectra, pointing to the presence of penicillanic acid core. In the ^{13}C NMR spectra, these carbons resonated at the expected chemical shift values in accordance with other 6-apa derivatives synthesized in this study. When compounds **18a**,**b** were treated with ethyl bromoacetate with the aim to obtain the corresponding 4-oxo-1,3-thiazolidine derivatives (**21a**,**b**), an unidentified viscous mixture was obtained. But still the synthesis of compound **21** was achieved by the reaction of 6-apa with the intermediate **20a** that was obtained from the condensation between **19a** and ethyl bromoacetate. It is interesting to note that the condensation between 6-apa and **20a** generated the diacylated product (**21**) in DMF, while similar condensation between 6-apa and **20a**,**b** in ethanolic solution resulted in the monoacylation; thus, compounds **22a**,**b** were obtained. The structures of compounds **21** and **22a**,**b** were elucidated by FT-IR, ^{1}H NMR, ^{13}C NMR, EI-MS spectroscopic methods, and elemental analysis.

To introduce the 1,3-thiazole nucleus into penicillanic acid molecular framework, compound **19a** was treated with 4-chlorophenacyl bromide, followed by the reaction with 6-apa; thus, compounds **22a**,**b** were obtained. ^{1}H NMR spectra of compounds **19a**,**b** revealed the presence of two NH groups due to the signals observed at 8.44 ppm (for **19a**) and 7.87 ppm (for **19b**) as D_2O exchangeable peaks, while ^{1}H NMR spectra of compounds **22a**,**b** displayed only one D_2O exchangeable peak at 9.23 (for **22a**) and 8.78 ppm (for **22b**) corresponding to one NH proton. Furthermore, in the NMR spectra of these compounds (**22a**,**b**), additional signals derived from 4-oxo-1,3-thiazolidine- and penicillanic acid moieties appeared at the related chemical shift values. In addition, elemental analyses were consistent with the assigned structures for these compounds, and the mass spectra of the cyclization products (**22a**,**b**) showed $[M]^+$ or $[M+H_2O]^+$ ion peaks at the corresponding *m*/*z* values, which match with their molecular formulas.

The cyclocondensation of **15** with 4-chlorophenacyl bromide produced 1,3-thiazol-2(3*H*)-ylidene-penicillanic acid derivative (**16**). On the other hand, the treatment of the same intermediate (**15**) with ethyl bromoacetate afforded the corresponding 4-oxo-3-phenyl-1,3-thiazolidine derivative (**17**). The disappearance of C=S stretching band in the FT-IR spectra of compounds **16** and **17** supported the cyclization. Moreover, in the NMR spectrum of **16**, the additional signals resonating for 8 protons in aromatic region were attributed to two chlorophenyl moieties. Elemental analysis of these compounds was in accordance with their structures, while these compound displayed reasonable fragmentation in the mass spectra including $[M-COOH]^+$, $[M+2-C_6H_5]^+$, $[M-PhCl]^+$, and/or $[M-PhCl-COOH]^+$ molecular ion peaks, which match with their molecular formulas.

Compounds **24a**,**b** were obtained by microwave irradiation of compounds **19a**,**b** with 4-chlorophenacylbromide, followed by the treatment of the obtained 1,3-thiazole derivatives (**23a**,**b**) with 6-apa.

As different from **23a** and **23b**, the NMR spectra of compounds **24a** and **24b** exhibited additional signals derived from penicillanic acid nucleus at the related chemical shift values. Moreover, the presence of $[M-2Cl]^+$ or $[M+1]^+$ ion peak in the mass spectra at the related *m*/*z* values and elemental analysis supported their molecular masses.

Biological activity

Antimicrobial activity

Compound **3** that contains a 4-amino-2-fluorophenylbenzoylamino moiety at the position 6 of penicillanic acid framework exhibited activity toward the test microorganisms except *Mycobacterium smegmatis* (Ms), *Candida albicans* (Ca), and *Saccharomyces cerevisiae* (Sc). With the conversion of amid function to amine group in the structures of compounds **4** and **6**, the antimicrobial activity diminished; only slight activity was observed on Ca and Sc, which are yeast-like fungi. Similarly, the introduction of piperazine nucleus into the structure of 6-apa resulted in no activity on the test microorganisms. On the other hand, compound **8** that contains a 4-(4-nitrophenyl)piperazine nucleus in the penicillanic acid structure displayed activity against *Pseudomonas aeruginosa* (Pa), *Staphylococcus aureus* (Sa), *Enterococcus faecalis* (Ef), which are Gram positive cocci, *Bacillus cereus* (Bc) that is Gram positive spore bacillus, *Mycobacterium smegmatis* (Ms) that is an atypical tuberculosis factor, and Ca. This compound (**8**) can be considered as the derivative of compound **7** including an additional 4-nitrophenyl group or a derivative of **6** with piperazine nucleus. Similarly, compound **9** that is sulfonamide derivative of **6** demonstrated moderate activity on the same test microorganisms with compound **8**. The carbo(thio)-

nylamino derivatives (**10a–e**) showed better antimicrobial activity than compounds **3–9**. Among these compounds, the activity of compound **10b** towards Sa was the same as that of standard drug ampicillin. **10d**, which contains a phenylamino nucleus in the carbonylamino side chain, displayed moderate activity against Ef with the inhibition zone of 8 mm. Other carbono(thio)ylamino compounds (**10a**, **10b**, **10c**, and **10d**) exhibited better activities than standard drug ampicillin, with the inhibition zones varying between 12 and 30 mm. Compounds **10a** and **10e** were found to possess the same activity on Bc as ampicillin. Among compounds **10a–e**, anti-Ca and -Sc activity was observed for compounds **10a** and **10c**, which are the compounds containing benzylamino (**10a**) or 4-methoxyphenylamino (**10c**) moiety in the carbonothioylamino side chain linked to penicillanic acid core. The activity of **10c** was better than **10a**. Other carbo(thio)nylamino compounds **18a**,**b** and **19a**,**b** displayed good-to-moderate inhibition activity on some of the test microorganisms. The penicillanic acid derivatives functionalized with 3,5-disubstituted 1,3-thiazole nucleus at the position 6 (**11a**) were found to be active on the test microorganisms except Ec, Yp, and Pa. On the other hand, compound **11b**, which contains a 1,3-oxazole ring instead of 1,3-thiazole moiety, displayed only slight activity on Sa with the inhibition zone of 8 mm. Among other 1,3-thiazole derivatives (**16**, **23a**, and **24a**) the best antibacterial activity was observed for compound **16** with the inhibition zones between 10 and 20 mm. Compound **24** carrying a 1,3-oxazole ring instead of 1,3-thiazole exhibited good-to-moderate activity on Ec, Yp, Pa, Bc, and Sc, while no inhibitory effect was observed for other 1,3-oxazole compound **23b** that did not contain any penicillanic acid framework. Among the compounds **12a**,**b**, **17**, **20a**,**b**, **21**, and **22a**,**b**, which are the 6-apa derivatives incorporating 5-oxo-1,3-thiazolidine (**12a**, **17**, **20a**, **21**, and **22a)** or 5-oxo-1,3-oxazolidine (**12b**, **20b**, and **22b**) moiety, compounds **17**, **21**, **22a**,**b** were found to be active against some of the test microorganisms. Compound **13** that can be considered as disubstituted derivative of **6** displayed moderate activity against the test microorganisms except Ef, Ca, and Sc. With the conversion of compound **13** to **14** by the reduction of two nitro groups, no activity was observed on Ec, Yp, and Ms; instead, additional activities were noted on Bc, Ca, and Sc. Compound **14** and its carbonothioylamino derivative **15** displayed the same activity on Sc with the inhibition zone 25 mm that is the same as with the standard drug fluconazole.

Anti-β-lactamase activity

The synthesized compounds were assayed for their *in vitro* inhibitory activity against *B. cereus* β-lactamase. Compounds **10a**, **10b**, and **10d**, which are 6-apa derivatives carrying an alkylaminocarbo(thio)nylamino side change, showed low β-lactamase inhibition. $HgCl_2$ with IC_{50} value 0.093 mM was used as standard inhibitor.

Anti-urease activity

The synthesized compounds were assayed for their *in vitro* inhibitory activity against Jack bean urease. Three of those compounds, **10c**, **10d**, and **10e**, which are alkylaminocarbono(thio)yl derivatives of 6-apa, showed potent urease inhibition. Thiourea with IC_{50} value $51.62 \pm 7.28\ \mu g\ mL^{-1}$ was used as standard inhibitor. Among tested compounds **10e** was found to have the maximum inhibitory effect against urease with an IC_{50} value of $54.14 \pm 1.45\ \mu g\ mL^{-1}$. Compounds **10c** and **10d** have moderate inhibitory activity. Dose-dependent inhibitory effects of compounds are depicted in Fig. 1. These compounds might be considered as potential antibiotics to treat infections. Urease inhibitor activity of the synthesized compounds may be associated with inhibition of *H. pylori*, a key cause of stomach infections.

Anti-lipase activity results

All compounds were evaluated with regard to pancreatic lipase activity, and **11a**, **18b**, and **20a** showed anti-lipase activities at various concentrations. No significant inhibitory effect was detected for other compounds. Dose-dependent pancreatic lipase activity is shown in Fig. 2. Among the tested compounds, **20a** showed the best anti-lipase activity. The compound inhibited pancreatic lipase activity by 62, 91, and 94% at concentrations of 1.25, 3.75, and $6.25\ \mu g\ mL^{-1}$, respectively. Orlistat, a known pancreatic lipase inhibitor used as anti-obesity drug, showed inhibitory effect by 80, 87, and 98% at the same concentrations. Orlistat and compound **20a** IC_{50} values were calculated as 0.61 and $0.84\ \mu g\ mL^{-1}$, respectively. Orlistat is the only approved anti-obesity medication [31] but it has some side effects, such as fecal incontinence, flatulence, and steatorrhea [32]. Compound **20a** can be considered as a good alternative to orlistat.

Conclusions

This study reports the successful synthesis of some new penicillanic acid derivatives containing piperazine, 1,3-oxazole, or 1,3-thiazole moiety. The antimicrobial, anti-β-lactamase, anti-urease, and anti-lipase activity screening studies were also performed in the study. Penicillanic acid nucleus is one of the active components present in many standard drugs and it is known to increase the pharmacological activity of the molecules. The presence of piperazine, 1,3-oxazol(idin)e, or 1,3-thiazol(idin)e moiety is also instrumental in contributing to the net biological activity of a system. Moreover, the therapeutic efficiency of hybrid molecules containing different pharmacophore groups was well documented for a number of pathological conditions.

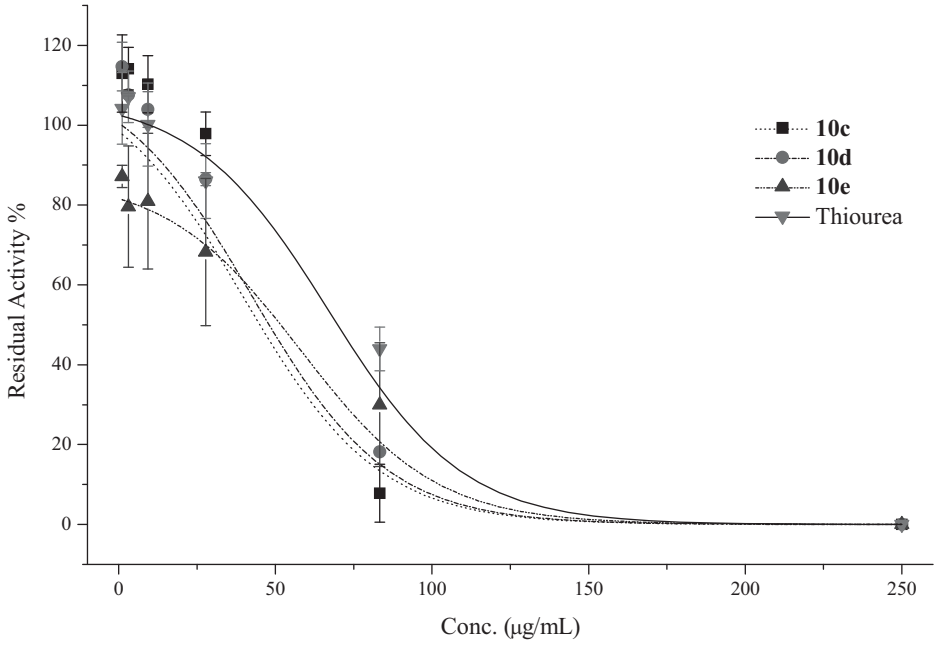


Figure 1. Dose-dependent inhibitory effect of some of the synthesized compounds. Thiourea was used as standard inhibitor. Inhibitory effect of all compounds and thiourea were measured in the range of 250–0.114 $\mu g\,mL^{-1}$ concentrations. Residual activities of compounds are expressed as the mean $\pm$ S.D. in triplicate.

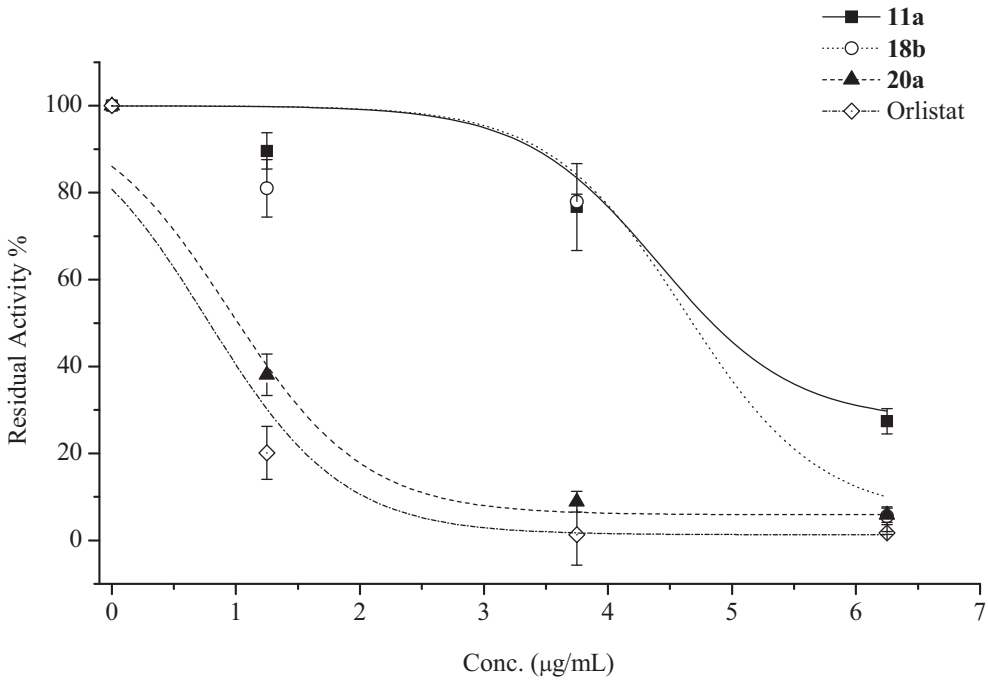


Figure 2. Dose-dependent inhibitory effects of the compounds. Orlistat was used positive control. All compounds and orlistat were measured at final concentrations of 1.25, 3.75, and 6.25 $\mu g\,mL^{-1}$. Residual activities of compounds are expressed as the mean $\pm$ S.D. in triplicate.

Hence, herein we combined all these potential units, namely penicillanic acid, piperazine, 1,3-oxazol(idin)e, and 1,3-thiazol(idin)e rings or sulfonamide function. Compounds **16** and **17**, which incorporate a phenyl substituent at the position 3 of 1,3-thiazole or 1,3-thiazolidine ring, could be obtained from condensation of compound **15** with 4-chlorophenacyl bromide (for compound **16**) or ethyl bromoacetate (for compound **17**). On the other hand, compounds **18a** and **18b**, which are the intermediates incorporating a benzyl substituent instead of phenyl, gave unidentified products at the same condensation conditions. It might be speculated that this result was obtained probably due to the steric hindrance of the benzyl group on C=S function of compounds **18a** and **18b**. In this way, penicillanic acid core, which already contains a stretched and unstable β-lactam ring, underwent decomposition in the reaction conditions. Thus, compounds **24a**,**b** were obtained by another way presented in Scheme 5.

The antimicrobial screening suggests that most of the synthesized compounds exhibited good-to-moderate activities against the test microorganisms. In addition, compounds **10a**, **10b**, and **10d**, which are 6-apa derivatives carrying an alkylaminocarbonylamino or alkylaminocarbonothioylamino side chain, showed low β-lactamase inhibition. Furthermore, three compounds, **10c**, **10d**, and **10e**, displayed perfect urease inhibition activity, while one compound, **20a**, demonstrated good anti-lipase activity.

Experimental

General

All the chemicals were purchased from Fluka Chemie AG Buchs (Switzerland) and used without further purification. Melting points of the synthesized compounds were determined in open capillaries on a Büchi B-540 melting point apparatus and are uncorrected. Reactions were monitored by thin-layer chromatography (TLC) on silica-gel 60 F254 aluminum sheets. The mobile phase was ethanol/ethyl ether 1:1, and detection was made using UV light. FT-IR spectra were recorded using a Perkin Elmer 1600 series FTIR spectrometer. ^{1}H NMR and ^{13}C NMR spectra were registered in DMSO-d_6 on a BRUKER AVENE II 400 MHz NMR spectrometer (400.13 MHz for ^{1}H and 100.62 MHz for ^{13}C) or Varian-Mercury 200 MHz NMR spectrometer (200 MHz for ^{1}H and 50 MHz for ^{13}C). The chemical shifts are given in ppm relative to Me_4Si as an internal reference; J values are given in Hz. The elemental analysis was performed on a Costech Elemental Combustion System CHNS-O elemental analyzer. All the compounds gave C, H, and N analysis within $\pm$0.4% of the theoretical values. The mass spectra were obtained on a Quattro LC-MS (70 eV) instrument. Compound **5** and 1-(2-fluoro-4-nitrophenyl)piperazine were obtained by the way reported earlier [34, 37].

*Ethyl (2S,5R,6R)-6-amino-penicillanate (**2**)*

The suspension of 6-apa (10 mmol) in ethanol was refluxed in the presence of catalytic amount of H_2SO_4 for 12 h. Then, the unreacted 6-apa was removed by filtration. After evaporating the solvent under reduced pressure, a solid was obtained. This crude product was recrystallized from ethyl acetate to give the pure compound. Yield 62%, m.p.: 140–141°C. FT-IR (υ_{max}, cm^{-1}): 3060 (aromatic C–H), 2902 (aliphatic C–H), 1722 (C=O), 1625 (C=O), 1235 (C–O). ^{1}H NMR (DMSO-d_6): δ (ppm) = 1.17 (brs, 3H, CH_3), 1.37 (brs, 3H, CH_3), 1.54 (s, 3H, CH_3), 3.42 (d, 2H, CH_2), 3.73 (s, 1H, CH), 4.41 (s, 2H, CH), 8.50 (brs, 2H, NH_2, D_2O exch.). ^{13}C NMR (DMSO-d_6): δ (ppm) = 12.11 (CH_3), 27.90 ($2CH_3$), 36.59 (CH_2), 61.38 (CH), 62.54 (CH), 65.63 (CH), 72.76 (C), 168.19 (C=O), 172.59 (C=O). EI MS m/z (%): 468.49 ([M+2]$^+$, 30), 467.55 ([M+1]$^+$, 100), 404.33 (23), 388.32 (25), 366.35 (35), 350.35 (32). Elemental analysis: for $C_{10}H_{16}N_2O_3S$ calcd. % C, 49.16; H, 6.60; N, 11.47. Found: % C, 48.86; H, 6.68; N, 11.46.

*(2S,5R,6R)-6-[(4-Amino-2-fluorobenzoyl)amino]-penicillanic acid (**3**)*

Ethylchloroformate (10 mmol) was added into the solution of 2-fluoro-4-aminobenzoic acid in dry tetrahydrofuran in the presence of triethylamine (10 mmol) at −15°C, drop by drop, and the mixture was stirred for 10 min. Then, the suspension of 6-apa (10 mmol) and triethylamine (10 mmol) in 10 mL of water was added into it, and the reaction was maintained at room temperature for 22 h. After the reaction was completed, water was added into the reaction mixture and acidified to pH 6 with diluted HCl. The acidified mixture was extracted with 15 mL of dichloromethane (three times). The combined organic layer was dried over sodium sulfate, and the solvent was evaporated under reduced pressure. The obtained solid was recrystallized from *n*-butyl acetate/diethyl ether (1:1) to give the pure product. Yield 21%, m.p.: 169°C. FT-IR (υ_{max}, cm^{-1}): 3200 (NH_2), 3104 (NH), 3063 (aromatic C–H), 2932 (aliphatic C–H), 1727 (C=O), 1655 (2C=O), 1233 (C–O). ^{1}H NMR (DMSO-d_6): δ (ppm) = 1.15 (brs, 6H, $2CH_3$), 1.97 (s, 1H, CH), 4.00 (brs, 2H, CH), 6.21–6.37 (m, 2H, NH_2, D_2O exch.), 7.53–7.58 (m, 3H Ar–H), 10.67 (brs, 1H, NH, D_2O exch.). ^{13}C NMR (DMSO-d_6): δ (ppm) = 27.19 (CH_3), 31.30 (CH_3), 58.79 (CH), 59.36(C), 65.57 (CH), 69.43 (CH), 112.45 (CH), 112.87 (CH), 125.19(CH), 137.03 (C), 142.3 (2C), 150.68 (C=O), 153.42 (C=O), 161.96 (C=O). EI MS m/z (%): 355.78 (42), 354.16 (37), 353.89 ([M]$^+$, 52), 265.19 (100). Elemental analysis: for $C_{15}H_{16}FN_3O_4S$ calcd. % C, 50.98; H, 4.56; N, 11.86. Found: % C, 51.01; H, 4.83; N, 11.56.

*Ethyl (2S,5R,6R)-6-[bis(2-fluoro-4-nitrophenyl)amino]-penicillanate (**4**)*

Triethylamine (30 mmol) was added to the suspension of 6-aminopenicillanic acid (6-apa) (10 mmol) and urea (20 mmol) in dry dichloromethane and the mixture was stirred at 10°C for 10 min, and at room temperature for 30 min. Then, trimethyl chlorosilane (30 mmol) was added dropwise at 5°C, the reaction content was stirred at room temperature for 30 min and an additional 1 h at 40°C. *n*-BuLi (10.3 mmol) was added dropwise to the solution of the obtained silyl ester of 6-apa [38] at −78°C in the presence of nitrogen atmosphere, and the mixture was stirred at the same temperature for 1 h. Then, the solution of 4-bromo-2-fluoronitrobenzene in dichloromethane was added into the reaction mixture and the stirring was continued at room temperature for additional 7 h. The reaction mixture was cooled to 0°C and 80 mL of water was added dropwise. The two layers were separated and the water layer was extracted with 40 mL of ethyl acetate two times. The combined organic layer was dried on

Na_2SO_4 and Na_2SO_4 was removed by filtration. Upon cooling the mixture overnight, a solid formed. This crude product was recrystallized from acetone to give the pure compound. Yield 57%, m.p.: 77–78°C. FT-IR (υ_{max}, cm^{-1}): 1725 (C=O), 1651 (C=O), 1517 and 1341 (NO_2), 1248 (C–O). ^1H NMR (DMSO-d_6): δ (ppm) = 1.33 (t, 3H, CH_3, J = 7.0 Hz), 1.71 (s, 3H, CH_3), 1.81 (s, 3H, CH_3), 4.26 (m, 5H, 3CH+ CH_2), 7.32 (d, 2H, Ar–H, J = 8.6 Hz), 7.60 (s, 2H, Ar–H), 7.83 (d, 2H, Ar–H, J = 8.6 Hz). ^{13}C NMR (DMSO-d_6): δ (ppm) = 14.19 ($3CH_3$), 65.70 (CH_2), 58.98 (CH), 59.58 (C), 65.51 (CH), 68.58 (CH), arC: [118.16 (2CH), 123.33 (2CH), 126.48 (2CH), 126.98 (2C), 127.42 (C), 132.30 (C), 138.58 (2C)], 151.90 (2C=O). EI MS m/z (%): 517.31 (21), 522.54 ($[M]^+$, 100), 523.54 ($[M+1]^+$, 26). Elemental analysis: for $C_{22}H_{20}F_2N_4O_7S$ calcd. % C, 50.57; H, 3.86; N, 10.72. Found: % C, 50.59; H, 3.83; N, 10.42.

*(2S,5R,6R)-2-[(2,2-Dimethylpropanoyl)oxy]-methylpenicillanat-6-aminium-4-methylbenzenesulfonate (**5**)*

Triethylamine (10 mmol) was added to the suspension of 6-apa (10 mmol) in 9 mL of anhydrous DMF and the mixture was stirred at room temperature for 30 min, then, chloromethylpivalate (20 mmol) was added into it. After stirring at 25°C for 4 h, the mixture was diluted with 50 mL of EtOAc. The precipitate was filtered off and the filtrate was washed with H_2O and petroleum ether to remove DMF and unreacted 6-apa. The organic layer was dried over Na_2SO_4 and freshly recrystallized *p*-toluenesulfonic acid (10 mmol) was added into it. The solid formed [39] was filtered and washed with cold EtOAc. Yield 43%, m.p.: 150–151°C. FT-IR (υ_{max}, cm^{-1}): 2972 (aliphatic C–H), 1750, 1763, and 1694 (3C=O). ^1H NMR (DMSO-d_6): δ (ppm) = 1.12 (brs, 9H, $3CH_3$), 1.41 (brs, 3H, CH_3), 1.59 (brs, 3H, CH_3), 2.26 (brs, 3H, CH_3), 4.57 (s, 1H, CH), 5.13 (s, 1H, CH), 5.52 (s, 1H, CH), 5.74–5.83 (m, 2H, CH_2), 7.12 (d, 2H, Ar–H, J = 7.4 Hz), 7.46 (d, 2H, Ar–H, J = 7.4 Hz), 8.85 (brs, NH_3^+, D_2O exch.). ^{13}C NMR (DMSO-d_6): δ (ppm) = 21.47 ($2CH_3$), 26.55 (CH_3), 27.21 (CH_3), 27.23 (CH_3), 34.12 (CH_3), 57.84 (CH), 60.17 (CH_2), 64.49 (CH), 71.71 (CH), 80.33 (2C), arC: [126.17 (CH), 126.75 (CH), 128.77 (2CH)], 146.21 (C=O), 168.31 (C=O), 169.61 (C=O).

*[(2,2-Dimethylpropanoyl)oxy]methyl (2S,5R,6R)-6-[(4-nitrophenyl)amino]-penicillanate (**6**)*

To a solution of compound **5** (2.2 mmol) in 1.7 mL of dimethyl formamide, triethylamine (2.2 mmol) was added at −5°C and the mixture was stirred at the same temperature for 30 min. The solution of 4-bromonitrobenzene in dichloromethane was added dropwise and the stirring was continued at room temperature for 24 h. Water was added into it and extracted with dichloromethane (3 × 5 mL). The combined organic layers were dried on Na_2SO_4 and evaporated under reduced pressure. The obtained oily product was recrystallized from ethyl acetate–hexane (1:1) to afford the desired compound. Yield 30%, m.p.: 114–115°C. FT-IR (υ_{max}, cm^{-1}): 3373 (NH), 3097 (aromatic C–H), 2976 (aliphatic C–H), 1749 and 1669 (2C=O), 1354 (C–O). ^1H NMR (DMSO-d_6): δ (ppm) = 1.12–1.25 (m, 15H, $5CH_3$), 1.46 (s, 2H, CH_2), 5.31 (s, 1H, CH), 5.60 (d, 1H, CH, J = 5.8 Hz), 5.87 (d, 1H, CH, J = 6.6 Hz), 7.84–7.90 (m, 2H, Ar–H), 8.10–8.17 (m, 2H, Ar–H). ^{13}C NMR (DMSO-d_6): δ (ppm) = 7.87 ($2CH_3$), 27.18 ($2CH_3$), 28.43 (CH_3), 46.12 (C), 52.74 (CH_2), 59.39 (C), 70.02 (CH), 73.05 (2CH), arC: [125.98 (2CH), 130.04 (C), 133.49 (2CH), 147.42 (C)], 165.61 (2C=O), 168.81 (C=O). EI MS m/z (%): 531.38 (65), 526.42 (38), 517.36 (37), 501.37 (38), 487.35 ($[M-2+K]^+$, 74), 473.34 ($[M+Na]^+$, 37), 457.37 (37), 443.33 (100), 429.31 (37), 413.31 (37). Elemental analysis: for $C_{20}H_{25}N_3O_7S$ calcd. % C, 53.20; H, 5.58; N, 9.31. Found: % C, 53.25; H, 5.59; N, 9.42.

*[(2,2-Dimethylpropanoyl)oxy]methyl (2S,5R,6R)-6-(piperazin-1-yl)aminopenicillanate (**7**)*

Piperazine (2.2 mmol) was added into the solution of compound **5** (2.2 mmol) in dichloromethane at 0°C and the mixture was stirred at room temperature for 5 h. Water was added, and two layers were separated. Water layer was extracted with 5 mL of dichloromethane two times and the combined organic layer was dried over $NaSO_4$. On evaporating the solvent under reduced pressure, a solid formed. This was recrystallized from ethyl acetate/hexane (1:1) to obtain the target compound. Yield 25%, m.p.: 94–95°C. FT-IR (υ_{max}, cm^{-1}): 3353 and 3300 (2NH), 2973 (aliphatic C–H), 1760, 1738 and 1642 (3C=O), 1279 (C–O). ^1H NMR (DMSO-d_6): δ (ppm) = 1.15 (s, 9H, $3CH_3$), 1.48 (s, 6H, $2CH_3$), 3.65–3.88 (m, 8H, $4CH_2$), 5.31–5.65 (m, 2H, 2CH), 5.90 (brs, 1H, CH). ^{13}C NMR (DMSO-d_6): δ (ppm) = 27.21 ($2CH_3$), 27.68 ($3CH_3$), 37.98 (C), 47.21 ($2CH_2$), 52.63 ($2CH_2$), 59.71 (C), 61.52 (CH), 71.90 (2CH), 80.11 (CH_2), 168.55 (C=O), 176.86 (C=O), 180.09 (C=O). EI MS m/z (%): 437.21 ($[M+Na]^+$, 32), 431.08 (30), 408.88 (29), 407.14 (100). Elemental analysis: for $C_{18}H_{30}N_4O_5S$ calcd. % C, 52.15; H, 7.29; N, 13.52. Found: % C, 52.25; H, 7.23; N, 13.41.

*[(2,2-Dimethylpropanoyl)oxy]methyl (2S,5R,6R)-6-{[4-(2-fluoro-4-nitrophenyl)piperazin-1-yl]aminopenicillanate (**8**)*

The solution of compound **5** (2.2 mmol) in 9 mL of anhydrous dichloromethane was stirred at 0°C in the presence of triethylamine (2.2 mmol) for 30 min. 1-(2-Fluoro-4-nitrophenyl)piperazine (2.2 mmol) was added and the mixture was stirred at room temperature for 10 h. Water was added into it and extracted with dichloromethane three times. The combined organic layers was dried on Na_2SO_4 and evaporated under reduced pressure. The obtained crude product was recrystallized from dichloromethane. Yield 25%, m.p.: 83°C. FT-IR (υ_{max}, cm^{-1}): 3287 (NH), 2970 (aliphatic C–H), 1751 (C=O), 1638 (2C=O), 1514 and 1330 (NO_2), 1234 (C–O). ^1H NMR (DMSO-d_6): δ (ppm) = 1.04 (brs, 9H, $3CH_3$), 1.46 (brs, 6H, $2CH_3$), 3.37 (brs, 6H, $3CH_2 + H_2O$), 3.61–3.87 (m, 4H, $2CH_2$), 4.80 (brs, 1H, CH), 5.71 (brs, 1H, CH), 5.87 (brs, 1H, CH), 7.20 (d, 1H, Ar–H, J = 8.8 Hz), 7.99–8.06 (m, 2H, Ar–H). ^{13}C NMR (DMSO-d_6): δ (ppm) = 26.81 (CH_3), 27.21 ($3CH_3$), 28.44 (CH_3), 38.91–41.37 (DMSO-d_6 + C-$(CH_3)_3$), 46.43 ($2CH_2$), 58.32 (CH_2), 59.52 (CH_2), 64.48 ($C(CH_3)_2$), 69.57 (CH), 70.35 (CH), 73.13 (CH), 78.92 (CH_2), arC: [114.51 and 114.94 (d, CH, J = 21.5 Hz), 119.02 and 119.40 (d, CH, J = 19.0 Hz), 122.13 and 122.23 (d, CH, J = 5.0 Hz), 126.17 and 128.72 (d, C, J = 127.5 Hz), 138.14 and 138.46 (d, C, J = 16.0 Hz), 147.64 and 152.01 (d, C, J = 218 Hz)], 165.61 (C=O), 166.97 (C=O), 176.77 (C=O). EI MS m/z (%): 554.27 ($[M+1]^+$, 63), 428.16 (25), 242.27 (27), 196.16 (42), 138.12 (100), 102.12 (50). Elemental analysis: for $C_{24}H_{32}FN_5O_7S$ calcd. % C, 52.07; H, 5.83; N, 12.65. Found: % C, 52.38; H, 6.09; N, 12.22.

*[(2,2-Dimethylpropanoyl)oxy]methyl (2S,5R,6R)-6-{[(4-nitrophenyl)sulfonyl]amino}penicillanate (**9**)*

Triethylamine (10 mmol) and 4-nitrobenzene sulfonyl chloride were added to a solution of compound **5** (10 mmol) in anhydrous dichloromethane at 0°C and the mixture was stirred at room temperature for 13 h. Water was added into it, two layers were separated, and the water layer was extracted with

dichloromethane two times and the combined organic layers were dried on Na_2SO_4. After evaporating the solvent under reduced pressure, a solid formed. This was recrystallized from ethanol/water (1:1) to afford the desired compound. Yield 50%, m.p.: 62°C. FT-IR (υ_{max}, cm^{-1}): 3108 (NH), 2976 (aliphatic CH), 1752 (2C=O), 1675 (C=O), 1530 and 1348 (NO_2), 1281 (C–O). ^{1}H NMR (DMSO-d_6): δ (ppm) = 1.12 (brs, 15H, 5CH_3), 3.59 (brs, 2H, CH_2), 5.38 (brs, 1H, NH), 5.70 (brs, 3H, CH), 8.07 (brs, 2H, Ar–H), 8.36 (brs, 2H, Ar–H). ^{13}C NMR (DMSO-d_6): δ (ppm) = 27.21 (CH_3), 31.23 (CH_3), 35.03 (3CH_3), 38.64 (C-(CH_3)$_3$), 41.86 (C-(CH_3)$_2$), 46.21 (CH_2), 59.90 (2CH), arC: [125.71 (2CH), 128.83 (2CH), 140.64 (2C)], 148.26 (C=O), 168.27 (C=O), 171.06 (C=O). EI MS *m/z* (%): 547.62 (25), 538.61 ([M+Na]$^+$, 21), 386.56 (68), 359.65 (31), 358.59 (100), 338.38 (34), 322.36 (37), 320.29 (71). Elemental analysis: for $C_{20}H_{25}N_3O_9S_2$ calcd. % C, 46.59; H, 4.89; N, 8.15. Found: % C, 46.51; H, 4.85; N, 8.42.

*General method for the synthesis of compounds **10a–e***

Triethylamine (35 mmol) was added to a solution of 6-apa (10 mmol) in dimethyl formamide at −5°C and stirred for 30 min. The solution of the corresponding alkyliso(thio)cyanate (10 mmol) in dimethyl formamide was added into it dropwise and stirred for additional 15 min. Then, temperature was allowed to reach to room temperature and the stirring was continued additional 3 h. The unreacted 6-apa was removed by filtration. Diethyl ether was added into the filtrate. The solution was stirred rapidly and left in cold overnight. The solid formed was filtered off and recrystallized from petroleum ether.

*Triethylammonium (2S,5R,6R)-6-{[(benzylamino)-carbonothioyl]amino}penicillanate (**10a**)*

Yield 55%, m.p.: 132°C. FT-IR (υ_{max}, cm^{-1}): 3125 (2NH), 3104 (NH), 3068 (aromatic C–H), 2932 (aliphatic C–H), 1727 (2C=O), 1108 (C=S), 1233 (C–O). ^{1}H NMR (DMSO-d_6): δ (ppm) = 1.11 (t, 9H, 3CH_3 J = 7.0 Hz), 1.46 (s, 3H, CH_3), 1.53 (s, 3H, CH_3), 2.92 (q, 6H, 3CH_2, J = 8.2 Hz), 4.67 (s, 2H, CH_2), 5.45 (s, 1H, CH), 5.83 (brs, 2H, CH), 7.29 (brs, 5H, Ar–H), 8.19 (brs, 1H, NH, D_2O exch.), 8.47 (s, 1H, NH, D_2O exch.). ^{13}C NMR (DMSO-d_6): δ (ppm) = 9.31 (3CH_3), 27.93 (CH_3), 31.57 (CH_3), 42.60 (CH_2), 45.78 (3CH_2), 59.44 (CH), 64.93 (C), 69.82 (CH), 73.59 (CH), arC: [126.74 (CH), 126.92 (CH), 127.01 (CH), 128.88 (CH), 128.95 (CH), 141.98 (C)], 157.52 (C=S), 170.13 (C=O), 172.59 (C=O). EI MS *m/z* (%): 468.49 ([M+2]$^+$, 30), 467.55 ([M+1]$^+$, 100), 404.33 (23), 388.32 (25), 366.35 (35), 350.35 (32). Elemental analysis: for $C_{22}H_{34}N_4O_3S_2$ calcd. % C, 56.62; H, 7.34; N, 12.01. Found: % C, 56.89; H, 7.73; N, 12.42.

*Triethylammonium (2S,5R,6R)-6-{[(benzylamino)carbonyl]-amino}penicillanate (**10b**)*

Yield 40%, m.p.: 188°C. FT-IR (υ_{max}, cm^{-1}): 3325 (3NH), 2980 (aliphatic C–H), 1764 (2C=O), 1679 (C=O), 1276 (C–O). ^{1}H NMR (DMSO-d_6): δ (ppm) = 1.12 (t, 9H, 3CH_3, J = 7.4 Hz), 1.46 (s, 3H, CH_3), 1.54 (s, 3H, CH_3), 2.93 (q, 6H, 3CH_2, J = 7.2 Hz), 3.97 (s, 1H, CH), 4.21 (s, 2H, CH), 5.39 (s, 2H, CH_2), 6.65 (d, 1H, NH, D_2O exch.), 6.86 (s, 1H, NH, D_2O exch.), 7.21–7.39 (m, 5H, Ar–H). ^{13}C NMR (DMSO-d_6): δ (ppm) = 9.32 (3CH_3), 27.92 (CH_3), 32.37 (CH_3), 43.61 (CH_2), 45.58 (3CH_2), 59.45 (CH), 64.83 (C), 68.62 (CH), 73.54 (CH), arC:[127.44 (CH), 127.62 (CH), 127.77 (CH), 128.83 (CH), 129.00 (CH), 140.91 (C)], 157.12 (C=O), 171.17 (C=O), 176.29 (C=O). EI MS *m/z* (%): 452.39 ([M+2]$^+$, 13), 449.23 (26), 447.22 (100), 432.27 (12), 405.26 (27), 354.23 (10). Elemental analysis: for $C_{22}H_{34}N_4O_4S$ calcd. % C, 58.64; H, 7.61; N, 12.43. Found: % C, 58.61; H, 7.69; N, 12.40.

*Triethylammonium (2S,5R,6R)-6-({[(4-methoxyphenyl)-amino]carbonothioyl}amino)penicillanate (**10c**)*

Yield 82%, m.p.: 139–140°C. FT-IR (υ_{max}, cm^{-1}): 3150 (3NH), 3077 (aromatic C–H), 2921 (aliphatic C–H), 1754 (C=O), 1609 (C=O), 1517 (C=S). ^{1}H NMR (DMSO-d_6): δ (ppm) = 1.357 (brs, 9H, 3CH_3), 1.47–1.55 (m, 6H, 2CH_3), 2.507 (s, 6H, 3CH_2 + DMSO-d_6), 3.01 (brs, 1H, CH), 3.51 (brs, 2H, 2CH + H_2O), 3.79 (s, 3H, OCH_3), 7.01 (d, 2H, Ar–H, J = 8.8 Hz), 7.19 (d, 2H, Ar–H, J = 7.6 Hz). ^{13}C NMR (DMSO-d_6): δ (ppm) = 8.63 (3CH_3), 23.13 (2CH_3), 22.07 (CH_2), 25.62 (CH_2), 31.26 (CH_2), 55.30 (OCH_3), 65.42 (CH), 68.96 (C), 69.22 (CH), 69.73 (CH), arC: [131.87 (2CH), 129.75 (2CH), 158.59 (C), 159.10 (C)], 162.40 (C=O), 172.32 (C=O), 183.66 (C=S). EI MS *m/z* (%): 500.98 ([M+H_2O]$^+$, 23), 483.71 ([M+1]$^+$, 40), 420.55 (100), 215.09 (23). Elemental analysis: for $C_{22}H_{34}N_4O_4S_2$ calcd. % C, 54.75; H, 7.10; N, 11.61. Found: % C, 54.39; H, 6.98; N, 11.52.

*Triethylammonium (2S,5R,6R)-6-[(anilinocarbonyl)amino]-penicillanate (**10d**)*

Yield 92%, m.p.: 170–171°C. FT-IR (υ_{max}, cm^{-1}): 3328 (2NH), 3290 (NH), 3137, 3091 (aromatic C–H), 2981 (aliphatic C–H), 1767 (2C=O), 1704 (C=O), 1212 (C–O). ^{1}H NMR (DMSO-d_6): δ (ppm) = 1.122 (t, 9H, 3CH_3, J = 7.2 Hz), 1.45 (s, 3H, CH_3), 1.59 (s, 3H, CH_3), 2.92 (t, 6H, 3CH_2, J = 7.2 Hz), 4.02 (s, 1H, CH), 5.47 (s, 2H, 2CH), 6.93 (t, 2H, Ar–H, J = 7.6 Hz), 7.24 (t, 2H, Ar–H, J = 8.0 Hz), 7.39 (t, 2H, Ar–H, J = 7.6 Hz), 9.04 (s, 2H, NH, D_2O exch.). ^{13}C NMR (DMSO-d_6): δ (ppm) = 8.90 (CH_3), 27.13 (CH_3), 31.95 (CH_3), 38.83 (3CH_2), 58.49 (CH), 64.23(C), 67.72 (CH), 72.67 (CH), arC: [121.56 (2CH), 126.99 (CH), 128.59 (2CH), 139.30 (C)], 153.40 (C=O), 170.56 (C=O), 174.41 (C=O). EI MS *m/z* (%): 438.49 ([M+2]$^+$, 32), 436.55 ([M]$^+$, 11), 404.59 (10), 369.35 (43), 350.35 (100). Elemental analysis: for $C_{21}H_{32}N_4O_4S$ calcd. % C, 57.77; H, 7.39; N, 12.83. Found: % C, 57.47; H, 7.21; N, 12.80.

*Triethylammonium (2S,5R,6R)-6-{[(tert-butylamino)-carbonothioyl]amino}penicillanate (**10e**)*

Yield 91%, m.p.: 129–130°C. FT-IR (υ_{max}, cm^{-1}): 3302 (2NH), 3173 (NH), 2927 (aliphatic C–H), 1792 (C=O), 1766 (C=O), 1506 (C=S). ^{1}H NMR (DMSO-d_6): δ (ppm) = 1.165 (t, 9H, 3CH_3, J = 7.2 Hz), 3.02 (t, 6H, 3CH_2, J = 7.2 Hz), 4.53 (d, 1H, CH, J = 4.2 Hz), 5.39 (d, 2H, 2CH, J = 4.2 Hz). ^{13}C NMR (DMSO-d_6): δ (ppm) = 8.46 (3CH_3), 26.49 (CH_3), 26.86 (CH_3), 27.63 (CH_3), 28.65 (CH_3), 33.45 (CH_3), 45.07 (3CH_2), 57.49 (C), 57.87 (CH), 62.29 (2CH), 63.08 (C), 170.61 (C=O), 172.42 (C=O), 178.45 (C=S). EI MS *m/z* (%): 431.70 ([M−1]$^+$, 28), 420.55 (10), 402.33 (54), 388.32 (25), 266.75 (35). Elemental analysis: for $C_{19}H_{36}N_4O_3S_2$ calcd. % C, 52.75; H, 8.39; N, 12.95. Found: % C, 52.68; H, 8.51; N, 12.78.

*General method for the synthesis of compounds **11a,b***

4-Chlorophenacyl bromide (10 mmol) and dried sodium acetate (200 mmol) were added to the solution of the corresponding compound **10** in absolute ethanol, and the reaction mixture was refluxed for 9 h. Then, the mixture was cooled to room temperature, poured into ice-cold water while stirring, and left overnight in cold. The formed solid was filtered, washed with water three times, and recrystallized from dimethyl formamide/diethylether (1:2) (for **11a**) or ethyl acetate (for **11b**) to afford the desired product.

*Triethylammonium (2S,5R,6R)-6-{2-[3-benzyl-5-(4-chlorobenzyl)-1,3-thiazol-2(3H)-ylidene]hydrazino}-penicillanate (**11a**)*

Yield 78%, m.p.: 95–96°C. FT-IR (υ_{max}, cm^{-1}): 3412 (2NH), 3170 (Ar–H), 2974 (aliphatic C–H), 1728 (C=O), 1659 (C=O), 1598 (C=N), 1342 (C–S). ^{1}H NMR (DMSO-d_6): δ (ppm) = 0.88–0.95 (m, 7H, $2CH_2 + CH_3$), 1.13 (brs, 3H, CH_3), 1.27–1.35 (m, 5H, $CH_2 + CH_3$), 1.76 (brs, 6H, $2CH_3$), 2.40–2.56 (m, 4H, 4CH), 4.88 (s, 2H, CH_2), 7.22–7.34 (m, 5H, Ar–H). ^{13}C NMR (DMSO-d_6): δ (ppm) = 15.08 ($2CH_3$), 44.71 ($3CH_3$), 53.03 ($4CH_2$), 55.15 (C), 67.71 (CH), 72.04 (2CH), 116.21 (C), arC: [126.99 (3CH), 128.59 (CH), 130.19 (2CH), 130.57 (2CH), 131.01(CH), 131.97 (C), 135.67 (C), 137.03 (CH), 138.30 (C)], 159.40 (N=C), 176.56 (C=O), 178.41 (C=O). EI MS *m/z* (%): 634.70 ($[M+H_2O]^+$, 25), 616.55 ($[M]^+$, 18), 489.33 (51), 258.32 (100), 241.75 (35). Elemental analysis: for $C_{30}H_{38}ClN_5O_3S_2$ calcd. % C, 58.47; H, 6.22; N, 11.36. Found: % C, 58.40; H, 6.35; N, 11.36.

*Triethylammonium (2S,5R,6R)-6-{2-[3-benzyl-5-(4-chlorobenzyl)-1,3-oxazol-2(3H)-ylidene]hydrazino}-penicillanate (**11b**)*

Yield 85%, m.p.: 110–111°C. FT-IR (υ_{max}, cm^{-1}): 3381 (2NH), 3170 (aromatic C–H), 2979 (aliphatic C–H), 1763 (C=O), 1634 (C=O), 1279 (C–O). ^{1}H NMR (DMSO-d_6): δ (ppm) = 1.12–1.68 (m, 21H, $5CH_3 + 3CH_2$), 3.82–4.18 (m, 6H, $CH_2 + 4CH$), 7.24 (brs, 9H, Ar–H). ^{13}C NMR (DMSO-d_6): δ (ppm) = 14.79 ($2CH_3$), 25.11 ($3CH_3$), 43.49 (CH_2), 51.18 (C), 63.85 (CH), 73.52 (2CH), 118.14 (C), 127.03 (CH), 127.20 (CH), 127.61 (CH), 127.93 (CH), 128.75 (CH), 128.85 (CH), 128.96 (CH), 129.08 (2CH), 130.92 (C), 134.87 (C), 141.86 (CH), 156.47 (C), 158.82 (N=C), 173.36 (C=O), 175.75 (C=O). EI MS *m/z* (%): 600.15 ($[M]^+$, 78), 586.33 (31), 326.52 (41), 245.32 (100). Elemental analysis: for $C_{30}H_{38}ClN_5O_4S$ calcd. % C, 60.04; H, 6.38; N, 11.67. Found: % C, 59.96; H, 6.36; N, 11.59.

*General method for the synthesis of compounds **12a**,**b***

Ethyl bromoacetate was added to the solution of the corresponding compound **10** in absolute ethanol (10 mmol) and the mixture was refluxed in the presence of dried sodium acetate (16.4 g, 200 mmol) for 20 h. Then, the mixture was cooled to room temperature, poured into ice-cold water while stirring and left overnight in cold. The solid formed was filtered, washed with water three times, and recrystallized from acetone.

*Triethylammonium (2S,5R,6R)-6-[(2-(3-benzyl)-5-oxo-1,3-thiazolidin-2-ylidene)hydrazino]penicillanate (**12a**)*

Yield 95%, m.p.: 100–101°C. FT-IR (υ_{max}, cm^{-1}): 3379 (NH), 3342 (NH), 3088, 3065, 3032 (aromatic C–H), 2970 (aliphatic C–H), 1713 (3C=O), 1535 (C=N), 1327 (C–S). ^{1}H NMR (DMSO-d_6): δ (ppm) = 0.93–1.28 (m, 15H, $5CH_3$), 1.56–1.86 (m, 6H, $3CH_2$), 2.56 (s, 4H, $2CH_2$), 4.34 (brs, 3H, 3CH), 7.22 (brs, 5H, Ar–H). ^{13}C NMR (DMSO-d_6): δ (ppm) = 12.21 ($2CH_3$), 30.63 (CH_2), 46.91 (CH_2), 47.21 ($3CH_3$), 52.21 (CH), 65.71 (CH), 72.05 (CH), arC: [127.21 (2CH), 128.61 (CH), 129.00 (2CH), 133.21 (C), 150.33 (C)], 167.03 (C=O), 170.51 (C=O), 176.21 (C=O). EI MS *m/z* (%): 523.63 ($[M+2]^+$, 41), 407.56 (74), 395.26 (32), 383.56 (82), 235.87 (100). Elemental analysis: for $C_{24}H_{35}N_5O_4S_2$ calcd. % C, 52.25; H, 6.76; N, 13.42. Found: % C, 52.24; H, 6.71; N, 13.49.

*Triethylammonium (2S,5R,6R)-6-[2-(3-benzyl)-5-oxo-1,3-oxazolidin-2-ylidene)hydrazino]penicillanate (**12b**)*

Yield 91%, m.p.: 225°C. FT-IR (υ_{max}, cm^{-1}): 3405 (2NH), 3170 (aromatic C–H), 2979 (aliphatic C–H), 1719 (C=O), 1634 (2C=O), 1548 (C=N), 1279 (C–O). ^{1}H NMR (DMSO-d_6): δ (ppm) = 2.17–2.37 (m, 21H, $3CH_2 + 5CH_3$), 3.90–4.01 (m, 7H, $2CH_2 + 3CH$), 7.65 (brs, 5H, Ar–H). ^{13}C NMR (DMSO-d_6): δ (ppm) = 13.15 (CH_3), 13.29 (CH_3), 44.32 ($3CH_3$), 51.69 ($2CH_2$), 54.71 ($2CH_2$), 55.67 (CH_2), 56.35 (CH), 65.41 (CH), 72.61 (CH), 117.15 (C), arC: [125.79 (3CH), 129.54 (CH), 130.89 (CH), 133.97 (C)], 161.21 (C=N), 174.64 (C=O), 176.45 (2C=O)]. EI MS *m/z* (%): 505.63 ($[M]^+$, 19), 487.58 ($[M-H_2O]^+$, 54), 389.65 (89), 234.27 (100). Elemental analysis: for $C_{24}H_{35}N_5O_5S$ calcd. % C, 57.01; H, 6.98; N, 13.85. Found: % C, 57.25; H, 6.99; N, 13.84.

*(2S,5R,6R)-6-[Bis(4-nitrobenzoyl)amino]penicillanic acid (**13**)*

The solution of K_2CO_3 (10 mmol) in 12 mL of acetone and 14 mL of water was added to the suspension of 6-apa (10 mmol) in 10 mL of acetone at −5°C and the mixture was stirred for 10 min. The solution of 4-nitrobenzoyl chloride (10 mmol) in 10 mL of acetone was added into it dropwise in 3 h. Then, temperature was allowed to reach to room temperature and the stirring was continued for an additional 1 h. Ethyl acetate was added and the reaction mixture was acidified to pH 3 with 37% HCl. The mixture was extracted with petroleum ether; the organic layer was separated and dried over Na_2SO_4. After evaporating the solvent under reduced pressure, a solid was obtained. The crude product was recrystallized from chloroform. Yield 33%, m.p.: 165–166°C. FT-IR (υ_{max}, cm^{-1}): 3163 (aromatic C–H), 2952 (aliphatic C–H), 1727 (C=O), 1655 (C=O), 1623 (C=O), 1475 and 1335 (NO_2), 1233(C–O). ^{1}H NMR (DMSO-d_6): δ (ppm) = 1.43 (brs, 6H, $2CH_3$), 4.26 (s, 1H, CH), 4.35 (s, 1H, CH), 5.47 (s, 1H, CH), 8.13 (d, 4H, H-2 and H-6, J = 8.6 Hz), 8.29 (d, 4H, H-3 and H-5, J = 8.6 Hz). ^{13}C NMR (DMSO-d_6): δ (ppm) = 27.04 (CH_3), 33.90 (CH_3), 57.69 (CH), 58.38 (C), 69.37 (CH), 71.41 (CH), arC: [124.40 (2CH), 131.37 (2CH), 137.03 (2C)], 150.68 (C=O), 161.96 (C=O), 166.50 (2C), 171.00 (C=O), 172.70 (C=O). EI MS *m/z* (%): 543.36 (29), 537.61 ($[M+Na]^+$, 10), 415.47 (30), 283.19 (100), 140.08 (69). Elemental analysis: for $C_{22}H_{18}N_4O_9S$ calcd. % C, 51.36; H, 3.53; N, 10.80. Found: % C, 51.01; H, 3.28; N, 10.77.

*(2S,5R,6R)-6-[Bis(4-aminobenzoyl)amino]penicillanic acid (**14**)*

The solution of K_2CO_3 (10 mmol) in 12 mL of acetone and 14 mL of water was added to the suspension of 6-apa (10 mmol) in 10 mL of acetone at −5°C and the mixture was stirred for 10 min. The solution of 4-aminobenzoyl chloride (10 mmol) in 10 mL of acetone was added into it dropwise in 3 h. Then, temperature was allowed to reach to room temperature and stirring was continued for an additional 1 h. Ethyl acetate was added and acidified to pH 3 with 37% HCl. The mixture was extracted with petroleum ether; the organic layer was separated and dried over Na_2SO_4. After evaporating the solvent under reduced pressure, a solid was obtained. The crude product was recrystallized from chloroform. Yield 55%, m.p.: 213–214°C. FT-IR (υ_{max}, cm^{-1}): 3459 (OH), 3381 and 3361 (NH_2), 3230 (aromatic C–H), 2967 (aliphatic C–H), 1659, 1635, and 1622 (3C=O). ^{1}H NMR (DMSO-d_6): δ (ppm) = 1.45 (brs, 6H, $2CH_3$), 4.28 (s, 1H, CH), 4,37 (s, 1H, CH), 5.55 (s, 1H, CH), 6.53 (d, 2H, NH_2, J = 8.2 Hz, exch. D_2O), 7.60 (d, 2H, NH_2, J = 7.0 Hz,

exch. D_2O), 8.10 (d, 4H, Ar–H, $J = 7.0$ Hz), 8.31 (d, 4H, Ar–H, $J = 6.0$ Hz). ^{13}C NMR (DMSO-d_6): δ (ppm) = 26.30 (CH_3), 34.50 (CH_3), 57.70 (CH), 58.38 (C), 71.41 (CH), 113.21 (CH), arC: [124.43 (4CH), 131.40 (4CH), 137.11 (C), 150.70 (C), 153.84 (C), 161.96 (C)], 166.52 (C=O), 168.19 (C=O), 171.01 (C=O), 172.71 (C=O). EI MS *m/z* (%): 575.39 (15), 519.05 ([M+2+K+Na]$^+$, 34), 443.32 (19), 399.29 (11), 305.14 (10), 242.27 (34), 212.11 (100), 108.07 (27). Elemental analysis: for $C_{22}H_{22}N_4O_5S$ calcd. % C, 58.14; H, 4.88; N, 12.33. Found: % C, 58.14; H, 4.81; N, 12.36.

*(2S,5R,6R)-6-(Bis{4-[(anilinocarbonothioyl)amino]-benzoyl}amino)penicillanic acid (**15**)*

The solution of compound **14** (10 mmol) in absolute ethanol was refluxed in the presence of phenylisothiocyanate (20 mmol) for 22 h. After evaporating the solvent under reduced pressure, a solid was obtained. This was recrystallized from ethyl acetate to afford the desired product. Yield 93%, m.p.: 149–150°C. FT-IR (υ_{max}, cm^{-1}): 3460, 3362, and 3207 (4NH), 3112 (aromatic C–H), 2985 (aliphatic C–H), 1686 and 1623 (C=O), 1311 and 1289 (C=S). ^{1}H NMR (DMSO-d_6): δ (ppm) = 1.22–1.32 (m, 6H, $2CH_3$), 4.48 (d, 2H, CH, $J = 6.6$ Hz), 6.55 (d, 1H, CH, $J = 8.2$ Hz), 7.12 (d, 2H, Ar–H, $J = 3.2$ Hz), 7.31 (d, 7H, Ar–H, $J = 6.6$ Hz), 7.43 (d, 3H, Ar–H, $J = 7.4$ Hz), 7.59–7.65 (m, 4H, Ar–H), 7.88 (d, 2H, Ar–H, $J = 8.2$ Hz), 9.70 (s, 1H, NH, D_2O exch.), 10.95 (s, 1H, NH, exch. D_2O), 11.24 (s, 1H, NH, D_2O exch.). ^{13}C NMR (DMSO-d_6): δ (ppm) = 13.91($2CH_3$), 112.50 (3CH), 116.80 (C), arC: [121.77 (CH), 121.97 (CH), 123.60 (2CH), 123.64 (2CH), 124.37 (CH), 124.65 (CH), 125.62 (C), 128.39 (CH), 128.48 (CH), 128.75 (CH), 128.96 (CH), 129.84 (2CH), 130.08 (CH), 131.17 (3CH), 139.18 (C), 139.41 (2C), 143.77 (C), 153.11 (C)], 166.74 (C=O), 166.91 (C=O), 167.45 (C=O), 179.32 (C=O), 179.56 (C=S), 187.49 (C=S). EI MS *m/z* (%): 531.38 ([(M+1)–PhN_2CH_2Ph]$^+$, 15), 461.31 (33), 399.29 (15), 242.26 (78), 212.10 (100). Elemental analysis: for $C_{37}H_{33}N_5O_5S_3$ calcd. % C, 61.39; H, 4.59; N, 9.67. Found: % C, 61.03; H, 4.51; N, 9.32.

*(2S,5R,6R)-6-[Bis(4-{[5-(4-chlorophenyl)-3-phenyl-1,3-thiazol-2(3H)-ylidene]amino}benzoyl)amino]penicillanic acid (**16**)*

A mixture of compound **15** (10 mmol) and 4-chlorophenacylbromide (20 mmol) in absolute ethanol was refluxed in the presence of dried sodium acetate (50 mmol) for 8 h. The reaction content was then cooled to room temperature and the salt was separated by filtration. After the solvent was evaporated under reduced pressure, the solid formed was recrystallized from ethyl acetate. Yield 45%, m.p.: 119–120°C. FT-IR (υ_{max}, cm^{-1}): 3065 (aromatic C–H), 2951 (aliphatic C–H), 1727, 1673, 1632, and 1606 (C=O), 1510 and 1467 (C=N). ^{1}H NMR (DMSO-d_6): δ (ppm) = 1.06 (s, 3H, CH_3), 1.32 (s, 3H, CH_3), 1.84 (s, 1H, CH), 2.09 (s, 1H, CH), 2.50 (s, 1H, CH + DMSO-d_6), 6.52 (s, 2H, Ar–H), 6.92 (s, 3H, Ar–H,), 7.02 (brs, 2H, Ar–H), 7.18 (brs, 4H, Ar–H), 7.30–7.40 (m, 15H, Ar–H), 9.63 (s, 2H, Ar–H). ^{13}C NMR (DMSO-d_6): δ (ppm) = 22.53 ($2CH_3$), 55.98 (C), 98.44 (CH), 98.58 (2CH), arC: [111.41 (2CH), 117.59 (2CH), 119.75 (2CH), 120.90 (2CH), 122.96 (2CH), 127.70 (2CH), 128.27 (2CH), 128.87 (2CH), 128.99 (2CH), 129.50 (2CH), 129.87 (2CH), 129.93 (2C), 130.00 (2CH), 130.62 (2CH), 130.98 (2CH), 133.04 (2C), 137.61 (2C), 137.86 (2C), 151.27 (2C), 152.44 (2C)], 159.00 (2C=N), 159.17 (2C=O), 169.42 (C=O). EI MS *m/z* (%): 883.45 ([M]–PhCl]$^+$, 13), 728.32 ([M]–PhCl–COOH]$^+$, 11), 707.38 (25), 663.36 (41), 567.04 (34), 501.08 (100). Elemental analysis: for $C_{52}H_{38}Cl_2N_6O_5S_3$ calcd. % C, 62.83; H, 3.85; N, 8.45. Found: % C, 62.74; H, 3.81; N, 8.49.

*(2S,5R,6R)-6-[Bis(4-{[4-oxo-3-phenyl-1,3-thiazolidin-2-ylidene]amino}benzoyl)amino]penicillanic acid (**17**)*

A mixture of compound **15** (10 mmol), dried sodium acetate (50 mmol), and ethylbromoacetate (20 mmol) in absolute ethanol was irradiated with CEM brand, microwave device at 200°C, 200 W, for 50 min. Then, the mixture was cooled to room temperature, poured into ice-cold water while stirring, and left overnight in cold. The formed solid was filtered and recrystallized from ethyl acetate/hexane (1:2). Yield 53%, m.p.: 181–182°C. FT-IR (υ_{max}, cm^{-1}): 3459 (OH), 3042 (aromatic C–H), 2986 (aliphatic C–H), 1727, 1675, 1637 (6C=O), 1420 (C=N). ^{1}H NMR (DMSO-d_6): δ (ppm) = 2.50 (s, 6H, $2CH_3$ + DMSO-d_6), 3.51 (s, 2H, $CH_2 + H_2O$), 4.78 (s, 3H, CH + CH_2), 6.58 (s, 2H, 2CH), 7.61–7.74 (m, 9H, Ar–H), 7.86–7.97 (m, 9H, Ar–H), 10.11 (s, 1H, OH, D_2O exch.). ^{13}C NMR (DMSO-d_6): δ (ppm) = 14.79 (CH_3), 15.09 (CH_3), 44.82 ($2CH_2$), 61.13 (C), 61.29 (CH), 62.44 (CH), 63.52 (CH), arC: [111.83 (2CH), 113.35 (CH), 117.93 (CH), 118.12 (2CH), 118.83 (CH), 119.03 (CH), 122.84 (2C), 131.12 (2CH), 131.31 (2CH), 131.48 (2CH), 131.69 (2CH), 131.87 (CH), 132.09 (CH), 144.99 (2C), 152.68 (2C)], 154.01 (2C=N), 165.61 (2C=O), 168.10 (2C=O), 168.56 (2C=O). EI MS *m/z* (%): 760.99 ([M]–COOH]$^+$, 11), 686.83 ([M+2]–C_6H_5]$^+$, 15), 410.96 (25), 331.86 (81), 317.86 (89), 260.81 (100). Elemental analysis: for $C_{54}H_{42}Cl_2N_6O_5S_3$ calcd. % C, 63.46; H, 4.14; N, 8.22. Found: % C, 63.44; H, 4.21; N, 8.12.

*General method for the synthesis of compounds **18a,b***

6-Apa (10 mmol) was added to the solution of K_2CO_3 (10 mmol) in water (8 mL) and acetone (6 mL) at −5°C and the mixture was stirred for 10 min. Then, the solution of the corresponding compound **19** in acetone (10 mL) was added in a period for 3 h. Temperature was allowed to reach to room temperature and the mixture was stirred for 13 h. 12 mL of water was added and stirred for an additional 1 h. The reaction content was acidified to pH 3 with diluted HCl and extracted with ethyl acetate (3 × 10 mL). Organic phase was dried on Na_2SO_4 and evaporated under reduced pressure. The obtained crude product was recrystallized from petroleum ether to afford the desired compound.

*(2S,5R,6R)-6-[Bis(4-{[(benzylamino)carbonothioyl]-amino}benzoyl)amino]penicillanic acid (**18a**)*

Yield 43%, m.p.: 184–185°C. FT-IR (υ_{max}, cm^{-1}): 3317, 3250, 3180 (4NH), 3064 (aromatic C–H), 2921 (aliphatic C–H), 1668 (4C=O), 1297, 1263 (2C=S). ^{1}H NMR (DMSO-d_6): δ (ppm) = 1.47 (s, 3H, CH_3), 1.94 (s, 3H, CH_3), 4.39 (s, 1H, CH), 4.73 (d, 4H, $2CH_2 + 2CH$, $J = 8.0$ Hz), 7.20–7.36 (m, 18H, Ar–H), 8.62 (brs, 2H, NH exch. D_2O), 9.49 (brs, 2H, NH exch. D_2O). ^{13}C NMR (DMSO-d_6): δ (ppm) = 26.35 (CH_3), 33.22 (CH_3), 46.90 (CH_2), 47.46 (CH_2), 51.42 (C), 57.08 (CH), 68.72 (CH), 70.73 (CH), arC: [126.52 (3CH), 126.83 (CH), 127.22 (5CH), 127.45 (CH), 128.10 (5CH), 128.39 (3CH), 139.07 (5C), 139.36 (C), 153.11 (C)], 159.62 (C=O), 161.23 (C=O), 170.28 (C=O), 170.62 (C=O), 171.99 (C=O), 182.62 (C=O). EI MS *m/z* (%): 752.95 ([M]$^+$, 21), 427.06 (13), 395.12 (41), 321.11 (73), 297.05 (100), 148.07 (66), 102.09 (89). Elemental analysis: for $C_{39}H_{37}N_5O_5S_3$ calcd. % C, 62.29; H, 4.96; N, 9.31. Found: % C, 62.57; H, 5.13; N, 9.69.

*(2S,5R,6R)-6-[Bis(4-{[(benzylamino)carbonyl]-amino}benzoyl)amino]penicillanic acid (**18b**)*

Yield 22%, m.p.: 218–219. FT-IR (υ_{max}, cm^{-1}): 3287 (4NH), 3064 (aromatic C–H), 2931 (aliphatic C–H), 1661 (3C=O), 1637 (3C=O). ^{1}H NMR (DMSO-d_6): δ (ppm) = 2.08 (s, 3H, CH_3), 2.50 (s, 3H, CH_3),

4.25 (s,1H, CH), 4.15 (d, 4H, $2CH_2$, $J = 5.9$ Hz), 6.85 (s, 1H, CH), 6.98 (s, 1H, CH), 7.21–7.36 (m, 10H, Ar–H), 7.51 (t, 4H, Ar–H, $J = 8.6$ Hz), 7.83 (t, 4H, Ar–H, $J = 8.6$ Hz), 8.94 (s, H, NH exch. D_2O), 9.03 (s, 2H, 2NH, exch. D_2O), 10.00 (s, 1H, NH, exch. D_2O), 12.57 (brs, H, OH, exch. D_2O). ^{13}C NMR (DMSO-d_6): δ (ppm) = 29.00 ($2CH_3$), 42.70 ($2CH_2$), 50.03 (C), 52.08 (CH), 65.72 (CH), 68.73 (CH), arC: [116.63 (3CH), 122.88 (C), 124.90 (C), 126.41 (2CH), 126.73 (4CH), 126.89 (2CH), 127.11 (3CH), 128.02 (2CH), 130.44 (2CH), 140.05 (C), 140.10 (2C), 144.72 (C)], 158.63 (2C=O), 158.74 (2C=O), 166.02 (C=S), 167.09 (C=S). EI MS *m/z* (%): 737.39 ($[M-1+H_2O]^+$, 13), 721.39 ($[M+1]^+$, 22), 707.38 (25), 663.36 (32), 619.34 (39), 567.04 (100), 531.29 (48). Elemental analysis: for $C_{38}H_{36}N_6O_7S$ calcd. % C, 63.32; H, 5.03; N, 11.66. Found: % C, 63.30; H, 5.01; N, 11.59.

*General method for the synthesis of compounds **19a,b***

Method 1: The solution of 4-aminobenzoyl chloride (10 mmol) in ethanol was refluxed in the presence of benzyl iso(thio)cyanate (10 mmol) for 8 h. The solid obtained upon cooling the reaction mixture in cold was filtered off and recrystallized from ethanol to give the pure compound.

Method 2: The mixture of 4-aminobenzoyl chloride (10 mmol) and benzyl iso(thio)cyanate (10 mmol) was irradiated with CEM brand, monopod microwave device at 125°C, 150 W, for 10 min. The obtained solid was recrystallized from ethanol.

*4-{[(Benzylamino)carbonothioyl]amino}benzoyl chloride (**19a**)*

Yield 70% (for method 1), 82% (for method 2), m.p.: 206–207°C. FT-IR (υ_{max}, cm^{-1}): 3247 and 3135 (NH), 3080 (aromatic C–H), 2977 (aliphatic C–H), 1668 (C=O), 1286 (C=S). ^1H NMR (DMSO-d_6): δ (ppm) = 4.72 (s, 2H, CH_2), 7.32 (brs, 5H, Ar–H), 7.64 (d, 2H, Ar–H, $J = 8.6$ Hz), 7.86 (d, 2H, Ar–H, $J = 8.6$ Hz), 8.44 (s, 1H, NH, exch. D_2O), 9.93(s, 1H, NH exch. D_2O). ^{13}C NMR (DMSO-d_6): δ (ppm) = 47.65 (CH_2), arC: [121.78 (CH), 127.72 (2CH), 128.18 (2CH), 129.05 (2CH), 130,72 (2CH), 139.21 (C), 144.33 (C), 144.44 (C)], 167.57 (C=O), 181.03 (C=S). EI MS *m/z* (%): 305.15 ($[M+1]^+$, 15), 301.14 (20), 293.09 (15), 289.16 ($[M+2-H_2O]^+$, 20), 271.10 (100). Elemental analysis: for $C_{15}H_{13}ClN_2OS$ calcd. % C, 59.11; H, 4.30; N, 9.19. Found: % C, 59.50, H, 4.54; N, 9.44.

*4-{[(Benzylamino)carbonyl]amino}benzoyl chloride (**19b**)*

Yield 44% (method 1), 47% (method 2), m.p.: 235–236°C. FT-IR (υ_{max}, cm^{-1}): 3285 and 3221 (NH), 3088 (aromatic C–H), 2929 (aliphatic C–H), 1786 and 1655 (C=O). ^1H NMR (DMSO-d_6): δ (ppm) = 4.29 (brs, 2H, CH_2), 7.29 (brs, 5H, Ar–H), 7.49 (d, 2H, Ar–H, $J = 8.2$ Hz), 7.80 (d, 2H, Ar–H, $J = 8.4$ Hz), 7.87 (s, 1H, NH, exch. D_2O), 8.98 (s, 1H, NH, exch. D_2O). ^{13}C NMR (DMSO-d_6): δ (ppm) = 43.25 (CH_2), arC: [117.43 (2CH), 123.53 (C), 127.55 (2CH), 127.79 (2CH), 129.06(CH), 131.21 (2CH), 140.57 (C), 145.20 (C)], 155.50 (C=O), 167.84 (C=O). EI MS *m/z* (%): 349.11 (19), 329.16 ($[M+2+K]^+$, 19), 321.11 (90), 315.01 (71), 305.09 ($[M-1+H_2O]^+$, 14), 301.08 (33), 293.03 (43), 271.05 ($[M+1-H_2O]^+$, 100). Elemental analysis: for $C_{15}H_{13}ClN_2O_2$ calcd. % C, 62.40; H, 4.54; N, 9.70. Found: % C, 62.78; H, 4.58; N, 9.94.

*General method for the synthesis of compounds **20a,b***

Ethyl bromoacetate was added to the solution of the corresponding compound **19** in absolute ethanol (10 mmol) and the mixture was refluxed in the presence of dried sodium acetate (200 mmol) for 14 h. Then, the mixture was cooled to room temperature, poured into ice-cold water while stirring, and left overnight in cold. The solid formed was filtered, washed with water three times, and recrystallized from ethyl acetate.

*4-{[3-Benzyl-4-oxo-1,3-thiazolidin-2-ylidene]amino}-benzoyl chloride (**20a**)*

Yield 65%, m.p.: 189–200°C. FT-IR (υ_{max}, cm^{-1}): 3065 (aromatic C–H), 2964 (aliphatic C–H), 1712 (C=O), 1674 (C=O), 1510 (C=N). ^1H NMR (DMSO-d_6): δ (ppm) = 4.10 (s, 2H, CH_2), 4.91 (s, 2H, CH_2), 6.76 (d, 2H, Ar–H, $J = 7.8$ Hz), 7.34 (s, 5H, Ar–H), 7.81 (d, 2H, Ar–H, $J = 8.2$ Hz. ^{13}C NMR (DMSO-d_6): δ (ppm) = 32.24 (CH_2), 33.03 (CH_2), 127.05 (CH), 127.43 (CH), 127.61 (CH), 127.89 (CH), 128.14 (CH), 128.26 (CH), 128.30 (CH), 128.32 (2CH), 135.02 (C), 136.44 (C), 139.05 (C), 171.74 (C=N), 171.98 (C=O), 178.49 (C=O). EI MS *m/z* (%): 347.13 (19), 344.52 ($[M]^+$, 19), 311.19 (13), 251.63 (100). Elemental analysis: for $C_{17}H_{13}ClN_2O_2S$ calcd. % C, 59.21; H, 3.80; N, 8.12. Found: % C, 59.24; H, 3.80; N, 8.10.

*4-{[3-Benzyl-4-oxo-1,3-oxazolidin-2-ylidene]amino}-benzoyl chloride (**20b**)*

Yield 67%, m.p.: 240–241°C. FT-IR (υ_{max}, cm^{-1}): 3086 (aromatic C–H), 2929 (aliphatic C–H), 1756 (C=O), 1656 (C=O), 1547 (C=N). ^1H NMR (DMSO-d_6): δ (ppm) = 4.22 (d, 4H, $2CH_2$, $J = 6.1$ Hz), 7.20 (brs, 2H, Ar–H), 7.28 (s, 5H, Ar–H), 7.78 (s, 2H, Ar–H). ^{13}C NMR (DMSO-d_6): δ (ppm) = 40.09 ($2CH_2$), arC: [126.41 (3CH), 126.94 (3CH), 128.02 (3CH), 140.57 (C=N), 158.74 (2C=O). EI MS *m/z* (%): 328.71 ($[M]^+$, 15), 321.19 (19), 259.47 (55), 209.21 (100). Elemental analysis: for $C_{17}H_{13}ClN_2O_3$ calcd. % C, 62.11; H, 3.99; N, 8.52. Found: % C, 62.01; H, 3.86; N, 8.51.

*(2S,5R,6R)-6-[Bis(4-{[3-benzyl-4-oxo-1,3-thiazolidin-2-ylidene]amino}benzoyl)amino]penicillanic acid (**21**)*

The solution of K_2CO_3 (10 mmol) in 3 mL of DMF and 14 mL of water was added to the suspension of 6-apa (10 mmol) in 3 mL of DMF at −5°C and the mixture was stirred for 10 min. The solution of **20a** (10 mmol) in 3 mL of DMF was added into it dropwise in 3 h and the mixture was stirred for 12 h. Then, temperature was allowed to reach to room temperature and stirring was continued for an additional 1 h. Ethyl acetate was added into it and acidified to pH 3 with 37% HCl. The mixture was extracted with ethylacetate; the organic layer was separated and dried over Na_2SO_4. The solvent was removed under reduce pressure and a solid was obtained. The crude product was recrystallized from ethyl acetate/*n*-hexane (1:2). Yield 53%, m.p.: 181–182°C. FT-IR (υ_{max}, cm^{-1}): 3459 (OH), 3068 (aromatic C–H), 2986 (aliphatic C–H), 1727, 1675, 1637 (6C=O), 1420 (C=N). ^1H NMR (DMSO-d_6): δ (ppm) = 1.19 (s, 3H, CH_3), 1.23 (s, 3H, CH_3), 3.51 (s, 2H, CH_2), 4.18 (s, 5H, $2CH_2 + CH$), 4.93 (s, 4H, $2CH + CH_2$), 6.97 (d, 4H, Ar–H, $J = 8.4$ Hz), 7.91 (d, 10H, Ar–H, $J = 8.4$ Hz), 7.99 (d, 1H, Ar–H, $J = 8.4$ Hz), 12.95 (s, 1H, OH, D_2O exch.). ^{13}C NMR (DMSO-d_6): δ (ppm) = 26.13 (CH_3), 30.51 (CH_3), 31.22 ($2CH_2$), 39.15 ($2CH_2$), 59.29 (C), 60.23 (CH), 61.88 (CH), 67.13 (CH), 125.75 (4CH), 128.06 (4CH), 129.45 (4CH), 130.53 (4CH), 132.81 (2C), 133.14 (2C), 158.01 (2C), 160.65 (2C=N), 166.54 (C=O), 171.27 (C=O), 171.53 (C=O), 171.77 (C=O). EI MS *m/z* (%): 833.70 ($[M]^+$, 22), 818.71 ($[M-CH_3]^+$, 15). Elemental analysis: for $C_{40}H_{32}N_6O_7S_3$ calcd. % C, 59.69; H, 4.01; N, 10.44. Found: % C, 59.51; H, 3.89; N, 10.61.

*(2S,5R,6R)-6-[(4-{[3-Benzyl-4-oxo-1,3-thiazolidin-2-ylidene]amino}benzoyl)amino]penicillanic acid (**22a**)*

The solution of K_2CO_3 (10 mmol) in 12 mL of acetone and 14 mL of water was added to the suspension of 6-apa (10 mmol) in 10 mL of acetone at −5°C and the mixture was stirred for 10 min. The solution of **20a** (10 mmol) in 10 mL of ethanol was added into it dropwise in 3 h and stirred at room temperature for 30 h. Ethyl acetate was added into it and acidified to pH 3 with 37% HCl. The mixture was extracted with petroleum ether; the organic layer was separated and dried over $NaSO_4$. The solvent was removed under reduce pressure and a solid was obtained. The crude product was recrystallized from ethyl acetate. Yield 57%, m.p.: 179–180°C. FT-IR (υ_{max}, cm^{-1}): 3456 (OH), 3168 (aromatic C–H), 2667 (aliphatic C–H), 1727, 1676, 1638 (4C=O), 1427 (C=N). ^{1}H NMR (DMSO-d_6): δ (ppm) = 1.20 (s, 3H, CH_3), 1.42 (s, 3H, CH_3), 3.52 (brs, 4H, $2CH_2 + H_2O$) 4.01 (s, 1H, CH), 4.96 (s, 2H, 2CH), 7.21–7.50 (m, 9H, Ar–H), 8,78 (s, 1H, NH). ^{13}C NMR (DMSO-d_6): δ (ppm) = 24.13 (CH_3), 29.51 (CH_3), 32.22 (CH_2), 39.65 (C), 45.15 (CH_2), 55.29 (CH), 58.88 (CH), 67.13 (CH), 126.75 (2CH), 127.06 (CH), 127.45 (CH), 127.53 (CH), 127.82 (CH), 127.95 (CH), 128.05 (CH), 128.16 (CH), 135.81 (C), 139.14 (C), 158.01 (C), 169.54 (C=O), 169.89 (C=N), 170.27 (C=O), 171.53 (C=O), 171.77 (C=O). EI MS *m/z* (%): 524.13 ($[M]^+$, 11), 321.08 (10), 245.47 (35), 206.51 (100). Elemental analysis: for $C_{25}H_{24}N_4O_6S$ calcd. % C, 57.24; H, 4.61; N, 10.68. Found: % C, 57.13; H, 4.61; N, 11.01.

*(2S,5R,6R)-6-[(4-{[3-Benzyl-4-oxo-1,3-oxazolidin-2-ylidene]amino}benzoyl)amino]penicillanic acid (**22b**)*

The solution of K_2CO_3 (10 mmol) in 3 mL of acetone and 14 mL of water was added to the suspension of 6-apa (10 mmol) in 3 mL of acetone at −5°C and the mixture was stirred for 10 min. **20b** (10 mmol) in 3 mL of acetone was added and the reaction mixture was irradiated under reflux with CEM brand, monopod microwave device at 150°C, 150 W, for 90 min. Then, temperature was allowed to reach room temperature and stirring was continued for an additional 1 h. Ethyl acetate was added and acidified to pH 3 with 37% HCl. The mixture was extracted with ethyl acetate; the organic layer was separated and dried over $NaSO_4$. The solvent was removed under reduce pressure and a solid was obtained. The crude product was recrystallized from methanol. Yield 57%, m.p.: 184–185°C. FT-IR (υ_{max}, cm^{-1}): 3286 (NH), 3030 (aromatic C-H), 2958 (aliphatic C-H), 1714 (2C=O), 1664 (2C=O), 1413 (C=N). ^{1}H NMR (DMSO-d_6): δ (ppm) = 1.18–1.54 (m, 6H, $2CH_3$), 1.82 (s, 4H, $2CH_2$), 3.99 (s, 1H, CH), 4.21 (s, 1H, CH), 4.38 (s, 1H, CH), 7.26 (brs, 9H, Ar–H), 9.23 (s, 1H, NH). ^{13}C NMR (DMSO-d_6): δ (ppm) = 25.73 (CH_3), 29.91 (CH_3), 33.26 (CH_2), 39.54 (C), 41.15 (CH_2), 59.26 (CH), 62.18 (CH), 67.23 (CH), 125.75 (2CH), 126.54 (CH), 128.45 (2CH), 129.53 (2CH), 129.98 (2CH), 135.11 (C), 139.14 (C), 158.01 (C), 162.23 (C=O), 169.54 (C=N), 170.29 (C=O), 171.58 (C=O), 172.89 (C=O). EI MS *m/z* (%): 526.35 ($[M+H_2O]^+$, 61), 451.67 (94), 421.30 (49), 243.49 (100). Elemental analysis: for $C_{25}H_{24}N_4O_6S$ calcd. % C, 59.04; H, 4.76; N, 11.02. Found: % C, 59.13; H, 4.78; N, 11.02.

4-{[3-Benzyl-5-(4-chlorophenyl)-1,3-thiazol-2(3*H*)-ylidene]amino}-benzoylchloride (**23a**). 4-Chlorophenacyl bromide (10 mmol) and dried sodium acetate (200 mmol) were added to the solution of the corresponding compound **19a** in absolute ethanol, and the reaction mixture was irradiated with CEM brand, microwave device at 125°C, 150 W, for 60 min. Then, the mixture was cooled to room temperature, after the solvent was evaporated under reduced pressure, poured into ice-cold water while stirring, and left overnight in cold. The formed solid was filtered and recrystallized from ethyl acetate. Yield 85%, m.p.: 240–241°C. FT-IR (υ_{max}, cm^{-1}): 3102 (aromatic C–H), 2668 (aliphatic C–H), 1674 (C=O), 1490 (C=N). ^{1}H NMR (DMSO-d_6): δ (ppm) = 5.08 (s, 2H, CH_2), 6.42 (s, 1H, CH), 6.98–7.23 (m, 4H, Ar–H), 7.26–7.48 (m, 5H, Ar–H), 7.46 (d, 2H, Ar–H, J = 8.2 Hz), 7.89 (d, 2H, Ar–H, J = 9.8 Hz). ^{13}C NMR (DMSO-d_6): δ (ppm) = 50.74 (CH_2), 100.93 (CH), 123.83 (CH), 127.60 (C), 129.26 (CH), 130.04 (CH), 130.40 (CH), 130.74 (CH), 130.90 (CH), 131.37 (CH), 131.98 (CH), 132.40 (CH), 132.63 (CH), 133.45 (CH), 134.00 (CH), 134.15 (CH), 137.01 (C), 139.72 (2C), 141.46 (C), 157.50 (C), 162.54 (C=N), 169.97 (C=O). EI MS *m/z* (%): 440.35 ($[M+1]^+$, 41), 351.48 (34), 421.35 ($[M-H_2O]^+$, 71), 245.49 (100). Elemental analysis: for $C_{23}H_{16}Cl_2N_2OS$ calcd. % C, 62.88; H, 3.67; N, 6.38. Found: % C, 62.82; H, 3.68; N, 8.39.

4-{[3-Benzyl-5-(4-chlorophenyl)-1,3-oxazol-2(3H)-ylidene]amino}-benzoyl chloride (**23b**). 4-Chlorophenacyl bromide (10 mmol) and dried sodium acetate (200 mmol) were added to the solution of compound **19b** in absolute ethanol, and the reaction mixture was refluxed for 14 h. Then, the mixture was cooled to room temperature, after the solvent was evaporated under reduced pressure, poured into ice-cold water while stirring, and left overnight in cold. The formed solid was filtered and recrystallized from ethyl acetate. Yield 65%, m.p.: 247–248°C. FT-IR (υ_{max}, cm^{-1}): 3065 (aromatic C–H), 2930 (aliphatic C–H), 1656 (C=O), 1454 (C=N). ^{1}H NMR (DMSO-d_6): δ (ppm) = 4.24 (d, 3H, CH_2 + CH), 6.98 (brs, 2H, Ar–H), 7.28 (s, 9H, Ar–H), 7.78 (s, 2H, Ar–H). ^{13}C NMR (DMSO-d_6): δ (ppm) = 32.24 (CH_2), 33.03 (CH_2), 126.41 (2CH), 126.97 (3CH), 128.02 (3CH), 127.99 (3CH), 128.87 (3CH), 137.02 (2C), 138.44 (2C), 140.05 (2C), 170.84 (C=N), 178.23 (C=O). EI MS *m/z* (%): 423.13 ($[M]^+$, 51), 402.08 (11), 344.79 (89), 308.51 (100). Elemental analysis: for $C_{23}H_{16}Cl_2N_2O_2$ calcd. % C, 65.26; H, 3.81; N, 6.62. Found: % C, 65.37; H, 3.84; N, 6.63.

*General method for the synthesis of compounds **24a**,**b***

The solution of K_2CO_3 (10 mmol) in 3 mL of DMF and 14 mL of water was added to the suspension of 6-apa (10 mmol) in 3 mL of DMF at −5°C and the mixture was stirred for 10 min. The corresponding compound **23** (10 mmol) was added into the reaction mixture and stirred at room temperature for 12 h. Then, temperature was allowed to reach room temperature and stirring was continued for an additional 1 h. Ethyl acetate was added and acidified to pH 3 with 37% HCl. The mixture was extracted with ethyl acetate; the organic layer was separated and dried over $NaSO_4$. The solvent was removed under reduce pressure and a solid was obtained. The crude product was recrystallized from ethyl acetate/hexane (1:2).

*(2S,5R,6R)-6-[Bis(4-{3-benzyl-5-(4-chlorophenyl)-1,3-thiazol-2(3H)-ylidene]amino}benzoyl)amino]penicillanic acid (**24a**)*

Yield 57%, m.p.: 165–166°C. FT-IR (υ_{max}, cm^{-1}): 3061 (aromatic C–H), 2957 (aliphatic C–H), 1795 (C=O), 1730 (C=O), 1674, (2C=O), 1492 (2C=N). ^{1}H NMR (DMSO-d_6): δ (ppm) = 2.73 (s, 6H, $2CH_3$), 3.34 (s, 4H, $2CH_2 + D_2O$), 4.76 (s, 2H, 2CH), 5.12 (s, 1H, CH), 5.71 (s, 1H, CH), 6.49 (s, 1H, CH), 7.01–7.22 (m, 3H, Ar–H), 7.24–7.38 (m, 13H, Ar–H), 7.47 (brs, 1H, Ar–H), 7.67 (d, 4H, Ar–H, J = 8.2 Hz), 7.89 (d, 4H, Ar–H, J = 7.6 Hz), 7.99 (s, 1H, Ar–H). ^{13}C NMR (DMSO-d_6): δ (ppm) = 39.56 ($2CH_3$), 47.84 (CH_2), 48.76 (CH_2), 67.84 (C), 121.57 (2CH), 121.88 (2CH), 126.08 (2C), 127.75 (2CH), 127.68 (3CH), 127.82 (CH), 128.22 (2CH), 129.70 (3CH), 129.27 (2CH), 129.87

(2CH), 129.99 (CH), 130.29 (2C), 130.87 (2CH), 130.67 (2CH), 131.00 (2CH), 131.62 (CH), 131.98 (2CH), 131.04 (2CH), 134.61 (2C), 136.86 (C), 139.26 (2C), 144.44 (3C), 158.00 (N=C), 161.20 (N=C), 167.72 (2C=O), 181.16 (2C=O). EI MS *m/z* (%): 953.16 ($[M-2Cl]^+$, 13), 527.81 (12), 707.38 (25), 420.70 (100), 359.83 (48). Elemental analysis: for $C_{54}H_{42}Cl_2N_6O_5S_3$ calcd. % C, 63.46; H, 4.14; N, 8.22. Found: % C, 63.50; H, 4.13; N, 8.18.

*(2S,5R,6R)-6-[Bis(4-{[3-benzyl-5-(4-chlorophenyl)-1,3-oxazol-2(3H)-ylidene]amino}benzoyl)amino]penicillanic acid (**24b**)*

Yield 70%, m.p.: 170–171°C. FT-IR (υ_{max}, cm^{-1}): 3032 (aromatic C–H), 2919 (aliphatic C–H), 1744 (C=O), 1692 (C=O), 1494 (2C=N). ^1H NMR (DMSO-d_6): δ (ppm) = 2.53(s, 6H, $2CH_3$), 3.34 (s, 4H, $2CH_2 + D_2O$), 4.86 (s, 1H, CH), 5.89 (s, 3H, 3CH), 5.98 (s, 1H, CH), 7.21–7.25 (m, 10H, Ar–H,), 7.35 (d, 2H, Ar–H, $J = 7.2$ Hz), 7.46 (d, 2H, Ar–H, $J = 7.8$ Hz), 7.63 (d, 4H, Ar–H, $J = 8.1$ Hz), 7.72 (d, 2H, Ar–H, $J = 7.2$ Hz), 7.81 (d, 2H, Ar–H, $J = 7.8$ Hz), 7.87 (d, 4H, Ar–H, $J = 7.2$ Hz). ^{13}C NMR (DMSO-d_6): δ (ppm) = 21.08 ($2CH_3$), 43.29 (CH_2), 43.42 (CH_2), 62.79 (CH), 67.06 (C), 68.22 (CH), 88.75 (CH), 117.33 (CH), 117.53 (CH), 121.75 (C), 125.63 (C), 127.13 (2CH), 127.45 (2CH), 127.61 (2CH), 127.69 (2CH), 127.83 (2CH), 128.73 (2CH), 129.00 (2CH), 129.26 (CH), 129.73 (2CH), 129.76 (2CH), 130.39 (2CH), 130.44 (2CH), 130.81 (CH), 131.12 (CH), 131,34 (CH), 133.23 (C), 139.52 (C), 140.72 (C), 140.78 (C), 141.27 (C), 144.37 (C), 145.31 (C), 146.22 (C), 155.48 (C), 155.55 (C), 159.46 (C=N), 165.64 (C=N), 167.95 (C=O), 170.55 (C=O), 192.67 (C=O), 192.91 (C=O). EI MS *m/z* (%): 990.16 ($[M+1]^+$, 23), 890.45 (15), 767.38 (30), 401.75 (100), 270.89 (23). Elemental analysis: for $C_{54}H_{42}Cl_2N_6O_7S$ calcd. % C, 65.52; H, 4.28; N, 8.49. Found: % C, 65.50; H, 4.29; N, 8.41.

Table 1. Screening for antimicrobial activity of the compounds (50 μL).

	Microorganisms and inhibition zone (mm)								
Comp. no	**Ec**	**Yp**	**Pa**	**Sa**	**Ef**	**Bc**	**Ms**	**Ca**	**Sc**
3	8	10	10	25	10	6	-	-	-
4	–	–	–	–	–	–	–	–	–
6	–	–	–	–	–	–	–	8	9
7	–	–	–	–	–	–	–	–	–
8	–	–	6	8	10	8	20	24	–
9	–	–	6	8	6	–	10	10	6
10a	–	–	12	22	12	14	13	6	
10b	8	8	12	35	30	8	–	–	20
10c	–	–	12	13	10	10	24	10	–
10d	8	8	12	30	8	10	–	–	–
10e	20	19	12	18	20	15	–	–	–
11a	–	–	–	20	8	11	10	8	6
11b	–	–	–	8	–	–	–	–	–
12a	–	–	–	–	–	–	–	–	–
12b	–	–	–	–	–	–	–	–	–
13	8	8	10	25	–	10	15	–	–
14	–	–	10	11	8	8	–	10	25
15	8	12	10	–	8	8	–	10	25
16	10	12	10	12	10	10	20	–	
17	10	10	8	12	8	12	25	–	
18a	10	10	8	13	8	10	10	12	25
18b	–	–	6	–	–	–	–	15	25
19a	–	–	10	10	10	12	20	–	–
19b	–	–	–	–	–	–	–	–	–
20a	–	–	6	–	–	–	–	10	10
20b	–	–	10	–	–	–	–	–	–
21	10	12	12	15	8	10	20	10	10
22a	10	10	8	20	8	10	15	10	–
22b	8	8	10	25		12	18	–	–
23a	–	–	12	–	–	–	6	–	–
23b	–	–	–	–	–	–	–	–	–
24a	10	12	8	–	–	6	–	–	10
24b	8	10	10	–	–	–	–	–	–
Amp. (10 μg)	10	18	18	35	10	15			
Strep. (10 μg)							35		
Flu. (5 μg)								25	25

Ec: *Escherichia coli* ATCC 25922, Yp: *Yersinia pseudotuberculosis* ATCC 911, Pa: *Pseudomonas aeruginosa* ATCC 43288, Sa: *Staphylococcus aureus* ATCC 25923, Ef: *Enterococcus faecalis* ATCC 29212, Bc: *Bacillus cereus* 702 Roma, Ms: *M. smegmatis* ATCC607, Ca: *Candida albicans* ATCC 60193, Sc: *Saccharomyces cerevisiae* RSKK 251, Amp.: ampicillin, Strep.: streptomycin, Flu.: fluconazole, (–): no activity.

Biological activity assessment

Antimicrobial activity

All test microorganisms were obtained from the Hifzissihha Institute of Refik Saydam (Ankara, Turkey) and were as follows: *Escherichia coli* (*E. coli*) ATCC25922, *Enterobacter aerogenes* (*E. aerogenes*) ATCC13048, *Yersinia pseudotuberculosis* (*Y. pseudotuberculosis*) ATCC911, *Pseudomonas aeruginosa* (*P. aeruginosa*) ATCC43288, *Staphylococcus aureus* (*S. aureus*) ATCC25923, *Enterococcus faecalis* (*E. faecalis*) ATCC29212, *Bacillus cereus* (*B. cereus*) 702 Roma, *Mycobacterium smegmatis* (*M. smegmatis*) ATCC607, *Candida albicans* (*C. albicans*) ATCC60193, *Candida tropicalis* (*C. tropicalis*) ATCC 13803, *Aspergillus niger* (*A. niger*) RSKK 4017, and *Saccharomyces cerevisiae* (*S. cerevisiae*) RSKK 251. All the newly synthesized compounds were weighed and dissolved in dimethylsulphoxide to prepare extract stock solution of 5.000 microgram/milliliter ($\mu g\,mL^{-1}$).

Agar-well diffusion method

Screening test using agar-well diffusion method [40] as adapted earlier was used for all newly synthesized compounds. Each microorganism was suspended in Mueller Hinton (MH) (Difco, Detroit, MI) broth and diluted approximately to 10^6 colony forming units (cfu) mL^{-1}. They were "flood-inoculated" onto the surface of MH agar and Sabouraud Dextrose Agar (SDA) (Difco, Detriot, MI) and then dried. For *C. albicans* and *S. cerevisiae*, SDA were used. Five-millimeter diameter wells were cut from the agar using a sterile cork-borer, and 50 mL of the extract substances was delivered into the wells. The plates were incubated for 18 h at 35°C. Antimicrobial activity was evaluated by measuring the zone of inhibition against the test organism. Ampicillin (10 μg), streptomycin (10 μg), and fluconazole (5 μg) were standard drugs. Dimethyl sulfoxide was used as solvent control. The antimicrobial activity results are summarized in Table 1.

β-Lactamase assay

In vitro β-lactamase (*Bacillus cereus* MBLs, Sigma) activity was determined by monitoring the hydrolysis of reporter substrate Nitrocefin (Calbiochem, Darmstadt, Germany) by β-lactamase, at 486 nm (Louie et al. 2012). Enzyme assays were performed in 25 mM piperazine-*N*,*N*′-bis(2-ethane sulfonic acid) (PIPES) buffer, pH 7.0, with 100 μM $ZnSO_4$. 50 μL of 1.5 μM enzyme solution, 50 μL of 150 μM Nitrocefin, and 50 μL of 1 mM synthesized compound were recorded continuously for 20 min at 37°C against the buffer alone by using microplate reader (Multiskan GO microplate spectrophotometer, Thermo Scientific) at 486 nm. The inhibitory activities of those compounds and $HgCl_2$, a positive control against β-lactamase, were measured at various concentrations. Residual activities were calculated by comparing to control without inhibitor (T+). The assays were done in triplicate. The IC_{50} value was determined as the concentration of compound that give 50% inhibition of maximal activity [41] and results are presented in Table 2.

Table 2. Inhibitory activities of the synthesized compounds against *B. cereus* β-lactamase. All compounds and $HgCl_2$ were assayed at final concentrations of 1 mM.

Compound	Inhibition (%)	IC_{50} (mM)
10a	10	nd
10b	18	nd
10d	22	nd
$HgCl_2$	76	0.093

Urease inhibition assay

Reaction mixtures comprising 25 μL of Jack bean urease, 55 μL of buffer (100 mM urea, 0.01 M K_2HPO_4, 1 mM EDTA, and 0.01 M LiCl, pH 8.2), and 100 mM urea were incubated with 5 μL of the test compounds at room temperature for 15 min in microtiter plates. The production of ammonia was measured by indophenol method and used to determine the urease inhibitory activity. The phenol reagent (45 μL, 1% w/v phenol and 0.005% w/v sodium nitroprusside) and alkali reagent (70 μL, 0.5% w/v sodium hydroxide and 0.1% v/v NaOCl) were added to each well and the increasing absorbance at 625 nm was measured after 20 min, using a microplate reader (Molecular Device, USA). The percentage inhibition was calculated from the equation $100 - (OD_{testwell}/OD_{control}) \times 100$. Thiourea was used as the standard inhibitor. In order to calculate IC_{50} values, different concentrations of synthesized compounds and standard were assayed at the same reaction conditions [42] and the obtained results are presented in Table 3.

Table 3. Inhibitory activities of the synthesized compounds against Jack bean urease. All compounds and thiourea were assayed at final concentrations of 250 and 100 $\mu g\,mL^{-1}$.

Compound	Inhibition (%)		$IC_{50} \pm$ S.D. ($\mu g\,mL^{-1}$)
	250 $\mu g\,mL^{-1}$	100 $\mu g\,mL^{-1}$	
5	44	–[a)]	nd[b)]
7	56	–	nd
10c	100	70.0	70.95 ± 7.14
10d	98	56.0	74.31 ± 5.83
10e	100	81.8	54.14 ± 1.45
18a	20	–	nd
18b	25	–	nd
20a	15	–	nd
21	31	–	nd
24a	6	–	nd
Thiourea	100	92.2	51.62 ± 7.28

[a)] –: No inhibition.
[b)] nd: Not determined.

Table 4. Residual lipase activity and IC_{50} values of synthesized chemical compounds. All compounds and orlistat were assayed at a final concentration of 6.25 $\mu g\,mL^{-1}$.

Compound no.	Inhibition (%) (6.25 $\mu g\,mL^{-1}$)	IC_{50} ($\mu g\,mL^{-1}$)
6	66	nd[a)]
10a	4	nd
10b	62	nd
11a	89	4.85
11b	8	nd
16	67	nd
18a	6	nd
18b	100	4.18
20a	98	0.84
20b	4	nd
23b	9	nd
24a	68	nd
Orlistat	100	0.61

a) : Not determined.

Anti-lipase activity assay

The inhibitory effects of those compounds were evaluated against porcine pancreatic lipase (PPL) (15 $ng\,mL^{-1}$). Lipase activity assays were done according to Pencreac'h et al. [43]. Microtiter plates were coated with purified tung oil TAGs. Compounds were mixed with PPL 1:2 v/v and incubated for 30 min. The microtiter plates containing purified tung oil, lipase solution, and assay buffer (10 mM Tris-HCl buffer, pH 8.0, containing 150 mM NaCl, 6 mM $CaCl_2$, 1 mM EDTA, and 3 $mg\,mL^{-1}$ β-cyclodextrin) were recorded continuously for 40 min against the buffer alone by using a microplate reader (SpectraMax M5, Molecular Devices) at 272 nm. The inhibitory activity of those compounds and orlistat, a positive control against pancreatic lipase, were measured at concentrations of 6.25, 2.08, and 1.04 $\mu g\,mL^{-1}$. Residual activities were calculated by comparing to control without inhibitor (T+). The assays were done in triplicate. The IC_{50} value was determined as the concentration of compound that gives 50% inhibition of maximal activity. The results are presented in Table 4.

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