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SYNTHESIS AND ANTIBACTERIAL ACTIVITY OF NOVEL *N*-CARBOXYALKYL-*N*-PHENYL-2-AMINOTHIA(OXA)ZOLE DERIVATIVES

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Abstract – *N*-Phenyl-*N*-thiocarbamoyl-α- and β-methyl-β-alanines were converted into a series of 1,3-thiazole derivatives by treatment with chloroacetaldehyde and haloketones. The reaction of *N*-phenyl-*N*-thiocarbamoyl-β-alanines and *N*-carbamoyl-*N*-phenyl-β-alanines with 2,3-dichloro-1,4-naphthoquinone and 2,3-dichloroquinoxaline provided naphthoquinone- and quinoxaline-fused thiazoles and oxazoles, respectively. A number of the synthesized compounds exhibited good antibacterial activity against *Staphylococcus aureus* and *Salmonella enteritidis* with MIC and MBC values (62.5 and 125 μg/mL, respectively) which are the same or even lower than those for the antibiotic oxytetracycline.

INTRODUCTION

Despite the rapid progress of science, the treatment of infectious diseases still remains a serious problem and concern to the scientific community, mainly because of the wide range of factors leading to the emergence of these diseases and also the increased number of pathogenic microorganisms with resistance towards multiple drugs.

The growing resistance of microorganisms requires the careful use of existing antimicrobial drugs.

Besides, there is a need for the design of novel antimicrobial agents, particularly for the treatment of the infections.[1-5] A potential approach to this problem is the design of innovative drugs with different mechanism of action in effort to avoid cross resistance to existing therapeuticals.[6]

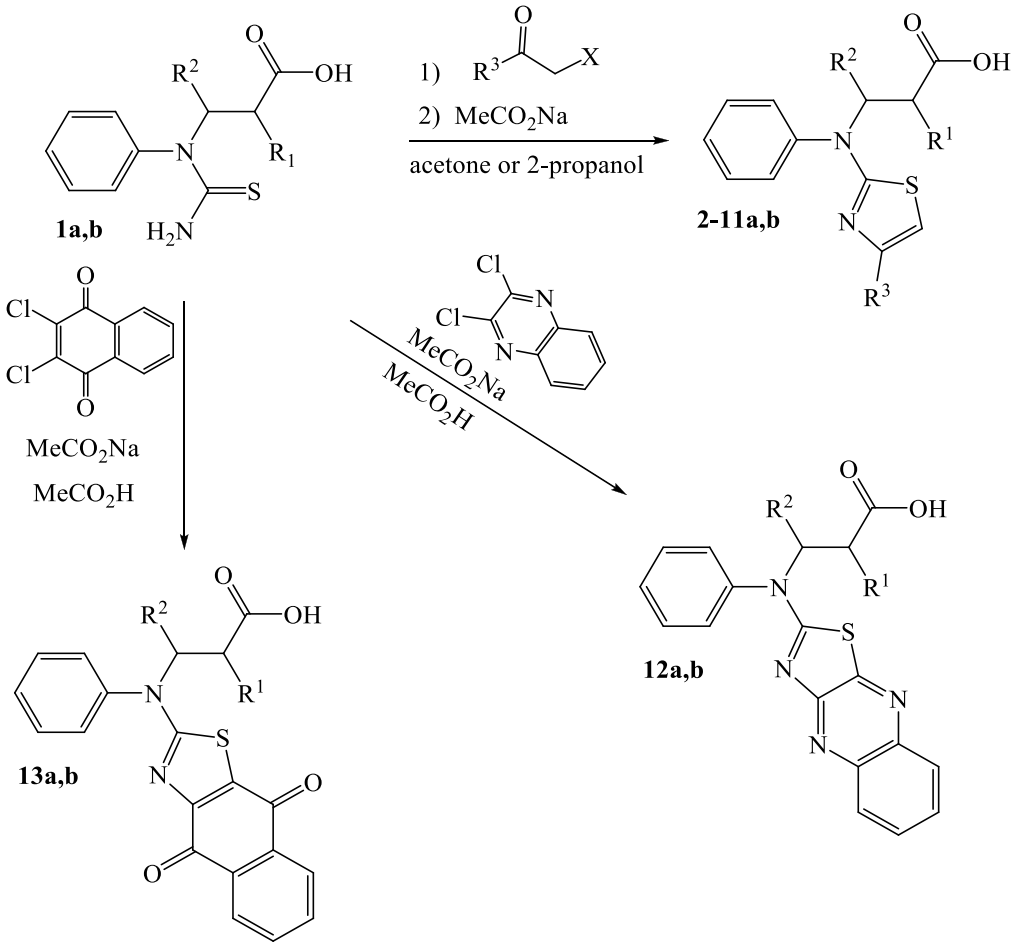
N-Substituted *β*-amino acids are used for synthesis of heterocyclic compounds such as dihydrouracils and their 2-thioanalogues,[7-10] quinolinones,[11-12] and quinazolinones.[13,14]

Heterocycles containing nitrogen and sulphur or oxygen atom constitute an important class of compounds in the field of medicinal chemistry.[15] The five-membered heterocyclic nucleus plays a vital role in many biological activities. For example, oxazoles are associated with antibacterial,[16] antifungal, antitubercular,[17] and antitumor[18] activities. Thiazoles and their derivatives are found to exhibit various biological activities such as antibacterial,[19-22] fungicidal,[23] anti-inflammatory,[24,25] antihypertensive,[26,27] anti-HIV,[28,29] antitumor,[30] and antioxidant.[31,32] Thiazole nucleus is also an integral part of all the available penicillins which have revolutionized the therapy of bacterial diseases.[33]

Recently, we have reported the application of *N*-aryl-*N*-thiocarbamoyl-*β*-alanines as excellent precursors for the synthesis of *N*-carboxyethylaminothiazoles.[21,34] Not so long ago, *N*-substituted thioureido acids were employed just for synthesis of 1-substituted thiodihydrouracils[7-9] or tetrahydropyridones.[20,35] Herein, we report the synthesis of novel aminothiazole and aminooxazole derivatives and investigation of structure activity relationship revealing the influence of the substituents in thiazole ring and aliphatic moiety on antimicrobial activity of the synthesized compounds. The data obtained enables the purposeful further synthesis of aminothiazole and aminooxazole derivatives in the search of effective antibacterial agents.

RESULTS AND DISCUSSION

One of the most convenient methods for preparation of thiazoles is Hantzsch synthesis, i.e. condensation of *α*-halocarbonyl derivatives with thioamides or thiocarbamides. Thus, *N*,*N*-disubstituted aminothiazoles **2a**,**b** were prepared by heating the corresponding *N*-phenyl-*N*-thiocarbamoyl-*α*- or *β*-methyl-*β*-alanines **1a**,**b** with chloroethanal at 80 °C for 2 h (Scheme 1). The reaction provided soluble in water amino acid hydrochlorides which were converted into insoluble bases by adding sodium acetate. When thioureido acids **1a,b** were treated with chloropropanone at reflux temperature, the reaction was completed already in 1 h. Thiazole derivatives **3a,b** were isolated by diluting the reaction mixture with water and treating the solution with sodium acetate.



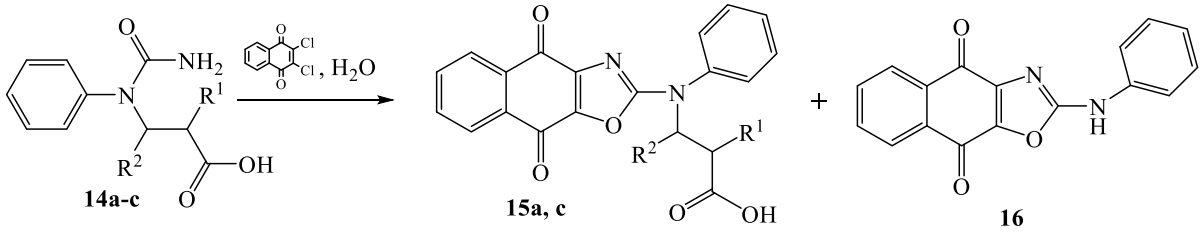
R[1] = a) Me, **b)** H; **R[2] = a)** H, **b)** Me; **X** = Cl, Br;
R[3] = 2) H, **3)** Me, **4)** C_6H_5, **5)** 4-F-C_6H_4, **6)** 4-Cl-C_6H_4, **7)** 4-NC-C_6H_4, **8)** 4-NO_2-C_6H_4,**9)** 3,4-Cl_2-C_6H_4, **10)** naphthalen-2-yl, **11)** chromen-3-yl.

Scheme 1

The structures of the synthesized compounds were confirmed by the data of elemental analysis, ^{1}H NMR, ^{13}C NMR and IR spectra. Formation of the thiazole ring in compounds **2a,b** has been proven by the doublets of SCH group proton at 7.15–7.17 ppm, whereas the same proton gave rise to singlets at 6.18–6.25 ppm in the ^{1}H NMR spectra of **3a,b**. In the ^{13}C NMR spectra of these compounds, the resonances ascribed to SCH group carbon are observed in the range of 101–108 ppm. The signals of NCH group carbon in the ^{13}C NMR spectra of **2a** and **2b** are observed at 139.1 ppm and 140.1 ppm, respectively, and the C=N group carbon gave rise to peaks at 169.5 ppm and 168.9 ppm in the spectra of **3a** and **3b**, respectively. In the IR spectrum of these compounds, the absorption band of the C=N group is observed in the range of 1506–1519 cm^{-1}.

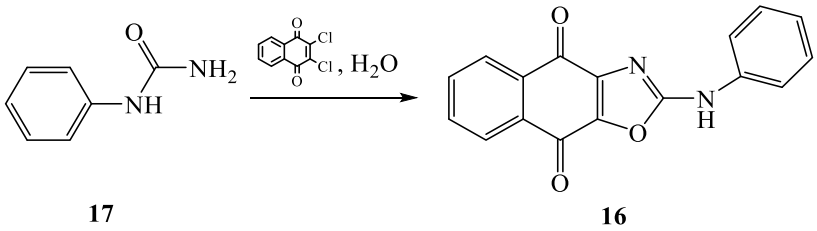
2,4-Disubstituted thiazole derivatives **4a,b–11a,b** were synthesized from thioureido acids **1a,b** and corresponding haloketones in 2-propanol at the reflux temperature of the reaction mixtures.

Naphthoquinone and quinoxaline-fused thiazoles **12a,b** and **13a,b** were prepared in the reactions of **1a,b** with 2,3-dichloroquinaxoline or 2,3-dichloro-1,4-naphthoquinone. These reactions were carried out in acetic acid in the presence of sodium acetate at 80 °C for 24 h. The synthesized compounds were purified by dissolving them in the aqueous KOH solution, filtering the obtained solution and acidifying it with acetic acid to pH 6.



R[1] = a) Me, **b)** H, **c)** H; **R[2] = a)** H, **b)** Me, **c)** H.

Scheme 2



Scheme 3

Reaction of *N*-carbamoyl-β-alanines **14a** and **14c** with 2,3-dichloro-1,4-naphthoquinone in water at reflux temperature provided a mixture of naphthoquinone-fused oxazoles **15a**,**c** and **16** which were isolated by column chromatography (Scheme 2). The analogues reaction of **14b** provided just 2-(phenylamino)naphtho[2,3-*d*]oxazole-4,9-dione (**16**) which was also prepared from *N*-phenylurea in order to prove the formation of such a compound and its structure (Scheme 3). In the ^{1}H NMR spectra for **12a,b**, **13a,b**, **15a,c** and **16**, the number of resonances in the aromatic region has increased in comparison with the spectra of precursors. In the ^{1}H NMR spectrum for **16**, the resonances in the aliphatic region are absent in comparison with the spectra of the analogous compounds. In the IR spectra for **12a,b** the absorption bands of three C=N groups are observed in the range of 1556–1683 cm^{-1} and three C=O absorption bands are present in the 1623–1710 cm^{-1} region in the IR spectra for **13a,b**.

ANTIBACTERIAL ACTIVITY

The antibacterial activity of the compounds **1a,b–13a,b**, **15a**,**c**, and **16** was screened by testing their different concentrations against Gram-positive cocci *Staphylococcus aureus* (ATCC 9144), Gram-negative

Table. Minimum inhibitory concentration (MIC, μg/mL) and minimum bactericidal concentration (MBC, μg/mL) values for the tested compounds **1a,b**–**13a,b**, **15a**, **15c**, and **16**

Compound	*Staphylococcus aureus*		*Escherichia coli*		*Salmonella enteritidis*		*Pseudomonas aeruginosa*	
	MIC, μg/mL	MBC, μg/mL	MIC, μg/mL	MBC, μg/mL	MIC, μg/mL	MBC, μg/mL	MIC, μg/mL	MBC, μg/mL
1a	250	250	+	+	250	250	+	+
1b	250	250	125	125	125	125	125	125
2a	250	250	250	250	250	250	+	+
2b	125	125	250	250	250	250	+	+
3a	62.5	62.5	250	250	250	250	+	+
3b	125	125	+	+	+	+	+	+
4a	+	+	250	500	500	500	+	+
4b	125	125	125	125	125	125	+	+
5a	62.5	62.5	250	500	500	500	+	+
5b	250	250	250	250	125	250	+	+
6a	125	125	250	250	125	250	500	500
6b	125	125	125	125	125	250	125	125
7a	62.5	62.5	250	250	125	125	250	250
7b	250	250	125	125	250	250	125	125
8a	250	250	125	125	125	125	125	125
8b	250	250	250	250	125	125	125	125
9a	125	125	125	125	125	125	125	125
9b	125	125	250	250	125	125	125	125
10a	250	250	125	125	250	250	125	125
10b	125	125	125	125	125	125	125	125
11a	125	125	125	125	250	500	125	125
11b	500	500	500	500	250	500	500	500
12a	500	500	500	500	250	500	250	250
12b	250	250	125	125	500	500	125	125
13a	250	250	250	250	500	500	250	500
13b	125	125	250	250	500	500	125	125
15a	62.5	125	500	500	500	500	500	500

15c	250	500	250	500	500	500	500	500
16	250	500	125	250	250	250	500	500
C*	62.5	62.5	62.5	250	250	250	62.5	250

* Oxytetracycline was used as a control.
+ – growth of microorganisms.

rods *Escherichia coli* (ATCC 8739), *Salmonella enteritidis* (ATCC 8739) and *Pseudomonas aeruginosa* (NCTC 6750) by the broth and spread-plate methods. A range of concentrations for each compound were prepared according to the experimental procedure described in Experimental. The minimum inhibition concentration (MIC, μg/mL) and minimum bactericidal concentration values (MBC, μg/mL) are listed in the Table. A broad-spectrum antibiotic oxytetracycline was used as a control in the antibacterial activity tests of the synthesized compounds.

As the screening data for antibacterial activity have shown, a number of the investigated compounds possess antibacterial properties. Compounds **3a**, **5a**, and **7a** have shown significant bactericidal activity against *S. aureus* with MIC and MBC values of 62.5 μg/mL, which are the same as the ones for oxytetracycline. Compound **5a** inhibits growth of this bacteria strain at 62.5 μg/mL and its MBC value is 125 μg/mL. Among tested compounds, just **4a** did not suppress growth of *S. aureus*. Compounds **1b**, **4b**, **6b**, **7b**, **8a**, **9a**, **10a**,**b**, **11a**, and **12b** have shown good activity against *E. coli* with MIC and MBC values of 125 μg/mL. Bacteristatic activity for **16** was also observed at 125 μg/mL, whereas its bactericidal action was noted at 250 μg/mL. Compounds **1b**, **4b**, **7a**, **8a**,**b**, **9a**,**b**, and **10b** have shown good bactericidal activity against *S. enteritidis* at 125 μg/mL, which is lower than MIC and MBC values for oxytetracycline. Compound **3b** did not suppress growth of *E. coli* and *S. enteritidis*. *P. aeruginosa* was resistant towards action of more compounds, i.e. **1a**, **2a**,**b**, **3a**,**b**, **4a**,**b**, and **5a**,**b**. On the other hand, this bacteria strain was sensitive to a number of compounds, i.e. **6b**, **7b**, **8a**,**b**, **9a**,**b**, **10a**,**b**, **11a**, **12b**, and **13b**, at 125 μg/mL.

CONCLUSIONS

A series of *N*-carboxyethylaminothiazoles and a few of their oxo analogues were synthesized. The analysis of the obtained data on antibacterial activity of the synthesized compounds has revealed that there is a relationship between structure of the synthesized compounds and their biological activity. Comparison of thus presented data with the ones published previously,[21] indicates that introduction of the methyl group into α- or β-position in the β-alanine fragment increases antibacterial efficacy of thiazole

derivatives. In the case of the action against Gram-positive cocci *S. aureus*, a very clear trend can be noticed as an introduction of the methyl group into α-position of the β-alanine fragment enhances significantly antibacterial properties of thiazole and oxazole derivatives in comparison with the ones containing the methyl group in β-position of the β-alanine fragment. Regarding the group of Gram-negative bacterial strains, it can be stated that derivatives with the methyl group in the β-position are more active and this tendency is strongly expressed for the efficacy against *P. aeruginosa*. As it could be expected, the antibacterial activity of thiazoles increases upon introduction of an aromatic substituent containing nitro group or halogen atom, and especially two of the latter as in the case of compounds **9a**,**b**, into thiazole ring as well its fusion with naphthoquinone. The comparison of the data on the antibacterial activity of naphthoquinone-fused thiazole **13a** and its oxo analogue **15a** has revealed that the oxygen-containing naphthoquinone derivative is more active against *S. aureus*, whereas Gram-negative bacterial strains are more sensitive to sulfur-containing compound.

The obtained data is valuable for the further synthesis of the compounds of similar structure and search for the more effective substances possessing antibacterial properties.

EXPERIMENTAL

The starting materials and solvents were obtained from Sigma-Aldrich Chemie GmbH (Munich, Germany) and Fluka Analyticals (Buchs, Switzerland) and were used without further purification. The methods used to follow the reactions were TLC and NMR. The NMR spectra were recorded on a Varian Unity Inova (300 MHz) (Varian, Inc., Palo Alto, CA, USA) and Bruker Avance III/400 (400 MHz) spectrometers. Chemical shifts are expressed as δ, ppm relative to TMS. The *J* constants are given in Hz. The IR spectra (ν, cm^{-1}) were recorded on a Perkin–Elmer BX FT–IR spectrometer (Perkin–Elmer Inc., Waltham, MA, USA) using KBr tablets. Elemental analyses were performed with a CE-440 elemental analyzer (Exeter Analytical Inc., North Chelmsford, MA, USA). Melting points were determined with a B-540 Melting Point Analyzer (Büchi Corporation, New Castle, DE, USA) and are uncorrected. TLC was performed using Silica gel 60 F254 (Kieselgel 60 F254) (Merck, Darmstadt, Germany) plates.

General procedure for preparation of 1,3-thiazoles 2a,b. A mixture of thioureido acid **1** (1.19 g, 5 mmol), 50% aqueous chloroethanal solution (0.79 g, 10 mmol), and water (20 mL) was heated at 80 °C for 2 h. Afterwards, sodium acetate (0.82 g, 10 mmol) was added and the mixture was stirred for 5 min. The precipitate was filtered off and washed with water. Purification was performed by dissolving the crystals in 10% aqueous Na_2CO_3 (25 mL), filtering, and acidifying the filtrate with acetic acid to pH 6 (the procedure was repeated twice).

2-Methyl-3-[phenyl(1,3-thiazol-2-yl)amino]propanoic acid (2a). White solid, yield 0.86 g (66%), mp 117–118 °C; IR (KBr) ν_{max} (cm^{-1}): 3435 (OH), 1695 (C=O), 1519 (C=N); ^{1}H NMR (400 MHz, DMSO-d_6) δ 1.07 (d, 3H, J = 7.1 Hz, CH_3), 2.76–2.84 (m, 1H_X, C*H*CH_3), 3.99 (dd, 1H_A, J^{AM} = 7.5 Hz, J^{AX} = 13.8 Hz, NCH_2), 4.09 (dd, 1H_M, J^{MA} = 7.2 Hz, J^{MX} = 13.8 Hz, NCH_2), 6.69 (d, 1H, J = 3.6 Hz, NCH), 7.17 (d, 1H, J = 3.6 Hz, SCH), 7.30–7.56 (m, 5H, H_{Ar}), 12.48 (s, 1H, OH); ^{13}C NMR (100 MHz, DMSO-d_6) δ 14.9 (CH_3), 38.1 (CH), 55.2 (CH_2), 108.0 (SCH), 126.7, 127.1, 130.0, 145.2 (C_{Ar}), 139.1 (NCH), 170.4 (C-N), 175.9 (C=O). Anal. Calcd for $C_{13}H_{14}N_2O_2S$: C, 59.52; H, 5.38; N, 10.68%. Found: C, 59.71; H, 5.53; N, 10.84%.

3-[Phenyl(1,3-thiazol-2-yl)amino]butanoic acid (2b). White solid, yield 1.05 g (80%), mp 167–168 °C; IR (KBr) ν_{max} (cm^{-1}): 3388 (OH), 1702 (C=O), 1507 (C=N); ^{1}H NMR (400 MHz, $(CD_3)_2CO$) δ 1.32 (t, 2H, J = 6.8 Hz, CH_2CO),), 2.49 (dd, 1H_A, J^{AM} = 7.9 Hz, J^{AX} = 15.6 Hz, CH_2CO), 2.93 (dd, 1H_M, J^{MA} = 6.6 Hz, J^{MX} = 15.6 Hz, CH_2CO), 5.05–5.13 (m, 1H_X, C*H*CH_3), 6.55 (d, 1H, J = 3.7 Hz, NCH), 7.15 (d, 1H, J = 3.7 Hz, SCH), 7.38–7.56 (m, 5H, H_{Ar}), 10.87 (br. s, 1H, OH); ^{13}C NMR (100 MHz, $(CD_3)_2CO$) δ 19.0 (CH_3), 40.0 (CH), 54.2 (CH_2), 108.2 (SCH), 129.4, 131.0, 131.2, 143.8 (C_{Ar}), 140.1 (NCH), 171.9 (C-N), 172.7 (C=O). Anal. Calcd for $C_{13}H_{14}N_2O_2S$: C, 59.52; H, 5.38; N 10.68%. Found: C, 59.73; H, 5.50; N 10.79%.

General procedure for preparation of 4-methyl-1,3-thiazoles 3a,b. A mixture of thioureido acid **1** (0.56 g, 2.5 mmol) and chloropropanone (0.29 g, 3 mmol) in acetone (10 mL) was refluxed for 1 h and diluted with water (50 mL). Afterwards, sodium acetate (0.98 g, 12 mmol) was added, and the reaction mixture was stirred for 5 min. The precipitate was filtered off and washed with water. Purification was performed by dissolving the crystals in 10% aqueous Na_2CO_3 (25 mL), filtering, and acidifying the filtrate with acetic acid to pH 6.

2-Methyl-3-[(4-methyl-1,3-thiazol-2-yl)(phenyl)amino]propanoic acid (3a). White solid, yield 0.56 g (81%), mp 95–96 °C; IR (KBr) ν_{max} (cm^{-1}): 3126 (OH), 1716 (C=O), 1519 (C=N); ^{1}H NMR (300 MHz, DMSO-d_6) δ 1.08 (d, 3H, J = 7.0 Hz, CHC*H*$_3$), 2.15 (s, 3H, CH_3), 2.71–2.85 (m, 1H_X, NCH), 3.97 (dd, 1H_A, J^{AM} = 7.4 Hz, J^{AX} = 13.7 Hz, NCH_2), 4.08 (dd, 1H_M, J^{MA} = 7.4 Hz, J^{MX} = 13.7 Hz, NCH_2), 6.25 (s, 1H, SCH), 7.30–7.49 (m, 5H, H_{Ar}), 12.29 (br. s, 1H, OH); ^{13}C NMR (75 MHz, DMSO-d_6) δ 14.9 (CH*C*H_3), 17.5 (CH_3), 37.9 (*C*HCO), 54.8 (NCH_2), 102.1 (SCH), 126.9, 127.2, 130.0, 148.3 (C_{Ar}), 144.9 (*C*CH_3), 169.5 (C=N), 175.8 (C=O). Anal. Calcd for $C_{14}H_{16}N_2O_2S$: C, 60.85; H, 5.84; N, 10.14%. Found: C, 60.72; H, 5.78; N, 10.26%.

3-[(4-Methyl-1,3-thiazol-2-yl)(phenyl)amino]butanoic acid (3b). White solid, yield 0.52 g (75%), mp 145–146 °C; IR (KBr) ν_{max} (cm^{-1}): 3123 (OH), 1717 (C=O), 1506 (C=N); ^{1}H NMR (300 MHz, DMSO-d_6) δ 1.20 (d, 3H, J = 6.8 Hz, CHC*H*$_3$), 2.15 (s, 3H, CH_3), 2.31 (dd, 1H_A, J^{AM} = 7.9 Hz, J^{AX} = 15.6

Hz, CH_2CO), 2.67 (dd, $1H_M$, J^{MA} = 6.7 Hz, J^{MX} = 15.6 Hz, CH_2CO), 4.92–5.03 (m, $1H_X$, C*H*CH_3), 6.18 (s, 1H, SCH), 7.30–7.54 (m, 5H, H_{Ar}), 12.31 (br. s, 1H, OH); ^{13}C NMR (75 MHz, DMSO-d_6) δ 16.8 (CH*C*H_3), 17.8 (CH_3), 37.9 (*C*H_2CO), 51.6 (NCH), 101.0 (SCH), 127.8, 129.3, 129.5, 147.5 (C_{Ar}), 140.8 (*C*CH_3), 168.9 (C=N), 171.6 (C=O). Anal. Calcd for $C_{14}H_{16}N_2O_2S$: C, 60.85; H, 5.84; N, 10.14%. Found: C, 60.63; H, 5.98; N, 10.32%.

General procedure for preparation of 4–11a,b. A mixture of thioureido acid **1** (1.12 g, 5 mmol), corresponding α-haloketone (5 mmol), sodium acetate (0.82 g, 10 mmol), and 2-propanol (10 mL) was refluxed for 5 h. Afterwards, it was diluted with water (30 mL). The precipitate was filtered off and washed with water. Purification was performed by dissolving the crystals in 10% aqueous Na_2CO_3 (30 mL), filtering, and acidifying the filtrate with acetic acid to pH 6.

2-Methyl-3-[phenyl(4-phenyl-1,3-thiazol-2-yl)amino]propanoic acid (4a) was prepared according to the general procedure from **1a** and 2-bromo-1-phenylethan-1-one to afford light yellow solid, yield 1.52 g (90%), mp 123–124 °C; IR (KBr) ν_{max} (cm^{-1}): 3111 (OH), 1703 (C=O), 1514 (C=N); ^{1}H NMR (300 MHz, DMSO-d_6) δ 1.15 (d, 3H, J = 7.0 Hz, CH_3), 2.86–2.98 (m, 1H, CHCO), 4.11 (dd, $1H_A$, J^{AM} = 7.3 Hz, J^{AX} = 13.8 Hz, NCH_2), 4.21 (dd, $1H_M$, J^{MA} = 7.3 Hz, J^{MX} = 13.7 Hz NCH_2), 7.12 (s, 1H, SCH), 7.26–7.89 (m, 10H, H_{Ar}), 12.22 (br. s, 1H, OH); ^{13}C NMR (75 MHz, DMSO-d_6) δ 15.0 (CH_3), 38.1 (*C*HCO), 55.2 (NCH_2), 102.5 (SCH), 125.6, 126.8, 127.3, 127.5, 128.5, 130.0, 134.6, 150.2 (C_{Ar}), 144.7 (NCH), 169.4 (C=N), 175.9 (C=O). Anal. Calcd for $C_{19}H_{18}N_2O_2S$: C, 67.43; H, 5.36; N, 8.28%. Found: C, 67.22; H, 5.15; N, 8.12%.

3-Phenyl(4-phenyl-1,3-thiazol-2-yl)amino]butanoic acid (4b) was prepared according to the general procedure from **1b** and 2-bromo-1-phenylethan-1-one to afford light yellow solid, yield 1.32 g (78%), mp 144–145 °C; IR (KBr) ν_{max} (cm^{-1}): 3122 (OH), 1711 (C=O), 1510 (C=N); ^{1}H NMR (300 MHz, DMSO-d_6) δ 1.30 (d, 3H, J = 6.8 Hz, CH_3), 2.45 (dd, $1H_A$, J^{AM} = 6.9 Hz, J^{AX} = 15.6 Hz, CH_2CO), 2.84 (dd, $1H_M$, J^{MA} = 6.9 Hz, J^{MX} = 15.6 Hz CH_2CO), 5.01–5.13 (m, 1H, C*H*CH_3), 7.09 (s, 1H, SCH), 7.26–7.88 (m, 10H, H_{Ar}), 12.33 (br. s, 1H, OH); ^{13}C NMR (75 MHz, DMSO-d_6) δ 18.4 (CH_3), 25.4 (*C*H_2CO), 53.1 (*C*HCH_3), 102.4 (SCH), 125.6, 127.4, 128.5, 128.6, 129.9, 130.1, 134.7, 150.2 (C_{Ar}), 141.8 (NCH), 169.4 (C=N), 172.4 (C=O). Anal. Calcd for $C_{19}H_{18}N_2O_2S$: C, 67.43; H, 5.36; N, 8.28%. Found: C, 67.20; H, 5.11; N, 8.10%.

3-{[4-(4-Fluorophenyl)-1,3-thiazol-2-yl](phenyl)amino}-2-methylpropanoic acid (5a) was prepared according to the general procedure from **1a** and 2-bromo-1-(4-fluorophenyl)ethan-1-one to afford white solid, yield 1.59 g (89%), mp 138–139 °C; IR (KBr) ν_{max} (cm^{-1}): 3119 (OH), 1699 (C=O), 1519 (C=N); ^{1}H NMR (400 MHz, $(CD_3)_2CO$) δ 1.25 (d, 3H, J = 7.1 Hz, CH_3), 3.09–3.15 (m, 1H, C*H*CH_3), 4.19 (dd, $1H_A$, J^{AM} = 7.2 Hz, J^{AX} = 13.8 Hz, NCH_2), 4.32 (dd, $1H_M$, J^{MA} = 7.2 Hz, J^{MX} = 13.8 Hz NCH_2), 6.94 (s, 1H, SCH), 7.13–7.98 (m, 9H, H_{Ar}), 10.65 (br. s, 1H, OH); ^{13}C NMR (100 MHz, $(CD_3)_2CO$) δ 15.6 (CH_3), 39.2

(NCH_2), 56.6 (*C*HCH_3), 102.6 (SCH), 116.0, 116.1, 128.2, 128.5, 128.7, 128.7, 131.0, 132.7, 151.0, 162.5, 163.9 (C_{Ar}), 146.4 (N-C), 171.1 (C=N), 176.3 (C=O). Anal. Calcd for $C_{19}H_{17}FN_2O_2S$: C, 64.03; H, 4.81; N, 7.86%. Found: C, 64.29; H, 4.87; N, 8.01%.

3-{[4-(4-Fluorophenyl)-1,3-thiazol-2-yl](phenyl)amino}butanoic acid (5b) was prepared according to the general procedure from **1b** and 2-bromo-1-(4-fluorophenyl)ethan-1-one to afford white solid, yield 1.60 g (90%), mp 158–159 °C; IR (KBr) ν_{max} (cm^{-1}): 3122 (OH), 1710 (C=O), 1513 (C=N); ^{1}H NMR (400 MHz, $(CD_3)_2CO$) δ 1.27 (d, 3H, J = 6.8 Hz, CH_3), 2.43 (dd, 1H_A, J^{AM} = 7.6 Hz, J^{AX} = 15.6 Hz, CH_2CO), 2.88 (dd, 1H_M, J^{MA} = 6.8 Hz, J^{MX} = 15.6 Hz CH_2CO), 5.01–5.12 (m, 1H_X, *CH*CH_3), 6.76 (s, 1H, SCH), 6.97–7.87 (m, 9H, H_{Ar}), 10.67 (br. s, 1H, OH); ^{13}C NMR (100 MHz, $(CD_3)_2CO$) δ 18.1 (CH_3), 40.0 (*C*H_2CO), 54.7 (*C*HCH_3), 102.5 (SCH), 116.0, 116.2, 128.7, 128.8, 129.6, 131.1, 131.3, 133.0, 151.1, 162.0, 164.4 (C_{Ar}), 143.6 (N-C), 171.1 (C=N), 172.8 (C=O). Anal. Calcd for $C_{19}H_{17}FN_2O_2S$: C, 64.03; H, 4.81; N, 7.86%. Found: C, 64.25; H, 4.73; N, 7.98%.

3-{[4-(4-Chlorophenyl)-1,3-thiazol-2-yl](phenyl)amino}-2-methylpropanoic acid (6a) was prepared according to the general procedure from **1a** and 2-bromo-1-(4-chlorophenyl)ethan-1-one to afford white solid, yield 1.70 g (91%), mp 128–129 °C; IR (KBr) ν_{max} (cm^{-1}): 3126 (OH), 1701 (C=O), 1508 (C=N); ^{1}H NMR (400 MHz, DMSO-d_6) δ 1.14 (d, 3H, J = 6.0 Hz, CH_3), 2.76–3.08 (m, 1H, C*H*CH_3), 3.92–4.43 (m, 2H, NCH_2), 7.20 (s, 1H, SCH), 7.30–7.97 (m, 9H, H_{Ar}), 12.43 (s, 1H, OH); ^{13}C NMR (100 MHz, DMSO-d_6) δ 14.9 (CH_3), 38.1 (CH), 55.2 (CH_2), 103.4 (SCH), 126.9, 127.3, 127.5, 128.6, 130.1, 131.9, 133.5, 149.0 (C_{Ar}), 144.7 (S-CH=*C*), 169.7 (N=C–N), 175.8 (C=O). Anal. Calcd for $C_{19}H_{17}ClN_2O_2S$: C, 61.20; H, 4.60; N, 7.51%. Found: C, 61.42; H 4.46; N, 7.68%.

3-{[4-(4-Chlorophenyl)-1,3-thiazol-2-yl](phenyl)amino}butanoic acid (6b) was prepared according to the general procedure from **1b** and 2-bromo-1-(4-chlorophenyl)ethan-1-one to afford white solid, yield 1.51 g (81%), mp 182.5–183.5 °C; IR (KBr) ν_{max} (cm^{-1}): 3125 (OH), 1709 (C=O), 1533 (C=N); ^{1}H NMR (400 MHz, $(CD_3)_2CO$) δ 1.42 (d, 3H, J = 6.8 Hz, CH_3), 2.58 (dd, 1H_A, J^{AM} = 7.6 Hz, J^{AX} = 15.6 Hz, CH_2CO), 3.02 (dd, 1H_M, J^{MA} = 6.8 Hz, J^{MX} = 15.6 Hz, CH_2CO), 5.16–5.28 (m, 1H, CH), 7.00 (s, 1H, SCH), 7.30–8.06 (m, 9H, H_{Ar}), 10.08 (s, 1H, OH); ^{13}C NMR (100 MHz, $(CD_3)_2CO$) δ 19.1 (CH_3), 40.0 (CH), 54.7 (CH_2), 103.5 (SCH), 128.4, 129.5, 129.7, 131.1, 131.3, 133.5, 135.2, 150.9 (C_{Ar}), 143.5 (S-CH=*C*), 171.2 (N=C–N), 172.8 (C=O). Anal. Calcd for $C_{19}H_{17}ClN_2O_2S$: C, 61.20; H, 4.60; N, 7.51%. Found: C, 61.39; H, 4.75; N, 7.67%.

3-{[4-(4-Cyanophenyl)-1,3-thiazol-2-yl](phenyl)amino}-2-methylpropanoic acid (7a) was prepared according to the general procedure from **1a** and 2-bromo-1-(4-isocyanophenyl)ethan-1-one to afford white solid, yield 1.47 g (81%), mp 173–174 °C; IR (KBr) ν_{max} (cm^{-1}): 3395 (OH), 2222 (C≡N), 1702 (C=O), 1513 (C=N); ^{1}H NMR (400 MHz, DMSO-d_6) δ 1.15 (d, 3H, J = 7.1 Hz, CH_3), 2.83–2.93 (m, 1H,

C*H*CH_3), 4.09 (dd, 1H_A, J^{AM} = 7.2 Hz, J^{AX} = 13.9 Hz, NCH_2), 4.21 (dd, 1H_M, J^{MA} = 7.4 Hz, J^{MX} = 13.8 Hz NCH_2), 7.38 (t, 1H, J = 7.2 Hz, H_{Ar}), 7.44 (s, 1H, SCH), 7.45–8.07 (m, 8H, H_{Ar}), 12.32 (s, 1H, OH); ^{13}C NMR (100 MHz, DMSO-d_6) δ 15.0 (CH_3), 38.0 (CH), 55.2 (CH_2), 106.4 (SCH), 109.6, 126.2, 126.9, 127.6, 130.2, 132.7, 138.7, 148.5 (C_{Ar}), 119.0 (C≡N), 144.5 (S-CH=*C*), 169.9 (N=C-N), 175.8 (C=O). Anal. Calcd for $C_{20}H_{17}N_3O_2S$: C, 66.10; H, 4.72; N, 11.56%. Found: C, 65.87; H, 4.56; N, 11.45%.

3-{[4-(4-Cyanophenyl)-1,3-thiazol-2-yl](phenyl)amino}butanoic acid (7b) was prepared according to the general procedure from **1b** and 2-bromo-1-(4-isocyanophenyl)ethan-1-one to afford white solid, yield 1.60 g (88%), mp 196–197 °C; IR (KBr) ν_{max} (cm^{-1}): 3396 (OH), 2222 (C≡N), 1707 (C=O), 1512 (C=N); ^{1}H NMR (400 MHz, DMSO-d_6) δ 1.30 (d, 3H, J = 6.8 Hz, CH_3), 2.41 (dd, 1H_A, J^{AM} = 7.6 Hz, J^{AX} = 15.5 Hz, CH_2CO), 2.78 (dd, 1H_M, J^{MA} = 6.9 Hz, J^{MX} = 15.5 Hz, CH_2CO), 5.00–5.15 (m, 1H, CH), 7.37–8.08 (s, 10H, SCH and H_{Ar}), 12.51 (s, 1H, OH); ^{13}C NMR (100 MHz, DMSO-d_6) δ 18.5 (CH_3), 40.0 (CH), 53.3 (CH_2), 106.2 (SCH), 109.5, 126.2, 128.8, 129.9, 130.2, 132.6, 138.9, 148.5 (C_{Ar}), 119.1 (C≡N), 141.7 (S-CH=*C*), 169.8 (N=C–N), 172.6 (C=O). Anal. Calcd for $C_{20}H_{17}N_3O_2S$: C, 66.10; H, 4.72; N, 11.56%. Found: C, 65.89; H, 4.59; N, 11.44%.

2-Methyl-3-{[4-(4-nitrophenyl)-1,3-thiazol-2-yl](phenyl)amino}propanoic acid (8a) was prepared according to the general procedure from **1a** and 2-bromo-1-(4-nitrophenyl)ethan-1-one to afford yellow solid, yield 1.55 g (81%), mp 183–184 °C; IR (KBr) ν_{max} (cm^{-1}): 3128 (OH), 1710 (C=O), 1506 (C=N); ^{1}H NMR (400 MHz, $(CD_3)_2CO$) δ 1.23 (d, 3H, J = 7.1 Hz, CH_3), 3.05–3.11 (m, 1H, C*H*CH_3), 4.18 (dd, 1H_A, J^{AM} = 7.4 Hz, J^{AX} = 13.9 Hz, NCH_2), 4.32 (dd, 1H_M, J^{MA} = 7.4 Hz, J^{MX} = 13.9 Hz, NCH_2), 7.30 (s, 1H, SCH), 7.47–8.25 (m, 9H, H_{Ar}), 10.81 (br. s, 1H, OH); ^{13}C NMR (100 MHz, $(CD_3)_2CO$) δ 15.6 (CH_3), 39.1 (CH), 56.6 (CH_2), 107.4 (SCH), 124.8, 127.5, 128.3, 128.7, 131.1, 146.1, 147.8, 149.9 (C_{Ar}), 142.0 (S-CH=*C*), 171.4 (N=C–N), 176.1 (C=O). Anal. Calcd for $C_{19}H_{17}N_3O_4S$: C, 59.52; H, 4.47; N, 10.96%. Found: C, 59.60; H, 4.34; N, 11.12%.

3-{[4-(4-Nitrophenyl)-1,3-thiazol-2-yl](phenyl)amino}butanoic acid (8b) was prepared according to the general procedure from **1b** and 2-bromo-1-(4-nitrophenyl)ethan-1-one to afford yellow solid, yield 1.71 g (89%), mp 175–176 °C; IR (KBr), ν_{max} (cm^{-1}): 3392 (OH), 1701 (C=O), 1595 (NO_2), 1504 (C=N); ^{1}H NMR (400 MHz, $(CD_3)_2CO$) δ 1.44 (d, 3H, J = 6.8 Hz, CH_3), 2.60 (dd, 1H_A, J^{AM} = 7.5 Hz, J^{AX} = 15.6 Hz, CH_2CO), 3.02 (dd, 1H_M, J^{MA} = 6.9 Hz, J^{MX} = 15.6 Hz, CH_2CO), 5.20–5.31 (m, 1H, CH), 7.32 (s, 1H SCH), 7.46–8.32 (s, 9H, H_{Ar}), 10.38 (s, 1H, OH); ^{13}C NMR (100 MHz, DMSO-d_6) δ 19.1 (CH_3), 39.9 (CH), 54.7 (CH_2), 107.3 (SCH), 124.8, 128.5, 129.9, 131.2, 131.3, 142.2, 147.8, 150.0 (C_{Ar}), 143.3 (S-CH=*C*), 171.5 (N=C–N), 172.8 (C=O). Anal. Calcd for $C_{19}H_{17}N_3O_4S$: C, 59.52; H, 4.47; N, 10.96%. Found: C, 59.33; H, 4.55; N, 11.17%.

3-{[4-(3,4-Dichlorophenyl)-1,3-thiazol-2-yl](phenyl)amino}-2-methylpropanoic acid (9a) was

prepared according to the general procedure from **1a** and 2-bromo-1-(3,4-dichlorophenyl)ethan-1-one to afford white solid, yield 1.65 g (81%), mp 136–137 °C; IR (KBr) ν_{max} (cm^{-1}): 3117 (OH), 1703 (C=O), 1590 (NO_2), 1506 (C=N); ^{1}H NMR (400 MHz, DMSO-*d*$_6$) δ 1.14 (d, 3H, J = 7.0 Hz, CH_3), 2.80–2.94 (m, 1H, C*H*CH_3), 4.07 (dd, 1H$_A$, J^{AM} = 7.1 Hz, J^{AX} = 13.8 Hz, NCH_2), 4.22 (dd, 1H$_M$, J^{MA} = 7.4 Hz, J^{MX} = 13.8 Hz NCH_2), 7.30–8.10 (m, 9H, H$_{Ar}$ and SCH), 12.30 (s, 1H, OH); ^{13}C NMR (100 MHz, DMSO-*d*$_6$) δ 14.9 (CH_3), 38.0 (CH), 55.17 (CH_2), 104.8 (SCH), 125.7, 127.1, 126.9, 127.5, 129.7, 130.1, 130.8, 131.3, 135.2, 147.6 (C_{Ar}), 144.5 (S-CH=*C*), 169.8 (N=C–N), 175.7 (C=O). Anal. Calcd for $C_{19}H_{16}Cl_2N_2O_2S$: C, 56.03; H, 3.96; N, 6.88%. Found: C, 56.25; H, 4.09; N, 6.93%.

3-{[4-(3,4-Dichlorophenyl)-1,3-thiazol-2-yl](phenyl)amino}butanoic acid (9b) was prepared according to the general procedure from **1b** and 2-bromo-1-(3,4-dichlorophenyl)ethan-1-one to afford white solid, yield 1.60 g (79%), mp 147–148 °C; IR (KBr) ν_{max} (cm^{-1}): 3387 (OH), 1703 (C=O), 1502 (C=N); ^{1}H NMR (400 MHz, $(CD_3)_2CO$) δ 1.39 (d, 3H, J = 6.9 Hz, CH_3), 2.55 (dd, 1H$_A$, J^{AM} = 7.5 Hz, J^{AX} = 15.6 Hz, CH_2CO), 2.79 (dd, 1H$_M$, J^{MA} = 6.9 Hz, J^{MX} = 15.6 Hz, CH_2CO), 5.19–5.27 (m, 1H, CH), 7.11 (s, 1H, SCH), 7.44–8.16 (m, 8H, H$_{Ar}$), 10.71 (s, 1H, OH); ^{13}C NMR (100 MHz, $(CD_3)_2CO$) δ 19.1 (CH_3), 39.9 (CH), 54.5 (CH_2), 104.8 (SCH), 126.5, 128.5, 129.7, 131.1, 131.2, 131.5, 132.9, 136.8, 149.4 (C_{Ar}), 143.2 (S-CH=*C*), 171.3 (N=C–N), 172.8 (C=O). Anal. Calcd for $C_{19}H_{16}Cl_2N_2O_2S$: C, 56.03; H, 3.96; N, 6.88%. Found: C, 56.27; H, 4.11; N, 6.99%.

2-Methyl-3-{[4-(naphthalen-2-yl)-1,3-thiazol-2-yl](phenyl)amino}propanoic acid (10a) was prepared according to the general procedure from **1a** and 2-bromo-1-(4-(naphthalen-2-yl)phenyl)ethan-1-one to afford yellow solid, 1.40 g (72%), mp 155–156 °C; IR (KBr), ν_{max} (cm^{-1}): 3355 (OH), 1740 (C=O), 1510 (C=N); ^{1}H NMR (400 MHz, DMSO-*d*$_6$) δ 1.10 (d, 3H, J = 7.0 Hz, CH_3), 2.40–2.50 (m, 1H, C*H*CH_3), 4.07–4.25 (m, 2H, NCH_2), 7.24 (s, 1H, SCH), 7.30–8.40 (m, 8H, H$_{Ar}$), 10.75 (s, 1H, OH); ^{13}C NMR (100 MHz, DMSO-*d*$_6$) δ 16.3 (CH_3), 25.5 (CH), 56.7, 62.0 (CH_2), 102.9 (SCH), 124.0, 124.2, 125.9, 126.4, 126.8, 127.6, 128.0, 128.1, 129.8, 132.3, 132.4, 133.1, 150.2 (C_{Ar}), 144.9 (S-CH=*C*), 170.0 (N=C–N), 177.7 (C=O). Anal. Calcd for $C_{23}H_{20}N_2O_2S$: C, 71.11; H, 5.19; N, 7.21%. Found: C, 69.86; H, 5.32; N, 7.06%.

3-{[4-(Naphthalen-2-yl)-1,3-thiazol-2-yl](phenyl)amino}butanoic acid (10b) was prepared according to the general procedure from **1b** and 2-bromo-1-(4-(naphthalen-2-yl)phenyl)ethan-1-one to afford white solid, yield 1.63 g (84%), mp 147–148 °C; IR (KBr) ν_{max} (cm^{-1}): 3387 (OH), 1703 (C=O), 1502 (C=N); ^{1}H NMR (400 MHz, $(CD_3)_2CO$) δ 1.39 (d, 3H, J = 6.9 Hz, CH_3), 2.55 (dd, 1H$_A$, J^{AM} = 7.5 Hz, J^{AX} = 15.6 Hz, CH_2CO), 2.79 (dd, 1H$_M$, J^{MA} = 6.9 Hz, J^{MX} = 15.6 Hz, CH_2CO), 5.19–5.27 (m, 1H, CH), 7.11 (s, 1H, SCH), 7.44–8.16 (m, 12H, H$_{Ar}$), 10.71 (s, 1H, OH); ^{13}C NMR (100 MHz, $(CD_3)_2CO$) δ 19.1 (CH_3), 39.9 (CH), 54.5 (CH_2), 104.8 (SCH), 126.5, 128.5, 129.7, 131.1, 131.2, 131.5, 132.9, 136.8, 149.4 (C_{Ar}), 143.2

(S-CH=*C*), 171.3 (N=C–N), 172.8 (C=O). Anal. Calcd for $C_{23}H_{20}N_2O_2S$: C, 71.11; H, 5.19; N, 7.21%. Found: C, 69.89; H, 5.34; N, 7.03%.

2-Methyl-3-{[4-(2-oxo-2*H*-chromen-3-yl)-1,3-thiazol-2-yl](phenyl)amino}propanoic acid (11a) was prepared according to the general procedure from **1a** and 3-(4-(2-bromoacetyl)phenyl)-2*H*-chromen-2-one to afford yellow solid, yield 1.81 g (89%), mp 198–199 °C; IR (KBr) ν_{max} (cm^{-1}): 3435 (OH), 1695 (C=O), 1492 (C=N); ^{1}H NMR (400 MHz, DMSO-d_6) δ 1.15 (d, 3H, J = 7.0 Hz, CH_3), 2.74–2.86 (m, 1H, C*H*CH_3), 4.10–4.24 (m, 2H, NCH_2), 7.30–7.90 (m, 10H, H_{Ar}), 8.62 (s, 1H, SCH); ^{13}C NMR (100 MHz, DMSO-d$_6$) δ 15.2 (CH_3), 38.30 (CH), 55.2 CH_2), 109.5 (SCH), 115.8, 119.2, 120.3, 124.7, 127.5, 127.0, 128.8, 130.1, 131.5, 138.4, 143.7, 152.2 ($C_{Ar\ and\ chrom.}$), 144.5 (S-CH=*C*), 158.7 (O-C=O), 168.9 (N=C–N), 176.1 (C=O). Anal. Calcd for $C_{22}H_{18}N_2O_4S$: C, 65.01; H, 4.46; N, 6.89%. Found: C, 64.77; H, 4.54; N, 6.80%.

3-{[4-(2-Oxo-2*H*-chromen-3-yl)-1,3-thiazol-2-yl](phenyl)amino}butanoic acid (11b) was prepared according to the general procedure from **1b** and 3-(4-(2-bromoacetyl)phenyl)-2*H*-chromen-2-one to afford yellow solid, yield 1.79 g (88%), mp 178–179 °C; IR (KBr) ν_{max} (cm^{-1}): 3517 (OH), 1718, 1703 (2C=O), 1531 (C=N); ^{1}H NMR (400 MHz, $(CD_3)_2CO$) δ 1.38 (d, J = 6.8 Hz, 3H, CH_3), 2.53 (dd, $1H_A$, J^{AM} = 7.3 Hz, J^{AX} = 15.6 Hz, CH_2CO), 2.95 (dd, $1H_M$, J^{MA} = 6.9 Hz, J^{MX} = 15.6 Hz, CH_2CO), 5.19–5.26 (m, 1H, CH), 7.30–7.75 (m, 10H, H_{Ar}), 8.70 (s, 1H, SCH); ^{13}C NMR (100 MHz, $(CD_3)_2CO$) δ 19.1 (CH_3), 39.3 (CH), 54.4 CH_2), 110.4 (SCH), 116.8, 120.7, 122.1, 125.5, 129.4, 129.7, 131.1, 131.3, 131.5, 132.2, 139.3, 143.1, 153.8 ($C_{Ar\ and\ chrom.}$), 145.4 (S-CH=*C*), 159.8 (O-C=O), 170.2 (N=C–N), 172.8 (C=O). Anal. Calcd for $C_{22}H_{18}N_2O_4S$: C, 65.01; H, 4.46; N, 6.89%. Found: C, 64.80; H, 4.53; N, 6.73%.

General procedure for preparation of 12a,b and 13a,b. A mixture of corresponding thioureido acid **1a,b** (1.19 g, 5 mmol), 2,3-dichloro-1,4-naphthoquinone (1.36 g, 6 mmol) or 2,3-dichloroquinoxaline (1.19 g, 6 mmol), sodium acetate (1,48 g, 18 mmol), and acetic acid (25 mL) was stirred at 80 °C for 24 h. Afterwards, it was cooled to room temperature and diluted with water (100 mL). The precipitate formed was filtered off and washed with water. Purification was performed by dissolving the crystals in 5% aqueous KOH (175 mL), filtering, and acidifying the filtrate with acetic acid to pH 6.

2-Methyl-3-[phenyl([1,3]thiazolo[4,5-*b*]quinoxalin-2-yl)amino]propanoic acid (12a). Yellow solid, yield 1.37 g (75%), mp 250–251 °C (decomp.); IR (KBr) ν_{max} (cm^{-1}): 3048 (OH), 1766 (C=O), 1683, 1614, 1595 (3 C=N); ^{1}H NMR (400 MHz, DMSO-d_6) δ 1.12 (d, 3H, J = 7.0 Hz, CH_3), 2.93–3.00 (m, 1H, C*H*CH_3), 3.72–3.85 (m, 2H, NCH_2), 7.04–7.43 (m, 9H, H_{Ar}), 11.89 (br. s, 1H, OH). Anal. Calcd for $C_{19}H_{18}N_4O_2S$: C, 62.28; H, 4.95; N, 15.29%. Found: C, 62.17; H, 5.09; N, 15.42%.

3-[Phenyl([1,3]thiazolo[4,5-*b*]quinoxalin-2-yl)amino]butanoic acid (12b). Yellow solid, yield 1.57 g (86%), mp 280–281 °C (decomp.); IR (KBr) ν_{max} (cm^{-1}): 3559 (OH), 1708 (C=O), 1608, 1590, 1556

(3C=N); ^{1}H NMR (400 MHz, DMSO-d_6) δ 1.22 (d, 3H, J = 7.0 Hz, CH_3), 2.88–2.97 (m, 2H, CH_2CO), 5.19–5.27 (m, 1H, C*H*CH_3), 7.07–8.04 (m, 9H, H_{Ar}), 11.90 (br. s, 1H, OH). Anal. Calcd for $C_{19}H_{18}N_4O_2S$: C, 62.28; H, 4.95; N, 15.29%. Found: C, 62.13; H, 5.12; N, 15.46%.

3-[(4,9-Dioxo-4,9-dihydronaphtho[2,3-*d*][1,3]thiazol-2-yl)(phenyl)amino]-2-methylpropanoic acid (13a). Dark brown solid, yield 1.44 g (73%), mp 181–182 °C (decomp.); IR (KBr) ν_{max} (cm^{-1}): 3062 (OH), 1707, 1642, 1626 (3C=O), 1522 (C=N); ^{1}H NMR (400 MHz, DMSO-d_6) δ 1.17 (d, 3H, J = 7.1 Hz, CH_3), 2.73–2.86 (m, 1H, C*H*CH_3), 4.23–4.30 (dd, 1H$_A$, J^{AB} = 7.2 Hz, J^{AX} = 13.8 Hz, NCH_2), 4.33–4.41 (dd, 1H$_B$, J^{BA} = 7.6 Hz, J^{BX} = 13.8 Hz, NCH_2), 7.32–7.99 (m, 9H, H_{Ar}), 12.19 (br. s, 1H, OH). Anal. Calcd for $C_{21}H_{16}N_2O_4S$: C, 64.27; H, 4.11; N, 7.14%. Found: C, 64.02; H, 4.29; N, 7.31%.

3-[(4,9-Dioxo-4,9-dihydronaphtho[2,3-*d*][1,3]thiazol-2-yl)(phenyl)amino]butanoic acid (13b). Dark brown solid, yield 1.57 g (80%), mp 195–196 °C (decomp.); IR (KBr) ν_{max} (cm^{-1}): 3375 (OH), 1710, 1637, 1623 (3C=O), 1519 (C=N); ^{1}H NMR (400 MHz, DMSO-d_6) δ 1.34 (d, 3H, J = 6.8 Hz, CH_3), 2.68–2.75 (dd, 1H$_A$, J^{AB} = 7.4 Hz, J^{AX} = 15.0 Hz, CH_2CO), 2.78–2.86 (dd, 1H$_B$, J^{BA} = 7.4 Hz, J^{BX} = 15.0 Hz, CH_2CO), 5.25–5.35 (m, 1H, C*H*CH_3), 7.46–7.98 (m, 9H, H_{Ar}), 12.45 (br. s, 1H, OH). Anal. Calcd for $C_{21}H_{16}N_2O_4S$: C, 64.27; H, 4.11; N, 7.14%. Found: C, 64.05; H, 4.30; N, 7.33%.

General procedure for preparation of 15a,c and 16. A mixture of corresponding ureido acid **14a-c** or *N*-phenylurea (4.5 mmol), 2,3-dichloro-1,4-naphthoquinone (1,04 g, 4.5 mmol, 98%) and water (30 mL) was stirred under reflux for 14 h. The precipitate was filtered off and washed with water. Products were isolated by column chromatography (acetone:hexane).

3-((4,9-Dioxo-4,9-dihydronaphtho[2,3-*d*]oxazol-2-yl)(phenyl)amino)-2-methylpropanoic acid (15a). Dark blue solid, yield 0.50 g (29%), mp 134–135 °C; R_f = 0.511 (acetone:hexane, 1:1); ^{1}H NMR (400 MHz, DMSO-d_6) δ 1.17 (d, 3H, J = 7.2 Hz, CH_3), 2.84–2.88 (m, 1H, C*H*CH_3), 3.75 (dd, 1H$_A$, J^{AB} = 4.6 Hz, J^{AX} = 15.3 Hz, NCH_2), 4.16 (dd, 1H$_B$, J^{BA} = 8.9 Hz, J^{BX} = 15.3 Hz, NCH_2), 6.91–7.40 (m, 5H, H_{Ar}), 7.92–8.09 (m, 4H, H_{Ar}), 12.31 (br.s., 1H, OH); ^{13}C NMR (100 MHz, DMSO-d_6) δ 15.89 (CH_3), 35.43 (CH), 54.19 (CH_2), 112.50, 117.03, 125.69, 126.24, 127.36, 129.10, 129.51, 131.88, 132.04, 134.72, 137.79, 146.62, 147.95 ($C_{Ar+oxazole}$), 176.89 (COOH), 178.33 (C=O), 180.84 (C=O); Anal. Calcd for $C_{21}H_{16}N_2O_5$: C, 67.02; H, 4.29; N, 7.44%. Found: C, 67.10; H, 4.15; N, 7.55%.

3-((4,9-Dioxo-4,9-dihydronaphtho[2,3-*d*]oxazol-2-yl)(phenyl)amino)propanoic acid (15c). Dark blue solid, yield 0.43 g (26%), mp 127-128 °C; R_f = 0.51 (acetone:hexane, 1:1); ^{1}H NMR (400 MHz, DMSO-d_6) δ 2.69 (t, 2H, J = 6.9 Hz, CH_2CO), 4.02 (t, 2H, J = 6.9 Hz, NCH_2), 6.87–7.25 (m, 5H, H_{Ar}), 7.88–8.10 (m, 4H, H_{Ar}), 12.27 (br.s., 1H, OH); ^{13}C NMR (100 MHz, DMSO-d_6) δ 33.32 (CH_2), 46.80 (CH_2), 117.10, 120.91, 126.79, 127.26, 129.57, 131.94, 132.09, 134.63, 134.70, 137.45, 145.80, 148.05 ($C_{Ar\ +oxazole}$), 173.48 (COOH), 178.43 (C=O), 181.05 (C=O); Anal. Calcd for $C_{20}H_{14}N_2O_5$: C, 66.30; H,

3.89; N, 7.73%. Found: C, 66.42; H, 3.95; N, 7.80%.

2-(Phenylamino)naphtho[2,3-*d*]oxazole-4,9-dione (16). Red solid, yield from **14a** – 0.31 g (24 %), from **14b** – 0,90 g (69%), from **14c** – 0.47 g (36%), from *N*-phenylurea – 1.01 g (77%), mp 179–180 °C; R_f= 0.53 (acetone:hexane, 1:2); ^{1}H-NMR (400 MHz, DMSO-d_6) δ 7.13–7.31 (m, 5H, H_{Ar}), 7.74–8.02 (m, 4H, H_{Ar}), 9.28 (s, 1H, NH); ^{13}C NMR (100 MHz, DMSO-d_6) δ 113.88, 123.59, 124.01, 125.71, 126.14, 127.54, 129.89, 131.59, 132.81, 134.41, 138.45, 142.76 ($C_{Ar\ +oxazole}$), 176.30 (C=O), 179.73 (C=O); Anal. Calcd for $C_{17}H_{10}N_2O_3$: C, 70.34; H, 3.47; N, 9.65%. Found: C, 70.41; H, 3.56; N, 9.74%.

Evaluation of antimicrobial activity. The following bacteria strains were used: Gram-positive cocci *Staphylococcus aureus* (ATCC 9144) Gram-negative rod *Escherichia coli* (ATCC 8739), *Salmonella enteritidis* (ATCC 8739), and *Pseudomonas aeruginosa* (NCTC 6750). Tryptic soy agar (TSA) and tryptic soy broth (TSB) were used for bacteria cultivation and antibacterial activity tests. Antibacterial activity of the compounds was determined by testing their different concentrations against *B. cereus*, *S. aureus*, *P. aeruginosa* and *E. coli* bacteria by the broth-dilution and spread plate methods.[36,37] A range of concentrations, 1000, 500, 250, 125, and 62.5 μg/mL, were prepared for each sample. They were streaked out on TSA plates and incubated at 37 °C for 24 h. A representative colony was placed in 5 mL of TBS and incubated at 37 °C for 24 h. *S. aureus*, *E. coli*, *S. enteritidis*, and *P. aeruginosa* cultures containing 108 CFU/mL (colony-forming units corresponding to McFarland's 0.5) were diluted with TSB and used for the antibacterial test. The test organisms (0.1 mL) were added to each tube and incubated at 37 °C for 24 h. At the end of this period, a small amount of the diluted mixture (different materials) from each tube was pulled out and spread on TSA. The plates were incubated at 37 °C for 48 h. The growth of bacterial cells was observed on agar plates. The lowest concentration of the bacterial material at which no growth was observed was considered as the minimum bactericidal concentration (MBC) value.[38] Minimum inhibitory concentration (MIC) is the lowest concentration of an antimicrobial that inhibits the visible growth of a microorganism after overnight incubation. Oxytetracycline inoculated with each test bacterium in the tubes and plates was used as a control. The growth of the test bacteria was observed in all plates as positive control.

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REFERENCES

1. F. C. Tenover and L. C. McDonald, *Curr. Opin. Infect. Dis.*, 2005, **18**, 300.
2. R. F. Pfeltz and B. J. Wilkinson, *Curr. Drug Targets: Infect. Disord.*, 2004, **4**, 273.
3. M. C. Roberts, *Curr. Drug Targets: Infect. Disord.*, 2004, **4**, 207.
4. A. Dessen, A. M. Di Guilmi, T. Vernet, and O. Dideberg, *Curr. Drug Targets: Infect. Disord.*, 2001, **1**, 63.
5. H. Muroi, K. Nihei, K. Tsujimoto, and I. Kubo, *Bioorg. Med. Chem.*, 2004, **12**, 583.
6. M. W. Khan, M. J. Alam, M. A. Rashid, and R. Chowdhury, *Bioorg. Med. Chem.*, 2005, **13**, 4796.
7. R. Vaickelioniene, V. Mickevicius, and G. Mikulskiene, *Molecules*, 2005, **10**, 407.
8. R. Vaickelioniene and V. Mickevicius, *Chem. Heterocycl. Compd.*, 2006, **6**, 862.
9. K. Anusevicius, R. Vaickelioniene, and V. Mickevicius, *Chem. Heterocycl. Compd.*, 2012, **48**, 1105.
10. S. K. Agrawal, M. Sathe, A. K. Halve, and M. P. Kaushik, *Tetrahedron Lett.*, 2012, **53**, 5996.
11. J. Liu, Y. Wang, Y. Sun, D. Marshall, S. Miao, G. Tonn, P. Anders, J. Tocker, H. L. Tang, and J. Medina, *Bioorg. Med. Chem. Lett.*, 2009, **19**, 6840.
12. J. Song, L. M. Jones, G. E. Chavarria, Amanda K. Charlton-Sevcik, A. Jantz, A. Johansen, L. Bayeh, V. Soeung, L. K. Snyder, Sh. D. Lade Jr., D. J. Chaplin, M. L. Trawick, and K. G. Pinney, *Bioorg. Med. Chem. Lett.*, 2013, **23**, 2801.
13. N. Katuoka, A. Tatara, M. Yatagai, and J. Yamanaka, Patent, US 20100204505, 2010.08.12.
14. A. Castro, M. J. Jereza, and C. Gila, *Eur. J. Med. Chem.*, 2008, **43**, 1349.
15. R. A. Hughes and C. J. Moody, *Angew. Chem. Int. Ed.*, 2007, **46**, 7930.
16. M. Kaspady, V. K. Narayanaswamy, M. Raju, and G. K. Rao, *Lett. Drug Des. Discov.*, 2009, **6**, 21.
17. A. C. Giddens, H. I. M. Boshoff, S. G. Franzblau, C. E. Barry, and B. R. Copp, *Tetrahedron Lett.*, 2005, **46**, 7355.
18. K. A. Z. Siddiquee, P. T. Gunning, M. Glenn, W. P. Katt, Sh. Zhang, Ch. Schroeck, S. M. Sebti, R. Jove, A. D. Hamilton, and J. Turkson, *ACS Chem. Biol.*, 2007, **2**, 787.
19. K. Liaras, A. Geronikaki, J. Glamoclija, A. Ciric, and M. Sokovic, *Bioorg. Med. Chem.*, 2011, **19**, 7349.
20. K. Anusevicius, I. Jonuskiene, and V. Mickevicius, *Monatsh. Chem.*, 2013, **144**, 1883.
21. V. Mickevicius, A. Voskiene, I. Jonuskiene, R. Kolosej, J. Siugzdaite, P. R. Venskutonis, R. Kazernaviciute, Z. Braziene, and E. Jakiene, *Molecules*, 2013, **18**, 15000.
22. F. Chimenti, B. Bizzarri, and A. Bolasco, *Eur. J. Med. Chem.*, 2011, **46**, 378.
23. K. C. Prakasha, G. M. Raghavendra, R. Harisha, and D. C. Gowda, *Int. J. Pharm. Sci.*, 2011, **3**, 120.
24. A. Zablotskaya, I. Segal, A. Geronikaki, T. Eremkina, S. Belyakov, M. Petrova, I. Shestakova, L. Zvejniece, and V. Nikolajeva, *Eur. J. Med. Chem.*, 2013, **70**, 846.

25. T. K. Venkatachalam, C. Mao, and F. M. Uckun, *Bioorg. Med. Chem.*, 2004, **12**, 4275.
26. W. C. Patt, H. W. Hamilton, M. D. Taylor, M. J. Ryan, D. G. Taylor Jr., C. J. C. Connolly, A. M. Doherty, S. R. Klutchko, and I. Sircar, *J. Med. Chem.*, 1992, **35**, 2562.
27. D. J. Carini and R. R. Wexler, Patent US4632930 A, 1986.12.30.
28. M. L. Barreca, J. Balzarini, A. Chimirri, E. De Clercq, L. De Luca, H. D. Höltje, M. Höltje, A. M. Monforte, P. Monforte, C. Pannecouque, A. Rao, and M. Zappalà, *J. Med. Chem.*, 2002, **45**, 5410.
29. S. Carradori, D. Secci, A. Bolasco, C. De Monte, and M. Yáñez, *Arch. Pharm.*, 2012, **345**, 973.
30. B. Jiang and X. H. Gu, *Bioorg. Med. Chem.*, 2000, **8**, 363.
31. F. N. Victoria, D. M. Martinez, M. Castro, A. M. Casaril, D. Alves, E. J. Lenardão, H. D. Salles, P. H. Schneider, and L. Savegnago, *Chem. Biol. Interact.*, 2013, **205**, 100.
32. N. M. Basha, G. Lavanya, A. Padmaja, and V. Padmavathi, *Arch. Pharm. Chem. Life Sci.*, 2013, **346**, 511.
33. S. Oncu, M. Punar, and H. Eraksoy, *Chemotherapy*, 2004, **39**, 546.
34. I. Parasotas, K. Anusevicius, I. Jonuskiene, and V. Mickevicius, *Chemija*, 2014, **25**, 107.
35. A. Voskiene, B. Sapijanskaite, V. Mickevicius, K. Kantminiene, M. Stasevych, O. Komarovska-Porokhnyavets, R. Musyanovych, and V. Novikov, *Monatsh. Chem.*, 2011, **142**, 529.
36. A. Franklin, J. Acar, F. Anthony, R. Gupta, T. Nicholls, Y. Tamura, S. Thompson, E. J. Thelfall, D. Vose, M. van Vuuren, D. G. White, H. C. Wegener, and M. L. Costarrica, *Rev. Sci. Tech. Off. Int. Epiz.*, 2001, **20**, 859.
37. B. Goodson, A. Ehrhardt, S. Ng, J. Nuss, K. Johnson, M. Giedlin, R. Yamamoto, A. Krebber, M. Ladner, M. B. Giacona, C. Vitt, and J. Winter, *Antimicrob. Agents Chemother.*, 1999, **43**, 1429.
38. A. Petersen, F. M. Aarestrup, M. Hofshagen, H. Sipilä, A. Franklin, and E. Gunnarsson, *Microb. Drug Resist.*, 2003, **9**, 381.