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# Synthesis and evaluation of the antibacterial, antioxidant activities of novel functionalized thiazole and bis(thiazol-5-yl)methane derivatives

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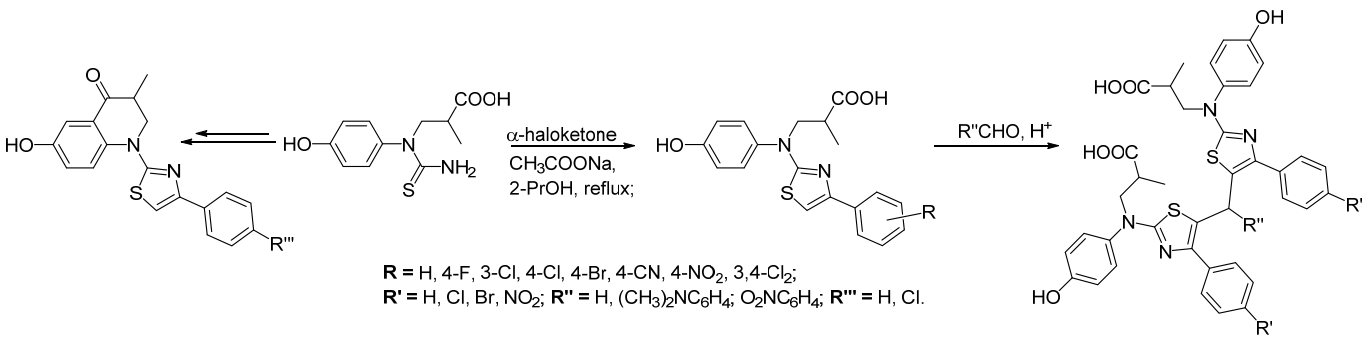
## Abstract

Novel functionalized thiazoles were prepared by the Hantzsch reaction from 3-[(4-hydroxyphenyl)carbamothioylamino]-2-methylpropanoic acid and the corresponding $\alpha$-halocarbonyl compounds in good yields. A series of chemical transformations of the obtained products were carried out, and new functionalized thiazole derivatives with aliphatic, aromatic and heterocyclic substituents were synthesized. 4-Phenyl-substituted *N*-(4-hydroxyphenyl)-*N*-carboxyalkylaminothiazoles were used as precursors for the synthesis of bis(thiazol-5-yl)methane derivatives, which then were screened for their antibacterial, antioxidant activities.



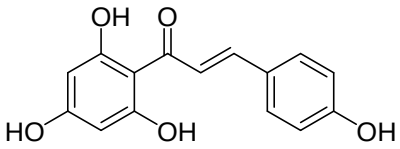

**Keywords:** Thiazole, dihydroquinolone, bis(thiazol-5-yl)methane, antibacterial, antioxidant activity

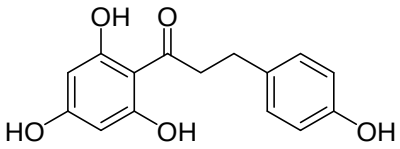
## Introduction

Thiazoles have found a wide spectrum of pharmacological and industrial applications. A series of synthesized thiazoles show biological activities: antioxidant,[1,2] antibacterial.[3-5] Aromatic ketone and enone compounds which form the central core for a variety of important biological compounds and which are known collectively as chalcones or chalconoids are typically found in plants. For example, **naringenin chalcone** found in the tomato peel shows the anti-allergic activity.[6] **Dihydrochalcone phlorizin** found in cherry, apple, pears inhibits SGLT1 and SGLT2 proteins which are responsible for glucose transport.[7] Because phlorizin can reduce glucose content in the blood,[8] it was studied as a potential pharmaceutical treatment for type II diabetes.[9] **Davidigenin** extracted from *Mascarenhasia arborescens* shows antispasmodic and antioxidant activities.[10] Chalcones have also good antibacterial,[11,12] anti-obesity,[13] immunosuppressant[14] properties.

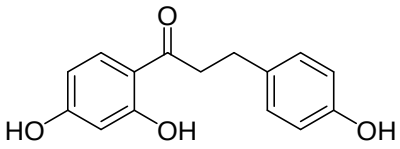
$\beta$-Amino acid fragments are widely encountered in living nature and show a variety of biological activities. For example, a dipeptide containing histidine and $\beta$-alanine fragments, called **anserine**, which can be found in the muscles and brain of mammals, shows an antitumor activity.[15] Anserine derivatives with $\beta$-cyclodextrin show good antioxidant properties.[16] $\beta$-Amino acid derivatives show antiseizure,[17] antimalarial[18] activities. Endomorphin-2 analogs containing *N*-methylated amino acids exhibit a strong analgesic effect.[19,20] As $\beta$-amino acid derivatives, compounds containing a thiazole ring also show strong bioactive properties, for example, vitamin $B_1$, penicillins.



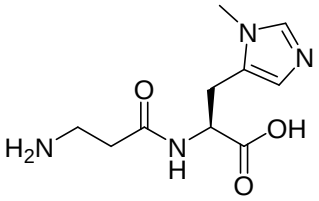
Naringenin chalcone



Phloretin



Davidigenin



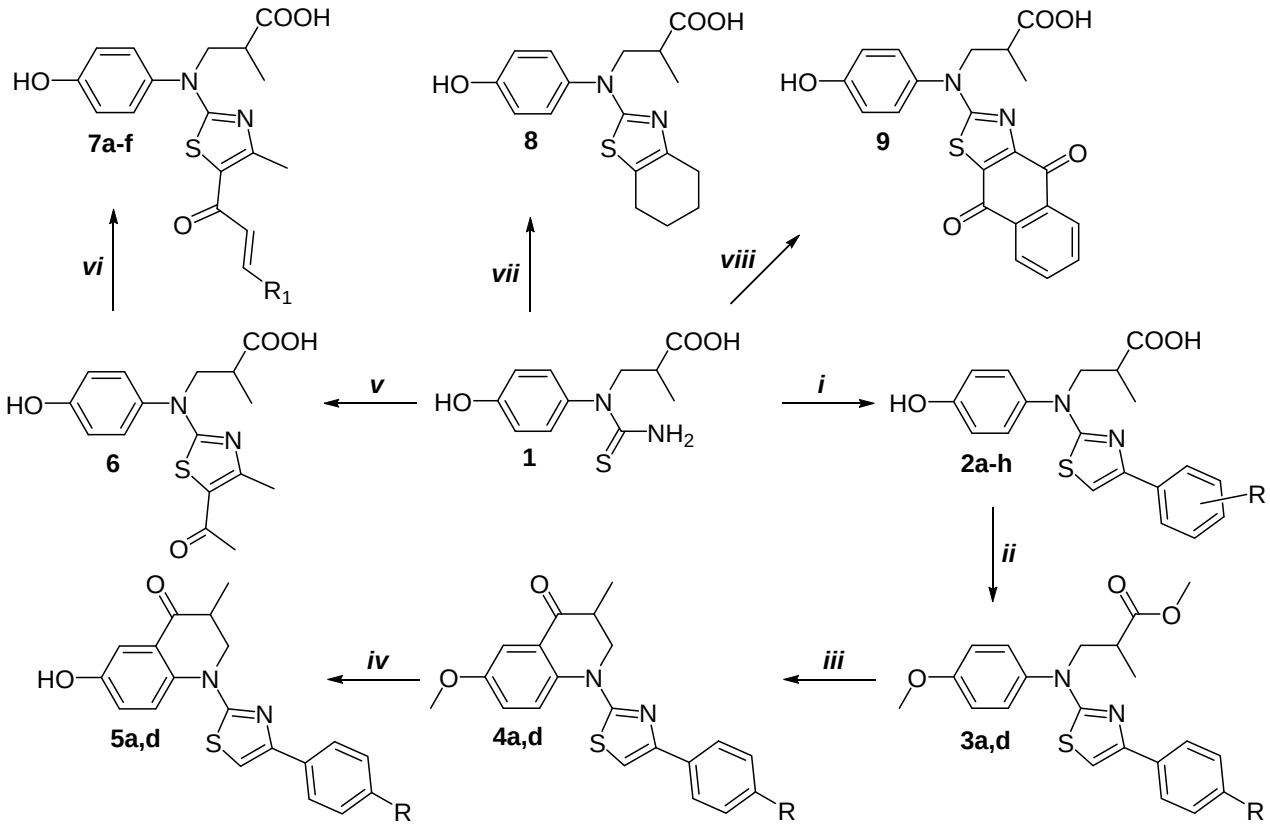
Anserine

In our previous papers,[21–23] we have reported the synthesis of *N*-aryl-*N*-carboxyethyl-2-aminothiazoles and their derivatives by the Hantzsch reaction, and some of the synthesized compounds showed antimicrobial activities. In continuation of our studies on the synthesis of functionalized thiazole derivatives, herein we report the synthesis of a new variety of functionalized thiazoles, bis(thiazol-5-yl)methanes and bis(thiazol-5-yl)phenylmethanes and the evaluation of their antibacterial and antioxidant activities.

## Results and Discussion

**Chemistry.** The synthetic sequence (Scheme 1) begins at the preparation of functionalized *N*,*N*-disubstituted 2-aminothiazoles **2a–h** from the thioureido acid **1** and various $\alpha$-haloketones. All reactions were carried out in refluxing 2-propanol, and the yield of the obtained products ranged within 59–87%.




**R = a)** H; **b)** 4-F; **c)** 3-Cl; **d)** 4-Cl; **e)** 4-Br; **f)** 4-CN; **g)** 4-$NO_2$; **h)** 3,4-$Cl_2$.
**$R_1$ = a)** 4-F-$C_6H_4$; **b)** 2-Cl-$C_6H_4$; **c)** 3-Cl-$C_6H_4$; **d)** 4-Cl-$C_6H_4$; **e)** 4-Br-$C_6H_4$; **f)** 2-thienyl.

***i*)** $\alpha$-haloketone, $CH_3COONa$, 2-PrOH, reflux, 4 h; ***ii*)** dimethyl sulphate, NaOH, acetone, reflux, 1 h; ***iii*)** PPA, 110 $^oC$, 16 h; ***iv*)** conc. HBr, $CH_3COOH$, reflux, 5 h; ***v*)** 3-chloro-2,4-pentanedione, acetone, reflux, 4 h, $H_2O$, $CH_3COONa$; ***vi*)** RCHO, MeOH, 10% NaOH aqueous solution, r.t., 24 h, $CH_3COOH$; ***vii*)** 2-bromocyclohexane-1-one, $CH_3COONa$, 2-PrOH, reflux, 2 h; ***viii*)** 2,3-dichloro-1,4-naphthoquinone, $CH_3COONa$, $CH_3COOH$, 80 $^oC$, 24 h.

**Scheme 1.** Synthesis of functionalized *N,N*-disubstituted 2-aminothiazole derivatives **2–9**.

The increasing interest to the dihydroquinolone-type compounds as potential therapeutic agents encouraged to investigate the synthesis of compounds with this core in the structure. The effort to synthesize the above-mentioned compounds according to the described method,[24] i.e. to cyclize those by heating in the polyphosphoric acid, failed probably because of the interaction of the hydroxy group with the phosphoric acid. Therefore, another pathway was chosen. Primarily, the hydroxy group was protected by alkylation with dimethyl sulphate, then the synthesized products **3a,d** were cyclized to **4a,d** using PPA, and subsequent cleavage of the ether bond by heating **4a,d** in the mixture of the acetic acid and hydrogen bromide afforded the desired products **5a,d** in high yields.

Treatment of thioureido acid **1** with 3-chloro-2,4-pentanedione yielded 57% of 3-((5-acetyl-4-methylthiazol-2-yl)(4-hydroxyphenyl)amino)-2-methylpropanoic acid **(6)**, which then was used for the synthesis of chalcone-type derivatives **7a–f**. In order to perform a more detailed investigation of the structure of the synthesized compounds **7** containing one asymmetric carbon atom, the X-ray analysis was carried out. The ORTEP diagram of the structure of compound **7c** is presented in Figure 1.

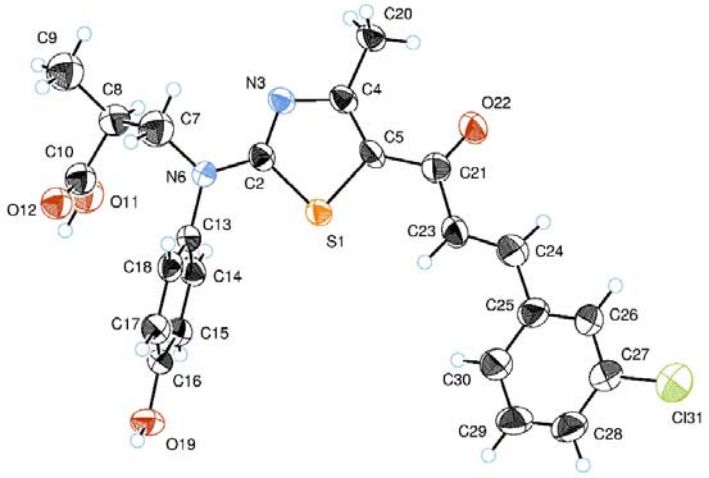


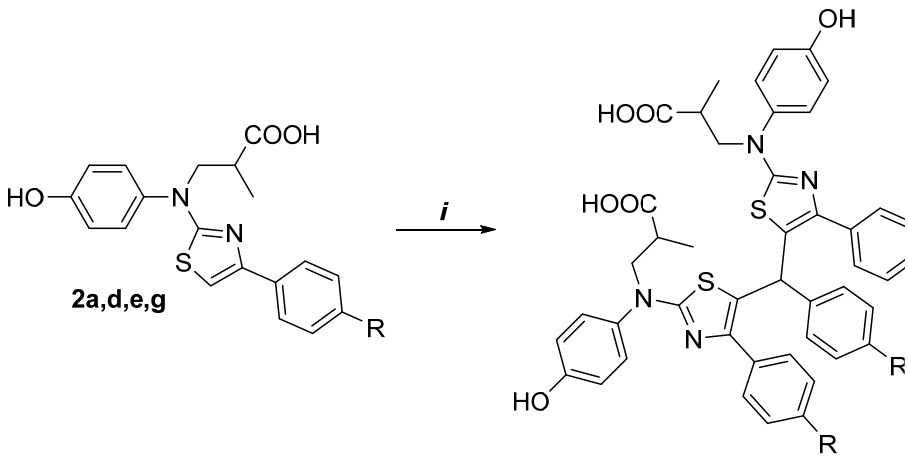

**Figure 1.** The ORTEP diagram of the structure **7c**.

It should be noted that in the crystalline state the compound **7c** exists in two conformations; this leads to a structural disorder. For the prevailing conformation (its occupation g-factor is equal to 0.75) the torsion angle of N6−C7−C8−C10 is 62.3(5)°; for the second conformation (with g 0.25) this angle is −93.4(9). Figure 1 shows the molecular structure in the dominant conformation.

The fused thiazole **8**, containing thiazole and cyclohexane fragments, was synthesized by the interaction of thioureido acid **1** and 2-bromocyclohexane-1-one in 2-propanol, in the presence of sodium acetate. The naphthoquinone-fused thiazole **9** was prepared from compound **1** and 2,3-dichloro-1,4-naphthoquinone by stirring them in acetic acid at 80 °C for 24 h, in the presence of sodium acetate. Efforts to obtain NMR spectrum data, due to the very poor solubility of the target substance **9** in organic solvents, were unsuccessful. Therefore, the structure of compound **9** was established by the data of MS and IR spectroscopy. The mass spectrum data show a positive molecular ion $[M+H]^+$ with 409.0854 *m/z*, the calculated monoisotopic mass for compound **9** ($C_{21}H_{16}N_2O_5S+H^+$) being 409.0853. The IR spectrum data show three strong absorption bands at about 1712, 1634, 1616 $cm^{-1}$, corresponding to two C=O groups in the naphthoquinone fragment and one in the carboxyl group.

On the strength of the raised purposes of this study, we tried to synthesize polyfunctionalized derivatives with thiazole fragments in the structure. The attempts were successful: the interaction of compounds **2** with aromatic aldehydes in the molar ratio of 2:1 resulted in a high yield of bis(thiazol-5-yl)phenyl methanes **10–15** (Scheme 2). The reactions were carried out in acetone under reflux for 17 h in the presence of a catalytic amount of hydrochloric acid. The resulting products precipitate in the form of insoluble salts in acetone. The microanalysis data showed that in the first case the double hydrochloric salts were formed. In the reactions with 4-(dimethylamino)benzaldehyde, the triple salts were obtained. These salts were converted to their base by dissolving them in 5% aqueous sodium carbonate and acidifying the solution with acetic acid to pH 6.

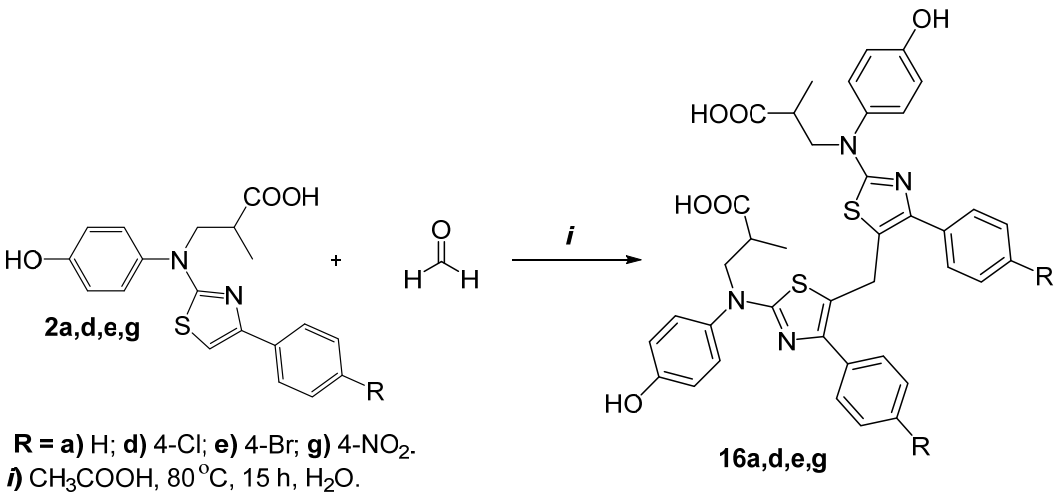
 




**R = a)** H; **d)** 4-Cl; **e)** 4-Br; **g)** 4-$NO_2$.
**R' = 10-12)** $NO_2$; **13-15)** $N(CH_3)_2$.
***i*)** R'CHO, HCl, acetone, reflux, 17 h; 5% aq. $Na_2CO_3$, $CH_3COOH$.

**Scheme 2.** Synthesis of polisubstituted bis(thiazolyl)phenylmethanes **10–15**.

The $^1$H NMR spectra of the synthesized compounds **10–15** showed a singlet at approx. 5.66 ppm, which was assigned to the SCCHCS group proton. A singlet at approx. 6.98 ppm characteristic of the CH group at the 5-position of the thiazole ring was not observed. The analysis of the aromatic region of the $^1$H NMR spectra showed the additional spectral peaks characteristic of a *p*-substituted phenyl ring. These data approve the formation of bis(thiazol-5-yl)phenyl methanes **10–15**. We have also found that thiazoles **2** easily react with formaldehyde to obtain bis(thiazol-5-yl)methanes **16a,d,e,g** (Scheme 3)**.** In this case, reactions were carried out in acetic acid, because in these conditions cleaner products are obtained.




**R = a)** H; **d)** 4-Cl; **e)** 4-Br; **g)** 4-$NO_2$.
***i*)** $CH_3COOH$, 80 °C, 15 h, $H_2O$.

**Scheme 3.** Synthesis of polisubstituted bis(thiazolyl)methanes **16**.

The structure of compounds **16** has been confirmed by methods of IR, $^1$H, $^{13}$C NMR spectroscopy and HRMS analysis.

**Biology.** All of the synthesized compounds (**2–16**) (50–1000 μg/mL) were evaluated for their antibacterial activity against the strains of *Rhizobium radiobacter*, *Xanthomonas campestris*, *Escherichia coli* by the diffusion technique.[25,26] Only some of them appeared to be active against the investigated strains of bacteria. The activities of the tested compounds were compared with those of the known antibacterial agent ampicillin (50 μg/mL). The most active were derivatives **10a**, **12e, 14e** against *Rhizobium radiobacter* and **12e**, **14e** against *Xanthomonas campestris* at the concentration of 50 μg/mL. The evaluation of the antibacterial activity against *Escherichia coli* revealed that the most active appeared to be derivatives **10a**, **12e**, **14e** at the concentration of 125 μg/mL.

The antioxidant properties of the compounds **10–16** were evaluated using different protocols including free radical scavenging (DPPH), the ferric reducing antioxidant power (FRAP), the reducing power assay (Figures 2–4).

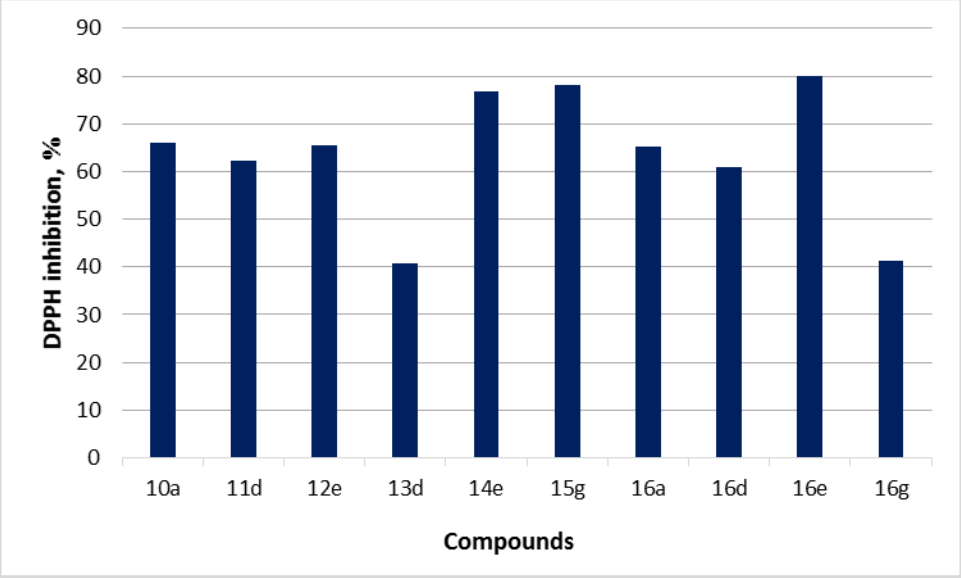



**Figure 2.** Antioxidant activity of the synthesized compounds **10–16** against DPPH.

The antioxidative activity of the synthesized compounds was evaluated by the 2,2-diphenyl-1-(2,4,6-trinitrophenyl)hydrazyl (DPPH) radical scavenging method. The results presented in Figure 2 showed that compounds **16e (**80.12%), **15g** (78.20%), **14e (**76.92%) possess very high radical scavenging abilities.

The ferric reducing antioxidant power (FRAP) method is based on the reduction of a ferroin analog, the $Fe^{3+}$ complex of tripyridyltriazine $Fe(TPTZ)^{3+}$, to the intensely blue coloured $Fe^{2+}$ complex $Fe(TPTZ)^{2+}$ by antioxidants in an acidic medium. The results are obtained as the absorbance increases at 593 nm and can be expressed as a $Fe^{2+}$μmol/L concentration.

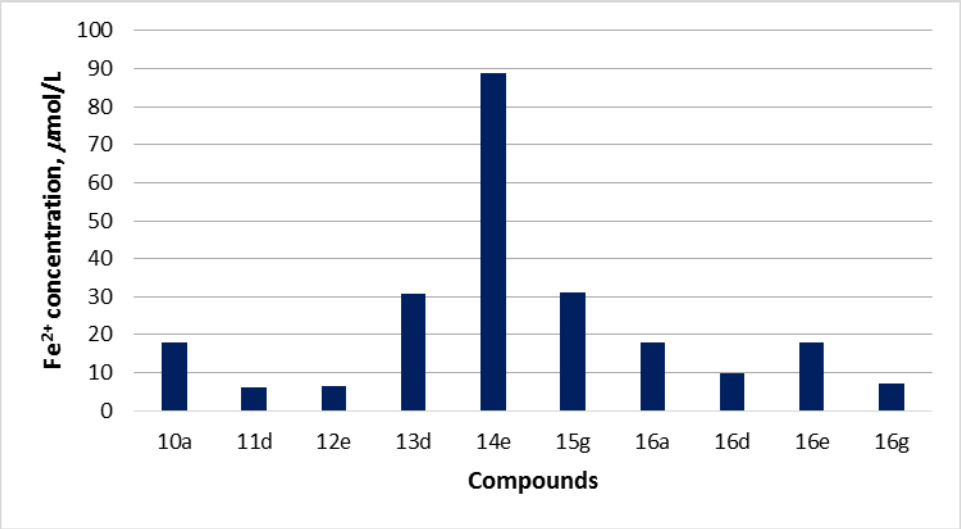



**Figure 3.** Antioxidant activity of the synthesized compounds **10–16** evaluated by FRAP method.

The results revealed (Figure 3) that compounds **14e** (88.72 $Fe^{2+}\mu$mol/L), **15g** (31.33 $Fe^{2+}\mu$mol/L), **13d** (30.67 $Fe^{2+}\mu$mol/L) showed the highest antioxidative activity evaluated by FRAP method.

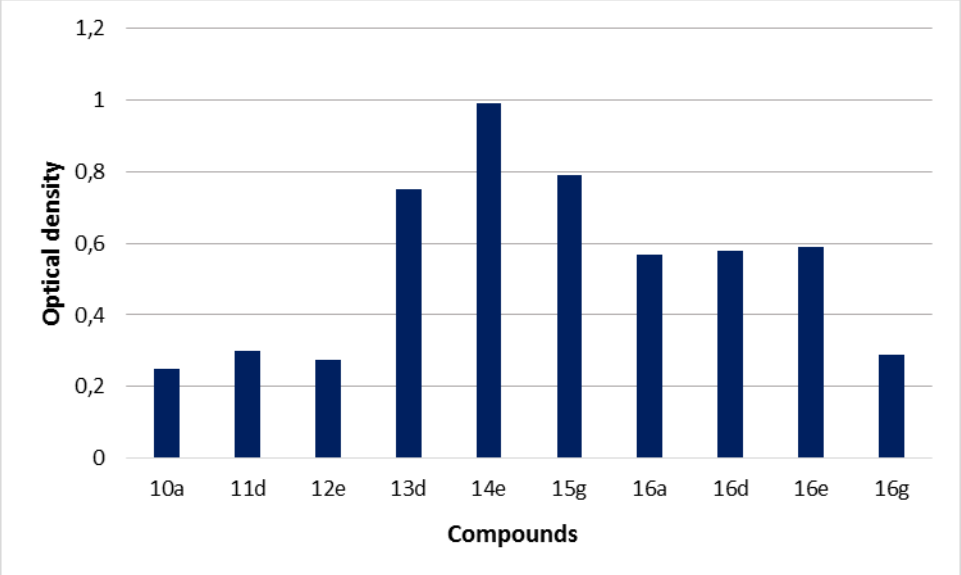



**Figure 4.** Reducing power of the synthesized compounds **10–16**.

In the reducing power assay, the presence of reductants (antioxidants) in a sample would result in the reducing of $Fe^{3+}$ to $Fe^{2+}$ by donating an electron. The amount of the $Fe^{2+}$ complex can then be monitored by measuring the formation of Perl's blue at 700 nm. The results of the reducing power assay (Figure 4) demonstrate that compounds **14e**, **15g**, **13d** exhibit antioxidant effect.

## Conclusions

Novel functionalized thiazoles with the $\alpha$-methyl-$\beta$-alanine fragment were synthesized from *N*-(4-hydroxyphenyl)-*N*-thiocarbamoyl-$\alpha$-methyl-$\beta$-alanine and $\alpha$-haloketones by the Hantzsch method. These

compounds were used for the synthesis of dihydroquinolone substituted thiazoles, symmetric polyfunctionalized bis(thiazol-5-yl)phenyl- and bis(thiazol-5-yl)methanes. Some of the bis(thiazol-5-yl)phenyl- and bis(thiazol-5-yl)methanes exhibited weak antibacterial and high antioxidant activity. It was found that bis(thiazol-5-yl)phenylmethanes exhibit a higher antibacterial and antioxidant activity in comparison with bis(thiazol-5-yl)methanes.

## Experimental Section

**General.** TLC was performed with Merck, Silica gel 60 F254 (Kieselgel 60 F254) silica gel plates. The $^{1}$H and $^{13}$C NMR spectra were recorded by the Bruker Ascend 400 ($^{1}$H 400 MHz, $^{13}$C 101 MHz) and Bruker Ascend ($^{1}$H 700 MHz, $^{13}$C 176 MHz) spectrometers. Chemical shifts are expressed as $\delta$, ppm relative to TMS. The IR spectra ($\nu$, cm$^{-1}$) were recorded on a Perkin Elmer Spectrum Bx FT-IR spectrometer using KBr tablets. Elemental analyses were performed with a CE-440 elemental analyzer. Melting points were determined with a B-540 Melting Point Analyzer (Buchi Corporation, USA) and are uncorrected. Mass spectra were measured with the Xevo TQ-S and Bruker maXis 4G mass spectrometers. The X-ray crystallographic analysis was performed with a Bruker-Nonius KappaCCD diffractometer using the graphite monochromated Mo-Kα radiation ($\lambda$ 0.71073 Å).

**General procedure for the synthesis of compounds 2a–h.** A mixture of compound **1** (0.64 g, 2.5 mmol), the corresponding $\alpha$-haloketone (2.75 mmol), sodium acetate (0.42 g, 5 mmol) and 2-propanol (10 mL) was refluxed for 4 h, cooled to room temperature and diluted with water (30 mL). The formed precipitate was filtered off, washed with water, dried. Purification was performed by dissolving crystals in 5% aqueous sodium hydroxide, filtering, and acidifying the filtrate with acetic acid to pH 6.

**3-((4-Hydroxyphenyl)(4-phenylthiazol-2-yl)amino)-2-methylpropanoic acid (2a).** White powder, yield 0.62 g (70%), mp 165–166 °C. IR (KBr, vmax, cm$^{-1}$): 1513 (C=N), 1699 (C=O), 3174 (O-H). $^{1}$H NMR(400 MHz, DMSO-$d_6$), $\delta$, ppm ($J$, Hz): 1.14 (3H, d, $J$ 7.0, $CH_3$), 2.77–2.92 (1H$_X$, m, CH), 4.02 (1H$_B$, dd, $J^{BA}$ 13.6, $J^{BX}$ 7.3, $CH_2$), 4.09 (1H$_A$, dd, $J^{AB}$ 13.6, $J^{AX}$ 7.2, $CH_2$), 6.86 (2H, d, $J$ 8.7, H$_{Ar}$), 7.07 (1H, s, SCH), 7.23 (2H, d, $J$ 8.7, H$_{Ar}$), 7.29 (1H, d, $J$ 7.3, H$_{Ar}$), 7.39 (2H, t, $J$ 7.6, H$_{Ar}$), 7.85 (2H, d, $J$ 7.2, H$_{Ar}$), 10.18 (1H, s, OH), 11.84 (1H, s, COOH). $^{13}$C NMR (101 MHz, DMSO-$d_6$), $\delta$, ppm: 15.0; 38.1; 55.3; 102.5; 116.6; 125.7; 127.5; 128.6; 129.0; 134.8; 136.0; 150.4; 156.9; 170.8; 176.0. Found, %: C 64.28; H, 5.25; N, 7.86. $C_{19}H_{18}N_2O_3S$. Calculated, %: C 64.39; H, 5.12; N, 7.90.

**3-((4-(4-Fluorophenyl)thiazol-2-yl)(4-hydroxyphenyl)amino)-2-methylpropanoic acid (2b).** White solid, yield 0.71 g (76%), mp 199–200 °C. IR (KBr, vmax, cm$^{-1}$): 1516 (C=N), 1699 (C=O), 3177 (O-H). $^{1}$H NMR (400 MHz, Acetone-$d_6$), $\delta$, ppm ($J$, Hz): 1.22 (3H, d, $J$ 7.1, $CH_3$), 2.94–3.11 (1H$_X$, m, CH), 4.08 (1H$_B$, dd, $J^{BA}$ 13.6, $J^{BX}$ 7.1, $CH_2$), 4.20 (1H$_A$, dd, $J^{AB}$ 13.6, $J^{AX}$ 7.3, $CH_2$), 6.85 (1H, s, SCH), 6.92 (2H, d, $J$ 8.7, H$_{Ar}$), 7.11 (2H, t, $J$ 8.9, H$_{Ar}$), 7.27 (2H, d, $J$ 8.7, H$_{Ar}$), 7.89–7.96 (2H, m, H$_{Ar}$), 8.73 (1H, s, OH). $^{13}$C NMR (101 MHz, Acetone-$d_6$), $\delta$, ppm: 15.5; 39.1; 56.6; 102.4; 116.1; 117.5; 128.7; 130.1; 132.8; 138.0; 151.0; 158.0; 161.9; 172.3; 176.2. Found, %: C, 61.44; H, 4.73; N, 7.43. $C_{19}H_{17}FN_2O_3S$. Calculated, %: C, 61.28; H, 4.60; N, 7.52.

**3-((4-(3-Chlorophenyl)thiazol-2-yl)(4-hydroxyphenyl)amino)-2-methylpropanoic acid (2c).** White powder, yield 0.57 g (59%), mp 148–149 °C. IR (KBr, vmax, cm$^{-1}$): 1511 (C=N), 1702 (C=O), 3144 (O-H). $^{1}$H NMR (400 MHz, DMSO-$d_6$), $\delta$, ppm ($J$, Hz): 1.13 (3H, d, $J$ 7.0, $CH_3$), 2.69–2.88 (1H$_X$, m, CH), 4.00 (1H$_B$, dd, $J^{BA}$ 13.6, $J^{BX}$ 7.4, $CH_2$), 4.09 (1H$_A$, dd, $J^{AB}$ 13.6, $J^{AX}$ 7.1, $CH_2$), 6.86 (2H, d, $J$ 8.7, H$_{Ar}$), 7.22 (1H, s, SCH), 7.23 (2H, d, $J$ 8.7, H$_{Ar}$), 7.33 (1H, d, $J$ 8.2, H$_{Ar}$), 7.42 (1H, t, $J$ 7.9, H$_{Ar}$), 7.82 (1H, d, $J$ 7.8, H$_{Ar}$), 7.89 (1H, t, $J$ 1.7, H$_{Ar}$), 11.03 (1H, s, COOH). $^{13}$C NMR (101 MHz, DMSO-d$_6$), $\delta$, ppm: 15.1; 38.2; 55.4; 104.0; 116.6; 124.2; 125.2; 127.2; 128.9; 130.5; 133.4; 135.8; 136.9; 148.7; 157.0; 171.0; 176.1. Found, %: C, 58.80; H, 4.48; N, 7.28. $C_{19}H_{17}ClN_2O_3S$. Calculated, %: C, 58.69; H, 4.41; N, 7.20.

**3-((4-(4-Chlorophenyl)thiazol-2-yl)(4-hydroxyphenyl)amino)-2-methylpropanoic acid (2d).** White powder, yield 0.77 g (79%), mp 200–201 °C. IR (KBr, vmax, $cm^{-1}$): 1515 (C=N), 1701 (C=O), 3111 (O-H). $^1$H NMR (400 MHz, DMSO-$d_6$), $\delta$, ppm ($J$, Hz): 1.14 (3H, d, $J$ 7.0, $CH_3$), 2.74–2.87 ($1H_X$, m, CH), 4.00 ($1H_B$, dd, $J^{BA}$ 13.6, $J^{BX}$ 7.2, $CH_2$), 4.09 ($1H_A$, dd, $J^{AB}$ 13.6, $J^{AX}$ 7.3, $CH_2$), 6.85 (2H, d, $J$ 8.7, $H_{Ar}$), 7.13 (1H, s, SCH), 7.23 (2H, d, $J$ 8.7, $H_{Ar}$), 7.45 (2H, d, $J$ 8.5, $H_{Ar}$), 7.86 (2H, d, $J$ 8.5, $H_{Ar}$), 9.77 (1H, s, OH), 12.31 (1H, s, COOH). $^{13}$C NMR (101 MHz, DMSO-$d_6$), $\delta$, ppm: 15.0; 38.1; 55.2; 103.3; 116.6; 127.4; 128.6; 129.0; 131.9; 133.7; 135.9; 149.1; 157.0; 171.0; 176.0. Found, %: C, 58.63; H, 4.35; N, 6.99. $C_{19}H_{17}ClN_2O_3S$. Calculated, %: C, 58.69; H, 4.41; N, 7.20.

**3-((4-(4-Bromophenyl)thiazol-2-yl)(4-hydroxyphenyl)amino)-2-methylpropanoic acid (2e).** White solid, yield 0.94 g (87%), mp 191–192 °C. IR (KBr, vmax, $cm^{-1}$): 1515 (C=N), 1700 (C=O), 3110 (O-H). $^1$H NMR (400 MHz, DMSO-$d_6$), $\delta$, ppm ($J$, Hz): 1.13 (3H, d, $J$ 7.0, $CH_3$), 2.70–2.89 ($1H_X$, m, CH), 4.01 ($1H_B$, dd, $J^{BA}$ 13.5, $J^{BX}$ 7.3, $CH_2$), 4.08 ($1H_A$, dd, $J^{AB}$ 13.5, $J^{AX}$ 7.2, $CH_2$), 6.85 (2H, d, $J$ 8.6, $H_{Ar}$), 7.14 (1H, s, SCH), 7.23 (2H, d, $J$ 8.6, $H_{Ar}$), 7.58 (2H, d, $J$ 8.4, $H_{Ar}$), 7.80 (2H, d, $J$ 8.4, $H_{Ar}$), 10.28 (1H, s, OH), 11.76 (1H, s, COOH). $^{13}$C NMR (101 MHz, DMSO-$d_6$), $\delta$, ppm: 15.1; 38.1; 55.2; 103.4; 116.6; 120.5; 127.7; 129.0; 131.5; 134.0; 135.9; 149.2; 157.0; 171.4; 176.1. Found, %: C, 52.56; H, 4.00; N, 6.27. $C_{19}H_{17}BrN_2O_3S$. Calculated, %: C, 52.67; H, 3.95; N, 6.46.

**3-((4-(4-Cyanophenyl)thiazol-2-yl)(4-hydroxyphenyl)amino)-2-methylpropanoic acid (2f).** Orange powder, yield 0.75 g (73%), mp 213–214 °C. IR (KBr, vmax, $cm^{-1}$): 1513 (C=N), 1708 (C=O), 2235 (C≡N), 3256 (O-H). $^1$H NMR (400 MHz, DMSO-$d_6$), $\delta$, ppm ($J$, Hz): 1.14 (3H, d, $J$ 6.9, $CH_3$), 2.73–2.89 ($1H_X$, m, CH), 4.01 ($1H_B$, dd, $J^{BA}$ 13.5, $J^{BX}$ 7.2, $CH_2$), 4.10 ($1H_A$, dd, $J^{AB}$ 13.5, $J^{AX}$ 7.3, $CH_2$), 6.86 (2H, d, $J$ 8.5, $H_{Ar}$), 7.24 (2H, d, $J$ 8.5, $H_{Ar}$), 7.38 (1H, s, SCH), 7.85 (2H, d, $J$ 8.2, $H_{Ar}$), 8.03 (2H, d, $J$ 8.2, $H_{Ar}$), 10.02 (1H, s, OH), 12.11 (1H, s, COOH). $^{13}$C NMR (101 MHz, DMSO-$d_6$), $\delta$, ppm: 15.0; 38.1; 55.3; 106.4; 109.5; 116.7; 119.2; 126.2; 129.0; 132.7; 135.8; 138.9; 148.7; 157.1; 171.2; 176.0. Found, %: C, 63.41; H, 4.52; N, 11.17. $C_{20}H_{17}N_3O_3S$. Calculated, %: C, 63.31; H, 4.52; N, 11.07.

**3-((4-Hydroxyphenyl)(4-(4-nitrophenyl)thiazol-2-yl)amino)-2-methylpropanoic acid (2g).** Bright orange powder. Yield 0.81 g (81%), mp 184–185 °C. IR (KBr, vmax, $cm^{-1}$): 1512 (C=N), 1713 (C=O), 3246 (O-H). $^1$H NMR (700 MHz, Acetone-$d_6$), $\delta$, ppm ($J$, Hz): 1.27 (3H, d, $J$ 7.0, $CH_3$), 3.01–3.15 ($1H_X$, m, CH), 4.14 ($1H_B$, dd, $J^{BA}$ 13.7, $J^{BX}$ 7.1, $CH_2$), 4.27 ($1H_A$, dd, $J^{AB}$ 13.7, $J^{AX}$ 7.3, $CH_2$), 6.97 (2H, d, $J$ 8.1, $H_{Ar}$), 7.30 (1H, s, SCH), 7.33 (2H, d, $J$ 8.1, $H_{Ar}$), 8.18 (2H, d, $J$ 8.3, $H_{Ar}$), 8.26 (2H, d, $J$ 8.3, $H_{Ar}$), 8.75 (1H, s, OH). $^{13}$C NMR (176 MHz, Acetone-$d_6$), $\delta$, ppm: 15.5; 39.0; 56.6; 107.3; 117.6; 124.7; 127.4; 130.2; 137.7; 142.2; 147.7; 150.0; 158.2; 172.6; 176.1. Found, %: C, 57.29; H, 4.38; N, 10.63. $C_{19}H_{17}N_3O_5S$. Calculated, %: C, 57.13; H, 4.29; N, 10.52.

**3-((4-(3,4-Dichlorophenyl)thiazol-2-yl)(4-hydroxyphenyl)amino)-2-methylpropanoic acid (2h).** White solid, yield 0.68 g (64%), mp 186–187 °C. IR (KBr, vmax, $cm^{-1}$): 1515 (C=N), 1702 (C=O), 3180 (O-H). $^1$H NMR (400 MHz, DMSO-$d_6$), $\delta$, ppm ($J$, Hz): 1.13 (3H, d, $J$ 7.0, $CH_3$), 2.68–2.87 ($1H_X$, m, CH), 3.99 ($1H_B$, dd, $J^{BA}$ 13.6, $J^{BX}$ 7.2, $CH_2$), 4.11 ($1H_A$, dd, $J^{AB}$ 13.6, $J^{AX}$ 7.3, $CH_2$), 6.86 (2H, d, $J$ 8.7, $H_{Ar}$), 7.23 (2H, d, $J$ 8.8, $H_{Ar}$), 7.29 (1H, s, SCH), 7.65 (1H, d, $J$ 8.4, $H_{Ar}$), 7.84 (1H, dd, $J$ 8.4, $H_{Ar}$), 8.07 (1H, d, $J$ 2.0, $H_{Ar}$), 9.84 (1H, s, OH), 12.25 (1H, s, COOH). $^{13}$C NMR (101 MHz, DMSO-$d_6$), $\delta$, ppm: 15.0; 38.0; 55.3; 104.7; 116.6; 125.7; 127.2; 129.0; 129.6; 130.8; 131.4; 135.4; 135.7; 147.8; 157.1; 171.1; 176.0. Found, %: C, 53.73; H, 3.76; N, 6.74. $C_{19}H_{16}Cl_2N_2O_3S$. Calculated, %: C, 53.91; H, 3.81; N, 6.62.

**General procedure for the synthesis of compounds 3a,d.** A mixture of compound **2a** or **2d** (2.8 mmol), dimethyl sulphate (0.66 ml, 7 mmol), sodium hydroxide (0.34 g, 8.4 mmol) and acetone (15 mL) was refluxed for 1 h. Then acetone was removed under reduced pressure, and the obtained oily mass was washed a few times with water and recrystallized from the MeOH/$H_2O$ mixture.

**Methyl 3-((4-methoxyphenyl)(4-phenylthiazol-2-yl)amino)-2-methylpropanoate (3a).** White solid, yield 0.99 g (92%), mp 163–164 °C. IR (KBr, vmax, $cm^{-1}$): 1509 (C=N), 1732 (C=O). $^1$H NMR (400 MHz, Acetone-$d_6$), $\delta$, ppm ($J$, Hz): 1.22 (3H, d, $J$ 7.1, $CH_3$), 3.00–3.14 ($1H_X$, m, CH), 3.51 (3H, s, $CH_3$), 3.85 (3H, s, $CH_3$), 4.11 ($1H_B$, dd, $J^{BA}$

13.7, $J^{BX}$ 6.2, $CH_2$), 4.25 (1$H_A$, dd, $J^{AB}$ 13.7, $J^{AX}$ 8.1, $CH_2$), 6.87–7.96 (10H, m, $H_{Ar}$, SCH). $^{13}$C NMR (101 MHz, Acetone-$d_6$), $\delta$, ppm: 15.2; 39.1; 51.7; 55.7; 56.6; 102.6; 115.9; 126.6; 128.1; 129.2; 129.9; 136.1; 138.7; 152.0; 159.9; 171.5; 175.5. Found, %: C, 65.79; H, 5.91; N, 7.22. $C_{21}H_{22}N_2O_3S$. Calculated, %: C, 65.95; H, 5.80; N, 7.32.

**Methyl 3-((4-(4-chlorophenyl)thiazol-2-yl)(4-methoxyphenyl)amino)-2-methylpropanoate (3d).** White solid, yield 1.03 g (88%), mp 154–155 °C. IR (KBr, vmax, $cm^{-1}$): 1508 (C=N), 1729 (C=O). $^1$H NMR (400 MHz, Acetone-$d_6$), $\delta$, ppm ($J$, Hz): 1.21 (3H, d, $J$ 7.1, $CH_3$), 2.99–3.13 (1$H_X$, m, CH), 3.51 (3H, s, $CH_3$), 3.85 (3H, s, $CH_3$), 4.09 (1$H_B$, dd, $J^{BA}$ 13.7, $J^{BX}$ 6.2, $CH_2$), 4.25 (1$H_A$, dd, $J^{AB}$ 13.7, $J^{AX}$ 8.1, $CH_2$), 6.85–8.09 (9H, m, $H_{Ar}$, SCH). $^{13}$C NMR (101 MHz, Acetone-$d_6$), $\delta$, ppm: 15.3; 39.2; 51.8; 55.8; 56.7; 103.5; 116.0; 128.3; 129.3; 130.0; 133.3; 134.9; 138.7; 150.8; 160.0; 171.8; 175.6. Found, %: C, 60.39; H, 5.15; N, 6.75. $C_{21}H_{21}ClN_2O_3S$. Calculated, %: C, 60.50; H, 5.08; N, 6.72.

**General procedure for the synthesis of compounds 4a,d.** A mixture of compound **3a** or **3d** (2.4 mmol) and polyphosphoric acid (15 mL) was stirred at 110 °C for 16 h. Then the reaction mixture was cooled to room temperature, and crushed ice was added. The formed yellow precipitate was filtered off, washed with water until the pH of the filtrate became neutral (pH 7), and recrystallized from the MeOH/$H_2O$ mixture.

**6-Methoxy-3-methyl-1-(4-phenylthiazol-2-yl)-2,3-dihydroquinolin-4(1*H*)-one (4a).** Yellow solid, yield 0.72 g (85%), mp 193–194 °C. IR (KBr, vmax, $cm^{-1}$): 1510 (C=N), 1715 (C=O). $^1$H NMR (400 MHz, acetone-$d_6$), $\delta$, ppm ($J$, Hz): 1.21 (3H, d, $J$ 7.0, $CH_3$), 2.88–2.95 (1$H_X$, m, CH), 3.84 (3H, s, $CH_3$), 3.98 (1$H_B$, dd, $J^{BA}$ 13.0, $J^{BX}$ 10.8, $CH_2$), 4.57 (1$H_A$, dd, $J^{AB}$ 13.0, $J^{AX}$ 4.8, $CH_2$), 7.17–8.16 (9H, m, $H_{Ar}$, SCH). $^{13}$C NMR (101 MHz, Acetone-$d_6$), $\delta$, ppm: 12.7; 42.2; 55.8; 56.0; 104.5; 110.4; 115.9; 122.4; 122.7; 126.7; 128.5; 129.3; 135.5; 140.6; 152.0; 156.3; 167.4; 195.9. Found, %: C, 68.48; H, 5.27; N, 8.10. $C_{20}H_{18}N_2O_2S$. Calculated, %: C, 68.55; H, 5.18; N, 7.99.

**1-(4-(4-Chlorophenyl)thiazol-2-yl)-6-methoxy-3-methyl-2,3-dihydroquinolin-4(1*H*)-one (4d).** Yellow solid, yield 0.76 g (83%), mp 173–174 °C. IR (KBr, vmax, $cm^{-1}$): 1491 (C=N), 1687 (C=O). $^1$H NMR (400 MHz, Acetone-$d_6$), $\delta$, ppm ($J$, Hz): 1.22 (3H, d, $J$ 7.0, $CH_3$), 2.86–3.07 (1$H_X$, m, CH), 3.86 (3H, s, $CH_3$), 4.02 (1$H_B$, dd, $J^{BA}$ 13.0, $J^{BX}$ 10.8, $CH_2$), 4.60 (1$H_A$, dd, $J^{AB}$ 13.0, $J^{AX}$ 4.7, $CH_2$), 7.16–8.18 (8H, m, $H_{Ar}$, SCH). $^{13}$C NMR (101 MHz, Acetone-$d_6$), $\delta$, ppm: 12.7; 42.3; 55.9; 56.0; 105.2; 110.4; 122.5; 122.7; 124.7; 128.3; 129.4; 133.7; 134.4; 140.6; 150.7; 156.5; 167.7; 195.9. Found, %: C, 62.53; H, 4.30; N, 7.39. $C_{20}H_{17}ClN_2O_2S$. Calculated, %: C, 62.41; H, 4.45; N, 7.28.

**General procedure for the synthesis of compounds 5a,d.** A mixture of compound **4a** or **4d** (1.4 mmol), concentrated hydrobromic (5 mL) and acetic (5 mL) acids was refluxed for 5 h. Then the acids were removed under reduced pressure, the residue was suspended in 10 % sodium carbonate aqueous solution and thoroughly mixed. The obtained yellow precipitate was filtered off, washed with water and recrystallized from the MeOH/$H_2O$ mixture.

**6-Hydroxy-3-methyl-1-(4-phenylthiazol-2-yl)-2,3-dihydroquinolin-4(1*H*)-one (5a).** Yellow solid, yield 0.38 g (81%), mp 162–163 °C. IR (KBr, vmax, $cm^{-1}$): 1493 (C=N), 1683 (C=O), 3419 (O-H). $^1$H NMR (400 MHz, Acetone-$d_6$), $\delta$, ppm ($J$, Hz): 1.22 (3H, d, $J$ 7.0, $CH_3$), 2.91–3.11 (1$H_X$, m, CH), 4.10 (1$H_B$, dd, $J^{BA}$ 13.3, $J^{BX}$ 7.0, $CH_2$), 4.60 (1$H_A$, dd, $J^{AB}$ 13.3, $J^{AX}$ 7.5, $CH_2$), 7.20–8.05 (9H, m, $H_{Ar}$, SCH). $^{13}$C NMR (101 MHz, Acetone-$d_6$), $\delta$, ppm: 12.6; 25.5; 57.2; 113.5; 118.2; 122.9; 123.0; 126.2; 127.0; 127.3; 127.8; 129.2; 129.3; 129.5; 138.5; 155.6; 195.8. Found, %: C, 67.93; H, 4.70; N, 8.39. $C_{19}H_{16}N_2O_2S$. Calculated, %: C, 67.84; H, 4.79; N, 8.33.

**1-(4-(4-Chlorophenyl)thiazol-2-yl)-6-hydroxy-3-methyl-2,3-dihydroquinolin-4(1*H*)-one (5d).** Yellow solid, yield 0.45 g (87%), mp 178–179 °C. IR (KBr, vmax, $cm^{-1}$): 1494 (C=N), 1669 (C=O), 3398 (O-H). $^1$H NMR (400 MHz, Acetone-$d_6$), $\delta$, ppm ($J$, Hz): 1.21 (3H, d, $J$ 7.0, $CH_3$), 2.89–3.08 (1$H_X$, m, CH), 4.01 (1$H_B$, dd, $J^{BA}$ 13.1, $J^{BX}$ 7.0, $CH_2$), 4.64 (1$H_A$, dd, $J^{AB}$ 13.1, $J^{AX}$ 4.7, $CH_2$), 7.12–8.00 (8H, m, $H_{Ar}$, SCH). $^{13}$C NMR (101 MHz, Acetone-$d_6$), $\delta$, ppm: 12.8; 42.3; 55.9; 104.8; 113.1; 122.6; 122.9; 125.3; 128.3; 129.3; 133.6; 134.3; 139.5; 150.6; 154.5; 167.9; 196.1. Found, %: C, 61.70; H, 4.15; N, 7.49. $C_{19}H_{15}ClN_2O_2S$. Calculated, %: C, 61.54; H, 4.08; N, 7.55.

**3-((5-Acetyl-4-methylthiazol-2-yl)(4-hydroxyphenyl)amino)-2-methylpropanoic acid (6).** A mixture of compound **1** (1.27 g, 5 mmol), 3-chloro-2,4-pentanedione (0.56 ml, 5 mmol) and acetone (15 mL) was heated under reflux for 4 h. Then the reaction mixture was cooled to room temperature, diluted with water (45 mL) and sodium acetate (0.41 g, 5 mmol) was added. The formed precipitate was filtered off, washed with water, dried. White powder, yield 0.95 g (57%), mp 183–184 °C (EtOH/$H_2O$). IR (KBr, vmax, $cm^{-1}$): 1513 (C=N), 1710 (C=O), 3149 (O-H). $^1$H NMR (400 MHz, DMSO-$d_6$), $\delta$, ppm ($J$, Hz): 1.08 (3H, d, $J$ 7.0, $CH_3$), 2.28 (3H, s, $CH_3$), 2.47 (3H, s, $CH_3$), 2.61–2.72 (1$H_X$, m, CH), 3.99 (1$H_B$, dd, $J^{BA}$ 13.7, $J^{BX}$ 7.3, $CH_2$), 4.05 (1$H_A$, dd, $J^{AB}$ 13.7, $J^{AX}$ 7.5, $CH_2$), 6.86 (2H, d, $J$ 8.7, $H_{Ar}$), 7.21 (2H, d, $J$ 8.8, $H_{Ar}$), 9.87 (1H, s, OH), 12.36 (1H, s, COOH). $^{13}$C NMR (101 MHz, DMSO-$d_6$), $\delta$, ppm: 14.8; 18.7; 29.6; 37.8; 54.4; 116.7; 122.3; 128.7; 134.6; 157.5; 157.6; 172.1; 175.6; 188.7. Found, %: C, 57.34; H, 5.30; N, 8.47. $C_{16}H_{18}N_2O_4S$. Calculated, %: C, 57.47; H, 5.43; N, 8.38.

**General procedures for the synthesis of compounds 7a–f.** A mixture of the corresponding aldehyde (3.84 mmol) and compound **6** (1.07 g, 3.2 mmol) was dissolved in a mixture of 10% aqueous NaOH and methanol 1:1 (15 mL) and stirred at room temperature for 24 h. Then the reaction mixture was diluted with water (20 mL) and acidified with acetic acid to pH 6. The formed chalcone was filtered off and washed with water, dried and recrystallized from 2-propanol.

**(*E*)-3-(5-(3-(4-Fluorophenyl)acryloyl)-4-methylthiazol-2-yl)(4-hydroxyphenyl)amino)-2-methylpropanoic acid (7a).** Bright yellow powder, yield 0.95 g (68%), mp 137–138 °C. IR (KBr, vmax, $cm^{-1}$): 1509 (C=N), 1711 (C=O), 3194 (O-H). $^1$H NMR (400 MHz, DMSO-$d_6$), δ, ppm ($J$, Hz): 1.11 (3H, d, $J$ 7.2, $CH_3$), 2.59 (3H, s, $CH_3$), 2.66–2.73 (1H, m, $CH_3C\underline{H}$), 3.97–4.17 (2H, m, $CH_2$), 6.89 (2H, d, $J$ 8.3, $H_{Ar}$), 7.15 (1H, d, $J$ 15.4, CH), 7.22 (2H, d, $J$ 8.7, $H_{Ar}$), 7.27 (2H, d, $J$ 8.1, $H_{Ar}$), 7.52 (1H, d, $J$ 15.4, CH), 7.70–7.89 (2H, m, $H_{Ar}$), 10.91 (1H, s, OH). $^{13}$C NMR (101 MHz, DMSO-$d_6$), δ, ppm: 14.9; 19.2; 38.0; 54.7; 115.8; 116.0; 116.8; 122.1; 124.6; 128.7; 130.85; 130.93; 131.2; 134.6; 140.3; 157.6; 159.0; 172.4; 180.2. Found, %: C, 62.56; H, 4.91; N, 6.29. $C_{23}H_{21}FN_2O_4S$. Calculated, %: C, 62.72; H, 4.81; N, 6.36.

**(*E*)-3-((5-(3-(2-Chlorophenyl)acryloyl)-4-methylthiazol-2-yl)(4-hydroxyphenyl)amino)-2-methylpropanoic acid (7b).** Yellow powder, yield 1.02 g (70%), mp 118–119 °C. IR (KBr, vmax, $cm^{-1}$): 1513 (C=N), 1709 (C=O), 3065 (O-H). $^1$H NMR (700 MHz, DMSO-$d_6$), $\delta$, ppm ($J$, Hz): 1.11 (3H, d, $J$ 7.0, $CH_3$), 2.60 (3H, s, $CH_3$), 2.66–2.73 (1$H_X$, m, CH), 4.04 (1$H_B$, dd, $J^{BA}$ 13.7, $J^{BX}$ 7.4, $CH_2$), 4.10 (1$H_A$, dd, $J^{AB}$ 13.7, $J^{AX}$ 7.4, $CH_2$), 6.89 (2H, d, $J$ 8.8, $H_{Ar}$), 7.25 (1H, d, $J$ 15.4, CH), 7.26 (2H, d, $J$ 8.7, $H_{Ar}$), 7.30–7.46 (4H, m, $H_{Ar}$), 7.81 (1H, d, $J$ 15.4, CH), 10.06 (1H, s, OH), 12.31 (1H, s, COOH). $^{13}$C NMR (176 MHz, DMSO-$d_6$), $\delta$, ppm: 14.8; 19.2; 37.8; 54.7; 116.7; 121.9; 127.5; 127.7; 128.4; 128.6; 129.9; 131.6; 132.2; 134.0; 134.5; 136.2; 157.6; 159.6; 172.6; 175.4; 179.8. Found, %: C, 60.38; H, 4.69; N, 6.21. $C_{23}H_{21}ClN_2O_4S$. Calculated, %: C, 60.46; H, 4.63; N, 6.13.

**(*E*)-3-(5-(3-(3-Chlorophenyl)acryloyl)-4-methylthiazol-2-yl)(4-hydroxyphenyl)amino)-2-methylpropanoic acid (7c).** Yellow powder, yield 0.86 g (64%), mp 129–130 °C. IR (KBr, vmax, $cm^{-1}$): 1509 (C=N), 1711 (C=O), 3181 (O-H). $^1$H NMR (700 MHz, DMSO-$d_6$), $\delta$, ppm ($J$, Hz): 1.10 (3H, d, $J$ 7.0, $CH_3$), 2.59 (3H, s, $CH_3$), 2.66–2.72 (1$H_X$, m, CH), 4.03 (1$H_B$, dd, $J^{BA}$ 13.7, $J^{BX}$ 7.3, $CH_2$), 4.09 (1$H_A$, dd, $J^{AB}$ 13.7, $J^{AX}$ 7.4, $CH_2$), 6.89 (2H, d, $J$ 8.7, $H_{Ar}$), 7.24–7.29 (3H, m, $H_{Ar}$ and CH), 7.39–7.46 (2H, m, $H_{Ar}$), 7.49 (1H, d, $J$ 15.4, CH), 7.69 (1H, d, $J$ 8.1, $H_{Ar}$), 7.86 (1H, s, $H_{Ar}$), 9.87 (1H, s, OH), 12.34 (1H, s, COOH). $^{13}$C NMR (176 MHz, DMSO-$d_6$), $\delta$, ppm: 14.8; 19.1; 37.8; 54.6; 116.7; 122.1; 126.3; 127.1; 127.9; 128.6; 129.8; 130.6; 133.7; 134.5; 136.8; 139.8; 157.5; 159.3; 172.5; 175.4; 180.1. Found, %: C, 60.51; H, 4.60; N, 6.23. $C_{23}H_{21}ClN_2O_4S$. Calculated, %: C, 60.46; H, 4.63; N, 6.13.

**(*E*)-3-(5-(3-(4-Chlorophenyl)acryloyl)-4-methylthiazol-2-yl)(4-hydroxyphenyl)amino)-2-methylpropanoic acid (7d).** Bright orange powder, yield 0.84 g (57%), mp 123–124 °C. IR (KBr, vmax, $cm^{-1}$): 1514 (C=N), 1711 (C=O), 3182 (O-H). $^1$H NMR (400 MHz, DMSO-$d_6$), $\delta$, ppm ($J$, Hz): 1.08 (3H, d, $J$ 7.0, $CH_3$), 2.56–2.67 (4H, m, $C\underline{H}_3CH$ and $CH_3C\underline{H}$), 4.04 (2H, d, $J$ 7.3, $CH_2$), 6.88 (2H, d, $J$ 8.7, $H_{Ar}$), 7.20 (1H, d, $J$ 15.5, CH), 7.24 (2H, d, $J$ 8.7, $H_{Ar}$), 7.45 (2H, d, $J$ 8.4, $H_{Ar}$), 7.50 (1H, d, $J$ 15.4, CH), 7.75 (2H, d, $J$ 8.4, $H_{Ar}$), 11.23 (1H, s, COOH). $^{13}$C NMR (101 MHz, DMSO-

$d_6$), $\delta$, ppm: 15.1; 19.2; 38.3; 55.1; 116.8; 121.9; 125.5; 128.7; 128.9; 130.3; 133.5; 134.5; 134.7; 140.0; 157.7; 159.3; 172.5; 175.9; 180.0. Found, %: C, 60.39; H, 4.71; N, 6.14. $C_{23}H_{21}ClN_2O_4S$. Calculated, %: C, 60.46; H, 4.63; N, 6.13.

**(*E*)-3-(5-(3-(4-Bromophenyl)acryloyl)-4-methylthiazol-2-yl)(4-hydroxyphenyl)amino)-2-methylpropanoic acid (7e).** Bright orange powder, yield 1.12 g (71%), mp 140–141 °C. IR (KBr, νmax, $cm^{-1}$): 1514 (C=N), 1709 (C=O), 3224 (O-H). $^1$H NMR (400 MHz, DMSO-$d_6$), $\delta$, ppm (*J*, Hz): 1.10 (3H, d, *J* 6.9, $CH_3$), 2.58 (3H, s, $CH_3$), 2.61–2.73 (1H, m, $CH_3C\underline{H}$), 3.97–4.14 (2H, m, $CH_2$), 6.89 (2H, d, *J* 8.6, $H_{Ar}$), 7.20 (1H, d, *J* 15.4, CH), 7.25 (2H, d, *J* 8.7, $H_{Ar}$), 7.48 (1H, d, *J* 15.5, CH), 7.58 (2H, d, *J* 8.3, $H_{Ar}$), 7.67 (2H, d, *J* 8.4, $H_{Ar}$), 10.90 (1H, s, OH). $^{13}$C NMR (101 MHz, DMSO-$d_6$), $\delta$, ppm: 14.9; 19.1; 38.0; 54.8; 116.8; 123.5; 125.5; 128.7; 130.4; 131.3; 131.8; 132.3; 133.8; 134.5; 140.1; 157.6; 159.2; 172.5; 180.0. Found, %: C, 55.23; H, 4.18; N, 5.69. $C_{23}H_{21}BrN_2O_4S$. Calculated, %: C, 55.10; H, 4.22; N, 5.59.

**(*E*)-3-9(4-Hydroxyphenyl)(4-methyl-5-(3-(thiophen-2-yl)acryloyl)thiazol-2-yl)amino)-2-methylpropanoic acid (7f).** Yellow powder, yield 1.23 g (90%), mp 121–122 °C. IR (KBr, νmax, $cm^{-1}$): 1513 (C=N), 1709 (C=O), 3026 (O-H). $^1$H NMR (400 MHz, DMSO-$d_6$), $\delta$, ppm (*J*, Hz): 1.10 (3H, t, *J* 6.8, $CH_3$), 2.57 (3H, s, $CH_3$), 2.63–2.77 ($1H_X$, m, CH), 4.02 ($1H_B$, dd, $J^{BA}$ 13.4, $J^{BX}$ 7.4, $CH_2$), 4.09 ($1H_A$, dd, $J^{AB}$ 13.6, $J^{AX}$ 7.6, $CH_2$), 6.82–7.75 (9H, m, $H_{Ar}$, thienyl, 2x CH), 9.95 (1H, s, OH), 12.37 (1H, s, COOH). $^{13}$C NMR (101 MHz, DMSO-$d_6$), $\delta$, ppm: 14.8; 19.1; 37.8; 54.6; 116.8; 122.1; 122.9; 128.7; 128.8; 129.7; 132.4; 134.4; 134.6; 139.6; 157.6; 158.7; 172.2; 175.5; 179.6. Found, %: C, 58.95; H, 4.82; N, 6.45. $C_{21}H_{20}N_2O_4S_2$. Calculated, %: C, 58.86; H, 4.70; N, 6.54.

**3-((4-Hydroxyphenyl)(4,5,6,7-tetrahydrobenzo[*d*]thiazol-2-yl)amino)-2-methylpropanoic acid (8).** A mixture of compound **1** (1.47 g, 5 mmol), 2-bromocyclohexane-1-one (0.89 g, 5 mmol), sodium acetate (0.42 g, 5 mmol) and 2-propanol was refluxed for 2 h, then cooled to room temperature. The formed precipitate was filtered off, washed with 2-propanol, dried. White powder, yield 1.45 g (87%), mp 219–220 °C (2-PrOH). IR (KBr, νmax, $cm^{-1}$): 1514 (C=N), 1619 (C=O), 3122 (O-H). $^1$H NMR (400 MHz, DMSO-$d_6$), $\delta$, ppm, (*J*, Hz): 1.06 (3H, d, *J* 7.0, $CH_3$), 1.69 (4H, s, 2x $CH_2$), 2.36–2.48 (4H, m, 2x $CH_2$), 2.64–2.76 ($1H_X$, m, CH), 3.84 ($1H_B$, dd, $J^{BA}$ 13.5, $J^{BX}$ 7.5, $CH_2$), 3.92 ($1H_A$, dd, $J^{AB}$ 13.5, $J^{AX}$ 7.3, $CH_2$), 6.80 (2H, d, *J* 8.7, $H_{Ar}$), 7.13 (2H, d, *J* 8.7, $H_{Ar}$), 10.97 (1H, s, OH). $^{13}$C NMR (101 MHz, DMSO-$d_6$), $\delta$, ppm: 15.4; 22.9; 23.1; 23.5; 27.0; 38.5; 55.1; 116.3; 116.8; 129.3; 136.8; 145.9; 157.1; 168.8; 176.5. Found, %: C, 61.59; H, 6.13; N, 8.58. $C_{17}H_{20}N_2O_3S$. Calculated, %: C, 61.42; H, 6.06; N, 8.43.

**3-(4,9-Dioxo-4,9-dihydronaphtho[2,3-*d*]thiazol-2-yl)(4-hydroxyphenyl)amino)-2-methylpropanoic acid (9).** A mixture of compound **1** (1.27 g, 5 mmol), 2,3-dichloro-1,4-naphthoquinone (1.14 g, 5 mmol), sodium acetate (0.62 g, 7.5 mmol) and acetic acid (10 mL) was stirred at 80 °C for 24 h. Then the reaction mixture was diluted with water (30 mL), the formed precipitate was filtered off, washed with water, dried.

Dark purple crystals, yield 1.26 g (62%), mp 231–232 °C. IR (KBr, νmax, $cm^{-1}$): 1535 (C=N), 1616, 1634, 1712 (C=O), 3386 (O-H). HRMS (ESI) for $C_{21}H_{16}N_2O_5S$, calcd 409.0854, found 409.0853 $[M+H]^+$. Found, %: C, 61.84; H, 3.90; N, 6.83. $C_{21}H_{16}N_2O_5S$. Calculated, %: C, 61.76; H, 3.95; N, 6.86.

**General procedures for the synthesis of compounds 10a, 11d, 12e, 13d, 14e, 15g.** A mixture of the corresponding compound **2** (2.8 mmol) and 4-nitrobenzaldehyde or 4-dimethylaminobenzaldehyde (2.8 mmol), concentrated hydrochloric acid (1 mL), and acetone (20 mL) was heated under reflux for 17 h. Then the formed compounds were filtered off, washed with acetone and transformed to the corresponding bases **10–15** by dissolving them in 5% aqueous $Na_2CO_3$ and acidifying with acetic acid to pH 6.

**3,3'-((((4-Nitrophenyl)methylene)bis(4-phenylthiazole-5,2-diyl))bis((4-hydroxyphenyl)azanediyl))bis(2-methylpropanoic acid) (10a).** White crystals, yield 0.93 g (78%), mp 214–215 °C. IR (KBr, νmax, $cm^{-1}$): 1511 (C=N), 1713 (C=O), 3104 (O-H). $^1$H NMR (400 MHz, DMSO-$d_6$), $\delta$, ppm, (*J*, Hz): 1.09 (6H, dd, *J* 7.0, 1.5, 2x$CH_3$), 2.78–2.64 ($2H_X$, m, 2xCH), 4.04 ($2H_A$, dd, $J^{AB}$ 13.7, $J^{AX}$ 7.4, 2x$CH_2$), 4.04 ($2H_B$, dd, $J^{BA}$ 14.0, $J^{BX}$ 7.1 Hz, 2x$CH_2$), 5.79

(1H, s, CH-methane), 8.20–6.77 (22H, m, $H_{Ar}$). $^{13}$C NMR (101 MHz, DMSO-$d_6$), $\delta$, ppm: 14.8; 15.0; 30.7; 37.9; 54.9; 116.7; 121.6; 121.7; 124.2; 128.1; 128.4; 128.7; 133.7; 135.2; 146.5; 146.9; 150.2; 157.3; 169.08; 169.14; 175.7. HRMS (ESI) for $C_{45}H_{40}N_5O_8S_2$, calcd 842.2318, found 842.2319 $[M+H]^+$.

**3,3'-((((4-Nitrophenyl)methylene)bis(4-(4-chlorophenyl)thiazole-5,2-diyl))bis((4-hydroxyphenyl)azanediyl))-bis(2-methylpropanoic acid) (11d).** White crystals, yield 1.28 g (79%), mp 226–227 °C. IR (KBr, vmax, $cm^{-1}$): 1511 (C=N), 1741 (C=O), 3060 (O-H). $^1$H NMR (400 MHz, DMSO-$d_6$), $\delta$, ppm, (*J*, Hz): 1.09 (6H, dd, *J* 7.0, 1.5, 2x$CH_3$), 2.69–2.73 (2$H_X$, m, 2xCH), 4.01 (2$H_B$, dd, $J^{BA}$ 14.0, $J^{BX}$ 7.1, 2x$CH_2$), 4.11 (2$H_A$, dd, $J^{AB}$ 13.7, $J^{AX}$ 7.4, 2x$CH_2$), 5.86 (1H, s, CH-methane), 6.96–8.14 (20H, m, $H_{Ar}$). $^{13}$C NMR (101 MHz, DMSO-$d_6$), $\delta$, ppm: 14.9; 15.7; 30.8; 37.3; 55.1; 116.7; 120.6; 121.7; 123.2; 127.2; 128.1; 128.5; 134.0; 135.5; 145.3; 146.1; 151.2; 158.1; 169.2; 168.2; 174.7. HRMS (ESI) for $C_{45}H_{38}Cl_2N_5O_8S_2$, calcd 910.1539, found 910.1532 $[M+H]^+$.

**3,3'-((((4-Nitrophenyl)methylene)bis(4-(4-bromophenyl)thiazole-5,2-diyl))bis((4-hydroxyphenyl)azanediyl))-bis(2-methylpropanoic acid) (12e).** White crystals, yield 1.23 g (88%), mp 232–234 °C. IR (KBr, vmax, $cm^{-1}$): 1511 (C=N), 1709 (C=O), 3093 (O-H). $^1$H NMR (400 MHz, DMSO-$d_6$), $\delta$, ppm, (*J*, Hz): 1.08 (6H, dd, *J* 6.9, 3.9, 2x$CH_3$), 2.60–2.83 (2$H_X$, m, 2xCH), 3.90 (2$H_B$, dd, $J^{BA}$ 13.9, $J^{BX}$ 7.3, 2x$CH_2$), 4.01 (2$H_A$, dd, $J^{AB}$ 13.6, $J^{AX}$ 7.2, 2x$CH_2$), 5.75 (1H, s, CH-methane), 6.71–6.90 (4H, m, $H_{Ar}$), 7.04–7.25 (8H, m, $H_{Ar}$), 7.41 (6H, ddd, *J* 8.9, 5.9, 3.1, $H_{Ar}$), 8.14 (2H, d, *J* 8.7, $H_{Ar}$), 9.51 (2H, s, 2xOH). $^{13}$C NMR (101 MHz, DMSO-$d_6$), $\delta$, ppm: 15.0; 30.7; 37.9; 41.2; 54.61; 54.8; 116.6; 121.3; 122.2; 122.3; 124.1; 128.8; 128.9; 130.0; 131.3; 133.4; 135.2; 135.3; 146.6; 150.0; 157.16; 157.19; 169.1; 169.2; 175.7; 175.9. HRMS (ESI) for $C_{45}H_{38}Br_2N_5O_8S_2$, calcd 998.0530, found 998.0524 $[M+H]^+$.

**3,3'-((((4-(Dimethylamino)phenyl)methylene)bis(4-(4-chlorophenyl)thiazole-5,2-diyl))bis((4-hydroxyphenyl)azanediyl))bis(2-methylpropanoic acid) (13d).** Pale blue crystals, yield 1.16 g (91%), mp 168–169 °C. IR (KBr, vmax, $cm^{-1}$): 1512 (C=N), 1706 (C=O), 3392 (O-H). $^1$H NMR (400 MHz, DMSO-$d_6$), $\delta$, ppm, (*J*, Hz): 1.08 (6H, dd, *J* 6.8, 3.3, 2x$CH_3$), 2.65–2.77 (2$H_X$, m, 2xCH), 3.84–4.05 (4H, m, 2x$CH_2$), 5.54 (1H, s, CH-methane), 6.54–7.39 (20H, m, $H_{Ar}$), 9.77 (2H, s, 2xOH). $^{13}$C NMR (101 MHz, DMSO-$d_6$), $\delta$, ppm: 14.9; 37.9; 40.4; 54.5; 54.7; 116.5; 128.07; 128.13; 128.15; 128.80; 128.82; 129.5; 132.2; 133.6; 135.5; 135.6; 145.4; 156.9; 157.0; 168.5; 168.6; 175.7; 175.8. HRMS (ESI) for $C_{47}H_{44}Cl_2N_5O_6S_2$, calcd 908.2110, found 908.2114 $[M+H]^+$.

**3,3'-((((4-(Dimethylamino)phenyl)methylene)bis(4-(4-bromophenyl)thiazole-5,2-diyl))bis((4-hydroxyphenyl)azanediyl))bis(2-methylpropanoic acid) (14e).** Pale blue crystals, yield 1.25 g (89%), mp 198–199 °C. IR (KBr, vmax, $cm^{-1}$): 1511 (C=N), 1726 (C=O), 3059 (O-H). $^1$H NMR (400 MHz, DMSO-$d_6$), $\delta$, ppm, (*J*, Hz): 1.08 (6H, dd, *J* 6.6, 3.5, 2x$CH_3$), 2.65–2.75 (2$H_X$, m, 2xCH), 3.88 (2$H_B$, dd, $J^{BA}$ 13.7, $J^{BX}$ 7.2, 2x$CH_2$), 4.01 (2$H_A$, dd, $J^{AB}$ 13.8, $J^{AX}$ 7.1, 2x$CH_2$), 5.66 (1H, s, CH-methane), 6.78–7.58 (20H, m, $H_{Ar}$), 9.32 (2H, s, 2xOH). $^{13}$C NMR (101 MHz, DMSO-$d_6$), $\delta$, ppm: 14.9; 30.7; 37.9; 44.1; 54.6; 54.8; 116.6; 121.2; 123.4; 128.8; 130.0; 131.2; 133.4; 133.41; 135.3; 135.4; 146.0; 157.2; 168.9; 169.0; 175.7. HRMS (ESI) for $C_{47}H_{44}Br_2N_5O_6S_2$, calcd 996.1100, found 996.1102 $[M+H]^+$.

**3,3'-((((4-(Dimethylamino)phenyl)methylene)bis(4-(4-nitrophenyl)thiazole-5,2-diyl))bis((4-hydroxyphenyl)azanediyl))bis(2-methylpropanoic acid) (15g).** Pale green crystals, yield 1.11 g (85%), mp 201–202 °C. IR (KBr, vmax, $cm^{-1}$): 1511 (C=N), 1736 (C=O), 3104 (O-H). $^1$H NMR (400 MHz, DMSO-$d_6$), $\delta$, ppm, (*J*, Hz): 1.07 (6H, dd, *J* 6.2, 3.0, 2x$CH_3$), 2.56–2.76 (2$H_X$, m, 2xCH), 3.81–4.09 (4H, m, 2x$CH_2$), 5.88 (1H, s, CH-methane), 6.85 (4H, dd, *J* 8.7, 2.2, $H_{Ar}$), 7.16 (4H, dd, *J* 8.6, 2.0, $H_{Ar}$), 7.35 (2H, d, *J* 5.7, $H_{Ar}$), 7.44 (6H, dd, *J* 8.4, 4.2, $H_{Ar}$), 7.94–8.07 (4H, m, $H_{Ar}$). $^{13}$C NMR (101 MHz, DMSO-$d_6$), $\delta$, ppm: 14.9; 37.9; 40.6; 44.1; 54.7; 116.7; 123.42; 128.8; 129.0; 135.3; 140.9; 146.3; 157.2; 169.2; 175.68; 175.72. HRMS (ESI) for $C_{47}H_{44}N_7O_{10}S_2$, calcd 930.2591, found 930.2587 $[M+H]^+$.

**General procedures for the synthesis of compounds 16a,d,e,g.** A mixture of the corresponding compound **2a,d,e,g** (3.5 mmol), 37% formaldehyde water solution (3.5 mmol, 0.26 mL) and acetic acid (20 mL) was

heated at 80 °C for 15 h. Then the reaction mixture was diluted with water, the formed crystals **16a,d,e,g** were filtered off and crystallized from the water – $CH_3COOH$ mixture (1:1).

**3,3'-((Methylenebis(4-phenylthiazole-5,2-diyl))bis((4-hydroxyphenyl)azanediyl))bis(2-methylpropanoic acid) (16a).** Blue crystals, yield 1.02 g (81%), mp 180–181 °C. IR (KBr, vmax, $cm^{-1}$): 1511 (C=N), 1707 (C=O), 3392 (O-H). $^1$H NMR (400 MHz, DMSO-$d_6$), $\delta$, ppm, ($J$, Hz): 1.08 (6H, d, $J$ 7.0, 2x$CH_3$), 2.61–2.85 ($2H_X$, m, 2xCH), 3.92 ($2H_B$, dd, $J^{BA}$ 13.6, $J^{BX}$ 7.3, 2x$CH_2$), 4.00 ($2H_A$, dd, $J^{AB}$ 13.6, $J^{AX}$ 7.2, 2x$CH_2$), 4.10 (2H, s, $CH_2$-methane), 6.81 (4H, d, $J$ 8.7, $H_{Ar}$), 7.19 (4H, d, $J$ 8.7, $H_{Ar}$), 7.25–7.38 (6H, m, $H_{Ar}$), 7.47 (4H, d, $J$ 8.7, $H_{Ar}$), 9.71 (2H, s, 2xOH), 12.22 (2H, s, 2xCOOH). $^{13}$C NMR (101 MHz, DMSO-$d_6$), $\delta$, ppm: 14.8; 24.6; 38.0; 54.6; 116.5; 120.2; 127.4; 128.1; 128.3; 128.8; 134.8; 135.8; 146.3; 156.8; 167.8, 175.8. HRMS (ESI) for $C_{39}H_{37}N_4O_6S_2$, calcd 721.2155, found 721.2152 $[M+H]^+$.

**3,3'-((Methylenebis(4-(4-chlorophenyl)thiazole-5,2-diyl))bis((4-hydroxyphenyl)azanediyl))bis(2-methylpropanoic acid) (16d).** Blue crystals, yield 0.99 g (72%), mp 173–174 °C. IR (KBr, vmax, $cm^{-1}$): 1512 (C=N), 1707 (C=O), 3415 (O-H). $^1$H NMR (400 MHz, DMSO-$d_6$), $\delta$, ppm, ($J$, Hz): 1.07 (6H, d, $J$ 7.0, 2x$CH_3$), 2.62–2.83 ($2H_X$, m, 2xCH), 3.91 ($2H_B$, dd, $J^{BA}$ 13.6, $J^{BX}$ 7.5, 2x$CH_2$), 3.98 ($2H_A$, dd, $J^{AB}$ 13.6, $J^{AX}$ 7.1, 2x$CH_2$), 4.09 (2H, s, $CH_2$-methane), 6.81 (4H, d, $J$ 8.7, $H_{Ar}$), 7.17 (4H, d, $J$ 8.7, $H_{Ar}$), 7.38 (4H, d, $J$ 8.5, $H_{Ar}$), 7.48 (4H, d, $J$ 8.5, $H_{Ar}$), 10.84 (2H, s, 2xOH). $^{13}$C NMR (101 MHz, DMSO-$d_6$), $\delta$, ppm: 15.0; 24.5; 54.6; 73.8; 116.5; 120.6; 128.3; 128.8; 129.8; 132.0; 133.6; 135.7; 145.4; 156.9; 167.9; 176.0. HRMS (ESI) for $C_{39}H_{35}Cl_2N_4O_6S_2$, calcd 789.1375, found 789.1373 $[M+H]^+$.

**3,3'-((Methylenebis(4-(4-bromophenyl)thiazole-5,2-diyl))bis((4-hydroxyphenyl)azanediyl))bis(2-methylpropanoic acid) (16e).** Blue crystals, yield 1.34 g (87%), mp 188–189 °C. IR (KBr, vmax, $cm^{-1}$): 1511 (C=N), 1705 (C=O), 3416 (O-H). $^1$H NMR (400 MHz, DMSO-$d_6$), $\delta$, ppm, ($J$, Hz): 1.07 (6H, d, $J$ 7.0, 2x$CH_3$), 2.59–2.77 ($2H_X$, m, 2xCH), 3.91 ($2H_B$, dd, $J^{BA}$ 13.5, $J^{BX}$ 7.6, 2x$CH_2$), 3.98 ($2H_A$, dd, $J^{AB}$ 13.5, $J^{AX}$ 7.0, 2x$CH_2$), 4.08 (2H, s, $CH_2$-methane), 6.81 (4H, d, $J$ 8.6, $H_{Ar}$), 7.17 (4H, d, $J$ 8.5, $H_{Ar}$), 7.41 (4H, d, $J$ 8.4, $H_{Ar}$), 7.51 (4H, d, $J$ 8.3, $H_{Ar}$), 10.82 (2H, s, 2xOH). $^{13}$C NMR (101 MHz, DMSO-$d_6$), $\delta$, ppm: 14.9; 24.5; 54.7; 116.5; 120.6; 120.7; 128.8; 130.1; 131.2; 133.9; 135.7; 145.1; 156.9; 168.0; 176.0. HRMS (ESI) for $C_{39}H_{35}Br_2N_4O_6S_2$, calcd 877.0365, found 877.0363 $[M+H]^+$.

**3,3'-((Methylenebis(4-(4-nitrophenyl)thiazole-5,2-diyl))bis((4-hydroxyphenyl)azanediyl))bis(2-methylpropanoic acid) (16g).** Green crystals, yield 1.12 g (79%), mp 175–176 °C. IR (KBr, vmax, $cm^{-1}$): 1513 (C=N), 1706 (C=O), 3425 (O-H). $^1$H NMR (400 MHz, DMSO-$d_6$), $\delta$, ppm, ($J$, Hz): 1.08 (6H, d, $J$ 7.0, 2x$CH_3$), 2.61–2.81 ($2H_X$, m, 2xCH), 4.10 ($2H_B$, dd, $J^{BA}$ 13.7 $J^{BX}$ 7.5, 2x$CH_2$), 4.20 ($2H_A$, dd, $J^{AB}$ 13.7, $J^{AX}$ 7.3, 2x$CH_2$), 4.27 (2H, s, $CH_2$-methane), 6.91 (4H, d, $J$ 8.6, $H_{Ar}$), 7.17 (4H, d, $J$ 8.6, $H_{Ar}$), 7.74 (4H, d, $J$ 8.8, $H_{Ar}$), 8.17 (4H, d, $J$ 8.8, $H_{Ar}$), 11.18 (2H, s, 2xOH). $^{13}$C NMR (101 MHz, DMSO-$d_6$), $\delta$, ppm: 15.3; 25.0; 55.1; 117.3; 124.3; 125.5; 129.0; 129.3; 129.5; 131.3; 134.7; 139.6; 148.5; 159.8; 168.6; 176.0. HRMS (ESI) for $C_{39}H_{35}N_6O_{10}S_2$, calcd 811.1856, found 811.1845 $[M+H]^+$.

**Crystallography.** *X-ray crystallographic analysis of compound **7c**.* Diffraction data were collected at −90 °C on a Bruker-Nonius KappaCCD diffractometer using the graphite monochromated Mo-Kα radiation (λ 0.71073 Å). The crystal structure of **7c** was solved by the direct method and refined by full-matrix least squares.[27] Crystal data for **7c**: monoclinic; $a$ 11.2244(5), $b$ 15.8918(8), $c$ 12.6457(6) Å, $\beta$ 102.351(3)°; $V$ 2203.5(2) $Å^3$, $Z$ 4, μ 0.301 $mm^{-1}$; space group is $P2_1/n$. A total of 8565 reflection intensities were collected up to $2\theta_{max}$ 55°; for structure refinement, 2603 independent reflections with $I > 2\sigma(I)$ were used. The final $R$-factor is 0.0839. For further details, see crystallographic data for **7c**, deposited with the Cambridge Crystallographic Data Centre as the Supplementary Publication Number CCDC 1455153. Copies of the data can be obtained free of charge on application to CCDC, 12 Union Road, Cambridge CB2 1EZ, UK.

**Antibacterial activity of the compounds.** Antibacterial activity was tested using the disk diffusion technique. The microorganisms *Rhizobium radiobacter, Escherichia coli, Xanthomonas campestris* were commercially available from the German Collection of Microorganisms and Cell Cultures (DSMZ). The zone of the inhibition

of bacterial growth was investigated. The main solution (1 mg/mL) of the synthesized compounds was prepared in DMSO and then diluted to various concentrations (50–1000 $\mu$g/mL) in DMSO. Cultures of *Rhizobium radiobacter, Xanthomonas campestris, Escherichia coli* were cultivated in Petri dishes for 24 h at 37 °C on the Luria–Bertani (LB) agar medium. A bacterial suspension was prepared from cultivated bacterial cultures, and 50 $\mu$L of the inoculum containing bacterial cells ($10^8$ CFU/mL) was spread over the LB agar medium. Filter paper disks were prepared by adding 25 $\mu$L of each compound solution, and then the disks were put on the LB agar medium. Ampicillin was used as the positive control, and DMSO was used as the negative control. The Petri dishes were incubated for 24 h at 37 °C, and the zones of inhibition were then ascertained for each sample.

**DPPH (1,1-diphenyl-2-picrylhydrazyl) radical scavenging assay.** The free radical scavenging activity of compounds was measured by DPPH using the widely used method.[26] Briefly, 1 mM solution of DPPH in ethanol was prepared, and this solution (1 mL) was added to the solutions of the tested compounds (1 mg/mL of dimethyl sulphoxide). The mixture was shaken vigorously and allowed to stand at room temperature for 20 min. Afterwards, the absorbance was measured at 517 nm with a UV-200-RS spectrophotometer (MRC Ltd., Israel).

The lower absorbance of the reaction mixture indicated a higher free radical scavenging activity. The capability to scavenge the DPPH radical was calculated according to the following equation:

$$\text{DPPH scavenging effect (\%)}\ (A_0 - A_1/A_0) \times 100,$$

where $A_0$ is the absorbance of the control reaction, and $A_1$ is the absorbance in the presence of the compounds.

**Reducing power assay.** The tested compounds (1000 $\mu$g/mL) were mixed with the phosphate buffer (2.5 mL, 0.2 mol/L, pH 6.6) and potassium ferricyanide $K_3[Fe(CN)_6]$, (2.5 mL, 1%). The mixture was incubated at 50 °C for 20 min. 10% TCA was added to the mixture, which was then centrifuged at 3000 rpm for 10 min. The upper layer of the solution was mixed with distilled water (2.5 mL), $FeCl_3$ (0.5 mL, 0.1%), and the absorbance was measured at 700 nm. The increased absorbance of the reaction mixture indicated an increased reducing power.[28]

**Ferric reducing antioxidant power assay (FRAP).** The principle of this method is based on the reduction of a ferric-tripyridyl triazine complex to its ferrous coloured form in the presence of antioxidants. Briefly, the FRAP reagent contained 2.5 mL of a 10 mmol/L TPTZ (2,4,6-tripyridyl-s-triazine) solution in 40 mmol/L HCl plus 2.5 mL of $FeCl_3$ (20 mmol/L) and 25 mL of acetate buffer (0.3 mol/L, pH 3.6). The aliquots of 100 $\mu$L tested compounds (1000 $\mu$g/mL) were mixed with 3 mL of the FRAP reagent, and the absorbance of the reaction mixture at 593 nm was measured spectrophotometrically after incubation at 37 °C for 10 min. For the construction of the calibration curve, five concentrations of $FeSO_4 \times 7\ H_2O$ (5, 10, 15, 20, 25 $\mu$mol/L) were used, and the absorbancies were measured as a sample solution.[29]

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