SYNTHESIS, TRANSFORMATION OF 3-[(4-ARYLTHIAZOL-2-YL)-(p-TOLYL)AMINO]PROPANOIC ACIDS, BIS(THIAZOL-5-YL)PHENYL-, BIS(THIAZOL-5-YL)METHANE DERIVATIVES, AND THEIR ANTIMICROBIAL ACTIVITY

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Abstract – Bis(thiazol-5-yl)phenyl- and bis(thiazol-5-yl)methanes were synthesized by the reaction of 2,4-disubstituted thiazoles with aromatic aldehydes or formaldehyde. In addition, modification of the carboxyl group of the molecule was carried out. The reactions afforded compounds bearing various heterocyclic fragments. All the synthesized compounds were tested for their effect on Escherichia coli, Staphylococcus aureus and Mycobacterium luteum bacteria and Aspergillus niger and Candida tenuis fungi strains. Some of them exhibited antibacterial activity against test-culture Mycobacterium luteum.

INTRODUCTION
The treatment of infectious diseases remains a challenging task because over time bacteria have become increasingly resistant to antibiotics. The wide use of antibiotics led to the mutation of microorganisms. They have acquired the ability to prevent the normal function of antibiotics. Mutated bacteria produce a novel mechanism to defeat the action of many antibiotics. This has induced new diseases and at the same time has become a reason for a very complicated problem. Hence, in spite of a large number of antibiotics applied for medical uses the emerging resistance to antibiotic substances has created a real need for novel classes of antimicrobial agents. Therefore, the design of new and effective molecules as antimicrobial agents is a target task nowadays.
Thiazole is a diverse scaffold in heterocyclic chemistry and is found in various natural products (e.g., epothilone, vitamin B1) and pharmacologically essential compounds including anti-inflammatory,\textsuperscript{1-4} anticonvulsant,\textsuperscript{5,6} antiviral,\textsuperscript{7} anticancer,\textsuperscript{8,9} antitubercular and antiplasmodial\textsuperscript{10} and others.\textsuperscript{11-18} It is a ubiquitous constituent in medicinal chemistry. Thiazole derivatives exhibit broad antibacterial and antifungal properties,\textsuperscript{19-23} i.e. kill or inhibit the growth of different strains of bacteria and fungi. The synthesis of bis(thiazol-5-yl)phenyl- and bis(thiazol-5-yl)methanes was encouraged by the fact that cystothiazoles, having a bisthiazole fragment, exhibit a potent antifungal activity.\textsuperscript{24} Furthermore, aryridenebis(thiazoles) show promising results as insecticidal agents.\textsuperscript{25} Taking all this into account, the task of the present work was to synthesize novel variously functionalized thiazole derivatives and to evaluate their antimicrobial activity.

RESULTS AND DISCUSSION
The starting compound thioureido acid 1 was prepared by the method described in literature.\textsuperscript{26} Using the convenient method for the synthesis of thiazoles, i.e. the action of compound 1 with different bromoacetophenones in refluxing acetone, aminothiazole hydrobromides were obtained, which then were transferred into the bases 2\textsuperscript{a-e} by boiling them in the aqueous sodium acetate (Scheme 1). The structures of compounds 2\textsuperscript{a-e} were established from their spectral data. For example, the IR spectra of compounds 2\textsuperscript{a} revealed a strong absorption band at 1721 and 1510 cm\textsuperscript{-1} characterized for C=O and C=N functional groups, respectively. Its \textsuperscript{1}H and \textsuperscript{13}C NMR spectra showed signals at 7.10 and 102.4 ppm, respectively, assignable to CH protons of the thiazole ring.

The interest to the synthesis of such new bis(thiazoles) 3 and 4 follows from the fact that analogous compounds show promising results for various applications. Refluxing compounds 2\textsuperscript{a–e} with aromatic aldehydes or formaldehyde in the molar ratio of 2:1 in acetone afforded bis(thiazol-5-yl)phenylmethanes 3\textsuperscript{a–s} and bis(thiazol-5-yl)methanes 4\textsuperscript{a–e}, respectively. The products were elucidated on the basis of their IR, NMR and mass spectroscopy data. The analysis of \textsuperscript{1}H NMR spectra of compounds 3\textsuperscript{a–s} revealed singlet at approx. 5.74 ppm, ascribed to the newly formed CCHC fragment which is clearly confirmed by the resonance line at approx. 40.8 ppm in the \textsuperscript{13}C NMR spectra. Both spectra also displayed an increased abundance of aromatic signals. An analogous situation is visible in the NMR spectra of compounds 4\textsuperscript{a–e}, only here the singlet of the methylene group is shifted to stronger magnetic fields and arises at approx. 4.18 (\textsuperscript{1}H NMR) and 24.4 (\textsuperscript{13}C NMR) ppm.
2a, 4a R = H; 2b, 4b R = F; 2c, 4c R = Cl; 2d, 4d R = CN; 2e, 4e R = NO₂; 3a–e R = H; 3a R¹ = F; 3b R¹ = Cl; 3c R¹ = Br; 3d R¹ = NMe₂; 3e R¹ = NO₂; 3f, g R = F; 3f R¹ = NMe₂; 3g R¹ = NO₂; 3h–l R = Cl; 3h R¹ = F; 3i R¹ = Cl; 3j R¹ = Br; 3k R¹ = NMe₂; 3l R¹ = NO₂; 3m R = CN; R¹ = Cl; 3n–s R = NO₂; 3n R¹ = F; 3o R¹ = Cl; 3p R¹ = Br; 3r R¹ = NMe₂; 3s R¹ = NO₂

i bromoacetophenone, acetone, Δ, 2 h, 4% aqueous AcONa, Δ, 5 min; ii aromatic aldehyde, acetone, conc. HCl, Δ, 18 h, 4% aqueous AcONa, Δ, 5 min; iii AcOH, formaldehyde, Δ, 16 h

**Scheme 1.** Synthesis of bis(thiazol-5-yl)phenyl- 3a–s and bis(thiazol-5-yl)methanes 4a–e

A supposed mechanism for the preparation of compounds 3 is presented in Scheme 2. First of all during the reaction between thiazoles 2 and aromatic aldehyde 3-({4- (4-substituted phenyl)-5-[((4-substituted phenyl)(hydroxy)methyl]thiazol-2-yl)(p-tolyl)amino)propanoic acid 2’ forms. Under the action of acidic medium the obtained compound 2’ transforms to the carbenium ion intermediate 2″, reaction of which with another thiazole derivative 2 results in the formation of bis(thiazol-5-yl)phenylmethanes 3.

**Scheme 2.** A supposed reaction mechanism for the formation of bis(thiazol-5-yl)phenylmethanes 3
Aminothiazoles 2c showed promising antimicrobial results; therefore, under the same reaction conditions compound 2c reacted with aminothiazole derivatives containing 4-fluoro-, 4-cyano- and 4-nitrophenyl substituent in the 4th position of the thiazole ring and different aromatic aldehydes to give products 5a–c (Scheme 3) with variously substituted phenyl fragments in the structure, hoping to enhance the biological properties of the molecule. The spectral data of compounds 5a–c confirmed the formation of the desired structures.

\[
2b, 5a \quad R = F, \quad R_1 = Cl; \quad 2d, 5b \quad R = CN, \quad R_1 = Cl; \quad 2e, 5c \quad R = NO_2, \quad R_1 = NMe_2
\]

\[ \text{i} 4\text{-chlorobenzaldehyde or 4-(dimethylamino)benzaldehyde, acetone, } \Delta, \text{ 16 h, 4\% aqueous AcONa, } \Delta, \text{ 5 min} \]

**Scheme 3.** Synthesis of bis(thiazol-5-yl)phenylmethanes 5a–c

The β-amino acid derivatives are known to have a variety of biological properties, for example, antimalarial,\textsuperscript{27} antiseizure,\textsuperscript{28} antibacterial and antifungal.\textsuperscript{29} We have tried to modify a carboxyl fragment by introducing various heterocycles into the molecule (Scheme 4). For this purpose, compound 2c first was esterified with methanol in the presence of the catalytic amount of sulphuric acid. Then the obtained ester 6 was converted to the acid hydrazide 7. The reaction was carried out in DMSO at 90–100 °C for 2 h. The next step of the work was the condensation of hydrazide 7 with 2,4-pentane- and 2,5-hexanediones. These reactions resulted in the formation of 3-[(4-(4-chlorophenyl)thiazol-2-yl)-p-tolylamino]-1-(3,5-dimethylpyrazol-1-yl)propan-1-one (8) and 3-[(4-(4-chlorophenyl)thiazol-2-yl)-p-tolylamino]-N-(2,5-dimethyl-1H-pyrrol-1-yl)propanamide (9). The $^{13}$C NMR spectrum of compound 8 exhibited three resonance lines at 111.2, 143.1 and 151.4 ppm, attributed to the pyrazole moiety. The protons of two methyl groups and the C–CH=CH fragment resonate in the expected area of the $^1$H NMR spectrum and also approve the presence of a pyrazole ring.
The $^1$H NMR spectrum of the pyrrole derivative 9 exhibited characteristic signals of the desired structures: intense singlets at 1.92 and 5.60 ppm assigned to CH$_3$ and CH groups of the pyrrole fragment were visible. The double intensity resonances at 10.9, 102.9 and 126.7 ppm in the $^{13}$C NMR spectrum demonstrate the existence of the pyrrole moiety. Despite the presence of a NH–CO fragment in the molecule, only a s-cis isomer with traces of the s-trans isomer is visible in the $^1$H and $^{13}$C NMR spectra of compound 9 in DMSO-$d_6$ solutions.

The interaction of carbohydrazide 7 with phenyl isothiocyanate in methanol at reflux gave thiosemicarbazide 10. The precipitate already forms during the reaction. In the $^1$H NMR spectrum of compound 10, one intense singlet at 9.53 ppm integrated for two protons, and one less intense at 9.97 ppm, integrated for one proton, show the presence of three NH groups. The formation of the
C(O)NHNHC(S)NHPh fragment finally approves resonances at 170.0 and 180.9 ppm assigned to C=O and C=S groups and additional spectral lines in the aromatic region in the $^{13}\text{C}$ NMR spectrum.

The last step of the study was ring closure reactions when the thiosemicarbazide 10 was heated under reflux in an aqueous sodium hydroxide solution or reacted with 2-bromo-4'-fluoroacetophenone in acetone. The first reaction afforded 3-substituted-1,2,4-triazole-5-thione derivative 11 and the other one resulted in the formation of the second thiazole cycle in the molecule and gave compound 12. A characteristic singlet at 13.75 (NH, $^1\text{H}$ NMR) and a resonance line at 167.7 (C=S, $^{13}\text{C}$ NMR) ppm in the NMR spectra of compound 11 show the existence of a 1,2,4-triazole-5-thione moiety. The absorption band 1267 cm$^{-1}$ in the IR spectrum proves this thiocarbonyl group formation. The absence of a signal of the proton of the SH group in the strong field of $^1\text{H}$ NMR spectrum ensure the presence of a thione form.

The structure of compound 12 was proved by spectroscopic techniques.

The synthesized compounds 2a–e, 3a–s, 4a–e, 5a–c, 6–12 were evaluated for their antibacterial and antifungal activity against strains of Escherichia coli B-906, Staphylococcus aureus 209-P, Mycobacterium luteum B-917, Candida tenuis VKM Y-70, and Aspergillus niger VKM F-1119 by the diffusion technique$^{30}$ and by the serial dilution technique (determination of MIC).$^{31}$ Their activities were compared with those of the known antibacterial agent Vancomycin and antifungal agent Nystatin (control C).

The test-cultures E. coli, C. tenuis, and A. niger appeared not to be sensitive or low sensitive to the tested compounds 2a–e, 3a–s, 4a–e, 5a–c, 6–12 investigated by the diffusion technique at concentrations of 0.1 and 0.5% (Table 1). S. aureus was moderately sensitive to compounds 2b and 2e at a concentration of 0.5%.

**Table 1.** Antimicrobial activity of the synthesized compounds determined by diffusion method (only compounds that gave positive results at least in one case are included in the table)

<table>
<thead>
<tr>
<th>Compound</th>
<th>Concentration, %</th>
<th>Inhibition diameter of microorganism growth, mm</th>
<th>Bactericidal activity</th>
<th>Fungicidal activity</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>E. coli</td>
<td>S. aureus</td>
</tr>
<tr>
<td>2b</td>
<td>0.5</td>
<td>7.0</td>
<td>12.7</td>
<td>24.0</td>
</tr>
<tr>
<td></td>
<td>0.1</td>
<td>6.0</td>
<td>0</td>
<td>10.0</td>
</tr>
<tr>
<td>2c</td>
<td>0.5</td>
<td>0</td>
<td>15.0</td>
<td>25.0</td>
</tr>
<tr>
<td></td>
<td>0.1</td>
<td>0</td>
<td>10.0</td>
<td>20.0</td>
</tr>
<tr>
<td>2e</td>
<td>0.5</td>
<td>0</td>
<td>10.0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>0.1</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>3m</td>
<td>0.5</td>
<td>0</td>
<td>0</td>
<td>10.0</td>
</tr>
<tr>
<td></td>
<td>0.1</td>
<td>0</td>
<td>0</td>
<td>7.0</td>
</tr>
<tr>
<td>C*</td>
<td>0.5</td>
<td>14.0</td>
<td>15.0</td>
<td>18.0</td>
</tr>
</tbody>
</table>

* Vancomycin was used as a control in the tests of antibacterial activity of the synthesized compounds and Nystatin was used in the tests of antifungal action.
Activity of compound 2c wholly coincided with the control agent Vancomycin. Other compounds were not active against this strain of bacteria. The *M. luteum* strain was highly sensitive to compounds 2b and 2c at a concentration of 0.5% (the diameter of the inhibition zone was 25.0 and 24.0 mm, respectively) and slightly-sensitive to compound 3m at the same concentration (the diameter of the inhibition zone was 10.0 mm). Evaluation of the antibacterial activity of synthesized compounds using the serial dilution technique (Table 2) showed that all the synthesized compounds had no inhibitory effect at the studied concentrations against *E. coli* and *C. tenuis*. The evaluations (Table 2) showed that only compounds 2b, 2e, 3s and 5a–c have MIC 31.2–125 µg/mL against the test-culture *S. aureus*, while derivatives 2b, 2c, 2e, 3d, 3m, 3s and 5a–c have MIC 7.8–125 µg/mL against *M. luteum*. Fungi strain *A. niger* shows low activity properties at MIC 31.2–500 µg/mL for compounds 5a–c. The other compounds did not exhibited any significant antifungal activity against fungal strains used in this study.

Table 2. Bactericidal and fungicidal activity of the synthesized compounds determined by serial dilution method (only compounds that gave positive results at least in one case are included in the table)

<table>
<thead>
<tr>
<th>Compound</th>
<th>Minimum inhibition concentration MIC (µg/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td><em>E. coli</em></td>
</tr>
<tr>
<td>2b</td>
<td>+</td>
</tr>
<tr>
<td>2c</td>
<td>+</td>
</tr>
<tr>
<td>2e</td>
<td>+</td>
</tr>
<tr>
<td>3d</td>
<td>+</td>
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<tr>
<td>3m</td>
<td>+</td>
</tr>
<tr>
<td>3s</td>
<td>+</td>
</tr>
<tr>
<td>5a</td>
<td>+</td>
</tr>
<tr>
<td>5b</td>
<td>+</td>
</tr>
<tr>
<td>5c</td>
<td>+</td>
</tr>
</tbody>
</table>

+ – growth of microorganisms

To our great surprise, it was found that modification of carboxyl fragment by introducing various heterocycles into the molecule did not yielded any positive results. Compounds 6–12 being derivatives of sufficiently effective compound 2c appeared to be completely inactive against tested bacteria and fungi strains.

The structure-activity relationship study of the investigated compounds has shown that thiazoles 2a–e and bis(thiazolylmethanes) 3a–s, 5a–c are inactive or show slight activity against *C. tenuis* and *A. niger*. The replacement of the nitro-group with halogen atom in thiazoles 2a–e intensifies the antibacterial activity against *M. luteum*. The comparison of the activity of thiazoles 2a–e with the one of bis(thiazol-5-yl)phenylmethanes 3a–s, 5a–c, and bis(thiazol-5-yl)methanes 4a–e has shown that the introduction a second thiazolyl moiety leads to the disappearance of the antibacterial effect against the
tested bacteria and fungi strains (the diffusion technique at concentrations of 0.1% and 0.5%). The presence of dimethylamino substituent at \( p \)-position in a one benzene ring when other two benzene rings are unsubstituted at this position in bis(thiazol-5-yl)phenylmethanes 3a–s increases the antibacterial activity against strain \( M. \) luteum than their analogue containing a substituent in \( p \)-position (serial dilution method).

**CONCLUSIONS**

In summary, a series of new bis(thiazol-5-yl)phenyl- and bis(thiazol-5-yl)methanes were designed and synthesized. In addition, a modification of the carboxyl group of the thiazole derivative was carried out and compounds containing various heterocyclic fragments were obtained. All the synthesized compounds were tested for their biological activity. The synthesized compounds 2b, 2c, 2e, 3d exhibited a promising antibacterial activity against bacteria \( M. \) luteum.

**EXPERIMENTAL**

The reaction course and the purity of the synthesized compounds were monitored by TLC using aluminium plates pre-coated with silica gel 60 F\(_{254}\) (MerckKGaA, Darmstadt, Germany). The melting points were determined with a B-540 melting point analyzer (Büchi Corporation, New Castle, DE, USA) and are uncorrected. IR spectra (\( \nu \), cm\(^{-1}\)) were recorded on a Perkin–Elmer Spectrum BX FT–IR spectrometer using KBr tablets. The \( ^1\)H and \( ^{13}\)C NMR spectra were recorded in DMSO-\( d_6 \) on a Bruker Ascend 400 (400, 101 MHz) spectrometer. Chemical shifts (\( \delta \)) were reported in ppm relative to internal TMS or the deuterated solvent. Mass spectra were measured on a Bruker maXis 4G mass spectrometer.

**Starting Materials.** All reagents and solvents were purchased from Sigma-Aldrich (St. Louis, MO, USA) and Fluka (Buchs, Switzerland).

**General Procedure for the Preparation of Thiazoles 2a–e.** A mixture of thioureido acid 1 (2 g, 8.4 mmol), the corresponding bromoacetophenone (10.4 mmol) and acetone (40 mL) was refluxed for 2–3 h. The formed \( N,N \)-disubstituted aminothiazole hydrobromides were filtered off, washed with plenty of acetone and then boiled in 4% aqueous sodium acetate for 5 min. The obtained appropriate product 2 was filtered off, washed with water and dried.

3-[(4-Phenythiazol-2-yl)-p-tolylamino]propanoic Acid (2a): a light-blue solid, yield 2.52 g (89%), mp 156–157 °C; IR (KBr) \( \nu_{\text{max}} \) (cm\(^{-1}\)): 1712 (C=O), 1510 (C=N); \( ^1\)H NMR (400 MHz, DMSO-\( d_6 \)): \( \delta \) 2.35 (3H, s, CH\(_3\)), 2.68 (2H, t, \( J = 7.1 \) Hz, CH\(_2\)CO), 4.18 (2H, t, \( J = 7.1 \) Hz, NCH\(_2\)), 7.12 (1H, s, CH), 7.29–7.88 (9H, m, H\(_{ar}\)), 12.27 (1H, s, OH); \( ^{13}\)C NMR (101 MHz, DMSO-\( d_6 \)): \( \delta \) 20.7 (CH\(_3\)), 32.4 (CH\(_2\)CO), 48.5 (NCH\(_2\)), 102.6, 125.7, 126.9, 127.5, 128.5, 130.6, 134.7, 137.1, 141.9, 150.4, 169.1, 172.7 (C=O, C=N,
3-[(4-(4-Chlorophenyl)thiazol-2-yl)-357.1089. 169.3, 172.6 (C
ar 7.30 C=N, C≡N, C=O); HRMS m/z calculated for C19H17F2O2S [M+H]+: 357.0995, found: 357.1089.

3-[(4-(4-Cyanophenyl)thiazol-2-yl)-48.5 (NCH 48.5 (NCH
2.34 (3H, s, CH 3
3-[(4-(4-Nitrophenyl)thiazol-2-yl)-176 °C; IR (KBr) ν
3-[(4-(4-Fluorophenyl)thiazol-2-yl)-198 °C; IR (KBr) ν
3.20 g (99%), mp 160–161 °C; IR (KBr) ν
3.10 g (99%), mp 160–161 °C; IR (KBr) ν
3.67 (2H, s, CH2CO), 4.17 (2H, t, J = 7.1 Hz, NCH2), 7.19 (1H, s, CH), 7.29–7.89 (8H, m, H ar), 12.28 (1H, s, OH); 13C NMR (101 MHz, DMSO-d6): δ 20.7 (CH3), 32.4 (CH2CO), 48.5 (NCH2), 103.4, 126.9, 127.3, 128.6, 130.6, 131.9, 133.5, 137.2, 141.9, 149.1, 169.3, 172.6 (C ar, C=N, C=O); HRMS m/z calculated for C19H17F2O2S [M+H]+: 364.1041, found: 364.1118.

3-[(4-(4-Nitrophenyl)thiazol-2-yl)-p-tolylamino]propanoic Acid (2e): a yellow solid, yield 2.87 g (89%), mp 181–182 °C; IR (KBr) ν
3-[(4-(4-Chlorophenyl)thiazol-2-yl)-p-tolylamino]propanoic Acid (2c): a light-blue solid, yield 3.10 g (99%), mp 160–161 °C; IR (KBr) ν
3-[(4-(4-Fluorophenyl)thiazol-2-yl)-p-tolylamino]propanoic Acid (2d): a light-green solid, yield 2.59 g (85%), mp 175–176 °C; IR (KBr) ν
3-[(4-(4-Cyanophenyl)thiazol-2-yl)-p-tolylamino]propanoic Acid (2b): a light-blue solid, yield 2.67 g (87%), mp 146–147 °C; IR (KBr) ν
3-[(4-(4-Fluorophenyl)thiazol-2-yl)-p-tolylamino]propanoic Acid (2a): a greenish solid, yield 0.5 g (44%), mp 197–198 °C; IR

General Procedure for the Preparation of Bis(thiazol-5-yl)phenylmethanes 3a–e. To a mixture of the corresponding compound 2a–e (3 mmol), the appropriate aromatic aldehyde (1.5 mmol) (molar ratio 2:1) and acetone (40 mL), the concentrated hydrochloric acid (0.5 mL) was added dropwise. The mixture was heated at reflux for 18 h and cooled down. The formed crystalline product was filtered off, washed with plenty of acetone and boiled in 4% aqueous sodium acetate for 5 min. The obtained appropriate product 3 was filtered off, washed with water and dried.

3-[(5-{[2-{(2-Carboxyethyl)-p-tolylamino]-4-phenylthiazol-5-yl}(4-fluorophenyl)methyl]-4-phenyl-
thiazol-2-yl)-p-tolylamino]propanoic Acid (3a): a greenish solid, yield 0.5 g (44%), mp 197–198 °C; IR
(KBr) $\nu_{max}$ (cm$^{-1}$): 1707 (C=O), 1509 (C=N); $^1$H NMR (400 MHz, DMSO-$d_6$): $\delta$ 2.32 (6H, s, 2xCH$_3$), 2.58 (4H, t, $J = 7.3$ Hz, 2xCH$_2$CO), 4.07 (4H, t, $J = 6.7$ Hz, 2xNCH$_2$), 5.74 (1H, s, CH), 7.08–7.28 (22H, m, H$_{ar}$); $^{13}$C NMR (101 MHz, DMSO-$d_6$): $\delta$ 20.7 (CH$_3$), 32.5 (CH$_2$CO), 40.7 (CCHC), 48.2 (NCH$_2$), 115.5, 115.7, 123.7, 126.9, 127.7, 127.9, 128.2, 129.3, 129.4, 130.6, 134.6, 137.2, 139.8, 141.7, 147.3, 159.8, 162.2, 167.1, 172.8 (C$_{ar}$, C=N, C=O); HRMS m/z calculated for C$_{45}$H$_{39}$FN$_{4}$O$_{5}$S$_2$ [M+H]$^+$: 783.2397, found: 783.2506.

3-{[(2-Carboxyethyl)-p-tolylamino]-4-phenylthiazol-5-yl}(4-chlorophenyl)methyl]-4-phenylthiazol-2-yl)-p-tolylamino)propanoic Acid (3b): a light-green solid, yield 0.5 g (42%), mp 168–169 °C; IR (KBr) $\nu_{max}$ (cm$^{-1}$): 1712 (C=O), 1512 (C=N); $^1$H NMR (400 MHz, DMSO-$d_6$): $\delta$ 2.31 (6H, s, 2xCH$_3$), 2.56 (4H, t, $J = 7.3$ Hz, 2xCH$_2$CO), 4.06 (4H, t, $J = 6.9$ Hz, 2xNCH$_2$), 5.73 (1H, s, CH), 7.10–7.34 (22H, m, H$_{ar}$), 12.48 (2H, s, 2xOH); $^{13}$C NMR (101 MHz, DMSO-$d_6$): $\delta$ 20.7 (CH$_3$), 32.7 (CH$_2$CO), 40.8 (CCHC), 48.4 (NCH$_2$), 123.2, 126.9, 127.7, 127.9, 128.2, 128.8, 129.2, 130.6, 131.7, 134.5, 137.1, 141.7, 142.6, 147.5, 167.1, 172.9 (C$_{ar}$, C=N, C=O); HRMS m/z calculated for C$_{45}$H$_{39}$ClN$_{4}$O$_{5}$S$_2$ [M+H]$^+$: 799.2101, found: 799.2201.

3-{[(4-Bromophenyl)-(2-carboxyethyl)-p-tolylamino]-4-phenylthiazol-5-yl}methyl]-4-phenylthiazol-2-yl)-p-tolylamino)propanoic Acid (3c): a light-green solid, yield 0.53 g (42%), mp 166–167 °C; IR (KBr) $\nu_{max}$ (cm$^{-1}$): 1713 (C=O), 1510 (C=N); $^1$H NMR (400 MHz, DMSO-$d_6$): $\delta$ 2.26 (6H, s, 2xCH$_3$), 2.46–2.48 (4H, m, 2xCH$_2$CO), 4.02 (4H, t, $J = 6.9$ Hz, 2xNCH$_2$), 5.71 (1H, s, CH), 7.04–7.45 (22H, m, H$_{ar}$); $^{13}$C NMR (101 MHz, DMSO-$d_6$): $\delta$ 20.6 (CH$_3$), 33.4 (CH$_2$CO), 40.9 (CCHC), 48.9 (NCH$_2$), 120.3, 123.0, 126.8, 127.7, 127.9, 128.2, 129.6, 130.5, 131.7, 134.6, 136.9, 141.8, 143.0, 147.5, 167.1, 173.6 (C$_{ar}$, C=N, C=O); HRMS m/z calculated for C$_{45}$H$_{39}$BrN$_{4}$O$_{5}$S$_2$ [M+H]$^+$: 843.1596, found: 843.1687.

3-{[(2-Carboxyethyl)-p-tolylamino]-4-phenylthiazol-5-yl}(4-dimethylaminophenyl)methyl]-4-phenylthiazol-2-yl)-p-tolylamino)propanoic Acid (3d): a greenish solid, yield 0.56 g (47%), mp 191–192 °C; IR (KBr) $\nu_{max}$ (cm$^{-1}$): 1713 (C=O), 1519 (C=N); $^1$H NMR (400 MHz, DMSO-$d_6$): $\delta$ 2.31 (6H, s, 2xCH$_3$), 2.58 (4H, t, $J = 7.1$ Hz, 2xCH$_2$CO), 2.82 (6H, s, N(CH$_3$)$_2$), 4.06 (4H, t, $J = 6.2$ Hz, 2xNCH$_2$), 5.63 (1H, s, CH), 6.59–7.29 (22H, m, H$_{ar}$), 12.45 (2H, br. s, 2xOH); $^{13}$C NMR (101 MHz, DMSO-$d_6$): $\delta$ 20.7 (CH$_3$), 32.5 (CH$_2$CO), 39.9 (N(CH$_3$)$_2$), 40.4 (CCHC), 48.2 (NCH$_2$), 112.2, 125.3, 126.9, 127.5, 127.9, 128.1, 130.6, 131.0, 134.8, 137.0, 141.9, 146.5, 149.1, 166.8, 172.7 (C$_{ar}$, C=N, C=O); HRMS m/z calculated for C$_{47}$H$_{45}$N$_5$O$_{5}$S$_2$ [M+H]$^+$: 808.2913, found: 808.3007.

3-{[(2-Carboxyethyl)-p-tolylamino]-4-phenylthiazol-5-yl}(4-nitrophenyl)methyl]-4-phenylthiazol-2-yl)-p-tolylamino)propanoic Acid (3e): a green solid, yield 0.56 g (47%), mp 167–168 °C; IR (KBr) $\nu_{max}$ (cm$^{-1}$): 1705 (C=O), 1513 (C=N); $^1$H NMR (400 MHz, DMSO-$d_6$): $\delta$ 2.32 (6H, s, 2xCH$_3$), 2.61 (4H, t, $J = 6.9$ Hz, 2xCH$_2$CO), 4.09 (4H, t, $J = 6.7$ Hz, 2xNCH$_2$), 5.85 (1H, s, CH), 7.19–8.15 (22H, m, H$_{ar}$); $^{13}$C NMR (101 MHz, DMSO-$d_6$): $\delta$ 20.7 (CH$_3$), 32.3 (CH$_2$CO), 41.3 (CCHC), 48.2 (NCH$_2$),
122.0, 124.1, 126.9, 128.0, 128.4, 128.8, 130.7, 134.2, 137.4, 141.6, 146.5, 147.8, 150.6, 167.4, 172.5 (C=ar, C=N, C=O); HRMS \( m/z \) calculated for C_{45}H_{39}N_{3}O_{6}S_{2} [M+H]^+: 810.2342, found: 810.2433.

3-[[2-[(2-Carboxyethyl)-p-tolylamino]-4-(4-fluorophenyl)thiazol-5-yl](4-dimethylaminophenyl)-methyl]-4-(4-fluorophenyl)thiazol-2-yl]-p-tolylamino)propanoic Acid (3f): a green-blue solid, yield 0.41 g (35%), mp 163–164 °C; IR (KBr): \( \nu_{\text{max}} \) (cm\(^{-1}\)): 1713 (C=O), 1512 (C=N); \(^1\)H NMR (400 MHz, DMSO-\(d_6\)): \( \delta \) 2.32 (6H, s, 2xCH\(_3\)), 2.58 (4H, s, 2xCH\(_2\)CO), 2.84 (6H, s, N(CH\(_3\))\(_2\)), 4.06 (4H, t, \( J = 6.7 \) Hz, 2xNCH\(_2\)), 5.53 (1H, s, CH), 6.68–7.27 (20H, m, H\(_{ar}\)), 12.26 (2H, s, 2xOH); \(^{13}\)C NMR (101 MHz, DMSO-\(d_6\)): \( \delta \) 20.7 (CH\(_3\)), 30.3 (CH\(_2\)CO), (N(CH\(_3\))\(_2\) overlaps with the solvent), 40.4 (CCHC), 48.0 (NCH\(_2\)), 112.6, 114.9, 115.1, 124.9, 126.9, 128.0, 129.8, 129.9, 130.6, 131.2, 137.2, 141.7, 145.6, 150.4, 160.2, 162.7, 166.9, 172.5 (C=ar, C=N, C=O); HRMS \( m/z \) calculated for C\(_{45}\)H\(_{43}\)F\(_2\)N\(_2\)O\(_4\)S\(_2\) [M+H]^+: 844.2725, found: 844.2801.

3-[[2-[(2-Carboxyethyl)-p-tolylamino]-4-(4-fluorophenyl)thiazol-5-yl](4-nitrophenyl)methyl]-4-(4-fluorophenyl)thiazol-2-yl]-p-tolylamino)propanoic Acid (3g): a green solid, yield 0.48 g (40%), mp 183–184 °C; IR (KBr) \( \nu_{\text{max}} \) (cm\(^{-1}\)): 1710 (C=O), 1513 (C=N); \(^1\)H NMR (400 MHz, DMSO-\(d_6\)): \( \delta \) 2.31 (6H, s, 2xCH\(_3\)), 2.60 (4H, t, \( J = 7.1 \) Hz, 2xCH\(_2\)CO), 4.08 (4H, t, \( J = 7.0 \) Hz, 2xNCH\(_2\)), 5.76 (1H, s, CH), 7.04–8.15 (20H, m, H\(_{ar}\)); \(^{13}\)C NMR (101 MHz, DMSO-\(d_6\)): \( \delta \) 20.7 (CH\(_3\)), 32.3 (CH\(_2\)CO), 41.2 (CCHC), 48.2 (NCH\(_2\)), 115.2, 115.4, 121.9, 124.1, 126.9, 128.9, 130.1, 130.2, 130.7, 137.5, 141.5, 146.6, 146.9, 150.3, 160.5, 162.9, 167.4, 172.5 (C=ar, C=N, C=O); HRMS \( m/z \) calculated for C\(_{45}\)H\(_{37}\)Cl\(_2\)F\(_2\)N\(_2\)O\(_4\)S\(_2\) [M+H]^+: 846.2153, found: 846.2242.

3-[[2-[(2-Carboxyethyl)-p-tolylamino]-4-(4-chlorophenyl)thiazol-5-yl][4-fluorophenyl)methyl]-4-(4-chlorophenyl)thiazol-2-yl]-p-tolylamino)propanoic Acid (3h): a white solid, yield 0.3 g (26%), mp 191–192 °C; IR (KBr) \( \nu_{\text{max}} \) (cm\(^{-1}\)): 1710 (C=O), 1510 (C=N); \(^1\)H NMR (400 MHz, DMSO-\(d_6\)): \( \delta \) 2.25 (6H, s, 2xCH\(_3\)), 2.44 (4H, s, 2xCH\(_2\)CO), 3.99 (4H, s, 2xNCH\(_2\)), 5.66 (1H, s, CH), 7.04–7.24 (20H, m, H\(_{ar}\)); \(^{13}\)C NMR (101 MHz, DMSO-\(d_6\)): \( \delta \) 20.6 (CH\(_3\)), 33.5 (CH\(_2\)CO), 40.6 (CCHC), 48.9 (NCH\(_2\)), 115.5, 115.7, 124.0, 126.8, 128.2, 129.6, 130.5, 132.4, 133.4, 136.9, 139.3, 141.7, 146.1, 159.8, 162.2, 167.1, 173.2 (C=ar, C=N, C=O); HRMS \( m/z \) calculated for C\(_{45}\)H\(_{37}\)Cl\(_2\)F\(_2\)N\(_2\)O\(_4\)S\(_2\) [M+H]^+: 851.1676, found: 851.1696.

3-[[2-[(2-Carboxyethyl)-p-tolylamino]-4-(4-chlorophenyl)thiazol-5-yl][4-chlorophenyl)methyl]-4-(4-chlorophenyl)thiazol-2-yl]-p-tolylamino)propanoic Acid (3i): a white solid, yield 0.33 g (28%), mp 181–182 °C; IR (KBr) \( \nu_{\text{max}} \) (cm\(^{-1}\)): 1711 (C=O), 1512 (C=N); \(^1\)H NMR (400 MHz, DMSO-\(d_6\)): \( \delta \) 2.25 (6H, s, 2xCH\(_3\)), 2.45 (4H, t, \( J = 7.5 \) Hz, 2xCH\(_2\)CO), 4.00 (4H, t, \( J = 7.1 \) Hz, 2xNCH\(_2\)), 5.66 (1H, s, CH), 7.14–7.31 (20H, m, H\(_{ar}\)); \(^{13}\)C NMR (101 MHz, DMSO-\(d_6\)): \( \delta \) 20.6 (CH\(_3\)), 33.4 (CH\(_2\)CO), 40.8 (CCHC), 48.9 (NCH\(_2\)), 123.5, 126.8, 128.3, 128.8, 129.3, 129.6, 130.5, 131.9, 132.5, 133.4, 137.0, 141.6, 142.1, 146.3, 167.2, 173.4 (C=ar, C=N, C=O); HRMS \( m/z \) calculated for C\(_{45}\)H\(_{37}\)Cl\(_3\)N\(_4\)O\(_4\)S [M+H]^+: 867.1322,
found: 867.1382.

3-[(4-Bromophenyl)[2-[(2-carboxyethyl)-p-tolylamino]-4-(4-chlorophenyl)thiazol-5-yl]methyl]-4-(4-chlorophenyl)thiazol-2-yl]-p-tolylamino]propanoic Acid (3j): a white solid, yield 0.57 g (47%), mp 158−159 °C; IR (KBr) νmax (cm−1): 1714 (C=O), 1512 (C=N); 1H NMR (400 MHz, DMSO-d6): δ 2.30 (6H, s, 2xCH3), 2.55 (4H, s, 2xCH2CO), 4.04 (4H, s, 2xNCH2), 5.64 (1H, s, CH), 7.09−7.48 (20H, m, Har); 13C NMR (101 MHz, DMSO-d6): δ 32.6 (CH2CO), 40.8 (CH2), 48.4 (NCH2), 120.5, 123.5, 126.9, 128.3, 129.6, 130.6, 131.8, 132.5, 133.3, 137.3, 141.6, 142.4, 146.4, 167.3, 172.8 (C=O); HRMS m/z calculated for C45H37BrClN4O4S [M+H]+: 911.0817, found: 911.0895.

3-[(5-[(2-(2-carboxyethyl)-p-tolylamino)-4-(4-chlorophenyl)thiazol-5-yl](4-dimethylaminophenyl)methyl]-4-(4-chlorophenyl)thiazol-2-yl]-p-tolylamino]propanoic Acid (3k): a green-blue solid, yield 0.43 g (37%), mp 162−163 °C; IR (KBr) νmax (cm−1): 1711 (C=O), 1510 (C=N); 1H NMR (400 MHz, DMSO-d6): δ 2.28 (6H, s, 2xCH3), 2.52 (4H, s, 2xCH2CO), 2.80 (6H, s, N(CH3)2), 4.03 (4H, s, 2xNCH2), 5.56 (1H, s, CH), 6.58−7.25 (20H, m, Har); 13C NMR (101 MHz, DMSO-d6): δ 20.6 (CH3), 32.9 (CH2CO), 39.8 (N(CH3)2), 40.4 (CH2HC), 48.5 (NCH2), 112.2, 125.6, 126.9, 127.9, 128.2, 129.6, 130.4, 130.5, 132.2, 133.6, 137.0, 141.7, 145.4, 149.1, 166.9, 173.1 (C=O); HRMS m/z calculated for C47H43ClN5O4S2 [M+H]+: 876.2134, found: 876.2229.

3-[(5-[(2-(2-carboxyethyl)-p-tolylamino)-4-(4-cyanophenyl)thiazol-5-yl](4-chlorophenyl)methyl]-4-(4-cyanophenyl)thiazol-2-yl]-p-tolylamino]propanoic Acid (3l): a greenish solid, yield 0.57 g (43%), mp 175−176 °C; IR (KBr) νmax (cm−1): 1711 (C=O), 1510 (C=N); 1H NMR (400 MHz, DMSO-d6): δ 2.28 (6H, s, 2xCH3), 2.53−2.55 (4H, m, 2xCH2CO), 4.04 (4H, t, J = 6.7 Hz, 2xNCH2), 5.80 (1H, s, CH), 7.20−8.13 (m, 20H, Har); 13C NMR (101 MHz, DMSO-d6): δ 21.1 (CH3), 33.1 (CH2CO), 41.7 (C=O), 48.9 (NCH2), 122.9, 124.6, 127.4, 128.8, 129.3, 130.2, 131.1, 133.1, 133.6, 137.8, 142.0, 147.0, 147.3, 150.6, 167.9, 173.3 (C=O); HRMS m/z calculated for C45H37ClN5O6S2 [M+H]+: 878.1562, found: 878.1637.

3-[(5-[(2-(2-carboxyethyl)-p-tolylamino)-4-(4-cyanophenyl)thiazol-5-yl]methyl]-4-(4-chlorophenyl)thiazol-2-yl]-p-tolylamino]propanoic Acid (3m): a grey solid, yield 0.52 g (41%), mp 217−218 °C; IR (KBr) νmax (cm−1): 1710 (C=O), 1511 (C=N); 1H NMR (400 MHz, DMSO-d6): δ 2.25 (6H, s, 2xCH3), 2.43 (4H, s, 2xCH2CO), 3.98 (4H, s, 2xNCH2), 5.76 (1H, s, CH), 7.17−7.67 (20H, m, H=H); 13C NMR (101 MHz, DMSO-d6): δ 20.6 (CH3), 33.3 (CH2CO), 40.7 (C=O), 48.9 (NCH2), 110.2, 118.6, 125.4, 126.7, 128.6, 128.9, 129.4, 129.5, 130.6, 130.7, 132.1, 132.2, 137.1, 138.9, 141.5, 145.8, 167.3, 173.3 (C=O); HRMS m/z calculated for C47H37ClN6O6S2 [M+H]+: 849.2006, found: 849.2075.
3-[[2-[(2-Carboxyethyl)-p-tolylamino]-4-(4-nitrophenyl)thiazol-5-yl](4-fluorophenyl)methyl]-4-(4-nitrophenyl)thiazol-2-yl]-p-tolylamino)propanoic Acid (3n): a yellow solid, yield 0.51 g (39%), mp 213–214 °C; IR (KBr): 1706 (C=O), 1511 (C=N) cm⁻¹; ¹H NMR (400 MHz, DMSO-d₆): δ 2.23 (s, 6H, 2xCH₃), 2.36 (s, 4H, 2xCH₂CO), 3.97 (s, 4H, 2xNCH₂), 5.86 (s, 1H, CH), 7.08–8.02 (m, 20H, Hₘ); ¹³C NMR (101 MHz, DMSO-d₆): δ 20.6 (CH₃), 34.2 (CH₂CO), 40.6 (CH₂CH₂), 49.5 (CH₂), 115.6, 115.8, 123.4, 126.3, 126.7, 128.9, 129.7, 130.5, 136.9, 138.6, 140.9, 141.6, 145.4, 146.3, 159.9, 167.3, 174.1 (Cₘ, C=N, C=O); HRMS m/z calculated for C₄₅H₃₇FN₆O₈S₂ [M+H]⁺: 873.2098, found: 873.2169.

3-[[2-[(2-Carboxyethyl)-p-tolylamino]-4-(4-nitrophenyl)thiazol-5-yl](4-chlorophenyl)methyl]-4-(4-nitrophenyl)thiazol-2-yl]-p-tolylamino)propanoic Acid (3o): a yellow solid, yield 0.56 g (42%), mp 197–198 °C; IR (KBr): v_max (cm⁻¹) 1712 (C=O), 1512 (C=N); ¹H NMR (400 MHz, DMSO-d₆): δ 2.23 (6H, s, 2xCH₃), 2.41 (4H, s, 2xCH₂CO), 3.99 (4H, s, 2xNCH₂), 5.86 (1H, s, CH), 7.15–8.02 (20H, m, Hₘ); ¹³C NMR (101 MHz, DMSO-d₆): δ 20.6 (CH₃), 33.7 (CH₂CO), 40.7 (CH₂CH₂), 49.2 (CH₂), 123.4, 125.8, 126.8, 128.9, 129.5, 130.1, 130.6, 132.1, 137.1, 140.8, 141.3, 141.5, 145.6, 146.3, 167.4, 173.8 (Cₘ, C=N, C=O); HRMS m/z calculated for C₄₅H₃₇ClN₆O₈S₂ [M+H]⁺: 889.1803, found: 889.1866.

3-[[4-(Bromophenyl)]2-[(2-carboxyethyl)-p-tolylamino]-4-(4-nitrophenyl)thiazol-5-yl]methyl]-4-(4-nitrophenyl)thiazol-2-yl]-p-tolylamino)propanoic Acid (3p): a yellow solid, yield 0.53 g (38%), mp 220–221 °C; IR (KBr) v_max (cm⁻¹): 1706 (C=O), 1512 (C=N); ¹H NMR (400 MHz, DMSO-d₆): δ 2.24 (6H, s, 2xCH₃), 2.43 (4H, s, 2xCH₂CO), 3.99 (4H, s, 2xNCH₂), 5.84 (1H, s, CH), 7.19–8.02 (20H, m, Hₘ); ¹³C NMR (101 MHz, DMSO-d₆): δ 20.6 (CH₃), 33.5 (CH₂CO), 40.8 (CH₂CH₂), 48.9 (CH₂), 120.8, 123.4, 125.8, 126.8, 128.9, 129.8, 130.6, 131.8, 137.2, 140.8, 141.5, 141.7, 145.6, 146.3, 167.4, 173.9 (Cₘ, C=N, C=O); HRMS m/z calculated for C₄₅H₃₇BrN₆O₈S₂ [M+H]⁺: 933.1298, found: 933.1363.

3-[[2-[(2-Carboxyethyl)-p-tolylamino]-4-(4-nitrophenyl)thiazol-5-yl](4-dimethylaminophenyl)methyl]-4-(4-nitrophenyl)thiazol-2-yl]-p-tolylamino)propanoic Acid (3r): a light-green solid, yield 0.55 g (41%), mp 212–213 °C; IR (KBr) v_max (cm⁻¹): 1708 (C=O), 1512 (C=N); ¹H NMR (400 MHz, DMSO-d₆): δ 2.23 (6H, s, 2xCH₃), 2.40 (4H, s, 2xCH₂CO), 2.79 (6H, s, N(CH₃)₂), 3.98 (4H, s, 2xNCH₂), 5.73 (1H, s, CH), 6.57–8.01 (20H, m, Hₘ); ¹³C NMR (101 MHz, DMSO-d₆): δ 20.6 (CH₃), 33.8 (CH₂CO), 40.5 (CH₂CH₂), 49.2 (CH₂), 112.3, 123.3, 126.8, 127.9, 128.2, 128.9, 129.6, 130.5, 136.9, 141.1, 141.6, 144.7, 146.2, 149.3, 167.1, 173.8 (Cₘ, C=N, C=O); HRMS m/z calculated for C₄₇H₄₃N₇O₈S₂ [M+H]⁺: 898.2615, found: 898.2685.

3-[[2-[(2-Carboxyethyl)-p-tolylamino]-4-(4-nitrophenyl)thiazol-5-yl](4-nitrophenyl)methyl]-4-(4-nitrophenyl)thiazol-2-yl]-p-tolylamino)propanoic Acid (3s): a yellow solid, yield 0.50 g (37%), mp 202–203 °C; IR (KBr) v_max (cm⁻¹): 1708 (C=O), 1513 (C=N); ¹H NMR (400 MHz, DMSO-d₆): δ 2.22 (6H, s, 2xCH₃), 2.44 (4H, s, 2xCH₂CO), 4.01 (4H, s, 2xNCH₂), 6.00 (1H, s, CH), 7.16–8.09 (20H, m, Hₘ); ¹³C NMR (101 MHz, DMSO-d₆): δ 20.6 (CH₃), 33.5 (CH₂CO), 41.2 (CH₂CH₂), 49.1 (CH₂), 123.5, 124.1,
3-[(5-[[2-[2-Carboxyethyl]-p-tolylamino]-4-phenylthiazol-5-ylmethyl]-4-phenylthiazol-2-yl]-p-tolylamino]propanoic Acid (4a): a light-blue solid, yield 0.35 g (34%), mp 237–238 °C; IR (KBr) ν_{max} (cm\(^{-1}\)): 1706 (C=O), 1516 (C≡N); \(^1\)H NMR (400 MHz, DMSO-d_6): δ = 2.32 (6H, s, 2xCH_3), 2.59 (4H, s, 2xCH_2CO), 4.07 (4H, d, \(J = 6.1\) Hz, 2xNCH_2), 4.14 (2H, s, CCH_2C), 7.27–7.49 (18H, m, H_\text{ar}), 12.25 (2H, s, 2xOH); \(^13\)C NMR (101 MHz, DMSO-d_6): δ = 20.7 (CH_3), 24.6 (CCH_2C), 32.3 (CH_2CO), 48.0 (NCH_2), 120.4, 126.9, 127.5, 128.1, 128.3, 130.6, 134.6, 137.1, 141.8, 146.3, 166.2, 172.6 (C_\text{ar}, C≡N, C=O); HRMS m/z calculated for C_{39}H_{34}N_4O_8S_2 [M+H]^+: 689.2178, found: 689.2269.

3-[[5-[[2-[2-Carboxyethyl]-p-tolylamino]-4-(4-fluorophenyl)thiazol-5-ylmethyl]-4-(4-fluorophenyl)thiazol-2-yl]-p-tolylamino]propanoic Acid (4b): a blue solid, yield 0.36 g (35%), mp 239–240 °C; IR (KBr) ν_{max} (cm\(^{-1}\)): 1707 (C=O), 1507 (C≡N); \(^1\)H NMR (400 MHz, DMSO-d_6): δ = 2.32 (6H, s, 2xCH_3), 2.58 (4H, s, 2xCH_2CO), 4.06 (4H, s, 2xNCH_2), 4.10 (2H, s, CCH_2C), 7.15–7.51 (16H, m, H_\text{ar}), 12.24 (2H, s, 2xOH); \(^13\)C NMR (101 MHz, DMSO-d_6): δ = 20.7 (CH_3), 24.4 (CCH_2C), 32.3 (CH_2CO), 47.9 (NCH_2), 115.1, 115.3, 120.1, 126.9, 130.1, 130.2, 130.6, 131.1, 133.2, 141.8, 145.3, 160.2, 162.7, 166.3, 172.5 (C_\text{ar}, C≡N, C=O); HRMS m/z calculated for C_{39}H_{34}F_2N_4O_8S_2 [M+H]^+: 725.1900, found: 725.2086.

3-[[5-[[2-[2-Carboxyethyl]-p-tolylamino]-4-(4-chlorophenyl)thiazol-5-ylmethyl]-4-(4-chlorophenyl)thiazol-2-yl]-p-tolylamino]propanoic Acid (4c): a white solid, yield 0.36 g (35%), mp 239–240 °C; IR (KBr) ν_{max} (cm\(^{-1}\)): 1710 (C=O), 1515 (C≡N); \(^1\)H NMR (400 MHz, DMSO-d_6): δ = 2.32 (6H, s, 2xCH_3), 2.58 (4H, t, \(J = 7.2\) Hz, 2xCH_2CO), 4.06 (4H, t, \(J = 7.2\) Hz, 2xNCH_2), 4.13 (2H, s, CCH_2C), 7.26–7.51 (16H, m, H_\text{ar}), 12.24 (2H, s, 2xOH); \(^13\)C NMR (101 MHz, DMSO-d_6): δ = 20.7 (CH_3), 24.4 (CCH_2C), 32.3 (CH_2CO), 47.9 (NCH_2), 120.8, 126.9, 128.3, 129.8, 130.6, 132.1, 133.4, 137.2, 141.7, 145.1, 166.4, 172.5 (C_\text{ar}, C≡N, C=O); HRMS m/z calculated for C_{39}H_{34}Cl_2N_4O_8S_2 [M+H]^+: 757.1399, found: 757.1479.

3-[[5-[[2-[2-Carboxyethyl]-p-tolylamino]-4-(4-cyanophenyl)thiazol-5-ylmethyl]-4-(4-cyanophenyl)thiazol-2-yl]-p-tolylamino]propanoic Acid (4d): a white solid, yield 0.36 g (35%), mp 239–240 °C; IR (KBr) ν_{max} (cm\(^{-1}\)): 1712 (C=O), 1513 (C≡N); \(^1\)H NMR (400 MHz, DMSO-d_6): δ = 2.32 (6H, s, 2xCH_3), 2.58 (4H, t, \(J = 7.2\) Hz, 2xCH_2CO), 4.06 (4H, t, \(J = 7.1\) Hz, 2xNCH_2), 4.23 (2H, s, CCH_2C), 7.26–7.81 (16H, m, H_\text{ar}), 12.25 (2H, s, 2xOH); \(^13\)C NMR (101 MHz, DMSO-d_6): δ = 20.7 (CH_3), 24.4 (CCH_2C), 32.3 (CH_2CO), 48.0 (NCH_2), 109.9, 118.8, 122.6, 126.9, 128.8, 130.7, 132.3, 137.4, 138.9, 141.6, 144.7, 166.5, 172.5 (C_\text{ar}, C≡N, C=O); HRMS m/z calculated for C_{41}H_{34}N_6O_8S_2 [M+H]^+: 739.2083, found: 739.2159.
3-[[5-[[2-[(2-Carboxyethyl)-p-tolylamino]-4-(4-nitrophenyl)thiazol-5-ylmethyl]-4-(4-nitrophenyl)thiazol-2-yl]-p-tolylamino]propanoic Acid (4e): a yellow solid, yield 0.11 g (11%), mp 226–227 °C; IR (KBr) $\nu_{\text{max}}$ (cm$^{-1}$): 1727 (C=O), 1514 (C=N); $^1$H NMR (400 MHz, DMSO-$d_6$): $\delta$ 2.32 (6H, s, 2xCH$_3$), 2.58 (4H, $t$, $J$ = 7.0 Hz, 2xCH$_2$CO), 4.07 (4H, $t$, $J$ = 7.1 Hz, 2xNCH$_2$), 4.30 (2H, s, CCH$_2$C), 7.26–8.19 (16H, m, H$_{\text{ar}}$), 12.27 (2H, s, 2xOH); $^{13}$C NMR (101 MHz, DMSO-$d_6$): $\delta$ 20.7 (CH$_3$), 24.4 (C$_{11}$H$_{11}$C), 32.3 (CH$_2$CO), 48.0 (NCH$_2$), 123.3, 123.6, 126.9, 129.1, 130.7, 137.4, 140.9, 141.5, 144.4, 146.2, 166.6, 172.5 (C$_{\text{ar}}$, C=N, C=O); HRMS m/z calculated for C$_{39}$H$_{34}$N$_6$O$_8$S$_2$ [M+H]$^+$: 779.1880, found: 779.1950.

General Procedure for the Preparation of Bis(thiazol-5-yl)phenylmethanes 5a–c. A mixture of compound 2c (0.26 g, 0.7 mmol), the corresponding thiazole derivative 2b, 2d or 2e (0.7 mmol), the appropriate aromatic aldehyde (1.4 mmol) and acetone was heated at reflux for 16 h. Then the mixture was cooled down, the formed crystalline product filtered off, washed with acetone and boiled in 4% aqueous sodium acetate for 5 min. Afterwards, the mixture was cooled down, the precipitate was filtered off, washed with water and dried.

3-[[5-[[2-[(2-Carboxyethyl)-p-tolylamino]-4-(4-fluorophenyl)thiazol-5-yl](4-chlorophenyl)methyl]-4-(4-chlorophenyl)thiazol-2-yl]-p-tolylamino]propanoic Acid (5a): a pale-green solid, yield 0.53 g (93%), mp 159–160 °C; IR (KBr) $\nu_{\text{max}}$ (cm$^{-1}$): 1710 (C=O), 1510 (C=N); $^1$H NMR (400 MHz, DMSO-$d_6$): $\delta$ 2.32 (6H s, 2xCH$_3$), 2.59 (4H, $t$, $J$ = 6.5 Hz, 2xCH$_2$CO), 4.06 (4H, s, 2xNCH$_2$), 5.65 (1H, s, CH), 7.04–7.36 (20H, m, H$_{\text{ar}}$), 12.14 (2H, br. s, 2xOH); $^{13}$C NMR (101 MHz, DMSO-$d_6$): $\delta$ 20.7 (CH$_3$), 32.3 (C$_{11}$H$_{11}$CO), 40.7 (C$_{11}$HC), 48.1 (NCH$_2$), 115.1, 115.3, 122.9, 123.1, 123.6, 123.8, 126.9, 128.3, 128.9, 129.3, 129.6, 129.9, 130.0, 130.7, 130.9, 131.9, 132.5, 133.3, 137.3, 141.6, 141.9, 142.1, 142.2, 144.2, 146.2, 146.3, 146.4, 146.5, 160.4, 167.2, 167.3, 172.5 (C$_{\text{ar}}$, C=N, C=O); HRMS m/z calculated for C$_{45}$H$_{37}$Cl$_2$FN$_2$O$_4$S$_2$ [M+H]$^+$: 851.1617, found: 851.1701.

3-[[5-[[2-[(2-Carboxyethyl)-p-tolylamino]-4-(4-cyanophenyl)thiazol-5-yl](4-chlorophenyl)methyl]-4-(4-chlorophenyl)thiazol-2-yl]-p-tolylamino]propanoic Acid (5b): a light-grey solid, yield 0.51 g (88%), mp 170–171 °C; IR (KBr) $\nu_{\text{max}}$ (cm$^{-1}$): 1709 (C=O), 1510 (C=N); $^1$H NMR (400 MHz, DMSO-$d_6$): $\delta$ 2.30 (6H, s, 2xCH$_3$), 2.55 (4H, d, $J$ = 5.4 Hz, 2xNCH$_2$), 5.66, 5.71, 5.76 (1H, s, CH), 7.15–7.69 (20H, m, H$_{\text{ar}}$); $^{13}$C NMR (101 MHz, DMSO-$d_6$): $\delta$ 20.7 (CH$_3$), 32.3 (CH$_2$CO), 40.7 (C$_{11}$HC), 48.5 (NCH$_2$), 110.1, 118.7, 123.4, 125.4, 126.9, 128.3, 128.6, 128.9, 129.4, 129.6, 130.6, 130.7, 132.2, 133.3, 137.2, 137.3, 138.9, 141.5, 141.6, 145.8, 146.3, 146.5, 167.4, 173.0 (C$_{\text{ar}}$, C=N, C=O); HRMS m/z calculated for C$_{46}$H$_{37}$Cl$_2$FN$_2$O$_4$S$_2$ [M+H]$^+$: 858.1664, found: 858.1740.

3-[[5-[[2-[(2-Carboxyethyl)-p-tolylamino]-4-(4-dimethylaminophenyl)thiazol-5-yl](4-nitrophenyl)methyl]-4-(4-chlorophenyl)thiazol-2-yl]-p-tolylamino]propanoic Acid (5c): a green solid, yield 0.48 g (88%), mp 178–179 °C; IR (KBr) $\nu_{\text{max}}$ (cm$^{-1}$): 1719 (C=O), 1512 (C=N); $^1$H NMR (400 MHz, DMSO-$d_6$): $\delta$ 2.30 (6H, s, 2xCH$_3$), 2.55 (4H, d, $J$ = 3.8 Hz, 2xCH$_2$CO), 2.82 (6H, s, N(CH$_3$)$_2$), 4.04 (4H, s, 2xNCH$_2$),
5.55, 5.65, 5.75 (1H, 3s, CH), 6.58–8.04 (20H, m, H\textsubscript{ar}); \textsuperscript{13}C NMR (101 MHz, DMSO-\textit{d}_6): \delta 20.7 (CH\textsubscript{3}), 32.6 (CH\textsubscript{2}CO), 39.9 (N(CH\textsubscript{3})\textsubscript{2}), 40.4 (CCH\textsubscript{2}), 48.3 (NCH\textsubscript{2}), 112.3, 123.4, 125.2, 125.6, 126.9, 127.0, 127.9, 128.0, 128.1, 128.2, 128.6, 128.8, 128.9, 129.6, 129.9, 130.6, 130.7, 132.3, 132.4, 133.6, 137.2, 137.3, 137.4, 141.1, 141.6, 141.7, 144.5, 145.4, 146.2, 149.3, 166.9, 167.1, 172.8 (C\textsubscript{ar}, C=N, C=O); HRMS \textit{m/z} calculated for C\textsubscript{47}H\textsubscript{43}ClN\textsubscript{6}O\textsubscript{6}S\textsubscript{2} [M+H]\textsuperscript{+}: 887.2374, found: 887.2447.

3-[(4-(4-Chlorophenyl)thiazol-2-yl)-p-tolylamino]propanoic Acid Methyl Ester (6). A mixture of compound 2c (5.09 g, 16 mmol), MeOH (90 mL), and sulfuric acid (2.5 mL) was refluxed for 4 h. Then the liquid fraction was evaporated under reduced pressure, the residue was boiled in 10% aqueous sodium carbonate (15 mL) for 5 min, then cooled down, filtered off, washed with water, Et\textsubscript{2}O, and dried to afford a white solid, yield 4.09 g (67%), mp 104–105 °C; IR (KBr) \nu\textsubscript{max} (cm\textsuperscript{-1}): 1726 (C=O), 1509 (C=N), 1088 (O-C); \textsuperscript{1}H NMR (400 MHz, DMSO-\textit{d}_6): \delta 2.35 (3H, s, CH\textsubscript{3}), 2.74 (2H, t, \textit{J} = 6.9 Hz, CH\textsubscript{2}CO), 3.51 (3H, s, OCH\textsubscript{3}), 4.20 (2H, t, \textit{J} = 6.9 Hz, NCH\textsubscript{2}), 7.19 (1H, s, CH), 7.29–7.89 (8H, m, H\textsubscript{ar}); \textsuperscript{13}C NMR (101 MHz, DMSO-\textit{d}_6): \delta 20.7 (CH\textsubscript{3}), 32.2 (CH\textsubscript{2}CO), 48.5 (NCH\textsubscript{2}), 51.4 (OCH\textsubscript{3}), 103.5, 126.9, 127.3, 128.6, 130.6, 131.9, 133.5, 137.3, 141.8, 149.1, 169.3, 171.6 (C\textsubscript{ar}, C=N, C=O); HRMS \textit{m/z} calculated for C\textsubscript{20}H\textsubscript{19}ClN\textsubscript{2}O\textsubscript{2}S [M+H]\textsuperscript{+}: 387.0856, found: 387.0943.

3-[(4-(4-Chlorophenyl)thiazol-2-yl)-p-tolylamino]propanoic Acid Hydrazide (7). The ester 6 (3.86 g, 10 mmol) was dissolved in DMSO (10 mL), and hydrazine monohydrate (100 g, 2 mol, large excess) was added. The mixture was stirred at 90–100 °C for 2 h, cooled down and diluted with water (20 mL). The precipitate was filtered off, washed with water and dried to afford a white solid, yield 3.37 g (87%), mp 138–139 °C; IR (KBr) \nu\textsubscript{max} (cm\textsuperscript{-1}): 3258, 3195 (NH, NH\textsubscript{2}), 1626 (C=O), 1510 (C=N); \textsuperscript{1}H NMR (400 MHz, DMSO-\textit{d}_6): \delta 2.35 (3H, s, CH\textsubscript{3}), 2.45–2.50 (2H, m, CH\textsubscript{2}CO), 3.90–4.38 (4H, m, NCH\textsubscript{2}, NH\textsubscript{2}), 7.18 (1H, s, CH), 7.28–7.94 (8H, m, H\textsubscript{ar}), 10.18 (1H, s, NH); \textsuperscript{13}C NMR (101 MHz, DMSO-\textit{d}_6): \delta 20.7 (CH\textsubscript{3}), 31.9 (CH\textsubscript{2}CO), 49.3 (NCH\textsubscript{2}), 51.4 (OCH\textsubscript{3}), 103.5, 126.9, 127.3, 128.6, 130.6, 131.9, 133.5, 137.3, 141.8, 149.1, 169.3, 171.6 (C\textsubscript{ar}, C=N, C=O); HRMS \textit{m/z} calculated for C\textsubscript{19}H\textsubscript{19}ClN\textsubscript{4}OS [M+H]\textsuperscript{+}: 387.0968, found: 387.0943.

3-[(4-(4-Chlorophenyl)thiazol-2-yl)-p-tolylamino]-1-(3,5-dimethylpyrazol-1-yl)propan-1-one (8). A mixture of hydrazide 7 (0.6 g, 1.6 mmol), pentane-2,4-dione (0.48 g, 4.8 mmol), and 1,4-dioxane (20 mL) was refluxed for 24 h and then cooled to room temperature. The liquid fraction was evaporated under reduced pressure, and residue was diluted with water (20 mL). The formed oily mass was three times washed with boiling water, the formed crystalline product was filtered off, washed with water, dried, recrystallized from propan-2-ol and washed with hot hexane to afford a yellowish solid, yield 0.57 g (81%), mp 114–115 °C; IR (KBr) \nu\textsubscript{max} (cm\textsuperscript{-1}): 1732 (C=O), 1505 (C=N); \textsuperscript{1}H NMR (400 MHz, DMSO-\textit{d}_6): \delta 2.15 (3H, s, CH\textsubscript{3}), 2.34, 2.35 (6H, 2s, 2xCH\textsubscript{3}), 3.42 (2H, t, \textit{J} = 6.8 Hz, CH\textsubscript{2}CO), 4.35 (2H, t, \textit{J} = 6.8 Hz, NCH\textsubscript{2}), 6.13 (1H, s, CH), 7.17 (1H, s, CH), 7.28–7.81 (8H, m, H\textsubscript{ar}); \textsuperscript{13}C NMR (101 MHz, DMSO-\textit{d}_6): \delta 13.5 (CCH\textsubscript{3}), 14.0 (CCH\textsubscript{3}), 20.6 (CH\textsubscript{3}), 33.7 (CH\textsubscript{2}CO), 48.5 (NCH\textsubscript{2}), 103.4, 111.2, 126.8, 127.3, 128.4,
130.6, 131.9, 133.4, 137.1, 141.8, 143.1, 148.9, 151.4, 169.2, 171.6 \text{ (C, C=N, C=O)}; \text{ HRMS } m/z \text{ calculated for C}_{24}H_{23}ClN_4OS [M+H]^+: 451.1281, \text{ found: } 451.1348.

3-\{4-(4-Chlorophenyl)thiazol-2-yl-p-tolylamino\}-N-(2,5-dimethyl-1H-pyrrol-1-yl)propanamide (9). A mixture of hydrazide 7 (0.6 g, 1.6 mmol), hexane-2,5-dione (0.37 g, 3.2 mmol), propan-2-ol (20 mL), and acetic acid (0.1 mL) was refluxed for 3 h, then cooled down, and a few drops of water were added. The mixture was vigorously stirred for 15 min and left in a refrigerator. The formed precipitate was filtered off, washed with a small amount of water and dried to afford a sand-colour solid, yield 0.57 g (81%), mp 143–144 °C; IR (KBr) ν\text{max} (cm^{-1}): 3261 (NH), 1678 (C=O), 1516 (C=N); \text{^1H NMR} (400 MHz, DMSO-\text{d}_6): \delta 1.92 (6H, s, 2xNCCH}_3), 2.36 (3H, s, CH}_3), 2.77 (2H, t, J = 7.0 Hz, CH}_2CO), 4.28 (2H, t, J = 7.0 Hz, NCH}_2), 5.60 (2H, s, 2xCH), 7.22–7.92 (9H, m, H\text{ar}, CH), 10.65 (1H, s, NH); \text{^13C NMR} (101 MHz, DMSO-\text{d}_6): \delta 10.9 (C\text{C}_H}_3), 11.0 (C\text{C}_H}_3), 20.7 (CH}_3), 31.7 (CH}_2CO), 48.7 (NCH}_2), 102.9, 103.5, 103.9, 126.5, 126.7, 126.8, 127.1, 127.4, 128.5, 130.6, 131.9, 133.5, 137.1, 141.9, 149.1, 169.3, 169.7 (C\text{ar}, C=N, C=O); \text{ HRMS } m/z \text{ calculated for C}_{25}H_{25}ClN_4OS [M+H]^+: 465.1438, \text{ found: } 465.1528.

2-(3-\{4-(4-Chlorophenyl)thiazol-2-yl\}-p-tolylamino)propanoyl-N-phenylhydrazine-1-carbothioamide (10). The hydrazide 7 (0.84 g, 2.2 mmol) was dissolved in MeOH (15 mL), and phenyl isothiocyanate (0.3 g, 2.2 mmol) was then added dropwise. The mixture was heated at reflux (crystals begin to form already during the reaction) for 1.5 h. After the completion of the reaction, the mixture was cooled down, the precipitate was filtered off, washed with plenty of water, then a small amount of MeOH, Et\text{2}O, and dried to afford a white solid, yield 1.06 g (94%), mp 171–172 °C; IR (KBr) ν\text{max} (cm^{-1}): 3314, 3230, 3200 (3NH), 1651 (C=O), 1519 (C=N), 1234 (C=S); \text{^1H NMR} (400 MHz, DMSO-\text{d}_6): \delta 2.35 (3H, s, CH}_3), 2.66–2.69 (2H, m, CH}_2CO), 4.19–4.23 (2H, m, NCH}_2), 7.19 (1H, s, CH), 7.14–7.78 (13H, m, H\text{ar}), 9.53 (2H, s, 2NH), 9.97 (1H, s, NH); \text{^13C NMR} (101 MHz, DMSO-\text{d}_6): \delta 20.7 (CH}_3), 31.7 (CH}_2CO), 48.4 (NCH}_2), 103.4, 125.1, 126.6, 127.1, 127.4, 128.0, 128.5, 130.7, 131.9, 133.5, 137.2, 139.1, 141.9, 149.1, 169.4, 170.0, 180.9 (C\text{ar}, C=N, C=O, C=S); \text{ HRMS } m/z \text{ calculated for C}_{26}H_{24}ClN_5OS_2 [M+H]^+: 522.1111, \text{ found: } 522.1190.

3-\{4-(4-Chlorophenyl)thiazol-2-yl\}-p-tolylamino-N-(4-phenyl-5-thioxo-4,5-dihydro-1H-[1,2,4]triazol-3-yl)propanamide (11). A mixture of thiosemicarbazide 10 (0.67 g, 1.3 mmol) and 4% aqueous sodium hydroxide (80 mL) was refluxed for 5 h, cooled down and acidified with concentrated hydrochloric acid to pH 4. The formed oily mass was left overnight in a refrigerator. Afterwards, the obtained crystalline product was filtered off, washed with water and dried to afford a white solid, yield 0.61 g (94%), mp 211–212 °C; IR (KBr) ν\text{max} (cm^{-1}): 2927 (NH), 1510 (C=N), 1267 (C=S); \text{^1H NMR} (400 MHz, DMSO-\text{d}_6): \delta 2.34 (3H, s, CH}_3), 2.92 (2H, t, J = 7.0 Hz, CH}_2CO), 4.05 (2H, t, J = 7.0 Hz, NCH}_2), 7.14 (1H, s, CH), 7.13–7.78 (13H, m, H\text{ar}), 13.75 (1H, s, NH); \text{^13C NMR} (101 MHz, DMSO-\text{d}_6): \delta 20.7 (CH}_3), 23.9 (CH}_2CO), 49.6 (NCH}_2), 103.5, 126.7, 127.4, 128.2, 128.5, 129.4, 129.5, 130.7, 131.9, 133.4,
133.6, 137.3, 141.5, 149.1, 150.0, 167.7, 169.1 (C\_ar, C=N, C=S); HRMS m/z calculated for C\_26H\_22ClN\_5S\_2 [M+H]^+: 504.1005, found: 504.1083.

3-[(4-(4-Chlorophenyl)thiazol-2-yl)(p-toly)amino]-1-[6-(4-fluorophenyl)-2-phenylamino-[1,3,4]-thiadiazin-4-yl]propan-1-one (12). To a solution of thiosemicarbazide 10 (0.68 g, 1.3 mmol) in acetone (25 mL), 2-bromo-4'-fluoroacetophenone (0.71 g, 3.25 mmol) was added, and the mixture was heated at reflux for 16 h. Then the reaction mixture was cooled overnight in a refrigerator, the formed crystalline solid was filtered off, boiled in sodium acetate (10 mL) for 5 min and cooled down. The obtained precipitate was filtered off, washed with water and dried to afford a white solid, yield 0.38 g (46%), mp 229–230 °C; IR (KBr) ν\_max (cm\(^{-1}\)): 3427 (NH), 1707 (C=O), 1505 (C=N); \(^1\)H NMR (400 MHz, DMSO-\(d_6\)): δ 2.32 (3H, s, CH\(_3\)), 2.54–2.74 (2H, m, CH\(_2\)CO), 3.93–4.17 (2H, m, NCH\(_2\)), 6.36 (1H, s, H\_ar), 6.82–7.54 (16H, m, H\_ar), 7.72–8.03 (2H, m, H\_ar), 11.06 (1H, s, NH); \(^13\)C NMR (101 MHz, DMSO-\(d_6\)): δ 20.7 (CH\(_3\)), 31.5 (CH\(_2\)CO), 48.4 (NCH\(_2\)), 103.5, 115.3, 115.5, 120.9, 123.1, 126.3, 127.0, 127.4, 128.5, 129.5, 129.8, 129.9, 130.5, 131.9, 133.5, 137.1, 138.1, 142.0, 149.0, 150.3, 156.4, 161.1, 163.6, 169.3, 169.8 (C\_ar, C\_Thiazol); HRMS m/z calculated for C\_34H\_27ClFN\_5OS\_2 [M+H]^+: 640.1330, found: 640.1425.

Microbiology
The antimicrobial activity of compounds was evaluated by diffusion in peptone on a nutrient medium (meat-extract agar for bacteria and wort agar for fungi). The microbial loading was 10^9 cells (spores)/1 mL. The required incubation periods were 24 h at 35 °C for bacteria and 48–72 h at 28–30 °C for fungi. The results were recorded by measuring the zones surrounding the disk. The control disk contained vancomycin (for bacteria) or nystatin (for fungi) was used as a standard. Testing was performed in a flat-bottomed 96-well tissue culture plate. The tested compounds were dissolved in DMSO applying the necessary concentration. The exact volume of the solution of compounds is brought into a nutrient medium. The bacteria and fungi were inoculated in a nutrient medium (meat-extract agar for bacteria and wort agar for fungi). The duration of incubation was 24–72 h at 37 °C for bacteria and 30 °C for fungi. The results were estimated according to the degree of the growth inhibition.

REFERENCES