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SYNTHESIS AND BIOLOGICAL EVALUATION OF NOVEL DI- AND TRISUBSTITUTED THIAZOLE DERIVATIVES

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Abstract – Novel di- and trisubstituted thiazole derivatives bearing heterocyclic, aromatic, chalcone, and carboxyalkyl-heterocyclic moieties were synthesized. Compounds possessing significant antibacterial activity, comparable to that of commercial antibacterial agent ampicillin, against *Rhizobium radiobacter*, *Xanthomonas campestris*, and *Escherichia coli* were identified. Some of the synthesized compounds exhibited a very high antioxidant activity.

INTRODUCTION

The growing environmental pollution and arising new pathogens, which can infect all types of life forms, prompt chemists of organic, medicinal, and pharmaceutical chemistry to combine forces for the search and design of new and effective compounds as pharmaceutical agents.

Thiazoles have been under investigation as therapeutic agents for a long time. Thiazole scaffold has been identified in a number of naturally occurring compounds, such as thiamine (vitamin B1), mycothiazole exhibiting cytotoxic activity,¹ and cystothiazole A possessing antifungal activity.² The examples of drugs bearing thiazole moiety are antibacterial sulfathiazole, antihelmintic tiabendazole, and antiviral ritonavir, and anti-inflammatory meloxicam.³ Thiazole derivatives have been shown to possess a broad spectrum of biological properties, such as anticonvulsant,⁴ antimicrobial,⁵⁻⁷ antituberculous,⁸ cytotoxic, anti-inflammatory and psychotropic,⁹ potent *in vivo* neuroprotection¹⁰ activities, they also are effective against allergies.⁹ 2-[(4-Chlorobenzyl)amino]-4-methyl-1,3-thiazole-5-carboxylic acid has been identified as potential antidiabetic, antioxidant and anti-inflammatory agent.¹¹ Recently, 2-(2-((1*H*-indol-5-yl)-

methylene)hydrazinyl)-4-methylthiazole and its 4-phenyl analogue have been reported to possess free radical scavenging potential higher than that of a commercial standard trolox.¹²

Quinolones are a very important class of heterocyclic compounds due to their broad spectrum of biological activity. They are prescribed for the treatment of tuberculosis,¹³ HIV-1,¹⁴ microbial¹⁵ and viral¹⁶ infections, and are also used as anticancer¹⁷ pharmaceuticals.

Chalcones and their derivatives are associated with a wide variety of different biological activities such as antimicrobial,¹⁸⁻²¹ antibacterial, anti-inflammatory,²² anticancer,²³ anticonvulsant,²⁴ antioxidant^{21,25} etc. Therefore, synthesis and biological evaluation of compounds containing thiazole, quinolone, and chalcone fragments is of great interest for organic and medicinal chemists.

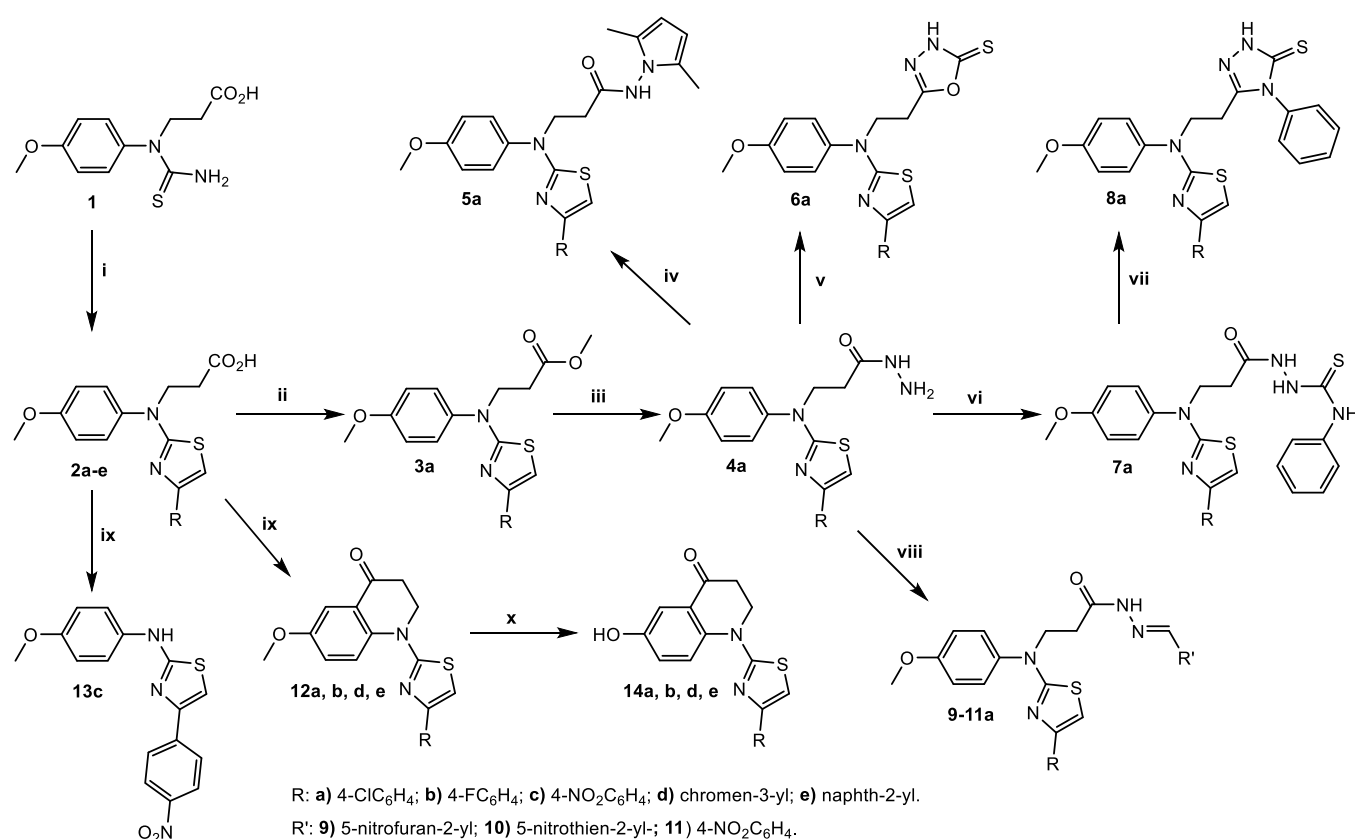
As a continuation of search of biologically active compounds and investigation of the influence of different substituents and molecule fragments on antibacterial^{26,27} and antioxidant²⁸ activity, herein we report on the synthesis and investigation of antibacterial and antioxidant activity of novel functionalized di- and trisubstituted thiazole derivatives bearing various aliphatic, aromatic, and heterocyclic substituents.

RESULTS AND DISCUSSION

Reaction of thioureido acid **1** with different 2-bromo-1-phenylethanones provided corresponding 3-[(4-methoxyphenyl)(4-arylsubstituted-1,3-thiazol-2-yl)amino]propanoic acids **2a-e** (Scheme 1). In the course of these reactions, corresponding thiazolium bromide salts were formed, which underwent facile conversion into 1,3-thiazoles **2a-e**. As an example, in the ¹H NMR spectrum for 3-[[4-(4-chlorophenyl)thiazol-2-yl](4-methoxyphenyl)amino]propanoic acid (**2a**), the singlet attributable to the SCH group proton is observed at 7.17 ppm, proving formation of the thiazole ring, and the increased number of aromatic proton peaks proves the presence of the second benzene ring in the molecule.

Among the compounds **2a-e** screened for the antibacterial activity against *Rhizobium radiobacter*, *Escherichia coli*, and *Xanthomonas campestris*, **2a** was identified as possessing the highest activity. Therefore, this compound was subjected to chemical transformations aiming to synthesize more active compounds. Ester **3a** was obtained according to the usual procedure for esterification of carboxylic acids by heating at reflux acid **2a** in methanol in the presence of concentrated sulfuric acid as a catalyst. Reaction of **3a** with hydrazine monohydrate in dimethyl sulfoxide provided 3-[[4-(4-chlorophenyl)-1,3-thiazol-2-yl](4-methoxyphenyl)amino}propanehydrazide (**4a**). Formation of hydrazide **4a** has been proven by the presence of the ¹H NMR resonances at 4.18 ppm and 9.11 ppm attributable to the protons of NH₂ and NH groups, respectively. Condensation reaction of hydrazide **4a** with hexane-2,5-dione in the presence of acetic acid resulted in formation of pyrrole derivative **5a**. Proton resonances assigned to the

CH₃ groups in the pyrrole moiety are observed at 1.95 ppm in the ¹H NMR spectrum for **5a**, whereas a singlet integrated for two protons at 5.63 ppm confirms the existence of a pyrrole ring in this compound. Compound **6a** bearing 1,3,4-oxadiazole moiety was synthesized in the reaction of **4a** with carbon disulfide in ethanol in the presence of potassium hydroxide. Formation of the oxadiazole ring has been confirmed by the presence of carbon resonances at 164.20 ppm, attributed to the C=N group, and 169.71 ppm, attributed to C=S group, in the ¹³C NMR spectrum. 2-(3-((4-(4-Chlorophenyl)thiazol-2-yl)(4-methoxyphenyl)amino)propanoyl)-*N*-phenylhydrazine-1-carbothioamide (**7a**) and hydrazones **9–11a** were synthesized by condensation reactions of **4a** with phenyl isothiocyanate or heterocyclic aldehydes.



i) 1) α -haloketone, acetone, reflux, 2-4 h, 2) 5% K₂CO₃, AcOH; ii) MeOH, H₂SO₄, reflux, 20 h; iii) N₂H₄·H₂O, DMSO, 90-100 °C, 2 h; iv) hexane-2,5-dione, 2-PrOH, AcOH, reflux, 4 h; v) 1) CS₂, KOH, EtOH, reflux, 72 h, 2) HCl; vi) PhNCS, MeOH, reflux, 3 h; vii) 1) 10% NaOH, reflux, 8 h, 2) HCl; viii) R'CHO, MeOH, reflux, 10 min or 1 h; ix) PPA, 110-120 °C, 4-6 h; x) 1) HBr, AcOH, 20-30 h, 2) NH₃·H₂O.

Scheme 1

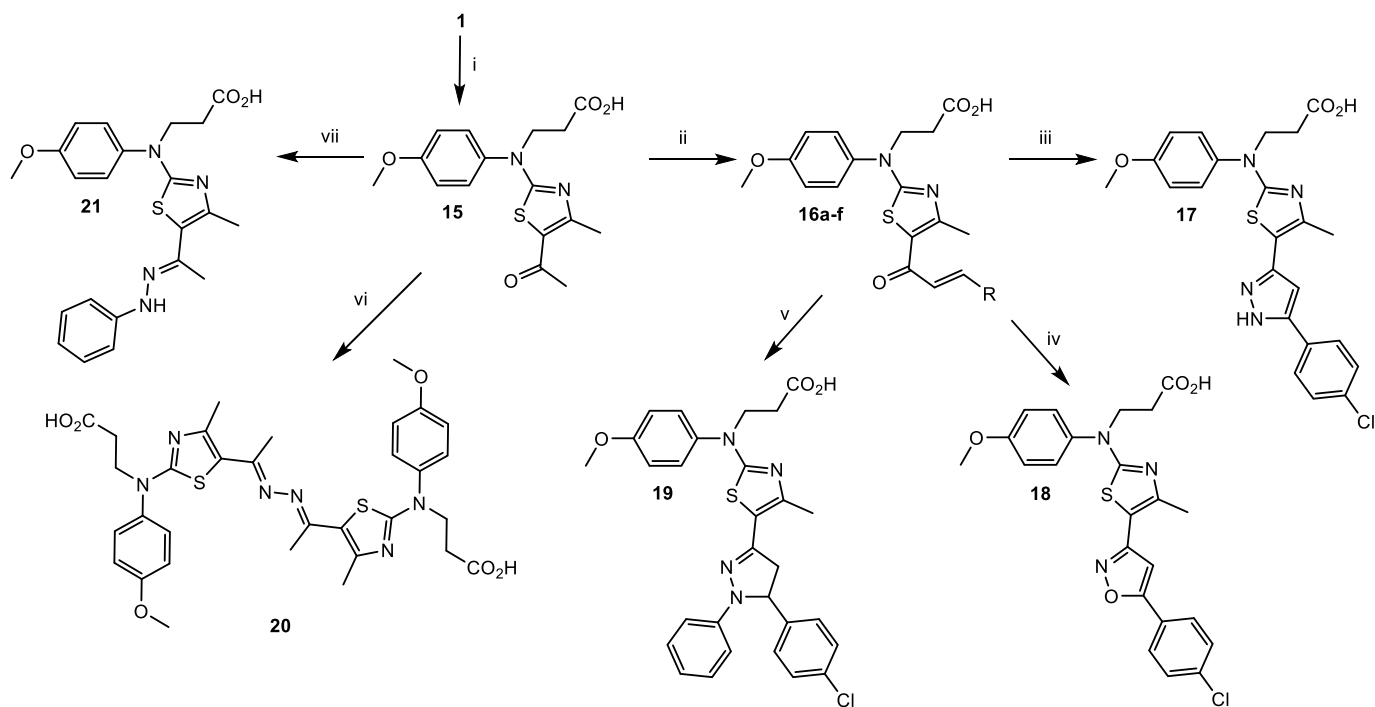
Presence of the amide group in the structure of these compounds determines the splitting of resonances in ¹H and ¹³C NMR spectra owing to the restricted rotation around the amide bond. In DMSO-*d*₆ solution these compounds exist as a mixture of *E/Z* isomers, where, in the majority of cases, the *Z* isomer predominates because of a hindered rotation around the CO–NH bond.^{29,30} Thiosemicarbazide **7a** underwent condensation to 5-(2-((4-(4-chlorophenyl)thiazol-2-yl)(4-methoxyphenyl)amino)ethyl)-4-phenyl-2,4-dihydro-3*H*-1,2,4-triazole-3-thione (**8a**) under treatment with 10% aqueous sodium

hydroxide solution. In the ^{13}C NMR spectrum for **8a**, the carbon resonance of the C=S group is observed at 169.62 ppm and is shifted downfield by approx. 10 ppm in comparison with the carbon signal attributed to the C=S group (180.86 ppm) in **7a**.

The quinolone core has long been recognized as one of the important structures for the design of antimicrobial agents. Therefore, β -alanine fragment in acids **2a**, **b**, **d**, **e** was employed in the synthesis of thiazole derivatives **12a**, **b**, **d**, **e** bearing quinolone moiety by heating **2a**, **b**, **d**, **e** at 110 °C in polyphosphoric acid. As an example, in the ^{13}C NMR spectrum for **12a**, the carbon resonance attributed to the C=O group at 192.78 ppm is shifted downfield in comparison with the corresponding carbon resonance in the β -alanine fragment in **2a** (172.63 ppm). In the ^1H NMR spectrum, the proton resonances in the aromatic region are integrated for one proton less proving formation of the quinolone ring. The analogous NMR resonances are observed in the spectra for other quinolone derivatives **12b**, **d**, **e**. However, attempts to synthesize the corresponding quinolone from **2c** under analogous reaction conditions failed due to the dealkylation reaction taking place. Instead, *N*-(4-methoxyphenyl)-4-(4-nitrophenyl)-1,3-thiazol-2-amine (**13c**) was isolated from the reaction mixture. Compounds **12a**, **b**, **d**, **e** were converted into hydroxyquinolones **14a**, **b**, **d**, **e** by cleaving the ether bond with HBr.

Further on, polysubstituted thiazoles **15–21** were synthesized. 3-[(5-Acetyl-4-methylthiazol-2-yl)(4-methoxyphenyl)amino]propanoic acid (**15**) was synthesized from thioureido acid **1** and 3-chloropentane-2,4-dione in acetone at reflux temperature (Scheme 2). Sodium acetate was used to convert the formed aminothiazolium chloride into the base. The synthesis of the target chalcones **16a–f** was accomplished by Claisen–Schmidt condensation of 5-acetyl-4-methylthiazole **15** with various aromatic aldehydes in methanol and 10% aqueous sodium hydroxide solution.³¹ The products were obtained in good and very good yields.

Structures of compounds **16a–f** were unambiguously confirmed by the data of IR, ^1H and ^{13}C NMR spectroscopy, and elemental analysis. IR spectra showed absorptions at 1731–1713 cm^{-1} (C=O) and sharp bands at 3436–3407 cm^{-1} (OH). In the ^1H NMR spectra, formation of chalcone moiety has been proven by the signals in the region of 2.55–2.64 ppm (CH_3), at ~ 7.08 ppm (CO–CH=), and 7.50 ppm (=CH–Ar). The CH=CH double bond in the enone moiety of chalcones can potentially adopt either a *Z* or an *E* configuration. The ^1H NMR spectrum of each compound **16a–f** displays the resonances attributable to the CH=CH protons in the region of 7.08–7.50 ppm, with $J > 15$ Hz. Thus, it can be assumed that chalcones **16a–f** were synthesized in the *E* configuration.³²



R: a) C₆H₅; b) 4-BrC₆H₄; c) 4-ClC₆H₄; d) 4-FC₆H₄; e) furan-2-yl; f) thien-2-yl.

i) 1) 3-chloro-2,4-pentanedione, acetone, reflux, 3 h, 2) 5% K₂CO₃, AcOH; ii) 1) RCHO, MeOH, 10% NaOH aq., 0-3 °C, 1.5 h, rt, 24 h, 2) 50% AcOH; iii) 1) N₂H₄·H₂O, 2-PrOH, KOH, 70-80 °C, 24 h, 2) AcOH; iv) 1) NH₂OH·HCl, 1,4-dioxane, reflux, 40 h, 2) 10% K₂CO₃, AcOH; v) 1) PhNHNH₂, 2-PrOH, KOH, 70-80 °C, 6 h, 2) AcOH; vi) N₂H₄·H₂O, MeOH, AcOH, reflux, 30 h; vii) PhNHNH₂, MeOH, AcOH, reflux, 22 h.

Scheme 2

Heterocyclization reactions of trisubstituted chalcone **16c** with nitrogen nucleophiles, such as hydrazine, phenylhydrazine, and hydroxylamine, were investigated. 3-([5-{5-(4-Chlorophenyl)-1*H*-pyrazol-3-yl}-4-methylthiazol-2-yl][4-methoxyphenyl]amino)propanoic acid (**17**) was synthesized by treating chalcone **16c** with hydrazine monohydrate in propan-2-ol in the presence of potassium hydroxide, whereas isoxazole derivative **18** was prepared by refluxing chalcone **16c** with hydroxylamine hydrochloride in 1,4-dioxane in the absence of basic catalyst. Dihydropyrazole derivative **19** was synthesized from phenylhydrazine according to the synthesis procedure of **17**. It should be noted that reactions of chalcone **16c** with hydrazine monohydrate and hydroxylamine hydrochloride provided pyrazole **17** and isoxazole **18** derivatives in oxidized form as the main reaction products. In the ¹³C NMR spectra for **17–19**, the carbon resonances of the newly formed pyrazole (**17**) and isoxazole (**18**) rings are shifted downfield in the aromatic region of the spectrum. In the ¹³C NMR spectrum for **19**, carbon resonances of the new 4,5-dihydropyrazole moiety are observed in the region characteristic of aliphatic carbon atoms, i.e. at 44.57 ppm (CH₂CH) and 62.62 ppm (CH₂CH).

Reaction of thiazole **15** with hydrazine and phenylhydrazine provided hydrazine type compounds **20** and **21**. The reactions were performed in methanol at reflux temperature in the presence of acetic acid as a catalyst. In the ¹³C NMR spectrum for **20**, the resonance line of C=O is absent in comparison with the spectrum for the initial compound **15**, whereas the signal at 150.37 ppm has proven the formation of

C=N-N=C bond. In the ^1H NMR spectrum for **21**, the singlet at 9.00 ppm ascribed to the NH group proton and the integral intensity of the signals of aromatic protons (9H) have proven formation of the target compound.

Some of the synthesized compounds (**2a–19**) (50–1000 $\mu\text{g/mL}$) were evaluated for their antibacterial activity against the strains of *R. radiobacter*, *X. campestris*, and *E. coli* by the diffusion technique (Tables 1–2). The activity of the tested compounds was compared with that of the known antibacterial agent ampicillin (50 $\mu\text{g/mL}$).

Table 1. The inhibition zone diameters of synthesized compounds **2a–19** at 1000 $\mu\text{g/mL}$ concentration

| Compound | The diameters of inhibition zone, mm | | |
|--------------------------------|---|---|--|
| | <i>E. coli</i> 1000 $\mu\text{g/mL}$ | <i>X. campestris</i> 1000 $\mu\text{g/mL}$ | <i>R. radiobacter</i> 1000 $\mu\text{g/mL}$ |
| 2a | 4.0 | 2.0 | 8.0 |
| 3a | 3.0 | – | 7.4 |
| 4a | – | 3.5 | 3.8 |
| 5a | 1 | 3.2 | 2.4 |
| 6a | 1.6 | 1.3 | 3 |
| 7a | 4.0 | – | 6.0 |
| 8a | 1.0 | 8.0 | 7.2 |
| 9a | 1.3 | 2 | 3.5 |
| 11a | – | 8.0 | 3.6 |
| 16c | 4.0 | 5.8 | 6.0 |
| 17 | 5.0 | 5.0 | – |
| 18 | 5.0 | 6.3 | 4.0 |
| 19 | 4.0 | 6.8 | 5.0 |
| Ampicillin 50 $\mu\text{g/mL}$ | 6.4 | 9.0 | 11.0 |

The evaluation of the antibacterial activity against *E. coli* has revealed that isoxazole derivative **18** is the most active (125 $\mu\text{g/mL}$). Chalcone derivative **16c** and pyrazole derivative **17** showed significant activity as well. Replacement of hydrogen atom in amino group of pyrazole ring with benzene ring in **19** decreased the activity against *E. coli*. Triazole-3-thione derivative **8a** and hydrazone **11a** bearing a 4-nitrobenzene moiety have been indicated as the most active against *X. campestris* (50 $\mu\text{g/mL}$). It is interesting to note that 3- $\{[4-(4\text{-chlorophenyl})-1,3\text{-thiazol-2-yl}]-4\text{-methoxyanilino}\}$ propanoic acid (**2a**)

has shown significant activity, whereas its ester **3a** is not active at all. On the other hand, acid **2a** and its ester **3a** suppressed growth of *R. radiobacter* at concentration of 50 $\mu\text{g/mL}$ the most among the tested compounds. Their activity was followed closely by the triazole-3-thione derivative **8a**. It should be noted that none of the tested compounds was more active than ampicillin.

Table 2. The inhibition zone diameters of selected compounds **2a–19** at 125 $\mu\text{g/mL}$ and 50 $\mu\text{g/mL}$ concentrations

| Compound | The diameters of inhibition zone, mm | | |
|--------------------------------|--|---|--|
| | <i>E. coli</i> 125 $\mu\text{g/mL}$ | <i>X. campestris</i> 50 $\mu\text{g/mL}$ | <i>R. radiobacter</i> 50 $\mu\text{g/mL}$ |
| 2a | | 1 | 3.8 |
| 3a | | – | 3.7 |
| 7a | | – | 3.0 |
| 8a | | 4.0 | 3.6 |
| 11a | | 4.0 | 1.8 |
| 16c | 3.0 | 0.6 | 2.0 |
| 17 | 3.0 | 0.8 | – |
| 18 | 4.0 | 0.7 | 2.0 |
| 19 | 2.0 | 0.8 | 2.5 |
| Ampicillin 50 $\mu\text{g/mL}$ | 6.4 | 9.0 | 11.0 |

The antioxidant properties of compounds **2a–19** were evaluated using different protocols including 2,2-diphenyl-1-(2,4,6-trinitrophenyl)hydrazyl (DPPH) radical scavenging method, the ferric reducing antioxidant power (FRAP), and the reducing power assay (Figures 1–3).

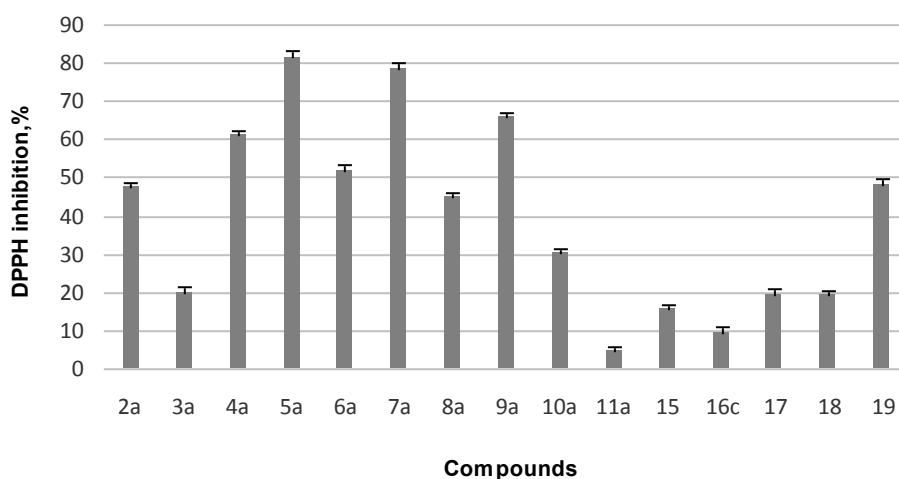


Figure 1. Antioxidant activity of compounds **2a–19** against DPPH

The DPPH assay is based on either a hydrogen atom transfer (HAT) or a single electron transfer (SET) mechanism. As seen from the results presented in Figure 1, propanamide **5a** bearing 2,5-dimethylpyrrole moiety (81.8%), thiosemicarbazide **7a** (78.6%), hydrazone **9a** bearing 5-nitrofuranyl moiety (65.9%), and hydrazide **4a** (61.6%) possess a very high DPPH radical scavenging ability. The higher free radical scavenging activities of these compounds could be attributed to the presence of an NH group, which can donate a hydrogen atom via a HAT mechanism leading to neutralization of the DPPH radical.³³

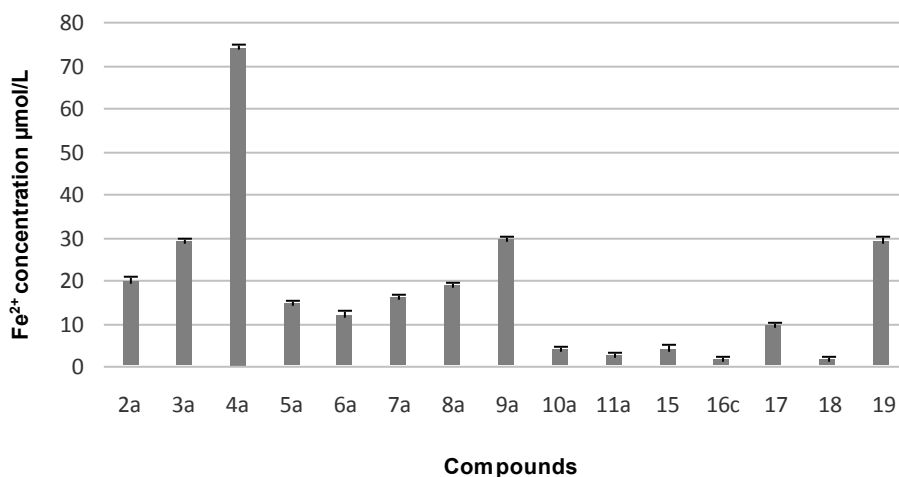


Figure 2. Antioxidant activity of compounds **2a–19** evaluated by FRAP method

The FRAP method is based on the reduction of a ferrioxalate analog, the Fe³⁺ complex of tripyridyltriazine Fe(TPTZ)³⁺, to the intensely blue coloured Fe²⁺ 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 Fe²⁺ µmol/L concentration. FRAP assay is based on a SET mechanism.

The results revealed (Figure 2) that hydrazide **4a** (74.11 Fe²⁺ µmol/L), hydrazone **9a** bearing 5-nitrofuranyl moiety (29.17 Fe²⁺ µmol/L), and pyrazole derivative **19** (29.06 Fe²⁺ µmol/L) showed the highest antioxidant activity evaluated by FRAP method.

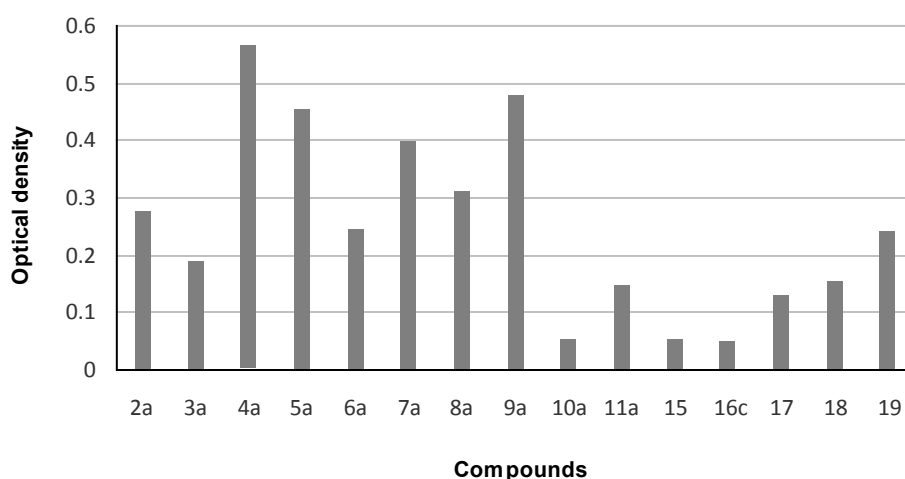


Figure 3. Reducing power of the compounds **2a–19**

In the reducing power assay, the presence of reductants (antioxidants) in a sample would result in the reduction of Fe^{3+} to Fe^{2+} by donating an electron (SET mechanism). 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 3) have demonstrated that compounds bearing fragments of hydrazide **4a**, propanamide **5a**, thiosemicarbazide **7a**, and hydrazone **9a** exhibit antioxidant effect. Interestingly, hydrazones **10a** and **11a** showed a very weak or moderate scavenging potential evaluated by any of the above assays.

CONCLUSIONS

In summary, various di- and trisubstituted thiazole derivatives with functionalized aromatic and heterocyclic substituents were synthesized. Screening of their antibacterial activity has revealed that *Rhizobium radiobacter* and *Xanthomonas campestris* were the most sensitive to chalcone derivative **16c**, isoxazole derivative **18**, and pyrazole derivative **19**, all containing chlorine substituent in the benzene ring, at the concentration of 50 $\mu\text{g/mL}$, whereas the same compounds at the concentration of 125 $\mu\text{g/mL}$ suppressed growth of *Escherichia coli*. Derivatives of 3- $\{[4-(4\text{-chlorophenyl})\text{-}1,3\text{-thiazol-}2\text{-yl}]\text{-}4\text{-methoxyanilino}\}$ propanoic acid bearing pyrrole **5a** and thiosemicarbazide **7a** moieties possess a very high DPPH free radical scavenging activity, 81.8% and 78.6% respectively. 3- $\{[4-(4\text{-Chlorophenyl})\text{-}1,3\text{-thiazol-}2\text{-yl}]\text{-}4\text{-methoxyanilino}\}$ propanohydrazide (**4a**) has been identified as possessing a significant antioxidant activity tested by FRAP method and reducing power assay.

EXPERIMENTAL

Synthesis

General Methods. The reagents and solvents were obtained from Sigma-Aldrich Chemie GmbH (Munich, Germany) and were used without further purification. The melting points were determined on a MEL-TEMP (Electrothermal, Bibby Scientific Company, Burlington, NJ, USA) melting point apparatus and are uncorrected. IR spectra (ν , cm^{-1}) were recorded on a Perkin-Elmer Spectrum BX FT-IR spectrometer (Perkin-Elmer Inc., Waltham, MA, USA) using KBr pellets. The ^1H and ^{13}C NMR spectra were recorded in $\text{DMSO-}d_6$ on a Bruker Avance III (400 MHz, 101 MHz) spectrometer. Chemical shifts (δ) are reported in parts per million (ppm) calibrated from TMS (0 ppm) as an internal standard for ^1H NMR, and $\text{DMSO-}d_6$ (39.43 ppm) for ^{13}C NMR. Elemental analyses (C, H, N) were performed on an Elemental Analyzer CE-440 (Exeter Analytical, Inc., North Chelmsford, MA, USA). The reaction course and the purity of the synthesized compounds was monitored by TLC using Silica gel 60 F254 (Kieselgel 60 F254) (Merck, Darmstadt, Germany) plates.

General procedure for preparation of 1,3-thiazoles 2a–e. To a solution of thioureido acid **1** (0.76 g, 3 mmol) in acetone (15 mL), the corresponding α -haloketone (3.6 mmol) was added, and the reaction mixture was refluxed for 2–4 h. Afterwards, the reaction mixture was cooled down, the precipitate was filtered off, washed with acetone, and dried. Purification was performed by dissolving the crystals in 5% aqueous K_2CO_3 (15 mL), filtering and acidifying the filtrate with acetic acid to pH 6.

3-{[4-(4-Chlorophenyl)-1,3-thiazol-2-yl]-4-methoxyanilino}propanoic acid (2a). White solid, yield 0.94 g (81%), mp 148–149 °C; IR (KBr) ν_{max} (cm^{-1}): 1726 (CO), 1508 (C=N); 1H NMR (400 MHz, DMSO- d_6) δ 2.65 (2H, t, $J = 7.2$ Hz, CH_2CO), 3.80 (3H, s, OCH_3), 4.14 (2H, t, $J = 7.1$ Hz, NCH_2), 7.05 (2H, d, $J = 8.7$ Hz, H_{Ar}), 7.17 (1H, s, SCH), 7.37 (2H, d, $J = 8.7$ Hz, H_{Ar}), 7.45 (2H, d, $J = 8.4$ Hz, H_{Ar}), 7.88 (2H, d, $J = 8.4$ Hz, H_{Ar}), 11.51 (1H, br. s, COOH); ^{13}C NMR (101 MHz, DMSO- d_6) δ 32.36 (CH_2CO), 48.55 (NCH_2), 55.36 (OCH_3), 103.42, 115.27, 127.34, 128.55, 129.00, 131.88, 133.51, 137.05, 149.09, 158.56 (C_{Ar} , C_{Th}), 169.97 (C=N), 172.63 (COOH). Anal. Calcd for $C_{19}H_{17}ClN_2O_3S$, %: C, 58.69; H, 4.41; N, 7.20. Found, %: C, 58.43; H, 4.35; N, 7.14.

3-{[4-(4-Fluorophenyl)-1,3-thiazol-2-yl]-4-methoxyanilino}propanoic acid (2b). Greenish solid, yield 0.89 g (80%), mp 128–129 °C; IR (KBr) ν_{max} (cm^{-1}): 1727 (CO), 1511 (C=N); 1H NMR (400 MHz, DMSO- d_6) δ 2.66 (2H, t, $J = 7.2$ Hz, CH_2CO), 3.80 (3H, s, OCH_3), 4.14 (2H, t, $J = 7.2$ Hz, NCH_2), 7.04 (2H, d, $J = 8.8$ Hz, H_{Ar}), 7.08 (1H, s, SCH), 7.22 (2H, t, $J = 8.8$ Hz, H_{Ar}), 7.37 (2H, d, $J = 8.7$ Hz, H_{Ar}), 7.90 (2H, dd, $J = 8.3$ Hz, $J = 5.7$ Hz, H_{Ar}), 12.24 (1H, br. s, COOH); ^{13}C NMR (101 MHz, DMSO- d_6) δ 32.37 (CH_2CO), 48.56 (NCH_2), 55.35 (OCH_3), 102.34, 115.25, 115.29, 115.41, 127.58, 127.63, 128.98, 131.30, 131.32, 137.13, 149.35, 158.51, 160.89, 162.28 (C_{Ar} , C_{Th}), 169.89 (C=N), 172.65 (COOH). Anal. Calcd for $C_{19}H_{17}FN_2O_3S$, %: C, 61.28; H, 4.60; N, 7.52. Found, %: C, 61.21; H, 4.56; N, 7.48.

3-{4-Methoxy[4-(4-nitrophenyl)-1,3-thiazol-2-yl]anilino}propanoic acid (2c). Yellow solid, yield 0.95 g (79%), mp 149–150 °C; IR (KBr) ν_{max} (cm^{-1}): 1710 (CO), 1509 (C=N); 1H NMR (400 MHz, DMSO- d_6) δ 2.66 (2H, t, $J = 7.3$ Hz, CH_2CO), 3.81 (3H, s, OCH_3), 4.17 (2H, t, $J = 7.2$ Hz, NCH_2), 7.06 (2H, d, $J = 8.9$ Hz, H_{Ar}), 7.38 (2H, d, $J = 8.8$ Hz, H_{Ar}), 7.48 (1H, s, SCH), 8.11 (2H, d, $J = 8.9$ Hz, H_{Ar}), 8.26 (2H, d, $J = 8.9$ Hz, H_{Ar}), 12.30 (1H, br. s, COOH); ^{13}C NMR (101 MHz, DMSO- d_6) δ 32.34 (CH_2CO), 48.54 (NCH_2), 55.38 (OCH_3), 107.48, 115.34, 124.05, 126.42, 129.06, 136.87, 140.69, 146.20, 148.39, 158.67 (C_{Ar} , C_{Th}), 170.26 (C=N), 172.63 (COOH). Anal. Calcd for $C_{19}H_{17}N_3O_5S$, %: C, 57.13; H, 4.29; N, 10.52. Found, %: C, 57.00; H, 4.24; N, 10.46.

3-[(4-Methoxyphenyl)(4-(2-oxo-2H-chromen-3-yl)thiazol-2-yl)amino]propanoic acid (2d). Yellow solid, yield 1.06 g (84%), mp 133–134 °C; IR (KBr) ν_{max} (cm^{-1}): 3381 (OH), 1718 (CO), 1510 (C=N); 1H NMR (400 MHz, DMSO- d_6) δ 2.66 (2H, t, $J = 7.2$ Hz, CH_2CO), 3.81 (3H, s, OCH_3), 4.20 (2H, t, $J = 7.3$ Hz, NCH_2), 7.06 (2H, d, $J = 8.9$ Hz, H_{Ar}), 7.35–7.40 (3H, m, H_{Ar}), 7.43 (1H, d, $J = 8.3$ Hz, H_{Ar}), 7.56 (1H,

s, SCH), 7.61 (1H, t, $J = 7.8$ Hz, H_{Ar}), 7.88 (1H, d, $J = 7.6$ Hz, H_{Ar}), 8.64 (1H, s, H_{Ar}), 12.27 (1H, br. s, COOH); ^{13}C NMR (101 MHz, DMSO- d_6) δ 32.42 (CH_2CO), 48.28 (NCH $_2$), 55.38 (OCH $_3$), 109.70, 115.32, 115.85, 119.28, 120.44, 124.71, 128.81, 129.12, 131.57, 136.91, 138.41, 143.89, 152.28, 158.66, 158.76 (C_{Ar} , C_{Th}), 169.29 (C=N), 172.70 (COOH). Anal. Calcd for $C_{22}H_{18}N_2O_5S$, %: C, 62.55; H, 4.29; N, 6.63. Found, %: C, 62.44; H, 4.21; N, 6.58.

3-{4-Methoxy[4-(2-naphthyl)-1,3-thiazol-2-yl]anilino}propanoic acid (2e). White solid, yield 0.97 g (80%), mp 152–153 °C; IR (KBr) ν_{max} (cm^{-1}): 1709 (CO), 1509 (C=N); 1H NMR (400 MHz, DMSO- d_6) δ 2.70 (2H, t, $J = 7.2$ Hz, CH_2CO), 3.81 (3H, s, OCH $_3$), 4.22 (2H, t, $J = 7.2$ Hz, NCH $_2$), 7.06 (2H, d, $J = 8.8$ Hz, H_{Ar}), 7.26 (1H, s, SCH), 7.40 (2H, d, $J = 8.8$ Hz, H_{Ar}), 7.47–7.54 (2H, m, H_{Ar}), 7.87–8.03 (4H, m, H_{Ar}), 8.40 (1H, s, H_{Ar}); ^{13}C NMR (101 MHz, DMSO- d_6) δ 32.46 (CH_2CO), 48.46 (NCH $_2$), 55.37 (OCH $_3$), 103.50, 115.28, 124.10, 124.11, 125.92, 126.39, 127.57, 128.01, 128.12, 129.08, 132.18, 132.46, 133.16, 137.09, 150.36, 158.56 (C_{Ar} , C_{Th}), 170.01 (C=N), 172.71 (COOH). Anal. Calcd for $C_{23}H_{20}N_2O_3S$, %: C, 68.30; H, 4.98; N, 6.93. Found, %: C, 68.26; H, 4.92; N, 6.89.

Methyl 3-{[4-(4-chlorophenyl)-1,3-thiazol-2-yl]-4-methoxyanilino}propanoate (3a). A mixture of acid **2a** (5.83 g, 0.015 mol), MeOH (50 mL), and sulfuric acid (0.8 mL, 1.47 g, 0.015 mol) was refluxed for 20 h. The liquid fraction was evaporated under reduced pressure, the residue was washed with 10% aqueous Na_2CO_3 (15 mL), and recrystallized from MeOH to afford white solid, yield 5.53 g (92%), mp 140–141 °C; IR (KBr) ν_{max} (cm^{-1}): 1736 (CO), 1510 (C=N); 1H NMR (400 MHz, DMSO- d_6) δ 2.75 (2H, t, $J = 7.2$ Hz, CH_2CO), 3.54 (3H, s, COOCH $_3$), 3.82 (3H, s, OCH $_3$), 4.19 (2H, t, $J = 7.2$ Hz, NCH $_2$), 7.06 (2H, d, $J = 8.8$ Hz, H_{Ar}), 7.19 (1H, s, SCH), 7.37 (2H, d, $J = 8.8$ Hz, H_{Ar}), 7.47 (2H, d, $J = 8.4$ Hz, H_{Ar}), 7.89 (2H, d, $J = 8.4$ Hz, H_{Ar}); ^{13}C NMR (101 MHz, DMSO- d_6) δ 32.95 (CH_2CO), 49.26 (NCH $_2$), 52.09 (COOCH $_3$), 56.05 (OCH $_3$), 104.17 (CH), 115.97, 128.01, 129.24, 129.69, 132.59, 134.21, 137.68, 149.86, 159.28 (C_{Ar} , C_{Th}), 170.59 (C=N), 172.27 (CO). Anal. Calcd for $C_{20}H_{19}ClN_2O_3S$, %: C, 59.62; H, 4.75; N, 6.95. Found, %: C, 59.55; H, 4.69; N, 6.89.

3-{[4-(4-Chlorophenyl)-1,3-thiazol-2-yl]-4-methoxyanilino}propanohydrazide (4a). A mixture of ester **3a** (4.02 g, 0.01 mol), hydrazine monohydrate (0.64 g, 0.02 mol), and DMSO (10 mL) was stirred at 90–100 °C for 2 h. The precipitate was filtered off and recrystallized from propan-2-ol to afford white solid, yield 3.26 g (81%), mp 144–145 °C; IR (KBr) ν_{max} (cm^{-1}): 3298 (NH), 1631 (CO), 1537 (C=N); 1H NMR (400 MHz, DMSO- d_6) δ 2.50 (2H, t, $J = 7.2$ Hz, CH_2CO), 3.82 (3H, s, OCH $_3$), 4.15 (2H, t, $J = 7.2$ Hz, NCH $_2$), 4.18 (1H, s, NH), 7.06 (2H, d, $J = 9.2$ Hz, H_{Ar}), 7.17 (1H, s, SCH), 7.37 (2H, d, $J = 9.2$ Hz, H_{Ar}), 7.47 (2H, d, $J = 8.8$ Hz, H_{Ar}), 7.92 (2H, d, $J = 8.8$ Hz, H_{Ar}), 9.11 (1H, s, NH); ^{13}C NMR (101 MHz, DMSO- d_6) δ 31.89 (CH_2CO), 49.22 (NCH $_2$), 55.26 (OCH $_3$), 103.21 (CH), 115.15, 127.30, 128.43, 128.87,

131.78, 133.51, 137.15, 149.13, 158.38 (C_{Ar} , C_{Th}), 169.34 ($C=N$), 169.80 (CO). Anal. Calcd for $C_{19}H_{19}ClN_4O_2S$, %: C, 56.64; H, 4.75; N, 13.91. Found, %: C, 56.54; H, 4.62; N, 13.87.

3-([4-(4-Chlorophenyl)-1,3-thiazol-2-yl]-4-methoxyanilino)-N-(2,5-dimethyl-1H-pyrrol-1-yl)propanamide (5a). A mixture of **4a** (0.60 g, 1.5 mmol), propan-2-ol (20 mL), hexane-2,5-dione (0.23 g, 0.16 mL, 2 mmol), and acetic acid (0.5 mL) was refluxed for 4 h. Then cold water (20 mL) was added. The precipitate was filtered off and recrystallized from propan-2-ol afforded pale taupe solid, yield 0.44 g (61%), mp. 131–132 °C; IR (KBr) ν_{max} (cm^{-1}): 3255 (NH), 1677 (CO), 1510 ($C=N$); 1H NMR (400 MHz, DMSO- d_6) δ 1.95 (6H, s, 2 CH_3), 2.78 (2H, t, $J = 6.8$ Hz, CH_2CO), 3.83 (3H, s, OCH_3), 4.28 (2H, t, $J = 6.8$ Hz, NCH_2), 5.63 (2H, s, 2 CH), 7.08 (2H, d, $J = 8.8$ Hz, H_{Ar}), 7.21 (1H, s, SCH), 7.38–7.53 (4H, m, H_{Ar}), 7.93 (2H, d, $J = 8.4$ Hz, H_{Ar}), 10.69 (1H, s, NH); ^{13}C NMR (101 MHz, DMSO- d_6) δ 10.80 (CH_3), 31.63 (CH_2CO), 48.67 (NCH_2), 55.29 (OCH_3), 102.81 (2 CH), 103.41 (CH), 115.19, 126.60, 127.29, 128.41, 128.84, 131.81, 133.47, 137.07, 149.07, 158.44 (C_{Ar} , C_{Th}), 169.60 ($C=N$), 169.87 (CO). Anal. Calcd for $C_{25}H_{25}ClN_4O_2S$, %: C, 62.43; H, 5.24; N, 11.65. Found, %: C, 62.32; H, 5.19; N, 11.62.

5-(2-([4-(4-Chlorophenyl)-1,3-thiazol-2-yl]-4-methoxyanilino)ethyl)-1,3,4-oxadiazole-2(3H)-thione (6a). To a mixture of KOH (0.08 g, 1.5 mmol) and EtOH (15 mL), CS_2 (0.18 mL, 0.23 g, 1.5 mmol) was added drop-wise and the mixture was stirred at room temperature for 15 min. Afterwards, a mixture of hydrazide **4a** (0.60 g, 1.5 mmol) and EtOH (10 mL) was added and the reaction mixture was refluxed for 72 h. The liquid fractions were removed under *vacuo*, the residue was dissolved in H_2O (15 mL) and acidified with HCl to pH 3–4. The precipitate was filtered off and recrystallized from propan-2-ol to afford white solid, yield 0.42 g (63%), mp 172–173 °C; IR (KBr) ν_{max} (cm^{-1}): 3477 (NH), 1511 ($C=N$); 1H NMR (400 MHz, DMSO- d_6) δ 3.32 (2H, t, $J = 6.4$ Hz, CH_2C), 3.82 (3H, s, OCH_3), 4.33 (2H, t, $J = 6.4$ Hz, NCH_2), 7.07 (2H, d, $J = 8.8$ Hz, H_{Ar}), 7.18 (1H, s, SCH), 7.35 (2H, d, $J = 8.8$ Hz, H_{Ar}), 7.47 (2H, d, $J = 8.4$ Hz, H_{Ar}), 7.86 (2H, d, $J = 8.4$ Hz, H_{Ar}), 9.09 (1H, s, NH); ^{13}C NMR (101 MHz, DMSO- d_6) δ 23.03 (CH_2C), 49.69 (NCH_2), 55.30 (OCH_3), 103.47 (CH), 115.30, 127.18, 128.45, 128.86, 131.78, 133.31, 136.64, 148.99, 154.28, 158.54 (C_{Ar} , C_{Th}), 164.20 ($C=N$), 169.71 ($C=S$). Anal. Calcd for $C_{20}H_{17}ClN_4O_2S_2$ %: C, 53.99; H, 3.85; N, 12.59. Found, %: C, 53.96; H, 3.80; N, 12.53.

2-(3-([4-(4-Chlorophenyl)-1,3-thiazol-2-yl]-4-methoxyanilino)propanoyl)-N-phenyl-1-hydrazinecarbothioamide (7a). A mixture of hydrazide **4a** (0.40 g, 1 mmol), phenyl isothiocyanate (0.20 g, 0.18 mL, 1.5 mmol), and MeOH (20 mL) was refluxed for 3 h until precipitate started forming. The precipitate was filtered off and recrystallized from DMF– H_2O mixture to afford white solid, yield 0.48 g (91%), mp 131–132 °C; IR (KBr) ν_{max} (cm^{-1}): 3168 (NH), 1691 (CO), 1509 ($C=N$), 1241 ($C=S$); 1H NMR (400 MHz, DMSO- d_6) δ 2.69 (2H, t, $J = 7.6$ Hz, CH_2CO), 3.82 (3H, s, OCH_3), 4.21 (2H, t, $J = 7.6$ Hz, NCH_2), 7.07 (2H, d, $J = 8.8$ Hz, H_{Ar}), 7.14–7.22 (2H, m, SCH + NH), 7.30–7.53 (7H, m, H_{Ar}), 7.92 (2H, d, $J = 8.4$ Hz,

H_{Ar}), 9.56 (1H, s, NH), 10.00 (1H, s, NH); ¹³C NMR (101 MHz, DMSO-*d*₆) δ 31.62 (CH₂CO), 48.43 (NCH₂), 55.30 (OCH₃), 103.31 (CH), 115.22, 125.05, 127.28, 127.96, 128.45, 128.61, 129.02, 131.80, 133.48, 137.10, 139.03, 149.12, 158.48 (C_{Ar}, C_{Th}), 169.34 (CN), 169.92 (CO), 180.86 (C=S). Anal. Calcd for C₂₆H₂₄ClN₅O₂S₂, %: C, 58.04; H, 4.50; N, 13.02. Found, %: C, 57.98; H, 4.45; N, 12.99.

5-(2-{[4-(4-Chlorophenyl)-1,3-thiazol-2-yl]-4-methoxyanilino}ethyl)-4-phenyl-2,4-dihydro-3H-1,2,4-triazole-3-thione (8a). A mixture of thiosemicarbazide **7a** (0.27 g, 0.5 mmol) and 10% aqueous KOH solution (20 mL) was refluxed for 8 h and cooled down. Then concentrated HCl was added to pH 4. The precipitate was filtered off, washed with water, and recrystallized from DMF–H₂O mixture to afford white solid, yield 0.18 g (69%), mp 230–231 °C; IR (KBr) ν_{\max} (cm⁻¹): 2920 (NH), 1509 (C=N), 1244 (C=S); ¹H NMR (400 MHz, DMSO-*d*₆) δ 2.93 (2H, t, *J* = 7.2 Hz, CH₂C), 3.82 (3H, s, OCH₃), 4.04 (2H, t, *J* = 7.2 Hz, NCH₂), 7.02 (2H, d, *J* = 8.8 Hz, H_{Ar}), 7.15 (1H, s, SCH), 7.21 (2H, d, *J* = 8.8 Hz, H_{Ar}), 7.31–7.37 (2H, m, H_{Ar}), 7.48 (2H, d, *J* = 8.4 Hz, H_{Ar}), 7.50–7.56 (3H, m, H_{Ar}), 7.79 (2H, d, *J* = 8.4 Hz, H_{Ar}), 13.77 (1H, s, NH); ¹³C NMR (101 MHz, DMSO-*d*₆) δ 23.82 (CH₂C), 49.52 (NCH₂), 55.32 (OCH₃), 103.42 (CH), 115.23, 127.27, 128.10, 128.42, 128.68, 129.31, 129.40, 131.79, 133.31, 133.48, 136.66, 149.07, 149.91, 158.47 (C_{Ar}, C_{Th}), 167.64 (C=N), 169.62 (C=S). Anal. Calcd for C₂₆H₂₂ClN₅OS₂, %: C, 60.05; H, 4.26; N, 13.47. Found, %: C, 60.00; H, 4.21; N, 13.43.

General procedure for preparation of compounds 9–11a. A mixture of hydrazide **4a** (0.10 g, 0.25 mmol), corresponding carbaldehyde (0.5 mmol), and MeOH (10 mL) was refluxed for 10 min (**10a**) or 1 h (**9**, **11a**). The precipitate was filtered off, washed with MeOH, and recrystallized from DMF–H₂O mixture.

3-{[4-(4-Chlorophenyl)-1,3-thiazol-2-yl]-4-methoxyanilino}-*N'*-[(5-nitro-2-furyl)methylidene]propanohydrazide (9a). Yellow solid, yield 0.09 g (69%), mp 184–185 °C; IR (KBr) ν_{\max} (cm⁻¹): 3204 (NH), 1676 (CO), 1510 (C=N); ¹H NMR (400 MHz, DMSO-*d*₆) δ (*Z/E* isomeric mixture, 70/30): 2.71 (0.6H, t, *J* = 6.8 Hz, CH₂CO), 3.06 (1.4H, t, *J* = 6.8 Hz, CH₂CO), 3.81 (3H, s, OCH₃), 4.21–4.31 (2H, m, NCH₂), 7.02–7.90 (11H, m, H_{Ar}), 7.95 (0.7H, s, N=CH), 8.11 (0.3H, s, N=CH), 11.76 (0.7H, s, NH), 11.84 (0.3H, s, NH); ¹³C NMR (101 MHz, DMSO-*d*₆) δ 29.92 (CH₂CO), 47.79 (NCH₂), 55.18 (OCH₃), 103.35 (CH), 114.54, 115.15, 127.29, 128.36, 128.38, 128.84, 128.89, 128.92, 131.72, 136.90, 137.46, 148.82, 151.55, 151.71, 160.16 (C_{Ar}, C_{Th}), 169.83 (C=N), 172.80 (CO). Anal. Calcd for C₂₄H₂₀ClN₅O₅S %: C, 54.81; H, 3.83; N, 13.32. Found, %: C, 54.78; H, 3.79; N, 13.29.

3-{[4-(4-Chlorophenyl)-1,3-thiazol-2-yl]-4-methoxyanilino}-*N'*-[(5-nitro-2-thienyl)methylidene]propanohydrazide (10a). Orange solid, yield 0.10 g (75%), mp 199–200 °C; IR (KBr) ν_{\max} (cm⁻¹): 3110 (NH), 1675 (CO), 1509 (C=N); ¹H NMR (400 MHz, DMSO-*d*₆) δ (*Z/E* isomeric mixture, 70/30): 2.70 (0.6H, t, *J* = 6.8 Hz, CH₂CO); 3.05 (1.4H, t, *J* = 6.8 Hz, CH₂CO), 3.80 (3H, s, OCH₃), 4.27 (2H, t, *J* = 6.8

Hz, NCH₂), 7.00–8.11 (11H, m, H_{Ar}), 8.14 (0.7H, s, N=CH), 8.41 (0.3H, s, N=CH), 11.78 (0.7H, s, NH), 11.82 (0.3H, s, NH); ¹³C NMR (101 MHz, DMSO-*d*₆) δ 30.71, 33.05 (CH₂CO), 48.67 (NCH₂), 55.19, 55.26 (OCH₃), 103.20, 103.29 (CH), 115.12, 115.16, 127.20, 127.27, 128.31, 128.37, 128.69, 128.80, 128.86, 130.31, 146.64, 146.77, 149.11, 150.20, 158.42 (C_{Ar}, C_{Th}), 167.21, 169.76 (C=N), 172.63 (CO). Anal. Calcd for C₂₄H₂₀ClN₅O₄S₂ %: C, 53.18; H, 3.72; N, 12.92. Found, %: C, 53.14; H, 3.68; N, 12.88.

3-{[4-(4-Chlorophenyl)-1,3-thiazol-2-yl]-4-methoxyanilino}-*N'*-[(4-nitrophenyl)methylidene]propa-nohydrazide (11a). Dark orange solid, yield 0.12 g (92%), mp 210–211 °C; IR (KBr) ν_{\max} (cm⁻¹): 2958 (NH), 1677 (CO), 1509 (C=N); ¹H NMR (400 MHz, DMSO-*d*₆) δ (Z/E isomeric mixture, 70/30): 2.71 (0.6H, t, *J* = 6.8 Hz, CH₂CO), 3.15 (1.4H, t, *J* = 6.8 Hz, CH₂CO), 3.80, 3.82 (3H, 2s, OCH₃), 4.26 (2H, t, *J* = 6.8 Hz, NCH₂), 7.05–8.30 (14H, m, H_{Ar}+N=CH), 11.68 (0.7H, s, NH), 11.76 (0.3H, s, NH); ¹³C NMR (101 MHz, DMSO-*d*₆) δ 30.77, 32.96 (CH₂CO), 49.02 (NCH₂), 55.24 (OCH₃), 103.28 (CH), 115.20, 123.69, 123.87, 127.20, 127.28, 127.36, 127.77, 128.38, 128.38, 128.88, 128.94, 131.74, 133.46, 137.08, 137.17, 140.29, 140.38, 140.60, 143.47, 147.37, 147.63, 149.10, 158.43 (C_{Ar}, C_{Th}), 167.05, 169.67 (C=N), 169.82, 172.79 (CO). Anal. Calcd for C₂₆H₂₂ClN₅O₄S %: C, 58.26; H, 4.14; N, 13.07. Found, %: C, 58.23; H, 4.11; N, 13.03.

General procedure for preparation of quinolones 12a, b, d, e and 1,3-thiazol-2-amine 13c. A mixture of corresponding thiazole **2a–e** (2 mmol) and polyphosphoric acid (15 mL) was heated with stirring at 110–120 °C for 4–6 h. Then the reaction mixture was cooled down and crushed ice was added up to 50 mL. The precipitate was filtered off, washed with 5% aqueous Na₂CO₃ solution and water. The precipitate was recrystallized from glacial acetic acid.

1-[4-(4-Chlorophenyl)-1,3-thiazol-2-yl]-6-methoxy-2,3-dihydroquinolin-4(1*H*)-one (12a). Yellow solid, yield 0.50 g (67%), mp 149–150 °C; IR (KBr) ν_{\max} (cm⁻¹): 1684 (CO), 1526 (C=N); ¹H NMR (400 MHz, DMSO-*d*₆) δ 2.85 (2H, t, *J* = 6.3 Hz, CH₂CO), 3.81 (3H, s, CH₃), 4.37 (2H, t, *J* = 6.3 Hz, NCH₂), 7.22–8.09 (8H, m, H_{Ar} and SCH); ¹³C NMR (101 MHz, DMSO-*d*₆) δ 37.57 (CH₂CO), 49.02 (NCH₂), 55.50 (OCH₃), 105.38, 109.31, 121.99, 122.23, 124.37, 127.45, 128.67, 132.26, 133.07, 139.52, 149.10, 155.19 (C_{Ar}, C_{Th}), 166.41 (C=N), 192.78 (C=O). Anal. Calcd for C₁₉H₁₅ClN₂O₂S, C, 61.54; H, 4.08; N, 7.55. Found, %: C, 61.49; H, 4.01; N, 7.47.

1-[4-(4-Fluorophenyl)-1,3-thiazol-2-yl]-6-methoxy-2,3-dihydroquinolin-4(1*H*)-one (12b). Yellow solid, yield 0.49 g (69%), mp 132–133 °C; IR (KBr) ν_{\max} (cm⁻¹): 1683 (CO), 1501 (C=N); ¹H NMR (400 MHz, DMSO-*d*₆) δ 2.85 (2H, t, *J* = 6.2 Hz, CH₂CO), 3.81 (3H, s, CH₃), 4.36 (2H, t, *J* = 6.2 Hz, NCH₂), 7.16–8.20 (8H, m, H_{Ar} and SCH); ¹³C NMR (101 MHz, DMSO-*d*₆) δ 37.56 (CH₂CO), 49.04 (NCH₂), 55.50 (OCH₃), 104.41, 109.28, 115.40, 115.61, 121.98, 122.25, 124.31, 127.82, 130.86, 139.58, 149.33, 155.14 (C_{Ar}, C_{Th}), 166.35 (C=N), 192.79 (C=O). Anal. Calcd for C₁₉H₁₅FN₂O₂S, C, 64.39; H, 4.27; N,

7.90. Found, %: C, 64.28; H, 4.20; N, 7.87.

6-Methoxy-1-[4-(2-oxo-2H-chromen-3-yl)-1,3-thiazol-2-yl]-2,3-dihydroquinolin-4(1H)-one (12d).

Yellow solid, yield 0.52 g (64%), mp 196–197 °C; IR (KBr) ν_{\max} (cm⁻¹): 1718, 1683 (2CO), 1523 (C=N); ¹H NMR (400 MHz, DMSO-*d*₆) δ 2.87 (2H, t, *J* = 6.3 Hz, CH₂CO), 3.82 (3H, s, CH₃), 4.45 (2H, t, *J* = 6.3 Hz, NCH₂), 7.27–8.73 (8H, m, H_{Ar} and H_{chr}), 7.86 (1H, s, SCH); ¹³C NMR (101 MHz, DMSO-*d*₆) δ 37.66 (CH₂CO), 48.86 (NCH₂), 55.54 (OCH₃), 109.39, 111.05, 115.90, 119.21, 120.07, 122.13, 122.34, 124.63, 124.76, 128.98, 131.84, 139.11, 139.46, 143.69, 152.41, 155.39, 158.76 (C_{Ar}, C_{Th}, C_{chr}), 165.75 (C=N), 192.84 (C=O). Anal. Calcd for C₂₂H₁₆N₂O₄S, C, 65.34; H, 3.99; N, 6.93. Found, %: C, 65.23; H, 4.01; N, 6.88.

6-Methoxy-1-[4-(naphthalen-2-yl)-1,3-thiazol-2-yl]-2,3-dihydroquinolin-4(1H)-one (12e).

Yellow solid, yield 0.47 g (61%), mp 132–133 °C; IR (KBr) ν_{\max} (cm⁻¹): 1687 (CO), 1523 (C=N); ¹H NMR (400 MHz, DMSO-*d*₆) δ 2.88 (2H, t, *J* = 6.3 Hz, CH₂CO), 3.82 (3H, s, CH₃), 4.43 (2H, t, *J* = 6.3 Hz, NCH₂), 7.26–8.49 (10H, m, H_{Ar}), 7.63 (1H, s, SCH); ¹³C NMR (101 MHz, DMSO-*d*₆) δ 37.63 (CH₂CO), 49.04 (NCH₂), 55.51 (OCH₃), 105.42, 109.31, 121.95, 122.35, 123.99, 124.33, 124.42, 126.12, 126.48, 127.60, 128.20, 131.66, 132.58, 133.15, 139.67, 150.32, 155.16 (C_{Ar}, C_{Th}, C_{chr}), 166.32 (C=N), 192.83 (C=O). Anal. Calcd for C₂₃H₁₈N₂O₂S, C, 71.48; H, 4.69; N, 7.25. Found, %: C, 71.33; H, 4.62; N, 7.22.

N-(4-Methoxyphenyl)-4-(4-nitrophenyl)-1,3-thiazol-2-amine (13c).

Brown solid, yield 0.38 g (58%), mp 146–147 °C; IR (KBr) ν_{\max} (cm⁻¹): 3374 (NH), 1508 (C=N); ¹H NMR (400 MHz, DMSO-*d*₆) δ 3.87 (3H, s, CH₃), 6.25 (1H, d, *J* = 7.8 Hz, SCH), 7.28–8.36 (8H, m, H_{Ar}), 8.68 (1H, s, NH); ¹³C NMR (101 MHz, DMSO-*d*₆) δ 55.56 (OCH₃), 105.58, 109.63, 119.06, 120.54, 122.31, 124.31, 126.84, 127.03, 134.32, 139.19, 142.04, 147.03, 149.40, 156.41 (C_{Ar}, C_{Th}), 160.76 (C=N). Anal. Calcd for C₁₆H₁₃N₃O₃S, C, 58.71; H, 4.00; N, 12.84; Found, %: C, 58.67; H, 3.96; N, 12.80.

General procedure for preparation of quinolones 14a, b, d, e. A mixture of corresponding quinolone **12a, b, d, e** (1 mmol), hydrobromic acid (3 mL), and glacial acetic acid (5 mL) was refluxed for 20–30 h. Then the reaction mixture was cooled down and neutralized with dilute aqueous ammonia to pH 7. The precipitate was filtered off, washed with water, and recrystallized from glacial acetic acid.

1-[4-(4-Chlorophenyl)-1,3-thiazol-2-yl]-6-hydroxy-2,3-dihydroquinolin-4(1H)-one (14a).

Yellow solid, yield 0.28 g (79%), mp 240–241 °C; IR (KBr) ν_{\max} (cm⁻¹): 3431 (OH), 1693 (CO), 1561 (C=N); ¹H NMR (400 MHz, DMSO-*d*₆) δ 2.79 (2H, t, *J* = 6.2 Hz, CH₂CO), 4.28–4.41 (2H, m, NCH₂), 7.05–7.96 (8H, m, H_{Ar} and SCH); ¹³C NMR (101 MHz, DMSO-*d*₆) δ 37.67 (CH₂CO), 48.85 (NCH₂), 91.09, 104.98, 111.78, 122.48, 122.56, 124.98, 127.48, 128.48, 128.69, 129.72, 132.24, 138.20, 149.07, 154.33 (C_{Ar}, C_{Th}), 166.76 (C=N), 193.01 (C=O). Anal. Calcd for C₁₈H₁₃ClN₂O₂S, C, 60.59; H, 3.67; N, 7.85. Found, %: C, 60.51; H, 3.62; N, 7.80.

1-[4-(4-Fluorophenyl)-1,3-thiazol-2-yl]-6-hydroxy-2,3-dihydroquinolin-4(1H)-one (14b). Greenish yellow solid, yield 0.28 g (82%), mp 144–145 °C; IR (KBr) ν_{\max} (cm⁻¹): 3344 (OH), 1669 (CO), 1506 (C=N); ¹H NMR (400 MHz, DMSO-*d*₆) δ 2.79 (2H, t, *J* = 6.0 Hz, CH₂CO), 4.36 (2H, t, *J* = 6.0 Hz, NCH₂), 7.06–7.99 (8H, m, H_{Ar} and SCH); ¹³C NMR (101 MHz, DMSO-*d*₆) δ 37.65 (CH₂CO), 48.84 (NCH₂), 103.98, 111.73, 115.40, 115.61, 122.23, 122.55, 124.91, 127.75, 127.83, 130.89, 138.24, 149.28, 153.64 (C_{Ar}, C_{Th}), 166.69 (C=N), 193.11 (C=O). Anal. Calcd for C₁₈H₁₃FN₂O₂S, C, 63.52; H, 3.85; N, 8.23. Found, %: C, 63.44; H, 3.79; N, 8.19.

6-Hydroxy-1-[4-(2-oxo-2H-chromen-3-yl)-1,3-thiazol-2-yl]-2,3-dihydroquinolin-4(1H)-one (14d). Yellow solid, yield 0.29 g (74 %), mp 269–270 °C; IR (KBr) ν_{\max} (cm⁻¹): 3417 (OH), 1722, 1681 (2CO), 1528 (C=N); ¹H NMR (400 MHz, DMSO-*d*₆) δ 2.81 (2H, t, *J* = 6.2 Hz, CH₂CO), 4.44 (2H, t, *J* = 6.2 Hz, NCH₂), 7.05–8.72 (9H, m, H_{Ar} and H_{chr} and SCH); ¹³C NMR (101 MHz, DMSO-*d*₆) δ 37.73 (CH₂CO), 48.63 (NCH₂), 110.62, 111.84, 115.87, 119.22, 120.07, 122.27, 122.58, 124.74, 125.14, 128.94, 131.77, 138.07, 139.02, 143.68, 152.38, 153.89, 158.75 (C_{Ar}, C_{Th}, C_{chr}), 166.00 (C=N), 193.12 (C=O). Anal. Calcd for C₂₁H₁₄N₂O₄S, C, 64.61; H, 3.61; N, 7.18. Found, %: C, 64.55; H, 3.57; N, 7.13.

6-Hydroxy-1-[4-(naphthalen-2-yl)-1,3-thiazol-2-yl]-2,3-dihydroquinolin-4(1H)-one (14e). Greenish yellow solid, yield 0.27 g (72%), mp 183–184 °C; IR (KBr) ν_{\max} (cm⁻¹): 3396 (OH), 1686 (CO), 1529 (C=N); ¹H NMR (400 MHz, DMSO-*d*₆) δ 2.83 (2H, t, *J* = 6.3 Hz, CH₂CO), 4.43 (2H, t, *J* = 6.2 Hz, NCH₂), 7.09–8.46 (11H, m, H_{Ar}); ¹³C NMR (101 MHz, DMSO-*d*₆) δ 37.71 (CH₂CO), 48.84 (NCH₂), 104.97, 111.76, 122.19, 122.62, 124.01, 124.39, 124.91, 126.10, 126.47, 127.60, 128.17, 128.20, 131.73, 132.58, 133.16, 138.32, 150.29, 153.65 (C_{Ar}, C_{Th}, C_{chr}), 166.65 (C=N), 193.14 (C=O). Anal. Calcd for C₂₂H₁₆N₂O₂S, C, 70.95; H, 4.33; N, 7.52. Found, %: C, 70.81; H, 4.29; N, 7.47.

3-[(5-Acetyl-4-methylthiazol-2-yl)(4-methoxyphenyl)amino]propanoic acid (15). A mixture of acid **1** (0.76 g, 3 mmol), 3-chloro-2,4-pentanedione (0.44 g, 3.3 mmol), and acetone (15 mL) was refluxed for 3 h. The precipitate was filtered off, washed with acetone, dried, and purified by dissolving it in 5% aqueous K₂CO₃ solution (15 mL), filtering and acidifying the filtrate with acetic acid to pH 6 to afford white solid, yield 0.72 g (72%), mp 156–157 °C; IR (KBr) ν_{\max} (cm⁻¹): 1713 (CO), 1510 (C=N); ¹H NMR (400 MHz, DMSO-*d*₆) δ 2.29 (3H, s, CH₃), 2.48 (3H, s, OCCH₃), 2.56 (2H, t, *J* = 7.3 Hz, CH₂CO), 3.80 (3H, s, OCH₃), 4.09 (2H, t, *J* = 7.3 Hz, NCH₂), 7.06 (2H, d, *J* = 8.9 Hz, H_{Ar}), 7.34 (2H, d, *J* = 8.9 Hz, H_{Ar}); ¹³C NMR (101 MHz, DMSO-*d*₆) δ 18.66 (CH₃), 29.66 (OCCH₃), 32.32 (CH₂CO), 48.07 (NCH₂), 55.53 (OCH₃), 115.55, 122.53, 129.01, 135.96, 157.71, 159.15 (C_{Ar}, C_{Th}), 171.24 (C=N), 172.48 (COOH), 188.83 (C=O). Anal. Calcd for C₁₆H₁₈N₂O₄S, %: C, 57.47; H, 5.43; N, 8.38. Found, %: C, 57.40; H, 5.38; N, 8.35.

General procedure for synthesis of chalcones 16a-f. To a solution of corresponding aldehyde (3.0

mmol) in 10% aqueous NaOH solution (2 mL) a solution of **15** (1.0 g, 3 mmol) in MeOH (12 mL) was added dropwise, and the reaction mixture was kept at 0–3 °C for 1.5 h. Then it was stirred at room temperature for 24 h. The reaction mixture was diluted with water (20 mL) and acidified with dilute acetic acid (1:1) to pH 6. The precipitate was filtered off, washed with water, dried, and purified by dissolving it in 5% aqueous NaOH solution (15 mL), filtering and acidifying the filtrate with dilute acetic acid (1:1) to pH 6. **16b**, **d**, **e**, **f** were additionally recrystallized from the indicated solvents.

3-[[5-[(2E)-3-Phenylprop-2-enoyl]-4-methyl-1,3-thiazol-2-yl](4-methoxyphenyl)amino]propanoic acid (16a) was prepared according to the general procedure from **15** and benzaldehyde to afford yellow solid, yield 0.66 g (52%), mp 186–187 °C; IR (KBr) ν_{\max} (cm⁻¹): 3407 (OH), 1713 (CO), 1510 (C=N); ¹H NMR (400 MHz, DMSO-*d*₆) δ 2.54–2.65 (5H, m, CH₃+CH₂CO), 3.82 (3H, s, OCH₃), 4.14 (2H, t, *J* = 7.2 Hz, NCH₂), 7.08 (2H, d, *J* = 8.8 Hz, H_{Ar}), 7.20 (1H, d, *J* = 15.5 Hz, CO-CH=CH), 7.37–7.42 (5H, m, H_{Ar}), 7.53 (1H, d, *J* = 15.4 Hz, CO-CH=CH), 7.67–7.73 (2H, m, H_{Ar}), 12.35 (1H, br. s, COOH); ¹³C NMR (101 MHz, DMSO-*d*₆) δ 19.05 (CH₃), 32.21 (CH₂CO), 48.19 (NCH₂), 55.42 (OCH₃), 115.48, 122.27, 124.65, 125.77, 127.96, 128.48, 128.90, 130.29, 134.49, 135.83, 141.56, 158.89, 159.09 (C_{Ar}, C_{Th}), 171.39 (C=N), 172.30 (COOH), 180.27 (C=O). Anal. Calcd for C₂₃H₂₂N₂O₄S, %: C, 65.38; H, 5.25; N, 6.63. Found, %: C, 65.33; H, 5.20; N, 6.59.

3-[[5-[(2E)-3-(4-Bromophenyl)prop-2-enoyl]-4-methyl-1,3-thiazol-2-yl](4-methoxyphenyl)amino]propanoic acid (16b) was prepared according to the general procedure from **15** and 4-bromobenzaldehyde to afford yellow solid, yield 0.93 g (62%), mp 200–201 °C (MeOH). IR (KBr) ν_{\max} (cm⁻¹): 1716 (CO), 1511 (C=N); ¹H NMR (400 MHz, DMSO-*d*₆) δ 2.53–2.69 (5H, m, CH₃+CH₂CO), 3.82 (3H, s, OCH₃), 4.13 (2H, t, *J* = 7.3 Hz, NCH₂), 7.08 (2H, d, *J* = 8.9 Hz, H_{Ar}), 7.22 (1H, d, *J* = 15.5 Hz, CO-CH=CH), 7.39 (2H, d, *J* = 8.9 Hz, H_{Ar}), 7.49 (1H, d, *J* = 15.4 Hz, CO-CH=CH), 7.58 (2H, d, *J* = 8.5 Hz, H_{Ar}), 7.68 (2H, d, *J* = 8.5 Hz, H_{Ar}), 12.34 (1H, br. s, COOH); ¹³C NMR (101 MHz, DMSO-*d*₆) δ 19.06 (CH₃), 32.26 (CH₂CO), 48.23 (NCH₂), 55.43 (OCH₃), 115.49, 122.15, 123.56, 125.45, 128.89, 130.43, 131.82, 133.79, 135.81, 140.20, 159.10, 159.17 (C_{Ar}), 171.48 (C=N), 172.33 (COOH), 180.06 (C=O). Anal. Calcd for C₂₃H₂₁BrN₂O₄S, %: C, 55.10; H, 4.22; N, 5.59. Found, %: C, 55.03; H, 4.19; N, 5.52.

3-[[5-[(2E)-3-(4-Chlorophenyl)prop-2-enoyl]-4-methyl-1,3-thiazol-2-yl](4-methoxyphenyl)amino]propanoic acid (16c) was prepared according to the general procedure from **15** and 4-chlorobenzaldehyde to afford yellow solid, yield 1.08 g (79%), mp 191–192 °C; IR (KBr) ν_{\max} (cm⁻¹): 3407 (OH), 1716 (CO), 1510 (C=N); ¹H NMR (400 MHz, DMSO-*d*₆) δ 2.55–2.63 (5H, m, CH₃+CH₂CO), 3.82 (3H, s, OCH₃), 4.13 (2H, t, *J* = 7.3 Hz, NCH₂), 7.08 (2H, d, *J* = 8.9 Hz, H_{Ar}), 7.20 (1H, d, *J* = 15.5 Hz, CO-CH=CH), 7.39 (2H, d, *J* = 8.8 Hz, H_{Ar}), 7.45 (2H, d, *J* = 8.5 Hz, H_{Ar}), 7.51 (1H, d, *J* = 15.5 Hz,

CO-CH=CH), 7.75 (1H, d, $J = 8.5$ Hz, H_{Ar}), 12.41 (1H, br. s, COOH); ^{13}C NMR (101 MHz, DMSO- d_6) δ 19.06 (CH₃), 32.29 (CH₂CO), 48.25 (NCH₂), 55.42 (OCH₃), 115.48, 122.15, 125.40, 128.89, 130.21, 133.47, 134.71, 135.81, 140.10, 159.09, 159.14 (C_{Ar} , C_{Th}), 171.47 (C=N), 172.35 (COOH), 180.06 (C=O). Anal. Calcd for C₂₃H₂₁ClN₂O₄S, %: C, 60.46; H, 4.62; N, 6.12. Found, %: C, 60.39; H, 4.58; N, 6.07.

3-[[5-[(2E)-3-(4-Fluorophenyl)prop-2-enoyl]-4-methyl-1,3-thiazol-2-yl](4-methoxyphenyl)amino]propanoic acid (16d) was prepared according to the general procedure from **15** and 4-fluorobenzaldehyde to afford yellow solid, yield 1.19 g (90%), mp 185–186 °C (EtOH); IR (KBr) ν_{max} (cm⁻¹): 3435 (OH), 1724 (CO), 1509 (C=N); 1H NMR (400 MHz, DMSO- d_6) δ 2.57–2.61 (5H, m, CH₃+CH₂CO), 3.82 (3H, s, OCH₃), 4.13 (2H, t, $J = 7.3$ Hz, NCH₂), 7.08 (2H, d, $J = 8.9$ Hz, H_{Ar}), 7.15 (1H, d, $J = 15.4$ Hz, CO-CH=CH); 7.22 (2H, t, $J = 8.6$ Hz, H_{Ar}); 7.39 (2H, d, $J = 8.9$ Hz, H_{Ar}); 7.53 (1H, d, $J = 15.4$ Hz, CO-CH=CH); 7.80 (2H, dd, $J = 7.3, 5.8$ Hz, H_{Ar}); 12.33 (1H, br. s, COOH); ^{13}C NMR (101 MHz, DMSO- d_6) δ 18.59 (CH₃), 19.09 (CH₃), 32.23 (CH₂CO), 48.20 (NCH₂), 55.45, 55.06 (OCH₃), 115.51, 115.84, 115.96, 122.22, 124.56, 128.94, 130.87, 130.92, 131.18, 131.19, 135.84, 140.41, 158.99, 159.12, 162.51, 163.92 (C_{Ar} , C_{Th}), 171.43 (C=N), 172.36 (COOH), 180.19 (C=O). Anal. Calcd for C₂₃H₂₁FN₂O₄S, %: C, 62.71; H, 4.81; N, 6.63. Found, %: C, 62.61; H, 4.75; N, 6.56.

3-[[5-[(2E)-3-Furanprop-2-enoyl]-4-methyl-1,3-thiazol-2-yl](4-methoxyphenyl)amino]propanoic acid (16e) was prepared according to the general procedure from **15** and furan-2-carbaldehyde to afford brown solid, yield 0.49 g (40%), mp 149–150 °C (MeOH); IR (KBr) ν_{max} (cm⁻¹): 1731 (CO), 1510 (C=N); 1H NMR (400 MHz, DMSO- d_6) δ 2.54–2.63 (5H, m, CH₃+CH₂CO), 3.82 (3H, s, OCH₃), 4.13 (2H, t, $J = 7.3$ Hz, NCH₂), 6.63 (1H, s, CH_{Fur}), 6.87 (1H, d, $J = 15.2$ Hz, CO-CH=CH), 6.98 (1H, s, CH_{Fur}), 7.08 (2H, d, $J = 8.3$ Hz, H_{Ar}), 7.33–7.43 (3H, m, H_{Ar} +CO-CH=CH), 7.81 (1H, s, OCH_{Fur}), 12.10 (1H, br. s, COOH); ^{13}C NMR (101 MHz, DMSO- d_6) δ 18.90 (CH₃), 30.61, 32.32 (CH₂CO), 48.28 (NCH₂), 55.42 (OCH₃), 112.94, 115.48, 116.45, 121.40, 121.95, 128.24, 128.88, 135.82, 145.81, 150.85, 158.79, 159.10 (C_{Ar} , C_{Th} , C_{Fur}), 171.20 (C=N), 172.39 (COOH), 179.39 (C=O). Anal. Calcd for C₂₁H₂₀N₂O₅S, %: C, 61.15; H, 4.89; N, 6.79. Found, %: C, 61.02; H, 4.80; N, 6.76.

3-(4-Methoxy{4-methyl-5-[(E)-3-(2-thienyl)-2-propenoyl]-1,3-thiazol-2-yl}anilino)propanoic acid (16f) was prepared according to the general procedure from **15** and 2-thiophenecarboxaldehyde to afford yellow solid, yield 0.54 g (46%), mp 167–168 °C (MeOH); IR (KBr) ν_{max} (cm⁻¹): 3436 (OH), 1713 (CO), 1504 (C=N); 1H NMR (400 MHz, DMSO- d_6) δ 2.54–2.62 (5H, m, CH₃+CH₂CO), 3.82 (3H, s, OCH₃), 4.13 (2H, t, $J = 7.3$ Hz, NCH₂), 6.87 (1H, d, $J = 15.2$ Hz, CO-CH=CH), 7.08 (2H, d, $J = 8.9$ Hz, H_{Ar}), 7.13 (1H, dd, $J = 4.8$ Hz, $J = 3.8$ Hz, CH_{Thioph}), 7.39 (2H, d, $J = 8.8$ Hz, H_{Ar}), 7.55 (1H, d, $J = 3.3$ Hz, CO-CH=CH), 7.70 (2H, dd, $J = 10.5$ Hz, $J = 5.0$ Hz, CH_{Thioph}), 12.38 (1H, br. s, COOH); ^{13}C NMR (101 MHz, DMSO- d_6) δ 18.96 (CH₃), 32.28 (CH₂CO), 48.23 (NCH₂), 55.42 (OCH₃), 115.48, 122.10, 122.93,

128.69, 128.87, 129.67, 132.32, 134.39, 135.81, 139.49, 158.68, 159.09 (C_{Ar} , C_{Th} , C_{Thioph}), 171.23 ($C=N$), 172.37 ($COOH$), 179.54 ($C=O$). Anal. Calcd for $C_{21}H_{20}N_2O_4S_2$, %: C, 58.86; H, 4.70; N, 6.54. Found, %: C, 58.79; H, 4.67; N, 6.50.

3-([5-{5-(4-Chlorophenyl)-1H-pyrazol-3-yl}-4-methylthiazol-2-yl][4-methoxyphenyl]amino)propanoic acid (17). A mixture of chalcone **16c** (0.46 g, 1 mmol), hydrazine monohydrate (0.15 g, 3 mmol), KOH (0.17 g, 3 mmol), and propan-2-ol (15 mL) was stirred at 70–80 °C for 24 h. The liquid fraction was evaporated under reduced pressure; the residue was dissolved in water and acidified with acetic acid to pH 6. The precipitate was filtered off, washed with water, dried, and recrystallized from propan-2-ol to afford white solid, yield 0.25 g (53%), mp 201–202 °C; IR (KBr) ν_{max} (cm^{-1}): 3329 (OH), 3156 (NH), 1717 (CO), 1524, 1511 ($C=N$); 1H NMR (400 MHz, $DMSO-d_6$) δ 2.38 (3H, s, CH_3), 2.60 (2H, t, $J = 7.3$ Hz, CH_2CO), 3.80 (3H, s, OCH_3), 4.06 (2H, t, $J = 7.3$ Hz, NCH_2), 6.81 (1H, s, $NHCCH$), 7.05 (2H, d, $J = 8.9$ Hz, H_{Ar}), 7.35 (2H, d, $J = 8.8$ Hz, H_{Ar}), 7.62 (2H, d, $J = 8.5$ Hz, H_{Ar}), 7.75 (2H, d, $J = 8.5$ Hz, H_{Ar}), 12.71 (2H, br. s, $NHCCH$ and OH); ^{13}C NMR (101 MHz, $DMSO-d_6$) δ 16.87 (CH_3), 32.40 (CH_2CO), 47.92 (NCH_2), 55.38 (OCH_3), 100.74, 115.25, 120.94, 125.15, 127.09, 127.14, 128.84, 129.09, 129.76, 131.75, 136.96, 145.22, 158.59 (C_{Ar} , C_{Th} , C_{Pyr}), 167.25 ($C=N$), 172.59 ($COOH$). Anal. Calcd for $C_{23}H_{21}ClN_4O_3S$, %: C, 57.82; H, 4.85; N, 11.24. Found, %: C, 57.78; H, 4.81; N, 11.19.

3-([5-{5-(4-Chlorophenyl)isoxazol-3-yl}-4-methylthiazol-2-yl][4-methoxyphenyl]amino)propanoic acid (18). A mixture of chalcone **16c** (0.46 g, 1 mmol), hydroxylamine hydrochloride (0.21 g, 3 mmol), and 1,4-dioxane (15 mL) was refluxed for 40 h. The liquid fraction was evaporated under reduced pressure. Then the residue was dissolved in 10% aqueous K_2CO_3 solution and acidified with acetic acid to pH 6. The precipitate was filtered off, washed with water, dried, and recrystallized from propan-2-ol to afford yellow solid, yield 0.28 g (60%), mp 198–199 °C; IR (KBr) ν_{max} (cm^{-1}): 3413 (OH), 1715 (CO), 1525, 1511 ($2C=N$); 1H NMR (400 MHz, $DMSO-d_6$) δ 2.47 (3H, s, CH_3), 2.60 (2H, t, $J = 7.3$ Hz, CH_2CO), 3.81 (3H, s, OCH_3), 4.10 (2H, t, $J = 7.3$ Hz, NCH_2), 7.05 (1H, s, $OCCH$), 7.08 (2H, d, $J = 8.9$ Hz, H_{Ar}), 7.39 (2H, d, $J = 8.9$ Hz, H_{Ar}), 7.56 (2H, d, $J = 8.6$ Hz, H_{Ar}), 7.91 (2H, d, $J = 8.6$ Hz, H_{Ar}), 12.36 (1H, br. s, OH); ^{13}C NMR (101 MHz, $DMSO-d_6$) δ 17.33 (CH_3), 32.31 (CH_2CO), 48.19 (NCH_2), 55.43 (OCH_3), 97.16 ($CH-C=N-O$), 105.73, 115.46, 127.35, 128.41, 129.09, 129.12, 134.89, 136.20, 150.81, 159.01 (C_{Ar} , C_{Th} , C_{Pyr}), 161.30 ($CH-C=N-O$), 164.09 ($C=N$), 169.74 ($N-O-C$), 172.44 ($COOH$). Anal. Calcd for $C_{23}H_{20}ClN_3O_4S$, %: C, 58.78; H, 4.29; N, 8.94. Found, %: C, 58.71; H, 4.22; N, 8.89.

3-([5-{5-(4-Chlorophenyl)-1-phenyl-4,5-dihydro-1H-pyrazol-3-yl}-4-methylthiazol-2-yl][4-methoxyphenyl]amino)propanoic acid (19). A mixture of chalcone **16c** (0.46 g, 1 mmol), phenylhydrazine (0.13 g, 1.2 mmol), KOH (0.17 g, 3 mmol), and propan-2-ol (15 mL) was stirred at 70–80 °C for 6 h. The liquid fraction was evaporated under reduced pressure; the residue was dissolved in water and acidified with

acetic acid to pH 6. The precipitate was filtered off, washed with water, dried, and recrystallized from propan-2-ol to afford brown solid, yield 0.40 g (73%), mp 121–122 °C; IR (KBr) ν_{\max} (cm⁻¹): 3416 (OH), 1714 (CO), 1511 (C=N); ¹H NMR (400 MHz, DMSO-*d*₆) δ 2.31 (3H, s, CH₃), 2.50–2.58 (2H, m, CH₂CO), 2.97–3.07 (1H, m, CHCH₂), 3.81 (3H, s, OCH₃), 3.84–3.96 (1H, m, CHCH₂), 3.97–4.11 (2H, m, NCH₂), 5.29–5.39 (1H, m, NCH), 6.64 (1H, t, *J* = 7.3 Hz, H_{Ar}), 6.76 (2H, d, *J* = 7.8 Hz, H_{Ar}), 7.06 (4H, dd, *J* = 12.5 Hz, *J* = 5.4 Hz, H_{Ar}), 7.19 (2H, d, *J* = 8.5 Hz, H_{Ar}), 7.35 (2H, d, *J* = 8.9 Hz, H_{Ar}), 7.51 (2H, d, *J* = 8.4 Hz, H_{Ar}); ¹³C NMR (101 MHz, DMSO-*d*₆) δ 17.16 (CH₃), 32.73 (CH₂CO), 44.57 (CH₂CH), 48.20 (NCH₂), 55.37 (OCH₃), 62.62 (CH₂CH), 112.63, 113.30, 115.29, 118.22, 120.42, 128.22, 128.83, 129.05, 131.84, 136.59, 141.73, 142.98, 144.10, 148.91, 158.72 (C_{Ar}, C_{Th}, N-N=C), 168.00 (C=N), 172.71 (COOH). Anal. Calcd for C₂₉H₂₇ClN₄O₃S, %: C, 63.67; H, 4.97; N, 10.24. Found, %: C, 63.61; H, 4.91; N, 10.21.

3-[(2-{1-[(*E*)-2-[(*E*)-1-{2-[(3-Hydroxy-3-oxopropyl)-4-methoxyanilino]-4-methyl-1,3-thiazol-5-yl}-ethylidene)hydrazono]ethyl}-4-methyl-1,3-thiazol-5-yl)-4-methoxyanilino]propanoic acid (20). A mixture of thiazole **15** (0.67 g, 2 mmol), hydrazine monohydrate (0.20 g, 4 mmol), MeOH (20 mL), and acetic acid (1 mL) was refluxed for 30 h. Then the reaction mixture was cooled down and diluted with water (25 mL). The precipitate was filtered off, washed with water, dried, and purified by dissolving it in 5% aqueous Na₂CO₃ solution (15 mL), filtering and acidifying the filtrate with dilute acetic acid (1:1) to pH 6 to afford yellow solid, yield 0.55 g (41%), mp 180–181 °C; IR (KBr) ν_{\max} (cm⁻¹): 1714 (CO), 1510 (C=N); ¹H NMR (400 MHz, DMSO-*d*₆) δ 2.20 (6H, s, 2CH₃), 2.41 (6H, s, 2CH₃-C=N), 2.53 (4H, t, *J* = 7.1 Hz, 2CH₂CO), 3.79 (6H, s, 2OCH₃), 4.03 (4H, t, *J* = 7.1 Hz, 2NCH₂), 7.03 (4H, d, *J* = 8.5 Hz, H_{Ar}), 7.32 (4H, d, *J* = 8.5 Hz, H_{Ar}), 12.34 (2H, br. s, OH); ¹³C NMR (101 MHz, DMSO-*d*₆) δ 16.20 (CH₃), 18.74 (CH₃-C=N), 32.49 (CH₂CO), 47.98 (NCH₂), 55.34 (OCH₃), 115.23, 121.47, 128.94, 136.49, 150.37, 156.56, 158.63 (C_{Ar}, C_{Th}), 168.47 (C=N), 172.62 (COOH). Anal. Calcd for C₃₂H₃₆N₃O₃S₂, %: C, 57.81; H, 5.46; N, 12.64. Found, %: C, 57.72; H, 5.33; N, 12.57.

3-[4-Methoxy(4-methyl-5-{1-[2-phenylhydrazono]ethyl}-1,3-thiazol-2-yl)anilino]propanoic acid (21). A mixture of thiazole **15** (0.67 g, 2 mmol), phenylhydrazine (0.43 g, 4 mmol), MeOH (20 mL), and acetic acid (1 mL) was refluxed for 22 h. Then the reaction mixture was cooled down and diluted with water (25 mL). The precipitate was filtered off, washed with water, dried, and dissolved it in 5% aqueous Na₂CO₃ solution (15 mL), filtered and the filtrate was acidified with dilute acetic acid (1:1) to pH 6. The precipitate was recrystallized from MeOH to afford brown solid, yield 0.29 g (35%), mp 171–172 °C; IR (KBr) ν_{\max} (cm⁻¹): 3331 (NH), 1709 (C=O), 1510 (C=N); ¹H NMR (400 MHz, DMSO-*d*₆) δ 2.29, 2.39 (6H, 2s, 2CH₃), 2.56 (2H, s, CH₂CO), 3.80 (3H, s, OCH₃), 3.97–4.14 (2H, m, 2NCH₂), 6.60–7.45 (9H, m, H_{Ar}), 9.00 (1H, s, NH), 12.33 (1H, br. s, OH); ¹³C NMR (101 MHz, DMSO-*d*₆) δ 18.53 (CH₃), 29.55

(CH₃-C=N), 32.42 (CH₂O), 48.06 (NCH₂), 55.41 (OCH₃), 112.40, 115.19, 115.43, 118.41, 121.67, 128.81, 128.87, 128.91, 135.90, 136.93, 137.90, 144.86, 145.93, 157.53, 158.44, 159.02, 166.44 (C_{Ar}, C_{Th}), 171.07 (C=N), 172.62 (COOH). Anal. Calcd for C₂₂H₂₄N₄O₃S, %: C, 62.24; H, 5.70; N, 13.20. Found, %: C, 62.18; H, 5.66; N, 13.14.

Microbiology

Antibacterial activity of the compounds. Antibacterial activity was tested using the disk diffusion technique. The microorganisms *Rhizobium radiobacter*, *Escherichia coli*, *Xanthomonas campestris* were purchased 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 μg/mL) in DMSO. Cultures of *R. radiobacter*, *X. campestris*, *E. 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 μL of the inoculum containing bacterial cells (10⁸ CFU/mL) was spread over the LB agar medium. Filter paper disks were prepared by adding 25 μL 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.^{34,35} Each experiment was repeated three times.

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.³⁶ Briefly, 1 mM solution of DPPH in EtOH was prepared, and this solution (1 mL) was added to the solutions of the tested compounds (1 mg/mL of DMSO). 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 A₀ is the absorbance of the control reaction and A₁ is the absorbance in the presence of the compounds. Each experiment was repeated three times.

Reducing power assay. The solutions of the tested compounds (1000 μg/mL) were mixed with the phosphate buffer (2.5 mL, 0.2 mol/L, pH 6.6) and potassium ferricyanide [K₃Fe(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), FeCl₃ (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.³⁷ Each experiment was repeated three times.

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 as well as 2.5 mL of FeCl₃ (20 mmol/L) and 25 mL of acetate buffer (0.3 mol/L, pH = 3.6). The aliquots of 100 μ L tested compounds (1000 μ 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 comprising of the calibration curve, five concentrations of FeSO₄·7 H₂O (5, 10, 15, 20, and 25 μ mol/L) were used, and the absorbancies were measured as a sample solution.³⁸ Each experiment was repeated three times.

Statistical analysis. Differences between means were assessed by the *Student's t-test* at $P = 0.05$. Values were expressed as mean \pm SD.³⁹

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