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Synthesis and Antimicrobial Evaluation of 2-[2-(9*H*-Fluoren-9-ylidene)hydrazin-1-yl]-1,3-thiazole Derivatives

Kazimieras Anusevičius [1,*], Ignė Stebrytė [1] and Povilas Kavaliauskas [2]

[1] Department of Organic Chemistry, Kaunas University of Technology, Radvilėnų, Rd. 19, LT-50254 Kaunas, Lithuania; igne.stebryte@gmail.com
[2] Division of Infectious Diseases, Department of Medicine, Weill Cornell Medicine of Cornell University, 527 East 68th Street, New York, NY 10065, USA; pok4001@med.cornell.edu
* Correspondence: kazimieras.anusevicius@ktu.lt; Tel.: +370-646-21841

Abstract: Fluorenyl-hydrazonothiazole derivatives **2–7** were synthesized by the *Hantzsch* reaction from 2-(9*H*-fluoren-9-ylidene)hydrazine-1-carbothioamide (**1**) and the corresponding α-halocarbonyl compounds in THF or 1,4-dioxane solvent. A base catalyst is not necessary for synthesising thiazoles, but it can shorten the reaction time. The antimicrobial properties of all synthesized compounds were screened for multidrug-resistant microorganism strains. The minimum inhibitory concentration of the tested compounds against Gram-positive bacteria and fungi was higher than 256 μg/mL, but several compounds had activity against Gram-positive strains.

Keywords: fluorenone; carbothioamide; thiazoles; antifungal; antibacterial

1. Introduction

Infectious diseases are nowadays a significant cause of morbidity and mortality throughout the world, and the ability of microorganisms to adapt to currently known antibiotics is a relevant problem. These factors lead to research on and the development of scaffolds for new antimicrobial compounds [1,2]. Heterocyclic compounds containing nitrogen and sulphur atoms have a significant role in medical chemistry, and they are widely used in the development of bioactive compounds, drugs, and industrial products [2–5]. Compounds containing a thiazole ring occupy an important place in the search for new derivatives with antimicrobial properties [6–14]. The *Hantzsch* thiazole synthesis is one of the most common methods for obtaining thiazole moieties in molecules [15]. For the synthesis of 1,3-thiazoles by reacting thiosemicarbazide derivatives with various haloketones, very different reaction conditions are chosen, e.g., aprotic solvents are used [16,17] and protic [18,19] solvents are used. Also, the reactions are carried out with a base catalyst [20–22] or without a base catalyst [23–25].

Molecules containing a fluorene scaffold are found in a variety of biologically significant compounds, such as those that are anticancer [26,27], antimicrobial [28], used for the treatment of Alzheimer's disease [29], and related to antioxidant activity [30]. According to Ray, S. et al., the introduction of a fluorene moiety into the molecule of some derivatives improves their pharmacological and pharmacokinetic properties [31].

In our previous works [13–32], 2-aminothiazoles and their derivatives were synthesized by the *Hantzsch* reaction, and some compounds showed promising antimicrobial activity. In the course of continuing research on the synthesis of 2-aminothiazole derivatives, several new fluorenyl-hydrazinthiazoles derivatives were synthesized and evaluated for their antimicrobial activity.

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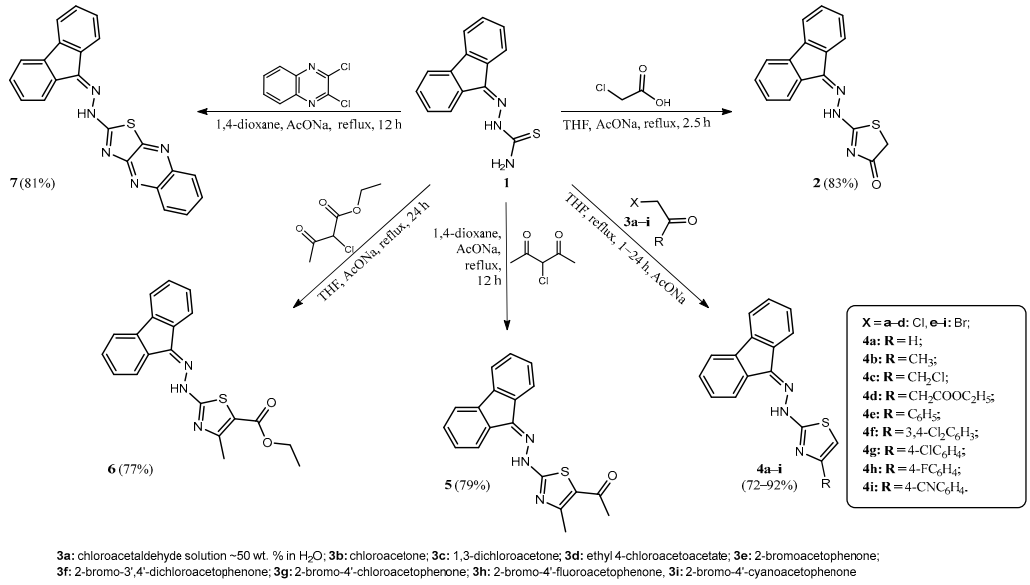
2. Results and Discussion

Synthesis and Characterization

In this work, the starting compound 2-(9*H*-fluoren-9-ylidene)hydrazine-1-carbothioamide (**1**) from fluorenone was synthesized by the method described in the literature [33,34]. The synthesis of compound **1** was carried out by the reaction of fluorenone and thiosemicarbazide in 1,4-dioxane in the presence of a catalytic amount of glacial acetic acid. In this project, 2-[2-(9*H*-fluoren-9-ylidene)hydrazinyl]-1,3-thiazol-4(5*H*)-one (**2**) was resynthesized by the method described in the literature [35,36]. However, tetrahydrofuran was observed to dissolve reagents better than alcoholic solvents and was therefore chosen for this work (Scheme 1). Analytical data for thiazolone derivative **2** are consistent with the literature [36]. The structure of all synthesized compounds **2–7** was confirmed by the data of the ^{1}H and ^{13}C NMR spectra (Supplementary Materials, Figures S1–S26).



Scheme 1. Synthesis of thiazole derivatives **2–7**.

2-[2-(9*H*-Fluoren-9-ylidene)hydrazinyl]-1,3-thiazole (**4a**) was synthesized by reacting carbothioamide **1** with chloroacetaldehyde in THF without a base catalyst. In the reaction, product **4a** was obtained as a hydrochloride salt due to the low solubility of the organic salts in THF solvents; during the synthesis, precipitates were formed. Product **4a** was obtained in a relatively pure form because unreacted reactants and by-products remained in the filtrate or were washed by filtering. The basic analogue of compound **4a** was obtained by dissolving the crystals in glacial acetic acid and diluting the solution with 10% aqueous sodium acetate. Other compounds **4b–i** were synthesized under analogous reaction conditions and shown in Scheme 1. A.P. Kaur et al. [36] performed the ultrasonic synthesis of compounds **4e** and **4g** in DMF solvent, and the reaction time was achieved very quickly, with excellent yields of 80–82%. In this work, the synthesis of compound **4e** was carried out in DMF solvent by heating the reaction mixture to 70–80 °C. Crystals from the reaction mixture were not formed, and resins appeared when the solution was diluted by water, which made it more complicated to separate and purify the products. However, crystals of the product were formed during the reaction or after the mixture had cooled, when tetrahydrofuran was used as a solvent for the synthesis with 72–92% yield.

In the ^{1}H NMR spectrum for thiazole **4a**, proton signals of the thiazole ring are visible at 6.64 and 7.21 ppm, and fluorene ring signals are identified as two multiplets at 7.26–7.54 and 7.69–7.91 ppm and one doublet at 8.67 ppm. The NH proton signal is not observed in this spectrum, which could be due to the rapid movement of the proton between the nucleophilic centers. Alternatively, it may be observed as a broad peak. For example, the NH proton signal is observed as a broad singlet at 10.18 ppm in the ^{1}H NMR spectrum

of compound **4b**. Analogous NMR spectral data are assigned for other compounds **4b–d** (Supplementary Materials, Figures S3–S20)

4,5-Disubstituted thiazole derivatives **5, 6** were synthesized by reacting carbothioamide **1** with 3-chloro-2,4-pentanedione or ethyl 2-chloroacetoacetate, as shown in Scheme 1. In this case, sodium acetate was used in the reactions as a catalyst; reactions can be performed without a base, but the progress of the reactions was slower. Also, the synthesis of compound **5** was slower in tetrahydrofuran than in 1,4-dioxane, so it was decided to change the solvent. Compounds **5** and **6** are the data of the ^{1}H and ^{13}C NMR spectra (Supplementary Materials, Figures S21–S24).

Quinoxaline-fused thiazole **7** was prepared by the reactions of carbothioamide **1** with 2,3-dichloroquinaxoline. This reaction was carried out in 1,4-dioxane in the presence of sodium acetate at reflux for 12 h. An initial attempt to synthesize thiazole **7** was carried out in acetic acid in the presence of sodium acetate [13], but product **7** was obtained with a 20% yield. 2,3-Dichloroquinoxaline is poorly soluble in tetrahydrofuran; therefore, compound **7** was synthesized in 1,4-dioxane with an 81% yield.

Fluorenyl-hydrazonothiazole derivatives **2–7** were tested against multidrug-resistant Gram-positive and Gram-negative bacteria or fungi strains. However, compounds **2–7** do not show antimicrobial activity against multidrug-resistant Gram-negative pathogens, e.g., *K. pneumoniae*, *P. aeruginosa*, and *E. coli*; also, they were not found to have any significant activity against drug-resistant virulent fungi, e.g., *C. auris*, and *C. albicans*. All synthesized compounds showed minimal inhibition concentration lower than 256 mg/mL.

Preliminary tests of synthesized compounds **2–7** against multidrug-resistant pathogens showed that thiazolone **2**, like thiazole **4d** and **4f,** has better than 256 mg/mL antibacterial activity against Gram-positive bacteria *S. aureus* and *E. faecalis*. In this work, the compounds **2, 4e**, and **4g** are known, as is their antimicrobial activity [36]. Comparing the results of the antimicrobial properties of known compounds, our test with multidrug-resistant virulent organisms has a minimum inhibitory concentration higher than that reported in the publication.

3. Materials and Methods

Chemistry

Reaction progress and compound purity-monitored TLC aluminum plates precoated with Silica gel with F 254 nm (Merck KGaA, Darmstadt, Germany) were used. Melting points in an open capillary with a B-540 melting point apparatus (Büchi Corporation, New Castle, DE, USA) were determined and uncorrected. A Perkin–Elmer Spectrum BX FT–IR spectrometer (Perkin–Elmer Inc., Waltham, MA, USA) was used to record the IR spectra (ν, cm^{-1}) from KBr tablets. The NMR spectra were recorded on Bruker Ascend 400 (^{1}H 400 MHz, ^{13}C 101 MHz). The chemical shifts (δ) were reported in parts per million (ppm) and calibrated from the standard of solvent the signal of DMSO-d$_6$ (2.50 ppm) for ^{1}H NMR and (39.43 ppm) for ^{13}C NMR. The Elemental Analyzer CE-440 was used for elemental analyses (C, H, N) in good agreement ($\pm$0.3%) with the calculated values. All reagents and solvents were purchased from Sigma-Aldrich (St. Louis, MO, USA) and were used without purification.

2-[2-(9*H*-Fluoren-9-ylidene)hydrazinyl]-1,3-thiazol-4(5*H*)-one (**2**) (See Figures S1 and S2 for NMR spectra)

A mixture of carbothioamide **1** (0.25 g, 1 mmol), monochloroacetic acid (0.12 g, 1.3 mmol), sodium acetate (0.25 g, 3 mmol), and 15 mL tetrahydrofuran was refluxed for 2.5 h. The reaction mixture was cooled down and diluted with 50 mL water; precipitate was filtered off and washed with water and propan-2-ol. 1,3-Thiazol-4(5*H*)-one **4** was purified by recrystallization method from methanol, yield 0.24 g (83%), m.p. 210–212 °C, data found in ref. 211–215 °C [35,36]. ^{1}H NMR (400 MHz, DMSO-d_6) δ: 3.98 (s, 2H, CH_2), 7.35 (t, 2H, J = 7.5 Hz, H_{Ar}); 7.40–7.58 (m, 4H, H_{Ar}), 7.75 (d, 1H, J = 7.4 Hz, H_{Ar}), 7.78–7.91 (m, 2H, H_{Ar}), 12.26 (br. s, 1H, NH); ^{13}C NMR (101 MHz, DMSO-d_6) δ: 33.15 (CH_2), 120.46, 120.49, 121.90, 128.23, 129.82, 130.85, 131.09, 131.38, 136.12, 140.26, 141.30 (C_{Ar}), 156.02

(C=N), 168.30 (C=O), 174.04 (N=C–S). Anal. Calcd for $C_{16}H_{11}N_3OS$: C 65.51%, H 3.78%, N 14.32%. Found: C 65.31%, H 3.64%, N 14.16%.

A general method for the synthesis of 1,3-thiazole derivatives 4a–i

A mixture of carbothioamide **1** (0.25 g, 1 mmol), corresponding α-haloketone (1.1 mmol), and 20 mL tetrahydrofuran was refluxed from 1 to 24 h. The reaction was monitored by TLC method (eluent–acetone/hexane 1:2). When the reaction finished, the reaction mixture was cooled down, and precipitate was filtered off and washed with diethyl ether. Products **4a–i** were purified by dissolving crystals in 10 mL glacial acetic acid, and appropriate solutions were diluted by 50 mL 10% sodium acetate aqueous solution. The precipitate was filtered off and washed with water and dried, then products were recrystallized from 1,4-dioxane.

2-[2-(9*H*-Fluoren-9-ylidene)hydrazinyl]-1,3-thiazole (**4a**) (See Figures S3 and S4 for NMR spectra)

The reaction of carbothioamide **1** (0.25 g, 1 mmol) with chloroacetaldehyde solution ~50 wt. % in H_2O (0.17 g, 2.2 mmol) was carried out in 20 mL tetrahydrofuran by heating under reflux for 12 h. The product **4a** yield was 0.21 g (76%), m.p. 198–199 °C. FTIR (KBr) ν_{max}: 3416 (NH), 1602, 1554 (2C=N) cm^{-1}. ^1H NMR (400 MHz, DMSO-d_6) δ: 6.64 (d, 1H, J = 4.4 Hz, $H_{Thiazol}$), 7.21 (d, 1H, J = 4.4 Hz, $H_{Thiazol}$); 7.26–7.54 (m, 4H, H_{Ar}) 7.69–7.91 (m, 3H, H_{Ar}), 8.67 (d, 1H, J = 7.5 Hz, H_{Ar}); ^{13}C NMR (101 MHz, DMSO-d_6) δ: 104.43, 105.10 (C–S), 120.08, 120.12, 120.23, 120.69, 127.59, 127.64, 127.79, 128.21, 128.71, 129.25, 129.51, 131.04, 137.02, 138.74, 139.99, 147.89 (C_{Ar} + $C_{Thiazol}$), 152.64 (C=N), 173.70 (N=C–S). Anal. Calcd for $C_{16}H_{11}N_3S$: C 69.29%, H 4.00%, N 15.15%. Found: C 69.24%, H 3.89%, N 15.09%.

2-[2-(9*H*-Fluoren-9-ylidene)hydrazinyl]-4-methyl-1,3-thiazole (**4b**) (See Figures S5 and S6 for NMR spectra)

The reaction of carbothioamide **1** (0.25 g, 1 mmol) with chloroacetone (0.10 g, 1.1 mmol) was carried out in 20 mL tetrahydrofuran by heating under reflux for 6 h. The product **4b** yield was 0.22 g (75%), m.p. 180–181 °C. FTIR (KBr) ν_{max}: 3417 (NH); 1609, 1585 (C=N) cm^{-1}. ^1H NMR (400 MHz, DMSO-d_6) δ: 2.16 (s, 3H, CH_3), 6.27 (s, 1H, $H_{Thiazol}$), 7.23–7.51 (m, 4H, H_{Ar}), 7.66–7.91 (m, 3H, H_{Ar}), 8.70 (d, 1H, J = 7.6 Hz H_{Ar}), 10.18 (br. s, 1H, NH). ^{13}C NMR (101 MHz, DMSO-d_6) δ: 13.95 (CH_3), 99.78 (C–S), 120.15, 120.87, 127.64, 127.68, 128.24, 128.87, 129.69, 130.90, 135.56, 136.96, 138.80, 140.12 (C_{Ar} + $C_{Thiazol}$), 148.19 (C=N), 173.63 (N=C–S). Anal. Calcd for $C_{17}H_{13}N_3S$: C 70.08%, H 4.50%, N 14.42%. Found: C 70.03%, H 4.43%, N 14.32%.

4-(Chloromethyl)-2-[2-(9*H*-fluoren-9-ylidene)hydrazinyl]-1,3-thiazole (4c) (See Figures S7 and S8 for NMR spectra)

The reaction of carbothioamide **1** (0.25 g, 1 mmol) with 1,3-dichloroacetone (0.14 g, 1.1 mmol) was carried out in 20 mL tetrahydrofuran by heating under reflux for 16 h. The product **4c** yield was 0.25 g (78%), m.p. 192–193 °C. FTIR (KBr) ν_{max}: 3419 (NH); 1608, 1501 (C=N) cm^{-1}. ^1H NMR (400 MHz, DMSO-d_6) δ: 4.66 (s, 2H, CH_2), 6.86 (s, 1H, $H_{Thiazol}$), 7.23–7.54 (m, 4H, H_{Ar}), 7.74 (d, 1H, J = 7.4 Hz, H_{Ar}), 7.82 (d, 1H, J = 7.4 Hz, H_{Ar}), 7.87 (d, 1H, J = 7.4 Hz H_{Ar}), 8.61 (d, 1H, J = 7.6 Hz, H_{Ar}), 11.40 (br. s, 1H, NH). ^{13}C NMR (101 MHz, DMSO-d_6) δ: 39.13 (CH_2), 106.62 (CH–S), 120.21, 120.24, 120.85, 127.79, 128.05, 129.13, 129.99, 130.57, 136.84, 138.84, 140.34 (C_{Ar} + $C_{Thiazol}$), 147.63 (C=N), 172.78 (N=C–S). Anal. Calcd for $C_{17}H_{12}ClN_3S$: C 62.67%, H 3.71%, N 12.90%. Found: C 62.51%, H 3.54%, N 12.73%.

Ethyl {2-[2-(9*H*-fluoren-9-ylidene)hydrazinyl]-1,3-thiazol-4-yl}acetate (**4d**) (See Figures S9 and S10 for NMR spectra)

The reaction of carbothioamide **1** (0.25 g, 1 mmol) with ethyl 4-chloroacetoacetate (0.18 g, 1.1 mmol) was carried out in 20 mL tetrahydrofuran by heating under reflux for 24 h. The product **4d** yield was 0.26 g (72%), m.p. 145–146 °C. FTIR (KBr) ν_{max}: 3411 (NH); 1732 (O=C–O); 1604, 1586 (C=N) cm^{-1}. ^1H NMR (400 MHz, DMSO-d_6) δ: 1.22 (t, 3H, J = 7.1 Hz, CH_3), 3.67 (s, 2H, CH_2), 4.14 (q, 2H, J = 7.1 Hz, $C\underline{H}_2CH_3$), 6.49 (s, 1H, $H_{Thiazol}$), 7.08–7.60 (m, 4H, H_{Ar}), 7.75 (d, 1H, J = 7.5 Hz, H_{Ar}), 7.81 (d, 1H, J = 7.4 Hz, H_{Ar}), 7.86 (d, 1H, J = 7.4 Hz, H_{Ar}), 8.66 (d, 1H, J = 7.6 Hz, H_{Ar}), 10.36 11.40 (br. s, 1H, NH). ^{13}C NMR

(101 MHz, DMSO-d_6) δ: 14.12 (CH_3), 33.88 (CH_2), 60.73 ($\underline{C}H_2CH_3$), 103.12 (CH–S), 120.15, 120.78, 127.66, 127.69, 128.15, 128.25, 128.85, 129.69, 130.86, 133.72, 136.96, 138.77, 140.12 (C_{Ar} + $C_{Thiazol}$), 147.79 (C=N), 169.01 (N=C–S) 173.26 (C=O). Anal. Calcd for $C_{20}H_{17}N_3O_2S$: C 66.10%, H 4.71%, N 11.56%. Found: C 65.95%, H 4.63%, N 11.47%.

2-[2-(9*H*-Fluoren-9-ylidene)hydrazinyl]-4-phenyl-1,3-thiazole (4e) (See Figures S11 and S12 for NMR spectra)

The reaction of carbothioamide **1** (0.25 g, 1 mmol) with ethyl 2-bromoacetophenone (0.22 g, 1.1 mmol) was carried out in 20 mL tetrahydrofuran by heating under reflux for 0.5 h. The product **4e** yield was 0.31 g (89%), m.p. 156–157 °C, data found in ref. [36] 156–157 °C. FTIR (KBr) ν_{max}: 3412 (NH); 1599, 1491 (C=N) cm^{-1}. ^{1}H NMR (400 MHz, DMSO-d_6) δ: 7.20 (s, 1H, $H_{Thiazol}$), 7.28–7.58 (m, 7H, H_{Ar}), 7.65–8.05 (m, 5H, H_{Ar}), 8.68 (d, 1H, J = 7.5 Hz, H_{Ar}). ^{13}C NMR (101 MHz, DMSO-d_6) δ: 102.47 (CH–S) 120.19, 120.22, 120.81, 125.44, 127.75, 127.78, 128.03, 128.34, 128.80, 129.06, 129.94, 130.50, 136.89, 138.77, 140.32 (C_{Ar} + $C_{Thiazol}$), 147.23 (C=N), 172.83 (N=C–S). Anal. Calcd for $C_{22}H_{15}N_3S$: C 74.76%, H 4.28%, N 11.89%. Found: C 74.59%, H 4.04%, N 11.84%.

4-(3,4-Dichlorophenyl)-2-[2-(9*H*-fluoren-9-ylidene)hydrazinyl]-1,3-thiazole (4f) (See Figures S13 and S14 for NMR spectra)

The reaction of carbothioamide **1** (0.25 g, 1 mmol) with ethyl 2-bromo-3′-chloroacetophenone (0.26 g, 1.1 mmol) was carried out in 20 mL tetrahydrofuran by heating under reflux for 5 h. The product **4f** yield was 0.30 g (85%), m.p. 205–206 °C. FTIR (KBr) ν_{max}: 3335 (NH); 1607,1596 (C=N) cm^{-1}. ^{1}H NMR (400 MHz, DMSO-d_6) δ: 7.14–7.59 (m, 6H, H_{Ar}), 7.68–7.93 (m, 4H, H_{Ar}), 7.97 (s, 1H, $H_{Thiazol}$), 8.61 (br. s, 1H, H_{Ar}), 12.12 (br. s, 1H, NH). ^{13}C NMR (101 MHz, DMSO-d_6) δ: 105.09 (CH–S) 120.22, 120.31, 120.85, 124.01, 125.16, 127.84, 129.24, 130.17, 130.64, 133.67, 136.77, 138.76, 140.49 (C_{Ar} + $C_{Thiazol}$), 148.77 (C=N), 171.54 (N=C–S). Anal. Calcd for $C_{22}H_{13}Cl_2N_3S$: C 62.57%, H 3.10%, N 9.95%. Found: C 62.35%, H 3.04%, N 9.67%.

4-(4-Chlorophenyl)-2-[2-(9*H*-fluoren-9-ylidene)hydrazinyl]-1,3-thiazole (4g) (See Figures S15 and S16 for NMR spectra)

The reaction of carbothioamide **1** (0.25 g, 1 mmol) with ethyl 2-bromo-4′-chloroacetophenone (0.26 g, 1.1 mmol) was carried out in 20 mL tetrahydrofuran by heating under reflux for 5 h. The product **4g** yield was 0.34 g (87%), m.p. 184–185 °C; found in ref. [36] 182–184 °C. FTIR (KBr) ν_{max}: 3401 (NH); 1591, 1559 (C=N) cm^{-1}. ^{1}H NMR (400 MHz, DMSO-d_6) δ: 7.21–8.03 (m, 2H, H_{Ar} + $H_{Thiazol}$), 8.56 (br. s, 1H, H_{Ar}), 12.15 (br. s, 1H, H_{Ar}). ^{13}C NMR (101 MHz, DMSO-d_6) δ: 105.10 (CH–S) 120.25, 120.35, 120.86, 127.22, 127.85, 127.88, 128.82, 129.24, 130.12, 130.29, 132.71, 136.81, 138.78, 140.45 (C_{Ar} + $C_{Thiazol}$), 158.50 (C=N), 165.55 (N=$\underline{C}$–S). Anal. Calcd for $C_{22}H_{14}ClN_3S$: C 68.12%, H 3.64%, N 10.83%. Found: C 68.25%, H 3.50%, N 10.68%.

2-[2-(9*H*-Fluoren-9-ylidene)hydrazinyl]-4-(4-fluorophenyl)-1,3-thiazole (4h) (See Figures S17 and S18 for NMR spectra)

The reaction of carbothioamide **1** (0.25 g, 1 mmol) with ethyl 2-bromo-3′-fluoroacetophenone (0.22 g, 1.1 mmol) was carried out in 20 mL tetrahydrofuran by heating under reflux for 3 h. The product **4h** yield was 0.30 g (82%), m.p. 196–197 °C. FTIR (KBr) ν_{max}: 3433 (NH); 1616, 1552 (C=N) cm^{-1}. ^{1}H NMR (400 MHz, DMSO-d_6) δ: 7.20 (s, 1H, $H_{Thiazol}$), 7.26–7.51 (m, 6H, H_{Ar}), 7.77 (d, 1H, J = 7.3 Hz, H_{Ar}), 7.84 (d, 1H, J = 7.4 Hz, H_{Ar}), 7.86–8.03 (m, 3H, H_{Ar}), 8.64 (d, 1H, J = 7.6 Hz, H_{Ar}), 12.11 (s, 1H, NH). ^{13}C NMR (101 MHz, DMSO-d_6) δ: 102.44 (CH–S) 115.54, 115.76, 120.16, 120.66, 127.55, 127.63, 127.69, 128.80, 129.71, 136.95, 138.53, 140.13, 160.68, 163.11 (C_{Ar} + $C_{Thiazol}$), 148.04 (C=N), 172.15 (N=C–S). Anal. Calcd for $C_{22}H_{14}FN_3S$: C 71.14%, H 3.80%, N 11.31%. Found: C 71.29%, H 3.94%, N 11.14%.

4-{2-[2-(9*H*-Fluoren-9-ylidene)hydrazinyl]-1,3-thiazol-4-yl}benzonitrile (4i) (See Figures S19 and S20 for NMR spectra)

The reaction of carbothioamide **1** (0.25 g, 1 mmol) with ethyl 2-bromo-4′-cyanoacetophenone (0.22 g, 1.1 mmol) was carried out in 20 mL tetrahydrofuran by heating under reflux for 4 h. The product **4i** yield was 0.35 g (92%), m.p. 209–210 °C. FTIR (KBr) ν_{max}: 3415 (NH); 1608, 1556 (C=N) cm^{-1}. ^{1}H NMR (400 MHz, DMSO-d_6) δ: 7.23–7.55 (m, 4H, H_{Ar})

7.63 (s, 1H, $H_{Thiazol}$), 7.77 (d, 1H, *J* = 7.4 Hz, H_{Ar}), 7.84 (d, 1H, *J* = 7.4 Hz, H_{Ar}), 7.91 (t, 3H, *J* = 7.6 Hz, H_{Ar}), 8.08 (d, 2H, *J* = 8.1 Hz, H_{Ar}), 8.56 (d, 1H, *J* = 7.6 Hz, H_{Ar}), 12.16 (s, 1H, NH). ^{13}C NMR (101 MHz, DMSO-d_6) δ: 107.57 (CH–S) 110.02, 115.06, 118.86, 120.21, 120.32, 120.76, 126.06, 127.54, 127.80, 129.10, 130.06, 132.77, 136.81, 138.58, 140.38, 142.77 (C_{Ar} + $C_{Thiazol}$), 146.26 (C=N), 172.61 (N=C–S). Anal. Calcd for $C_{23}H_{14}N_4S$: C 73.00%, H 3.73%, N 14.80%. Found: C 72.85%, H 3.61%, N 14.78%.

1-{2-[2-(9*H*-Fluoren-9-ylidene)hydrazinyl]-4-methyl-1,3-thiazol-5-yl}ethan-1-one (5) (See Figures S21 and S22 for NMR spectra)

A mixture of carbothioamide **1** (0.25 g, 1 mmol), 3-chloro-2,4-pentanedione (0.15 g, 1.1 mmol), sodium acetate (0.25 g, 3 mmol), and 20 mL tetrahydrofuran was refluxed for 12 h. Then, the reaction mixture was cooled down and diluted with 20 mL water; precipitate was filtered off and washed with diethyl ether. Product **5** was purified by recrystallization method from 1,4-dioxane, m.p. 253–254 °C, yield 0.26 g (79%). FTIR (KBr) ν_{max}: 3405 (NH); 1652, 1604 (C=N) cm^{-1}. ^{1}H NMR (400 MHz, DMSO-d_6) δ: 2.40 (s, 3H, CH_3), 2.61 (s, 3H, CH_3), 7.12–7.62 (m, 4H, H_{Ar}) 7.65–8.06 (m, 3H, H_{Ar}), 8.68 (d, 1H, *J* = 7.6 Hz, H_{Ar}). ^{13}C NMR (101 MHz, DMSO-d_6) δ: 14.77 (CH_3), 29.07 (CH_3), 116.57, 120.23, 120.25, 121.23, 127.83, 127.85, 128.92, 129.54, 130.21, 131.22, 136.66, 139.34, 140.53, 145.54 (C_{Ar} + $C_{Thiazol}$), 151.15 (C=N); 169.81 (N=C–S), 188.72 (C=O). Anal. Calcd for $C_{19}H_{15}N_3OS$: C 68.45%, H 4.53%, N 12.60%. Found: C 68.32%, H 4.37%, N 12.36%.

Ethyl 2-[2-(9*H*-fluoren-9-ylidene)hydrazinyl]-4-methyl-1,3-thiazole-5-carboxylate (6) (See Figures S23 and S24 for NMR spectra)

A mixture of carbothioamide **1** (0.25 g, 1 mmol), ethyl 2-chloroacetoacetate (0.18 g, 1.1 mmol), sodium acetate (0.25 g, 3 mmol), and 20 mL tetrahydrofuran was refluxed for 24 h. Then, the reaction mixture was cooled down and diluted with 20 mL water; precipitate was filtered off and washed with diethyl ether. The product **6** was purified by recrystallization method from 1,4-dioxane, m.p. 185–186 °C, yield 0.30 g (77%). FTIR (KBr) ν_{max}: 3411 (NH); 1715 (C=O); 1605, 1582 (C=N) cm^{-1}. ^{1}H NMR (400 MHz, DMSO-d_6) δ: 1.27 (t, 3H, *J* = 7.1 Hz, $CH_2C\underline{H}_3$), 2.47 (s, 2H, CH_3), 4.21 (q, 2H, *J* = 7.1 Hz, $C\underline{H}_2CH_3$), 7.16–7.54 (m, 5H, H_{Ar}), 7.71–7.92 (m, 3H, H_{Ar}), 8.66 (d, 1H, *J* = 7.5 Hz, H_{Ar}), 10.95 (br. s, 1H, NH). ^{13}C NMR (101 MHz, DMSO-d_6) δ: 13.82 ($CH_2\underline{C}H_3$), 14.82 (CH_3), 60.52 ($\underline{C}H_2CH_3$), 103.62, 120.23, 121.21, 127.83, 128.84, 129.50, 130.18, 131.19, 136.66, 139.30, 140.51, 147.21 (C_{Ar} + $C_{Thiazol}$), 150.89 (C=N); 161.39 (C=O), 170.23 (N=C–S). Anal. Calcd for $C_{20}H_{17}N_3O_2S$: C 66.10%, H 4.72%, N 11.56%. Found: C 66.93%, H 4.57%, N 11.38%.

2-[2-(9*H*-Fluoren-9-ylidene)hydrazinyl][1,3]thiazolo [4,5-*b*]quinoxaline (7) (See Figures S25 and S26 for NMR spectra)

A mixture of carbothioamide **1** (0.25 g, 1 mmol), 2,3-dichloroquinoxaline (0.24 g, 1.2 mmol), sodium acetate (0.25 g, 3 mmol), and 20 mL 1,4-dioxane was refluxed for 12 h. Then, the reaction mixture was cooled down and diluted with 20 mL water; precipitate was filtered off and washed with diethyl ether. Product **7** was purified by recrystallization method from 1,4-dioxane, m.p. 251–252 °C, yield 0.31 g (81%). FTIR (KBr) ν_{max}: 3428 (NH); 1627, 1608, 1573 (C=N) cm^{-1}. ^{1}H NMR (400 MHz, DMSO-d_6) δ: 7.10–8.10 (m, 12H, H_{Ar}), 9.93 (s, 1H, NH). ^{13}C NMR (101 MHz, DMSO-d_6) δ: 120.97, 123.83, 127.36, 127.56, 127.94, 127.97, 129.04, 129.40, 130.08, 133.51, 133.82, 134.86, 139.10, 139.44, 141.44, 141.90, 142.49, 142.94, 143.17, 145.11, (C_{Ar} + $C_{Thiazol}$), 148.68, 148.78, 156.80 (C=N); 163.87 (N=C–S). Anal. Calcd for $C_{22}H_{13}N_5S$: C 69.64%, H 3.45%, N 18.46%. Found: C 69.45%, H 3.50%, N 18.21%.

4. Conclusions

A series of 2-aminothiazole derivatives containing fluorenyl hydrazone moiety in the molecules were synthesized by the *Hantzsch* reaction mechanism and evaluated for their in vitro antimicrobial activity using strains of multidrug-resistant bacterial and fungal pathogens preliminarily. Thiazolone **2** possesses the most potent activity against the tested strains of multidrug-resistant *S. aureus* and *E. faecalis* ATCC.

Supplementary Materials: The following are available online, Figure S1: ^{1}H-NMR of compound **2**, Figure S2: ^{13}C-NMR of compound **2**, Figure S3: ^{1}H-NMR of compound **4a**, Figure S4: ^{13}C-NMR of compound **4a**, Figure S5: ^{1}H-NMR of compound **4b**, Figure S6: ^{13}C-NMR of compound **4b**, Figure S7: ^{1}H-NMR of compound **4c**, Figure S8: ^{13}C-NMR of compound **4c**, Figure S9: ^{1}H-NMR of compound **4d**, Figure S10: ^{13}C-NMR of compound **4d**, Figure S11: ^{1}H-NMR of compound **4e**, Figure S12: ^{13}C-NMR of compound **4d**, Figure S13: ^{1}H-NMR of compound **4f**, Figure S14: ^{13}C-NMR of compound **4f**, Figure S15: ^{1}H-NMR of compound **4g**, Figure S16: ^{13}C-NMR of compound **4g**, Figure S17: ^{1}H-NMR of compound **4h**, Figure S18: ^{13}C-NMR of compound **4h**, Figure S19: ^{1}H-NMR of compound **4i**, Figure S20: ^{13}C-NMR of compound **4i**, Figure S21: ^{1}H-NMR of compound **5**, Figure S22: ^{13}C-NMR of compound **5**, Figure S23: ^{1}H-NMR of compound **6**, Figure S24: ^{13}C-NMR of compound **6**, Figure S25: ^{1}H-NMR of compound **7**, Figure S26: ^{13}C-NMR of compound **7**.

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