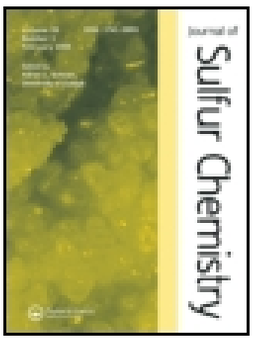

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Synthesis and antimicrobial activity of new thiazole and thiadiazole derivatives *via* ethyl pyruvate

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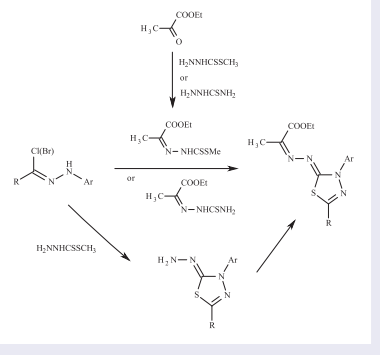
ABSTRACT

Reaction of ethyl 2-(2-((methylthio)carbonothioyl)hydrazono)propanoate (**2**) and ethyl 2-(2-carbamothioyl-hydrazono)propanoate (**3**) with hydrazonoyl halides **4a-e** afforded the corresponding thiadiazoles **5a-e**. Also, treatment of compound **2** with α-ketohydrazonoyl halides **12a-f** in absolute ethanol and in the presence of triethylamine gave the corresponding thiadiazoles **13a-f**; while reaction of ethyl 2-(2-carbamothioyl-hydrazono)propanoate (**3**) with α-ketohydrazonoyl halide **12** gave thiazole derivatives **15**. Also, the reaction of compound **3** with hydrazonoyl chloride **12f** afforded thiazolone derivative **18**. Antimicrobial studies are performed using two-gram positive bacteria and two-gram negative bacteria. Data revealed that thiazole derivative **17a** achieved the lowest MIC values (high efficient derivative) against the sensitive bacterial strain *S. aureus* with MIC value 160 µg/ml.



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Ethyl pyruvate; thiosemicarbazide; methyl hydrazinecarbodithioate; hydrazonoyl halides; thiadiazoles; thiazoles

Introduction

Ethyl pyruvate is a simple ester of pyruvate and stable lipophilic ester obtained from the endogenous metabolite pyruvate and has been exhibited to protect against inflammation [1] and to mitigate organ dysfunction in several animal models of diverse disorders, including severe sepsis, burns and acute pancreatitis [2,3]. Also, ethyl pyruvate has been revealed

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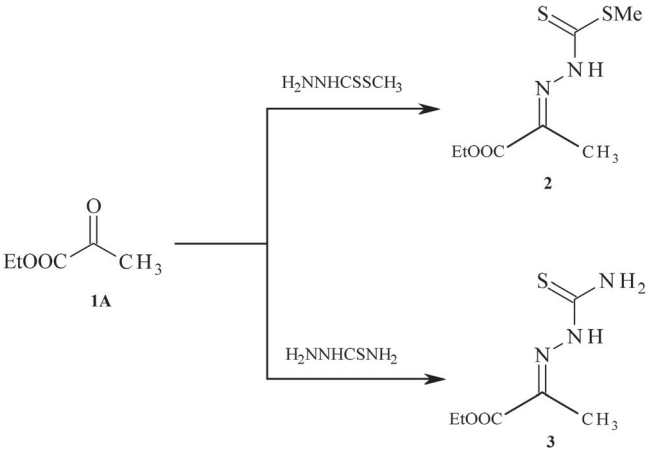
to possess a protective effect on acute lung injury (ALI), probably through the inhibition of the mitogen-activated protein kinase (MAPK) pathway in lungs [4–6]. Ethyl pyruvate is an important intermediate of organic synthesis with wide applications inhibits the growth of cells relying predominantly on glycolysis [7], antitumor [8], antitrypanosome activity [9], and other industrial and pharmaceutical applications [2,10–14]. The oxazolone synthesized from ethyl pyruvate widely used for the synthetic fungicides [15,16]. It is well known that thiadiazole derivatives are formed *via* reaction of hydrazonoyl halides with potassium thiocyanate [17–21] or by coupling of 1-substitutedl-2-thiocyanatoethan-1-one with *arenediazonium chlorides* [22,23]. Also, thiazole derivatives were prepared *via* reaction of thiourea with α-ketohydrazonoyl halide [24] or with phenacyl bromide [24]. We report herein the utility of ethyl pyruvate in the synthesis of new thiadiazole and thiazole having many functional groups and study the biological activities of some new synthesized compounds.

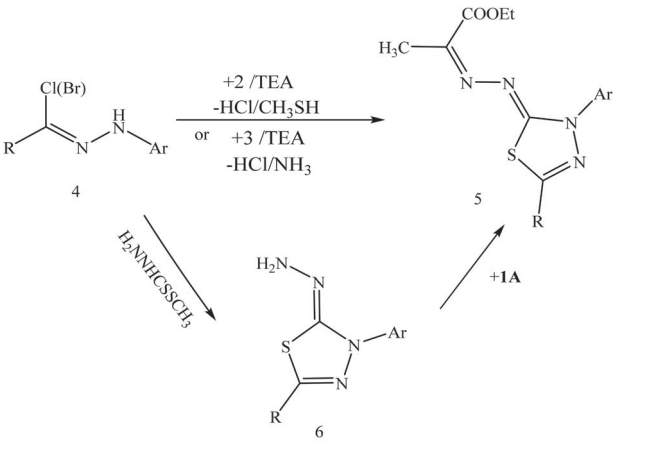
Results and discussion

Refluxing of ethyl pyruvate (**1A**) with methyl hydrazinecarbodithioate in 2-propanol afforded ethyl 2-(2-((methylthio)carbonothioyl)hydrazono)propanoate (**2**) [25–27] (Scheme 1). Stirring of compound **2** with the appropriate hydrazonoyl halides **4a–e** in absolute ethanol in the presence of triethylamine at room temperature afforded the corresponding thiadiazoles **5a–e**, respectively (Scheme 2). The structures of isolated products **5a–e** were established by their spectral data and elemental analyses.

Also, treatment of ethyl 2-(2-carbamothioylhydrazono)propanoate (**3**) [28] with hydrazonoyl halides **4a–e** in refluxing ethanol in the presence of triethylamine gave, in each case, one isolated product which proved identical in all respects (mp, mixed mp, IR, ^{1}H NMR, ^{13}C NMR and MS spectra) with the corresponding derivatives **5** (Scheme 2). Moreover, the structures of **5a–e** were confirmed by alternative synthesis. For example, thiadiazole **5a** was obtained *via* refluxing 2-hydrazono-1,3,4-thiadiazole derivative **6a** (prepared from

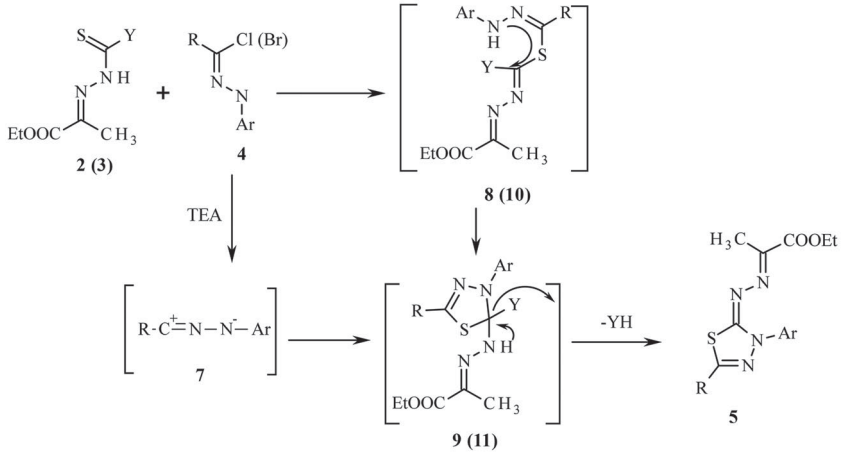


Scheme 1. Synthesis of ethyl 2-(2-((methylthio)carbonothioyl)hydrazono)propanoate (**2**) and ethyl 2-(2-carbamothioyl-hydrazono)propanoate (**3**).



4,5,6: R (Ar): a , C_6H_5 (C_6H_5); b, $C_6H_5CH{=}CH$ (C_6H_5); c, 2-C_4H_3O (4-$NO_2C_6H_4$); d, 2-C_4H_3S (4-$NO_2C_6H_4$); e, $C_6H_5NHCO(C_6H_5)$

Scheme 2. Synthesis of 1,3,4-thiadiazoles **5a–e**.

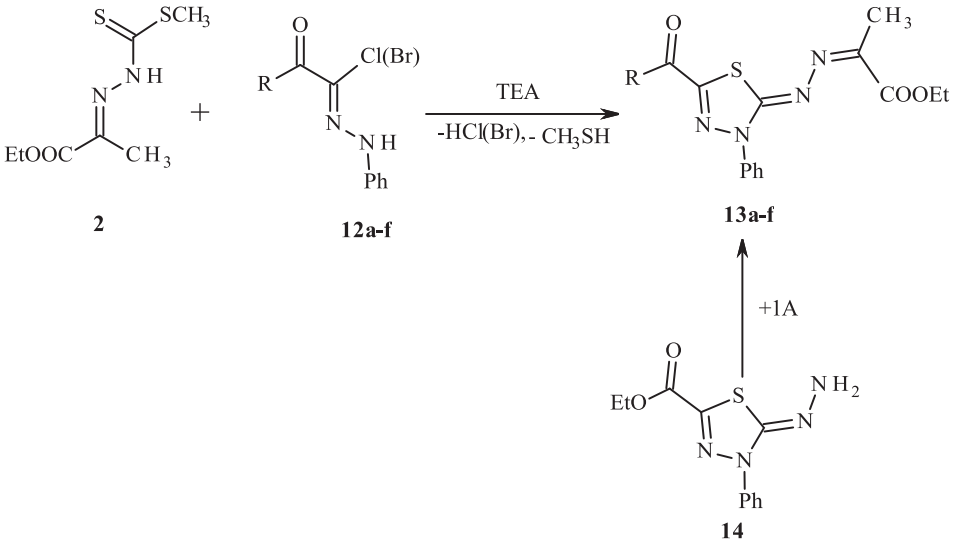


2, 8,9 (Y= SCH_3)
3, 10, 11 (Y= NH_2)

Scheme 3. A plausible mechanism for the formation 1,3,4-thiadiazole derivatives **5**.

reaction of methyl hydrazinecarbodithioate (**2**) with hydrazonoyl halides **4a** [29–32]) with ethyl pyruvate in 2-propanol.

In light of these results, the mechanism outlined in Scheme 3 seems to be the most plausible pathway for the formation of **5a–e** from the reaction of the **3** or **2** with **4**. The reaction involves the initial formation of thiohydrazonates **8**, **10** *via* 1,3-addition, which undergoes intramolecular cyclization as soon as it is formed to yield the intermediate **9**, **11** or *via* 1,3-dipolar cycloaddition of nitrilimines **7** to CS double bond of **3** or **2**, which afforded intermediate **9** or **11** which gave a final product **5** by elimination of ammonia or methyl mercaptan, respectively [33,34].

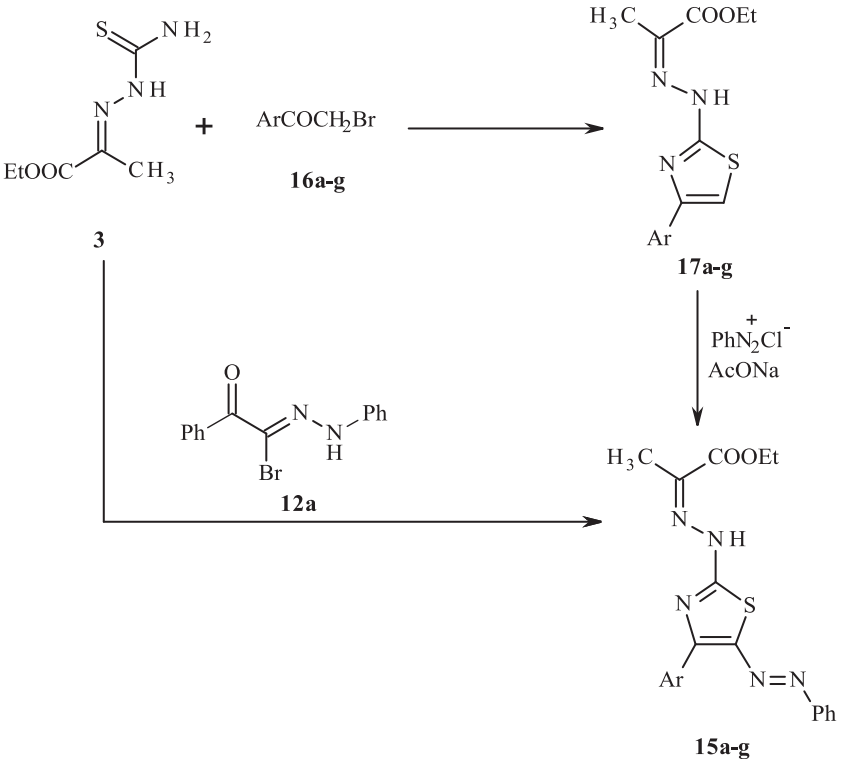


12, 13 R: a, C_6H_5; b, 2-$C_{10}H_7$; c, CH_3; d, 2-C_4H_3S; e, 2-C_4H_3O; f, OC_2H_5

Scheme 4. Synthesis of 1,3,4-thiadiazoles **13a–f**.

Analogously, treatment of α-ketohydrazonoyl halides **12a–f** with compound **2** in ethanolic triethylamine yielded 1,3,4-thiadiazoles **13a–f** in a good yield (Scheme 4). Structures of **13a–f** were confirmed based on elemental analysis, spectral data and alternative synthetic route. For example, treatment of ethyl 5-hydrazono-4-phenyl-4,5-dihydro-1,3,4-thiadiazole-2-carboxylate (**14**) [32] with **1A** in 2-propanol gave product identical in all respect mp, mixed mp, and spectra with compound **13f**.

In contrast, treatment of α-ketohydrazonoyl halide **12a** with compound **3** in boiling ethanol and in the presence of triethylamine gave one isolable product in each case, and the products formulated as: ethyl 2-(2-(4-substituted-5-(phenyldiazenyl)-thiazol-2-yl)hydrazono)propanoate (**15a**) (Scheme 5). Structures **15** were elucidated on the base of elemental analyses, spectral data and alternative synthetic route. Thus, reaction of ethyl 2-(2-carbamothioylhydrazono)propanoate (**3**) with ω-bromoacetophenone (**16a**) in boiling ethanol gave the corresponding thiazoles **17a**. Coupling of the latter compound **17a** with benzenediazonium chloride in ethanolic sodium acetate solution gave a product identical in all respect (mp, mixd mp and spectra) with **15a** (Scheme 5). Its mass spectrum showed an intense peak, base peak, at m/z = 393 which corresponding to its molecular weight. The IR spectrum showed two characteristic bands at ν 3429 and 1697 cm^{-1} assignable to NH and C = O stretching frequencies, respectively. Also, the ^{1}H NMR spectrum revealed signals at δ 1.31 ppm (t, J = 7 Hz, 3H) and 4.27 ppm (q, J = 7 Hz, 2H) corresponding to ethoxycarbonyl group, singlet signal at 2.14 ppm (CH_3) and a singlet signal at δ 11.38 ppm exchangeable with deuterium oxide assignable to NH proton, in addition to signals of aromatic moiety. Similarly, compounds **17b–g** were prepared *via* reaction of ω-bromoacetophenone derivatives **16b–g** with ethyl 2-(2-carbamothioylhydrazono)propanoate (**3**) in boiling ethanol. Coupling of the latter compounds **17b–g** with benzenediazonium chloride in ethanolic sodium acetate solution



15, 16 Ar: a, C_6H_5; b, 4-$CH_3C_6H_4$; c, 4-$CH_3OC_6H_4$; d, 4-$NO_2C_6H_4$; e, 4-ClC_6H_4; f, 4-BrC_6H_4; g, 4-CNC_6H_4

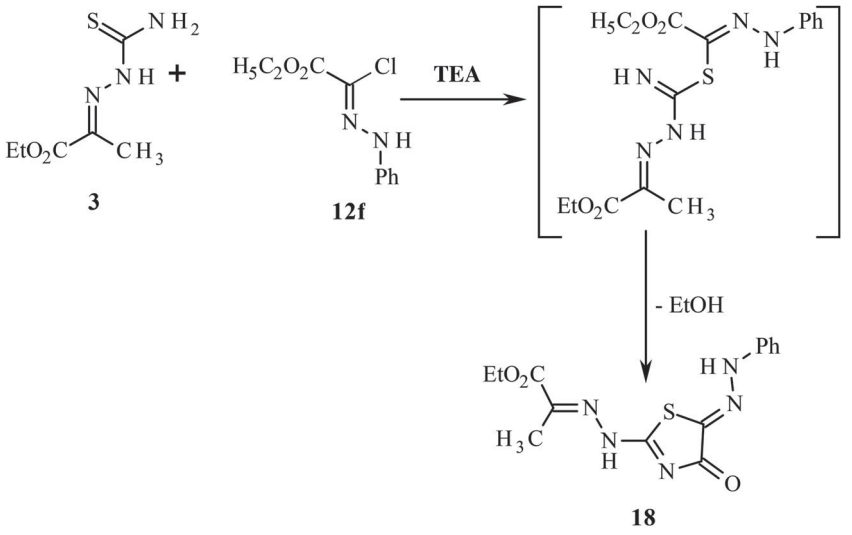
Scheme 5. Synthesis of ethyl 4-(aryl)-5-(2-phenylhydrazono)thiazol-2(5*H*)-ylidene)hydrazono)-propanoate **15a–g**.

gave ethyl 4-(aryl)-5-(2-phenylhydrazono)thiazol-2(5*H*)-ylidene)hydrazono)-propanoate **15b–g** (Scheme 5).

However, refluxing of compound **3** with *N*-phenyl-*C*-ethoxycarbonylmethano-hydra zonoyl chloride **12f** in ethanol in the presence of triethylamine yielded ethyl 2-((4-oxo-5-(2-phenylhydrazono)thiazolidin-2-ylidene)hydrazono)-propanoate (**18**) (Scheme 6). The structure of compound **18** based on elemental analysis and spectral data (see Experimental). Yield% of all new synthesized compounds **5–18** was mentioned in Table 1.

Antimicrobial screening

The antibacterial and antifungal activities of new synthesized compounds **5, 13, 15, 17,** and **18** were studied by the disc diffusion method. The antibacterial activities were done on the following pathogenic organisms; the gram-positive bacteria *Staphylococcus aureus* and *Bacillus subtilis*, the gram negative bacteria *Escherichia coli* and *Pseudomonas aeruginosa*. Moreover, antifungal activities aganist *Aspergillus flavus* and *Candida albicans* were studied. The synthesized compounds were used at the concentration of 20 mg/mL using



Scheme 6. Synthesis of ethyl 2-(2-(4-oxo-5-(2-phenylhydrazono)-4,5-dihydrothiazol-2-yl)hydrazono) propanoate **18**.

Table 1. Yield% of all new synthesized compounds **5–18**.

Compounds	Yield %	Compounds	Yield %	Compounds	Yield %
5a	65	**13e**	70	**17a**	77
5b	72	**13f**	73	**17b**	70
5c	69	**15a**	66	**17c**	66
5d	73	**15b**	63	**17d**	74
5e	63	**15c**	60	**17e**	73
13a	67	**15d**	67	**17f**	77
13b	66	**15e**	64	**17g**	73
13c	68	**15f**	60	**18**	70
13d	70	**15g**	68		

DMSO as a solvent. The *Ampicillin* 10 µL/disc was used as a standard antibacterial agent and the *Amphotericin B* 20 µg/mL as standard antifungal agent. The results presented in Table 2 all compounds show no antibacterial and antifungal activities except compounds **15e, 15f, 17a, 17b, 17c,** and **18** however, the most potent compound exhibited antibacterial and antifungal activities was compound **17a**.

Compound **17a** showed high potentiality in growth inhibition of tested microorganisms with relative activity of standard antibiotics with relative activity of 42% in *B. subtilis*, 100% in *S. aureus*, 60% in *E. coli*, 50% in *P. aeruginosa*, and 66.7% in *C. albicans*. Regarding the structure–activity relationship the parent compound (Ar = Ph) **17a** showed high antimicrobial activity, while the presence of electron donating groups leads to decrease the antimicrobial activity.

In vitro susceptibility tests were performed to evaluate minimum inhibitory concentration (MIC) was measured by a broth dilution method [35–37]. MIC values were determined for the highly efficient antibacterial compounds using the most sensitive microorganisms. Compound **17a** achieved the lowest MIC value (high efficient derivative) against the sensitive bacterial strain *S. aureus* with MIC value 160 µg/ml, followed by

Table 2. In vitro antibacterial and antifungal activities of compounds **15, 17** and **18** (Inhibition zone in mm).[a]

		Antimicrobial activity									
		Bacterial species (G^+)				Bacterial species (G^-)				Fungi	
		Bacillus subtilis		*Staphylococcusaureus*		*Escherichia coli*		*Pseudomonasaeruginosa*		*Candida albicans*	
Compounds		IZ	RA%	IZ	RA%	IZ	RA%	IZ	RA%	IZ	RA%
	Control: DMSO	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Standard	Ampicillin	26	100	21	100	25	100	26	100		
	Amphotericin B									21	100
15e		0.0	0.0	11.0	52.4	0.0	0.0	0.0	0.0	0.0	0.0
15f		9.0	34.6	15	71.4	9.0	36.0	9.0	34.6	0.0	0.0
17a		11	42.0	21	100.0	15	60.0	13	50.0	14.0	66.7
17b		0.0	0.0	11	52.4	0.0	0.0	0.0	0.0	0.0	0.0
17c		00.0	0.0	11.0	52.4	11.0	44.0	10.0	38.5	14.0	66.7
18		9.0	34.6	14.0	66.7	0.0	0.0	0.0	0.0	0.0	0.0

[a]G^- = Gram negative; G^+ = gram positive; R. A. = relative activity.

bacterial strain *E. coli* with MIC value 380 μg/ml and the highest MIC values against *C. albicans* MIC with value 420 μg/ml.

Experimental

Melting points were measured with a Stuart melting point apparatus and are uncorrected. The IR spectra were recorded using a FTIR Bruker–vector 22 spectrophotometer as KBr pellets. The ^{1}H and ^{13}C NMR spectra were recorded in $CDCl_3$ or DMSO–d_6 as solvent on Varian Gemini NMR spectrometer at 300 and 75 MHz, respectively, using TMS as internal standard. Chemical shifts are reported as δ values in ppm. Mass spectra were recorded with a Shimadzu GCMS–QP–1000 EX mass spectrometer in EI (70 eV) model. The elemental analyses were performed at the Micro analytical center, Cairo University.

Synthesis of ethyl (aryl-1,3,4-thiadiazol-2(3H)-ylidene)hydrazono)propanoate (5, 13)

Method A: A mixture of ethyl 2-(2-((methylthio)carbonothioyl)hydrazono)-propanoate (**2**) [25] (0.44 gm, 5 mmol) and the appropriate hydrazonoyl halides **4a–e** and **12a–f** (5 mmol) was dissolved in ethanol (50 mL). To the resulting solution triethylamine (2 mL) was added and reaction mixture was stirred for 6 h at room temperature. The resulting solid product that precipitated was collected, washed with ethanol and crystallized from a suitable solvent to afford the corresponding thiadiazole derivatives **5a–e** and **13a–f**. The products **5, 13** prepared together with their physical constants are listed below in yield (73%–63%).

Method B: To a solution of ethyl 2-(2-carbamothioylhydrazono)propanoate (**3**) [28] (0.95 gm, 5 mmol) and the appropriate hydrazonoyl chlorides **4a–f** (5 mmol) was dissolved in ethanol (50 mL), triethylamine (2 mL) was added and reaction mixture was refluxed for 6 h. The resulting solid product that precipitated was collected, washed with ethanol and crystallized from a suitable solvent to afford the corresponding thiadiazole derivatives **5a–f** in yield (60%–52%).

***Ethyl 2-((3,5-diphenyl-1,3,4-thiadiazol-2(3H)-ylidene)hydrazono)propanoate* (5a):** Yellow crystals (EtOH), mp 143–144°C; IR (KBr) ν 1702 (C = O) cm^{-1};^{1}H NMR (300 MHz, $CDCl_3$) δ 1.39 (t, J = 7 Hz, 3H, CH_3), 2.31 (s, 3H, CH_3), 4.33 (q, J = 7 Hz, 2H, CH_2) and 7.27–8.17 (m, 10H);^{13}C NMR (75 MHz, $CDCl_3$) δ 14.2, 14.8, 61.3, 121.2, 122.8, 128.6, 128.9, 129.1, 130.6, 130.8, 137.9, 151.8, 152.2, 165.3, 167.4. MS (EI, 70 eV) *m/z* (%): 366 (M^+, 14.8), 91 (100). Anal. Calcd. for $C_{19}H_{18}N_4O_2S$ (366.4): C, 62.28; H, 4.95; N, 15.29; S, 8.75. Found: C, 62.16; H, 4.88; N, 15.24; S, 8.80.

***Ethyl 2-((3-phenyl-5-((E)-styryl)-1,3,4-thiadiazol-2(3H)-ylidene)hydrazono)propanoate* (5b):** Yellow crystals (EtOH), mp 137–138°C; IR (KBr) ν 1699 (C = O) cm^{-1};^{1}H NMR (300 MHz, $CDCl_3$) δ 1.40 (t, J = 7 Hz, 3H, CH_3), 2.30 (s, 3H, CH_3), 4.33 (q, J = 7 Hz, 2H, CH_2), 7.02 (d, J = 14 Hz, 1H, CH), 7.13 (d, J = 14 Hz, 1H, CH), 7.27–7.54 (m, 8H) and 8.10 (d, 2H);^{13}C NMR (75 MHz, $CDCl_3$) δ 14.3, 14.7, 61.4, 118.5, 121.9, 126.4, 127.2, 128.7, 128.9, 129.5, 135.1, 137.6, 139.4, 151.9, 152.1, 165.1, 167.8. MS (EI, 70 eV) *m/z* (%): 392 (M^+, 100). Anal. Calcd. for $C_{21}H_{20}N_4O_2S$ (392.5): C, 64.27; H, 5.14; N, 14.28; S, 8.17. Found: C, 64.13; H, 5.20; N, 14.20; S, 8.23.

***Ethyl 2-((5-(furan-2-yl)-3-(4-nitrophenyl)-1,3,4-thiadiazol-2(3H)-ylidene)hydrazono)-propan-oate* (5c):** Yellow crystals (DMF), mp 192–194°C; IR (KBr) ν 1689 (C = O) cm^{-1};^{1}H NMR (300 MHz, $CDCl_3$) δ 1.39 (t, J = 7 Hz, 3H, CH_3), 2.34 (s, 3H, CH_3), 4.33 (q, J = 7 Hz, 2H, CH_2) and 6.60–8.47 (m, 7H);^{13}C NMR (75 MHz, $CDCl_3$) δ 14.1, 14.6, 61.4, 108.2, 108.8, 110.2, 122.8, 135.9, 139.6, 140.6, 142.2, 150.7, 151.0, 153.9, 163.2. MS (EI, 70 eV) m/z (%): 401 (M^+, 100). Anal. Calcd. for $C_{17}H_{15}N_5O_5S$ (401.4): C, 50.87; H, 3.77; N, 17.45; S, 7.99. Found: C, 50.93; H, 3.70; N, 17.38; S, 7.85.

***Ethyl 2-((3-(4-nitrophenyl)-5-(thien-2-yl)-1,3,4-thiadiazol-2(3H)-ylidene)hydrazono)-propanoate* (5d):** Yellow crystals (DMF), mp 186–188°C; IR (KBr) ν 1700 (C = O) cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.40 (t, J = 7 Hz, 3H, CH_3), 2.34 (s, 3H, CH_3), 4.34 (q, J = 7 Hz, 2H, CH_2) and 7.13–8.47 (m, 7H);^{13}C NMR (75 MHz, $CDCl_3$) δ 14.0, 14.6, 61.5, 109.0, 109.2, 110.5, 123.9, 136.5, 139.3, 141.0, 142.8, 150.9, 151.5, 154.2, 163.5. Anal. Calcd. for $C_{17}H_{15}N_5O_4S_2$ (417.5): **C,** 48.91; H, 3.62; N, 16.78; S, 15.36. Found: C, 49.00; H, 3.69; N, 16.70; S, 15.39.

***Ethyl 2-((3-phenyl-5-(phenylcarbamoyl)-1,3,4-thiadiazol-2(3H)-ylidene)hydrazono)-propanoate* (5e):** Yellow crystals (EtOH), mp 145–146°C; IR (KBr) ν 3410 (NH), 1690, 1683 (2C = O) cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.25 (t, J = 7 Hz, 3H, CH_3), 2.26 (s, 3H, CH_3), 4.30 (q, J = 7 Hz, 2H, CH_2), 7.20–8.00 (m, 10H) and 8.49 (s, 1H, NH);^{13}C NMR (75 MHz, $CDCl_3$) δ 14.2, 14.4, 61.5, 120.6, 122.3, 122.5, 126.8, 127.0, 128.1, 134.7, 135.1, 140.8, 150.5, 151.2, 163.5, 164.2. MS (EI, 70 eV) m/z (%): 409 (M^+, 21.3), 77 (100). Anal. Calcd. for $C_{20}H_{19}N_5O_3S$ (409.5): C, 58.67; H, 4.68; N, 17.10; S, 7.83. Found: C, 58.55; H, 4.78; N, 17.04; S, 7.88.

***Ethyl 2-((5-benzoyl-3-phenyl-1,3,4-thiadiazol-2(3H)-ylidene)hydrazono)propanoate* (13a):** Yellow crystals (EtOH), mp 162–164°C; IR (KBr) ν 1712, 1697 (2C = O) cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.39 (t, J = 7 Hz, 3H, CH_3), 2.28 (s, 3H, CH_3), 4.32 (q, J = 7 Hz, 2H, CH_2) and 7.27–8.35 (m, 10H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 14.1, 14.7, 61.5, 122.4, 127.4, 128.5, 128.9, 130.5, 133.9, 134.3, 138.9, 152.8, 153.8, 164.9, 168.6, 182.3. MS (EI, 70 eV) m/z (%): 394 (M^+, 11.7), 105 (100). Anal. Calcd. for $C_{20}H_{18}N_4O_3S$ (394.4): C, 60.90; H, 4.60; N, 14.20; S, 8.13. Found: C, 60.99; H, 4.58; N, 14.28; S, 8.05.

***Ethyl 2-((5-(2-naphthoyl)-3-phenyl-1,3,4-thiadiazol-2(3H)-ylidene)hydrazono)-propanoate* (13b):** Yellow crystals (CH_3CN), mp 150–152°C; IR (KBr) ν 1720, 1709 (2C = O) cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.40 (t, J = 7 Hz, 3H, CH_3), 2.30 (s, 3H, CH_3), 4.33 (q, J = 7 Hz, 2H, CH_2) and 7.27–9.02 (m, 12H);^{13}C NMR (75 MHz, $CDCl_3$) δ 14.0, 14.4, 61.3, 120.2, 121.3, 122.9, 125.3, 126.7, 127.2, 127.5, 128.0, 128.9, 130.2, 131.2, 131.3, 133.5, 136.7, 140.5, 155.6, 157.2, 168.2, 183.9. Anal. Calcd. for $C_{24}H_{20}N_4O_3S$ (444.5): C, 64.85; H, 4.54; N, 12.60; S, 7.21. Found: C, 64.93; H, 4.60; N, 12.78; S, 7.15.

***Ethyl 2-((5-acetyl-3-phenyl-1,3,4-thiadiazol-2(3H)-ylidene)hydrazono)propanoate* (13c):** Yellow crystals (EtOH), mp 139–140°C; IR (KBr) ν 1712, 1690 (2C = O) cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.37 (t, J = 7 Hz, 3H, CH_3), 2.26 (s, 3H, CH_3), 2.65 (s, 3H, $\underline{CH_3}CO$), 4.30 (q, J = 7 Hz, 2H, CH_2) and 7.27–8.03 (m, 5H);^{13}C NMR (75 MHz, $CDCl_3$) δ 14.1, 14.7, 25.1, 61.5, 122.4, 127.4, 128.9, 138.8, 152.0, 153.7, 164.9, 169.2, 189.4. MS (EI, 70 eV) m/z (%): 332 (M^+, 78.8), 259 (100). Anal. Calcd. for $C_{15}H_{16}N_4O_3S$ (332.4): C, 54.20; H, 4.85; N, 16.86; S, 9.65. Found: C, 54.08; H, 4.78; N, 16.91; S, 9.70.

***Ethyl 2-((3-phenyl-5-(thien-2-oyl)-1,3,4-thiadiazol-2(3H)-ylidene-hydrazono)-propanoate* (13d):** Yellow crystals (CH_3CN), mp 149–150°C; IR (KBr) ν 1710, 1699 (2C = O)

cm^{-1};^{1}H NMR (300 MHz, $CDCl_3$) δ 1.40 (t, J = 7 Hz, 3H, CH_3), 2.28 (s, 3H, CH_3), 4.37 (q, J = 7 Hz, 2H, CH_2) and 7.22–8.41 (m, 8H);^{13}C NMR (75 MHz, $CDCl_3$) δ 14.1, 14.7, 61.5, 122.3, 127.3, 128.4, 128.9, 136.1, 136.4, 138.5, 139.0, 152.2, 153.8, 164.9, 168.5, 173.9. Anal. Calcd. for $C_{18}H_{16}N_4O_3S_2$ (400.5): C, 53.99; H, 4.03; N, 13.99; S, 16.01. Found: C, 53.87; H, 4.11; N, 13.87; S, 16.14.

***Ethyl 2-((5-(furan-2-oyl)-3-phenyl-1,3,4-thiadiazol-2(3H)-ylidene)hydrazono) propanoate* (13e):** Yellow crystals (CH_3CN), mp 190–192°C; IR (KBr) ν 1715, 1691 (2C = O) cm^{-1};^{1}H NMR (300 MHz, $CDCl_3$) δ 1.38 (t, J = 7 Hz, 3H, CH_3), 2.27 (s, 3H, CH_3), 4.31 (q, J = 7 Hz, 2H, CH_2) and 6.65–8.05 (m, 8H);^{13}C NMR (75 MHz, $CDCl_3$) δ 14.0, 14.6, 61.5, 121.0, 127.1, 128.8, 128.9, 135.9, 136.3, 138.1, 139.0, 152.0, 153.9, 164.4, 168.2, 173.9. MS (EI, 70 eV) *m/z* (%): 384 (M^+, 55.4), 95 (100). Anal. Calcd. for $C_{18}H_{16}N_4O_4S$ (384.4): C, 56.24; H, 4.20; N, 14.58; S, 8.34. Found: C, 56.18; H, 4.09; N, 14.64; S, 8.43.

***Ethyl 5-((1-ethoxy-1-oxopropan-2-ylidene)hydrazono)-4-phenyl-4,5-dihydro-1,3,4-thiadiazole-2-carboxylate* (13f):** Yellow crystals (EtOH), mp 109–110°C; IR (KBr) ν 1702, 1692 (2C = O) cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.37 (t, J = 7 Hz, 6H, $2CH_3$), 2.25 (s, 3H, CH_3), 4.30 (q, J = 7 Hz, 2H, CH_2), 4.43 (q, J = 7 Hz, 2H, CH_2) and 7.27–8.02 (m, 5H);^{13}C NMR (75 MHz, $CDCl_3$) δ 14.1, 14.6, 53.5, 61.5, 63.0, 122.5, 127.4, 128.8, 138.8, 144.1, 153.4, 158.2, 164.8, 168.9. MS (EI, 70 eV) *m/z* (%): 362 (M^+, 100). Anal. Calcd. for $C_{16}H_{18}N_4O_4S$ (362.4): C, 53.03; H, 5.01; N, 15.46; S, 8.85. Found: C, 53.11; H, 4.85; N, 15.33; S, 8.88.

Synthesis of ethyl 2-(2-(4-(aryl)thiazol-2-yl)hydrazono)propanoate (17)

To a solution ethyl 2-(2-carbamothioyl-hydrazono)propanoate **3** (0.95 gm, 5 mmol) in ethanol (50 mL), the appropriate phenacyl bromides **16a–g** (5.0 mmol) was added and reaction mixture was refluxed for 6 h. The resulting solid product that precipitated was collected and crystallized from a suitable solvent to afford the corresponding thiazole derivatives **17a–g**.

***Ethyl 2-(2-(4-phenylthiazol-2-yl)hydrazono)propanoate* (17a):** Yellow crystals (CH_3 CN), mp 131–133°C; IR (KBr) ν 1700 (C = O) cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.34 (t, J = 7 Hz, 3H, CH_3), 2.18 (s, 3H, CH_3), 4.22 (q, J = 7 Hz, 2H, CH_2), 7.04–7.38 (m, 6H) and 9.22 (s, 1H, NH);^{13}C NMR (75 MHz, $CDCl_3$) δ 11.5, 14.1, 61.5, 118.2, 120.1, 122.3, 129.3, 131.2, 147.7, 150.1, 151.3, 165.7; MS (EI, 70 eV) *m/z* (%): 289 (M^+, 26), 216 (100). Anal. Calcd. for $C_{14}H_{15}N_3O_2S$ (289.4): C, 58.11; H, 5.23; N, 14.52; S, 11.08. Found: C, 58.02; H, 5.31; N, 14.59; S, 10.97.

***Ethyl 2-(2-(4-(p-tolyl)thiazol-2-yl)hydrazono)propanoate* (17b):** Yellow crystals (EtOH), mp 149–150°C; IR (KBr) ν 1706 (C = O) cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.37 (t, J = 7 Hz, 3H, CH_3), 1.96 (s, 3H, CH_3), 2.36 (s, 3H, 4-$CH_3C_6H_4$), 4.30 (q, J = 7 Hz, 2H, CH_2), 6.91 (s, 1H, CH), 7.18 (d, J = 8.5 Hz, 2H, Ar), 7.64 (d, J = 8.5 Hz, 2H, Ar) and 8.89 (s, 1H, NH);^{13}C NMR (75 MHz, $CDCl_3$) δ 11.7, 14.1, 21.1, 61.5, 104.2, 125.7, 129.4, 131.4, 137.9, 138.3, 151.2, 164.2, 168.6. MS (EI, 70 eV) *m/z* (%): 303 (M^+, 38), 230 (100). Anal. Calcd. for $C_{15}H_{17}N_3O_2S$ (303.4): C, 59.39; H, 5.65; N, 13.85; S, 10.57. Found: C, 59.22; H, 5.73; N, 13.79; S, 10.63.

***Ethyl 2-(2-(4-(4-methoxyphenyl)thiazol-2-yl)hydrazono)propanoate* (17c):** Yellow crystals (MeOH), mp 107–108°C; IR (KBr) ν 1697 (C = O) cm^{-1}; ^{1}H NMR (300 MHz,

$CDCl_3$) δ 1.34 (t, J = 7 Hz, 3H, CH_3), 2.03 (s, 3H, CH_3), 3.80 (s, 3H, 4-$C\underline{H_3}OC_6H_4$), 4.27 (q, J = 7 Hz, 2H, CH_2), 6.80 (s, 1H, CH), 6.90 (d, J = 8.5 Hz, 2H, Ar), 7.66 (d, J = 8.5 Hz, 2H, Ar) and 8.97 (s, 1H, NH);^{13}C NMR (75 MHz, $CDCl_3$) δ 12.0, 14.0, 55.2, 61.6, 102.8, 114.1, 126.1, 127.1, 139.4, 149.7, 159.6, 163.9, 168.7. MS (EI, 70 eV) *m/z* (%): 319 (M^+, 45), 246 (100). Anal. Calcd. for $C_{15}H_{17}N_3O_3S$ (319.4): C, 56.41; H, 5.37; N, 13.16; S, 10.04. Found: C, 56.29; H, 5.41; N, 13.20; S, 9.97.

Ethyl 2-(2-(4-(4-nitrophenyl)thiazol-2-yl)hydrazono)propanoate **(17d):** Yellow crystals (CH_3CN), mp 198–200°C; IR (KBr) ν 1690 (C = O) cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.31 (t, J = 7 Hz, 3H, CH_3), 2.14 (s, 3H, CH_3), 4.24 (q, J = 7 Hz, 2H, CH_2) 7.02 (s, 1H, CH), 7.45 (d, J = 9 Hz, 2H, Ar), 7.87 (d, J = 9 Hz, 2H, Ar) and 9.28 (s, 1H, NH);^{13}C NMR (75 MHz, $CDCl_3$) δ 11.7, 14.0, 61.6, 119.8, 123.7, 127.8, 130.9, 133.1, 138.5, 150.6, 164.2, 168.4. MS (EI, 70 eV) *m/z* (%): 334 (M^+, 17), 261 (100). Anal. Calcd. for $C_{14}H_{14}N_4O_4S$ (334.3): C, 50.29; H, 4.22; N, 16.76; S, 9.59. Found: C, 50.17; H, 4.30; N, 16.68; S, 9.66.

Ethyl 2-(2-(4-(4-chlorophenyl)thiazol-2-yl)hydrazono)propanoate **(17e):** Yellow crystals (EtOH), mp 167–168°C; IR (KBr) ν 1701 (C = O) cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.33 (t, J = 7 Hz, 3H, CH_3), 1.91 (s, 3H, CH_3), 4.27 (q, J = 7 Hz, 2H, CH_2), 6.94 (s, 1H, CH), 7.31 (d, J = 8 Hz, 2H, Ar), 7.65 (d, J = 8 Hz, 2H, Ar) and 9.20 (s, 1H, NH);^{13}C NMR (75 MHz, $CDCl_3$) δ 11.6, 14.1, 61.6, 105.5, 127.0, 128.8, 132.7, 133.7, 138.3, 150.0, 164.1, 168.5. MS (EI, 70 eV) *m/z* (%):326 (M + 2, 2), 325 (M + 1, 10) 324 (M^+, 6), 250 (100). Anal. Calcd. for $C_{14}H_{14}ClN_3O_2S$ (323.8): C, 51.93; H, 4.36; Cl, 10.95; N, 12.98; S, 9.90. Found: C, 51.80; H, 4.41; Cl, 10.88; N, 12.91; S, 9.98.

Ethyl 2-(2-(4-(4-bromophenyl)thiazol-2-yl)hydrazono)propanoate **(17f):** Yellow crystals (CH_3CN), mp 169–170°C; IR (KBr) ν 1689 (C = O) cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.31 (t, J = 7 Hz, 3H, CH_3), 2.14 (s, 3H, CH_3), 4.22 (q, J = 7 Hz, 2H, CH_2), 6.91 (s, 1H, CH), 7.27 (d, J = 8.5 Hz, 2H, Ar), 7.55 (d, J = 8.5 Hz, 2H, Ar) and 9.18 (s, 1H, NH); ^{13}C NMR (75 MHz, $CDCl_3$) δ 11.5, 14.2, 61.3, 115.2, 126.1, 127.9, 133.1, 134.7, 138.5, 150.1, 164.2, 168.6. MS (EI, 70 eV) *m/z* (%): 370 (M + 2, 4), 369 (M + 1, 22), 368 (M^+, 5), 296 (100). Anal. Calcd. for $C_{14}H_{14}BrN_3O_2S$ (368.2): C, 45.66; H, 3.83; Br, 21.70; N, 11.41; S, 8.71. Found: C, 45.57; H, 3.90; Br, 21.79; N, 11.33; S, 8.65.

Ethyl 2-(2-(4-(4-cyanophenyl)thiazol-2-yl)hydrazono)propanoate **(17 g):** Yellow crystals (CH_3CN), mp 171–172°C; IR (KBr) ν 2218 (CN), 1700 (C = O) cm^{-1};^{1}H NMR (300 MHz, $CDCl_3$) δ 1.35 (t, J = 7 Hz, 3H, CH_3), 2.10 (s, 3H, CH_3), 4.29 (q, J = 7 Hz, 2H, CH_2), 7.12 (s, 1H, CH), 7.64 (d, J = 8.5 Hz, 2H, Ar) 7.85 (d, J = 8.5 Hz, 2H, Ar) and 9.16 (s, 1H, NH);^{13}C NMR (75 MHz, $CDCl_3$) δ 11.5, 14.1, 61.8, 108.3, 111.1, 118.8, 126.2, 132.5, 132.5, 138.3, 138.4, 149.5, 163.9, 168.0. MS (EI, 70 eV) *m/z* (%): 314 (M^+, 20), 241 (100). Anal. Calcd. for $C_{15}H_{14}N_4O_2S$ (314.4): C, 57.31; H, 4.49; N, 17.82; S, 10.20. Found: C, 57.19; H, 4.57; N, 17.90; S, 10.07.

Synthesis of ethyl 4-(aryl)-5-(2-phenylhydrazono)thiazol-2(5H)-ylidene)hydrazono)-propanoate 15a–g

Method A: To the appropriate solution of thiazoles **17a–g** (5.0 mmol) in ethanol (100 mL), sodium acetate (0.41 g, 5.0 mmol) was added. To the resulting cold mixture, the prepared as usual by diazotizing aniline (0.5 mL, 5.0 mmol) in hydrochloric acid (6, 3 mL) with sodium nitrite (0.35 g, 5.0 mmol) in water (5 mL), was added portion wise over 30 min.

The reaction mixture was stirred for further 1 h while cooling in an ice-bath. The solid that precipitated was filtered, washed with water, dried and finally crystallized from suitable solvent to give **15a–g**.

***Ethyl 2-(2-(4-phenyl-5-(phenyldiazenyl)thiazol-2-yl)hydrazono)propanoate* (15a):** Red crystals (CH_3CN), mp 178–180°C; IR (KBr) ν 3429 (NH), 1697 (C = O) cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 1.31 (t, $J = 7$ Hz, 3H, CH_3), 2.14 (s, 3H, CH_3), 4.27 (q, $J = 7$ Hz, 2H, CH_2), 7.22–8.98 (m, 10H) and 11.38 (s, 1H, NH); ^{13}C NMR (75 MHz, $CDCl_3$) δ 14.1, 14.5, 55.0, 109.3, 118.2, 125.8, 128.2, 128.5, 129.6, 130.1, 132.5, 140.8, 148.3, 150.8, 163.8, 164.9. MS (EI, 70 eV) *m/z* (%): 393 (M^+, 65), 77 (100). Anal. Calcd. for $C_{20}H_{19}N_5O_2S$ (393.5): C, 61.05; H, 4.87; N, 17.80; S, 8.15. Found: C, 60.89; H, 4.77; N, 17.91; S, 8.07.

***Ethyl 2-(2-(5-(phenyldiazenyl)-4-(p-tolyl)thiazol-2-yl)hydrazono)propanoate* (15b):** Red crystals (EtOH), mp 129–130°C; IR (KBr) ν 3423 (NH), 1700 (C = O) cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 1.36 (t, $J = 7$ Hz, 3H, CH_3), 2.21 (s, 3H, CH_3), 2.42 (s, 3H, 4-$\underline{CH_3}C_6H_4$), 3.90 (s, 1H, NH), 4.34 (q, $J = 7$ Hz, 2H, CH_2) and 7.07–8.37 (m, 9H);^{13}C NMR (75 MHz, $CDCl_3$) δ 13.9, 14.1, 19.7, 21.4, 21.5, 61.8, 61.9, 122.6, 128.9, 129.0. 129.2, 129.6, 130.1, 131.2, 139.5, 162.7, 168.2. MS (EI, 70 eV) *m/z* (%): 407 (M^+, 96), 77 (100). Anal. Calcd. for $C_{21}H_{21}N_5O_2S$ (407.5): C, 61.90; H, 5.19; N, 17.19; S, 7.87. Found: C, 61.79; H, 5.04; N, 17.24; S, 7.80.

***Ethyl 2-(2-(4-(4-methoxyphenyl)-5-(phenyldiazenyl)thiazol-2-yl)hydrazono)propanoate* (15c):** Red crystals (EtOH), mp 77–78°C; IR (KBr) ν 3427 (NH), 1695 (C = O) cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 1.30 (t, $J = 7$ Hz, 3H, CH_3), 2.14 (s, 3H, CH_3), 3.22 (s, 3H, 4-$\underline{CH_3}OC_6H_4$), 4.28 (q, $J = 7$ Hz, 2H, CH_2), 7.19–8.66 (m, 9H) and 11.77 (s, 1H, NH);^{13}C NMR (75 MHz, $CDCl_3$) δ 14.1, 14.3, 55.1, 55.9, 109.8, 117.6, 125.3, 128.8, 129.0, 129.7, 130.9, 132.4, 141.3, 148.8, 150.1, 163.0, 164.5. MS (EI, 70 eV) *m/z* (%): 423 (M^+, 100). Anal. Calcd. for $C_{21}H_{21}N_5O_3S$ (423.5): C, 59.56; H, 5.00; N, 16.54; S, 7.57. Found: C, 59.44; H, 5.12; N, 16.48; S, 7.49.

***Ethyl 2-(2-(4-(4-nitrophenyl)-5-(phenyldiazenyl)thiazol-2-yl)hydrazono)propanoate* (15d):** Red crystals (CH_3CN), mp 236–238°C; IR (KBr) ν 3422 (NH), 1690 (C = O) cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 1.31 (t, $J = 7$ Hz, 3H, CH_3), 2.14 (s, 3H, CH_3), 4.30 (q, $J = 7$ Hz, 2H, CH_2), 7.09–8.56 (m, 9H) and 11.78 (s, 1H, NH);^{13}C NMR (75 MHz, $CDCl_3$) δ 13.9, 14.1, 55.7, 122.4, 124.6, 127.8, 127.9, 128.0, 128.5, 134.2, 138.9, 143.5, 145.9, 150.1, 163.4, 166.8. MS (EI, 70 eV) *m/z* (%): 438 (M^+, 48), 77 (100). Anal. Calcd. for $C_{20}H_{18}N_6O_4S$ (438.5): C, 54.79; H, 4.14; N, 19.17; S, 7.31. Found: C, 54.66; H, 4.02; N, 19.25; S, 7.40.

***Ethyl 2-(2-(4-(4-chlorophenyl)-5-(phenyldiazenyl)thiazol-2-yl)hydrazono)propanoate* (15e):** Red crystals (EtOH), mp 123–124°C; IR (KBr) ν 3429 (NH), 1699 (C = O) cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 1.30 (t, $J = 7$ Hz, 3H, CH_3), 2.15 (s, 3H, CH_3), 4.29 (q, $J = 7$ Hz, 2H, CH_2), 7.23–8.57 (m, 9H) and 11.67 (s, 1H, NH);^{13}C NMR (75 MHz, $CDCl_3$) δ 14.0, 14.1, 55.8, 124.7, 125.0, 125.2, 127.8, 128.0, 128.6, 130.2, 132.5, 135.1, 143.4, 150.2, 163.2, 166.3. MS (EI, 70 eV) *m/z* (%): 430 (M + 2, 4), 429 (M + 1), 428 (M^+, 18), 77 (100). Anal. Calcd. for $C_{20}H_{18}ClN_5O_2S$ (427.9): C, 56.14; H, 4.24; Cl, 8.28; N, 16.37; S, 7.49. Found: C, 56.02; H, 4.17; Cl, 8.17; N, 16.45; S, 7.38.

***Ethyl 2-(2-(4-(4-bromophenyl)-5-(phenyldiazenyl)thiazol-2-yl)hydrazono)propanoate* (15f):** Red crystals (EtOH), mp 129–130°C; IR (KBr) ν 3424 (NH), 1690 (C = O) cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 1.32 (t, $J = 7$ Hz, 3H, CH_3), 2.17 (s, 3H, CH_3), 4.20

(q, $J = 7$ Hz, 2H, CH_2), 7.39–8.49 (m, 9H) and 11.70 (s, 1H, NH);^{13}C NMR (75 MHz, $CDCl_3$) δ 14.0, 14.2, 55.7, 123.1, 124.7, 125.1, 126.9, 127.4, 128.1, 130.5, 132.8, 135.7, 142.9, 150.5, 163.1, 166.7. MS (EI, 70 eV) *m/z* (%): 474 (M + 2, 7), 473 (M + 1, 28), 472 (M^+, 23), 77 (100). Anal. Calcd. for $C_{20}H_{18}BrN_5O_2S$ (472.4): C, 50.86; H, 3.84; Br, 16.92; N, 14.83; S, 6.79. Found: C, 50.77; H, 3.78; Br, 16.85; N, 14.79; S, 6.68.

***Ethyl 2-(2-(4-(4-cyanophenyl)-5-(phenyldiazenyl)thiazol-2-yl)hydrazono)propanoate* (15 g):** Red crystals (CH_3CN), mp 198–200°C; IR (KBr) ν 3423 (NH), 2215 (CN), 1697 (C = O) cm^{-1};^{1}H NMR (300 MHz, $CDCl_3$) δ 1.25 (t, $J = 7$ Hz, 3H, CH_3), 2.12 (s, 3H, CH_3), 4.18 (q, $J = 7$ Hz, 2H, CH_2), 7.52–8.40 (m, 9H) and 11.76 (s, 1H, NH); ^{13}C NMR (75 MHz, $CDCl_3$) δ 13.9, 14.0, 55.6, 112.2, 118.3, 120.7, 124.1, 126.4, 127.4, 128.7, 130.1, 132.3, 135.8, 142.3, 150.4, 163.5, 166.5. MS (EI, 70 eV) *m/z* (%): 418 (M^+, 76), 77 (100). Anal. Calcd. for $C_{21}H_{18}N_6O_2S$ (418.5): C, 60.27; H, 4.34; N, 20.08; S, 7.66. Found: C, 60.12; H, 4.25; N, 20.17; S, 7.58.

Alternative method for synthesis of Ethyl 2-(2-(4-phenyl-5-(phenyldiazenyl) thiazol-2-yl)hydrazono)propanoate (15a)

A mixture of 2-oxo-*N*,2-diphenylacetohydrazonoyl bromide **12a** (0.3 g, 1 mmol), compound **3** (0.13 g, 1 mmol) and triethylamine in ethanol (20 mL) was refluxed for 3 h. The solid was collected and crystallized from ethanol afforded identical product mp, mixed mp and spectra with compound **15a**.

Synthesis of ethyl 2-((4-oxo-5-(2-phenylhydrazono)thiazolidin-2- ylidene) hydrazono)-propanoate (18)

To a solution ethyl 2-(2-carbamothioylhydrazono)propanoate **3** (0.95 gm, 5 mmol) and *N*-phenyl-*C*-ethoxycarbonylmethanohydrazonoyl chloride **12f** (1.13 gm, 5 mmol) was dissolved in ethanol (50 mL), triethylamine (2 mL) was added and reaction mixture was refluxed for 6 h. The resulting solid product that precipitated was collected and crystallized from ethanol to afford ethyl 2-((4-oxo-5-(2-phenylhydrazono)thiazolidin-2-ylidene)hydrazono)propanoate **(14):** Yellow crystals, mp 176–178°C; IR (KBr) ν 3422 (NH), 1697, 1686 (2C = O) cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.29 (t, $J = 7$ Hz, 3H, CH_3), 2.17 (s, 3H, CH_3), 4.20 (q, $J = 7$ Hz, 2H, CH_2), 7.01–7.37 (m, 5H), 11.55 (s, 1H, NH) and 11.70 (s, 1H, NH);^{13}C NMR (75 MHz, $CDCl_3$) δ 14.1, 14.2, 56.4, 112.9, 121.2, 128.5, 136.9, 142.3, 146.8, 156.3, 163.5, 166.0. MS (EI, 70 eV) *m/z* (%): 333 (M^+, 10), 307 (100). Anal. Calcd. for $C_{14}H_{15}N_5O_3S$ (333.4): C, 50.44; H, 4.54; N, 21.01; S, 9.62. Found: C, 50.32; H, 4.67; N, 20.93; S, 9.49.

Antimicrobial assay

Antimicrobial activity of the tested compounds was determined using a modified Kirby-Bauer disc diffusion method [38]. Briefly, 100 µl of the test bacteria/fungi were grown in 10 ml of fresh media until they reached a count of approximately 10^8 cells/ml for bacteria or 10^5 cells/ml for fungi [39]. 100 µl of microbial suspension was spread onto agar plates corresponding to the broth in which they were maintained.

Of the many media available, NCCLS recommends Mueller-Hinton agar due to: it results in good batch-to-batch reproducibility. Disc diffusion method for filamentous fungi tested by using approved standard method (M38-A) developed by the (NCCLS, 2002) [40] for evaluating the susceptibilities of filamentous fungi to antifungal agents. Disc diffusion method for yeasts developed by using approved standard method (M44-P) by the (NCCLS, 2003) [41].

Minimum inhibitory concentration study

The MIC values were measured by the broth dilution method [37,42]. Five hundred micro liters of a stock solution (10.24 mg/mL) of each tested compound in dimethyl sulfoxide (DMSO) were prepared and then diluted with Mueller-Hinton broth to 1024 µg/mL. The strains were grown briefly at 37°C in Mueller-Hinton media. After 5 h of bacterial growth, the bacterial culture was diluted to obtain a concentration of 5×10^5 cells/mL. Then, 150 µL bacterial suspensions were added to each well of the flat-bottomed 96-well tissue culture plate. Two-fold serial dilutions were carried out from the fist well to the tenth well; the final concentrations of the compounds ranged from 1 to 512 µg/mL; and excess media (150 µL) were discarded from the last well. The plates were incubated at 37°C for 24 h in an electro-heating standing temperature cultivator and were read visually. The MIC of the sample showing no turbidity was recorded as the lowest concentration of compound that inhibited bacterial growth completely. Each assay was run in triplicate.

Conclusion

In summary, ethyl pyruvate proved to be useful precursor for convenient synthesis of new thiadiazoles and thiazoles having many functional groups that have not been reported hitherto. As antimicrobial results showed that ethyl 2-(2-(4-phenylthiazol-2-yl)hydrazono)propanoate **(17a)** achieved the lowest MIC value (high efficient derivative) against the sensitive bacterial strain *S. aureus* with MIC value 160 µg/ml.

Disclosure statement

No potential conflict of interest was reported by the authors.

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