European Journal of Chemistry

View Journal Online
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A series of 1,3-imidazoles and triazole-3-thiones based thiophene-2-carboxamides as anticancer agents: Synthesis and anticancer activity

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RESEARCH ARTICLE

10.5155/eurjchem.9.2.99-106.1701

Received: 18 March 2018
Received in revised form: 09 April 2018
Accepted: 16 April 2018
Published online: 30 June 2018
Printed: 30 June 2018

KEYWORDS

Thiophene
1,3-Imidazole
Isothiocyanates
1,3,4-Thiadiazoles
Triazole-3-thiones
Anticancer activity

ABSTRACT

By addition of semicarbazide or phenylhydrazine hydrochloride to thienoylisothiocyanate (1) resulted in building of thiosemicarbazide derivative (2), triazole derivative (4) and thiophene-2-carboxamide (5), respectively. Basic cyclization of compound 2 led to formation of oxadiazine (3). Synthesis of thiadiazine derivative (6) was achieved via reaction of compound 5 and maleic anhydride in triethyl amine. Heating of compound 5 with ethyl chloroacetate or sodium ethoxide produced thiadiazine derivative (7) and triazolethione (8), respectively. Thiosemicarbazide derivative 11 was synthesized by addition of nicotinic hydrazide to compound 1. Refluxing of compound 11 with lead acetate afforded triazole (13). Moreover, acid and base mediated cyclizations of compound 11 gave thiadiazole (12) and 1,2,4-triazolethione (14) throughout thiophene intermediate, respectively. Addition of ethyl 2-aminothiophene-3-carboxylate to compound 1 formed thiourea (15) which was refluxed with ethoxide giving thiophene-3-carboxylic acid (16). Lastly, nucleophilic addition of amino phenol or ethylene diamine to compound 1 yielded oxazine structure (18) and imidazole derivative (19), respectively. The yields of the synthesized compounds were 61-95%. The detailed synthesis and spectroscopic data of the new compounds are reported.

Cite this: *Eur. J. Chem.* **2018**, *9(2)*, 99-106 **Journal website**: www.eurjchem.com

1. Introduction

Aromatic five-membered nitrogen heterocycles have been potential targets of investigations because of their medicinal properties. In the last few decades, the chemistry of 1,2,4-triazole-3-thiones, 1,3,4-thiadiazoles and their fused heterocyclic derivatives has received considerable attention owing to their effective biological activities as anticonvulsant [1,2] and antioxidant [3]. Several imidazole derivatives have been published as antiviral [4], anticoagulant [5] and anticancer [6,7] agents. The 1,2,4-triazole rings have been incorporated into a wide variety of therapeutically interesting drug candidates including antiviral (Ribavarin), antimigraine (Rizatriptan) (Figure 1) [8-10], CNS stimulants sedatives and antianxiety [11,12] agents. Compounds connecting to 1,2,4-triazole moiety are famous for powerful antitumorial and anti-HIV agents [13-15]. The synthetic nucleoside ribavirin involving a 1,2,4-triazole nuclus has become an outstanding drug when combined with the pegylated interferon-α, for the treatment of hepatitis C virus infections [16]. We have published some papers containing the synthesis of some new 1,2,4-triazoles attached with several alkyl as well as their activities towards various cyclizing agents [17-20]. As a result, our interest has been recently directed towards the synthesis of some novel azoles and azines of expected antitumor activity [21,22].

2. Experimental

2.1. Materials

All experiments were performed using drying solvents. Thin-layer chromatography (TLC) was performed on a Merck Silica Gel 60F_{254} with detection by UV light. Products were purified by crystallization.

2.2. Instrumentation

We measured the melting points that are uncorrected by an Electro thermal IA 9100 apparatus with open capillary tubes. The IR spectra (KBr disc) were recorded on a Pye Unicam Sp-3-300 or a Shimadzu FTIR 8101 PC infrared spectrophotometer.

ISSN 2153-2249 (Print) / ISSN 2153-2257 (Online)
http://dx.doi.org/10.5155/eurjchem.9.2.99-106.1701

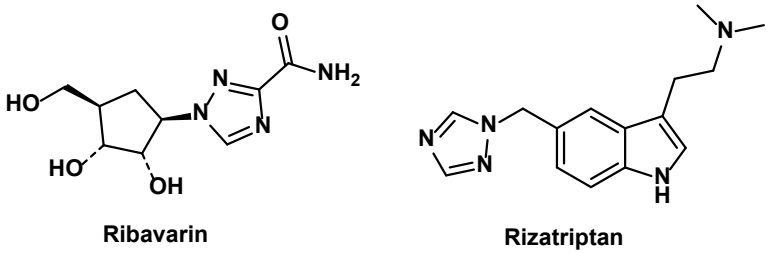


Figure 1. Structure of ribavirin and rizatriptan.

The ^{1}H- and ^{13}C-NMR spectra were recorded at a Varian Mercury VX-300 NMR (1H, 300 MHz, ^{13}C, 75.4 MHz) spectrometer using DMSO-d_6 as a solvent. All chemical shifts were expressed on the δ (ppm) scale using TMS as an internal standard reference. The coupling constant (*J*) values are given in Hz. Mass spectrometry and analytical data were obtained from the Microanalysis Center at Cairo University, Giza, Egypt.

2.3. Synthesis

2.3.1. Synthesis of N-((thiophene-2-carbonyl)carbamothioyl)hydrazinecarboxamide hydrochloride (2)

A mixture of thiophene-2-carbonylisothiocyanate (**1**) (3.40 mol) and semicarbazide hydrochloride (3.40 mol) in dioxane: water (1:1, *v*:*v*) (50 mL) was stirred at room temperature for 1 h. The reaction mixture was poured into cold water. The formed precipitate was filtered off, washed with cold water, dried and crystallized from ethanol to give compound **2** (Scheme 1). Color: White. Yield: 33%. M.p.: 210-211 °C. FT-IR (KBr, ν, cm^{-1}): 3433, 3317 (NH_2), 3156 (NH), 3207, 1671 (C=O), 1667 (C=C), 1267 (C-O). ^{1}H NMR (300 MHz, DMSO-d_6, δ, ppm): 7.12-7.86 (m, 3H, thiophene protones), 8.33 (s, 1H, NH), 8.34 (s, 1H, NH), 8.99 (s, 1H, NH), 11.63 (s, 2H, NH_2). ^{13}C NMR (75.4 MHz, DMSO-d_6, δ, ppm): 167.1 (1C, NCS), 165.2, 164.3 (2C, 2C=O), 129.8, 128.4, 128.0, 127.5 (Ar-C). MS (EI, *m/z* (%)): 280 (M^+, 100). 128 (4), 110 (100), 83 (30), 69 (35), 57 (60).

2.3.2. Synthesis of 2-hydrazinyl-6-(thiophen-2-yl)-4H-1,3,5-oxadiazine-4-thione (3)

A mixture of *N*-((thiophene-2-carbonyl)carbamothioyl) hydrazinecarboxamide hydrochloride (**2**) (1.70 mol) and sodium ethoxide (1.70 mol) in ethanol (20 mL) was heated under reflux for 2 h. The reaction mixture was neutralized by 10% HCl. The formed precipitate was filtered off, dried and crystallized from ethanol to give compound **3** (Scheme 1). Color: White. Yield: 89%. M.p.: 279-280 °C. FT-IR (KBr, ν, cm^{-1}): 3411, 3325 (NH_2, NH), 1599 (C=C), 1235 (C-O). ^{1}H NMR (300 MHz, DMSO-d_6, δ, ppm): 7.75-7.19 (m, 3H, thiophene protons), 13.61 (s, 2H, D_2O exchangeable, NH_2), 13.91 (1H, D_2O exchangeable, s, NH). ^{1}H NMR (300 MHz, DMSO-d_6, δ, H/D-exchange, ppm): 7.75-7.19 (m, 3H, thiophene protons). ^{13}C NMR (75.4 MHz, DMSO-d_6, δ, ppm): 168.1 (1C, NCS), 163.6 (1C, OCN), 155.3 (1C, NCN), 129.5, 128.7, 128.3, 127.2 (Ar-C). MS (EI, *m/z* (%)): 226 (M^+, 15), 183 (100), 124 (35), 110 (40), 70 (25), 69 (30). Anal. calcd. for $C_7H_6N_4OS_2$: C, 37.16; H, 2.67; N, 24.76. Found: C, 37.10; H, 2.53; N, 24.64%.

2.3.3. Synthesis of 1,2-dihydro-1-phenyl-5-(thiophen-2-yl)-1,2,4-triazole-3-thione (4)

A mixture of thiophene-2-carbonylisothiocyanate (**1**) (3.40 mol) and phenyl hydrazine (3.40 mol) in dioxane (50 mL) was heated under reflux for 2 h. The reaction mixture was poured into water. The formed precipitate was filtered off, washed with water, dried and crystallized from ethanol to give compound **4** (Scheme 1). Color: Yellow. Yield: 63%. M.p.: 175-176 °C. FT-IR (KBr, ν, cm^{-1}): 3313 (NH), 1635 (C=N), 1615 (C=C). ^{1}H NMR (300 MHz, DMSO-d_6, δ, ppm): 7.93-6.72 (m, 8H, ArH's + thiophene protons), 10.37 (s, 1H, D_2O exchangeable, NH). ^{1}H NMR (300 MHz, DMSO-d_6, δ, H/D-exchange, ppm): 7.93-6.72 (m, 8H, ArH's + thiophene protons). ^{13}C NMR (75.4 MHz, DMSO-d_6, δ, ppm): 166.6 (1C, NCS), 156.4 (1C, NCN), 136.5, 129.4, 129.0, 128.6, 128.1, 127.7, 121.1, 119.2 (Ar-C). MS (EI, *m/z* (%)): 258 (M^+, 20), 217 (30), 110 (100), 77 (20). Anal. calcd. for $C_{12}H_9N_3S_2$: C, 55.57; H, 3.50; N, 16.20. Found: C, 55.49; H, 3.45; N, 15.90%.

2.3.4. Synthesis of N-(1-phenylhydrazinecarbonothioyl)thiophene-2-carboxamide hydrochloride (5)

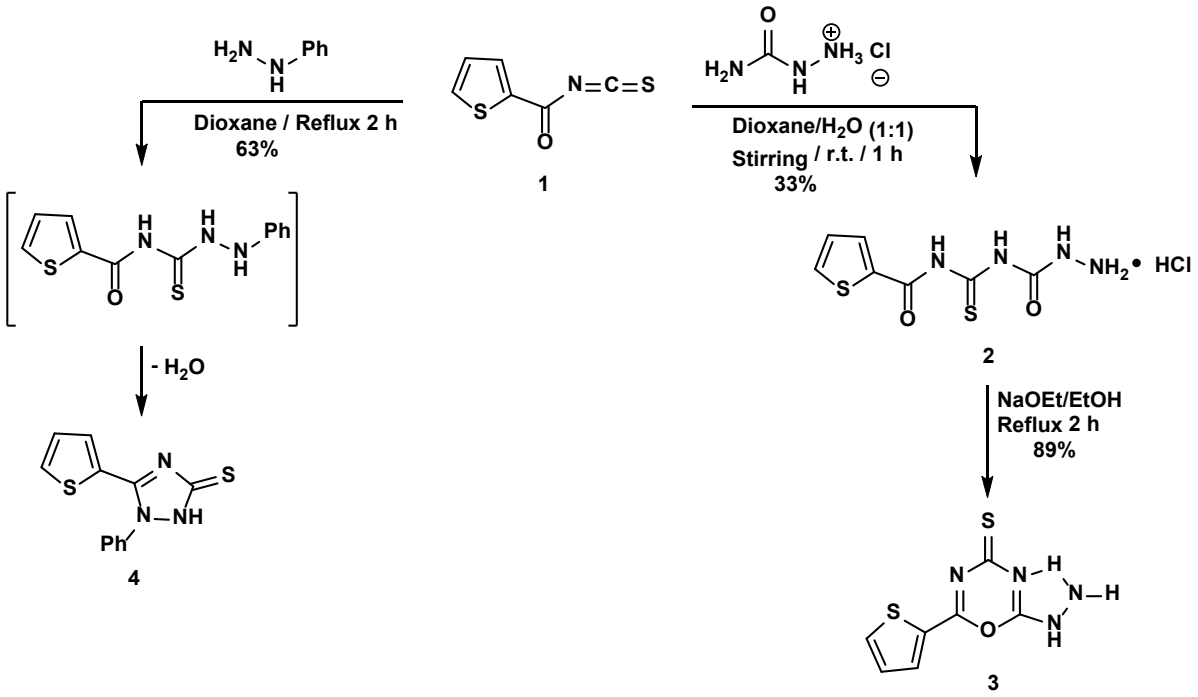
A mixture of thiophene-2-carbonylisothiocyanate (**1**) (10.0 mol) and phenyl hydrazine hydrochloride (10.0 mol) in dioxane:water (1:1, *v*:*v*) (50 mL) was stirred at room temperature for 1 h. The reaction mixture was poured into cold water. The formed precipitatet was filtered off, washed with cold water, dried and crystallized from ethanol to give compound **5** (Scheme 2). Color: White. Yield: 54%. M.p.: 268-269 °C. FT-IR (KBr, ν, cm^{-1}): 3406, 3280, (NH_2, NH), 1665 (C=O), 1635 (C=C). ^{1}H NMR (300 MHz, DMSO-d_6, δ, ppm): 7.98-7.24 (m, 8H, ArH's + thiophene protons), 11.62 (s, 2H, NH_2), 14.41 (s, 1H, NH).

2.3.5. Synthesis of N-(6-oxo-2-phenyl-5,6-dihydro-1H-furo[2,3-e][1,3,4]thiadiazin-3(2H)-ylidene)thiophene-2-carboxamide (6)

A mixture of *N*-(1-phenylhydrazinecarbonothioyl)thiophene-2-carboxamide hydrochloride (**5**) (1.50 mol), maleic anhydride (1.50 mol) and triethyl amine (1.50 mole) in dimethyl formamide (20 mL) was heated under reflux for 6 h. The reaction mixture was neutralized by 10% HCl. The formed precipitate was filtered off, dried and crystallized from ethanol to give compound **6** (Scheme 2 and 3). Color: Brown. Yield: 61%. M.p.: 288-289 °C. FT-IR (KBr, ν, cm^{-1}): 3631, 3217 (NH), 1723 (C=O) (ester), 1667 (C=O) (amide), 1631 (C=N), 1617 (C=C), 1244 (C-O). ^{1}H NMR (300 MHz, DMSO-d_6, δ, ppm): 2.95 (s, 2H, CH_2CO), 7.98-7.15 (m, 8H, ArH's + thiophene protons), 12.65 (s, 1H, D_2O exchangeable, NH). ^{13}C NMR (75.4 MHz, DMSO-d_6, δ, ppm): 168.3, 165.2 (2C, 2C=O), 156.7 (1C, NCN), 143.1, 146.0, 136.9, 129.7, 129.4, 128.8, 128.2, 127.1, 122.1, 121.2, (Ar-C), 23.8 (1C, CH_2). MS (EI, *m/z* (%)): 357 (M^+, 20), 259 (100), 215 (10), 91 (25). Anal. calcd. for $C_{16}H_{11}N_3O_3S_2$: C, 53.77; H, 3.10; N, 11.76. Found: C, 53.68; H, 2.99; N, 11.70%.

2.3.6. Synthesis of N-(5-hydroxy-3-phenyl-3,6-dihydro-2H-1,3,4-thiadiazin-2-ylidene)thiophene-2-carboxamide (7)

A mixture of *N*-(1-phenylhydrazinecarbonothioyl)thiophene-



Scheme 1

2-carboxamide hydrochloride (**5**) (1.50 mol), ethyl chloroacetate (1.50 mol) and triethyl amine (1.50 mole) in ethanol (20 mL) was heated under reflux for 6 h. The reaction mixture was neutralized by 10% HCl. The formed precipitate was filtered off, dried and crystallized from ethanol to give compound **7** (Scheme 2). Color: White. Yield: 79%. M.p.: 280-281 °C. FT-IR (KBr, ν, cm^{-1}): 3631 (OH), 3117 (CH arom.), 1667 (C=O) (amide), 1631 (C=N) 1220 (C-O). ^{1}H NMR (300 MHz, DMSO-d_6, δ, ppm): 2.23 (s, 2H, CH_2), 8.28-7.15 (m, 8H, ArH's + thiophene protons), 12.95 (s, 1H, D_2O exchangeable, OH). ^{13}C NMR (75.4 MHz, DMSO-d_6, δ, ppm): 165.8 (1C, C=O), 159.1, 156.4 (2C, C=N), 136.1, 129.2, 128.8, 128.4, 127.9, 127.0, 123.1, 121.8 (Ar-C), 34.3 (1C, SCH_2). MS (EI, *m/z* (%)): 317(M^+, 100), 271 (20), 200 (30), 110 (30), 91 (10), 77 (50). Anal. calcd. for $C_{14}H_{11}N_3O_2S_2$: C, 52.98; H, 3.49; N, 13.24. Found: C, 52.93; H, 3.40; N, 13.16%.

2.3.7. Synthesis of 1,2-dihydro-2-phenyl-5-(thiophen-2-yl)-1,2,4-triazole-3-thione (8)

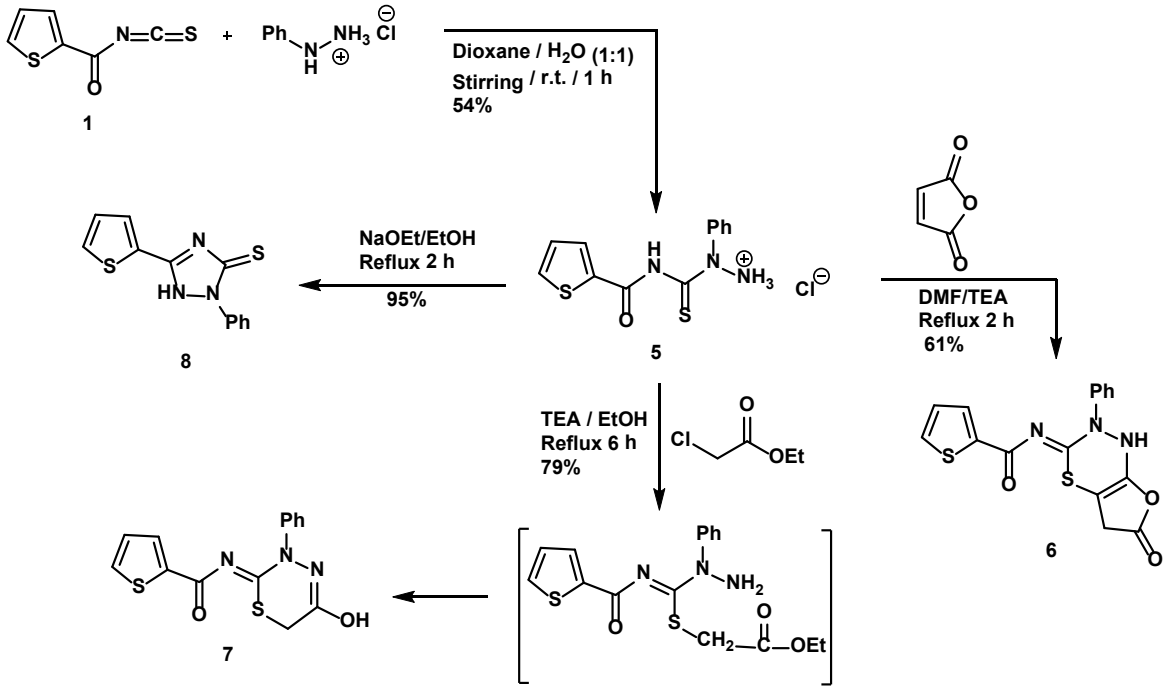
A mixture of *N*-(1-phenylhydrazinecarbonothioyl)thiophene-2-carboxamide hydrochloride (**5**) (1.50 mol) and sodium ethoxide (1.50 mol) in ethanol (20 mL) was heated under reflux for 2 h. The reaction mixture was neutralized by 10% HCl. The formed precipitate was filtered off, dried and crystallized from ethanol to give compound **8** (Scheme 2). Color: Pale white. Yield: 95%. M.p.: 290-291 °C. FT-IR (KBr, ν, cm^{-1}): 3424 (NH), 1635 (C=N), 1597 (C=C). ^{1}H NMR (300 MHz, DMSO-d_6, δ, ppm): 8.06-7.18 (m, 8H, ArH's + thiophene protons), 10.41 (s, 1H, NH). ^{13}C NMR (75.4 MHz, DMSO-d_6, δ, ppm): 167.1 (1C, NCS), 156.1 (1C, NCN), 135.8, 129.7, 129.1, 128.4, 128.3, 127.1, 121.4, 120.2 (Ar-C). MS (EI, *m/z* (%)): 259 (M^+, 100), 200 (10), 149 (5), 108 (10), 91 (25). Anal. calcd. for $C_{12}H_9N_3S_2$: C, 55.57; H, 3.50; N, 16.20. Found: C, 55.46; H, 3.39; N, 16.10%.

2.3.8. Synthesis of N-(2-isonicotinoylhydrazinecarbonothioyl)thiophene-2-carboxamide (11)

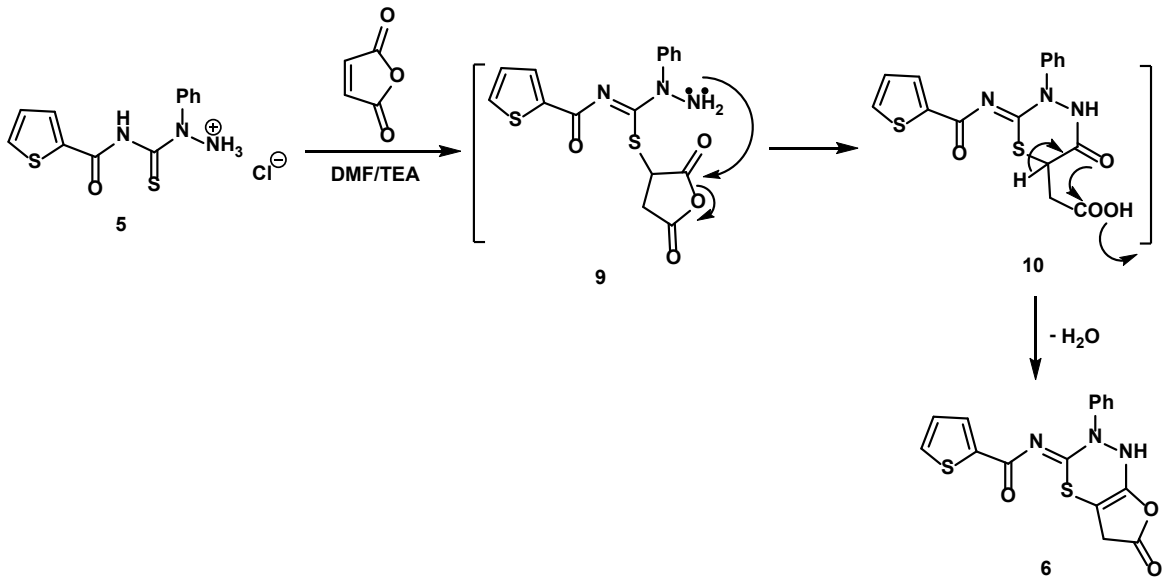
A mixture of thiophene-2-carbonylisothiocyanate (**1**) (10.0 mol) isonicotinohydrazide (10.0 mol) in dioxane (50 mL) was stirred at room temperature for 1 h. The reaction mixture was poured into water. The formed precipitate was filtered off, washed with water, dried and crystallized from ethanol to give compound **11** (Scheme 4). Color: Yellow. Yield: 58%. M.p.: 212-213 °C. FT-IR (KBr, ν, cm^{-1}): 3419, 3252 (NH), 3018 (CH arom.), 1671, 1666 (2C=O). ^{1}H NMR (300 MHz, DMSO-d_6, δ, ppm): 7.98-7.24 (m, 7H, ArH's + thiophene protons), 11.41 (s, 1H, D_2O exchangeable, NH), 11.85 (s, 1H, D_2O exchangeable, NH), 12.15 (s, 1H, D_2O exchangeable, NH). ^{13}C NMR (75.4 MHz, DMSO-d_6, δ, ppm): 167.1 (1C, NCS), 156.1 (1C, NCN), 135.8, 129.7, 129.1, 128.4, 128.3, 127.1, 121.4, 120.2 (Ar-C). MS (EI, *m/z* (%)): 306 (M^+, 100), 273 (15), 170 (5), 137 (5), 110 (10), 78 (30).

2.3.9. Synthesis of N-(5-(pyridin-4-yl)-1,3,4-thiadiazol-2-yl)thiophene-2-carboxamide (12)

A mixture of *N*-(2-isonicotinoylhydrazinecarbonothioyl) thiophene-2-carboxamide (**11**) (1.20 mol) and sulfuric acid (1.20 mol) in ethanol (20 mL) was heated under reflux for 6 h. The reaction mixture was poured into water. The formed precipitate was filtered off, dried and crystallized from ethanol to give compound **12** (Scheme 4). Color: White. Yield: 95%. M.p.: 320-321 °C. FT-IR (KBr, ν, cm^{-1}): 3386 (NH), 1662 (C=O) (amide), 1637 (C=N), 1628 (C=C). ^{1}H NMR (300 MHz, DMSO-d_6, δ, ppm): 8.97-7.30 (m, 7H, ArH's + thiophene protons), 8.98 (s, 1H, D_2O exchangeable, NH). ^{13}C NMR (75.4 MHz, DMSO-d_6, δ, ppm): 162.4 (1C, C=O), 156.4, 152.1 (2C, triazole ring), 149.0, 135.3, 121.4, 137.8, 129.2, 128.4, 127.0 (Ar-C). MS (EI, *m/z* (%)): 288 (M^+, 60), 261 (100), 250 (50), 173 (55), 160 (50), 112 (60). Anal. calcd. for $C_{12}H_8N_4OS_2$: C, 49.98; H, 2.80; N, 19.43. Found: C, 49.87; H, 2.69; N, 19.38%.



Scheme 2



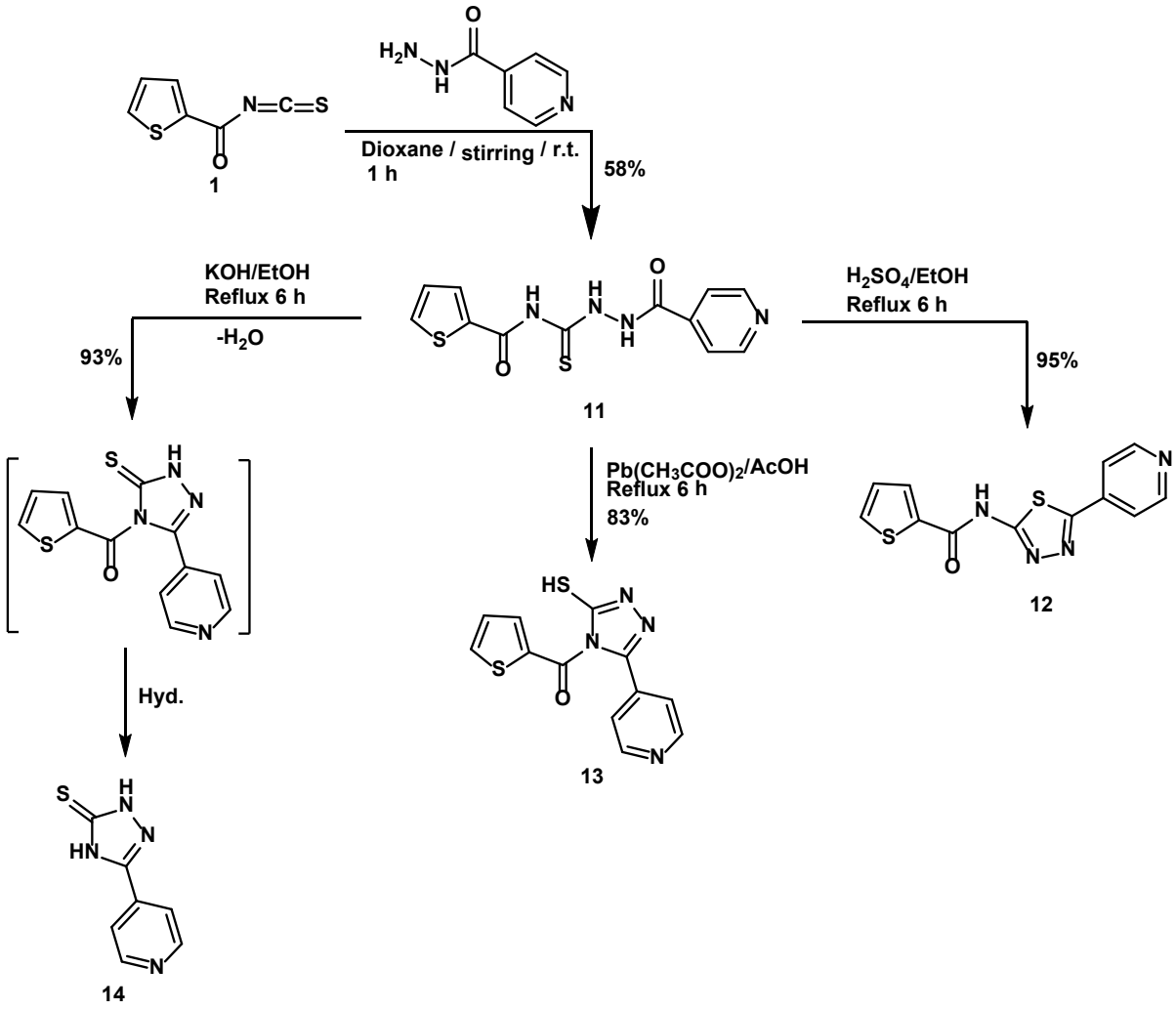
Scheme 3

2.3.10. Synthesis of (3-mercapto-5-(pyridin-4-yl)-4H-1,2,4-triazol-4-yl)(thiophen-2-yl)methanone (13)

A mixture of *N*-(2-isonicotinoylhydrazinecarbonothioyl) thiophene-2-carboxamide (**11**) (1.20 mol) and lead acetate (1.20 mol) in acetic acid (20 mL) was heated under reflux for 6 h. The reaction mixture was poured into water. The formed precipitate was filtered off, dried and crystallized from ethanol to give compound **13** (Scheme 4). Color: Grey. Yield: 83%. M.p.: 327-328 °C. FT-IR (KBr, ν, cm^{-1}): 3108 (CH arom.), 1661 (C=O), 1624 (C=C). ^{1}H NMR (300 MHz, DMSO-d_6, δ, ppm): 8.72-7.27 (m, 7H, ArH's + thiophene protons), 13.08 (s, 1H, D_2O exchangeable, SH). ^{13}C NMR (75.4 MHz, DMSO-d_6, δ, ppm): 168.4 (1C, C=O), 157.6, 152.8 (2C, triazole ring), 149.6, 134.3, 124.2, 129.2, 128.1, 127.6, 120.0 (Ar-C). MS (EI, *m/z* (%)): 288 (M^+, 100), 259 (55), 193 (15), 127 (20), 110 (95), 63 (15). Anal. calcd. for $C_{12}H_8N_4OS_2$: C, 49.98; H, 2.80; N, 19.43. Found: C, 49.91; H, 2.75; N, 19.35%.

2.3.11. Synthesis of 5-(pyridin-4-yl)-2H-1,2,4-triazole-3(4H)-thione (14)

A mixture of *N*-(2-isonicotinoylhydrazinecarbonothioyl) thiophene-2-carboxamide (**11**) (1.20 mol) and potassium hydroxide (1.20 mol) in ethanol (20 mL) was heated under reflux for 6 h.



Scheme 4

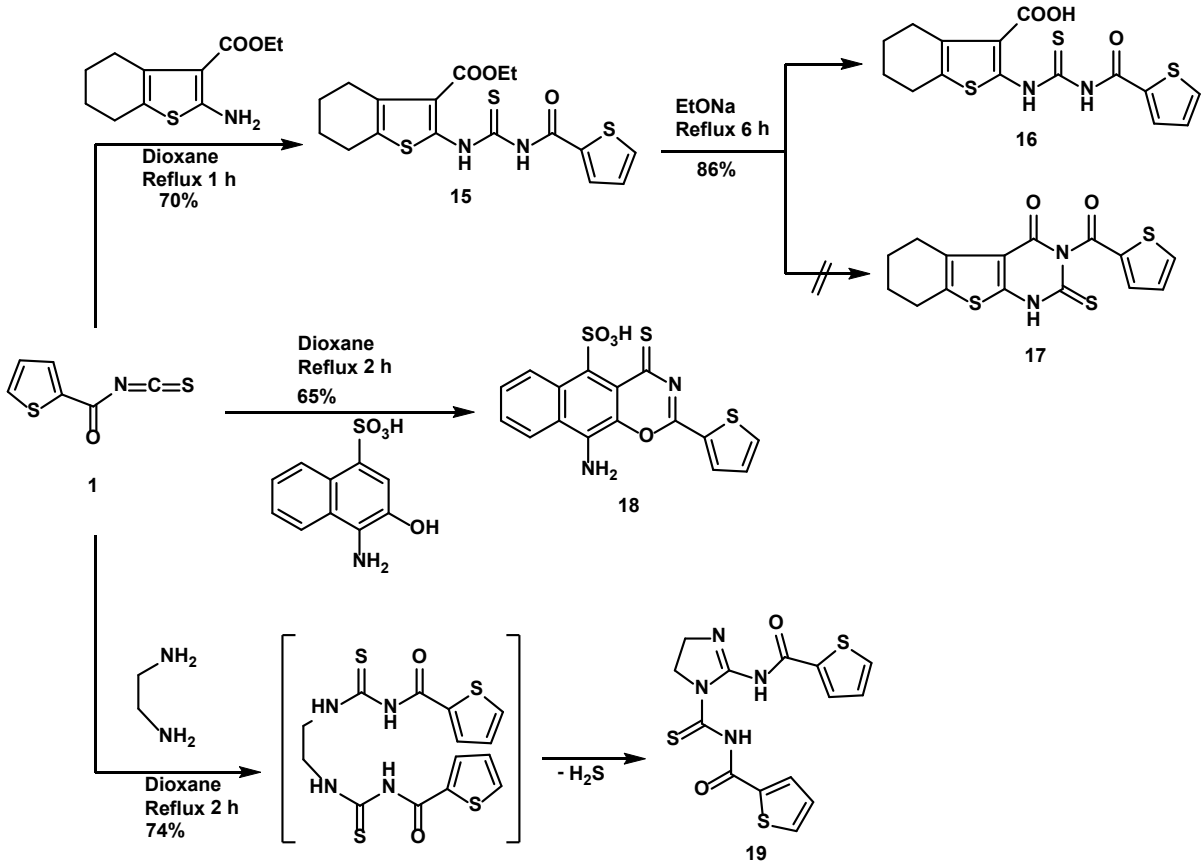
The reaction mixture was poured into water. The formed precipitate was filtered off, dried and crystallized from ethanol to give compound **14** (Scheme 4). Color: Yellow. Yield: 93%. M.p.: 318-319 °C. FT-IR (KBr, ν, cm^{-1}): 3445 (NH), 3087 (CH arom.), 1648 (C=N). ^{1}H NMR (300 MHz, DMSO-d_6, δ, ppm): 8.46-7.15 (m, 4H, ArH's), 8.97 (s, 1H, D_2O exchangeable, NH), 8.98 (s, 1H, D_2O exchangeable, NH). ^{13}C NMR (75.4 MHz, DMSO-d_6, δ, ppm): 169.6, 151.5 (2C, triazole ring), 148.1, 134.8, 123.0 (Ar-C). MS (EI, *m/z* (%)): 178 (M^+, 10), 127 (60), 110 (100), 82 (15), 56 (35). Anal. calcd. for $C_7H_6N_4S$: C, 47.18; H, 3.39; N, 31.44. Found: C, 47.10; H, 3.26; N, 31.33%.

2.3.12. Synthesis of ethyl 2-(3-(thiophene-2-carbonyl)thioureido)-4,5,6,7-tetrahydrobenzo[b]thiophene-3-carboxylate (15)

A mixture of thiophene-2-carbonylisothiocyanate (**1**) (3.3 mol) and ethyl 2-amino-4,5,6,7-tetrahydrobenzo[*b*]thiophene-3-carboxylate (3.3 mol) in dioxane (50 mL) was heated under reflux for 1 h. The reaction mixture was poured into water. The formed precipitate was filtered off, washed with water, dried and crystallized from acetic acid to give compound **15** (Scheme 5). Color: Yellow. Yield: 70%. M.p.: 236-237 °C. FT-IR (KBr, ν, cm^{-1}): 3412, 3261(2NH), 1735 (C=O) (ester), 1677 (C=O) (amide), 1649 (C=C). ^{1}H NMR (300 MHz, DMSO-d_6, δ, ppm): 1.34 (t, *J* = 2.3 Hz, 3H, CH_3), 1.72 (m, 4H, cyclohexane-$2CH_2$), 2.63 (t, *J* = 5.6 Hz, 2H, cyclohexane-CH_2), 2.75 (t, *J* = 5.6 Hz, 2H, cyclohexane-CH_2), 4.37 (q, *J* = 2.3 Hz, 2H, CH_2), 7.24 (d, *J* = 3.6 Hz, 1H, thiophene-H_c), 8.06 (d, *J* = 4.8 Hz, 1H, thiophene-H_a), 8.38 (t, *J* = 4.8 Hz, *J* = 3.6 Hz, 1H, thiophene-H_b), 11.84 (s, 1H, D_2O exchangeable, NH), 14.61 (s, 1H, D_2O exchangeable, NH). ^{13}C NMR (75.4 MHz, DMSO-d_6, δ, ppm): 167.3 (1C, C=S), 162.4, 161.7 (2C, 2C=O), 150.1, 132.6, 130.8, 129.2, 128.4, 126.2, 125.4, 114.2 (Ar-C), 61.2 (1C, OCH_2), 25.8, 23.9, 23.2, 22.5 (4C, $4CH_2$-cyclohexane), 14.3 (1C, CH_3). MS (EI, *m/z* (%)): 394 (M^+, 100), 267 (10), 225 (40), 179 (65), 150 (30), 110 (25). Anal. calcd. for $C_{17}H_{18}N_2O_3S_3$: C, 51.75; H, 4.60; N, 7.10. Found: C, 51.66; H, 4.52; N, 7.02%.

2.3.13. Synthesis of 2-(3-(thiophene-2-carbonyl)thioureido)-4,5,6,7-tetrahydrobenzo[b]thiophene-3-carboxylic acid (16)

A mixture of ethyl 2-(3-(thiophene-2-carbonyl)thioureido)-4, 5, 6, 7-tetrahydrobenzo[b]thiophene-3-carboxylate (**15**) (1.26 mol) and sodium ethoxide (1.26 mol) in ethanol (20 mL) was heated under reflux for 6 h. The reaction mixture was neutralized by 10% HCl. The formed precipitate was filtered off, dried and crystallized from ethanol to give compound **16** (Scheme 5). Color: Yellow. Yield: 86%. M.p.: 282-283 °C. FT-IR (KBr, ν, cm^{-1}): 3654 (OH), 3424 (NH), 1697 (C=O) (acid), 1676 (C=O) (amide).



Scheme 5

^{1}H NMR (300 MHz, DMSO-d_6, δ, ppm): 1.75 (m, 4H, cyclohexane-2CH_2), 2.67 (t, J = 5.6 Hz, 2H, cyclohexane-CH_2), 2.71 (t, J = 5.6 Hz, 2H, cyclohexane-CH_2), 7.23 (d, J = 3.6 Hz, 1H, thiophene-H_c), 8.01 (d, J = 4.8 Hz, 1H, thiophene-H_a), 8.29 (t, J = 4.8 Hz, J = 3.6 Hz, 1H, thiophene-H_b), 11.76 (s, 1H, D_2O exchangeable, NH), 13.29 (s, 1H, D_2O exchangeable, NH), 14.61 (s, 1H, D_2O exchangeable, OH). ^{13}C NMR (75.4 MHz, DMSO-d_6, δ, ppm): 167.4 (1C, C=S), 165.6, 164.7 (2C, 2C=O), 149.8, 132.0, 130.3, 129.4, 128.1, 126.6, 125.0, 114.7 (Ar-C), 25.0, 24.1, 23.4, 22.7 (4C, 4CH_2-cyclohexane). MS (EI, m/z (%)): 366 (M^+, 100), 287(20), 237(95), 178(45), 150(40), 110(10). Anal. calcd. for $C_{15}H_{14}N_2O_3S_3$: C, 49.16; H, 3.85; N, 7.64. Found: C, 49.09; H, 3.72; N, 7.54%.

2.3.14. Synthesis of 10-amino-2-(thiophen-2-yl)-4-thioxo-4H-naphtho[2,3-e][1,3]oxazine-5-sulfonic acid (18)

A mixture of thiophene-2-carbonylisothiocyanate (**1**) (3.34 mol) and 4-amino-3-hydroxynaphthalene-1-sulfonic acid (3.34 mmol) in dioxane (20 mL) was heated under reflux for 2 h. The reaction mixture was poured into water. The formed precipitate was filtered off, washed with water, dried and crystallized from ethanol to give compound **18** (Scheme 5). Color: Brown. Yield: 65%. M.p.: 322-323 °C. FT-IR (KBr, ν, cm^{-1}): 3652 (OH), 3237 (NH_2), 1352(C-O). ^{1}H NMR (300 MHz, DMSO-d_6, δ, ppm): 8.80-7.38 (m, 7H, ArH's + thiophene protons), 9.78-9.74 (br s, 2H, D_2O exchangeable, NH_2), 11.00 (s, 1H, D_2O exchangeable, OH). MS (EI, m/z (%)): 390(M^+, 100), 280 (70), 168 (50), 159 (90), 129 (40), 112 (20). Anal. calcd. for $C_{16}H_{10}N_2O_4S_3$: C, 49.22; H, 2.58; N, 7.17. Found: C, 49.11; H, 2.53; N, 7.14%.

2.3.15. Synthesis of N-(4,5-dihydro-1-(sulfanylene(thiophene-2-carboxamido)methyl)-1H-imidazol-2-yl)thiophene-2-carboxamide (19)

A mixture of thiophene-2-carbonylisothiocyanate (**1**) (3.34 mol) and ethylene diamine (3.34 mol) in dioxane (20 mL) was heated under reflux for 2 h. The reaction mixture was poured into water. The formed precipitate was filtered off, washed with water, dried and crystallized from ethanol to give compound **19** (Scheme 5). Color: White. Yield: 74%. M.p.: 328-329 °C. FT-IR (KBr, ν, cm^{-1}): 3391, 3290 (NH), 1674, 1665 (2C=O) (amide), 1537 (C=N). ^{1}H NMR (300 MHz, DMSO-d_6, δ, ppm): 3.33 (t, J = 2.6 Hz, 2H, CH_2), 3.94 (t, J = 2.6 Hz, 2H, CH_2), 8.65-7.13 (m, 6H, thiophene protons), 10.80 (s, 1H, D_2O exchangeable, NH), 11.40 (s, 1H, D_2O exchangeable, NH). ^{13}C NMR (75.4 MHz, DMSO-d_6, δ, ppm): 168.41 (1C, C=S), 161.4 (2C, 2C=O), 153.8 (1C, NCN), 130.8, 129.0, 128.3, 119.7 (2Ar-C), 54.4, 52.7 (1C, 2CH_2-imidazole). MS (EI, m/z (%)): 366 (M^+, 100), 364(M^+, 15), 261(10), 185(5), 110(100), 82(20), 57(25). Anal. calcd. for $C_{14}H_{12}N_4O_2S_3$: C, 46.14; H, 3.32; N, 15.37. Found: C, 46.31; H, 3.20; N, 15.26%.

2.4. Antitumor activity test - tumor cell growth assay

Human tumor cell line, MCF-7 (breast adenocarcinoma) MCF-7 was kindly provided by the National Cancer Institute (NCI, Cairo, Egypt). The effects of compounds **6**, **7**, **8**, **12**, **13**, **15**, **16** and **18** on the *in vitro* growth of human tumor cell line were evaluated according to the method adopted by the National Cancer Institute (NCI, USA) [22-24].

Table 1. The inhibitory activities of the synthesized compounds on the growth of MCF-7 cell line.

Compound	GI_{50} (µg/L)
	MCF-7
8	10.29±5.44
12	0.01±0.004
13	0.01±0.008
15	0.01±0.001
16	0.01±0.003
18	2.70±1.24
Doxorubicin	0.04±0.008

Table 2. Evaluation of cytotoxicity of the obtained compounds against MCF-7 cell line.

Compound	Viability rate (%)			IC_{50} (mg/mL)
	0.1 µg/mL	1 µg/mL	10 µg/mL	
8	87.26±6.29	82.21±3.98	80.23±5.56	10.49±7.44
12	56.52±4.42	50.89±5.50	55.23±3.90	0.01±0.90
13	54.62±4.53	58.09±6.50	53.34±3.81	0.01±0.50
15	51.32±4.42	55.89±5.50	51.23±3.90	0.01±0.008
16	51.62±4.53	58.09±6.50	51.34±3.81	0.01±0.009
18	93.26±4.19	91.20±3.66	82.09±5.71	2.69±0.04

3. Results and discussion

3.1. Chemistry

This research aims at presenting importance of isothiocyanates as synthetic intermediate for building biologically active heterocycles [25,26]. To begin with addition of semicarbazide hydrochloride to thienoylisothiocyanate (**1**) [27] in dioxane:water (1:1, *v*:*v*) (50 mL) with stirring at room temperature for 1 h afforded thiosemicarbazide derivative **2** as a result of nucleophilic addition to the electrophilic carbon of the isothiocyanate group. An intramolecular basic cyclization of thiophene derivative **2** in sodium ethoxide resulted in oxadiazine nuclus **3** in 89% yield (Scheme 1). Moreover, addition of phenylhydrazine to the heteroallene **1** led to formation of the expected triazole **4** in 63% yield after loss of H_2O molecule (Scheme 1).

The new structures of the prepared compounds were elucidated on the bases of their spectroscopic data. As an example, the IR spectrum of oxadiazine derivative **3** exhibited characteristic absorption bands at 3411, 3325, 1599 and 1235 cm^{-1} for NH, NH_2, C=N and C=S, respectively. In addition to, absence of carbonyl stretching peak is observed. The ^1H NMR of compound **3** showed two singlets with different integration at δ 13.91 and 13.61 ppm for NH and NH_2 protons, respectively. There is a multiplet in between δ 7.75-7.19 ppm for three protons of the thiophene ring. Disappearance the two peaks at δ 13.91 and 13.61 ppm in D_2O spectrum of compound **3** emphasized that the NH and NH_2 protons were exchangeable with D_2O and this is proof for the correct suggested structure. Also, the mass spectrometry of compound **3** is in agreement with its structure as well as its molecular weight. The ^1H NMR spectrum of compound **4** revealed a sharp singlet at δ 10.37 ppm for NH proton which was D_2O exchangeable.

Addition of phenylhydrazine hydrochloride to thiophene-2-carbonyl isothiocyanate (**1**) in dioxane:water (1:1, *v*:*v*) produced thiosemicarbazide **5** [23-25]. The synthesis of thiadiazine derivative **6** in 61% yield was achieved via one pot three component reaction of compound **5**, maleic anhydride and triethyl amine in DMF (Scheme 2). A mixture of ethyl chloroacetate and thiophene-2-carboxamide hydrochloride **5** was heated for 6 h to yield thiadiazine **7** in 79% yield. Upon heating **5** with sodium ethoxide for 2 h, the triazole **8** was obtained in 95% yield as in Scheme 2.

The IR spectrum of compound **8** showed strong bands at 3424, 1597 and 1252 cm^{-1} for NH, C=N and C=S, respectively. The ^1H NMR spectrum of triazole **8** displayed a singlet at δ 14.41 ppm for NH proton which was exchangeable with D_2O as well. A multiplet peak is at δ 8.06-7.18 ppm for aromatic protons. The mass spectrometry of compound **8** is in agreement with its suggested structure.

The suggested mechanism for establishment of thienoyl-thiadiazine **6** showed that formation of acyclic thienoylimine intermediate **9**. Subsequently, the intermediate **9** underwent an intramolecular nucleophilic cyclization to form thiadiazine derivative **10** that was dehydrated giving thienoylthiadiazine structure **6** (Scheme 3).

Thiosemicarbazide derivative **11** was synthesized by the addition of nicotinic hydrazide to thiophene-2-carbonyl isothiocyanate (**1**) in dioxane as outlined in (Scheme 4) [23-25]. Depending on reaction condition compound **11** underwent several kinds of heterocyclizations. So, acid mediated cyclization of compound **11** resulted in thiadiazole ring **12** in 95% yield. Heating of compound **11** with lead acetate in acetic acid produced triazole derivative cyclization **13** in 83% yield. On the other hand, base mediated cyclization of compound **11** afforded triazolethione **14** in 93% yield via thiophene intermediate (Scheme 4).

The thiourea derivative of type **15** was obtained in 70% yield as a result of addition of ethyl 2-amino-4,5,6,7-tetra hydrobenzo[*b*]thiophene-3-carboxylate in dioxane to thienoyl-isothiocyante **1** (Scheme 5). Upon heating of compound **15** with ethoxide produced thiophene-3-carboxylic acid **16** in 86% yield but the other cyclized product **17** was not formed. Moreover, addition of amino phenol sulfonic acid derivative to heteroallene **1** in dioxane resulted in building the oxazine structure **18** in 65% yield. On the other hand, nucleophilic addition of ethylene diamine in dioxane to thiophene-2-carbonyl isothiocyanate (**1**) led to formation of imidazole derivative **19** in 74% yield as outlined in Scheme 5.

The ^1H NMR spectrum of thiophene carboxylic acid **16** revealed three sharp singlets at δ 14.61, 13.29 and 11.76 ppm for OH and 2NH protons which were D_2O exchangeable. A triplet peak at δ 8.29 ppm with J = 4.8 and 3.6 Hz for thiophene-H_b. There are two doublets at δ 8.01 and 7.23 ppm for thiophene-H_a and thiophene-H_c.

3.2. Antitumor activity

The inhibitory activities of the compounds **8**, **12**, **13**, **15**, **16** and **18** were evaluated on the *in vitro* growth of cancer human cell line breast adenocarcinoma MCF-7. Nearly, all the screened compounds were able to inhibit the growth of the tested human tumor cell line. The results tabulated in Table 1 indicated that Compounds **12**, **13**, **15** and **16** showed the highest inhibitory effect against MCF-7 cell line compared to the reference doxorubicin. Meanwhile compounds **18** and **8** exhibited high inhibitory effects, which are less than the standard reference in Table 1.

The cytotoxic and antitumor activities of the compounds **8**, **12**, **13**, **15**, **16** and **18** were tested against MCF-7 cells line. The inhibitory activities were detected by using different concentrations as shown in Table 2. Results presented in Table 2 displayed that, compounds **15**, **16**, **13** and **12** had very strong cytotoxic antitumor activity, while compounds **18** and **8** have strong cytotoxic antitumor activity against MCF-7 cell line.

4. Conclusion

In conclusion, we were able to realize the synthesis of a series of new 1,2,4-triazolethione, 1,3,4-thiadiazole, 1,3-imidazole and oxadiazine derivatives bearing thiophene ring system in high yields 61-95%. Compounds were screened for their anticancer activity on cancer human cell lines like breast cancer MCF-7. Most of selected compounds showed remarkable antitumor activity. Compounds **12**, **13**, **15** and **16** are the most active ones on the cancer cell line due to their reactivity is more than that of the standard doxorubicin on the same cell line. Accordingly, these preliminary results of the newly synthesized thiophene derivatives can serve as a starting point for the development of potent anti-cancer agents.

Disclosure statement

Conflict of interests: The authors declare that they have no conflict of interest.
Author contributions: All authors contributed equally to this work.
Ethical approval: All ethical guidelines have been adhered.
Sample availability: Samples of the compounds are available from the author.

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