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Facile synthesis of some condensed 1,3-thiazines and thiazoles under conventional conditions: antitumor activity

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Abstract 1,3-Thiazine **3** was obtained from cinnamoyl thiourea derivative **2** as the kinetic control product. Refluxing of **2** with sodium ethoxide afforded pyrimidine derivative **4**. Moreover, stirring of **2** with bromine/acetic acid gave thiazole **5** that was condensed with *o*-phenylene diamine forming benzimidazole **6**. Heating of arylthiourea **8** with maleic anhydride or phenacyl chloride afforded thiazole derivatives **9** and **10**, respectively. Condensation of compound **10** with *o*-phenylene diamine gave benzimidazole **11**. Reaction of *p*-amino benzoic acid with chloro acetyl isothiocyanate, acetylacetone and ethylacetoacetate produced imidazole **14**, enaminone **15** and crotonate **16** derivatives, respectively. Stirring a mixture of benzoyl isothiocyanate with **15** and/or **16** resulted in pyridine-2-thione **17**. The yields of the prepared compounds were 41–93%. The experimental section is simple and easy. The detailed synthesis, spectroscopic data, IC_{50} and antitumor activity of the synthesized compounds were reported. The cytotoxicity of the newly synthesized products showed that compound **4** is the most active compound towards the cancer cell line at which its reactivity is higher than that of the standard doxorubicin (anticancer reference drug).

Keywords 1,3-Thiazines · Benzothiazoles · Isothiocyanate · Imidazoles · Pyrimidines and antitumor activity

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Introduction

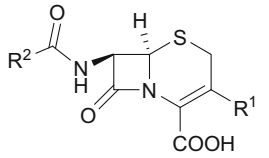
Synthetic heterocyclic compounds especially containing heteroatoms N, S, O have enormous potential primarily as agrochemicals and drugs. 1,3-Thiazines with *N–C–S* linkage have promising pharmacological activities which have drawn the attention of scientists. 1,3-Thiazines and subsequently their derivatives have been used as antitubercular, antiradiation [1] and antitumor [2] agents. Further, they are used in various organic synthesis and transformations as reaction intermediates [1, 3]. Moreover, the core moiety of 1,3-thiazines is present in the fused form with a β-lactam ring in major class of antibiotics like cephalosporins (Fig. 1) [4, 5].

Benzothiazoles are widely found in bioorganic and medicinal chemistry with applications in drug discovery and are of great scientific interest. Benzothiazole moities are a part of compounds showing numerous biological activities such as anticancer [6–9], antihelmintic [10] and antidiabetic [11] activities. Several benzimidazoles have been reported as antiviral [12], anticoagulant [13] and anticancer agents [14, 15]. As a result, our research project was directed towards the synthesis of some new fused azoles and azines of expected antitumor activity. As a part of our programme, we published a paper on azoles and azines derivatives of expected biological activity [16].

Experimental

Melting points were measured using an Electro thermal IA 9100 apparatus with open capillary tubes and are uncorrected. All experiments were carried out using drying solvents. Thin-layer chromatography (TLC) was performed on a Merck Silica Gel 60F254 with detection by UV light. Products were purified by crystallization. The IR spectra (KBr disc) were recorded on a Pye Unicam Sp-3-300 or a Shimadzu FTIR 8101 PC infrared spectrophotometer. The $^{1}H/(^{13}C)$ NMR spectra were recorded at a Varian Mercury VX-300 NMR 300 (75.4) MHz spectrometer using DMSO-d6 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 spectrometery and analytical data were obtained from the Microanalysis Center at Cairo University, Giza, Egypt.

*(E)-4-(3-cinnamoylthioureido)benzoic acid (**2**)* [21] A mixture of 4-aminobenzoic acid (**1**) (24.0 mmol) and cinnamoyl isothiocyanate (24.0 mmol) in dry acetone



R^1 = -CH_2COOMe, -Me, -CH_2Cl
R^2 = -CH_2CN, p-$(NH_2)C_6H_4$, p-$(OH)C_6H_4$

Fig. 1 Structure of cephalosporins

(50 mL) was stirred at room temperaure for 1 h. The reaction mixture was poured into water. The formed product was filtered off, washed with water, dried and crystallized from methanol to give a yellow powder in 82% yield. mp 260–261 °C; ν_{max} (KBr) 3454, 3163, 3151, 1286 cm^{-1}; δ_H (300 MHz, DMSO-d_6) 12.90 (1H, D_2O exchangeable, s, OH), 11.68 (1H, D_2O exchangeable, s, NH), 11.65 (1H, D_2O exchangeable, s, NH), 6.93–7.95 (11H, m, ArH's + vinylic protone); *m/z* (EI, 70 eV) 326(98), 293(15), 179(10), 131(98), 103(65), 77(40); Elem. Anal. Calcd for $C_{17}H_{14}N_2O_3S$ (326.37): C, 62.56; H, 4.32; N, 8.58. Found: C, 62.43; H, 4.18; N, 8.42.

*4-(4-oxo-6-phenyl-1,3-thiazinan-2-ylideneamino)benzoic acid (**3**)* A mixture of (E)-4-(3-cinnamoylthioureido) benzoic acid (**2**) (3.00 mmol) and sodium ethoxide (3.00 mmol) in ethanol (20 mL) was stirred overnight at room temperature. The reaction mixture was neutralized by HCl. The formed product was filtered off, dried and crystallized from ethanol to give a yellow powder in 58% yield. mp 232–233 °C; ν_{max} (KBr) 3720, 3450, 1710, 1648 cm^{-1}; δ_H (300 MHz, DMSO-d_6) 12.89 (1H, br s, COOH), 10.52 (1H, br s, NH), 7.24–8.22 (9H, m, ArH's), 4.89 (2H, d, $J = 2.4$ Hz, CH_2), 2.93 (1H, t, $J = 2.4$ Hz, CH); *m/z* (EI, 70 eV) 326(20), 147(25), 131(98), 103(70), 91(80), 77(50); Elem. Anal. Calcd for $C_{17}H_{14}N_2O_3S$ (326.37): C, 62.56; H, 4.32; N, 8.58. Found: C, 62.33; H, 4.21; N, 8.36.

*4-(4-oxo-6-phenyl-2-thioxotetrahydropyrimidin-1(2H)-yl)benzoic acid (**4**)* A mixture of (E)-4-(3-cinnamoylthioureido) benzoic acid (**2**) (3.00 mmol) and sodium ethoxide (3.00 mmol) in ethanol (20 mL) was heated under reflux for 3 h. The reaction mixture was cooled and neutralized by HCl. The formed product was filtered off, washed with water, dried and crystallized from ethanol to give yellow crystals in 41% yield. mp 319–320 °C; ν_{max} (KBr) 3429, 3266, 1712, 1240 cm^{-1}; δ_H (300 MHz, DMSO-d_6) 12.53 (1H, br, D_2O exchangeable, COOH), 11.33 (1H, s, D_2O exchangeable, NH), 7.19–7.90 (9H, m, ArH's), 4.73 (2H, d, $J = 2.4$ Hz, CH_2), 2.98 (1H, t, $J = 2.4$ Hz, CH); *m/z* (EI, 70 eV) 326(15), 131(30), 103(15), 91(98), 80(15), 65(20); Elem. Anal. Calcd for $C_{17}H_{14}N_2O_3S$ (326.37): C, 62.56; H, 4.32; N, 8.58. Found: C, 62.44; H, 4.16; N, 8.45.

*2-cinnamamidobenzo[d]thiazole-6-carboxylic acid (**5**)* A mixture of (E)-4-(3-cinnamoylthioureido) benzoic acid (**2**) (3.00 mmol) and bromine (3.00 mmol) in acetic acid (20 mL) was stirred overnight at room temperature. The reaction mixture was poured into cold water. The formed product was filtered off, dried and crystallized from acetic acid to give white crystals in 65% yield. mp > 360 °C; ν_{max} (KBr) 3443, 3233, 1710 and 1679 cm^{-1}; δ_H (300 MHz, DMSO-d_6) 12.76 (1H, br, COOH), 11.06 (1H, s, NH), 6.83–6.87 (2H, dd, $J = 14.8$ Hz, vinylic protons), 7.44–7.90 (8H, m, ArH's),; *m/z* (EI, 70 eV) 324(15), 146(98), 131(98), 103(85), 77(65), 65(30); Elem. Anal. Calcd for $C_{17}H_{12}N_2O_3S$ (324.35): C, 62.95; H, 3.73; N, 8.64. Found: C, 62.74; H, 3.56; N, 8.48.

*N-(6-(1H-benzo[d]imidazol-2-yl)benzo[d]thiazol-2-yl)cinnamamide (**6**)* A mixture of thiazole carboxylic acid **5** (1.00 mmol) and *o*-phenylene diamine (1.00 mmol) in ethanol (10 mL) was heated under reflux for 6 h. The reaction mixture was cooled

and poured into ice-cold water. The formed product was filtered off, dried and crystallized from ethanol to give a green powder in 62% yield. mp 220–221 °C; ν_{max} (KBr) 3414, 1684 cm^{-1}; δ_H (300 MHz, DMSO-d_6) 10.98 (1H, s, D_2O exchangeable, NH), 8.04 (1H, s, D_2O exchangeable, NH), 7.25–7.85 (12H, m, ArH's), 6.83–6.87 (2H, dd, $J = 14.8$ Hz, vinylic protons); *m/z* (EI, 70 eV) 397(10), 146(30), 131(20), 108(85), 80(98), 64(35); Elem. Anal. Calcd for $C_{23}H_{16}N_4OS$ (396.56): C, 69.68; H, 4.07; N, 14.13. Found: C, 69.57; H, 3.98; N, 14.01.

*4-(3-benzoylthioureido)benzoic acid (**7**)* [22] A mixture of 4-aminobenzoic acid (**1**) (71.0 mmol) and benzoyl isothiocyanate (71.0 mmol) in dry acetone (50 mL) was stirred at room temperature for 1 h. The reaction mixture was poured into water. The formed product was filtered off, washed with water, dried and crystallized from methanol to give yellow crystals in 73% yield. mp 229–230 °C; ν_{max} (KBr) 3766, 3373, 3256, 1690, 1592, 1261 cm^{-1}; δ_H (300 MHz, DMSO-d_6) 12.93 (1H, s, COOH), 9.14 (1H, s, NH), 7.25–7.95 (9H, m, ArH's), 3.48 (1H, s, NH); *m/z* (EI, 70 eV) 300(60), 179(25), 137(25), 120(25), 105(98), 77(60); Elem. Anal. Calcd for $C_{15}H_{12}N_2O_3S$ (300.33): C, 59.99; H, 4.03; N, 9.33. Found: C, 59.82; H, 3.85; N, 9.22.

*4-thioureidobenzoic acid (**8**)* [23] A mixture of 4-(3-benzoylthioureido) benzoic acid (**7**) (10.0 mmol) and 10% sodium hydroxide (10.0 mmol) in water (20 mL) was heated under reflux for 30 min. The reaction mixture was cooled and neutralized by HCl. The formed product was filtered off, washed with water, dried and crystallized from butanol to give a white powder in 93% yield. mp > 300 °C; ν_{max} (KBr) 3440, 3339, 3279, 3170, 1684, 1291 cm^{-1}; δ_H (300 MHz, DMSO-d_6) 12.72 (1H, br s, COOH), 9.96 (1H, s, NH), 7.54–7.90 (4H, m, ArH's), 3.37 (1H, s, NH_2); *m/z* (EI, 70 eV) 196(10), 179(98), 162(98), 134(75), 120(95), 92(35); Elem. Anal. Calcd for $C_8H_8N_2O_2S$ (196.23): C, 48.97; H, 4.11; N, 14.28. Found: C, 48.84; H, 4.07; N, 14.24.

*4-(5-(carboxymethyl)thiazol-2-ylamino)benzoic acid (**9**)* A mixture of 4-thioureidobenzoic acid (**8**) (5.00 mmol) and maleic anhydride (5.00 mmol) in dry pyridine (10 mL) was heated under reflux for 6 h. The reaction mixture was cooled and poured into ice-cold water. The formed product was filtered off, washed with water, dried and crystallized from ethanol to give brown crystals in 47% yield. mp 279–280 °C; ν_{max} (KBr) 3323, 3194, 3173, 1690 and 1713 cm^{-1}; δ_H (300 MHz, DMSO-d_6) 12.72 (1H, s, COOH), 10.52 (1H, s, COOH), 9.19 (1H, s, NH), 7.53–7.92 (5H, m, ArH's), 1.95 (2H, s, CH_2); *m/z* (EI, 70 eV) 278(15), 241(98), 186(55), 146(65), 105(98), 77(55); Elem. Anal. Calcd for $C_{12}H_{10}N_2O_4S$ (278.28): C, 51.79; H, 3.62; N, 10.07. Found: C, 51.65; H, 3.41; N, 10.01.

4-(4-phenylthiazol-2-ylamino) benzoic acid (**10**) A mixture of 4-thioureidobenzoic acid (**8**) (5.00 mmol) and phenacyl chloride (5.00 mmol) in dry pyridine (10 mL) was heated under reflux for 6 h. The reaction mixture was cooled and poured into ice-cold water. The formed product was filtered off, washed with water, dried and crystallized from ethanol to give brown crystals in 78% yield. mp. 249–250 °C; ν_{max} (KBr) 3395, 3263, 1675, cm^{-1}; δ_H (300 MHz, DMSO-d_6) 12.58 (1H, br s,

COOH), 10.67 (1H, s, NH), 7.29–7.92 (10H, m, ArH's); *m/z* (EI, 70 eV) 296(98), 134(55), 104(25), 90(25), 77(25), 65(20); Elem. Anal. Calcd for $C_{16}H_{12}N_2O_2S$ (296.34): C, 64.85; H, 4.08; N, 9.45. Found: C, 64.67; H, 3.95; N, 9.23.

*N-(4-(1H-benzo[d]imidazol-2-yl) phenyl)-4-phenylthiazol-2-amine (**11**)* A mixture of 4-(4-phenylthiazol-2-ylamino) benzoic acid (**10**) (1.00 mmol) and *o*-phenylene diamine (1.00 mmol) in ethanol (10 mL) was heated under reflux for 6 h. The reaction mixture was cooled and poured into ice-cold water. The formed product was filtered off, dried and crystallized from ethanol to give yellow crystals in 66% yield. mp 170–171 °C; ν_{max} (KBr) 3440, 3315 cm^{-1}; δ_H (300 MHz, DMSO-d_6) 10.72 (1H, s, D_2O exchangeable, NH), 7.41–7.93 (14H, m, ArH's + SCH =), 4.22 (1H, s, D_2O exchangeable, NH); *m/z* (EI, 70 eV) 368(98), 324(98), 295(25), 279(25), 134(45), 90(30); Elem. Anal. Calcd for $C_{22}H_{16}N_4S$ (368.45): C, 71.71; H, 4.38; N, 15.21. Found: C, 71.57; H, 4.31; N, 15.07.

*4-(2-chloroacetamido) benzoic acid (**12**)* [24] To a mixture of 4-aminobenzoic acid (**1**) (15.0 mmol) and pyridine (15.0 mmol) in benzene (20 mL) chloroacetyl chloride (15.0 mmol) was added dropwise. The formed product was filtered off, washed with water, dried and crystallized from ethanol to give a white powder in 77% yield. mp 274–275 °C; ν_{max} (KBr) 3401, 3279, 1688, 1603 cm^{-1}; δ_H (300 MHz, DMSO-d_6) 12.73 (1H, br, COOH), 10.58 (1H, s, NH), 7.66–7.88 (4H, m, ArH's), 4.39 (2H, s, CH_2); *m/z* (EI, 70 eV) 213(35), 164(25), 137(98), 120 (65), 77(20), 65(35); Elem. Anal. Calcd for $C_9H_8ClNO_3$ (213.62): C, 50.60; H, 3.77; N, 6.56. Found: C, 50.47; H, 3.60; N, 6.41.

*4-(2-imino-4-oxothiazolidin-3-yl) benzoic acid (**13**)* A mixture of 4-(2-chloroacetamido) benzoic acid (**11**) (5.00 mmol), ammonium thiocyanate (5.00 mmol) and sodium ethoxide (5.00 mmol) in ethanol (20 mL) was heated under reflux for 3 h. The reaction mixture was cooled and neutralized by HCl. The formed product was filtered off, washed with water, dried and crystallized from ethanol to give a white powder in 51% yield. mp 288–289 °C; ν_{max} (KBr) 3411, 3346, 1708, 1674 cm^{-1}; δ_H (300 MHz, DMSO-d_6) 12.64 (1H, br s, COOH), 10.69 (1H, s, NH), 7.64–7.89 (4H, m, ArH's), 4.16 (2H, s, CH_2); *m/z* (EI, 70 eV) 236(55), 163(35), 137(90), 120(98), 90(40), 65(70); Elem. Anal. Calcd for $C_{10}H_8N_2O_3S$ (236.25): C, 50.84; H, 3.41; N, 11.86. Found: C, 50.72; H, 3.27; N, 11.76.

*4-(4-oxo-2-thioxoimidazolidin-1-yl) benzoic acid (**14**)* A mixture of 4-aminobenzoic acid (**1**) (29.0 mmol) and chloroacetylisothiocyanate (29.0 mmol) in dry acetone (20 mL) was heated under reflux for 1 h. The reaction mixture was cooled and poured into cold water. The formed product was filtered off, washed with water, dried and crystallized from ethanol to give yellow crystals in 48% yield. mp 228–229 °C; ν_{max} (KBr) 3400, 3278, 1689, 1602, 1251, cm^{-1}; δ_H (300 MHz, DMSO-d_6) 12.75 (1H, br s, COOH), 10.59 (1H, s, NH), 7.66–7.89 (4H, m, ArH's), 4.26 (2H, s, CH_2); *m/z* (EI, 70 eV) 236(15), 202(15), 162(10), 100(10), 80(20), 56(25); Elem. Anal. Calcd for $C_{10}H_8N_2O_3S$ (236.25): C, 50.84; H, 3.41; N, 11.86. Found: C, 50.68; H, 3.28; N, 11.63.

*4-((4-oxopent-2-en-2-yl) amino) benzoic acid (**15**)* [25] A mixture of 4-aminobenzoic acid (**1**) (29.0 mmol), acetylacetone (29.0 mmol) and 3 drops of acetic acid in ethanol (10 mL) was heated under reflux for 2 h. The reaction mixture was cooled and poured into ice-cold water. The formed product was filtered off, washed with water, dried and crystallized from ethanol to give yellow crystals in 55% yield. mp 172–173 °C; ν_{max} (KBr) 3450, 3384, 1696, 1683 cm^{-1}; δ_H (300 MHz, DMSO-d_6) 12.56 (1H, s, COOH), 8.90 (1H, s, NH), 7.22–7.88 (4H, m, ArH's), 5.27 (1H, s, = CH), 2.47 (3H, s, CH_3), 2.09 (3H, s, CH_3); *m/z* (EI, 70 eV) 219(75), 204(98), 160(65), 132(40), 117(30), 65(70); Elem. Anal. Calcd for $C_{12}H_{13}NO_3$ (219.24): C, 65.74; H, 5.98; N, 6.39. Found: C, 65.55; H, 5.81; N, 6.24.

*4-(4-ethoxy-4-oxobut-2-en-2-yl) amino) benzoic acid (**16**)* A mixture of 4-aminobenzoic acid (**1**) (29.0 mmol), ethylacetoacetate (29.0 mmol) and 3 drops of acetic acid in ethanol (10 mL) was heated under reflux for 2 h. The reaction mixture was cooled and poured into ice-cold water. The formed product was filtered off, washed with water, dried and crystallized from ethanol to give white crystals in 59% yield. mp 146–148 °C; ν_{max} (KBr) 3465, 3383, 1705 and 1683 cm^{-1}; δ_H (300 MHz, DMSO-d_6) δ 12.10 (1H, s, COOH), 10.74 (1H, s, NH), 6.64–8.04 (4H, m, ArH's), 4.79 (1H, s, = CH), 4.14 (2H, q, $J = 2.2$ Hz, CH_2) 2.26 (3H, s, CH_3), 1.27 (3H, t, $J = 2.2$ Hz, CH_3); *m/z* (EI, 70 eV) 249(60), 203(30), 176(40), 162(98), 130(45), 65(50); Elem. Anal. Calcd for $C_{13}H_{15}NO_4$ (249.26): C, 62.64; H, 6.07; N, 5.62. Found: C, 62.49; H, 6.00; N, 5.46.

*4-((2-mercapto-6-phenylpyridin-4-yl) amino) benzoic acid (**17**)* A mixture of compounds **15** or **16** (5.00 mmol), benzoyl isothiocyanate (5.00 mmol) and and few drops of triethylamine in dry acetone (50 mL) was stirred at room temperature for 30 min. The reaction mixture was poured into water. The formed product was filtered off, washed with water, dried and crystallized from ethanol/acetic acid (1:3) to give yellow crystals in 55% and 85% yield, respectively. mp 226–228 °C; ν_{max} (KBr) 3380, 3257, 1697 cm^{-1}; δ_H (300 MHz, DMSO-d_6) 12.74 (1H, s, D_2O exchangeable, NH), 11.65 (1H, s, D_2O exchangeable, NH), 7.51–7.95 (11H, m, ArH's); *m/z* (EI, 70 eV) 323(5), 300(25), 179(20), 137 (20), 105(98), 77(55); Elem. Anal. Calcd for $C_{18}H_{14}N_2O_2S$ (322.38): C, 67.06; H, 4.38; N, 8.69. Found: C, 66.91; H, 4.31; N, 8.55.

Antitumor activity tests

Reagents

Fetal bovine serum (FBS) and L-glutamine, were from Gibco Invitrogen (UK). RPMI-1640 medium was from Cambrex (NJ, USA). Dimethyl sulfoxide (DMSO), doxorubicin, penicillin, streptomycin and sulforhodamine B (SRB) were from Sigma Chemical (Saint Louis, USA).

Cell cultures

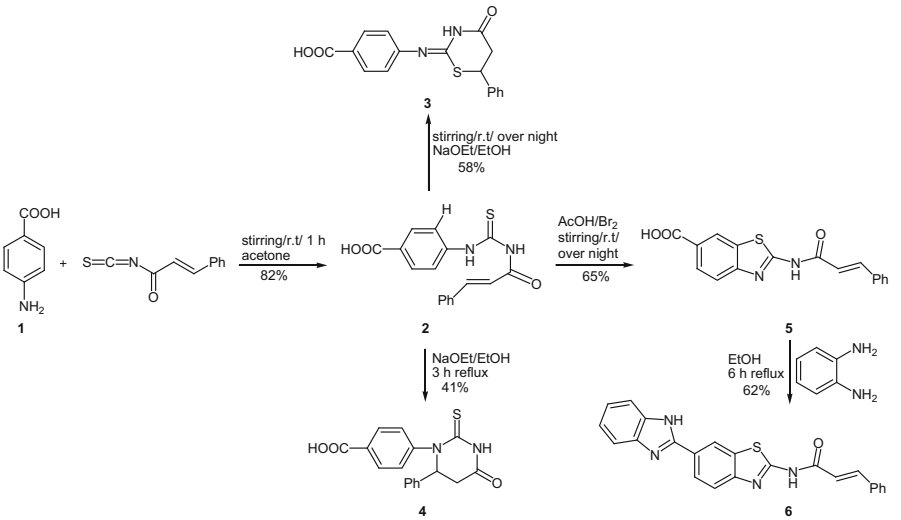
Human tumor cell line MCF-7 (breast adenocarcinoma) was kindly provided by the National Cancer Institute (NCI, Cairo, Egypt). They grow as a monolayer and were routinely maintained in RPMI-1640 medium supplemented with 5% heat-inactivated FBS, 2 mM glutamine and antibiotics (penicillin 100 U/mL, streptomycin 100 mg/mL), at 37 °C in a humidified atmosphere containing 5% CO_2. Exponentially growing cells were obtained by plating 1.5*105 cells/mL for MCF-7 followed by 24 h of incubation. The effect of the vehicle solvent (DMSO) on the growth of these cell line was evaluated in all the experiments by exposing untreated control cells to the maximum concentration (0.5%) of DMSO used in each assay.

Tumor cell growth assay

The effects of compounds **3, 4, 10, 13** and **14** on the in vitro growth of the human tumor cell line were evaluated according to the procedure adopted by the National Cancer Institute (NCI, USA) in the 'In vitro Anticancer Drug Discovery Screen' which uses the protein-binding dye sulforhodamine B to assess cell growth [26]. Briefly, exponentially, cells growing in 96-well plates were then exposed for 48 h to five serial concentrations of each compound, starting from a maximum concentration of 150 μM. Following this exposure period, adherent cells were fixed, washed, and stained. The bound stain was solubilized and the absorbance was measured at 492 nm in a plate reader (Power wave XS; Bio-Tek Instruments, Wincoski, USA). For each test compound and cell line, a dose–response curve was obtained and the growth inhibition of 50% (GI_{50}), and IC_{50} (minimum inhibitory concentration) corresponding to the concentration of the compounds that inhibited 50% of the net cell growth was calculated, as described elsewhere [27]. Doxorubicin was used as a positive control and tested in the same manner.

Results and discussion

This report presents the synthesis of some novel fused azoles and azines via isothiocyanates that are versatile reagents and have been used as synthetic intermediates to prepare biologically active heterocyclic compounds [17, 18]. Initially, the addition of *p*-aminobenzoic acid (**1**) to cinnamoyl isothiocyanate [19] in acetone with stirring at room temperature for 1 h gave cinnamoyl thiourea derivative **2** (Scheme 1). Base-induced intramolecular ring closing of **2** led to 1,3-thiazine cyclization **3** as the kinetic control product. On the other hand, refluxing of **2** with sodium ethoxide afforded pyrimidine derivative **4** as a thermodynamic stable product via the addition of nucleophilic nitrogen to the electrophilic cinnamoyl carbon. Stirring of cinnamoyl derivative **2** with bromine/acetic acid resulted in thiazole **5** in 65% yield through oxidative cyclization. Condensation of the carboxylic function **5** with *o*-phenylene diamine produced the polycyclic-bearing benzimidazole ring system **6** in 62% yield (Scheme 1).



Scheme 1 Reaction of cinnamoyl thiourea **2** with ethoxide or bromine/acetic acid

The chemical structures of the synthesized compounds were elucidated by analysis of their spectroscopic data. In the IR spectrum of compound **2**, there are absorption bands at 3454, 3163, 3151 and 1286 cm^{-1} for OH, 2NH and C=S, respectively. Its ^{1}H NMR displayed three broad singlets at $\delta = 12.90$, 11.68 and 11.65 ppm for COOH and 2NH protons, respectively. In addition, a doublet of doublet is in between $\delta = 6.93$–7.95 ppm for vinylic protons (Scheme 1). The IR spectrum of compound **3** exhibited absorption bands at 3720, 3450, 1710 and 1684 cm^{-1} for OH, NH, acidic C=O and amidic carbonyl, respectively. The ^{1}H NMR spectrum of **3** showed two singlets at $\delta = 12.89$ and 10.52 ppm for COOH and NH protons, respectively. Moreover, there is a doublet at $\delta = 4.89$ ppm for $CO\underline{CH_2}$ and a triplet at $\delta = 2.93$ ppm for the $\underline{CH}$Ph group. (Scheme 1). The IR spectrum of **4** showed absorption bands at 3429, 3266, 1710, 1682 and 1240 cm^{-1} for OH, NH, 2C=O and C=S, respectively. Its ^{1}H NMR explained two singlets at $\delta = 12.53$ and 11.33 ppm for COOH and NH protons, respectively. There is a doublet at $\delta = 4.73$ ppm for $CO\underline{CH_2}$ and a triplet at $\delta = 2.98$ ppm for the $\underline{CH}$Ph proton with coupling constant $J = 2.4$ Hz. The IR spectrum of **5** showed absorption bands at 3443, 3233, 1710 and 1679 cm^{-1} for OH, NH, acidic C=O and amidic carbonyl, respectively, while its ^{1}H NMR spectrum revealed two sharp singlets at $\delta = 12.76$ and 11.06 ppm for COOH and NH protons, respectively. In addition, doublet of doublet is in between $\delta = 6.83$–6.87 ppm for vinylic protons with coupling constant $J = 14.8$ Hz. The structure of compound **6** was elucidated from its IR spectrum which showed that the acidic carbonyl band disappeared at 1710 cm^{-1}. However, there are absorption bands at 3414 and 1684 cm^{-1} for 2NH and amidic C=O, respectively. Its ^{1}H NMR spectrum displayed two broad singlets at $\delta = 10.98$ ppm and $\delta = 8.05$ ppm for 2NH protons (Fig. 2).

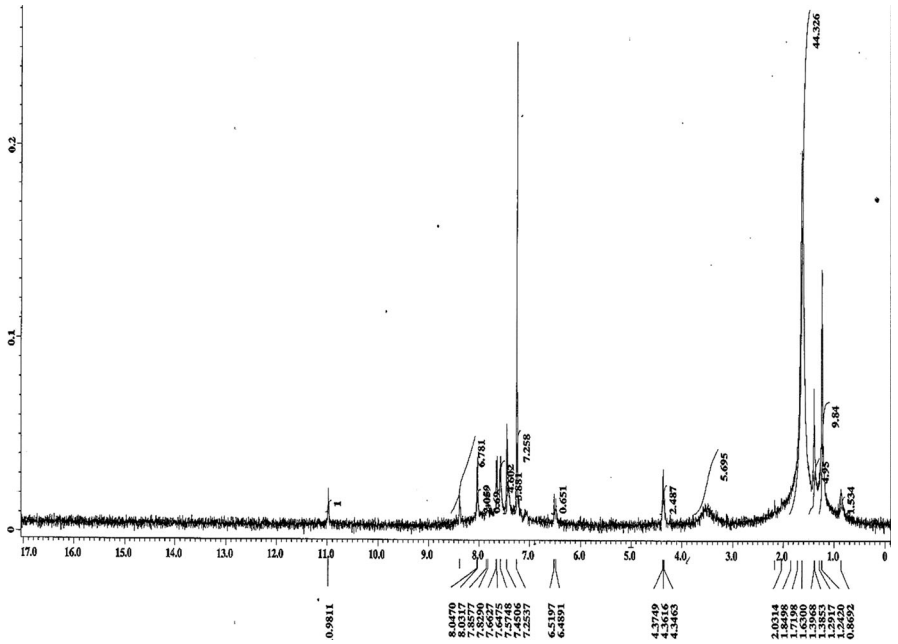


Fig. 2 The ^{1}H NMR spectrum of **6**

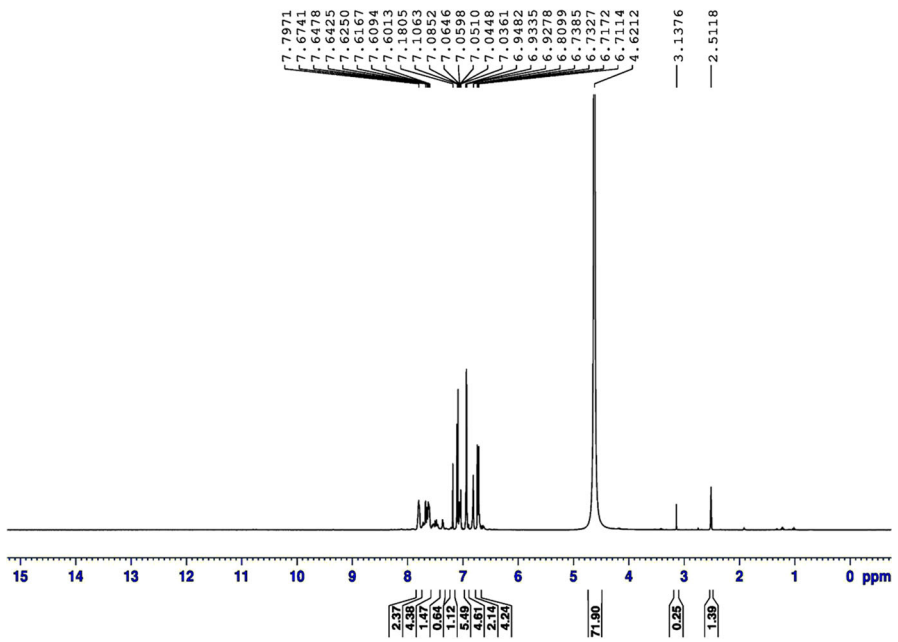


Fig. 3 The D_2O spectrum of **6**

The disappearance of the two peaks at $\delta = 10.98$ and 8.05 ppm in the D_2O spectrum of **6** (Fig. 3) confirmed that the 2NH protons were exchangable with D_2O and this is evidence for the suggested structure.

Moreover, the mass spectrometry of compound **6** is in agreement with its structure as well as its molecular weight (Fig. 4).

Upon addition of *p*-aminobenzoic acid (**1**) to benzoyl isothiocyanate [20] in acetone with stirring at room temperature for 1 h led to the formation of benzoyl thiourea **7** in 73% yield which in turn underwent basic hydrolysis by 10% NaOH producing arylthiourea **8** in 93% yield (Scheme 2). Heating of compound **8** with maleic anhydride in the presence of pyridine for 6 h produced a cycloaddition reaction to form thiazole derivative **9** in 47% yield via a thiourea derivative intermediate. Arylthiourea **8** was reacted with phenacyl chloride in pyridine for 6 h to afford the thiazole ring **10** in 78% yield. Condensation of compound **10** with *o*-phenylene diamine in ethanol gave benzimidazole derivative **11** in 66% yield (Scheme 2).

The chemical structure of compound **9** was elucidated by analysis of its IR and ^{1}H NMR spectra. Its IR spectrum showed absorption bands at 3323, 3194, 3173, 1690 and 1713 cm^{-1} for 2OH, NH, and 2C=O, respectively. The ^{1}H NMR spectrum of **9** gave three singlet signals at $\delta = 12.72$, 10.52 and 9.19 ppm for 2COOH and NH protons, respectively. Moreover, there is another singlet at $\delta = 1.95$ ppm for $C\underline{H_2}CO$ protons. The structure of compound **11** was confirmed by its IR spectrum which exhibited the disappearance of the acidic carbonyl band at 1710 cm^{-1}. This indicates that the carboxylic group was condensed with the amino group to form an

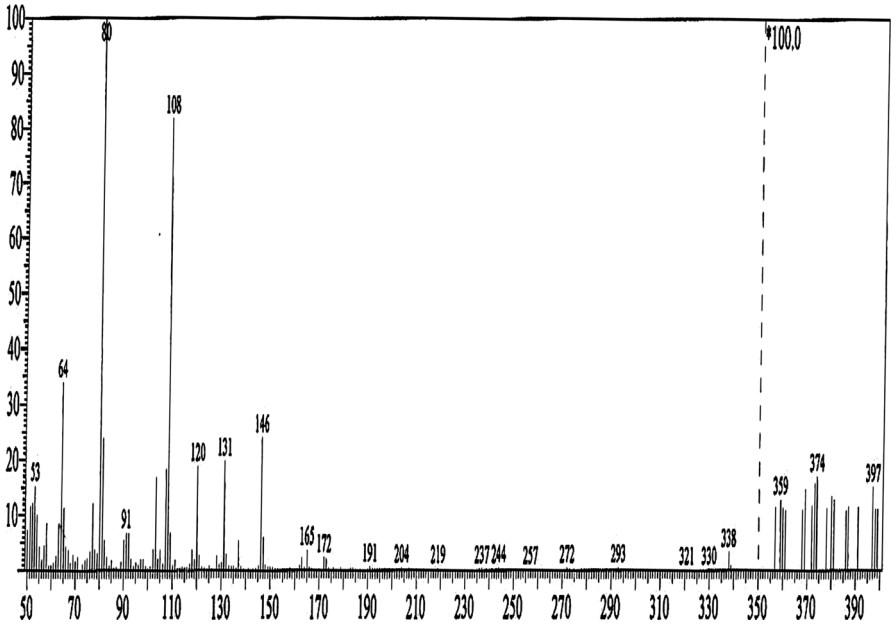
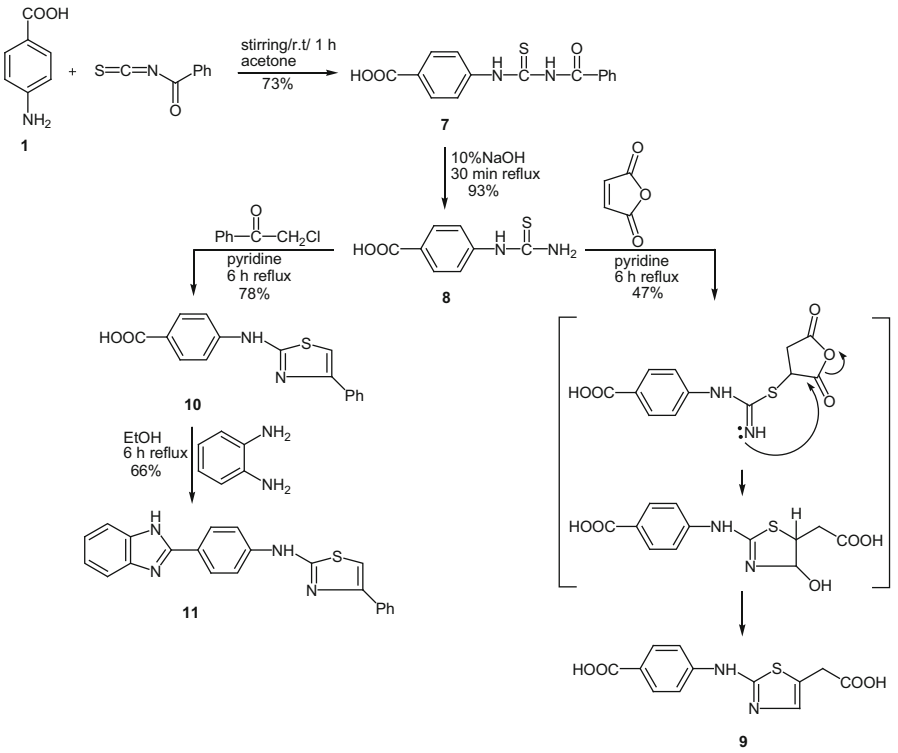


Fig. 4 The MS spectrum of **6**



Scheme 2 Synthesis of thiazole **9** and benzimidazole derivatives **11**

imidazole ring. In addition, there were two bands at 3440 and 3315 cm^{-1} for the 2NH groups. The ^1H NMR spectrum of **11** showed a singlet signal at $\delta = 10.72$ ppm for NH which was exchangable with D_2O. There was an inferred multiplet at $\delta = 7.41–7.93$ ppm for the aromatic protons and SC<u>H</u> = group (Fig. 5).

Acetylation of *p*-amino benzoic acid (**1**) with chloroacetyl chloride resulted in arylchloroacetyl chloride **12** in 77% yield. Reflux of compound **12** with ammonium thiocyanate in the presence of sodium ethoxide produced thiazole derivative **13** in 51% yield (Scheme 3). Refluxing of **1** with chloro acetyl isothiocyanate in dry acetone for 1 h gave imidazole derivative **14** in 48% yield. Condensation of compound **1** with acetylacetone and/or ethylacetoacetate in the presence of ethanol and acetic acid produced enaminone **15** and crotonate **16** derivatives in 55 and 59% yields, respectively (Scheme 3).

The IR spectrum of compound **13** showed absorption bands at 3411, 3346, 1674 and 1708 cm^{-1} for OH, NH, amidic and acidic C=O, respectively. Its ^1H NMR spectrum exhibited three singlet signals at $\delta = 12.64$, 10.69 and 4.16 ppm for COOH, NH and CH_2 protons, respectively. The aromatic protons appeared as a multiplet at $\delta = 7.64–7.89$ ppm. The structures of compounds **14** and **15** are in agreement with their analysis data. The IR spectrum of **16** showed absorption bands

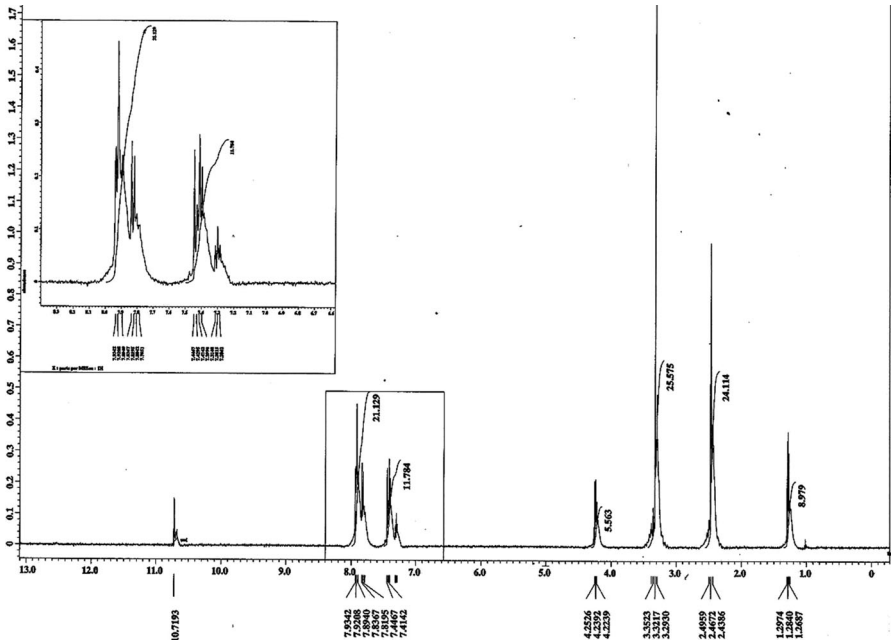
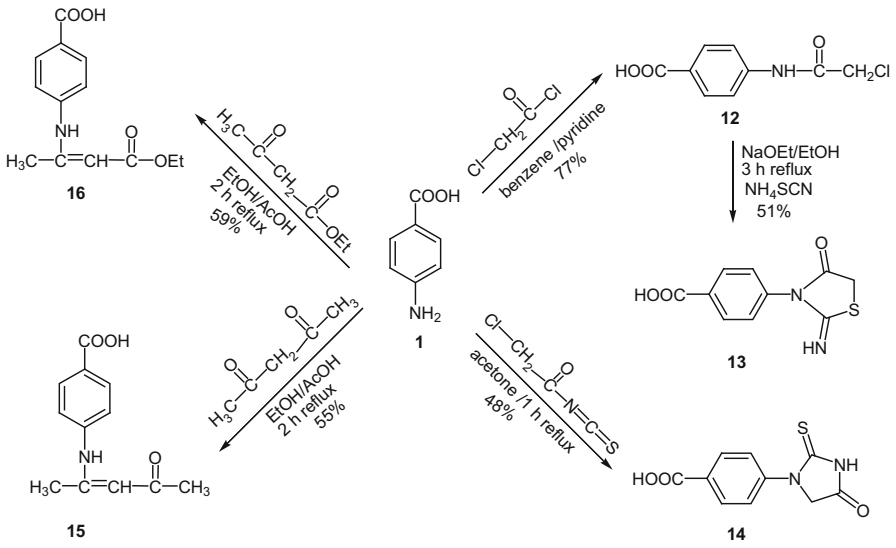


Fig. 5 The ^{1}H NMR spectrum of **11**



Scheme 3 Acetylation and condensation of *p*-amino benzoic acid **1** with different reagents

at 3465, 3383, 1705 and 1683 cm^{-1} for OH, NH and 2C=O, respectively. In its ^{1}H NMR spectrum, there is no signal for the NH_2 group but a singlet signal $\delta = 10.74$ ppm for NH. This emphasizes that the reaction occurred at the amino

group not the carboxylic one. Moreover, there was another singlet at $\delta = 4.79$ ppm for the =CH proton (Scheme 3).

Stirring a mixture of benzoyl isothiocyanate and enamine **15** and/or crotonate derivatives **16** in acetone and a few drops of TEA for 30 min, pyridine-2-thione derivative **17** was obtained in 55 and 85% yields, respectively (Scheme 4).

The IR spectrum of compound **17** showed absorption bands at 3380, 3257 and 1697 cm^{-1} for OH, NH, SH and C=O, respectively. The ^1H NMR spectrum of **17** displayed two broad singlets at $\delta = 12.74$ and 11.65 for 2NH protons which were also exchangable with D_2O (Fig. 6). There was a multiplet at $\delta = 7.51$–7.95 ppm for aromatic protons.

Moreover, the mass spectrometry of **17** is in agreement with its structure as well as with its molecular weight (Fig. 7).

The proposed mechanism for the building of pyridine-2-thione derivative **17** showed the formation of an acyclic thioamide intermediate. Subsequently, dehydration followed by deacetylation took place (Scheme 5).

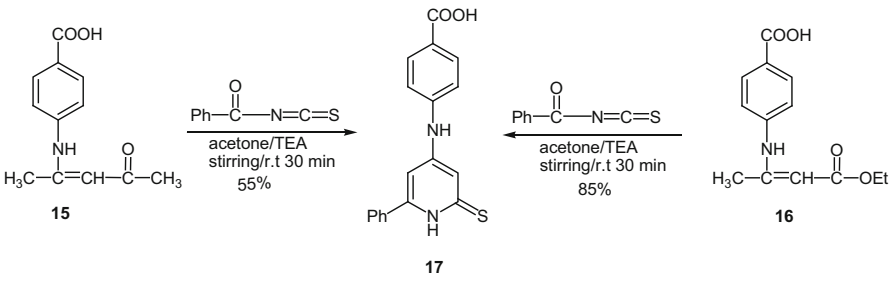
Pharmacological studies

Antitumor activity tests

The effect of the newly synthesized compounds **3**, **4**, **10**, **13** and **14** was evaluated on the in vitro growth of a human tumor cell line, namely breast adenocarcinoma (MCF-7), after a continuous exposure for 48 h. The results are introduced in Table 1 and Fig. 8.

The results are given in concentrations that were able to cause 50% of cell growth inhibition (GI_{50}) after a continuous exposure of 48 h and show mean ± SEM of three-independent experiments performed in duplicate.

All the examined compounds were able to inhibit the growth of the tested human tumor cell line in a dose-dependent manner. The results indicated in Table 1 and Fig. 8 reveal that compound **4** showed the highest inhibitory effect against the MCF-7 cell line, and that such activity is higher than the reference doxorubicin. Meanwhile, compound **14** showed high inhibitory effects against the MCF-7 cell line, which is less than the corresponding reference doxorubicin. Compound **13**



Scheme 4 Synthesis of pyridine-2-thione derivative **17**

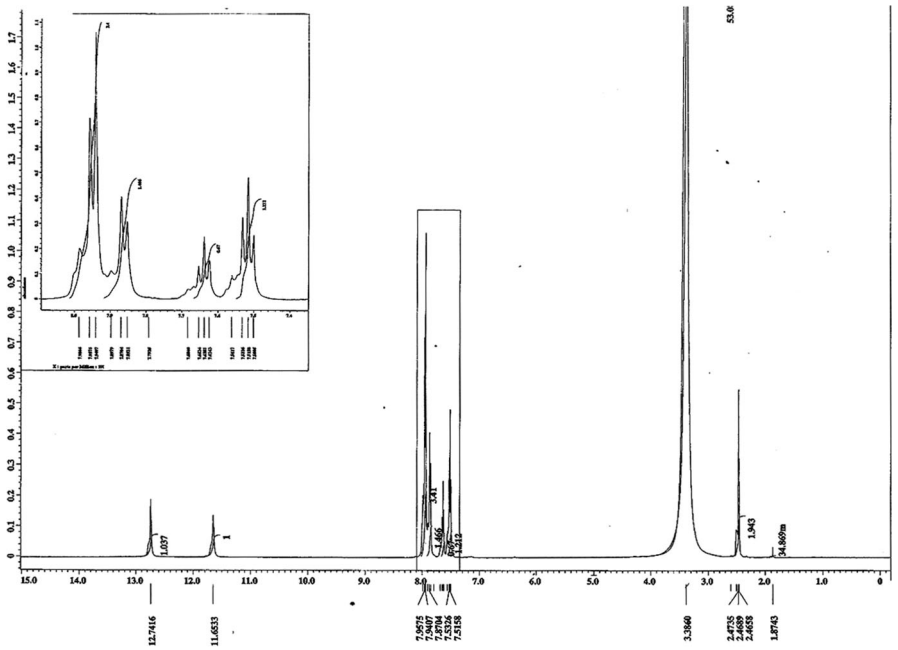


Fig. 6 ^{1}H NMR spectrum of compound **17**

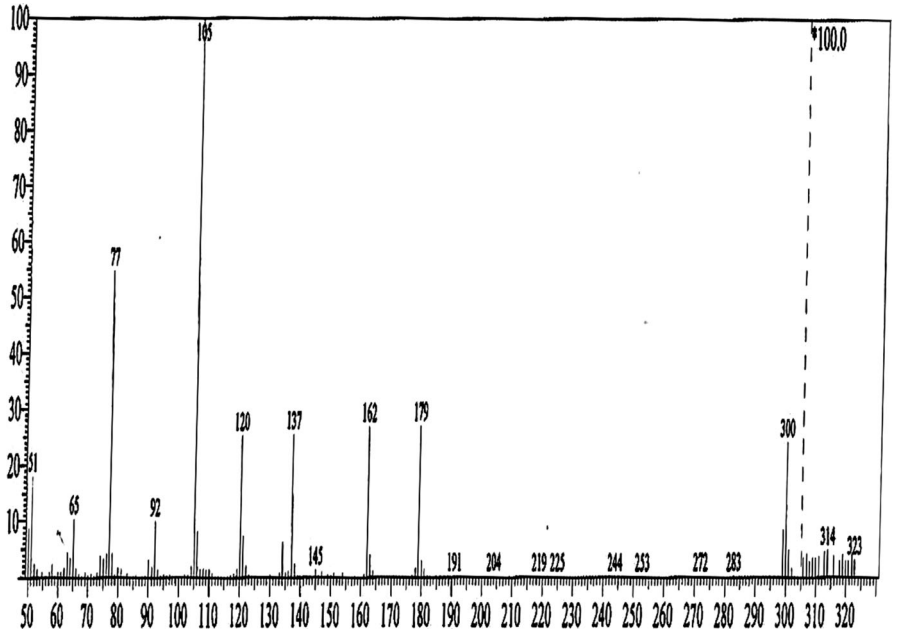
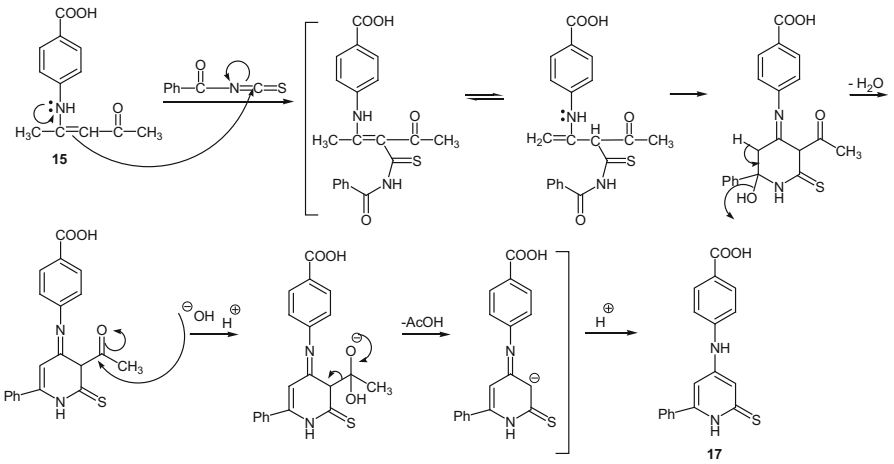


Fig. 7 MS spectrum of **17**



Scheme 5 Suggested mechanism for the building of pyridine-2-thione derivative **17**

Table 1 Effect of the obtained compounds on the growth of MCF-7 cell line

Compound	MCF-7 GI_{50} (μg L^{-1})
3	20.4 ± 6.8
4	0.01 ± 0.001
10	22.22 ± 4.12
13	30.7 ± 8.60
14	2.6 ± 1.14
Doxorubicin	0.04 ± 0.008

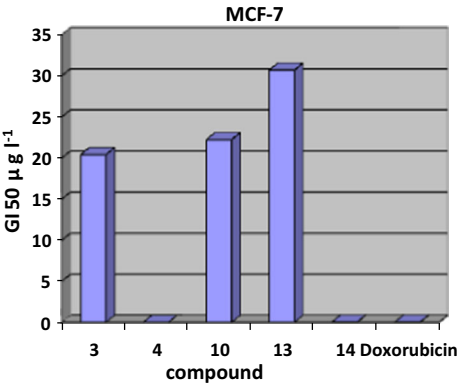


Fig. 8 The inhibitory activities against the MCF-7 cell line

showed the lowest inhibitory effect, while the compounds **3**, **10** exhibited a moderate growth inhibitory effect.

The cytotoxic and antitumor activities of compounds **3**, **4**, **10**, **13** and **14** were tested against MCF-7 cell line. The inhibitory activities were detected by using different concentrations of the tested compounds (0.10, 1.00 and 10.0 μg) and the

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	
3	72.56 ± 2.44	70.12 ± 4.60	63.31 ± 4.22	20.32 ± 3.55
4	52.32 ± 4.69	56.99 ± 6.50	52.29 ± 3.94	0.01 ± 0.006
10	78.26 ± 8.22	77.44 ± 4.29	70.18 ± 4.68	22.50 ± 2.35
13	78.26 ± 6.29	72.21 ± 3.98	70.23 ± 5.56	30.29 ± 5.44
14	93.26 ± 4.22	90.20 ± 3.66	82.09 ± 6.41	2.59 ± 0.03

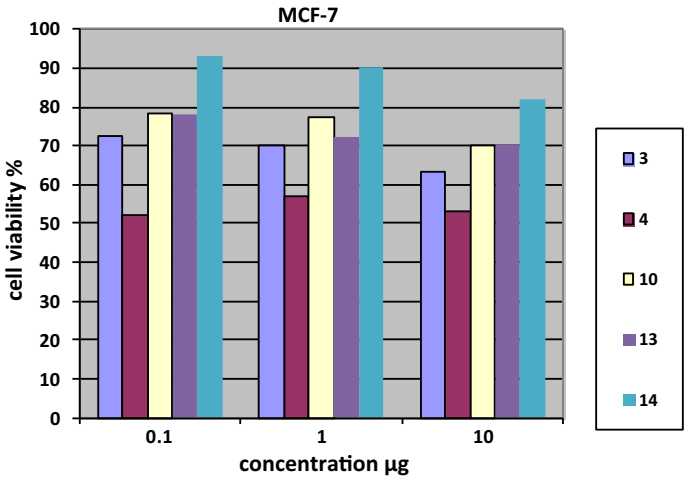


Fig. 9 The cytotoxic activities against the MCF-7 cell line

viability cells (%) were determined by colorimetric method. Also, the IC_{50} was calculated (Table 2; Fig. 9).

The results presented in Table 2 and Fig. 9 reveal that compounds **4** and **14** have very strong cytotoxic antitumor activity, compounds **3** and **10** have strong cytotoxic antitumor activity and compound **13** has moderate cytotoxic antitumor activity against the MCF-7 cell line.

Conclusion

In this study, we succeeded in synthesizing some novel condensed azoles and azines via the addition of *p*-aminobenzoic acid to a heteroallene such as isothiocyanate. Some of the compounds exhibited very good antitumor activity in the MCF-7 cell line. The cytotoxicity of compound **4** is the most active one towards the cancer cell line where its reactivity is more than that of the standard doxorubicin (anticancer reference drug). The obtained results are considered as a promotive force to encourage us for further work on such a ring system.

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