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Heterocyclization of Isoniazid: Synthesis and Antimicrobial Activity of Some New Pyrimidine, 1, 3-Thiazole, 1, 2, 4-Thiadiazole, and 1, 2, 4-Triazole Derivatives Derived from Isoniazid

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THE REACTION of isonicotinic hydrazide (**1**) (isoniazid) with cinnamoyl isothiocyanate (**2**) afforded cinnamoyl thiosemicarbazide derivative **3**. Treatment of **3** with lead acetate in acetic acid, sodium ethoxide, sulphuric acid, chloroacetylchloride and sodium hypochlorite and sodium hydroxide gave the corresponding dihydropyrimidine **4**, triazolethiazine **5**, 1,3,4-thiadiazole **6**, 1,3-thiazole **7** and 1,2,4-thiadiazole **8**, respectively. The reactivity of isoniazid **1** towards ammonium thiocyanate, cyclohexanone and acetophenone to give 1,2,4-triazole thione **10**, hydrazones **9** and **12** was studied. Treatment of hydrazones **9** and **12** with carbon disulfide and aryl isothiocyanates gave 1,3,4-thiadiazolidine **11** and 1,2,4-triazole thione derivatives **13a, b**. The antimicrobial activity of these new compounds has been evaluated against 6 microbial strains. Some of the newly synthesized compounds showed moderate activity.

Keywords: Isonicotinichydrazide, Triazolothiazine, Thiadiazole, Triazolethione, Dihydropyrimidine, Thiazole, Antimicrobial activity.

Introduction

Isoniazid is a manufactured antimicrobial and a standout amongst the most vital first-line drugs utilized as a part of the treatment of tuberculosis (TB). Indeed, even after its discovery more than 60 years ago, the drug stays at the front line of the anti-tuberculosis treatment mainly due to its potency and high selectivity against *Mycobacterium tuberculosis* [1-4]. In 2016, the World Health Organization (WHO) announced that there were around 10.4 million new TB cases in worldwide with approximately 1.3 million TB deaths among HIV negative people and 0.374 million deaths among HIV-positive individuals[5-7]. Therefore, there is a critical need to synthesise new anti-tubercular drugs (ATDs), which will be powerful against all forms of TB, in all individuals and in all locations of the world.

A survey of literature reveals that the heterocyclic compounds that contain 1,2,4-triazole rings and 1,3,4-thiadiazole rings possess antimicrobial activity [8-11]. For instance, substituted 1,2,4-triazole ring such as vorozole and anastraole had been used in chemotherapy for treatment of breast cancer [12-15]. In light of above facts, The aim of the present work is to elucidate the chemistry of thiocarbamoyl derivatives **3**, which prepared by the reaction of isonicotinic acid hydrazide **1** (Isoniazid) and cinnamoyl isothiocyanate **2**, and also to study its importance as versatile reagent in the synthesis of such functionalized new heterocyclic compounds. This study reports on the synthesis of several new thiazole, thiadiazoles, triazoles, triazolothiazine and pyrimidine derivatives by the reaction of thiocarbomyl derivatives **3** with various reagents. These newly synthesized compounds have not been reported previously, and were prepared in good yields under very mild conditions.

Experimental

Chemistry

Melting points were measured using an Electrothermal IA 9100 apparatus with open capillary tube and are uncorrected. All experiments were carried out using drying solvents. Products were purified by recrystallization. The IR spectrum (KBr disc) was recorded on a Pye Unicam Sp-3-300 or a Shimadzu FTIR 8101 PC infrared spectrophotometer. The ^{1}H NMR 400 MHz and ^{13}CNMR 100 MHz spectrum were measured on a JEOL-JNM-LA spectrometer using DMSO 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. Analytical data were obtained from the Microanalysis Center at Cairo. The mass

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spectra were recorded on an MS-S988 instrument operating at 70 eV.

N-(2-isonicotinoylhydrazinecarbonothioyl) cinnamamide (3)

A mixture of cinnamoyl isothiocyanate **2** (18.92 g, 0.1 mol) and isonicotinic acid hydrazide **1** (13.71 g, 0.1 mol) in dioxane (50 mL) was heated under reflux for 1 hr. The precipitate obtained after pouring onto water was crystallized from ethanol to give compound **3** as yellow powder. Yield 71% m.p. 278-280 °C. Elem. Anal. Calcd. (%) for $C_{16}H_{14}N_4O_2S$ (326.37): C, 58.88; H, 4.32; N, 17.17; S, 9.82. Found: C, 58.9; H, 4.40; N, 17.2; S, 9.83. IR (KBr, υ, cm^{-1}): 3194 (NH), 2653 (SH), 1686 (C=O), 1668 (C=O), 1631 (C=N), 1215 (C=S). ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm): 13.62 (br. s, 1H, SH), 12.26 (s, 1H, NH), 11.76 (s, 1H, NH), 11.42 (s, 1H, NH), 8.81- 8.77 (d, 1H, J = 16 Hz, CH=CH), 8.70-7.48 (m, 9H, CH of phenyl ring and pyridine ring), 7.089-7.049 (d, 1H, J = 16 Hz, CH=CH).

N-(4-oxo-6-phenyl-2-thioxo-3,4-dihydropyrimidin-1(2H)-yl)isonicotinamide (4)

A mixture of compound **3** (0.326 g, 1 mmol) and lead acetate (0.321 g, 1 mmol), in acetic acid (30 mL)was refluxed for 2 hr then the mixture was poured onto water and stirred for 15 min. The white powder of compound **4** was obtained after recrystallization from acetic acid. Yield 66%, m.p. 359-360 °C. Elem. Anal. Calcd. (%) for: $C_{16}H_{12}N_4O_2S$ (324.07): C, 59.25; H, 3.73; N, 17.27; S, 9.89. Found: C, 59.30; H, 3.80; N, 17.30; S, 9.90. IR (KBr, υ, cm^{-1}): 3150 (NH), 1689 (C=O), 1634 (C=O), 1217 (C=S). ^{1}HNMR (400 MHz, DMSO-d_6) δ (ppm): 12.01 (br. s, 1H, NH), 12.00 (br. s, 1H, NH), 8.75-8.74 (d, 2H, J = 4.8Hz, CH of C_2 and C_6 of pyridine ring), 7.94-7.93 (d, 2H, J = 4.8 Hz, CH of C_3 and C_5 of pyridine ring), 7.85-6.95 (m, 6H, CH of phenyl ring, CH of pyrimidine ring). ^{13}CNMR (100 MHz, DMSO-d_6) δ (ppm): 172.06 (C=S), 163.64 (C=O), 160.00 (C=O), 150.74, 143.72, 137.16, 134.03, 130.68, 129.142, 128.18, 120.80, 118.90 (Ar-C).

7-Phenyl-3-(pyridin-4-yl)-5H-[1,2,4] triazolo[3,4-b][1,3]thiazin-5-one (5)

A mixture of compound **3** (0.326 g, 1 mmol) and sodium ethoxide (0.015 mol in 30 mL ethanol) was heated under reflux for 2 hr. the content of flask was poured onto water to give a yellow powder that collected by filtration and recrystallized from acetic acid to give compound **5** as yellow powder. Yield 80%, m.p. 358-360 °C. Elem. Anal. Calcd. (%) for $C_{16}H_{10}N_4OS$ (306.34): C, 62.73; H, 3.29; N, 18.29; S, 10.47. Found: C, 62.8; H, 3.3; N, 18.3; S, 10.5. IR (KBr, υ, cm^{-1}): 1641 (C=O), 1554 (C=N). ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm): 8.52-8.53 (d, 2H, J = 1.5 Hz, CH of C_2 and C_6 of pyridine ring), 8.51-8.52 (d, 2H, J = 1.5 Hz, CH of C_3 and C_5 of pyridine ring), 7.77-6.48 (m, 6H, CH of phenyl ring, CH of thiazine ring). ^{13}C NMR (100 MHz, DMSO-d_6) δ (ppm): 172.55 (C=O), 169.09, 167.23, 150.92, 149.69, 135.57, 139.21, 128.92, 128.72, 127.45, 125.13, 119.48 (Ar-C).

N-(5-(pyridin-4-yl)-1,3,4-thiadiazol-2-yl) cinnamamide (6)

A solution of compound **3** (1 mmol) and sulphuric acid (5 mL) in ethanol (15 mL was refluxed for 2 hr. The precipitate was obtained after pouring onto water and recrystallized from acetic acid and to give compound **6** as a white powder. Yield 55%, m.p. 338- 340°C. Elem. Anal. Calcd. (%) for $C_{16}H_{12}N_4OS$ (308.36): C, 62.32; H, 3.92; N, 18.17; S, 10.40. Found: C, 62.4; H, 3.93; N, 18.2; S, 11.0. IR (KBr, υ, cm^{-1}): 3431 (NH), 1689 (C=O), 1634 (C=N). ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm): 11.94 (br. s, 1H, NH), 8.75- 8.74 (d, 2H, J = 4Hz, CH of C_2 and C_6 of pyridine ring), 7.94-7.93 (d, 2H, J = 4 Hz, CH of C_3 and C_5 of pyridine ring), 7.85-7.81 (d, 1H, J = 16 Hz, CH=CH), 7.68-7.48 (m, 5H, CH of phenyl ring), 6.99-6.95 (d, 1H, J =16 Hz, CH=CH). ^{13}C NMR (100 MHz, DMSO-d_6) δ (ppm): 163.57 (C=O), 159.96 (C=N), 159.89 (C=N) 143.82, 150.75, 134.00, 137.13, 130.72, 129.72, 128.20, 120.82, 118.78 (Ar-C and ethene-C) .

N'-(3-cinnamoyl-4-hydroxythiazol-2(3H)-ylidene)isonicotinohydrazide (7)

A mixture of compound **3** (1 mmol) and chloroacetylchloride in the presence of sodium acetate (1 mmol), in dioxane (15 mL) was left at room temperature overnight, then the content of flask was poured onto water and stirred for 15 min. The yellow powder of compound **7** was obtained after recrystallization from ethanol. Yield 54%, m.p.253-255°C. Elem. Anal. Calcd. (%) for $C_{18}H_{14}N_4O_3S$ (366.39): C, 59.01; H, 3.85; N, 15.29; S, 8.75. found: C, 60; H, 3.9; N, 15.3; S, 8.8. IR (KBr, υ, cm^{-1}): 3394 (NH), 1681 (C=O), 1627 (C=O). ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm): 13.17 (s, 1H, OH), 12.93 (s, 1H, NH), 8.81-8.79 (d, 2H, J = 6 Hz, CH of C_2 and C_6 of pyridine ring), 8.06-8.05 (d, 2H, J = 6 Hz, CH of C_3 and C_5 of pyridine ring), 7.87-7.83 (d, 1H, J =

15Hz, CH=CH), 7.83-7.47 (m, 5H, CH of phenyl ring), 6.99-6.95 (d, 1H, *J* = 15 Hz, CH=CH), 5.18 (s, 1H, CH of thiazole ring). ^{13}CNMR (100 MHz, DMSO-d_6) δ (ppm): 164.18 (OH-C=), 164.06 (C=O), 161.10 (C=N), 148.19, 144.56, 144.25, 134.44, 134.39, 131.28, 131.17, 129.59, 119.18, 58.72(Ar-C, ethene-C and -CH=).

N'-(3-((E)-styryl)-1, 2, 4-thiadiazol-5(4H)-ylidene) isonicotino hydrazide (8)

A solution of compound **3** (1 mmol), sodium hypochlorite (5 mL), in the presence of NH_4OH (10 mL), and NaOH (5 mL) was left at room temperature overnight, The precipitate was obtained after pouring onto water and recrystallized from ethanol and to give compound **8** as a yellow powder. Yield 45%, m.p. 135-137 °C. Elem. Anal. Calcd. (%) for $C_{16}H_{13}N_5OS$ (323.37): C, 59.43; H, 4.05; N, 21.66; S, 9.92. Found: C, 59.5; H, 4.1; N, 21.7; S, 10.1. IR (KBr, υ, cm^{-1}): 3055 (NH), 1685 (C=O), 1631 (C=N). ^{1}HNMR (400MHz, DMSO-d_6) δ (ppm): 12.41 (br. s, 2H, 2NH), 7.70-7.69 (d, 2H, *J* = 3 Hz, CH of C_2 and C_6 of pyridine ring), 7.68-7.68 (d, 2H, *J* = 3 Hz, CH of C_3 and C_5 of pyridine ring), 7.62-7.58 (d, 1H, *J* = 16 Hz, CH=CH), 7.43- 7.42 (m, 5H, CH of phenyl ring), 6.56-6.52(d, 1H, *J* = 16 Hz, CH=CH), ^{13}C NMR (100 MHz, DMSO-d_6) δ (ppm): 168.91 (C=O), 144.45 (C=N), 150.01(C=N), 144.56, 144.25, 134.44, 134.60, 134.39, 131.28, 130.74, 129.41, 119.59 (Ar-C and ethene C).

N'-(1-phenylethylidene)isonicotinohydrazide (9) [16]

A mixture of compound **1** (10 mol) and acetophenone (10 mol) in ethanol were refluxed for 5 hrs. The precipitate obtained was filtered off, washed and recrystallized from ethanol to give compound **9** as pale yellow crystals. Yield 90%, m.p.182-185°C.

5-Methyl-5-phenyl-2-thioxo-1,3,4-thiadiazolidin-3-yl)(pyridin-4-yl)methanone (10)

A mixture of compound **9** (1 mmol), carbon disulfide (1 mmol) and KOH (1 mmol) was boiled under reflux for 1 hr the content of the flask, and then acidified by dilute acetic acid (20 mL, 1:10) orange powder of compound **10** was obtained after recrystallization from of ethanol. Yield 62%, m.p. 159-160 °C. Elem. Anal. Calcd. (%) for $C_{15}H_{13}N_3OS_2$ (315.41): C, 57.12; H, 4.15; N, 13.32; S, 20.33. Found: C, 57.3; H, 4.2; N, 13.4; S, 20.4. IR (KBr, υ, cm^{-1}): 1646 (C=O), 1282 (C=S). ^{1}HNMR (400 MHz, DMSO-d_6) δ (ppm): 11.02 (s, 1H, NH), 8.81-8.79 (d, 2H, *J* = 6 Hz, CH of C_2 and C_6 of pyridine ring), 8.77-8.76 (d, 2H, *J* = 6 Hz, CH of C_3 and C_5 of pyridine ring), 7.91-7.35 (m, 5H, CH of phenyl ring), 2.27 (s, 3H, CH_3 out of the plane), 2.38 (s, 3H, CH_3 in the plane).

5-(Pyridin-4-yl)-3H-1,2,4-triazole-3-thione (11)

A solution of compound **1** (0.01mol) and ammonium thiocyanate (0.01mol**)** in acetic acid was heated under reflux for 2 hr, the content of the flask was poured on $NaHCO_3$/H_2O (1:10), concentrated and left at room temperature overnight to give a white powder of compound **11**, that was filtered, dried and recrystallized from acetic acid. Yield 45%, m.p. 338-340°C. Elem. Anal. Calcd. (%) for $C_7H_4N_4S$ (176.20): C, 47.72; H, 2.29; N, 31.80; S, 18.20. Found: C, 47.8; H, 2.3; N, 31.9; S, 18.3. IR (KBr, υ, cm^{-1}): 1666 (C=N), 1302 (C=S). ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm): 8.73-8.72 (d, 2H, *J* = 5.6Hz, CH of C_2 and C_6 of pyridine ring), 7.81-7.80 (d, 2H, *J* = 5.6 Hz, CH of C_3 and C_5 of pyridine ring), 18.3. MS: *m/z*: 176(M^+, 5%), 51(100%).

N'-cyclohexylidenebenzohydrazide (12) [17]

A mixture of compound **1** (1 mmol) and cyclohexanone (1 mmol) in ethanol were refluxed for 5 hrs. The reaction mixture was concentrated and left at room temperature. The crude product obtained on re-crystallization from alcohol to give the hydrazone of isonicotinic acid as yellow powder **12**. Yield 60%, m.p. 180-182°C.

(3-Phenyl-5-thioxo-4,5-dihydro-1H-1,2,4-triazol-1-yl)(pyridin-4-yl)methanone (13a)

A mixture of compound **12** (1 mmol), benzoyl isothiocyanate (1 mmol) in acetone was heated under reflux for 1 hr, then keep at refrigerator overnight and then the mixture was filtered off and recrystallized from ethanol to give a white powder of compounds **13a**. Yield 51%**,** m.p. 278-280 °C. Elem. Anal. Calcd. (%) for $C_{14}H_{12}N_4OS$ (284.34): C, 59.14; H, 4.25; N, 19.70; S, 11.28. Found: C, 59.2; H, 4.35; N, 19.80; S, 11.38. IR (KBr, υ, cm^{-1}): 3204 (NH), 1668 (C=O), 1217 (C=S). ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm): 12.32 (s, 1H, SH), 11.81 (s, 1H, NH), 8.81-8.80 (d, 2H, *J* = 2 Hz, CH of C_2 and C_6 of pyridine ring), 8.79-8.78 (d, 2H, *J* = 2 Hz, CH of C_3 and C_5 of pyridine ring), 8.00-7.53 (m, 5H, CH of phenyl ring). ^{13}C NMR (100 MHz, DMSO-d_6) δ (ppm): 162.56 (C=S), 157.94 (C=O), 149.94 (C=N), 149.74, 138.12, 135.00, 130.71, 128.14, 127.19, 119.70 (Ar-C).

Pyridin-4-yl(3-styryl-5-thioxo-2,5-dihydro-1H-1,2,4-triazol-1-yl)methanone (13b)

This compound was prepared by the same method used in the synthesis of 13a by using cinnamoylisothiocyanate instead of benzoyl isothiocyanate. Yield 51%, m.p. 327-330°C. Elem. Anal. Calcd. (%) for $C_{16}H_{12}N_4OS$ (308.36): C, 62.32; H, 3.92; N, 18.17; S, 10.40. found: C, 62.4; H, 4; N, 18.2; S, 11. IR (KBr, υ, cm^{-1}): 3463 (NH), 1687 (C=O). ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm): 13.09 (s, 1H, NH), 8.75- 8.74 (d, 1H, CH=CH), 7.95-7.48 (m, 9H, CH of phenyl ring, CH of pyridine ring), 6.99-6.95 (d, 1H, CH=CH). ^{13}C NMR (100 MHz, DMSO-d_6) δ (ppm): 163.55 (C=S), 159.95 (C=O), 159.87(C=N), 150.74, 137.12, 134.00, 134.60, 130.71, 129.14, 128.19, 120.70, 118.77 (Ar-C and ethene-C).

Antimicrobial activity

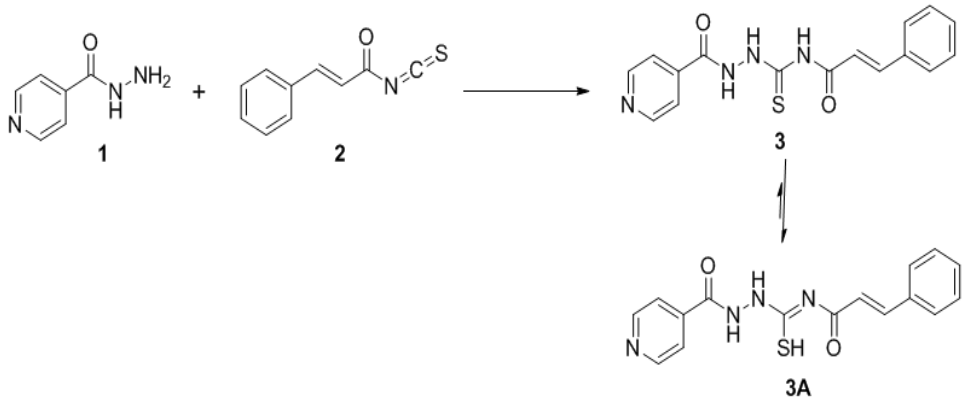
The Susceptibility tests were performed according to the National Committee for Clinical Laboratory Standards (NCCLS) recommendations. Screening tests regarding the inhibition zone were carried out by the agar-well diffusion method [18]. The inoculum suspension was prepared from colonies grown overnight on an agar plate and inoculated into Mueller-Hinton broth (fungi using malt broth). A sterile swab was immersed in the suspension and used to inoculate Mueller-Hinton agar plates (fungi using malt agar plates). Six millimeter (6.0 mm) diameter wells were cut from the agar using a sterile cork-borer, and then 0.05 ml of concentration (5 mg/1 mL) of different tested compounds was transferred into each well. The inhibition zone was measured around each well after 24 h at 37 °C. Antimicrobial activity was evaluated by measuring the mean zone of inhibition against the test organism. Gentamycin and Ketoconazole were used as standard drugs. Dimethyl sulfoxide used as solvent controls.

Results and Discussion

Chemistry

N-(2-isonicotinoylhydrazinecarbonothioyl) cinnamamide [19] (**3**) was obtained by the reaction of isonicotinic acid hydrazide **(1)** (isoniazid) with cinnamoyl isothiocyanate (**2**) (prepared through reaction of cinnamoyl chloride with ammonium thiocyanate in dioxane) [20] *via* michael addition followed by hydrogen transfer and subsequent 1,3-H shift as depicted in the following mechanism (**Scheme 1**). The ^{1}H NMR spectrum of compound **3** showed a deshielded broad signal due to –SH group at 13.626 ppm (D_2O-exchangeable). It is interesting to note that thiocarbonyl compound is present in their thion-thiol tautomeric form solution as indicated by IR and ^{1}H NMR spectra. D_2O-exchangeable NH signals were observed at 12.264 ppm, 11.761 ppm and 11.429 ppm in different electronic density. The downfield vinyl proton was shown at δ = 8.808 ppm with J = 16 Hz. The multiplet signal at δ = 7.479-8.796 ppm was assigned to aromatic and pyridine protons. The second *trans* vinyl proton was resonated at δ = 7.089 ppm with J = 16 Hz. The IR spectrum of compound **3** displayed signals for NH, SH, 2 C=O and C=N at 3194, 2653, 1686, 1668 and 1631 cm^{-1}.

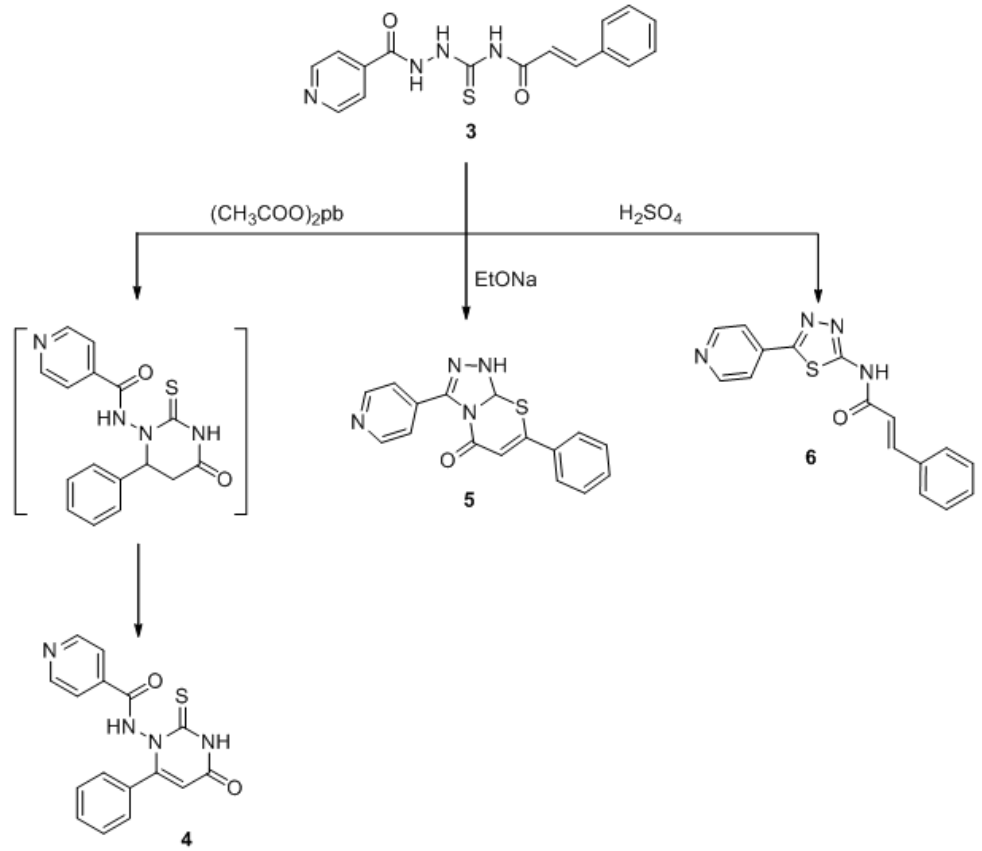
The pyrimidine **4** was obtained from cyclization of cinnamoyl thiosemicarbazide **3** by heating with lead acetate in acetic acid as shown in **Scheme 2**. ^{1}H NMR was in agreement with the pyrimidine structure which showed two NH signals at 12.01 ppm and 12.00 ppm (controlled by changing D_2O) in addition to the aromatic protons. IR spectrum showed two carbonyls



Scheme1. Synthesis of N-(2-isonicotinoylhydrazine-1-carbonothioyl) cinnamamide (3).

stretching frequency at 1689 and 1634 cm^{-1} in addition to C=S function at 1217 cm^{-1}. ^{13}C NMR of compound **4** showed signals at 172.06, 163.64 and 160 ppm of the carbons of C=S and 2 C=O sp^2 carbon. The cyclization reaction of compound **3** using sodium ethoxide was also studied. Thus, heating of thiosemicarbazide with sodium ethoxide furnished triazolothiazine **5** through thiazine cyclization followed by cyclocondensation and subsequent dehydration as demonstrated in **Scheme 2**. ^1H NMR of condensed compound **5** revealed no NH signals. Aromatic hydrogens were observed as a multiplet signal at 8.53-6.47 ppm. ^{13}C NMR of compound **5** showed signals at 172 and 169 ppm due to the C=O and C=N sp^2 carbons. The 1,3,4- thiadiazole derivative **6** was obtained as a result of treatment of compound 3 by H_2SO_4 *via* an attack of SH nucleophilc to the electrophilic carbonyl carbon of nictonyl moiety followed by dehydration as shown in **Scheme 2**. ^1H NMR detected the triazole structure by the presence of D_2O-exchangeable NH signal at 11.94 ppm and also the appearance of C=O function in IR spectrum at 1689 cm^{-1} potentiates the suggested structure. In^{13}C NMR of compound **6** the C=O, C=S and C=N SP^2 carbon showed signals at 163 ppm, 159.9 ppm and 159.8 ppm.

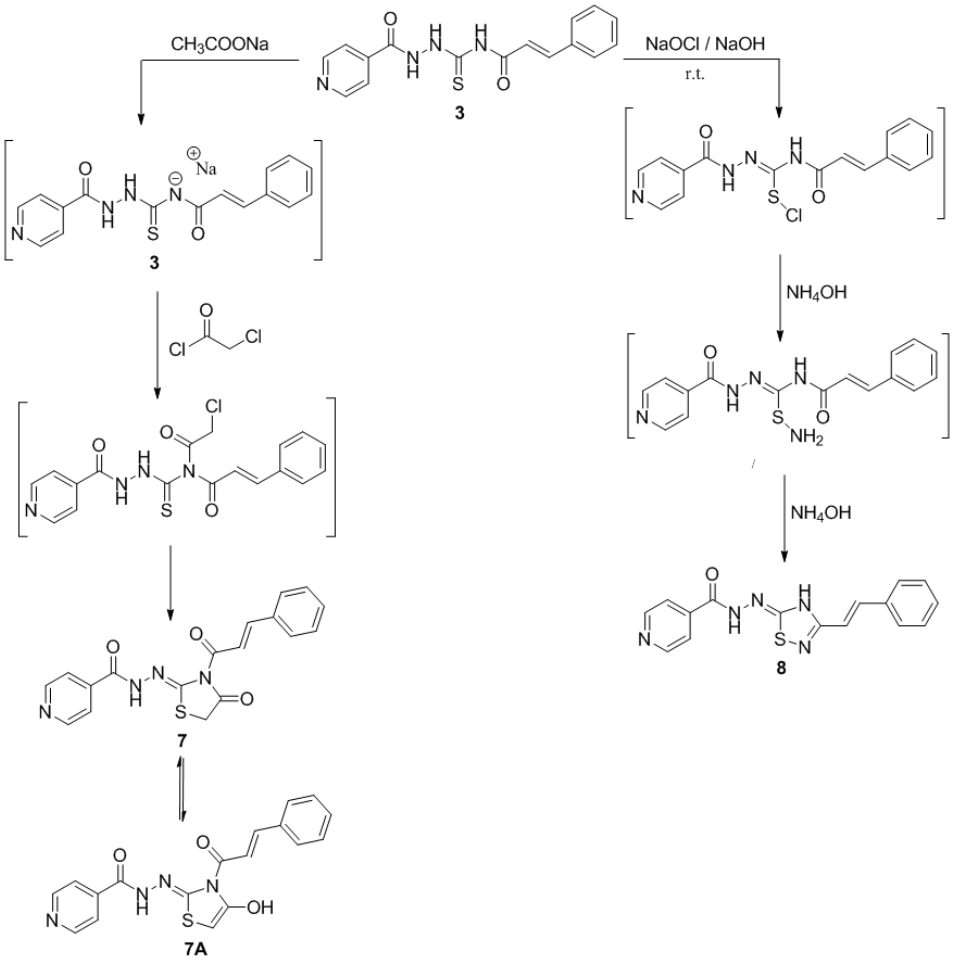
Acylation of cinnamoyl thiosemicarbazide **3** with chloroacetyl chloride in presence of sodium acetate formed thiazole derivative **7**. The reaction may proceed *via* the formation of the anion **A** of the more acidic NH followed by chloroacetylation and subsequent enolization forming the final product **7** as depicted in **Scheme 3**. The IR spectrum of compound **7** showed the characteristic broad band of the hydroxyl group at 3394 cm^{-1} also displayed two different carbonyls at 1683 and 1627cm^{-1}. The ^1H NMR spectrum of thiazole derivative **7** revealed two downfield signals at 13.17 and 12.93 ppm for OH and NH protons (D_2O-exchangeable). The deshielded vinyl proton resonated at δ = 7.866 ppm with J = 15 Hz, aromatic protons appeared at 7.031-8.062 ppm as a multiplet singnal and the other vinylic proton located at δ = 6.989 ppm with J = 15 Hz as doublet. The thiazole proton was observed at δ = 5.183 ppm as a singlet signal. Moreover, 2 C=O and C=N groups resonated at 164.19, and 160.88 ppm



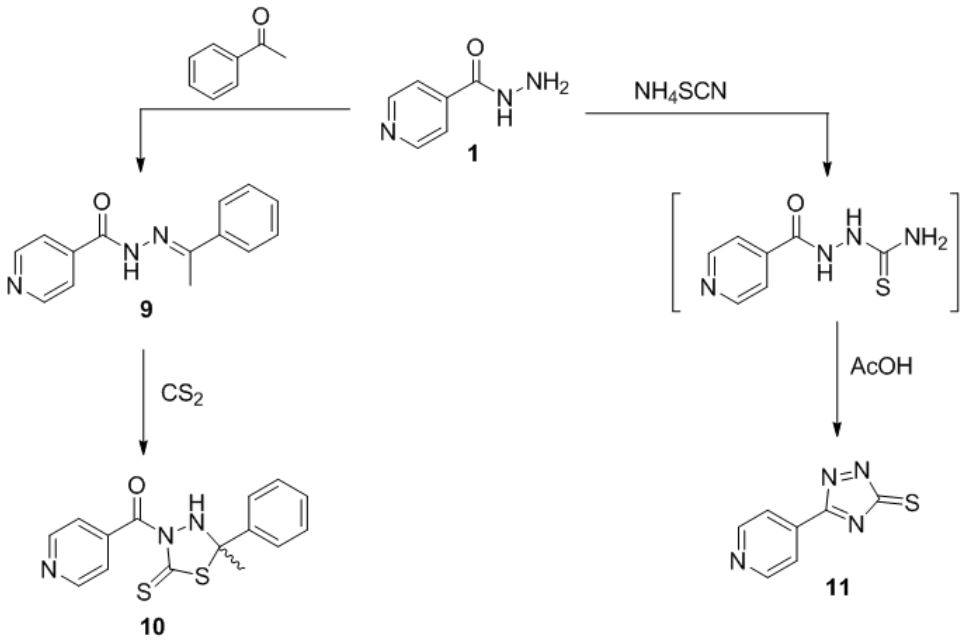
Scheme 2. Synthesis of pyrimidine 4, triazolothiazine 5 and 1,3,4-thiadiazole 6.

in the ^{13}C NMR spectrum of compound **7**. The reaction of thiosemicarbazide derivative **3** with sodium hypochlorite in the presence of NH_4OH and NaOH resulted in thiadiazole cyclization affording compound **8** as shown in **Scheme 3** *via* the formation of the non-isolable sulphenyl chloride, amination forming sulphenamide which in turn undergo intramolecular cyclodehydration [21]. All spectral data are consistent with the title thiadiazole derivative **8**. Thus ^1H NMR revealed broad signal at 12.41 for two acidic NH signals (D_2O-exchangeable). Two doublets signals at 6.557 ppm (J = 16 Hz) and 7.617 (J = 16 Hz) ppm are assigned to vinyl protons indicating that the ethylene in the styryl moiety is in *trans*-conformation in the compound **8**. In addition, a multiplet signal was observed at 7.432-7.416 ppm due to aromatic protons. IR spectrum of compound **8** revealed the presence of bands for NH at 3055 cm^{-1}, for C=O at 1685 cm^{-1}, at 1631 cm^{-1} due to C=N cm^{-1} and 1577 cm^{-1} C=C. ^{13}C NMR of compound **10** showed signals at 168.09 and 144.4 ppm of C=O and C=N sp^2 carbons.

Cyclization of Schiff base **9** was achieved upon the treatment of compound **9** with carbon disulfide and KOH to afford thiadiazolothione **10** as shown in **Scheme 4**. ^1H NMR of **10** showed a downfield signal at 11.020 ppm for NH (controlled by changing D_2O) in addition to aromatic and pyridine protons which observed as a multiplet at 8.807-7.349 ppm. Also, the ^1H NMR spectrum of **10** revealed two signals for CH_3 at 2.274 and 2.384 ppm. IR spectrum of **10** showed C=O band at 1646 cm^{-1} and C=S at 1282 cm^{-1}. Upon addition of hydrazide **1** to ammonium thiocyanate furnished triazolothione **11** in one step through thiosemicarbazide by *N*-cyclization followed by oxidation as illustrated in **Scheme 4**. ^1H NMR showed only the pyridine protons. IR also showed C=N band at 1666 cm^{-1} and C=S at 1302 cm^{-1}. The mass spectrum of **11** showed a peak at *m/z* 176 (M^+) corresponding to its molecular ion.

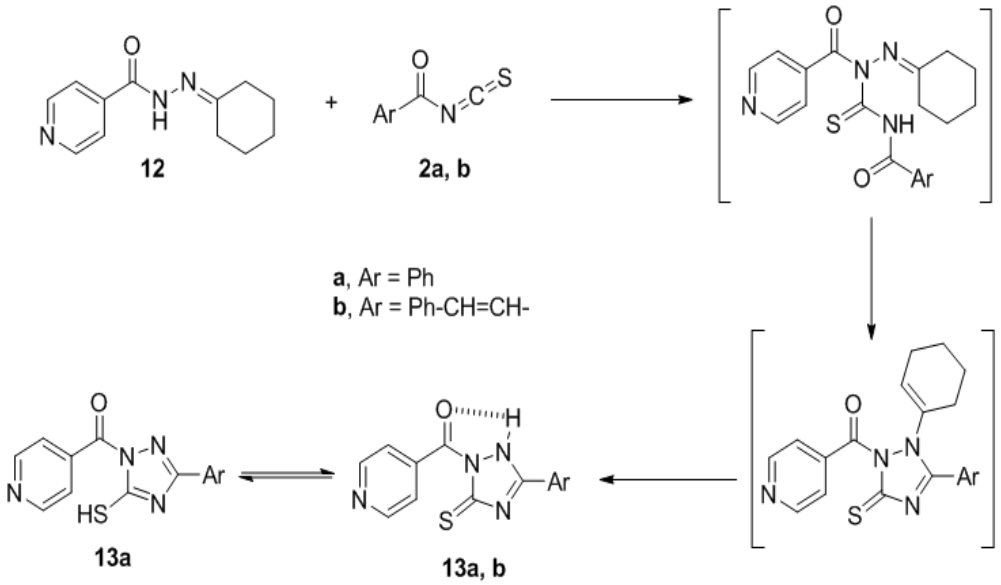


Scheme 3. Synthesis of 1,3-thiazole 7 and 1,2,4-thiadiazole 8.



Scheme 4. Synthesis of 1,3,4-thiadiazole 10 and 1,3,4-triazole 11.

The hydrazone **12** was reacted with arylisothiocyanates (Ar = Ph, PhCH=CH) to give triazolothione **13a** and **13b**. The reaction may be preceded through the formation of thiosemicarbazone followed by cyclodehydration and subsequent enaminc hydrolysis [22, 23] as illustrated in **Scheme 5**. ^{1}H NMR spectrum of **13a** showed downfield signal at 12.324 ppm for SH and 11.439 ppm for NH (controlled by changing D_2O) that indicate the compound **13a** present in thion–thiol tautameric form. Also, the C=O absorption stretching was observed in IR spectrum at 1668 cm^{-1}. ^{1}H NMR spectrum of **13b** showed downfield deshielded signal at 13.3089 ppm for NH (controlled by changing D_2O) due to intra-molecular hydrogen bond. Also, the C=O absorption stretching was observed in IR spectrum at 1687cm^{-1}. ^{13}C NMR spectra of compound **13a** and **13b** are given in the experimental section.



Scheme 5. Synthesis of 1,3,4-triazoles 13a, b.

Antimicrobial activity

All bacterial and fungi strains were obtained from the Regional Center for Mycology and Biotechnology, AL-Azhar University (Cairo, Egypt) and were as follows: *Escherichia coli* ATCC 25955, *Salmonella typhimurium* ATCC14028, *Staphylococcus aureus* (RCMB010010), *Bacillus subtilies* NRRLB-543, *Aspergillus falvus* (RCMB002002) and *Candida albicans* ATCC 10231. All the newly synthesized compounds were dissolved in dimethyl sulfoxide (DMSO) to prepare final concentration 5 mg/1 mL. The antimicrobial activity results are summarized in **Table 1**. The synthesized compounds showed weak to moderate anti-microbial activity.

TABLE 1. Screening for antimicrobial activity of synthesized compounds

Compound number	Mean inhibition zone (M. IZ mm) and ± Standard deviation (S.D.)					
	Bacteria strains				Fungi strains	
	Gram +ve		*Gram -ve*			
	S. aureus	*B. s.*	*Sal. t.*	*E.coli*	*A. falvus*	*C.albicans*
3	–	–	–	–	–	–
4	10±0.2	–	11±0.1	–	12±0.1	15±0.7
5	–	–	9±0.2	–	12±0.2	–
6	–	–	8±0.4	–	–	–
7	6±0.2	–	7±0.3	–	–	14±0.6
8	11±0.1	–	–	–	10±0.2	–
10	–	16±0.6	14±0.6	–	15±0.6	12±0.4
11	–	–	–	–	–	–
13a	14±0.4	–	–	–	–	–
13b	13±0.3	–	12±0.5	–	–	–
Gentamycin	23±0.01	16±0.02	27±0.01	33±0.01	–	–
Ketoconazole	–	–	–	–	26±0.01	30±0.01

(–) = No activity. ***S. aureus*** = *Staphylococcus aureus*, ***B. s.*** = *Bacillus subtilies,* ***Sal. t.*** = *Salmonella typhimurium*, *E.* ***coli*** = *Escherichia coli. A.* ***falvus*** =*Aspergillus falvus,* ***C. albicans.*** = *Candida albicans.*

Conclusions

This study reports the synthesis of some heterocyclic compounds containing triazolothiazine, 1,2,4-thiadiazole, 1,2,4-triazole-thione and dihydropyrimidine rings. Spectral and analytical data of the newly synthesized compounds were in good agreement with proposed chemical structure. The antimicrobial activity study revealed that compounds **4**, **5**, **6**, **7**, **8**, **10**, **13a** and **13b** showed moderate activity except compounds **3** and **11**.

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الحولقه الغير متجانسه للايزونيكوتينك هيدارزيت :تخليق و تقييم النشاط البيولوجى لمشتقات الثيازين، البيرميدين، الترايازول، الثيازول

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تفاعل الايزونيكوتينك هيدازيت **١** مع الثناموبل ايزوثيونات **٢** اعطى مشتق الثناموبل ثيوسيميهيدازيت **٣**. تفاعل المركب ٣ مع خلات الرصاص في حمض الخليك، الإيثوكسيد الصوديوم على الساخن، حمض الكبريتيك، الكلوا رواسيتل كلوريد، فى وجود صوديوم هيبو كلوريد وصوديوم هيدروكسيدو امونيوم هيدروكسيد ادى الى تخليق كل من مشتق بيريدين ٤ ، مشتق الترياز ولوثيازين **٥**و مشتق الثيادايازول٦, مشتق الثيازول **٧**، مشتق ثيادايازول ٨. تم دراسه نشاط الهيدرازيت عن طريق تفاعله مع الامونيوثيوثيانات، السيكلوهكسان و الاسيتوفينون حيث اعطى تريازولو ثيون **١٠**وهيدرازونات **٩،١٢**.فمعالجة هذه الهيدرازونات **٩،١٢** مع الكربون داى سلفيد و الاريل ايزوثيوثيانات يعطى ثيا ديازوليدين**١١**، مشتق التريازولوثيون **١٣أ ،١٣ب**. دراسه النشاط الميكروبى للمركبات اوضحت ان مركبات ٤ ،٥ ، ٦،٧،٨،١٠ ، ١٣أ، **١٣ب** لها نشاط متوسط ماعدا مركبات **٣،١١**