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Synthesis of Some Novel Substituted Nicotines and Evaluation of Their Antimicrobial Activity

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A VARIETY of some novel substituted nicotines were synthesized and characterized by spectroscopic means where nicotinic acid hydrazide **(6a)** and/or **(6b)** are used as a key intermediate in the synthesis of hydrazones **(8a-l)** and **(10a-f)** by the reaction with different aromatic aldehydes **(7a-f)** and isatines **(9a-e)**, respectively. Moreover, nicotinic acid hydrazide reacted with pentane-2,4-dione **(11)**, 2-ethoxymethylene-malononitrile **(13)** and thiosemicarbazone **(15)** generating pyrazoles **(12)**, **(14)** and 1,2,4-triazole **(16)**, respectively. The newly synthesized compounds were tested against nine microbial strains. Some of these compounds showed a significant activity against several of the microorganisms. Compounds **(8d)**, **(10b)** and **(10c)** were determined to be the most active compounds. Compound **(8d)** showed activity against *S. aureus*, *B. subtilis* and *A. flavus* with IZ = 2.4, 3.2 and 2.6 mm, respectively when compared with reference drugs (Amoxicillin, IZ = 2.3, 3.5 and Griseofulvin, IZ = 2.4 mm). Additionally, compound **(10b)** showed potent activity against *P. aeruginosa* and *P. expansum* with IZ = 2.6 and 2.8, respectively when compared with reference drugs (Amoxicillin, IZ = 2.2 mm and Griseofulvin, IZ = 2.8 mm), respectively, while **(10c)** showed the same activity as Griseofulvin against *P. expansum* with IZ = 2.8 mm. The biological activity (SAR) of the evaluated compounds and the relationship between the functional group variations are discussed.

Keywords: Antimicrobial activity, Enaminone, Isatin, Nicotines, Pyridine.

Introduction

It is well known that microbes have resisted prophylaxis which makes the infectious microbial disease a pressing problem worldwide. In recent decades, issues of multidrug-resistant microorganisms have reached a frightful level. Resistance to a variety of anti-microbial agents (β-lactam antibiotics, macrolides, quinolones, and vancomycin) among a range of clinically vital species of microorganism is turning into more and more vital world drawback [1]. It has been discovered that when active pharmacophores coupled together, they would produce novel molecular templates which are likely showed interesting biological properties [2].

One of the most important chemical compounds is pyridine and its derivatives due to its tremendous applications in the various fields [3]. Furthermore, the pyridine nucleus is an interesting building block in drug discovery. In recent times, pyridine derivatives have been shown a range of pharmacological activities, such as antibacterial, antitumor, anti parasite, and analgesic activity [4-12].

Another chemical compound which exhibit various biological activities such as anti-tuberculosis [13], anticancer [14, 15], antitumor [16, 17], antibacterial [18], anti-inflammatory [19], anticonvulsant [20], antiviral [21], antifungal [22] as well as anti-HIV activity [23]

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is orindole-1*H*-2,3-dione, Isatin. Isatin is one of the natural products found in plants of Isatis and Couroupita guianensis Aubl [24] and an important heterocyclic system, which is a core constituent of many alkaloids and drugs as well as dyes, pesticides, and analytical reagents. [25].

Interestingly, a recent literature survey has shown that attachment of a furan or thiophene moiety can considerably enhance the biological activity of candidate compounds [26]. Therefore, they are a key structural segment in many pharmaceutical and chemical compounds [27]. Thiophenes and furans compounds have been also found to show insecticidal, antibacterial, antifungal, and antioxidant activity [28, 29]. Based on the previous data which demonstrate that thiophenes, furans, pyridines, and isatins have activity against microbes and in continuation of our interest in the synthesis of biologically active heterocycles specially pyridines [30-36], the aim of this study is the synthesis of some new thiophene/furan based pyridines linked to isatin or pyrazole moiety to evaluate their possible anti-microbial potential.

Experimental

Chemistry

All melting points are measured on a SMP3 melting point apparatus and are uncorrected. IR spectra were recorded in KBr disc on a Perkin-Elmer 1650 spectrophotometer at faculty of science, CairoUniversity.^{1}H NMR (400 MHz) and ^{13}C NMR (100 MHz) spectra were measured in deuterated DMSO-d_6 or $CDCl_3$as solvent at room temperature using Bruker Avance (III)-400 MHz. Chemical shifts (δ) were reported in *ppm* to scale calibrated for tetramethylsaline (TMS), which is used as an internal standard at the Ain Shams University, Cairo, Egypt. Follow up of the reactions and checking the purity of the compounds were made by TLC on silica gel coated aluminum sheets (Type 60 GF254,Merck) and the spots were detected by exposure to UV lamp at λ 254 nm for few seconds.

General procedure for the synthesis of enaminones (3a, b)

To a solution of 1-(furan-2-yl)ethanone **(1a)** (1.1 g, 10 mmol) or 1-(thiophen-2-yl)ethanone **(1b)** (1.26 g, 10 mmol), 1,1-dimethoxy-*N,N*-dimethylmethanamine (DMF-DMA) **(2)** (1.31 g, 11 mmol) was added. The reaction mixture was refluxed for 5 h, then left to cool and treat the residue with diethyl ether. The formed precipitate was filtered off to afford the corresponding enaminone 3-(dimethylamino)-1-(furan-2-yl) prop-2-en-1-one **(3a)** [33] and 3-(dimethylamino)-1-(thiophen-2-yl)prop-2-en-1-one **(3b)** [34], respectively.

General procedure for the synthesis of nicotinic acid esters (5a, b)

To a solution of glacial acetic acid (25 ml), ethyl acetoacetate **(4)** (1.3 g, 10 mmol) and ammonium acetate (7.7 g, 100 mmol), enaminone **(3a**, **b)** (10 mmol) was added. The reaction mixture was heated under reflux for 5 h. After cooling and pouring into ice/water mixture, the residue obtained was filtered and washed with water and finally crystallized from ethanol to give pyridine derivative **(5a)** [35] and **(5b)** [28],respectively.

General procedure for the synthesis nicotinic acid hydrazide derivatives (6a,b)

A mixture of the ester (**5a**, **b)** (10 mmol) and 99% hydrazine hydrate (5 mL) was refluxed for 4 h. The solid product obtained upon cooling was filtered off and recrystallized from 1,4-dioxan to afford the corresponding 6-(furan-2-yl)-2-methylnicotinohydrazide (**6a)** and 2-methyl-6-(thiophen-2-yl)nicotinohydrazide (**6b)**, respectively.

6-(2-Furan-2-yl)-2-methylnicotinohydrazide (6a): White powder; yield 70%; m.p. 154-157°C; IR ν_{max}/cm^{-1}: (3194-3296) (NH +NH_2), 1643 (C=O), 1601 (C=N); ^{1}H NMR ($CDCl_3$-d_6,400 MHz) δ *ppm*: 2.69 (s, 3H, CH_3), 5.24 (br.s, 2H, NH_2, D_2O exchangeable), 6.53 (m, 1H, furan H4), 7.11 (d, J = 4.4 Hz, 1H, furan-H3), 7.46 (d, J = 8.8 Hz,, 1H, pyridine-H5), 7.54 (s, 1H, furan-H5), 7.69 (d, J = 8.0 Hz,, 1H, pyridine H4), 10.43 (s, 1H, NH, D_2O exchangeable); Anal. Calcd. For $C_{11}H_{11}N_3O_2$ (217.22), C, 60.82; H, 5.10; N, 19.34. Found: C, 60.97; H, 5.03; N, 19.47.

2-Methyl-6-(thiophen-2-yl)nicotinohydrazide (6b): White powder; yield (70%); m.p. 173-175°C [27].

General procedure for the synthesis of hydrazones (8a-l)

To a solution of **(6a**, **b)** (10 mmol), the appropriate aromatic aldehyde **(7a-e)** (10 mmol) in absolute ethanol (30 mL), glacial acetic acid was added (0.3 mL). The reaction mixture was refluxed for 5 h. The solid product obtained upon cooling was filtered off and recrystallized from ethanol/DMF to afford the corresponding hydrazones **(8a-l)**.

N'-(4-Chlorobenzylidene)-6-(furan-2-yl)-2-methylnicotinohydrazide (8a): White crystals; yield 52%; m.p. 203-207°C; IR ν_{max}/cm^{-1}: 3212 (NH), 1652 (C=O), 1602 (C=N); ^{1}H NMR (DMSO-d_6, 400 MHz) δ *ppm*: 2.61 (s, 3H, CH_3), 6.68-6.69 (m, 1H, Ar-H), 7.18-7.21 (m, 1H, Ar-H), 7.40-8.10 (m, 7H, Ar-H), 8.32 (s, 1H, -C*H*=N), 11.98 (s, 1H, NH, D_2O exchangeable); ^{13}C NMR (DMSO-d_6, 100 MHz) δ *ppm*: 23.36 (CH_3), 110.13, 110.46, 112.96, 115.34, 115.55, 128.71, 128.83, 129.27, 129.43, 133.56, 135.15, 137.17, 147.03, 149.18, 153.06, 156.77, 164.37; Anal. Calcd. For $C_{18}H_{14}ClN_3O_2$ (339.78), C, 63.63; H, 4.15; N, 12.37. Found: C, 63.87; H, 4.1; N, 12.46.

N'-(2,4-Dichlorobenzylidene)-6-(furan-2-yl)-2-methylnicotinohydrazide (8b): Buff powders; yield 54%; m.p. 210-215°C; IR ν_{max}/cm^{-1}: 3172 (NH), 1651 (C=O), 1616 (C=N); ^{1}H NMR (DMSO-d_6, 400 MHz) δ *ppm*: 2.62 (s, 3H, CH_3), 6.69-7.20 (m, 2H, Ar-H), 7.36-7.69 (m, 4H, Ar-H), 7.89-8.00 (m, 2H, Ar-H), 8.69 (s, 1H, -C*H*=N), 12.17 (s, 1H, NH, D_2O exchangeable); Anal. Calcd. For $C_{18}H_{13}Cl_2N_3O_2$ (374.22), C, 57.77; H, 3.50; N, 11.23. Found: C, 57.64; H, 3.55; N, 11.30.

N'-(4-Fluorobenzylidene)-6-(furan-2-yl)-2-methylnicotinohydrazide (8c): Buff powders; yield 57%; m.p. 195-200°C; IR ν_{max}/cm^{-1}: 3215 (NH), 1651 (C=O), 1604 (C=N); ^{1}H NMR (DMSO-d_6, 400 MHz) δ *ppm*: 2.61 (s, 3H, CH_3), 6.68-6.69 (m, 1H, Ar-H), 7.18-7.49 (m, 4H, Ar-H), 4.64-7.95 (m, 3H, Ar-H) 8.33 (s, 1H, -C*H*=N), 11.92 (s, 1H, NH, D_2O exchangeable); Anal. Calcd. For $C_{18}H_{14}FN_3O_2$ (323.32), C, 66.87; H, 4.36; N, 13.00. Found: C, 66.65; H, 4.41; N, 13.17.

6-(Furan-2-yl)-N'-(4-hydroxy-3-methoxybenzylidene)-2-methylnicotinohydrazide (8d): Buff powers; yield 70%; m.p. 215-220°C; IR ν_{max}/cm^{-1}: 3405 (OH), 3216 (NH), 1645 (C=O), 1600 (C=N); ^{1}H NMR (DMSO-d_6, 400 MHz) δ *ppm*: 2.61 (s, 3H, CH_3), 3.86 (m, 3H, OCH_3), 6.68 (s, 1H, Ar-H), 6.84-6.88 (m, 1H, Ar-H), 7.08-7.34 (m, 3H, Ar-H), 7.63-7.67 (m, 1H, Ar-H), 7.79-7.97 (m, 2H, Ar-H), 8.18 (s, 1H, -C*H*=N), 8.57 (s, 1H, OH, D_2O exchangeable), 11.73 (s, 1H, NH, D_2O exchangeable); Anal. Calcd. For $C_{19}H_{17}N_3O_4$ (351.36), C, 64.95; H, 4.88; N, 11.96. Found: C, 66.08; H, 4.85; N, 12.05.

N'-(3,4-Dimethoxybenzylidene)-6-(furan-2-yl)-2-methylnicotinohydrazide (8e): Buff powders; yield 51%; m.p. 195-200°C; IR ν_{max}/cm^{-1}: 3222 (NH), 1654 (C=O), 1623 (C=N); ^{1}H NMR (DMSO-d_6, 400 MHz) δ *ppm*: 2.61 (s, 3H, CH_3), 3.84 (s, 6H, $2OCH_3$), 7.07-8.00 (m, 8H, Ar-H), 8.64 (s,1H, -C*H*=N), 11.80 (s, 1H, NH, D_2O exchangeable). Anal. Calcd. For $C_{20}H_{19}N_3O_4$ (365.38), C, 65.74; H, 5.24; N, 11.50. Found: C, 65.44; H, 5.20; N, 11.62.

N'-(2,5-Dimethoxybenzylidene)-6-(furan-2-yl)-2-methylnicotinohydrazide (8f): Buff powders; yield 56%; m.p. 222-228°C; IR ν_{max}/cm^{-1}: 3180 (NH), 1651 (C=O) and 1584 (C=N); ^{1}H NMR (DMSO-d_6, 400 MHz) δ *ppm*: 2.61 (s, 3H, CH_3), 3.78 (s, 3H, OCH_3), 3.81 (s, 3H, OCH_3), 6.94-7.96 (m, 8H, Ar-H), 8.66 (s, 1H, -C*H*=N), 11.90 (s, 1H, NH, D_2O exchangeable); Anal. Calcd. For $C_{20}H_{19}N_3O_4$ (365.38), C, 65.74; H, 5.24; N, 11.50. Found: C, 65.88; H, 5.31; N, 11.32.

N'-(4-Chlorobenzylidene)-2-methyl-6-(thiophen-2-yl)nicotinohydrazide (8g): White crystals; yield 72%; m.p. 210-215°C; IR ν_{max}/cm^{-1}: 3225 (NH), 1651 (C=O), 1585 (C=N); ^{1}H NMR (DMSO-d_6, 400 MHz) δ *ppm*: 2.60 (s, 3H, CH_3), 7.20-7.21 (m, 1H, Ar-H), 7.41-7.46 (m, 1H, Ar-H), 7.53 (d, *J* = 8.0 Hz, 1H, Ar-H), 7.68-7.94 (m, 6H, Ar-H), 8.31 (s, 1H, -C*H*=N), 11.96 (s, 1H, NH, D_2O exchangeable); Anal. Calcd. For $C_{18}H_{14}ClN_3OS$ (355.84), C, 60.76; H, 3.97; N, 11.81; S, 9.01. Found: C, 61.01; H, 4.01;N, 11.72; S, 9.07.

N'-(2,4-Dichlorobenzylidene)-2-methyl-6-(thiophen-2-yl)nicotinohydrazide (8h): Yellow powders; yield 68%; m.p. 225-230°C; IR ν_{max}/cm^{-1}: 3167 (NH), 1651 (C=O) and 1586 (C=N); ^{1}H NMR (DMSO-d_6, 400 MHz) δ *ppm*: 2.61 (s, 3H, CH_3), 7.19-8.06 (m, 8H, Ar-H), 8.69 (s, 1H, -C*H*=N), 12.16 (s, 1H, NH, D_2O exchangeable); Anal. Calcd. For $C_{18}H_{13}Cl_2N_3OS$ (390.29), C, 55.39; H, 3.36; N, 10.77; S, 8.22.Found: C, 55.59; H, 3.31; N, 10.85; S, 8.31.

N'-(4-Fluorobenzylidene)-2-methyl-6-(thiophen-2-yl)nicotinohydrazide (8i): White crystals; yield 76%; m.p. 197-202°C; IR ν_{max}/cm^{-1}: 3224 (NH), 1651 (C=O), 1601 (C=N); ^{1}H NMR ($CDCl_3$-d_6, 400 MHz) δ *ppm*: 2.68 (br.s, 3H, CH_3), 7.01-7.78 (m, 9H, Ar-H), 7.98 (s, 1H, -C*H*=N), 10.74 (s, 1H, NH, D_2O exchangeable); Anal. Calcd. For $C_{18}H_{14}FN_3OS$ (339.39), C, 63.70; H, 4.16; N, 12.38; S, 9.45. Found: C, 63.66; H, 4.21; N, 12.25; S, 9.51.

N'-(4-Hydroxy-3-methoxybenzylidene)-2-methyl-6-(thiophen-2-yl)nicotinohydrazide (8j): White crystals; yield 78%; m.p. 218-222°C; IR

ν_{max}/cm^{-1}: 3414 (OH), 3187 (NH), 1651 (C=O), 1584 (C=N); ^{1}H NMR (DMSO-d_6, 400 MHz) δ *ppm*: 2.59 (s, 3H, CH_3), 3.85 (s, 3H, OCH_3), 6.85-6.91 (m, 1H, Ar-H), 7.09-7.11 (m, 1H, Ar-H), 7.18-7.21 (t, *J* = 9.2 Hz, 1H, Ar-H), 7.35 (s, 1H, Ar-H), 7.67-7.70 (m, 1H, Ar-H), 7.78-7.91 (m, 3H, Ar-H), 8.21 (s, 1H, -C*H*=N), 9.59 (s, H, OH, D_2O exchangeable), 11.61 (s, 1H, NH, D_2O exchangeable); Anal. Calcd. For $C_{19}H_{17}N_3O_3S$ (367.42), C, 62.11; H, 4.66; N, 11.44; S, 8.73. Found: C, 62.26; H, 4.71; N, 11.50; S, 8.62.

N'-(3,4-Dimethoxybenzylidene)-2-methyl-6-(thiophen-2-yl)nicotinohydrazide (8k): White powders; yield 80%; m.p. 212-217°C; IR ν_{max}/cm^{-1}: 3201 (NH), 1645 (C=O), 1597 (C=N); ^{1}H NMR (DMSO-d_6, 400 MHz) δ *ppm*: 2.60 (s, 3H, CH_3), 3.82 (s, 3H, OCH_3), 3.84 (s, 3H, OCH_3), 6.95-8.00 (m, 8H, Ar-H), 8.24 (s, 1H, -C*H*=N), 11.77 (s, 1H, NH, D_2O exchangeable); Anal. Calcd. For $C_{20}H_{19}N_3O_3S$ (381.45), C, 62.97; H, 5.02; N, 11.02; S, 8.41.Found: C, 62.75; H, 5.1; N, 11.15; S, 8.39.

N'-(2,5-Dimethoxybenzylidene)-2-methyl-6-(thiophen-2-yl)nicotinohydrazide (8l): Yellow powders; yield 81%; m.p. 220-225°C; IR ν_{max}/cm^{-1}: 3179 (NH), 1646 (C=O), 1581 (C=N); ^{1}H NMR (DMSO-d_6, 400 MHz) δ *ppm*: 2.60 (s, 3H, CH_3), 3.78 (s, 3H, OCH_3), 3.81 (s, 3H, OCH_3), 6.94-7.94 (m, 8H, Ar-H), 8.65 (s, 1H, -C*H*=N), 11.88 (s, 1H, NH, D_2O exchangeable); Anal. Calcd. For $C_{20}H_{19}N_3O_3S$ (381.45), C, 62.97; H, 5.02; N, 11.02; S, 8.41.Found: C, 63.12; H, 5.08; N, 11.15; S, 8.55.

General procedure for the synthesis of compounds (10a-f)

To a solution of **(6a)** or **(6b)** (10 mmol), a variety of isatin derivatives **(9a-f)** (10 mmol) was added in the presence of glacial acetic acid (0.3 mL). The reaction mixture was heated under reflux for 7h in each case. The obtained solid product from the different reactions was filtered off and recrystallized from ethanol/DMF to afford the corresponding hydrazones **(10a-f)**.

6-(Furan-2-yl)-2-methyl-N'-(2-oxoindolin-3-ylidene)nicotinohydrazide (10a): Orange powders; yield 79%; m.p. 160-165°C; IR ν_{max}/cm^{-1}: 3187 (2NH), 1680 (2C=O), 1618 (C=N); ^{1}H NMR (DMSO-d_6, 400 MHz) δ *ppm*: 2.64 (s, 3H, CH_3), 6.69-6.71 (m, 1H, Ar-H), 6.94 (d, *J* = 8.0 Hz, 1H, Ar-H), 7.08 (s, 1H, Ar-H), 7.23 (d, *J* = 3.6 Hz, 1H, Ar-H), 7.38 (m, 2H, Ar-H), 7.71 (d, *J* = 8.8 Hz, 1H, Ar-H), 7.91 (s, 1H, Ar-H), 8.02-8.04 (m, 1H, Ar-H), 11.33 (s, 1H, NH, D_2O exchangeable), 13.45 (s, 1H, NH, D_2O exchangeable); ^{13}C NMR (DMSO-d_6, 100 MHz) δ 23.17 (CH_3), 11.06, 111.69 (2C), 113.05 (2C), 116.00, 120.15, 121.00, 123.20 (2C), 127.08, 132.37, 143.02 (2C), 145.54, 152.92, 163.00, 168.50; Anal. Calcd. For $C_{19}H_{14}N_4O_3$ (346.34), C, 65.89; H, 4.07; N, 16.18. Found: C, 65.77; H, 4.06; N, 16.30.

N'-(5-Chloro-2-oxoindolin-3-ylidene)-6-(furan-2-yl)-2-methylnicotinohydrazide (10b): Orange crystals; yield 72%; m.p. 310-315°C; IR ν_{max}/cm^{-1}: 3216 (2NH), 1703 (C=O), 1677 (C=O), 1621 (C=N); ^{1}H NMR (DMSO-d_6, 400 MHz) δ *ppm*: 2.64 (s, 3H, CH_3), 6.70-6.71 (m, 1H, Ar-H), 6.96 (d, *J* = 9.6 Hz, 1H, Ar-H), 7.24 (d, *J* = 3.6 Hz, 1H, Ar-H), 7.42 (d, *J* = 8.8 Hz, 2H, Ar-H), 7.72 (d, *J* = 8.4 Hz, 1H, Ar-H), 7.92 (s, 1H, Ar-H), 8.02 (d, *J* = 8.8 Hz, 1H, Ar-H) 11.45 (s, 1H, NH, D_2O exchangeable), 13.31 (s, 1H, NH, D_2O exchangeable); Anal. Calcd. For $C_{19}H_{13}ClN_4O_3$ (380.78), C, 59.93; H, 3.44; N, 14.71. Found: C, 59.80; H, 3.44; N, 14.89.

N'-(5-Bromo-2-oxoindolin-3-ylidene)-6-(furan-2-yl)-2-methylnicotinohydrazide (10c): Orange crystals; yield 75%; m.p. 244-250°C; IR ν_{max}/cm^{-1}: 3221 (2NH), 1703 (C=O), 1678 (C=O), 1617 (C=N); ^{1}H NMR (DMSO-d_6, 400 MHz) δ *ppm*: 2.64 (s, 3H, CH_3), 6.70-7.23 (m, 3H, Ar-H), 7.28-8.04 (m, 5H, Ar-H), 10.82 (s, 1H, NH, D_2O exchangeable),11.44 (s, 1H, NH, D_2O exchangeable); Anal. Calcd. For $C_{19}H_{13}BrN_4O_3$ (425.24), C, 53.67; H, 3.08; N, 13.18. Found: C, 53.87; H, 3.05; N, 13.05.

6-(Furan-2-yl)-2-methyl-N'-(1-methyl-2-oxoindolin-3-ylidene)nicotinohydrazide (10d): Orange crystals; yield 70%; m.p. 140-145°C; IR ν_{max}/cm^{-1}: 3227 (NH), 1702 (2C=O), 1618 (C=N); ^{1}H NMR (DMSO-d_6, 400 MHz) δ ppm: 2.64 (s, 3H, CH_3), 3.23 (s, 3H, CH_3), 6.70-6.71 (m, 1H, Ar-H), 7.14 (d, *J* = 8.4 Hz, 2H, Ar-H), 7.23 (d, *J* = 4.8 Hz, 1H, Ar-H), 7.45-7.48 (m, 2H, Ar-H), 7.71 (d, *J* = 8.8 Hz, 1H, Ar-H), 7.91 (s, 1H, Ar-H), 8.02-8.03 (m, 1H, Ar-H), 13.33 (s, 1H, NH, D_2O exchangeable); Anal. Calcd. For $C_{20}H_{16}N_4O_3$ (360.37), C, 66.66; H, 4.48; N, 15.55. Found: C, 66.47; H, 4.40; N, 15.36.

2-Methyl-N'-(1-methyl-2-oxoindolin-3-ylidene)-6-(thiophen-2-yl)nicotinohydrazide (10e): Orange crystals; yield 69%; m.p. 195-200 °C; IR ν_{max}/cm^{-1}: 3101 (NH), 1685 (2C=O), 1617 (C=N); ^{1}H NMR (DMSO-d_6, 400 MHz) δ ppm:

2.63 (s, 3H, CH_3), 3.19 (s, 3H, CH_3), 7.10-7.13 (m, 2H, Ar-H), 7.19 (t, *J* = 9.6 Hz, 1H, Ar-H), 7.41 (t, *J* = 15.2 Hz, 2H, Ar-H), 7.71 (d, *J* = 5.2 Hz, 1H, Ar-H), 7.88-7.90 (m, 2H, Ar-H) 7.98 (d, *J* = 8.0 Hz, 1H, Ar-H) 13.06 (s, 1H, NH, D_2O exchangeable); Anal. Calcd. For $C_{20}H_{16}N_4O_2S$ (376.43), C, 63.81; H, 4.28; N, 14.88; S, 8.52. Found: C, 63.65; H, 4.33; N, 14.79; S, 8.50.

N'-(1-benzyl-2-oxoindolin-3-ylidene)-2-methyl-6-(thiophen-2-yl)nicotinohydrazide (10f): Yellow crystals; yield 73%; m.p. 180-186 °C; IR ν_{max}/cm^{-1}: 3101 (NH), 1702 (C=O), 1682 (C=O), 1608 (C=N); ^{1}H NMR (DMSO-d_6, 400 MHz) δ *ppm*: 2.67 (s, 3H, CH_3), 5.01 (s, 2H, CH_2), 7.06-7.40 (m, 10H, Ar-H), 7.72 (d, *J* = 4.0 Hz, 1H, Ar-H), 7.91-7.93 (m, 2H, Ar-H), 8.03-8.05 (m, 1H, Ar-H), 13.31 (s, 1H, NH, D_2O exchangeable); Anal. Calcd. For$C_{26}H_{20}N_4O_2S$ (452.53), C, 69.01; H, 4.45; N, 12.38; S, 7.09. Found: C, 69.23; H, 4.45; N, 12.58;S, 7.21.

Synthesis of (3,5-dimethyl-1H-pyrazol-1-yl)(2-methyl-6-(thiophen-2-yl)pyridin-3-yl) methanone (12)

To a solution of nicotinic hydrazide (6b) (0.233 g, 1 mmol) and acetyl acetone **(11)** (0.13 g, 1 mmol) in absolute ethanol (30 mL), glacial acetic acid was added (0.3 mL). The reaction mixture was refluxed for 7h. The solid product obtained upon cooling was filtered off and recrystallized from ethanol to afford the corresponding pyrazole **(12)** as yellow powders; yield 66%; m.p. 125-130°C; IR ν_{max}/cm^{-1}: 1701 (C=O), 1585 (C=N); ^{1}H NMR (DMSO-d_6, 400 MHz) δ *ppm*: 2.11 (s, 3H, CH_3), 2.40 (s, 3H, CH_3), 2.61 (s, 3H, CH_3), 6.32 (s, 1H, Ar-H), 7.20-7.22 (m, 1H, Ar-H), 7.12 (d, *J* = 4.0 Hz, 1H, Ar-H), 7.84-7.89 (m, 3H, Ar-H); ^{13}C NMR (DMSO-d_6, 100 MHz) δ: 13.78 (CH_3), 14.30 (CH_3), 23.24 (CH_3), 112.32, 115.59, 126.92, 128.88, 129.06, 129.96, 138.34, 144.12, 144.55, 152.85, 152.03, 155.82, 168.57; Anal. Calcd. For $C_{16}H_{15}N_3OS$ (297.37), C, 64.62; H, 5.08; N, 14.13; S, 10.78. Found: C, 64.35; H, 5.0; N, 14.25; S, 10.92.

Synthesis of 5-amino-1-(2-methyl-6-(thiophen-2-yl)nicotinoyl)-1H-pyrazole-4-carbonitrile (14)

A mixture of hydrazide **(6b)** (0.233 g, 1 mmol) and 2-ethoxymethylene-malononitrile **(13)** (0.122 g, 10 mmol), which was purchased from Alfa Aesar, Inc. (Germany), in absolute ethanol (30 mL) was heated under reflux for 7h. The solid product obtained was filtered off and recrystallized from ethanol/DMF to afford the corresponding pyrazole **(14)** as white powder; yield 61%; m.p. 230-235 °C; IR ν_{max}/cm^{-1}: 3428 (NH), 3289 (NH), 2230 (C≡N), 1702 (C=O), 1637 (C=N); ^{1}H NMR (DMSO-d_6, 400 MHz) δ *ppm*: 2.46 (s, 3H, CH_3), 7.20-8.16 (m, 6H, Ar-H); ^{13}C NMR (DMSO-d_6, 100 MHz) δ *ppm*: 23.27 (CH_3), 73.19, 114.06, 115.44, 127.25, 127.33, 129.11, 130.25, 138.77, 143.95, 145.11, 153.31, 155.69, 156.02, 170.13; Anal. Calcd. For $C_{15}H_{11}N_5OS$ (309.35), C, 58.24; H, 3.58; N, 22.64; S, 10.37.Found: C, 58.0; H, 3.53; N, 22.77; S, 10.45.

Synthesis of 2-(2-methyl-6-(thiophen-2-yl) nicotinoyl)-N-phenylhydrazinecarbothio-amide (15)

A mixture of compound **(6b)** (2.33 g, 10 mmol) and phenylisothiocyanate (1.35 g, 1 mmol) in absolute ethanol (50 mL) was heated under reflux for 7h. The solid product obtained upon cooling was filtered off and recrystallized from ethanol to afford the corresponding thiosemicarbazone **(15)** as white powder; yield 63%; m.p. 180-185°C; IR ν_{max}/cm^{-1}: 3276, 3195, 3140 (3NH), 1678 (C=O), 1582 (C=N); ^{1}H NMR (DMSO-d_6, 400 MHz) δ *ppm*: 2.64 (s, 3H, CH_3), 7.19-7.21 (m, 2H, Ar-H), 7.35 (t, *J* = 14.8 Hz, 2H, Ar-H), 7.48 (s, 2H, Ar-H), 7.69 (d, *J* = 4.8 Hz, 1H, Ar-H), 7.85-7.89 (m, 2H, Ar-H), 8.09 (s, 1H, Ar-H), 9.75-9.82 (m, 2H, NH, D_2O exchangeable), 10.43 (s, 1H, NH, D_2O exchangeable); Anal. Calcd. For $C_{18}H_{16}N_4OS_2$ (368.48), C, 58.67; H, 4.38; N, 15.21; S, 17.40. Found: C, 58.87; H, 4.31; N, 15.33; S, 17.56.

Synthesis of 5-(2-methyl-6-(thiophen-2-yl) pyridin-3-yl)-4-phenyl-4H-1,2,4-triazole-3-thiol (16)

A mixture of thiosemicarbazide **(15)** (0.368 g, 1 mmol) and sodium hydroxide (10%, 25 mL) was refluxed for 6 h, and then allowed to cool, filtered, and finally the filtrate was acidified with dilute hydrochloric acid. The precipitated solid was filtered off, washed with water, and recrystallized from EtOH/DMF to afford triazole derivative **(16)** as yellow powder; yield 47%; m.p. 320-325°C; IR ν_{max}/cm^{-1}: 1593 (C=N); ^{1}H NMR (DMSO-d_6, 400 MHz) δ *ppm*: 2.40 (s, 3H, CH_3), 7.13 (t, *J* = 8.8 Hz, 1H, Ar-H), 7.37-7.45 (m, 5H, Ar-H), 7.66 (d, *J* = 2.0 Hz, 1H, Ar-H), 7.71 (s, 2H, Ar-H), 7.80 (d, *J* = 2.8 Hz, 1H, Ar-H), 14.26 (s, 1H, SH, D_2O exchangeable); ^{13}C NMR (DMSO-d_6, 100 MHz) δ23.49 (CH_3), 115.87, 123.64, 126.10, 127.46, 128.58 (2C), 128.68 (2C), 128.90, 129.16, 137.48, 139.01, 144.45, 148.99, 151.20, 157.05, 168.48; Anal. Calcd. For $C_{18}H_{14}N_4S_2$ (350.46), C, 61.69; H, 4.03; N, 15.99; S, 18.30.Found: C, 61.99; H, 3.08; N, 15.79; S, 18.0.

Anti-microbial activity

Microorganisms

Nine clinical strains employed for this investigation include four filamentous fungi (*Aspergillus fumigatus, Aspergillus flavus, Syncephlastarum racemosum* and *Penicillium expansum*), one yeast (*Candida albicans*), two Gram-positive (*Staphylococcus aureus* and *Bacillus subtilis*) and two Gram-negative (*Klebsilla pnumonia* and *Pseudomonas aeruginosa*) bacteria. All strains were kindly provided from culture collection of Regional Center for Mycology and Biotechnology (RCMB), Al-Azhar University, Cairo, Egypt.

Antimicrobial assay

By diffusion agar technique, the antifungal and antibacterial potentialities against the tested species were expressed as the measurement of diameter of their inhibition zone. Hole-plate diffusion method was used; six equidistant (1 cm diameter) holes were made using sterile cork borer in malt extract agar and Nutrient agar sterile plates (10x10 cm), which had previously been seeded with tested fungal and bacterial isolates, respectively. Holes were filled with 100 µl of three concentrations 5, 2.5 and 1 mg/mL of each of the synthesized compounds after completely dissolving in ethyl acetate. Control holes were filled with ethyl acetate solvent.

Plates were left in a cooled incubator at 4 (±2) ^{o}C for 1 h and then incubated at 37 (±2) ^{o}C for bacterial isolates and incubated at 28 (±2) ^{o}C for fungal isolates used. Inhibition zones developed due to active ingredients were measured after 24–48 h of incubation time. Griseofulvin was used as a standard antifungal agent while Amoxicillin was used as a standard antibacterial agent [43].

Minimum inhibitory concentration (MIC) assays: Determination of MIC was performed by a serial dilution technique described. Appling ethyl acetate solvent of the synthesized compounds started with a maximum concentration of 500 mg/ml and then reduced it by successive twofold dilutions of that stock solution using a calibrated micropipette. MIC of the sample determination was carried out by inoculation of their serial dilutions with test organisms and measurement of inhibition zones using diffusion agar technique. MIC was expressed as the lowest concentration inhibiting test

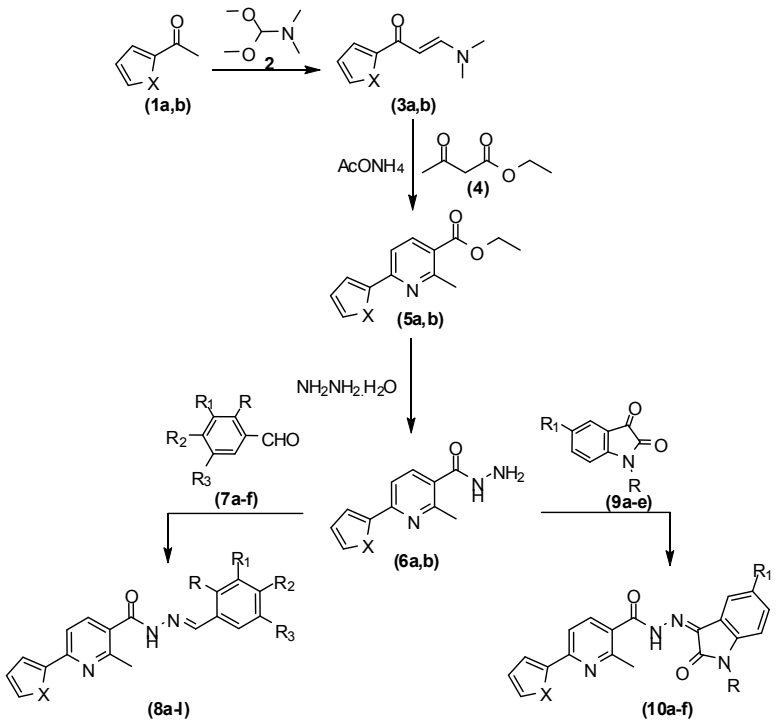
Microbial cells (70 ml, 10^6 CFU/ml) of each tested pathogens were spread onto the nutrient agar plates. The wells (6 mm diameter) were dug on the inoculated agar plates and 100 µl of the so-synthesized target molecule was suspended in DMSO at 200 mg/ml and subsequently was poured in the wells. The plates were allowed to stand at 4 ^{o}C for 2 h before incubation for the diffusion. The plates were incubated at 37 ^{o}C for 24 h except yeast strain were incubated at 28 °C. Incubation step was followed by measuring of the diameter of inhibition zone (IZ) in mm and three replicates were averaged [44].

Results and Discussion

Chemistry

The synthetic pathways of the newly prepared compound are depicted in Schemes 1 and 2. In Scheme 1, the key intermediates enaminones **(3a**,**b)** were prepared by the reaction of 1-(furan-2-yl)ethan-1-one **(1a)** [37] or 1-(thiophen-2-yl) ethan-1-one **(1b)** with *N*,*N*-dimethylformamide-dimethylacetal (DMF-DMA) **(2)** [38]. Ethyl 2-methyl-6-arylnicotinate **(5a)** [39] and **(5b)** [30] was obtained *via* the reaction of enaminones **(3a)** and **(3b)**, respectively, with ethyl acetoacetate **(4)** in the presence of ammonium acetate. Hydrazinolysis of the latter esters resulted in the formation of nicotinic acid hydrazides **(6a**,**b)**, respectively. The IR spectrum of the newly prepared hydrazide **(6a)** showed an absorption band due to the carbonyl group at 1643 cm^{-1} in addition to bands in the region 3194-3296 cm^{-1} due to NH and NH_2 groups. The ^{1}H NMR spectra of **(6a)** showed broad D_2O-exchangeable singlet signal attributed to NH_2 protons at δ 5.24 *ppm* while the methyl protons appeared as singlet signal at δ 2.69 *ppm*.

Next, nicotinic acid hydrazides **(6a**,**b)** were reacted with different aromatic aldehydes **(7a-f)** or isatines **(9a-e)**, in ethanol in the presence of acetic acid to yield the corresponding hydrazones **(8a-l)** and **(10a-f)**, respectively (Scheme 1). IR spectra of the newly synthesized compounds **(8a-l)** showed an absorption bands due to the NH groups in the region 3179-3222 cm^{-1}, in addition to a carbonyl band in the region 1645-1651 cm^{-1}. The ^{1}H NMR spectra of **(8a-l)** showed D_2O-exchangeable singlet signal attributed to NH protons in the region δ 9.59-12.17 ppm, and the methyl protons appeared as singlet signals in the region δ 2.59-2.68 ppm. The ^{1}H NMR spectrum of **(10a-f)** revealed singlet signals at δ 2.64 due to methyl group. The IR spectra of **(10a-f)** exhibited a band in the region 1680-1703 cm^{-1} due to two carbonyl groups whereas compounds **(10a-c)** showed absorption bands due to the 2NH groups in the region 3187-3221 cm^{-1}.



8	X	R	R_1	R_2	R_3
a	O	H	H	Cl	H
b	O	Cl	H	Cl	H
c	O	H	H	F	H
d	O	H	OMe	OH	H
e	O	H	OMe	OMe	H
f	O	OMe	H	H	OMe
g	S	H	H	Cl	H
h	S	Cl	H	Cl	H
i	S	H	H	F	H
j	S	H	OMe	OH	H
k	S	H	OMe	OMe	H
l	S	OMe	H	H	OMe

10	X	R	R_1
a	O	H	H
b	O	H	Cl
c	O	H	Br
d	O	Me	H
e	S	Me	H
f	S	$-CH_2Ph$	H

Scheme 1. Synthesis of hydrazones (8a-l) and (10a-f).

It has been reported that compounds containing arylidene–hydrazide structure may exist as E/Z geometrical isomers about –C=N– double bond and cis/trans amide conformers around amide function (-CONH-). These compounds were present in higher percentage in DMSO-d_6 in the form of geometrical E isomer about –C=N– bond. The presence of cis/trans conformers can be established by observing the signal of the amide group (–CONH–) which appeared as two singlets. In the present study, it could be suggested that the signals of *Z* isomer have not appeared, whereas the cis/trans conformers of *E* isomer were clearly observed [40].

In the light of reported antimicrobial activity of pyrazoles, we aimed to synthesis pyrazoles **(12)** and **(14)** which incorporating pyridine moiety [41, 42]. Thus, 3,5-dimethylpyrazole **(12)** was prepared by the reaction of 6-(2-thiophen-2-yl)-2-methylnicotinohydrazide **(6b)** with pentane-2,4-dione **(11)**. The structure of **(12)** was established on the basis of its spectral data. The ^{1}H NMR spectrum of pyrazole **(12)** revealed three singlet signals at δ 2.11, 2.40 and 2.61due to three methyl groups. The IR spectrum of **(12)** revealed an absorption bands at 1701 and 1585 cm^{-1} corresponding to C=O and C=N, respectively. In addition, pyrazole-4-carbonitrile **(14)** was prepared by the reaction of **(6b)** with 2-ethoxymethylene-malononitrile **(13)**. The IR spectrum of pyrazole **(14)** revealed an absorption bands at 2230 and 1750 cm^{-1} corresponding to C≡N and C=O, respectively, in addition to the absorption band of NH_2 group in the region 3289-3428 cm^{-1}.

In addition, thiosemicarbazone **(15)** was obtained by the reaction of nicotinic hydrazide derivative **(6b)** with phenylisothiocyanate. The ^{1}H NMR spectrum of **(15)** revealed the D_2O-exchangeable signal at δ 9.75-10.43 *ppm* of NH

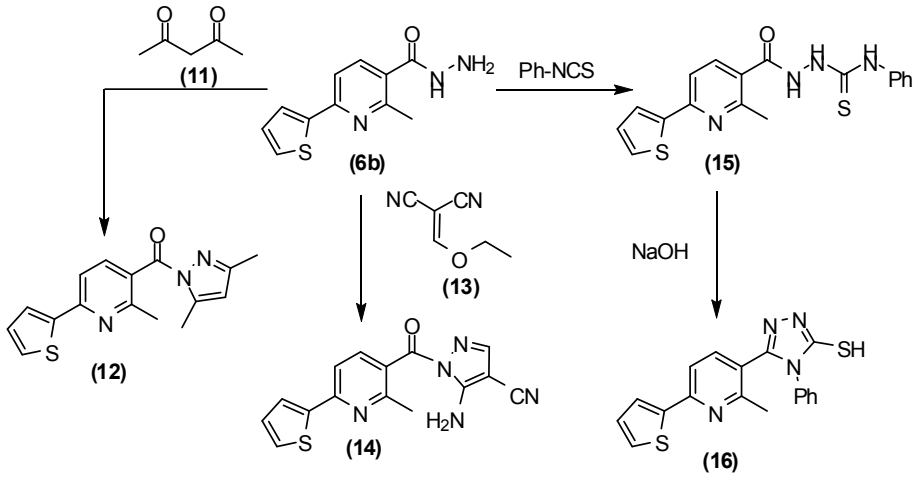
proton while the methyl protons appeared as singlet signal at δ 2.4 ppm. The IR spectrum of **(15)** revealed an absorption bands in the region 3276-3140 cm^{-1} due to 3NH function and an absorption band at 1678 cm^{-1} of C=O function. Intermolecular cyclization of thiosemicarbazone **(15)** *via* sodium hydroxide afforded 1,2,4-triazole **(16)**. The ^1H NMR spectrum of **(16)** showed the D_2O-exchangeable signal at *δ* 14.26 *ppm* of SH proton. The IR spectra of **(16)** exhibited an absorption bands at 3093 and 1593 cm^{-1} corresponding to NH and C=N, respectively (Scheme 2).

Biological activity

Anti-microbial activity

Antibacterial and antifungal activities were performed at the Region Center for Mycology and Biotechnology (RCMB), Al-Azhar University, Cairo, Egypt. All synthesized compounds and reference drug were evaluated *in vitro* for their antimicrobial and antifungal activities by inhibition zone technique and minimum inhibitory concentration (MIC) using nine clinical strains include four filamentous fungi (*Aspergillus fumigatus, Aspergillus flavus, Syncephlastarum racemosum* and *Penicillium expansum*), one yeast (*Candida albicans*), two Gram-positive (*Staphylococcus aureus* and *Bacillus subtilis*) and two Gram-negative (*Klebsilla pnumonia* and *Pseudomonas aeruginosa*) bacteria.

The anti-bacterial activities of **(8a-l)**, **(10a-f)**, **(12)**, **(14)**, **(15)** and **(16)** are represented in Table 1. It is noticed that, compounds **(8a)** (X = O, R = R_1= R_3 = H, R_2 = Cl), **(8b)** (X = O,R_1= R_3 = H, R =R_2 = Cl), **(8d)** (X = O, R =R_3 = H, R_1 = OMe, R_2 = OH), **(8e)** (X = O, R = R_3 = H, R_1 = R_2 = OMe), **(10b)** (X = O, R = H, R_1 = Cl) and **(15)** revealed a moderate antibacterial activity against *Staphylococcus aureus* with inhibition zones 2.1, 0.9, 2.4, 1.2, 1.0 and 1.2 mm, respectively, when compared with that of reference drug Amoxicillin (IZ = 2.3 mm) whereas compounds **(8c)** (X = O, R = R_1= R_3 = H, R_2 = F), **(8d)** (X = O, R = R_3 = H, R_1 = OMe, R_2 = OH), **(8e)** (X = O, R = R_3 = H, R_1 = R_2 = OMe), **(8j)** (X = S, R = R_3 = H, R_1 = OMe, R_2 = OH), **(10a)** (X = O, R = R_1 = H), **(10b)** (X = O, R = H, R_1 = Cl), **(10c)** (X = O, R = H, R_1 = Br) and **(15)** exhibited a moderate antibacterial activity against *Bacillus subtilis* with inhibition zones in the range of 0.9, 3.2, 0.6, 1.2, 1.0, 1.4, 1.5 and 1.0 mm, respectively, when compared with that of reference drug Amoxicillin (IZ = 3.5 mm). Additionally, compounds **(8c)** (X = O, R = R_1= R_3 = H, R_2 = F), **(8d)** (X = O, R = R_3 = H, R_1 = OMe, R_2 = OH), **(8k)** (X = S, R = R_3 = H, R_1 = R_2 = OMe) and **(10b)** (X = O, R = H, R_1 = Cl) revealed antibacterial activity against *Klebsilla pneumonia* with inhibition zones in the range of 1.2, 0.8, 0.9 and 0.9 mm, respectively, when compared with that of reference drug Amoxicillin (IZ = 2.4 mm). whereas compounds **(8a)** (X = O, R = R_1= R_3 = H, R_2 = Cl), **(8b)** (X = O, R_1 = R_3 = H, R = R_2 = Cl), **(8d)** (X = O, R = R_3 = H, R_1 = OMe, R_2 = OH), **(8j)** (X = S, R = R_3 = H, R_1 = OMe, R_2 = OH), **(8k)** (X = S, R = R_3 = H, R_1 = R_2 = OMe), **(10b)** (X = O, R = H, R_1 = Cl), **(10c)** (X = O, R = H, R_1 = Br) and **(15)** exhibited antibacterial activity against *Pseudomonas aeruginosa* with inhibition zones in the range of 1.8, 0.5, 1.7, 1.8, 2.0, 2.6, 1.0 and 2.3 mm, respectively, when compared with that of reference drug Amoxicillin (IZ = 2.2 mm).



Scheme 2. Synthesis of pyrazoles (12), (14) and 1,2,4-triazole (16) .

The anti-fungal activities of the synthesized compounds are represented in Table 1. From the data, it is observed that compounds **(8b)** (X = O,R_1 = R_3 = H, R = R_2 = Cl), **(8d)** (X = O, R = R_3 = H, R_1 = OMe, R_2 = OH), **(10b)** (X = O, R = H, R_1 = Cl), **(10c)** (X = O, R = H, R_1 = Br) and **(16)** revealed a moderate antifungal action against *Aspergillus fumigates* with inhibition zones in the range of 1.3, 1.2, 2.2, 0.8 and 1.5 mm respectively, when compared with that of reference drug Griseofulvin (IZ= 3.3 mm). Compounds **(8a)** (X = O, R = R_1= R_3 = H, R_2 = Cl), **(8b)** (X = O, R_1 = R_3 = H, R = R_2 = Cl), **(8c)** (X = O, R = R_1 = R_3 = H, R_2 = F), **(8d)** (X = O, R = R_3 = H, R_1 = OMe, R_2 = OH), **(8e)** (X = O, R = R_3 = H, R_1 = R_2 = OMe), **(8f)** (X = O, R = R_3 = OMe, R_1 = R_2 = H), **(8l)** (X = S, R = R_3 = OMe, R_1 = R_2 = H), **(10b)** (X = O, R = H, R_1 = Cl), **(10c)** (X = O, R = H, R_1 = Br) and **(15)** revealed a moderate antifungal action against *Aspergillus flavus* with inhibition zones 1.5, 0.7, 1.2, 2.6, 0.8, 0.9, 1.3, 1.4, 1.0 and 0.7 mm, respectively, when compared with that of reference drug Griseofulvin (IZ= 2.4 mm). Compounds **(8b)** (X = O, R_1 = R_3 = H, R = R_2 = Cl), **(8d)** (X = O, R = R_3 = H, R_1 = OMe, R_2 = OH) and **(10b)** (X = O, R = H, R_1 = Cl) revealed a moderate antifungal action against *Syncephlastarum racemosum* with inhibition zones 1.0, 0.8 and 1.8 mm respectively, when compared with that of reference drug Griseofulvin (IZ= 2.1 mm). Compounds **(8a)** (X = O, R = R_1= R_3 = H, R_2 = Cl), **(8d)** (X = O, R = R_3 = H, R_1 = OMe, R_2 = OH), **(8f)** (X = O, R = R_3 = OMe, R_1 = R_2 = H), **(8l)** (X = S, R = R_3 = OMe, R_1 = R_2 = H), **(10a)** (X = O, R = R_1 = H), **(10b)** (X = O, R = H, R_1 = Cl), **(10c)** (X = O, R = H, R_1 = Br) and **(16)** revealed a moderate antifungal action against *Penicillium expansum* with inhibition zones 1.1, 1.0, 1.0, 0.7, 0.7, 2.8, 2.8 and 1.2 mm, respectively when compared with that of reference drug Griseofulvin (IZ = 2.8 mm).

TABLE 1. The anti-microbial activity of the newly synthesized compounds against 9 microbial species

Comp. (Conc. 5 mg/ mL)	**Inhibition Zone *(IZ mm)***								
	Antibacterial activity				**Antifungal activity**				
	S. aureus	***B. subtilis***	***K. pnumonia***	***P. aeruginosa***	***A. fumigatus***	***A. flavus***	***S. racemosum***	***P. expansum***	***C. albicans***
8a	2.1	-	-	1.8	-	1.5	-	1.1	0.9
8b	0.9	-	-	0.5	1.3	0.7	1.0	-	-
8c	-	0.9	1.2	-	-	1.2	-	-	`1.5
8d	2.4	3.2	0.8	1.7	1.2	2.6	0.8	1.0	2.0
8e	1.2	0.6	-	-	-	0.8	-	-	0.5
8f	-	-	-	-	-	0.9	-	1.0	-
8g	-	-	-	-	-	-	-	-	-
8h	-	-	-	-	-		-	-	-
8i	-	-	-	-	-	-	-	-	-
8j	-	1.2	-	1.8	-	-	-	-	-
8k	-	-	0.9	2.0	-		-	-	-
8l	-	-	-	-	-	1.3	-	0.7	1.5
10a	-	1.0	-	-	-	-	-	0.7	1.2
10b	1.0	1.4	0.9	2.6	2.2	1.4	1.8	2.8	0.8
10c	-	1.5	-	1.0	0.8	1.0	-	2.8	1.1
10d	-	-	-	-	-	-	-	-	-
10e	-	-	-	-	-	-	-	-	-
10f	-	-	-	-	-	-	-	-	-
12	-	-	-	-	-	-	-	-	-
14	-	-	-	-	-	-	-	-	-
15	1.2	1.0	-	2.3	-	0.7	-	-	1.0
16	-	-	-	-	1.5	-	-	1.2	-
Std.*	2.3	3.5	2.4	2.2	3.3	2.4	2.1	2.8	

*Antibacterial reference drug = Amoxicillin and antifungal reference drug = Griseofulvin

Minimum inhibitory concentration (MIC)

The results of minimum inhibitory concentration were reported in Table 2. Nicotine derivatives **(8d)** and **(10c)** (MIC: 0.25 and 0.5mg/mL, respectively) showed better results when compared with **(10b)** (MIC: 0.75 mg/mL) as revealed from their MIC values.

TABLE 2. MIC (mg/mL) of compounds (8d), (10c) and (10b).

Compound	MIC mg/mL
8d	0.25
10c	0.5
10b	0.75

Structure activity relationship (SAR)

Structure activity relationship (SAR) was performed to determine how the substituents on the hydrazones **(8a-l)**, **(10a-f)**, pyrazoles **(12)**, **(14)** and triazole **(16)** affected the antimicrobial activity. Firstly, structure–activity relationships of hydrazones **(8a-l)** substitutions displayed that compounds with *para* electron-donating substituents **(8d)** showed more potent activities than those with electron withdrawing substituents **(8g-i)**. On the other hand **(10a-f)** revealed that the presence of electron withdrawing (Cl and Br) attached to isatin showed more potent activities than those of un-substituted derivative **(10d-f)**. *N*-substituted isatines **(10d-f)** showed no enhancement in the activity. Furthermore, the furan derivatives showed antimicrobial activity higher than that of thiophene derivatives. Pyrazoles **(12)**, **(14)** and triazole **(16)** showed no activity when compared with hydrazones **(8a-l)** or **(10a-f)**.

Conclusion

This study presents a facile and convenient route for the synthesis of certain novel substituted nicotines by the reaction of nicotinic acid hydrazide with either aromatic aldehydes, isatines, pentane-2,4-dione, 2-ethoxymethylene-malononitrile or thiosemicarbazone **(15)**, and evaluation of their antimicrobial activity. Hydrazones **(8d)**, **(10b)** and **(10c)** showed a promising antimicrobial activity. The combination between furan and pyridine in these hydrazones could be considered to be a new strategy for discovering of new antimicrobial agents.

Conflict of Interest

The authors declare that they have no conflict of interest.

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تحضير بعض النيكوتينات المستبدلة وتقييم نشاطهم المضاد للميكروبات

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تم تشييد مجموعة متنوعة من بعض النيكوتين المستبدلة الجديدة وحدد الشكل الكيميائي لها بواسطة الوسائل الطيفية حيث يستخدم حمض النيكوتين هيدرازيد 6a / أو 6b كوسيط رئيسي في تخليق هيدرازون **(8a-l)** و**(10a-f)** بالإضافة إلى البيرازولات **(12)** و **(14)** و**(16)**. تم اختبار المركبات المصنعة حديثا ضد تسعة سلالات ميكروبية. أظهرت بعض هذه المركبات نشاطًا كبيرًا ضد العديد من الكائنات الحية الدقيقة. تم تحديد المركبات **8d** و **10b** و **10c** لتكون أكثر المركبات نشاطًا. أظهر المركب **8d** نشاطًا ضد *S. aureus* و *B. subtilis* و *A. flavus* (مع IZ = 2.4, 3.2 and 2.6) ، على التوالي عند مقارنته بالعقار المرجعي (Amoxicillin مع 3.5 و Griseofulvin، IZ = 2.3 مع IZ = 2.4). ،كما أظهر المركب **10b** نشاطًا قويًا ضد *P. aeruginosa* و *P. expansum* مع 2.8 و IZ = 2.6 ، على التوالي عند مقارنته بالعقار المرجعي (Amoxicillin IZ = 2.2 مع Griseofulvin IZ=2.8 ، على التوالي، في حين أظهر **10** نفس نشاط Griseofulvin ضد *P expansum* مع مع IZ = 2.8. وتم نقاش النشاط البيولوجي (SAR) للمركبات المقررة والعلاقة بين المجموعة الوظيفية المختلفه.