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SYNTHESIS AND BIOLOGICAL EVALUATION OF SOME NEW PYRIDINES, ISOXAZOLES AND ISOXAZOLOPYRIDAZINES BEARING 1,2,3-TRIAZOLE MOIETY

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Abstract: Some new isoxazole derivatives **3a-d** were synthesized via the reaction of 3-(dimethylamino)-1-(5-methyl-1-(3-nitrophenyl)-1*H*-1,2,3-triazol-4-yl)prop-2-en-1-one (**1**) with different hydroximoyl chloride derivatives **2a-d**. From these new isoxazoles **3a-d** a new series of isoxazolopyridazines **4a-d** was derived using hydrazine hydrate. In addition, enaminone **1** was reacted with ethyl acetoacetate to afford the corresponding ester derivative **6**, the latter was submitted to react with different chemical reagents to obtain a variety of bioactive substituted pyridine derivatives. The azido derivative **14**, was used as the key molecule for the synthesis of new urea and aryl carbamate derivatives upon its reaction with different amines and phenol through Curtius rearrangement. The chemical compositions of all the new compounds were investigated from their spectral and microanalytical data. The synthesized compounds were tested for their pharmacological potency as, anti-hepatic cancer and anti-microbial agents. Most of the tested compounds showed good anti-hepatic cancer results comparing with the standard drug doxorubicin especially when their toxic effects on the normal cell lines were studied. Referring to the anti-microbial test most of the compounds showed strong effects.

Keywords: 1,2,3-Triazoles, Hepatic cancer, Antimicrobial activity, Pyridines, Isoxazoles, Hydroximoyl chlorides, Isoxazolopyridazines, Curtius rearrangement

The emersion of drugs resistance in the disease treatment calls for the continuing investigation of new chemotherapeutic agents with higher activity and lower toxicity able to solve this problem. In the last decade, we have been involved in a program aiming to synthesize functionally substituted heterocycles using cheaply available laboratory starting materials with expected pharmaceutical activity (1-9). Recently, in the frame of our program, some new functionally substituted pyridines, isoxazoles and their fused derivatives incorporating 1,2,3-triazole moiety were required to be screened for their biological potency as antitumor agents. It seemed to us that the combination of different molecules in one entity may lead to enhanced biological activity owing to the synergistic effect of these rings.

In subsequent investigations, 1,2,3-triazoles were found to be one of the most important nitrogenous heterocyclic systems. Recently, the literature has reported a large number of systems containing 1,2,3-triazole moiety that have been incorporated into a wide spectrum of pharmaceutically and therapeutically interesting drug candidates including, GABA-antagonists (10), synthetic intermediates for antibiotics (11), anti-proliferative agents (12), cytostatic (13), antihistaminic agents (14), muscarinic agonists for the treatment of Alzheimer's disease (15), rotaxanes (16), chemi-luminescent compounds (17), nucleosides(18), virostatics (19), polyheterocyclic compounds with neuroleptic activity (20), aromatase inhibitors (21), c-Met kinase inhibitors (22), antimycobacterial agents (23), 5α-reductase

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inhibitors (24), antimicrobial agents (25), new inhibitors of HIV type 1 protease (26), antimalarial agents (27) and, glycosidase inhibitors (28), anti-cancer compound CAI (29), β-lactum antibiotic Tazobactam, the nucleoside derivative non-nucleoside reverse transcriptase inhibitor known as TSAO (30) and the cephalosporin Cefatrizine (Figure 1).

On the other hand, isoxazoles are found in many natural products as ibotenic acid. They are an important part of a number of drugs, for example, cox-2 inhibitor and nitric oxide donor–furaxan. Isoxazole and pyrazole nucleus blocks are used widely in the pharmaceutical industry (31, 32). Their derivatives were mostly utilized with high pharmacology applications, as anti-bacterial, hypoglycemic, anti-inflammatory, analgesic, anti-HIV and, anti-cancer agents (33, 34). Also, they are used in hypertension, schizophrenia and, Alzheimer disease (35-37). Pyrazoles can be used recently as anti-parasite drugs and kinase inhibitors (38, 39).

Moreover, among the most important nitrogen-containing six-member heterocycles, the pyridines or piperidines are mostly found in naturally occurring bioactive compounds like alkaloids (40).

In addition, pyridazines are one of the most important classes of six-membered heterocyclic compounds due to their diverse pharmacological activities. Pharmacologically pyridazines have been found to inhibit the activities of cAMP-PDE-4 and cGMP phosphodiesterase (PDE-3) enzymes (41).

In this paper, we report a new series of potent anti-hepatic cancer agents, through a combination of different bioactive heterocycles.

EXPERIMENTAL

Instrumentation

All melting points were determined on an electrothermal apparatus and are uncorrected. IR spectra were recorded (KBr discs) on a Shimadzu FT-IR 8201 PC spectrophotometer. ^{1}H NMR and ^{13}C NMR spectra were recorded in $CDCl_3$ and $(CD_3)_2SO$ solutions on BRUKER 400 FT-NMR system spectrometer and chemical shifts are expressed in ppm units using TMS as an internal reference. Mass spectra were recorded on a GC-MS QP1000 EX Shimadzu. Elemental analyses, anti-microbial were carried out at the Microanalytical Center of Cairo University. Anticancer activity was carried out in the Institute of Cancer, Cairo, Egypt.

3-(Dimethylamino)-1-(5-methyl-1-(3-nitrophenyl)-1*H*-1,2,3-triazol-4-yl)prop-2-en-1-one (1)

A mixture of 1-(5-methyl-1-(3-nitrophenyl)-1*H*-1,2,3-triazol-4-yl)ethanone (2.46 g, 10 mmol) and dimethylformamide dimethyl acetal (11.9 g, 14 mL, 10 mmol) were heated under reflux in dry xylene (15 mL) for 4 h. The hot solution was evaporated to its half volume, then cooled. The resulting solid was collected and crystallized from benzene to give **1** as orange crystals. Yield: 89%, m.p.: 200-202°C; FT-IR (KBr, cm^{-1}): 1665 ν (C=O), 1620 ν (C=N), 1585 ν (C=C); ^{1}H NMR (400 MHz, DMSO-d_6): δ = 2.47 (s, 6H, 2CH_3), 3.12 (s, 3H, CH_3); 5.12 (d, 1H, CH=), 6.52 (d, 1H, CH=), 7.22-7.51 (m, 4H, Ar'Hs); ^{13}C NMR (100 MHz, DMSO-d_6): δ = 15.02, 22.3, 69, 82, 106, 112.4, 122.6, 130.5, 148.9, 158.6;

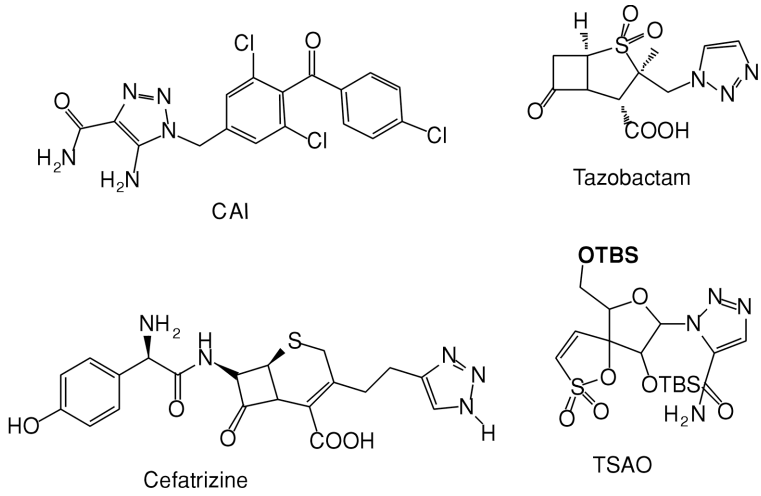


Figure 1.

Analysis: calcd. for $C_{14}H_{15}N_5O_3$ (301) C, 55.81; H, 5.02; N, 23.24%; found: C, 55.92; H, 4.89; N, 23.19%.

Isoxazole derivatives 3a-d
General procedure

Method A: A mixture of 3-(dimethylamino)-1-(5-methyl-1-(3-nitrophenyl)-1*H*-1,2,3-triazol-4-yl)prop-2-en-1-one (**1**) (3 g, 10 mmol), the appropriate hydroximoyl chlorides **2a-d** (10 mmol), and triethylamine (1.5 mL, 1 g, 10 mmol) in dry toluene (20 mL) was stirred at 0°C for 4 h. The reaction mixture was evaporated under reduced pressure and the product was triturated with petroleum ether 40/60. The resulting solid was collected and crystallized from the proper solvent to give the corresponding isoxazoles **3a-d**, respectively.

Method B: A mixture of **1** (3 g, 10 mmol) and the appropriate hydroximoyl chlorides **2a-d** (10 mmol) in dry toluene (20 mL) containing triethylamine was heated under reflux for 3 h. The solvent was evaporated under reduced pressure and the product was triturated with petroleum ether 40/60. The resulting solid was collected and crystallized from the proper solvent to give the corresponding isoxazoles **3a-d**, respectively.

4-(3-Benzoylisoxazol-4-carbonyl)-5-methyl-1-(3-nitrophenyl)-1*H*-1,2,3-triazole (3a)

Beige crystals from acetic acid. Yield: 65[a],74[b]%, m.p.: 150-152°C; FT-IR (KBr, cm^{-1}): 3078 *v* (CH), 1689 *v* (C=O), 1643 *v* (C=N),1593 *v* (C=C); ^{1}H NMR (400 MHz, DMSO-d_6): δ = 2.58 (s, 3H, CH_3), 7.32-7.85 (m, 9H, ArH's), 8.5 (s, 1H, isoxazole H-5); ^{13}C NMR (100 MHz, DMSO-d_6): δ = 10.05, 100.25, 121.03, 124.3, 128.9, 129.4, 129.7, 132.7, 135.14, 136.07, 145.5, 146.3, 148.7, 154.8; MS (EI, m/z (%)): 403 (M^+, 12), 401 (5), 370(15), 362 (22), 359 (5.9), 320(3), 260(6), 240(9), 227 (5), 105 (60), 77 (100), 50(21); Analysis: calcd. for $C_{20}H_{13}N_5O_5$ (403) C, 59.56; H, 3.25; N, 17.36%; found: C, 59.50; H, 3.13; N, 17.23%.

4-[3-(Furan-2-carbonyl)-isoxazol-4-carbonyl]-5-methyl-1-(3-nitrophenyl)-1*H*-1,2,3-triazole (3b)

Brown crystals from benzene. Yield: 58[a], 72[b]%, m.p.: >300°C; FT-IR (KBr, cm^{-1}): 2937, 2867 *v* (CH), 1680 *v* (C=O); ^{1}H NMR (400 MHz, DMSO-d_6): δ = 2.5 (s, 3H, CH_3), 7.12-7.48 (m, 7H, ArH's), and 8.32 (s, 1H, isoxazole H-5); ^{13}C NMR (100 MHz, DMSO-d_6): δ = 7.5, 100.25, 107.3, 124.26, 128.5, 129.07, 136.22, 145.13, 146.12, 151.02, 158.02; MS (EI, m/z (%)): 395(M^++2, 20), 393 (M^+, 100), 350 (7), 348 (8), 337 (18), 278 (15), 268 (1), 230 (17), 209 (82), 160 (2), 158 (4), 150 (13), 127 (38), 105 (17), 95 (100), 77 (6), 69 (39), 63 (18); Analysis: calcd. for $C_{18}H_{11}N_5O_6$ (393) C, 54.97; H, 2.82; N, 18.81%; found: C, 54.89; H, 2.75; N, 18.72%.

4-[3-(Thiophen-2-carbonyl)isoxazol-4-carbonyl]-5-methyl-1-(3-nitrophenyl)-1*H*-1,2,3-triazole (3c)

Gray crystals from benzene. Yield: 62[a],70[b]% m.p.: 290-292°C; FT-IR (KBr, cm^{-1}): 1687 *v* (C=O); ^{1}H NMR (400 MHz, DMSO-d_6): δ = 2.58 (s, 3H, CH_3), 7.13-7.58 (m, 7H, ArH's), and 8.8 (s, 1H, isoxazole H-5); MS(EI, m/z (%)): 409 (M^+, 4), 390(11), 376 (65), 368 (3), 364 (9), 248 (15), 230 (23), 140 (41), 137 (18), 109 (17), 111 (100), 77 (30), 61 (9); Analysis: calcd. for $C_{18}H_{11}N_5O_5S$ (409) C, 52.81; H, 2.71; N, 17.11%; found: C, 52.90; H, 2.65; N, 17.3%.

4-[3-(Naphthalen-2-carbonyl)-isoxazol-4-carbonyl]-5-methyl-1-(3-nitrophenyl)-1*H*-1,2,3-triazole (3d)

Beige crystals from dioxane. Yield: 68[a],91[b]% m.p.:160-162°C; FT-IR(KBr, cm^{-1}): 1698 *v* (C=O), 1622 *v* (C=N), 1590 *v* (C=C); ^{1}H NMR (400 MHz, DMSO-d_6): δ = 2.58 (s, 3H, CH_3), 7.22-7.78 (m, 11H, ArH's), 8.9 (s, 1H, isoxazole H-5); MS (EI, m/z (%)): 453(M^+, 12), 402(50), 390 (12), 380 (9), 377 (62), 260 (8), 230 (5), 227 (6), 176 (1), 150 (100), 77 (2); Analysis: calcd. for $C_{24}H_{15}N_5O_5$ (453) C, 63.58; H, 3.33; N, 15.45%; found: C, 63.64; H, 3.22; N, 15.32%.

Isoxazolo[3,4-*d*]pyridazines 4a-d
General procedure

A mixture of the appropriate isoxazoles **3a-d** (5 mmol) and hydrazine hydrate (1 g, 1 mL, 10 mmol) in ethanol (20 mL) was heated under reflux for 3 h. The reaction mixture was cooled and the resulting solid was collected and crystallized from the proper solvent to give isoxazolo[3,4-*d*]pyridazines **4a-d**, respectively.

7-(5-Methyl-1-(3-nitrophenyl)-1*H*-1,2,3-triazol-4-yl)-4-phenylisoxazolo[3,4-*d*]-pyridazine (4a)

White crystals from acetic acid. Yield: 85%, m.p.: 292-294 °C; FT-IR (KBr, cm^{-1}): 1620 *v* (C=N), 1508 *v* (C=C); ^{1}H NMR (400 MHz, DMSO-d_6): δ = 2.5 (s, 3H, CH_3), 7.32-7.51 (m, 9H, ArH's), 8.92 (s, 1H, isoxazole H-5); MS (EI, m/z (%)): 401 (M^+, 7), 390(18), 362(12), 357 (6), 354 (51), 340 (1), 337 (2), 327 (6), 320 (16), 305 (37), 267 (12), 257 (4), 160 (11), 150 (100), 70 (89); Analysis: calcd. for $C_{20}H_{13}N_7O_3$ (401) C, 59.85; H, 3.77; N, 24.43%; found: C, 59.92; H, 3.65; N, 24.36%.

4-(Furan-2-yl)-7-(5-methyl-1-(3-nitrophenyl)-1*H*-1,2,3-triazol-4-yl)isoxazolo[3,4-*d*]-pyridazine (4b)

Beige crystals from acetic acid. Yield: 90% m.p.: 250-252°C; FT-IR (KBr, cm^{-1}): 1600 *v* (C=C); ^{1}HNMR(400 MHz, DMSO-d_6): δ = 2.60 (s, 3H, CH_3), 7.37-8.11 (m, 7H, ArH's), 8.91 (s, 1H, isoxazole H-5); MS (EI, m/z (%)): 389 (M^+, 5), 374 (82), 354 (37), 353 (3), 345 (9), 327 (2), 297 (7), 267 (9), 245 (21), 240 (29), 239 (26), 237 (5), 107 (37), 97 (100), 50 (16); Analysis: calcd. for $C_{18}H_{11}N_7O_4$ (389) C, 55.53; H, 2.85; N, 25.18%; found: C, 55.62; H, 2.80; N, 25.02%.

7-(5-Methyl-1-(3-nitrophenyl)-1*H*-1,2,3-triazol-4-yl)-4-(thiophen-2-yl)isoxazolo[3,4-*d*]-pyridazine (4c)

Beige crystals from ethanol. Yield: 91%, m.p.: >300°C; FT- IR (KBr, cm^{-1}): 1602 *v* (C=N), 1509 *v* (C=C); ^{1}H NMR (400 MHz, DMSO-d_6): δ = 2.57 (s, 3H, CH_3), 7.13-7.79 (m, 7H, ArH's), 8.95 (s, 1H, isoxazole H-5); ^{13}C NMR (100 MHz, DMSO-d_6): δ = 7.35, 110.01, 121.63, 124.05, 128.2,129.43, 134.01,136.8, 142.5, 144.7, 152.3, 155.9; MS (EI, m/z (%)): 405 (5), 403 (9), 390 (16), 378 (16), 369 (3), 218 (14), 195 (31), 165 (12), 150 (26), 147 (8), 133 (18), 120 (9), 90 (1), 77 (100), 66 (9); Analysis: calcd. for $C_{18}H_{11}N_7O_3S$ (405) C, 53.33; H, 2.73; N, 24.19%; found: C, 53.39; H, 2.65; N, 24.10%.

7-(5-Methyl-1-(3-nitrophenyl)-1*H*-1,2,3-triazol-4-yl)-4-(naphthalen-6-yl)isoxazolo[3,4-*d*]pyridazine (4d)

Yellow crystals from acetic acid .Yield: 95% m.p.: 240-242°C; FT-IR (KBr, cm^{-1}): 1698 *v* (C=O), 1620 *v* (C=N), 1590 *v* (C=C); ^{1}H NMR (400 MHz, DMSO-d_6): δ = 2.57 (s, 3H, CH_3), 7.21-8.11 (m, 11H, ArH's) ,8.95 (s, 1H, isoxazole H-5); MS (EI, m/z (%)): 449 (M^+, 18), 448 (M^+-l, 1), 435 (5), 420 (29), 395 (19), 327 (5), 308 (19), 287 (8), 248 (17), 237 (6), 211 (3), 194 (18), 178 (13), 177 (19), 155 (10), 125 (2), 70 (89); Analysis: calcd. for $C_{24}H_{15}N_7O_3$ (449) C, 64.14; H, 3.36; N, 21.82%; found: C, 64.19; H, 3.25; N, 21.79%.

Ethyl 2-methyl-6-(5-methyl-1-(3-nitrophenyl)-1*H*-1,2,3-triazol-4-yl)pyridin-3-carboxylate (6)

A mixture of **1** (1.5 g, 5mmol) and ethyl acetoacetate (0.65 mL, 5mmol) in acetic acid (30 mL) containing ammonium acetate (0.37g, 5mmol) was refluxed for 4 h. The resulting solid was collected and crystallized to give **6** as yellow crystals from acetic acid. Yield (92%), m.p.: 120-122°C; FT-IR (KBr, cm^{-1}): 1735 *v* (C=O, ester); ^{1}H NMR (400 MHz, DMSO-d_6): δ = 1.22 (t, 3H, CH_2CH_3), 2.58 (s, 3H, CH_3). 2.69 (s, 3H, CH_3), 4.12 (q, 2H, CH_2CH_3), 7.18-7.89 (m, 6 H, ArH's); MS (EI, m/z (%)): 367 (M^+, 18), 352 (14), 337 (11), 323 (5), 269 (14), 250 (9), 197 (26), 177 (29), 150 (100), 127 (17), 105 (2), 103 (2), 77 (80), 66 (19); Analysis: calcd. for $C_{18}H_{17}N_5O_4$ (367) C,58.85; H,4.66; N,19.06%; found: C, 58.94; H, 4.56; N, 18.97%.

2-Methyl-6-(5-methyl-1-(3-nitrophenyl)-1*H*-1,2,3-triazol-4-yl)pyridin-3-carbohydrazide (7)

Equimolar amounts of ethyl 2-methyl-6-(5-methyl-l-(3-nitrophenyl)-l*H*-1,2,3-triazol-4-yl)pyridin-3-carboxylate (**6**) and hydrazine hydrate (5 mmol for each) in ethanol (10 mL) were refluxed for 3 h. The resulting solid, was cooled and crystallized from ethanol to give **7** as white crystals. Yield (79%), m.p.: 160-162°C. FT-IR (KBr, cm^{-1}): 3390 *v* (br., NH, NH_2), 1662 *v* (C=O), 1630 *v* (C=N), 1596 *v* (C=C); ^{1}H NMR (400 MHz, DMSO-d_6): δ = 2.53 (s, 3H, CH_3), 2.69 (s, 3H, CH_3), 5.5 (s, br., 2H, NH_2), 7.11-7.89 (m, 6 H, ArH's), 8.53(s, 1H, NH); ^{13}C NMR (100 MHz, DMSO-d_6): δ = 6.95, 18.8, 121.6, 122.5, 124.7, 129.3, 134, 136.5, 138.6, 144.1, 148.05, 153.2, 158.06; MS (EI, m/z (%)): 354 (M^++1, 15), 353(M^+, 14), 336 (6), 322 (45), 277 (12), 250 (6), 217 (3), 165 (20), 123 (6), 50 (12), 28 (100); Analysis: calcd. for $C_{16}H_{15}N_7O_3$ (353) C, 54.39; H, 4.28; N, 27.75%; found: C, 54.45; H, 4.15; N, 27.64%.

(3,5-Dimethyl-1*H*-pyrazol-1-yl)(2-methyl-6-(5-methyl-1-(3-nityrophenyl)-1*H*-1,2,3-triazol-4-yl) pyridin-3-yl)methanone (8) and 5-Methyl-2-[2-methyl-6-(5-methyl-1-phenyl-1*H*-1,2,3-triazol-4-yl)pyridin-3-carbonyl]-2,4-dihydro-pyrazol-3-one (9)

Equimolar amounts of 2-methyl-6-(5-methyl-l-(3-nitrophenyl)-l*H*-l,2,3-triazol-4-yl)-pyridme-3-carbohydrazide (**7**) and acetylacetone or ethyl acetoacetate (10 mmol for each) in ethanol (20 mL) with few drops of acetic acid were refluxed for 2 h. The resulting solid, so formed, was cooled and crystallized from the proper solvent to give the corresponding **8** and **9**, respectively.

(3,5-Dimethyl-1*H*-pyrazol-1-yl)(2-methyl-6-(5-methyl-1-(3-nitrophenyl)-1*H*-1,2,3-triazol-4-yl)pyridin-3-yl)methanone (8)

White crystals from acetic acid. Yield (85%), m.p.: 190-192°C; FT-IR (KBr, cm^{-1}): 1695 *v* (C=O), 1589 *v* (C=C); ^{1}H NMR (400 MHz, DMSO-d_6): δ = 2.42 (s, 3H, CH_3), 2.53 (s, 3H, CH_3), 2.67 (s, 3H, CH_3), 2.70 (s, 3H, CH_3), 6.52 (s, 1H, pyrazole H-4), 7.18-7.72 (m, 6 H, ArH's); MS (EI, m/z (%)): 418 (M^++l, 14), 417 (M^+, 100), 402 (81), 387 (15), 372 (19), 357 (18), 285 (18), 257 (10), 227 (9), 199 (6),

150 (7), 145 (11), 115 (43), 96 (6); Analysis: calcd. for $C_{21}H_{19}N_7O_3$ (417) C, 60.42; H, 4.59: N, 23.49%; found: C, 60.35; H,4.65: N, 23.32%.

5-Methyl-2-[2-methyl-6-(5-methyl-1-(3-nitrophenyl)-1*H*-1,2,3-triazol-4-yl)-pyridin-3-carbonyl]-2,4-dihydropyrazol-3-one (9)

White crystals from acetic acid. Yield (80%), m.p.: 250-252°C; FT-IR (KBr, cm^{-1}): 1720, 1670 *v* (CO), 1620 *v* (C=C); ^{1}H NMR (400 MHz, DMSO-d_6): δ = 2.28 (s, 3H, CH_3), 2.52 (s, 3H. CH_3), 2.62 (s, 3H, CH_3), 3.72 (s, 2H, CH_2 (pyrazole)), 7.11-7.79 (m, 6 H, ArH's); ^{13}C NMR (100 MHz, DMSO-d_6): δ = 7.12, 18.8, 24.5, 42.9, 121.3, 122.5, 124.9, 129.8, 134.2, 136.1, 138.2, 144, 148.6, 153.1, 159.7, 162; MS (EI, m/z (%)): 419 (M^+, 30), 417(5), 404(12), 389(5), 285(3), 197(7), 153 (6). 135 (10), 105 (100), 95 (8), 77 (29), 69 (15); Analysis: calcd. for $C_{20}H_{17}N_7O_4$ (419) C, 57.28; H, 4.09; N, 23.38%; found: C, 57.36; H, 3.97; N, 23.28%.

(4-Arylazo-3,5-dimethylpyrazol-1-yl)-[2-methyl-6-(5-methyl-1-(3-nitrophenyl)-1*H*-1,2,3-triazol-4-yl)-pyridin-3-yl]methanone (10) and 4-(Arylhydrazono)-5-methyl-2-[2-methyl-6-(5-methyl-1-(3-nitrophenyl)-1*H*-1,2,3-triazol-4-yl)pyridin-3-carbonyl]-2,4-dihydropyrazol-3-one (11)

Method A: Dropwise addition of arene diazonium chlorides (5 mmole), which is prepared *via* reaction of the appropriate aniline or *p*-toluidene (5 mmole), hydrochloric acid (3 mL, 6 M), and sodium nitrite (0.37 g, 5 mmol) at 0-5°C, to a mixture of the appropriate **8** or **9** (5 mmol) and sodium acetate trihydrate (0.66 g, 5 mmole) in ethanol (20 mL) at 0-5°C, while stirring. The reaction mixture was stirred for 3 h. The resulting solid was collected, washed with water and crystallized to give **10a,b** and **11a,b**, respectively.

Method B: A mixture of **7** and the appropriate 3-(2-arylhydrazono)pentane-2,4-dione **12** or ethyl 2-arylazo-3-oxo-4-butanoate **13** (5 mmol for each) in ethanol (20 mL) and catalytic amount of acetic acid (2-5 drops) was refluxed for 2 h. The resulting solid, so formed, was collected and crystallized from acetic acid to give product identical in all aspects with corresponding products obtained in Method A.

(4-Phenylazo-3,5-dimethylpyrazol-1-yl)[2-methyl-6-(5-methyl-1-(3-nitrophenyl)-1*H*-1,2,3- triazol-4-yl)pyridin-3-yl]methanone (10a)

Brown crystals from ethanol. Yield (75%), m.p.: 230-232°C; FT-IR (KBr, cm^{-1}): 1710 *v* (C=O), 1647 *v* (C=N), 1547 *v* (C=C); ^{1}H NMR (400 MHz, DMSO-d_6): δ =2.29 (s, 3H, CH_3), 2.37 (s, 3H, CH_3), 2.64 (s, 3H, CH_3). 2.77 (s, 3H, CH_3), 7.23-8.14 (m, 9 H, ArH's), 8.81 (d, 1H, ArH), 9.17 (d, 1H, ArH); MS (EI, m/z (%)): 521 (M^+, 51), 506 (11), 491(23), 476 (15), 430 (17), 422 (1), 377 (17), 365 (17), 350 (7), 312 (27), 277 (35), 237 (37), 191 (15), 181 (3), 165 (100), 120 (14), 70 (7), 43 (39); Analysis: calcd. for $C_{27}H_{23}N_9O_3$ (521) C, 62.18; H, 4.45; N, 24.17%; found: C, 62.12; H, 4.38; N, 24.11%.

(4-*p*-Tolylazo-3,5-dimethyl-pyrazol-1-yl)[2-methyl-6-(5-methyl-1-(3-nitrophenyl)-1*H*-1,2,3-triazol-4-yl)pyridin-3-yl]methanone (10b)

Brown crystals from acetic acid. Yield (89%), m.p.: 228-230°C. FT-IR (KBr, cm^{-1}): 1698 *v* (C=O), 1622 *v* (C=N), 1590 *v* (C=C); ^{1}H NMR (400 MHz, DMSO-d_6): δ = 2.21 (s, 3H, CH_3), 2.32 (s, 3H, CH_3), 2.37 (s, 3H, CH_3), 2.68 (s, 3H, CH_3), 2.72 (s, 3H, CH_3), 7.15-7.72 (m, 8 H, ArH's), 8.26 (d, 1H, ArH's), 9.15 (d, 1H, ArH); ^{13}C NMR (100 MHz, DMSO-d_6): δ = 7.1, 9.6, 12.2, 18.4, 31.3, 104.4, 107.6, 123.9, 125.7, 128.9, 129, 130.9, 137.6, 138.5, 140, 143.8, 155.3; MS (EI, m/z (%)): 535 (M^+, 81), 534 (M^+-1, 16), 520(7), 504(3), 491(12), 456(23), 432(15), 420 (17), 396 (2), 370 (10), 309 (9), 290 (11), 277 (17), 257 (15). 225 (17), 170 (19), 156 (5), 145 (27), 127 (19), 123 (4), 117 (28), 97 (5), 96 (48), 77 (72), 60 (99); Analysis: Calcd. for $C_{28}H_{25}N_9O_3$ (535) C, 62.79; H, 4.71; N, 23.54%; found: C, 62.63; H, 4.82; N, 23.61%.

4-(Phenylhydrazono)-5-methyl-2-[2-methyl-6-(5-methyl-1-(3-nitrophenyl)-1*H*-1,2,3-triazol-4-yl)pyridin-3-carbonyl]-2,4-dihydropyrazol-3-one (11a)

Yellowish brown crystals from acetic acid. Yield (64%), m.p.: >300°C; FT-IR (KBr, cm^{-1}): 3290 *v* (NH), 1720, 1668 *v* (C=O), 1593 *v* (C=C); ^{1}H NMR (400 MHz, DMSO-d_6): δ = 2.21 (s, 3H, CH_3), 2.64 (s, 3H, CH_3), 2.71 (s, 3H, CH_3), 7.10-7.65 (m, 11 H, ArH's), 11.8 (s, 1H, NH); MS (EI, m/z (%)): 523 (M^+, 7), 508(19), 493(5), 478 (4), 450(6), 412(12), 396(3), 365(17), 355 (2), 327 (4), 305 (9), 295 (6), 277 (26), 272 (25), 250 (49), 238 (24), 221 (15), 205 (6), 179 (13), 166 (29), 140 (14), 125 (14), 77 (6), 30 (100); Analysis: calcd. for $C_{26}H_{21}N_9O_4$ (523) C, 59.65; H, 4.04; N, 24.08%; found: C, 59.76; H, 4.95; N, 23.97%.

4-(*p*-Tolylhydrazono)-5-methyl-2-[2-methyl-6-(5-methyl-1-(3-nitrophenyl)-1*H*-1,2,3-triazol-4-yl)pyridin-3-carbonyl]-2,4-dihydropyrazol-3-one (11b)

Yellow crystals from acetic acid. Yield (72%). m.p.: 260-262°C; FT-IR (KBr, cm^{-1}): 3341 *v* (NH),

1715, 1663 *v* (C=O), 1602 *v* (C=C); ^{1}H NMR (400 MHz, DMSO-d_6): δ = 2.22 (s, 3H, CH_3), 2.37 (s, 3H, CH_3), 2.65 (s, 3H, CH_3), 2.75 (s, 3H, CH_3), 7.11-7.68 (m, 10 H, ArH's), 11.02(s, 1H, NH); MS (EI, m/z (%)): 539 (M^++2.9), 537(100), 522(9), 507(13), 485(12), 397 (19), 387 (19), 367 (19), 350 (17), 332 (1), 325 (17), 305 (14), 287 (17), 197 (29), 137 (15), 125 (3), 98 (18), 66 (26); Analysis: calcd. for $C_{27}H_{23}N_9O_4$ (537) C. 60.33; H, 4.31; N, 23.45%; found: C, 60.27; H, 4.21: N, 23.36%.

Azido(2-methyl-6-(5-methyl-1-(3-nitrophenyl)-1*H*-1,2,3-triazol-4-yl)pyridin-3-yl)-methanone (14)

To a stirred solution of 2-methyl-6-(5-methyl-1-(3-nitrophenyl)-1*H*-1,2,3-triazol-4-yl)-pyridin-3-carbohydrazide (**7**) (5 mmole) in acetic acid (15 mL) at 0-5°C, sodium nitrite was added portion-wise till effervescence ended. The reaction mixture was stirred for 2 h. The resulting solid, was collected, filtered, washed with water, and crystallized to give the corresponding **14**. Beige crystals, from acetic acid. Yield (86%), m.p.: 180-184°C; FT-IR (KBr, cm^{-1}): 3090 *v* (CH), 2137 *v* (azide group) (42), 1687 *v* (C=O); ^{1}H NMR (400 MHz, DMSO-d_6): δ = 2.53 (s, 3H, CH_3), 2.68 (s, 3H, CH_3), 7.66-8.11 (m, 6 H, ArH's); MS (EI, m/z (%)): 364 (M^+, 15), 349(11), 334(6), 319 (45), 292(6), 278(14), 264 (16), 257 (45), 245 (10), 227 (19), 197 (18), 177 (22), 165 (12), 105(100), 77 (17), 50 (23); Analysis: calcd. for $C_{16}H_{12}N_8O_3$ (364) C, 52.75; H, 3.32; N, 30.76%; found: C, 52.66; H, 3.25; N, 30.62%.

1-(2-Methyl-6-(5-methyl-1-(3-nitrophenyl)-1*H*-1,2,3-triazol-4-yl)pyridin-3-yl)-3-substituted urea 15a-c and 16

A mixture of **14** (1.85 g, 5 mmol) and appropriate aniline, *p*-toluidine, *p*-Anisidine or 3-amino-5-phenylpyrazole (5 mmol) in dry dioxane (20 mL) was refluxed for 4 h. The resulting solid, so formed, was collected and recrystallized to give **15a-c** and, **16**, respectively.

1-(2-Methyl-6-(5-methyl-1-(3-nitrophenyl)-1*H*-1,2,3-triazol-4-yl)pyridin-3-yl)-3-phenylurea (15a)

Gray crystals, from *N*,*N*-dimethylformamide. Yield (75%). m.p.: 243-245°C; FT-IR (KBr, cm^{-1}): 3385 *v* (NH), 1695 *v* (C=O); ^{1}H NMR (400 MHz, DMSO-d_6): δ = 2.27 (s, 3H, CH_3), 2.58 (s, 3H, CH_3), 7.62-7.89 (m, 11 H, ArH's), 9.25 (s, 2H, 2NH); MS (EI, m/z (%)): 429 (M^+, 12), 414 (15), 399(1), 333(19), 276 (23), 237 (35), 137 (1), 91 (100), 77 (18); Analysis: calcd. for $C_{22}H_{19}N_7O_3$ (429) C, 61.53; H, 4.46; N, 22.83%; found: C, 61.59; H, 6.35; N, 22.78%.

1-(2-Methyl-6-(5-methyl-1-(3-nitrophenyl)-1*H*-1,2,3-triazol-4-yl)pyridin-3-yl)-3-*p*-tolylurea (15b)

Off-white crystals, from *N*,*N*-dimethylformamide, Yield (91%), m.p.: 260-262°C. FT-IR (KBr, cm^{-1}): 3306 *v* (NH), 1710 *v* (C=O), 1620 *v* (C=N), 1590 *v* (C=C); ^{1}HNMR (400 MHz, DMSO-d_6): δ = 2.27 (s, 3H, CH_3), 2.32 (s, 3H, CH_3), 2.65 (s, 3H, CH_3), 7.68-8.21 (m, 10 H, ArH's), 9.54 (s, 2H. 2NH); ^{13}C NMR (100 MHz, DMSO-d_6): δ = 9.6, 16.8, 107.02, 121.3, 123.6, 128.3, 129.9, 133.5, 135.7, 137.5, 139.8, 141.3, 143.9, 144.1, 146.2, 152.1; MS (EI, m/z (%)): 443 (M^+, 3), 328 (11), 313(25), 287 (45), 268 (13), 277 (63), 150 (18), 105 (100), 77 (17); Analysis: calcd. for $C_{23}H_{21}N_7O_3$ (443) C, 62.29; H, 4.77; N, 22.11%; found: C, 62.38; H, 4.72; N, 22.02%.

1-(4-Methoxyphenyl)-3-(2-methyl-6-(5-methyl-1-(3-nitrophenyl)-1*H*-1,2,3-triazol-4-yl)-pyridin-3-yl)urea (15c)

Yellow crystals, from *N*,*N*-dimethylformamide. Yield (83%), m.p.: >300°C; FT-IR (KBr, cm^{-1}): 3320 *v* (NH), 1697 *v* (C=O), 1620 *v* (C=N), 1605 *v* (C=C); ^{1}H NMR (400 MHz, DMSO-d_6): δ = 2.22 (s, 3H, CH_3), 2.46 (s, 3H, CH_3), 3.92 (s, 3H, OCH_3), 7.14-7.82 (m, 10 H, ArH's), 9.65 (s, 1H, NH), 9.75 (s, 1H. 1NH); MS (EI, m/z (%)): 459 (M^+, 18), 444 (19),428 (5), 413 (11), 398 (14), 332 (12), 316 (14), 295 (32), 258 (19), 257 (55), 217 (11), 205 (11), 195 (8), 190 (14), 184 (100), 170 (11), 127(8); Analysis: calcd. for $C_{23}H_{21}N_7O_4$ (459) C, 60.12; H, 5.61; N, 21.34%; found: C, 60.22; H, 5.69; N, 21.23%.

1-(2-Methyl-6-(5-methyl-1-(3-nitrophenyl)-1*H*-1,2,3-triazol-4-yl)pyridine-3-yl)-3-(3-phenyl-1*H*-pyrazol-5-yl)urea (16)

Yellow crystals, from ethanol. Yield (85%), m.p.: 230-232°C; FT-IR (KBr, cm^{-1}): 3316 *v* (NH), 1693 *v* (C=O); ^{1}H NMR (400 MHz, DMSO-d_6): δ = 2.42 (s, 3H, CH_3), 2.58 (s, 3H, CH_3), 6.63 (s, 1H, pyrazole H-4), 7.18-7.99 (m, 11 H, ArH's), 8.95 (s, 1H, 1NH), 9.58 (s, 2H, 2NH); ^{13}C NMR (100 MHz, DMSO-d_6): δ = 8.9, 16.5, 87.8, 105.8, 121.6, 122.1, 123.9, 127.5, 128.7, 129.2, 133.05, 137, 141.4, 143.2, 144.01, 146.9, 151.8, 153.2; MS (EI, m/z (%)): 495 (M^+, 37), 480 (51), 465(12), 399(9), 377 (17), 365 (4), 337(7), 320 (31), 320 (15), 308 (27), 280 (3), 237 (7), 220 (17), 165 (100), 119 (14), 66 (17)57 (11), 50 (36); Analysis: calcd. for $C_{25}H_{21}N_9O_3$ (495) C, 60.60; H, 4.27; N, 25.44%; found: C,60.78; H, 4.17; N, 25.32%.

3-(2-Methyl-6-(5-methyl-1-(3-nitrophenyl)-1*H*-1,2,3-triazol-4-yl)pyridin-3-yl)quinazolin-2,4(1*H*, 3*H*)dione (17)

A mixture of **14** (1.85 g, 5 mmol) and appropriate anthranilic acid or methyl anthranilate (5 mmol) in dry dioxane (20 mL) was refluxed for 4 h. The resulting solid formed, was collected and crystallized to give **17** as gray crystals, from acetic acid. Yield (87%), m.p.: 220-222°C; FT-IR (KBr, cm^{-1}): 3323 *v* (NH), 1668 *v* (C=O), 1587 *v* (C=C); ^{1}H NMR (400 MHz, DMSO-d_6): δ = 2.37 (s, 3H, CH_3), 2.52 (s, 3H, CH_3), 7.29-8.12 (m, 10 H, ArH's), 9.23 (s, 1H. 1NH); MS (EI, m/z (%)): 455 (M^+, 71), 397 (19), 382 (90), 207 (24), 198 (20), 177 (25), 108 (20), 90 (23), 77 (26), 60 (12), 55 (9); Analysis: calcd. for $C_{23}H_{17}N_7O_4$ (455) C, 60.66; H, 3.76; N, 21.53%; found: C, 60.78; H, 3.82; N, 21.64%

Phenyl 2-Methyl-6-(5-methyl-1-(3-nitrophenyl)-1*H*-1,2,3-triazol-4-yl)pyridin-3-yl-carbamate (18)

A mixture of **14** (1.85 g, 5 mmol) and phenol (5 mmol) in dry benzene (20 mL) was refluxed for 4 h. The resulting solid formed was collected and crystallized from dioxane to give **18** as white crystals, yield (65%) m.p.: 212-214°C; FT-IR (KBr, cm^{-1}): 1672 *v* (C=O), 1621 *v* (C=N); ^{1}H NMR (400 MHz, DMSO-d_6): δ = 2.35 (s, 3H, CH_3), 2.51 (s, 3H, CH_3), 7.11-8.12 (m, 11H, ArH's), 9.48 (s, 1H, 1NH); MS (EI, m/z (%)): 430 (M+, 35), 412 (9), 385 (23), 383 (100), 290 (15), 270 (28), 195 (30), 177 (14), 170 (90), 165 (22), 157 (15), 150 (28), 127 (10), 122 (15); Analysis: calcd. for $C_{22}H_{18}N_6O_4$ (430) C,61.39; H, 4.22; N,19.53%; found: C, 61.45; H, 4.18; N, 19.45%.

Anti-proliferative Activity Cells

Cell lines: Hep-G2 (human hepatic cancer) and BALB/3T3 (murine fibroblast) were obtained from American Type Culture Collection (Rockville, Maryland, USA) and maintained in the Institute of Cancer, Cairo, Egypt. HepG2 cells were cultured in Eagle's Minimum Essential Medium (EMEM) supplemented with 10% FBS; DMEM and RPMI1640 are also alternatives that work well. Aspirate and add fresh culture medium every 2-3 days. HepG2 cell doubling time is 48 h. BALB/3T3 cell line was cultured in DMEM (Gibco, UK) supplemented with 2 mM L-glutamine, 10% fetal bovine serum (GE Healthcare, Logan, UT, USA). To passage cells, rinse cell monolayer with 1x PBS twice and add prewarmed (37°C) 0.05% Trypsin-EDTA solution to cover the bottom of the flask; incubate for 5-7 min. As cells detach, neutralize the Trypsin by adding 4x volume of complete growth medium with 10% FBS and gently suspend the cells by pipetting. To avoid clumping do not agitate the cells by shaking the flask while waiting for detachment. Split cells 1:4 every 3 days or 1 : 8 every 6 days. Cultures should be incubated at 37°C in a humidified atmosphere with 5% CO_2.

Compounds

All compounds were dissolved in DMSO (stock solution 10 mg/mL) and subsequently diluted in culture medium to reach the required concentrations (ranging from 100 to 0,1 µg/mL).

An anti-proliferative assay in vitro

24 h before addition of the tested compounds, the cells were plated in 96-well plates (Sarstedt, Germany) at a density of 1×10^4 cells per well. The assay was performed after 72 h exposure to varying concentrations of the tested compounds. The *in vitro* cytotoxic effect of all compounds was examined using the SRB assay.

Cytotoxic test SRB

The details of this technique were described by Skehan et al. (43, 44). The cells were attached to the bottom of plastic wells by fixing them with cold 50% TCA (trichloroacetic acid, Sigma-Aldrich Chemie GmbH, Steinheim, Germany) on the top of the culture medium in each well. The plates were incubated at 4°C for 1 h and then washed five times with tap water. The cellular material fixed with TCA was stained with 0.4% sulphorhodamine B (SRB, Sigma-Aldrich Chemie GmbH, Steinheim, Germany) dissolved in 1% acetic acid for 30 min. Unbound dye was removed by rinsing (five times) in 1% acetic acid. The protein-bound dye was extracted with 10 mM unbuffered Tris base for determination of the optical density (λ = 540 nm) in Synergy H4 multi-mode microplate reader (BioTek Instruments USA).

Antimicrobial activity

The samples were prepared by dissolving 2 mg in 2 mL of DMSO and 100 µL (containing 100 µg) was used in this test. The antimicrobial activity of different samples was investigated by the agar cup plate method. Four different test microbes namely: *Pseudomonas aeruginosa* (Gram-negative), *Staphylococcus aureus* (Gram-positive), *Aspergillus niger* (fungus) *and Candida albicans* (yeast) were used. Nutrient agar plates were heavily seeded uniformly with 1mL of 10^5-10^6 cells/mL in case of bacteria and yeast. A Czapek-Dox agar plate seeded by the fungus was used to evaluate the antifungal activities.

Then a hole was made in media by gel cutter (Cork borer no.4) in a sterile condition. Then, one drop of melted agar was poured into the hole and allowed to solidify to make a base layer. After that specific amount of culture filtrate (0.1 mL) was poured into the hole. Then plates were kept at low temperature (4°C) for 2-4 h to allow maximum diffusion. The plates were then incubated at 37°C for 24 h for bacteria and at 30°C for 48 h in the upright position to allow maximum growth of the organisms. The antimicrobial activity of the test agent was determined by measuring the diameter of zone of inhibition expressed in millimeter (mm). The experiment was carried out more than once and mean of reading was recorded (45).

RESULTS AND DISCUSSION

Chemistry

The synthetic procedures adopted for the preparation of the target compounds **1-18** were outlined in Schemes 1-3.

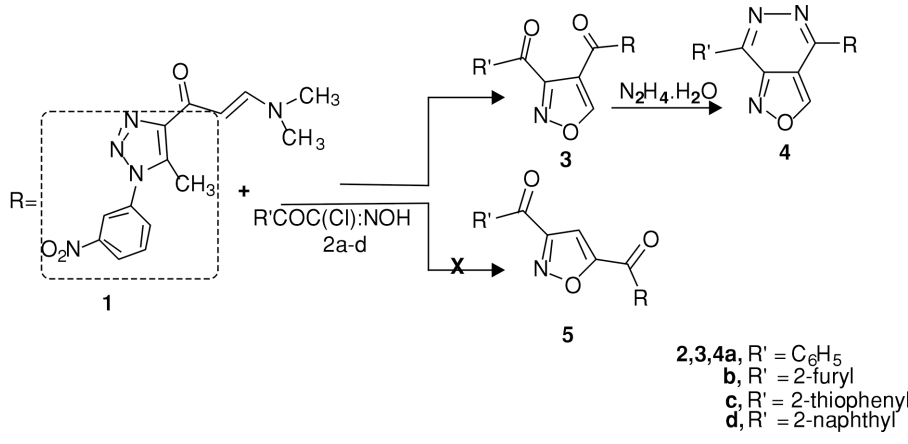
3-(Dimethylamino)-1-(5-methyl-1-(3-nitrophenyl)-1*H*-1,2,3-triazol-4-yl)prop-2-en-1-one (**1**) was prepared through the reaction of the previously synthesized 1-(5-methyl-1-(3-nitrophenyl)-1*H*-1,2,3-triazol-4-yl)ethanone (5) with dimethylformamide dimethyl acetal in dry xylene (Scheme 1).

Boiling of the enamine **1** with 2-chloro-2-(hydroxyimino)-1-phenylethanone **2a** in dry toluene containing triethylamine, gave 4-(3-benzoylisoxazol-4-carbonyl)-5-methyl-1-(3-nitrophenyl)1*H*-1,2,3-triazole (**3a**), not 4-(3-benzoylisoxazol-5-carbonyl)5-methyl-1-(3-nitrophenyl)1*H*-1,2,3-triazole **5a** based on the spectral data of the obtained product, where the ^{1}H NMR spectrum of **3a** showed singlet signal at δ = 8.5 ppm, which indicated the formation of isoxazole ring. The structure of **3a** also confirmed through the chemical transformation to **4a** by reaction with hydrazine hydrate.

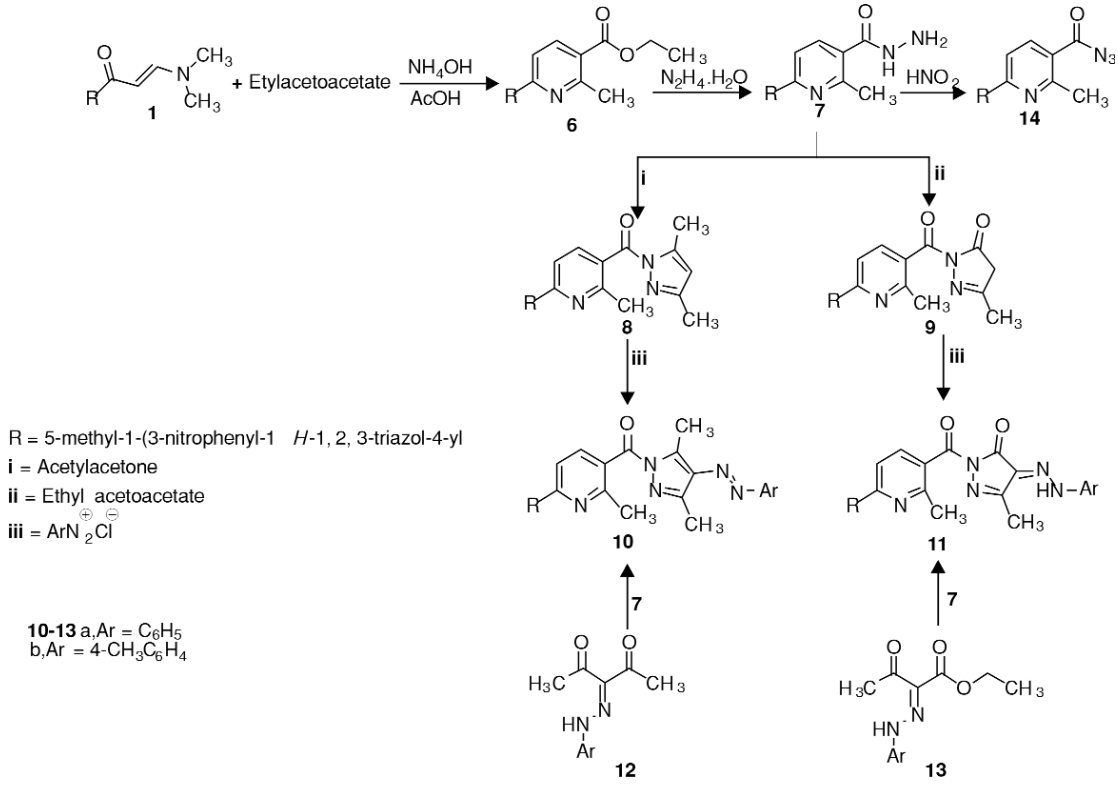
On the other hand, structure of compound **3a** was confirmed via alternative synthetic pathway in which stirring of the enamine **1** with **2a** in dry toluene containing catalytic amount of triethylamine at room temperature affords product identical in all aspects (m.p., mixed m.p., IR, NMR, mass spectra) with that obtained from the previous method.

Analogously, **1** was reacted with the appropriate hydroximoyl chlorides **2b-d** to give the corresponding isoxazoles **3b-d**, respectively. Refluxing of **3b-d** with hydrazine hydrate in boiling ethanol afforded the corresponding isoxazolopyridazines **4b-d** (46, 47) (Scheme 1).

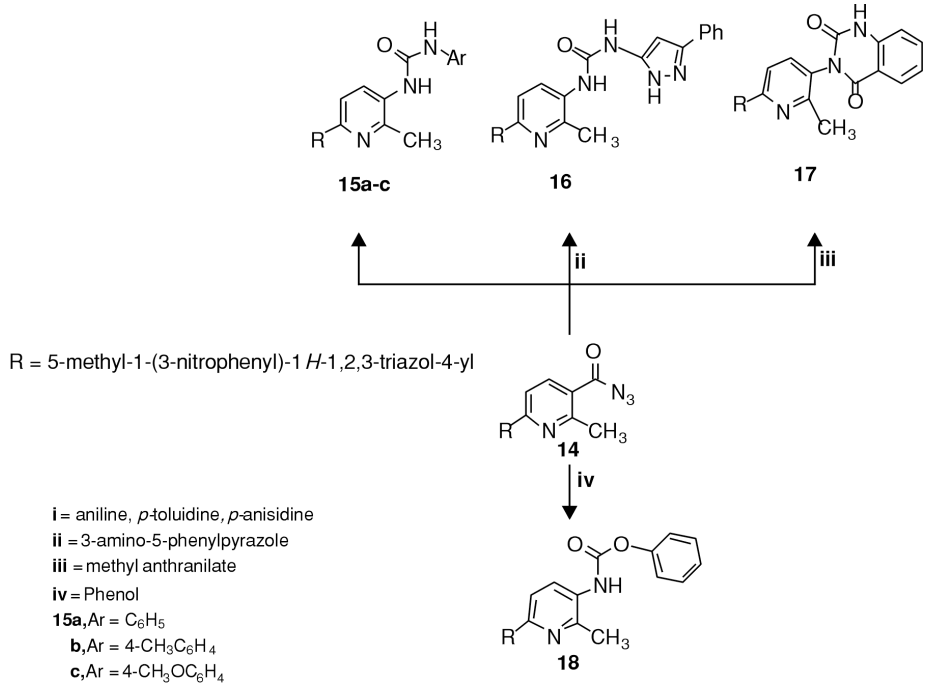
Then, enamine **1** was submitted to react with ethyl acetoacetate in boiling acetic acid containing ammonium acetate in the catalytic amount under reflux for 5 h to give ethyl 2-methyl-6-(5-methyl-1-(3-nitrophenyl)-1*H*-1,2,3-triazole-4-yl)pyridine-3-carboxylate (**6**). The chemical composition of **6** was concluded from its spectral data, where, its IR spectra revealed strong absorption band at ν 1735 cm^{-1} for the carbonyl ester group, its ^{1}H NMR spectrum revealed triplet signal at δ = 1.22 ppm and quartet signal at δ = 4.12 ppm for three protons and two protons of the ester group, respectively. Also, the chemical structure of **6** was supported by its mass spectrum which agrees with its molecular formula (see the experimental part). Then, **6** was reacted with hydrazine hydrate to give 2-methyl-6-(5-methyl-1-(3-nitrophenyl)-1*H*-1,2,3-triazole-4-yl)pyridine-3-



Scheme 1.



Scheme 2.



Scheme 3.

carbohydrazide (**7**). The IR spectrum of **7** showed the disappearance of the absorption band of the carbonyl ester group beside the appearance of a new band at v1662 cm^{-1} for the carbonyl amid group. Also, a strong abroad band appeared at v 3390 cm^{-1} for NH, NH_2 groups. ^{1}H NMR spectrum of **7** revealed singlet signal at δ = 5.5 ppm for NH_2 group and singlet signal at δ = 8.53 ppm for NH group. Also, the ^{1}H NMR spectra of **7** devoid of any signals for the protons of the ester group which indicated its involvement in the reaction.

The later was submitted to react with acetylacetone and, ethyl acetoacetate to afford (3,5-dimethyl-1*H*-pyrazol-1-yl)(2-methyl-6-(5-methyl-1-(3-nitrophenyl)-1*H*-1,2,3-triazol-4-yl)pyridine-3-yl)methanone (**8**) and 5-methyl-2-[2-methyl-6-(5-methyl-1-(3-nitrophenyl)-1*H*-1,2,3-triazol-4-yl)pyridine-3-carbonyl]-2,4-dihydropyrazol-3-one (**9**).

Chemical structures of **8** and **9** were deduced from its spectral data. ^{1}H NMR spectrum of **8** revealed the disappearance of any signals for NH or NH_2 groups in addition to the appearance of singlet signal at δ = 6.52 ppm for H-4 of pyrazole. On the other hand, IR spectrum of compound **9** showed a strong absorption band at v 1670 cm^{-1} for the carbonyl of pyrazolone ring. Also, its ^{1}H NMR spectrum showed singled signals at δ = 3.72 ppm for the two protons of CH_2 of pyrazolone ring.

In addition, chemical structures of compounds **8** and **9** were confirmed via chemical transformation, in which they reacted with arene diazonium chlorides in a cold solution of ethanol in the presence of sodium acetate as a buffer solution to give **10** and **11**.

The chemical composition of compounds **10** and **11** was confirmed using an alternative synthetic

Table 1. The anti-proliferative potency of the newly prepared derivatives towards hepatic cancer and normal cell lines.

Compound	Hep-G2 IC_{50} ± SD [µg/mL]	BALAB/3T3 IC_{50} ± SD [µg/mL]
Doxorubicin	3.57 ± 0.48	3.21 ± 0.37
1	Nd	Nd
3a	63.32 ± 11.83	34.09 ± 12.00
3b	59.20 ± 9.37	Nd
3c	53.32 ± 10.83	Nd
3d	Nd	Nd
4a	Nd	Nd
4b	37.52 ± 6.81	Nd
4c	53.24 ± 8.65	56.72 ± 4.14
4d	Nd	Nd
6	11.93 ± 3.08	Nd
7	53.51 ± 4.48	1.24 ± 3.01
8	Nd	Nd
9	42.73 ± 5.36	28.34 ± 7.61
10a	Nd	Nd
10b	Nd	Nd
11a	32.01 ± 0.21	2.03 ± 2.01
11b	Nd	Nd
14	Nd	Nd
15a	46.37 ± 6.17	3.04 ± 6.02
15b	Nd	Nd
15c	23.14 ± 5.65	Nd
16	78.24 ± 6.65	23.04 ± 6.12
17	61.73 ± 13.28	63.04 ± 8.52
18	15.93 ± 32.11	4.04 ± 5.52

Table 2. Antimicrobial activity of the newly synthesized derivatives against different test microbes.

Clear zone (mm)				
Tested microorganism / Sample	*Pseudomonas aeruginosa*	*Staphylococcus aureus*	*Aspergillus niger*	*Candida albicans*
Neomycin 100 µg	23	22	0	25
Cycloheximide 100 µg	0	0	35	0
1	5	0	12	3
3a	15	14	0	10
3b	9	0	20	14
3c	8	20	25	15
3d	14	15	19	15
4a	0	10	18	0
4b	17	18	29	17
4c	12	5	11	21
4d	0	14	0	12
6	0	10	37	2
7	5	7	3	4
8	0	15	8	17
9	15	2	14	15
10a	20	0	25	14
10b	2	0	27	16
11a	11	12	28	10
11b	5	11	0	9
14	25	18	13	28
15a	4	3	10	15
15b	0	0	2	10
15c	23	0	0	10
16	12	23	15	11
17	6	2	12	14
18	17	4	8	0

method and put via refluxing of **7** with the appropriate of 3-(2-arylhydrazono)pentane-2,4-dione **12** or ethyl 2-arylazo-3-oxo-4-butanoate **13** in ethanol in the presence of few drops of acetic acid (catalytic amount) yielded products found to be identical to the obtained products of the previous synthetic pathway.

When **7** was allowed to react with nitrous acid it afforded the target (azido(2-methyl-6-(5-methyl-1-(3-nitrophenyl)1*H*-1,2,3-triazol-4-yl)pyridine-3-yl)methanone) (**14**) (Scheme 2).

Finally, the azido derivative **14** was utilized as the versatile material for the synthesis of a new series of heterocycles via its reaction with a variety of active reagents via Curtius rearrangement (48).

Compound **14** was submitted to react with different amines as aniline derivatives, 3-amino-5-phenylpyrazole and methyl anthranilate in dry dioxane to afford the corresponding urea derivatives **15-17** in excellent yields. The chemical compositions of the newly synthesized urea derivatives were derived from its spectra. IR spectrum of compound **15a** exhibited strong absorption band at ν 3385cm^{-1} for the two NH groups and a strong absorption band at ν1695 cm^{-1} for carbonyl group. Its ^{1}H NMR spectrum showed singlet signal at $\delta = 9.25$ ppm for protons of the two NH groups.

Also, **14** was allowed to react with phenol in dry benzene to afford the phenyl carbamate **18** (Scheme 3).

Pharmacology

Anti-proliferative activity

The data calculated as the concentration of the tested samples needed to inhibit half of the cancer cells population IC_{50} values were calculated for each compound separately and mean values ± SD are presented in Table 1, the antiproliferative potency of the screened compounds are illustrated in Table 1.

Note: Compounds were tested in concentration from 100 to 0.1 µg/mL; Nd: not detected in used concentrations; concentration of DMSO: 1%.

The results stated in Table 1 revealed that many compounds showed good antiproliferative activity against hepatic cancer cell lines (HepG-2) with no toxicity on normal cell lines. These compounds are **3b**, **3c**, **4b**, **6** and **15c**. On the other hand, compounds **7**, **11a**, **15a**, **18** exhibited good antiproliferative activity against the hepatic cancer cell lines (HepG-2) comparing with the standard drug doxorubicin in used range of concentration and they have low toxicity on the normal cell lines.

Antimicrobial activity

Antibiotics resistance is a serious problem. It has become a global increasingly pressing problem. Around the world, microbes have acquired resistance mechanisms to antibiotic or intrinsically resist antimicrobial drugs and hence the number of patients with diseases which resist these substances continuing to increase (49-51). So, there is a critical demand to investigate new antibiotics.

The newly synthesized derivatives were screened for their *in vitro* antimicrobial potency on four microbial strains, assigned as *Pseudomonas aeruginosa* (Gram-negative), *Staphylococcus aureus* (Gram-positive), *Aspergillus niger* (fungus) and *Candida albicans* (yeast). Neomycin was used as a standard antibacterial drug and Cycloheximide was used as a standard antifungal drug to make a comparison between the different effects of the tested newly synthesized derivatives under the same condition. Results found in Table (2) showed the antimicrobial activities of different tested derivatives. From the results it was found that compounds **14** and **15c** have antibacterial activity against *Pseudomonas aeruginosa* exceeding the effect of the Neomycin (25, 23 mm, respectively), referring to the structure-activity relationship (SAR) the azido derivative**14** have pyridine ring in addition to the 1,2,3-triazole ring which may be responsible for the reactivity of this compound. On the other hand, the chemical composition of compound **15c** (it has 1,2,3-triazole ring, thiophenyl and isoxazole rings) may be responsible for the high activity of this compound. Compound **16** exhibited strong antibacterial activity against *Staphylococcus aureus* exceeding the activity of Neomycin (23 mm), its reactivity may be owing to the combination between different rings (pyrazole, 1,2,3-triazole and pyridine) in the same compound. Strong antifungal activity against *Aspergillus niger* has been reported with compound **6** which has pyridine ring with 1,2,3-triazole ring in addition to ester group in its chemical composition may be the reason for its reactivity. The azido derivative **14** has been revealed the highest inhibitory effect against *Candida albicans***.**

CONCLUSION

The newly synthesized derivatives seemed to be preferred for pharmaceutical studies. The results obtained from the tested compounds showed reasonable medical indices especially those of potent activities and this beside their lower possible side effects due to no or weak action on normal cell lines. Compounds **3b**, **3c**, **4b**, **6** and **15c** revealed good potency as anti-hepatic cancer compounds with no effect on the normal cells. On the other hand, compounds **7**, **11a**, **15a**, **18** exhibited good anti-proliferative potency against hepatic cancer cell lines with week effect on normal cell lines. As well, compounds **6**, **14**, **15c**, **16** revealed excellent antimicrobial activities against the tested microorganisms, their activities exceeded the tested standard drugs themselves. Referring to compound **14** it showed a strong antimicrobial effect against both *Pseudomonas aeruginosa* and *Candida albicans* exceeding the effect of Neomycin.

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