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Study of the Biological Activity of Microwave Synthesized of Some New Pyridine Derivatives Fused With Sulfonamide Moiety

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Abstract

Alkylation of pyridine **1a,b** with 3-chloropropanol, 1,3-dichloroisopropanol, epichlorohydrin and methyl bromoacetate under microwave irradiation afforded N-(4-(5-cyano-6-(3-hydroxypropoxy)-4-(4-isopropylphenyl)pyridin-2-yl)phenyl)-4-methylbenzenesulfonamide **(2a),** N-(4-(5-cyano-6-(3-hydroxypropoxy)-4-(thiophen-2-yl)pyridin-2-yl)phenyl)-4-methylbenzenesulfonamide **(2b),** N-(4-(6-(3-chloro-2-hydroxypropoxy)-5-cyano-4-(4-isopropylphenyl)-pyridin-2-yl)phenyl)-4-methylbenzenesulfonamide **(3a),** N-(4-(6-(3-chloro-2-hydroxypropoxy)-5-cyano-4-(thiophen-2-yl)pyridin-2-yl)phenyl)-4-methylbenzenesulfonamide **(3b),** N-(4-(5-cyano-4-(4-isopropylphenyl)-6-(oxiran-2-ylmethoxy)pyridin-2-yl)phenyl)-4-methylbenzenesulfonamide **(4a),** N-(4-(5-cyano-6-(oxiran-2-ylmethoxy)-4-(thiophen-2-yl)pyridin-2-yl)phenyl)-4-methylbenzenesulfonamide **(4b),** Methyl 2-(3-cyano-4-(4-isopropylphenyl)-6-(4-(4-methylphenylsulfon-amido)-phenyl)pyridin-2-yloxy)acetate **(5a),** Methyl 2-(3-cyano-6-(4-(4-methylphenylsulfonamido)phenyl)-4-(thiophen-2-yl)pyridin-2-yloxy)acetate **(5b)** respectively. Hydrazenolysis of pyridine **5a,b** with hydrazine hydrate afforded N-(4-(5-cyano-6-(2-hydrazinyl-2-oxoethoxy)-4-(4-isopropylphenyl)-pyridin-2-yl)phenyl)-4-methylbenzenesulfonamide **(6a)** and N-(4-(5-cyano-6-(2-hydrazinyl-2-oxoethoxy)-4-(thiophene-2-yl)-pyridin-2-yl)phenyl)-4-methylbenzenesulfonamide **(6b).** Reaction of pyridine **1a,b** with methyl bromide derivatives **7** resulted in 2-((3-Cyano-4-(4-isopropylphenyl)-6-(4-(4-methylphenylsulfon-amido)phenyl)-2-oxopyridin-1(2H)-yl)methoxy)ethyl acetate **(8a)** and 2-((3-Cyano-6-(4-(4-methylphenylsulfonamido)phenyl)-4-(thiophen-2-yl)pyridin-2-yloxy)methoxy)ethyl acetate **(10b),** respectively, while reaction of pyridine **1a,b** with 4-bromobutyl acetate **12** yielded 4-(3-Cyano-4-(4-isopropylphenyl)-6-(4-(4-methylphenylsulfonamido)-phenyl)pyridin-2-yloxy)butyl acetate **(13a)** and 4-(3-Cyano-6-(4-(4-methylphenylsulfonamido)phenyl)-4-(thiophen-2-yl)pyridin-2-yloxy)butyl acetate **(13b).** Deacetylation of pyridine **8a, 10b** and **13a,b** afforded N-(4-(5-cyano-1-((2-hydroxyethoxy)methyl)-4-(4-isopropylphenyl)-6-oxo-1,6-dihydropyridin-2-yl)phenyl)-4-methylbenzenesulfonamide **(9a),** N-(4-(5-cyano-6-((2-hydroxyethoxy)methoxy)-4-(thiophene-2-yl)-pyridin-2-yl)phenyl)-4-methylbenzenesulfonamide **(11b**), N-(4-(5-cyano-6-(4-hydroxybutoxy)-4-(4-isopropylphenyl)pyridin-2-yl)-phenyl)-4-methylbenzenesulfonamide **(14a)** and N-(4-(5-cyano-6-(4-hydroxybutoxy)-4-(thiophene-2-yl)pyridin-2-yl)phenyl)-4-methylbenzenesulfonamide **(14b)..** pyridine **1a,b** was reacted with allyl bromide to gave a mixture of N-(4-(6-(allyloxy)-5-cyano-4-(4-isopropylphenyl)pyridin-2-yl)phenyl)-4-methylbenzenesulfonamide **(15a**), N-(4-(6-(allyloxy)-5-cyano-4-(thiophen-2-yl)pyridin-2-yl)phenyl)-4-methylbenzenesulfonamide **(15b),** N-(4-(1-allyl-5-cyano-4-(4-isopropylphenyl)-6-oxo-1,6-dihydropyridin-2-yl)phenyl)-4-methylbenzenesulfonamide **(16a)** and N-(4-(1-allyl-5-cyano-6-oxo-4-(thiophen-2-yl)-1,6-dihydropyridin-2-yl)phenyl)-4-methylbenzenesulfonamide **(16b).**while alkylation with propargyl bromide gave a mixture of N-(4-(5-cyano-4-(4-isopropylphenyl)-6-(prop-2-ynyloxy)pyridin-2-yl)phenyl)-4-methylbenzenesulfonamide **(17a),** N-(4-(5-cyano-6-(prop-2-ynyloxy)-4-(thiophen-2-yl)pyridin-2-yl)phenyl)-4-methylbenzenesulfonamide **(17b),** N-(4-(5-cyano-4-(4-isopropylphenyl)-6-oxo-1-(prop-2-ynyl)-1,6-dihydro-pyridin-2-yl)phenyl)-4-methylbenzenesulfonamide **(18a)** and N-(4-(5-cyano-6-oxo-1-(prop-2-ynyl)-4-(thiophen-2-yl)-1,6-dihydro-pyridin-2-yl)phenyl)-4-methylbenzenesulfonamide **(18b).** .Antitumor activity and cytotoxicity of the pyridine **4a**, **4b**, **13a**, **15b** and **17b** were evaluated. Additionally, the antimicrobial activity of pyridine **2a, 3a, 4b, 15a and 18b** were evaluated.

Keywords: Synthesis; Sulfonamide; Microwave; pyridine; Antimicrobial activity; Cytotoxicity

1. Introduction

Pyridine derivatives a heterocyclic compound that have a wide range of biological activities[1-4], including antimicrobial[5, 6], antitumor[7, 8], antiviral[9], anticancer[10], antituberculosis, anti-inflammatory[11] [12], and heart treatment properties[13, 14]. Sulfonamides have also shown strong pharmacological effects[14]. Therefore, combining pyridine with sulfonamides was expected to result in compounds with high biological activity[15]. Microwave heating was utilized in our study to expedite reactions and improve product yields compared to traditional heating methods[15].

Experimental

An Electro thermal IA 9100 apparatus is used to measure melting points which are uncorrected. Ultraviolet light (UV) detected TLC and Conducted at Merck Silica Gel 60F254. The analytical and spectral data were carried out at the Microanalysis Center at Cairo University, Giza, Egypt. IR spectra were measured on a Pye Unicam Sp-3–300 or a

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Shimadzu FTIR 8101 PC infrared spectrophotometer using KBr discs. DMSO-d_6 is used as solvent to detect ^{1}H NMR and ^{13}C NMR spectra which were measured on a JEOL-JNM-LA 400MHz spectrometer. A microwave oven (ME731K, 800 W and operating at 2450MHz) was used to synthesize compounds. The Regional Center for Mycology & Biotechnology, Al-Azhar University and the Microbiology Department, Faculty of Pharmacy, Zagazig University produced the Cytotoxicity Evaluation and the antimicrobial activities**.** Compounds **1a** and **1b** were used as starting materials and prepared according to literature procedures [16-18].

2-1. preparation of pyridine derivatives:

General procedure for preparation of alkylated pyridine 2a,b, 3a,b, 4a,b, 5a,b, 8a, 10a, 13a,b 15a,b, 16a,b, 17a,b and 18a,b.

Method A:

A mixture of an equimolar amounts (10mmole) of pyridine **1a,b,** 3-chloro-1-propanol, 1,3dichloro-2-propanol, methyl bromoacetate, 4-bromobutylacetate, (2-acetoxyethoxy)methyl bromide, and/ or epichlorohydrine in dry DMF (10 mL) containing anhydrous K_2CO_3 (11 mmol), was stirred under reflux for 5h then poured onto ice. The solid formed was filtered, dried and recrystallized from ethanol.

Method B:

A mixture of pyridine **1a,b** (10 mmol) , allyl bromide and/ or proargyl bromide (10 mmol) in acetone (10 mL) containing anhydrous K_2CO_3 (11 mmol) was stirred under reflux for 5h. The solvent was evaporated and the solid formed was filtered and dried then separated using eluent [CH_2Cl_2 (9.9ml)/ MeOH (0.1ml) and silica gel chromatography (200-400 mesh).

Method C:

A mixture of pyridine **1a,b** (1 mmol), 3-chloro-1-propanol, 1,3dichloro-2-propanol, 4-bromobutylacetate, (2-acetoxyethoxy)methyl bromide, and epichlorohydrine, methyl bromoacetate, allyl bromide and proargyl bromide (1 mmol) in presence of dry K_2CO_3 was stirred for 2 min then irradiated with microwave in open flask for 2 min. In a four step mode with interval 30 s. The mixture was separated and purified as shown in the ordinary method.

N-(4-(5-cyano-6-(3-hydroxypropoxy)-4-(4-isopropylphenyl)pyridin-2-yl)phenyl)-4-methylbenzenesulfonamide (2a)

Method A and C: Yield 70 % (0.379 g), as white crystals, mp 200-203^{0}C. IR (υ, cm^{-1}): 3443 (OH) and 2222 (C≡N). ^{1}H NMR (DMSO-d_6 , δ, ppm): 1.25 (d, 6H, (C<u>H</u>$_3$)$_2$CH), 1.95 (m, 2H, C<u>H</u>$_2$ (b)), 2.33 (s, 3H, p-C<u>H</u>$_3$), 2.97 (m, 1H, C<u>H</u>(CH$_3$)$_2$), 3.38 (t, 2H, C<u>H</u>$_2$ (c)), 3.61 (t, 2H, C<u>H</u>$_2$ (a)), 4.43 (t, 1H, , OH, exchange with D_2O), 7.20 -8.22 (m, 12H, Ar-H and pyridine-H-5); Anal. Calcd. for $C_{31}H_{31}N_3O_4S$ (541.66): C, 68.74; H, 5.77; N, 7.76. Found: C, 68.71; H, 5.79; N, 7.73.

N-(4-(5-cyano-6-(3-hydroxypropoxy)-4-(thiophen-2-yl)pyridin-2-yl)phenyl)-4-methylbenzenesulfonamide (2b).

Method A and C: Yield 72 % (0.364 g), as white crystals, mp 196-198^{0}C. IR (υ, cm^{-1}): 3424 (OH) and 2218 (C≡N). ^{1}H NMR (DMSO-d_6 , δ, ppm) : 1.95 (m, 2H, C<u>H</u>$_2$ (b)), 2.32 (s, 3H, p-C<u>H</u>$_3$), 3.31 (t, 2H, C<u>H</u>$_2$ (c)), 3.62 (t, 2H, C<u>H</u>$_2$ (a)), 4.69 (t, 1H, OH, exchange with D_2O), 7.21-8.12 (m, 12H, Ar-H, thiophene-H, and pyridine-H-5); Anal. Calcd for $C_{26}H_{23}N_3O_4S_2$ (505.61): C, 61.76; H, 4.59; N, 8.31. Found: C, 61.77; H, 4,60; N, 8.33.

N-(4-(6-(3-chloro-2-hydroxypropoxy)-5-cyano-4-(4-isopropylphenyl)-pyridin-2-yl)phenyl)-4-methylbenzenesulfonamide (3a).

Method A and C: Yield 66 % (0.380 g), as white crystals, mp 96-98^{0}C. IR (υ, cm^{-1}): 3434 (OH) and 2223 (C≡N). ^{1}H NMR (DMSO-d_6 , δ, ppm): 1.26 (d, 6H, (C<u>H</u>$_3$)$_2$CH), 2.39 (s, 3H, p-CH$_3$), 3.01 (m, 1H, CH(CH$_3$)$_2$), 4.06 - 4.31 (m, 2H, CH$_2$Cl), 4.45 – 4.56 (m, 2H, OCH$_2$), 4.81 – 4.89 (m, 1H, C<u>H</u>OH), 5.28 (t, 1H, OH, exchange with D_2O), 7.20- , 8.40 (m, 12H, Ar-H and pyridine-H-5); Anal. Calcd for $C_{31}H_{30}ClN_3O_4S$ (576.11): C, 64.63; H, 5.25; N, 7.29. Found: C, 64.65; H, 5.26; N, 7.31.

N-(4-(6-(3-chloro-2-hydroxypropoxy)-5-cyano-4-(thiophen-2-yl)pyridin-2-yl)phenyl)-4-methylbenzenesulfonamide (3b).

Method A and C: Yield 60 %(0.324 g), as white crystals, mp 92-95^{0}C. IR (υ, cm^{-1}): 3435 (OH) and 2222 (C≡N). ^{1}H NMR (DMSO-d_6 , δ, ppm): 2.39 (s, 3H, p-CH$_3$), 3.96 - 4.08 (m, 2H, CH$_2$Cl), 4.45 – 4.71 (m, 2H, OCH$_2$), 4.81 – 4.89 (m, 1H, C<u>H</u>OH), 5.38 (t, 1H, OH, exchange with D_2O), 7.20 – 8.24(m, 12H, Ar-H, thiophene-H and pyridine-H-5); Anal. Calcd for $C_{26}H_{22}ClN_3O_4S_2$ (540.05): C, 57.82; H, 4.11; N, 7.78. Found: C, 57.79; H, 4.09; N, 7.76.

N-(4-(5-cyano-4-(4-isopropylphenyl)-6-(oxiran-2-ylmethoxy)pyridin-2-yl)phenyl)-4-methylbenzenesulfonamide (4a).

Method A and C: Yield 60% (0.323 g), colourless crystals, m.p. 90 - 92^{0}C. IR (υ, cm^{-1}): 3445 (NH), 2221 (C≡N). ^{1}H NMR (DMSO-d_6 , δ, ppm): 1.22 (d, 6H, (C<u>H</u>$_3$)$_2$CH), 2.39 (s, 3H, p-C<u>H</u>$_3$), 2.65 – 3.06 (m, 3H, OCH$_2$ oxiran ring and C<u>H</u>(CH$_3$)$_2$), 3.82 (m, 1H, OCH oxiran ring), 4.36 (2d, 1H, OCH<u>H</u>), 4.93 (d, 1H, OC<u>H</u>H), 7.18 - 8.23 (m, 12H, Ar-H and pyridine-H-5); Anal. Calcd. for $C_{31}H_{29}N_3O_4S$ (539.64):

C, 69.00; H, 5.42; N, 7.79. Found: C, 69.04; H, 5.45; N, 7.83.

N-(4-(5-cyano-6-(oxiran-2-ylmethoxy)-4-(thiophen-2-yl)pyridin-2-yl)phenyl)-4-methylbenzenesulfonamide (4b).

Method A and C: Yield 63% (0.317 g), colorless crystals, m.p. 95 - 97^{0}C. IR (υ, cm^{-1}): 3478 (NH), 2218 (C≡N). ^{1}H NMR (DMSO-d$_6$, δ, ppm): 2.39 (s, 3H, p-C$\underline{H}_3$), 2.36 – 2.90 (m, 2H, OCH$_2$ oxiran ring), 3.91 (m, 1H, OCH oxiran ring), 4.41 (2d, 1H, OCH$\underline{H}$), 4.97 (dd, 1H, 2.89 Hz, OC$\underline{H}$H), 7.27 -8.24 (m, 10H, Ar-H, thiophene-H and pyridine-H-5); Anal. Calcd. for $C_{26}H_{21}N_3O_4S_2$ (503.59): C, 62.01; H, 4.20; N, 8.34. Found: C, 62.02; H, 4.23; N, 8.30.

Methyl 2-(3-cyano-4-(4-isopropylphenyl)-6-(4-(4-methylphenylsulfon-amido)-phenyl)pyridin-2-yloxy)acetate (5a).

Method B and c: Yield 77 % (0.427 g), as yellow crystals, mp 150-152^{0}C. IR (υ, cm^{-1}): 2222 (C≡N) and 1755 (C=O, ester). ^{1}H NMR (DMSO-d$_6$, δ, ppm): 1.25 (d, 6H, (C$\underline{H}_3$)$_2$CH), 2.33 (s, 3H, p-C$\underline{H}_3$), 2.98 (m, 1H, C$\underline{H}$(CH$_3$)$_2$), 3.71 (s, 3H, OC$\underline{H}_3$), 5.18 (s, 2H, OC$\underline{H}_2$), 7.31-8.10 (m, 12H, Ar-H and pyridine-H-5); Anal. Calcd. for $C_{31}H_{29}N_3O_5S$ (555.64): C, 67.01; H, 5.26; N, 7.56. Found: C, 67.03; H, 5.23; N, 7.52.

Methyl 2-(3-cyano-6-(4-(4-methylphenylsulfonamido)phenyl)-4-(thiophen-2-yl)pyridin-2-yloxy)acetate (5b).

Method B and C: Yield 80 % (0.415 g), as yellow crystals, mp 146-148^{0}C. IR (υ, cm^{-1}): 2222 (C≡N) and 1757 (C=O, ester). ^{1}H NMR (DMSO-d$_6$, δ, ppm): 2.37 (s, 3H, p-C$\underline{H}_3$), 3.72 (s, 3H, OC$\underline{H}_3$), 5.12 (s, 2H, OC$\underline{H}_2$), 7.21 - 8.12 (m, 12H, Ar-H, thiophene-H and pyridine-H-5). Anal. Calcd. for $C_{26}H_{21}N_3O_5S_2$ (519.59): C, 60.10; H, 4.07; N, 8.09. Found: C, 60.07; H, 4.10; N, 8.11.

2-((3-Cyano-4-(4-isopropylphenyl)-6-(4-(4-methylphenylsulfon-amido)phenyl)-2-oxopyridin-1(2H)-yl)methoxy)ethyl acetate (8a).

Method A and C: Yield 60% (0.359 g), white crystal, mp 90-92^{0}C. IR (υ, cm^{-1}): 3434 (NH), 2221 (C≡N), 1739 (C=O, acetoxy) and 1643 (C=O, amidic). ^{1}H NMR (DMSO-d$_6$, δ, ppm): 1.24 (d, 6H, (C$\underline{H}_3$)$_2$CH), 1.95 (s, 3H, C$\underline{H}_3$CO), 2.30 (s, 3H, p-C$\underline{H}_3$), 2.98 (m, 1H, C$\underline{H}$(CH$_3$)$_2$), 3.66 (t, 2H, OC$\underline{H}_2$ (b)), 4.56 (t, 2H, C$\underline{H}_2$O (c)), 4.80 (s, 2H, NC$\underline{H}_2$ (a)), 7.14 -8.04 (m, 12H, Ar-H and pyridine-H-5); Anal. Calcd. for $C_{33}H_{33}N_3O_6S$ (599.70): C, 66.09; H, 5.55; N, 7.01. Found: C, 66.11; H, 5.58; N, 7.03.

2-((3-Cyano-6-(4-(4-methylphenylsulfonamido)phenyl)-4-(thiophen-2-yl)pyridin-2-yloxy)methoxy)ethyl acetate (10b).

Method A and C: Yield 62% (0.349 g), white crystal, mp 88-90^{0}C. IR (υ, cm^{-1}): 3448 (NH), 2220 (C≡N), 1739 (C=O, acetoxy). ^{1}H NMR (DMSO-d$_6$, δ, ppm): 1.90 (s, 3H, C$\underline{H}_3$CO), 2.35 (s, 3H, p-C$\underline{H}_3$), 3.78 (t, 2H, OC$\underline{H}_2$(b)), 4.25 (t, 2H, C$\underline{H}_2$O(c)), 4.43 (s, 2H, OC$\underline{H}_2$(a)O), 7.27 – 8.25 (m, 12H, Ar-H, pyridon-H-5 and thiophene-H). Anal. Calcd. for $C_{28}H_{25}N_3O_6S_2$ (563.64): C, 59.67; H, 4.47; N, 7.46. Found: C, 59.65; H, 4.49; N, 7.44.

4-(3-Cyano-4-(4-isopropylphenyl)-6-(4-(4-methylphenylsulfonamido)-phenyl)pyridin-2-yloxy)butyl acetate (13a).

Method A and C: Yield 80%(0.478 g), white crystal, mp 88-90^{0}C. IR (υ, cm^{-1}): 3459 (NH), 2221 (C≡N), 1736 (C=O, acetoxy). ^{1}H NMR (DMSO-d$_6$, δ, ppm): 1.21 (d, 6H, (C$\underline{H}_3$)$_2$CH), 1.62 (m, 2H, C$\underline{H}_2$ (c)), 1.84 (m, 2H, C$\underline{H}_2$ (b)), 1.96 (s, 3H, C$\underline{H}_3$CO), 2.40 (s, 3H, p-C$\underline{H}_3$), 2.97 (m, 1H, C$\underline{H}$(CH$_3$)$_2$), 4.03 (t, 2H, C$\underline{H}_2$ (a)), 4.20 (t, 2H, C$\underline{H}_2$ (d)), 7.20 -8.22 (m, 12H, Ar-H and pyridine-H-5); Anal. Calcd. for $C_{34}H_{35}N_3O_5S$ (597.72): C, 68.32; H, 5.90; N, 7.03. Found: C, 68.33; H, 5.88; N, 7.05.

4-(3-Cyano-6-(4-(4-methylphenylsulfonamido)phenyl)-4-(thiophen-2-yl)pyridin-2-yloxy)butyl acetate (13b).

Method A and C: Yield 82%(0.460 g), white crystal, mp 85-89^{0}C. IR (υ, cm^{-1}): 3442 (NH), 2219 (C≡N), 1735 (C=O, acetoxy). ^{1}H NMR (DMSO-d$_6$, δ, ppm): 1.60 (m, 2H, C$\underline{H}_2$ (c)), 1.81 (m, 2H, C$\underline{H}_2$ (b)), 1.96 (s, 3H, C$\underline{H}_3$CO), 2.39 (s, 3H, p-C$\underline{H}_3$), 4.02 (t, 2H, C$\underline{H}_2$ (a)), 4.20 (t, 2H, C$\underline{H}_2$ (d)), 7.34-8-29 (m, 12H, Ar-H, thiophene-H and pyridon-H-5); Anal. Calcd. for $C_{29}H_{27}N_3O_5S_2$ (561.67): C, 62.01; H, 4.85; N, 7.48. Found: C, 62.04; H, 4.83; N, 7.46.

N-(4-(6-(allyloxy)-5-cyano-4-(4-isopropylphenyl)pyridin-2-yl)phenyl)-4-methylbenzenesulfonamide (15a)

Method B and C: Yield 70% (0.366 g), colorless crystals, m.p. 103 - 105^{0}C. IR (υ, cm^{-1}): 3444 (NH), 2220 (C≡N). ^{1}H NMR (DMSO-d$_6$, δ, ppm): 1.10 (d, 6H, (C$\underline{H}_3$)$_2$CH), 2.24 (s, 3H, p-C$\underline{H}_3$), 2.84 (m, 1H, C$\underline{H}$(CH$_3$)$_2$), 4.95 (d, 2H, OCH$_2$), 5.16 (d, 1H, =CH$\underline{H}$), 5.39 (d, 1H, =C$\underline{H}$H), 6.01 (m, 1H, -C$\underline{H}$=), 7.10-8.08 (m, 12H, Ar-H and pyridine-H-5);. ^{13}C NMR (DMSO-d$_6$, δ, ppm): 21.4, 24.1 and 33.8 (p.CH$_3$, (C$\underline{H}_3$)$_2$CH and C$\underline{H}$(CH$_3$)$_2$), 67.8 (OC$\underline{H}_2$), 92.7, 114.3, 115.8 (C≡N), 118.5, 119.4, 127.2, 127.7, 128.6,129.1, 130.2, 133.3, 133.6, 135.8, 141.3, 144.1, 151.1, 156.5, 156.8 and 164.1 (CH=CH$_2$, Ar-C and C=N). Anal. Calcd. for $C_{31}H_{29}N_3O_3S$ (523.65): C, 71.10; H, 5.58; N, 8.02. Found: C, 71.13; H, 5.55; N, 8.06.

N-(4-(6-(allyloxy)-5-cyano-4-(thiophen-2-yl)pyridin-2-yl)phenyl)-4-methylbenzenesulfonamide (15b).

Method B and C: Yield 73% (0.355 g), colorless crystals, m.p. 108 - 110^{0}C. IR (υ, cm^{-1}): 3117 (NH), 2219 (C≡N). ^{1}H

NMR (DMSO-d_6 , δ, ppm): 2.39 (s, 3H, p-C<u>H</u>$_3$), 5.09 (d, 2H, OC<u>H</u>$_2$), 5.33 (d, 1H, =CH<u>H</u>), 5.52 (d, 1H, =C<u>H</u>H), 6.12 (m, 1H, -C<u>H</u>=), 7.23 -8-20 (m, 12H, Ar-H, thiophene-H and pyridon-H-5); Anal. Calcd. for $C_{26}H_{21}N_3O_3S_2$ (487.49): C, 64.04; H, 4.34; N, 8.62. Found: C, 64.08; H, 4.32; N, 8.65.

N-(4-(1-allyl-5-cyano-4-(4-isopropylphenyl)-6-oxo-1,6-dihydropyridin-2-yl)phenyl)-4-methylbenzenesulfonamide (16a).

Method B and C: Yield 15% (0.078 g), colorless crystals, m.p. 107 - 109^{0}C. IR (υ, cm^{-1}): 3478 (NH), 2220 (C≡N), 1652 (C=O amidic). ^{1}H NMR (DMSO-d_6 , δ, ppm): 1.10 (d, 6H, (C<u>H</u>$_3$)$_2$CH), 2.24 (s, 3H, p-C<u>H</u>$_3$), 2.84 (m, 1H, C<u>H</u>(CH$_3$)$_2$), 5.01 (d, 2H, N.C<u>H</u>$_2$), 5.19 (d, =CH<u>H</u>), 5.39 (d, 1H, =C<u>H</u>H), 6.01 (m, 1H, -C<u>H</u>=CH$_2$), 7.10- 8.05 (m, 12H, Ar-H and pyridine-H-5). ^{13}C NMR (DMSO-d_6 , δ, ppm): 21.4, 24.1 and 33.8 (p.CH$_3$, (<u>C</u>H$_3$)$_2$CH and <u>C</u>H(CH$_3$)$_2$), 52.6 (N<u>C</u>H$_2$), 92.7, 114.3, 115.8 (C≡N), 118.5, 119.4, 127.7, 128.6, 129.1,129.1, 130.2, 133.3, 133.4, 137.7, 135.8, 135.8, 141.3, 144.1, 151.2, 156.6, 156.9 and 164.1 (CH=CH$_2$, Ar-C, C=N and C=O). Anal. Calcd. for $C_{31}H_{29}N_3O_3S$ (523.65): C, 71.10; H, 5.58; N, 8.02. Found: C, 71.14; H, 5.54; N, 8.00.

N-(4-(1-allyl-5-cyano-6-oxo-4-(thiophen-2-yl)-1,6-dihydropyridin-2-yl)phenyl)-4-methylbenzenesulfonamide (16b).

Method B and C: Yield 9% (0.043 g), colorless crystals, m.p. 109 - 111^{0}C. IR (υ, cm^{-1}): 3444 (NH), 2216 (C≡N), 1645 (C=O amidic). Anal. Calcd. for $C_{26}H_{21}N_3O_3S_2$ (487.59): C, 64.04; H, 4.34; N, 8.62. Found: C, 64.05; H, 4.38; N, 8.66.

N-(4-(5-cyano-4-(4-isopropylphenyl)-6-(prop-2-ynyloxy)pyridin-2-yl)phenyl)-4-methylbenzenesulfonamide (17a)

Method B and C: Yield 65% (0.339 g), colorless crystals, m.p. 98 - 100^{0}C. IR (υ, cm^{-1}): 3444 (NH), 2220 (C≡N). ^{1}H NMR (DMSO-d_6 , δ, ppm): 1.27 (d, 6H, (C<u>H</u>$_3$)$_2$CH), 2.33 (s, 3H, p-C<u>H</u>$_3$), 2.98 (m, 1H, C<u>H</u>(CH$_3$)$_2$), 3.42 (s, 1H, ≡ C-<u>H</u>), 5.37 (s, 2H, OCH$_2$), 7.69 (s, 1H, pyridon-H-5),7.19-7.79 (m, 12H, Ar-H); Anal. Calcd. for $C_{31}H_{27}N_3O_3S$ (521.63): C, 71.38; H, 5.22; N, 8.06. Found: C, 71.35; H, 5.20; N, 8.09.

N-(4-(5-cyano-6-(prop-2-ynyloxy)-4-(thiophen-2-yl)pyridin-2-yl)phenyl)-4-methylbenzenesulfonamide (17b)

Method B and c: Yield 68% (0.330 g), colorless crystals, m.p. 106 - 108^{0}C. IR (υ, cm^{-1}): 3117 (NH), 2219 (C≡N). ^{1}H NMR (DMSO-d_6 , δ, ppm): 2.39 (s, 3H, p-C<u>H</u>$_3$), 3.62 (s, 1H, ≡ C-<u>H</u>), 5.27 (s, 2H, OCH$_2$), 7.96 (s, 1H, pyridon-H-5), 8.05(t, 1H, thiophen-H), 7.32–8.29 (m, 10H, Ar-H); Anal. Calcd. for $C_{26}H_{19}N_3O_3S_2$ (485.85): C, 64.31; H, 3.94; N, 8.65. Found: C, 64.35; H, 3.92; N, 8.63.

N-(4-(5-cyano-4-(4-isopropylphenyl)-6-oxo-1-(prop-2-ynyl)-1,6-dihydro-pyridin-2-yl)phenyl)-4-methylbenzenesulfonamide (18a).

Method B and C: Yield 13% (0.067 g), colorless crystals, m.p. 103 - 105^{0}C. IR (υ, cm^{-1}): 3478 (NH), 2222 (C≡N), 1642 (C=O amidic). ^{1}H NMR (DMSO-d_6 , δ, ppm): 1.23 (d, 6H, (C<u>H</u>$_3$)$_2$CH), 2.33 (s, 3H, p-C<u>H</u>$_3$), 2.98 (m, 1H, C<u>H</u>(CH$_3$)$_2$), 3.28 (s, 1H, ≡ C-<u>H</u>), 4.61 (s, 2H, N-CH$_2$), 7.19-7.77 (m, 12H, Ar-H and pyridine-H-5); Anal. Calcd. for $C_{31}H_{27}N_3O_3S$ (521.63): C, 71.38; H, 5.22; N, 8.06. Found: C, 71.41; H, 5.25; N, 8.10.

N-(4-(5-cyano-6-oxo-1-(prop-2-ynyl)-4-(thiophen-2-yl)-1,6-dihydro-pyridin-2-yl)phenyl)-4-methylbenzenesulfonamide (18b).

Method B and C: Yield 15% (0.072 g), colorless crystals, m.p. 110 - 112^{0}C. IR (υ, cm^{-1}): 3444 (NH), 2216 (C≡N), 1642 (C=O amidic). ^{1}H NMR (DMSO-d_6 , δ, ppm): 2.39 (s, 3H, p-C<u>H</u>$_3$), 3.29 (s, 1H, ≡ C-<u>H</u>), 4.59 (s, 2H, N-CH$_2$), 7.32–8.29 (m, 10H, Ar-H, thiophene-H and pyridon-H-5); Anal. Calcd. for $C_{26}H_{19}N_3O_3S_2$ (485.58): C, 64.31; H, 3.94; N, 8.65. Found: C, 64.34; H, 3.96; N, 8.68.

General procedure for preparation of acid hydrazide 6a,b:

Method A: A mixture of an equimolar amounts (10mmole) pyridine **5a,b** and NH_2NH_2 in methanol (20 mL), was stirred under reflux for 4 hours. The mixture was poured onto ice. The formed solid was filtered, drided then recrystallized from methanol

Method B: A mixture of pyridine **5a,b** (10 mmol) and NH_2NH_2 (20 mmol) was stirred for 2 min then irradiated with microwave for 2 min. The mixture was separated and purified as shown in the ordinary method.

N-(4-(5-cyano-6-(2-hydrazinyl-2-oxoethoxy)-4-(4-isopropylphenyl)-pyridin-2-yl)phenyl)-4-methylbenzenesulfonamide (6a).

Yield 83 % (0.461 g), as white crystals, mp 220-223^{0}C. IR (υ, cm^{-1}): 3429 (broad, NH, NH$_2$), 2223 (C≡N) and 1635 (C=O, amidic). Anal. Calcd. for $C_{30}H_{29}N_5O_4S$ (555.65): C, 64.85; H, 5.26; N, 12.60. Found: C, 64.88; H, 5.25; N, 12.58.

N-(4-(5-cyano-6-(2-hydrazinyl-2-oxoethoxy)-4-(thiophene-2-yl)-pyridin-2-yl)phenyl)-4-methylbenzenesulfonamide (6b).

Yield 85 % (0.441 g), as white crystals, mp 217-219^{0}C. IR (υ, cm^{-1}): 3427 (broad, NH, NH$_2$), 2223 (C≡N) and 1632

(C=O, amidic). Anal. Calcd. for $C_{25}H_{21}N_5O_4S_2$ (519.60): C, 57.79; H, 4.07; N, 13.48. Found: C, 57.80; H, 4.09; N, 13.46.

General procedure for preparation of pyridine (9a, 11b and 14a,b)

Method A: A mixture of pyridine **9a, 11b and/or 14a,b** (10 mmol) and TEA (1 mL) in methanol:H_2O (20:1 mL) was stirred under reflux for 4 hours. The mixture was poured on ice and few drops of acetic acid. The formed solid was filtered, dried then recrystallized from methanol

Method B: A mixture of pyridine **9a, 11b and/or 14a,b** (10 mmol) and TEA (1 mL) in methanol (1 mL) was stirred for 2 min then irradiated with microwave for 2 min. The mixture Yield 88% (0.490 g), colorless crystals, m.p. 123-125^{0}C. IR (υ, cm^{-1}): 3425 (broad, OH), 2220 (C≡N), 1643 (C=O, amidic). ^{1}H NMR (DMSO-d_6 , δ, ppm): 1.24 (d, 6H, ($C\underline{H}_3$)$_2$CH), 2.31 (s, 3H, p-CH_3), 2.98 (m, 1H, $C\underline{H}(CH_3)_2$), 3.42 (m, 2H, $C\underline{H}_2$OH(c)), 3.66 (t, 2H, OCH_2(b)), 4.55 (s, 2H, NCH_2O(a)), 5.17 (t, 1H, OH exchange with D_2O), 8.01 (s, 1H,) 7.12-8.20 (m, 12H, Ar-H and pyridon-H-5); Anal. Calcd. for $C_{31}H_{31}N_3O_5S$ (557.66): C, 66.77; H, 5.60; N, 7.54. Found: C, 66.74; H, 5.58; N, 7.59.

N-(4-(5-cyano-1-((2-hydroxyethoxy)methyl)-4-(4-isopropylphenyl)-6-oxo-1,6-dihydropyridin-2-yl)phenyl)-4-methylbenzenesulfonamide (9a).

Yield 88% (0.490 g), colorless crystals, m.p. 123-125^{0}C. IR (υ, cm^{-1}): 3425 (broad, OH), 2220 (C≡N), 1643 (C=O, amidic). ^{1}H NMR (DMSO-d_6 , δ, ppm): 1.24 (d, 6H, ($C\underline{H}_3$)$_2$CH), 2.31 (s, 3H, p-CH_3), 2.98 (m, 1H, $C\underline{H}(CH_3)_2$), 3.42 (m, 2H, $C\underline{H}_2$OH(c)), 3.66 (t, 2H, OCH_2(b)), 4.55 (s, 2H, NCH_2O(a)), 5.17 (t, 1H, OH exchange with D_2O), 8.01 (s, 1H,) 7.12-8.20 (m, 12H, Ar-H and pyridon-H-5); Anal. Calcd. for $C_{31}H_{31}N_3O_5S$ (557.66): C, 66.77; H, 5.60; N, 7.54. Found: C, 66.74; H, 5.58; N, 7.59.

N-(4-(5-cyano-6-((2-hydroxyethoxy)methoxy)-4-(thiophene-2-yl)-pyridin-2-yl)phenyl)-4-methylbenzenesulfonamide (11b).

Yield 86% (0.448 g), colorless crystals, m.p. 120-122^{0}C. IR (υ, cm^{-1}): 3459 cm^{-1} (broad, OH), 3290 cm^{-1} (NH), 2214 cm^{-1} (C≡N). ^{1}H NMR (DMSO-d_6 , δ, ppm): 2.32 (s, 3H, p-CH_3), 3.50 (m, 2H, CH_2OH(c)), 3.59 (t, 2H, OCH_2(b)), 4.52 (t, 1H, OH, exchange with D_2O), 5.42 (s, 2H, OCH_2O(a)), 7.12 – 8.12 (m, 12H, Ar-H, thiophene-H and pyridon-H-5). Anal. Calcd. for $C_{26}H_{23}N_3O_5S_2$ (521.61): C, 59.87; H, 4.44; N, 8.06. Found: C, 59.84; H, 4.48; N, 8.09.

N-(4-(5-cyano-6-(4-hydroxybutoxy)-4-(4-isopropylphenyl)pyridin-2-yl)-phenyl)-4-methylbenzenesulfonamide (14a).

Yield 86% (0.477 g), colorless crystals, m.p. 120-122^{0}C. IR (υ, cm^{-1}): 3410 cm^{-1} (broad, OH), 2221 cm^{-1} (C≡N). ^{1}H NMR (DMSO-d_6 , δ, ppm): 1.21 (d, 6H, ($C\underline{H}_3$)$_2$CH), 1.62 (m, 2H, $C\underline{H}_2$ (c)), 1.86 (m, 2H, $C\underline{H}_2$ (b)), 2.39 (s, 3H, p-$C\underline{H}_3$), 3.00 (m, 1H, $C\underline{H}(CH_3)_2$), 3.46 (t, 2H, CH_2 (d)), 3.60 (t, 2H, $C\underline{H}_2$ (a)), 4.58 (t, 1H, O$\underline{H}$, exchange with D_2O), 7.21-8.22 (m, 12H, Ar-H and pyridone-H-5); Anal. Calcd. for $C_{32}H_{33}N_3O_4S$ (555.69): C, 69.17; H, 5.99; N, 7.56. Found: C, 69.19; H, 5.98; N, 7.55.

N-(4-(5-cyano-6-(4-hydroxybutoxy)-4-(thiophene-2-yl)pyridin-2-yl)phenyl)-4-methylbenzenesulfonamide (14b).

Yield 88% (0.457 g), colorless crystals, m.p. 116-118^{0}C. IR (υ, cm^{-1}): 3416 cm^{-1} (broad, OH), 2218 cm^{-1} (C≡N). ^{1}H NMR (DMSO-d_6 , δ, ppm): 1.65 (m, 2H, $C\underline{H}_2$ (c)), 1.85 (m, 2H, $C\underline{H}_2$ (b)), 2.39 (s, 3H, p-$C\underline{H}_3$), 3.32 (t, 2H, $C\underline{H}_2$ (d)), 3.49 (t, 2H, $C\underline{H}_2$ (a)), 4.39(t, 1H, O$\underline{H}$, exchange with D_2O), 7.22-8.22 (m, 12H, Ar-H, thiophene-H and pyridon-H-5). Anal. Calcd. for $C_{27}H_{25}N_3O_4S_2$ (519.64): C, 62.41; H, 4.85; N, 8.09. Found: C, 62.43; H, 4.83; N, 8.07.

2-2. Evaluation of Cytotoxic Effects of pyridine derivatives

Mammalian cell lines: HepG-2 cells (human Hepatocellular cancer cell line). were obtained from the American Type Culture Collection (ATCC, Rockville, MD).

Chemicals Used: trypan blue dye, Dimethyl sulfoxide (DMSO) and MTT were purchased from Sigma (St. Louis, Mo., USA)., DMEM, RPMI-1640, Fetal Bovine serum HEPES buffer solution, , gentamycin L-glutamine and 0.25% Trypsin-EDTA were purchased from Lonza (Belgium).

Cell line Propagation:

The cells were grown on RPMI-1640 medium supplemented with 10% inactivated fetal calf serum and 50µg/ml gentamycin. The cells were maintained at 37ºC in a humidified atmosphere with 5% CO_2 and were subcultured two to three times a week.

Cytotoxicity evaluation using viability assay:

For the antitumor assays, the tumor cell lines were prepared by suspending them in medium at a concentration of $5x10^4$ cells/well in Corning® 96-well tissue culture plates. The plates were then incubated for 24 hours. After the incubation period, the tested compounds were added to the 96-well plates, with three replicates for each compound. Twelve different concentrations were used for each compound. Additionally, six vehicle controls with media or 0.5% DMSO were included on each 96-well plate as control groups.

After further incubating for 24 hours, the number of viable cells was determined using the MTT test. The procedure involved removing the media from the 96-well plates and replacing it with 100 µl of fresh RPMI 1640 medium without phenol red. Then, 10 µl of a 12 mM MTT stock

solution (5 mg of MTT in 1 mL of PBS) was added to each well, including the untreated controls. The plates were incubated at 37°C and 5% CO_2 for 4 hours.
Following the incubation period, 85 µl of the media was removed from each well, and 50 µl of DMSO was added to each well and thoroughly mixed using a pipette. The plates were then incubated at 37°C for 10 minutes. Subsequently, the optical density was measured at 590 nm using a microplate reader (SunRise, TECAN, Inc, USA) to determine the number of viable cells.

The percentage of viability was calculated as [(ODt/ODc)]x100%, where ODt represents the mean optical density of wells treated with the tested sample, and ODc represents the mean optical density of untreated cells. The survival curve of each tumor cell line after treatment with the specified compound was plotted by analyzing the relationship between surviving cells and drug concentration. The 50% inhibitory concentration (IC50), which indicates the concentration required to cause toxic effects in 50% of intact cells, was estimated by analyzing the dose-response curve using Graphpad Prism software (San Diego, CA, USA).[19]

2. Dissection

This work describes a continuation of our reactions on pyridine **1a,b** for synthesis of new derivatives [20]. Alkylation of pyridine **1a,b** with 3-chloropropanol in the presence of dry K_2CO_3 under MW irradiation for 2min lead to pyridine derivatives **2a,b** in 70% - 72% yield respectively (Scheme1).

IR spectra showed that the amidic C=O band is absent which meaning the synthesis of O-product not N-analogues, in addition to bands at 3443 and 3424 cm^{-1} for OH.

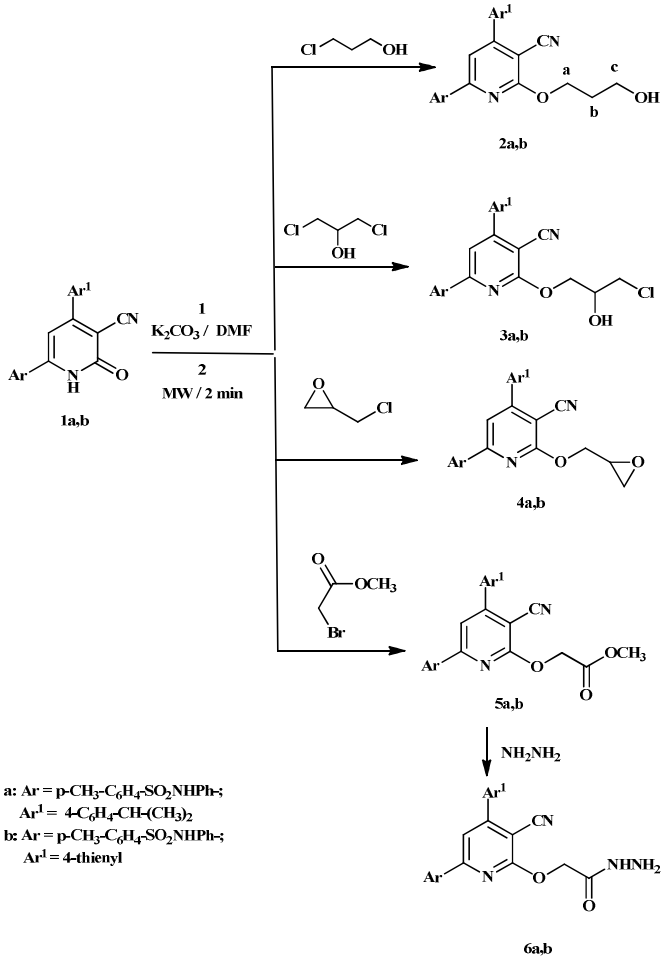
^{1}H NMR spectra of pyridine **2a,b** showed a multiplet and two triplet signals at 1.95, 3.31 – 3.38 and 3.41 – 3.62 ppm for CH_2(b), CH_2(c) and CH_2(a), respectively, in addition to, triplet signals at 4.43 and 4.69 ppm (exchangeable with D_2O) with coupling constant 5.10 and 5.29 Hz,.

Pyridine **1a,b** reacted with 1,3-dichloroisopropanol to give pyridine derivatives **3a,b** in 66% - 60% yield respectively. IR spectra showed that amidic C=O band is absent which meaning the synthesis of O-product, not N-analogues, in addition to bands at 3434 and 3435 cm^{-1} for OH. ^{1}H NMR spectra of compounds **3a,b** showed three multiplet signals in between 3.96 – 4.31 and 4.81 – 4.89 ppm for CH_2Cl, OCH_2 and CHOH groups respectively, in addition to the hydroxyl group protons appear as triplet signals (exchangeable with D_2O) at 5.28 and 5.38 ppm with J = 5.1 Hz respectively.

Reaction of pyridine **1a,b** with epichlorohydrin gave pyridine **4a,b** in 60% and 63% yields, respectively. IR spectra showed that amidic C=O band is absent which meaning the synthesis of O-product, not N-analogues. ^{1}H NMR spectra of **4a,b** showed two multiplet signals at 2.36 – 3.06 and 3.82 – 3.91 ppm for OCH_2 and OCH of oxiran ring, in addition to, two doublet of doublet at 4.36 – 4.41 and 4.93 – 4.97 ppm corresponding to O-CH_2O protons, respectively.

Reaction of compound **1a,b** with methyl bromoacetate afforded ethyl ester derivatives **5a,b** respectively. The IR spectrum of **5a,b** showed that the amidic C=O band is absent which meaning the synthesis of O-alkylated derivatives, not N-analogues, in addition to bands at 1755 and 1757 cm^{-1} for $COOCH_3$.
^{1}H NMR spectra of **5a,b** showed singlet signals at 3.71, 3.72 and 5.18 ppm corresponding to 2 OCH_3 and 2 OCH_2 protons, respectively.

Hydrazenolysis of compound **5a,b** with NH_2NH_2 afforded acid hydrazide derivatives **6a,b**. IR spectrum of **6a,b** showed broad absorption bands at 1635, 1632, and 3427 cm^{-1} characterized amide carbonyl, NH and NH_2 groups and ester carbonyl group is absent (Scheme 1).



scheme 1 Alkylation of pyridine

Pyridine **1a** reacted with (2-acetoxyethoxy)methyl bromide **7** to give N-acyclonucleoside **8a** in 60% yield. IR spectrum showed band at 1643 cm^{-1} for amide C=O which meaning the synthesis of N-alkylated derivatives, not O-analogues, in addition to bands at 2221 cm^{-1} for C≡N and 1739 for C=O. ^{1}HNMR spectra of **8a** showed singlet signals at 1.95 and 4.80 ppm corresponding to CH_3CO and NCH_2(a) and triplet signals at 3.66 and 4.56 ppm for OCH_2(b) and CH_2O(c), respectively.

Deacetylation of N-acyclonucleoside **8a** with triethyl amine followed by TLC afforded sulfonamide **9a** in yield 88%. IR spectra showed band at 3425 cm^{-1} for OH group. ^{1}H NMR spectrum of **9a** revealed disappearance of signal at 1.95 ppm of acetoxy protons and the presence of a multiplet and triplet at 3.42 and 3.66 ppm for CH_2OH(c) and OCH_2(b), respectively, in addition to, triplet at 5.17 ppm (exchangeable with D_2O) corresponding to OH group with coupling constant J = 5.4 Hz and singlet signal at 4.55 ppm corresponding to NCH_2O(a).

Treatment of pyridine **1b** with compound **7** gave the corresponding O- alkylated derivatives **10b** in 62% yield . IR spectra of **10b** showed the absence of amidic C=O band which indicate the synthesis of O-alkylation and not N-alkylation. In addition to bands at 2220 cm^{-1} for C≡N and 1739 cm^{-1} for C=O of acetoxy group. ^{1}H NMR spectra of **10b** showed singlet signals at 1.90 and 4.43 ppm for CH_3CO group protons and OCH_2O(a) and triplet signals at 3.78 and 4.25 ppm corresponding to OCH_2(b) and OCH_2(c) with coupling constant 5.7 and 6.0 Hz, respectively.

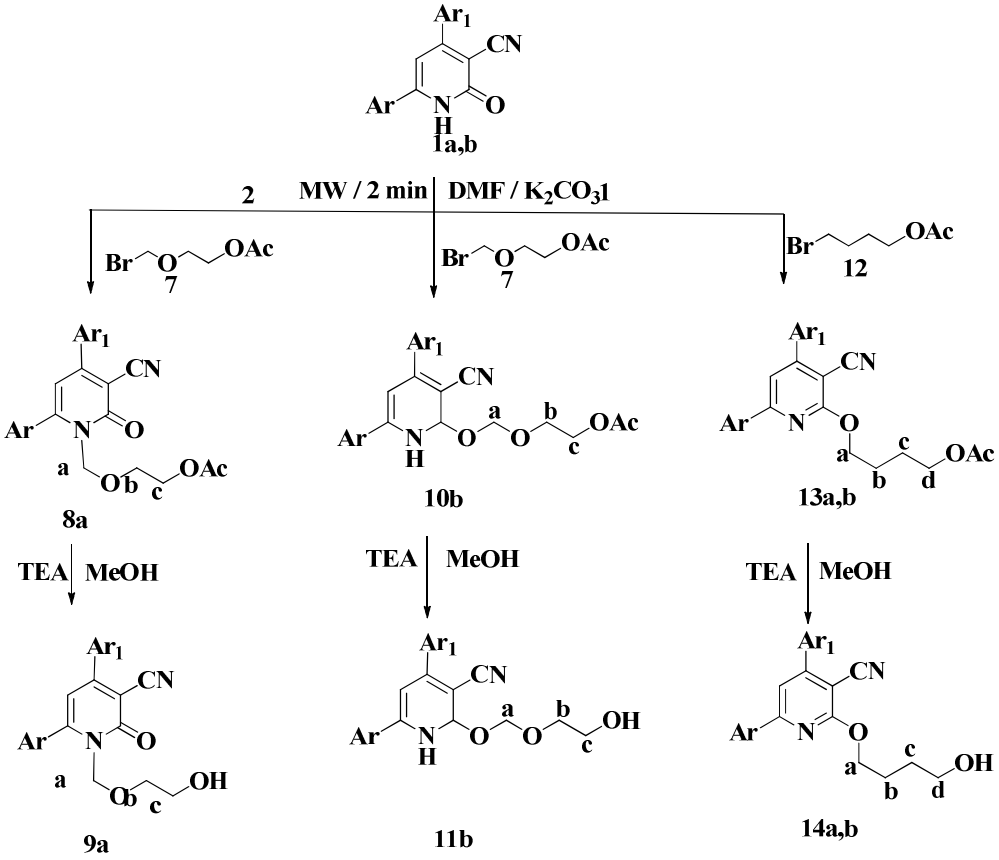
Deacetylation of O- alkylated derivatives **10b** with triethyl amine/methanol followed by TLC gave sulfonamide **11b** in 86% yield. IR spectrum showed broad band at 3459 cm^{-1} for OH and 2214 cm^{-1} for C≡N. ^{1}HNMR spectrum of **11b** showed the disappearance of OAc protons and the presence of a multiplet and triplet at 3.50 and 3.59 ppm corresponding to CH_2OH(c) and OCH_2(b), respectively, in addition to, triplet (exchangeable with D_2O) at 4.52 ppm corresponding to OH group with coupling constant J = 5.4 Hz, and singlet signal at 5.42 for OCH_2O(a) protons.

Reactions of pyridine **1** with alkylated reagent **12** gave O-alkylated derivatives **13**. IR spectra of **13a,b** showed bands at 3459, 3442, 1736 and 1735 cm^{-1} for NH groups and the C=O groups respectively, with absence of amidic band indicate the O-alkylation not N-alkylation. ^{1}H NMR spectra

showed two multiplet signals in between 1.60 – 1.62 and 1.82 – 1.84 ppm corresponding to the protons of CH_2(c) and CH_2(b), respectively, singlet at 1.96 ppm for CH_3CO and triplet signals in between 4.02 – 4.03 and 4.20 ppm corresponding to OCH_2(a) and CH_2OCO(d), respectively.

Deacetylation of compounds **13a,b** with triethyl amine/methanol followed by TLC gave sulfonamide derivatives **14a,b** in 86% and 88% yield, respectively. IR spectra of **14a,b** showed broad bands at 3410 and 3416 cm^{-1} for OH respectively and the absent of acetoxy C=O groups.

^{1}H NMR spectra of compounds **14a,b** showed multiplet signals in between 1.62 – 1.65 ppm and 1.85 – 1.86 ppm corresponding to CH_2(c) and CH_2(b), respectively, and the absence of the signals at 1.96 ppm of acetoxy groups, in addtition to signals at 3.32 – 3.46 ppm and 3.49 – 3.60 ppm as triplet corresponding to CH_2(OH)(d) and OCH_2(a) respectively and the OH group appear as a triplet at 4.58 and 4.39 ppm with j = 5.10 and 5.10 Hz, respectively. (scheme 2)



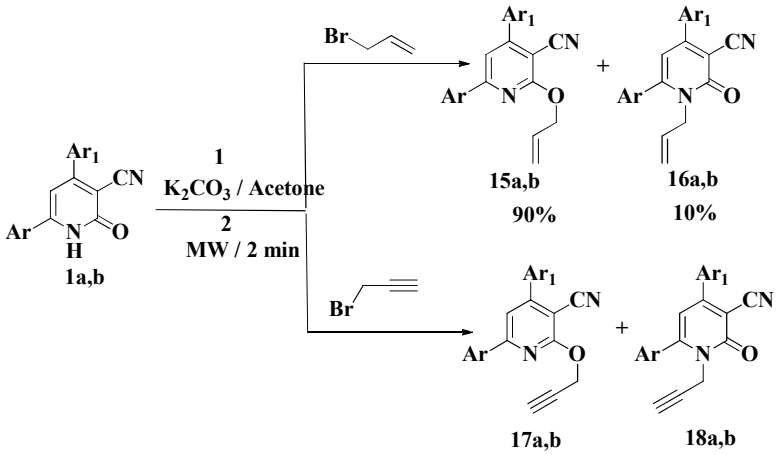
scheme 2 Alkylation and Deacetylation of pyridine

Alkylation of pyridine **1** with allyl bromide afforded the two isomer O- allyl and N- allyl product **15a,b** and **16a,b** in ratio 70 : 15% yield, respectively. The isomers were isolated by column chromatography using eluent [(9.9 ml) CH_2Cl_2 : (0.1) MeOH] . IR spectrum of **15a,b** showed disappearance of the amide C=O which indicating synthesis of O-derivatives and not N-analogues. While, in compounds **16a,b** the amidic carbonyl appear at 1652 and 1645 cm^{-1} respectively, which meaning the synthesis of N-derivatives and not O-analogues. ^{1}HNMR spectrum of **15a** showed doublet signals at 4.95, 5.16 and 5.39 ppm corresponding to OCH_2, =CHH and =CHH, respectively, additionally multiplet signals at 6.01 ppm for CH=CH_2 proton. ^{13}C NMR spectrum of **15a** showed signals at 67.8, 114.3 and118.5 ppm corresponding to OCH_2, =CH_2 and CH= carbons, respectively, in addition to, the aromatic carbons appear in between 119.4 – 164.1 ppm and C≡N carbon at 115.8 ppm. ^{1}H NMR spectrum of **15b** showed signals at 2.39, 5.09, 5.33, 5.52, 6.12, 7.23 -7.52, 7.89 and 7.89 – 8.20 ppm corresponding to -CH_3, OCH_2, =CHH, =CHH, CH= , Ar-H, pyridon-H- 5 and Ar-H respectively.

^{1}H NMR spectra of **16a** showed doublet signals at 5.01, 5.19 and 5.39 ppm corresponding to NCH_2, =CHH and =CHH,

respectively, in addition to, multipet signals at 6.01 ppm for (–C<u>H</u>=CH_2). Its ^{13}C NMR showed three signals at 52.6, 92.7 and 114.3 ppm corresponding to NCH_2 and $CH=CH_2$ carbons, while the aromatic carbons and amidic carbonyl appear in between 118.5 – 164.1 ppm.

Pyridine **1** reacted with propargyl bromide afforded mixture of prop-2-ynyloxy pyridine **17a,b** in 65% and 68% yield and N-prop-2-ynyl pyridine **18a,b** (Scheme 3). IR spectrum of **17a,b** showed disappearance of the amidic C=O, which indicating the synthesis of O-alkyl derivatives and not N-alkyl derivatives. While in compounds **18a,b**, the amidic carbonyl appear at 1642 cm^{-1} which confirm the synthesis of N-alkyl derivatives. ^1H NMR spectrum of compounds **17a,b** and **18a,b** showed singlet signals at 3.42, 3.62, 3.25 and 3.29 ppm for ≡C-H acetyenic proton, respectively, in other hand, the OCH_2 and NCH_2 protons in compound **17a,b** and **18a,b** appears as singlet at 5.37, 5.27, 4.61 and 4.59 ppm, respectively.



scheme 3 Alkylation of pyridine and formation a mixure of O and N isomers

3. Biological Activity:

4-1. Antimicrobial Activity

Amoxycillin is used as standard to detect antibacterial activity of Compounds **2a, 3a, 4b, 15a and 18b** by well diffusion method[21, 22] . The tested compounds **2a,** and **15a** showed **the** highest activity while Compounds **3a, 18b** and **4b showed medium** activity than Amoxycillin against Gram positive and Gram negative. (Table 1). Antifungal Activity of Compounds 2a**, 3a, 4b, 15a and 18b** showed **higher** activity than the standard drug Amphotericin B against (Asperigillus niger) and (Candida albicans) which detected in Table (1). The higher activity for the tested compounds owing to the presence of sulfonamide moiety in the pyridine ring.

Table 1: Antibacterial and Antifungal activities of compounds2a, 3a, 15a, 18b and 4b

Comp. No.	Inhibition Zone (mm)						
	Bacteria					fungi	
	Gram (+ve)		Gram (-ve)				
	S. aureus ATCC 6538	S.epidermidis ATCC12228	Escherichia Coli ATCC 10536	Klebsiella Pneumoniae ATCC 27736	Pseudomonas Aeruginosae ATCC 9022	**Candida albicans ATCC10231**	**Aspergillus niger ATCC 16404**
2a	21	20	16	17	17	18	21
3a	20	19	17	20	18	18	20
15a	21	20	19	19	18	18	20
18b	20	20	17	16	17	18	20
4b	18	19	19	20	15	20	18
Amoxycillin (300µg/mL)	20	19	16	15	15		
Amphotericin B (300µg/mL)						16	14

4-2.Cytotoxicity Evaluation

Antitumor activity and cytotoxicity of the compounds **4a**, **4b**, **13a**,**15b** and **17b** was evaluated on the in vitro growth of human (HepG-2) Hepatocellular cancer cell line. The selected compounds showed an Inhibitory activity against Hepatocellular carcinoma cells by using MTT assay [19, 23-25] under the experimental conditions. Compounds **17b** and **15b** showed high inhibitory effect while compound **13a** showed moderate inhibitory effects and Compounds **4a** and **4b** showed low inhibitory effects against the HepG-2 cell line. (Table 2 and 3) (Figure 1 and 2).

Table 2: Evaluation of cytotoxicity against HepG-2 cell line

Evaluation of cytotoxicity against HepG-2 cell line															
Sample Code	4a			4b			13a			15b			17b		
Sample conc. (µg/ml)	Viability %	Inhibitory %	S.D. (±)	Viability %	Inhibitory %	S.D. (±)	Viability %	Inhibitory %	S.D. (±)	Viability %	Inhibitory %	S.D. (±)	Viability %	Inhibitory %	S.D. (±)
500	17.54	82.46	1.28	15.46	84.54	1.28	11.78	88.22	2.14	6.79	93.21	0.37	3.68	96.32	0.16
250	48.71	51.29	2.87	39.54	60.46	2.12	32.95	67.05	3.79	20.31	79.69	0.75	11.46	88.54	0.28
125	83.95	16.05	2.13	61.29	38.71	2.37	47.56	52.44	2.42	36.88	63.12	1.46	26.13	73.87	1.79
62.5	98.13	1.87	0.79	83.12	16.88	1.46	80.94	19.06	2.78	45.23	54.77	2.19	38.51	61.49	2.35
31.25	100	0		98.05	1.95	0.91	98.71	1.29	0.93	71.39	28.61	2.57	54.28	45.72	2.44
15.6	100	0		100	0		100	0		89.57	10.43	1.21	78.06	21.94	1.72
7.8	100	0		100	0		100	0		97.04	2.96	0.68	92.31	7.69	0.95
3.9	100	0		100	0		100	0		100	0		96.88	3.12	0.64
0	100	0		100	0		100	0		100	0		100	0	

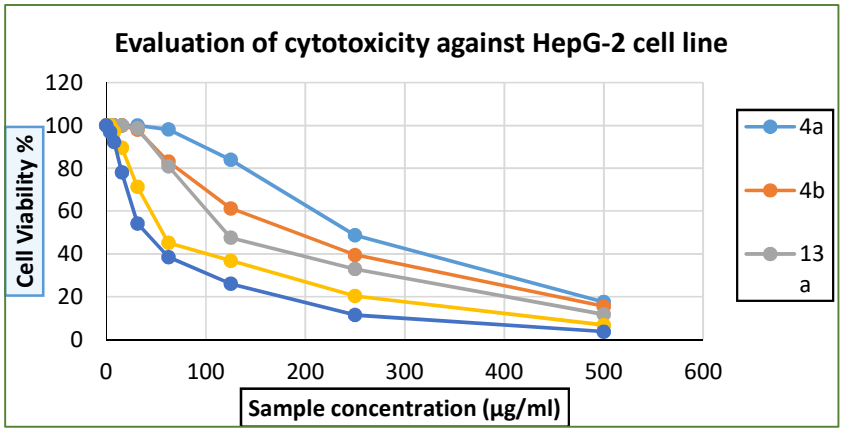


Figure 1: cell viability % of tested compound

Table 3: IC_{50} of tested compound

Sample Code:	4a	4b	13a	15b	17b
IC50 (µg/ml)	245.42 ± 6.18 µg/ml.	189.88 ± 5.08 µg/ml.	120.43 ± 2.89 µg/ml.	56.81 ± 2.13 µg/ml.	39.73 ± 1.45 µg/ml.

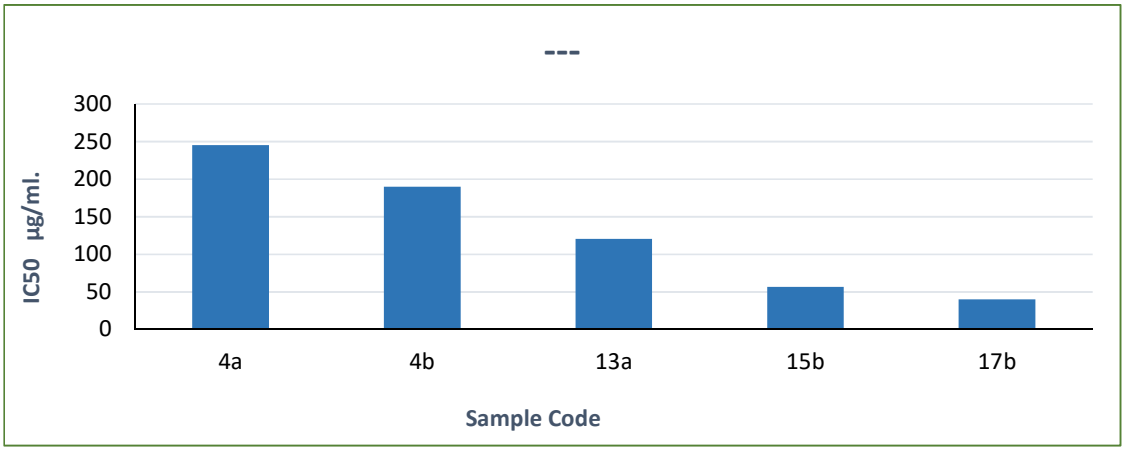


Figure 2: IC_{50} of tested compound

5. Conclusions

New derivatives of pyridine, fused with Sulfonamide Moiety, have been synthesized through the alkylation of pyridine 1a and 1b. These pyridine derivatives have demonstrated high effectiveness as antibacterial and antifungal agents. Additionally, the new pyridine derivatives were tested as cytotoxic agents and exhibited inhibitory effects against the HepG-2 cell line.

6. Conflicts of interest

There are no conflicts to declare**.**

7. Formatting of funding sources

Qassim University is the funding source.

8. Author Contributions

I.R., and R. A.E. Conceived, designed performed the experiments, analyzed the data and contributed reagents/materials/analysis tools. I.R. Wrote and reviewed the paper.

9. Reference

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