Available online at www.derpharmachemica.com

ISSN 0975-413X
CODEN (USA): PCHHAX

Der Pharma Chemica, 2018, 10(5): 6-10
(http://www.derpharmachemica.com/archive.html)

A Facile Synthesis of *N*- and *O*-alkylated Nicotinonitriles and its 2-methoxy 1,2,3-triazole Candidates as Potential Anticancer and Antimicrobial Agents

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ABSTRACT

A series of N- and O- alkylated nicotinonitriles was reported. Base mediate alkylation of nicotinonitriles 1a-c with chloroacetonitrile, allyl bromide and propargyl bromide produced N- and O- alkylated nicotinonitrile derivatives 3-6. Reaction of compounds 6a,b with ethyl 2-azidoacetate in the presence of $CuSO_4$ and sodium ascorbate afforded 1,4-disubstituted triazoles 7a,b. The anticancer activity against RPE-1 and MCF-7 human cell lines showed that all compounds did not showed big variation in their cytotoxicity activities at concentrations of 25, 50 and 100 µM against both cell types. The antimicrobial activity of the new compounds showed that all the tested compounds do not showed significant antibacterial and antifungal activities against the mention microorganisms, except compounds 4b, 5c, 5d and 6b showed moderate antimicrobial activity towards Bacillus subtilis.

Keywords: 2-Oxo-nicotinonitrile, Alkylation, Click chemistry, 1,2,3-triazole, anticancer

INTRODUCTION

The 2-oxo-nicotinonitriles are important intermediates in the synthesis of functionalized heterocyclic systems of biological significance, as manifested in the antitumor agents, camptothecin [1,2]. They are also structural cores of naturally occurring products such as the heterocyclic annelated pyridone alkaloid Cerpegin, analgesic, antiulcer and anti-inflammatory agents [3,4]. Funiculosine is another example of biologically active 2-pyridones, which possesses antifungicidal properties [5]. The regioselectivity alkylation of alkali metal salt of 2-pyridone with alkyl halides was depend on some factors such as the solvent, cation, structure of alkyl halide and structure of substrate. The literature reported that 6-substituted-2-pyridones prefer the *O*- over *N*- alkylation, which may be attributed to steric nature of the C-6 substituted group [6-9]. The studies presented here are a continuous of our previous efforts for synthesis of nicotinonitrile analogues from simple available reagents for biological study [10-13].

EXPERIMENTAL SECTION

General

The elemental analyses were obtained on a Perkin Elmer 240. The mass spectra (Ms) were measured with Shimadzu GCMS-QP 1000 EX mass spectrometer. The IR spectra were acquired in KBr on a Pye Unicam Sp-3–300 infrared spectrophotometer. The ^{1}H-NMR spectra were measured on a Bruker Avance 400 spectrometer at 400.0 MHz. The chemical shifts were measured relative to Deuterated Dimethyl Sulfoxide (DMSO-d_6) proton signal. The melting points were determined on an Electro thermal IA 9100 apparatus and are uncorrected.

General procedure for preparation of pyridin-2-(1*H*)-one-3-carbonitriles (1a-c)

A mixture of 2-acetylnaphthalene (10 mmol), 4-bromoacetophenone (10 mmol), aromatic aldehydes namely (*p*-tolualdehyde, 3-methyl-2-thiophenecarboxaldehyde and 4-bromoacetophenone) (10 mmol), ethyl cyanoacetate (10 mmol), and excess from ammonium acetate (80 mmol), in absolute ethanol (30 ml) was refluxed for 29 h, the reaction mentioned by Thin Layer Chromatography (TLC) using (Methylene chloride/MeOH 10:1), leave to cooling at room temperature, the formed precipitate was filtered off, washed with ethanol, dried and crystallized from methanol/acetic acid (1:2) ratio.

6-(Naphthalen-2-yl)-2-oxo-4-(*p*-tolyl)-1,2-dihydro-pyridine-3-carbonitrile (1a): Yellow powder; yield 39%; m.p. 303-305°C. IR (KBr): 3455 cm^{-1} (NH), 2219 cm^{-1} (C≡N) and 1682 cm^{-1} (C=O, amide); ^{1}H-NMR (DMSO-d_6): δ=2.41 (s, 3H, CH_3), 6.96 (s, 1H, pyridone H-5), 7.39 (d, 2H,

J=7.6 Hz, Ar-H), 7.63 (m, 4H, A-H), 8.01 (m, 4H, A-H), 8.55 (s, 1H, Ar-H), 12.64 (br, 1H, NH); ^{13}C-NMR (DMSO-d_6): δ=20.94 (CH_3), 97.99, 106.5, 116.6, 124.3, 127.0, 127.6, 127.9, 128.1, 128.2, 128.5, 128.8, 129.3, 132.4, 133.2, 133.8, 140.4, 151.2, 159.6, 162.1 and 172.0, (C≡N, Ar-C and C=O). Anal. Calcd for $C_{23}H_{16}N_2O$ (336.39): C, 82.12; H, 4.79; N, 8.33. Found: C, 82.01; H, 4.83; N, 8.29.

4-(3-Methylthien-2-yl)-6-(naphthalen-2-yl)-2-oxo-1,2-dihydropyridine-3-carbonitrile (1b): Yellow powder; yield 33.5%; m.p. 283-285°C. IR (KBr): 3442 cm^{-1} (NH), 2217 cm^{-1} (C≡N) and 1640 cm^{-1} (C=O, amide); ^{1}H-NMR (DMSO-d_6): δ=2.32 (s, 3H, CH_3), 6.95 (s, 1H, pyridone H-5), 7.13 (d, 1H, *J*=4.80 Hz, thiophene), 7.63 (m, 2H, Ar-H), 7.79 (d, 1H, *J*=4.80 Hz, thiophene), 8.03 (m, 4H, Ar-H), 8.53 (s, 1H, Ar-H), 12.92 (s, 1H, NH); ^{13}C-NMR (DMSO-d_6): δ=15.20 (CH_3), 116.2, 124.2, 127.0.127.6, 127.9, 128.1, 128.2, 128.5, 128.9, 131.0, 131.1, 132.3, 133.8, 138.1, 160.2 (C≡N, Ar-C and C=O). Anal. Calcd for $C_{21}H_{14}N_2OS$ (342.41): C, 73.66; H, 4.12; N, 8.18. Found: C, 73.77; H, 4.18; N, 8.09.

6-(4-Bromophenyl)-4-(4-chlorophenyl)-2-(prop-2-yn-1-yloxy)nicotine-nitrile (1c). Yellow powder; yield 43.5%; m.p. 317-319°C. IR (KBr): 3434 cm^{-1} (NH), 2216 cm^{-1} (C≡N) and 1632 cm^{-1} (C=O, amide); ^{1}H-NMR (DMSO-d_6): δ=6.90 (s, 1H, pyridone, H-5), 7.63 (d, 2H, *J*=8.40 Hz, Ar-H), 7.74 (d, 2H, *J*=8.80 Hz, Ar-H), 7.77 (d, 2H, *J*=8.80 Hz, Ar-H), 7.84 (d, 2H, *J*=8.00 Hz, Ar-H), 12.91 (br, 1H, NH exchange with D_2O); ^{13}C-NMR (DMSO-d_6): δ=98.34, 106.6, 116.2, 125.0, 128.8, 129.7, 130.2, 131.8, 134.6, 135.3, 150.8, 158.2, 162.0, (C≡N, Ar-C and C=O). Anal. Calcd for $C_{18}H_{10}BrClN_2O$ (385.64): C, 56.06; H, 2.61; N, 7.26. Found: C, 55.91; H, 2.65; N, 7.22.

General procedure for synthesis of compounds 2-5

A mixture of pyridin-2-(1H)-one-3-carbonitriles 1a-c (10 mmol) and (11 mmol) potassium carbonate or potassium hydroxide was stirred in dry DMF (20 ml) for 1h, followed by the addition of the appropriate alkyl halide (10 mmol) namely allyl/propargyl bromides, chloroacetonitrile and acetic anhydride. Allyl derivatives and chloroacetonitrile, the reaction mixture was stirred at room temperature for 24 h, propagy derivatives, the reaction mixture was stirred at room temperature for 32 h then poured into ice-water to give the crude product as precipitate, which in turn was filtered off and dried. Except for acetic anhydride, the reaction mixture was refluxed from 5 h, cooling, then poured into ice-water to give the crude product as precipitate, which in turn was filtered off and dried. The product was crystallized from methanol.

1-Acetyl-6-(naphthalen-2-yl)-2-oxo-4-(*p*-tolyl)-1,2-dihydro-pyridine-3-carbonitrile (2a): Yellow powder; yield 74.5%; m.p. 160-162°C. IR (KBr): 2222 cm^{-1} (C≡N), 1769 cm^{-1} (C=O, acetyl) and 1633 cm^{-1} (C=O, amide); ^{1}H-NMR (DMSO-d_6): δ=2.39 (s, 3H, CH_3CO), 2.42 (s, 3H, CH_3), 7.44 (d, 2H, *J*=8.0 HZ, Ar-H), 7.60 (m, 2H, Ar-H), 7.74 (d, 2H, *J*=8.0 Hz, Ar-H), 7.79-8.01 (m, 3H, Ar-H), 8.32 (d, 1H, *J*=6.08 Hz, Ar-H), 8.38 (s, 1H, Ar-H), 8.87 (s, 1H, Ar-H). Anal. Calcd for $C_{25}H_{18}N_2O_2$ (378.42): C, 79.35; H, 4.79; N, 7.40. Found: C, 79.27; H, 4.83; N, 7.33.

1-Acetyl-4-(3-methylthien-2-yl)-6-(naphthalen-2-yl)-2-oxo-1,2-dihydropyridine-3-carbonitrile (2b): yellow powder; yield 51%; m.p. decomposed 280-283°C. IR (KBr): 2218 cm^{-1} (C≡N) and 1773, 1699 cm^{-1} (2C=O); ^{1}H-NMR (DMSO-d_6): δ=1.89, 2.30 (2s, 6H, 2CH_3), 6.89 (s, 1H, pyridine-H), 7.12 (d, 1H, *J*=5.5 Hz, thiophene-H), 7.60-8.05 (m, 6H, Ar-H and thiophene-H), 8.28 (d, 1H, *J*=8.5 Hz, Ar-H) and 8.34 (s, 1H, Ar-H). Anal. Calcd for $C_{23}H_{16}N_2O_2S$ (384.45): C, 71.85; H, 4.19; N, 7.29. Found C, 71.80; H, 4.23; N, 7.34.

6-(Naphthalen-2-yl)-2-oxo-4-(p-tolyl)-1,2-dihydropyridine-3-carbonitrile (3a): White powder; yield 87%; m.p. 138-140°C. IR (KBr): 2220 cm^{-1} (2C≡N) and 1647 cm^{-1} (C=O, amide); ^{1}H-NMR (DMSO-d_6): δ=2.41 (s, 3H, CH_3), 5.57 (s, 2H, NCH_2), 7.40 (d, 2H, *J*=7.60 Hz, Ar-H), 7.59 (m, 2H, Ar-H), 7.67 (d, 2H, *J*=7.20 Hz, Ar-H), 7.96 - 8.05 (m, 4H, Ar-H), 8.39 (d, 1H, *J*=8.40 Hz, Ar-H), 8.90 (s, 1H, Ar-H); ^{13}C-NMR (DMSO-d_6): δ=20.89 (CH_3), 51.96 (OCH_2), 92.14, 114.7, 115.3, 116.5, 124.2, 126.7, 127.6, 127.9, 128.4, 128.6, 128.9, 129.4, 132.5, 132.7, 133.4, 133.9, 140.2, 156.7, 156.8 and 161.7 (2C≡N and Ar-C). Anal. Calcd for $C_{25}H_{17}N_3O$ (375.42): C, 79.98; H, 4.56; N, 11.19. Found: C, 79.85; H, 4.60; N, 11.24.

2-(Cyanomethoxy)-4-(3-methylthien-2-yl)-6-(naphthalen-2-yl)nicotinonitrile (3b): White powder; yield 85%; m.p. 158-160°C. IR (KBr): 2217 cm^{-1} (2C≡N); ^{1}H-NMR (DMSO-d_6): δ=2.31 (s, 3H, CH_3), 5.60 (s, 2H, OCH_2), 7.16 (d, 1H, *J*=7.20 Hz, Ar-H), 7.61 (d, 2H, *J*=4.0, thiophene), 7.81 (d, 1H, *J*=8.4 Hz, Ar-H), 8.40 (d, 1H, *J*=8.4 Hz, Ar-H), 8.94 (s, 1H, Ar-H); ^{13}C-NMR (DMSO-d_6): δ=14.85 (CH_3), 52.14 (OCH_2), 93.98, 114.3, 116.4, 116.7, 124.2, 126.8, 127.6, 127.8, 128.1, 128.2, 128.5, 128.9, 130.1, 131.0, 132.7, 133.1, 134.0, 138.1, 150.3, 156.8, 161.7, (2C≡N and Ar-C). Anal. Calcd for $C_{23}H_{15}N_3OS$ (381.45): C, 72.42; H, 3.96; N, 11.02. Found: C, 72.32; H, 4.01; N, 10.93.

1-Allyl-6-(naphthalen-2-yl)-2-oxo-4-(*p*-tolyl)-1,2-dihydropyridine-3-carbonitrile (4a): White powder; yield 90.5%; m.p. 104-105°C. IR (KBr): 2220 cm^{-1} (C≡N), 1645 cm^{-1} (C=O); ^{1}H-NMR (DMSO-d_6): δ=2.58 (s, 3H, CH_3), 5.26 (s, 2H, $NCH_{2(a)}$), 5.44 (d, 1H, *J*=10.4 Hz, $H_{(c)}$), 5.65 (d, 1H, *J*=17.2 Hz, $H_{(c')}$), 6.27 (m, 1H, $H_{(b)}$), 7.48 (d, 2H, *J*=7.60 Hz, Ar-H), 7.68 (m, 2H, Ar-H), 7.75 (d, 2H, *J*=7.6 Hz, Ar-H), 8.04 (s, 2H, Ar-H), 8.11 (d, 2H, *J*=8.40 Hz, Ar-H), 8.42 (m, 1H, Ar-H), 8.94 (s, 1H, Ar-H). Anal. Calcd for $C_{26}H_{20}N_2O$ (376.45): C, 82.95; H, 5.35; N, 7.44. Found: C, 82.83; H, 5.31; N, 7.38.

2-(Allyloxy)-4-(3-methylthien-2-yl)-6-(naphthalen-2-yl)-1,2-dihydropyridine-3-carbonitrile (4b): Pale yellow powder; yield 47%; m.p. 90-91°C. IR (KBr): 2215 cm^{-1} (C≡N); ^{1}H-NMR (DMSO-d_6): δ=2.29 (s, 3H, CH_3), 5.15 (d, 2H, *J*=4.85 Hz, $OCH_{2(a)}$), 5.33 (d, 1H, *J*=10.40 Hz, $H_{(c')}$), 5.53 (d, 1H, *J*=17.20 Hz, $H_{(c)}$), 6.20 (m, 1H, $H_{(b)}$)), 7.13 (d, 1H, *J*=4.80 Hz, thiophene), 7.56 (m, 2H, Ar-H), 7.77 (d, 1H, *J*=5.20 Hz, thiophene), 7.82-8.30 (m, 5H, Ar-H), 8.80 (s, 1H, Ar-H); ^{13}C-NMR (DMSO-d_6): δ=14.83 (CH_3), 67.51 (OCH_2), 93.8, 114.9, 115.2, 118.1, 124.1, 126.2, 127.5, 127.6, 127.7, 128.3, 128.9, 130.5, 130.9, 132.7, 132.8, 133.5, 133.9, 137.7, 149.7, 156.9, 163.4 (C≡N and Ar-C). Anal. Calcd for $C_{24}H_{18}N_2OS$ (382.48): C, 75.37; H, 4.74; N, 7.32. Found: C, 75.48; H, 4.69; N, 7.40.

1-Allyl-6-(4-bromophenyl)-4-(4-chlorophenyl)-2-oxo-1,2-dihydropyridine-3-carbonitrile (4c): White powder; yield 92%; m.p. 218-220°C. IR (KBr): 2221 cm^{-1} (C≡N) and 1649 cm^{-1} (C=O, amide); ^{1}H-NMR (DMSO-d_6): δ=5.12 (s, 2H, $NCH_{2(a)}$), 5.32 (d, 1H, *J*=10.4 Hz, $H_{(c)}$), 5.49 (d, 1H, *J*=17.2 Hz, $H_{(c')}$), 6.61 (m, 1H, $H_{(b)}$), 7.67 (d, 2H, *J*=6.80 Hz, Ar-H), 7.73 (d, 2H, *J*=7.20 Hz, Ar-H), 7.81 (d, 2H, *J*=7.20 Hz, Ar−H), 7.88 (s, 1H, pryidone, H-5), 8.20 (d, 2H, *J*=7.60 Hz, Ar-H). Anal. Calcd for $C_{21}H_{14}BrClN_2O$ (425.71): C, 59.25; H, 3.13; N, 6.58. Found: C, 59.13; H, 3.16; N, 6.67.

6-(Naphthalen-2-yl)-2-oxo-1-(prop-2-yn-1-yl)-4-(p-tolyl)-1,2-dihydropyridine-3-carbonitrile (5a): Yellow powder; yield 85.5%; m.p. 138-140°C. IR (KBr): 2220 cm^{-1} (C≡N) and 1651 cm^{-1} (C=O, amide); ^{1}H-NMR (DMSO-d_6): δ=2.42 (s, 3H, CH_3), 3.67 (s, 1H, ≡C-H), 5.34 (s, 2H, OCH_2), 7.41 (d, 2H, *J*=7.60 Hz, Ar-H), 7.60 (m, 2H, A-H), 7.69 (d, 2H, *J*=7.20 HZ, Ar-H), 7.97-8.07 (m, 4H, Ar-H), 8.39 (d, 1H, *J*=8.4 Hz, Ar-H), 8.90 (s, 1H, Ar-H); ^{13}C-NMR (DMSO-d_6): δ=20.88 (CH_3), 54.73 (OCH_2), 78.05, 78.90 (C≡C), 92.05, 114.4, 115.1, 124.3, 126.6, 127.5, 127.6, 127.7, 128.3, 128.5, 128.8, 129.3, 132.7, 133.7, 133.8, 140.0, 156.4, 156.8 and 162.7 (C≡N and Ar-C). Anal. Calcd for $C_{26}H_{18}N_2O$ (374.43): C, 83.40; H, 4.85; N, 7.48. Found: C, 83.52; H, 4.78; N, 7.55.

4-(3-Methylthiophen-2-yl)-6-(naphthalen-2-yl)-2-(prop-2-yn-1-yloxy)nicotinonitrile (5b): Yellow powder; yield 89%; m.p. 163-164°C. IR (KBr): 2216 cm^{-1} (C≡N); ^{1}H-NMR (DMSO-d_6): δ=2.30 (s, 3H, CH_3), 3.69 (s, 1H, ≡CH), 5.35 (s, 2H, NCH_2), 7.15 (d, 1H, *J*=4.40 Hz, thiophene), 7.59 (m, 2H, Ar-H), 7.79 (d, 1H, *J*=4.40 Hz, thiophene), 7.96 (s, 1H, Ar-H), 8.06 (m, 4H, Ar-H), 8.36 (d, 1H, *J*=7.60 Hz, Ar-H), 8.89

(s, 1H, Ar-H); ^{13}C-NMR (DMSO-d_6): δ=14.48 (CH_3), 54.93 (NCH_2), 78.20 78.80 (C≡C), 93.86, 114.7, 115.8, 124.2, 126.8, 127.5, 127.9, 128.4, 128.9, 130.3, 131.0, 132.7, 133.4, 133.9, 149.9, 156.9, 162.6 (C≡N, Ar-C and C=O). Anal. Calcd for $C_{24}H_{16}N_2OS$ (380.46): C, 75.77; H 4.24; N, 7.36. Found: C, 75.89; H 4.19; N, 7.43.

6-(4-Bromophenyl)-4-(4-chlorophenyl)-2-oxo-1-(prop-2-yn-1-yl)-1,2-dihydropyridine-3-carbonitrile (5c) Yellow powder; yield 93%; m.p. 249-250°C. IR (KBr): 2223 cm^{-1} (C≡N) and 1660 cm^{-1} (C=O, amide); ^{1}H-NMR (DMSO-d_6): δ=3.64 (s, 1H, ≡C−H), 5.28 (d, 2H, *J*=2.0 Hz, NCH_2), 7.66 (d, 2H, *J*=8.40 Hz Ar-H), 7.76 (d, 2H, *J*=8.40 Hz, Ar-H), 7.81 (s, 2H, *J*=7.20 Hz, Ar-H), 7.93 (s, 1H, pyridone, H-5), 8.26 (d, 2H, *J*=8.80 Hz, Ar-H); ^{13}C-NMR (DMSO-d_6): δ=54.84 (NCH_2), 78.14, 78.71 (C≡C), 114.3, 114.8, 124.7, 128.8, 129.5, 130.6, 131.8, 134.3, 135.3, 135.2, 135.5, 155.4, 156.0, 162.6 (C≡N, Ar-C and C=O). Anal. Calcd for $C_{21}H_{12}BrClN_2O$ (423.69): C, 59.53; H, 2.85; N, 6.61. Found: C, 59.39; H, 2.78; N, 6.75.

General procedure for preparation of 1,2,3-triazole derivatives 6a,b

Ethyl 3-azidopropanoate (0.011 mol) and alkylated 2-pyridone derivatives 5a and 5b (0.01 mol) were dissolved in H_2O/DMF (30:70 (10 ml)). The reaction mixture was stirred at room temperature for 10 min, while an aqueous solution of $CuSO_4.5H_2O$ (2.0 ml, 5%) and an aqueous solution of sodium ascorbate (2.0 ml, 10%) were added. The reaction mixture was stirred until complete consumption of the starting material indicated by thin layer chromatography (TLC; 3-5 h.). The reaction mixture was evaporated under reduced pressure, extracted with dichloromethane and the organic phase was dried over anhydrous Na_2SO_4. After filtration, the solvent was evaporated to dryness under reduced pressure and the residue was crystallized from methanol/acetic acid (5:2).

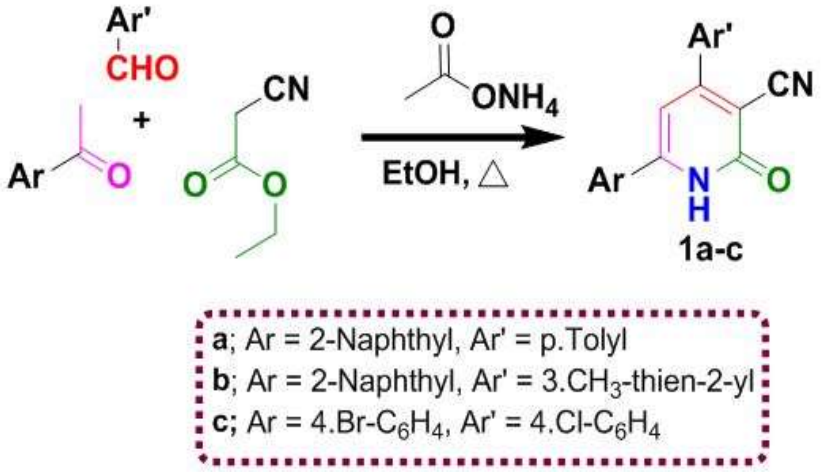
Ethyl 2-(4-((3-cyano-6-(naphthalen-2-yl)-2-oxo-4-(p-tolyl)pyridin-1(2H)-yl)methyl)-1H-1,2,3-triazol-1-yl)acetate (6a): Yellow powder; yield 82%; m.p. 106-108°C. IR (KBr): 2220 cm^{-1} (c≡N), 1750 Cm^{-1} (C=O, estr), 1648 cm^{-1} (C=O, amide); ^{1}H-NMR (DMSO-d_6): δ=1.19 (t, 2H, *J*=7.84 Hz, H-ethyl), 2.42 (s, 3H, CH_3), 4.17 (q, 2H, *J*=7.84 Hz, H-ethyl), 5.30 (s, 2H, CH_2N), 5.83 (s, 2H, CH_2N), 7.40-8.93 (m, 13H, Ar-H, pyridine-H, H-triazol); ^{13}C-NMR (DMSO-d_6): δ=13.38, 21.52 ($2CH_3$), 50.94 (O-CH_2), 60.58, 61.95 ($2CH_2N$), 92.61, 114.6, 124.9, 126.6, 127.1, 128.0, 128.07, 128.2, 128.9, 129.0, 129.4, 129.8, 130.1, 133.3, 133.4, 134.4, 140.5, 142.7, 156.9, 157.5, 163.9, 167.6, 172.4 (C≡N, Ar-C and 2C=O). Anal. Calcd for $C_{30}H_{25}N_5O_3$ (503.55): C, 71.56; H, 5.00; N, 13.91. Found C, 71.63; H, 4.96; N, 13.97.

Ethyl-2-(4-(((3-cyano-4-(3-methylthiophen-2-yl)-6-(naphthalen-2-yl)pyridin-2-yl)oxy)methyl)-1H-1,2,3-triazol-1-yl)acetate (6b): Yellow powder; yield 76.5%; m.p. 120-122°C. IR (KBr): 2219 cm^{-1} (C≡N), 1747 cm^{-1} (C=O, estr); ^{1}H-NMR (DMSO-d_6): δ=1.19 (t, 2H, *J*=7.12 Hz, H-ethyl), 2.30 (s, 3H, CH_3-ring), 4.17 (q, 2H, *J*=7.12 Hz, H-ethyl), 5.44 (s, 2H, CH_2N), 5.85 (s, 2H, CH_2N), 7.12-8.95 (m, 13H, Ar-H, pyridine-H, H-triazol); ^{13}C-NMR (DMSO-d_6): δ= 15.33 (CH_3), 21.5 (CH_3), 50.94 (OCH_2), 60.75, 61.95 ($2CH_2N$), 94.4, 115.3, 116.0, 124.8, 126.6, 127.1, 128.0, 128.3, 128.4, 129.0, 129.5, 130.9, 131.4, 133.3, 134.1, 134.4, 138.2, 142.6, 150.5, 157.5, 163.7, 167.6, 172.4 (C≡N, Ar-C and 2C=O). Anal. Calcd for $C_{28}H_{23}N_5O_3S$ (509.58): C, 66.00; H, 4.55; N, 13.74. Found C, 66.08; H, 4.59; N, 13.69.

RESULTS AND DISCUSSION

Chemistry

4-Aryl-6-naphth-2-yl-2-oxo-1,2-dihydronicotinonitrile 1a-c were selected as starting precursor for this study. They were synthesized as reported method [13] *via* one pot multicomponent condensation of suitable acetyl derivatives, araldehyde (Namely, 4-methyl benzaldehyde and 3-methyl thiophene-2-carboxaldehyde), ethyl cyanoacetate and ammonium acetate in refluxing ethanol (Scheme 1). The spectroscopic data and microanalysis were agreed with the assigned structure. Refluxing of nicotinonitriles 1a,b with acetic anhydride tolerated the corresponding *N*-acetyl products 2a,b. Their IR bands showed carbonyl bands at 1769, 1773, 1633, 1699 cm^{-1}. Its ^{1}H-NMR signals showed two singlets at 2.39 and 2.30 ppm for two acetyl groups protons.

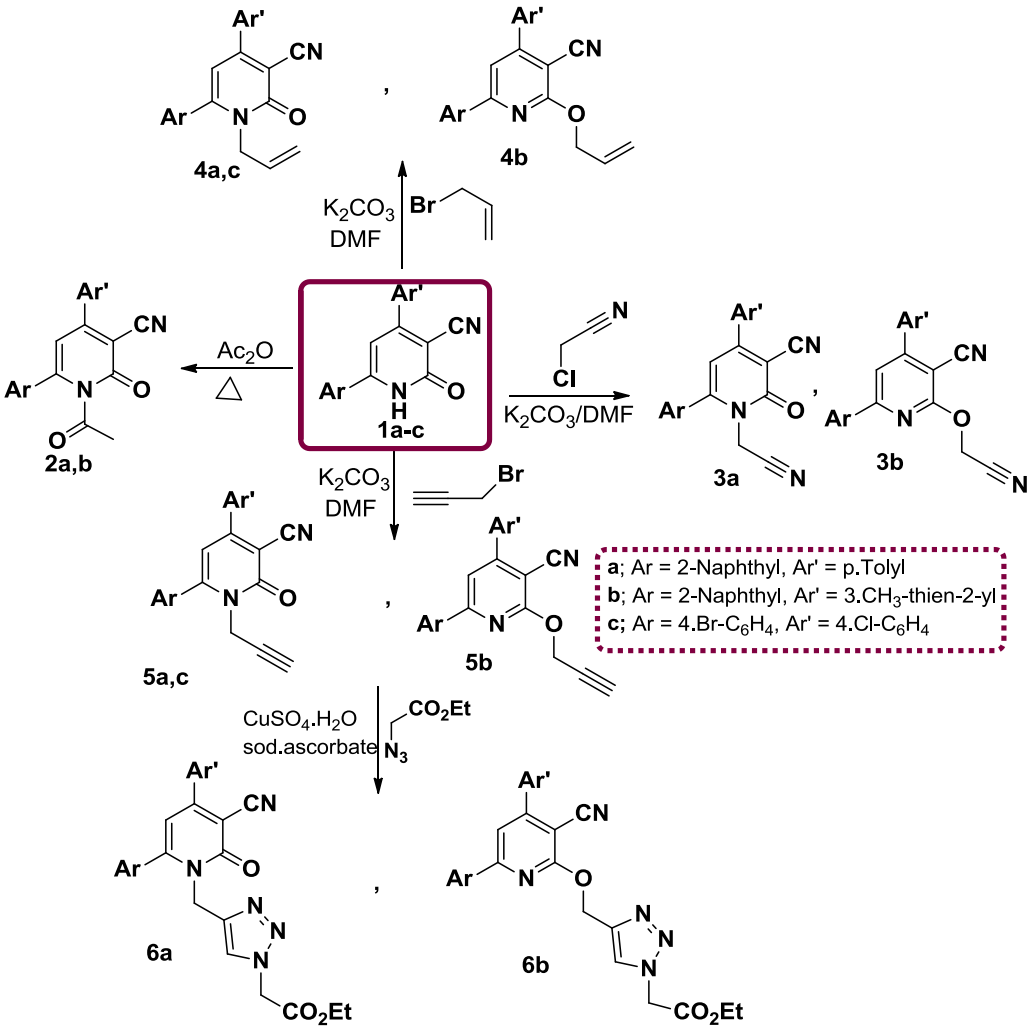


Scheme 1: One pot synthesis of nicotinonitriles 1a-c

Base mediate alkylation of nicotinonitriles 1a-c with chloroacetonitrile, allyl bromide and propargyl bromide produced *N*- and *O*- alkylated nicotinonitrile derivatives 3-6 (Scheme 2). The *N*- and *O*- alkylated products were identified from IR bands, where the *N*-derivatives showed absorption bands at between 1645-1660 cm^{-1}, which are absent in *O*-derivatives. ^{1}H-NMR data of compounds 3-6 were agreed with the assigned structure.

Click chemistry is one of the important methodology for synthesis of 1,2,3-triazole analogs. Thus reaction of compounds 6a,b with ethyl-2-

azidoacetate in the presence of $CuSO_4$ and sodium ascorbate afforded 1,4-disubstituted triazoles 7a,b (Scheme 2). The spectroscopic data of compounds 7a,b were agreed with their structure (see the experimental part).



Scheme 2: The presence of $CuSO_4$ and sodium ascorbate afforded 1,4-disubstituted triazoles 7a,b.

Biology

Antitumor activity

Seven compounds were examined *in vitro* for their antitumor activities against Retinal Pigmented Epithelial Cells Page 1 (RPE-1) and Human Breast Adenocarcinoma Cell Line (MCF-7) using 3-(4,5-dimethythiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) assay. The percentage of the intact cells was measured and compared to the control. The activities of these compounds against the three carcinoma cells were compared with that of Doxorubicin®.

The obtained results showed that all compounds did not showed big variation in their cytotoxicity activities at concentrations of 25, 50 and 100 µM against both cell types. From Table 1 we can deduce that, at 100 µM, all compounds showed good cytotoxicity activities against both types of cells.

Table 1: The cytotoxicity IC_{50} values of the seven compounds using MTT assay against two cell types

Compound	MCF-7	RPE-1
	IC_{50} (µM) 48-70	
2a	22.8	19.9
2b	22.6	20.9
4a	26.6	22.8
4b	21.9	19.2
5a	22.0	19.2
5c	28.1	21.4
6a	22.0	19.0
Doxorubicin	20.9	19.1

Antimicrobial activity

The antimicrobial activity of new compounds were investigated using the agar well diffusion method as modified from [14], compared with cefotaxime as control. For antifungal, nystatin was used as standard drug. It is clearly observed that, from the obtained data in Table 2, all the tested compounds do not showed significant antibacterial and antifungal activities against the mention microorganisms, except compounds 4b, 5c, 5d and 6b showed moderate antimicrobial activity towards *Bacillus subtilis*.

Table 2: Antimicrobial activities of some new synthesized compounds (Inhibition zones mm). Diameter (mm) of inhibition zones against the corresponding standard microbial strains

Compound. No.	Gram-positive bacteria		Gram-negative bacteria		Fungi
	B. subtilis	***B. cereus***	***P. aeruginosa***	***E. coli***	***Aspergillus niger***
2a	21	22	25	18	0
2b	15	22	23	18	15
4a	11	29	15	22	11
4b	22	27	16	21	18
5a	2	2	1	0	21
5c	22	14	12	24	22
6a	46	18	22	19	19
Cefotaxime	32	28	32	34	10
Nystatin	-	-	-	-	20
DMSO	-	-	-	-	-

CONCLUSION

A series of *N*- and *O*- alkylated nicotinonitriles was synthesized via reaction of nicotinonitriles 1a-c with chloroacetonitrile, allyl bromide and propargyl bromide. The anticancer activity against RPE-1 and MCF-7 human cell lines showed that all compounds did not showed big variation in their cytotoxicity activities at concentrations of 25, 50 and 100 µM against both cell types. The antimicrobial activity of the new compounds showed that all the tested compounds do not showed significant antibacterial and antifungal activities against the mention microorganisms, except compounds 4b, 5c, 5d and 6b showed moderate antimicrobial activity towards *B. subtilis*.

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