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Synthesis of Some New Aryl-azo Derivatives Clubbed with Pyridone and Evaluating their Biological Broadcast

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SYNTHESIS of some new Aryl-azopyridone analogues was synthesized through coupling of diverse Diazonium salts with 1-ethyl-4-methyl-2,6-dioxopiperidine-3-carbonitrile. The proper structures for all incorporated derivatives were demonstrated to be confirmed *via* various physical and spectral analyses performed. The synthesized analogues were probed as cytotoxic agents toward four dissimilar cell lines, in which the results displayed persuasive activities relative to the results of antibiotic standards. In addition, the antioxidant activity of the synthesized analogues was evaluated using ABTS•+ method.

Keywords: Anticancer activities, Antioxidant, Cytotoxicity, Diazonium salts, Pyridones.

Introduction

In the most recent years scientific active researches target a kind of organic dyes which weren't generally synthesized for dyeing textile and different materials only but also have a great significant in hi-tech industries of electrons such as sensitized solar cells, photochromic dyes, fluorescent sensors dyes, pharmaceutical chemicals and biomedical treatment like photodynamic therapy for treating cancer disease and anti-oxidant issues [1-4]. These dyes are called functional dyes. Functional dyes come to make breakthrough in synthesizing field of dyes and increasing the great value of dyes by adding different functionality to their application and performance [5]. Heterocyclic compounds are representing these kinds of functional dyes as it plays an important role in bioscience and textile industries [6]. Pyridones is considered as one of the heterocyloc moieties with interesting biological activities [7]. Where, 2-pyridone skeleton "dominant tautomer of 2-hydroxypyridine" is correspondingly existent in DNA and RNA particles [8]. Meanwhile, Pyridone analogues have pharmaceutical and an extensive assortment of pharmacological efficacies [9]. They display antibacterial [10], antifungal [11], anti-HIV [12], anticancer activities [13]. Pyridones act through many mechanisms including inhibition of tyrosine kinases such as FGFR and VEGFR, Met and TAM family kinases [14]. They were also identified as inhibitors of the serine/threonine kinase PIM-1, which plays an important role in cell cycle progression, signal transduction pathways, and apoptosis [15]. Our work will represent new series of dyes based on pridone moiety and use them to dyes polyester fabric .also different activity of the final dyes like antimicrobial activity, antioxidant activity and anticancer are evaluated.

Experimental

Instruments

Melting points were detected by "Gallenkamp" apparatus. IR spectra were registered on a "Nicolet 5000 FT-IR" spectrophotometer. 1H NMR spectra were obtained by "Bruker WP 400 MHz" in DMSO-d6. Mass spectra were enumerated by "Quadrupole GC/MS Thermo Scientific Focus/ DSQII" at 70 eV. Elemental analyses were measured by "Perkin Elmer 2400 analyzer".

Synthesis

Synthesis of 1-ethyl-6-hydroxy-4-methyl-2-oxo-5-(phenylazo)-1,2-dihydropyridine-3-carbonitrile 7a-i

The compounds were prepared in the light of reported procedure in the literature [7] *via*

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diazo-coupling reaction of diazotized o-,m-,p-nitroanilne, o-,m-,p-aminophenol or o,m-, *p*-anisidine with 1-ethyl-6-hydroxy-4-methyl-2-oxo-1,2-dihydropyridine-3-carbonitrile.

1-ethyl-6-hydroxy-4-methyl-5-((2-nitrophenyl) diazenyl)-2-oxo-1,2-dihydropyridine-3-carbonitrile (7a)

Orange powder; yield = 80%; m.p. = 243-245°C, IR: 3413(OH), 3157(CH=), 2221 (CN), 1685 (C=O) cm^{-1}. ^1H NMR: 1.43 (t, 3H, CH_3), 2.33 (s, 3H, CH_3-pyridone), 3.42 (q, 2H, CH_2), 7.26-7.57 (m, 4H, Ar-H), 10.34 (s, 1H, OH). EIMS m/z, 327 (55) $[M]^+$, 312 (65), 310 (17), 213 (33), 155 (13), 92 (94), 77 (100).

1-ethyl-6-hydroxy-4-methyl-5-((3-nitrophenyl) diazenyl-2-oxo-1,2-dihydropyridine-3-carbonitrile (7b)

Yellow solid, yield = 66%, m.p. = 260-262°C. IR: 3382(OH), 3112(CH=), 22207(CN), 1645 (C=O) cm^{-1}. ^1H NMR: 1.39 (t, 3H, CH_3), 2.24 (s, 3H, CH_3-pyridone), 3.23 (q, 2H, CH_2), 7.22-7.26 (d.d, 2H, Ar-H), 7.74-7.76 (d.d, 2H, Ar-H), 7.81 (s, 1H, Ar-H), 10.23 (s, 1H, OH). EIMS m/z, 327 (27) $[M]^+$, 312 (33), 297 (14), 213 (12), 170 (07), 92 (93), 64 (100).

1-ethyl-6-hydroxy-4-methyl-5-((4-nitrophenyl)diazenyl-2-oxo-1,2-dihydropyridine-3-carbonitrile (7c)

Brown solid, yield = 71%, m.p. = 270-272°C. IR: 3372(OH), 3182(CH=), 2207 (CN), 1664 (C=O) cm^{-1}. 1H NMR: 1.39 (t, 3H, CH3), 2.36 (s, 3H, CH_3-pyridone), 3.48 (q, 2H, CH_2), 7.21-7.64 (m, 4H, Ar-H), 10.29 (s, 1H, OH). EIMS m/z, 327 (82) $[M]^+$, 282 (18), 181 (14), 176 (78), 149 (28), 92 (53), 78 (100).

1-ethyl-6-hydroxy-5-((2-hydroxyphenyl) diazenyl)-4-methyl-2-oxo-1,2-dihydropyridine-3-carbonitrile (7d)

Orange powder; yield = 69%; m.p. = 256-258°C, IR: 34073(OH), 3123(CH=), 2190 (CN), 1647 (C=O) cm^{-1}. ^1H NMR: 1.27 (t, 3H, CH_3), 2.25 (s, 3H, CH_3-pyridone), 3.26 (q, 2H, CH_2), 7.17-7.49 (m, 4H, Ar-H), 10.28 (s, 1H, OH). EIMS m/z, 398 (15) $[M]^+$, 267 (06), 213 (08), 121 (16), 108 (100), 92 (50), 77 (59).

1-ethyl-6-hydroxy-5-((3-hydroxyphenyl) diazenyl)-4-methyl-2-oxo-1,2-dihydropyridine-3-carbonitrile (7e)

Yellow solid, yield = 68%, m.p. = 232-234°C, lit. IR: 3402(OH), 3111(CH=), 2210(CN), 1661 (C=O) cm^{-1}. ^1H NMR: 1.42 (t, 3H, CH_3), 2.42 (s, 3H, CH_3-pyridone), 3.28 (q, 2H, CH_2),7.33-7.69 (m,3H, Ar-H), 7.85 (s, 1H, Ar-H), 10.63 (s, 1H, OH). EIMS m/z, 298 (100) [M]+, 279 (37), 267 (19), 118 (108), 108 (52), 77 (84), 53 (68).

1-ethyl-6-hydroxy-5-((4-hydroxyphenyl) diazenyl)-4-methyl-2-oxo-1,2-dihydropyridine-3-carbonitrile (7f)

Violet solid, yield = 64%, m.p. = 227-229°C. IR: 3371(OH), 3129(CH=), 2215 (CN), 1651 (C=O) cm^{-1}. 1H NMR: 1.29 (t, 3H, CH3), 2.19 (s, 3H, CH_3-pyridone), 3.26 (q, 2H, CH2), 7.09-7.47 (d.d, 2H, Ar-H), 7.56-7.81 (d.d, 2H, Ar-H), 10.61 (s, 1H, OH). EIMS m/z, 298 (15) $[M]^+$, 267 (03), 177 (07), 118 (15), 108 (45), , 77 (65), 53 (100).

1-ethyl-6-hydroxy-5-((2-methoxyphenyl) diazenyl)-4-methyl-2-oxo-1,2-dihydropyridine-3-carbonitrile (7g)

Orange powder; yield = 75%; m.p. = 268-270°C, IR: 3393(OH), 3107(CH=), 2218 (CN), 1643 (C=O) cm^{-1}. ^1H NMR: 1.26 (t, 3H, CH_3), 2.47 (s, 3H, CH_3-pyridone), 3.19 (q, 2H, CH_2), 7.47-7.81 (m, 4H, Ar-H), 10.88 (s, 1H, OH). EIMS m/z, 321 (23) $[M]^+$, 267 (19), 213 (27), 198 (47), 107 (65) , 77 (100), 64 (87).

1-ethyl-6-hydroxy-5-((3-methoxyphenyl) diazenyl)-4-methyl-2-oxo-1,2-dihydropyridine-3-carbonitrile (7h)

yellow solid, yield = 73%, m.p. = 215-217°C, lit. IR: 3401(OH), 3132(CH=), 2221(CN), 1677 (C=O) cm^{-1}. ^1H NMR: 1.37 (t, 3H, CH_3), 2.19 (s, 3H, CH_3-pyridone), 3.25 (q, 2H, CH_2), 7.47-7.89 (m, 3H, Ar-H), 8.72 (s, 1H, Ar-H), 10.90 (s, 1H, OH). EIMS m/z, 321 (100) $[M]^+$, 267 (30), 213 (29), 198 (23), 107 (27), , 77 (25), 64 (14).

1-ethyl-6-hydroxy-5-((4-methoxyphenyl) diazenyl)-4-methyl-2-oxo-1,2-dihydropyridine-3-carbonitrile (7i)

Violet solid, yield = 55%, m.p. = 228-230°C. IR: 3413(OH), 3141(CH=), 2216 (CN), 1675 (C=O) cm^{-1}. ^1H NMR: 1.53 (t, 3H, CH_3), 2.49 (s, 3H, CH_3-pyridone), 3.62 (s, 2H, CH_2), 7.22-7.53 (d.d, 2H, Ar-H), 7.69-8.17 (d.d, 2H, Ar-H), 10.56 (s, 1H, OH). EIMS m/z, 321 (11) $[M]^+$, 267 (48), 213 (17), 198 (39), 107 (100) , 77 (71), 64 (33).

1-ethyl-6-hydroxy-4-methyl-5-((2-nitrophenyl) diazenyl)-2-oxo-1,2-dihydropyridine-3-carbonitrile (10a)

Orange powder; yield = 68%; m.p. = 210-212°C, IR: 3511(OH), 3126(CH=), 2214 (CN), 1651 (C=O) cm^{-1}. ^1H NMR: 1.56 (t, 3H, CH_3),

2.37 (s, 3H, CH_3-pyridone), 3.28 (q, 2H, CH_2), 7.24-8.27 (m, 4H, Ar-H), 11.61 (s, 1H, OH). EIMS m/z, 341 (11) $[M]^+$, 326 (12), 293 (17), 267 (09), 138 (16) , 91 (11), 65 (05).

1-ethyl-6-hydroxy-4-methyl-5-((3-nitrophenyl) diazenyl-2-oxo-1,2-dihydropyridine-3-carbonitrile (10b)

Yellow solid, yield = 63%, m.p. = 292-294°C, lit. IR: 3486(OH), 3167(CH=), 2211(CN), 1648 (C=O) cm^{-1}. ^1H NMR: 1.19 (t, 3H, CH_3), 2.52 (s, 3H, CH_3-pyridone), 3.61 (q, 2H, CH_2), 7.07-7.73 (m, 3H, Ar-H), 8.32 (s, 1H, Ar-H), 11.23 (s, 1H, OH). EIMS m/z, 341 (100) $[M]^+$, 326 (37), 293 (18), 267 (12), 138 (29) , 91 (68), 65 (18).

1-ethyl-6-hydroxy-4-methyl-5-((4-nitrophenyl) diazenyl-2-oxo-1,2-dihydropyridine-3-carbonitrile (10c)

Yellow solid, yield = 59%, m.p. = 237-139°C. IR: 3511(OH), 3136(CH=), 2214 (CN), 1657 (C=O) cm^{-1}. ^1H NMR: 1.27 (t, 3H, CH_3), 2.44 (s, 3H, CH_3-pyridone), 3.35 (q, 2H, CH2), 7.35-7.63 (d.d, 2H, Ar-H), 7.86-8.09 (d.d, 2H, Ar-H), 10.77 (s, 1H, OH). EIMS m/z, 341 (66) $[M]^+$, 326 (49), 293 (53), 267 (28), 138 (64) , 91 (100), 65 (73).

1,4-diethyl-6-hydroxy-5-((2-hydroxyphenyl) diazenyl)-2-oxo-1,2-dihydropyridine-3-carbonitrile (10d)

Orange powder; yield = 55%; m.p. = 148-150°C, IR: 3470(OH), 3139(CH=), 2120 (CN), 1648 (C=O) cm^{-1}. ^1H NMR: 1.35 (t, 3H, CH_3), 2.54 (s, 3H, CH_3-pyridone), 3.12 (q, 2H, CH_2), 7.10-7.62 (m, 4H, Ar-H), 10.96 (s, 1H, OH). EIMS m/z, 313 (66) $[M]^{+1}$, 312 (100) $[M]^+$,273 (62), 220 (63), 190 (19), 131 (52) , 91 (38), 77 (56).

1,4-diethyl-6-hydroxy-5-((3-hydroxyphenyl) diazenyl)-2-oxo-1,2-dihydropyridine-3-carbonitrile (10e)

Brown solid, yield = 75%, m.p. = 207-209°C, lit. IR: 3521(OH), 3139(CH=), 2233(CN), 1641 (C=O) cm^{-1}. ^1H NMR: 1.16 (t, 3H, CH_3), 2.48 (s, 3H, CH_3-pyridone), 3.52 (q, 2H, CH_2), 7.14-7.37 (m, 3H, Ar-H), 7.72 (s, 1H, Ar-H), 11.13 (s, 1H, OH). EIMS m/z, 312 (47) $[M]^+$,281 (23), 266 (17), 253 (08), 128 (06) , 91 (81), 77 (100).

1,4-diethyl-6-hydroxy-5-((4-hydroxyphenyl) diazenyl)-2-oxo-1,2-dihydropyridine-3-carbonitrile (10f)

Yellow solid, yield = 67%, m.p. = 189-191°C. IR: 3492(OH), 3144(CH=), 2223(CN), 1659 (C=O) cm^{-1}. ^1H NMR: 1.23 (t, 3H, CH_3), 2.49 (s, 3H, CH_3-pyridone), 3.46 (q, 2H, CH_2), 7.09- 750(d.d, 2H, Ar-H), 7.69-7.84 (d.d, 2H, Ar-H), 11.17 (s, 1H, OH). EIMS m/z, 312 (100) $[M]^+$,281 (49), 266 (38), 253 (41), 128 (25) , 91 (19), 77 (34).

1,4-diethyl-6-hydroxy-5-((2-methoxyphenyl) diazenyl)-2-oxo-1,2-dihydropyridine-3-carbonitrile (10g)

Orange powder; yield = 63%; m.p. = 233-235°C, IR: 3413(OH), 3157(CH=), 2221 (CN), 1685 (C=O) cm^{-1}. ^1H NMR: 1.23 (t, 3H, CH_3), 2.43 (s, 3H, CH_3-pyridone), 3.40 (q, 2H, CH_2), 7.11-7.69 (m, 4H, Ar-H), 10.67 (s, 1H, OH). EIMS m/z, 330 (100) $[M]^+$,301 (82), 296 (19), 265 (28), 176 (42) , 91 (28), 77 (63).

1,4-diethyl-6-hydroxy-5-((3-methoxyphenyl) diazenyl)-2-oxo-1,2-dihydropyridine-3-carbonitrile (10h)

Yellow solid, yield = 74%, m.p. = 226-228°C. IR: 3492(OH), 3143(CH=), 2230(CN), 1637 (C=O) cm^{-1}. ^1H NMR: 1.51 (t, 3H, CH_3), 2.40(s, 3H, CH_3-pyridone), 3.29 (q, 2H, CH_2), 7.29-7.68 (m, 3H, Ar-H), 7.84 (s, 1H, Ar-H), 7.81 (s, 1H, Ar-H), 10.68 (s, 1H, OH). EIMS m/z, 330 (49) $[M]^+$,301 (29), 296 (52), 265 (06), 176 (17) , 91 (68), 77 (37).

1,4-diethyl-6-hydroxy-5-((4-methoxyphenyl) diazenyl)-2-oxo-1,2-dihydropyridine-3-carbonitrile (10i):

Yellow solid, yield = 55%, m.p. = 169-171°C. IR: 3502(OH), 3110(CH=), 2227 (CN), 1649 (C=O) cm^{-1}. ^1H NMR: 1.36 (t, 3H, CH_3), 2.44 (s, CH_3-pyridone), 3.09 (q, 2H, CH_2), 7.29- 758(d.d, 2H, Ar-H), 7.77-7.93 (d.d, 2H, Ar-H), 10.87 (s, 1H, OH). EIMS m/z, 330 (55) $[M]^+$,301 (67), 296 (88), 265 (100), 176 (36) , 91 (29), 77 (18).

Antioxidant assay

ABTS was obtained and L-ascorbic acid were purchased from Sigma Aldrich. To (25 mg/ml) of MnO_2 solution, 60 µM of $ABTS^{\bullet+}$ and 2 mL of each tested sample in the presence of "pH 7, 0.1 M" phosphate buffer solution. The solution was centrifuged and filtered. The absorbance of the appeared green-blue solution was measured at λ= 543 nm. Next, a solution of the tested sample (50 µL, 2 mM) in "phosphate buffer/ MeOH (1:1)" was added. The inhibition % was calculated from the measured absorbance for each tested sample. L- Ascorbic acid was used as a reference [16] and a blank sample was measured using "phosphate buffer/ MeOH (1:1)" instead of the tested sample without the addition of $ABTS^{\bullet+}$. The -ve control was prepared with the addition of phosphate

buffer/ MeOH (1:1) and $ABTS^{\bullet+}$. The % inhibition for each tested sample was calculated according to Eq. (2) [17].

Inhibition %= [A (control) – A (test) /A (control)] × 100 Eq. (2)

DNA damage experiment

To a mixture of 0.5 mg / mL of DNA, 5 mM magnesium chloride, 50 mM ferric chloride and 0.05 mg / mL bleomycin sulfate was prepared. The test sample was prepared in concentration "0.1 mg / mL" and added to the mixture. After incubation of the mixture for one hour at 37 °C, "0.05 mL" of EDTA "0.1 M" was added to terminate the reaction. In the next step, 0.5 mL of TBA "1% w / v" and 0.5 mL of HCl "25% v / v" were added for color development and the mixture was heated for 10 minutes at 80 °C and centrifuged. The amount of damage to the DNA was measured at λ = 540- 550 nm [18].

Cytotoxicity assay

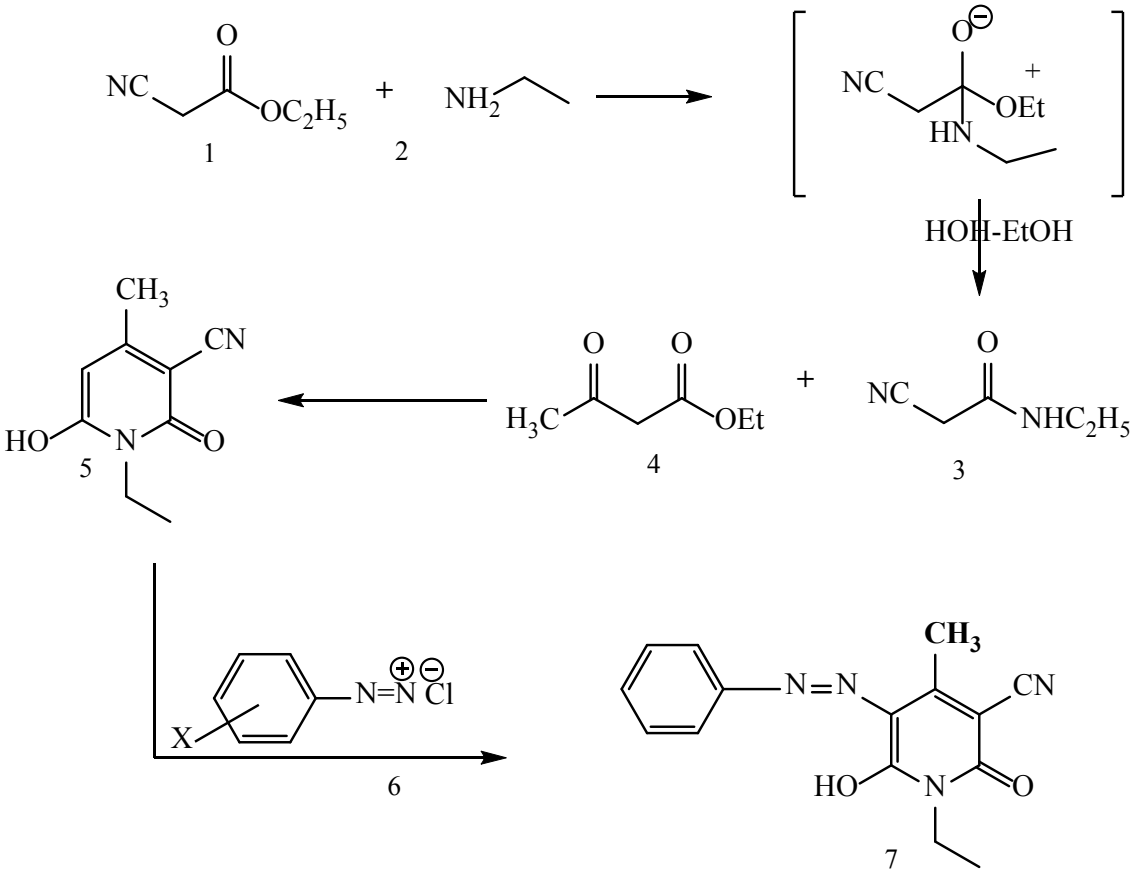
The tested samples were solvated in "50 mL DMSO" and diluted in sterile culture medium "0.4 mg / mL". The suspension were further diluted to "0.02 mg/ mL", in which both solutions were used in reserve to test samples at "100, 50, 20, 10, 5, 2 and 1 mg / mL" in microtiter plates and the further steps of the experiment were proceeded according to Patel et al. [19].

Results and Discussion

The strategic starting 1-ethyl-4-methyl-2,6-dioxopiperidine-3-carbonitrile 5 was prepared via reaction of ethyl cyanoacetate 1 with etylamine 2 to afford the corresponding 2-cyano-N-ethylacetamide 3 then intramolecular cyclization with ethyl acetoacetate 4 rendering to the beforehand conveyed methodology[20]. Diazotization by different aromatic Diazonium salts with the corresponding 1-ethyl-4-methyl-2,6-dioxopiperidine-3-carbonitrile 5 was demonstrated 1-ethyl-4-methyl-6-hydroxy-2-oxo-5-(azophenyl)-1,2,3,4-tetrahydropyridine-3-carbonitriles 7a-i (Scheme 1). The IR spectrum of 7a (as an example) exhibited absorption peak at 3413 cm^{-1} for the hydroxy group (OH), 3157 cm^{-1} for olifinic group, 2221 cm^{-1} for the nitrile (CN)group in the position 3 and 1645 cm^{-1} the carbonyl group (C=O). Meanwhile, the ^1H NMR spectrum of 3a displayed doublet signals at 1.43 for the methylene moiety in the ethyl group and supported by the triplet signal for the methyl moiety at 3.42 cm^{-1}. Where, the methyl group on the pyridone ring was confirmed via singlet signal, which appeared at 2.33 ppm. Furthermore, the aromatic protons were displayed multiplet signal at the range from 7.26-7.57 ppm revealed to four adjacent aromatic protons on the benzene ring. Moreover, the mass spectra for derivative 7a were assigned for the molecular ion peak at 327 with abundant ratio 65%. On the other hand, The IR spectrum of derivative 7e demonstrated absorption peak at 3402cm^{-1} for the hydroxy group (OH), 3111cm^{-1} for olifinic group, 2210 cm^{-1} for the nitrile (CN)group in the position 3 and 1661 cm^{-1} the carbonyl group (C=O). Meanwhile, the ^1H NMR spectrum of 7e showed doublet signals at 1.42ppm for the methylene moiety in the ethyl group and supported by the triplet signal for the methyl moiety at 3.26 ppm. Where, the methyl group on the pyridone ring was established via singlet signal, which appeared at 2.42 ppm. Likewise, the aromatic protons were exhibited multiplet signal at the range from 7.33-7.69 ppm revealed to three adjacent aromatic protons and one singlet signal at 7.85 for one aromatic protons on the benzene ring. Also, the mass spectra for derivative 7e were assigned for the molecular ion peak at 298 with abundant ratio 100%.

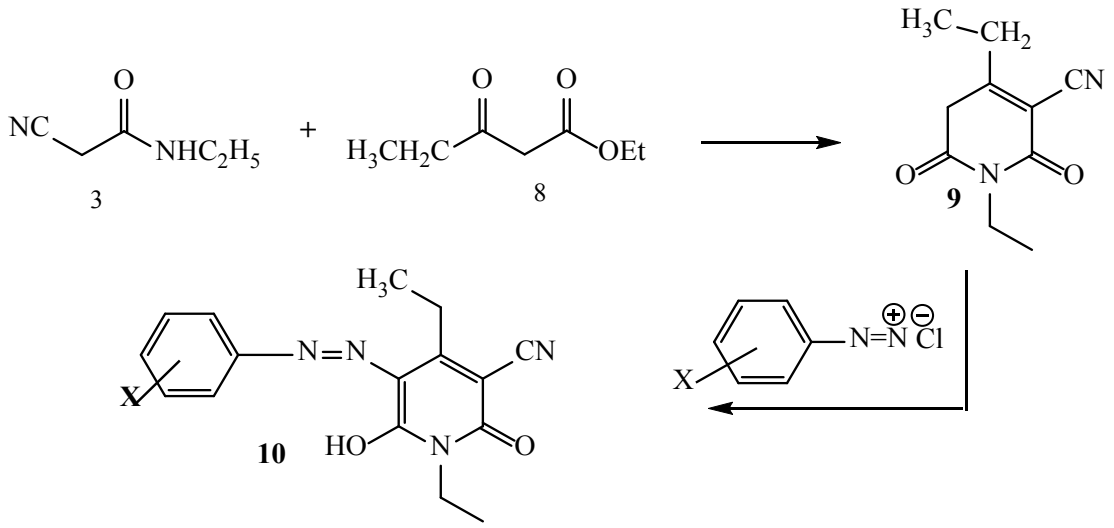
Meanwhile, the reaction of 2-cyano-N-ethylacetamide **3** with methyl porpionylacetate **8** was presented 1,4-diethyl-2,6-dioxo-1,2,5,6-tetrahydropyridine-3-carbonitrile **9** [20]. Furthermore, Diazotization of **8** with diverse aromatic Diazonium salts was afforded the corresponding 1,4-diethyl-6-hydroxy-2-oxo-5-(azophenyl)-1,2,3,4-tetrahydropyridine-3-carbonitriles **10** (Scheme 2).

Finally, derivative **10i** was revealed IR spectra at 3502cm^{-1} for the hydroxy group (OH), 3110cm^{-1} for olifinic group, 2227 cm^{-1} for the nitrile (CN)group in the position 3 and 1649 cm^{-1} the carbonyl group (C=O). Meanwhile, the ^1H NMR spectrum of **10i** presented doublet signals at 1.36 ppm for the methylene moiety in the ethyl group and supported by the triplet signal for the methyl moiety at 3.09 ppm. Where, the methyl group on the pyridone ring was established via singlet signal, which appeared at 2.40 ppm. Similarly, the aromatic protons were demonstrated two doublet of doublet signals at 7.29 and 7.77 ppm revealed to four aromatic, respectively. This is a good indication foe the presence of the methoxy group in the para position. In addition, the mass spectra for derivative **10i** were consigned for the molecular ion peak at 330 with abundant ratio 55%.



Where, a, x = o-NO_2, b, x = m-NO_2, c, x = p- NO_2
d, x =o-OH, e, x = m-OH, f, x = p- OH
g, x = o-OCH_3, h, x = m-OCH_3, i, x = p-OCH_3

Scheme 1.



Where, a, x = o-NO_2, b, x = m-NO_2, c, x = p- NO_2
d, x =o-OH, e, x = m-OH, f, x = p- OH
g, x = o-OCH_3, h, x = m-OCH_3, i, x = p-OCH_3

Scheme 2.

Biological evaluation

Antimicrobial activity

Antibacterial efficacy of the preps derivatives was evaluated through a wide spectrum using disc-agar procedure [21] as pronounced in the experimental section. Where, some positive controls were employed to evaluate the activity of the synthesized derivatives towards both of different bacterial strains, such as Chloramphenicol for Gram-positive bacteria and Cephalothin for Gram-negative bacteria.

Antimicrobial effecacies of the preps Aryl-azopyridone analogues were epitomised in Table 1. Initially, all of the synthesized methyl pyridine derivatives **7a-i** exhibited appropriate antibacterial efficacy. Where, the nitro derivatives in the different positions ortho, meta and para were displayed good activities toward gram positive bacteria as the following order *P*-> *O*- > *m*-. While, hydroxyl substituents were displayed respectable results than the methoxy substituents. All of these results were observed in correspondence to Chloramphenicol as a positive control. Meanwhile, the ethyl pyridone derivatives **10a-i** were exhibited the same or less reactivates towards the gram positive bacteria in comparison to the metyl derivatives, i.e the slightly increasing of the aliphatic chain on the pyridine ring demonstrated less reactivity toward the gram positive bacteria. Moreover, both of the methyl and the ethyl derivatives of pyridone ring were displayed less reactivates toward gram negative bacteria such as *S. typhimurium* and *E. coli* toward Cephalothin as a positive control (Table 1, Fig. 1). Likewise, derivatives **7c** presented better efficacy toward both of Gram-ve bacterial strains "*S. typhimurium* and *E. coli*" in appraisal to the outcomes of Cephalothin, The higher activity is owing to the incorporation of nitro moiety.

Meanwhile, antifungal activity for all derivatives with concentrations "0.5 and 1.0 mg/mL"was screened toward *"Candida albicans*, *Aspergillus fumigatus*" and Cycloheximide as a reference. All the synthesized derivatives revealed better inhibition toward *"C. albicans*". The achieved data for antifungal efficacy in Table 2 demonstrated effective antifungal activity toward fungal strains. Amongst the derivatives **7a-10a-i,** derivatives **7b, and I 10a** and, **10b** have persuasive activity toward *"C. albicans*". Consequantly, all the synthesized analogues exhibited low activities toward *A. fumigatus* in assessment to Cycloheximide as a reference drug (Table 2, Fig. 4). In the light of chemical structure and observed results, it had been seen that the existence of ethyl pyridone moieties **10a -f** increased their efficaciousness against fungous strains. Furthermore, the presence of various substituents like nitro and methoxy group on the phenyl rings were improved the bioavailability and lipophilicity.

MIC values for both of the synthesized methyl and ethyl pyridones derivatives were studied according to *"S. aureus*, *B. subtilis*, *S. typhi*, *E. coli* and *C. albicans*" relative to "Chloramphenicol, Cephalothin and Cycloheximide" as references (Table 3). Where, the comparable study for the effectiveness of the screened derivatives toward the bacterial (+ve) strain, it was observable that derivatives **7a-i** have a good activity on the bacterial growth with minimal concentrations "MIC = ≤54 - ≤73 µg/mL» against *S. aureus* and "MIC = ≤49 - ≤70 µg/mL» against *B. subtilis*. Whereas, derivative **7c** has the utmost attention on *S. typhimurium and E. coli* "MIC = ≤36 µg/mL», «MIC = ≤129 µg/mL», respectively, accorded to Cephalothin as standard. Likewise, derivative **7b** "MIC = ≤1219 µg/mL» and **7a** "MIC = ≤217 µg/mL» were represented decent antifungal efficacy against *C. albicans* with refereed to Cycloheximide outcome Table 3).

Antioxidant activity using ABTS method

All the prepared methyl and ethyl pyridone derivatives apportioned to calculate their antioxidant efficacy in contrast to "ascorbic acid" as a control over applying ABTS procedure [22]. Where, ABTS method is depending on detection and consumption of free radical from the medium. The stable free radical consumption was calculated *via* Eq. (1):

$$XH + Y^{\cdot} \longrightarrow X^{\cdot} + YH \quad \text{Eq. (1)}$$

Where, the descending in concentration of $X^{\cdot}$ was recognized the capability of antioxidant analogues to catch the free radicals. The free radical consumption was designated *via* vanishing of blue color from the medium. The variation of color was assessed spectrophotometry at λ= 470- 550nm.

TABLE 1. Inhibition zone for the synthesized compounds.

Organism	Gram-positive bacteria				Gram-negative bacteria			
	S. aureus		*B. subtilis*		*S. typhimurium*		*E. coli*	
	0.5 mg/mL	**1.0 mg/mL**	**0.5 mg/mL**	**1.0 mg/mL**	**0.5 mg/mL**	**1.0 mg/mL**	**0.5 mg/mL**	**1.0 mg/mL**
7a	53.38±0.31	56.79±0.13	38.36±0.52	46.85±0.53	30.72±0.37	34.85±0.07	24.83±0.54	43.75±0.41
7b	49.18±0.50	53.56±0.29	39.25±0.11	48.74±0.36	28.36±0.08	29.47±0.40	19.68±0.23	43.39±0.39
7c	54.63±0.38	57.46±0.33	40.19±0.02	47.73±0.29	31.62±0.43	39.89±0.51	27.36±0.12	44.31±0.13
7d	31.28±0.17	37.19±0.46	28.87±0.24	36.70±0.24	21.78±0.20	29.61±0.28	25.71±0.25	36.59±0.29
7e	28.14±0.44	36.32±0.25	27.54±0.10	34.53±0.37	19.76±0.39	30.54±0.34	26.22±0.41	36.71±0.04
7f	34.57±0.27	39.32±0.62	31.00±0.06	37.62±0.16	24.49±0.19	33.24±0.16	28.00±0.29	38.53±0.18
7g	46.61±0.22	48.78±0.23	39.68±0.29	38.26±0.09	24.54±0.28	30.36±0.04	26.52±0.41	32.84±0.34
7h	42.65±0.31	46.61±0.08	35.95±0.12	37.61±0.31	26.65±0.38	31.83±0.32	28.49±0.63	34.39±0.21
7i	47.00±0.34	51.38±0.14	42.55±0.01	39.44±0.28	29.24±0.03	34.65±0.13	29.51±0.08	36.82±0.19
10a	53.27±0.23	55.61±0.47	37.86±0.03	45.09±0.33	30.12±0.29	30.26±0.44	23.66±0.41	43.48±0.12
10b	48.67±0.04	52.62±0.11	38.67±0.45	47.82±0.46	27.26±0.17	27.47±0.04	18.25±0.19	43.83±0.33
10c	53.88±0.17	56.68±0.34	39.29±0.51	45.73±0.18	30.36±0.64	32.65±0.30	26.44±0.38	44.91±0.26
10d	29.56±0.48	35.01±0.38	27.86±0.26	35.78±0.02	22.64±0.10	28.12±0.39	24.32±0.23	36.46±0.19
10e	27.38±0.36	34.63±0.42	25.62±0.07	31.38±0.37	18.62±0.24	29.31±0.11	25.83±0.26	35.47±0.06
10f	32.81±0.42	38.76±0.19	28.35±0.37	36.40±0.15	23.63±0.22	33.89±0.38	26.92±0.27	37.94±0.27
10g	45.38±0.19	45.36±0.03	37.54±0.37	36.75±0.28	24.79±0.41	31.36±0.04	26.67±0.12	32.27±0.16
10h	42.18±0.24	43.78±0.47	33.95±0.12	34.61±0.31	26.48±0.09	33.83±0.32	27.71±0.06	34.06±0.27
10i	46.37±0.07	48.69±0.29	41.55±0.01	38.56±0.19	27.52±0.25	38.65±0.46	28.37±0.31	36.60±0.04
Chloramphenicol	24.33±0.26	35.63±0.13	28.27±0.23	31.55±0.23	–	–	–	–
Cephalothin	–	–	–	–	30.64±.05	39.46±0.23	33.65±0.04	43.66±01

Notes: Chloramphenicol and Cephalothin as positive control for Gram-positive and Gram-negative bacteria.

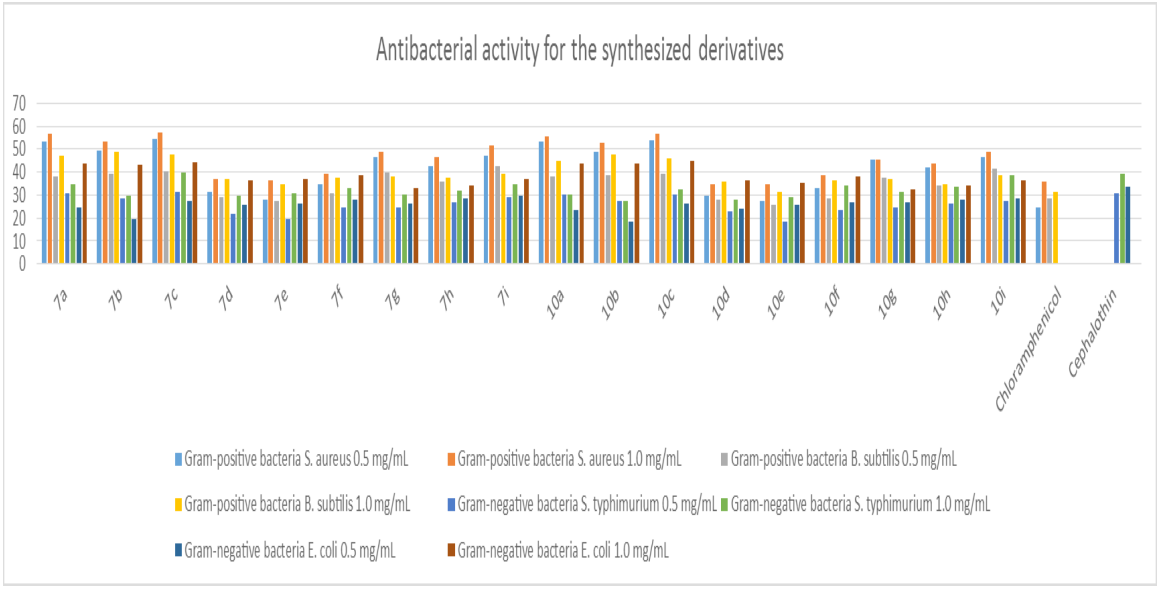


Fig. 1. Comparison of the antibacterial activity results of the tested compounds toward Gram (-ve) and (+ve) bacteria.

TABLE 2. Inhibitory zone for the synthesized compounds against various fungal strains (7a-c >7d-f > g-I m>o>p).

Sample	Yeasts and fungi			
	Candida albicans		*Aspergillus fumigatus*	
	0.5 mg/mL	1.0 mg/mL	0.5 mg/mL	1.0 mg/mL
7a	43.73±0.37	46.08±0.23	31.79±0.22	35.72±0.12
7b	45.82±0.19	47.64±0.31	34.45±0.36	39.19±0.32
7c	42.61±0.40	44.84±0. 20	28.76±0.19	33.43±0.21
7d	38.77±0.44	40.73±0.29	29.66±0.24	35.96±0.41
7e	41.89±0.29	43.89±0.17	32.88±0.19	34.69±0.01
7f	37.93±،،38	40.19±0.25	27.65±0.17	32.82±0.29
7g	34.66±0.12	33.68±0.31	26.49±0.36	33.65±0.03
7h	36.76±0.29	37.56±0.23	26.66±0.15	36.74±0.11
7i	33.87±0.15	35.42±0.33	24.51±0.26	29.35±0.36
10a	44.74±0.16	46.29±0.08	25.36±0.28	34.87±0.34
10b	46.69±0.38	49.13±0.25	27.93±،،04	34.66±0.02
10c	43.78±0.53	43.90±0.43	22.86±0.35	32.71±0.27
10d	39.45±0.05	41.65±0.36	27.54±0.33	34.30±0.06
10e	43.42±0.33	45.27±0.51	25.62±0.01	35.29±0.37
10f	38.46±0.42	41.23±0.11	23.18±0.47	33.34±0.14
10g	34.39±0.26	34.28±0.22	24.71±0.52	31.48±0.39
10h	37.59±0.16	39.18±0.03	27.74±0.46	35.22±0.25
10i	34.18±0.47	35.71±0.43	23.83±0.12	28.83±0.29
Cycloheximide	36.87±0.05	38.65±0.44	28.86±0.29	39.78±0.19

Notes: Cycloheximide as reference for fungal strain.

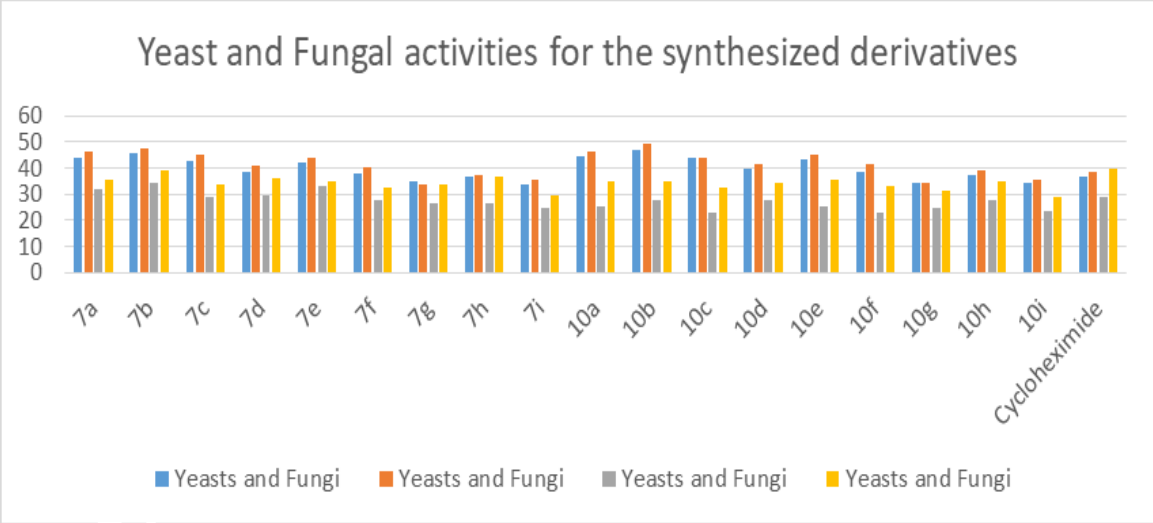


Fig. 2. The antifungal activity for the synthesized compounds against different fungal strains.

TABLE 3. Minimal inhibition concentrations for the synthesized compounds.

Sample	Gram-positive bacteria		Gram-negative bacteria		Yeasts and fungi	
	S. aureus	***B. subtilis***	***S. typhimurium***	***E. coli***	***C. albicans***	***A. fumigatus***
7a	≤71	≤70	≤35	≤127	≤217	–
7b	≤68	≤69	≤33	≤125	≤219	–
7c	≤73	≤72	≤36	≤129	≤216	–
7d	≤65	≤61	≤30	≤123	≤211	–
7e	≤63	≤59	≤28	≤121	≤214	–
7f	≤66	≤63	≤31	≤126	≤209	–
7g	≤57	≤53	≤24	≤115	≤197	–
7h	≤55	≤51	≤22	≤112	≤199	–
7i	≤60	≤56	≤27	≤118	≤196	–
10a	≤69	≤68	≤34	≤126	≤215	–
10b	≤67	≤67	≤31	≤124	≤216	–
10c	≤72	≤69	≤35	≤128	≤213	–
10d	≤64	≤57	≤29	≤122	≤208	–
10e	≤61	≤57	≤26	≤119	≤212	–
10f	≤65	≤61	≤29	≤123	≤207	–
10g	≤55	≤50	≤23	≤114	≤195	–
10h	≤54	≤49	≤19	≤109	≤197	–
10i	≤58	≤54	≤24	≤116	≤191	–
Chloramphenicol	≤ 42	≤ 51	–	–	–	–
Cephalothin	–	–	≤ 34	≤ 127	–	–
Cycloheximide	–	–	–	–	≤ 200	–

Notes: Chloramphenicol and Cephalothin as a positive control for of Gram+ve and Gram-ve bacteria ; Cycloheximide in the case of fungi "*C. albicans* (ATCC 10231) and *A. fumigatus*".

Table 4 signified the antioxidant effectiveness of the pyridone derivatives that contain diverse positions of nitro, hydroxyl and methoxy moieties. The achieved results were revealed that both of derivatives **7g-i** and **10g-i** were displayed strong effectiveness than the rest derivatives in association to the reference (Fig. 3). The achieved results may described by the existence of the methoxy groups on the phenyl ring. Likewise, derivatives **7d-f** and **10d-f** were exhibited good activity owing to it possess hydroxy groups in their structure. Furthermore, the structure activity relationship demonstrated the following consequences: (1) Both of derivatives **7g-i** and **10g-i** that possesses methoxy group demonstrated higher efficacy than the rest derivatives **1**. (2) The incorporation of electron releasing moieties as in derivatives **7g-i** and **10g-i** facilitate the free radical trapping. (3) The connection of nitro nucleus which act as electron attracting group like **7a-c** and **10a-c** derivatives were demonstrated less controller of antioxidant capacity than the rest derivatives for trapping the free radicals. (4) The ethyl pyridine derivatives **10a-I** were recorded a slightly effect than the methyl derivatives **10a-i**. Finally, the order of reactivity of the pyridones analogues toward the different cell lines is MCF-7> PC3> HePG2> HEp-2.

Bleomycin-dependent DNA damage

The synthesized 3-cyanopyridone analogues were screened toward bleomycin-dependent DNA damage. Table 5 represented the capability of the synthesized derivatives to defend DNA from damage *via* a convinced mechanism. Compounds **7g-i** and **10g-i** were exhibited enhanced efficacies to defend the DNA from destruction *via* bleomycin (Fig. 4).

TABLE 4. Antitumor activities for the synthesized derivatives.

Dye	Cytotoxicity IC_{50} (µg/L)			
	HePG2	**HEp-2**	**PC3**	**MCF-7**
7a	18.12±0.17	17.27±0.016	24.56±0.01	33.29±0.17
7b	19.37±0.31	18.78±0.25	27.64±0.43	34.20±0.31
7c	15.71±0.23	16.11±0.04	25.74±0.33	32.72±0.08
7d	19.68±0.43	20.27±0.12	28. 51±0.06	36.43±0.13
7e	23.66±0.12	22.11±0.09	29.23±0.09	38.42±0.04
7f	22.36±0.19	21.43 ± 0.44	31.32 ± 0.38	36.51±0.22
7g	27.55±0.04	26.52 ± 0.22	33.25± 0.43	42.37 ± 0.24
7h	29.57±0.41	28.36 ± 0.23	35.82 ± 0.17	44.83±0.19
7i	32.62±0.03	29.18±0.37	36.66 ± 0.21	48.09±0.34
10a	21.62±0.23	20.39±0.37	25.17 ± 0.51	37.267±0.28
10b	22.34 ± 0.05	23.45±0.15	29.84±0.32	38.72±0.06
10c	19.91±0.40	19.64±0.27	26.19±0.07	33.49±0.17
10d	24.97±0.11	20.76±0.23	29.14±0.26	38.11±0.34
10e	23.86±0.33	21.85±0.46	30.09±0.34	40.88±0.26
10f	25.45±0.28	21.69±0.17	32.42±0.22	39.62±0.08
10g	29.17±0.26	25.66±0.39	33.87±0.19	44.36±0.40
10h	30.74±0.09	28.97±0.40	34.73±0.51	45.91±0.05
10i	33.57±0.37	29.47±0.03	36.89±0.01	49.48±0.15
5-Fu[a]	5.0±0.32	5.0±0.67	5.0±0.19	10.0±0.26

Notes: a 5-Flurouracil (5-Fu) is used as a standard antibiotic for antitumor tests; IC_{50}: 1-10 very strong, 11-20 strong, 21-50 moderate, 51-100 weak and > 100 non-cytotoxic.

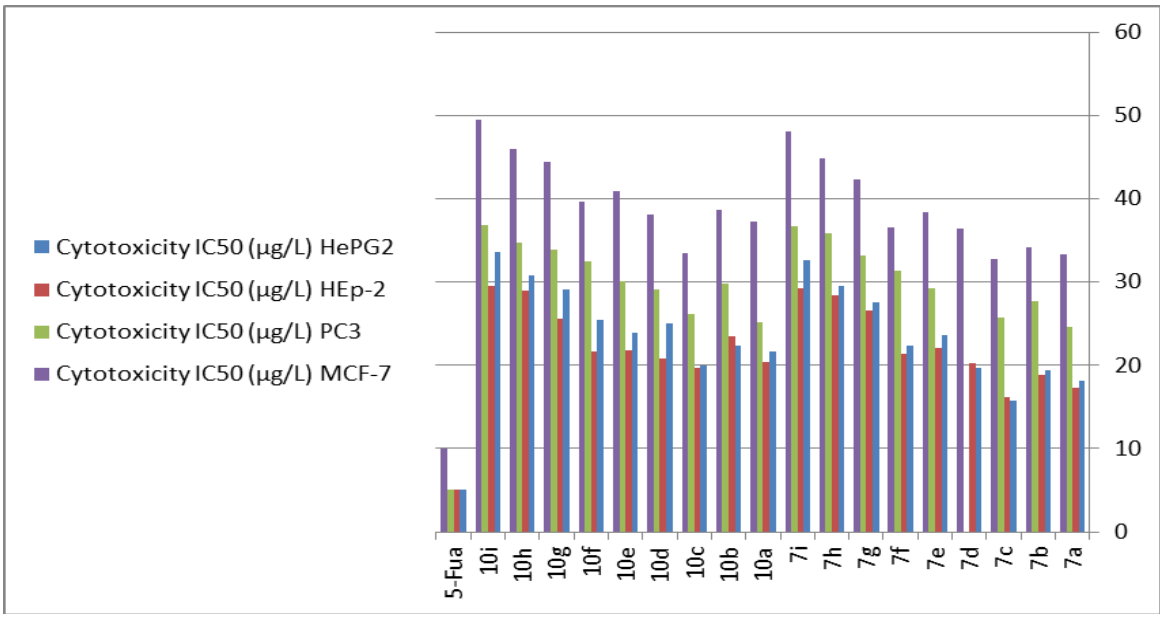


Fig. 3. Comparison of the results of cytotoxic activity (IC_{50}, µg/L) relative to the different tested cells.

TABLE 5. Antioxidant activities and Bleomycin-dependent DNA damage for compounds 4-7a-c .

Samples	Antioxidant activity (ABTS procedure)		Bleomycin-dependent DNA damage
	Absorbance	Inhibition (%)	
7a	0.155±0.30	93.02±0.16	0.149±0.52
7b	0.158±0.19	95.80±0.35	0.152±0.10
7c	0.147±0.16	89.61±0.07	0.139±0.13
7d	0.152±0.04	91.38±0.43	0.143±0.07
7e	0.161±0.36	98.13±0.22	0.155±0.12
7f	0.138±0.19	86.77±0.11	0.099 ± 0.35
7g	0.141±0.39	88.34±0.06	0.084 ± 0.21
7h	0.132±0.26	83.71±0.23	0.083 ± 0.12
7i	0.098±0.04	78.66±0.21	0.079±0.03
10a	0.153±0.29	92.65±0.16	0.147±0.41
10b	0.157±0.36	93.46±0.27	0.150±0.32
10c	0.099±0.15	79.26±0.34	0.080±0.27
10d	0.096±0.18	72.85±0.05	0.093 ± 0.18
10e	0.086±0.29	69.44±0.36	0.086 ± 0.27
10f	0.092±0.18	70.65±0.44	0.088 ± 0.05
10g	0.099±0.15	79.26±0.34	0.080±0.27
10h	0.131±0.18	83.71±0.21	0.083 ± 0.33
10i	0.097±0.23	78.66±0.27	0.079±0.26
Ascorbic Acid [a]	0.073±0.21	84.73±0.33	0.078±0.24

Notes: [a] Ascorbic acid is used as a standard for antioxidant tests.

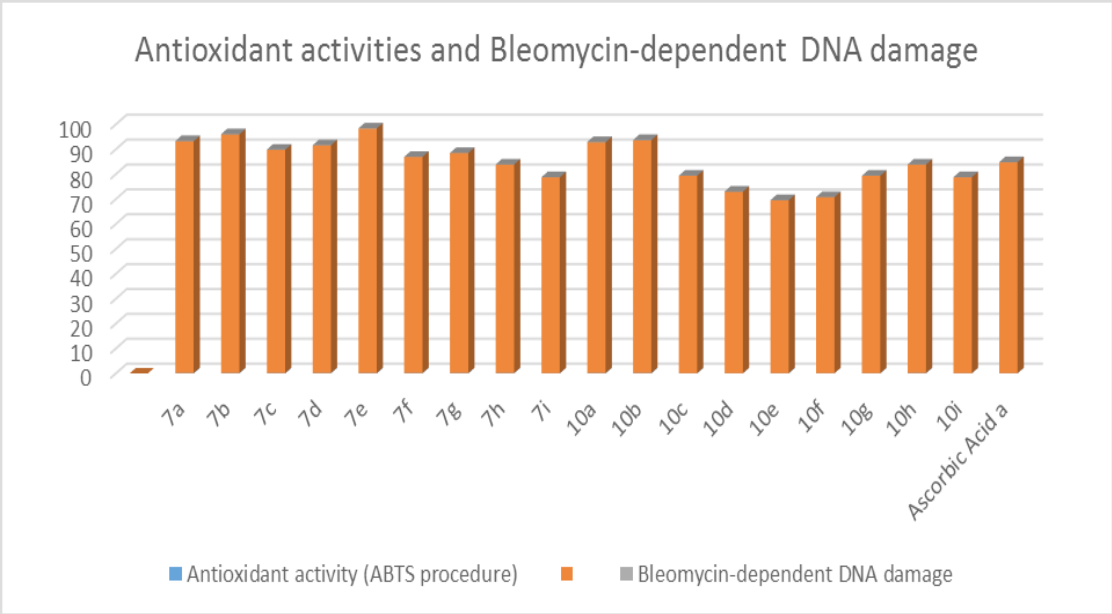


Fig. 4. Comparison of the antioxidant results "inhibition %" of the synthesized compounds relative to Vitamin C.

Conclusion

New arylazo derivatives clubbed with 3-cyanopyridones analogues were synthesized successfully through coupling of diverse Diazonium salts with 1-ethyl-4-methyl-2,6-dioxopiperidine-3-carbonitrile. Spectroscopic analyses such as IR, ^{1}HNMR and mass spectroscopy were utilized to reveal the accurate structures and, their antitumor and antioxidant efficacies were screened by diverse techniques. Meanwhile, biological results were demonstrated a respectable efficacy toward antioxidant and cytotoxic activity. Where, series of **derivatives 7a-i** were displayed a good effectiveness toward the bacterial growth with minimal concentrations "MIC = ≤54 - ≤73 µg/mL» against S. aureus and «MIC = ≤49 - ≤70 µg/mL» against B. subtilis. Whereas, derivative **7c** has the utmost attention on S. typhimurium and E. coli "MIC = ≤36 µg/mL», «MIC = ≤129 µg/mL», respectively, accorded to Cephalothin as standard. Likewise, derivative 7b «MIC = ≤1219 µg/mL» and 7a «MIC = ≤217 µg/mL» were represented decent antifungal efficacy against C. albicans with refereed to Cycloheximide as reference. **7g-i** and **10g-i** were exhibited strong activity than the rest derivatives in comparison to 5- fluorouracil as a reference. Meanwhile, derivatives **7g-i** and **10g-i** were exhibited a remarkable efficacies to defend the DNA from destruction *via* bleomycin. However, there is much degree in this promising moiety as various diverse sub- molecular targets is accessible for 3-cyanopyridones analogues and their methods for synthesis have taken full consideration for those interested in this field.

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