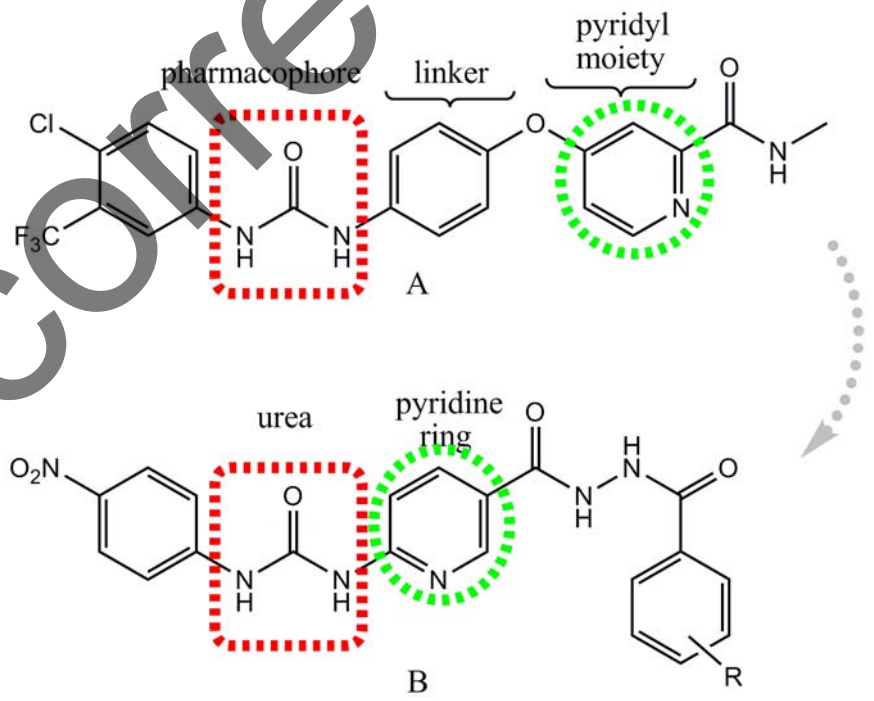
Design, synthesis and evaluation of biological activities of some new carbohydrazide and urea derivatives

INTRODUCTION

Cancer is one of the major causes of death worldwide. Development of identification and treatment has been important steps for cancer treatment. However, effective and selective treatment methods are insufficient against some types of cancer. The scientists continue their studies to find effective molecules for cancer treatment.[1,2] The compounds bearing nitrogen, sulfur and oxygen are significant role by hydrogen bonds with DNA.[3] Therefore heterocyclic compounds such as pyridine, pyrimidine showed anticancer,[4] antibacterial,[5] antifungal,[6] analgesic, anti-inflammatory activity.[7]

Sorafenib carrying pyridine ring and urea group was confirmed by Food and Drug Administration for treatment of renal cell carcinoma in 2005. Sorafenib which has broad-spectrum for anticancer therapy, inhibits some kinases such as vascular endothelial and platelet-derived growth factor. Therefore sorafenib could be used numerous types of cancer. Chemical structures of compounds were envisioned consisting of three parts: pyridyl moiety, a linker and urea functional group as a pharmacophore (Scheme 1).[8]



Scheme 1: The similarity of synthesized compounds (A) and sorafenib (B).

Wang et al.[9] studied new benzimidazole-2-urea derivatives decreasing the proliferation of some cancer cells (HeLa, K562, HepG2) and described that these molecules could be used tubulin inhibitors. In another study, Fortin et al.[10] discovered that compounds containing urea derivatives were more antiproliferative activity than amide groups from structure activity relationship. De et al.[11] synthesized N'-(2-(4-substitue)cyclopropanecarbonyl)isonicotinohydrazide containing carbohydrazide and pyridine ring and evaluated their cytotoxic activity against A549, PC3 and U373 cells.

Pyridine is an important ring system to have numerous biological activities. For example Kurumurthy et al.[12] selected pyridine derivatives to be able to get cytotoxic activity against THP1, U937, HL60 and B16-F10 cells.

Through the results to be obtained within the literature's data and pharmacophore analysis (scheme 1), target molecules which are carrying urea and carbohydrazide derivatives were synthesized from metyl-6-aminopyridine-3-carboxylate and evaluated their cytotoxic activity against some cancer cells (HCC1937, Capan1, MCF-7, HeLa and MRC5).

MATERIALS AND METHODS

Chemistry

All chemicals reagents and solvents were obtained from Sigma-Aldrich (St. Louis, MO, USA), and Merck (Darmstadt, Germany). The homogeneity and purity of the compounds were checked by thin layer chromatography (TLC), performed on commercially available silica gel (Kieselgel 60, F254) coated aluminum sheets (Merck) by using petroleum ether:ethyl acetate (10:90 v/v) as the solvent system. Visualization on TLC was done by both ultra-violet (UV) light (λ= 254 nm) and iodine indicator. Melting points were determined by Schmelzpunktbestimmer SMP II. IR spectra were recorded with a Shimadzu FTIR-8400S (Japan). ^{1}H-NMR spectra were recorded on a Bruker Avance 400 MHz (USA) in DMSO-d_6 using tetramethylsilane (TMS) as the internal reference. Chemical shifts (δ) were expressed in parts per million relative to TMS and the following abbreviations were used to describe the peak patterns when appropriate: s, (singlet); d, (doublet); t, (triplet); m, (multiplet); Elemental analysis (C,

H and N) was performed on a CHNS-Thermo Scientific Flash 2000 (Waltham, MA USA).

Synthesis of the urea derivatives

Methyl 6-aminopyridine-3-carboxylate was dissolved in acetone, at 80 °C. Then, a solution of the corresponding equimolar isocyanate in dry acetone was added as two parts, per 30 minutes. The reaction mixture was refluxed for 6 hours. The reaction was finalized by TLC control and left overnight. The precipitate was filtered off, dried and purified with acetone.[13]

Methyl 6-(3-(4-nitrophenyl)ureido)nicotinate (1)

Yellow solid; Yield: 70%; m.p. 237-239 °C; IR (ν_{max} cm^{-1}): 3365, 3211 (N-H), 3080 (=C-H stretching), 2983, 2843 (C-H), 1708 (urea C=O), 1604, 1562, 1506, 1491, 1411 (C=C, NO_2, N-H bending, C-N), 1273 (C-O), 839 (=C-H). ^{1}H-NMR (DMSO-d_6/TMS, 400 MHz, δ in ppm): 3.83 (s, 3H, -OCH_3), 7.69-8.84 (m, 7H, Ar-H), 9.99 (s, 1H, NH), 10.73 (s, 1H, NH). For $C_{14}H_{12}N_4O_5$ (M.W.: 316.27 g/mol) calculated (%): C:53.17, H:3.82, N:17.71. Found: C:54.46, H:3.66, N:17.95.

Synthesis of the hydrazide derivatives

Methyl 6-(3-(4-nitrophenyl)ureido)nicotinate was dissolved in ethanol on a magnetic stirrer. Then, hydrazine monohydrate (1 mL) was added. The reaction mixture was refluxed for 6 hours. The mixture was filtered and washed with methanol.[14]

1-(5-(Hydrazinecarbonyl)pyridin-2-yl)-3-(4-nitrophenyl)urea (2)

Yellow solid; Yield: 65%; m.p. 274-275 °C; IR (ν_{max} cm^{-1}): 3338, 3221 (N-H), 3078 (=C-H stretching), 1712 (C=O urea), 1678 (C=O hydrazide), 1620 (C=N), 1562, 1510, 1491, 1431 (C=C, NO_2, N-H, C-N), 815 (=C-H). ^{1}H-NMR (DMSO-d_6/TMS, 400 MHz, δ in ppm): 4.48 (s, 2H, NH_2), 7.71-8.87 (m, 7H, Ar-H), 9.93 (s, 1H, NH), 10.04(s, 1H, NH), 10.78 (s, 1H, NH). $C_{13}H_{12}N_6O_4$ (M.W.: 316.27 g/mol) calculated (%): C:49.37, H:3.82, N:26.57. Found: C:51.05, H:3.97, N:25.25.

Synthesis of the carbohydrazide derivatives

To a solution of hydrazide (1 mmol) **(2)** and trimethylamine (2 mmol) in dry CH_2Cl_2 (5 mL), a solution of previously prepared benzoyl chloride (1 mmol) was added dropwise at room temperature. The reaction mixture was stirred on a magnetic stirrer for 3 hours. Then the precipitate was washed with distilled water and filtered. The purity of compounds was checked with thin layer chromatography (TLC).[15]

1-(5-(2-(4-Fluorobenzoyl)hydrazinecarbonyl)pyridin-2-yl)-3-(4-nitrophenyl)urea (3a)

Yellow solid; Yield: 70%; m.p. 257-259 °C; IR (ν_{max} cm^{-1}): 3304, 3207, 3122 (N-H), 3080 (=C-H stretching), 1712 (C=O), 1612, 1562, 1508, 1492 (C=C, NO_2, N-H, C-N), 842 (=C-H). ^{1}H-NMR (DMSO-d_6/TMS, 400 MHz, δ in ppm): 7.67-8.84 (m, 11H, Ar-H), 9.69 (s, 1H, NH), 10.04(s, 1H, NH), 10.84 (s, 2H, NH). $C_{20}H_{15}FN_6O_5$ (M.W.: 438.37 g/mol) calculated (%): C:54.80, H:3.45, N:19.17. Found: C:55.24, H:3.22, N:18.65.

1-(5-(2-(4-Chlorobenzoyl)hydrazinecarbonyl)pyridin-2-yl)-3-(4-nitrophenyl)urea (3b)

Yellow solid; Yield: 75%; m.p. 202-203 °C; IR (ν_{max} cm^{-1}): 3304, 3207, 3122 (N-H), 3080 (=C-H stretching), 1712 (C=O), 1612, 1562, 1508, 1492, 1431 (C=C, NO_2, N-H, C-N), 842 (=C-H). ^{1}H-NMR (DMSO-d_6/TMS, 400 MHz, δ in ppm): 7.71-8.85 (m, 11H, Ar-H), 9.98 (s, 2H, NH), 10.73 (s, 2H, NH). $C_{20}H_{15}ClN_6O_5$ (M.W.: 454.82 g/mol) calculated (%): C:52.81, H:3.32, N:18.48. Found: C:52.75, H:3.48, N:18.44.

1-(5-(2-(4-Nitrobenzoyl)hydrazinecarbonyl)pyridin-2-yl)-3-(4-nitrophenyl)urea (3c)

Yellow solid; Yield: 80%; m.p. 273-275 °C; IR (ν_{max} cm^{-1}): 3215, 3124 (N-H), 3082 (=C-H stretching), 1714 (C=O), 1612, 1564, 1510, 1492 (C=C, NO_2, N-H, C-N), 846 (=C-H). ^{1}H-NMR (DMSO-d_6/TMS, 400 MHz, δ in ppm): 7.68-8.85 (m, 11H, Ar-H), 9.62 (s, 1H, NH), 10.00 (s, 1H, NH), 10.74 (s, 2H, NH). $C_{20}H_{15}N_7O_7$ (M.W.: 465.38 g/mol) calculated (%): C:51.62, H:3.25, N:21.07. Found: C:52.33, H:3.12, N:20.66.

1-(5-(2-(2,6-Dichlorobenzoyl)hydrazinecarbonyl)pyridin-2-yl)-3-(4-nitrophenyl)urea (3d)

Yellow solid; Yield: 70%; m.p. 258-260 °C; IR (ν_{max} cm^{-1}): 3369, 3215, 3122 (N-H), 3084 (=C-H), 1712 (C=O), 1612, 1566, 1492, 1481 (C=C, NO_2, N-H, C-N), 1031 (C-Cl), 844 (=C-H). ^{1}H-NMR (DMSO-d_6/TMS, 400 MHz, δ in ppm): 7.78-8.68 (m, 10H, Ar-H), 9.43 (s, 1H, NH), 10.00 (s, 1H, NH), 10.75 (s, 2H, NH). $C_{20}H_{14}Cl_2N_6O_5$ (M.W.: 489.27 g/mol) calculated (%): C:49.10, H:2.88, N:17.18. Found: C:50.35, H:2.97, N:16.85.

1-(5-(2-(4-Methylbenzoyl)hydrazinecarbonyl)pyridin-2-yl)-3-(4-nitrophenyl)urea (3e)

Yellow solid; Yield: 65%; m.p. 275-277 °C; IR (ν_{max} cm^{-1}): 3369, 3205, 3122 (N-H), 3049 (=C-H stretching), 2987 (C-H), 1712 (C=O), 1610, 1562, 1508, 1492, 1431 (C=C, NO_2, N-H, C-N), 844 (=C-H). ^{1}H-NMR (DMSO-d_6/TMS, 400 MHz, δ in ppm): 2.39 (s, 3H, -CH_3), 7.47-8.81 (m, 11H, Ar-H), 9.45 (s, 1H, NH), 10.06 (s, 1H, NH), 10.77 (s, 2H, NH). $C_{21}H_{18}N_6O_5$ (M.W.: 434.41 g/mol) calculated (%): C:58.06, H:4.98, N:19.15. Found: C:57.23, H:5.05, N:18.56.

1-(5-(2-(4-Bromobenzoyl)hydrazinecarbonyl)pyridin-2-yl)-3-(4-nitrophenyl)urea (3f)

Yellow solid; Yield: 75%; m.p. 240-241 °C; IR (ν_{max} cm^{-1}): 3207, 3122 (N-H), 3082 (=C-H stretching), 1712 (C=O), 1610, 1562, 1508, 1491, 1431 (C=C, NO_2, N-H, C-N), 844 (=C-H). ^{1}H-NMR (DMSO-d_6/TMS, 400 MHz, δ in ppm): 7.45-8.81 (m, 11H, Ar-H), 9.36 (s, 1H, NH), 10.20 (s, 1H, NH), 10.84 (s, 2H, NH). $C_{20}H_{15}BrN_6O_5$ (M.W.: 499.27 g/mol) calculated (%): C:48.11, H:3.03, N:16.83 Found: C:47.48, H:3.25, N:16.92.

1-(4-nitrophenyl)-3-(5-(2-(4-(trifluoromethyl)benzoyl)hydrazinecarbonyl)pyridin-2-yl)urea (3g)

Yellow solid; Yield: 70%; m.p. 299-300 °C; IR (ν_{max} cm^{-1}): 3333, 3271, 3213 (N-H), 3080 (=C-H stretching), 1712 (C=O), 1680 (C=O hydrazide), 1614 (C=N), 1564, 1489, 1431, 1411 (C=C, NO_2, N-H, C-N), 1261 (C-F), 890 (=C-H). ^{1}H-NMR (DMSO-d_6/TMS, 400 MHz, δ in ppm): 7.64-8.84 (m, 11H, Ar-H), 9.75 (s, 1H, NH), 9.97 (s, 1H, NH), 10.72 (s, 2H, NH). $C_{21}H_{15}F_3N_6O_5$ (M.W.: 488.38 g/mol) calculated (%): C:51.65, H:3.10, N:17.21 Found: C:51.48, H:3.25, N:16.92.

1-(5-(2-(4-methoxybenzoyl)hydrazinecarbonyl)pyridin-2-yl)-3-(4-nitrophenyl)urea (3h)

Yellow solid; Yield: 75%; m.p. 222-223 °C; IR (ν_{max} cm^{-1}): 3275, 3171, 3117 (N-H), 3080 (=C-H stretching), 2978 (C-H asymmetric stretching), 2841 (C-H symmetric stretching), 1703 (C=O), 1662 (C=O hydrazide), 1633 (C=N), 1537, 1506, 1499, 1471 (C=C, NO_2, N-H, C-N), 1327 (C-O), 824 (=C-H). ^{1}H-NMR (DMSO-d_6/TMS, 400 MHz, δ in ppm): 3.84 (s, 3H, OCH_3), 7.05-8.84 (m, 11H, Ar-H), 9.79 (2s, 1H, NH), 10.45 (2s, 1H, NH), 10.72 (s, 2H, NH). $C_{21}H_{18}N_6O_6$ (M.W.: 450.40 g/mol) calculated (%): C:56.00, H:4.03, N:18.66 Found: C:56.48, H:3.25, N:18.92.

1-(5-(2-benzoylhydrazinecarbonyl)pyridin-2-yl)-3-(4-nitrophenyl)urea (3i)

Yellow solid; Yield: 70%; m.p. 203-204 °C; IR (ν_{max} cm^{-1}): 3271, 3213, 3124 (N-H), 3045 (=C-H stretching), 1712 (C=O), 1666 (C=O hydrazide), 1641 (C=N), 1602, 1562, 1506, 1489, 1431 (C=C, NO_2, N-H, C-N), 846 (=C-H). ^{1}H-NMR (DMSO-d_6/TMS, 400 MHz, δ in ppm): 7.45-8.84 (m, 11H, Ar-H), 9.80 (2s, 1H, NH), 10.68 (2s, 1H, NH), 11.48 (s, 2H, NH). $C_{20}H_{16}N_6O_5$ (M.W.: 420.38 g/mol) calculated (%): C:57,14 H:3.84, N:19.99 Found: C:57.48, H:3.65, N:19.92.

1-(4-nitrophenyl)-3-(5-(2-(4-(trifluoromethylthio)benzoyl)hydrazinecarbonyl)pyridin-2-yl)urea (3j)

Yellow solid; Yield: 65%; m.p. 277-278 °C; IR (ν_{max} cm^{-1}): 3333, 3221 (N-H), 3080 (=C-H stretching), 1714 (C=O urea), 1680 (C=O hydrazide), 1641 (C=N), 1564, 1510, 1492 (C=C, NO_2, N-H, C-N), 844 (=C-H). ^{1}H-NMR (DMSO-d_6/TMS, 400 MHz, δ in ppm): 7.63-8.83 (m, 11H, Ar-H), 9.79 (2s, 1H, NH), 9.97 (s, 1H, NH), 10.72 (s, 2H, NH). $C_{21}H_{15}F_3N_6O_5S$ (M.W.: 520.44 g/mol) calculated (%): C:48.46, H:2.91, N:16.15 Found: C:48.76, H:3.05, N:16.92.

Biology

Cell culture

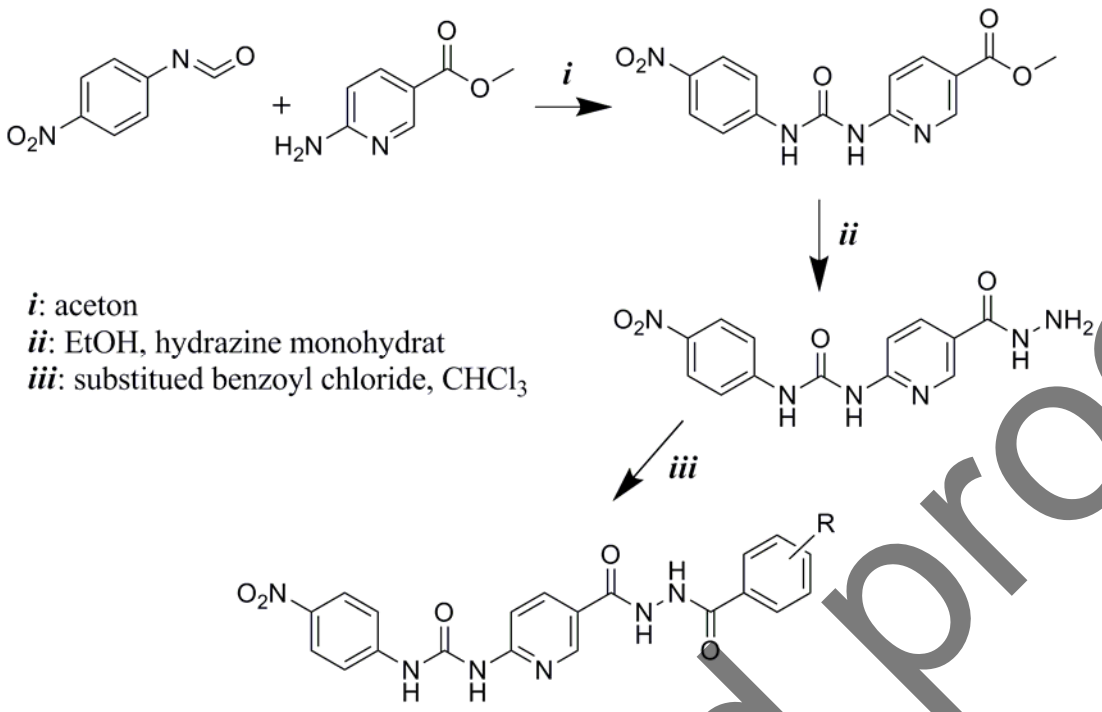
Human pancreatic adenocarcinoma Capan1 cell line, human cervix carcinoma HeLa cell line, human lung fibroblasts MRC5 cell line, human breast adenocarcinoma MCF7 and HCC1937 cell lines were obtained from American Type Culture Collection (ATCC, Bethesda) maintained as exponentially growing monolayers by culturing according to the supplier's instructions in a humidifier incubator at 37 °C supplied with 5% CO_2. All cell culture reagents were purchased from Biological Industries (Israel).

Cytotoxicity test

The cytotoxic potencies of test compounds were determined by using WST1 Cell Protliferation Assay (Roche) according to the manufacturer's instructions. All tested compounds were dissolved in DMSO. Cells were seeded into 96-well plate at a density of 5000 cells/well for HeLa, MCF7 and HCC1937, 7500 cells/well for Capan1 and 10000 cells per well for MRC5. Next day, cells were treated with compounds with the final concentrations of 1, 2, 5, 10, 25 μM and incubated for 48 hours at conventional cell culture conditions. While DMSO was used negative solvent control, doxorubicin was included in the study as positive cytotoxic control compound. The ratio of surviving cells after compound treatment was determined by the colorimetric WST-1 assay (Roche) as indicated in the protocol provided by manufacturer. The absorbance was measured by using Varioscan microplate reader (Thermo) at 450 nm and a 620-nm reference filter. To determine the IC_{50} values, a sigmoid-dose response curve was fitted to the data using nonlinear regression in GraphPad Prism 5 software.

RESULTS AND DISCUSSION

The synthetic route to the target compounds is outlined in Scheme 2. The structures of the compounds (**1, 2, 3a-3j**) were confirmed by IR, ^{1}H-NMR and elemental analysis. IR spectra of the compounds (**1, 2, 3a-3j**) afforded N-H stretching (3115-3369) bands. IR spectra of all compounds (**1, 2, 3a-3j**) were described C-H stretching (3045-3082), urea and carbohydrazide C=O stretching (1662-1714) bands, aromatic rings C=C stretching and NO_2 stretching (1411-1604) bands. The NH protons of carbohydrazide and urea groups resonated as two different singlet peak at 9.36-11.48 ppm. The aromatic protons displayed a multiplet at 7.05-8.85 ppm. The elemental analysis of compounds was in agreement with the proposed structures of the compounds.



Scheme 2: The synthesis route of the compounds.

It is known that some of PARP inhibitors are highly selective promising agents against cancer cells with homologous recombination (HR) DNA repair pathway deficiencies such as those harboring mutations on tumor suppressors BRCA1 or BRCA2 via generation of chromatid breaks, cell cycle arrest and apoptosis.[16,17] Therefore, we included HCC1937 and Capan1, which are defective in BRCA1 and BRCA2, respectively. We aimed to compare the cytotoxicity of tested compounds on these cell lines to their activities on HR-proficient cancer cell lines (HeLa and MCF7) and also non-tumoral MRC5 fibroblast cell line.

When the substitution pattern at the phenyl ring were determined, effect of electron donor and electron acceptor groups on activity was considered. Therefore methyl, methoxy and halogens like F, Cl, Br were selected as electron donor and nitro group was selected as electron acceptor. Our data suggests that only 3a having fluoro, 3b having chloro, 3c having nitro and 3d having 2,6 dichloro substituents have cytotoxic activities at the tested concentrations. The IC_{50} values of these compounds are given in Table 1. The compounds **3e-3j** showed less cytotoxic activity on all cancer cells compared to **3a-3d**. However, our results suggest that compounds do not possess any selectivity toward HR defective Capan1 and HCC1937 cells harboring BRCA mutations compared to MCF7 and HeLa cells with intact HR pathway.

Table 1: Cytotoxic activity of compounds.

	IC_{50} (µM)				
Compounds	**HCC1937**	**Capan1**	**MCF7**	**HeLa**	**MRC5**
3a	7.6 ± 0.09	7.4 ± 0.62	7.3 ± 0.86	6.6 ± 0.53	15.4 ± 1.42
3b	8.9 ± 0.07	8.4 ± 0.26	9.2 ± 0.49	11.7 ± 1.02	19.6 ± 1.06
3c	10.4 ± 0.6	9.3 ± 0.79	9.6 ± 0.56	9.8. ± 1.86	18.3 ± 1.54
3d	7.8 ± 0.82	7.3 ± 0.75	7.5 ± 0.13	7.9 ± 1.68	17.4 ± 1.12
3e	> 25	> 25	> 25	> 25	> 25
3f	> 25	> 25	> 25	> 25	> 25
3g	> 25	> 25	> 25	> 25	> 25
3h	> 25	> 25	> 25	> 25	> 25
3i	> 25	> 25	> 25	> 25	> 25
3j	> 25	> 25	> 25	> 25	> 25
Doxorubicin	1.05 ± 0.07	0.98 ± 0.08	1.13 ± 0.12	0.73 ± 0.13	7.2 ± 1.37

CONCLUSION

In the present paper, we reported the synthesis of some new urea and carbohydrazide derivatives from methyl 6-aminopyridine-3-carboxylate. Synthesized compounds have been evaluated their cytotoxic activity. Our data indicate that **3a-3d** are more selective to cancer cells compared to nontumoral fibroblasts, however these compounds are not more potent on HR defective cells with BRCA mutants.

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