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ORIGINAL ARTICLE

Design, Synthesis and Evaluation of the Biological Activities of Some New Carbohydrazide and Urea Derivatives

Bazı Yeni Karbohidrazit ve Üre Türevlerinin Tasarımı, Sentezi ve Biyolojik Aktivitelerinin Değerlendirilmesi

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ABSTRACT

Objectives: Urea and carbohydrazide derivatives are important compounds exhibiting cytotoxic activities. In this study, a series of new urea and carbohydrazide derivatives containing an pyridine ring were synthesized and evaluated for cytotoxic activity.

Materials and Methods: The proposed structures of the synthesized compounds were confirmed using elemental analysis, IR, and ^{1}H-NMR spectroscopic techniques. The cytotoxic potencies of synthesized compounds were determined using a 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide assay (MTT) on *BRCA* mutant-carrying HCC1937 and Capan-1 cell lines, as well as on MCF7, HeLa, and MRC5 cells.

Results: 3a, 3b, 3c and 3d showed cytotoxic activity against all cancer cell lines.

Conclusion: Our data indicate that compounds 3a-d are more selective to cancer cells compared with nontumoral fibroblasts; however, these compounds are not more potent on HR defective cells with *BRCA* mutants.

Key words: Carbohydrazide, urea, cytotoxic activity

ÖZ

Amaç: Üre ve karbohidrazit türevleri önemli sitotoksik etkinlik gösteren bileşiklerdir. Bu çalışmada, bir yeni seri piridin halkası taşıyan üre ve karbohidrazit türevleri sentezlenmiş ve sitotoksik etkileri araştırılmıştır.

Gereç ve Yöntemler: Sentezlenen bileşiklerin önerilen yapıları elemental analiz, IR, ^{1}H-NMR spektroskopik yöntemleriyle doğrulanmıştır. Sentezlenen bileşiklerin sitotoksik etki güçleri, 3-(4,5-dimetiltiyazol-2-il)-2,5-difeniltetrazolyum bromür (MTT) kullanılarak *BRCA* mutasyonu taşıyan HCC1937 ve Capan-1 hücre hatları olan MCF7, HeLa ve MRC5 hücreleri üzerinde tespit edilmiştir.

Bulgular: 3a, 3b, 3c ve 3d tüm kanser hücre hatlarına karşı sitotoksik etki göstermiştir.

Sonuç: Verilerimiz, 3a-d bileşiklerinin kanser hücrelerinde tümöral olmayan fibroblastlarla kıyaslandığında daha seçici olduğunu, ancak bu bileşiklerin *BRCA1* mutant homolog rekombinasyonal (HR) DNA onarımı hatalı hücrelerde ise daha fazla etkili olmadığını göstermektedir.

Anahtar kelimeler: Karbohidrazit, üre, sitotoksik aktivite

INTRODUCTION

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

Sorafenib, which carries a pyridine ring and urea group was confirmed by the United States Food and Drug Administration for the treatment of renal cell carcinoma in 2005. Sorafenib, which

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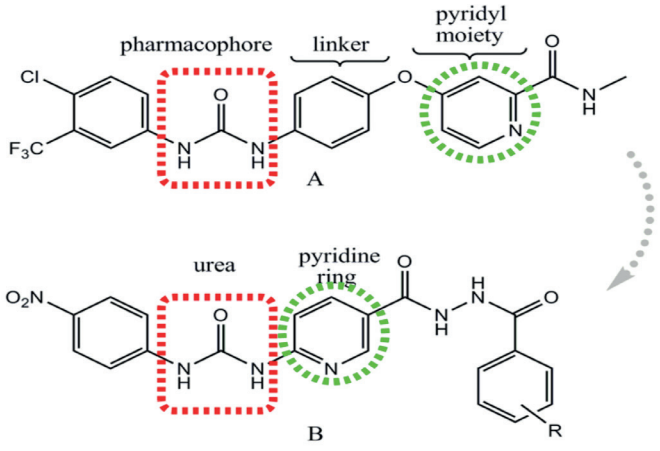
has a broad spectrum for anticancer therapy, inhibits some kinases such as vascular endothelial and platelet-derived growth factor. Therefore, sorafenib could be used with numerous types of cancer. Chemical structures of compounds were envisioned consisting of three parts: pyridyl moiety, a linker, and urea functional group as a pharmacophore (Scheme 1).[8]

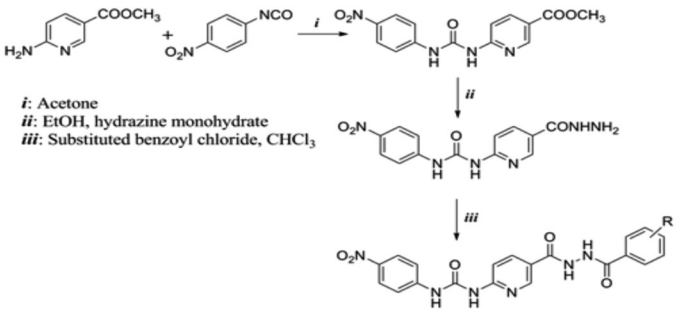


Scheme 1. The similarity of synthesized compounds (A) and sorafenib (B)

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

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

Using results obtained in the literature and pharmacophore analysis (Scheme 1), target molecules carrying urea and carbohydrazide derivatives were synthesized from methyl 6-aminopyridine-3-carboxylate and evaluated their cytotoxic activity against cancer cells (HCC1937, Capan1, MCF-7, HeLa and MRC5).



Scheme 2. The synthesis route of the compounds

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 using 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 performed using ultraviolet light (λ=254 nm) and an iodine indicator. Melting points (MP) were determined using a 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 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, every 30 minutes. The reaction mixture was refluxed for 6 hours. The reaction was finalized by checking with TLC 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%; MP: 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%; MP: 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 triethylamine (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. The precipitate was then washed with distilled water and filtered. The purity of compounds was checked with TLC.[15]

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

Yellow solid; Yield: 70%; MP: 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%; MP: 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%; MP: 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%; MP: 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%; MP: 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%; MP: 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%; MP: 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%; MP: 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%; MP: 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%; MP: 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

A 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 line were obtained from American Type Culture Collection (Bethesda), and 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 the test compounds were determined using a WST1 Cell Protliferation Assay (Roche) according to the manufacturer's instructions. All tested compounds were dissolved in DMSO. Cells were seeded into a 96-well plate at a density of 5000 cells/well for HeLa, MCF7 and HCC1937, 7500 cells/well for Capan1 and 10,000 cells per well for MRC5. The 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. DMSO was used a negative solvent control, and doxorubicin was included in the study as a positive cytotoxic control compound. The ratio of surviving cells after compound treatment was determined using a colorimetric WST-1 assay (Roche) as indicated in the protocol provided by manufacturer. The absorbance was measured using a Varioscan microplate reader (Thermo) at 450 nm with a 620-nm reference filter. To determine the IC_{50} values, a sigmoid-dose response curve was fitted to the data using nonlinear regression on GraphPad Prism 5 software.

This study did not need ethics committee approval because *in vitro* methods were used for biologic activity processes.

RESULTS AND DISCUSSION

The synthetic route to the target compounds is outlined in Scheme 2. The structures of the compounds (1, 2, 3a-j) were confirmed using IR, ^{1}H-NMR, and elemental analysis. IR spectra of the compounds (1, 2, 3a-j) afforded N-H stretching (3115-3369) bands. IR spectra of all compounds (1, 2, 3a-j) 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.

It is known that some 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 the compounds on these cell lines with their activities on HR-proficient cancer cell lines (HeLa and MCF7) and also a non-tumoral MRC5 fibroblast cell line.

When the substitution pattern at the phenyl ring was determined, the effect of electron donor and electron acceptor groups on activity was considered. Therefore, methyl, methoxy, and halogens such as F, Cl, Br were selected as electron donors and a nitro group was selected as an electron acceptor. Our data suggest that only 3a having fluoro, 3b having chloro, 3c having nitro and 3d having 2,6 dichloro substituents had cytotoxic activities at the tested concentrations. The IC_{50} values of these compounds are given in Table 1. Compounds 3e-j showed less cytotoxic activity on all cancer cells compared with 3a-d. However, our results suggest that the compounds possessed no selectivity toward HR defective Capan1 and HCC1937 cells harboring *BRCA* mutations compared with MCF7 and HeLa cells with intact HR pathways.

CONCLUSIONS

In the present paper, we reported the synthesis of some new urea and carbohydrazide derivatives from methyl 6-aminopyridine-

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

3-carboxylate. The synthesized compounds were evaluated for their cytotoxic activity. Our data indicate that 3a-d are more selective to cancer cells compared with nontumoral fibroblasts; however, these compounds are not more potent on HR defective cells with *BRCA* mutants.

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Conflict of Interest: No conflict of interest was declared by the authors.

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