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Synthesis and Anticancer Activity of Novel Benzofurancarboxamides

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Abstract: In our present work, we presented an efficient synthesis and anticancer activity evaluation of some novel benzofurancarboxamides. Our proposed approaches provide the possibility to design benzofurans diversity with a considerable chemical novelty. The synthesized substances were selected by the National Cancer Institute (NCI) Developmental Therapeutics Program for the in vitro cell line screening to investigate their anticancer activity. The compounds with significant levels of anticancer activities have been found that can be used for further optimization.

Keywords: organic synthesis; benzofurancarboxamides; NMR spectroscopy; anticancer activity.

1. Introduction

The problem of finding effective low-toxic antitumor drugs is very important in modern medicine and pharmacy. According to the World Health Organization, more than 10 million new cancer patients appear annually.

The incidence of developing cancer is 27.7% for men and 18.5% for women. In recent years, pharmacotherapy of tumor pathology has been enriched with numerous new drugs that increase its effectiveness and safety [1].

However, despite significant advances in the chemotherapy of malignant tumors, many types of cancer remain incurable, and the availability of antitumor drugs is insufficient. Therefore, the search for new organic compounds with anticancer activity is an urgent problem of our time. The benzofuran derivatives have attracted many researchers due to the broad scope of their biological activity, which includes anticancer, antimicrobial, antiviral, antioxidant, anti-inflammatory, and other properties [1-5].

However, the study of benzofurancarboxamides is quite ambiguous. In particular, among this class of compounds, compounds exhibiting antimicrobial and anticancer were found [6-10]. Some of their analogues were recognized as inhibitors Sirtuin 2 [11], inosine 5'-monophosphate dehydrogenase [12], matrix metallopeptidase 13 [13], ligands of adenosine A2A [14] and metabotropic glutamate 4 receptors [15].

In this article, which is the part of our researching biologically active heterocycles [16-55], we described the synthesis and anticancer activities of new benzofurancarboxamides.

2. Materials and Methods

2.1. Materials.

The reagents used in the synthesis were commercially available and of analytical grade. All solvents and reagents were used without further purification.

2.2. Chemistry.

All melting points were determined in an open capillary. The NMR-spectra were recorded using Varian Mercury 400 (400 MHz) at 298 K. Chemical shifts are reported as δ (ppm). Chemical shifts are reported as δ (ppm) relative to tetramethylsilane (TMS) as an internal standard. The coupling constant J is expressed in Hz. Elemental analysis was performed on a Vario MICRO cube automatic CHNS analyzer. This experimental analysis data on contents of Carbon, Hydrogen, and Nitrogen were within ±0.3% of the theoretical values.

General procedure for the synthesis of benzofurancarboxamides (12-17): In 30 ml of dry dioxane dissolved 0.01 mol of the chloroanhydride (**1a** or **1b**) and 0.01 mol of the corresponding amine. After the complete dissolution of the mixture, 1 ml of triethylamine is added to the solution and left for 30 minutes. The mixture formed at this stage is precipitated with water and left for 12 h to form a precipitate and then filtered. The resulting substances on the filter are washed with cold water and dried.

N-(4-isopropylphenyl)-1-benzofuran-2-carboxamide **(12a).** Yield 81%, mp 125-126°C. ^{1}H NMR (400 MHz, DMSO) δ 10.40 (s, 1H, NH), 7.80 (d, *J* = 7.7 Hz, 1H, benzofuran), 7.75 – 7.66 (m, 4H, benzofuran+C_6H_4), 7.48 (t, *J* = 7.4 Hz, 1H, benzofuran), 7.34 (t, *J* = 7.4 Hz, 1H, benzofuran), 7.21 (d, *J* = 8.4 Hz, 2H, C_6H_4), 2.84 (dt, *J* = 13.7, 6.8 Hz, 1H, CH), 1.18 (d, J = 6.9 Hz, 6H, $2CH_3$). Anal. Calcd. for $C_{18}H_{17}NO_2$: C, 77.40; H, 6.13; N, 5.01. Found: C, 77.78; H, 6.08; N, 5.09.

N-(2-benzoylphenyl)-1-benzofuran-2-carboxamide **(12b).** Yield 73%, mp 169-170°C. ^{1}H NMR (400 MHz, DMSO) δ 11.21 (s, 1H, NH), 8.01 (d, *J* = 7.9 Hz, 1H, ArH), 7.75 (d, *J* = 7.4 Hz, 1H, benzofuran)), 7.73 – 7.60 (m, 4H, benzofuran + ArH), 7.60 – 7.54 (m, 2H, ArH), 7.51 – 7.42 (m, 4H, ArH, benzofuran+ ArH), 7.30 (dd, *J* = 15.4, 7.7 Hz, 2H, benzofuran + ArH). Anal. Calcd. for $C_{22}H_{15}NO_3$: C, 77.41; H, 4.43; N, 4.10. Found: C, 77.19; H, 4.36; N, 4.13.

Methyl 2-[(1-benzofuran-2-ylcarbonyl)amino]-4-chlorobenzoate **(12c).** Yield 88%, mp 133-1134°C. ^{1}H NMR (400 MHz, DMSO) δ 11.98 (s, 1H, NH), 8.68 (d, *J* = 2.0 Hz, 1H, C_6H_3), 7.98 (d, *J* = 8.5 Hz, 1H, benzofuran), 7.79 (d, *J* = 7.6 Hz, 1H, benzofuran), 7.74 – 7.66 (m, 2H, benzofuran+ C_6H_3), 7.50 (t, *J* = 7.3 Hz, 1H, benzofuran), 7.34 (t, *J* = 7.4 Hz, 1H, benzofuran), 7.27 (dd, *J* = 8.5, 2.0 Hz, 1H, C_6H_3), 3.90 (s, 3H, CH_3). Anal. Calcd. for $C_{17}H_2ClNO_4$: C, 61.92; H, 3.67; N, 4.25. Found: C, 61.85; H, 3.55; N, 4.33.

Methyl 2-[(1-benzofuran-2-ylcarbonyl)amino]-4,5-dimethoxybenzoate **(12d).** Yield 91%, mp 147-148°C. ^{1}H NMR (400 MHz, DMSO) δ 12.02 (s, 1H, NH), 8.37 (s, 1H, C_6H_2), 7.77 (d, *J* = 7.6 Hz, 1H, benzofuran), 7.67 (d, *J* = 8.3 Hz, 1H, benzofuran), 7.62 (s, 1H, benzofuran), 7.48 (t, *J* = 7.5 Hz, 1H, benzofuran), 7.39 – 7.30 (m, 2H, ArH, benzofuran + C_6H_2), 3.87 (s, 3H, CH_3), 3.80 (s, 3H, CH_3), 3.73 (s, 3H, CH_3). Anal. Calcd. for $C_{19}H_{17}NO_6$: C, 64.22; H, 4.82; N, 3.94. Found: C, 64.43; H, 4.90; N, 3.98.

5-Chloro-N-(4-methylphenyl)-1-benzofuran-2-carboxamide **(12e).** Yield 88%, mp 156-157°C. ^{1}H NMR (400 MHz, DMSO): δ = 10.46 (s, 1H, NH), 7.88 (d, *J* = 7.9 Hz, 1H,

benzofuran), 7.73-7.63 (m, 4H, benzofuran+C_6H_4), 7.47 (dd, 8.8, 2.2 Hz,, 1H, benzofuran), 7.13 (d, *J* = 8.3 Hz, 2H, C_6H_4), 2.25 (s, 3H, CH_3). Anal. Calcd. for $C_{16}H_{12}ClNO_2$: C, 67.26; H, 4.23; N, 4.90. Found: C, 67.44; H, 4.16; N, 4.85.

***Methyl 2-[(1-benzofuran-2-ylcarbonyl)amino]-4,5-dimethylthiophene-3-carboxylate* (13).** Yield 85%, mp 147-148°C. ^{1}H NMR (400 MHz, DMSO): δ = 12.08 (s, 1H, NH), 7.81 (d, *J* = 7.8 Hz, 1H, benzofuran), 7.74 (d, *J* = 9.0 Hz, 2H, benzofuran), 7.53 (t, *J* = 7.9 Hz, *J* = 8.0 Hz, 1H, benzofuran), 7.38 (t, *J* = 7.4 Hz, 1H, benzofuran), 3.90 (s, 3H, O-CH_3), 2.22 (s, 3H, CH_3), 2.17 (s, 3H, CH_3). Anal. Calcd. for $C_{17}H_{15}NO_4S$: C, 61.99; H, 4.59; N, 4.25. Found: C, 61.87; H, 4.66; N, 4.12.

***N-(2-oxo-2H-chromen-6-yl)-1-benzofuran-2-carboxamide* (14).** Yield 85%, mp 242-243°C. ^{1}H NMR (400 MHz, DMSO): δ = 10.73 (s, 1H, NH), 8.20 (s, 1H, chromen), 8.07 (d, *J* = 8.3 Hz, 1H, chromen), 7.89 (d, *J* = 6.9 Hz, 1H, benzofuran), 7.80-7.68 (m, 3H, benzofuran+chromen), 7.48-7.34 (m, 3H, benzofuran+chromen), 6.47 (d, *J* = 8.5 Hz, 1H, chromen). Anal. Calcd. for $C_{18}H_{11}NO_4$: C, 70.82; H, 3.63; N, 4.59. Found: C, 70.70; H, 3.74; N, 4.66.

***5-Chloro-N-1,3-thiazol-2-yl-1-benzofuran-2-carboxamide* (15).** Yield 78%, mp 143-144°C. ^{1}H NMR (400 MHz, DMSO): δ 12.99 (s, 1H, NH), 8.01 – 7.87 (m, 2H, benzofuran), 7.80 – 7.69 (m, 1H, benzofuran), 7.61 – 7.43 (m, 2H, benzofuran+thiazole), 7.27 (s, 1H, thiazole). Anal. Calcd. for $C_{12}H_7ClN_2O_2S$: C, 51.71; H, 2.53; N, 10.05. Found: C, 51.60; H, 2.48; N, 10.11.

***N-1,3-Benzothiazol-2-yl-1-benzofuran-2-carboxamide* (16)**. Yield 81%, mp 256-257°C. ^{1}H NMR (400 MHz, DMSO): δ = 13.32 (s, 1H, NH), 8.30 (s, 1H, benzothiazol), 8.14 (s, 1H, benzothiazol), 7.87 (d, *J* = 7.9 Hz, 1H, benzofuran), 7.77 - 7.69 (m, 3H, benzofuran+benzothiazol), 7.61 (dd, *J* = 8.6, 1.9 Hz, 1H, benzothiazol), 7.54 (t, *J* = 8.2 Hz, *J* = 7.4 Hz, 1H, benzofuran), 7.39 (t, *J* = 7.6 Hz, 1H, benzofuran). Anal. Calcd. for $C_{16}H_{10}N_2O_2S$: C, 65.29; H, 3.42; N, 9.52. Found: C, 65.34; H, 3.55; N, 9.44.

***N-[5-(3-methylbenzyl)-1,3-thiazol-2-yl]-1-benzofuran-2-carboxamide* (17a).** Yield 84%, mp 163-164°C. ^{1}H NMR (400 MHz, DMSO): δ = 12.80 (s, 1H, NH), 7.96 (s, 1H, benzofuran), 7.80 (d, *J* = 7.7 Hz, 1H, benzofuran), 7.69 (d, *J* = 8.3 Hz, 1H, benzofuran), 7.48 (t, *J* = 8.1 Hz, 1H, benzofuran), 7.33 (s, 2H, thiazole+C_6H_4), 7.19 (t, *J* = 7.5 Hz, 1H, benzofuran), 7.08-7.02 (m, 3H, C_6H_4), 4.05 (s, 2H, CH_2), 2.26 (s, 3H, CH_3). Anal. Calcd. for $C_{20}H_{16}N_2O_2S$: C, 68.95; H, 4.63; N, 8.04. Found: C, 69.08; H, 4.77; N, 8.08.

***N-[5-(2-chlorobenzyl)-1,3-thiazol-2-yl]-1-benzofuran-2-carboxamide* (17b).** Yield 84%, mp 188-189°C. ^{1}H NMR (400 MHz, DMSO): δ = 12.85 (s, 1H, NH), 7.99 (s, 1H, C_6H_4), 7.82 (d, J = 7.8 Hz, 1H, benzofuran), 7.71 (d, *J* = 8.5 Hz, 1H, benzofuran), 7.52-7.45 (m, 3H, benzofuran+C_6H_4), 7.37-7.28 (m, 4H, benzofuran+thiazole+C_6H_4), 4.23 (s, 2H, CH_2). Anal. Calcd. for $C_{19}H_{13}ClN_2O_2S$: C, 61.87; H, 3.55; N, 7.59. Found: C, 62.02; H, 3.49; N, 7.64.

***N-[5-(2,3-dichlorobenzyl)-1,3-thiazol-2-yl]-1-benzofuran-2-carboxamide* (17c).** Yield 88%, mp 173-174°C. ^{1}H NMR (400 MHz, DMSO): δ = 12.84 (s, 1H, NH), 7.96 (s, 1H, C_6H_4), 7.80 (d, *J* = 7.5 Hz, 1H, benzofuran), 7.69 (d, J = 8.2 Hz, 1H, benzofuran), 7.53 (d, *J* = 7.9 Hz, 1H, benzofuran), 7.48 (t, *J* = 7.9 Hz, 1H, benzofuran), 7.42 (d, *J* = 7.6 Hz, 1H, C_6H_4), 7.36-7.31 (m, 3H, benzofuran+thiazole+C_6H_4), 4.27 (s, 2H, CH_2). Anal. Calcd. for $C_{19}H_{12}Cl_2N_2O_2S$: C, 56.59; H, 3.00; N, 6.95. Found: C, 56.52; H, 3.06; N, 6.89.

***N-{5-[4-chloro-3-(trifluoromethyl)benzyl]-1,3-thiazol-2-yl}-1-benzofuran-2-carboxamide* (17d).** Yield 79%, mp 205-206°C. ^{1}H NMR (400 MHz, DMSO): δ =12.83 (s, 1H, NH), 7.96 (s, 1H, C_6H_4), 7.82 – 7.78 (m, 2H, benzofuran), 7.70 – 7.66 (m, 2H, benzofuran+

C_6H_4), 7.60 (d, *J* = 8.1 Hz, 1H, C_6H_4), 7.48 (t, *J* = 8.0 Hz, 1H, benzofuran), 7.38-7.32 (m, 2H, benzofuran+thiazole), 4.22 (s, 2H, CH_2). Anal. Calcd. for $C_{20}H_{12}ClF_3N_2O_2S$: C, 54.99; H, 2.77; N, 6.41. Found: C, 54.85; H, 2.82; N, 6.55.

***Synthesis of N-[3-(2-oxopropyl)-1,2,4-thiadiazol-5-yl]-1-benzofuran-2-carboxamide* (21)**. 0,005 mol Benzofuran-2-carbonyl chloride (**1a**), was dissolved in 10 ml of anhydrous acetonitrile and 0,005 mol of potassium thiocyanate was added under stirring then the resulting mixture at 60°C was heated for 30 min. Next 0,0034 mol of 5-methyl-1,2-oxazol-3-amine was added, and the mixture was heated under stirring for 2 h and poured into water. The precipitate was filtered off, washed with several portions of water, and purified by recrystallization from ethanol. Yield 64%, mp 108-109 °C. ^{1}H NMR (400 MHz, DMSO): δ = 13.79 (s, 1H, NH), 7.91 (s, 1H, benzofuran), 7.83 (d, *J* = 7.6 Hz, 1H, benzofuran), 7.71 (d, *J* = 8.2 Hz, 1H, benzofuran), 7.51 (t, *J* = 7.8 Hz, 1H, benzofuran), 7.35 (t, *J* = 7.3 Hz, 1H, benzofuran), 4.02 (s, 3H, CH_2), 2.17 (s, 3H, CH_3). Anal. Calcd. for $C_{14}H_{11}N_3O_3S$: C, 55.81; H, 3.68; N, 13.95. Found: C, 55.66; H, 3.79; N, 13.88.

2.3. Pharmacology.

The tested compounds were added to the culture at a single concentration (10^{-5}M),and the cultures were incubated for 48 h. Endpoint determinations were made with a protein-binding dye, sulforhodamine B (SRB). Results for each tested compound were reported as the percent growth of the treated cells when compared to the untreated control cells.

The percent growth was evaluated spectrophotometrically versus not treated controls. The cytotoxic and/or growth inhibitory effects of the most active compounds were tested *in vitro* against the full panel of about 60 human tumor cell lines at 10-fold dilutions of five concentrations ranging from 10^{-4} to 10^{-8}M. The 48-h continuous drug exposure protocol was followed, and an SRB protein assay was used to estimate cell viability or growth.

Using the seven absorbance measurements [time zero, (Tz), control growth in the absence of drug, (C), and test growth in the presence of drug at the five concentration levels (Ti)], the percent growth was calculated at each of the drug concentrations levels. Percent growth inhibition was calculated as:

$[(Ti - Tz)/(C - Tz)] \times 100$ for concentrations for which $Ti \geq Tz$;

$[(Ti - Tz)/Tz] \times 100$ for concentrations for which $Ti < Tz$.

Three dose-response parameters were calculated for each compound. Growth inhibition of 50% (GI_{50}) was calculated from $[(Ti - Tz)/(C - Tz)] \times 100 - 50$, which is the drug concentration resulting in a 50% lower net protein increase in the treated cells (measured by SRB staining) as compared to the net protein increase seen in the control cells.

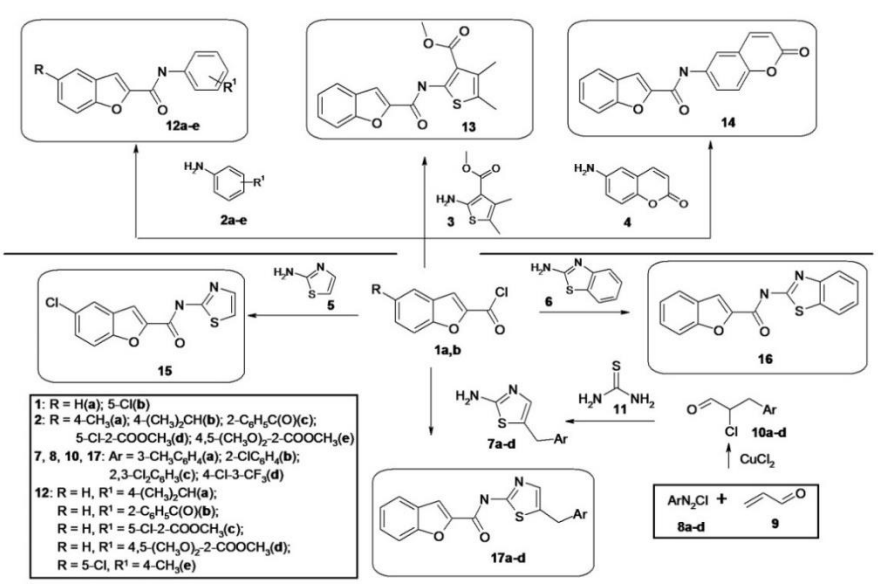
The drug concentration resulting in total growth inhibition (TGI) was calculated from Ti = Tz. The LC_{50} (concentration of drug resulting in a 50% reduction in the measured protein at the end of the drug treatment as compared to that at the beginning) indicating a net loss of cells following treatment was calculated from $[(Ti - Tz)/Tz] \times 100 = -50$.

Values were calculated for each of these three parameters if the level of activity was reached; however, if the effect was not reached or was exceeded, the value for that parameter was expressed as more or less than the maximum or minimum concentration was tested.

3. Results and Discussion

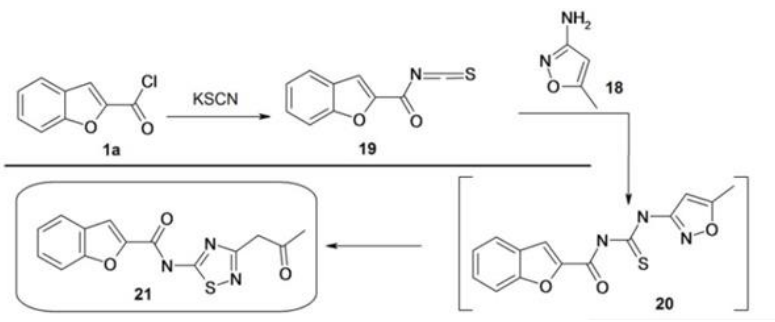
3.1.Synthesis of some benzofurancarboxamides.

We were continuing the systematic study of benzofurane derivatives as potential drug candidates we spent synthesis and anticancer activity screening of among the specified class of compounds. The target benzofurancarboxamides were synthesized from chloroanhydrides of commercially available benzofuran-2-carboxylic acids (**1a, b**) and arylamines (**2a-e**), Gewald amine (**3**), 6-aminocoumarine (**4**), 2-aminothiazole (**5**), 2-aminobenzothiazole (**6**), and 2-amino-5-arylmethylthiazoles (**7a-d**). 2-Amino-5-arylmethylthiazoles (**7a-d**) were prepared from diazonium salts (**8a-d**) and acrolein (**9**) according to procedures described earlier [56]. At first stage (**8a-d**) and acrolein (**9**) in condition of Meerwein reaction [57] to form 3-aryl-2-chloropropanals (**10a-d**) which, by the reaction with thiourea (**11**), were transformed into target 2-amino-5-arylmethylthiazoles (**7a-d**). The acylation reaction was performed in dry dioxane at room temperature in the presence triethylamine (**Scheme 1**).



Scheme 1. Synthesis of benzofurancarboxamides (**12-17**).

The amide **21** was prepared according to scheme 2. Acyl isothiocyanates **19** generated in situ from acid chlorides **1a** and potassium thiocyanate reacted with 5-methyl-1,2-oxazol-3-amine **18**. The expected products of this reaction, N-acylthioureas were not isolated, for they underwent recyclization involving the opening of the 1,2-oxazole ring and closure of 1,2,4-thiadiazole ring [58] to produce finally N-[3-(2-oxopropyl)-1,2,4-thiadiazol-5-yl]-1-benzofuran-2-carboxamide.



Scheme 2. Synthesis of *N*-[3-(2-oxopropyl)-1,2,4-thiadiazol-5-yl]-1-benzofuran-2-carboxamide (**21**).

The structures of the synthesized compounds and their composition were confirmed by quantitative elemental analysis and ^{1}H NMR spectroscopy. The spectroscopic data of all new compounds correspond to the proposed structures.

3.2. Pharmacology.

The synthesized compounds were selected by the National Cancer Institute (NCI) Developmental Therapeutics Program (www.dtp.nci.nih.gov) for the *in vitro* cell line screening to investigate their anticancer activity. The primary anticancer assay was performed at approximately sixty human tumor cell lines panel derived from nine neoplastic diseases, the protocol of the Drug Evaluation Branch, National Cancer Institute, Bethesda [59-63].

Results for each tested compound were reported as the percentage of growth of the treated cells when compared to the untreated control cells. The percentage of growth was evaluated spectrophotometrically versus controls not treated with test agents. The screening results are shown in Table 1.

Table 1. Cytotoxic activity of the tested compounds in the concentration 10^{-5} M against 60 cancer cell lines.

Test compounds	Average growth, %	Range of growth, %	Most sensitive cell line (cancer line/type) GP, %
12a	85.61	56.95 – 110.16	CAKI-1 (Renal Cancer) 56.95 HOP-92 (Non-Small Cell Lung Cancer) 57.53 SF-295 (CNS Cancer) 63.71
12b	77.40	17.09 – 108.35	SNB-75 CNS Cancer 17.09 OVCAR-4 Ovarian Cancer 27.58 HS 578T Breast Cancer 28.88
12c	89.84	56.02 – 110.81	CAKI-1 Renal Cancer 56.02 UO-31 Renal Cancer 56.16 HOP-92 Non-Small Cell Lung Cancer 60.93
12d	83.30	51.53 – 104.89	HOP-92 Non-Small Cell Lung Cancer 51.53 MCF7 Breast Cancer 59.06 OVCAR-8 Ovarian Cancer 61.95
12e	100.39	80.85 – 122.53	HOP-92 (Non-Small Cell Lung Cancer) 80.85
13	60.31	17.47 – 112.78	UO-31 Renal Cancer 63.22 HOP-92 Non-Small Cell Lung Cancer 68.80
14	91.38	65.20 – 126.53	NCI-H522 Non-Small Cell Lung Cancer 65.20 UO-31 Renal Cancer 74.91
15	97.73	69.54 – 133.30	HCT-116 Colon Cancer 69.54
16	102.14	79.49 – 120.17	HOP-92 Non-Small Cell Lung Cancer 79.49 CCRF-CEM Leukemia 80.88
17a	28.76	-54.45 – 71.84	MDA-MB-435 Melanoma -54.45 NCI-H522 Non-Small Cell Lung Cancer -32.39 RXF 393 Renal Cancer -8.33 OVCAR-3 Ovarian Cancer -5.09 SF-295 CNS Cancer 4.95 SF-539 CNS Cancer 4.21
17b	81.21	14.65 – 105.61	SR Leukemia 14.65 MDA-MB-435 Melanoma 31.21 T-47D Breast Cancer 33.04 MCF7 Breast Cancer 33.60
17c	-4.31	-74.59 – 44.93	TK-10 Renal Cancer -74.59 OVCAR-3 Ovarian Cancer -60.14 ACHN Renal Cancer -60.23 HCT-116 Colon Cancer -60.26 786-0 Renal Cancer -56.06 HT29 Colon Cancer -54.29
17d	44.38	-42.84 – 79.81	SK-MEL-5 Melanoma -42.84 UACC-62 Melanoma 7.33 KM12 Colon Cancer 9.26 HCC-2998 Colon Cancer 11.92

The screening results are shown in Table 1. The synthesized compounds display a different level of activity in the *in vitro* screening on the tested cell lines. Compounds **12a, 13,**

17b showed moderate activity with GP = 79.40 – 89.84% while **17a, c, d** high –with GP = -4.31 – 44.38%. To the most active compound, **17c** was susceptible to cell line TK-10 ACHN and 786-0 (Renal Cancer), OVCAR-3 (Ovarian Cancer), HCT-116, and HT29 (Colon Cancer). In all mentioned cases, a high cytotoxic effect was observed.

Finally, compound **17a, c, d** was selected for an advanced assay against a panel of approximately sixty tumor cell lines at 10-fold dilutions of five concentrations (100 µM, 10 µM, 1.0 µM, 0.1 µM, and 0.01 µM) (Table 2).

The percentage of growth was evaluated spectrophotometrically versus controls not treated with test agents after 48-h exposure and using SRB protein assay to estimate cell viability or growth. Dose-response parameters were calculated for each cell line:

GI50 – molar concentration of the compound that inhibits 50% net cell growth;

TGI – molar concentration of the compound leading to the total inhibition;

LC50– molar concentration of the compound leading to 50% net cell death.

Furthermore, mean graph midpoints (MG_MID) were calculated for GI_{50} and TGI, giving an average activity parameter over all cell lines for the tested compound. For the MG_MID calculation, insensitive cell lines were included with the highest concentration tested. Compounds **17a** showed high inhibition activity (GI_{50} <10 µM) against all 58 human tumor cell lines with MG-MID GI_{50} values of 2.03 µM **17c** - against 56 of 58 (MG-MID = 3.26 µM) and **17d** against 53 of 58 (MG-MID = 3.26 µM) human tumor cell lines. The colon cancer subpanel demonstrated the highest sensitivity to compound **17a** and **17c** with a mean GI50 value of 0.87µM and 0.84 µM, respectively. The most sensitive line was T-47D (Breast Cancer, GI_{50} = 0.088 µM).

Table 2. Influence of compound **17a, c, d** on the growth of tumor cell lines.

Panel/Cell Line	Growth inhibitory concentration µM					
	17a		17c		17d	
	GI_{50}	TGI	GI_{50}	TGI	GI_{50}	TGI
Leucemia						
CCRF-CEM	1.32	11.4	11.9	37.5	2.42	> 100
HL-60 (TB)	0.318	2.09	0.666	> 100	> 100	> 100
K-562	0.357	11.8	0.427	> 100	0.963	> 100
MOLT-4	2.42	25.2	63.0	> 100	> 100	> 100
RPMI-8226	1.34	6.61	8.54	> 100	28.6	> 100
SR	0.309	2.68	0.368	> 100	15.4	> 100
Non-Small Cell Lung Cancer						
A549/ATCC	0.541	28.9	1.27	9.38	0.738	> 100
EKVX	-	-	3.12	> 100	4.20	> 100
HOP-62	1.16	16.7	1.30	4.16	6.13	27.5
HOP-92	0.268	3.98	-	-	1.77	5.57
NCI-H226	7.76	55.0	3.15	56.9	6.93	74.3
NCI-H23	4.35	> 100	2.43	25.5	9.29	> 100
NCI-H322M	2.99	94.7	1.74	34.5	9.86	> 100
NCI-H460	0.388	11.0	1.33	5.11	0.570	70.1
NCI-H522	0.401	8.15	0.420	7.01	5.13	38.4
CNS Cancer						
SF-268	3.12	21.6	2.73	34.9	2.95	34.5
SF-295	9.53	32.9	0.756	3.54	0.527	11.7
SF-539	1.68	9.33	2.07	6.90	-	-
SNB-19	5.60	42.4	2.17	25.0	4.54	27.3
SNB-75	2.11	13.9	1.60	3.57	2.07	5.02
U251	0.739	14.9	1.08	3.53	3.29	18.5
Colon cancer						
COLO 205	0.573	4.04	2.28	> 100	8.87	> 100
HCC-2998	4.05	> 100	0.925	9.43	0.142	> 100
HCT-116	0.530	62.4	1.13	3.23	2.47	> 100
HCT-15	0.0522	> 100	0.0547	> 100	0.0667	> 100
HT29	0.394	13.0	0.501	4.07	11.9	> 100

Panel/Cell Line	Growth inhibitory concentration μM					
	17a		17c		17d	
	GI_{50}	TGI	GI_{50}	TGI	GI_{50}	TGI
KM12	0.0749	11.0	0.0855	3.47	0.0364	> 100
SW-620	0.440	24.6	0.920	> 100	6.54	> 100
Melanoma						
LOX IMVI	1.11	20.6	1.99	> 100	2.12	> 100
MALME-3M	1.99	18.3	1.42	25.5	3.81	> 100
M14	0.667	> 100	0.858	> 100	1.04	> 100
MDA-MB-435	0.208	0.525	0.245	0.771	0.527	> 100
SK-MEL-2	0.704	12.1	0.138	3.02	3.16	33.1
SK-MEL-28	4.07	> 100	2.69	> 100	16.5	> 100
SK-MEL-5	0.190	1.61	0.339	3.47	2.64	8.36
UACC-257	1.15	24.3	5.79	> 100	9.77	> 100
UACC-62	0.565	14.9	0.139	4.60	-	-
Ovarian Cancer						
IGROV1	2.75	35.8	1.96	21.2	7.54	> 100
OVCAR-3	0.715	3.18	1.30	3.04	2.72	10.1
OVCAR-4	2.03	13.9	0.0396	> 100	8.39	> 100
OVCAR-5	6.95	52.9	-	-	-	-
OVCAR-8	2.69	17.0	2.61	> 100	3.49	55.1
NCI/ADR-RES	0.760	27.7	0.673	> 100	8.48	> 100
SK-OV-3	2.88	21.9	1.63	4.17	5.16	22.2
Prostate Cancer						
PC-3	1.53	15.4	2.45	18.4	2.88	51.8
DU-145	4.03	59.9	4.67	> 100	8.45	> 100
Renal Cancer						
786-0	2.72	15.0	1.66	3.65	2.56	11.2
A498	2.19	8.72	1.40	4.71	2.59	9.07
ACHN	2.44	22.6	-	-	1.01	14.0
CAKI-1	1.24	17.5	1.38	20.4	0.372	50.9
RXF 393	3.32	18.9	1.96	8.90	5.47	25.4
SN12C	2.57	> 100	3.64	> 100	22.2	> 100
TK-10	1.39	11.2	1.63	4.59	3.72	19.3
UO-31	2.78	14.2	2.44	9.54	0.534	65.0
Breast Cancer						
MCF7	0.0767	29.2	0.0651	> 100	1.69	> 100
MDA-MB-231/ATCC	1.55	22.0	0.573	5.73	2.80	> 100
HS 578T	1.76	13.9	2.00	8.29	3.79	33.3
BT-549	4.00	74.3	10.6	66.7	18.4	> 100
T-47D	0.0282	13.1	0.0255	21.0	-	-
MDA-MB-468	0.769	11.0	0.591	6.34	3.36	20.7

The selectivity index (SI) obtained by dividing the full panel MG-MID (μM) of the compound **17a** by its sub-panel MG-MID (μM) was considered as a measure of compound's selectivity. Ratios between 3 and 6 refer to moderate selectivity, ratios greater than 6 indicate high selectivity toward the corresponding cell line, while compounds not meeting either of the criteria are rated non-selective [59]. In this context, the active compounds **17c** and **17d** demonstrates the same moderate selectivity toward colon and CNS cancer cell lines (Table 3).

Table 3. Anticancer selectivity pattern of the most active compounds **17a, c, d**.

Cpd	Parameters	Subpanel tumor cell lines								
		L	NSCLC	ColC	CNSC	M	OV	RC	PC	BC
17a	GI_{50}	1.01	2.23	0.87	3.80	1.18	2.68	2.33	2.78	1.36
	SI*	2.01	0.91	2.33	0.53	1.72	0.76	0.87	0.73	1.49
	TGI	9.96	39.80	45.01	22.51	32.48	24.63	26.02	37.65	27.25
	SI**	2.96	0.74	0.65	1.31	0.91	1.20	1.13	0.78	1.08
17c	GI_{50}	14.15	1.85	0.84	1.73	1.51	1.37	2.06	3.56	2.31
	SI*	0.23	1.76	3.88	1.88	2.16	2.38	1.58	0.92	1.41
	TGI	89.58	30.32	45.74	12.91	48.60	54.74	21.68	59.20	34.68
	SI**	0.49	1.46	0.97	3.42	0.91	0.81	2.04	0.75	1.27
17d	GI_{50}	41.23	4.96	4.29	2.68	4.95	5.96	4.81	5.67	6.01
	SI*	0.22	1.80	2.09	3.34	1.81	1.50	1.86	1.58	1.49

Cpd	Parameters	Subpanel tumor cell lines								
		L	NSCLC	ColC	CNSC	M	OV	RC	PC	BC
	TGI	100.00	68.43	100.00	19.40	80.18	64.57	36.86	75.90	70.80
	SI**	0.68	1.00	0.68	3.52	0.85	1.06	1.86	0.90	0.97

L – leukemia, NSCLCC – non-small cell lung cancer, ColC – colon cancer, CNSC – CNS cancer, M – melanoma, OV– ovarian cancer, RC – renal cancer, PC – prostate cancer, BC – breast cancer.
*Selectivity index at the GI_{50} level. **Selectivity index at the TGI level

In table 4 the results of the mean growth inhibitory concentration (GI50, μM) of compound 17a, c, d in comparison with 5-FU, Cisplatin, and Curcumin.

Table 4. Mean growth inhibitory concentration (GI50, μM) of compound 17a, c, d in comparison with 5-FU, Cisplatin, and Curcumin.

	Subpanel tumor cell lines									
Cpd	L	NSCLC	ColC	CNSC	M	OV	RC	PC	BC	MG-MID
17a	1.01	2.23	0.87	3.80	1.18	2.68	2.33	2.78	1.36	2.03
17c	14.15	1.85	0.84	1.73	1.51	1.37	2.06	3.56	2.31	3.26
17d	41.23	4.96	4.29	2.68	4.95	5.96	4.81	5.67	6.01	8.95
5-FU	15.1	>100	8.4	72.1	70.6	61.4	45.6	22.7	76.4	52.5
Cisplatin	6.3	9.4	21.0	4.7	8.5	6.3	10.2	5.6	13.3	9.48
Curcumin	3.7	9.2	4.7	5.8	7.1	8.9	10.2	11.2	5.9	7.41

The tested compound **17a, c, d** is effective against all of the cell lines, as the full panel mean-graph shows it. MG-MID (μM) values for **17a, c, d** are less than those for 5-Fluorouracil, Curcumin, and Cisplatin when tested in the same manner.

4. Conclusions

To develop novel anticancer agents, a series of novel benzofurancarboxamides were synthesized. The structures of the obtained compounds were confirmed by 1H NMR spectroscopy and elemental analysis. Anticancer activity screening was carried out within the framework of the Developmental Therapeutic Program of the National Cancer Institute's (DTP, NCI, Bethesda, Maryland, USA). The synthesized compounds were exhibited high activity against most of the cancer cells. The results prove the necessity of further investigations to clarify the features underlying the antitumor effect of tested compounds.

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Conflicts of Interest

The authors declare no conflict of interest.

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