Visnagin (4-methoxy-7-methyl-5H-furo[3,2-g][1]benzofuran-5-one) is one of the essential chemical constituents of the fruits and seeds of *Ammi visnaga*, family *Umbelliferae* (1, 2, 3) and is known to possess antispasmodic properties to the ureter and bile duct, treats angina, whooping cough, gall bladder and renal colic. It is considered as a potent coronary vasodilator as well as its role in treating bronchial asthma (4, 5).

Visnagin (4-methoxy-7-methyl-5H-furo[3,2-g][1]benzofuran-5-one) is one of the essential chemical constituents of the fruits and seeds of *Ammi visnaga*, family *Umbelliferae* (1, 2, 3) and is known to possess antispasmodic properties to the ureter and bile duct, treats angina, whooping cough, gall bladder and renal colic. It is considered as a potent coronary vasodilator as well as its role in treating bronchial asthma (4, 5).

It also possesses desirable lipid altering activities (it decreases the VLDL secretion and the atherogenic cholesterol fraction) (6–10). It induces skin pigmentation upon UV irradiation thus is used in the treatment of psoriasis and vitiligo (4–10). A series of synthesized benzofuran based compounds inhibited the farnesyl transferase and acted as antitumor agents in human cancer (2), the most potent of which has an enzyme inhibitory activity IC50 = 1.1 nM. Benzofuran-5-carbonyl derivatives are also a new series of potent antitumor derivatives (1).

A total of five 1H-cyclopenta[b]benzofuran ligands inhibited the growth of human cancer cells in culture (4). New derivatives of (benzofuran-2-yl)-3-phenyl-3-methyl cyclobutyl ketoxime were tested for their antimicrobial activity against *Staphylococcus aureus* ATCC 6538, *Staph. epidermidis* ATCC12228, *Escherichia coli* ATCC8739, *Klebsiella pneumonia* ATCC4352, *Salmonella typhi*, *Shigella flexneri* and *Candida albicans* ATCC10231 (5).

A series of 2-substituted N-acylphenothiazines showed very good antibacterial and antifungal activities (7).
The present work deals with the synthesis and characterization of some benzofuran derivatives obtained from visnaginone, which is derived from the naturally occurring visnagin. Our goal to prepare semisynthetic derivatives with potent anticancer and antimicrobial activities led us to combine and incorporate the benzofuran nucleus with pyranyl, pyridinyl, pyrimidinyl and thiazolyl moieties to allow for a high structural diversity and subsequently, promising cancer chemotherapy and antimicrobial drug candidates.

MATERIALS AND METHODS

All melting points are uncorrected. Elemental analyses were carried out in the micro analytical unit of the National Research Centre. IR spectra were recorded on a Mattson-5000 FTIR spectrometer using KBr disc technique. 1H-NMR spectra were determined on a Varian-Gemini-300 MHz and Jeol-Ex-300 MHz NMR spectrometer using TMS as an internal standard with chemical shift (δ) = 0 ppm. Mass spectra were determined on Finnigan Mat SSQ 7000 apparatus, mode: EI, 70 Ev (Thermo Inst. Sys. Inc., USA). The melting points were determined using Buchi 510 apparatus. The purity of the synthesized compounds was tested by thin layer chromatography (TLC), on silica gel 60 F254 25 aluminium sheets 20 × 20 cm (Merck).

Cytotoxic and biological effects of the novel derivatives derived from benzofurans against liver cancer cell line

Based on the reports and findings that many benzofuran derivatives are active as antibacterial (7), antifungal (7), anticoagulant, anti-inflammato-ry, anti angiogenesis and antitumor agents (1), in the present work, the proliferative effect of the newly synthesized compounds in the field of liver cancer were studied. Since all the selected benzofuran derivatives were soluble in DMSO at concentrations high enough to allow cell experiments, the in vitro biological activity of these compounds was evaluated by their growth inhibitory potency in liver HEPG2 cancer cell lines. The cytotoxic potency of compounds 2-5a, 6a, 8a-b-9b, 10a-b-11a, 12a,c, 13a,b, 14a, 15a, 16c and 17b,c were studied in comparison to the known anticancer drugs 5-fluorouracil (5-FU) and doxorubicin (DOX).

Antimicrobial evaluation of some synthesized compounds

Antibacterial as well as antifungal activities of eleven tested compounds were evaluated in vitro using agar well diffusion test (11–13) using two different concentrations of the compounds (300 µg/mL) dissolved in 1 mL of DMSO as a qualitative method for studying the antimicrobial activity of the tested compounds against the following tested strains: i) bacterial strains: S. typhimurium, L. monocytogenes, S. aureus, P. aeruginosa, B. cereus, E. coli O119. ii) Fungal strains were Candida albicans and Aspergillus flavus, control positive: tobramycin (0.0213 µM) and fluconazole (0.0382 µM) were used as standard antibacterials while fluconazole (0.0816 µM) was used as standard antifungal, dimethylsulfoxide (DMSO) was used as control negative.

Strains selected for being tested were isolated from feed byproducts of animal origin including poultry byproduct (E. coli O119 and S. typhimu-rium) as well as from mastitic cow milk (L. monocytogenes, and B. cereus) and from minced meat (S. aureus and P. aeruginosa). These strains are commonly accused of being a cause of food intoxication in human consuming animal byproducts.

EXPERIMENTAL

1-(2,7-Dibromo-6-hydroxy-4-methoxybenzofuran-5-yl)ethanone (2)

Bromine (0.286 mL) was added dropwise to a solution of 1 (0.260 mL) in acetic acid (40 mL). The solution was stirred for 2 h at room temperature. The reaction mixture was poured into water and treated with 5% sodium bisulfate solution to remove the excess bromine. The solid so obtained was filtered, washed with water and recrystallized from ethanol to give compound 2.

Pale green powder, m.p. 185–187°C, yield 90%. Analysis: for C12H10Br2O4, m.w. 378.01, calcd.: C 38.13, H 2.67, Br 42.28, O 16.93%; found: C 38.16, H 2.10, Br 42.67, O 16.67%. 1H-NMR (DMSO-d6, δ, ppm): 2.70 (s, 3H, COCH 3), 4.17 (s, 3H, OCH 3), 6.92 (s, 1H furan H-3), 13.92 (s, 1H, OH) exchanged with D2O.

1-(2,7-Dibromo-4,6-dimethoxybenzofuran-5-yl)ethanone (3)

A mixture of 2 (2 g), acetone (100 mL), methyl iodide (7 mL) and anhydrous potassium carbonate (7 g) was refluxed for 12 h and was then filtered while hot. The acetone solution was evaporated to dryness, and the residue was crystallized from ethanol to give compound 3.

Yellow powder, m.p. 110–112°C, yield 85%. Analysis: for C12H10Br2O4, m.w. 378.01, calcd.: C 38.13, H 2.67, Br 42.28, O 16.93%; found: C 38.16, H 2.54, Br 42.25, O 16.98%. 1H-NMR (DMSO-d6, δ,
ppm): 2.50 (s, 3H, COCH₃), 3.84, 4.01 (s, 6H, 2OCH₃), 6.91 (s, 1H furan H-3) and absence of OH signal.

(E)-1-(2,7-Dibromo-4,6-dimethoxybenzofuran-5-yl)-3-(dimethylamino)prop-2-en-1-one (4)

Compound 3 (0.01 mol) and dimethylformamide dimethylacetal (0.015 mol) are heated at 90–100°C for 2 h. The reaction mixture was cooled to room temperature and the excess methanol and dimethylformamide dimethylacetal were removed in vacuo then the residue crystallized from ethanol to yield 4.

Pale yellow powder, m.p. 145–147°C, yield 80%. Analysis: for C₁₅H₁₅Br₂NO₄, m.w. 433.09, calcd.: C 41.60, H 3.49, Br 36.90, N 3.23, O 14.78%; found: C 41.52, H 3.51, Br 36.75, N 3.15, O 14.76%. 1H-NMR (DMSO-d₆, δ, ppm): 2.98 (d, 6H, N(CH₃)₂), 3.87, 3.98 (s, 6H, 2OCH₃), 5.35, 7.21 (2d, 2H CH=CH) and 6.88 (s, 1H furan H-3).

2-Amino-6-(2,7-dibromo-4,6-dimethoxybenzofuran-5-yl)-4-(substituted) nicotinonitrile (5a-c)

2-Amino-6-(2,7-dibromo-4,6-dimethoxybenzofuran-5-yl)-4-(4-hydroxy-3-methoxyphenyl)nicotinonitrile (5a)

Crystallized from propan-2-ol, white powder, m.p. 245–247°C, yield 80%. Analysis: for C₂₃H₁₇Br₂N₃O₅, m.w. 575.21, calcd.: C 48.03, H 2.98, Br 27.78, N 7.31, O 16.91%; found: C 47.97, H 2.74, Br 27.83, N 4.94, O 16.73%. IR (KBr, cm⁻¹): 1609 (C=N), 1681 (CO), 2269 (CN), 3410 (OH). 1H-NMR (DMSO-d₆, δ, ppm): 3.77, 3.86, 4.02 (s, 9H, 3OCH₃), 6.86 (s, 1H furan H-3), 7.18–7.23 (m, 3H, aromatic protons), 7.20 (s, 2H, NH₂) exchanged by D₂O, 7.45 (s, 1H of pyridine ring).

2-Amino-6-(2,7-dibromo-4,6-dimethoxybenzofuran-5-yl)-4-phenylnicotinonitrile (5b)

Crystallized from propan-2-ol, pale white powder, m.p. 190–194°C, yield 85%. Analysis: for C₂₂H₁₅Br₂N₃O₃, m.w. 529.18, calcd.: C 49.93, H 2.86, Br 30.20, N 7.94, O 9.07%; found: C 49.98, H 2.97, Br 30.10, N 8.12, O 9.12%. IR (KBr, cm⁻¹): 1609 (C=N), 1681 (CO), 2269 (CN), 3410 (OH).

2-Amino-6-(2,7-dibromo-4,6-dimethoxybenzofuran-5-yl)-4-(3-hydroxy-4-methoxyphenyl)nicotinic acid (6a)

A mixture of compound 5a (0.01 mol, 5.7521 g) and sulfuric acid (15 mL) was stirred at room temperature for 24 h and then poured on ice. The precipitate formed was filtered off, dried then crystallized from propan-2-ol to give 6a.

Black powder, m.p. 115–116°C, yield 80%. Analysis: for C₂₃H₁₈Br₂N₂O₇, m.w. 576.19, calcd.: C 46.49, H 3.07, Br 28.69, N 4.71, O 18.85%; found: C 46.40, H 2.94, Br 27.01, N 4.94, O 17.73%. IR (KBr, cm⁻¹): 1639 (C=N), 1704 (CO of COOH), 3300–3550 (OH of COOH) and absence of CN band.

6-(2,7-Dibromo-4,6-dimethoxybenzofuran-5-yl)-2-hydroxy-4-(substituted) nicotinonitrile (7a-c)

General procedure

A mixture of compound 3 (0.01 mol), malononitrile (0.01 mol), anhydrous ammonium acetate (0.8 mol) and the appropriate aldehydes, namely, vanillin, benzaldehyde or 3-anisaldehyde (0.01 mol) in n-butanol (30 mL) was refluxed for 3–5 h. After cooling, the reaction mixture was filtered off, and recrystallized from the appropriate solvent to give compounds 7a-c.

2-Amino-6-(2,7-dibromo-4,6-dimethoxybenzofuran-5-yl)-4-(4-hydroxy-3-methoxyphenyl)nicotinonitrile (7a)

Crystallized from propan-2-ol, white powder, m.p. 115–117°C, yield 80%. Analysis: for C₂₃H₁₆Br₂N₂O₆, m.w. 576.19, calcd.: C 47.94, H 2.80, Br 27.74, N 4.86, O 16.66%; found: C 47.97, H 2.74, Br 27.83, N 4.94, O 16.73%. IR (KBr, cm⁻¹): 1609 (C=N), 1681 (CO), 2269 (CN), 3410 (OH) and absence of CN band.
m.p. 255–257°C, yield 80%. Analysis: for C\textsubscript{22}H\textsubscript{14}Br\textsubscript{2}N\textsubscript{2}O\textsubscript{4}, m.w. 530.17, calcd.: C 49.84, H 2.66, Br 30.14, N 5.28, O 12.07%; found: C 49.96, H 2.73, Br 30.23, N 5.32, O 11.97%. IR (KBr, cm\textsuperscript{-1}): 1625 (C=N), 1690 (CO), 2211 (CN), 3426 (OH).

6-(2,7-Dibromo-4,6-dimethoxybenzofuran-5-yl)-2-hydroxy-4-(3-methoxyphenyl)nicotinonitrile (7c)
Crystallized from propan-2-ol, white powder, m.p. 275–277°C, yield 85%. Analysis: for C\textsubscript{23}H\textsubscript{16}Br\textsubscript{2}N\textsubscript{2}O\textsubscript{5}, m.w. 560.19, calcd.: C 49.31, H 2.88, Br 28.53, N 5.00, O 14.28%; found: C 49.21, H 2.93, Br 28.63, N 5.21, O 14.11%. IR (KBr, cm\textsuperscript{-1}): 1611 (C=N), 1699 (CO), 2204 (CN), 3423 (OH).

1H-NMR (DMSO-d\textsubscript{6}, \textit{δ}, ppm): 3.60, 3.72, 3.77 (s, 9H, 3OCH\textsubscript{3}), 6.91 (s, 1H furan H-3), 7.01–7.34 (m, 4H, aromatic protons), 7.36 (s, 1H of pyridine ring), 10.60 (s, 1H, OH) exchanged by D\textsubscript{2}O.

(1-(1-(2,7-Dibromo-4,6-dimethoxybenzofuran-5-yl)ethylidene)-2-substituted hydrazine (8a,b)

**General procedure**
A mixture of compound 3 (0.005 mol) and phenylhydrazine derivatives (0.005 mol, 98%) in acetic acid (5 mL) was refluxed for 6 h. After cooling, ice-cold water was added and the formed solid was filtered off, washed with water, air dried and crystallized from ethanol to give compounds 8a,b (14).

1-(1-(2,7-Dibromo-4,6-dimethoxybenzofuran-5-yl)ethylidene)hydrazine (8a)

Dark yellow powder, m.p. 100–101°C, yield 85%. Analysis: for C\textsubscript{12}H\textsubscript{12}Br\textsubscript{2}N\textsubscript{2}O\textsubscript{3}, m.w. 392.04, calcd.: C 36.76, H 3.09, Br 40.76, N 7.15, O 12.24%; found: C 36.68, H 3.07, Br 40.65, N 7.10, O 12.32%. IR (KBr, cm\textsuperscript{-1}): 1618 (C=N), 3151, 3127 (NH\textsubscript{2}) and absence of CO signal. 1H-NMR (DMSO-d\textsubscript{6}, \textit{δ}, ppm): 3.90, 4.02 (s, 6H, 2OCH\textsubscript{3}), 6.80 (s, 1H furan H-3), 7.59 (s, 2H, NH\textsubscript{2}) exchanged by D\textsubscript{2}O.

(1-(2,7-Dibromo-4,6-dimethoxybenzofuran-5-yl)-3-(phenylamino)thiazolidin-4-one (9b)
To a well stirred solution of compound 8b (0.01 mol) in 50 mL of dry benzene, thioglycolic acid (0.01 mol) was added and the reaction mixture was refluxed for 5–7 h on a water bath. The reaction mixture was concentrated to 1/3 of its volume in vacuum and the precipitate formed after cooling was filtered off, dried and crystallized from ethanol to give compound 9b.

Brown powder, m.p. 150–152°C, yield 80%. Analysis: for C\textsubscript{20}H\textsubscript{18}Br\textsubscript{2}N\textsubscript{2}O\textsubscript{4}S, m.w. 542.24, calcd.: C 44.30, H 3.35, Br 29.47, N 5.17, O 11.80, S 5.91%; found: C 44.31, H 3.36, Br 30.00, N 5.10, O 11.92, S 5.86%. IR (KBr, cm\textsuperscript{-1}): 1700 (C=O), 3329 (NH), 3423 (OH). 1H-NMR (DMSO-d\textsubscript{6}, \textit{δ}, ppm): 1.70 (s, 1H, CH\textsubscript{3} of thiazolidinone), 3.90, 4.01 (s, 6H, 2OCH\textsubscript{3}), 3.86 (d, 2H, CH\textsubscript{2} of thiazolidinone), 6.82 (s, 1H furan H-3), 7.01–7.86 (m, 4H, aromatic protons), 7.01 (s, 1H, NH) exchanged by D\textsubscript{2}O.

(E)-N-(1-(2,7-dibromo-4,6-dimethoxybenzofuran-5-yl)ethylidene)-2,4-dinitroaniline (10a,b)

**General procedure**
A mixture of 3 (0.001 mol) and the appropriate amines (0.001 mol) in absolute ethanol (25 mL) was refluxed for 8 h. The solid that formed was filtered off, dried and crystallized from ethanol to give 10a and 10b.

(E)-4-(1-(2,7-Dibromo-4,6-dimethoxybenzofuran-5-yl)ethylideneamino)-1,5-di methyl-2-phenyl-1H-pyrazol-3(2H)-one (10a)

White powder, m.p. 90–92°C, yield 85%. Analysis: for C\textsubscript{23}H\textsubscript{21}Br\textsubscript{2}N\textsubscript{3}O\textsubscript{4}, m.w. 563.24, calcd.: C 49.05, H 3.76, Br 28.37, N 7.46, O 11.36%; found: C 49.21, H 3.65, Br 28.42, N 7.32, O 11.21%. IR (KBr, cm\textsuperscript{-1}): 1605 (C=N), 1699 (CO).

(E)-N-(1-(2,7-dibromo-4,6-dimethoxybenzofuran-5-yl)ethylidene)-2,4-dinitroaniline (10b)

Orange powder, m.p. 200–201°C, yield 75%. Analysis: for C\textsubscript{18}H\textsubscript{13}Br\textsubscript{2}N\textsubscript{3}O\textsubscript{7}, m.w. 543.12, calcd.: C 39.81, H 2.41, Br 29.42, N 7.74, O 20.62%; found: C 39.75, H 2.52, Br 29.53, N 7.65, O 20.55%. IR (KBr, cm\textsuperscript{-1}): 1637 (C=N) and absence of CO signal. 1H-NMR (DMSO-d\textsubscript{6}, \textit{δ}, ppm): 2.51 (s, 3H, CH\textsubscript{3}), 3.91, 4.06 (s, 6H, 2OCH\textsubscript{3}), 6.80 (s, 1H furan H-3), 7.59 (s, 2H, NH\textsubscript{2}) exchanged by D\textsubscript{2}O.

1-(2,7-Dibromo-4,6-dimethoxybenzofuran-5-yl)ethylidene-2-phenyldiazine (8b)

Pale brown powder, m.p. 120–122°C, yield 80%. Analysis: for C\textsubscript{21}H\textsubscript{14}Br\textsubscript{2}N\textsubscript{2}O\textsubscript{2}, m.w. 468.14, calcd.: C 46.18, H 3.44, Br 34.14, N 5.98, O 10.25%; found: C 46.23, H 3.53, Br 34.23, N 6.11, O 10.31%. IR (KBr, cm\textsuperscript{-1}): 1600 (C=N), 3262 (NH) and absence of CO signal.

(E)-1-(2,7-Dibromo-4,6-dimethoxybenzofuran-5-yl)-2-methyl-1-(phenylamino)thiazolidin-4-one (9b)

To a well stirred solution of compound 8b (0.01 mol) in 50 mL of dry benzene, thioglycolic acid (0.01 mol) was added and the reaction mixture was refluxed for 5–7 h on a water bath. The reaction mixture was concentrated to 1/3 of its volume in vacuum and the precipitate formed after cooling was filtered off, dried and then crystallized from the proper solvent to give compounds (IIa–c).
(E)-1-(2,7-Dibromo-4,6-dimethoxybenzofuran-5-yl)-3-(4-hydroxy-3-methoxyphenyl)prop-2-en-1-one (11a)

Crystallized from propan-2-ol, brown powder, m.p. 90–92°C, yield 85%. Analysis: for C_{20}H_{16}Br_{2}O_{6}, m.w. 512.15, calcd.: C 46.90, H 3.15, Br 31.20, O 18.74%; found: C 46.87, H 3.21, Br 31.07, O 18.85%. IR (KBr, cm^{-1}): 1667 (C=O), 3433 (OH).

\[ \delta, \text{ppm} \]
- 3.71, 3.92, 4.03 (s, 9H, \text{3OCH}_3), 5.36 (s, 1H, \text{OH}) exchanged by D\text{2O}, 6.74 (s, 1H, \text{furan H-3}), 6.84–7.39 (m, 5H, aromatic protons + 2 olefinic protons).

(E)-1-(2,7-Dibromo-4,6-dimethoxybenzofuran-5-yl)-3-phenylprop-2-en-1-one (11b)

Crystallized from propan-2-ol, brown powder, m.p. 214–216°C, yield 80%. Analysis: for C_{19}H_{14}Br_{2}O_{4}, m.w. 466.12, calcd.: C 48.96, H 3.03, Br 34.28, O 13.73%; found: C 48.85, H 4.12, Br 34.34, O 13.65%. \[ \delta, \text{ppm} \]
- 3.92, 4.04 (s, 6H, \text{2OCH}_3), 6.86 (s, 1H, \text{furan H-3}), 7.33–8.06 (m, 7H, aromatic protons + 2 olefinic protons).

(E)-1-(2,7-Dibromo-4,6-dimethoxybenzofuran-5-yl)-3-(3-methoxyphenyl)prop-2-en-1-one (11c)

Crystallized from propan-2-ol, pale yellow powder, m.p. > 300°C, yield 80%. Analysis: for C_{20}H_{16}Br_{2}O_{5}, m.w. 496.15, calcd.: C 48.42, H 3.25, Br 32.21, O 16.12%; found: C 48.54, H 3.34, Br 32.11, O 16.00%. \[ \delta, \text{ppm} \]
- 3.75, 3.92, 4.01 (s, 9H, \text{3OCH}_3), 6.91 (s, 1H, \text{furan H-3}), 7.61–8.06 (m, 6H, aromatic protons + 2 olefinic protons).

4-(2,7-Dibromo-4,6-dimethoxybenzofuran-5-yl)-6-(substituted)pyrimidine-2(1H)-thione (12a-c)

General procedure

A mixture of compound 11a-c (0.001 mol), thiourea (0.001 mol) and sodium hydroxide (0.1 g) in 25 mL of 80% ethanol was refluxed for 6 h. The reaction mixture was then concentrated under vacuum, cooled and neutralized with ammonium hydroxide. The formed solid was filtered off, washed with water, dried and then crystallized from proper solvent to give compounds 12a-c.

4-(2,7-Dibromo-4,6-dimethoxybenzofuran-5-yl)-6-(4-hydroxy-3-methoxyphenyl)pyrimidine-2(1H)-thione (12a)

Crystallized from propan-2-ol, white powder, m.p. 140–141°C, yield 75%. Analysis: for C_{21}H_{16}Br_{2}N_{2}O_{5}S, m.w. 568.26, calcd.: C 48.43, H 2.84, Br 28.12, N 4.93, O 14.08, S 5.64%; found: C 48.43, H 2.75, Br 28.23, N 4.87, O 14.12, S 5.54%. IR (KBr, cm^{-1}): 1637 (C=N), 3350–3500 (OH), 3153 (NH), 1325 (C=S) and absence of CO signal. \[ \delta, \text{ppm} \]
- 3.88, 3.91, 4.05 (s, 9H, \text{3OCH}_3), 5.72 (s, 1H, \text{OH}) exchanged by D\text{2O}, 6.84 (s, 1H, CH of pyrimidine), 6.94 (s, 1H, CH of pyrimidine), 8.46 (s, 1H, NH) exchanged by D\text{2O}.

4-(2,7-Dibromo-4,6-dimethoxybenzofuran-5-yl)-6-phenylpyrimidine-2(1H)-thione (12b)

Crystallized from propan-2-ol, white powder, m.p. 110–112°C, yield 80%. Analysis: for C_{20}H_{14}Br_{2}N_{2}O_{3}S, m.w. 522.21, calcd.: C 46.00, H 2.65, Br 30.55, N 0.98, S 6.29%. IR (KBr, cm^{-1}): 1609 (C=N), 3350–3500 (OH), 3368 (NH), 1324 (C=S) and absence of CO signal. \[ \delta, \text{ppm} \]
- 3.87, 4.04 (s, 6H, \text{2OCH}_3), 6.84 (s, 1H, CH of pyrimidine), 7.43–8.55 (m, 5H, aromatic protons), 8.62 (s, 1H, NH) exchanged by D\text{2O}.

4-(2,7-Dibromo-4,6-dimethoxybenzofuran-5-yl)-6-(3-methoxyphenyl)pyrimidine-2(1H)-thione (12c)

Crystallized from propan-2-ol, brown powder, m.p. 95–97°C, yield 75%. Analysis: for C_{21}H_{16}Br_{2}N_{2}O_{4}S, m.w. 552.24, calcd.: C 45.67, H 2.65, Br 30.55, N 0.98, S 5.76%. IR (KBr, cm^{-1}): 1606 (C=N), 3147 (NH), 1325 (C=S) and absence of CO signal.

Ethyl 2-amino-6-(2,7-dibromo-4,6-dimethoxybenzofuran-5-yl)-4-(substituted)-4H-pyran-3-carboxylate (13a,b)

General procedure

One mol of compound 11a or 11b and (1.0 mol) of butyric cyanoanhydride in 50 mL of pyridine were refluxed for 12 h, then the reaction mixture was poured into ice water and neutralized with hydrochloric acid, then filtered and crystallized from the appropriate solvent to give compounds 13a,b.

Ethyl 2-amino-6-(2,7-dibromo-4,6-dimethoxybenzofuran-5-yl)-4-(4-hydroxy-3-methoxyphenyl)-4H-pyran-3-carboxylate (13a)

Crystallized from propan-2-ol, pale yellow powder, m.p. 100–102°C, yield 85%. Analysis: for C_{25}H_{23}Br_{2}N_{2}O_{8}, m.w. 625.26, calcd.: C 45.76, H 3.12, Br 30.87, N 4.89, S 5.76%. IR (KBr, cm^{-1}): 1701 (COOC\text{2H}_5), 3375 (OH), 3129, 3153 (NH\text{2}) and absence of CO signal.
absence of CO signal. \( ^1H\)-NMR (DMSO-d\(_6\), \( \delta \), ppm): 1.37 (t, 3H, CH\(_3\)), 3.88, 3.98, 4.06 (s, 9H, 3OCH\(_3\)), 4.33 (q, 2H, CH\(_2\)), 4.41 (s, 1H, CH of pyran), 5.72 (s, 1H, OH) exchanged by D\(_2\)O, 6.86 (s, 1H furan H-3), 6.98–7.85 (m, 3H, aromatic protons), 8.14 (s, 2H, NH\(_2\)) exchanged by D\(_2\)O.

Ethyl 2-amino-6-(2,7-dibromo-4,6-dimethoxybenzofuran-5-yl)-4-phenyl-4H-pyran-3-carboxylate (13b)

Crystallized from propan-2-ol, pale yellow powder, m.p. 107–108°C, yield 70%. Analysis: for C\(_{24}\)H\(_{21}\)Br\(_2\)NO\(_6\), m.w. 579.23, calcd.: C 49.77, H 3.65, Br 27.59, N 2.42, O 16.57%; found: C 49.64, H 3.87, Br 27.87, N 2.22, O 16.42%. IR (KBr, cm\(^{-1}\)): 1700 (COOC\(_2\)H\(_5\)), 3132, 3389 (NH\(_2\)) and absence of CO signal. \( ^1H\)-NMR (DMSO-d\(_6\), \( \delta \), ppm): 1.26 (t, 3H, CH\(_3\)), 3.83, 4.07 (s, 6H, 2OCH\(_3\)), 4.23 (q, 2H, CH\(_2\)), 4.41 (s, 1H, CH of pyran), 6.86 (s, 1H furan H-3), 6.99–7.50 (m, 5H, aromatic protons), 7.65 (s, 2H, NH\(_2\)) exchanged by D\(_2\)O.

(Z)-Ethyl 2-amino-6-(2,7-dibromo-4,6-dimethoxybenzofuran-5-yl)-N-(2,4-dinitrophenyl)-4-(4-hydroxy-3-methoxyphenyl)-4H-pyran-3-carboximidate (14a)

A mixture of 3 (0.01 mol) and 2,4-dinitroaniline (0.01 mol) in absolute ethanol (25 mL) was refluxed for 8 h. The solid formed was filtered off, dried and crystallized from ethanol to give 14a. Brown powder, m.p. 145–147°C, yield 75%. Analysis: for C\(_{29}\)H\(_{22}\)Br\(_2\)N\(_4\)O\(_{11}\), m.w. 790.37, calcd.: C 45.69, H 2.91, Br 20.96, N 7.35, O 23.09%; found: C 46.03, H 3.00, Br 20.12, N 6.97, O 22.03%. IR (KBr, cm\(^{-1}\)): 3396 (OH), 3343, 3456 (NH\(_2\)), 1660 (C=O) and absence of COOC\(_2\)H\(_5\) signal.

3-(2,7-Dibromo-4,6-dimethoxybenzofuran-5-yl)-5-(substituted)-4,5-dihydroisoxazole (15a,b)

General procedure

A mixture of compound 11a or 11b (0.005 mol) and hydroxylamine hydrochloride (0.005 mol) in sodium hydroxide solution (0.5 g NaOH in 2.5 mL of water) in ethanol (60 mL) was refluxed for 6–8 h. After cooling, the reaction mixture was diluted with ice-cold water and the formed solid was filtered off, washed with water, air dried and crystallized from ethanol (14).

4-(3-(2,7-Dibromo-4,6-dimethoxybenzofuran-5-yl)-4,5-dihydroisoxazol-5-yl)-2-methoxyphenol (15a)

Yellow powder, m.p. 185–187°C, yield 75%. Analysis: for C\(_{20}\)H\(_{17}\)O\(_3\)N\(_3\)S, m.w. 527.16, calcd.: C 45.57, H 3.25, Br 30.31, N 2.66, O 18.21%; found: C 45.39, H 3.00, Br 30.45, N 2.75, O 18.41%. IR (KBr, cm\(^{-1}\)): 3262 (OH), 1630 (C=N) and absence of CO signal. \( ^1H\)-NMR (DMSO-d\(_6\), \( \delta \), ppm): 3.80 (d, 2H, CH\(_2\)), 3.92, 3.97, 4.01 (s, 9H, 3OCH\(_3\)), 6.91 (s, 1H furan H-3).
C₃H₂Br₂N₄O₁₁, m.w. 790.37, calcd.: C 53.98, H 3.62, Br 28.73, O 8.63%; found: C 53.76, H 3.49, Br 28.55, O 8.39%. IR (cm⁻¹): 1598 cm⁻¹ (C=N), and absence of CO signal.

3-(2,7-Dibromo-4,6-dimethoxybenzofuran-5-yl)-5-(3-methoxyphenyl)-1-phenyl-4,5-dihydro-1H-pyrazole (17c)

Crystallized from propan-2-ol, white powder, m.p. 125–127°C, yield 70%. Analysis: for C₂₆H₂₂Br₂N₂O₄, m.w. 586.27, calcd.: C 53.27, H 3.78, Br 27.26, N 4.78, O 10.92%; found: C 53.42, H 3.54, Br 27.44, N 4.86, O 11.04%. IR (KBr, cm⁻¹): 1599 (C=N) and absence of CO signal.

**Anticancer testing**

**Measurement of potential cytotoxicity by SRB assay**

The selected benzofuran derivatives (compounds 2–5a, 6a, 8a,b–9b, 10a,b–11a, 12a,c, 13a,b, 14a, 15a, 16c, 17b,c) were subjected to a screening system for evaluation of their antitumor activity against liver HEPG2 cancer cell lines in comparison to the known anticancer drugs: 5-FU and DOX. Potential cytotoxicity of the selected coumarin derivatives was tested using the method of Skehan et al. (15) as follows:

Cells were plated in 96-multiwell plate (10⁴ cells/well) for 24 h before treatment with the compound(s) to allow attachment of cells to the wall of the plate. Different concentrations of the compounds under test (0, 1, 2.5, 5, 10 µg/mL) were added to the cell monolayer. Triplicate wells were prepared for each individual dose. Monolayer cells were incubated with the compound(s) for 48 h at 37°C and in an atmosphere of 5% CO₂. Cultures were then fixed with trichloroacetic acid and stained for 30 min with 0.4% (w/v) sulforhodamine B (SRB) dissolved in 1% acetic acid. Unbound dye was removed by four washes with 1% acetic acid, and protein-bound dye was extracted with 10 mM unbuffered Tris base [tris(hydroxymethyl)amino methane] for determination of optical density in a computer-interfaced, 96-well micro titer plate reader. The SRB assay results were linear with the number of cells and with values for cellular protein measured by both the Lowry and Bradford assays at densities ranging from sparse subconfluence to multilayered supraconfluence. The signal-to-noise ratio at 564 nm was approximately 1.5 with 1,000 cells per well. The relation between surviving fraction and drug concentration was plotted to get the survival curve of both cancer cell lines after the specified compound.

**Antimicrobial testing**

Müller Hinton agar plates were inoculated with bacterial strains while Sabouraud dextrose agar plates were inoculated with fungal strains prepared in conc. equivalent with 0.5 MacFarland for bacterial strains and 2 ×10⁰ and streaked onto the agar plates using sterile swabs, and then 50 µg of the dissolved compound in DMSO were placed into the wells under sterile conditions. All plates were incubated at 37°C/24 h for bacterial growth and at 28°C/48–72 h for fungal growth. Zone of inhibition were measured in mm using a ruler. The experiment was carried out in duplicate and the mean of the zone of inhibition was tabulated in Tables 2 and 3.

**RESULTS AND DISCUSSION**

**Chemistry**

The present work deals with the synthesis of some benzopyran derivatives derived from the naturally occurring visnaginone of expected anticancer and antibacterial activities.

### Table 1. Effect of some selected newly synthesized compounds on liver carcinoma cell line (HEPG2).

<table>
<thead>
<tr>
<th>Compound</th>
<th>IC₅₀ [µM]</th>
</tr>
</thead>
<tbody>
<tr>
<td>5-Fluorouracil</td>
<td>0.0384</td>
</tr>
<tr>
<td>Doxorubicin</td>
<td>0.00654</td>
</tr>
<tr>
<td>2</td>
<td>0.0102</td>
</tr>
<tr>
<td>3</td>
<td>0.0224</td>
</tr>
<tr>
<td>4</td>
<td>0.0147</td>
</tr>
<tr>
<td>5a</td>
<td>0.0114</td>
</tr>
<tr>
<td>6a</td>
<td>0.0061</td>
</tr>
<tr>
<td>8a</td>
<td>0.0208</td>
</tr>
<tr>
<td>8b</td>
<td>0.0182</td>
</tr>
<tr>
<td>9b</td>
<td>0.0121</td>
</tr>
<tr>
<td>10a</td>
<td>0.0166</td>
</tr>
<tr>
<td>10b</td>
<td>0.0176</td>
</tr>
<tr>
<td>11a</td>
<td>0.0145</td>
</tr>
<tr>
<td>12a</td>
<td>0.0114</td>
</tr>
<tr>
<td>12c</td>
<td>0.0124</td>
</tr>
<tr>
<td>13a</td>
<td>0.0065</td>
</tr>
<tr>
<td>13b</td>
<td>0.0122</td>
</tr>
<tr>
<td>14a</td>
<td>0.0066</td>
</tr>
<tr>
<td>15a</td>
<td>0.0156</td>
</tr>
<tr>
<td>16c</td>
<td>0.0070</td>
</tr>
<tr>
<td>17b</td>
<td>0.0054</td>
</tr>
<tr>
<td>17c</td>
<td>0.0159</td>
</tr>
</tbody>
</table>

IC₅₀ = dose of the compound which reduces survival by 50%.
When the naturally occurring visnagin was heated with potassium hydroxide it yielded 4-methoxy-7-methyl-5H-furo[3,2-g]chromen-5-one visnaginone (1). When 1 reacted with bromine, it gave the dibromo derivative 1-(2,7-dibromo-6-hydroxy-4-methoxybenzofuran-5-yl)ethanone (2). The derivative 2 was methylated using methyl iodide in acetone to give 1-(2,7-dibromo-4,6-dimethoxybenzofuran-5-yl)ethanone (3). When 3 was condensed with dimethylformamide dimethylacetal it gave (E)-1-(2,7-dibromo-4,6-dimethoxybenzofuran-5-yl)-3-(dimethylamino)prop-2-en-1-one (4).

Reaction of 3 with aromatic aldehydes, namely: vanillin, benzaldehyde and 3-anisaldehyde in ammonium acetate, malononitrile and/or butyric cyanoanhydride gave 2-amino-6-(2,7-dibromo-4,6-dimethoxybenzofuran-5-yl)-4-(substituted)nicotinonitriles (5a-c) and 6-(2,7-dibromo-4,6-dimethoxybenzofuran-5-yl)-2-hydroxy-4-(substituted)nicotinonitriles (7a-c), respectively. 5a was hydrolyzed with sulfuric acid on cold to give 2-amino-6-(2,7-dibromo-4,6-dimethoxybenzofuran-5-yl)-4-(3-hydroxy-4-methoxyphenyl)nicotinic acid (6a). The structures of compounds 5a-c and 7a-c were assigned based on the analogy with the structure referred to in previous work (16, 17).

When the dimethoxybenzofuran derivative 3 reacted with hydrazine hydrate and phenylhydrazine it yielded the hydrazine derivatives 8a and 8b, respectively. On the other hand, 8b reacted with thioglycolic acid to give the thiazolidinone derivative 9b.

When 3 was heated with aromatic amines, namely: 4-aminoantipurin and 2,4-dinitroaniline, it
gave the Schiff bases (E)-N-(1-(2,7-dibromo-4,6-dimethoxybenzofuran-5-yl)ethylidene)-2,4-dinitroaniline (10a, b) (Scheme 1).

The reaction of 3 with the previously used aromatic aldehydes under different conditions gave the Schiff bases (11a-c). When compounds 11a-c reacted with thiourea and sodium hydroxide they gave the pyrimidines (12a-c). Compounds 11a, b reacted with butyric cyanoanhydride in pyridine and gave ethyl 2-amino-6-(2,7-dibromo-4,6-dimethoxybenzofuran-5-yl)-4-(substituted)-4H-pyran-3-carboxylate (13a, b).

The carboxylate ester 13a reacted with 2,4-dinitroaniline in ethanol to form the amide 14a.

Compounds 11a and 11b also reacted with hydroxylamine hydrochloride to give 3-(2,7-dibromo-4,6-dimethoxybenzofuran-5-yl)-5-(substituted)-4,5-dihydroisoxazole (15a, b).

On the other hand, when compounds 11b and 11c were allowed to react with hydrazine hydrate...
and phenylhydrazine, they yielded compounds 16b,c and 17b,c, respectively (Scheme 2).

**Anticancer activity**

The selected compounds showed reasonable antitumor activity in comparison to 5-FU and DOX. Cytotoxic drugs remain the mainstay of cancer chemotherapy and are being administered with novel ways of therapy such as inhibitors of signals (18). It is therefore important to discover novel cytotoxic agents with spectra of activity and toxicity that differ from those current agents (19). It is well known that chemotherapy aims to destroy the cancer cells with various types of chemicals (20). The substances used are supposed to target mainly the cancer cells and doses are calculated to minimize the collateral damage to surrounding tissues, which nevertheless occurs (21). This kind of treatment increases the entropy of the organism, suppresses the immune system, and forms a toxic cell environment which may destroy surrounding healthy cells. It is important to use minimum effective doses in a hope to minimize the side effects of chemotherapeutic drugs.

Preliminary screening of the selected benzofuran derivatives showed that all selected compounds exhibited a moderate to strong growth inhibition activity on the tested cell line between 1–10 µg/mL.

### Table 2. Antibacterial activity of chemical compounds.

| Tested strains → |
|------------------|------------------|------------------|------------------|------------------|------------------|------------------|------------------|------------------|
| Tested chemicals |
| S. typhi-         | L. Monocytogenes | S. aureus        | P. aeruginosa    | B. cereus        | E. coli          | Mean zone of inhibition |
| O119 (0.824 µM)  | 15 13 12 13 18 14 14.2 |
| 5a (0.521 µM)    | 18 15 20 13 21 18 17.5 |
| 10a (0.532 µM)   | 23 17 15 20 18 24 19.5 |
| 8b (0.640 µM)    | 28 17 16 10 16 18 17.5 |
| 12c (0.511 µM)   | 12 17 16 10 16 14 14.2 |
| 17b (0.543 µM)   | 28 23 15 12 24 22 20.7 |
| 4 (0.692 µM)     | 13 17 15 14 17 12 14.7 |
| 7a (0.520 µM)    | 18 25 16 12 13 18 17.0 |
| 3 (0.793 µM)     | 21 18 18 10 18 13 16.3 |
| 10b (0.552 µM)   | 30 34 28 14 20 30 26.0 |
| Tobramycin       | 22 20 23 21 20 23 21.38 |
| Flumox           | 19 20 19 19 18 19 18.75 |
| DMSO             | - - - - - - - |

### Table 3. Antifungal activity of five chemical compounds.

<table>
<thead>
<tr>
<th>Tested chemical</th>
<th>C. albicans</th>
<th>A. flavus</th>
<th>Mean zone of inhibition [mm]</th>
</tr>
</thead>
<tbody>
<tr>
<td>10a (0.532 µM)</td>
<td>20 39</td>
<td>29.5</td>
<td></td>
</tr>
<tr>
<td>8b (0.640 µM)</td>
<td>- 60</td>
<td>30</td>
<td></td>
</tr>
<tr>
<td>12c (0.511 µM)</td>
<td>- 48</td>
<td>24</td>
<td></td>
</tr>
<tr>
<td>3 (0.793 µM)</td>
<td>10 12</td>
<td>11</td>
<td></td>
</tr>
<tr>
<td>10b (0.552 µM)</td>
<td>- 40</td>
<td>20</td>
<td></td>
</tr>
<tr>
<td>Fluconazole</td>
<td>17 19</td>
<td>18</td>
<td></td>
</tr>
<tr>
<td>DMSO</td>
<td>- -</td>
<td>-</td>
<td></td>
</tr>
</tbody>
</table>

− means no inhibition for a microbe by the tested compound.
concentrations in comparison to the known anticancer drugs: 5-fluorouracil and doxorubicin. Table 1 indicated the cytotoxic activity of the newly synthesized benzofuran derivatives (compounds 2–5a, 6a, 8a–b, 9a, 10a–b, 11a, 12a,c, 13a,b, 14a, 15a, 16c, 17b,c) on liver HepG2 cancer cell lines in comparison to the traditional anticancer drugs: 5-FU and DOX. It can be deduced from the results that compounds 2, 6a, 13a, 14a, 16c and 17b were the most active and induced a reasonable growth inhibition, in a dose-dependent manner against HepG2 when compared to 5-FU and DOX (IC$_{50}$ equals 0.0102, 0.0061, 0.0065, 0.0066, 0.0070 and 0.0054 µM, while for 5-FU and DOX were 0.0384 and 0.0065 µM).

Novel derivatives of benzofuran possessing a broader spectrum of antitumor activity and fewer toxic side effects than 5-FU and DOX have been sought. The antitumor activities of such compounds were assessed against HepG2 cancer cell line in comparison to the traditional anticancer drugs: 5-FU and DOX.

**Antimicrobial activity**

Results revealed that compound 10b (0.552 µM) gives the highest antibacterial activity against all tested strains with a mean zone of inhibition equal to 26.0 mm followed by 17c (0.543 µM); 20.7 mm, then 10a (0.532 µM); 19.5 mm, 5a (0.521 µM) and 8b (0.640 µM); 17.5 mm each and finally 7a (0.520 µM); 17.0 mm and 3 (0.793 µM) and 17b (0.379 µM); 16.3 and 16.2 mm, respectively, and 4 (0.692 µM) giving zone of inhibition 14.7 mm, then 12c (0.511 µM) and 2 (0.824 µM) with zone of inhibition 14.2 mm each as shown in Table 2.

Results revealed that compound 8b (0.640 µM) gives the highest antifungal activity against all tested strains with a mean zone of inhibition equal to 30 mm followed by 10a (0.532 µM), 29.5 mm, then 12c (0.511 µM), 24 mm and 10b (0.552 µM), 20 mm and finally 3 (0.793 µM), 11 mm keeping in consideration that the tested compounds give higher hindrance activity against A. flavus than against C. albicans as shown in Table 3.

Antimicrobial activity revealed that the tested compounds have better antimycotic activities than the reference drug – fluconazole.

**REFERENCES**


Received: 17. 04. 2011