

Synthesis, antimicrobial, and antiproliferative activities of substituted phenylfuranylnicotinamidines

Magdy M Youssef^{1,2}

Reem K Arafa^{3,4}

Mohamed A Ismail^{1,2}

¹Department of Chemistry, College of Science, King Faisal University, Hofuf, Saudi Arabia; ²Department of Chemistry, Faculty of Science, Mansoura University, Mansoura,

³Department of Pharmaceutical Chemistry, Faculty of Pharmacy, Cairo University, Cairo, ⁴Biomedical Sciences Program, University of Science and Technology, Zewail City of Science and Technology, Cairo, Egypt

Abstract: This research work deals with the design and synthesis of a series of substituted phenylfuranylnicotinamidines **4a–i**. Facile preparation of the target compounds was achieved by Suzuki coupling-based synthesis of the nitrile precursors **3a–i**, followed by their conversion to the corresponding nicotinamidines **4a–i** utilizing $\text{LiN}(\text{TMS})_2$. The antimicrobial activities of the newly synthesized nicotinamide derivatives were evaluated against the Gram-negative bacterial strains *Escherichia coli* and *Pseudomonas aeruginosa* as well as the Gram-positive bacterial strains *Staphylococcus aureus* and *Bacillus megaterium*. The minimum inhibitory concentration values of nicotinamidines against all tested microorganisms were in the range of 10–20 μM . In specific, compounds **4a** and **4b** showed excellent minimum inhibitory concentration values of 10 μM against *Staphylococcus aureus* bacterial strain and were similar to ampicillin as an antibacterial reference. On the other hand, selected nicotinamide derivatives were biologically screened for their cytotoxic activities against a panel of 60 cell lines representing nine types of human cancer at a single high dose at National Cancer Institute, Bethesda, MD, USA. Nicotinamidines showing promising activities were further assessed in a five-dose screening assay to determine their compound concentration causing 50% growth inhibition of tested cell (GI_{50}), compound concentration causing 100% growth inhibition of tested cell (TGI), and compound concentration causing 50% lethality of tested cell (LC_{50}) values. Structure-activity relationship studies demonstrated that the activity of members of this series can be modulated from cytostatic to cytotoxic based on the substitution pattern/nature on the terminal phenyl ring. The most active compound was found to be **4e** displaying a submicromolar GI_{50} value of 0.83 μM , with TGI and LC_{50} values of 2.51 and 100 μM , respectively. Finally, the possible underlying mechanism of action of this series of compounds was investigated by determining their nuclease-like DNA degradation ability in addition to their antioxidant power and all monocations proved to be effective in all assays.

Keywords: substituted phenylfuranylnicotinamidines, Suzuki coupling, antiproliferative, antibacterial, antioxidant

Introduction

Over the past few years, the development of bacterial resistance to antibiotics has been reported and resistance to multiple antimicrobial agents has become a major health problem.¹ A continuous discovery of new anti-infectious agents is urgently needed owing to the progress of antibiotic resistance in almost all clinically significant pathogens.² Cancer is a disease that still intrigues the scientific community stimulating their interest in finding novel chemotherapeutic agents effective for the management and remediation of this life-threatening ailment.³ Many factors compromise the rate of success of cancer chemotherapy, with the emergence of drug resistance to currently clinically useful agents being the most worrisome in the fight against cancer.⁴ Heterocycle-based chemical entities have been explored as backbone scaffolds for the design of novel antiproliferative

Correspondence: Mohamed A Ismail
Department of Chemistry, Faculty of
Science, Mansoura University, Mansoura
35516, Egypt
Tel +20 10 9684 9505
Email mabouelenan@kfu.edu.sa



agents. Pyridine heterocycles are of particular interest because in this class of compounds, the pyridine ring mostly contributes an effective role in the pharmacokinetic and/or the pharmacodynamic properties of these bioactive molecules. Literature has demonstrated the ability of synthetic pyridine-containing compounds to elicit a wide spectrum of biological activities with emphasis on their usefulness as anticancer therapeutic agents.⁵ Furthermore, furan-containing molecules are found in both natural products and pharmaceuticals; for example, Zantac is one of the most widespread drugs, as well as being on the World Health Organization's list of essential medicines.⁶ On the other hand, the dicationic compound pentamidine has been known for its antimicrobial activity for over 50 years and remains the only aromatic diamidine that is still in clinical use.^{7,8} However, recent studies have shown that pentamidine can also act as an anticancer agent eliciting variable modes of action.^{9,10} Also, furamidine, the furanyl analog of pentamidine, was found to elicit an anticancer activity.^{11,12} Aza-furamidine derivatives have been launched as potent antiprotozoal agents;¹³ from this aza-furamidine series, 2,5-bis(5-amidino-2-pyridyl)furan (DB829; CPD-0802) is a preclinical candidate for treatment of second stage human African trypanosomiasis.¹⁴⁻¹⁷ Recently, several aryl-2,2'-bichalcophene monoamidines, including their aza-analogs, were synthesized and shown to exhibit potent antibacterial,¹⁸⁻²⁰ antimutagenic,^{21,22} and anticancer activities.²³

As part of a research program directed to drug discovery and due to the promising anticancer activities of our recently reported aromatic monoamidines, we have decided to design and synthesize a series of furanylnicotinamidines derivatives,

including phenyl, monosubstituted phenyl, and disubstituted phenyl ring analogs. Molecular manipulations have also included both electron donating and electron withdrawing substitutions. The newly synthesized nicotinamidines and one nicotinonitrile compound have been subjected to in vitro cytotoxic evaluation against a panel of 60 cell lines representing nine types of cancer. Finally in this report, antibacterial, DNA cleavage, and antioxidant activities of these novel compounds have also been carried out.

Experimental section

Chemistry

Melting points were recorded using a Gallenhampt melting point apparatus and are uncorrected. Thin-layer chromatography analysis was carried out on silica gel 60 F₂₅₄ precoated aluminum sheets and detected under ultraviolet light. Infrared (IR) spectra were recorded using KBr wafer technique on a Shimadzu 5800 Fourier transform (FT)-IR spectrometer. ¹H NMR spectra were recorded employing a Varian Mercury VX-300 spectrometer and ¹³C NMR were recorded employing Bruker Avance 400 MHz spectrometer, and chemical shifts (δ) were in parts per million relative to that of the solvent. Mass spectra were recorded on a gas chromatography mass spectrometry (Shimadzu Qp-2010 Plus) spectrometer. Elemental analyses were performed at the microanalytical laboratories of the Faculty of Science, Cairo University, Cairo, Egypt, on a Perkin-Elmer 2400 analyzer and are within ± 0.4 of the theoretical values (Table 1). 6-(5-Bromofuran-2-yl)nicotinonitrile was prepared as per the reported literature method.¹³

Table 1 Elemental analysis data

Compound	Calculated			Found		
	C	H	N	C	H	N
3a (C ₁₆ H ₁₀ N ₂ O)	78.03	4.09	11.38	77.81	4.17	11.19
3b (C ₁₇ H ₁₂ N ₂ O ₂)	73.90	4.38	10.14	73.84	4.45	10.01
3c (C ₁₈ H ₁₄ N ₂ O ₃)	70.58	4.61	9.15	70.31	4.49	9.09
3d (C ₁₈ H ₁₅ N ₃ O)	74.72	5.23	14.52	74.64	5.31	14.59
3e (C ₁₆ H ₉ ClN ₂ O)	68.46	3.23	9.98	68.61	3.14	10.12
3f (C ₁₆ H ₉ FN ₂ O)	72.72	3.43	10.60	72.57	3.50	10.47
3g (C ₁₆ H ₈ Cl ₂ N ₂ O)	60.98	2.56	8.89	61.12	2.49	8.93
3h (C ₁₆ H ₈ F ₂ N ₂ O)	68.09	2.86	9.93	68.24	2.71	9.79
3i (C ₁₆ H ₈ F ₂ N ₂ O)	68.09	2.86	9.93	67.82	2.89	10.01
4a (C ₁₆ H ₁₃ N ₃ O-2.0 HCl)	57.16	4.50	12.49	57.29	4.63	12.22
4b (C ₁₇ H ₁₅ N ₃ O ₂ -2.0 HCl-0.75 H ₂ O)	53.76	4.91	11.06	53.88	4.83	11.27
4c (C ₁₈ H ₁₇ N ₃ O ₃ -2.0 HCl-1.0 H ₂ O)	52.18	5.11	10.14	52.24	4.94	10.35
4d (C ₁₈ H ₁₈ N ₄ O-3.0 HCl-1.5 H ₂ O)	48.82	5.46	12.65	48.50	5.23	12.37
4e (C ₁₆ H ₁₂ ClN ₃ O-2.0 HCl)	51.84	3.81	11.33	52.17	3.89	11.06
4f (C ₁₆ H ₁₂ FN ₃ O-2.0 HCl-0.5 H ₂ O)	52.90	4.16	11.56	52.70	4.31	11.56
4g (C ₁₆ H ₁₁ Cl ₂ N ₃ O-2.0 HCl-0.75 H ₂ O)	45.90	3.49	10.04	46.10	3.46	10.20
4h (C ₁₆ H ₁₁ F ₂ N ₃ O-2.0 HCl-0.5 H ₂ O)	50.41	3.70	11.02	50.55	3.91	11.21
4i (C ₁₆ H ₁₁ F ₂ N ₃ O-2.0 HCl-0.5 H ₂ O)	50.41	3.70	11.02	50.42	3.78	11.03

The experiments were conducted under the approval of Deanship of Scientific Research, King Faisal University according to the university regulations (Project No. 150122).

Preparation of (substituted) phenylfuranlynicotinonitrile derivatives **3a-i**

6-(5-Phenylfuran-2-yl)nicotinonitrile (3a): To a stirred solution of 6-(5-bromofuran-2-yl)nicotinonitrile (1.24 g, 5 mmol) and Pd(PPh₃)₄ (150 mg) in toluene (20 mL) was added an aqueous solution of Na₂CO₃ (2 M, 5 mL), followed by phenylboronic acid (0.73 g, 6 mmol) in methanol (5 mL). The stirred reaction mixture was warmed at 80°C for 12 hours, after which the reaction mixture was partitioned between an aqueous solution containing concentrated ammonia (5 mL) and ethyl acetate (250 mL, 3×). The organic layer was dried (anhydrous Na₂SO₄), and then evaporated to dryness under reduced pressure to furnish the nicotinonitrile derivative **3a** as a pale-yellow solid in 81% yield, melting point (mp) 187°C–187.5°C (EtOH/DMF). Rate of flow (R_f)=0.54, petroleum ether (60°C–80°C)-EtOAc (8:2). IR (KBr) ν̄ 3,050, 3,031 (CH), 2,224 (CN), 1,598, 1,586, 1,551 (C=C) cm⁻¹. ¹H NMR (dimethylsulfoxide [DMSO]-d₆); δ 7.25 (d, J=3.3 Hz, 1H), 7.38–7.51 (m, 4H), 7.89–7.92 (m, 2H), 8.07 (d, J=8.4 Hz, 1H), 8.36 (dd, J=8.4, 2.4 Hz, 1H), 9.01 (d, J=2.4 Hz, 1H). ¹³C NMR (DMSO-d₆); δ 106.8, 109.5, 115.3, 117.8, 118.5, 124.6, 129.1, 129.5, 129.8, 141.2, 151.1, 151.6, 153.3, 156.1. MS (EI) m/e relative intensity (rel. int.); 246 (M⁺, 100), 218 (11), 115 (46). Analysis (Anal.) (C₁₆H₁₀N₂O) C, H, N.

6-[5-(4-Methoxyphenyl)furan-2-yl]nicotinonitrile (3b): Obtained as a golden-yellow solid in 74% yield, mp 203°C–204°C, R_f=0.36, petroleum ether (60°C–80°C)-EtOAc (8:2). IR (KBr) ν̄ 3,066, 3,037, 2,983, 2,947 (CH), 2,228 (CN), 1,641, 1,615, 1,598, 1,589 (C=N, C=C) cm⁻¹. ¹H NMR (DMSO-d₆); δ 3.80 (s, 3H), 7.01 (d, J=8.4 Hz, 2H), 7.06 (d, J=3.3 Hz, 1H), 7.40 (d, J=3.3 Hz, 1H), 7.81 (d, J=8.4 Hz, 2H), 8.01 (d, J=8.4 Hz, 1H), 8.30 (dd, J=8.4, 2.4 Hz, 1H), 9.01 (d, J=2.4 Hz, 1H). MS (EI) m/e (rel. int.); 276 (M⁺, 100), 261 (64), 233 (13). Anal. (C₁₇H₁₂N₂O₂) C, H, N.

6-[5-(3,5-Dimethoxyphenyl)furan-2-yl]nicotinonitrile (3c): Obtained as a golden-yellow solid in 80% yield, mp 211°C–212.5°C. R_f=0.30, petroleum ether (60°C–80°C)-EtOAc (8:2). IR (KBr) ν̄ 3,119, 3,070, 2,993, 2,942 (CH), 2,230 (CN), 1,672, 1,591, 1,554 (C=N, C=C) cm⁻¹. ¹H NMR (DMSO-d₆); δ 3.80 (s, 6H), 6.50 (s, 1H), 7.01 (s, 2H), 7.23 (d, J=3.3 Hz, 1H), 7.39 (d, J=3.3 Hz, 1H), 8.03 (d, J=8.4 Hz, 1H), 8.31 (dd, J=8.4, 2.4 Hz, 1H), 8.96 (d, J=2.4 Hz, 1H). MS (EI) m/e (rel. int.); 306 (M⁺, 100), 277 (6). Anal. (C₁₈H₁₄N₂O₃) C, H, N.

6-[5-(4-(Dimethylamino)phenyl)furan-2-yl]nicotinonitrile (3d): Obtained as a yellow solid in 77% yield,

mp 213°C–214°C. R_f=0.43, petroleum ether (60°C–80°C)-EtOAc (8:2). IR (KBr) ν̄ 2,893, 2,808 (CH), 2,221 (CN), 1,615, 1,596 (C=N, C=C) cm⁻¹. ¹H NMR (DMSO-d₆); δ 2.98 (s, 6H), 6.79 (d, J=8.7 Hz, 2H), 6.93 (d, J=3.6 Hz, 1H), 7.39 (d, J=3.6 Hz, 1H), 7.71 (d, J=8.7 Hz, 2H), 7.93 (d, J=8.4 Hz, 1H), 8.28 (dd, J=8.4, 2.1 Hz, 1H), 8.94 (d, J=2.1 Hz, 1H). MS (EI) m/e (rel. int.); 289 (M⁺, 100), 274 (10), 158 (34). Anal. (C₁₈H₁₅N₃O) C, H, N.

6-[5-(4-Chlorophenyl)furan-2-yl]nicotinonitrile (3e): Obtained as a yellowish-green solid in 80% yield, mp 218.5°C–220°C. R_f=0.50, petroleum ether (60°C–80°C)-EtOAc (8:2). IR (KBr) ν̄ 3,116 (CH), 2,227 (CN), 1,638, 1,589 (C=N, C=C) cm⁻¹. ¹H NMR (DMSO-d₆); δ 7.27 (d, J=3.9 Hz, 1H), 7.44 (d, J=3.9 Hz, 1H), 7.54 (d, J=8.4 Hz, 2H), 7.92 (d, J=8.4 Hz, 2H), 8.06 (d, J=8.1 Hz, 1H), 8.35 (dd, J=8.1, 2.1 Hz, 1H), 8.99 (d, J=2.1 Hz, 1H). ¹³C NMR (DMSO-d₆); δ 106.9, 110.2, 115.3, 117.8, 118.6, 126.3, 128.7, 129.6, 133.5, 141.3, 151.0, 151.9, 153.3, 154.9. MS (EI) m/e (rel. int.); 280, 282 (M⁺, 100, 34: chlorine isotopes), 252 (17), 149 (45). Anal. (C₁₆H₉ClN₂O) C, H, N.

6-[5-(4-Fluorophenyl)furan-2-yl]nicotinonitrile (3f): Obtained as a yellowish-green solid in 76% yield, mp 228°C–229.5°C. R_f=0.48, petroleum ether (60°C–80°C)-EtOAc (8:2). IR (KBr) ν̄ 3,139, 3,062 (CH), 2,229 (CN), 1,640, 1,589 (C=N, C=C) cm⁻¹. ¹H NMR (DMSO-d₆); δ 7.21 (d, J=3.6 Hz, 1H), 7.30–7.36 (m, 2H), 7.45 (d, J=3.6 Hz, 1H), 7.93–7.98 (m, 2H), 8.06 (d, J=8.1 Hz, 1H), 8.36 (dd, J=8.1, 1.8 Hz, 1H), 8.99 (d, J=1.8 Hz, 1H). MS (EI) m/e (rel. int.); 264 (M⁺, 100), 236 (23), 133 (78). Anal. (C₁₆H₉FN₂O) C, H, N.

6-[5-(3,5-Dichlorophenyl)furan-2-yl]nicotinonitrile (3g): Obtained as a brown-yellow solid in 79% yield, mp 291°C–293°C. R_f=0.57, petroleum ether (60°C–80°C)-EtOAc (8:2). IR (KBr) ν̄ 3,130, 3,060 (CH), 2,230 (CN), 1,642, 1,596 (C=N, C=C) cm⁻¹. ¹H NMR (DMSO-d₆); δ 7.47 (s, 2H), 7.59 (d, J=3.6 Hz, 1H), 7.97–8.01 (m, 2H), 8.23 (d, J=8.4 Hz, 1H), 8.37 (dd, J=8.4, 2.4 Hz, 1H), 9.01 (d, J=2.4 Hz, 1H). MS (EI) m/e (rel. int.); 314, 315, 316 (M⁺, 100, 20, 64: two chlorine isotopes), 286 (11), 103 (66). Anal. (C₁₆H₈Cl₂N₂O) C, H, N.

6-[5-(3,5-Difluorophenyl)furan-2-yl]nicotinonitrile (3h): Obtained as a yellowish-green solid in 65% yield, mp 271°C–273°C. R_f=0.50, petroleum ether (60°C–80°C)-EtOAc (8:2). IR (KBr) ν̄ 3,122, 3,091 (CH), 2,230 (CN), 1,661, 1,624, 1,589 (C=N, C=C) cm⁻¹. ¹H NMR (DMSO-d₆); δ 7.18–7.25 (m, 1H), 7.40 (d, J=3.6 Hz, 1H), 7.45 (d, J=3.6 Hz, 1H), 7.64–7.69 (m, 2H), 8.19 (d, J=8.4 Hz, 1H), 8.37 (dd, J=8.4, 2.1 Hz, 1H), 8.99 (d, J=2.1 Hz, 1H). MS (EI) m/e (rel. int.); 282 (M⁺, 31), 80 (100). Anal. (C₁₆H₈F₂N₂O) C, H, N.

6-[5-(2,4-Difluorophenyl)furan-2-yl]nicotinonitrile (3i): Obtained as a pale-yellow solid in 74% yield, mp 203°C–204.5°C. $R_f=0.52$, petroleum ether (60°C–80°C)-EtOAc (8:2). IR (KBr) ν' 3,050, 3,031 (CH), 2,224 (CN), 1,598, 1,586, 1,551 (C=C) cm^{-1} . ^1H NMR (DMSO- d_6); δ 7.06 (d, $J=3.9$ Hz, 1H), 7.23–7.49 (m, 3H), 8.07–8.15 (m, 2H), 8.38 (dd, $J=8.4, 2.1$ Hz, 1H), 9.01 (d, $J=2.1$ Hz, 1H). MS (EI) m/e (rel. int.); 282 (M^+ , 100), 254 (28), 151 (49). Anal. ($\text{C}_{16}\text{H}_8\text{F}_2\text{N}_2\text{O}$) C, H, N.

Preparation of (substituted) phenylfuranlynicotinamide derivatives 4a–i

6-(5-Phenylfuran-2-yl)nicotinamide hydrochloride salt (4a): The furanylnicotinonitrile derivative 3a (638 mg, 3 mmol) was treated with $\text{LiN}(\text{TMS})_2$ (1 M solution in tetrahydrofuran [THF], 9 mL, 9 mmol) and the reaction was permitted to stir for 12 hours. The reaction mixture was then cooled, after which hydrogen chloride ethanolic solution (25 mL, 1.25 M) was added, whereupon a precipitate started forming. The mixture was left to run for 8 hours, after which it was diluted with ether and the resultant solid was collected through filtration. The furanylnicotinamide derivative was purified by neutralization with 1 N NaOH followed by filtration of the subsequent solid and washing with water. At the end, the nicotinamide free base was stirred with hydrogen chloride ethanolic solution for 8 hours, diluted with ether, and the solid formed was filtered and dried to afford the hydrochloride salt of nicotinamide derivative **4a**, as a golden-yellow solid in 72% yield, mp 261°C–263°C. IR (KBr) ν' 3,363, 3,200 (NH, NH_2), 3,059 (C–H stretch), 1,677, 1,599, 1,547 (C=N, C=C stretch, NH bend) cm^{-1} . ^1H NMR (DMSO- d_6); δ 7.23 (d, $J=3.3$ Hz, 1H), 7.38–7.52 (m, 4H), 7.90–7.93 (m, 2H), 8.10 (d, $J=8.4$ Hz, 1H), 8.33 (dd, $J=8.4, 2.4$ Hz, 1H), 9.02 (d, $J=2.4$ Hz, 1H), 9.32 (br s, 2H, exchangeable with D_2O), 9.59 (br s, 2H, exchangeable with D_2O). ^{13}C NMR (DMSO- d_6); δ 109.4, 114.8, 118.1, 122.3, 124.6, 129.0, 129.5, 129.9, 137.7, 149.8, 151.9, 152.3, 155.9, 164.0. MS (EI) m/e (rel. int.); 263 (M^+ , 100), 246 ($M^+-\text{NH}_3$, 15), 115 (27). Anal. ($\text{C}_{16}\text{H}_{13}\text{N}_3\text{O}-2.0\text{HCl}$) C, H, N.

6-[5-(4-Methoxyphenyl)furan-2-yl]nicotinamide hydrochloride salt (4b): Obtained as an orange solid in 78% yield, mp 276°C–278°C. IR (KBr) ν' 3,300, 3,235 (NH, NH_2), 3,083, 3,011, 2,994 (C–H stretch), 1,665, 1,629, 1,602, 1,550 (C=N, C=C stretch, NH bend) cm^{-1} . ^1H NMR (DMSO- d_6); δ 3.84 (s, 3H), 7.02 (d, $J=8.4$ Hz, 2H), 7.07 (d, $J=3.9$ Hz, 1H), 7.42 (d, $J=3.9$ Hz, 1H), 7.83 (d, $J=8.4$ Hz, 2H), 8.05 (d, $J=8.4$ Hz, 1H), 8.28 (dd, $J=8.4, 2.1$ Hz, 1H), 8.97 (d, $J=2.1$ Hz, 1H), 9.33 (br s, 2H, exchangeable with

D_2O), 9.62 (br s, 2H, exchangeable with D_2O). ^{13}C NMR (DMSO- d_6); δ 55.3, 107.3, 114.5, 114.9, 117.5, 121.5, 122.2, 125.8, 137.4, 148.9, 150.3, 151.4, 155.9, 159.6, 163.4. MS (EI) m/e (rel. int.); 293 (M^+ , 100), 276 ($M^+-\text{NH}_3$, 17). Anal. ($\text{C}_{17}\text{H}_{15}\text{N}_3\text{O}_2-2.0\text{HCl}-0.75\text{H}_2\text{O}$) C, H, N.

6-[5-(3,5-Dimethoxyphenyl)furan-2-yl]nicotinamide hydrochloride salt (4c): Obtained as an orange solid in 84% yield, mp 285.5°C–287°C. IR (KBr) ν' 3,363, 3,200 (NH, NH_2), 3,059 (C–H stretch), 1,677, 1,599, 1,547 (C=N, C=C stretch, NH bend) cm^{-1} . ^1H NMR (DMSO- d_6); δ 3.80 (s, 6H), 6.51 (s, 1H), 7.03 (s, 2H), 7.26 (d, $J=3.9$ Hz, 1H), 7.43 (d, $J=3.9$ Hz, 1H), 8.10 (d, $J=8.4$ Hz, 1H), 8.31 (dd, $J=8.4, 2.1$ Hz, 1H), 8.99 (d, $J=2.1$ Hz, 1H), 9.31 (br s, 2H, exchangeable with D_2O), 9.62 (br s, 2H, exchangeable with D_2O). MS (EI) m/e (rel. int.); 323 (M^+ , 100), 306 ($M^+-\text{NH}_3$, 34), 286 (39). ^{13}C NMR (DMSO- d_6); δ 55.5, 100.6, 102.4, 109.7, 114.5, 117.8, 122.0, 131.2, 137.4, 149.2, 151.3, 151.6, 155.3, 161.0, 163.6. Anal. ($\text{C}_{18}\text{H}_{17}\text{N}_3\text{O}_3-2.0\text{HCl}-1.0\text{H}_2\text{O}$) C, H, N.

6-[5-(4-(Dimethylamino)phenyl)furan-2-yl]nicotinamide hydrochloride salt (4d): Obtained as a brown solid in 66% yield, mp 278°C–280°C. IR (KBr) ν' 3,390, 3,300 (NH, NH_2), 3,024, 2,800 (C–H stretch), 1,681, 1,618, 1,596 (C=N, C=C stretch, NH bend) cm^{-1} . ^1H NMR (DMSO- d_6); δ 3.04 (s, 6H), 7.10 (d, $J=3.6$ Hz, 1H), 7.20 (br s, 1H, exchangeable with D_2O), 7.46 (d, $J=3.6$ Hz, 1H), 7.86 (d, $J=8.7$ Hz, 2H), 8.07 (d, $J=8.4$ Hz, 1H), 8.37 (dd, $J=8.4, 2.4$ Hz, 1H), 9.03 (d, $J=2.4$ Hz, 1H), 9.42 (br s, 2H, exchangeable with D_2O), 9.70 (br s, 2H, exchangeable with D_2O). MS (EI) m/e (rel. int.); 306 (M^+ , 100), 289 ($M^+-\text{NH}_3$, 60), 158 (42). Anal. ($\text{C}_{18}\text{H}_{18}\text{N}_4\text{O}-3.0\text{HCl}-1.5\text{H}_2\text{O}$) C, H, N.

6-[5-(4-Chlorophenyl)furan-2-yl]nicotinamide hydrochloride salt (4e): Obtained as a yellow solid in 79% yield, mp 299°C–301°C. IR (KBr) ν' 3,300, 3,240 (NH, NH_2), 3,016 (C–H stretch), 1,666, 1,627, 1,600 (C=N, C=C stretch, NH bend) cm^{-1} . ^1H NMR (DMSO- d_6); δ 7.27 (d, $J=3.6$ Hz, 1H), 7.47 (d, $J=3.6$ Hz, 1H), 7.55 (d, $J=9.0$ Hz, 2H), 7.93 (d, $J=9.0$ Hz, 2H), 8.11 (d, $J=8.4$ Hz, 1H), 8.37 (dd, $J=8.4, 2.4$ Hz, 1H), 9.04 (d, $J=2.4$ Hz, 1H), 9.42 (br s, 2H, exchangeable with D_2O), 9.69 (br s, 2H, exchangeable with D_2O). MS (EI) m/e (rel. int.); 297, 299 (M^+ , 100, 36: chlorine isotopes), 280 ($M^+-\text{NH}_3$, 62), 149 (50). ^{13}C NMR (DMSO- d_6); δ 110.4, 115.7, 119.0, 122.7, 126.7, 128.9, 130.0, 134.0, 138.4, 149.6, 152.0, 152.3, 155.3, 163.4. Anal. ($\text{C}_{16}\text{H}_{12}\text{ClN}_3\text{O}-2.0\text{HCl}$) C, H, N.

6-[5-(4-Fluorophenyl)furan-2-yl]nicotinamide hydrochloride salt (4f): Obtained as a golden-yellow solid in 65% yield, mp 309°C–310.5°C. IR (KBr) ν' 3,240, 3,170 (NH, NH_2), 3,016 (C–H stretch), 1,666, 1,627, 1,604 (C=N,

C=C stretch, NH bend) cm^{-1} . ^1H NMR ($\text{DMSO}-d_6$); δ 7.21 (d, $J=3.6$ Hz, 1H), 7.31–7.37 (m, 2H), 7.45 (d, $J=3.6$ Hz, 1H), 7.93–7.99 (m, 2H), 8.10 (d, $J=8.4$ Hz, 1H), 8.36 (dd, $J=8.4$, 2.4 Hz, 1H), 9.03 (d, $J=2.4$ Hz, 1H), 9.40 (br s, 2H, exchangeable with D_2O), 9.67 (br s, 2H, exchangeable with D_2O). MS (EI) m/e (rel. int.); 281 (M^+ , 100), 264 (M^+-NH_3 , 27), 133 (68). Anal. ($\text{C}_{16}\text{H}_{12}\text{FN}_3\text{O}-2.0\text{HCl}-0.5\text{H}_2\text{O}$) C, H, N.

6-[5-(3,5-Dichlorophenyl)furan-2-yl]nicotinamide hydrochloride salt (4g): Obtained as a golden-yellow solid in 80% yield, mp $319^\circ\text{C}-321.5^\circ\text{C}$. IR (KBr) ν 3,348, 3,270 (NH, NH_2), 3,085 (C–H stretch), 1,681, 1,597 (C=N, C=C stretch, NH bend) cm^{-1} . ^1H NMR ($\text{DMSO}-d_6$); δ 7.53 (s, 2H), 7.54 (s, 1H), 7.97 (s, 2H), 8.23 (d, $J=8.4$ Hz, 1H), 8.35 (dd, $J=8.4$, 2.1 Hz, 1H), 9.03 (d, $J=2.1$ Hz, 1H), 9.40 (br s, 2H, exchangeable with D_2O), 9.68 (br s, 2H, exchangeable with D_2O). MS (EI) m/e (rel. int.); 332, 333, 334 (M^+ , 50, 20, 94; two chlorine isotopes), 315 (M^+-NH_3 , 30), 238 (100). ^{13}C NMR ($\text{DMSO}-d_6$); δ 111.6, 114.2, 118.2, 122.37, 122.41, 127.4, 132.6, 134.9, 137.3, 149.2, 151.4, 152.3, 152.4, 163.5. Anal. ($\text{C}_{16}\text{H}_{11}\text{Cl}_2\text{N}_3\text{O}-2.0\text{HCl}-0.75\text{H}_2\text{O}$) C, H, N.

6-[5-(3,5-Difluorophenyl)furan-2-yl]nicotinamide hydrochloride salt (4h): Obtained as an orange solid in 73% yield, mp $287.5^\circ\text{C}-289^\circ\text{C}$. IR (KBr) ν 3,355, 3,239 (NH, NH_2), 3,112, 3,077, 3,015 (C–H stretch), 1,667, 1,599, 1,554 (C=N, C=C stretch, NH bend) cm^{-1} . ^1H NMR ($\text{DMSO}-d_6$); δ 7.19–7.25 (m, 1H), 7.42 (d, $J=3.6$ Hz, 1H), 7.47 (d, $J=3.6$ Hz, 1H), 7.67–7.70 (m, 2H), 8.23 (d, $J=8.4$ Hz, 1H), 8.37 (dd, $J=8.4$, 2.4 Hz, 1H), 9.04 (d, $J=2.4$ Hz, 1H), 9.41 (br s, 2H, exchangeable with D_2O), 9.68 (br s, 2H, exchangeable with D_2O). MS (EI) m/e (rel. int.); 299 (M^+ , 52), 282 (M^+-NH_3 , 100), 151 (60). Anal. ($\text{C}_{16}\text{H}_{11}\text{F}_2\text{N}_3\text{O}-2.0\text{HCl}-0.5\text{H}_2\text{O}$) C, H, N.

6-[5-(2,4-Difluorophenyl)furan-2-yl]nicotinamide hydrochloride salt (4i): Obtained as an orange solid in 68% yield, mp $286^\circ\text{C}-288^\circ\text{C}$. IR (KBr) ν 3,359, 3,237 (NH,

NH_2), 3,079, 3,014 (C–H stretch), 1,666, 1,629, 1,603, 1,548 (C=N, C=C stretch, NH bend) cm^{-1} . ^1H NMR ($\text{DMSO}-d_6$); δ 7.02–7.05 (m, 1H), 7.25–7.44 (m, 2H), 7.47 (d, $J=3.6$ Hz, 1H), 8.07–8.14 (m, 2H), 8.37 (dd, $J=8.4$, 2.4 Hz, 1H), 9.05 (d, $J=2.4$ Hz, 1H), 9.43 (br s, 2H, exchangeable with D_2O), 9.70 (br s, 2H, exchangeable with D_2O). MS (EI) m/e (rel. int.); 299 (M^+ , 68), 282 (M^+-NH_3 , 100), 151 (91). Anal. ($\text{C}_{16}\text{H}_{11}\text{F}_2\text{N}_3\text{O}-2.0\text{HCl}-0.5\text{H}_2\text{O}$) C, H, N.

Biology

Antimicrobial activities

Inhibition zone determination

The antimicrobial activity of the novel nicotinamide derivatives was verified toward the standard Gram-negative bacterial strains *Escherichia coli* (*E. coli*) ATCC 25922 and *Pseudomonas aeruginosa* (*P. aeruginosa*) ATCC 27853 and Gram-positive bacterial strains *Staphylococcus aureus* (*S. aureus*) ATCC 25923 and *Bacillus megaterium* (*B. megaterium*) ATCC 14591. The antimicrobial screening was assessed by determining bacterial growth using agar well diffusion technique.²⁴ The verified nicotinamide derivatives **3d** and **4a-i** were liquefied at 20 mM concentration in DMSO. The Luria–Bertani agar (LB) medium was utilized for bacterial growth.²⁵ Solutions of the nicotinamide derivatives equal to 100 μM were employed independently in wells, previously cut in the growth medium. The microorganisms were incubated with the tested nicotinamide derivatives overnight at 37°C . The subsequent inhibition zones (mm) were recorded (Table 2) and the antimicrobial activities of the tested nicotinamides against the tested microorganisms were analyzed.

Minimum inhibitory concentrations

The minimum inhibitory concentration (MIC) of nicotinamide derivatives **3d** and **4a-i** was measured according

Table 2 Effect of the novel furanylnicotinamide derivatives on Gram-negative and Gram-positive microorganisms

100 μM - ($\mu\text{g/mL}$)	Gram-negative		Gram-positive	
	<i>Escherichia coli</i>	<i>Pseudomonas aeruginosa</i>	<i>Staphylococcus aureus</i>	<i>Bacillus megaterium</i>
4a- (33.62)	14	12	12	14
4b- (37.98)	13	11	11	13
4c- (41.43)	12	11	12	14
3d- (28.93)	6	4	4	5
4d- (44.28)	12	11	11	13
4e- (37.07)	13	11	12	13
4f- (36.32)	10	12	10	10
4g- (41.86)	13	12	12	13
4h- (38.12)	11	11	12	12
4i- (38.12)	11	10	9	10
Ampicillin- (34.94)	17	14	13	16

Note: The results expressed as zone inhibition in mm diameter.

Table 3 Minimum inhibitory concentration μM ($\mu\text{g}/\mu\text{L}$) of furanylnicotinamide derivatives against Gram-negative and Gram-positive microorganisms

Compound number	Gram-negative μM ($\mu\text{g}/\mu\text{L}$)		Gram-positive μM ($\mu\text{g}/\mu\text{L}$)	
	<i>Escherichia coli</i>	<i>Pseudomonas aeruginosa</i>	<i>Staphylococcus aureus</i>	<i>Bacillus megaterium</i>
4a	10 (3.36)	10 (3.36)	10 (3.36)	15 (5.40)
4b	10 (3.79)	15 (5.69)	10 (3.79)	15 (5.69)
4c	15 (6.21)	15 (6.21)	15 (6.21)	15 (6.21)
3d	35 (10.12)	40 (11.56)	35 (10.12)	35 (10.12)
4d	15 (6.65)	15 (6.65)	15 (6.65)	15 (6.65)
4e	15 (5.56)	20 (7.41)	20 (7.41)	15 (5.56)
4f	15 (5.45)	20 (7.26)	20 (7.26)	20 (7.26)
4g	15 (6.28)	15 (6.28)	15 (6.28)	10 (4.19)
4h	15 (5.72)	20 (7.62)	20 (7.62)	15 (5.72)
4i	15 (5.72)	20 (7.62)	20 (7.62)	20 (7.62)
Ampicillin	5 (1.75)	5 (1.75)	10 (3.49)	5 (1.75)

to the processes detailed in Clinical and Laboratory Standards Institute/National Committee for Clinical Laboratory Standards techniques.²⁶ Various concentrations (1–100 μM) of the tested nicotinamide derivatives in DMSO were added independently to the previously autoclaved LB liquid medium. The prepared LB cultures *E. coli*, *P. aeruginosa*, *S. aureus*, and *B. megaterium* microorganisms were used for the current assay. All microorganisms were separately cultured in 50 μL liquid LB medium having nearly 5×10^4 colony forming units overnight. The tested derivatives were added and the optical density of the examined aliquot was maintained at ≈ 0.5 OD and the test cultures were incubated overnight at 37°C . Controls (broth alone and culture) were preserved, subsequently. Following the overnight incubation, the optical density of the cultures was measured at 600 nm. The concentration at which the overnight culture (optical density is 0.5) turns turbid has been measured as MICs of the examined nicotinamide samples (Table 3). Experiments were performed as triplicates.

In vitro antiproliferative screening

Eight of the newly synthesized compounds represented by seven nicotinamides and one nicotinonitrile were chosen by National Cancer Institute (NCI), Bethesda, MD, USA, for evaluation of their anticancer activity. All selected compounds were subjected to a primary in vitro one-dose (10 μM) anti-cancer assay using the standard NCI 60 cancer cell line panel. The data shown in Table 4 were obtained from the percent

growth graphs of treated cells and are presented as mean percent growth inhibition (MPGI). Consequently, compounds displaying promisingly high growth inhibition percentage values were further progressed to the five-dose screen study against the panel of 60 human cancer cell lines, and their compound concentrations causing 50% growth inhibition of tested cell (GI_{50}) values against the individual cell lines are presented in Table 5. Finally, the median GI_{50} , compound concentration causing 100% growth inhibition of tested cell (TGI) and compound concentration causing 50% lethality of tested cell (LC_{50}) values were determined for the tested monocations against all the 60 tested cell lines as determined from the mean graph midpoints (Table 6).

The experimental procedure used to estimate cell viability or growth was carried out employing a 48-hour continuous drug exposure protocol adopting the sulforhodamine B protein assay and following the previously published standard method.^{27–29}

DNA binding and degradation assay

The DNA binding and degradation test of the nicotinamide derivatives was done as described.³⁰ Briefly, the tested nicotinamides were liquefied in DMSO (20 mM) and 0.5, 1, 2, 3, and 4 μM were added individually to 2 μg DNA purified from *E. coli*. The controls for this test were used as DNA alone and DNA in DMSO. The assays were carried out at 37°C for 1 hour and the DNA degradation was evaluated via agarose gel electrophoresis.³¹ Ethidium bromide was

Table 4 In vitro MPGI of the novel furanylnicotinamide derivatives against a panel of 60 cell lines at a single dose level (10 μM) (NCI, Bethesda, MD, USA)

Compound number	3d	4a	4b	4c	4d	4e	4f	4g
MPGI	25.48	20.02	33.75	–30.47	–56.15	–74.08	–52.59	–49.88

Abbreviations: MPGI, mean percent growth inhibition; NCI, National Cancer Institute.

Table 5 In vitro antiproliferative activity of the novel furanylnicotinamides against a panel of 60 cell lines at a five-dose level (National Cancer Institute, Bethesda, MD, USA)

Cancer type/cell line	4b	4c	4d	4e	4f	4g
Leukemia						
CCRF-CEM	3.20	2.06	1.80	0.856	1.51	1.93
HL-60 (TB)	2.14	2.12	1.76	0.219	1.52	2.42
K-562	3.97	1.32	0.541	0.314	0.934	1.50
MOLT-4	4.40	1.74	1.59	0.681	1.89	2.46
RPMI-8226	2.78	1.92	1.30	0.310	1.20	2.03
SR	3.11	1.73	0.378	0.275	1.32	1.78
Non-small cell lung cancer						
A549/ATCC	5.10	1.86	1.89	1.06	1.50	1.90
HOP-62	10.5	1.70	1.74	1.35	1.50	1.98
HOP-92	1.31	1.42	1.27	0.401	0.579	1.56
NCI-H226	12.9	1.81	1.88	1.52	1.40	2.13
NCI-H23	11.6	1.90	1.77	1.40	1.76	1.96
NCI-H322M	6.00	1.60	1.44	0.838	1.73	1.74
NCI-H460	3.69	1.63	1.35	0.293	1.61	1.90
NCI-H522	6.80	1.73	1.84	1.61	1.51	1.98
Colon cancer						
Colo 205	1.82	1.62	1.04	0.201	0.817	2.01
HCC-2998	2.45	1.69	1.07	0.212	1.22	1.92
HCT-116	3.26	1.66	1.47	0.405	1.40	1.97
HCT-15	4.62	1.84	1.36	0.486	1.30	1.80
HT29	3.40	1.39	0.479	0.319	0.736	1.76
KMI2	4.41	1.78	1.43	0.388	1.69	1.85
SW-620	4.05	1.67	0.774	0.492	1.61	1.78
CNS cancer						
SF-268	11.2	1.86	1.77	1.30	1.55	1.82
SF-295	NT	1.86	2.00	1.79	1.54	1.92
SF-539	4.74	1.55	1.54	1.19	1.41	1.76
SNB-19	11.7	1.85	1.87	1.56	1.72	1.63
SNB-75	10.5	1.48	1.59	1.35	1.24	1.64
U251	4.70	1.62	1.53	1.28	1.49	1.76
Melanoma						
LOX IMVI	2.58	1.63	1.53	0.306	1.48	1.77
MALME-3M	3.25	1.82	2.04	1.35	1.94	2.42
M14	2.12	1.90	1.85	1.31	1.54	1.90
MDA-MB-435	2.18	1.55	1.24	0.381	1.36	1.76
SK-MEL-2	4.91	2.01	1.96	1.95	2.02	2.09
SK-MEL-28	8.23	1.65	1.76	1.52	1.59	1.80
SK-MEL-5	3.43	1.60	1.58	1.48	1.46	1.77
UACC-257	2.18	1.76	1.77	1.31	1.71	1.84
UACC-62	10.3	1.77	1.76	1.57	1.69	1.78
Ovarian cancer						
IGROV-1	5.29	1.81	1.89	1.50	1.70	2.09
OVCAR-3	8.90	1.89	1.75	1.34	1.87	1.88
OVCAR-4	10.4	1.74	1.56	1.17	1.40	1.90
OVCAR-5	10.9	1.74	1.62	1.19	1.58	1.73
OVCAR-8	11.3	1.71	1.74	1.67	1.70	1.88
NCI/ADR-RES	14.1	6.53	2.03	1.63	1.69	1.93
SK-OV-3	10.2	1.91	1.88	1.53	1.40	1.95
Renal cancer						
786-0	6.02	1.72	1.79	1.27	1.46	1.95
A498	12.8	1.22	1.09	0.770	1.26	1.47
ACHN	6.56	1.71	1.75	1.23	1.66	1.81
CAKI-1	6.79	NT	NT	NT	1.30	1.76
RXF 393	7.01	1.60	1.58	1.16	1.19	1.73

(Continued)

Table 5 (Continued)

Cancer type/cell line	4b	4c	4d	4e	4f	4g
SN12C	8.01	1.62	1.58	1.23	1.69	1.62
TK-10	10.7	1.79	1.95	1.94	1.74	1.87
UO-31	2.68	1.59	1.48	0.960	1.60	1.54
Prostate cancer						
PC-3	4.18	1.65	1.50	1.03	1.48	1.74
DU-145	11.3	1.76	1.72	0.834	1.70	1.87
Breast cancer						
MCF-7	3.17	1.74	1.61	0.484	1.37	1.73
MDA-MB-231/ATCC	3.34	1.83	1.80	0.469	NT	1.65
HS-578T	10.9	1.66	1.90	1.31	1.57	1.87
BT-549	10.7	1.78	1.80	1.56	1.40	1.89
T-47D	5.13	1.72	1.73	0.379	NT	2.18
MDA-MB-468	2.04	1.51	1.39	0.507	1.37	1.69

Note: Data represent the compounds' GI_{50} in μM against the tested cell lines.**Abbreviations:** GI_{50} , drug concentration that causes 50% inhibition of cell growth; NT, not tested; CNS, central nervous system.

used to stain agarose gels, which visualized by an ultraviolet transilluminator.

Antioxidant activity

Super oxide dismutase (SOD) mimetic catalytic activity assay

The SOD-like activity of the tested furanylnicotinamides was measured via nitroblue tetrazolium (NBT)/reduced nicotinamide adenine dinucleotide (NADH)/phenazine methosulfate to photo-generate $O_2^{\cdot-}$ in phosphate buffer (pH = 8.3). The reduced NBT to blue formazan was utilized as a marker of $O_2^{\cdot-}$ generation and detected at 560 nm. The reaction in the presence of the nicotinamide derivatives and controls was recorded for 5 minutes.³² The percentages of inhibition of free radical generation from SOD-like activity were measured via the equation:

$$I\% = \frac{\Delta A_{\text{control}} - \Delta A_{\text{sample}}}{\Delta A_{\text{control}}} \times 100 \quad (1)$$

where A_{control} is the absorbance of the control (comprising all reagents except the nicotinamide derivatives) and A_{sample} is the absorbance of the nicotinamide derivatives.

Table 6 Median GI_{50} , TGI, and LC_{50} (μM) for the most active furanylnicotinamides against a panel of 60 cell lines at a five-dose level (NCI, Bethesda, MD, USA)

MG-MID	4b	4c	4d	4e	4f	4g
GI_{50}	5.24	1.66	1.51	0.83	1.44	1.86
TGI	15.48	3.47	3.16	2.51	2.95	3.63
LC_{50}	43.65	7.58	7.24	100	6.02	7.94

Abbreviations: MG-MID, mean graph midpoint representing mean sensitivity of all examined cell lines to the test compound; GI_{50} , compound concentration causing 50% growth inhibition of tested cells; TGI, compound concentration causing 100% growth inhibition of tested cells; LC_{50} , compound concentration causing 50% lethality of tested cells; NCI, National Cancer Institute.

DPPH free radical scavenging activity

The 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radical scavenging activities of furanylnicotinamide derivatives were carried out as reported by Al-Omar et al.³³ The reduction of stable DPPH free radical from a purple to a yellow colored diphenylpicryl hydrazine was measured by the electron donor competence of the tested nicotinamide derivatives. After an electron was moved to the odd electron in DPPH•, the absorbance at 517 nm reduced steadily to increase nonradical DPPH forms. Briefly, 1 mL of the test compound (2 µM) in ethanol/DMSO (1:1 v:v) or standard (vitamin C) was added to 4 mL of 0.004% (w/v) ethanol solution of DPPH and vortexed carefully. After a 30-minute incubation at 30°C, the absorbance was recorded against control (methanol/DMSO 1:1 v:v) at 517 nm. Consequently, after an electron was transferred to the odd electron in DPPH•, the absorbance at 517 nm reduced steadily due to the increase of the nonradical DPPH forms. The percentage of inhibition of DPPH free radical was calculated by the equation:

$$I\% = \frac{A_{\text{control}} - A_{\text{sample}}}{A_{\text{control}}} \times 100 \quad (2)$$

where A_{control} is the absorbance of the control (comprising all substances except the nicotinamide derivatives) and A_{sample} is the absorbance of the furanylnicotinamide derivatives.

NO scavenging assay

The scavenging activity method of nitric oxide (NO) was achieved according to the assay of Green et al.³⁴ where sodium

nitroprusside generates NO radicals, which cooperate with oxygen to generate nitrite ions. The latter can be evaluated by utilizing the Griess reagent (2% H_3PO_4 , 1% sulfanilamide, and 0.1% N-(1-naphthyl)ethylenediamine dihydrochloride). The NO scavengers participate with O_2 to minimize the generation of nitrite ions. Sodium nitroprusside (10 mM) in phosphate buffer (PB) saline was mixed with the tested nicotinamide derivatives (2 µM) and standard. The reaction mixtures were kept for 2.5 hours at 25°C after which Griess reagent (0.5 mL) was added.³⁵ The absorbance of the pink color generated was read at 546 nm and referred to the absorbance of standard solutions of NaNO_2 treated in the same way with Griess reagent. NO scavenging activity was considered according to the equation:

$$\% \text{ NO scavenging activity} = \frac{A_{\text{control}} - A_{\text{sample}}}{A_{\text{control}}} \times 100 \quad (3)$$

where A_{control} is the absorbance of the control (comprising all reagents except the nicotinamide derivatives) and A_{sample} is the absorbance of the nicotinamide derivatives.

All data about total antioxidant activity are the average of triplicate analyses. All procedures were approved by the University of King Faisal Committee of Scientific Research Ethics.

Results and discussion

Chemistry

The syntheses of the novel furanylnicotinamides **4a–i** were initiated by the Suzuki coupling reaction of 6-(5-bromofuran-2-yl)nicotinonitrile with the corresponding phenylboronic

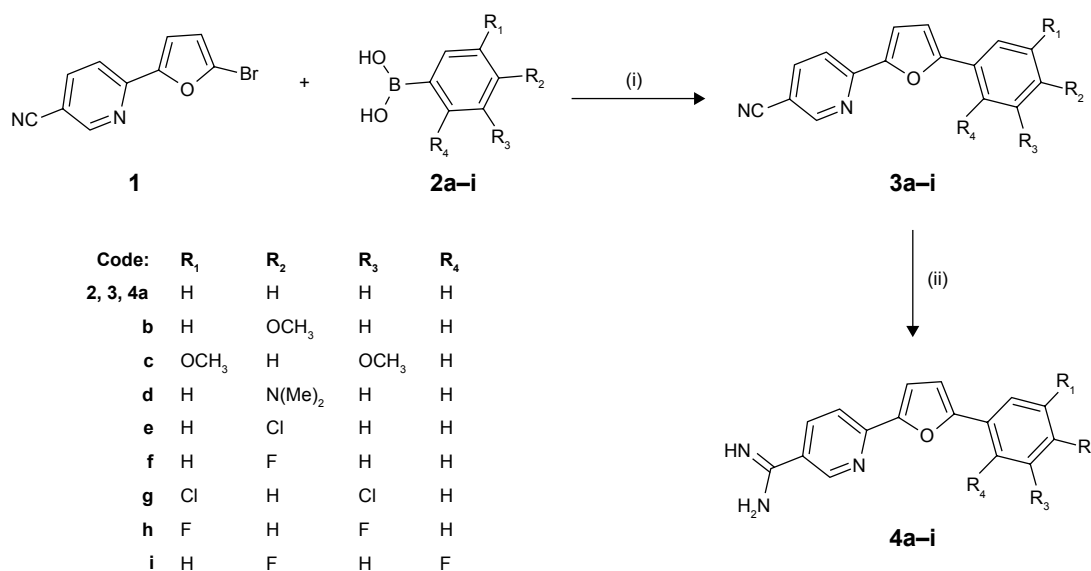


Figure 1 Synthesis scheme for the new furanylnicotinamide derivatives.

Notes: (i) $\text{Pd}(\text{PPh}_3)_4$, Na_2CO_3 , toluene, 80°C; (ii) a) $\text{LiN}(\text{TMS})_2$, THF, r.t., overnight, b) HCl (gas), dry ethanol, r.t., overnight.

Abbreviation: r.t., room temperature.

acid derivatives to furnish the phenylfuranylnicotinonitrile derivatives **3a–i** (Figure 1). The nicotinonitriles were converted to the corresponding nicotinamide derivatives by the action of $\text{LiN}(\text{TMS})_2$ solution in THF. The structures of the newly synthesized nicotinamide derivatives **4a–i** and **3a–i** were established based on their spectral and elemental analyses. Thus, ^1H NMR spectrum of the nicotinamide derivative **4b** displayed two deuterium exchangeable singlet signals at δ 9.33 (2H) and δ 9.62 (2H) characteristic for the cationic amidine group in addition to the signals corresponding to the 1,4-disubstituted benzene ring, furan, and 2,6-disubstituted pyridyl moieties. ^{13}C NMR spectrum showed 15 carbon lines that assigned to the carbon network of compound **4b** structure. Furthermore, mass spectrum of nicotinamide **4b** furnished an m/z peak at 293 of its molecular ion peak (M^+).

Biology

Antimicrobial activities

Inhibition zone determination

The nicotinamides **4a–i** and nicotinonitrile derivative **3d** were tested against the growth of both Gram-negative and Gram-positive microorganisms. The zone of inhibition diameter for the tested nicotinamide derivatives against the growth of the verified microorganisms is presented in Table 2. From these results, it is apparent that the nicotinamide derivatives **4a**, **4b**, **4c**, **4d**, **4e**, and **4g** exhibit the most antimicrobial activity toward the wholly considered microorganisms compared to ampicillin as an antibacterial control. The antimicrobial activity of the nicotinamide derivatives that have electron donating groups in the phenyl ring as *p*- OCH_3 , 3,5-dimethoxy, and *p*- $\text{N}(\text{CH}_3)_2$ in compounds **4b–d** is lower than that recorded with the parent compound **4a**. This finding was also the case for the nicotinamide derivatives that have electron withdrawing atoms substituents on the phenyl ring. The nicotinamide compounds **4f**, **4h**, and **4i** reveal good antimicrobial activity toward the examined microorganisms, but still less than the parent compound **4a**. The nicotinamides **4e** and **4g** substituted with chloro/dichlorophenyl ring have greater antibacterial activity than their fluoro/difluoro counterparts **4f**, **4h**, and **4i**. However, the nicotinonitrile derivative **3d** reveals the lowest antimicrobial activity of all, over the study. From a structure point of view, it is noticeable that the existence of amidine group in compounds **4a–i** increased the antimicrobial activities of the nicotinamides compared to the nicotinonitrile derivative **3d**. Due to poor water insolubility of our furanylnicotinamide derivatives, the antimicrobial activities of these compounds were assessed using DMSO as a solvent. To ensure that the

results obtained were reflective of the antimicrobial activity of the test compounds without interference of the solvent used, a blank experiment was performed against all tested organisms where compound-free DMSO was used to evaluate the microbial growth under the same experimental conditions. Blank experiment results showed that the tested microorganisms were able to grow in the presence of DMSO alone. Also, a literature survey showed that while DMSO can act as a chaotropic or hydrophobic stressor disordering the macromolecular systems of the cell, this holds true only at much higher concentrations than those used in our experiments employing DMSO as a solvent.³⁶ Moreover, the concentration ranges in which the novel compounds inhibit bacterial growth are consistent with those at which inhibitors with a specific toxic mode of action (rather than stressors) inhibit the microbial growth.³⁷

Minimum inhibitory concentrations

The MIC value is the minimum concentration of the nicotinamide derivative at which no microbial growth is recognized. The comparison of the MICs (μM) of nicotinamide derivatives and ampicillin as a typical medication against *E. coli*, *P. aeruginosa*, *S. aureus*, and *B. megaterium* microorganisms is shown in Table 3. Interestingly, the nicotinamide derivatives **4a** and **4b** displayed an MIC value ($10\ \mu\text{M}$) similar to that recorded for the reference standard ampicillin against *S. aureus*. Compounds **4a** and **4b** showed excellent MIC values ($10\ \mu\text{M}$) against both *E. coli* and *S. aureus*. Equally, compound **4a** exhibited the same MIC ($10\ \mu\text{M}$) value against *P. aeruginosa*. Additionally, the nicotinamide derivative **4g** displayed an excellent MIC value of $10\ \mu\text{M}$ against *B. megaterium* and is more potent than that recorded for the other tested nicotinamides for the same bacterial strain. The nicotinamide derivatives **4c–i** demonstrated good antimicrobial activities against *E. coli* with an MIC value of $15\ \mu\text{M}$. In addition, a good MIC value of $20\ \mu\text{M}$ was verified with nicotinamides **4e**, **4f**, **4h**, and **4i** against *P. aeruginosa*. On the other hand, the nicotinonitrile derivative **3d** showed the lowest MICs in the range of $35\text{--}40\ \mu\text{M}$ against the tested microorganisms.

In vitro antiproliferative screening

A series of eight compounds representing seven nicotinamides and one nicotinonitrile were subjected to an in vitro antiproliferative screening against a panel of 60 cell lines representing nine types of human cancer. The standard practice is testing the chosen compounds at an initial high dose ($10\ \mu\text{M}$). Thereafter, the compounds showing a satisfactorily high MPGI, (six compounds in this research work),

are subjected to a five-dose screen against the full panel of 60 cancer cell lines and their GI_{50} values are determined.

Table 4 shows the MPGI results for the initial single dose screen against the panel of 60 cancer cell lines exhibited by the seven nicotinamides and one nicotinonitrile. First, it was found that the nicotinonitrile precursor **3d** displayed an MPGI of 25.48 being far less active than its nicotinamide counterpart **4d** that elicited an MPGI value of -56.15. For this reason the nicotinonitrile derivative was not progressed to the five-dose screen; these primary results clearly indicate that the presence of the amidine group plays a fundamental role in the antiproliferative activity of this class of compounds.

The structure-activity relationship findings among the seven nicotinamide derivatives gave an insight on the structural features associated with the enhanced antiproliferative activity of this class of compounds. First, it was shown that the electron donating groups 4-methoxy, 3,5-dimethoxy-, and *p*-N(CH₃)₂ substitution on the phenyl ring led to a profound enhancement of antiproliferative activity as shown for the isosteres **4b**, **4c**, and **4d**, even enhancing the biological activity of these derivatives by being better cytostatics as in the case of **4b** (MPGI = 33.75) to being cytotoxic **4c** and **4d** (MPGIs = -30.47, -56.15, respectively) as compared to the prototype compound **4a** (MPGI = 20.02). Second, introducing withdrawing groups *p*-chloro-, *p*-fluoro-, and 3,5-dichloro- substitution on the phenyl ring led to a great enhancement of cytotoxic activity of these analogs as well. Indeed, this was the case for the *p*-chlorophenyl derivative **4e** (MPGI = -74.08) and the *p*-fluorophenyl analog **4f** (MPGI = -52.59), showing cytotoxic abilities compared to cytostatic activity for *p*-methoxyphenyl derivative **4b**. Finally, bioisosteric replacement of the 3,5-dimethoxyphenyl ring in **4c** by 3,5-dichlorophenyl ring in **4g** led to a significant increase in the MPGI value (-49.88 for **4g**).

Table 5 displays the individual GI_{50} values of the six furanylnicotinamides that were promoted for a five-dose screen due to their high antiproliferative profile demonstrated in the primary single dose screening assay. Among the tested six nicotinamides, the most active compound was **4e**, as anticipated from the single dose screen results, showing an IC_{50} value less than 0.5 μ M against some cancer cell lines belonging to five types of cancer, namely, leukemia, non-small cell lung, colon, melanoma, and breast cancers. The most responsive to the antiproliferative effect of the tested nicotinamide **4e** were cancer cell lines HL-60(TB) leukemia, Colo 205 colon, and HCC-2998 colon with an IC_{50} s less than 0.25 μ M. In addition, nicotinamide **4d** showed good activity against SR leukemia and

HT29 colon cancer cell lines with GI_{50} values of 0.37 and 0.47 μ M, respectively.

Finally, Table 6 depicts the median GI_{50} , TGI, and LC_{50} of the tested compounds in the five-dose screen. The most active compound, and in compliance with the primary single dose cell growth percent inhibition assay, was found to be **4e** displaying a submicromolar GI_{50} value of 0.83 μ M. All the other tested compounds exhibited GI_{50} values below 2 μ M except for **4b** (GI_{50} = 5.24 μ M), which was the least active in the primary single dose screen assay. The most potent derivative was the chloro nicotinamide **4e** showing a micromolar TGI of 2.51 μ M and an LC_{50} of 100 μ M. The *p*-fluoro derivative **4f** comes second with a TGI of 2.95 μ M and an LC_{50} of 6.02 μ M. In spite of the fact that the *p*-chloro derivative **4e** showed high potency with regard to its growth inhibitory abilities reflected in its GI_{50} and TGI values (0.83 and 2.51 μ M, respectively), it was the least cytotoxic in this series with an LC_{50} of 100 μ M. Finally, derivative **4b** which had the lowest MPGI ability among the six nicotinamides selected for the five-dose screening assay was still the least potent with regard to the two measured parameters, GI_{50} and TGI.

DNA binding and degradation

The ability of the novel nicotinamides **4a-i** and nicotinonitrile derivative **3d** to bind and degrade DNA was considered side by side to that of controls (DNA alone and DNA in DMSO) applying a horizontal electrophoresis procedure. In the present study, the tested nicotinamides exhibited a DNA degradation effect in a dose-dependent manner, which proves their binding ability to the DNA as shown in Figure 2. Once the DNA was permitted to interrelate with the nicotinamide derivatives at doses of 0.5, 1, 2, 3, and 4 μ M, DNA cleavage was found to increase in a concentration proportional fashion as presented in Figure 2. The data show that the nicotinamide derivative **4a** (parent compound) is able to carry out an effective degradation for the DNA at 1 μ M. Moreover, nicotinamide derivatives **4b** and **4g** elicited effective DNA degradation at 2 μ M. Whereas, the tested compounds **4c**, **4d**, and **4i** at 3 μ M are found to exhibit strong nuclease-like activity on the genomic DNA starting at a 3 μ M concentration level. In addition, at 4 μ M concentration of the nicotinamides **4a-i**, an efficient cleavage of DNA was observed. On the other hand, the nicotinonitrile derivative **3d** did not reveal any degradation effect on the studied DNA at the tested concentrations range (0.5–4 μ M).

These results specify that DNA degradation efficiency depends on the concentration and the structural features of

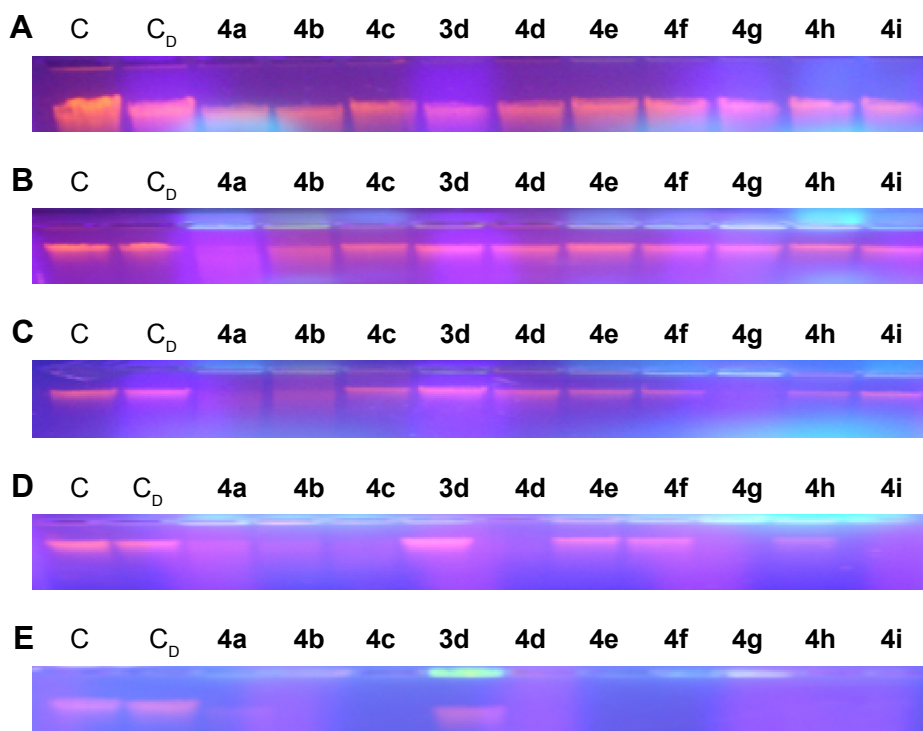


Figure 2 A figure showing the degradation effect of 0.5 μM (**A**), 1 μM (**B**), 2 μM (**C**), 3 μM (**D**), and 4 μM (**E**) of the novel furanylnicotinamide derivatives (lanes 3–12) on the genomic DNA isolated from *Escherichia coli*. Lane 1 *E. coli* DNA and lane 2 *E. coli* DNA + DMSO.

Abbreviation: DMSO, dimethylsulfoxide.

the studied compounds. It was observed that the presence of the amidine group as in derivatives **4a–i**, compared to the cyano group of the nicotinonitrile **3d**, results in a significant DNA cleavage affinity. Thus, the newly synthesized nicotinamides have attracted special attention as endonuclease simulators due to their significant structures. Finally, the current study validates that the nicotinamides **4a–i** have a substantial nuclease action toward the degradations of DNA molecule in the absence of any additional ingredients at 4 μM concentration. The DNA binding and degradation action is a potential feature for the furanylnicotinamides as promising chemotherapeutic mediators in tumor cures.

Antioxidant activity

Superoxide dismutase mimetic catalytic activity

The DNA binding studies so far revealed that the tested nicotinamides showed good DNA binding affinity; consequently, it was considered valuable to study the antioxidant activity of this promising class of nicotinamide derivatives. In the SOD-like activity assessment, the verified nicotinamide derivatives participate with NBT for oxidation of the produced superoxide ions. The more effective nicotinamides, the minor the concentration that resembles to 50% inhibition of NBT reduction.

The results presented in Table 7 report the SOD-like inhibition percent of each nicotinamide derivative producing an effective quenching of the $\text{O}_2^{\cdot-}$ in the reaction mixture. The parent nicotinamide derivative **4a** and its isosteres substituted on the phenyl ring with *p*-methoxy- (**4b**), 3,5-dimethoxy- (**4c**), and *p*-dimethylamino- (**4d**) groups displayed a substantial SOD-like efficiency and caused an inhibition percentage of 59.7, 57.4, 54.6, and 52.9 for these compounds in the same order. Also, the halogenated derivatives **4g** (3,5-dichloro-), **4h** (3,5-difluoro-), **4e** (*p*-chloro-),

Table 7 Superoxide dismutase mimetic catalytic activity of furanylnicotinamide derivatives as an antioxidant enzyme

	% inhibition
Control	–
Horse radish	69.2
4a	59.7
4b	57.4
4c	54.6
3d	33.6
4d	52.9
4e	52.0
4f	51.4
4g	56.1
4h	52.4
4i	51.5

4i (2,4-difluoro-), and **4f** (*p*-fluoro-) exhibited a substantial SOD-like effect and contribute an inhibition percentage of 56.1%, 52.4%, 52%, 51.5%, and 51.4%, respectively. However, the nicotinonitrile compound **3d** displayed weak SOD-like activity as denoted by an inhibition percent of 33.6%.

DPPH free radical scavenging activity

The DPPH is frequently utilized as a substrate to estimate antioxidant activities of compounds.³⁸ The DPPH• radical scavenging activity assessment is a representative assay in antioxidant activity studies and comprises a quick method for screening the radical scavenging activity of nicotinamide compounds. The interaction of the tested nicotinamide derivatives with steady DPPH free radical specifies their free radical scavenging capability. The tested nicotinamides displayed antiradical activity by inhibiting DPPH radical (Figure 3). Most of the verified compounds in nicotinamide series revealed high to moderate interaction with the DPPH radical at 2 μ M concentration. Significant antioxidant activities were observed for nicotinamides and were in the following order **4a** > **4b** > **4g** > **4c** > **4d**, which all displayed more than 55% inhibition comparable to that of standard vitamin C tested in a parallel concentration as shown in Figure 3. It was found that substitution on the phenyl ring with *p*-methoxy-, 3,5-dimethoxy-, 3,5-dichloro-, and *p*-dimethylamino- might play a functional role in the tested nicotinamide activity. Introduction of two chlorine atoms in *m*-positions of the phenyl ring (**4g**) increases the antioxidant activity when compared to the electron donating dimethoxy groups in the same position (**4c**). However, the

presence of a cyano group (**3d**) instead of an amidine group (**4d**) in the same position decreased the antioxidant activity. The other nicotinamides **4e**, **4f**, **4h**, and **4i** exhibited good radical scavenging activity, but still less than the parent compound **4a** as presented in Figure 3. Antioxidant activity of these compounds is related to their electron or hydrogen radical donating ability to DPPH radical. It seems that nicotinamides retain hydrogen donating aptitudes and perform as antioxidants.

NO scavenging assay

The “novel” nicotinamide derivatives were studied for their antioxidant property through NO scavenging activity as illustrated in Figure 4. The tested nicotinamide derivatives demonstrated NO radical scavenging activity in the subsequent order **4a** > **4b** > **4g**. The other nicotinamides **4c**, **4d**, **4e**, **4f**, **4h**, and **4i** showed good antioxidant activity, but less than the parent compound **4a**. The nicotinamide derivative **4b** (*p*-MeO) exhibited greater antioxidant activity when compared to the corresponding monohalogenated compounds **4e** (*p*-Cl) and **4f** (*p*-F). However, the nicotinonitrile **3d** exhibited feeble NO scavenging activity when compared to the control. The tested nicotinamides possibly have the material to neutralize the effect of NO establishment and in effect possess significant ability to prevent the severe effects of dangerous NO generation in the living organism. NO produced from sodium nitroprusside binds with O₂ to form NO₂. Consequently, nicotinamides may prevent NO₂ establishment by competing with O₂ to react through NO. In biological systems, NO radical has a short lifetime yet causes harm to most biomolecules, including DNA, protein,

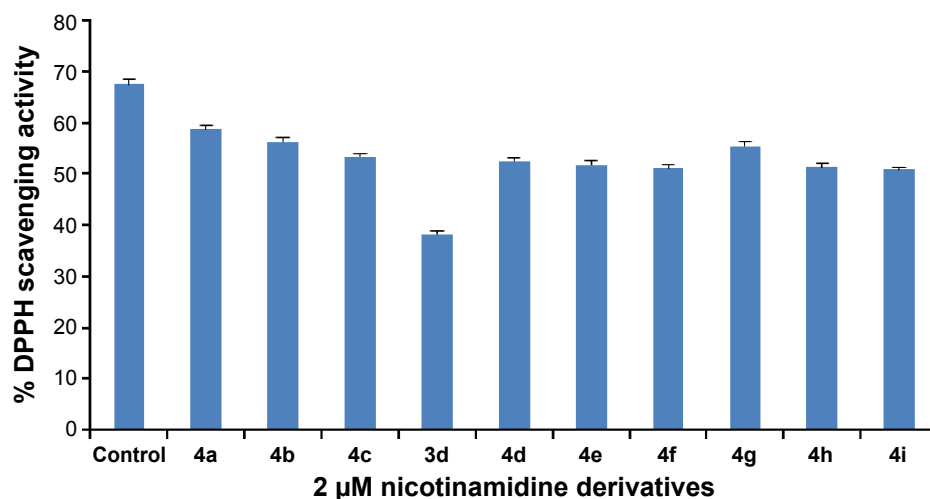


Figure 3 Antioxidant activities of the furanylnicotinamide derivatives using 2,2-diphenyl-1-picrylhydrazyl (DPPH).

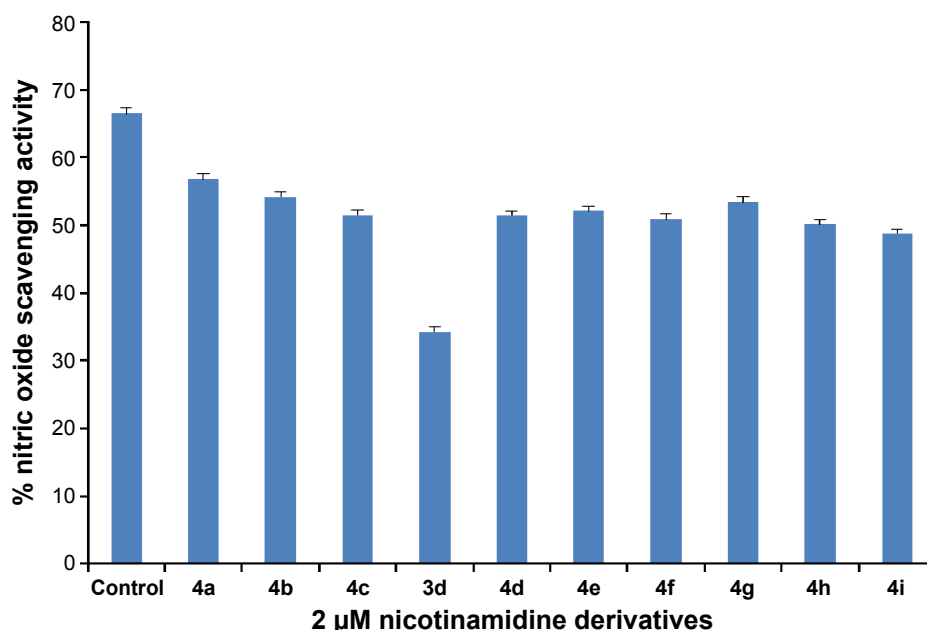


Figure 4 Antioxidant activities of the furanylnicotinamide derivatives using nitric oxide.

and lipids, thus playing a serious role in some human diseases, including tumor and inflammation.³⁹

Conclusion

This research endeavor has led to the synthesis of a series of substituted phenylfuranylnicotinamides using facile synthesis procedure. Investigation into the biological activities of this class led to discovery of their anticancer, antimicrobial, DNA cleavage, and antioxidant ability. The antiproliferative activity screening was performed at NCI against 60 cancer cell lines representing nine types of cancer and revealed that the activity is linked with the substitution pattern on the terminal phenyl ring where the introduction of electron withdrawing groups was much more favorable than the electron donating ones, imparting to these derivatives not only a cytostatic property but a cytotoxic power as well. On the other hand, these nicotinamides demonstrated good antimicrobial activity when tested against representative Gram-positive and Gram-negative bacteria. The nuclease-like activity of these nicotinamides was also investigated and showed a dose-dependent DNA degradation ability. Finally, the antioxidant effect of the newly synthesized compounds was tested by SOD, DPPH, and NO scavenging assays and all nicotinamides demonstrated a good antioxidant capacity. In conclusion, this class of nicotinamides represents an easily synthesized chemotype effective in cancer management not only by virtue of their nuclease-like activity on DNA, which is useful in case of cancer cells, but also by

virtue of their cytoprotective antioxidant activity in the case of normal cells.

Acknowledgments

The authors acknowledge the financial support from Deanship of Scientific Research, King Faisal University, Saudi Arabia, Project No 150122. The in vitro anticancer screening was done at the National Cancer Institute (NCI, Bethesda, MD, USA).

Disclosure

The authors report no conflicts of interest in this work.

References

- Kennedy B, O'Connor B, Korn B, Gibbons N, O'Connor T, Keane J. Multi-drug resistant tuberculosis: experiences of two tertiary referral centres. *Ir Med J.* 2011;104(6):182–185.
- Kutty N. Treating children without antibiotics in primary healthcare. *Oman Med J.* 2011;26(5):303–305.
- Levy SB. Factors impacting on the problem of antibiotic resistance. *J Antimicrob Chemother.* 2002;49(1):25–30.
- Lind MJ. Principles of cytotoxic chemotherapy. *Medicine.* 2008;36(1):19–23.
- Summers LA. *The Bipyridinium Herbicides*. London: Academic Press; 1980.
- World Health Organization. *WHO Model List of Essential Medicines*. Geneva: World Health Organization; October 2013. Retrieved April 22, 2014. Available from: http://apps.who.int/iris/bitstream/10665/112738/1/9789240692671_eng.pdf. Accessed February 18, 2016.
- Libman MD, Miller MA, Richards GK. Antistaphylococcal activity of pentamidine. *Antimicrob Agents Chemother.* 1990;34(9):1795–1796.
- Werbovetz K. Diamidines as antitrypanosomal, antileishmanial and antimalarial agents. *Curr Opin Investig Drugs.* 2006;7(2):147–157.
- Pathak MK, Dhawan D, Lindner DJ, Borden EC, Farver C, Yi T. Pentamidine is an inhibitor of PRL phosphatases with anticancer activity. *Mol Cancer Ther.* 2002;1(14):1255–1264.

10. Smith J, Stewart BJ, Glaysher S, et al. The effect of pentamidine on melanoma. *Anticancer Drugs*. 2010;21(2):181–185.
11. Lansiaux A, Tanious F, Mishal Z, et al. Distribution of furamidine analogues in tumor cells targeting of the nucleus or mitochondria depending on the amidine substitution. *Cancer Res*. 2002;62(24):7219–7229.
12. Antony S, Marchand C, Stephen AG, et al. Novel high-throughput electrochemiluminescent assay for identification of human tyrosyl-DNA phosphodiesterase (Tdp1) inhibitors and characterization of furamidine (NSC 305831) as an inhibitor of Tdp1. *Nucleic Acids Res*. 2007;35(13):4474–4484.
13. Ismail MA, Brun R, Easterbrook JD, Tanious FA, Wilson WD, Boykin DW. Synthesis and antiprotazoal activity of aza-analogues of furamidine. *J Med Chem*. 2003;46(22):4761–4769.
14. Wenzler T, Boykin DW, Ismail MA, Hall JE, Tidwell RR, Brun R. New treatment option for second-stage African sleeping sickness: in vitro and in vivo efficacy of aza analogs of DB289. *Antimicrob Agents Chemother*. 2009;53(10):4185–4192.
15. Thuita JK, Wolf KK, Murilla GA, et al. Safety, pharmacokinetic, and efficacy studies of oral DB868 in a first stage vervet monkey model of human African trypanosomiasis. *PLoS Negl Trop Dis*. 2013;7(6):e2230.
16. Wenzler T, Yang S, Braissant O, Boykin DW, Brun R, Wang MZ. Pharmacokinetics, Trypanosoma brucei gambiense, efficacy, and time of drug action of DB829, a preclinical candidate for treatment of second-stage human African trypanosomiasis. *Antimicrob Agents Chemother*. 2013;57(11):5330–5343.
17. Thuita JK, Wolf KK, Murilla GA, et al. Chemotherapy of second stage human African trypanosomiasis: comparison between the parenteral diamidine DB829 and its oral prodrug DB868 in vervet monkeys. *PLoS Negl Trop Dis*. 2015;9(2):e0003409.
18. Youssef MM, Al-Omar MA, Ismail MA. Synthesis, DNA affinity, and antimicrobial activity of 4-substituted phenyl-2,2'-bichalcophenes and aza-analogues. *Med Chem Res*. 2012;21:4074–4082.
19. Hussin WA, Ismail MA, El-Sayed WM. Novel 4-substituted phenyl-2,2'-bichalcophenes and aza-analogues as antibacterial agents: a structural activity relationship. *Drug Des Devel Ther*. 2013;7:185–193.
20. El-Sayed WM, Hussin WA, Ismail MA. Efficacy of two novel 2,2'-bifurans to inhibit methicillin-resistant *Staphylococcus aureus* infection in male mice in comparison to vancomycin. *Drug Des Devel Ther*. 2012;6:279–287.
21. Hussin WA, Ismail MA, Alzahrani AM, El-Sayed WM. Evaluation of the biological activity of novel monocationic fluoroaryl-2,2'-bichalcophenes and their analogues. *Drug Des Devel Ther*. 2014;8:963–972.
22. El-Sayed WM, Hussin WA. Antimutagenic and antioxidant activity of novel 4-substituted phenyl-2,2'-bichalcophenes and aza-analogs. *Drug Des Devel Ther*. 2013;7:73–81.
23. Ismail MA, Arafa RK, Youssef MM, El-Sayed WM. Anticancer, antioxidant activities and DNA affinity of novel monocationic bithiophenes and analogues. *Drug Des Devel Ther*. 2014;8:1659–1672.
24. Bauer W, Kirby WM, Sherris JC, Turck MJ. Antibiotic susceptibility testing by a standardized single disk method. *Clinic Pathol*. 1966;45(4):493–496.
25. Youssef MM, Mahmoud AA, Al-Faiyz YS. Antimicrobial and antioxidant activities of *Artemisia abyssinica* extracts and DNA degradation effects. *Asian J Biochem*. 2015;10(1):31–41.
26. CLSI/NCCLS. *Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria That Grow Aerobically*, Approved Standard, 7th ed. Wayne, PA: CLSI; 2006.
27. Skehan P, Storeng R, Scudiero D, et al. New colorimetric cytotoxicity assay for anticancer-drug screening. *J Natl Cancer Inst*. 1990;82(13):1107–1112.
28. McCaffrey TA, Agarwal LA, Weksler BB. A rapid fluorometric DNA assay for the measurement of cell density and proliferation in vitro. *In Vitro Cell Dev Biol*. 1988;24(3):247–252.
29. Monks A, Scudiero D, Skehan P, et al. Feasibility of a high-flux anticancer drug screen using a diverse panel of cultured human tumor cell lines. *J Natl Cancer Inst*. 1991;83(11):757–766.
30. Sayed AR, Youssef MM, Al-Faiyz YS. Synthesis, characterization, and biological evaluation of novel thiadiazoline sulfonamides and metal complexes. *J Appl Sci*. 2015;15(6):884–893.
31. Sambrook J, Fritsch EF, Maniatis T. *Molecular Cloning: A Laboratory Manual*. Cold Spring Harbor, NY: Cold Spring Harbor Laboratory Press; 1989.
32. Bridges SM, Salin ML. Distribution of iron-containing superoxide dismutase in vascular plants. *Plant Physiol*. 1981;68(2):275–278.
33. Al-Omar MA, Sayed AR, Youssef MM. Synthesis of novel triazoles, tetrazine, thiadiazoles and their biological activities. *Molecules*. 2015;20:2591–2610.
34. Green LC, Wagner DA, Glogowski J, Skipper PL, Wishnok JS, Tannenbaum SR. Analysis of nitrate, nitrite, and [15N] nitrate in biological fluids. *Anal Biochem*. 1982;126(1):131–138.
35. Sreejayan N, Rao MNA. Nitric oxide scavenging by curcuminoids. *J Pharm Pharmacol*. 1997;49(1):105–107.
36. Cray JA, Bell ANW, Bhaganna P, Mswaka AY, Timson DJ, Hallsworth JE. Hydrophobic substances induce water stress in microbial cells. *Microb Biotechnol*. 2013;6:453–492.
37. Cray JA, Stevenson A, Ball P, et al. Chaotropicity: a key factor in product tolerance of biofuel-producing microorganisms. *Curr Opin Biotechnol*. 2015;33:228–259.
38. Oyaizu M. Studies on product of browning reaction prepared from glucose amine. *Jpn J Nutr*. 1986;44:307–315.
39. Moncada S, Palmer RM, Higgs EA. Nitric oxide: physiology, pathophysiology, and pharmacology. *Pharmacol Rev*. 1991;43(2):109–142.

Drug Design, Development and Therapy

Publish your work in this journal

Drug Design, Development and Therapy is an international, peer-reviewed open-access journal that spans the spectrum of drug design and development through to clinical applications. Clinical outcomes, patient safety, and programs for the development and effective, safe, and sustained use of medicines are a feature of the journal, which

Submit your manuscript here: <http://www.dovepress.com/drug-design-development-and-therapy-journal>

Dovepress

has also been accepted for indexing on PubMed Central. The manuscript management system is completely online and includes a very quick and fair peer-review system, which is all easy to use. Visit <http://www.dovepress.com/testimonials.php> to read real quotes from published authors.