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Design, Synthesis and Antibacterial Activity of *N*-Aryl-3-(arylamino)-5-(((5-substituted furan-2-yl)methylene)amino)-1*H*-pyrazole-4-carboxamide as Nitrofurantoin® Analogues

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Abstract

Nitrofurantoin® is an effective drug and used for treating urinary infectious diseases. A series of nitrofurantoin® analogues bearing furan and pyrazole scaffolds as *N*-aryl-3-(arylamino)-5-(((5-substituted furan-2-yl)methylene)amino)-1*H*-pyrazole-4-carboxamide (**7a-g** and **9a-f**) were designed and synthesized by the condensation of 5-aminopyrazole with 5-nitrofuran-2-carbaldehyde (**6**) or 5-methylfuran-2-carbaldehyde **(8)** for evaluation of their antibacterial properties against Gram +ve and Gram -ve bacteria then comparing with nitrofurantoin® as standard drug.

Keywords: Furan scaffold; Pyrazole scaffold; 5-Aminopyrazole; Nitrofurantoin® analogues; Antibacterial

1. Introduction

Microbial resistance is one of the biggest public health challenges of our time. Therefore, there is an urgent need for the co-operation between the organic and medicinal researchers to find new drugs to face this problem.

Nitrofurantoin® **(A)**, 1-(5-nitrofurfurylidenamino) hydantoin, is an effective drug that acts on a number of Gram-positive and Gram-negative microorganisms (*staphylococci*, *streptococci*, *dysentery bacillus*, *colon bacillus*, *paratyphoid bacillus* and others). It is primarily used for treating infectious diseases of the urinary tract (pyelitis, pyelonephritis, cystitis, and urethritis). Also, we found in the literature survey some drugs bearing furan moiety. For example, Rofecoxib **(B)**, 4-(4-methylsulfonylphenyl)-3-phenyl-5*H*-furan-2-one, is a nonsteroidal anti-inflammatory drug (NSAID) and a selective COX-2 inhibitor [1].

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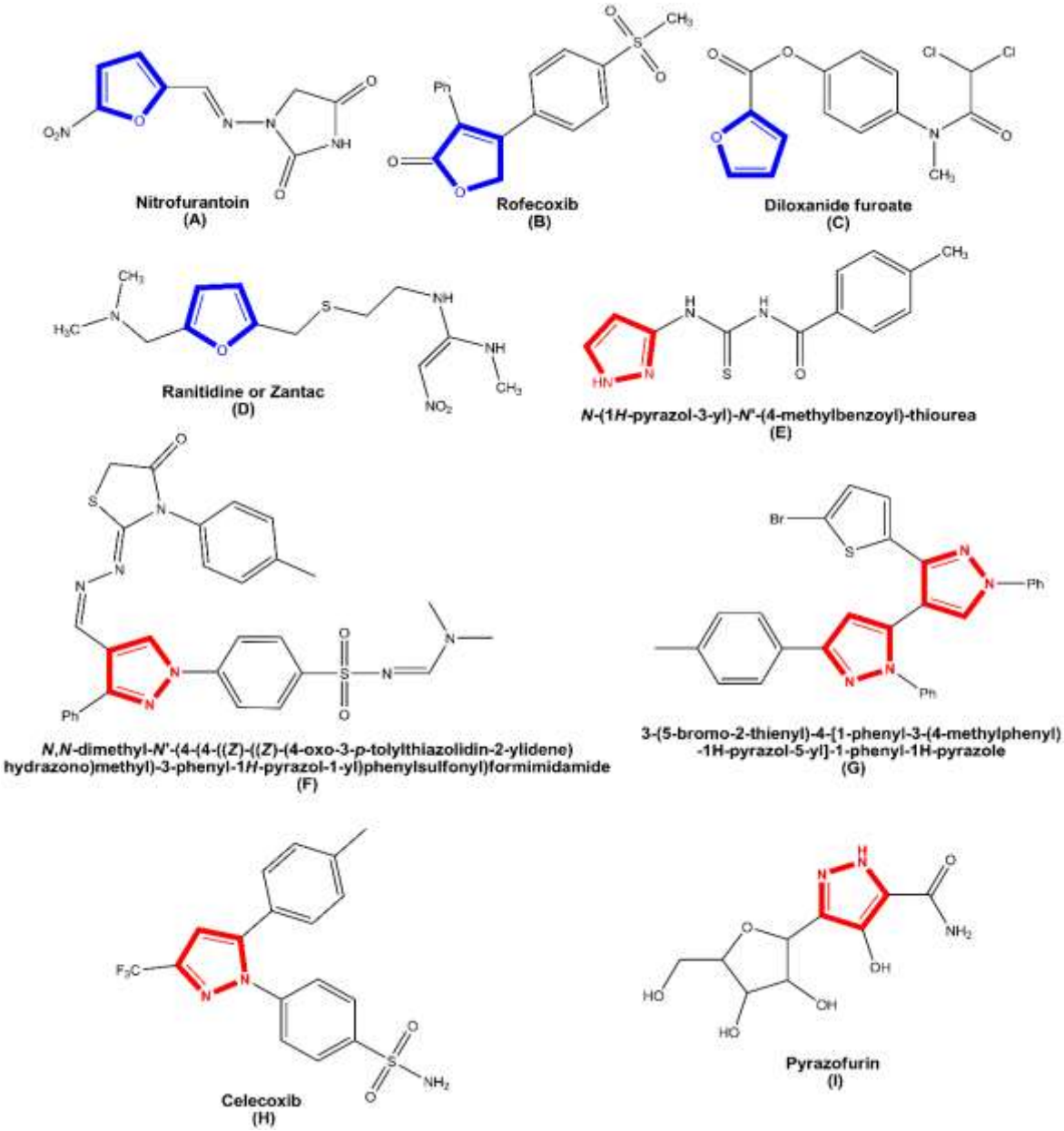


Figure 1. Furan or pyrazole-based drugs

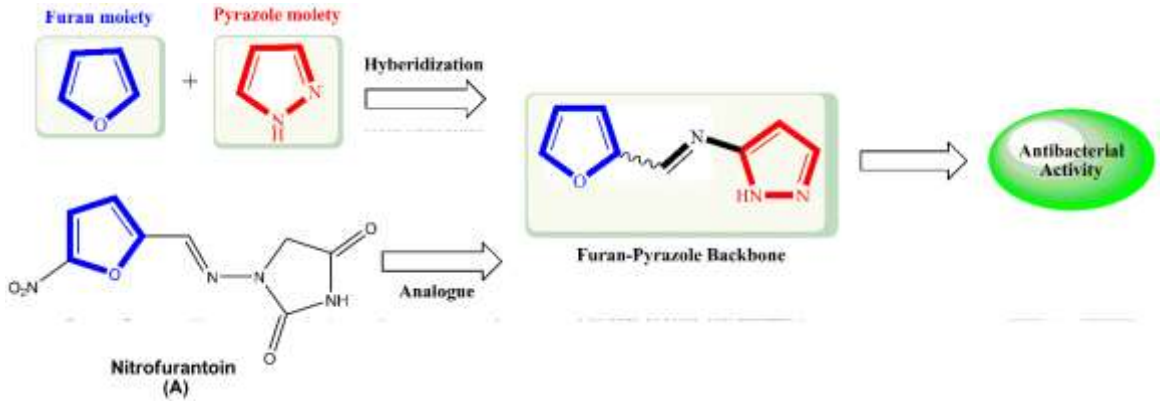


Figure 2. Design of furan-pyrazole derivatives as antibacterial agents

Diloxanide furoate **(C)**, 4-[(dichloroacetyl)(methyl)amino]phenyl furan-2-carboxylate, is an anti-protozoal drug used in the treatment of *Entamoeba histolytica* [2]. Ranitidine or Zantac **(D)**, *N*-(2-[(5-(dimethyl amino methyl)furan-2-yl)methylthio]ethyl)-*N*-methyl-2-nitroethene-1,1-diamine, is a histamine H2-receptor antagonist that inhibits stomach acid production [3]. Pyrazole derivatives have wide biological activities [4-6] e.g. *N*-(1*H*-pyrazol-3-yl)-*N*'-(4-methylbenzoyl)-thiourea **(E)** displayed cytotoxic activity [7]. *N*,*N*-dimethyl-*N*'-(4-(4-((*Z*)-((*Z*)-(4-oxo-3-*p*-tolylthiazolidin-2-ylidene)hydrazono)methyl)-3-phenyl-1*H*-pyrazol-1-yl) phenylsulfonyl)formimidamide **(F)** demonstrated antibacterial activity against *E. coli* [8]. 3-(5-Bromo-2-thienyl)-4-[1-phenyl-3-(4-methylphenyl)-1*H*-pyrazol-5-yl]-1-phenyl-1*H*-pyrazole **(G)** displayed anti-inflammatory activity with selective COX-2 inhibitor and antibacterial activity against *S. aureus* [9]. Furthermore, there are some pyrazole-based drugs such as celecoxib (**H**) is anti-inflammatory with potent COX-2 inhibitor [10]. Pyrazofurin (**I**) is potential of antiviral activity [11]. **Figure 1**

In view of these facts and in continuation of our efforts for preparation of bioactive compounds [12-35], we have designed series of *N*-aryl-3-(arylamino)-5-(((5-substituted furan-2-yl)methylene)amino)-1*H*-pyrazole-4-carboxamide (**7a-g** and **9a-f**) as Nitrofurantoin® analogues and evaluated their antibacterial activities against various microbes. **Figure 2**

Results and discussion

1. Chemistry

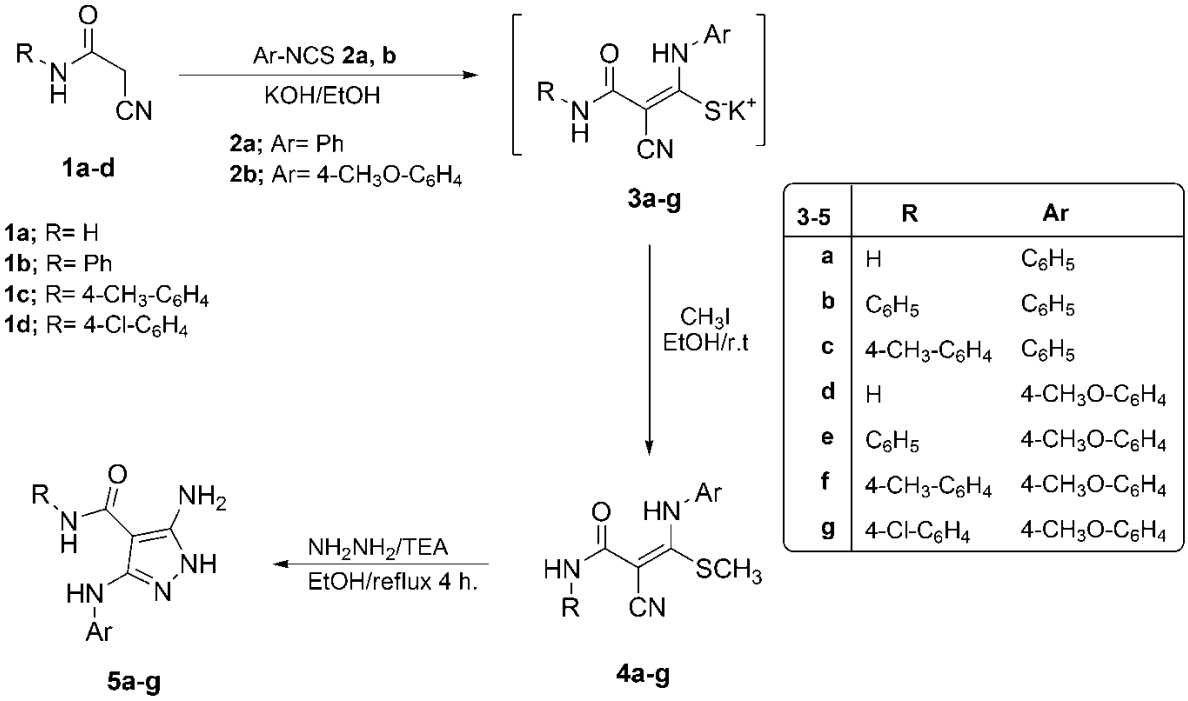
5-Amino-1*H*-pyrazole-4-carboxamides **5a-g** were prepared according to the literature procedure [36-38] as in **Scheme 1.**

Nitrofurantoin® analogues **7a-g,** *N*-aryl-3-(arylamino)-5-((5-nitrofuran-2-yl)methyleneamino)-1*H*-pyrazole-4-carboxamide, were synthesized through the condensation of 5-amino-1*H*-pyrazole-4-carboxamides **5a-g** with 5-nitrofuran-2-carbaldehyde (**6**) in absolute ethanol in the presence of a catalytic amount of glacial CH_3COOH (**Scheme 2**).

The structures of nitrofurantoin® analogues **7a-g** were characterized and confirmed by their spectral data.

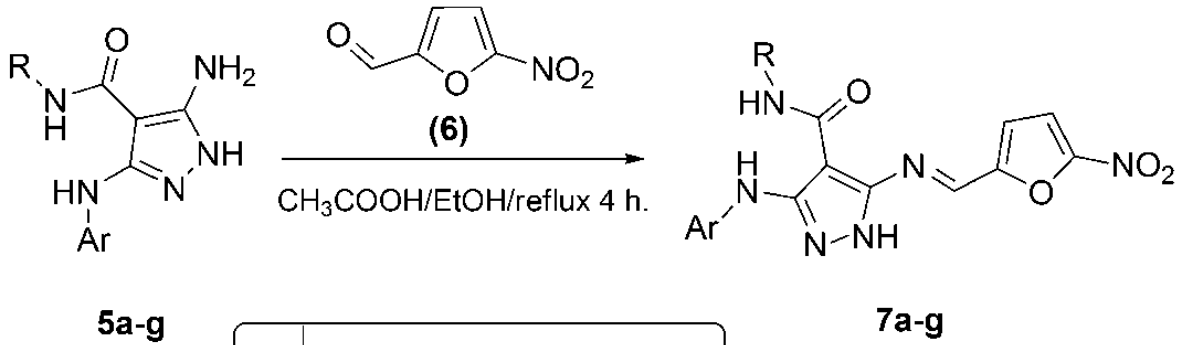
^{1}H NMR spectrum of compound **7e**, 3-(4-methoxyphenylamino)-5-((5-nitrofuran-2-yl)methyleneamino)-*N*-phenyl-1*H*-pyrazole-4-carboxamide, proved that this compound exist in the *E* form, since *trans* (*E*) isomer is more stable than *cis* (*Z*) isomer [39]. Also, this spectrum displayed singlet signals at δ 3.75, 8.68, 9.02, 10.42 and 12.65 ppm for protons of methoxy group, –N=CH–, NH, NH and NH, respectively. The aromatic and furan rings appeared at 6.94 (d, 2H, Aromatic-H, J_{HH} = 8.8 Hz), 7.10 (t, 1H, Aromatic-H), 7.35-7.42 (m, 4H, Aromatic-H), 7.68 (d, 1H, furan H-3, J_{HH} = 2.6 Hz), 7.88 (d, 2H, Aromatic-H, J_{HH} = 8.0 Hz) and 7.90 (d, 1H, furan H-4, J_{HH} = 3.4 Hz).

Moreover, ^{1}H NMR spectrum of compound **7c**, 5-((5-nitrofuran-2-yl)methyleneamino)-3-(phenylamino)-*N*-*p*-tolyl-1*H*-pyrazole-4-carboxamide, showed the presence of *E* and *Z* forms of compound **7c** in 54% and 46% ratio, respectively (**Figure 3**).



3-5	R	Ar
a	H	C_6H_5
b	C_6H_5	C_6H_5
c	4-CH_3-C_6H_4	C_6H_5
d	H	4-CH_3O-C_6H_4
e	C_6H_5	4-CH_3O-C_6H_4
f	4-CH_3-C_6H_4	4-CH_3O-C_6H_4
g	4-Cl-C_6H_4	4-CH_3O-C_6H_4

Scheme 1. Synthesis of compounds **5a-g**



7	R	Ar
a	H	C_6H_5
b	C_6H_5	C_6H_5
c	4-CH_3-C_6H_4	C_6H_5
d	H	4-CH_3O-C_6H_4
e	C_6H_5	4-CH_3O-C_6H_4
f	4-CH_3-C_6H_4	4-CH_3O-C_6H_4
g	4-Cl-C_6H_4	4-CH_3O-C_6H_4

Scheme 2. Synthesis of nitrofurantoin® analogues **7a-g**

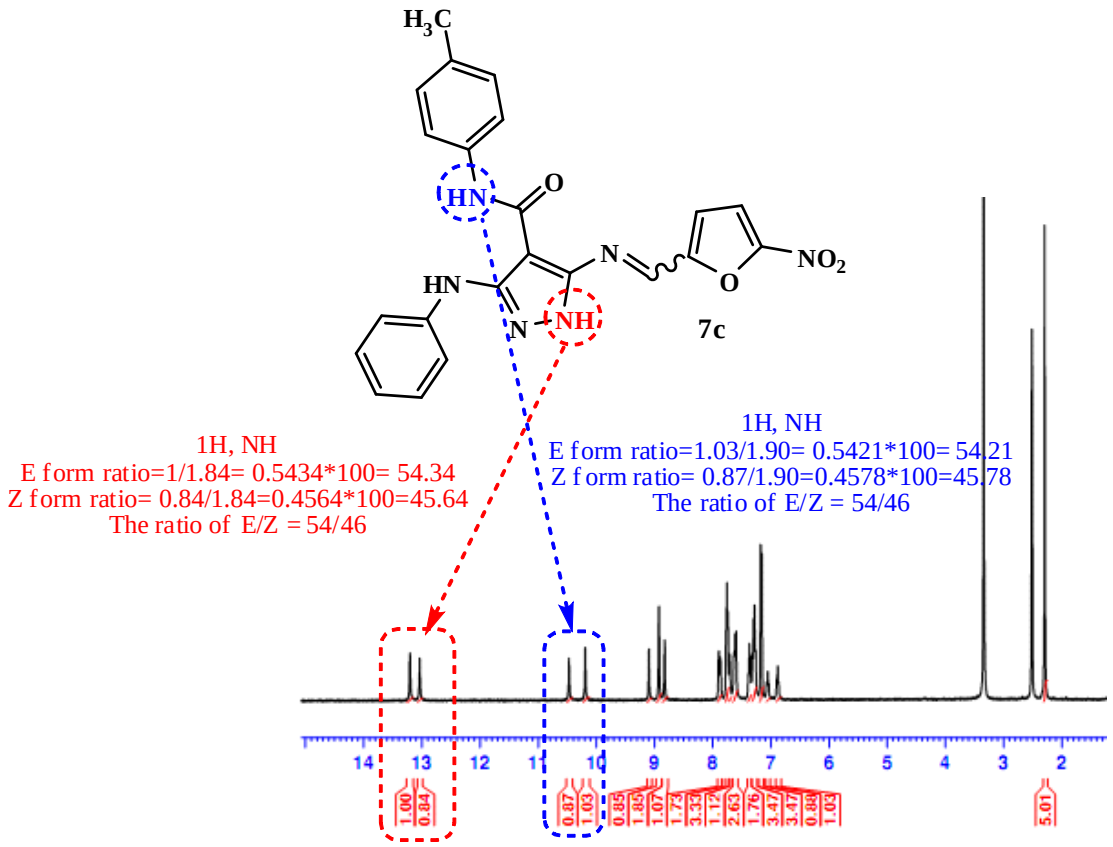
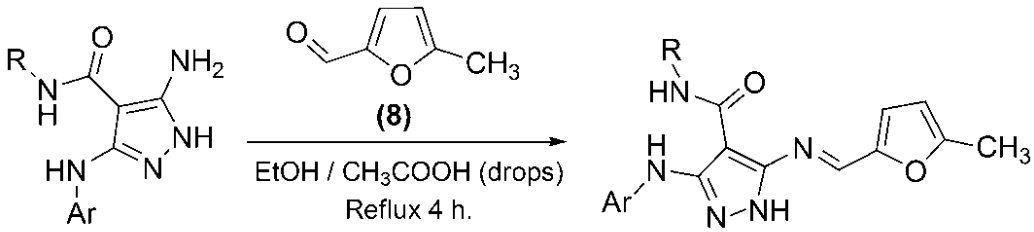


Figure 3. The ^{1}H NMR spectrum of compound **7c**

Moreover, 5-aminopyrazoles **5a** and **5c-g** were condensed with 5-methylfuran-2-carbaldehyde **(8)** in absolute ethanol with glacial CH_3COOH to furnish the target compounds, *N*-aryl-3-(arylamino)-5-((5-methylfuran-2-yl)methyleneamino)-1*H*-pyrazole-4-carboxamide **9a-f** (**Scheme 3**).

IR spectrum of the compound **9b** showed bands at 3222 and 1651 cm^{-1} corresponding to NH and C=O groups, respectively. The mass spectrum revealed a peak at *m/z* = 399 (relative abundance = 81.25 %) equivalent to [M^+, $C_{23}H_{21}N_5O_2$ (399.45)].

Its ^{1}H NMR spectrum displayed two singlet signals at δ 2.26 and 3.37 ppm for two methyl groups. Also, four signals at δ 8.63, 8.89, 10.51 and 12.85 ppm for protons of NH, –N=CH–, NH and NH, respectively. ^{1}H NMR spectrum displayed the aromatic and furan rings at 6.50 (s, 1H, Aromatic-H), 6.86 (s, 1H, furan H-3), 7.16 (d, 2H, Aromatic-H, J_{HH} = 8.3 Hz), 7.28 (t, 2H, Aromatic-H), 7.36 (s, 1H, furan H-4) and 7.61 (d, 4H, Aromatic-H, J_{HH} = 8.3 Hz). ^{13}C NMR of **9b** revealed three signals for two methyl ($2CH_3$) and one carbonyl (C=O) carbons at δ 13.78, 20.45 and 162.59 ppm, respectively.



5a, 5c-g **9a-f**

9	R	Ar
a	H	C_6H_5
b	4-CH_3-C_6H_4	C_6H_5
c	H	4-CH_3O-C_6H_4
d	C_6H_5	4-CH_3O-C_6H_4
e	4-CH_3-C_6H_4	4-CH_3O-C_6H_4
f	4-Cl-C_6H_4	4-CH_3O-C_6H_4

Scheme 3. Synthesis of compounds **9a-f**

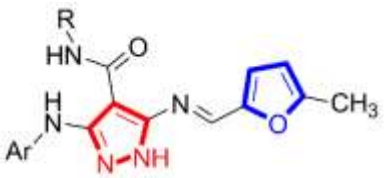
2. Antibacterial activity

The bacterial cultures used in this study were *Escherichia coli* and *Salmonella typhimurium* as representatives for Gram-negative bacteria. Also, *Staphylococcus aureus* and *Streptococcus faecium* were as representatives for Gram-positive bacteria. The test organisms were kindly provided by the Department of Microbiology and Immunology, National Research Centre, Dokki, Egypt. The antibacterial activity of the Nitrofurantoin® analogues (**7a-g** and **9a-f**) expressed as the diameter of the inhibition zones (cm) according to the agar diffusion test [40] and the results are shown in **Table 1**. Nitrofurantoin® antibiotic discs were used as positive control.

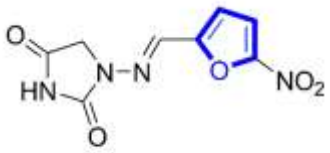
The results revealed that, the four compounds (**7a**, **7d**, **7f** and **7g**) exhibited good antibacterial activities against *Escherichia coli*, while, the compounds **7b**, **7c** and **7e** showed moderate activities in comparison with Nitrofurantoin®. The compounds **7a-g** showed good activities (inhibition zone (IZ) in the range from 1.2 to 1.6 cm) against *Salmonella typhimurium*. The two compounds **9b** and **9d** displayed moderate activities with inhibition zone 0.9 and 0.8 cm, respectively, against *Salmonella typhimurium*. The compounds **7a-g** and **9a-f** were biologically inactive against the two Gram-positive bacteria in this study (*Staphylococcus aureus* and *Streptococcus faecium*). Also, the positive control, Nitrofurantoin®, was inactive against the Gram-positive bacteria.

Table 1: The bacterial activity (inhibition zone (IZ) in cm) of Nitrofurantoin® analogues (**7a-g** and **9a-f**) using the agar diffusion test:-

7a-g



9a-f



Nitrofurantoin

Compounds	R	Ar	Inhibition Zone (IZ, cm)			
			Gram-negative bacteria		Gram-positive bacteria	
			Escherichia coli	*Salmonella typhimurium*	*Staphylococcus aureus*	*Streptococcus faecium*
7a	H	C_6H_5	1.6	1.4	-	-
7b	C_6H_5	C_6H_5	0.9	1.6	-	-
7c	4-CH_3-C_6H_4	C_6H_5	0.75	1.2	-	-
7d	H	4-CH_3O-C_6H_4	1.4	1.2	-	-
7e	C_6H_5	4-CH_3O-C_6H_4	0.7	1.3	-	-
7f	4-CH_3-C_6H_4	4-CH_3O-C_6H_4	1.4	1.3	-	-
7g	4-Cl-C_6H_4	4-CH_3O-C_6H_4	1.6	1.4	-	-
9a	H	C_6H_5	-	-	-	-
9b	4-CH_3-C_6H_4	C_6H_5	-	0.9	-	-
9c	H	4-CH_3O-C_6H_4	-	-	-	-
9d	C_6H_5	4-CH_3O-C_6H_4	-	0.8	-	-
9e	4-CH_3-C_6H_4	4-CH_3O-C_6H_4	-	-	-	-
9f	4-Cl-C_6H_4	4-CH_3O-C_6H_4	-	-	-	-
Nitrofurantoin®			2.00	2.1	-	-

Conclusions

The aim of our work was to design, synthesize, characterize and evaluate the antibacterial activities of *N*-aryl-3-(arylamino)-5-(((5-substituted furan-2-yl)methylene)amino)-1*H*-pyrazole-4-carboxamide (**7a-g** and **9a-f**) as Nitrofurantoin® analogues.

The evaluation showed that the three compounds **7a**, **7b** and **7g** have the highest antibacterial activities against *Escherichia coli* and *Salmonella typhimurium* bacteria. In the future, the project will be extended to study the action mechanism of the three compounds **7a**, **7b** and **7g** with modification to obtain more potent antibacterial agents.

Experimental

1. Chemistry

All melting points were measured on a Gallenkamp melting point apparatus and are uncorrected. The IR spectra were recorded (KBr disk) on a Perkin Elmer 1650 FT-IR instrument. ^{1}H NMR (400 or 500 MHz) and ^{13}C NMR (100 or 125 MHz) spectra were recorded on a Varian spectrometer using DMSO-d_6 as solvent and TMS as an internal standard. Chemical shifts are reported in ppm. Coupling constants (*J*) are expressed in Hz. Mass spectra were recorded on a Varian MAT 112 spectrometer at 70 eV. Elemental analyses were performed at the Microanalytical Center, Cairo University, Egypt.

Progress of the reactions was monitored by thin-layer chromatography (TLC) using aluminum sheets coated with silica gel F_{254} (Merck), viewing under a short-wavelength UV lamp effected detection. All evaporations were carried out under reduced pressure at 40 ^{o}C.

Synthesis of Nitrofurantoin® analogues, *N*-aryl-3-(arylamino)-5-((5-nitrofuran-2-yl)methyleneamino)-1*H*-pyrazole-4-carboxamide, 7a-g

A mixture of compounds **5a-g** (0.01 mol) with 5-nitrofuran-2-carbaldehyde (**6**) (0.01 mol) and a catalytic amount of glacial CH_3COOH (1 mL) in absolute ethanol (25 mL) was refluxed for 1 hour and the solid obtained was collected and recrystallized from EtOH.

5-((5-Nitrofuran-2-yl)methyleneamino)-3-(phenylamino)-1*H*-pyrazole-4-carboxamide (7a)

Reddish-brown crystals, m.p. 280-282 ºC, yield (70%). IR (KBr) ν_{max}/cm^{-1} 3358, 3181 (NH, NH_2), 1651 (C=O). ^{1}H NMR (400 MHz, δ ppm) (*E* form) 6.88 (s, 1H, Aromatic-H), 7.21-7.53 (m, 6H, Aromatic-H + NH_2 exchanged with D_2O), 7.74 (d, 1H, furan H-3, J_{HH} = 3.9 Hz), 7.87 (d, 1H, furan H-4, J_{HH} = 3.1 Hz), 8.81 (s, 1H, NH exchanged with D_2O), 9.10 (s, 1H, –N=CH–), 12.95 (s, 1H, NH exchanged with D_2O). Anal. Calcd. (%) for $C_{15}H_{12}N_6O_4$ (340.29): C, 52.94; H, 3.55; N, 24.70. Found: C, 53.00; H, 3.50; N, 24.75 %.

5-((5-Nitrofuran-2-yl)methyleneamino)-*N*-phenyl-3-(phenylamino)-1*H*-pyrazole-4-carboxamide (7b)

Reddish-brown crystals, m.p. 268-270 ºC, yield (75%). IR (KBr) ν_{max}/cm^{-1} 3324 (NH), 1647 (C=O). ^{1}H NMR (400 MHz, δ ppm) (the ratio of *E*/*Z* = 50/50) 6.88 (t, 1H, Aromatic-H), 7.04-7.13 (m, 2H, Aromatic-H), 7.26-7.42 (m, 3H, Aromatic-H), 7.60 (d, 2H, Aromatic-H, J_{HH} = 7.9 Hz), 7.64, 7.72 (d, 1H, furan H-3, J_{HH} = 3.6 Hz, *E/Z form*), 7.85 (d, 2H, Aromatic-H, J_{HH} = 7.0 Hz), 7.88, 7.90 (d, 1H, furan H-4, J_{HH} = 3.8 Hz, *E/Z form*), 8.85, 8.90 (s, 1H, -N=CH-, *E/Z form*), 8.92, 9.11 (s, 1H, NH exchanged with D_2O, *E/Z form*), 10.25, 10.54 (s, 1H, NH

exchanged with D_2O, *E/Z form*), 13.03, 13.22 (s, 1H, NH exchanged with D_2O, *E/Z form*). Anal. Calcd. (%) for $C_{21}H_{16}N_6O_4$ (416.39): C, 60.57; H, 3.87; N, 20.18. Found: C, 60.49; H, 3.92; N, 20.10 %.

5-((5-Nitrofuran-2-yl)methyleneamino)-3-(phenylamino)-*N*-*p*-tolyl-1*H*-pyrazole-4-carboxamide (7c)

Orange crystals, m.p. 266-268 ºC, yield (75%). IR (KBr) ν_{max}/cm^{-1} 3261 (NH), 1648 (C=O). ^{1}H NMR (400 MHz, δ ppm) (the ratio of *E/Z* = 54/46) 2.26 (s, 3H, CH_3, *E/Z form*) 6.88, 7.05 (t, 1H, Aromatic-H, *E/Z form*), 7.16 (d, 2H, Aromatic-H, J_{HH} = 8.1 Hz, *E/Z form*), 7.27, 7.37 (t, 2H, Aromatic-H, *E/Z form*), 7.60 (d, 2H, Aromatic-H, J_{HH} = 7.8 Hz, *E/Z form*), 7.62, 7.70 (d, 1H, furan H-3, J_{HH} = 3.3 Hz, *E/Z form*), 7.74 (d, 2H, Aromatic-H, J_{HH} = 7.1 Hz, *E/Z form*), 7.85, 7.89 (d, 1H, furan H-4, J_{HH} = 2.9 Hz, *E/Z form*), 8.83, 8.92 (s, 1H, -N=CH-, *E/Z form*), 8.92, 9.08 (s, 1H, NH, exchanged with D_2O, *E/Z form*), 10.19, 10.46 (s, 1H, NH, exchanged with D_2O, *E/Z form*), 13.03, 13.21 (s, 1H, NH, exchanged with D_2O, *E/Z form*). Anal. Calcd. (%) for $C_{22}H_{18}N_6O_4$ (430.42): C, 61.39; H, 4.22; N, 19.53. Found: C, 61.45; H, 4.17; N, 19.60 %.

3-(4-Methoxyphenylamino)-5-((5-nitrofuran-2-yl)methyleneamino)-1*H*-pyrazole-4-carboxamide (7d)

Reddish-brown crystals, m.p. 270-272 ºC, yield (77%). IR (KBr) ν_{max}/cm^{-1} 3220 (NH), 1649 (C=O). ^{1}H NMR (400 MHz, δ ppm) (the ratio of *E/Z* = 50/50) 3.70, 3.74 (s, 3H, OCH_3, *E/Z form*), 6.86, 6.93 (d, 2H, Aromatic-H, J_{HH} = 8.2 & 7.9 Hz, *E/Z form*), 7.17, 7.47 (d, 2H, Aromatic-H, J_{HH} = 8.2 & 8.4 Hz, *E/Z form*), 7.24, 7.32 (s, 1H, NH_2 exchanged with D_2O, *E/Z form*), 7.37, 7.50 (s, 1H, NH_2, *E/Z form*), 7.64, 7.73 (d, 1H, furan H-3, J_{HH} = 3.0 & 3.8 Hz, *E/Z form*), 7.83, 7.87 (d, 1H, furan H-4, J_{HH} = 2.8 Hz, *E/Z form*), 8.77, 8.78 (s, 1H, -N=CH-, *E/Z form*), 8.88, 8.99 (s, 1H, NH exchanged with D_2O, *E/Z form*), 12.60, 12.84 (s, 1H, NH exchanged with D_2O, *E/Z form*). Anal. Calcd. (%) for $C_{16}H_{14}N_6O_5$ (370.32): C, 51.89; H, 3.81; N, 22.69. Found: C, 51.80; H, 3.86; N, 22.75 %.

(*E*)-3-(4-methoxyphenylamino)-5-((5-nitrofuran-2-yl)methyleneamino)-*N*-phenyl-1*H*-pyrazole-4-carboxamide (7e)

Reddish-brown crystals, m.p. 265-267 ºC, yield (80%). IR (KBr) ν_{max}/cm^{-1} 3253 (NH), 1650 (C=O). ^{1}H NMR (400 MHz, δ ppm) (*E* form) 3.75 (s, 3H, OCH_3), 6.94 (d, 2H, Aromatic-H, J_{HH} = 8.8 Hz), 7.10 (t, 1H, Aromatic-H), 7.35-7.42 (m, 4H, Aromatic-H), 7.68 (d, 1H, furan H-3, J_{HH} = 2.6 Hz), 7.88 (d, 2H, Aromatic-H, J_{HH} = 8.0 Hz), 7.90 (d, 1H, furan H-4, J_{HH} = 3.4 Hz), 8.68 (s, 1H, -N=CH-), 9.02 (s, 1H, NH exchanged with D_2O), 10.42 (s, 1H, NH exchanged with D_2O), 12.65 (s, 1H, NH exchanged with D_2O). Anal. Calcd. (%) for $C_{22}H_{18}N_6O_5$ (446.42): C, 59.19; H, 4.06; N, 18.83. Found: C, 59.10; H, 4.12; N, 18.90 %.

(*E*)-3-(4-Methoxyphenylamino)-5-((5-nitrofuran-2-yl)methyleneamino)-*N*-*p*-tolyl-1*H*-pyrazole-4-carboxamide (7f)

Reddish-brown crystals, m.p. 258-260 ºC, yield (81%). IR (KBr) ν_{max}/cm^{-1} 3225 (NH), 1651 (C=O). ^{1}H NMR (400 MHz, δ ppm) (*E* form) 2.29 (s, 3H, CH_3), 3.77 (s, 3H, OCH_3), 6.92 (d, 2H, Aromatic-H, J_{HH} = 6.8 Hz), 7.14 (d, 2H, Aromatic-H, J_{HH} = 6.0 Hz), 7.34 (s, 2H, Aromatic-H), 7.61 (d, 1H, furan H-3, J_{HH} = 4.8 Hz), 7.73 (d, 2H, Aromatic-H, J_{HH} = 7.6 Hz), 7.85 (d, 1H, furan H-4, J_{HH} = 4.0 Hz), 8.69 (s, 1H, -N=CH-), 8.94 (s, 1H, NH exchanged with D_2O), 10.30 (s, 1H, NH exchanged with D_2O), 12.65 (s, 1H, NH exchanged with D_2O). Anal. Calcd. (%) for

$C_{23}H_{20}N_6O_5$ (460.44): C, 60.00; H, 4.38; N, 18.25. Found: C, 59.91; H, 4.46; N, 18.31 %.

(*E*)-*N*-(4-Chlorophenyl)-3-(4-methoxyphenylamino)-5-((5-nitrofuran-2-yl)methyleneamino)-1H-pyrazole-4-carboxamide (7g)

Orange crystals, m.p. 240-242 ºC, yield (79%). IR (KBr) ν_{max}/cm^{-1} 3230 (NH), 1659 (C=O). ^{1}H NMR (400 MHz, δ ppm) (*E* form) 3.75 (s, 3H, OCH_3), 6.95 (d, 2H, Aromatic-H, J_{HH} = 7.3 Hz), 7.38 (d, 2H, Aromatic-H, J_{HH} = 8.5 Hz), 7.46 (d, 1H, furan H-3, J_{HH} = 3.9 Hz), 7.81 (d, 1H, furan H-4, J_{HH} = 3.9 Hz), 7.87 (d, 4H, Aromatic-H, J_{HH} = 8.6 Hz), 8.72 (s, 1H, -N=CH-), 9.07 (s, 1H, NH exchanged with D_2O), 10.60 (s, 1H, NH exchanged with D_2O), 12.72 (s, 1H, NH exchanged with D_2O). Anal. Calcd. (%) for $C_{22}H_{17}ClN_6O_5$ (480.86): C, 54.95; H, 3.56; N, 17.48. Found: C, 55.60; H, 3.51; N, 17.40 %.

Synthesis of *N*-aryl-3-(arylamino)-5-((5-methylfuran-2-yl)methyleneamino)-1*H*-pyrazole-4-carboxamide 9a-f

A mixture of compounds **5a, 5c-g** (0.01 mol) with 5-methylfuran-2-carbaldehyde **(8)** (0.01 mol) in absolute ethanol (30 mL) and a catalytic amount of glacial acetic acid (four drops) was refluxed for 1 hour. The solvent was concentrated under reduced pressure and the solid obtained was collected and recrystallized from ethanol to give **9a-f**.

5-{[(5-Methylfuran-2-yl)methylidene]amino}-3-(phenylamino)-1*H*-pyrazole-4-carboxamide (9a):

Yellow crystals, m.p. 235-238 °C [41].

(*E*)-5-((5-methylfuran-2-yl)methyleneamino)-3-(phenylamino)-*N*-(4-methylphenyl)-1*H*-pyrazole-4-carboxamide (9b)

Yellow crystals, m.p. 248-250 ºC, yield (82%). IR (KBr) ν_{max}/cm^{-1} 3222 (NH), 1651 (C=O). ^{1}H NMR (500 MHz, δ ppm) (*E* form) 2.26 (s, 3H, CH_3), 3.37 (s, 3H, CH_3), 6.50 (s, 1H, Aromatic-H), 6.86 (s, 1H, furan H-3), 7.16 (d, 2H, Aromatic-H, J_{HH} = 8.3 Hz), 7.28 (t, 2H, Aromatic-H), 7.36 (s, 1H, furan H-4), 7.61 (d, 4H, Aromatic-H, J_{HH} = 8.3 Hz), 8.63 (s, 1H, NH exchanged with D_2O), 8.89 (s, 1H, –N=CH–), 10.51 (s, 1H, NH exchanged with D_2O), 12.85 (s, 1H, NH exchanged with D_2O). ^{13}C NMR (125 MHz, δ ppm) 13.78, 20.45 (2C, $2CH_3$), 93.92 (C_4, pyrazole), 110.9, 116.28, 119.59, 122.19, 123.85, 129.02, 129.49, 132.02, 136.38, 141.40, 145.52, 147.96, 149.60, 157.39, 159.37 (19C), 162.59 (C=O). MS (*m/z*, %): 399 (M^+, 81.25). Anal. Calcd. (%) for $C_{23}H_{21}N_5O_2$ (399.45): C, 69.16; H, 5.30; N, 17.53. Found: C, 69.10; H, 5.35; N, 17.60 %.

3-[(4-Methoxyphenyl)amino]-5-{[(5-methylfuran-2-yl)methylidene]amino}-1H-pyrazole-4-carboxamide(9c)

Buff crystals, m.p. 232-234 °C [41].

3-(4-Methoxyphenylamino)-5-((5-methylfuran-2-yl)methyleneamino)-*N*-phenyl-1*H*-pyrazole-4-carboxamide (9d)

Yellow crystals, m.p. 200-202 ºC [42].

3-(4-Methoxyphenylamino)-5-((5-methylfuran-2-yl)methyleneamino)-*N*-(4-methylphenyl)-1*H*-pyrazole-4-carboxamide (9e)

Yellow crystals, m.p. 202-204 ºC [42].

3-(4-Methoxyphenylamino)-5-((5-methylfuran-2-yl)methyleneamino)-*N*-(4-chlorophenyl)-1*H*-pyrazole-4-carboxamide (9f)

Yellow crystals, m.p. 202-204 ºC [42].

2. Screening for the antibacterial activity

The bacterial cultures used in this study were *Staphylococcus aureus* and *Streptococcus faecium* as representatives for Gram-positive bacteria and *Escherichia coli* and *Salmonella typhimurium* as representatives for Gram-negative bacteria. The test organisms were kindly provided by the Department of Microbiology and Immunology, National Research Centre, Dokki, Egypt. The test organisms were maintained on agar slant at 4 °C and subcultured on a fresh agar plates. Bacterial liquid cultures were initiated by placing a loop of bacteria from the slant into 10 mL of lysogeny broth (LB) media. Agar diffusion test was conducted to detect the bacterial susceptibility to the prepared compounds [40]. A volume of 100 µL of cell culture suspension matching with 0.5 Mc-Farland of each test organism were spread onto the surface of solid agar medium (Muller Hinton agar). The prepared compounds were adjusted to a concentration of 20 mg/mL using DMSO as solvent. Filter paper discs with a diameter of 7 mm each were impregnated with 15 µL of each of the different compounds (equivalent to ≈ 300 µg) which is comparable to the antibiotic concentration in the antibiotic discs used as control. Then the soaked discs were placed on the surface of agar plates containing the microorganisms and incubated at 37±0.1 °C for 24 hours. The inhibition of bacterial growth was evaluated by measuring the diameter (cm) of the clear zone around each disc. Nitrofurantoin® antibiotic discs were used as positive control. Filter paper discs impregnated with 15 µL of DMSO were also used as control for the solvent.

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Conflict of interest

The authors declare that they have no competing interests.

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