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# New 5-arylamino-4-(5-nitrofuran-2-yl)pyrimidines as promising antibacterial agents

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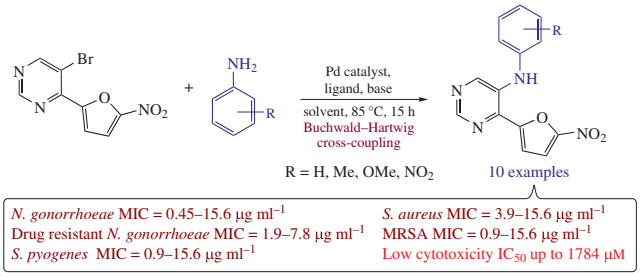
**A facile synthetic approach to 5-arylamino-4-(5-nitrofuran-2-yl)pyrimidines by the Buchwald–Hartwig cross-coupling with various anilines has been developed. All synthesized compounds demonstrated a significant level of *in vitro* antibacterial activity against *Neisseria gonorrhoeae*, *Streptococcus pyogenes* and *Staphylococcus aureus*, including their drug-resistant strains, which is much higher than that of the commercial drug Spectinomycin.**



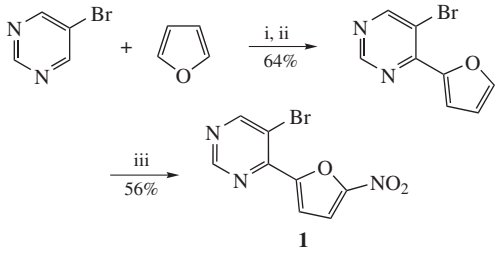

An increasing bacterial resistance to wide range of commercial antibiotics has caused life-threatening infectious diseases.[1,2] More recently the multiple drug resistant (MDR) *Staphylococcus strains* have become the phenomena number one in case of hospital-associated infections.[3–5] Moreover, there is an ever growing concern on our ability to treat infections, caused by *Neisseria gonorrhoeae*, as highlighted in the statement of the World Health Organization in July 2017, concerning the emergence and spread of alleles, conferring resistance to multiple antimicrobials in naturally competent bacteria.[6,7]

Nitrofuran derivatives have been used as antibacterials since the beginning of 1940s, commencing from introduction of nitrofurazone.[8] This substance has given a rise to the next generation of nitrofuran antibacterial agents[9–12] (*e.g.*, antitubercular nitroimidazoles PA-824[13] and OPC-67683,[14] as well as other related compounds[15]) (Figure S1, see Online Supplementary Materials). In addition, 4-(5-nitrofuranyl) substituted pyrimidines proved to be antibacterial agents with a broad-spectrum activity against both Gram-negative and Gram-positive bacteria.[16–22]

Motivated by these findings, we have adopted the strategy[23,24] of using the consecutive nucleophilic aromatic substitution of hydrogen ($S_N^H$) and Pd-catalyzed cross-coupling, in order to incorporate the fragments of substituted anilines into the structure of 4-(5-nitrofuran-2-yl)pyrimidine to obtain novel compounds, in anticipation of enhancement of antimicrobial activity.

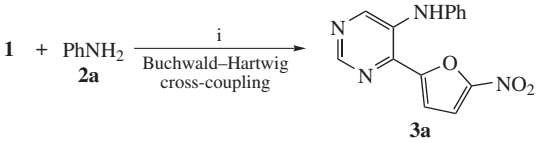
In this communication, we intend to describe arylamination of 5-bromo-4-(5-nitrofuran-2-yl)pyrimidine **1** with various anilines through the Buchwald–Hartwig cross-coupling, and to present the data on evaluation of their antimicrobial activities *in vitro* against *M. tuberculosis* $H_{37}Rv$, gram-negative (*N. gonorrhoeae*, *E. coli*, *C. braakii*, *S. flexneri*, *P. vulgaris*, *S. marcescens*, *K. pneumoniae*, *P. aeruginosa*) and gram-positive (*S. pyogenes* and *S. aureus*) bacteria.

5-Bromo-4-(5-nitrofuran-2-yl)pyrimidine **1**,[22] used as the starting material, has previously been obtained from 5-bromopyrimidine *via* the $S_N^H$ methodology[25] (Scheme 1).




**Scheme 1** *Reagents and conditions:* i, $CF_3COOH$, ~20 °C, 24 h; ii, $K_3Fe(CN)_6$, KOH, $H_2O$, ~20 °C, 24 h; iii, $HNO_3$, $H_2SO_4$, $CH_2Cl_2$, –10 °C.

In our initial studies, we have been trying to define suitable conditions for the Pd-catalyzed coupling of 5-bromo-4-(5-nitrofuran-2-yl)pyrimidine **1** with aniline **2a** (Scheme 2, Table 1). Palladium catalysts derived from several bidentate phosphines, including dppf and Xantphos, have been shown to afford high




**Scheme 2** *Reagents and conditions:* i, [Pd], ligand, base, solvent, 85 °C, 15 h (see Table 1).

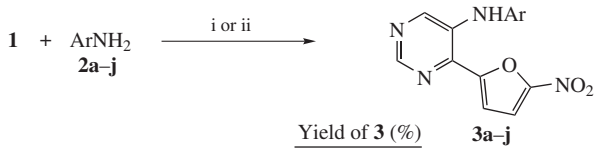
**Table 1** Optimization of the Buchwald–Hartwig cross-coupling reaction conditions.[a]

| Entry | Palladium source (mol%) | Ligand (equiv.) | Base (2.5 equiv.) | Solvent | Yield (%) |
|---|---|---|---|---|---|
| 1 | $Pd_2(dba)_3$ (10) | Xantphos (0.2) | $Bu^tONa$ | toluene | 13 |
| 2 | $Pd_2(dba)_3$ (10) | Xantphos (0.2) | $Bu^tONa$ | 1,4-dioxane | 41 |
| 3 | $Pd_2(dba)_3$ (10) | Xantphos (0.2) | $K_3PO_4$ | 1,4-dioxane | 58 |
| 4 | $Pd(PPh_3)_4$ (10) | – | $K_3PO_4$ | 1,4-dioxane | 0 |
| 5 | $Pd(OAc)_2$ (10) | Xantphos (0.2) | $K_3PO_4$ | 1,4-dioxane | 76 |
| 6 | $Pd(OAc)_2$ (10) | dppf (0.2) | $K_3PO_4$ | 1,4-dioxane | 67 |
| 7 | $Pd(OAc)_2$ (20) | dppf (0.4) | $K_3PO_4$ | 1,4-dioxane | 56 |
| 8 | $Pd(OAc)_2$ (10) | dppf (0.2) | AcOK | 1,4-dioxane | 0 |

[a]Xantphos is 4,5-bis(diphenylphosphino)-9,9-dimethylxanthene; dppf is (1,1′-ferrocenediyl)bis(diphenylphosphine); $Pd_2(dba)_3$ is tris(dibenzylideneacetone)dipalladium(0).

yields of the coupled product **3a** (entries 5 and 6). Both dppf and Xantphos have been exploited as supporting ligands in palladium-catalyzed C–N cross-coupling reactions,[26] however cheaper dppf was chosen for further studies.

At the next step, we have examined the scope of varying the aniline moiety in the Pd-catalyzed arylamination of bromide **1** with anilines **2a–j** (Scheme 3, conditions i). Unfortunately, the most of *N*-aryl-4-(5-nitrofuran-2-yl)pyrimidin-5-amines **3**, except for **3a** and **3g**, were obtained in poor yields (2–45%). The structures of products **3a**,**g** were established unequivocally by X-ray crystallography analysis (Figures 1 and 2).†




| | Ar | Yield of **3** (%) i | ii |
|---|---|---|---|
| **a** | Ph | 67 | 56 |
| **b** | 3-$MeC_6H_4$ | 33 | 47 |
| **c** | 4-$MeC_6H_4$ | 41 | 67 |
| **d** | 3-$MeOC_6H_4$ | 9 | 35 |
| **e** | 4-$MeOC_6H_4$ | 23 | 45 |
| **f** | 3,4-$(MeO)_2C_6H_3$ | 17 | 55 |
| **g** | 3,4,5-$(MeO)_3C_6H_2$ | 59 | 50 |
| **h** | 2-$NO_2C_6H_4$ | 2 | 29 |
| **i** | 3-$NO_2C_6H_4$ | 36 | 53 |
| **j** | 4-$NO_2C_6H_4$ | 45 | 58 |

**Scheme 3** *Reagents and conditions:* i, $Pd(OAc)_2$ (10 mol%), dppf (20 mol%), $K_3PO_4$ (2.5 equiv.), 1,4-dioxane, 85 °C; ii, $Pd(OAc)_2$ (20 mol%), dppf (40 mol%), $K_3PO_4$ (2.5 equiv.), 1,4-dioxane, 85 °C.

† *Crystallographic data for* **3a**: crystals of $C_{14}H_{10}N_4O_3$ ($M$ = 282.26) are monoclinic, space group $P12_1/c1$, at 295 K: $a$ = 17.5800(6), $b$ = 19.9566(7) and $c$ = 17.3295(17) Å, $\beta$ = 99.411(4)°, $V$ = 2562.11(18) Å$^3$, $Z$ = 8, $d_{calc}$ = = 1.463 g cm$^{-3}$, $\mu$(MoKα) = 0.107 mm$^{-1}$, $F$(000) = 1168. Intensities of 6900 reflections were measured and 4669 independent reflections ($R_{int}$ = = 0.0457) were used in a further refinement. The refinement converged to $wR_2$ = 0.1278 and GOF = 1.010 for all independent reflections [$R_1$ = 0.0457 was calculated against $F$ for 3190 observed reflections with $I > 2\sigma(I)$]. The measurements were made on an Xcalibur 3 diffractometer with graphite-monochromated MoKα radiation ($\lambda$ = 0.71073 Å).

*Crystallographic data for* **3g**: crystals of $C_{17}H_{16}N_4O_6$ ($M$ = 372.34) are monoclinic, space group $P2_1/c$, at 295 K: $a$ = 10.112(10), $b$ = 11.582(5) and $c$ = 14.365(11) Å, $\beta$ = 95.37(7)°, $V$ = 1675(2) Å$^3$, $Z$ = 4, $d_{calc}$ = = 1.477 g cm$^{-3}$, $\mu$(CuKα) = 0.968 mm$^{-1}$, $F$(000) = 776. Intensities of 17688 reflections were measured and 2895 independent reflections ($R_{int}$ = = 0.0925) were used in a further refinement. The refinement converged to $wR_2$ = 0.0763 and GOF = 1.005 for all independent reflections [$R_1$ = 0.0435 was calculated against $F$ for 1328 observed reflections with $I > 2\sigma(I)$]. The measurements were made on an Xcalibur 3 diffractometer diffractometer with graphite-monochromated CuKα radiation ($\lambda$ = 1.54184 Å).

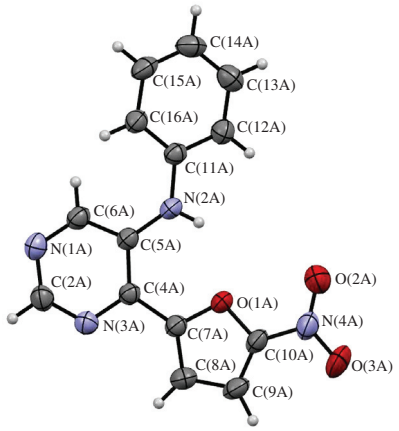



**Figure 1** ORTEP representation of the X-ray crystal structure of **3a** with thermal ellipsoids of 50% probability.

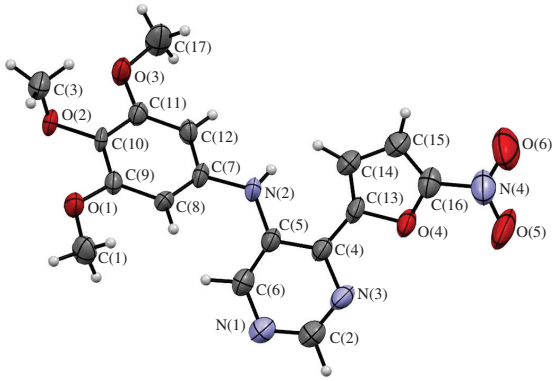



**Figure 2** ORTEP representation of the X-ray crystal structure of **3g** with thermal ellipsoids of 50% probability.

To improve yields of the desired products **3a–j**, quantities of both phosphine ligand and $Pd(OAc)_2$ were doubled. Indeed, this step enabled to obtain the corresponding products **3b–f**,**h–j** in reasonable (29–67%) yields (see Scheme 3, conditions ii).‡ It is a curious fact, but yields of products **3a** and **3g** went down from 67 to 56% and from 59 to 50%, respectively. Notably, both electron-rich (**2b–g**) and electron-deficient (**2h–j**) anilines underwent cross-coupling in a similar manner. In case of sterically encumbered *ortho*- (**2h**) and *meta*-substituted (**2b**,**d**,**i**) anilines, the expected diarylamines **3** were formed in lower yields compared to those with unsubstituted **2a** and *para*-substituted **2c**,**e**,**j** anilines, regardless of the reaction conditions.

The structures were solved by direct methods, and the non-hydrogen atoms were located from the trial structure and then refined anisotropically with SHELXTL using a full-matrix least-squares procedure based on $F^2$.[27] All non-hydrogen atoms were refined anisotropically, the positions of the hydrogen atoms were calculated as a riding model in isotropic approximation.

CCDC 1587557 and 1587558 contain the supplementary crystallographic data for this paper. These data can be obtained free of charge from The Cambridge Crystallographic Data Centre *via* http://www.ccdc.cam.ac.uk.

‡ N-*Aryl-4-(5-nitrofuran-2-yl)pyrimidin-5-amines* **3** *(typical procedure).* A stirred mixture of 5-bromo-4-(5-nitrofuran-2-yl)pyrimidine **1** (270 mg, 1.0 mmol), corresponding aniline **2** (1.2 mmol), (1,1′-ferrocenediyl)bis(diphenylphosphine) (222 mg, 40 mol%), $Pd(OAc)_2$ (45 mg, 20 mol%) and $K_3PO_4$ (531 mg, 2.5 mmol) in deaerated 1,4-dioxane (25 ml) was heated in Schlenk tube at 85 °C under nitrogen for 15 h. The reaction mixture was cooled, filtered, and dissolved with a mixture of AcOEt and water (1 : 1, 50 ml) and the organic layer was separated. The aqueous layer was extracted with AcOEt (2×25 ml). The combined organic extracts were dried with $MgSO_4$ and the solvents were evaporated. The residue was purified by flash column chromatography (hexane–ethyl acetate, 1 : 3) to afford the desired cross-coupling products **3a–j**.

Pyrimidines **3a–j** were screened for their activity *in vitro* against fourteen strains of gram-negative (*N. gonorrhoeae*, *E. coli*, *C. braakii*, *S. flexneri*, *P. vulgaris*, *S. marcescens*, *K. pneumoniae*, *P. aeruginosa*) and gram-positive (*S. pyogenes* and *S. aureus*) bacteria. The data on minimum inhibitory concentrations (MICs) for these compounds are summarized in Table S1 (see Online Supplementary Materials). Clinical drug Spectinomycin was taken as a positive control.

The general trend is that $NO_2$-phenyl substituted derivatives **3b–g** proved to have lower antibacterial activities than those of Me or OMe substituted analogues **3h–j**. Pyrimidines **3b**,**d** with *meta* Me or OMe groups show much higher activities against strains *S. pyogenes* and *S. aureus*, which are up to 278 times higher than that of Spectinomycin (see Table S1, entries 2 and 4). On the other hand, 4-(5-nitrofuran-2-yl)-*N*-(3,4,5-trimethoxyphenyl)-pyrimidin-5-amine **3g** was found to be the most effective (up to 0.45 μg ml$^{-1}$) against *N. gonorrhoeae* including multidrug-resistant strains (see Table S1, entry 7).

Five compounds **3a**,**c**,**e–g**, exhibiting a high level of activity against *N. gonorrhoeae* NCTC 8375/ATCC19424 (MIC from 0.9 to 3.9 μg ml$^{-1}$), have also been elucidated against *M. tuberculosis* $H_{37}$Rv. It has been found that only 4-(5-nitrofuran-2-yl)-*N*-phenylpyrimidin-5-amine **3a** demonstrates a relatively high level of tuberculostatic activity (MIC = 1.5 μg ml$^{-1}$), while all other compounds **3c**,**e–g** exhibit a low level of activity against *M. tuberculosis* $H_{37}$Rv (MIC = 12.5 μg ml$^{-1}$).

*In vitro* cytotoxicity of compounds **3a–j** has been evaluated against mouse fibroblast-like cell line (McCoy B) using colorimetric assay with *p*-Nitro-Blue tetrazolium chloride.[28] The $IC_{50}$ values obtained for these compounds are given in Table 2. The majority of the tested compounds **3c–h** exhibit a low cytotoxic effect on McCoy B cells, as compared to Spectinomycin as a standard.

Interestingly, six compounds showing the highest antibacterial potency and low cytotoxicity **3c–h** proved to bear Me or OMe substituents in the arylamino moiety, the only variation being the position in the phenyl group. It can be used as a basis for design of the next generation analogues (using a scaffold-hopping approach[29]), where the pharmacophoric 5-nitrofuran-2-yl and arylamino substituents could be affixed in an azine scaffold.

In conclusion, it is worth mentioning, that we have designed and synthesized novel scaffolds of pyrimidine conjugating nitrofurans, which enable to effectively inhibit the strains of *N. gonorrhoeae*, *S. pyogenes* and *S. aureus* with the activity level much higher than that of Spectinomycin. According to the above data, novel *N*-aryl-4-(5-nitrofuran-2-yl)pyrimidin-5-amines can be regarded as promising antibacterial agents for treatment of urinary tract infections, as well as purulent-inflammatory infections of skin.

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*Online Supplementary Materials*

Supplementary data associated with this article can be found in the online version at doi: 10.1016/j.mencom.2018.07.017.

*4-(5-Nitrofuran-2-yl)*-N-*phenylpyrimidin-5-amine* **3a**: Yield 160 mg (56%), dark red crystals, mp 123–124 °C. $^1$H NMR (500 MHz, DMSO-$d_6$) $\delta$: 8.89 (s, 1H), 8.82 (s, 1H), 8.30 (s, 1H), 7.83 (d, 1H, *J* 4.0 Hz), 7.47 (d, 1H, *J* 4.0 Hz), 7.28 (m, 2H), 7.05 (d, 2H, *J* 7.7 Hz), 6.95 (m, 1H). $^{13}$C NMR (126 MHz, DMSO-$d_6$) $\delta$: 151.8, 151.6, 151.1, 150.5, 141.8, 141.5, 135.0, 129.4, 121.7, 117.9, 116.2, 114.2. GC $t_R$ 26.40 min; MS, *m/z* (%): 282 ($M^+$, 100). HRMS (ESI), *m/z*: 283.0822 $[M+H]^+$ (calc. for $C_{14}H_{11}N_4O_3$, *m/z*: 283.0826). Found (%): C, 59.40; H, 3.48; N, 19.82. Calc. for $C_{14}H_{10}N_4O_3$ (%): C, 59.57; H, 3.57; N, 19.85.

**Table 2** Levels of cytotoxicity induced by selected compounds **3a–j** on MacCoy B cells.

| Compounds | Molecular weight | $IC_{50}$/μg ml$^{-1}$ | $IC_{50}$/μM |
|---|---|---|---|
| **3a** | 282.26 | 9.39 | 33.27 |
| **3b** | 296.29 | 42.90 | 144.79 |
| **3c** | 296.29 | 528.70 | 1784.40 |
| **3d** | 312.29 | 127.70 | 408.91 |
| **3e** | 312.29 | 119.70 | 383.30 |
| **3f** | 342.31 | 220.20 | 643.28 |
| **3g** | 372.34 | 458.90 | 1232.48 |
| **3h** | 327.26 | 100.80 | 308.01 |
| **3i** | 327.26 | 9.36 | 28.60 |
| **3j** | 327.26 | 11.23 | 34.38 |
| SPEC | 332.33 | >500 | >1504.43 |

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