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Synthesis and antituberculosis activity of novel 5-styryl-4-(hetero)aryl-pyrimidines *via* combination of the Pd-catalyzed Suzuki cross-coupling and S_N^H reactions

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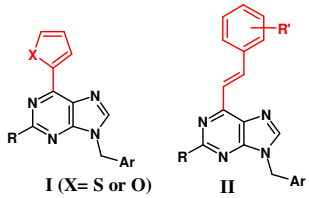
ABSTRACT

Combination of the Suzuki cross-coupling and nucleophilic aromatic substitution of hydrogen (S_N^H) reactions proved to be a convenient method for the synthesis of 5-styryl-4-(hetero)aryl substituted pyrimidines from commercially available 5-bromopyrimidine. All intermediate 5-bromo-4-(hetero)aryl substituted pyrimidines and also the targeted 5-styryl-4-(hetero)arylpyrimidines were found to be active in micromolar concentrations *in vitro* against *Mycobacterium tuberculosis* $H_{37}Rv$, *avium, terrae*, and multi-drug-resistant strain isolated from tuberculosis patients in Ural region (Russia). It has been found that some of these compounds possess a low toxicity and have a bacteriostatic effect, comparable and even higher with that of first-line antituberculosis drugs.

Tuberculosis* (TB) is still a very serious disease, which claims approximately two millions of deaths per year world wide.[1] The wide spread of TB is due to the following major factors: the susceptibility of people infected with the acquired immune deficiency syndrome (AIDS), which enhances the risk of developing TB in 100 times, and increasing resistance to the existing drugs.[2]

Although a long term solution of the problem is associated with the development of a better vaccine, in the nearest future the major role will certainly belong to the chemotherapy,[3] requiring novel, effective, non-toxic antituberculosis agents.[1,3,4]

It has been established that some pyrimidines,[5-9] and purine derivatives[1,10-12] possess a high antituberculosis activity. Screening of the series of 6-substituted and 2,6-disubstituted 9-benzylpurines revealed several compounds with a promising level of activity against M. tuberculosis, with a general trend that 6-substituted derivatives to be more effective than 2,6-disubstituted compounds. Indeed, a high inhibitory activity was found for purines of general formula **I** and **II** (Fig. 1), bearing *trans*-styryl or heteroaryl substituents in the 6-position; the most promising compound containing the furyl substituent was active at the minimal inhibitory concentration (MIC) comparable with that for rifampicin.[12]



I (X= S or O) **II**

Figure 1. General structures of antimycobacterial 9-substituted purines

Our research studies were focused on the synthesis and biological testing of novel 4,5-disubstituted pyrimidines containing both 2-thienyl- (2-furyl-) and *trans*-styryl fragments. The target 5-styryl-4-(hetero)arylpyrimidines **7a-d** were synthesized from commercially available 5-bromo-pyrimidine **(1)**, as shown in Scheme 1. At the first stage, we obtained 5-bromo-4-(hetero)arylpyrimidines **3a-d** by using the reaction of nucleophilic aromatic substitution of hydrogen (S_N^H). It has previously been shown that 5-bromopyrimidine **(1)** reacts with thiopene **(2a)**, bithiophene **(2b)** and 2-phenylthiophene **(2c)** in CF_3COOH to afford the corresponding

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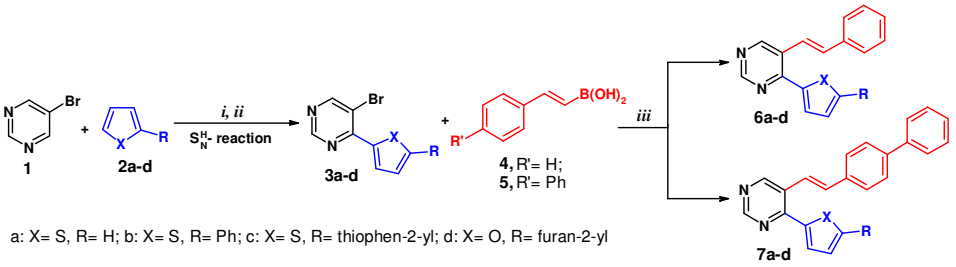
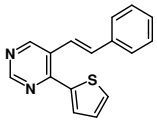
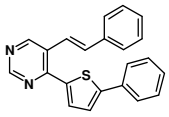
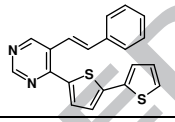
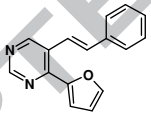
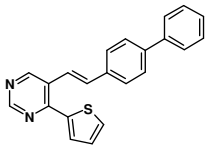
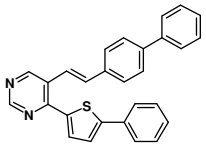
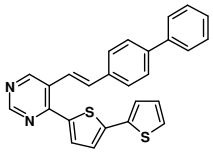
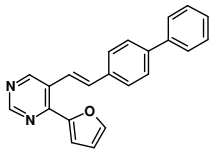


Scheme 1. Reagents and conditions: (*i*) CF_3COOH, r.t., 24 hours; (*ii*) $K_3Fe(CN)_6$, KOH, H_2O, r.t., 6 hours; (*iii*) $Pd(PPh_3)_4$, K_2CO_3, H_2O, THF, 155 °C, MW, 20 min.

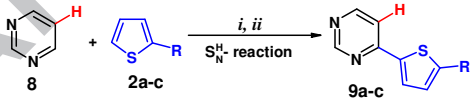
σ^H-adducts – 5-bromo-4-(hetero)aryl-3,4-dihydropyrimidinium salts.[13] Oxidation of these compounds with $K_3Fe(CN)_6$ in an aqueous solution of KOH gave 5-bromo-4-(hetero) arylpyrimidines **3a-c**. The reaction 5-bromopyrimidine **(1)** with furan **(2d)** under the same conditions leads to the corresponding 5-bromo-4-(furyl-2'l)pyrimidine **(3d)** in a good yield (64%).

Table 1. The microwave-assisted Suzuki cross-coupling reaction of 5-bromo-4-(hetero)arylpyrimidines **(3a-d)** with *trans*-2-styrilboronic acids **(4** and **5)**

Entry	Reaction	Isolated yield (%)	Products
1	**3a + 4**	**6a** – 88	
2	**3b + 4**	**6b** – 71	
3	**3c + 4**	**6c** – 80	
4	**3d + 4**	**6d** – 95	
5	**3a + 5**	**7a** – 54	
6	**3b + 5**	**7b** – 52	
7	**3c + 5**	**7c** – 62	
8	**3d + 5**	**7d** – 52	

Bromo derivatives **3a-d** have been involved in the Suzuki reaction with *trans*-2-styrylboronic acids **4** and **5** under microwave irradiation (155 °C, 20 min). The corresponding cross-coupling products, (*E*)-5-arylethenyl-4-(hetero)aryl-pyrimidines **6a-d** and **7a-d,** have been obtained in high yields (Scheme 1, Table 1).

5-Bromo- **(3a-d)** and 5-arylethenyl- **(6a-d** and **7a-d)** substituted 4-(hetero)arylpyrimidines were screened for their activity *in vitro* against *Mycobacterium tuberculosis* $H_{37}Rv$, *avium, terrae,* and multi-drug-resistant strains. The antimycobacterial activities for 5-bromo- **(3a-c)** and 5-arylethenyl- **(6a-c** and **7a-c)** substituted 4-(hetero)arylpyrimidines were correlated with activities of recently obtained 5-unsubsituted 4-(hetero)arylpyrimidines (Scheme 2).[13]



Scheme 2. Reagents and conditions: (*i*) CF_3COOH, r.t., 24 hours; (*ii*) $K_3Fe(CN)_6$, KOH, H_2O, r.t., 6 hours.

The synthesized pyrimidine derivatives were compared to the commercially available drugs Isoniazid and Pyrazinamide under the same experimental conditions (Table 2).

All 5-unsubsituted 4-thiehylpyrimidines **9a-c** demonstrated a low level of tuberculostatic activity (MIC = 12.5 μg/mL). However, 5-bromo substituted pyrimidines **3a-d** proved to exhibit significant inhibitory properties against *Mycobacterium tuberculosis* $H_{37}Rv$ (MIC from 6.25 to 0.7 μg/mL). As a rule, substitution of bromo at C-5 with an arylethenyl fragment results in a decrease of the tuberculostatic activity to 12.5 μg/mL. Introduction of [2,2′]bithiophenyl substituent into C-4 position is accompanied with a considerable increase in anti-TB activities of pyrimidines **6c** and **7c** (MIC= 1.5 μg/mL). For the most active compound **3a**, **3b** and **3d** the acute toxicity on white mice was determined (Table 2). These compounds were less toxic for white mice than Isoniazid. For compounds **6c** and **7c** acute oral toxicity was three or four times higher than that for compounds **3a,b,d**. Since the ability of some synthesized 5-substituted pyrimidines to inhibit the growth of mycobacteria is comparable with Pyrazinamide or even higher, these structures can be regarded as potential prototypes for design of new antituberculosis drugs.

In summary, we have obtained a novel series of 5-arylethenyl-4-(hetero)arylpyrimidines by using a convenient two step procedure. All compounds have exhibited a reasonable level of activity against tuberculosis and a low toxicity. Being not active enough to be therapeutics, they certainly can be considered as promising structures for further studies aimed at the development of novel effective agents to combat resistant forms of TB.

Table 2. *In vitro* antituberculosis activity of 5-bromo-4-(hetero)arylpyrimidines **(3a-d)**, (*E*)-5-arylethenyl-4-(hetero)arylpyrimidines **(6a-d** and **7a-d)**, 4-(hetero)arylpyrimidines **(9a-c)** and their acute *in vivo* toxicity in mice

Entry	Compound	Antimycobacterial activity against different strains of *Mycobacterium tuberculosis* (MIC in μg/mL)				LD_{50} mouse, mg/kg
		$H_{37}Rv$	*M. avium*	*M. terrae*	MDR[a]	
1	**3a**	0.7	0.3	0.3	0.7	315
2	**3b**	1.5	0.7	0.7	1.5	225
3	**3c**	6.25	n.d.	n.d.	n.d.	n.d.
4	**3d**	1.5	0.7	0.7	1.5	405
5	**6a**	12.5	n.d.	n.d.	n.d.	n.d.
6	**6b**	12.5	n.d.	n.d.	n.d.	n.d.
7	**6c**	1.5	0.7	0.7	1.5	100
8	**6d**	6.25	n.d.	n.d.	n.d.	n.d.
9	**7a**	12.5	n.d.	n.d.	n.d.	n.d.
10	**7b**	12.5	n.d.	n.d.	n.d.	n.d.
11	**7c**	1.5	0.7	0.7	1.5	78
12	**7d**	12.5	n.d.	n.d.	n.d.	n.d.
13	**9a**	12.5	n.d.	n.d.	n.d.	n.d.
14	**9b**	12.5	n.d.	n.d.	n.d.	n.d.
15	**9c**	12.5	n.d.	n.d.	n.d.	n.d.
	PZA	12.5	n.d.	n.d.	-	n.a[b] 1680[c]
	INH	0.1	0.1	0.1	-	133[b] 151[c]

n.d. - not determined; n.a. - data not available; INH – Isoniazid; PZA – Pyrazinamide;
[a]MDR (multi-drug-resistant tuberculosis strain) – Rifampin and Isoniazid resistant *Mycobacterium tuberculosis* strain having Beijing genotype with a combination of mutations *Ser 531 - Leu 315* and *Ser-Thr* in *rpoB* and *katG* genes, respectively.
[b]LD_{50} Oral mouse[14]
[c]LD_{50} Intraperitoneal mouse[14]

Acknowledgments

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Supplementary Material

Supplementary material (experimental procedures, characterization of final compounds and biological assays protocols) associated with this article can be found in the online version.

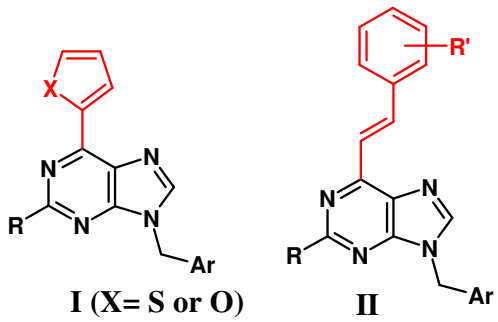
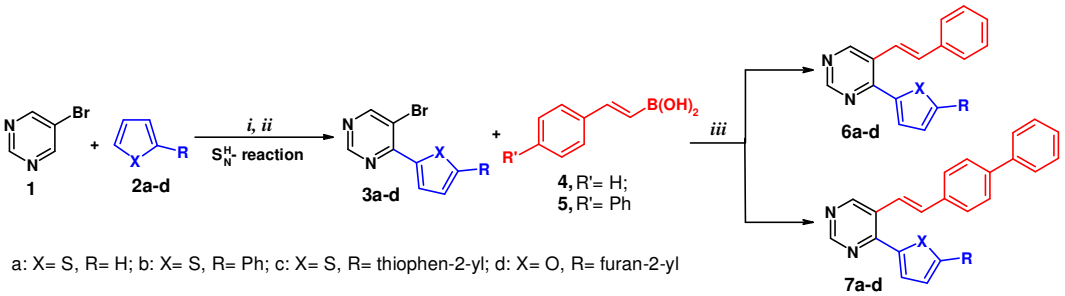
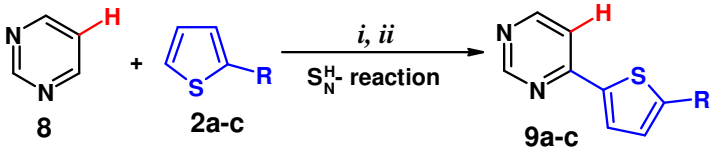


Figure 1. General structures of antimycobacterial 9-substituted purines



a: X= S, R= H; b: X= S, R= Ph; c: X= S, R= thiophen-2-yl; d: X= O, R= furan-2-yl

Scheme 1. Reagents and conditions: (*i*) CF_3COOH, r.t., 24 hours; (*ii*) $K_3Fe(CN)_6$, KOH, H_2O, r.t., 6 hours; (*iii*) $Pd(PPh_3)_4$, K_2CO_3, H_2O, THF, 155 ºC, MW, 20 min.



a: R= H; b: R= Ph; c: R= thiophen-2-yl

Scheme 2. Reagents and conditions: (*i*) CF_3COOH, r.t., 24 hours; (*ii*) $K_3Fe(CN)_6$, KOH, H_2O, r.t., 6 hours.

Table 1. The microwave-assisted Suzuki cross-coupling reaction of 5-bromo-4-(hetero)arylpyrimidines **(3a-d)** with *trans*-2-styrilboronic acids **(4** and **5)**

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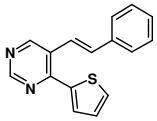
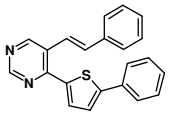
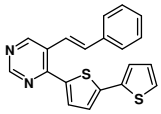
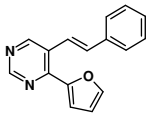
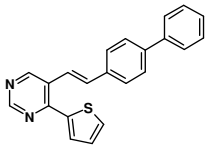
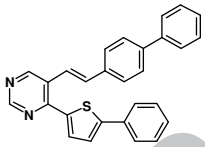
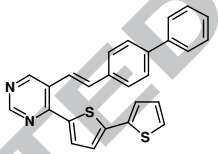
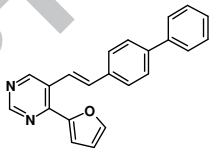
1	**3a + 4**	**6a** – 88	
2	**3b + 4**	**6b** – 71	
3	**3c + 4**	**6c** – 80	
4	**3d + 4**	**6d** – 95	
5	**3a + 5**	**7a** – 54	
6	**3b + 5**	**7b** – 52	
7	**3c + 5**	**7c** – 62	
8	**3d + 5**	**7d** – 52	

Table 2. *In vitro* antituberculosis activity of 5-bromo-4-(hetero)arylpyrimidines **(3a-d)**, (*E*)-5-arylethenyl-4-(hetero)arylpyrimidines **(6a-d** and **7a-d)**, 4-(hetero)arylpyrimidines **(9a-c)** and their acute *in vivo* toxicity in mice

Entry	Compound	Antimycobacterial activity against different strains of *Mycobacterium tuberculosis* (MIC in μg/mL)				LD_{50} mouse, mg/kg
		$H_{37}Rv$	*M. avium*	*M. terrae*	MDR[a]	
1	**3a**	0.7	0.3	0.3	0.7	315
2	**3b**	1.5	0.7	0.7	1.5	225
3	**3c**	6.25	n.d.	n.d.	n.d.	n.d.
4	**3d**	1.5	0.7	0.7	1.5	405
5	**6a**	12.5	n.d.	n.d.	n.d.	n.d.
6	**6b**	12.5	n.d.	n.d.	n.d.	n.d.
7	**6c**	1.5	0.7	0.7	1.5	100
8	**6d**	6.25	n.d.	n.d.	n.d.	n.d.
9	**7a**	12.5	n.d.	n.d.	n.d.	n.d.
10	**7b**	12.5	n.d.	n.d.	n.d.	n.d.
11	**7c**	1.5	0.7	0.7	1.5	78
12	**7d**	12.5	n.d.	n.d.	n.d.	n.d.
13	**9a**	12.5	n.d.	n.d.	n.d.	n.d.
14	**9b**	12.5	n.d.	n.d.	n.d.	n.d.
15	**9c**	12.5	n.d.	n.d.	n.d.	n.d.
	PZA	12.5	n.d.	n.d.	-	n.a[b] 1680[c]
	INH	0.1	0.1	0.1	-	133[b] 151[c]

n.d. - not determined; n.a. - data not available; INH – Isoniazid; PZA – Pyrazinamide;
[a]MDR (multi-drug-resistant tuberculosis strain) – Rifampin and Isoniazid resistant *Mycobacterium tuberculosis* strain having Beijing genotype with a combination of mutations *Ser 531 - Leu 315* and *Ser-Thr* in *rpoB* and *katG* genes, respectively.
[b]LD_{50} Oral mouse[14]
[c]LD_{50} Intraperitoneal mouse[14]

Graphical Abstract

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Synthesis and antituberculosis activity of novel 5-styryl-4-(hetero)arylpyrimidines *via* combination of the Pd-catalyzed Suzuki cross-coupling and S_N^H reactions

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