Synthesis and biological evaluations of new families of histidine kinases inhibitors

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SYNTHESIS AND BIOLOGICAL EVALUATIONS OF NEW FAMILIES OF HISTIDINE KINASES INHIBITORS

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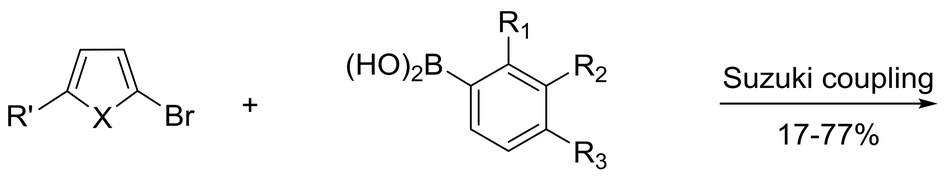
I. INTRODUCTION

Nowadays, the emergence of multidrug resistant bacteria requires interest in the medical field and thereby an urgent need to develop efficient and specific methods in targeting unique bacterial cellular processes.[1,2] Two-component signal transduction systems (TCS) are widely used in bacteria to allow adaptation, osmosis and resistance appearance by translating an external signal into a cellular response. In order to attenuate these signaling pathways, we aimed at targeting the TCS signal transducer histidine kinase by focusing on their highly conserved ATP-binding domain.[3] Preliminary modeling work carried out in our laboratory led to a series of thiophene derivatives. Twenty-one new thiophene derivatives were synthesized and evaluated *in vitro* on bacterial histidine kinases (HK) PhoR, ResE and WalK, from which eight derivatives showed significant inhibitory activity. One compound exhibited broad-spectrum antimicrobial activity with the particularity to restore antibacterial activity of antibiotics ineffective on resistant bacteria treated alone,[4] With the purpose to improve biological activity, amino thiophene derivatives have been developed.

II. SYNTHESIS



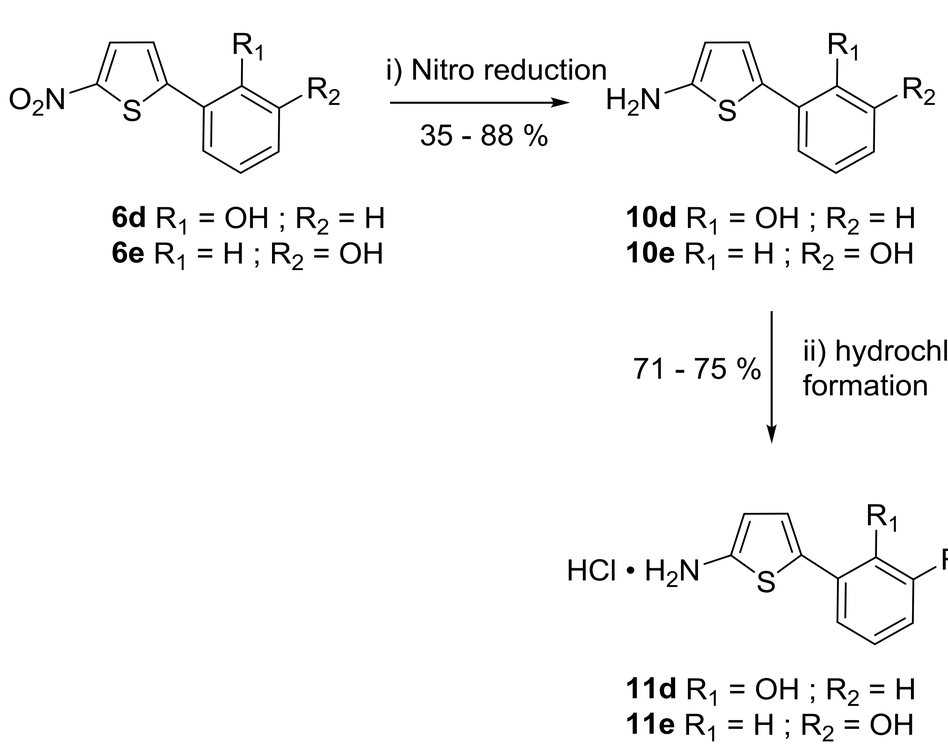
1 X = S ; R' = NO_2
2 X = S ; R' = CH_3CO
3 X = S ; R' = $NHCOCH_3$
4 X = O ; R' = NO_2

5a R_1 = NH_2 ; R_2 = H ; R_3 = H
5b R_1 = H ; R_2 = NH_2 ; R_3 = H
5c R_1 = H ; R_2 = H ; R_3 = NH_2
5d R_1 = OH ; R_2 = H ; R_3 = H
5e R_1 = H ; R_2 = OH ; R_3 = H
5f R_1 = H ; R_2 = H ; R_3 = OH
5g R_1 = OCH_3 ; R_2 = OCH_3 ; R_3 = H

6a-6g X = S ; R' = NO_2
7a-7g X = S ; R' = CH_3CO
8a-8g X = S ; R' = $NHCOCH_3$
9d-9g X = O ; R' = NO_2

Reagents and conditions : $Pd(PPh_3)_4$ or $PdCl_2(PPh_3)_2$, Na_2CO_3 or K_2CO_3 or Cs_2CO_3, Toluene/EtOH or 1,4-dioxane/H_2O or DMF/H_2O or DMF, 70-90°C.

Scheme 1: Synthesis of thiophene and furanes derivatives.



6d R_1 = OH ; R_2 = H
6e R_1 = H ; R_2 = OH

10d R_1 = OH ; R_2 = H
10e R_1 = H ; R_2 = OH

11d R_1 = OH ; R_2 = H
11e R_1 = H ; R_2 = OH

Reagents and conditions : i) Pd/C, $NaBH_4$, MeOH, 0°C to r.t., 30 min; ii) conc. HCl, THF, 0°C, 1h

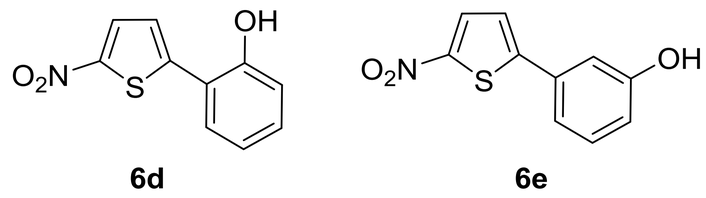
Scheme 2: Synthesis of hydrochloride salts **11d** and **11e**.

All the derivatives were characterized by FT-IR, ^1H, ^{13}C NMR, UPLC and HRMS.

III. BIOLOGICAL EVALUATIONS

Evaluations have been realized on other proteins of the GHKL (DNA Gyrase, Hsp90, Histidine Kinase, MutL) superfamily. The 8 potent molecules are:

- Specific towards tested HK (WalK, PhoR and ResE)
- Inactive towards Ser/Thr kinase (IreK)
- Inactive towards DNA gyrase
- No hemolytical activity



6d **6e**

Figure 1: Structures of molecules **6d** and **6e**.

6d exhibits **adjuvant activity** in presence of antibiotics whereas **6e** showed antibacterial activity.

Table 1. IC_{50} values against histidine kinase PhoR, ResE and WalK; Ser/Thr kinase IreK and DNA-gyrase for compounds **6d**, **6e** and **11d**.

Compounds	PhoR (µM)	ResE (µM)	WalK (µM)	IreK (µM)	DNA-gyrase (µM)
6d	122.6	124.3	196.9	> 800	> 400
6e	13.11	89.36	52.81	> 800	> 800
11d	50.53	/	/	/	/

IV. CONCLUSION

In this work, we have designed and synthesized a series of 24 molecules with thiophene and furane ring by Suzuki-Miyaura coupling. On the other hand, two hydrochloride salts of nitrothiophene derivatives were synthesized to increase solubility, stability and therefore biodisponibility. All compounds were characterized by ^1H and ^{13}C NMR and analyzed by LC-MS and HRMS. Two of them (**6d** and **6e**) exhibit significant inhibition of multiple HK and exhibited broad-spectrum antimicrobial activity. Moreover they do not inhibit the others GHKL superfamily proteins in addition to not hydrolyzing sheep red blood cells. More soluble hydrochloride salts of first designed amines (**6a-8c**) do not improve histidine kinase inhibition. The compounds were also evaluated as adjuvant against *Escherichia coli extended spectrum* β-*lactamase* (ESBL) and *Methicillin resistant Staphylococcus aureus* (MRSA). From this latter, derivative **6d** showed adjuvant properties because of its tendency to restore antibacterial activity in presence of antibiotics on resistant bacteria.

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