An efficient and convenient route for the synthesis of thiophene-2- carboxamidines as potential inhibitors of nitric oxide synthase (NOS)

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Graphical Abstract

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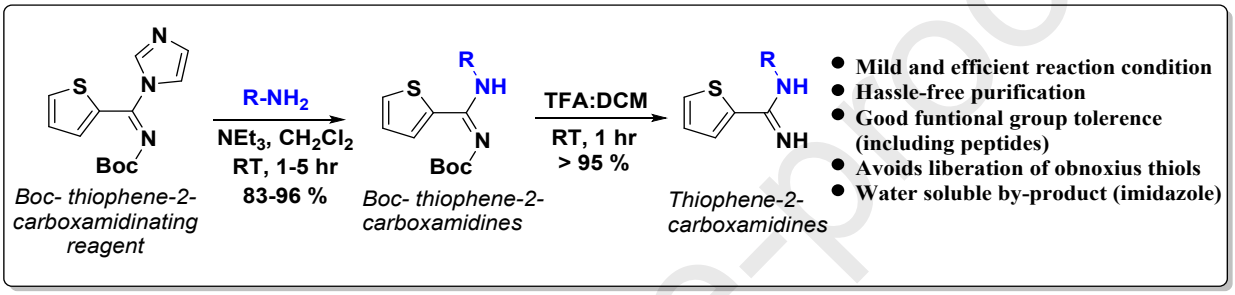
PP. Rashid,[a,b] Dharmendra Singh,[a,b] and Gangadhar J. Sanjayan[a,b]*



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An efficient and convenient route for the synthesis of thiophene-2-carboxamidines as potential inhibitors of nitric oxide synthase (NOS).

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ABSTRACT

A mild and efficient synthesis of substituted thiophene-2-carboxamidines which are potent inhibitors of nitric oxide synthase (NOS) is reported herein. The key step involves reaction of a BOC-protected imidazolyl thiophene-2-carboxamidine reactive intermediate with various primary amines to form BOC- thiophene-2-carboxamidines which could be readily deprotected using TFA to furnish free carboxamidines. The method is very mild and tolerates diverse substituents including sensitive peptide and amino acid fragments. This new methodology represents a substantial improvement to the literature method owing to its simplicity and hassle-free purification procedures.

Introduction

Nitric oxide (NO) is a vital important signaling molecule in the human body, having a crucial role in cell and neuronal communication, regulation of blood pressure, and in immune activation. Nitric oxide synthase (NOS) is a class of heme-dependent enzymes which generate NO from L-arginine. Overproduction of NO by a neuronal isoform of NOS (nNOS) could cause spinal transmission of pain, migraine and chronic tension-type headaches, Parkinson's disease, Alzheimer's disease and neurodegeneration during stroke.[1a-e]

Among the various potent NOS inhibitors developed in recent years, thiophene-2-carboxamidine has emerged as a "privileged structure" which is found in many recently developed NOS inhibitors with high isoform selectivity. Thiophene-2-carboxamidine-based NOS inhibitors are known to selectively bind to the neuronal isoform of NOS over endothelial NOS (eNOS) and inducible NOS (iNOS) and regulate the overproduction of NO. The selective inhibition of nNOS over the other isoforms is critical because each isoform has individual biological implications.[2a-b]

Several nNOS inhibitors are known featuring thiophene-2-carboxamidine as a "privileged structure" (Fig. 1). It is noteworthy that thiophene-2-carboxamidine-based nNOS inhibitors also feature good pharmacological profiles showing promise of developing drug candidates. Despite the widespread interest in thiophene-2-carboxamidines as potent nNOS inhibitors, their synthesis is often met with diverse challenges.

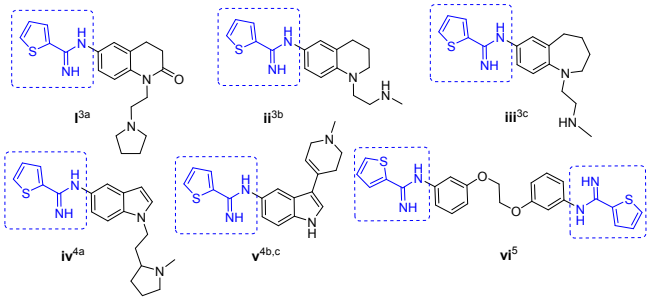
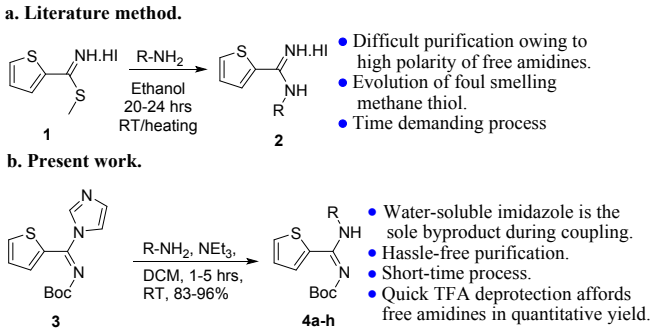


Fig 1. Thiophene-2- carboxamidine-based nNOS inhibitors.

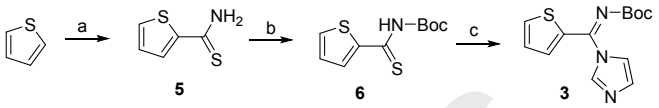


Scheme 1. a) Literature method and b) present method for the synthesis of substituted thiophene-2- carboxamidines.

A comparison between the literature and present methods for thiophene-2-carboxamidine synthesis is illustrated in scheme-1 (*vide supra*). The traditional method[6a,b] involves the reaction of thiomethyl thiophene carboxamidine **1** (scheme-1) with amine forming free amidines **2** which are highly polar - rendering purification cumbersome. Further disadvantage with this method

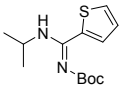
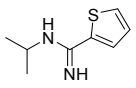
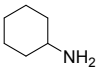
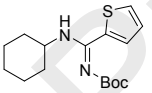
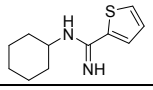
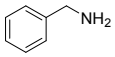
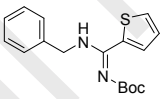
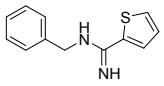
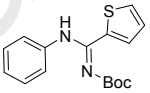
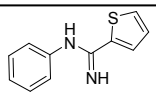
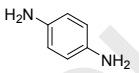
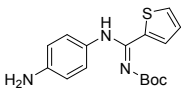
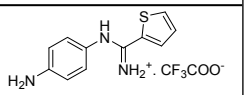
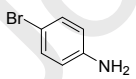
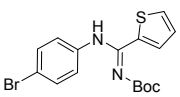
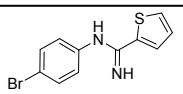
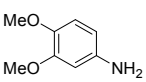
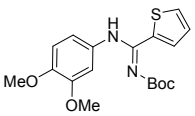
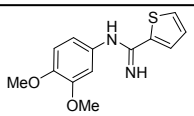
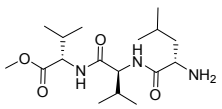
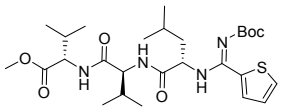
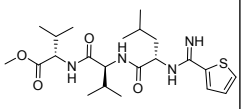
is a time demanding process. Overall, the traditional method warranted a substantial modification for the efficient synthesis of thiophene carboxamidines which are potent NOS inhibitors. As part of a programme to develop dual acting CNS agents, we needed a hassle-free procedure to synthesize thiophene-2-carboxamidines. In this connection, we discovered a new protocol for the efficient synthesis of thiophene-2-carboxamidines. The present method, outlined in scheme-1 (*vide supra*) involves the reaction of Boc-protected imidazolyl thiophene-2-carboxamidine reactive intermediate **3** with various primary amines to form non-polar Boc-thiophene-2-carboxamidines **4** which could be readily purified. The reactive intermediate **3** could be readily synthesized as per the protocol detailed in scheme-2. This method is also very mild and tolerates diverse substituents - including sensitive peptide fragments (table 1). This new methodology represents a substantial improvement to the literature method owing to its simplicity and hassle-free purification procedures.

The reactive thiophene-imidazole conjugate **3** is the key intermediate for the preparation of thiophene-2-carboxamidines (scheme 2). The reactive intermediate **3** could be readily synthesized from thiophene in two steps, following literature procedures[7]. Thus, thiophene-2-thiocarboxamide **5**, obtained by reacting thiophene with potassium-thiocyanate in methane sulfonic acid was Boc-protected to yield **6**, which was converted to the intermediate **3** by coupling with imidazole, mediated by $AgNO_3$ in DMF.



Scheme 2. Synthesis of reactive intermediate **3**. Reagents and conditions: (a) KSCN, $MeSO_3H$, 27 °C, 8 h, 75% (b) Boc_2O, NaH, THF, 27 °C, 6 h, 60% (c) Imidazole, $AgNO_3$, NEt_3, DMF, 90 °C, 6 h, 70%.

Table 1. Thiophene-2-carboxamidines prepared *via* **Scheme 1** (see page 1).

Entry	R-NH_2	Product	Boc-protected amidines[a]	Time in hrs	Isolated yield of **4a-h** (%)	Product	Boc- deprotected amidines[b]
1		**4a**		2.5	95	**7a**	
2		**4b**		2.5	96	**7b**	
3		**4c**		2.5	92	**7c**	
4		**4d**		4.5	86	**7d**	
5		**4e**		4	90	**7e**	
6		**4f**		4.5	83	**7f**	
7		**4g**		4	90	**7g**	
8		**4h**		3	90	**7h**	

[a]Reagents and conditions: **3** (1 mmol), amine (1.2 mmol) and triethyl amine (1.5 mmol) in CH_2Cl_2 under open-flask conditions. [b]Boc deprotection of **4a-h** was carried by TFA/DCM (1hr, RT) to afford **7a-h**; quantitative yield.

The key intermediate **3** could be readily converted to the target thiophene-2-carboxamidines **4a-h** by simply reacting with various amines, at room temperature, as illustrated in scheme 1 (*vide supra*). The expected Boc-protected thiophene-2-

carb H.

temperature and in good yields (Table 1). The reaction worked equally well for aromatic (**4d-g**) as well as aliphatic amines (**4a-c, 4h**). It is noteworthy that sensitive peptide amines could also be used in this reaction - forming peptide-conjugated thiophene-2-carboxamidines (**4h**). When desired, free amidines of **4** could be quickly made by its Boc-deprotection with TFA in DCM (see SI 28-45).

In summary, we have developed a hassle-free and high-yielding methodology for the preparation of thiophene-2-carboxamidines which are a privileged class of potent NOS inhibitors. This new methodology avoids the release of foul-smelling thiols and renders purification of the amidines easy, owing to their non-polar nature. Imidazole, which is the sole side product formed during the coupling reaction, could be readily washed away with water, rendering easy isolation of the target molecules in good yield. Notably, thiophene-2-carboxamidines conjugated with sensitive peptide fragments / protected amino acids could also be synthesized efficiently by this method. Overall, this new methodology represents a substantial improvement to the existing route for the synthesis of pharmacologically important thiophene-2-carboxamidines. Currently, we are exploring the synthetic potential of this methodology for developing dual acting NOS inhibitors featuring thiophene-2- carboxamidine , and the results will be published in due course.

Acknowledgments

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Supplementary Material

Full experimental procedures, characterization data, HR-MS, ^{1}H and ^{13}C NMR spectra for all compounds. Supplementary data ass

Click here to remove instruction text...

- Mild and efficient reaction condition.
- Hassle-free purification.
- Good funtional group tolerence (including peptides).
- Avoids liberation of obnoxius thiols.
- Water soluble by-product (imidazole)