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Exploring Kinase Inhibition Properties of 9*H*-pyrimido[5,4-*b*]- and [4,5-*b*]indol-4-amine Derivatives

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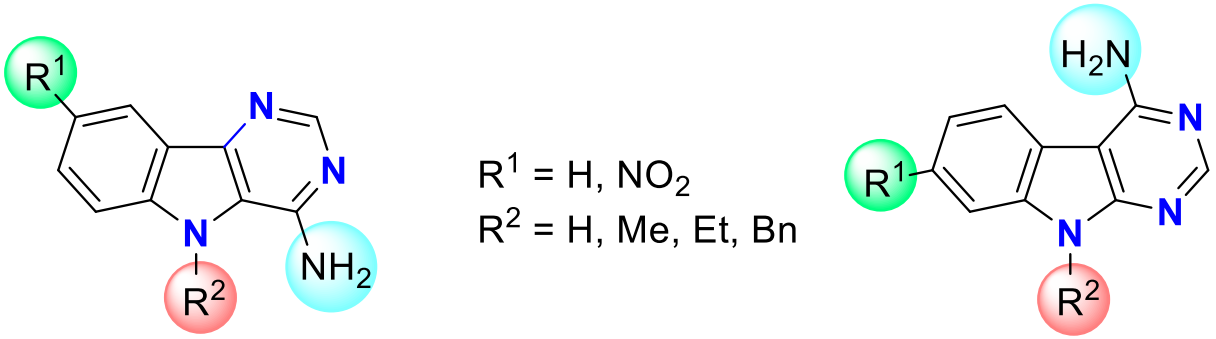
Graphical Abstract



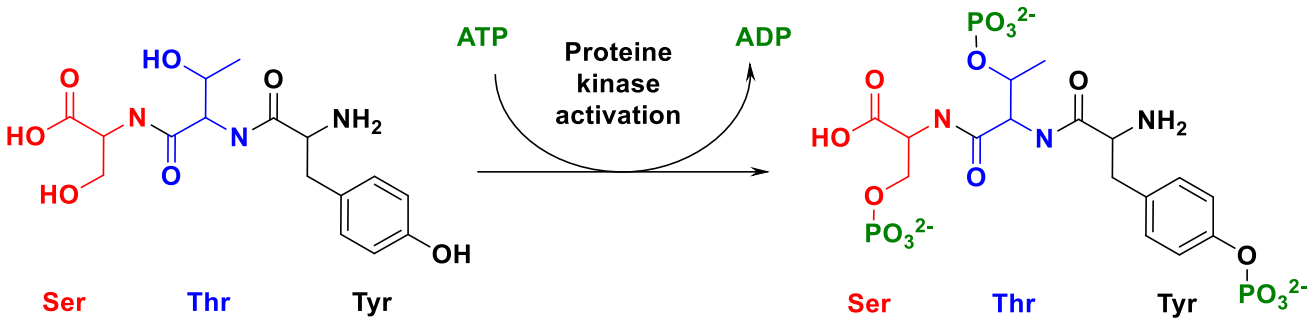
Kinase inhibitory potency: 0.6 to 4.0 µM for CK1δ/ε and 2.2 to 7.6 µM for DYRK1A

Abstract: We previously highlighted the interest of 6,5,6-fused tricyclic analogues of 4-aminoquinazolines as kinase inhibitors in the micromolar to the nanomolar range of IC_{50} values. For the generation of the chemical libraries, the formamide-mediated cyclization of the cyanoamidine precursors was carried out under microwave irradiation in an eco-friendly approach. In order to explore more in depth the pharmacological interest of such tricyclic skeletons, the central five member ring, *i.e.* thiophene or furan, was replaced by a pyrrole to afford 9*H*-pyrimido[5,4-*b*]- and [4,5-*b*]indol-4-amine derivatives inspired from harmine. The inhibitory potency of the final products was determined against four protein kinases (CDK5/p25, CK1δ/ε, GSK3α/β and DYRK1A). As a result, we have identified promising compounds targeting CK1δ/ε and DYRK1A and displaying micromolar and submicromolar IC_{50} values.

Keywords: Microwave-assisted chemistry; protein kinases; CK1; DYRK1A; CDK5; GSK-3

Introduction

Protein kinases are an important family of enzymes able to phosphorylate tyrosine (Tyr) and serine (Ser)/threonine (Thr) residues present in various proteins. Abnormal protein kinase regulation and phosphorylation are now associated with numerous diseases including cancer and neurodegenerative disorders.

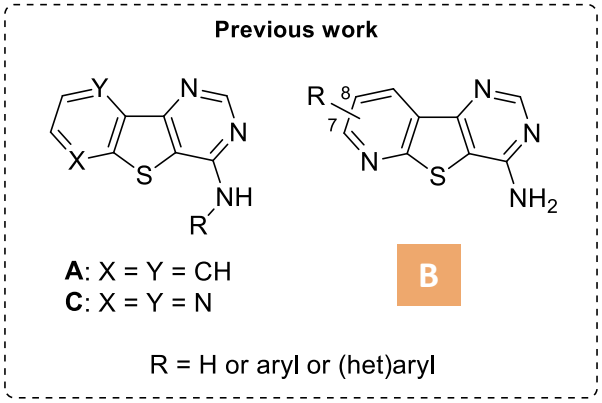


In the last decade, about 300 protein kinase inhibitors were involved in clinical trials and 49 have been recently approved by the US Food and Drug Administration (FDA), mostly tyrosine kinase inhibitors and mainly for cancer therapy.

- Manning *et al. Science* **2002**, *298*, 1912−1934. Doi: 10.1126/science.1075762
- Ionescu *et al. Mini-Rev. Med. Chem.* **2012**, *12*, 1315−1329. Doi: 10.2174/138955712804586639
- Fernandez-Martinez *et al. Mol. Cell. Oncol.* **2015**, *2*, e970048. Doi: 10.4161/23723548.2014.970048
- Duchon *et al. Front. Behav. Neurosci.* **2016**, *10*, 104-120. Doi: 10.3389/fnbeh.2016.00104
- Branca *et al. Aging Cell* **2017**, *16*, 1146-1154. Doi: 10.1111/acel.12648
- Stotani *et al. Future Med. Chem.* **2016**, *8*, 681−696. Doi: 10.4155/fmc-2016-0013
- Roskoski *et al. Pharmacol. Res.* **2020**, *152*, 104609. Doi: 10.1016/j.phrs.2019.104609

Our groups have been particularly invested in the development of efficient and eco-compatible chemical methodologies allowing rapid access to libraries of potent bioactive arenes and their heteroarenes analogues. Studying ancestral thermal-sensitives reactions for which usual methods require forcing conditions or prolonged reaction times (e.g. **Niementowski reaction** and **Dimroth transposition**), microwave-assisted syntheses of novel benzo[*b*]thieno[3,2-*d*]pyrimidin-4-amines (**series A**) and their pyrido (**series B**) and pyrazino analogues (**series C**) have been successfully described.

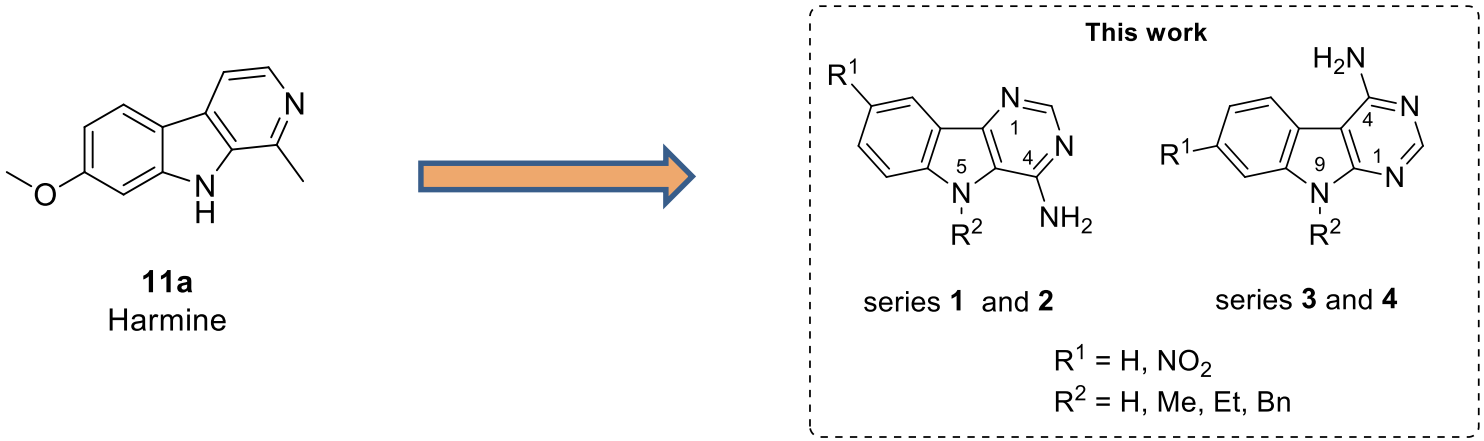
> 100 derivatives



Inhibitory potency towards CK1δ/ε over the other tested enzymes (CDK5/p25, GSK3α/β, Dyrk1A)

- Alexandre *et al. Tetrahedron Lett.* **2003**, *44*, 4455-4458. Doi:10.1016/S0040-4039(03)01026-8
- Loidreau *et al. Tetrahedron* **2011**, *67*, 4852-4857. Doi: 10.1016/j.tet.2011.05.010
- Foucourt *et al, Tetrahedron* **2010**, *66*, 4495-4502. Doi: 10.1016/j.tet.2010.04.066
- Loidreau *et al. J. Heterocycl. Chem.* **2013**, *50*, 1187-1197. Doi: 10.1002/jhet.1716
- Loidreau *et al. Eur. J. Med. Chem*. **2013**, *59*, 283-295. Doi: 10.1016/j.ejmech.2012.11.030
- Loidreau *et al. Eur. J. Med. Chem.* **2012**, *58*, 171-183. Doi: 10.1016/j.ejmech.2012.10.006
- Loidreau *et al. Eur. J. Med. Chem.* **2015**, *92*, 124-134. Doi: 10.1016/j.ejmech.2014.12.038

In an effort to expand the chemical space and to highlight efficient kinase inhibitors, the synthesis of indole counterparts of the previous described series has been envisaged and novel compounds were derived from **harmine 11a**, a natural alkaloid that still generates a lot of work in the hope of developing therapies for Alzheimer's disease (AD) and Down syndrome (DS).

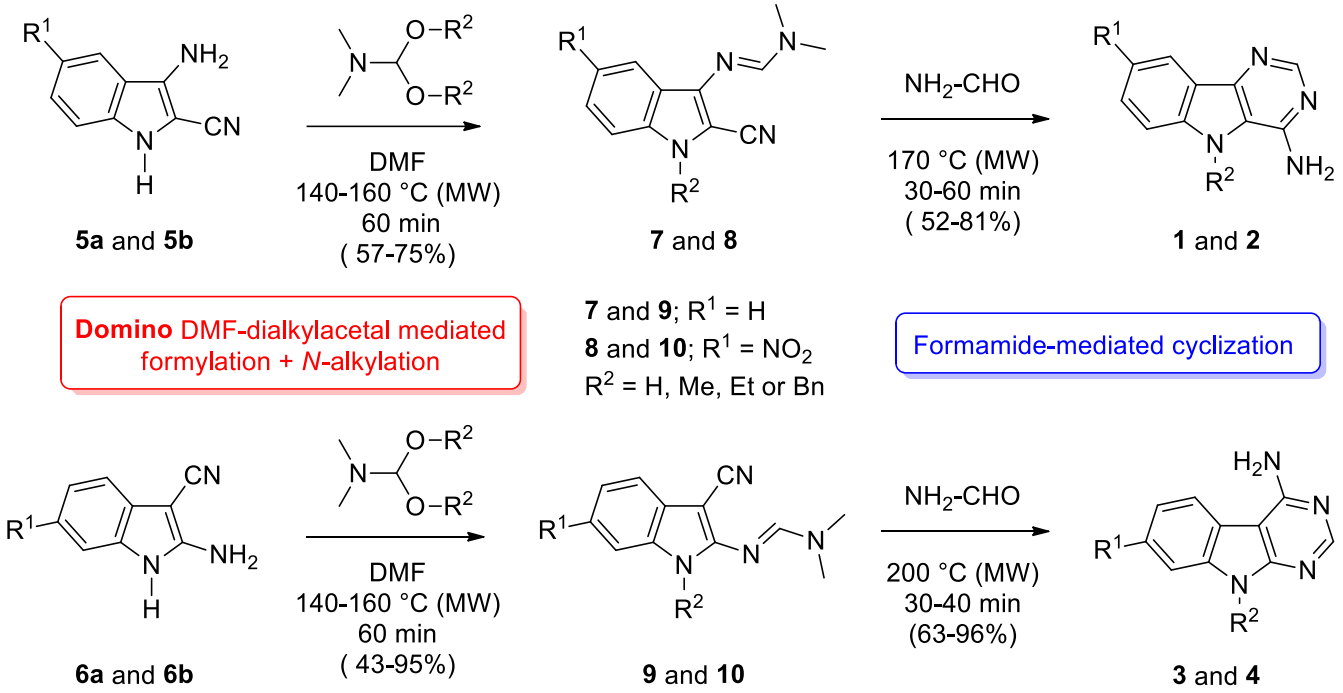


Kinase inhibition of the products obtained was evaluated on an array of four Ser/Thr kinases (CDK5/p25, CK1δ/ε, DYRK1A and GSK3α/β), all members of the CMGC kinase family, chosen for their strong implication in various cellular regulation processes.

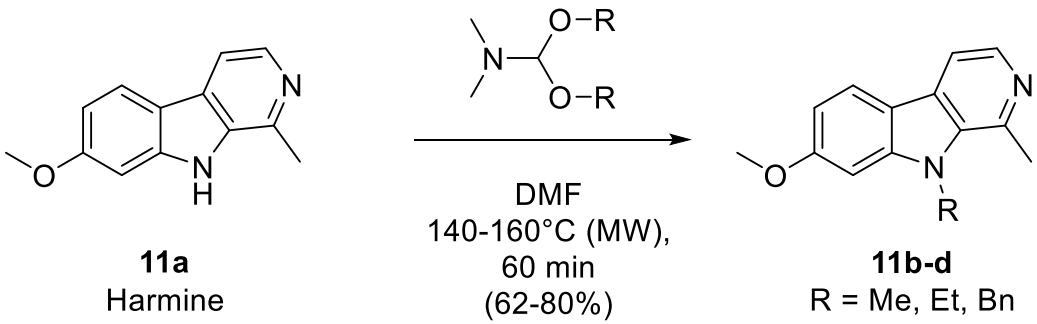
- Jarhad *et al. J. Med. Chem.* **2018**, *61*, 9791-9810. Doi: 10.1021/acs.jmedchem.8b00185
- Czarna *et al. J. Med.Chem.* **2018**, *61*, 7560-7572. Doi: 10.1021/acs.jmedchem.7b01847

Results and discussion: chemistry

The *N'*-(cyano-1*H*-indolyl)-*N,N*-dimethyl formimidamide precursors (**7a-d**, **8a-d, 9a-d** and **10a-d**) were heated at 170-200 °C under microwaves in the presence of an excess of formamide (40 equiv.). Functionalized indoles (**7a-d**, **8a-d, 9a-d** and **10a-d)** were previously obtained from 3-amino-1*H*-indole-2-carbonitriles (**5a** and **5b**) or their 5-nitro-3-amino-1*H*-indole-2-carbonitrile isomers (**6a** and **6b**) which were condensed with 10 equiv. of DMF-dialkylacetals like *N,N*-dimethylformamide dimethyl acetal (DMF-DMA), *N,N*-dimethylformamide diethyl acetal (DMF-DEA) and *N,N*-dimethylformamide dibenzyl acetal (DMF-DBA), respectively.



To compare the biological results of harmine (**11a**) and some of its *N9*-alkylated derivatives (**11b-d**) with the pyrimidoindoles prepared in this work (series **1**, **2**, **3**, and **4**), we decided to explore the capacity of DMF-dialkylacetals to transfer an alkylgroup to nucleophilic atoms, as an interesting alternative to previous methods [see refs]. Then, harmine was heated under controlled microwave-assisted heating in sealed vials (10 mL) in the presence of 10 equiv. of DMF-DMA, DMF-DEA or DMF-DBA. The corresponding *N9*-alkylated 7-methoxy-1-methyl-β-carbolines (**11b-d**) were obtained in good yields.



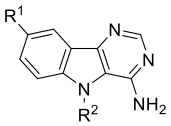
11a
Harmine

11b-d
R = Me, Et, Bn

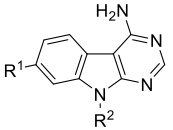
- Cao *et al. Eur. J. Med. Chem.*, **2013**, *60*, 135-143. Doi: 10.1016/j.ejmech.2012.11.045
- Cao *et al. Bioorg. Med. Chem.* **2004**, *12*, 4613-4623. Doi: 10.1016/j.bmc.2004.06.038
- Bálint *et al. ChemMedChem* **2017**, *12*, 932-939. Doi: 10.1002/cmdc.201600539
- Du *et al. Bioorg. Med. Chem. Lett*. **2016**, *26*, 4015-4019. Doi: 10.1016/j.bmcl.2016.06.087

Results and discussion: kinase inhibition

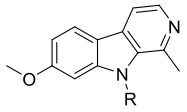
Kinases inhibitory potencies (IC_{50} in μM)[1] for compounds of the series **1**, **2**, **3** and **4**.



series **1** and **2**



series **3** and **4**



series **11**

Compound	R^1	R^2	CDK5/p25	CK1δ/ε	DYRK1A	GSK-3α/β
1a	H	H	>10	2.0	2.2	>10
1b	H	Me	>10	4.0	5.8	>10
1c	H	Et	>10	2.8	4.1	>10
1d	H	Bn	>10	**0.6**	>10	>10
2a	NO_2	H	>10	>10	7.6	>10
2b	NO_2	Me	>10	>10	>10	>10
2c	NO_2	Et	>10	>10	>10	>10
2d	NO_2	Bn	>10	>10	>10	>10
3a	H	H	6	**0.7**	3.1	>10
3b	H	Me	>10	2.5	>10	>10
3c	H	Et	>10	1.6	9.8	>10
3d	H	Bn	>10	2.7	>10	>10
4a	NO_2	H	>10	3.5	7.6	>10
4b	NO_2	Me	>10	2.8	>10	>10
4c	NO_2	Et	>10	1.6	5.9	>10
4d	NO_2	Bn	>10	>10	>10	>10
11a (Harmine)	R = H		>10	1.5	**0.029**	>10
11b	R = Me		>10	>10	**0.13**	>10
11c	R = Et		>10	>10	**0.037**	>10
11d	R = Bn		>10	>10	**0.059**	>10

[1] Average of triplicate determination (<10% variation among values).

General trends for kinase inhibition

CK1δ/ε kinase inhibition

- No activity against CDK-5/p25 and GSK3α/β.
- 8-Nitro-5*H*-pyrimido[5,4-*b*]indol-4-amines (**2a-d)** also inactive against the two other tested kinases.
- Similar inhibitory activity for all the series against the array of four kinases.
- Values are mainly in the micromolar range against CK1δ/ε, except compounds **1d** and **3a** which disclose submicromolar IC_{50} values (0.6 and 0.7 μM, respectively).
- It can be denoted that **1d** seems more specific for CK1δ/ε.
- Considering CK1δ/ε kinase inhibition, the introduction of a nitro group is deleterious for series **1** *vs* **2** whereas inhibitory activity remains for series **3** *vs* **4**, depending of the orientation of the aminopyrimidine ring in the fused system and its relative position to the nitro group.

General trends for kinase inhibition

DYRK1A kinase inhibition for harmine derivatives

- A loss of DYRK1A inhibitory activity was observed for the compounds bearing a *N*-methyl or a *N*-benzyl chain compared to their NH or *N*-ethyl analogues, except for the inactive series **2** and the very active compounds **11a-d**.

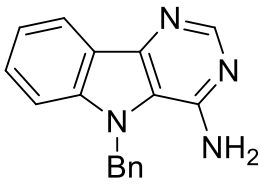
- Tested as a positive control under the same conditions as series **1**-**4**, harmine (**11a**) was definitely inactive against CDK5/p25 and GSK3α/β. This natural product shows interesting activity against DYRK1A and a weak inhibition of CK1δ/ε (IC_{50} = 1.5 µM) as previously mentioned in various papers relating the use of harmine for treatment of neurodegenerative diseases.

- The *N*-methyl, *N*-ethyl and *N*-benzyl derivatives (**11b**, **11c** and **11d**) are totally inactive against the three kinases CDK5/p25, CK1δ/ε and GSK3α/β. Their affinity is focused on DYRK1A with interesting IC_{50} values, quite close to the nanomolar range IC_{50} obtained for the lead harmine (**11a**), confirming recently published results.

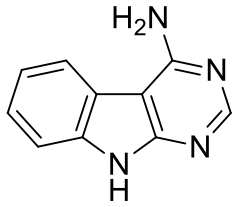
- Patel *et al. Asian Pac. J. Trop. Biomed.* **2012**, *2*, 660-664. Doi: 10.1016/S2221-1691(12)60116-6
- Adayev *et al. Arch. Biochem. Biophys*. **2011**, *507*, 212-218. Doi: 10.1016/j.abb.2010.12.024
- Bálint *et al. ChemMedChem* **2017**, *12*, 932-939. Doi: 10.1002/cmdc.201600539

Conclusion

This medicinal chemistry work allowed us to highlight very promising compounds as kinase inhibitors with good activity against CK1δ/ε and DYRKA protein kinases.
Interestingly, that kinases play a central role in cancer and neurodegenerative diseases and showed the biological interest of such series of compounds.

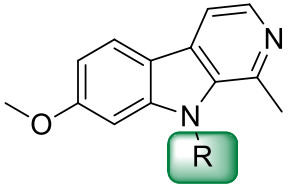


1d



3a

CK1δ/ε inhibitors at submicromolar ranges



11b-d
R = Me, Et, Bn

DYRK1A inhibitors at nanomolar range

For details on the experimental part see: Loidreau, Y. *et al. Pharmaceuticals* **2020**, *13*, 89; https://doi.org/10.3390/ph13050089

Acknowledgments

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