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β-Carbolines as Specific Inhibitors of Cyclin-Dependent Kinases

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Abstract—Harmine (**3**), 7-fluoro-1-methyl β-carboline (**35**) and 1-(5-methyl-imidazol-4-yl) β-carboline (**41**) were potent and specific inhibitors of cyclin-dependent kinases. The degree of aromaticity of the tricyclic ring and the positioning of substituents are important for inhibitory activity. While most β-carbolines inhibited CDK2 and CDK5 to the same extent, selective inhibition against CDK2 was observed in 1-(2-chlorophenyl)- (**12**), 1-(2-fluorophenyl)- (**15**), and 1-(2-chloro-5-nitrophenyl)- (**28**) β-carbolines.
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Cyclin-dependent kinases (CDKs) play essential roles in the regulation of cell division cycle.[1] Much evidence has shown that CDKs are deregulated in human cancers. CDK5, a major protein kinase which causes hyperphosphorylation of a microtubule-binding protein tau, is closely linked to Alzheimer disease.[2] Several classes of CDK inhibitors have been reported, including staurosporine, olomoucine, flavopiridol, indirubins, paullones, and a series of 2,6,9-substituted purines.[3] X-ray crystallographic studies showed that these compounds compete with ATP-Mg^{2+} for the ATP-binding site of CDKs.

In our continuing search for traditional Chinese medicinal herbs-derived anticancer agents, harmine (**3**), a β-carboline alkaloid, was first identified as a hit in the screen towards CDK5 inhibition inhibition (data to be published elsewhere). β-Carbolines are widely distributed in many plants and mammals and they exhibit a wide spectrum of biological activities.[4,5] Chemical and pharmacological studies in China showed that harmine, originally isolated from the plant *Peganum harmala*, was effective in suppressing the growth and proliferation of tested tumour cells.[6] More recently, harmine was reported to exhibit strong cytotoxicity against a number of human tumor cell lines.[7] However, the underlying mechanism and the cellular target molecules responsible for such activity were not identified. Although some β-carbolines were reported to have DNA intercalating activity[8] and inhibitory activity of topoisomerase,[9] the weak activities indicated that they are unlikely to be the targets for the potent inhibition of cell growth. To the best of our knowledge, a few enzymes, including cytochrome P450[10] and monoamine oxidase A,[11] have been found to be potently inhibited by β-carbolines in nanomolar scales, but none of them is directly involved in the cell proliferation cycle. Chemical synthesis of harmine analogues was subsequently carried out to understand the structure–activity relationships (SARs) and to further improve the activity of harmine.

Oxidation and decarboxylation of crude 1,2,3,4-tetrahydro-3-carboxyl-β-carboline (synthesized from Pictet Spengler reaction) with 10% $K_2Cr_2O_7$ (or SeO_2 for compounds **40**–**42**) gave the desired β-carboline (Fig. 1).[12] When tryptamine was employed as the starting material, the corresponding tetrahydro-β-carboline failed to undergo dehydrogenation reaction under similar condition, thus suggesting the importance of 3-carboxylic acid in facilitating the aromatization process. In one exceptional case, tryptamine was refluxed with the trifluoroacetaldehyde ethyl hemiacetal in 1,4-dioxane without any catalyst for 5 h to afford the corresponding

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1-trifluoromethyl - 1,2,3,4 - tetrahydro-β-carboline. The optimized dehydrogenation was achieved by refluxing the intermediate with activated MnO_2 in benzene for 1 h to give compound **44.**

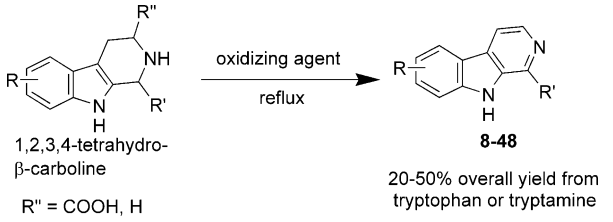


Figure 1. General preparation of β-carbolines.

An amino group was also introduced at the 4-position of the β-carboline ring.[13] 1-Propyl-1,2,3,4-tetrahydro-β-carboline prepared from tryptamine was *N*-acylated to give **49a**. It was subjected to a DDQ mediated oxidation in aqueous THF to give the corresponding 4-oxo-substituted product, which upon further reaction with hydrazine at 120 °C for 7 h, yielded the fully aromatized 1-propyl-4-amino-β-carboline (**49**) (Fig. 2). 7-Substituted β-carboline was prepared by either reacting harmol with a variety of alkyl halide or aliphatic isocyanates (Fig. 3). ^{1}H–^{13}C HMQC and HMBC NMR experiments were also carried out to ensure that the products were *O*-alkylated and not *N*-alkylated.

The structures of β-carbolines (**1**–**62**) and their inhibitory activities against CDK5 and CDK2 were summarized in Table 1. The inhibition assay was carried out in duplicate and evaluated at 50 μM of each test compound and 15 μM of ATP-Mg^{2+}.[14–18] In general, the inhibition profiles of β-carbolines towards CDK5 and CDK2 are similar. This is not surprising, as the proteins have been known to exhibit similar substrate specificity. For easy understanding, the SAR discussion shall focus primarily on the CDK2 inhibition result.

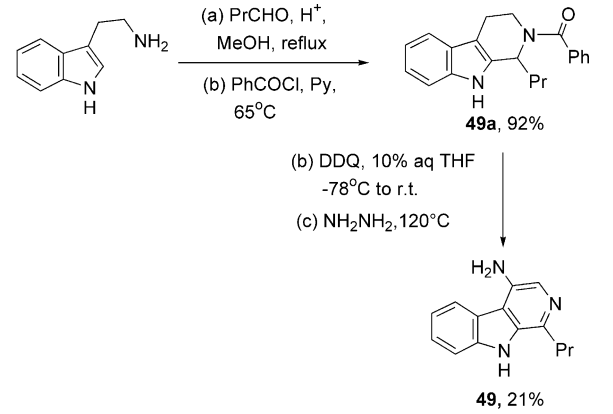


Figure 2. Synthesis of 1-propyl-4-amino-β-carboline.

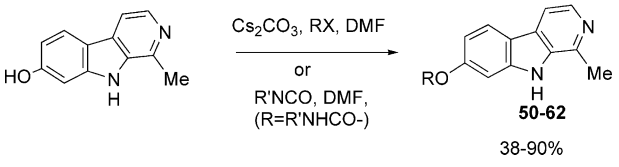


Figure 3. Synthesis of 7-substituted β-carbolines.

Firstly, while all compounds contain the core structure of a tricyclic ring, full aromaticity of the tricyclic ring is required for good CDK inhibitory activities. Dihydro-β-carbolines **4** (10%) and **6** (13%) invariably showed much lower activity than their fully aromatic counterparts, β-carbolines **3** (62%) and **5** (69%) respectively. The tetrahydro β-carboline **7** (12%) was also inactive.

Secondly, substitution at the 6-position reduces the potency of β-carboline. CDK inhibition was completely abolished when the methoxy group at the 7-position (**3**) was moved to the 6-position (**47**, 2%). 6-Benzyloxyl β-carboline (**48**, 2%) was equally inactive. The same trend was also observed in 7-methyl- (**31,** 23%), 6-methyl- (**33**, 15%) and 5-methyl-β-carbolines (**34**, 30%), as well as in 7-fluoro- (**35**, 65%), 6-fluoro- (**45**, 46%) and 5-fluoro-β-carbolines (**46**, 55%).

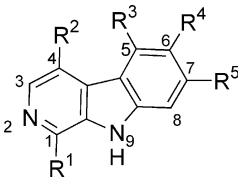
Thirdly, among the 1-methyl β-carbolines, compounds that showed at least comparable inhibition as harmine **3** were those having substituents at the 7-position: harmol (**5**, 69%), fluoro- (**35**, 65%) and *N*-propyl-aminocarbonyloxy-β-carbolines (**58**, 61%). Harman **2** and all other 7-substituted compounds (**31**, **50**–**57, 59**–**62**) showed lower inhibitory activities than harmine **3**. In addition, 1-methyl group was the optimized substituent among the 7-fluoro β-carbolines (**35**–**40**). On the contrary, there was no obvious steric or electronic requirement for C-1 substituent (**1**–**2**, **8**–**30**, **41**–**44**). The best compound was the 1-(5-methylimidazol-4-yl) β-carboline (**41**, 89%). Although synthesis was further carried out to make a β-carboline containing 7-fluoro and 1-(5-methylimidazol-4-yl) substituents, the resulted compound (**40**) exhibited lower activity than the parent compounds **26** and **41**.

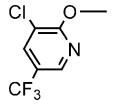
Although most β-carbolines showed similar inhibitory activities towards CDK5 and CDK2, 1-(2-chlorophenyl)- (**12**), 1-(2-fluorophenyl)- (**15**), 1-(2-chloro-5-nitrophenyl)-β-carbolines (**28**) were found to display potent and selective inhibition towards CDK2 (>60%). Finally, the 4-amino β-carboline (**49**) was also inactive.

Of all active compounds (>60%), compounds **3**, **28**, **35**, and **41** were selected for IC_{50} determination (Table 2). All except compound **28** inhibited CDC2/cyclin B, CDK2/cyclin A and CDK5/$p25^{nck5a}$ in micromolar range. Consistent with the data in Table 1, compound **28** showed selective inhibition towards CDK2. These compounds had negligible inhibition towards other tested kinases, including cAMP-dependent protein kinase, protein kinase C, mitogen-activated protein kinase, and the Src-related protein tyrsine kinases Lck, Lyn and Fyn (>250 μM).

In conclusion, we have identified harmine **3** and compound **41** as potent and specific CDK inhibitors (data to be published elsewhere). SAR analysis demonstrated that a complete aromatized tricyclic ring and the positioning of substituents are important for inhibitory activity. As CDC2 and CDK2 are known to control the cell division cycle and CDK5 is involved in the pathological development of Alzheimer's disease, development of specific CDK inhibitors is believed to have potential pharmaceutical applications.

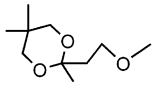
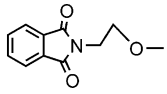
Table 1. Inhibition of CDK5/p25^{nck5a} and CDK2 by β-carbolines



Compd[a]	R′	R^2	R^3	R^4	R^5	Inhibition of CDK5 (%) at 50 μM	Inhibition of CDK2 (%) at 50 μM
1 (Norharmane)	H	H	H	H	H	35	37
2 (Harmane)	Me	H	H	H	H	65	47
3 (Harmine)	Me	H	H	H	MeO–	68	62
4[b] (Harmaline)	Me	H	H	H	MeO–	7	10
5 (Harmol)	Me	H	H	H	HO–	72	69
6[c] (Harmalol)	Me	H	H	H	HO–	15	13
7	–COOH	H	H	MeO–	H	9	12
8	Pr	H	H	H	H	25	43
9	*i*Pr	H	H	H	H	27	44
10	*t*Bu	H	H	H	H	30	50
11	Ph	H	H	H	H	5	13
12	2-Chlorophenyl	H	H	H	H	36	69
13	3-Chlorophenyl	H	H	H	H	17	32
14	4-Chlorophenyl	H	H	H	H	44	48
15	2-Fluorophenyl	H	H	H	H	37	64
16	3-Fluorophenyl	H	H	H	H	5	6
17	4-Fluorophenyl	H	H	H	H	26	20
18	2-Bromophenyl	H	H	H	H	4	10
19	3-Methoxyphenyl	H	H	H	H	2	5
20	4-Methoxyphenyl	H	H	H	H	5	6
21	2-Cyanophenyl	H	H	H	H	4	8
22	3-Cyanophenyl	H	H	H	H	37	31
23	4-Cyanophenyl	H	H	H	H	41	29
24	2-Nitrophenyl	H	H	H	H	22	43
25	3-Nitrophenyl	H	H	H	H	16	19
26	4-Nitrophenyl	H	H	H	H	32	31
27	2,4-Dimethylphenyl	H	H	H	H	1	5
28	2-Chloro-5-nitrophenyl	H	H	H	H	19	70
29	3-Chloro-4-nitrophenyl	H	H	H	H	3	4
30	2,4-Difluorophenyl	H	H	H	H	45	56
31	Me	H	H	H	Me	18	23
32	2-Fluorophenyl	H	H	H	Me	19	36
33	Me	H	H	Me	H	13	15
34	Me	H	Me	H	H	32	30
35	Me	H	H	H	F	69	65
36	Et	H	H	H	F	55	54
37	Pr	H	H	H	F	48	60
38	Ph	H	H	H	F	40	57
39	2-Fluorophenyl	H	H	H	F	28	55
40	5-Methylimidazol-4-yl	H	H	H	F	55	51
41	5-Methylimidazol-4-yl	H	H	H	H	94	89
42	Imidazol-4-yl	H	H	H	H	55	30
43	CH_2COOCH_3	H	H	H	H	2	11
44	CF_3	H	H	H	H	45	39
45	Me	H	H	F	H	46	46
46	Me	H	F	H	H	64	55
47	Me	H	H	MeO–	H	0	2
48	Me	H	H	BzO–	H	0	2
49	Pr	NH_2	H	H	H	9	22
50	Me	H	H	H	EtO–	41	43
51	Me	H	H	H	$HOCH_2CH_2O$–	8	4
52	Me	H	H	H	*n*BuO	40	50
53	Me	H	H	H	NH_2COCH_2O	13	15
54	Me	H	H	H	$CH_3COOCH_2CH_2O$–	24	23
55	Me	H	H	H	$PhCOOCH_2CH_2O$–	11	13
56	Me	H	H	H	$BnOOCCH_2O$–	59	57
57	Me	H	H	H	$(EtO)_2CHCH_2O$–	2	1
58	Me	H	H	H	PrNCOO–	69	61
59	Me	H	H	H	c-HexNCOO–	64	53
60	Me	H	H	H		2	10

(*continued on next page*)

Table 1 (*continued*)

Compd[a]	R′	R^2	R^3	R^4	R^5	Inhibition of CDK5 (%) at 50 μM	Inhibition of CDK2 (%) at 50 μM
61	Me	H	H	H		22	27
62	Me	H	H	H		2.5	8

[a]Compounds **1**–**7** were obtained from Aldrich.
[b]3,4-dihydro-β-carbolines.
[c]1,2,3,4-tetrahydro-β-carboline.

Table 2. IC_{50} (μM) of harmine (**3**), β-carbolines **28**, **35** and **41**

Compd	CDC2/cyclin B	CDK2/cyclin A	CDK5/p25^{nck5a}
3	18	35	21
28	45	22	>1200
35	42	30	24
41	25	6	5
Olomoucine	—	—	7
Roscovitine	—	—	0.8

Acknowledgements

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References and Notes

1. (a) Morgan, D. O. *Annu. Rev. Cell Dev. Biol.* **1997**, *13*, 261. (b) Stein, G. S.; Baserga, R.; Giordano, A.; Denhardt, D. T. *The Molecular Basis of Cell Cycle and Growth Control*; Wiley-Liss Inc.: New York, 1999.
2. Lew, J.; Wang, J. H. *Trends. Biochem. Sci.* **1995**, *20*, 33.
3. (a) See reviews and the references cited therein: Gray, N.; Détivaud, L.; Doerig, C.; Meijer, L. *Curr. Med. Chem.* **1999**, *6*, 859. (b) Senderowicz, A. M.; Sausville, E. A. *J. Natl. Cancer Inst.* **2000**, *92*, 376. (c) Sielecki, T. M.; Boylan, J. F.; Benfield, P. A.; Trainor, G. L. *J. Med. Chem.* **2000**, *43*, 1.
4. Torreilles, J.; Guerin, M. C.; Previero, A. *Biochimie* **1985**, *67*, 929.
5. de Meester, C. *Mutat. Res.* **1995**, *339*, 139.
6. Zhan, T.; Li, C. *J. Beijing Med. Univ.* **1990**, *22*, 382.
7. Ishida, J.; Wang, H.-K.; Bastow, K. F.; Hu, C.-Q.; Lee, K.-H. *Bioorg. Med. Chem. Lett.* **1999**, *9*, 3319.
8. Taira, Z.; Kanzawa, S.; Dohara, C.; Ishida, S.; Matsumoto, M.; Sakiya, Y. *Jpn. J. Toxicol. Environ. Health* **1997**, *43*, 83.
9. Funayama, Y.; Nishio, K.; Wakabayashi, K.; Nagao, M.; Shimoi, K.; Ohira, T.; Hasegawa, S.; Saijo, N. *Mutat. Res.* **1996**, *349*, 183.
10. Stawowy, P.; Bonnet, R.; Rommelspacher, H. *Biochem. Pharmacol.* **1999**, *57*, 511.
11. (a) Kim, H.; Sablin, S. O.; Ramsay, R. R. *Arch. Biochem. Biophys.* **1997**, *337*, 137. (b) de Arriba, A. F.; Lizcano, J. M.; Balsa, M. D.; Unzeta, M. *J. Pharm. Pharmacol.* **1994**, *46*, 809.
12. Preparation of β-carboline: To a solution of (substituted) tryptophan (1 mmol) in deionised water (5 mL) and H_2SO_4 (1 mmol) was added the aldehyde (1.5 mmol). The reaction mixture was heated at 65°C for 12 h before adding glacial AcOH (1.5 mL) and 10% $K_2Cr_2O_7$ (4 mL). It was refluxed for 3 min and then cooled to rt. Saturated Na_2SO_3 was added to reduce the excess oxidant and solid Na_2CO_3 was added to neutralize the solution. The product was extracted into CH_2Cl_2 (3×20 mL) and dried over Na_2SO_4. After removal of the solvent, the residue was subjected to preparative TLC to yield the pure β-carboline.
13. Trudell, M. L.; Fukada, N.; Cook, J. M. *J. Org. Chem.* **1987**, *52*, 4293.
14. Protein kinase assays: Radioactive isotopes, [γ-^{32}P]ATP (3000 Ci/mmol, 1 mCi/mL) and [^{3}H]Thymidine (1 mCi/mL), were purchased from Amersham. Other chemicals were obtained from Sigma Chemicals. The CDK5/p25^{nck5a} enzyme was prepared from recombinant GST-Cdk5 and GST-p25^{nck5a} by reconstitution, thrombin cleavage of the GST moiety, and then isolation of CDK5/p25^{nck5a}. CDK2/cyclin A was prepared by reconstitution of bacterially expressed CDK2 and cyclin A and then activation of the complex by phosphorylation of CDK2 with CAK.[15–17] Cdc2/cyclin B was purchased from New England BioLabs. The kinase reaction of CDK5/p25^{nck5a}, CDC2/cyclin B, and CDK2/cyclin A was carried out as previously described.[18]
15. Fesquet, D.; Labbe, J. C.; Derancourt, J.; Capony, J. P.; Galas, S.; Girard, F.; Lorca, T.; Shuttleworth, J.; Doree, M.; Cavadore, J. C. *EMBO J.* **1993**, *12*, 3111.
16. Poon, R. Y.; Yamashita, K.; Adamczewski, J. P.; Hunt, T.; Shuttleworth, J. *EMBO J.* **1993**, *12*, 3123.
17. Solomon, M. J.; Harper, J. W.; Shuttleworth, J. *EMBO J.* **1993**, *12*, 3133.
18. Qi, Z.; Huang, Q.-Q.; Lee, K.-Y.; Lew, J.; Wang, J. H. *J. Biol. Chem.* **1995**, *270*, 10847.