Design, synthesis of new β-carboline derivatives and their selective anti-HIV-2 activity

Penta Ashok, Subhash Chander, Jan Balzarini, Christophe Pannecouque, Sankaranarayanan Murugesan

PII: S0960-894X(15)00078-5
DOI: http://dx.doi.org/10.1016/j.bmcl.2015.01.058
Reference: BMCL 22398

To appear in: *Bioorganic & Medicinal Chemistry Letters*

Received Date: 15 December 2014
Revised Date: 10 January 2015
Accepted Date: 23 January 2015

Please cite this article as: Ashok, P., Chander, S., Balzarini, J., Pannecouque, C., Murugesan, S., Design, synthesis of new β-carboline derivatives and their selective anti-HIV-2 activity, *Bioorganic & Medicinal Chemistry Letters* (2015), doi: http://dx.doi.org/10.1016/j.bmcl.2015.01.058

Design, synthesis of new β-carboline derivatives and their selective anti-HIV-2 activity

Penta Ashok[a], Subhash Chander[a], Jan Balzarini[b], Christophe Pannecouque[b], Sankaranarayanan Murugesan[a]*

[a]Medicinal Chemistry Research Laboratory, Department of Pharmacy, Birla Institute of Technology & Science, Pilani-333031, India. E. mail: penta.ashok@gmail.com, murugesan@pilani.bits-pilani.ac.in
[b]Rega Institute for Medical Research, KU Leuven, Minderbroedersstraat 10, B-3000 Leuven, Belgium.

Abstract: In the present study, a new series of β-carboline derivatives were synthesized and evaluated for inhibition activity against both HIV-1 and HIV-2 strains. Among these reported analogues, surprisingly (1-phenyl-9*H*-pyrido[3,4-*b*]indol-3-yl)(4-p-tolylpiperazin-1-yl)methanone (**7b**), (4-(2-methoxyphenyl)piperazin-1-yl)(1-phenyl-9*H*-pyrido[3,4-*b*]indol-3-yl)methanone (**7f**), (4-(4-fluorophenyl)piperazin-1-yl)(1-phenyl-9*H*-pyrido[3,4-*b*]indol-3-yl)methanone (**7k**), (4-(2-fluorophenyl)piperazin-1-yl)(1-phenyl-9*H*-pyrido[3,4-*b*]indol-3-yl)methanone (**7l**) displayed selective inhibition of HIV-2 strain with EC_{50} values of 3.3, 3.2, 2.6 and 5.4 μM respectively, which are comparable with nucleoside reverse transcriptase inhibitors lamivudine and dideoxyinosine. As these analogues have not shown *in-vitro* HIV-2 reverse transcriptase inhibition, it could be excluded as potential target for their specific anti-HIV-2 activity.

Key words: β-carboline, HIV-1, HIV-2, Reverse Transcriptase

Human Immunodeficiency Virus (HIV), a pathogenic lentivirus which belongs to the family of *Retroviridae*, is responsible for one of the most dreadful disease Acquired Immune Deficiency Syndrome (AIDS). HIV species are majorly classified as two types such as HIV-1 and HIV-2. HIV-1 is more pathogenic, widely distributed throughout the world and further classified into different groups and sub-groups [1], whereas HIV-2 is endemic in West Africa with little spread over other parts like Africa, Europe, India and United States. HIV-2 has low transmission efficiency and requires more time to produce immune suppression when compared to HIV-1 [2, 3].

FDA approved anti HIV drugs are classified into seven different classes, Nucleoside Reverse Transcriptase Inhibitors (NRTIs), Nucleotide Reverse Transcriptase Inhibitors (NtRIs), Non-Nucleoside Reverse Transcriptase Inhibitors (NNRTIs), Protease Inhibitors (PIs), Integrase

Inhibitors (INIs), Fusion Inhibitors (FIs) and Entry Inhibitors or CCR5 co-receptor inhibitors (CRIs) [4]. All these drugs are highly effective against HIV-1, but unfortunately till now no such efforts are made to develop inhibitors active against HIV-2. Unlike HIV-1, susceptibility of HIV-2 to these anti HIV-1 drugs is variable [5]. Therapeutic efficacy of NRTIs is comparable against HIV-2 and HIV-1 with low resistance barrier to HIV-2 [6, 7], while inhibition potency of PIs against HIV-2 is uneven with only a few drugs like lopinavir, saquinavir and darunavir are active against HIV-2 [8, 9]. Integrase inhibitors [10] and CCR5 antagonist, maraviroc (MVC) exhibited activity against some HIV-2 isolates even though role of CCR5 receptors in HIV-2 lifecycle has yet to be understood [11]. Moreover anti-HIV drugs such as, NNRTIs and fusion inhibitors are therapeutically inactive against HIV-2 infection [11-13]. Despite of uneven therapeutic efficacy of anti-HIV drugs against HIV-2 infection, considerable efforts have not made to develop selective anti-HIV-2 inhibitors.

β-carboline represents a tricyclic pyrido[3,4-*b*]indole ring system present in a large number of natural products isolated from different sources like territorial plants [14], marine sponges [15], food [16] and mammals [17]. The β-carboline ring has a privileged position in medicinal chemistry, as compounds containing β-carboline skeleton displayed various biological activities like anti-cancer, anti-thrombotic, anti-microbial, anti-malarial, anti-leishmanial, anti-tubercular and anti-viral activity [18]. Natural as well as synthetic β-carboline derivatives displayed significant anti-HIV activity (Figure 1) through multiple mechanisms such as, interfering with the early stages of the HIV life cycle by inhibiting cell to cell transmission [19, 20] or by blocking the Tat–TAR interaction [21, 22]. Initially natural β-carboline alkaloid, 1-methoxycanthinone reported for its anti-HIV activity (EC_{50} 1.02 μM) in 2000 [23], followed by alkaloids harman (10.7 μM) [14], drymaritin (2.8 μM) [24], and flazin (2.36 μM) [25] exhibited moderate anti-HIV activity. Manzamines are a unique group of β-carboline alkaloids, among these manzamine alkaloids, 8-hydroxymanzamine A displayed significant anti-HIV activity with EC_{50} value 0.56 μM and manzamine A, X, F, E, *neo*-kaulamine etc., exhibited moderate anti-HIV activity [18, 26]. Besides these natural β-carboline alkaloids, synthetic β-carboline derivatives were also evaluated for anti-HIV activity, among the synthetic β-carboline derivatives, semi-synthetic analogues of flazin displayed moderate to significant activity. Especially 3-formido derivative, flazinamide showed increased anti-HIV activity against both

HIV-1 and HIV-2 strains with EC_{50} values 0.38 and 0.57 µM respectively [25, 27]. Synthetic β-carboline derivatives with different substitution on position 1,3 and 9 were explored in the literature as anti-HIV agents, among these reported synthetic derivatives, β-carboline derivatives having alkaline (amine and guanidine) side chain substitutions on position-3 of β-carboline skeleton displayed anti-HIV activity by inhibiting Tat-TAR interactions [21, 22]. From the literature reports on anti-HIV activity of β-carboline derivatives and with our interest on biological evaluation of β-carboline derivatives, we have reported design, synthesis of 3-piperazinoyl-β-carbolines based on ligand based drug design approach and their *in-vitro* activity against both HIV-1 and HIV-2 strains.

Figure 1: Structures of reported anti-HIV β-carboline derivatives

The synthetic protocol of the designed bisheteroarylpiperazine derivatives is illustrated in scheme 1. The compounds were synthesized from the starting material, DL-Tryptophan **(1)** in a sequence of reactions. Initial esterification of DL-Tryptophan **(1)** using thionylchloride in ethanol to obtain ethyl ester of tryptophan **(2)**, was followed by cyclization in the presence of trifluoroacetic acid to afford tricyclic ethyl 2,3,4,9-tetrahydro-1-phenyl-1*H*-pyrido[3,4-*b*]indole-3-carboxylate **(3).** Upon oxidation with potassium permanganate, ethyl 1-phenyl-9*H*-pyrido[3,4-*b*]indole-3-carboxylate **(4)** was obtained, continued by 9-*N* methylation with methyl iodide in the presence of potassium hydroxide to obtain ethyl 9-methyl-1-phenyl-9*H*-pyrido[3,4-*b*]indole-3-carboxylate **(5)**, followed by alkaline ester hydrolysis to afford 9-methyl-1-phenyl-9*H*-pyrido[3,4-*b*]indole-3-carboxylic acid **(6)** as key intermediate. Then **6** was treated with appropriate amines (aryl-substituted piperazines) in the presence of 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride (EDCI) and hydroxybenzotriazole (HOBt) to obtain desired products **(7a-p)** in good to excellent yields [28, 29].

Scheme 1: Reagents and conditions: i) thionyl chloride, ethanol, reflux, 30 min, 76 % ii) benzaldehyde, trifluoroacetic acid, DCM, rt, 3h, 82 % iii) $KMnO_4$, THF, rt, 24 h, 68 % iv) methyl iodide, KOH, DMSO, rt, 30 min, 72 % v) 50 % aq. NaOH, reflux, 30 min, 78 % vi) EDCI, HOBt, THF, piperazines, 0 ºC-rt 6h, 62-82 %

In-vitro Anti-HIV Activity

All the synthesized β-carboline derivatives were first evaluated for their cytotoxicity using MTT based cell viability assay against HeLa cell line. All compounds were evaluated for *in-vitro* anti-HIV activity against both HIV-1 (III_B) and HIV-2 (ROD) at below their cytotoxic concentration. Nevirapine (NVP), lamivudine (3TC), zidovudine (AZT) and dideoxyinosine (DDI) were used as reference drugs to compare the inhibition potency of reported analogues.

Among these synthesized analogues, **7b** (1-phenyl-9*H*-pyrido[3,4-*b*]indol-3-yl)(4-p-tolylpiperazin-1-yl)methanone, **7f** (4-(2-methoxyphenyl)piperazin-1-yl)(1-phenyl-9*H*-pyrido[3,4-*b*]indol-3-yl)methanone, **7k** (4-(4-fluorophenyl)piperazin-1-yl)(1-phenyl-9*H*-pyrido[3,4-*b*]indol-3-yl)methanone and **7l** (4-(2-fluorophenyl)piperazin-1-yl)(1-phenyl-9*H*-pyrido[3,4-*b*]indol-3-yl)methanone were exhibited significant activity with EC_{50} values of 3.3**,** 3.2**,** 2.6 and 5.4 μM, respectively and selectivity indices of 9, 8, ≥72 and 9, respectively. Compound **7a** (1-phenyl-9*H*-pyrido[3,4-*b*]indol-3-yl)(4-phenylpiperazin-1-yl)methanone displayed moderate activity with EC_{50} 29.4 μM against HIV-2 strain. Surprisingly, these compounds showed specific inhibitory activity against HIV-2 (ROD) and in particular compound **7k** proved quite selective in its anti-HIV-2 action (ratio CC_{50}/EC_{50}: >72).

Table 1: *In vitro* anti-HIV activity of the synthesized compounds

[1] EC_{50}: concentration of compound required to achieve 50 % protection of MT-4 cell cultures against HIV induced cytotoxicity, as determined by MTT method.

[2] CC_{50}: concentration required to reduce the viability of mock-infected cells by 50 %, as determined by MTT method.

[3] SI: selectivity index (CC_{50}/EC_{50} against HIV-2).

In the present study, we explored the substitution pattern at phenyl ring attached to the piperazine moiety with various electron donating and electron withdrawing groups. Among the synthesized compounds, the unsubstituted phenyl derivative **7a** exhibited moderate anti-HIV activity against HIV-2 with selectivity index (SI > 8). Further substitutions were made with the intention to increase the anti-viral potency with a better selectivity index. Electron donating groups on the phenyl ring increased anti-HIV activity significantly. Derivatives with electron donating substitutions such as **7b** (4-methyl) and **7f** (2-methoxy) were much more potent than **7a**.

Substitution with electron withdrawing groups like chloro and nitro on the phenyl ring decreases the potency drastically. However, when substitutions were made with fluorine (strong electron withdrawing and less steric), an enhanced potency was observed with **7k** and **7l**. Especially compound **7k** (4-(4-fluorophenyl)piperazin-1-yl)(1-phenyl-9*H*-pyrido[3,4-*b*]indol-3-yl)methanone exhibited a significant increase in potency with a much better selectivity index (≥72). Replacement of the phenyl ring by benzyl and heteroaryl rings (i.e. pyridine), resulted in a significant increase in cytotoxicity with no significant effect on anti-HIV activity. These results indicated that, substitutions on position 2 and 4 of the phenyl attached to piperazine are favorable for anti-HIV activity especially with electron-donating groups and electron-withdrawing groups at respective positions. Substitution on position 4 with less steric and electron-withdrawing groups favors the anti-HIV potency with increased selectivity. Substitutions on position 3 of the phenyl ring either with electron donating or electron withdrawing groups are not favorable for anti-HIV activity. Further lead optimization yet to be done with evaluation of di-substituted derivatives having favorable substitutents on their respective positions. In the present series of compounds, interestingly none of these compounds showed activity against HIV-1 below their cytotoxic concentration in contrast to their anti-HIV-2 activity. This is a rather unusual anti-HIV activity spectrum for heterocyclic compounds.

HIV-Reverse Transcriptase inhibition Activity

Compounds (**7a, 7b, 7f, 7k, 7l**) were evaluated further to find out their exact mechanism of action. When investigated for their potential inhibitory activity against HIV-2 reverse transcriptase (RT), none of these compounds (**7a, 7b, 7f, 7k, 7l**) were displayed inhibitory activity against HIV-2 RT at 200 μg/ml, regardless the nature of the used template-primer (poly rA.dT or poly rC.dG). Therefore, specific anti-HIV-2 activity of these β-carboline derivatives was not due to inhibition of HIV-2 reverse transcriptase and further extensive studies are required to understand the exact mechanism of action.

In summary, a series of new 3-piperazinoyl-β-carboline derivatives were designed and evaluated for inhibitory activity against HIV-1 and HIV-2 strains. Among the designed analogues, compounds **7a, 7b, 7f, 7k** and **7l** showed selective inhibition against HIV-2 (ROD). Especially **7k** (4-(4-fluorophenyl)piperazin-1-yl)(1-phenyl-9*H*-pyrido[3,4-*b*]indol-3-yl)methanone exhibited significant inhibition (EC_{50} 2.6 μM) of HIV-2 which is comparable to standard NRTIs such as

Lamivudine and dideoxyinosine with good selectivity index (>72). The mechanism of this unusual selectivity is currently unclear.

Acknowledgments

We are grateful to SAIF, Panjab University, Chandigarh, India for providing NMR spectra. One of the authors Ashok Penta, acknowledges Council of Scientific and Industrial Research, New Delhi, India for financial support in the form of Senior Research Fellowship. The anti-viral and enzyme studies were supported by the KU Leuven (GOA 10/014). The technical help of Mrs. K. Erven and Mrs. L. van Berckelaer was gratefully acknowledged.

References

[1] Buonaguro, L.; Tornesello, M.; Buonaguro, F. *J. Virol.* **2007,** *81,* 10209.

[2] Campbell-Yesufu, O. T.; Gandhi, R. T. *Clin. Infect. Dis.* **2011,** *52,* 780.

[3] Kanki, P.; Travers, K. U.; Marlink, R.; Essex, M.; MBoup, S.; Gueye-Ndiaye, A.; Siby, T.; Thior, I.; Sankale, J.; Hsieh, C. *The Lancet* **1994,** *343,* 943.

[4] De Clercq, E. *J. Med. Chem.* **1995,** *38,* 2491.

[5] Jallow, S.; Alabi, A.; Sarge-Njie, R.; Peterson, K.; Whittle, H.; Corrah, T.; Jaye, A.; Cotten, M.; Vanham, G.; McConkey, S. J. *J. Clin. Microbiol.* **2009,** *47,* 2200.

[6] Smith, R. A.; Anderson, D. J.; Pyrak, C. L.; Preston, B. D.; Gottlieb, G. S. *J. Infect. Dis.* **2009,** *199,* 1323.

[7] Boyer, P. L.; Sarafianos, S. G.; Clark, P. K.; Arnold, E.; Hughes, S. H. *PLoS Path.* **2006,** *2,* e10.

[8] Desbois, D.; Roquebert, B.; Peytavin, G.; Damond, F.; Collin, G.; Bénard, A.; Campa, P.; Matheron, S.; Chêne, G.; Brun-Vézinet, F. *Antimicrob. Agents Chemother.* **2008,** *52,* 1545.

[9] Hao, G. F.; Yang, G. F.; Zhan, C. G. *J. Phys. Chem. B* **2010,** *114,* 9663.

[10] Roquebert, B.; Damond, F.; Collin, G.; Matheron, S.; Peytavin, G.; Benard, A.; Campa, P.; Chene, G.; Brun-Vezinet, F.; Descamps, D. *J. Antimicrob. Chemother.* **2008,** *62,* 914.

[11] Owen, S. M.; Ellenberger, D.; Rayfield, M.; Wiktor, S.; Michel, P.; Grieco, M. H.; Gao, F.; Hahn, B. H.; Lal, R. B. *J. Virol.* **1998,** *72,* 5425.

[12] Witvrouw, M.; Pannecouque, C.; Switzer, W. M.; Folks, T. M.; De Clercq, E.; Heneine, W. *Antivir. Ther.* **2004,** *9,* 57.

[13] Poveda, E.; Rodes, B.; Toro, C.; Soriano, V. *AIDS Res. Hum. Retroviruses* **2004,** *20*, 347.

[14] Ishida, J.; Wang, H. K.; Oyama, M.; Cosentino, M. L.; Hu, C. Q.; Lee, K. H. *J. Nat. Prod.* **2001,** *64*, 958.

[15] Sakai, R.; Higa, T.; Jefford, C. W.; Bernardinelli, G. *J. Am. Chem. Soc.* **1986,** *108*, 6404.

[16] Adachi, J.; Mizoi, Y.; Naito, T.; Ogawa, Y.; Uetani, Y.; Ninomiya, I. *J. Nutr.* **1991,** *121*, 646.

[17] Brossi, A.; Focella, A.; Teitel, S. *J. Med. Chem.* **1973,** *16*, 418.

[18] Rao, K. V.; Kasanah, N.; Wahyuono, S.; Tekwani, B. L.; Schinazi, R. F.; Hamann, M. T. *J. Nat. Prod.* **2004,** *67*, 1314.

[19] Brahmbhatt, K. G.; Ahmed, N.; Sabde, S.; Mitra, D.; Singh, I. P.; Bhutani, K. K. *Bioorg. Med. Chem. Lett.* **2010,** *20*, 4416.

[20] Wang, Y. H.; Tang, J. G.; Wang, R. R.; Yang, L. M.; Dong, Z. J.; Du, L.; Shen, X.; Liu, J. K.; Zheng, Y.-T. *Biochem. Biophys. Res. Commun.* **2007,** *355*, 1091.

[21] Yu, X.; Lin, W.; Li, J.; Yang, M. *Bioorg. Med. Chem. Lett.* **2004,** *14*, 3127.

[22] Yu, X.; Lin, W.; Pang, R.; Yang, M. *Eur. J. Med. Chem.* **2005,** *40*, 831.

[23] Xu, Z.; Chang, F. R.; Wang, H. K.; Kashiwada, Y.; McPhail, A. T.; Bastow, K. F.; Tachibana, Y.; Cosentino, M.; Lee, K. H. *J. Nat. Prod.* **2000,** *63*, 1712.

[24] Hsieh, P. W.; Chang, F. R.; Lee, K. H.; Hwang, T. L.; Chang, S. M.; Wu, Y. C. *J. Nat. Prod.* **2004,** *67*, 1175.

[25] Tang, J. G.; Wang, Y. H.; Wang, R. R.; Dong, Z. J.; Yang, L. M.; Zheng, Y. T.; Liu, J. K. *Chem. Biodivers.* **2008,** *5*, 447.

[26] Rao, K. V.; Donia, M. S.; Peng, J.; Garcia-Palomero, E.; Alonso, D.; Martinez, A.; Medina, M.; Franzblau, S. G.; Tekwani, B. L.; Khan, S. I.; Wahyuono, S.; Willett, K. L.; Hamann, M. T. *J. Nat. Prod.* **2006,** *69*, 1034.

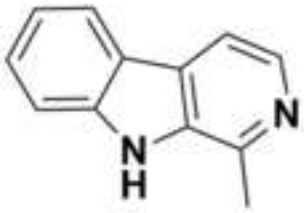
[27] Wang, P.; Kong, F.; Wei, J.; Wang, Y.; Wang, W.; Hong, K.; Zhu, W. *Mar. Drugs* **2014,** *12*, 477.

[28] Hutait, S.; Nayak, M.; Penta, A.; Batra, S. *Synthesis* **2011,** *03*, 419.

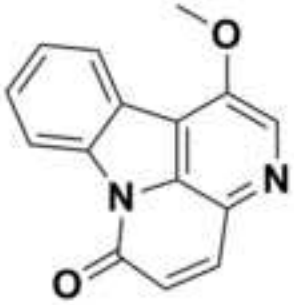
[29] Mahesh, R.; Devadoss, T.; Pandey, D. K.; Bhatt, S. *Bioorg. Med. Chem. Lett.* **2011,** *21*, 1253.

Table 1: *In vitro* anti-HIV activity of the synthesized compounds

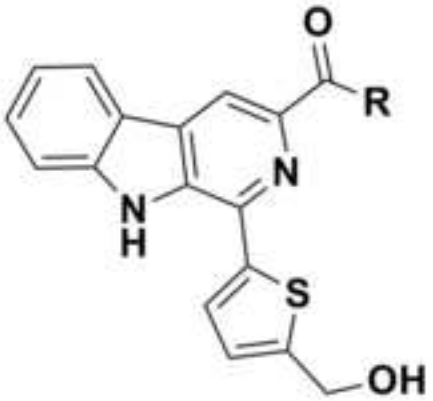
S. No	Comp. Code	R	$EC_{50}(\mu M)^1$		$CC_{50}(\mu M)^2$	HIV-2 SI^3
			HIV-1 (III_B)	HIV-2 (ROD)		
1.	7a	Phenyl	>224	29.4 ± 5.9	>224	>8
2.	7b	4-Methylphenyl	>29.6	3.3 ± 0.02	29.6 ± 0.88	9
3.	7c	2-Methylphenyl	>16.9	>16.9	16.9 ± 1.55	<1
4.	7d	4-Methoxyphenyl	>210.0	>210.0	>210.0	-
5.	7e	3-Methoxyphenyl	>133.9	>133.9	133.9 ± 12.46	<1
6.	7f	2-Methoxyphenyl	>25.3	3.2 ± 0.22	25.3 ± 0.54	8
7.	7g	4-Chlorophenyl	>208.3	>208.3	>208.3	-
8.	7h	3-Chlorophenyl	>110.6	>110.6	110.6 ± 8.36	<1
9.	7i	2-Chlorophenyl	>23.6	>23.6	23.6 ± 0.44	<1
10.	7j	4-Nitrophenyl	>203.6	>203.6	>203.6	-
11.	7k	4-Fluorophenyl	>189	2.6 ± 0.02	>189.0	>72
12.	7l	2-Fluorophenyl	>50.6	5.4 ± 0.23	50.6 ± 4.00	9
13.	7m	2,3-Dichlorophenyl	>63.9	>63.9	63.9 ± 4.13	<1
14.	7n	Benzyl	>21.7	>21.7	21.7 ± 1.33	<1
15.	7o	4-Pyridyl	>4.02	>4.02	4.02 ± 0.05	<1
16.	7p	2-Pyridyl	>19.2	>19.2	19.2 ± 2.46	<1
17.	Nevirapine		0.027 ± 0.002	>4	>4	-
18.	Lamivudine		0.89 ± 0.66	3.56 ± 2.56	>20	>5.6
19.	Dideoxyinosine		2.56 ± 0.79	2.27 ± 0.49	>50	>21.7
20.	Zidovudine		0.0019 ± 0.0001	0.0016 ± 0.0002	>25	>12500



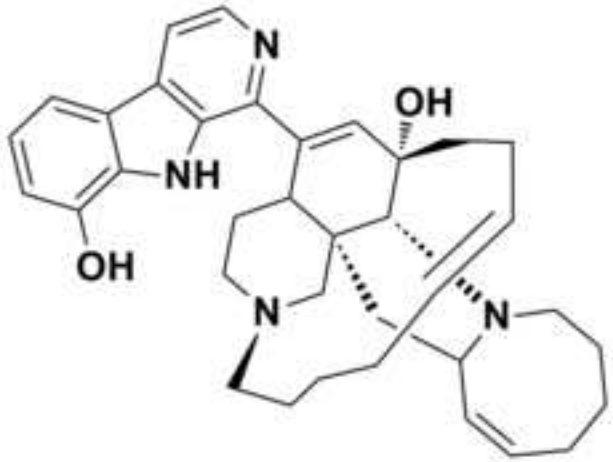
Harman



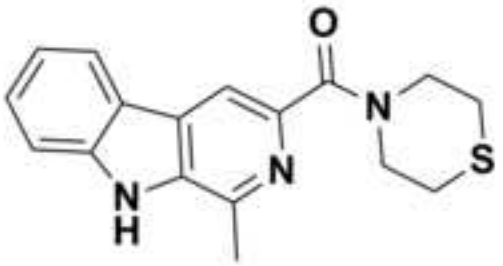
1-methoxycanthinone

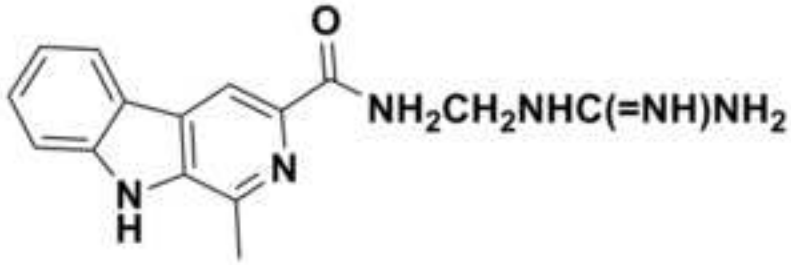


Flazin R = OH
Flazinamide R = NH_2

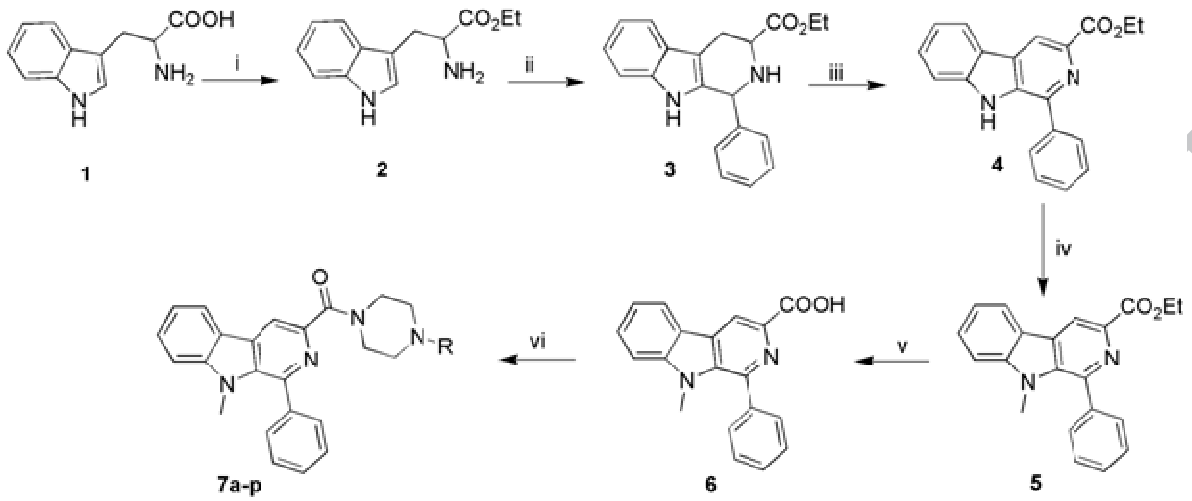


8-hydroxymanzamine A





Synthetic β–carboline derivatives



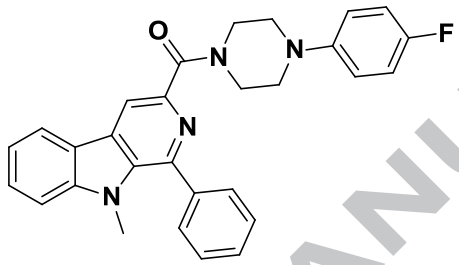
Scheme 1

Graphical Abstract

Design, synthesis of new β-carboline derivatives and their selective anti-HIV-2 activity

Penta Ashok[a], Subhash Chander[a], Jan Balzarini[b], Christophe Pannecouque[b], Sankaranarayanan Murugesan[a]*

[a,]Medicinal Chemistry Research Laboratory, Department of Pharmacy, Birla Institute of Technology & Science, Pilani-333031, India. E. mail: murugesan@pilani.bits-pilani.ac.in*
[b]Rega Institute for Medical Research, KU Leuven, Minderbroedersstraat 10, B-3000 Leuven, Belgium.



7l, potent anti-HIV-2 activtiy with EC_{50} 2.6 μM