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Synthesis and evaluation of β-carboline derivatives as inhibitors of human immunodeficiency virus

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ABSTRACT

A series of β-carboline derivatives were synthesized by utilizing aromatization and chemoselective alkylation method recently reported from our laboratory. Synthesized derivatives were evaluated for anti-HIV activity in human CD4+ T cell line (CEM-GFP) infected with HIV-1 $NL_{4.3}$ virus. 1-Formyl-β-carboline-3-carbxylic acid methyl ester (**15**) showed inhibition of human immunodeficiency virus at IC_{50} = 2.9 μM.

An urgent need for the discovery of newer agents for the treatment of AIDS demands that all approaches to drug discovery should be exploited aggressively. Among the possible approaches, the one from natural products has made unique and vital contributions to drug discovery in many disease areas. Natural products are rich source for biologically active compounds. The conversion of these biologically active compounds into derivatives better suited for medicinal use has become a useful strategy for many modern drug discovery programs. Many natural products have shown promising anti-HIV activities which have been elaborately reviewed by our group.[1] Several natural products have been used as lead molecules for further modifications to improve their anti-HIV activities.[2–5]

β-Carboline derivatives, obtained from various natural and synthetic sources, show variety of important biological activities;[6–11] including recent reports on their anti-HIV potential (Fig. 1). *N*9-butylharmine (**1**), a semi-synthetic derivative of widely occurring alkaloid harmine, has been found to inhibit HIV replication in H9 lymphocytes.[12] Presence of alkyl group at N9 and methyl group at C-1 are essential for better anti-HIV activity. 1-Methyl-β-carboline-3-carboxamide derivative (**4**) bearing guanidinium group has been known to inhibit replication of HIV-1 by hindering the essential interaction of regulatory protein Tat to trans-activation response region (TAR).[13,14] Furthermore, C-1 substituted alkaloid flazine (**2**) and its amide derivative (**3**) have also been reported for their anti-HIV activity.[15]

This led us to study various β-carboline analogues as anti-HIV agents as a part of our ongoing work. In this Letter, we report synthesis and evaluation of several β-carboline derivatives for anti-HIV activity using human CD4+ reporter T cell line (CEM-GFP) infected with HIV-1 $NL_{4.3}$.

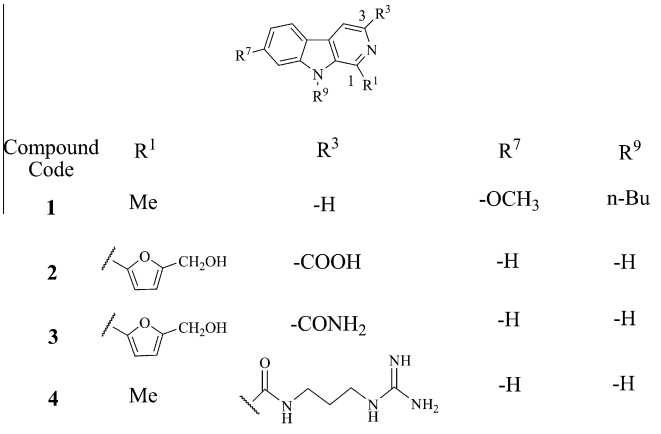

Figure 1. Anti-HIV β-carboline derivatives.

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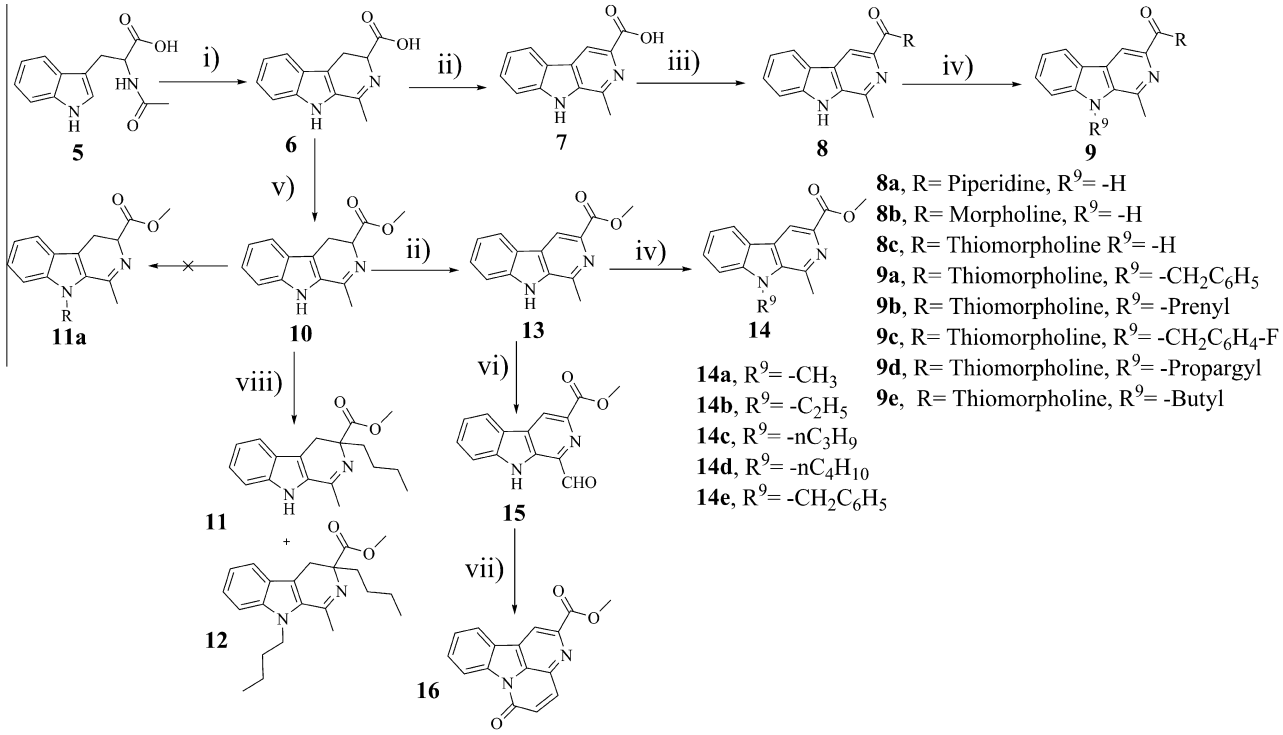

Scheme 1. Synthesis of β-carboline derivatives: Reagents and conditions: (i) dicyclohexylcarbodiimide (DCC), CH_2Cl_2, 20 °C, 3 h then CF_3COOH, 50 °C, 1 h, 85%; (ii) NaH, anhydrous DMF, 28 °C (**7** 62% 4 h; **13** 80% 30 min); (iii) $MeSO_2Cl$, Et_3N, CH_2Cl_2, 0 °C, 2 h then amine, 20 °C, 6 h (**8a** 70%; **8b** 68%; **8c** 72%); (iv) NaH, anhydrous DMF, 0 °C, 30 min then, RX (X = Br or I), 28 °C, 3 h (**9a** 82%; **9b** 80%; **9c** 83%; **9d** 78%; **9e** 75%; **14a** 85%; **14b** 78%; **14c** 80%; **14d** 75%; **14e** 85%); (v) $SOCl_2$, MeOH, 4 h, 30 °C, 96%; (vi) SeO_2, dioxane, reflux, 67%; (vii) Ac_2O, pyridine, reflux, 3 h, 60%; (viii) inverse addition: *n*-BuI, anhydrous DMF, degassing by freeze-pump–thaw then NaH, 28 °C, oxygen-free argon atmosphere, 30 min (**11** 77%; **12** 13%).

The overall strategy followed for the synthesis of β-carboline derivatives is shown in Scheme 1. *N*-acetyl tryptophan (**5**), obtained by the Schotten–Baumann method from commercially available dl-tryptophan, was cyclized by a modified Bischler–Napieralski condensation to afford 1-methyl-3,4-dihydro-β-carboline-3-carboxylic acid (**6**) in 85% yield.[16] Methyl ester derivative (**10**) was synthesized by reacting **6** with thionyl chloride in methanol.[17] Compounds **6** and **10** were aromatized by employing sodium hydride in anhydrous DMF to yield **7** and **13** in 62% and 80%, respectively.[18] Compound **7** was reacted with methanesulfonyl chloride in dichloromethane in the presence of triethyl amine to obtain reactive mesyl ester which was reacted in situ with piperidine, morpholine or thiomorpholine to generate **8a–c** in 70%, 68% and 72% yield, respectively.[19] 1-Methyl-β-carboline-3-carboxylic acid methyl ester (**13**) and thiomorpholine derivative (**8c**) were alkylated to afford N9-alkyl derivatives **9a–e**[20] and **14a–e**,[18] respectively. Synthesis of **11a** was attempted by alkylation of **10**, however, these attempts resulted in formation of C-3 alkylated derivative (**11**) in 77% yield along with **12** as a result of chemoselective alkylation in oxygen free conditions.[18] Mixture of **11** and **12** was separated by dry column vacuum chromatography (DCVC).

Table 1
Anti-HIV activity of synthesized compounds

Entry	Compound code	Non-cytotoxic concentration[e] (μM)	% Inhibition p24 ELISA at non-cytotoxic concentration	Entry	Compound code	Non-cytotoxic concentration[e] (μM)	% Inhibition p24 ELISA at non-cytotoxic concentration
1	**6**	17.54	16.48	12	**12**	14.12	NI
2	**8a**	1.71	NI[a]	13	**13**	4.17	21.3
3	**8b**	27.12	NI	14	**14a**	19.66	NI
4	**8c**	1.61	16.9	15	**14b**	3.73	NI
5	**9a**	6.22	NI	16	**14c**	3.54	NI
6	**9b**	19.76	NI	17	**14d**	1.69	NI
7	**9c**	23.84	NI	18	**14e**	15.13	NI
8	**9d**	21.46	NI	19	**15**	9.83	78.17[b,c]
9	**9e**	1.36	NI	20	**16**	3.59	NI
10	**10**	18.59	17.26	21	**AZT**	4.98	89.75[b,d]
11	**11**	8.39	12.17				

[a] NI = no inhibition.
[b] IC_{50} = concentration of compound required to achieve 50% protection of CEM-GFP cells ($n = 3$) from virus, as determined by p24 ELISA assay, CC_{50} = concentration of compound required to reduce proliferation of CEM-GFP cells ($n = 3$) by 50%, as determined by MTT assay and T.I.(therapeutic index) = CC_{50}/IC_{50}.
[c] IC_{50} = 2.9 μM, CC_{50} = 20.3 μM and TI = 7.
[d] IC_{50} = 1.04 μM, CC_{50} = 23.04 μM and TI = 23.07.
[e] Non-cytotoxic concentration = highest concentration where the cell viability is more than 95%.

Compound **13** underwent oxidation upon treatment with selenium dioxide to yield 1-formyl derivative (**15**) in 67% which was refluxed with acetic anhydride and pyridine (10:1) to obtain canthin-6-one-3-carboxylic acid methyl ester (**16**) in 60% yield.[21]

All synthesized analogues were first evaluated for their cytotoxicity in MTT based cell viability assay[22] in CEM-GFP cells. Based on the results of MTT assay, non-cytotoxic concentration of each analogue was used for determination of in vitro anti-HIV activity in CEM-GFP cells[23–26] as shown in Table 1. Only the active compounds showing more than 60% inhibition at non-cytotoxic concentration were further evaluated to determine their CC_{50} and IC_{50} values. The results are shown in Table 1.

In our preliminary screenings, compounds **6**, **8c**, **10** and **13** were found to show inhibition of HIV-1 $NL_{4.3}$ (Table 1, entries 1, 4, 10 and 13). N-Alkylated derivatives of harmine have been reported to show better activity than harmine.[12] Based on this observation, **8c** and **13** were alkylated to afford **9a–e** and **14a–e**, respectively, whereas, alkylation of **10** resulted in formation of **11** and **12**. It is noteworthy that N-alkylation of **8c** and **13** was detrimental and resulted in loss of activity (Table 1, entries 5–9 and 14–18). This observation suggested a different mode of action and/or a different binding site for β-carboline-3-carboxylic acid derivatives as compared to harmine (7-methoxy-β-carboline) derivatives. Interestingly, 1-formyl-β-carboline-3-carboxylic acid methyl ester derivative (**15**) showed inhibition of HIV at IC_{50} of 2.9 μM and CC_{50} of 20.3 μM. Anti-HIV potential of **15** is encouraging as there are no reports of 1-formyl derivative of β-carboline showing anti-HIV activity in the literature.

In conclusion, we have described synthesis and biological evaluation of β-carboline derivatives for anti-HIV activity. Out of various compound tested, 1-formyl-β-carboline-3-carboxylic acid methyl ester derivative (**15**) was found to be active against HIV. Further modification of the selected lead is under progress, and results will be published in a due course of time.

Acknowledgment

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19. *General procedure for preparation of amide derivatives* (**8a–c**): To a mixture of **7** (1.60 g, 7.08 mmol) and triethyl amine (2.86 g, 28.3 mmol) in anhydrous dichloromethane (10 ml), methanesulfonyl chloride (0.97 g, 8.5 mmol) was added dropwise at 0 °C under positive argon atmosphere. The reaction mixture was allowed to stir at 28 °C for 2 h followed by addition of amine [**a**: piperidine (0.66 g, 7.79 mmol), **b**: morpholine (0.68 g, 7.79 mmol) or **c**: thiomorpholine (0.80 g, 7.79 mmol)]. The resulting mixture was allowed to stir for another 6 h at 28 °C. The reaction mixture was poured into cold water, and extracted with chloroform (3 × 100 ml). The combined organic phase was washed with water (3 × 200 ml) and with brine (3 × 200 ml), then dried over anhydrous sodium sulfate and evaporated in vacuo. The crude product obtained was purified by column chromatography (Silica Gel #60–120, hexane/EtOAc-gradient) to obtain compound **8a**, **8b** or **8c** in 70%, 68% and 72% yield, respectively depending on the amine used.
(1-Methyl-β-carboline-3-yl)(piperidin-1-yl)methanone (**8a**): ^{1}H NMR (400 MHz, $CDCl_3$ ppm): δ 9.41 (br s, 1H), 7.99 (s, 1H), 7.92 (d, 1H, *J* = 7.84 Hz), 7.45–7.51 (m, 2H), 7.20–7.23 (m, 1H), 3.81 (br s, 2H), 3.54 (br s, 2H), 2.69 (s, 3H), 2.74 (br s, 2H), 2.69 (s, 3H), 1.70 (br s, 4H), 1.56 (br s, 2H). ^{13}C NMR (100 MHz, $CDCl_3$, ppm): δ 168.5, 142.2, 139.9, 139.7, 133.7, 127.3, 127.2, 120.9, 120.7, 119.1, 111.8, 110.9, 47.7, 42.7, 25.5, 24.6, 23.7, 19.2. MS (APCI): *m/z* 294.3 $[M+1]^+$.
(1-Methyl-β-carboline-3-yl)(morpholino)methanone (**8b**): ^{1}H NMR (300 MHz, DMSO-d_6, ppm): δ 8.08 (d, 2H, *J* = 9.38), 7.34–7.45 (m, 2H), 7.07 (t, 1H, *J* = 7.30), 3.43 (br s, 8H), 2.58 (s, 3H). ^{13}C NMR (75.5 MHz, DMSO-d_{6s}, ppm): δ 168.4, 142.2, 141.1, 141.0, 134.9, 128.7, 127.6, 122.4, 121.5, 120.1, 113.9, 112.5, 48.0, 42.7, 20.7. MS (APCI): *m/z* 296.1 $[M+1]^+$.
(1-Methyl-β-carboline-3-yl)(thiomorpholino)methanone(**8c**): ^{1}H NMR (400 MHz, $CDCl_3$, ppm): δ 9.48 (br s, 1H), 8.09 (s, 1H), 7.92 (d, 1H, *J* = 7.60 Hz), 7.49–7.45 (m, 2H), 7.24–7.20 (m, 1H), 4.12 (br s, 2H), 3.92 (br s, 2H), 2.85 (br s, 2H), 2.74 (br s, 2H), 2.69 (s, 3H). ^{13}C NMR (100 MHz, $CDCl_3$, ppm): δ 169.8; 142.4, 140.8, 140.6, 134.9, 128.4, 128.3, 121.8, 121.7, 120.3, 113.7, 111.9, 50.3, 45.3, 28.1, 27.4, 20.3. MS (APCI): *m/z* 312.2 $[M+1]^+$.
20. *General procedure for alkylation of (1-methyl-β-carboline-3-yl)(thiomorpholino)methanone* (**8c**): Compound **8c** (250 mg, 0.80 mmol) was stirred in anhydrous DMF (10 ml) at room temperature in argon atmosphere. It was cooled to 0–5 °C and then sodium hydride (60%) (38.6 mg, 0.96 mmol) was added. The reaction mixture was allowed to stir for 30 min at 28 °C. Alkyl halide (3 mmol) was added dropwise and the reaction mixture was stirred for 3 h at 28 °C. The resulting mixture was then poured into cold water, and extracted with ethyl acetate (3 × 50 ml). The organic phase was washed with water (2 × 25 ml) and brine (2 × 25 ml), then dried over anhydrous sodium sulfate and evaporated in vacuo. The crude product was purified by column chromatography (Silica Gel #60–120, hexane/EtOAc-gradient) to obtain compound **9a–e** in 82%, 80%, 83%, 78% and 75%, respectively.
(9-Benzyl-1-methyl-β-carboline-3-yl)(thiomorpholino)methanone(**9a**): ^{1}H NMR (400 MHz, DMSO-d_6, ppm): δ 8.51 (s, 1H), 8.43 (d, 1H, *J* = 7.24), 7.74 (d, 1H, *J* = 7.78), 7.67 (d, 1H, *J* = 6.60), 7.38 (d, 1H, *J* = 6.68), 7.25–7.30 (m, 3H), 6.99 (d, 2H, *J* = 6.16), 5.98 (s, 2H), 3.96 (br s, 2H), 3.76 (br s, 2H), 2.87 (s, 3H), 2.71(br s, 4H). ^{13}C NMR (100 MHz, DMSO-d_6, ppm): δ 166.5, 142.5, 140.6, 140.0, 138.3, 134.5, 129.6, 129.4, 128.9, 127.3, 125.3, 122.3, 120.8, 120.4, 113.8, 110.8, 49.6, 47.4, 44.4, 27.1, 26.6, 21.6. MS (APCI): *m/z* 402.2 $[M+1]^+$.
(9-Prenyl-1-methyl-β-carboline-3-yl)(thiomorpholino)methanone (**9b**): ^{1}H NMR (400 MHz, $CDCl_3$, ppm): δ 8.29 (s, 1H), 8.14 (d, 1H, *J* = 7.80 Hz), 7.60 (dt, 1H, J_1 = 8.28, J_2 = 1.00 Hz), 7.43 (d, 1H, *J* = 8.36 Hz), 7.31 (dt, 1H, J_1 = 7.72, J_2 = 0.44 Hz), 5.18–5.22 (m, 3H), 4.12 (br s, 2H), 4.01 (br s, 2H), 3.03 (s, 3H, $-CH_3$), 2.81 (br s, 2H), 2.76 (br s, 2H), 1.91 (s, 3H), 1.74 (s, 3H). ^{13}C NMR (100 MHz, $CDCl_3$, ppm): δ 169.0, 141.7, 139.8, 135.2, 134.7, 129.3, 128.4, 121.5, 121.3, 120.8, 120.1, 114.2, 109.8, 63.5, 50.2, 45.2, 43.3, 27.8, 27.1, 24.9, 23.0, 18.1. MS (APCI): *m/z* 380.3 $[M+1]^+$.
9-(4-Fluorobenzyl)-1-methyl-β-carboline-3-yl(thiomorpholino)methanone(**9c**): ^{1}H NMR (400 MHz, $CDCl_3$, ppm): δ 8.33 (s, 1H), 8.18 (d, 1H, *J* = 7.88 Hz), 7.59 (dt, 1H, J_1 = 7.24, J_2 = 1.00), 7.38 (m, 2H, *J* = 8.38. Hz), 6.97 (m, 4H), 5.81 (s, 2H,), 4.11 (br s, 2H), 3.97 (br s, 2H), 2.93 (s, 3H, $-CH_3$), 2.83 (br s, 2H), 2.75 (br s, 2H). ^{13}C NMR (100 MHz, $CDCl_3$, ppm): δ 163.1, 160.6, 142.2, 141.2, 139.7, 135.1, 133.0, 129.8, 129.1, 126.7, 126.6, 121.5, 120.9, 120.6, 115.7, 115.5, 114.0, 109.4, 63.3, 50.1, 45.1, 28.4, 27.5, 24.6. MS (APCI): *m/z* 420.3 $[M+1]^+$.
(9-Propargyl-1-methyl-β-carboline-3-yl)(thiomorpholino)methanone(**9d**): ^{1}H NMR (400 MHz, $CDCl_3$, ppm): δ 8.28 (s, 1H), 8.12 (d, 1H, *J* = 7.80 Hz), 7.65 (dt, 1H, J_1 = 7.16, J_2 = 1.16 Hz), 7.54 (d, 1H, *J* = 8.36 Hz), 7.35 (dt, 1H, J_1 = 7.88, J_2 = 0.80 Hz), 5.30 (d, 2H, *J* = 2.44), 4.12 (br s, 2H), 3.99 (br s, 2H), 3.14 (s, 3H, $-CH_3$), 2.82 (br s, 2H), 2.76 (br s, 2H), 2.38 (t, 1H, *J* = 2.44). ^{13}C NMR (100 MHz, $CDCl_3$, ppm): δ 168.8, 143.1, 141.4, 139.9, 135.0, 130.0, 128.9, 121.8, 121.8, 121.0, 114.4, 109.4, 78.3, 73.7, 50.4, 45.4, 34.7, 28.1, 27.4, 23.2. MS (APCI): *m/z* 350.3 $[M+1]^+$.
*(9-Butyl-1-methyl-β-carboline-3-yl)(thiomorpholino)methanone (**9e**):* ^{1}H NMR (400 MHz, $CDCl_3$, ppm): δ 8.29 (s, 1H), 8.14 (d, 1H, *J* = 7.76 Hz), 7.61 (dt, 1H, J_1 = 7.32, J_2 = 1.12 Hz), 7.49 (d, 1H, *J* = 8.36 Hz), 7.31 (dt, 1H, J_1 = 7.76, J_2 = 0.60 Hz), 4.56 (t, 2H, *J* = 7.84), 4.12 (br s, 2H), 4.02 (br s, 2H), 3.05 (s, 3H), 2.81 (br s, 2H), 2.76 (br s, 2H), 1.81–1.89 (m, 2H), 1.47 (m, 2H), 1.00 (t, 3H, *J* = 7.36). ^{13}CNMR (100 MHz, $CDCl_3$, ppm): δ 168.8, 141.6, 141.4, 139.3, 134.9, 129.2, 128.3, 121.3, 121.0, 120.0, 114.0, 109.6, 63.2, 63.0, 44.4, 32.6, 24.7, 24.5, 22.9, 19.8, 13.4. MS (APCI): *m/z* 368.2 $[M+1]^+$.

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26. *General procedure for evaluation of anti-HIV activity*: CEM-GFP is a human CD4+ reporter T cell line which expresses green fluorescent protein (GFP) upon HIV infection. GFP expression is due to trans-activation by Tat protein of stably integrated long terminal repeat regulated GFP gene. This cell line is used widely for determination of anti-HIV activity due to easy visualization of infected cells.[23] CEM-GFP cells were infected with HIV-1 $NL_{4.3}$ virus at a multiplicity of infection (MOI) of 0.05 using the standard protocol previously published.[24,25] The cells were then incubated with samples for up to 8 days post infection. Virus production was assayed in the culture supernatant on day-8 post infection by p24 antigen capture ELISA (Perkin–Elmer, USA).