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Synthesis of 2-Methylsulfanyl-1*H*-imidazoles as Novel Non-nucleoside Reverse Transcriptase Inhibitors (NNRTIs)

α-Aminoketone hydrochlorides **2 a–d** were synthesized by Dakin-West reaction from *L*-phenylalanine and *L*-cyclohexylalanine followed by hydrolysis in acidic medium. Treatment of **2 a–d** with aqueous potassium thiocyanate afforded 1,3-imidazole-2-thiones **3 a–d** which were alkylated with methyl iodide to give 2-methylsulfanyl-1*H*-imidazoles **4 a–d** with 4-benzyl/4-cyclohexylmethyl and 5-ethyl/5-isopropyl substituents. Coupling of **4 a–d** with ethoxymethyl chloride or benzyloxymethyl chloride furnished N-1 **5 a–d** and N-3 **6 a–h** alkylated products. The synthesised compounds were tested for their activity against HIV-1. The most active compounds have a cyclohexylmethyl group in the 5-position of **6** and showed an activity against HIV-1 comparable to the activity of Nevirapine.

Keywords: α-Aminoketones; Imidazole-2-thiones; Non-nucleoside reverse transcriptase inhibitors; Human immunodeficiency virus

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Introduction

In the late 1980s, compounds from the corporate library were screened at Janssen Pharmaceutica, for their ability to inhibit HIV replication as a part of their anti-HIV program. This was the first opportunity to discover unique inhibitors of the key multifunctional HIV-1 enzyme, reverse transcriptase. Compounds of the tetrahydroimidazo[4,5,1-*jk*][1,4]-benzodiazepin-2(1*H*)-one (TIBO) series were found to be noncompetitive inhibitors. Crystallographic studies later showed that they inhibited the enzyme's action by binding to a hydrophobic pocket close to, but distinct from, the active site [1–5]. Subsequently, many new structural classes of non-nucleoside resverse transcriptase inhibitors (NNRTIs) were found to specifically inhibit the reverse transcriptase of HIV-1 by binding at this site [6–8]. Although less likely to cause deleterious side effects than nucleoside inhibitors, NNRTIs were found to be more vulnerable to HIV's high mutation rate, leading to a rapid selection of strains that are resistant to inhibition [9, 10]. In spite of this shortcoming, nevirapine and delavirdine were developed and approved for sale as important adjuncts to the accepted multidrug therapies used to treat HIV-positive patients. A third NNRTI, efavirenz, has been approved and can be considered as a true second generation NNRTI because of its ability to inhibit some of the mutant strains resistant to its predecessors [11].

A variety of potential drug candidates of the NNRTI type has been developed over the recent years, *e.g.* imidazole derivatives. 2-Carbamoyloxymethyl-5-(3,5-dichlorophenyl)thio-4-isopropyl-1-(pyridin-4-yl)methyl-1*H*-imidazole (Capravirine, AG 1549) is in clinical testing [12], but its use has recently been restricted due to vasculitis (an inflammation of the blood vessels), in animals that received high doses of Capravirine [13]. Therefore, further research is needed in order to find new drug candidates within this class of compounds.

Chemistry

1,3-Dihydroimidazole-2-thiones have been synthesized by treatment of K-aminoketones with potassium thiocyanate. We have synthesized α-acylaminoketones by the Dakin-West reaction [14, 15] by refluxing *L*-phenylalanine and *L*-cyclohexylalanine propionic anhydride and isobutyric anhydride in the presence of pyridine to induce the acylation of the chiral CH group and subsequent decarboxylation in the same manner as described by Cleland and Niemann for synthesis of **1 a** [16] and Loksha *et al.* for synthesis of **1 b** [17]. α-Acylaminoketones **1 a–d** were hydrolysed by 6M hydrochloric acid to

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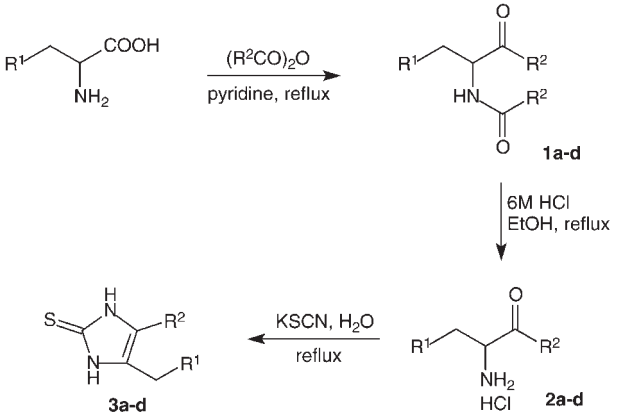
afford α-aminoketone hydrochlorides **2 a–d**. Compounds **2 a**, **b** have been previously synthesised by Sheppard *et al.* [18] and Loksha *et al.* [17]. 5-Alkyl-4-cyclohexylmethyl-1,3-dihydroimidazole-2-thiones **3 c**, **d** were prepared directly without separation of compounds **1 c**, **d** and **2 c**, **d** by treatment of the residual oil with aqueous potassium thiocyanate. Compounds **3 a**, **b** have been previously synthesized by Bullerwell and Lawson [19] and Loksha *et al.* [17].

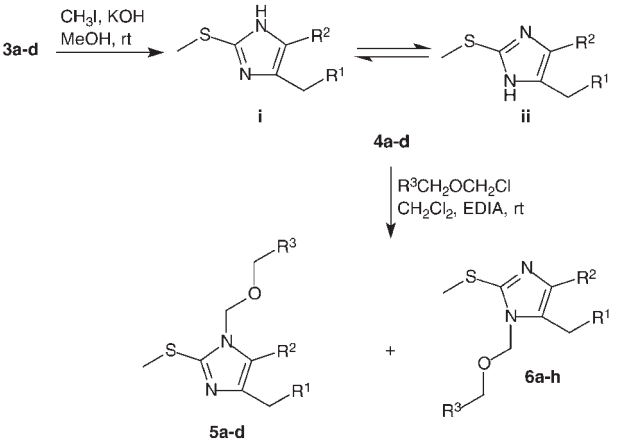


1-3	R^1	R^2
a	Ph	Et
b	Ph	*i*-Pr
c	C_6H_{11}	Et
d	C_6H_{11}	*i*-Pr

Scheme 1

2-Methylsulfanylimidazoles **4 a–d** were obtained by formation of the potassium salt of compounds **3 a–d** using methanolic potassium hydroxide solution and the salt was *S*-alkylated with methyl iodide. Compounds **4 a–d** were found to be in an equilibrium between the two tautomeric forms **i** and **ii** (Scheme 2). For some compounds the equilibrium was very fast as shown in ^{1}H-NMR spectra of compounds **4 a–c** where signals of the two tautomers collapsed, whereas for **4 d** the equilibrium between the two tautomers was slower, the peaks of the two tautomers appearing as individual signals. For the same reason, C-4 and C-5 in compounds **4 a**, **d** were not observed in the ^{13}C-NMR spectra, whreas C-2 and the methylene carbon atom in the benzyl group showed significant broadening of the peaks.

Alkylation of compounds **4 a–d** with ethoxymethyl chloride and benzyloxymethyl chloride using *N*-ethyldiisopropylamine (EDIA) as a bulky base afforded coupling at N^1 or N^3 depending on R^2. When R^2 was an ethyl group, the coupling occurred at both N1 (compounds **5 a–d**) and N^3 (compounds **6 a–d)**, while steric hinderance of



4	R^1	R^2
a	Ph	Et
b	Ph	*i*-Pr
c	C_6H_{11}	Et
d	C_6H_{11}	*i*-Pr

5,6	R^1	R^2	R^3
a	Ph	Et	Me
b	Ph	Et	Ph
c	C_6H_{11}	Et	Me
d	C_6H_{11}	Et	Ph

6	R^1	R^2	R^3
e	Ph	*i*-Pr	Me
f	Ph	*i*-Pr	Ph
g	C_6H_{11}	*i*-Pr	Me
h	C_6H_{11}	*i*-Pr	Ph

Scheme 2

R^2, being an isopropyl group, induced coupling only at N^3 to afford the compounds **6 e–h**.

The assignment of structures of **5 a–d** and **6 a–h** was confirmed by NOE. Irradiation of NCH_2O in compound **5 a** showed 1.8 % NOE in CH_3CH_2-C^5, and no NOE in CH_2Ph was detected, while the same irradiation in compounds **6 a** and **6 e** showed no NOE in CH_3CH_2-C^5 and $(CH_3)_2CH$, respectively, but showed 1 % and 1.36 % NOE, respectively, in CH_2Ph.

Biological properties

The test for activity against HIV-1 was performed in MT4 cell cultures infected with wild type HIV-1 (strain IIIB).

Table. Antiviral activity of compounds **6 a–h** against HIV-1 in MT-4 cells[a].

Compd	IC_{50} (μM)[b]	CC_{50} (μM)[c]	Compd	IC_{50} (μM)	CC_{50} (μM)
6 a	>100	34	**6 e**	>100	29
6 b	>100	34	**6 f**	3.3	29
6 c	4.1	25	**6 g**	0.25	39
6 d	0.40	25	**6 h**	0.26	31
Nevirapine	0.38	>100			

[a] All data represent mean values for three separate experiments. [b] Inhibitory concentration of compound achieving 50 % inhibition of HIV-1 multiplication in MT-4-infected cells. [c] Cytotoxic concentration of compound required to reduce the viability of normal uninfected MT-4 cells by 50 %.

The compounds **5 a–d** and **6 a**, **b**, **e** were inactive at 100 µM whereas compounds **6 c**, **d**,**f–h** showed activity against wild type HIV-1 as shown in the table. Compounds with a 4-isopropyl group (**6 f–h**) were found more active than the corresponding compounds **6 b–d** with a 4-ethyl group. Also, a cyclohexylmethyl group in the 5-position (**6 c**, **d**, **g**, **h**) improved the activity against HIV when compared with compounds with a benzyl group (**6 a**, **b**, **e**, **f**). In fact, the activity of the most active cyclohexylmethyl derivatives **6 d**, **g**, **h** were comparable to the activity of Nevirapine, which is about 10-fold less active against HIV-1 than Capravirine [12].

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Experimental section

NMR spectra were recorded on a Varian Gemini 2000 NMR spectrophotometer at 300 MHz for ^{1}H and 75 MHz for ^{13}C with TMS as an internal standard. EI mass spectra were recorded on a Finnigan MAT SSQ 710. MALDI spectra were recorded on a 4.7 Tesla Ultima Fourier transform Mass spectrometer (IonSpec, Irvine, CA). Melting points were determined in a Büchi melting point apparatus. The silica gel (0.040–0.063 mm) used for column chromatography was purchased from Merck. Microanalyses were carried out at Atlantic Microlab, Inc., Norcross, Georgia, USA.

General procedure for the synthesis of 5-alkyl 4-cyclohexylmethyl-1,3-dihydro-imidazole-2-thiones **3 c**, **d**

A mixture of *L*-cyclohexylalanine (8.55 g, 50 mmol), anhydrous pyridine (41 mL, 500 mmol) and the appropriate acid anhydride (propionic and isobutyric anhydride) (500 mmol) was heated in an oil bath at 150 °C for 12 h until carbon dioxide was no longer evolved. After that, the excess of pyridine, acid anhydride, and the acid formed were removed under reduced pressure, the residue obtained was treated with an aqueous saturated solution of sodium bicarbonate (20 mL) to remove the acidic components and then extracted with ether (3 × 50 mL). The combined ethereal extracts were dried over sodium sulfate (5 g), filtered and evaporated till dryness under vacuum. The residue was treated with 6M hydrochloric acid (220 mL) and ethanol (120 mL). The mixture was refluxed for 7 h, cooled and the solvents were removed under reduced pressure, potassium thiocyanate (4.9 g, 50 mmol) in water (50 mL) was added to the residual material and the mixture was refluxed for 5 h. After cooling to room temperature, the solid product formed was filtered off, washed with ether (50 mL), and dried to afford compounds **3 c**, **d**.

4-Cyclohexylmethyl-5-ethyl-1,3-dihydroimidazole-2-thione (**3 c**)

Yield 6.9 g (62 %) as a white solid; mp 268–270 °C. ^{1}H-NMR ([D$_6$]DMSO): δ (ppm = 0.84–0.88 (m. 2 H, H$_{cy}$), 1.07–1.21 (m, 5 H, C*H*$_3$CH$_2$ and H$_{cy}$), 1.47–1.60 (m, 7 H, H$_{cy}$), 2.10–2.31 (m. 4 H, C*H*$_3$CH$_2$ and C*H*$_2$Cy), 11.50 (bs, 1 H, NH), 11.62 (bs, 1 H, NH). – ^{13}C-NMR ([D$_6$]DMSO): δ (ppm) = 13.97 (*C*H$_3$CH$_2$), 16.45 (CH$_3$*C*H$_2$), 25.86, 25.54, 32.18, 37.04 (C$_{cy}$), 30.44 (*C*H$_2$Cy), 121.85 (C-4), 126.05 (C-5), 158.46 (C=S). – EI MS: *m/z* = 224 (M$^+$). – Anal. Calcd for C$_{12}$H$_{20}$N$_2$S × 0.25 H$_2$O (228.87): C, 62.98; H, 9.03; N, 12.24. Found: C, 62.95; H, 8.77; N, 12.27.

4-Cyclohexylmethyl-5-isopropyl-1,3-dihydroimidazole-2-thione (**3 d**)

Yield 2.4 g (20 %) as a white solid; mp 275–277 °C. ^{1}H-NMR ([D$_6$]DMSO): δ (ppm = 0.85–0.96 (m, 2 H, H$_{cy}$), 1.16 (d, 6 H, *J* = 7.0 Hz, (C*H*$_3$)$_2$CH), 1.23–1.29 (m, 2 H, H$_{cy}$), 1.46–1.54 (m, 1 H, H$_{cy}$), 1,59–1.71 (m, 6 H, H$_{cy}$), 2.23 (d, 2 H, *J* = 7.3 Hz, C*H*$_2$Cy), 2.81 (hept., 1 H, *J* = 7.0 Hz, (CH$_3$)$_2$C*H*), 11.53 (s, 1 H, NH), 11.69 (s, 1 H, NH). – ^{13}C-NMR ([D$_6$]DMSO): δ (ppm) = 21.87 ((*C*H$_3$)$_2$CH), 23.38 ((CH$_3$)$_2$*C*H), 25.52, 25.84, 32.16, 37.02 (C$_{cy}$), 30.46 (*C*H$_2$Cy), 120.75 (C-4), 130.18 (C-5), 158.60 (C=S). – HiResMALDI MS: *m/z* = 239.1569 (MH$^+$, C$_{13}$H$_{23}$N$_2$S); requires 239.1581.

General procedure for the synthesis of 2-methylsulfanyl-1H-imidazoles **4 a–d**

To a solution of potassium hydroxide (0.28 g, 5 mmol) in MeOH (15 mL), **3 a–d** (5 mmol) was added and the mixture was stirred for 0.5 h. Methyl iodide (0.31 mL, 5 mmol) was added to the reaction mixture, and stirred at room temperature for an additional 1 h. The solvent was removed under reduced pressure, and water (25 mL) was added to the residual material. The solid product was filtered off and recrystallized from ethanol/water to give compounds **4 a–d**.

4-Benzyl-5-ethyl-2-methylsulfanyl-1H-imidazole (**4 a**)

Yield 0.94 g (81 %) as a white solid; mp 148–150 °C. ^{1}H-NMR ([D$_6$]DMSO): δ (ppm) = 1.06 (t, 3 H, *J* = 7.5 Hz, C*H*$_3$CH$_2$), 2.42–2.49 (m, 5 H, CH$_3$S and CH$_3$C*H*$_2$), 3.76 (s, 2 H, C*H*$_2$Ph), 7.14–7.28 (m, 5 H, Ph). – ^{13}C NMR ([D$_6$]DMSO): δ (ppm) = 14.53 (*C*H$_3$CH$_2$), 15.75 (CH$_3$S), 18.11 (broad, CH$_3$*C*H$_2$), 31.37 (broad, *C*H$_2$Ph), 125.62, 128.09, 128.12, 137.33 (C$_{arom}$), 140.74 (broad, C-2). – EI MS: *m/z* = 232 (M$^+$). – Anal. Calcd for C$_{13}$H$_{16}$N$_2$S × 0.25 H$_2$O (236.85): C, 65.92; H, 7.02; N, 11.83. Found: C, 65.96; H, 6.89; N, 11.83%.

4-Benzyl-5-isopropyl-2-methylsulfanyl-1H-imidazole (**4 b**)

Yield 1.15 g (93 %) as a white solid; mp 118–120 °C. ^{1}H-NMR ([D$_6$]DMSO): δ (ppm) = 1.11 (d, 6 H, *J* = 6.9 Hz, (C*H*$_3$)$_2$CH), 2.46 (s, 3 H, CH$_3$S), 2.93 (hept., 1 H, *J* = 6.9 Hz, (CH$_3$)$_2$C*H*), 3.77 (s, 2 H, C*H2*Ph), 7.15–7.28 (m, 5 H, Ph). – ^{13}C-NMR ([D$_6$]DMSO): δ (ppm) = 15.75 (CH$_3$S), 22.70 ((*C*H$_3$)$_2$CH), 24.58 (broad, (CH$_3$)$_2$*C*H), 31.32 (broad, *C*H$_2$Ph), 125.61, 128.08, 137.39 (C$_{arom}$), 140.75 (broad, C-2). – EI MS: *m/z* = 246 (M$^+$).

4-Cyclohexylmethyl-5-ethyl-2-methylsulfanyl-1H-imidazole (**4 c**)

Yield: 1 g (88 %) as a white solid; mp 140–142 °C. ^{1}H-NMR ([D$_6$]DMSO): δ (ppm = 0.81–0.92 (m, 2 H, H$_{cy}$), 1.05–1.21 (m, 6 H, C*H*$_3$CH$_2$ and H$_{cy}$), 1.43–1.64 (m, 6 H, H$_{cy}$), 2.27 (d, 2 H, *J* = 6.9 Hz, C*H*$_2$Cy), 2.39 (q, 2 H, CH$_3$C*H*$_2$), 2.47 (s, 3 H, CH$_3$S). – ^{13}C-NMR ([D$_6$]DMSO): δ (ppm) = 14.52 (*C*H$_3$CH$_2$), 15.88 (CH$_3$S), 18.17 (CH$_3$*C*H$_2$), 25.96, 25.64, 32.45, 37.86 (C$_{cy}$), 32.34 (broad, *C*H$_2$Cy), 129.77 (C-4), 134.28 (broad, C-2), 137.05 (C-5). – EI MS: *m/z* = 238 (M$^+$). – Anal. Calcd for C$_{13}$H$_{22}$N$_2$S × 0.2 H$_2$O (242.00): C, 64.52; H, 9.33; N, 11.58. Found: C, 64.60; H, 9.17; N, 11.60 %.

4-Cyclohexylmethyl-5-isopropyl-2-methylsulfanyl-1H-imidazole (**4 d**)

Yield 0.71 g (56 %) as a white solid; mp 172–174 °C. ^{1}H-NMR ([D$_6$]DMSO) (two tautomers): δ (ppm) = 0.82–0.93 (m, 4 H, H$_{cy}$), 1.09–1.21 (m, 18 H, 2 × (C*H*$_3$)$_2$CH and H$_{cy}$), 1.45–1.63 (m, 12 H, H$_{cy}$), 2.21 (d, 2 H, *J* = 6.6 Hz, C*H*$_2$Cy), 2.31 (d, 2 H, *J* = 6.9 Hz,

CH_2Cy), 2.44 (s, 6 H, 2 × CH_3S), 2.74 (hept., 1 H, J = 6.8 Hz, (CH_3)$_2$CH), 2.86 (hept., 1 H, J = 6.9 Hz, (CH_3)$_2$CH), 11.51 (s, 1 H, NH), 11.57 (s, 1 H, NH). – ^{13}C-NMR ([D$_6$]DMSO) (two tautomers): δ (ppm) = 15.83 (CH_3S), 22.71, 23.19 ((CH_3)$_2$CH), 24.01, 25.37 ((CH_3)$_2$CH), 25.62, 25.73, 25.93, 26.09, 32.44, 32.65, 37.92 (Ccy), 31.27, 34.24 (CH_2Cy), 124.34, 133.55 (C-4), 134.22, 143.20 (C-5), 136.54, 136.85 (C-2). – EI MS: *m/z* = 252 (M^+). – Anal. Calcd for $C_{14}H_{24}N_2S$ × 0.6 H_2O (263.23): C, 63.88; H, 9.64; N, 10.64. Found: C, 63.89; H, 9.24; N, 10.64 %.

General procedure for preparation of compounds **5 a–d** *and* **6 a–h**

Each of the compounds **4 a–d** (2 mmol) was dissolved in methylene chloride (20 mL) under nitrogen. *N*-Ethyldiisopropylamine (EDIA) (0.36 mL, 2 mmol) was added to the above solution followed by addition of ethoxymethyl chloride or benzyloxymethyl chloride (2 mmol). The reaction mixture was stirred for 3 h at room temperature and quenched with water (20 mL). Methylene chloride (30 mL) was added and the two layers were separated. The organic layer was dried over sodium sulfate (5 g), filtered and the solvent was removed under reduced pressure. The residual material was chromatographed on a column of silica gel with CH_2Cl_2:EtOAc (*v:v* = 6:1) to afford compounds **5 a–d** and **6 a–h**.

4-Benzyl-1-ethoxymethyl-5-ethyl-2-methylsulfanyl-1H-imidazole (**5 a**)

Yield 58 mg (10 %) as an oil. ^{1}H-NMR ($CDCl_3$): δ (ppm) = 1.04 (t, 3 H, J = 7.5 Hz, CH_3CH$_2$), 1.18 (t, 3 H, J = 7.0 Hz, CH_3CH$_2$O), 2.54 (s, 3 H, CH_3S), 2.57 (q, 2 H, J = 7.5 Hz, CH_3CH_2), 3.49 (q, 2 H, J = 7.0 Hz, CH_3CH_2O), 3.89 (s, 2 H, CH_2Ph), 5.26 (s, 2 H, NCH_2O), 7.13–7.25 (m, 5 H, Ph). – ^{13}C-NMR ($CDCl_3$): δ (ppm) = 14.43 (CH_3CH$_2$), 14.85 (CH_3CH$_2$), 16.91 (CH_3CH_2), 17.37 (CH_3S), 33.57 (CH_2Ph), 63.71 (CH_3CH_2O), 73.04 (NCH_2O), 125.73, 128.16, 128.45, 137.38 (C_{arom}), 131.46 (C-4), 137.38 (C-5), 140.66 (C-2). – HiResMALDI MS: *m/z* = 291.1510 (MH^+, $C_{16}H_{23}N_2OS$); requires 291.1531.

4-Benzyl-1-benzyloxymethyl-5-ethyl-2-methylsulfanyl-1H-imidazole (**5 b**)

Yield 115 mg (16 %) as an oil. ^{1}H-NMR ($CDCl_3$): δ (ppm) = 1.02 (t, 3 H, J = 7.6 Hz, CH_3CH$_2$), 2.50–2.60 (m, 5 H, CH_3S and CH_3CH_2), 3.89 (s, 2 H, CH_2Ph), 4.50 (s, 2 H, OCH_2Ph), 5.33 (s, 2 H, NCH_2O), 7.15–7.33 (m, 10 H, 2 Ph). – ^{13}C NMR ($CDCl_3$): δ (ppm = 14.43 (CH_3CH$_2$), 16.88 (CH_3CH_2), 17.34 (CH_3S), 33.56 (CH_2Ph), 70.24 (OCH_2Ph), 72.77 (NCH_2O), 125.73, 127.91, 128.16, 128.42, 128.43, 136.85, 137.50 (C_{arom}), 131.47 (C-4), 140.59 (C-5), 141.08 (C-2). – HiResMALDI MS: *m/z* = 353.1670 (MH^+, $C_{21}H_{25}N_2OS$); requires 353.1687.

4-Cyclohexylmethyl-1-ethoxymethyl-5-ethyl-2-methylsulfanyl-1H-imidazole (**5 c**)

Yield 53 mg (9 %) as an oil. ^{1}H-NMR ($CDCl_3$): δ (ppm) = 0.86–0.97 (m, 2 H, H_{cy}), 1.07–1.28 (m, 9 H, CH_3CH$_2$ and CH_3CH$_2$O and H_{cy}), 1.67–1.71 (m, 6 H, H_{cy}), 2.34 (d, 2 H, J = 6.6 Hz, CH_2Cy), 2.52 (s, 3 H, CH_3S), 2.58 (q, 2 H, CH_3CH_2), 3.48 (q, 2 H, J = 7.2 Hz, CH_3CH_2O), 5.28 (s, 2 H, NCH_2O). – ^{13}C-NMR ($CDCl_3$): δ (ppm) = 14.73 (CH_3CH$_2$), 14.84 (CH_3CH$_2$O), 16.77 (CH_3CH_2), 17.67 (CH_3S), 26.59, 26.25, 33.28, 38.03 (C_{cy}), 34.91 (CH_2Cy), 63.54 (CH_3CH_2O), 73.01 (NCH_2O), 131.16 (C-4), 137.85 (C-5), 140.37 (C-2). – HiResMALDI MS: *m/z* = 297.1987 (MH^+, $C_{16}H_{29}N_2OS$); requires 297.1995.

1-Benzyloxymethyl-4-cyclohexylmethyl-5-ethyl-2-methylsulfanyl-1H-imidazole (**5 d**)

Yield 150 mg (15 %) as an oil. ^{1}H-NMR ($CDCl_3$): δ (ppm) = 0.87–0.98 (m, 2 H, H_{cy}), 1.07–1.25 (m, 7 H, CH_3CH$_2$ and H_{cy}), 1.65–1.72 (m, 5 H, H_{cy}), 2.35 (d, 2 H, J = 7.0 Hz, CH_2Cy), 2.53 (s, 3 H, CH_3S), 2.59 (q, 2 H, J = 7.5 Hz, CH_3CH_2), 4.50 (s, 2 H, CH_2Ph), 5.35 (s, 2 H, NCH_2O), 7.25–7.37 (m, 5 H, Ph). – ^{13}C-NMR ($CDCl_3$): δ (ppm = 14.78 (CH_3CH$_2$), 16.78 (CH_3CH_2), 17.68 (CH_3S), 26.61, 26.28, 33.31, 38.06 (C_{cy}), 34.94 (CH_2Cy), 70.16 (OCH_2Ph), 72.83 (NCH_2O), 127.73, 127.89, 128.42, 136.99 (C_{arom}), 131.20 (C-4), 138.04 (C-5), 140.58 (C-2).- HiResMALDI MS: *m/z* = 359.2149 (MH^+, $C_{21}H_{31}N_2OS$); requires 359.2147.

5-Benzyl-1-ethoxymethyl-4-ethyl-2-methylsulfanyl-1H-imidazole (**6 a**)

Yield 120 mg (21 %) as an oil. ^{1}H-NMR ($CDCl_3$): δ (ppm) = 1.08 (t, 3 H, J = 7.0 Hz, CH_3CH$_2$O), 1.23 (t, 3 H, J = 7.5 Hz, CH_3CH$_2$), 2.54–2.61 (m, 5 H, CH_3S and CH_3CH_2), 3.36 (q, 2 H, J = 7.0 Hz, CH_3CH_2O), 3.99 (s, 2 H, CH_2Ph), 5.05 (s, 2 H, NCH_2O), 7.08–7.28 (m., 5 H, Ph). – ^{13}C-NMR ($CDCl_3$): δ (ppm) = 14.64 (CH_3CH$_2$), 14.69 (CH_3CH$_2$O), 17.09 (CH_3S), 20.43 (CH_3CH_2), 29.19 (CH_2Ph), 63.58 (CH_3CH_2O), 73.13 (NCH_2O), 126.14 (C-4), 126.34, 127.92, 128.49, 138.68 (C_{arom}), 141.64 (C-2), 142.27 (C-5). – HiResMALDI MS: *m/z* = 291.1515 (MH^+, $C_{16}H_{23}N_2OS$); requires 291.1531.

5-Benzyl-1-benzyloxymethyl-4-ethyl-2-methylsulfanyl-1H-imidazole (**6 b**)

Yield 210 mg (29 %) as an oil. ^{1}H-NMR ($CDCl_3$): δ (ppm) = 1.23 (t, 3 H, J = 7.5 Hz, CH_3CH$_2$), 2.54–2.61 (m, 5 H, CH_3S and CH_3CH_2), 3.98 (s, 2 H, CH_2Ph), 4.39 (s, 2 H, OCH_2Ph), 5.11 (s, 2 H, NCH_2O), 7.03–7.32 (m, 10 H, 2 Ph). – ^{13}C-NMR ($CDCl_3$): L (ppm) = 14.66 (CH_3CH$_2$), 17.06 (CH_3S), 20.47 (CH_3CH_2), 29.19 (CH_2Ph), 70.06 (OCH_2Ph), 72.85 (NCH_2O), 126.18 (C-4), 126.39, 127.64, 127.95, 128.38, 128.54, 136.94, 138.59 (C_{arom}), 141.86 (C-2), 142.36 (C-5). – HiResMALDI MS: *m/z* = 353.1666 (MH^+, $C_{21}H_{25}N_2OS$); requires 353.1687.

5-Cyclohexylmethyl-1-ethoxymethyl-4-ethyl-2-methylsulfanyl-1H-imidazole (**6 c**)

Yield 80 mg (14 %) as an oil. ^{1}H-NMR ($CDCl_3$): δ (ppm) = 0.86–0.98 (m, 2 H, H_{cy}), 1.11–1.23 (m, 9 H, CH_3CH$_2$, CH_3CH$_2$O and H_{cy}), 1.44–1.68 (m, 6 H, H_{cy}), 2.43–2.52 (m, 4 H, CH_2Cy and CH_3CH_2), 2.54 (s, 3 H, CH_3S), 3.47 (q, 2 H, J = 7.0 Hz, CH_3CH_2O), 5.25 (s, 2 H, NCH_2O). – ^{13}C-NMR ($CDCl_3$): δ (ppm) = 14.29 (CH_3CH$_2$), 14.83 (CH_3CH$_2$O), 17.35 (CH_3S), 20.52 (CH_3CH_2), 26.34, 26.24, 33.21, 38.43 (C_{cy}), 31.18 (CH_2Cy), 63.54 (CH_3CH_2O), 72.99 (NCH_2O), 127.17 (C-4), 140.75 (C-2), 141.52 (C-5). – HiResMALDI MS: *m/z* = 297.2002 (MH^+, $C_{16}H_{29}N_2OS$); requires 297.2006.

1-Benzyloxymethyl-5-cyclohexylmethyl-4-ethyl-2-methylsulfanyl-1H-imidazole (**6 d**)

Yield 140 mg (18 %) as an oil. ^{1}H-NMR ($CDCl_3$): δ (ppm) = 0.84–0.94 (m, 2 H, H_{cy}), 1.09–1.16 (m, 3 H, H_{cy}), 1.21 (t, 3 H, J = 7.5 Hz, CH_3CH$_2$), 1.39–1.65 (m, 6 H, H_{cy}), 2.42 (d, 2 H, J = 7.3 Hz, CH_2Cy), 2.49 (q, 2 H, J = 7.5 Hz, CH_3CH_2), 2.55 (s, 3 H, CH_3S), 4.50 (s, 2 H, OCH_2Ph), 5.31 (s, 2 H, NCH_2O), 7.37–7.26 (m, 5 H, Ph). – ^{13}C-NMR ($CDCl_3$): δ (ppm) = 14.31 (CH_3CH$_2$), 17.36 (CH_3S), 20.55 (CH_3CH_2), 26.32, 26.16, 33.21, 38.47 (C_{cy}), 31.18 (CH_2Cy), 70.16 (OCH_2Ph), 72.76 (NCH_2O), 127.22 (C-4), 127.74, 127.89, 128.40, 136.98 (C_{arom}), 140.93 (C-2), 141.65 (C-5). – HiResMALDI MS: *m/z* = 359.2157 (MH^+, $C_{21}H_{31}N_2OS$); requires 359.2157.

5-Benzyl-1-ethoxymethyl-4-isopropyl-2-methylsulfanyl-1H-imidazole (**6 e**)

Yield 250 mg (41 %) as an oil. ^{1}H-NMR ($CDCl_3$): δ (ppm) = 1.08 (t, 3 H, *J* = 7.0 C*H*$_3$CH$_2$), 1.26 (d, 6 H, *J* = 6.9 Hz, (C*H*$_3$)$_2$CH), 2.52 (s, 3 H, CH_3S), 2.93 (hept., 1 H, *J* = 6.9 Hz, (CH$_3$)$_2$C*H*), 3.37 (q, 2 H, *J* = 7.0 Hz, CH$_3$C*H*$_2$O), 4.02 (s, 2 H, C*H*$_2$Ph), 5.07 (s, 2 H, NCH_2O), 7.07–7.28 (m, 5 H, Ph). – ^{13}C-NMR ($CDCl_3$): δ (ppm) = 14.71 (*C*H$_3$CH$_2$), 17.77 (CH_3S), 22.84 ((*C*H$_3$)$_2$CH), 26.37 ((CH$_3$)$_2$*C*H), 29.10 (*C*H$_2$Ph), 63.56 (CH$_3$*C*H$_2$O), 73.14 (NCH_2O), 125.21 (C-4), 126.30, 127.88, 128.49, 138.81 (C_{arom}), 141.23 (C-2), 146.28 (C-5). – HiResMALDI MS: *m/z* = 305.1673 (MH^+, $C_{17}H_{25}N_2OS$); requires 305.1687.

5-Benzyl-1-benzyloxymethyl-4-isopropyl-2-methylsulfanyl-1H-imidazole (**6 f**)

Yield 400 mg (55 %) as an oil. ^{1}H NMR ($CDCl_3$): δ (ppm) = 1.27 (d, 2 H, *J* = 6.9 Hz, (C*H*$_3$)$_2$CH), 2.53 (s, 3 H, CH_3S), 2.93 (hept., 1 H, *J* = 6.9 Hz, (CH$_3$)$_2$C*H*), 3.99 (s, 1 H, C*H*$_2$Ph), 4.40 (s, 2 H, OC*H*$_2$Ph), 5.12 (s, 2 H, NCH_2O), 7.02–7.36 (m, 10 H, 2 Ph). – ^{13}C-NMR ($CDCl_3$): δ (ppm) = 17.72 (CH_3S), 22.85 ((*C*H$_3$)$_2$CH) 26.38 ((CH$_3$)$_2$*C*H), 29.08 (*C*H$_2$Ph), 70.12 (O*C*H$_2$Ph), 72.00 (NCH_2O), 125.25 (C-4), 126.34, 127.64, 127.85, 127.91, 128.39, 128.53, 136.99, 138.69 (C_{arom}), 141.45 (C-2), 146.35 (C-5). – HiResMALDI MS: *m/z* = 367.1829 (MH^+, $C_{22}H_{27}N_2OS$); requires 367.1844.

5-Cyclohexylmethyl-1-ethoxymethyl-4-isopropyl-2-methylsulfanyl-1H-imidazole (**6 g**)

Yield 500 mg (80 %) as an oil. ^{1}H-NMR ($CDCl_3$): δ (ppm) = 0.88–1.03 (m, 2 H, H_{cy}), 1.15–1.30 (m, 12 H, C*H*$_3$CH$_2$, (C*H*$_3$)$_2$CH and H_{cy}), 1.43–1.69 (m, 6 H, H_{cy}), 2.45 (d, 2 H, *J* = 7.4 Hz, C*H*$_2$Cy), 2.51 (s, 3 H, CH_3S), 2.83 (hept., 1 H, *J* = 6.9 Hz, (CH$_3$)$_2$C*H*), 3.48 (q, 2 H, *J* = 7.0 Hz, CH$_3$C*H*$_2$O), 5.27 (s, 2 H, NCH_2O). – ^{13}C-NMR ($CDCl_3$): δ (ppm = 14.88 (*C*H$_3$CH$_2$), 18.09 (CH_3S), 22.79 ((*C*H$_3$)$_2$CH), 26.17 ((CH$_3$)$_2$*C*H), 26.37, 26.31, 33.26, 38.43 (C_{cy}), 31.13 (*C*H$_2$Cy), 63.54 (CH$_3$*C*H$_2$O), 73.05 (NCH_2O), 126.30 (C-4), 140.44 (C-2), 145.63 (C-5). – HiResMALDI MS: *m/z* = 311.2155 (MH^+, $C_{17}H_{31}N_2OS$); requires 311.2157.

1-Benzyloxymethyl-5-cyclohexylmethyl-4-isopropyl-2-methylsulfanyl-1H-imidazole (**6 h**)

Yield 600 mg (82 %) as an oil. ^{1}H-NMR ($CDCl_3$): δ (ppm) = 0.83–0.95 (m, 2 H, H_{cy}), 1.09–1.28 (m, 9 H, (C*H*$_3$)$_2$CH and H_{cy}), 1.38–1.65 (m, 6 H, H_{cy}), 2.43 (d, 2 H, *J* = 7.4 Hz, C*H*$_2$Cy), 2.51 (s, 3 H, CH_3S), 2.82 (hept., 1 H, *J* = 6.9 Hz, (CH$_3$)$_2$C*H*), 4.51 (s, 2 H, OC*H*$_2$Ph), 5.33 (s, 2 H, NCH_2O), 7.25–7.36 (m, 5 H, Ph). – ^{13}C-NMR ($CDCl_3$): δ (ppm) = 18.06 (CH_3S), 22.77 ((*C*H$_3$)$_2$CH), 26.19 ((CH$_3$)$_2$*C*H), 26.33, 26.19, 33.21, 38.39 (C_{cy}), 31.08 (*C*H$_2$Cy), 70.18 (O*C*H$_2$Ph), 72.81 (NCH_2O), 126.31 (C-2), 145.71 (C-5). – HiResMALDI MS: *m/z* = 373.2314 (MH^+, $C_{22}H_{33}N_2OS$); requires 373.2308.

Viruses and cells

The HIV-1 strains HTLV-IIIB [20] was propagated in H9 cells [21] at 37 °C, 5 % CO_2 using RPMI 1640 with 10 % heat-inactivated fetal calf serum (FCS) and antibiotics (growth medium). Culture supernatant was filtered (0.45 nm), aliquotted, and stored at –80 °C until use. The HIV-1 strain was obtained from NIH AIDS Research and Reference Program.

Inhibition of HIV-1 replication

Compounds were examined for possible antiviral activity against HIV-1 using MT4 cells as target cells. MT4 cells were incubated with virus (0.005 MOI) and growth medium containing the test dilutions of compound for six days in parallel with virus-infected and uninfected control cultures without compound added. Expression of HIV in the cultures was indirectly quantified using the MTT assay [22]. Compounds mediating less than 30 % reduction of HIV expression were considered without biological activity. Compounds were tested in parallel for cytotoxic effect in uninfected MT4 cultures containing the test dilutions of compound as described above. A 30 % inhibition of cell growth relative to control cultures was considered significant. The 50 % inhibitory concentration (IC_{50}) and the 50 % cytotoxic concentration (CC_{50}) were determined by interpolation from the plots of percent inhibition versus concentration of compound.

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