3-Benzamido, Ureido and Thioureidoimidazo[1,2-*a*]pyridine Derivatives as Potential Antiviral Agents

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Received May 9, 2001; accepted August 10, 2001

This work reports the synthesis and the antiviral activities of 3-benzamido, 3-phenylureido and 3-phenylthioureido derivatives in the imidazo[1,2-*a*]pyridine series. The structure was proven by NMR spectroscopy. The synthesized compounds were evaluated against a large number of viruses. The 3-phenylthioureido derivative 7 showed moderate activity against human cytomegalovirus (HCMV) *in vitro*. The crystallographic data for 8 are also reported and explain the absence of activity against human immunodeficiency virus (HIV).

Key words imidazo[1,2-*a*]pyridine; cytomegalovirus; phenylthioureido; crystallographic data

The first antiretroviral agents approved for the treatment of AIDS were nucleosidic inhibitors of reverse transcriptase such as Zidovudine. Later, the human immunodeficiency virus (HIV) protease inhibitors (*e.g.* saquinavir) were introduced as therapeutics. Recently, a third class of antiretroviral agents, the non-nucleosidic reverse transcriptase inhibitors (NNRTIs) (*i.e.* nevirapine, delavirdine, efavirenz) have been marketed. Since the discovery of the anti-HIV activity of 1-[(2-hydroxyethoxy)methyl]-6-(phenylthio)thymidine (HEPT)[1] and tetrahydroimidazo[4,5,1-*jk*][1,4]benzodiazepin-2(1*H*)-one and thione (TIBO)[2] as the first NNRTIs, numerous additional compounds have been reported as NNRTIs.[3]

From all the active compounds, some common characteristics have emerged. First, NNRTIs interact with a non-substrate binding site that is located in the close vicinity of the substrate binding site of HIV-1. As a consequence, all these compounds are deprived of activity against HIV-2.

Furthermore, structural studies have shown that these derivatives contain a central hydrophilic part and two hydrophobic moieties, generally an aromatic cycle forming a butterfly-like conformation.[4] Finally, most of these compounds contain an amide, thioamide, urea or thiourea function in their structure.[5] In continuation of our studies on the antiviral activities of bridgehead nitrogen heterocycles,[6] we were interested in the synthesis of amide, urea and thiourea derivatives within the imidazo[1,2-*a*]pyridine series. In a first approach, we studied the introduction of these functions in the 3-position.

Chemistry

The 2-aminopyridine **1a** was reacted with bromoacetone or bromopinacolone in refluxing ethanol to give the imidazo[1,2-*a*]pyridines **2a**, **b** (Chart 1). It is now well established that in this series, electrophilic substitution occurs in the 3-position.[7] Thus a cooled solution (−5 °C) of **2a**, **b** in concentrated sulfuric acid was treated with nitric acid, the temperature not rising above 5 °C. Then the reaction mixture was allowed to stand at 20 °C to give the 3-nitro compounds **3a**, **b** in good yields. Reduction of the nitro compounds using tin in hydrochloric acid at room temperature gave the amino

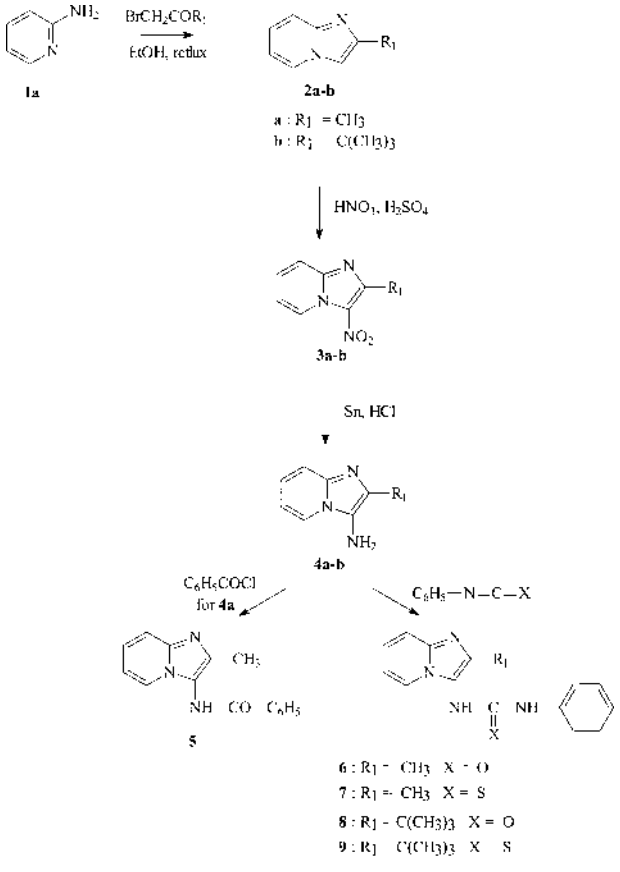

Chart 1

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derivatives **4a**, **b**. First, the amide **5** was obtained by reaction of the amine **4a** with benzoyl chloride using usual methods. Then the ureas and thioureas **6**—**9** were obtained by nucleophilic addition of **4a**, **b** on phenylisocyanate or isothiocyanate at room temperature in acetonitrile following the conditions reported by Cantrell *et al.*[8] The ureas and thioureas were obtained in 65 to 75% yields. Proof of the structures was obtained from ^{1}H- and ^{13}C-NMR spectra. Surprisingly, in the case of the thiourea derivative **9**, the spectrum in deuteriodimethylsulfoxide (DMSO-d_6) showed the presence of two species at a ratio of 34—66%. This result was interpreted as indicating the existence under the experimental conditions of two configurations of the thiourea due to the restricted rotation around the C–N bond as reported by Tompa *et al.*[9] From the dissymmetry of the thiourea, the (*Z*) and (*E*)-conformations could concern either the NH linked to the phenyl ring or the imidazo[1,2-*a*]pyridinic moiety. Using ^{1}H–^{1}H correlation spectroscopy (COSY) and X–H correlation spectroscopy (XHCOR) all the resonances could be assigned. In order to determine the two species present in the solution, nuclear Overhauser effect (NOE) studies were made. Unfortunately, neither differential nuclear Overhauser enhancement (NOEDIFF) nor two-dimensional (2D) nuclear Overhauser enhancement spectroscopy (NOESY) with mixing times of 250 and 400 ms led to spatial interaction fulfilling our goal.

With a view to studying the interaction of these compounds with reverse transcriptase, we were then interested in the crystallographic data for one of the synthesized compounds, and a crystal structure was obtained for compound **8**.

Crystal Structure The thermal ellipsoid representation and the labelling of non-hydrogen atoms[10] for both conformations are presented in Fig. 1.

The imidazo[1,2-*a*]pyridine ring ∅1 (atoms N1 to C8A), urea ∅2 (atoms N9, C10, O1 and N11), and phenyl ring ∅3 (atoms C1″ to C6″) are planar.

There are numerous weak van der Waals interactions so the crystal cohesion is assumed through several hydrogen bonds (Fig. 2). The molecule I is involved in hydrogen bonding between the NH groups of I and O atom of a neighbouring molecule II.

Antiviral Activities The activities of the synthesized compounds were evaluated against a large number of viruses. None of the synthesized compounds showed any activity against HIV [HIV-1 (III_B) or HIV-2 (strain ROD)] in MT-4 cells. A partial explanation for the inactivity could be found in the crystal data that showed no butterfly-like conformation for the described compounds. The compounds were also devoid of activity against herpes simplex virus [HSV-1 (strain KOS), thymidine kinase deficient (TK^-) HSV-1 resistant to aciclovir (ACV^R), HSV-2 (G)], vaccinia virus (VV), vesicular stomatitis virus (VSV) in embryonic skin-muscle (E_6SM) cell cultures, VSV, Coxsackie virus B4, respiratory syncytial virus in Hela cell cultures, and parainfluenza-3 virus, Sindbis virus, Coxsackie virus B4 and Punta Toro virus in Vero cell cultures. However, some of the compounds showed moderate activity against human cytomegalovirus (HCMV) (Table 1).

Structure Activity Relationship From the activity data against HCMV it appeared that amide is less active than urea and thiourea. A bulky group in position 2 decreased the antiviral activity. Finally, thioureas were more potent than

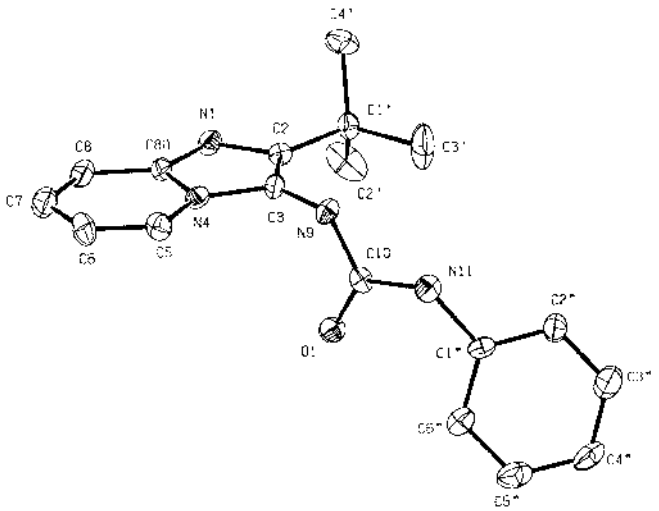

Fig. 1. Ellipsoïd Representation of the Non-hydrogen Atoms (Probability 20%) of One Molecule and Atomic Labelling of **8** (X-Ray Numbering)

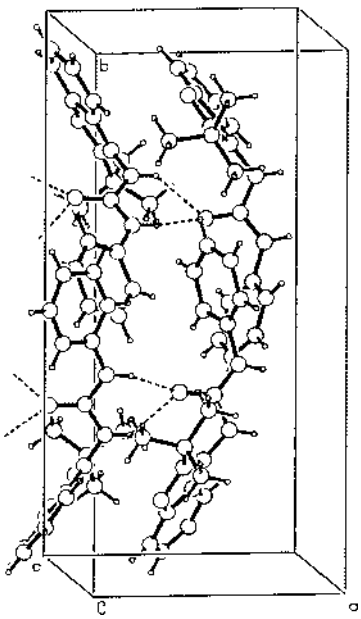

Fig. 2. Packing Representation of **8**

Table 1. Anti-HCMV Activities and Cytotoxic Properties in Human Embryonic Lung (HEL) Cells

Compound	Antiviral activity IC_{50} (μg/ml)[a]		Cytotoxicity (μg/ml)	
	AD 169 strain	Davis strain	Cell morphology (MCC)[b]	Cell growth (CC_{50})[c]
5 (U3)	>50	>50	>50	>50
6 (U1)	50	33	>50	>50
7 (U2)	13	10	>50	>50
8 (U5)	>50	32	>50	>50
9 (U6)	>50	27	>50	>50

a) Inhibitory concentration required to reduce virus-induced cytopathicity by 50%. Virus input was 100 plaque forming units[2] (PFU). *b*) Minimum cytotoxic concentration causing a microscopically detectable alteration of normal cell morphology. *c*) Cytotoxic concentration required to reduce cell growth by 50%.

ureas. These findings appeared compatible with our previously reported results, which indicated that the 3-aralkylthiomethyl derivatives are more potent than the corresponding ethers.[11] Interestingly, the compounds described herein exhibited very weak if any, cytotoxicity, as exemplified by a selectivity index higher than 5 for compound **7**. Further developments in these series of compounds are under progress in order to enhance their potency against HCMV.

Experimental

Melting points were determined on a Köfler hotstage apparatus and are uncorrected. NMR spectra were recorded on a Bruker DPX 200 or a Bruker

Table 1a. Atomic Coordinates and Equivalent U_{eq} Factors for Molecule I

	x/*a*	*y*/*b*	*z*/*c*	U_{eq}
O1	0.4833 (6)	0.3212 (2)	0.7154 (5)	0.0510 (14)
N1	0.4458 (7)	0.1089 (3)	0.8853 (6)	0.0486 (16)
C2	0.5380 (8)	0.1676 (4)	0.9032 (7)	0.0400 (18)
C3	0.5930 (8)	0.1898 (4)	0.7904 (8)	0.044 (2)
N4	0.5318 (7)	0.1441 (3)	0.6949 (6)	0.0408 (16)
C5	0.5515 (9)	0.1409 (4)	0.5641 (8)	0.055 (2)
C6	0.4773 (10)	0.0895 (5)	0.4967 (8)	0.066 (3)
C7	0.3822 (10)	0.0396 (4)	0.5583 (9)	0.066 (3)
C8	0.3638 (10)	0.0427 (4)	0.6877 (9)	0.061 (2)
C8A	0.4442 (8)	0.0961 (4)	0.7598 (7)	0.0414 (19)
N9	0.6884 (6)	0.2473 (3)	0.7533 (5)	0.0421 (16)
C10	0.6243 (8)	0.3134 (4)	0.7376 (6)	0.0378 (19)
N11	0.7230 (7)	0.3684 (3)	0.7493 (6)	0.0511 (18)
C1′	0.5694 (8)	0.1942 (4)	1.0401 (7)	0.048 (2)
C2′	0.4135 (10)	0.2086 (6)	1.1001 (9)	0.098 (4)
C3′	0.6650 (15)	0.2619 (6)	1.0450 (9)	0.109 (4)
C4′	0.6501 (12)	0.1354 (5)	1.1150 (9)	0.095 (4)
C1″	0.6809 (8)	0.4417 (4)	0.7643 (7)	0.0433 (19)
C2″	0.7521 (11)	0.4795 (5)	0.8612 (8)	0.066 (2)
C3″	0.7144 (13)	0.5498 (5)	0.8784 (10)	0.081 (3)
C4″	0.6080 (12)	0.5825 (5)	0.8031 (11)	0.075 (3)
C5″	0.5369 (11)	0.5441 (5)	0.7063 (11)	0.080 (3)
C6″	0.5705 (11)	0.4736 (5)	0.6881 (9)	0.070 (3)

Table 1b. Atomic Coordinates and Equivalent U_{eq} Factors for Molecule II

	x/*a*	*y*/*b*	*z*/*c*	U_{eq}
O1	0.9879 (6)	0.2901 (3)	0.8454 (5)	0.0529 (15)
N1	0.9411 (7)	0.1253 (3)	0.5499 (6)	0.0476 (17)
C2	1.0350 (8)	0.1831 (4)	0.5580 (7)	0.044 (2)
C3	1.0968 (8)	0.1910 (4)	0.6796 (7)	0.0413 (19)
N4	1.0394 (7)	0.1355 (3)	0.7532 (6)	0.0422 (16)
C5	1.0650 (9)	0.1165 (5)	0.8790 (7)	0.054 (2)
C6	0.9905 (10)	0.0577 (5)	0.9230 (9)	0.065 (3)
C7	0.8890 (11)	0.0187 (5)	0.8424 (10)	0.070 (3)
C8	0.8657 (9)	0.0364 (4)	0.7176 (9)	0.060 (2)
C8A	0.9411 (9)	0.0980 (4)	0.6698 (7)	0.046 (2)
N9	1.1901 (7)	0.2427 (3)	0.7420 (5)	0.0435 (16)
C10	1.1259 (9)	0.2929 (4)	0.8197 (7)	0.0346 (18)
N11	1.2206 (7)	0.3434 (3)	0.8662 (6)	0.0490 (17)
C1′	1.0581 (10)	0.2292 (4)	0.4407 (8)	0.053 (2)
C2′	1.0666 (16)	0.1809 (5)	0.3208 (9)	0.113 (4)
C3′	1.2071 (12)	0.2717 (6)	0.4453 (10)	0.100 (4)
C4′	0.9232 (15)	0.2765 (7)	0.4233 (12)	0.145 (6)
C1″	1.1791 (8)	0.3985 (4)	0.9508 (7)	0.046 (2)
C2″	1.2371 (10)	0.4662 (4)	0.9310 (8)	0.062 (2)
C3″	1.1968 (12)	0.5206 (5)	1.0131 (11)	0.079 (3)
C4″	1.0952 (13)	0.5095 (6)	1.1089 (11)	0.086 (3)
C5″	1.0472 (12)	0.4425 (6)	1.1312 (10)	0.084 (3)
C6″	1.0827 (10)	0.3874 (5)	1.0513 (8)	0.061 (2)

Table 2. Bond Lengths (Å) and Standard Deviations in Brackets

	Molecule I	Molecule II
O1–C10	1.246 (8)	1.234 (8)
N1–C8A	1.323 (9)	1.344 (9)
N1–C2	1.370 (9)	1.357 (9)
C2–C3	1.341 (10)	1.365 (10)
C2–C1′	1.523 (10)	1.510 (10)
C3–N4	1.402 (9)	1.390 (8)
C3–N9	1.419 (9)	1.410 (9)
N4–C5	1.373 (9)	1.365 (9)
N4–C8A	1.367 (8)	1.389 (9)
C5–C6	1.345 (10)	1.363 (10)
C6–C7	1.411 (11)	1.404 (12)
C7–C8	1.356 (10)	1.346 (10)
C8–C8A	1.421 (10)	1.423 (11)
N9–C10	1.366 (9)	1.368 (8)
C10–N11	1.344 (8)	1.334 (9)
N11–C1″	1.433 (9)	1.409 (9)
C1′–C4′	1.510 (12)	1.476 (12)
C1′–C3′	1.517 (12)	1.518 (11)
C1′–C2′	1.527 (10)	1.541 (12)
C1″–C2″	1.364 (11)	1.368 (10)
C1″–C6″	1.362 (11)	1.384 (11)
C2″–C3″	1.371 (11)	1.381 (12)
C3″–C4″	1.341 (13)	1.361 (14)
C4″–C5″	1.368 (14)	1.346 (14)
C5″–C6″	1.368 (12)	1.365 (12)

Table 3. Bond Angles (°) and Standard Deviations in Brackets

	Molecule I	Molecule II
C8A–N1–C2	105.6 (6)	105.2 (6)
N1–C2–C3	110.6 (6)	111.3 (6)
C3–C2–C1′	130.7 (7)	128.7 (7)
N1–C2–C1′	118.6 (6)	120.0 (7)
C2–C3–N4	106.8 (6)	106.8 (6)
C2–C3–N9	134.1 (7)	134.8 (7)
N4–C3–N9	119.0 (6)	118.2 (7)
C5–N4–C8A	122.8 (7)	122.9 (6)
C5–N4–C3	132.1 (6)	132.0 (7)
C8A–N4–C3	105.1 (6)	105.1 (6)
C6–C5–N4	118.4 (8)	117.7 (8)
C5–C6–C7	121.1 (8)	121.2 (8)
C8–C7–C6	120.3 (8)	121.2 (8)
C7–C8–C8A	118.8 (8)	118.5 (8)
N1–C8A–N4	111.9 (6)	111.5 (6)
N1–C8A–C8	129.6 (7)	130.0 (8)
N4–C8A–C8	118.5 (7)	118.3 (7)
C10–N9–C3	119.0 (6)	120.5 (6)
O1–C10–N11	123.0 (7)	122.9 (7)
O1–C10–N9	121.5 (6)	120.6 (6)
N11–C10–N9	115.5 (6)	116.5 (6)
C10–N11–C1″	125.7 (6)	125.5 (6)
C4′–C1′–C3′	110.5 (8)	108.7 (7)
C4′–C1′–C2	108.0 (7)	111.0 (8)
C3′–C1′–C2	112.9 (6)	114.0 (7)
C4′–C1′–C2′	108.8 (8)	108.1 (9)
C3′–C1′–C2′	109.1 (8)	106.0 (9)
C2–C1′–C2′	107.5 (6)	108.9 (7)
C2″–C1″–C6″	119.6 (7)	118.9 (7)
C2″–C1″–N11	117.9 (7)	118.8 (7)
C6″–C1″–N11	122.4 (7)	122.3 (7)
C1″–C2″–C3″	119.5 (9)	119.3 (9)
C4″–C3″–C2″	121.7 (9)	121.0 (9)
C3″–C4″–C5″	118.4 (9)	118.5 (9)
C6″–C5″–C4″	121.1 (10)	121.8 (10)
C5″–C6″–C1″	119.6 (9)	120.0 (9)

Table 4. Significative Torsion Angles (°) with Standard Deviations in Brackets

	Molecule I	Molecule II
N1–C2–C1′–C2′	−56.2 (10)°	−37.7 (10)
N1–C2–C1′–C4″	−61.0 (9)°	78.8 (10)
N4–C3–N9–C10	96.8 (8)°	−74.3 (8)
C3–N9–C10–N11	156.7 (6)	−174.5 (6)
N9–C10–N11–C1″	−166.0 (6)	−177.9 (6)
C10–N11–C1″–C2″	130.6 (8)	−139.3 (7)

Table 5. Table of Dihedral Angles (°) with Standard Deviations in Brackets

	Molecule I	Molecule II
∅2/∅1	70.9 (2)°	55.9 (2)°
∅3/∅1	87.6 (2)°	74.0 (2)°
∅3/∅2	42.1 (3)°	41.5 (3)°

Table 6. Table of Hydrogen Bond

D–H ··· A	*d* (D ··· A) (Å)	D–H–A (°)
N9–H9-I ··· O1-II	2.856	128.45
N11–H11-I ··· O1-II	2.880	139.26
N9–H9-II ··· O1-I*	2.957	139.10
N11–H11-II ··· O1-I*	2.827	153.58

AM 400 WB spectrometer. The *J* values are expressed in hertz. Elemental microanalyses were performed by the microanalytical center, Ecole Normale Supérieure de Montpellier (ENSCM), Montpellier. 2-Methylimidazo[1,2-*a*]pyridine **2a** was obtained according to a described procedure.[12)]

2-*tert*-Butylimidazo[1,2-*a*]pyridine (2b) A solution of 2-aminopyridine (2.6 g, 2.8 mmol) and bromopinacolone (5 g, 2.6 mmol) was refluxed in ethanol (50 ml) for 5 h. After cooling, the solution was concentrated *in vacuo*, poured into water and made alkaline with sodium carbonate. The aqueous layer was extracted with dichloromethane. The combined and dried organic phases were evaporated to dryness and the residue chromatographed on neutral alumina eluting with dichloromethane to give colorless crystals (77%); mp 80 °C; ^{1}H-NMR ($CDCl_3$) δ: 1.43 (9H, s, $3CH_3$), 6.72 (1H, td, $J_{6,7}=J_{5,6}=6.8$, $J_{6,8}=1.2$, H-6), 7.12 (1H, ddd, $J_{7,8}=9.1$, $J_{5,7}=1.2$, H-7), 7.36 (1H, s, H-3), 7.59 (1H, dd, H-8), 8.06 (1H, dt, $J_{5,8}=1.2$, H-5).

General Procedure for Nitration The imidazo[1,2-*a*]pyridine (0.04 mol) was slowly added to concentrated sulfuric acid (60 ml) cooled to −5 °C without the temperature rising above 5 °C. To the solution was added nitric acid (6 ml, $d=1.41$), also without allowing the temperature to rise above 5 °C. At the end of the addition the mixture was allowed to stand until it reached room temperature and then stirred for a further 2 h. The mixture was poured onto ice (400 g) and the formed precipitate was collected and dissolved in dichloromethane. The organic solution was dried on calcium chloride and chromatographed on neutral alumina eluted with dichloromethane.

2-Methyl-3-nitroimidazo[1,2-*a*]pyridine (**3a**): 60% yield; mp 142 °C [lit.[13)] mp 142—144 °C]; ^{1}H-NMR ($CDCl_3$, 200 MHz) δ: 2.82 (3H, s, CH_3), 7.23 (1H, td, $J_{6,7}=J_{5,6}=6.9$, $J_{6,8}=1.4$, H-6), 7.61 (1H, ddd, $J_{7,8}=9.0$, $J_{5,7}=1.4$, H-7), 7.73 (1H, dt, $J_{5,8}=1.4$, H-8), 9.40 (1H, dt, H-5).

2-*tert*-Butyl-3-nitroimidazo[1,2-*a*]pyridine (**3b**): 70% yield; mp 173 °C; ^{1}H-NMR ($CDCl_3$, 200 MHz) δ: 1.56 (9H, s, $3CH_3$), 7.27 (1H, t, $J_{6,7}=J_{5,6}=6.9$, H-6), 7.65 (1H, dd, $J_{7,8}=8.8$, H-7), 7.86 (1H, br d, H-8), 9.51 (1H, br d, H-5). *Anal.* Calcd for $C_{11}H_{13}N_3O_2$: C, 60.27; H, 5.94; N, 19.18. Found: C, 60.12; H, 6.07; N, 19.13.

General Procedure for Reduction To concentrated hydrochloric acid (60 ml) cooled to −15 °C was added tin (4 g, 33.6 mmol), then the nitro derivative **3a**, **b** (22.5 mmol) was added portionwise without the temperature rising above 0 °C. The reaction mixture was allowed to stand at room temperature and stirred for a further 2 h. The suspension was diluted with ice cooled water (20 ml), made alkaline with concentrated ammonia and extracted with dichloromethane. After the usual workup, the dried residue was chromatographed on neutral alumina eluted with dichloromethane/methanol (98/2 v/v).

3-Amino-2-methylimidazo[1,2-*a*]pyridine (**4a**): 85% yield; mp 134 °C [lit.[14)] mp 134 °C]; ^{1}H-NMR ($CDCl_3$, 200 MHz) δ: 2.38 (3H, s, CH_3), 3.04 (2H, br s, NH_2), 6.76 (1H, td, $J_{5,6}=J_{6,7}=6.8$, $J_{6,8}=1.2$, H-6), 7.01 (1H, ddd, $J_{7,8}=9$, $J_{5,7}=1.4$, H-7), 7.38 (1H, dd, $J_{7,8}=9$, H-8), 7.92 (1H, dd, H-5).

3-Amino-2-*tert*-butylimidazo[1,2-*a*]pyridine (**4b**): Oil (66%); ^{1}H-NMR ($CDCl_3$, 200 MHz) δ: 1.46 (9H, s, $3CH_3$) 3.10 (2H, br s, NH_2), 6.69 (1H, td, $J_{5,6}=J_{6,7}=6.8$, $J_{6,8}=1.2$, H-6), 6.99 (1H, ddd, $J_{7,8}=9$, $J_{5,7}=1.2$, H-7), 7.43 (1H, dt, $J_{5,8}=1.2$, H-8), 7.94 (1H, dt, H-5). *Anal.* Calcd for $C_{11}H_{15}N_3$: C, 69.84; H, 7.94; N, 22.22. Found: C, 69.72; H, 7.83; N, 22.13.

***N*-(2-Methylimidazo[1,2-*a*]pyridin-3-yl)benzamide (5)** To a solution of **4a** (200 mg, 1.36 mmol) in dichloromethane (113 ml) was added benzoyl chloride (0.158 ml, 191 mg, 1.36 mmol). The mixture was stirred for 30 min at room temperature, then evaporated to dryness. The residue was dissolved in water, basified with sodium carbonate, then extracted with dichloromethane. The organic layers were dried over calcium chloride and evaporated *in vacuo*. The residue was chromatographed on neutral alumina eluted with dichloromethane to give the amide **5** (302 mg, 89%); mp 211—214 °C; ^{1}H-NMR ($CDCl_3$, 200 MHz) δ: 2.19 (3H, s, CH_3), 6.77 (1H, td, $J_{5,6}=J_{6,7}=6.8$, $J_{6,8}=0.8$, H-6), 7.14 (1H, ddd, $J_{7,8}=9$, $J_{5,7}=1.2$, H-7), 7.42 (1H, br d, H-8), 7.60 (3H, m, H-3′, 4′, 5′), 7.78 (1H, br d, H-5), 8.15 (2H, d, $J=7.1$, H-2′, 6′), 9.16 (1H, br s, NH); ^{13}C-NMR ($CDCl_3$, 50 MHz) δ: 12.3 (CH_3), 111.8 (C-6), 115.5 (C-3), 116.2 (C-8), 122.8 (C-5), 124.3 (C-7), 128.0 (C-3′, 5′), 128.7 (C-2′, 6′), 132.4 (C-1′), 132.8 (C-4′), 137.4 (C-2), 142.3 (C-8a), 167.2 (C=O). *Anal.* Calcd for $C_{15}H_{13}N_3O$: C, 71.71; H, 5.18; N, 16.73. Found: C, 71.82; H, 5.23; N, 16.63.

3-(2-Methylimidazo[1,2-*a*]pyridin-3-yl)-1-phenylurea (6) A solution of amine **4a** (250 mg, 1.7 mmol) in acetonitrile (3.5 ml) was treated with phenylisocyanate (0.185 ml, 1.7 mmol) and the mixture was stirred for 2 h. The precipitate which was formed was filtered off and washed with ether to give **6** (331 mg, 73%); mp 217—219 °C; ^{1}H-NMR (DMSO-d_6, 200 MHz) δ: 2.28 (3H, s, CH_3), 6.90 (1H, ps.t, $J_{5,6}=J_{6,7}=6.7$, H-6), 7.00 (1H, m, H-7), 7.26 (3H, m, H-3′, 4′, 5′), 7.49 (3H, m, H-2′, 6′, 8), 8.05 (1H, br d, H-5), 8.21 (1H, br s, NH), 9.11 (1H, br s, NH); ^{13}C-NMR (DMSO-d_6, 50 MHz) δ: 13.2 (CH_3), 111.8 (C-6), 116.6 (C-8), 116.7 (C-3), 118.9 (C-2′, 6′), 122.4 (C-5), 123.6 (C-7), 124.1 (C-4′), 129.1 (C-3′, 5′), 137.7 (C-1′), 140.1 (C-2), 141.7 (C-8a), 154.1 (C=O). *Anal.* Calcd for $C_{15}H_{14}N_4O$: C, 67.67; H, 5.26; N, 21.05. Found: C, 67.58; H, 5.30; N, 21.13.

3-(2-Methylimidazo[1,2-*a*]pyridin-3-yl)-1-phenylthiourea (7) This compound was obtained according to the above procedure using phenyl isothiocyanate (0.2 ml, 1.7 mmol) in 67% yield; mp 240—243 °C; ^{1}H-NMR (DMSO-d_6, 200 MHz) δ: 2.29 (3H, s, CH_3), 6.93 (1H, ps.t., $J_{5,6}=J_{6,7}=6.6$, H-6), 7.22 (2H, m, H-7, 4′), 7.38 (2H, t, $J=7.4$, H-3′, 5′), 7.38 (1H, br d, $J_{7,8}=9$, H-8), 7.54 (2H, m, H-2′, 6′), 8.03 (1H, br d, H-5), 9.46 (1H, br s, NH), 10.23 (1H, br s, NH); ^{13}C-NMR (DMSO-d_6, 50 MHz) δ: 13.4 (CH_3), 111.8 (C-6), 116.6 (C-8, 2′, 6′, 3), 123.6 (C-5), 124.3 (C-7), 125.4 (C-4′), 128.9 (C-3′, 5′), 138.3 (C-1′), 139.5 (C-2), 141.9 (C-8a), 182.1 (C=S). *Anal.* Calcd for $C_{15}H_{14}N_4S$: C, 63.83; H, 4.96; N, 19.86. Found: C, 63.72; H, 5.04; N, 19.77.

1-Phenyl-3-(2-*tert*-butylimidazo[1,2-*a*]pyridin-3-yl)urea (8) The urea **8** was obtained from **4b** according to the procedure of **6** in 75% yield; mp >260 °C; ^{1}H-NMR (DMSO-d_6, 200 MHz) δ: 1.41 (9H, s, $3CH_3$), 6.90 (1H, ps.t, $J_{5,6}=J_{6,7}=6.7$, H-6), 6.99 (1H, t, $J=7.4$, H-4′), 7.24 (1H, ddd, $J_{7,8}=9.1$, $J_{5,7}=1.2$, H-7), 7.30 (2H, dd, $J=7.7$, H-3′, 5′), 7.51 (2H, d, $J=7.7$, H-2′, 6′), 7.52 (1H, d, H-8), 8.04 (1H, d, H-5), 8.08 (1H, br s, NH), 9.06 (1H, br s, NH); ^{13}C-NMR (DMSO-d_6, 50 MHz) δ: 30.8 (CH_3), 33.5 (C), 112.3 (C-6), 114.9 (C-3), 117.3 (C-8), 119.2 (C-2′, 6′), 122.8 (C-4′), 123.8 (C-5), 124.8 (C-7), 129.6 (C-3′, 5′), 140.6 (C-1′), 141.3 (C-8a), 149.3 (C-2), 154.7 (C=O). *Anal.* Calcd for $C_{18}H_{20}N_4O$: C, 70.13; H, 6.49; N, 18.18. Found: C, 70.22; H, 6.56; N, 18.03.

1-Phenyl-3-(2-*tert*-butylimidazo[1,2-*a*]pyridin-3-yl)thiourea (9) Compound **9** was obtained from **4b** according to the procedure of **6** in 69% yield; mp >260 °C; ^{1}H-NMR (DMSO-d_6, 200 MHz) δ: 1.39 (s, $3CH_3$ maj), 1.41 (s, $3CH_3$ min), 6.89 (t, $J_{5,6}=J_{6,7}=6.7$, H-6 maj), 6.96 (t, $J_{5,6}=J_{6,7}=6.6$, H-6 min), 7.17 (t, $J=7.5$, H-4′ maj), 7.19—7.31 (m, H-2′, 6′ min, H-3′, 5′ min, H-4′ min, H-7 min+maj), 7.38 (dd, $J=7.8$, H-3′, 5′ maj), 7.50 (d, $J_{7,8}=9$, H-8 maj), 7.54 (d, $J_{7,8}=9$, H-8 min), 7.64 (d, H-2′, 6′ maj), 7.95 (d, H-5 maj), 7.96 (d, H-5 min), 9.24 (br s, NH maj), 9.34 (br s, NH min), 9.73 (br s, NH min), 10.39 (br s, NH maj); ^{13}C-NMR (DMSO-d_6, 50 MHz) δ: 30.6 ($3CH_3$ min+maj), 33.6 (C min+maj), 112.1 (C-6 maj), 113.1 (C-6 min), 113.4 (C-3 min), 116.6 (C-3 maj), 117.1 (C-8 maj), 117.6 (C-8 min), 123.3 (C-5 min), 124.2 (C-5 maj), 124.4 (C-2′, 6′ maj), 125.1 (C-7 maj), 125.6 (C-7 min),

125.8 (C-4′ maj), 126.2 (C-4′ min), 127.3 (C-2′, 6′ min), 128.7 (C-3′, 5′ min), 129.6 (C-3′, 5′ maj), 139.9 (C-1′ maj), 140.4 (C-1′ min), 141.3 (C-8a maj), 142.2 (C-8a min), 149.0 (C-2 maj), 150.1 (C-2 min), 182.7 (C=S maj), 183.1 (C=S min). *Anal.* Calcd for $C_{18}H_{20}N_4S$: C, 66.67; H, 6.17; N, 17.28. Found: C, 66.52; H, 6.12; N, 17.34.

X-Ray Diffraction of Compound 8 Colourless crystals of **8** were grown by slow evaporation of ethanol–chloroform solution at 293 °K. The crystal used for X-ray measurement was lamellar, with dimensions: 0.05×0.28×0.38 mm. The studied compound, $C_{18}H_{20}N_4O$, Mx=308.4 $g \cdot mol^{-1}$ crystallized in the monoclinic system, space group P_c (Z=4) two independent molecules per asymmetric unit. The unit cell parameters were obtained by a least-squares fit of the setting angles of 25 reflections with θ between 16 and 33° and were as follows: a=8.665(5), b=18.765(12), c=10.370(6) Å and β=91.55(5)° with a cell volume of 1686 Å^3. The calculated density was 1.215 $g \cdot cm^{-3}$. The linear absorption coefficient was μ=0.623 mm^{-1} for the Cu$K\alpha$ radiation (λ=1.54178 Å).

The diffracted intensities were collected with a CAD-4 Enraf–Nonius diffractometer equipped with a graphite monochromator for θ_{max}=50°: 0≤h≤8, 0≤k≤18, −10≤l≤10 and an $\omega-2\theta$ scan. Three standard reflections were used to monitor the data collected and to detect any decrease of intensity (−3 7 1, 3 3 −3, 3 7 −1); the transmission factor $T(hkl)$ lay between 0.884 and 1.0; nevertheless, the crystal absorption correction was performed using the Ψ scan technique.[15] There were 1697 independent reflections, 1359 of which were considered as observed [$I>2\sigma(I)$, R_{int}=0.023].

The crystal structure was solved by application of direct methods and refined by full-matrix least squares on F^2 using the SHELX97 program.[16] Scattering factors were taken from the International Tables for Crystallography.[17] The hydrogen atoms were introduced in their theoretical positions and allowed to ride with the atoms to which they are attached. The final reliability factors were: R=0.046, wR=0.105 and the goodness of fit was equal to s=1.05. The weight was equal to: $w=1/[\sigma^2(F_o^2)+(0.0752P)^2]$, where $P=(F_o^2+2F_c^2)/3$. The minimum and maximun residual densities were equal to −0.157 and 0.160 e · Å^{-3}, respectively.

Inhibition of HIV-1-Induced Cytopathicity in MT-4 and CEM Cells The methodology of the anti-HIV assays has been described previously.[18] Briefly, human MT-4 (*ca.* 4×10^5 cell ml^{-1}) cells were infected with 100 $CCID_{50}$ (the amount of virus that is infective to 50% of a series of identical cell cultures) of HIV-1 (III_B)/ml or HIV-2(ROD)/ml and seeded in 200-μl wells of a microtiter plate containing appropriate dilutions of the test compounds. After 5 d of incubation at 37 °C, the number of viable (MT-4) cells was determined by an automated MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide] dye staining of living cells.

Antiviral Assays The antiviral assays, other than HIV-1, were based on inhibition of virus-induced cytopathicity in either E_6SM cells (HSV-1, HSV-2, VV, VSV), Human Embryonic Lung (HEL) cells [Varicella Zoster Virus (VZV), HCMV], Hela cells (respiratory syncytial virus) or Vero cells (Coxsackie B4 virus, parainfluenza-3 virus, Sindbis virus, Punta Toro virus, reovirus-1), following previously established procedures.[19] Briefly, confluent cell cultures in microtiter 96-well plates were inoculated with 100 $CCID_{50}$ of virus, 1 $CCID_{50}$ being the virus dose required to infect 50% of the cell cultures. After a 1-h virus adsorption period, residual virus was removed, and the cell cultures were incubated in the presence of varying concentrations (400, 200, 100, … μg/ml) of the test compounds. Viral cytopathicity was recorded as soon as it reached completion in the control virus-infected cell cultures that had not been treated with the test compounds.

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