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MEDICINAL CHEMISTRY RESEARCH

ORIGINAL RESEARCH

Synthesis and antiviral activity of new 4-(phenylamino)/4-[(methylpyridin-2-yl)amino]-1-phenyl-1*H*-pyrazolo[3,4-*b*]pyridine-4-carboxylic acids derivatives

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Abstract The synthesis of new 4-(phenylamino)-1-phenyl-1*H*-pyrazolo[3,4-*b*]pyridine-4-carboxylic acid **(3a-l)** derivatives and the new 4-[(methylpyridin-2-yl)amino]-1-phenyl-1*H*-pyrazolo[3,4-*b*]pyridine-4-carboxylic acid **(5a–c)** derivatives was achieved with an efficient synthetic route. Ethyl 4-chloro-1-phenyl-1*H*-pyrazolo[3,4-*b*]pyridine-5-carboxylate **(1)** on fusion with appropriate substituted anilines or aminopicolines gave the required new ethyl 4-(phenylamino)-1-phenyl-1*H*-pyrazolo[3,4-*b*]pyridine-5-carboxylates **(2a–l)** (52–82%) or new ethyl 4-[(methylpyridin-2-yl)amino]-1-phenyl-1*H*-pyrazolo[3,4-*b*]pyridine-5-carboxylates **(4a–c)** (50–60%), respectively. Subsequent hydrolysis of the esters afforded the corresponding carboxylic acids **(3a–l)** (86–93%) and **(5a–c)** in high yield (80–93%). Inhibitory effects of 4-(phenylamino)/4-[(methylpyridin-2-yl)amino]-1-phenyl-1*H*-pyrazolo[3,4-*b*]pyridine-4-carboxylic acids. Derivatives on Herpes simplex virus type 1 (HSV-1), Mayaro virus (MAY) and vesicular stomatitis virus (VSV) were investigated. Compounds **2d**, **3f**, **3a**, and **3c** exhibited antiviral activity against

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HSV-1, MAY, and VSV virus with EC_{50} values of 6.8, 2.2, 4.8, 0.52, 2.5, and 1.0. None of these compounds showed toxicity for Vero cells.

Keywords 1*H*-pyrazolo[3,4-*b*]pyridine · antiviral · HSV-1 · MAY · VSV

Introduction

Among the various important parent ring systems of pyrazolopyridine, substituted 1*H*-pyrazolo[3,4-*b*]pyridine is known to have several biological activities such as anxyolitic, anticonvulsant, anti-inflammatory, analgesic, hypoglycemic, antipyretic, vasodilators, and antileishmanial (Hohn *et al*., 1972; Lynch *et al*., 1988; Bernardino *et al*., 1996a; Ahluwalia and Goyal, 1996; Quiroga *et al*., 1998; Bernardino *et al*., 1999; Mello *et al*., 2004). Recently our research group described the synthesis and antiviral activity of derivatives of the 1*H*-pyrazolo[3,4-*b*]pyridine and thieno[2,3-*b*]pyridine systems with promising results (Bernardino *et al*., 1996b; Azevedo *et al*., 2002a; Pinheiro *et al*., 2004; Bernardino *et al*., 2007).

In a attempt to explore the antiviral potential of 1*H*-pyrazolo[3,4-*b*]pyridine derivatives, we have studied their effects of these compounds on Herpes simplex virus type 1 (HSV-1), Mayaro virus (MAY), and vesicular stomatitis virus (VSV). Herpes simplex type 1 (HSV-1) infects mucocutaneous epithelial cells and establishes latency in sensory ganglions (Whitley and Roizman, 2001). HSV-1 causes gingivostomatitis, cold sores, keratoconjunctivitis, and encephalitis. Although any viral protein essential for viral replication is a potential target, nearly all currently available drugs for herpes viruses are primarily inhibitors of viral DNA polymerase (Eizuru, 2003). Among the anti-herpes-virus agents, acyclovir, valaciclovir, penciclovir, famciclovir, idoxuridine, and trifluridine (applied topically) as well as brivudin are used in the treatment of herpes simplex virus (De Clercq, 2005).

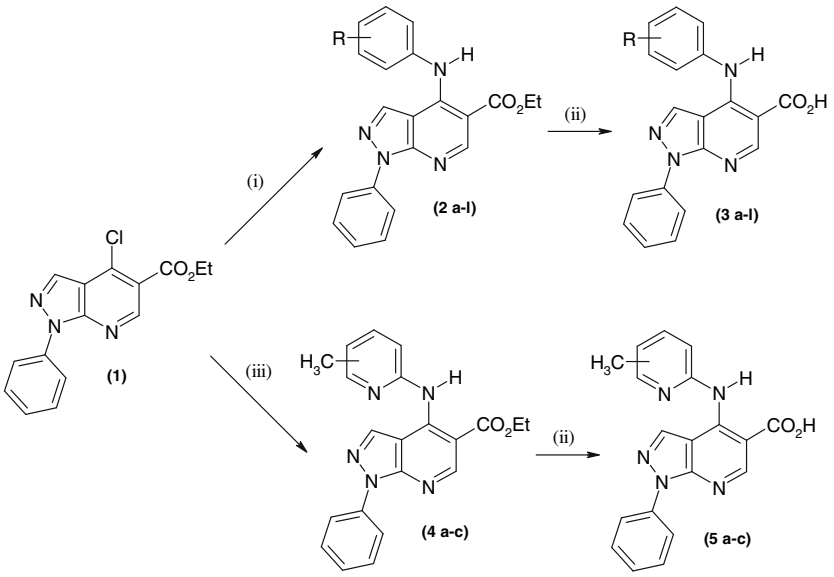
Mayaro virus is a member of the Alphavirus genus, Togaviridae family and is antigenically closely related to Semiliki Forest virus (Casals and Whitman, 1957). Mayaro virus is an arbovirus, isolated for the first time in Trinidad, in 1954 and has since been reported as the cause of several epidemic outbreaks in Brazil, Bolivia, and other regions, mainly at colonial borders in the Amazon region (Causey and Maroja, 1957). Clinical manifestations of human infection have been described as feverish illness accompanied by headache, chills, nausea, photophobia, myalgia, and arthralgia. In some cases there is true arthritis that persists for months (Strauss and Strauss, 1994). Vesicular stomatitis virus (VSV) is a single-strand RNA virus of the Rhabdoviridae family. VSV has been isolated from a variety of animals and causes nonfatal disease of significant economic importance in cattle and swine (Rose and Whitt, 2007).

In order to identify the 1*H*-pyrazolo[3,4-*b*]pyridine system as promising compounds for the development of new antiviral agents, we synthesized a new set of derivatives 4-(phenylamino)/4-[(methylpyridin-2-yl)amino]-1-phenyl-1*H*-pyrazolo[3,4-*b*]pyridines **(2–5)**. The aim of this study was to assess their cytotoxicity and the potential antiviral activity.

Results and discussion

Chemistry

The synthesis of new 4-(phenylamino)-1-phenyl-1*H*-pyrazolo[3,4-*b*]pyridine-4-carboxylic acid **(3a–l)** derivatives and the new 4-[(methylpyridin-2-yl)amino]-1-phenyl-1*H*-pyrazolo[3,4-*b*]pyridine-4-carboxylic acid **(5a–c)** derivatives was achieved with an efficient synthetic route outlined in Scheme 1. A halogen in the C-4 position and an ester group in the C-5 position of 1*H*-pyrazolo[3,4-*b*]pyridine react readily with nucleophilic compounds to form a good precursor for the synthesis of these derivatives. Ethyl 4-chloro-1-phenyl-1*H*-pyrazolo[3,4-*b*]pyridine-5-carboxylate **(1)** was available in our laboratory and could be easily prepared from 5-aminopyrazoles through condensation with diethyl ethoxymethylenemalonate followed by chlorocyclization with phosphorus oxychloride (Hohn *et al*., 1971; Azevedo *et al*., 2002a; Azevedo *et al*., 2002b). Ethyl 4-chloro-1-phenyl-1*H*-pyrazolo[3,4-*b*]pyridine-5-carboxylate **(1)** on fusion with appropriate substituted anilines or aminopicolines gave the required new ethyl 4-(phenylamino)-1-phenyl-1*H*-pyrazolo[3,4-*b*]pyridine-5-carboxylates **(2a–l)** (52–82%) or new ethyl 4-[(methylpyridin-2-yl)amino]-1-phenyl-1*H*-pyrazolo[3,4-*b*]pyridine-5-carboxylates **(4a–c)** (50–60%), respectively. Subsequent hydrolysis of the ester afforded the corresponding 4-(phenylamino)-1-phenyl-1*H*-pyrazolo[3,4-*b*]pyridine-4-carboxylic acids **(3a–l)** in high yields (86–93%) and 4-[(methylpyridin-2-yl)amino]-1-phenyl-1*H*-pyrazolo[3,4-*b*]pyridine-4-carboxylic acids **(5a–c)** in high yields (80–93%) (Azevedo *et al*., 2002b).



(i) suitable aniline, DMF, 4h, reflux; (ii) NaOH 20%, ethanol, reflux, 1-3 h; (iii) suitable aminopicoline, DMF, 4h, reflux.

Scheme 1 **2a, 3a** R = H, **2b, 3b** R = *p*-CH_3, **2c, 3c** R = *m*-Cl, **2d, 3d** R = *m*-NO_2, **2e, 3e** R = *p*-NO_2, **2f, 3f** R = *m*-Br, **2g, 3g** R = *p*-Br, **2h, 3h** R = *m*-CH_3, **2i, 3i** R = *p*-Cl, **2f, 3f** R = *m*-OCH_3, **2g, 3g** R = *p*-OCH_3

The structures of esters and acids were determined by infrared (IR), ^{1}H and ^{13}C nuclear magnetic resonance (NMR), and mass spectroscopy. In the IR spectra, the carbonyl group absorptions were observed in the acids and esters derivatives at 1649–1678 cm^{-1} and 1672–1696 cm^{-1}, respectively. In the 3435–3125 cm^{-1} region, NH bands were observed. The OH bands for the acid compounds were observed in the 3491–2500 cm^{-1} region. ^{1}H NMR spectra indicated a chemical shift of the NH in the range of δ = 10.98–9.46 ppm as singlet signals. The aromatic protons signals in aniline and aminopicoline moieties appeared as a multiplet in *orto* derivatives or a double doublet in *para* derivatives. The quartet and triplet signals of the esters derivatives **(2a–l** and **4 a-c)** appeared in the ranges δ = 4.62–4.42 ppm and 1.58–1.50 ppm, respectively. The singlet signals of the acids **(3a–l** and **5a–c)** appeared in the range δ = 13.00–11.36 ppm.

Biological evaluation

Initially, the anti-herpetic effect of all compounds investigate was evaluated in a primary screening using the 50% end-point titration method described by Reed and Muench (1938).

As shown in Table 1, compounds **2a**, **2d**, **2h**, **2i**, **2j**, **4b,** and **4c** exhibited the highest anti-HSV-1 activity, with the esters being, in general, more effective inhibitors than the corresponding acids. In conclusion, the introduction of ester group at C-5 in the 1*H*-pyrazolo[3,4-*b*]pyridine system increased activity against HSV-1 virus.

To calculate the selective or therapeutic index of each compound for antiviral activity we infected Vero cells with HSV-1, Mayaro, and VSV to determine the EC_{50} value by plaque-reducing assay. The cytotoxicity of the compounds was determined in parallel by the MTT 3-(4,5-dimethylthiazol-2-yl)-2-5-diphenyltetrazolium bromide colorimetric method (Mossmann, 1983) (Table 2). As shown in the Table 3, the effects of the compounds **3a** and **3c**, selected on the basis of preliminary screening (data not shown), exhibit the best EC_{50} values against VSV and Mayaro virus replication cycle.

Conclusion

In summary, 15 new derivatives of the 1*H*-pyrazolo[3,4-*b*]pyridine system **2a–l**, **3a–l**, **4a–c,** and **5a–c** with different substituents were synthesized and exhibited a range of significant anti-herpes, anti-Mayaro, and anti-VSV activities, suggesting that these compounds are potential antivirus agents. Compounds **2d**, **3f**, **3a**, and **3c** exhibited antiviral activity against HSV-1, MAY, and VSV virus, with EC50 values of 6.8, 2.2, 4.8, 0.52, 2.5, and 1.0. None of the compounds showed toxicity for Vero cells. The mechanism of antiviral activity is unknown and requires further study, which is in progress in our laboratory.

Table 1 Anti-HSV-1 virus activity of 1*H*-pyrazolo[3,4-*b*]pyridine derivatives **2a–l**, **3a–l**, **4a–c,** and **5a–c**

Compounds	R	HSV-1 virus yield inhibition (%)
2a	H	90.0 ± 3.3
2b	*p*-CH_3	69.2 ± 2.2
2c	*m*-Cl	60.0 ± 5.2
2d	*m*-NO_2	90.0 ± 1.8
2e	*p*-NO_2	0
2f	*m*-Br	70.0 ± 2.7
2g	*p*-Br	75.0 ± 3.4
2h	*m*-CH_3	90.0 ± 5.7
2i	*p*-Cl	92.0 ± 5.5
2j	*m*-OCH_3	98.0 ± 2.3
2k	*p*-OCH_3	75.5 ± 5.5
2l	*m*-F	0
3a	H	0
3b	*p*-CH_3	58.4 ± 4.2
3c	*m*-Cl	68.0 ± 7.4
3d	*m*-NO_2	20.6 ± 3.6
3e	*p*-NO_2	80.0 ± 6.3
3f	*m*-Br	84.0 ± 2.4
3g	*p*-Br	37.0 ± 5.8
3h	*m*-CH_3	38.5 ± 4.3
3i	*p*-Cl	68.0 ± 6.0
3j	*m*-OCH_3	75.5 ± 5.5
3k	*p*-OCH_3	80.6 ± 3.4
3l	*m*-F	68.0 ± 3.7
4a	4-CH_3	60.0 ± 2.0
4b	5-CH_3	90.0 ± 3.2
4c	6-CH_3	90.0 ± 3.7
5a	4-CH_3	75.0 ± 4.0
5b	5-CH_3	60.0 ± 1.5
5c	6-CH_3	21.0 ± 2.1
ACV	–	96.0 ± 1.0

The experimental concentration of 1*H*-pyrazolo[3,4-*b*]pyridine derivatives **2a–l**, **3a–l**, **4a–c**, and **5a–c** was 50 μM and that for ACV was 10 μM

Results are presented as the mean of triplicate experiments

ACV: acyclovir has been included for comparison purposes

Experimental section

^{1}H and ^{13}C NMR spectra were obtained at 300 MHz and 75 MHz, respectively, using a Varian Unity Plus instrument with tetramethylsilane as an internal standard. The chemical shifts (δ) are reported in ppm and the coupling constants (J) in Hertz. Fourier-transform infrared (FT-IR) spectra were recorded in a Perkin–Elmer Spectrum One instrument. The solid samples were determined in potassium bromide (KBr) pellets. Melting points (m.p.) were determined with a Fisher–Johns apparatus. Thin-layer chromatography (TLC) was carried out using silica gel F-254 glass

Table 2 Anti-HSV-1 virus activity, cytotoxicity, and selectivity index in Vero cells for 1*H*-pyrazolo[3,4-*b*]pyridine derivatives

Compounds	**R**	EC_{50}^{a}(μM)	CC_{50}^{b}(μM)	S.I.[c]
2d	*m*-NO_2	6.8 ± 0.1	1000 ± 100	141.4
3f	*m*-Br	2.2 ± 0.05	600 ± 50	272.4
ACV		1.09 ± 0.25	960 ± 156	880

ACV: acyclovir has been included for comparison purposes

[a] 50% effective concentration or concentration required to inhibit HSV-1 virus yield

[b] 50% cytotoxic concentration or concentration required to reduce the viability of host cells by 50%

[c] Selective index (CC_{50}/EC50)

Table 3 Anti-Mayaro and VSV virus activity, cytotoxicity, and selectivity index in Vero cells for 1*H*-pyrazolo[3,4-*b*]pyridine derivatives

Compounds	**R**	EC_{50}^{a}(μM)		
		May	VSV	
3a	*m*-H	4.80 ± 0.05	2.5 ± 0.05	
3c	*m-Cl*	0.52 ± 0.01	1.00 ± 0.07	
Compounds	**R**	CC_{50}^{b}(μM)	S.I.[c]	
			May	VSV
3a	*m*-H	135 ± 0.9	28	54
3c	*m-Cl*	37.4 ± 0.5	71	37
ACV	–	960 ± 156	880	

ACV: acyclovir has been included for comparison purposes

[a] 50% effective concentration or concentration required to inhibit HSV-1 virus yield

[b] 50% cytotoxic concentration or concentration required to reduce the viability of host cells by 50%

[c] Selective index (CC_{50}/EC50)

plate (20 × 20 cm). All reagents and solvents used were analytical grade. The electron-ionization mass spectrometry (EI-MS) spectra were recorded using a Finingan MAT 711 A instrument. The ionization energy was 70 eV with a source temperature of 200°C and an accelerative voltage of 8 kV. Samples were introduced by using a standard direct-insertion probe. High-resolution data were obtained with the instrument using 10000 resolution).

Ethyl 4-chloro-1-phenyl-1*H*-pyrazolo[3,4-*b*]pyridine-5-carboxylate **(1)**

The procedure developed for the synthesis of **1** started from the previously prepared 5-amino-1-phenyl-1*H*-pyrazole. The condensation of aminopyrazole with diethyl ethoxymethylenemalonate, based on the Gould–Jacobs method, produced ethyl α-carboetoxi-β-(N-5-pyrazolylamino)acrylate, which was recrystallized from

anhydrous ethanol. The resulting acrylate was mixed with phosphorus oxychloride and refluxed for 5 hours. The excess solvent was removed under reduced pressure and the resulting material was poured onto crushed ice and the product collected by filtration. The solid material was recrystallized from ethanol to yield ethyl 4-chloro-1-phenyl-1*H*-pyrazolo[3,4-*b*]pyridine-5-carboxylate **(1)** (Azevedo *et al*., 2002b).

General procedure for the preparation of the ethyl 4-(arylamino)-1-phenyl-1*H*-pyrazolo[3,4-*b*]pyridine-5-carboxylates **(2a–l)**

An equimolar mixture of **1** (1,2 g, 4 mmol) and a slight excess of the appropriate aniline (5 mmol), in 10 ml N,N-dimethylformamide (DMF) was heated in a silicone oil bath under reflux for 4 hours. The reaction mixture, after cooling, was poured into 50 ml of ice-cold water. The precipitate was filtered, dried, and recrystallized from a mixture of ethanol and water. The compounds were isolated in good yields (52–82%). The structures of the compounds were elucidated by IR, ^{1}H and ^{13}C NMR, and mass spectrometry.

2a. *Ethyl 4-(phenylamino)-1-phenyl-1H-pyrazolo[3,4-b]pyridine-5-carboxylate*

Yield: 1.08g (76%), m.p.: 197°C; IR (KBr, cm^{-1}): (*v* NH 3420; *v* C = O 1678); ^{1}H NMR (DMSO-d$_6$, *J* in Hz, δ in ppm) 6,81(s, H-3); 9,04(s, H-6); 8,08(d, 8.7, H-2′,6′), 7,51-7,26(10H, m), 4,42(q, 7.1, CH_2), 1,42(t, 7.1, CH_3), 10,61(s, N-H);^{13}C NMR (DMSO-d$_6$, δ in ppm) 135.7(C-3), 99.7(C-3a), 137.4(C-4), 105.1(C-5), 149.5(C-6), 150.3(C-7a), 131.9(C-1′), 121.5(C-2′, 6′), 128.3(C-3′, 5′), 126.8(C-4′), 158.1(C-1″), 120.4(C-2″), 128.5(C-3″), 140.1(C-4″), 128.5(C-5″), 120.4(C-6″), 164.9($\underline{C}O_2$Et), 60.5(CH_2), 13.7(CH_3);

2b. *Ethyl 4-(4′-methylphenylamino)-1-phenyl-1H-pyrazolo[3,4-b]pyridine-5-carboxylate*

Yield: 0.93 g (63%), m.p.: 155°C; IR (KBr, cm^{-1}): (*v* NH 3435; *v* C = O 1678); ^{1}H NMR (DMSO-d$_6$, *J* in Hz, δ in ppm) 6.73(s, H-3), 9.04(s, H-6), 8.23(d, 8.7, H-2′,6′), 7.67(dd, 7.5, H-3′,5′), 7.54–7.46(5H, m), 4.53(q, 7.2, CH_2), 1.53(t, 7.2, CH_3), 10.52(s, N-H), 3.38(s, Ar-CH_3); ^{13}C NMR (DMSO-d$_6$, δ in ppm) 135.5(C-3), 101.7(C-3a), 139.6(C-4), 105.4(C-5), 153.6(C-6), 151.7(C-7a), 137.3(C-1′), 122.4(C-2′, 6′), 131.3(C-3′, 5′), 130.0(C-4′), 158.6(C-1″), 122.4(C-2″), 128.1(C-3″), 138.6(C-4″), 128.1(C-5″), 122.4(C-6″), 168.6($\underline{C}O_2$Et), 21.7(Ar-$\underline{C}H_3$), 61.6(CH_2), 15.1(CH_3).

2c. *Ethyl 4-(3′-chlorophenylamino)-1-phenyl-1H-pyrazolo[3,4-b]pyridine-5-carboxylate*

Yield: 1.06 g (68%), m.p.: 182°C; IR (KBr, cm^{-1}): (*v* NH 3208; *v* C = O 1681); ^{1}H NMR (DMSO-d$_6$, *J* in Hz, δ in ppm) 6.92(s, H-3), 9.03(s, H-6), 8.20(d, 8.7, H-2′,6′),

7.68–7.56(5H, m), 7.47(dd, 7.5, H-4′), 7.73(dd, 8.7, H-5″), 4.50(q, 7.2, CH_2), 1.47(t, 7.2, CH_3),10.57(s, N-H); ^{13}C NMR (DMSO-d_6, δ in ppm) 133.9(C-3), 101.(C-3a), 138.3(C-4), 104.4(C-5), 152.2(C-6), 152.0(C-7a), 133.5(C-1′), 121.1(C-2′, 6′), 130.4(3′, 5′), 128.6(C-4′), 158.8(C-1″), 124.8(C-2″), 149.1(C-3″), 140.4(C-4″), 127.0(C-5″), 126.1(C-6″), 167.0($\underline{C}O_2$Et), 60.3(CH_2), 13.7(CH_3); EI (70 eV) m/z (%): $M^{+\cdot}$ 392.10435 (95), 346.06091 (100), 311.09595 (25), 283.09918 (15), 173.03200 (7), 110.99878 (4), 77.03763 (10)

2d. *Ethyl 4-(3′-nitrophenylamino)-1-phenyl-1H-pyrazolo[3,4-b]pyridine-5-carboxylate*

Yield: 0.96 g (60%), m.p.: 173°C; IR (KBr, cm^{-1}): (ν NH 3207; ν C = O 1681); ^{1}H NMR (DMSO-d_6, J in Hz, δ in ppm) 7.25(s, H-3), 9.13(s, H-6), 8.30(d, 8.7, H-2′,6′), 7.74(dd, 7.5, H-3′,5′), 7.55(dd, 7.5, H-4′), 8.50(s, H-2″), 8.44(d, 8.1, H-4″), 7.97(dd, 8.7, H-5″), 8.10(d, 8.7, H-6″), 4.55(q, 7.2, CH_2), 1.55(t, 7.2, CH_3), 10.75(s, N-H); ^{13}C NMR (DMSO-d_6, δ in ppm) 134.8(C-3), 102.7(C-3a), 138.8(C-4), 105.2(C-5), 153.0(C-6), 152.6(C-7a), 133.2(C-1′), 122.1(C-2′, 6′), 129.4(C-3′, 5′), 126.9(C-4′), 158.9(C-1″), 120.9(C-2″), 148.9(C-3″), 140.9(C-4″), 121.9(C-5″), 120.9(C-6″), 167.6($\underline{C}O_2$Et), 61.2(CH_2), 14.5(CH_3); EI (70 eV) m/z (%): $M^{+\cdot}$ 403.12863 (100), 357.09045 (70), 311.09466 (30), 283.10117 (30), 256.08533 (7), 77.03626 (11).

2e. *Ethyl 4-(4′-nitrophenylamino)-1-phenyl-1H-pyrazolo[3,4-b]pyridine-5-carboxylate*

Yield: 1.11 g (69%), m.p.: 176°C; IR (KBr, cm-1): (ν NH 3208; ν C = O 1681); ^{1}H NMR (DMSO-d6, J in Hz, δ in ppm) 7.18(s, H-6), 9.09(s, H-6), 8.25(d, 8.7, H-2′,6′), 7.68(dd, 7.5, H-3′,5′), 7.50(dd, 7.5, H-4′), 7.74(d, 8.7, H-2″), 8.43(d, 8.7, H-3″), 8.43(d, 8.7, H-5″), 7.74(d, 8.7, H-6″), 4.45(q, 7.2, CH_2), 1.46(t, 7.2, CH_3), 10.67(s, N-H); ^{13}C NMR (DMSO-d_6, δ in ppm) 134.4(C-3), 104.1(C-3a), 138.3(C-4), 105.6(C-5), 152.1(C-6), 152.0(C-7a), 133.6(C-1′), 121.1(C-2′, 6′), 128.8(C-3′, 5′), 126.3(C-4′), 158.7(C-1″), 123.3(C-2″), 124.9(C-3″), 144.0(C-4″), 124.9(C-5″), 124.9(C-6″), 166.7($\underline{C}O_2$Et), 60.7(CH_2), 13.8(CH_3); EI (70 eV) m/z (%): $M^{+\cdot}$ 403.12461 (100), 357.07820 (45), 311.08910 (80), 283.08977 (30), 256.08447 (7), 77.03500 (11).

2f. *Ethyl 4-(3′-bromophenylamino)-1-phenyl-1H-pyrazolo[3,4-b]pyridine-5-carboxylate*

Yield: 1.31 g (75%), m.p.: 180°C; IR (KBr, cm^{-1}): (ν NH 3224; ν C = O 1679); ^{1}H NMR (DMSO-d_6, J in Hz, δ in ppm) 6.88(s, H-3), 8.99(s, H-6), 8.17(d, 8.7, H-2′,6′), 7.64–7.57(3H, m), 7.43(dd, 7.5, H-4′), 7.82(s, H-2″), 7.75(dd, 7.5, H-5″), 7.75(d, 7.5, H-6″), 4.44(q, 7.5, CH_2), 1.44(t, 7.5, CH_3),10.53(s, N-H); ^{13}C NMR (DMSO-d_6, δ in ppm) 134.7(C-3), 102.0(C-3a), 138.9(C-4), 104.9(C-5), 153.0(C-6), 153.2(C-7a), 133.0(C-1′), 121.8(C-2′, 6′), 129.1(C-3′, 5′), 126.8(C-4′), 158.0(C-1‴),

120.0(C-2″), 150.1(C-3″), 139.0(C-4″), 121.0(C-5″), 120.0(C-6″), 167.8(CO_2Et), 61.0(CH_2), 14.4(CH_3); EI (70 eV) m/z (%): $M^{+\cdot}$ 436.05399 (85), 392.00998 (55), 358.13943 (10), 311.09562 (100), 283.09590 (30), 256.08710 (8), 222.57337 (15), 155.54822 (18), 95.07887 (23).

2g. *Ethyl 4-(4′-bromophenylamino)-1-phenyl-1H-pyrazolo[3,4-b]pyridine-5-carboxylate*

Yield: 1.31 g (75%), m.p.: 187°C; IR (KBr, cm^{-1}): (*v* NH 3219; *v* C = O 1683); ^{1}H NMR (DMSO-d_6, *J* in Hz, *δ* in ppm) 6.88(s, H-3), 8.99(s, H-6), 8.15(d, 8.7, H-2′,6′), 7.60–7.50(7H, m), 4.45(q, 7.5, CH_2), 1.40(t, 7.5, CH_3), 10.50(s, N-H); ^{13}C NMR (DMSO-d_6, *δ* in ppm) 134.1(C-3), 101.4(C-3a), 138.4(C-4), 104.5(C-5), 153.3(C-6), 152.1(C-7a), 132.6(C-1′), 121.4(C-2′, 6′), 129.0(C-3′, 5′), 128.8(C-4′), 158.2(C-1″), 120.1(C-2″), 126.4(C-3″), 138.5(C-4″), 126.4(C-5″), 120.1(C-6″), 167.4(CO_2Et), 60.5(CH_2), 13.5(CH_3); EI (70 eV) m/z (%): $M^{+\cdot}$ 436.05358 (65), 390.01168 (15), 311.09221 (100), 283.09354 (20), 256.08226 (4), 155.54525 (12), 77.17351(10).

2h. *Ethyl 4-(3′-methylphenylamino)-1-phenyl-1H-pyrazolo[3,4-b]pyridine-5-carboxylate*

Yield: 0.99 g (67%), m.p.: 187°C; IR (KBr, cm^{-1}): (*v* NH 3210; *v* C = O 1680); ^{1}H NMR (DMSO-d_6, *J* in Hz, *δ* in ppm) 6.75(s, H-3), 9.03(s, H-6), 8.21(d, 8.7, H-2′,6′), 7.68–7.41(7H, m), 4.51(q, 7.2, CH_2), 1.51(t, 7.2, CH_3), 10.53(s, N-H), 3.37(s, Ar-CH_3); ^{13}C NMR (DMSO-d_6, *δ* in ppm) 135.5(C-3), 101.1(C-3a), 138.4(C-4), 105.1(C-5), 153.0(C-6), 152.9(C-7a), 133.0(C-1′), 122.0(C-2′, 6′), 128.7(C-3′, 5′), 126.1(C-4′), 158.1(C-1″), 120.5(C-2″), 129.0(C-3″), 138.5(C-4″), 124.0(C-5″), 120.5(C-6″), 166.9(CO_2Et), 1.7(Ar-CH_3), 61.6(CH_2), 15.1(CH_3).

2i. *Ethyl 4-(4′-chlorophenylamino)-1-phenyl-1H-pyrazolo[3,4-b]pyridine-5-carboxylate*

Yield: 1.00 g (64%), m.p.: 159°C; IR (KBr, cm^{-1}): (*v* NH 3218; *v* C = O 1681); ^{1}H NMR (DMSO-d_6, *J* in Hz, *δ* in ppm) 6.94(s, H-3), 9.05(s, H-6), 8.08(d, 8.7, H-2′,6′), 7.51–7.25(7H, m), 4.42(q, 7.1, CH_2), 1.42(t, 7.1, CH_3), 10.51(s, N-H); ^{13}C NMR (DMSO-d_6, *δ* in ppm) 134.4(C-3), 104.1(C-3a), 138.3(C-4), 105.6(C-5), 152.3(C-6), 152.6(C-7a), 133.0(C-1′), 121.2(C-2′, 6′), 128.8(3′, 5′), 126.3(C-4′), 158.4(C-1″), 123.3(C-2″), 126.6(C-3″), 145.7(C-4″), 126.6(C-5″), 123.3(C-6″), 166.7(CO_2Et), 60.7(CH_2), 13.8(CH_3); EI (70 eV) m/z (%): $M^{+\cdot}$ 392.10854 (95), 346.06119 (100), 311.08943 (25), 283.08789 (15), 173.03286 (7), 110.99432 (4), 77.03839 (10)

2j. *Ethyl 4-(3′-methoxyphenylamino)-1-phenyl-1H-pyrazolo[3,4-b]pyridine-5-carboxylate*

Yield: 0.97 g (63%), m.p.: 135°C; IR (KBr, cm^{-1}): (*v* NH 3435; *v* C = O 1678); ^{1}H NMR (DMSO-d_6, *J* in Hz, *δ* in ppm) 6.77(s, H-3), 8.95(s, H-6), 8.16(d, 8.7, H-2′,6′),

7.13(dd, 7.5, H-3′,5′), 7.41(dd, 7.5, H-4′), 7.51(s, H-2″), 7.55(d, 7.8, H-4″), 7.60(dd, 7.5, H-5″), 7.53(d, 7.8, H-6″),4.43(q, 7.0, CH_2), 1.43(t, 7.0, CH_3), 10.51(s, N-H), 3.86(s, Ar-OCH_3); ^{13}C NMR (DMSO-d_6, δ in ppm) 135.8(C-3), 98.1(C-3a), 137.3(C-4), 101.6(C-5), 147.4(C-6), 149.9(C-7a), 131.8(C-1′), 118.6(C-2′, 6′), 127.8(C-3′, 5′), 126.3(C-4′), 157.6(C-1″), 120.2(C-2″), 148.5(C-3″), 109.8(C-4″), 123.5(C-5″), 110.9(C-6″), 164.6($\underline{C}O_2$Et), 57.9(Ar-O$\underline{C}H_3$), 69.4(CH_2), 11.4(CH_3); EI (70 eV) m/z (%): $M^{+\cdot}$ 388.15321 (100), 342.11180 (100), 271.10042 (5), 244.08777(2), 171.06055 (7), 77.04092 (6).

2k. *Ethyl 4-(4′-methoxyphenylamino)-1-phenyl-1H-pyrazolo[3,4-b]pyridine-5-carboxylate*

Yield: 0.80 g (52%), m.p.: 148°C; IR (KBr, cm^{-1}): (*v* NH 3435; *v* C = O 1672); ^{1}H NMR (DMSO-d_6, *J* in Hz, δ in ppm) 6.60(s, H-3), 8.98(s, H-6), 8.19(d, 8.7, H-2′,6′), 7.63(dd, 7.5, H-3′,5′), 7.45(dd, 7.5, H-4′), 7.22(d, 8.7, H-2″), 7.50(d, 8.7, H-3″), 7.22 (d, 8.7, H-5″), 7.22(d, 8.7, H-6″), 4.49(q, 7.2, CH_2), 1.48(t, 7.2, CH_3), 10.46(s, N-H), 3.96(s, Ar-OCH_3); ^{13}C NMR (DMSO-d_6, δ in ppm) 134.0(C-3), 99.9(C-3a), 138.1(C-4), 103.9(C-5), 152.2(C-6), 150.8(C-7a), 131.0(C-1′), 120.9(C-2′, 6′), 128.6(C-3′, 5′), 125.9(C-4′), 158.5(C-1″), 114.6(C-2″), 128.5(C-3″), 151.8(C-4″), 128.5(C-5″), 114.6(C-6″), 167.1($\underline{C}O_2$Et), 55.0(Ar-O$\underline{C}H_3$), 60.1(CH_2), 13.7(CH_3); EI (70 eV) m/z (%): $M^{+\cdot}$ 372.15870 (85), 326.12064 (100), 300.13490 (9), 163.06130 (7), 77.04092 (4).

2l. *Ethyl 4-(3′-fluorophenylamino)-1-phenyl-1H-pyrazolo[3,4-b]pyridine-5-carboxylate*

Yield: 1.23 g (82%), m.p.: 206°C; IR (KBr, cm^{-1}): (*v* NH 3249; *v* C = O 1676); ^{1}H NMR (DMSO-d_6, *J* in Hz, δ in ppm) 7.03(s, H-3), 9.13(s, H-6), 8.30(d, 8.7, H-2′,6′), 7.77–7.53(7H, m), 4.57(q, 7.5, CH_2), 1.55(t, 7.0, CH_3), 10.67(s, N-H); ^{13}C NMR (DMSO-d_6, δ in ppm) 134.2(C-3), 104.0(C-3a), 138.4(C-4), 105.7(C-5), 153.0(C-6), 152.1(C-7a), 135.0(C-1′), 121.0(C-2′, 6′), 128.0(C-3′, 5′), 127.0(C-4′), 158.7(C-1″), 123.2(C-2″), 148.0(C-3″), 142.1(C-4″), 120.3(C-5″), 112.6(C-6‴), 167.0($\underline{C}O_2$Et), 60.5(CH_2), 13.5(CH_3); EI (70 eV) m/z (%): $M^{+\cdot}$ 376.12275 (90), 330.08058 (100), 303.07368 (10), 236.20421 (7), 165.03244 (11), 83.07803 (14).

General procedure for the preparation of the 4-(arylamino)-1-phenyl-1*H*-pyrazolo[3,4-*b*]pyridine-4-carboxylic acids **(3a–l)**

A mixture of 3 mmol of **(2a–g)**, 10 ml of 20% sodium hydroxide solution, and 10 ml of ethanol was heated under reflux for 1–3 hours. On cooling the mixture was acidified with diluted hydrochloric acid (1:3), and the precipitate was filtered and recrystallized from a mixture of DMF and water. The compounds were isolated in

excellent yields (86–93%). The structures of the compounds were elucidated by IR, ^{1}H and ^{13}C NMR, and mass spectrometry.

3a. *4-Phenylamino-l-phenyl-1H-pyrazolo[3,4-b]pyridine-5-carboxylic acid*

Yield: 0.85 g (86%), m.p.: 229°C; IR (KBr, cm^{-1}): (ν OH 3435−2598; ν C = O 1654); ^{1}H NMR (DMSO-d_6, *J* in Hz, δ in ppm) 7.01(s, H-3), 9.02(s, H-6), 8.23(d, 8.1, H-2′,6′), 7.86−7.45(8H, m), 10.78(s, N-H); ^{13}C NMR (DMSO-d_6, δ in ppm) 135.4(C-3), 100.0(C-3a), 151.7(C-4), 105.5(C-5), 153.1(C-6), 153.5(C-7a), 114.2(C-1′), 122.2(C-2′, 6′), 131.5(C-3′, 5′), 129.1(C-4′), 140.2(C-1″), 126.4(C-2″), 139.5(C-3″), 126.9(C-4″), 139.5(C-5″), 126.4(C-6″), 170.2($\underline{C}O_2H$).

3b. *4-(4′-Methylphenylamino)-1-phenyl-1H-pyrazolo[3,4-b]pyridine-5-carboxylic acid*

Yield: 0.94 g (92%), m.p.: 258°C; IR (KBr, cm^{-1}): (ν OH 3430−2603; ν C = O 1654); ^{1}H NMR (DMSO-d_6, *J* in Hz, δ in ppm) 6.67(s, H-3), 8.95(s, H-6), 8.18(d, 7.5, H-2′,6′), 7.61−7.40(7H, m), 10.73(s, N-H), 2.47(s, Ar-CH_3); ^{13}C NMR (DMSO-d_6, δ in ppm) 134.8(C-3), 100.1(C-3a), 151.3(C-4), 104.5(C-5), 153.5(C-6), 152.8(C-7a), 139.1(C-1′), 121.7(C-2′, 6′), 130.7(C-3′, 5′), 129.3(C-4′), 139.1(C-1″), 126.6(C-2″), 137.8(C-3″), 136.7(C-4″), 137.8(C-5″), 126.6(C-6″), 170.3($\underline{C}O_2H$), 21.0(Ar-$\underline{C}H_3$); EI (70 eV) m/z (%): $M^{+\cdot}$ 344.13367 (95), 326.12041 (100), 297.10439 (5), 270.11045 (3), 199.93153 (4), 73.36787 (4).

3c. *4-(3′-Chlorophenylamino)-1-phenyl-1H-pyrazolo[3,4-b]pyridine-5-carboxylic acid*

Yield: 1.01 g (93%), m.p.: 236°C; IR (KBr, cm^{-1}): (ν OH 3488−2766; ν C = O 1649); ^{1}H NMR (DMSO-d_6, *J* in Hz, δ in ppm) 7.01(s, H-3), 9.07(s, H-6), 8.27(d, 7.5, H-2′,6′), 7.74−7.62 (5H, m), 7.52(dd, 7.5, H-4′), 7.79(s, H-2″), 10.92(s, N-H); ^{13}C NMR (DMSO-d_6, δ in ppm) 132.9(C-3), 100.5(C-3a), 148.6(C-4), 103.2(C-5), 151.6(C-6), 150.8(C-7a), 120.1(C-1′), 125.2(C-2′, 6′), 130.0(C-3′, 5′), 127.7(C-4′), 139.3(C-1″), 124.0(C-2″), 137.2(C-3″), 126.1(C-4″), 132.3(C-5″), 120.1(C-6″), 168.4($\underline{C}O_2H$); EI (70 eV) m/z (%): $M^{+\cdot}$ 364.07926 (100), 311.10332 (20), 283.10468 (9), 236.22030 (3), 155.55160 (7), 77.17758 (20).

3d. *4-(3′-Nitrophenylamino)-1-phenyl-1H-pyrazolo[3,4-b]pyridine-5-carboxylic acid*

Yield: 1.01 g (90%), m.p.: 255 °C, IR (KBr, cm^{-1}): (ν OH 3401−2603; ν C = O l674); ^{1}H NMR (DMSO-d_6, *J* in Hz, δ in ppm) 7.12(s, H-3), 9.04(s, H-6), 8.22(d, 7.5, H-2′,6′), 7.65(dd, 7.5, H-3′,5′), 7.46(dd, 7.5, H-4′), 8.42(s, H-2″), 8.35(d, 8.7, H-

4″), 7.88(d, 8.7, H-5″), 8.03(d, 8.7, H-6″), 10.99(s, N-H); ^{13}C NMR (DMSO-d_6, δ in ppm) 138.2(C-3), 102.1(C-3a), 148.8(C-4), 104.3(C-5), 152.7(C-6), 152.0(C-7a), 120.2(C-1′), 126.1(C-2′, 6′), 132.0(C-3′, 5′), 130.5(C-4′), 148.1(C-1″), 121.3(C-2″), 140.0(C-3″), 128.7(C-4″), 134.0(C-5″), 120.9(C-6″), 168.2($\underline{C}O_2H$); EI (70 eV) m/z (%): $M^{+\cdot}$ 375.09687 (100); 310.09034 (27); 211.20865 (8); 178.57242 (23); 145.62161 (9); 121.49006 (10); 77.17135 (9).

3e. *4-(4′-Nitrophenylamino)-1-phenyl-1H-pyrazolo[3,4-b]pyridine-5-carboxylic acid*

Yield: 1.04 g (93%), m.p.: 256°C; IR (KBr, cm^{-1}): (ν OH 3409−2603; ν C = O 1678); ^{1}H NMR (DMSO-d_6, *J* in Hz, δ in ppm) 7.16(s, H-3), 9.06(s, H-6), 8.23(d, 7.5, H-2′,6′), 7.65(dd, 7.5, H-3′,5′), 7.46(dd, 7.5, H-4′), 7.74(d, 8.7, H-2″), 8.40(d, 8.7, H-3″), 8.40(d, 8.7, H-5″), 7.74(d, 8.7, H-6″), 10.98(s, N-H); ^{13}C NMR (DMSO-d_6, δ in ppm) 139.5(C-3), 130.3(C-3a), 148.2(C-4), 104.9(C-5), 153.7(C-6), 153.1(C-7a), 120.3(C-1′), 126.0(C-2′, 6′), 133.2(C-3′, 5′), 129.9(C-4′), 148.1(C-1″), 121.3(C-2″), 139.4(C-3″), 127.3(C-4″), 131.7(C-5″), 120.3(C-6″), 170.4($\underline{C}O_2H$); EI (70 eV) m/z (%): $M^{+\cdot}$ 375.09575 (100), 311.09014 (45), 283.09583 (26), 204.83632 (23), 138.51953 (12), 116.02373 (20), 83.21144 (14).

3f. *4-(3′-Bromophenylamino)-1-phenyl-1H-pyrazolo[3,4-b]pyridine-5-carboxylic acid*

Yield: 1.10 g (90%), m.p.: 246°C; IR (KBr, cm^{-1}): (ν OH 3490−2580; ν C = O 1650); ^{1}H NMR (DMSO-d_6, *J* in Hz, δ in ppm) 6.65(s, H-3), 8.72(s, H-6), 7.93(d, 7.5, H-2′,6′), 7.50−7.31(5H, m), 7.17(dd, 7.5, H-4′), 7.57(s, H-2″), 12.90(s, CO_2H), 10.57(s, N-H); ^{13}C NMR (DMSO-d_6, δ in ppm) 134.4(C-3), 102.0(C-3a), 150.0(C-4), 104.7(C-5), 153.3(C-6), 152.6(C-7a), 120.5(C-1′), 125.9(C-2′, 6′), 130.5(C-3′, 5′), 129.6(C-4′), 141.0(C-1″), 121.5(C-2″), 138.8(C-3″), 126.6(C-4″), 131.7(C-5″), 120.5(C-6″), 170.0($\underline{C}O_2H$); EI (70 eV) m/z (%): $M^{+\cdot}$ 410.00421 (100), 339.37886 (8), 311.07759 (100), 210.05296 (7), 76.04006 (5).

3g. *4-(4′-Bromophenylamino)-1-phenyl-1H-pyrazolo[3,4-b]pyridine-5-carboxylic acid*

Yield: 1.10 g (90%), m.p.: 265°C; IR (KBr, cm^{-1}): (ν OH 3431−2580; ν C = O 1651); ^{1}H NMR (DMSO-d_6, *J* in Hz, δ in ppm) 6.87(s, H-3), 8.95(s, H-6), 8.16(d, 7.5, H-2′,6′), 7.59(dd, 7.5, H-3′,5′), 7.40(dd, 7.5, H-4′), 7.50(d, 8.7, H-2″), 7.78(d, 8.7, H-3″), 7.78(d, 8.7, H-5″), 7.50(d, 8.7, H-6″), 13.00(s, CO_2H), 10.73(s, N-H); ^{13}C NMR (DMSO-d_6, δ in ppm) 132.5(C-3), 101.5(C-3a), 149.9(C-4), 104.5(C-5), 153.0(C-6), 152.2(C-7a), 115.0(C-1′), 120.0(C-2′, 6′), 128.7(C-3′, 5′), 126.3(C-4′), 138.4(C-1″), 120.0(C-2″), 134.2(C-3″), 121.2(C-4″), 128.9(C-5″), 115.0(C-6″),

169.7($\underline{C}O_2H$); EI (70 eV) m/z (%): $M^{+\cdot}$ 410.00732 (13), 386.21067 (4), 311.08624 (100), 283.04687 (13), 82.30021 (5).

3h. *4-(3′-Methylphenylamino)-l-phenyl-1H-pyrazolo[3,4-b]pyridine-5-carboxylic acid*

Yield: 0.92 g (90%), m.p.: 237°C; IR (KBr, cm^{-1}): (*v* OH 3482−2592; *v* C = O 1650); ^{1}H NMR (DMSO-d_6, *J* in Hz, δ in ppm) 6.71(s, H-3), 8.95(s, H-6), 8.16(d, 7.5, H-2′,6′), 7.60(dd, 7.5, H-3′,5′), 7.52(dd, 7.5, H-4′), 7.44−7.32(4H, m), 10.81(s, N-H), 2.21(s, Ar-CH_3); ^{13}C NMR (DMSO-d_6, δ in ppm) 138.9(C-3), 101.4(C-3a), 151.1(C-4), 104.7(C-5), 153.3(C-6), 152.6(C-7a), 124.4(C-1′), 127.8(C-2′, 6′), 130.0(C-3′, 5′), 129.4(C-4′), 140.0(C-1″), 126.7(C-2″), 139.1(C-3″), 128.9(C-4″), 134.8(C-5″), 121.7(C-6″), 170.3($\underline{C}O_2H$), 21.3(Ar-$\underline{C}H_3$); EI (70 eV) m/z (%): $M^{+\cdot}$ 344.13178 (95), 326.11951 (100), 297.11572 (5), 270.10950 (3), 199.93871 (4), 73.35493 (4).

3i. *4-(4′-Chlorophenylamino)-l-phenyl-1H-pyrazolo[3,4-b]pyridine-5-carboxylic acid*

Yield: 0.97 g (89%), m.p.: 268°C; IR (KBr, cm^{-1}): (*v* OH 3430−2603; *v* C = O 1654); ^{1}H NMR (DMSO-d_6, *J* in Hz, δ in ppm) 6.87(s, H-3), 8.97(s, H-6), 8.17(d, 7.5, H-2′,6′), 7.61(dd, 7.5, H-3′,5′), 7.42(dd, 7.5, H-4′), 7.58(d, 8.7, H-2″), 7.67(d, 8.7, H-3″), 7.67(d, 8.7, H-5″), 7.58(d, 8.7, H-6″), 10.77(s, N-H); ^{13}C NMR (DMSO-d_6, δ in ppm) 131.7(C-3), 101.4(C-3a), 150.0(C-4), 104.3(C-5), 153.0(C-6), 152.2(C-7a), 121.2(C-1′), 128.5(C-2′, 6′), 128.9(C-3′, 5′), 128.9(C-4′), 138.4(C-1″), 126.3(C-2″), 134.2(C-3″), 137.9(C-4″), 129.6(C-5″), 121.2(C-6″), 169.6($\underline{C}O_2H$); EI (70 eV) m/z (%): $M^{+\cdot}$ 364.07828 (100), 311.10296 (20), 283.11744 (9), 236.22865 (3), 155.54781 (7), 77.17234 (20).

3j. *4-(3′-Methoxyphenylamino)-l-phenyl-1H-pyrazolo[3,4-b]pyridine-5-carboxylic acid*

Yield: 0.97 g (90%), m.p.: 237°C; IR (KBr, cm^{-1}): (*v* OH 3330−2590; *v* C = O 1654); ^{1}H NMR (DMSO-d_6, *J* in Hz, δ in ppm) 6.91(s, H-3), 9.05(s, H-6), 8.27(d, 7.5, H-2′,6′), 7.69(dd, 7.5, H-3′,5′), 7.51(dd, 7.5, H-4′), 7.66−7.61(4H, m), 10.88(s, N-H), 3.95(s, Ar-OCH_3); ^{13}C NMR (DMSO-d_6, δ in ppm) 139.3(C-3), 101.8(C-3a), 151.1(C-4), 105.0(C-5), 153.7(C-6), 153.0(C-7a), 114.3(C-1′), 122.0(C-2′, 6′), 131.3(C-3′, 5′), 129.7(C-4′), 140.7(C-1″), 119.6(C-2″), 161.0(C-3″), 127.0(C-4″), 135.2(C-5″), 113.1(C-6″), 170.6($\underline{C}O_2H$), 56.1(Ar-O$\underline{C}H_3$); EI (70 eV) m/z (%): $M^{+\cdot}$ 360.12379 (100), 298.08825 (10), 230.57425 (4), 171.06160 (8), 77.17826 (13).

3k. *4-(4′-Methoxyphenylamino)-l-phenyl-1H-pyrazolo[3,4-b]pyridine-5-carboxylic acid*

Yield: 0.98 g (91%), m.p.: 261°C; IR (KBr, cm^{-1}): (*v* OH 3430−2588; *v* C = O 1654); ^{1}H NMR (DMSO-d_6, *J* in Hz, δ in ppm) 6.70(s, H-3), 9.03(s, H-6), 8.26(d,

7.5, H-2′,6′), 7.69(dd, 7.5, H-3′,5′), 7.51(dd, 7.5, H-4′), 7.28(d, 8.7, H-2″), 7.56(s, H-3″), 7.56(d, 8.7, H-5‴″), 7.28(d, 8.7, H-6″), 10.77(s, N-H), 4.02(s, Ar-OCH_3); ^{13}C NMR (DMSO-d_6, δ in ppm) 131.3(C-3), 100.5(C-3a), 151.3(C-4), 104.1(C-5), 152.9(C-6), 152.2(C-7a), 114.1(C-1′), 121.1(C-2′, 6′), 128.7(C-3′, 5′), 126.1(C-4′), 138.5(C-1″), 120.0(C-2″), 134.2(C-3″), 158.7(C-4″), 128.8(C-5″), 114.0(C-6″), 169.8($\underline{C}O_2H$), 55.2(Ar-$O\underline{C}H_3$); EI (70 eV) m/z (%): $M^{+\cdot}$ 360.12451 (100), 298.08978 (10), 230.58327 (4), 171.07122 (8), 77.17399 (13).

3l. *4-(3′-Fluorophenylamino)-l-phenyl-1H-pyrazolo[3,4-b]pyridine-5-carboxylic acid*

Yield: 0.97 g (93%), m.p.: 258°C; IR (KBr, cm^{-1}): (ν OH 3412−2586; ν C = O 1675); ^1H NMR (DMSO-d_6, J in Hz, δ in ppm) 7.04(s, H-3), 9.08(s, H-6), 8.28(d,7.5, H-2′,6′), 7.79−7.54(6H, m), 7.49(dd,7.5, H-4′), 10.98(s, N-H); ^{13}C NMR (DMSO-d_6, δ in ppm) 134.8(C-3), 102.3(C-3a), 150.4(C-4), 105.0(C-5), 153.6(C-6), 152.9(C-7a), 115.0(C-1′), 121.9(C-2′, 6′), 129.6(C-3′, 5′), 126.9(C-4′), 141.4(C-1″), 121.9(C-2″), 139.2(C-3″), 123.3(C-4″), 131.9(C-5″), 114.8(C-6″), 170.4($\underline{C}O_2H$); EI (70 eV) m/z (%): $M^{+\cdot}$ 348.10136 (100), 304.11069 (7), 274.07411 (4), 198.04128 (4), 165.04218 (10), 77.17166 (12).

General procedure for the preparation of the ethyl 4-[(methylpyridin-2-yl)amino]-1-phenyl-1*H*-pyrazolo[3,4-*b*]pyridine-5-carboxylates **(4a–c)**

An equimolar mixture of **1** (1.08 g, 4 mmol) and a slightly excess of the appropriate aminopicoline (5 mmoles) in 10 ml DMF was heated in a silicone oil bath under reflux for 4 hours. The reaction mixture, after cooling, was poured into 50 ml of ice-cold water. The precipitate was filtered, dried, and recrystallized from a mixture of ethanol and water. The compounds were isolated in good yields (50–60%). The structures of the compounds were elucidated by IR, ^1H and ^{13}C NMR, and mass spectrometry.

4a. *Ethyl 4-[(4′-methylpyridin-2-yl)amino]-1-phenyl-1H-pyrazolo[3,4-b]pyridine-5-carboxylate*

Yield: 0.72 g (60%), m.p.: 180°C; IR (KBr, cm^{-1}): (ν NH 3110; ν C = O 1696); ^1H NMR (DMSO-d_6, J in Hz, δ in ppm) 8.12(d, 7.5, H-2′,6′), 7.52(dd, 7.5, H-3′,5′), 7.33(dd, 7.5, H-4′), 7.33(dd, 7.5, H-3″), 7.52(dd, 7.5, H-4″), 8.13(d, 7.5, H-6″), 4.38(q, 7.0, CH_2), 1.40(t, 7.0, CH_3), 9.56 (s, N-H), 3.30 (s, Ar-CH_3); ^{13}C NMR (DMSO-d_6, δ in ppm) 136.7(C-3), 107.0(C-3a), 145.5(C-4), 108.0(C-5), 152.7(C-6), 152.5(C-7a), 134.0(C-1′), 121.7(C-2′, 6′), 129.6(C-3′, 5′), 126.5(C-4′), 157.1(C-2″), 121.6(C-3″), 139.4(C-4″), 119.4(C-5″), 152.0(C-6″), 167.1($\underline{C}O_2Et$), 26.1(Ar-$\underline{C}H_3$), 60.9(CH_2), 14.6(CH_3); EI (70 eV) m/z (%): $M^{+\cdot}$ 373.4102 (100)

4b. *Ethyl 4-[(5′-methylpyridin-2-yl)amino]-1-phenyl-1H-pyrazolo[3,4-b]pyridine-5-carboxylate*

Yield: 0.70 g (50%), m.p.: 193°C; IR (KBr, cm^{-1}): (ν NH 3110; ν C = O 1695); ^1H NMR (DMSO-d_6, J in Hz, δ in ppm) RMN 8.90 (s, H-3), 9.26 (s, H-6), 8.38 (d, 7.5, H-2′,6′), 7.82(dd, 7.5, H-3′,5′), 7.63(dd, 7.5, H-4′), 8.38(d, 7.5, H-6″), 4.62(q, 7.0, CH_2), 1.58(t, 7.0, CH_3), 3.50(s, Ar-CH_3); ^{13}C NMR (DMSO-d_6, δ in ppm) 135.1(C-3), 101.2(C-3a), 145.4(C-4), 103.4(C-5), 152.4(C-6), 152.3(C-7a), 139.0(C-1′), 122.2(C-2′, 6′), 130.0(C-3′, 5′), 127.8(C-4′), 157.5(C-2″), 117.8(C-3″), 139.1(C-4″), 120.3(C-5″), 152.1(C-6″), 164.2($\underline{C}O_2Et$), 21.9(Ar-$\underline{C}H_3$), 62.5(CH_2), 14.8(CH_3); EI (70 eV) m/z (%): $M^{+\cdot}$ 373.4139 (100)

4c. *Ethyl 4-[(6′-methylpyridin-2-yl)amino]-1-phenyl-1H-pyrazolo[3,4-b]pyridine-5-carboxylate*

Yield: 0.70 g (50%), m.p.: 165°C; IR (KBr, cm^{-1}): (ν NH 3125; ν C = O 1693); ^1H NMR (DMSO-d6, J in Hz, δ in ppm) 9.40 (s, H-3), 9.50 (s, H-6), 9.10 (d, 7.5, H-2′,6′), 8.30−7.20(6H, m), 4.45(q, 7.0, CH_2), 1.45(t, 7.0, CH_3), 2.20 (s, Ar-CH_3); ^{13}C NMR (DMSO-d_6, δ in ppm) 136.2(C-3), 104.0(C-3a), 145.9(C-4), 102.8(C-5), 152.1(C-6), 152.9(C-7a), 136.7(C-1′), 122.4(C-2′, 6′), 129.1(C-3′, 5′), 126.9(C-4′), 156.8(C-2″), 120.2(C-3″), 139.7(C-4″), 119.6(C-5″), 152.9(C-6″), 164.9($\underline{C}O_2Et$), 25.2(Ar-$\underline{C}H_3$), 60.5(CH_2), 14.1(CH_3); EI (70 eV) m/z (%): $M^{+\cdot}$ 373.4186 (100)

General procedure for the preparation of the 4-[(methylpyridin-2-yl)amino]-1-phenyl-1*H*-pyrazolo[3,4-*b*]pyridine-4-carboxylic acids **(5a–c)**

A mixture of 3 mmol of **(4a–c)**, 10 ml of 20% sodium hydroxide solution, and 10 ml of ethanol was heated under reflux for 1–3 hours. On cooling mixture was acidified with diluted hydrochloric acid (1:3), and the precipitated was filtered and recrystallized from a mixture of DMF and water. The compounds were isolated in excellent yields (80–93%). The structures of the compounds were elucidated by IR, ^1H and ^{13}C NMR, and mass spectrometry.

5a. *4-[(4′-Methylpyridin-2-yl)amino]-l-phenyl-1H-pyrazolo[3,4-b]pyridine-5-carboxylic acid*

Yield: 0.73 g (80%), m.p.: > 300°C; IR (KBr, cm^{-1}): (ν OH 3491−2580; ν C = O 1674); ^1H NMR (DMSO-d_6, J in Hz, δ in ppm) 8.42(s, H-3), 9.07(s, H-6), 8.22(d, 8.0, H-2′,6′), 7.69(dd, 7.5, H-3′,5′), 7.51(dd, 7.5, H-4′), 7.90(d, 7.5,H-3″), 7.42(d, 7.5, H-4″), 7.73(s, H-6″), 11.37(s, CO_2H), 3.56(s, Ar-CH_3); ^{13}C NMR (DMSO-d_6, δ in ppm) 135.8(C-3), 102.4(C-3a), 145.3(C-4), 106.1(C-5), 152.5(C-6), 151.1(C-7a), 137.0(C-1′), 121.9(C-2′, 6′), 129.9(C-3′, 5′), 126.8(C-4′), 157.3(C-2″), 118.6(C-3″),

139.7(C-4″), 119.8(C-5″), 152.6(C-6″), 21.7(Ar-$\underline{C}H_3$), 170.9($\underline{C}O_2H$); EI (70 eV) m/z (%): $M^{+\cdot}$ 345.3629 (100)

5b. *4-[(5′-Methylpyridin-2-yl)amino]-l-phenyl-1H-pyrazolo[3,4-b]pyridine-5-carboxylic acid*

Yield: 0.85 g (93%), m.p.: 225°C; IR (KBr, cm^{-1}): (ν OH 3426−2600; ν C = O 1678); ^{1}H NMR (DMSO-d_6, J in Hz, δ in ppm) 8.81(s, H-3), 9.08(s, H-6), 8.23(d, 8.0, H-2′,6′), 7.61(dd, 7.5, H-3′,5′), 7.44(dd, 7.5, H-4′), 7.95(s, H-3″), 7.70(d, 7.5, H-5″), 8.43(d, 7.5, H-6″), 11.36(s, CO_2H), 9.46(s, N-H), 3.27(s, Ar-CH_3); ^{13}C NMR (DMSO-d_6, δ in ppm) 135.6(C-3), 102.8(C-3a), 145.7(C-4), 104.2(C-5), 152.1(C-6), 152.5(C-7a), 138.5(C-1′), 121.6(C-2′, 6′), 129.2(C-3′, 5′), 127.2(C-4′), 157.8(C-2″), 117.6(C-3″), 139.0(C-4″), 119.6(C-5″), 152.2(C-6″), 21.2(Ar-$\underline{C}H_3$), 170.2($\underline{C}O_2H$); EI (70 eV) m/z (%): $M^{+\cdot}$ 345.3678 (100)

5c. *4-[(6′-Methylpyridin-2-yl)amino]-l-phenyl-1H-pyrazolo[3,4-b]pyridine-5-carboxylic acid*

Yield: 0.78 g (85%), m.p.: 252°C; IR (KBr, cm^{-1}): (ν OH 3400−2500; ν C = O 1678); ^{1}H NMR (DMSO-d_6, J in Hz, δ in ppm) 8.86(s, H-3), 9.10(s, H-6), 8.25(d, 8.0, H-2′,6′), 7.65(dd, 7.5, H-3′,5′), 7.46(dd, 7.5, H-4′), 7.36(d, 7.5, H-4″), 7.77(dd, 7.5, H-5″), 8.35(d, 7.5, H-6″), 11.45(s, CO_2H), 9.51(s, NH), 3.31(s, Ar-CH_3); ^{13}C NMR (DMSO-d_6, δ in ppm) 135.4(C-3), 102.2(C-3a), 145.1(C-4), 103.7(C-5), 152.8(C-6), 152.0(C-7a), 138.9(C-1′), 121.0(C-2′, 6′), 129.8(C-3′, 5′), 127.6(C-4′), 157.1(C-2″), 116.8(C-3″), 139.7(C-4″), 119.2(C-5″), 152.8(C-6″), 21.7(Ar-$\underline{C}H_3$), 171.2($\underline{C}O_2H$); EI (70 eV) m/z (%): $M^{+\cdot}$ 345.3634 (100)

Cell culture and virus

Vero cells were cultured in Dulbecco's modified Eagle's medium (DMEM) supplemented with 5% fetal bovine serum (FBS; HyClone, Logan, Utah), 100 U/mL penicillin and 100 ug/mL streptomycin, at 37°C in 5% CO_2.

Cytotoxicity assay

A monolayer of about 104 Vero cells in 96-multiwell plates was treated with various concentrations of the compounds for the 72 h. Then, 50 µl of a 1 mg/mL solution of 3-(4,5-dimethylthiazol-2-yl)-2,5- diphenyl tetrazolium bromide (MTT; Sigma) was added to evaluate cell viability according to procedures described elsewhere (Mossmann, 1983). The 50% cytotoxic concentration (CC_{50}) was calculated by linear regression analysis of the dose–response curves.

Plaque-reduction assay

The assay followed procedures described previously (Lucero *et al*., 2006). Acyclovir was used as a positive control. Vero cells in six-well plates were exposed to different dilutions of the supernatant from the assay described above for 1 h at 37°C. Residual viruses were then rinsed with PBS, and DMEM containing 5% FBS and 1% methylcellulose (Fluka) (overlay medium) was added to cells. After 72 h at 37°C, the monolayers were fixed with 10% formaldehyde in PBS and stained with a 0.1% solution of crystal violet in 70% methanol, and the virus titers were calculated by scoring the plaque-forming units (PFU). The 50% antiviral concentration (EC_{50}) was calculated by linear regression analysis of dose–response curves.

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