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ORIGINAL PAPER

Synthesis and antiproliferative activity of multisubstituted N-fused heterocycles against the Hep-G2 cancer cell line

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Abstract Pyrrolo[1,2-*a*]imidazole and pyrrolo[2,1-*b*]thiazole derivatives were synthesized in a one-pot procedure by [3 + 2] cycloaddition reactions of the corresponding imidazolium ylides and thiazolium ylides with an alkene followed by oxidative aromatization of the primary cycloadducts under the action of the mild oxidant tetrakispyridinecobalt(II) dichromate. Antiproliferative activity of 14 new bicyclic N-fused heterocycles against the human hepatocellular liver carcinoma (Hep-G2) cell line were examined by the MTT method. Some of the compounds showed favorable antiproliferative activity, especially compound **3i** displayed potent antiproliferative activities with an IC_{50} value of 0.36 μg/cm^3.

Keywords N-fused heterocycles · Antiproliferative activity · Structure–activity relationship · Hep-G2 cell line

Introduction

Heteroaromatic molecules with an N-fused pyrrole unit or their reduced analogues are an important structural motif, many of which are biologically active substances [1–3]. In particular, the fused 5–5 bicyclic structural frameworks of pyrrolo[2,1-*b*]thiazole [3] and pyrrolo[1,2-*a*]imidazole with a bridgehead nitrogen atom are widely found in naturally occurring and synthetic biologically active molecules. The pyrrolo[2,1-*b*]thiazole derivatives display a wide range of biological activities such as antileukemic [4], anticonvulsant [5], anti-inflammatory [6, 7], antagonistic [8], and hypoglycemic activities [9]. Some of the pyrrolo[1,2-*a*]imidazole derivatives are potent kinase inhibitors with improved affinity and selectivity, and are useful in designing new kinase inhibitors [10–12]. Pyrrolo[1,2-*a*]imidazoles also serve as potent partial agonists of the α1A adrenergic receptor with good selectivity over the α1B, α1D, and α2A receptor subtypes [13]. As a result, the synthesis of pyrrolo[2,1-*b*]thiazole [14–19] and pyrrolo[1,2-*a*]imidazole [20–23] derivatives has attracted much recent research interest.

Hepatocellular carcinoma (HCC) is the most commonly seen histological type of primary liver carcinoma. The worldwide incidence of HCC is one of the highest of all cancer types [24–27]. However, there are few reports on the search for potential drugs with antitumor activity against HCC, and the bioactivity of pyrrolo[2,1-*b*]thiazoles and pyrrolo[1,2-*a*]imidazoles against HCC [28] has not been investigated. With the aim of searching for new pharmaceuticals against HCC, a series of multifunctionalized pyrrolo[2,1-*b*]thiazoles and pyrrolo[1,2-*a*]imidazoles were synthesized and their activity in inhibiting the Hep-G2 cell line was examined.

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Results and discussion

Synthesis and chemical characterization

1,3-Dipolar cycloadditions of thiazolium ylides derived from the thiazolium salts **1a**–**1c** [29] (Table 1, Scheme 1) with the electron deficient alkenes **2a**–**2e** were carried out in *N*,*N*-dimethylformamide (DMF) solution in the presence of triethylamine as a base and tetrakispyridinecobalt(II) dichromate [$Py_4Co(HCrO_4)_2$] (TPCD) [30] as an oxidant.

During the reactions, the primary cycloadducts, tetrahydropyrrolo[2,1-*b*]thiazoles, were oxidatively dehydrogenated by TPCD to give the products. Pyrrolo[2,1-*b*]thiazoles **3a**–**3i** were prepared from simple starting materials in satisfactory yields by similar one-pot reactions (Scheme 2, Table 2).

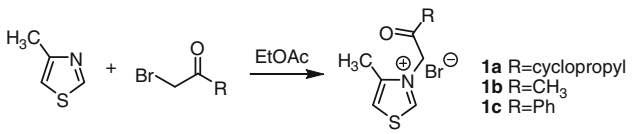
The structures of the products were assigned on the basis of their spectral (IR, ^{1}H and ^{13}C NMR, MS) and elemental analysis data and were further confirmed by X-ray crystallographic analyses of product **3c** (Fig. 1).

It can be seen from Table 2 that the yield of compound **3g** (79%) in which R is a phenyl group is significantly higher than those when R is a cyclopropyl group or methyl group. The yield of compound **3h** is lower than **3g** because the ester group is hydrolyzed under the reaction conditions to give the by-product **3h′** (Scheme 3).

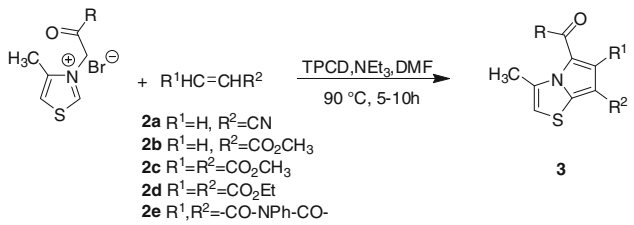
Similarly, the 1,3-dipolar cycloadditions of imidazolium ylides derived from 1-methylimidazole and *β*-bromoketones **4a** and **4b** with the alkenes **2a**, **2b**, and **2d** in the presence of triethylamine and TPCD afforded the pyrrolo[1,2-*a*]imidazoles **5a**–**5e** (Scheme 4, Table 2). The only difference in these cases is that the ylides were prepared in situ from *N*-methylimidazole and bromopropanone prior to the 1,3-cycloadditions without separation because these imidazolium salts are highly hygroscopic. Beside the normal product **5**, we obtained the corresponding indolizine derivatives **5′** as by-products (Fig. 2).

Table 1 Yields and melting points of the thiazolium salts

Compd.	R	m.p. (°C) (lit.)	Yield (%)
1a	Cyclopropyl	180–181	62
1b	CH_3	172–174	94
1c	Ph	215 (210 [29])	81



Scheme 1



Scheme 2

Table 2 Synthesis of pyrrolo[2,1-*b*]thiazoles

Product	R	R^1	R^2	Yield (%)
3a	Cyclopropyl	H	CN	52
3b	Cyclopropyl	H	CO_2CH_3	49
3c	Cyclopropyl	CO_2CH_3	CO_2CH_3	28
3d	CH_3	H	CN	49
3e	CH_3	H	CO_2CH_3	23
3f	CH_3	CO_2Et	CO_2Et	16
3g	Ph	H	CN	79
3h	Ph	H	CO_2CH_3	38
3i	Cyclopropyl	–CO–NPh–CO–		51

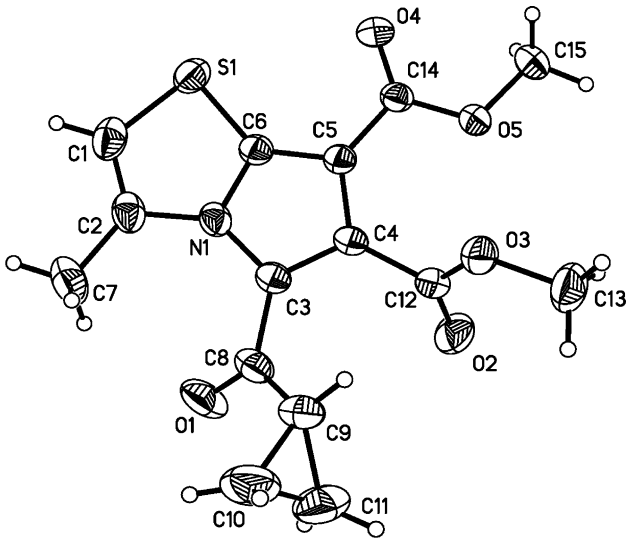
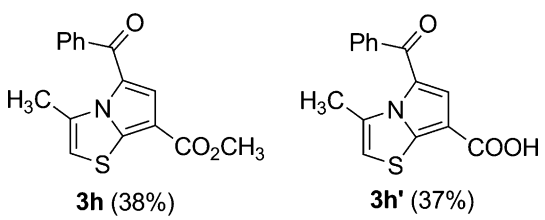
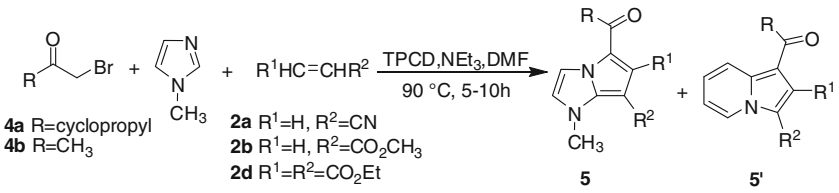


Fig. 1 Oak Ridge thermal ellipsoid plot (ORTEP) drawing of compound **3c**



Scheme 3

Scheme 4



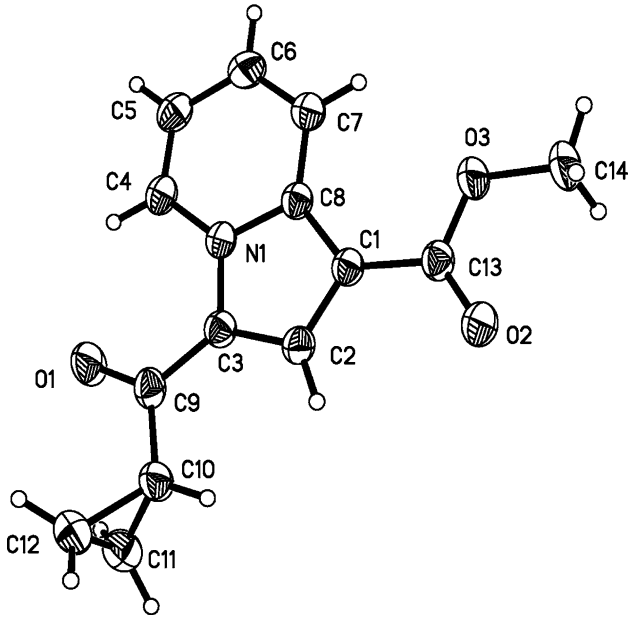


Fig. 2 ORTEP drawing of compound **5b′**

N-Methylimidazole ($pK_a = 7.33$) is a stronger base than pyridine ($pK_a = 5.2$) [31]. The exchange between the imidazole in the imidazolium ylides and the pyridine in TPCD results in the formation of pyridinium ylides which took part in cycloadditions with the alkenes to give the indolizine derivatives **5′**. Therefore, when we used freshly prepared manganese dioxide as an alternative oxidant to replace TPCD, the products **5′** were no longer obtained (Table 3). However, in this case, the yields of the compounds **5** were not improved. Therefore, TPCD seems to be a more suitable oxidant in these reactions because it is more easily prepared and can be stored for a longer time without losing activity.

Antiproliferative activity against the Hep-G2 cell line

The in vitro antiproliferative activities of the synthesized pyrrolo[2,1-*b*]thiazoles and pyrrolo[1,2-*a*]imidazoles against the human liver cancer cell line Hep-G2 were studied by applying the MTT colorimetric assay. Compounds were tested over a range of concentrations from 0.1 to 40 μg/cm^3, and the calculated IC_{50} values (the IC_{50} value refers to the concentration (μg/cm^3) of a compound able to cause 50% cell death with respect to the control culture) are reported in Table 4.

Table 3 Synthesis of pyrrolo[1,2-*a*]imidazoles using TPCD and MnO_2

Compd.	R	R^1	R^2	Yield (%)	
				TPCD	MnO_2
5a	Cyclopropyl	H	CN	21	16
5a′				6	–
5b	Cyclopropyl	H	CO_2CH_3	20	15
5b′				8	–
5c	CH_3	H	CN	17	11
5c′				18	–
5d	CH_3	H	CO_2CH_3	12	10
5d′				15	–
5e	CH_3	CO_2Et	CO_2Et	9	7
5e′				10	–

Table 4 Antiproliferative effect of pyrrolo[2,1-*b*]thiazoles and pyrrolo[1,2-*a*]imidazoles against Hep-G2

Compd.	IC_{50} (μg/cm^3)	Compd.	IC_{50} (μg/cm^3)
3a	1.1 ± 0.6	**3h**	5.2 ± 0.6
3b	37 ± 0.6	**3i**	0.36 ± 0.09
3c	1.4 ± 0.6	**5a**	15 ± 0.9
3d	19 ± 0.9	**5b**	31 ± 0.8
3e	25 ± 0.7	**5c**	26 ± 0.9
3f	37 ± 0.6	**5d**	23 ± 0.9
3g	3.8 ± 0.3	**5e**	35 ± 0.9
5-Fluorouracil	0.25 ± 0.07	–	–

As shown in Table 4, compound **3i**, which contains a cyclopropylcarbonyl group and *N*-phenylmaleimide group, exhibits the most potent antiproliferative activity with an IC_{50} value of 0.36 μg/cm^3, which is comparable to the reference compound 5-fluorouracil (IC_{50} 0.25 μg/cm^3). The pyrrolo[2,1-*b*]thiazole compounds usually have better antiproliferative activity than the pyrrolo[1,2-*a*]imidazole derivatives. Furthermore, pyrrolo[2,1-*b*]thiazole derivatives with a 7-CN group showed better activity than the corresponding 7-ester compounds (e.g., **3a** vs. **3b**, **3d** vs. **3e**, **3g** vs. **3h**). The same trend was also found for the pyrrolo[1,2-*a*]imidazole series (e.g., **5a** vs. **5b**, **5c** vs. **5d**).

Conclusion

A series of pyrrolo[1,2-*a*]imidazole and pyrrolo[2,1-*b*]thiazole derivatives were synthesized in a one-pot procedure. Antiproliferative activity of the 14 new bicyclic N-fused heterocycles (compounds **3a**–**3i** and **5a**–**5e**) against the human hepatocellular liver carcinoma (Hep-G2) cell line was examined. Some of the compounds (e.g., **3a**, **3c**, and **3i**) show favorable antiproliferative activity, with compound **3i**, which contains a cyclopropylcarbonyl group and an *N*-phenylmaleimide group, having the strongest activity with an IC_{50} value of 0.36 μg/cm^3.

Experimental

General methods

Melting points were measured on a X-4 (Taike Corp., Beijing, China) microscopic melting point apparatus. ^{1}H NMR spectra were recorded on a Bruker ACF-400 spectrometer with $CDCl_3$ as solvent unless otherwise specified. ^{13}C NMR spectra were measured on a Bruker ACF-400 spectrometer at 100 MHz with $CDCl_3$ as solvent. The chemical shifts (δ) are reported in ppm relative to the residual undeuterated solvent signal, and coupling constants (J) are given in Hz. IR spectra were measured with a Nicolet FT-IR 5DX spectrometer by attenuated total reflection Fourier transform infrared (ATR-FTIR) spectroscopy. Mass spectra (EI) were recorded with a VG ZAB-HS spectrometer. Elemental analyses were obtained using a Heraeus CHN-O-Rapid analyzer, and results agreed with calculated values.

For X-ray crystallographic analysis, the X-ray diffraction intensities and the unit cell parameters were determined on a Bruker SMART APEXII CCD diffractometer employing graphite-monochromated (Mo-Kα) radiation ($\lambda = 0.71073$ Å) and operating in the $\omega/2\theta$ scan mode. Data collection and cell refinement were performed with APEX2 software. Structures were solved by direct methods and refined by full-matrix least-squares on F^2 with SHELXTL. Non-hydrogen atoms were refined by anisotropic displacement parameters, and the positions of all hydrogen atoms were fixed geometrically and included in estimated positions using a riding model. Crystallographic data (excluding structure factors) for the structures in this paper have been deposited with the Cambridge Crystallographic Data Centre as supplementary publication numbers CCDC 745819 and 745820. Copies of the data can be obtained, free of charge, on application to CCDC, 12 Union Road, Cambridge, CB2 1EZ, UK (fax: +44 1223 336033 or e-mail: deposit@ccdc. cam.ac.uk).

*General procedure for the synthesis of **1a–1c***

A mixture of 9.9 g 4-methylthiazole (100 mmol) and 16.3 g 2-bromo-1-cyclopropylethanone (100 mmol) in 50 cm^3 EtOAc was stirred at room temperature for 0.5 h. After it stood for another 48 h, the precipitated solid was collected and rinsed with 50 cm^3 EtOAc to give **1a**. Salts **1b** and **1c** were prepared by the same procedure and directly used in the next step without any further purification.

*General procedure for the synthesis of **3a–3i***

A mixture of the thiazolium salt (10 mmol), olefin (40 mmol), 2 cm^3 triethylamine, and 4 g TPCD in 40 cm^3 DMF was heated at 90 °C for 5–10 h. The reaction course was monitored by TLC. After the reaction was completed, the solution was cooled and the reaction mixture was poured into an aqueous hydrochloric acid solution (5%, 100 cm^3). The precipitated crude product was collected by filtration and further purified by silica gel column chromatography with petroleum ether (b.p. 60–90 °C)/ethyl acetate as eluents (gradient elution).

*General procedure for the synthesis of **5a–5e***

A mixture of 1-methyl-1*H*-imidazole (10 mmol), bromopropanone (10 mmol), olefin (40 mmol), 2 cm^3 triethylamine, and 4 g TPCD in 40 cm^3 DMF was heated at 90 °C for 5–10 h. The reaction course was monitored by TLC. After the reaction was completed, the solution was cooled and the reaction mixture was poured into an aqueous hydrochloric acid solution (5%, 100 cm^3). The precipitated crude product was collected by filtration and further purified by silica gel column chromatography with petroleum ether (b.p. 60–90 °C)/ethyl acetate as eluents (gradient elution).

3-(2-Cyclopropyl-2-oxoethyl)-4-methylthiazol-3-ium bromide (**1a**, $C_9H_{12}NOS$)
White powder; IR (ATR): $\bar{\nu} = 3{,}131,\ 1{,}660$ cm^{-1}. ^{1}H NMR (D_2O, 400 MHz): $\delta = 1.32$ (t, 2H, $J = 2.0$ Hz), 1.32 (t, 2H, $J = 3.2$ Hz), 2.44 (t, 1H, $J = 2.9$ Hz), 2.54 (s, 3H), 4.80 (s, 2H), 5.94 (s, 1H), 7.99 (s, 1H) ppm; ^{13}C NMR (D_2O, 100 MHz): $\delta = 12.2, 13.1, 36.2, 60.5, 120.6, 146.9, 159.4, 202.2$ ppm.

4-Methyl-3-(2-oxopropyl)thiazol-3-ium bromide (**1b**, $C_7H_{10}NOS$)
White powder; IR (ATR): $\bar{\nu} = 3{,}016,\ 1{,}723$ cm^{-1}. ^{1}H NMR (D_2O, 400 MHz): $\delta = 2.55$ (s, 6H), 5.79 (s, 2H), 8.00 (s, 1H), 9.89 (s, 1H) ppm; ^{13}C NMR (D_2O, 100 MHz): $\delta = 12.1, 19.1, 60.4, 120.5, 146.9, 159.4, 204.6$ ppm.

4-Methyl-3-(2-oxo-2-phenylethyl)thiazol-3-ium bromide (**1c**) [29]
White needles. ^{1}H NMR (D_2O, 400 MHz): $\delta = 2.59$ (s, 3H), 4.80 (s, 2H), 6.41 (d, 1H, $J = 7.6$ Hz), 7.77 (t, 2H, $J = 7.6$ Hz), 7.94 (t, 1H, $J = 7.2$ Hz), 8.06 (s, 1H), 8.22 (d, 2H, $J = 7.6$ Hz) ppm; ^{13}C NMR (D_2O, 100 MHz): $\delta = 12.2$, 58.4, 120.7, 128.5, 129.2, 132.8, 135.6, 147.2, 192.2 ppm.

5-Cyclopropylcarbonyl-3-methylpyrrolo[2,1-b]thiazole-7-carbonitrile (**3a**, $C_{12}H_{10}N_2OS$)
Yellow powder; m.p.: 158–159 °C; IR (ATR): $\bar{\nu} = 3{,}127$, 3,010, 2,219, 1,657, 1,452, 965, 735 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$): $\delta = 0.99$–1.04 (m, 2H), 1.16–1.20 (m, 2H), 2.40–2.46 (m, 1H), 2.65 (s, 3H), 6.59 (s, 1H), 7.67 (s, 1H) ppm; ^{13}C NMR (100 MHz, $CDCl_3$): $\delta = 10.8$, 17.1, 18.3, 83.7, 110.8, 114.8, 127.0, 128.3, 136.0, 146.6, 187.7 ppm; MS: *m/z* (%) = 230 (M^+, 100), 189 (44), 175 (7), 161 (8).

Methyl 5-cyclopropylcarbonyl-3-methylpyrrolo[2,1-b]-thiazole-7-carboxylate (**3b**, $C_{13}H_{13}NO_3S$)
Yellow powder; m.p.: 176 °C; IR (ATR): $\bar{\nu} = 3{,}086$, 2,932, 1,708, 1,643, 1,223, 966, 765, 734 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$): $\delta = 0.97$ (s, 2H), 1.15 (s, 2H), 2.50 (s, 1H), 2.67 (s, 3H), 3.91 (s, 3H), 6.56 (s, 1H), 7.86 (s, 1H) ppm; ^{13}C NMR (100 MHz, $CDCl_3$): $\delta = 10.4$, 17.1, 18.0, 51.6, 105.6, 111.2, 125.1, 128.4, 135.7, 146.2, 163.8, 188.0 ppm; MS: *m/z* (%) = 263 (M^+, 100), 222 (39), 204 (14), 164 (7).

Diethyl 5-cyclopropylcarbonyl-3-methylpyrrolo[2,1-b]-thiazole-6,7-dicarboxylate (**3c**, $C_{15}H_{15}NO_5S$)
Yellow powder; m.p.: 118–119 °C; IR (ATR): $\bar{\nu} = 2{,}957$, 2,360, 1,720, 1,662, 1,219, 739 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$): $\delta = 0.98$–1.03 (m, 2H), 1.22–1.26 (m, 2H), 2.37 (d, 1H, $J = 3.9$ Hz), 2.45 (d, 3H, $J = 0.8$ Hz), 3.90 (s, 3H), 3.97 (s, 3H), 6.62 (d, 1H, $J = 0.8$ Hz) ppm; ^{13}C NMR (100 MHz, $CDCl_3$): $\delta = 11.9$, 16.4, 20.1, 51.8, 53.0, 103.5, 112.1, 125.7, 128.1, 134.7, 143.9, 162.7, 166.0, 190.0 ppm; MS: *m/z* (%) = 321 (M^+, 100), 289 (92), 258 (23), 203 (32), 175 (23).

X-ray structure analysis: $C_{15}H_{15}NO_5S$, $M = 243.25$. Orthorhombic, space group *Iba*2, $a = 10.9785(16)$ Å, $b = 34.405(6)$ Å, $c = 8.0074(13)$ Å, $\alpha = 90°$, $\beta = 90°$, $\gamma = 90°$, $V = 3024.6(8)$ Å^3, $Z = 8$, $D_c = 1.411$ g cm^{-3}, $F(000) = 1{,}344.0$, absorption coefficient 0.237 mm^{-1}, scan range for data collection $1.93 \leq \theta \leq 27.52°$, 9,467 measured reflections, 3,028 independent reflections, 2,248 reflections with $I > 2\sigma(I)$, $R_{int} = 0.0262$, 200 refinable parameters, $R[F^2 > 2\sigma(F^2)] = 0.0361$, $wR_2(F^2) = 0.0990$.

5-Acetyl-3-methylpyrrolo[2,1-b]thiazole-7-carbonitrile (**3d**, $C_{10}H_8N_2OS$)
Yellow powder; m.p.: 198–199 °C; IR (ATR): $\bar{\nu} = 3{,}133$, 3,098, 2,221, 1,652, 952, 734 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$): $\delta = 2.50$ (s, 3H), 2.72 (s, 3H), 6.60 (s, 1H), 7.51 (s, 1H) ppm; ^{13}C NMR (100 MHz, $CDCl_3$): $\delta = 17.4$, 27.3, 83.9, 110.9, 114.6, 127.9, 136.3, 147.1 ppm; MS: *m/z* (%) = 204 (M^+, 25), 189 (100), 161 (8).

Methyl 5-acetyl-3-methylpyrrolo[2,1-b]thiazole-7-carboxylate (**3e**, $C_{11}H_{11}NO_3S$)
Yellow powder; m.p.: 142–144 °C; IR (ATR): $\bar{\nu} = 3{,}100$, 2,949, 2,362, 1,702, 1,665, 1,221, 943, 758, 705 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$): $\delta = 2.50$ (s, 3H), 2.73 (d, 3H, $J = 0.8$ Hz), 3.90 (s, 3H), 6.56 (s, 1H), 7.69 (d, 1H, $J = 1.2$ Hz) ppm; ^{13}C NMR (100 MHz, $CDCl_3$): $\delta = 17.4$, 27.1, 51.6, 105.6, 111.3, 125.9, 127.9, 135.9, 146.7, 163.6, 185.5 ppm; MS: *m/z* (%) = 237 (M^+, 43), 222 (100), 206 (12).

Diethyl 5-acetyl-3-methylpyrrolo[2,1-b]thiazole-6,7-dicarboxylate (**3f**, $C_{15}H_{17}NO_5S$)
Yellow powder; m.p.: 114–115 °C; IR (ATR): $\bar{\nu} = 3{,}104$, 2,923, 1,727, 1,696, 1,662, 1,421, 1,208, 966, 778 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$): $\delta = 1.37$ (t, 3H, $J = 7.0$ Hz), 1.43 (t, 3H, $J = 7.2$ Hz), 2.47 (s, 3H), 2.58 (s, 3H), 4.35 (q, 2H, $J = 7.1$ Hz), 4.47 (q, 2H, $J = 7.2$ Hz), 6.62 (s, 1H) ppm; ^{13}C NMR (100 MHz, $CDCl_3$): $\delta = 14.0$, 14.4, 17.1, 28.2, 60.6, 62.4, 103.9, 112.3, 125.1, 129.6, 135.6, 145.1, 162.1, 165.6, 186.1 ppm; MS: *m/z* (%) = 323 (M^+, 31), 277 (47), 234 (22), 177 (100).

5-Benzoyl-3-methylpyrrolo[2,1-b]thiazole-7-carbonitrile (**3g**, $C_{15}H_{10}N_2OS$)
Yellow powder; m.p.: 177–179 °C; IR (ATR): $\bar{\nu} = 3{,}087$, 2,925, 2,225, 1,628, 910, 709 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$): $\delta = 2.66$ (s, 3H), 6.69 (s, 1H), 7.24 (s, 1H), 7.52 (t, 2H, $J = 7.6$ Hz), 7.63 (t, 1H, $J = 7.4$ Hz), 7.91 (d, 2H, $J = 7.6$ Hz) ppm; ^{13}C NMR (100 MHz, $CDCl_3$): $\delta = 16.5$, 83.8, 111.0, 114.7, 126.6, 128.5, 129.78, 129.82, 132.9, 135.6, 137.9, 146.6, 182.5 ppm; MS: *m/z* (%) = 266 (M^+, 100), 265 (75), 189 (19), 105 (18), 77 (38).

Methyl 5-benzoyl-3-methylpyrrolo[2,1-b]thiazole-7-carboxylate (**3h**, $C_{16}H_{13}NO_3S$)
Yellow powder; m.p.: 139–141 °C; IR (ATR): $\bar{\nu} = 3{,}098$, 2,929, 1,696, 1,633, 1,246, 901, 700 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$): $\delta = 2.68$ (d, 3H, $J = 0.8$ Hz), 3.88 (s, 3H), 6.67 (d, 1H, $J = 0.8$ Hz), 7.41 (d, 1H, $J = 0.8$ Hz), 7.48–7.52 (m, 2H), 7.58-7.62 (m, 1H), 7.91 (d, 2H,

$J = 1.6$ Hz) ppm; ^{13}C NMR (100 MHz, $CDCl_3$): $\delta = 16.6$, 29.7, 51.6, 105.8, 111.4, 126.9, 128.1, 128.4, 129.8, 132.4, 135.4, 138.6, 146.4, 163.7, 183.1 ppm; MS: *m/z* (%) = 299 (M^+, 100), 298 (24), 268 (19), 222 (12), 105 (11), 77 (20).

5-Benzoyl-3-methylpyrrolo[2,1-b]thiazole-7-carboxylic acid (**3h′**, $C_{15}H_{11}NO_3S$)
Yellow powder; m.p.: 115–117 °C; IR (ATR): $\bar{\nu} = 3{,}262$, 2,955, 1,726, 1,629, 1,556, 1,215, 759, 698 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$): $\delta = 3.85$ (s, 3H), 7.31 (q, 1H, $J = 1.3$ Hz), 7.49–7.53 (m, 2H), 7.59–7.63 (m, 1H), 7.72 (q, 1H, $J = 1.6$ Hz), 7.91–7.94 (m, 2H), 10.11 (s, 1H) ppm; ^{13}C NMR (100 MHz, $CDCl_3$): $\delta = 51.46$, 51.51, 118.5, 119.3, 128.57, 128.63, 128.7, 129.0, 131.5, 132.5, 137.4, 164.3, 185.0 ppm; MS: *m/z* (%) = 241 (M^+ − COOH, 0.14), 229 (100), 198 (86), 170 (19), 152 (20), 120 (41), 105 (13), 77 (21).

5-Cyclopropylcarbonyl-3-methyl-7-phenyl-6H-pyrrolo-[3′,4′:3,4]pyrrolo-[2,1-b]thiazole-6,8(7H)-dione (**3i**, $C_{19}H_{14}N_2O_3S$)
Yellow powder; m.p.: 177–179 °C; IR (ATR): $\bar{\nu} = 2{,}925$, 2,853, 1,757, 1,699, 1,656, 895, 761, 730, 699 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$): $\delta = 1.12$–1.17 (m, 2H), 1.22–1.26 (m, 2H), 2.59 (d, 3H, 1.2), 3.37–3.43 (m, 1H), 6.71 (d, 1H, $J = 1.2$ Hz), 7.37–7.42 (m, 3H), 7.47–7.51 (m, 2H) ppm; ^{13}C NMR (100 MHz, $CDCl_3$): $\delta = 12.7$, 17.0, 20.7, 108.9, 112.9, 124.7, 127.1, 128.0, 129.0, 130.2, 132.3, 135.6, 136.4, 161.5, 163.2, 189.8 ppm; MS: *m/z* (%) = 350 (M^+, 100), 322 (18), 258 (14), 203 (14).

5-Cyclopropylcarbonyl-1-methyl-1H-pyrrolo[1,2-a]-imidazole-7-carbonitrile (**5a**, $C_{12}H_{11}N_3O$)
Yellow powder; m.p.: 168–170 °C; IR (ATR): $\bar{\nu} = 3{,}163$, 2,919, 2,207, 1,620, 1,604, 1,498, 702 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$): $\delta = 0.95$ (q, 2H, $J = 3.6$ Hz), 1.17 (t, 2H, $J = 3.6$ Hz), 2.29–2.35 (m, 1H), 3.88 (s, 3H), 6.84 (s, 1H), 7.47 (s, 1H), 7.94 (s, 1H) ppm; ^{13}C NMR (100 MHz, $CDCl_3$): $\delta = 9.9$, 16.5, 33.5, 67.0, 111.5, 116.2, 119.6, 122.3, 124.2, 140.6, 187.7 ppm; MS: *m/z* (%) = 213 (M^+, 100), 172 (38), 144 (31).

1-Cyclopropylcarbonylindolizine-3-carbonitrile (**5a′**, $C_{13}H_{10}N_2O$)
White crystals; m.p.: 163–164 °C; IR (ATR): $\bar{\nu} = 3{,}114$, 2,924, 2,213, 1,637, 1,628, 942, 758 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$): $\delta = 1.04$ (s, 2H), 1.25 (s, 2H), 2.51 (s, 1H), 7.06 (s, 1H), 7.42 (s, 1H), 7.79 (s, 1H), 7.94 (s, 1H), 9.90 (br, 1H) ppm; ^{13}C NMR (100 MHz, $CDCl_3$): $\delta = 10.7$, 18.2, 84.4, 115.3, 115.6, 117.4, 123.8, 125.7, 127.2, 129.5, 140.7, 189.5 ppm; MS: *m/z* (%) = 210 (M^+, 100), 169 (46), 141 (15), 114 (8).

Methyl 5-cyclopropylcarbonyl-1-methyl-1H-pyrrolo-[1,2-a]imidazole-7-carboxylate (**5b**, $C_{13}H_{14}N_2O_3$)
Yellow powder; m.p.: 115–117 °C; IR (ATR): $\bar{\nu} = 2{,}949$, 1,689, 1,611, 1,496, 1,203, 1,089, 686 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$): $\delta = 0.89$–0.93 (m, 2H), 1.14–1.17 (m, 2H), 2.39 (d, 1H, $J = 3.9$ Hz), 3.84 (s, 3H), 4.12 (s, 3H), 6.77 (d, 1H, $J = 0.8$ Hz), 7.69 (s, 1H), 7.94 (d, 1H, $J = 2.4$ Hz) ppm; ^{13}C NMR (100 MHz, $CDCl_3$): $\delta = 9.5$, 16.3, 35.6, 51.0, 92.5, 110.9, 118.8, 122.6, 124.0, 140.1, 164.1, 187.8 ppm; MS: *m/z* (%) = 246 (M^+, 100), 215 (7), 205 (25).

Methyl 1-cyclopropylcarbonylindolizine-3-carboxylate (**5b′**, $C_{14}H_{13}NO_3$)
White powder; m.p.: 135–136 °C; IR (ATR): $\bar{\nu} = 3{,}113$, 2,951, 1,697, 1,618, 756 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$): $\delta = 0.97$–1.02 (m, 2H), 1.21–1.25 (m, 2H), 2.56–2.62 (m, 1H), 3.94 (s, 3H), 6.99 (t, 1H, $J = 7.0$ Hz), 7.38 (t, 1H, $J = 7.8$ Hz), 8.17 (s, 1H), 8.34 (d, 1H, $J = 9.2$ Hz), 9.90 (d, 1H, $J = 6.8$ Hz) ppm; ^{13}C NMR (100 MHz, $CDCl_3$): $\delta = 10.3$, 18.0, 51.3, 105.4, 115.0, 119.2, 123.3, 125.4, 127.0, 129.1, 139.2, 164.5, 189.8 ppm; MS: *m/z* (%) = 243 (M^+, 100), 212 (10), 202 (19).

X-ray structure analysis: $C_{14}H_{13}NO_3$, $M = 243.25$. Triclinic, space group $P\bar{1}$, $a = 7.2548(6)$ Å, $b = 8.2019(8)$ Å, $c = 11.0324(10)$ Å, $\alpha = 89.636(7)$, $\beta = 71.956(6)$, $\gamma = 70.945(6)$, $V = 586.69(9)$ Å^3, $Z = 2$, $D_c = 1.377$ g cm^{-3}, $F(000) = 256.0$, absorption coefficient 0.098 mm^{-1}, scan range for data collection $1.95 \leq \theta \leq 27.62°$, 9,170 measured reflections, 2,715 independent reflections, 1,821 reflections with $I > 2\sigma(I)$, $R_{int} = 0.0262$, 163 refinable parameters, $R[F^2 > 2\sigma(F^2)] = 0.0452$, $wR_2(F^2) = 0.1367$.

5-Acetyl-1-methyl-1H-pyrrolo[1,2-a]imidazole-7-carbonitrile (**5c**, $C_{10}H_9N_3O$)
Yellow powder; m.p.: 188–190 °C; IR (ATR): $\bar{\nu} = 3{,}107$, 2,203, 1,626, 1,501, 703 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$): $\delta = 2.40$ (s, 3H), 3.89 (s, 3H), 6.85 (q, 1H, $J = 0.9$ Hz), 7.33 (d, 1H, $J = 1.2$ Hz), 7.96 (d, 1H, $J = 2.4$ Hz) ppm; ^{13}C NMR (100 MHz, $CDCl_3$): $\delta = 25.0$, 33.5, 67.1, 111.6, 116.0, 119.3, 122.3, 125.0, 185.4 ppm; MS: *m/z* (%) = 187 (M^+, 60), 172 (100), 144 (74).

1-Acetylindolizine-3-carbonitrile (**5c′**, $C_{11}H_8N_2O$)
White powder; m.p.: 199–201 °C; IR (ATR): $\bar{\nu} = 3{,}116$, 2,925, 2,218, 1,647, 1,206, 753 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$): $\delta = 2.59$ (s, 3H), 7.08–7.11 (m, 1H), 7.45 (dd, 1H, $J = 8.4$, 7.2 Hz), 7.78 (d, 1H, $J = 1.6$ Hz), 7.81 (s, 1H), 9.90 (d, 1H, $J = 7.2$ Hz) ppm; ^{13}C NMR (100 MHz, $CDCl_3$): $\delta = 27.3$, 84.3, 115.2, 115.8, 117.4, 123.2,

126.4, 127.5, 129.5, 140.8, 187.4 ppm; MS: *m/z* (%) = 184 (M^+, 31), 169 (100), 141 (23), 114 (6).

Methyl 5-acetyl-1-methyl-1H-pyrrolo[1,2-a]imidazole-7-carboxylate (**5d**, $C_{11}H_{12}N_2O_3$)
Yellow powder; m.p.: 158–160 °C; IR (ATR): $\bar{\nu}$ = 3,093, 2,955, 1,686, 1,619, 1,198, 691 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): δ = 2.42 (s, 3H), 3.84 (s, 3H), 4.12 (s, 3H), 6.79 (s, 1H), 7.55 (s, 1H), 7.96 (d, 1H, J = 2.0 Hz) ppm; ^{13}C NMR (100 MHz, $CDCl_3$): δ = 24.9, 35.6, 51.1, 92.7, 111.0, 118.4, 122.7, 125.0, 140.2, 164.0, 185.5 ppm; MS: *m/z* (%) = 220 (M^+, 100), 205 (81), 189 (21), 177 (8).

Methyl 1-acetylindolizine-3-carboxylate (**5d′**, $C_{12}H_{11}NO_3$)
White crystals; m.p.: 151–152 °C; IR (ATR): $\bar{\nu}$ = 3,116, 2,952, 1,694, 1,623, 1206, 745 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): δ = 2.60 (s, 3H), 3.94 (s, 3H), 7.02–7.06 (m, 1H), 7.39–7.43 (m, 1H), 8.01 (s, 1H), 8.35 (d, 1H, J = 9.2 Hz), 9.90–9.92 (m, 1H) ppm; ^{13}C NMR (100 MHz, $CDCl_3$): δ = 27.2, 51.2, 105.4, 115.2, 119.3, 122.8, 126.1, 127.2, 129.1, 139.3, 164.4, 187.8 ppm; MS: *m/z* (%) = 217 (M^+, 100), 202 (86), 186 (67), 174 (8).

Diethyl 5-acetyl-1-methyl-1H-pyrrolo[1,2-a]imidazole-6,7-dicarboxylate (**5e**, $C_{15}H_{18}N_2O_5$)
Yellow powder; m.p.: 78–80 °C; IR (ATR): $\bar{\nu}$ = 2,984, 1,724, 1,689, 1,628, 1,200, 730, 694 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): δ = 1.34 (t, 3H, J = 7.2 Hz), 1.43 (t, 3H, J = 7.2 Hz), 2.38 (s, 3H), 4.08 (s, 3H), 4.29 (q, 2H, J = 7.1 Hz), 4.44 (q, 2H, J = 7.2 Hz), 6.81 (d, 1H, J = 2.0 Hz), 8.03 (d, 1H, J = 2.4 Hz) ppm; ^{13}C NMR (100 MHz, $CDCl_3$): δ = 14.1, 14.3, 26.4, 36.0, 60.2, 62.1, 91.1, 111.0, 115.6, 123.4, 129.4, 138.4, 162.6, 166.4, 185.5 ppm; MS: *m/z* (%) = 306 (M^+, 100), 261 (7), 217 (15), 189 (33).

Diethyl 1-acetylindolizine-2,3-dicarboxylate (**5e′**, $C_{16}H_{17}NO_5$)
White crystals; m.p.: 65–67 °C; IR (ATR): $\bar{\nu}$ = 2,988, 1,731, 1,693, 1,626, 1,507, 1,197, 784, 757 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): δ = 1.41 (t, 3H, J = 7.0 Hz), 1.45 (t, 3H, J = 7.2 Hz), 2.55 (s, 3H), 4.39 (q, 2H, J = 7.1 Hz), 4,51 (q, 2H, J = 7.2 Hz), 7.06–7.09 (m, 1H), 7.42–7.46 (m, 1H), 8.38 (d, 1H, J = 9.2 Hz), 9.98 (d, 1H, J = 7.2 Hz) ppm; ^{13}C NMR (100 MHz, $CDCl_3$): δ = 14.0, 14.4, 28.5, 60.5, 62.5, 104.0, 116.1, 119.7, 119.9, 128.1, 129.3, 131.6, 137.9, 162.9, 166.8, 187.7 ppm; MS: *m/z* (%) = 303 (M^+, 100), 258 (15), 230 (17), 214 (41), 186 (43).

Antiproliferative activities assay

The antiproliferative activities of the pyrrolo[2,1-*b*]thiazole and pyrrolo[1,2-*a*]imidazole derivatives were determined using a standard MTT-based colorimetric assay (Sigma). Briefly, cell lines were seeded at a density of 7×10^3 cells/well in 96-well microtiter plates (Costar). After 12 h, exponentially growing cells were exposed to the indicated compounds at final concentrations ranging from 0.1 to 40 $\mu g/cm^3$. After 48 h, cell survival was determined by the addition of an MTT solution (25 mm^3 of 4 mg/cm^3 MTT in PBS). After 6 h, 100 mm^3 of 10% SDS in 0.01 N HCl was added, and the plates were incubated at 37 °C for a further 12 h; optical absorbance was measured at 570 nm on an LX300 Epson Diagnostic microplate reader. Survival ratios are expressed in percentages with respect to untreated cells. IC_{50} values were determined from replicates of six wells from at least two independent experiments.

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