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Synthesis and *in vitro* cytotoxic evaluation of 2-hydrazinylpyrido[2,3-*b*]pyrazin-3(4*H*)-one derivatives

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Abstract

A series of novel 2-hydrazinylpyrido[2,3-*b*]pyrazin-3(4*H*)-one derivatives were synthesized and evaluated for their cytotoxic activities against A549, MDA-MB-231 and HT-29 cell lines *in vitro*. Pharmacological data indicated that compounds **5b**, **5c**, **10a** and **10g** possessed marked cytotoxicity, especially **10a** (with IC_{50} values of 0.81, 2.56 and 1.63 μmol/L against A549, MDA-MB-231 and HT29 cell lines, respectively), which had emerged as a lead compound.

Keywords: 2-Hydrazinylpyrido[2,3-*b*]pyrazin-3(4*H*)-ones; Synthesis; Cytotoxicity

Cancer is a major worldwide health problem. According to the American Cancer Society, 7.6 million people died from cancer in the world during 2008 [1]. In order to develop more effective and reliable anticancer agents, a large number of compounds bearing nitrogen-containing fused heterocyclics keletons, such as quinoxalines, pyrrolopyrimidines, 4-anilinoquinazolines, pyridopyrimidines, and pyrazolopyridazines, have been reported and many of them exhibited excellent anticancer activity [2–6].

Recently, pyrido[2,3-*b*]pyrazin-3(4*H*)-ones have aroused increasing attentions from chemical and biological view points since they were proved to be the promising anticancer agents with mechanisms of BRAF inhibition [7]. On another hand, compounds containing arylhydrazine were reported for their good cytotoxicity [8,9], which have inspired us largely to develop the related derivatives. With an aim to develop potent pyrido[2,3-*b*]pyrazin-3(4*H*)-one derivatives a series of new molecules containing various arylhydrazones on C-2 position of the scaffold were designed and synthesized. Further modifications were performed by introducing phenyl or 4-trifluoromethoxyl-phenyl group into *N*-4 position on the pyrido[2,3-*b*]pyrazin-3(4*H*)-one core. In this paper, we would like to report the synthesis and cytotoxicity of a series of novel 2-hydrazinylpyrido[2,3-*b*]pyrazin-3(4*H*)-ones represented by the generable structures of **5a**–**5g** and **10a**–**10g**.

The title 2-hydrazinylpyrido[2,3-*b*]pyrazin-3(4*H*)-one derivatives **5a**–**5g** and **10a**–**10g** were synthesized as shown in Scheme 1. The commercially available 2-chloro-3-nitropyridine and aniline were treated with *N*,*N*-diisopropylethylamine in isopropanol to give the compound **1**. Next, reduction of **1** with zinc powder was carried out in 95% ethanol at reflux to afford the N^2-phenylpyridine-2,3-diamine intermediate, which was converted to **2** by a

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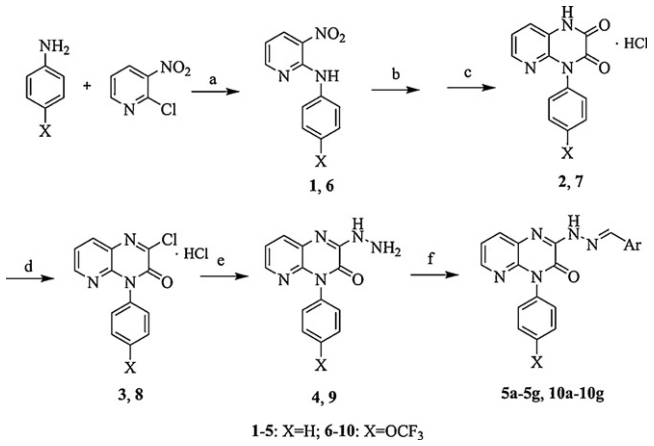

Scheme 1. Reagents and conditions: (a) DIPEA/MeOH, r.t., 1 h, 60 °C, 8 h, yield: 53–75%; (b) zinc powder/NH_4Cl/EtOH, r.f., 5 h; (c) oxalic acid/4 mol/L HCl, r.f., 15 h, yield: 33–52%; (d) $POCl_3$, r.f., 3 h, yield: 75–82%; (e) 80% $NH_2NH_2{\cdot}H_2O$, 60 °C, 1 h, yield: 85–91%; (f) EtOH, 60 °C, 5 h, yield: 45–65%.

cyclization reaction with oxalic acid. Subsequent treatment of **2** with phosphorus oxychloride and acetonitrile afforded intermediate **3** [10], which was reacted with an excess of 80% hydrazine hydrate in ethanol to furnish **4**. Another important intermediate **9** was obtained according to the same method as described for compound **4** when aniline was replaced by 4-(trifluoromethoxy)aniline, respectively. Finally, the target compounds **5a–5g** and **10a–10g** were successfully obtained *via* the reaction of intermediate **4** and **9** with different aromatic aldehydes in the refluxing ethanol, respectively. The products were purified by silica gel column chromatography, using EtOAc/petroleum ether as eluent and the structures of the target compounds were confirmed by MS, ^{1}H NMR and ^{13}C NMR [11].

Cytotoxicity of compounds **5a–5g** and **10a–10h** against A549, MDA-MB-231 and HT29 cell lines were determined by MTT assay, procaspase activating compound **1** (PAC-1) [9], a well-known hydrazine-contain anticancer agent, as positive control and the results expressed as IC_{50} are summarized in Table 1. As shown in Table 1, compounds **5b**, **10a** and **10g** exhibited good cytotoxicity, *in vitro* against HT-29 cell lines. Compound **10a** was of particular interest because of its marked activity (IC_{50} values of 0.81, 1.56 and 2.63 μmol/L against A549, MDA-MB-231 and HT29 cell lines, respectively) and had emerged as a lead compound.

The data indicated that substituents on *N*-4 position of pyrido[2,3-*b*]pyrazin-3(4*H*)-one scaffold had a very important effect on antitumor activity, and variation of arylidene on hydrazine at C-2 position would optimize the

Table 1
Cytotoxicity of the tested compounds against A549, MDA-MB-231 and HT-29 cell lines *in vitro*.

Compd.	X	Ar	IC_{50} (μmol/L)		
			A549	MDA-MB-231	HT-29
5a	H	4-Fluorobenzyl	47.32 ± 5.28	14.76 ± 2.33	38.63 ± 3.92
5b	H	2-Hydroxynaphthalen-1-yl	1.69 ± 0.15	2.87 ± 0.32	3.45 ± 0.41
5c	H	4-Hydroxy-3-methoxybenzyl	25.25 ± 3.61	2.98 ± 0.45	6.81 ± 0.49
5d	H	1*H*-Pyrrol-2-yl	74.21 ± 6.84	11.26 ± 1.32	87.48 ± 8.74
5e	H	1*H*-Indol-3-yl	60.74 ± 7.13	14.75 ± 2.31	63.40 ± 8.10
5f	H	2,3,4-Trimethoxybenzyl	19.46 ± 2.03	9.58 ± 0.87	13.53 ± 2.16
5g	H	3-Nitrobenzyl	>100	>100	>100
10a	–OCF_3	2-Hydroxynaphthalen-1-yl	0.81 ± 0.09	1.56 ± 0.12	2.63 ± 0.35
10b	–OCF_3	3,4-Difluorobenzyl	>100	34.71 ± 3.59	22.8 ± 1.96
10c	–OCF_3	2,4-Dimethoxybenzyl	14.39 ± 1.87	18.84 ± 3.21	15.62 ± 2.11
10d	–OCF_3	4-(Methylsulfonyl)benzyl	80.01 ± 7.24	>100	>100
10e	–OCF_3	3-Hydroxy-4-methoxybenzyl	18.56 ± 1.78	11.04 ± 1.02	13.22 ± 1.54
10f	–OCF_3	Imidazo[1,2-*a*]pyridin-3-yl	22.38 ± 2.16	5.64 ± 0.77	81.47 ± 8.15
10g	–OCF_3	4-Hydroxy-3-methoxybenzyl	14.21 ± 0.19	1.52 ± 0.24	2.77 ± 0.31
PAC-1			0.66 ± 0.08	6.63 ± 0.58	1.64 ± 0.13

activity dramatically. Contrast to benzyl group, 4-(triflouromethyl)benzyl group was more potent substituent which produced the compounds with excellent activity. A case in point is that compound **5c** with benzyl group at *N*-4 position had lower activity, whereas compound **10g** bearing 4-triflouromethylbenzyl group provided about a 2-fold increase in potency against three tumor cell lines relative to the **5c**. On the other hand, introduction of heterocyclidene (*e.g.* 1*H*-pyrrol-2-ylmethylene, 1*H*-indol-3-ylmethylene, and imidazo[1,2-*a*]pyridine-3-yl-methylene) exhibited the selectivity of MDA-MB-231 cell line, while introduction of 2-hydroxynaphthalen-1-yl showed enhanced antitumor activity against all three cell lines. In addition, electron-donating group on benzylidene such as hydroxy and methoxy groups had good contributions to the anti-tumor activity. Compounds **10c**, **10e** and **10g** showed more potent cytotoxicity superior to the corresponding compounds bearing electron-withdraw group on the benzylidene.

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References

[1] A. Jemal, F. Bray, M.M. Center, et al. CA Cancer J. Clin. 61 (2011) 69.
[2] J. Porter, S. Lumb, F. Lecomte, et al. Bioorg. Med. Chem. Lett. 19 (2009) 397.
[3] Z. Wu, J.C. Hartnett, L.A. Neilson, et al. Bioorg. Med. Chem. Lett. 18 (2008) 1274.
[4] S. Madapa, Z. Tusi, A. Mishra, et al. Bioorg. Med. Chem. 17 (2009) 222.
[5] G.A. Gfesser, E.K. Bayburt, M. Cowart, et al. Eur. J. Med. Chem. 38 (2003) 245.
[6] J.F. Blake, N.C. Kallan, D. Xiao, et al. Bioorg. Med. Chem. Lett. 20 (2010) 5607.
[7] A. Zambon, D. Menard, B.M. Suijkerbuijk, et al. J. Med. Chem. 53 (2010) 5639.
[8] T. Horiuchi, J. Chiba, K. Uoto, et al. Bioorg. Med. Chem. Lett. 19 (2009) 305.
[9] Q.P. Peterson, D.C. Hsu, D.R. Goode, et al. J. Med. Chem. 52 (2009) 5721.
[10] B.D. Louis, O.D. Rhys, P. Christopher, et al., World Patent 2007020521 (C.A. 2007, 146:274402).
[11] Physical and spectral data for target compounds. **5a**: mp 168–170 °C; ESI-MS (*m/z*, %): 360.1 $(M+H)^+$; ^{1}H NMR (300 MHz, DMSO-d_6): δ 8.70 (s, 1H), 8.11 (dd, 1H, *J* = 4.7, 1.5 Hz); 8.03 (dd, 1H, *J* = 7.9, 1.5 Hz), 7.90 (dd, 2H, *J* = 8.6, 5.7 Hz), 7.51 (t, 2H, *J* = 7.3 Hz), 7.44 (t, 1H, *J* = 7.2 Hz), 7.39–7.30 (m, 4H), 7.24 (dd, 1H, *J* = 7.7, 4.7 Hz). **5b**: mp 173–175 °C; ESI-MS (*m/z*, %): 408.1 $(M+H)^+$; ^{1}H NMR (300 MHz, DMSO-d_6): δ 13.16 (s, 1H), 11.93 (s, 1H), 9.76 (s, 1H), 8.15 (m, 2H), 7.93 (m, 3H), 7.61 (t, 1H, *J* = 7.5 Hz), 7.51 (t, 2H, *J* = 7.4 Hz), 7.44–7.40 (m, 2H), 7.35 (d, 2H, *J* = 7.2 Hz), 7.27–7.22 (m, 2H). **5c**: mp 181–183 °C; ESI-MS (*m/z*, %): 388.1 $(M+H)^+$; ^{1}H NMR (300 MHz, DMSO-d_6): δ 11.28 (s, 1H), 9.60 (s, 1H), 8.52 (s, 1H), 8.02 (s, 1H), 7.94 (d, 1H, *J* = 7.6 Hz), 7.56 (t, 2H, *J* = 7.4 Hz), 7.48 (t, 1H, *J* = 7.3 Hz), 7.39 (d, 3H, *J* = 7.4 Hz), 7.25 (s, 1H), 7.17 (s, 1H), 6.87 (d, 1H, *J* = 8.1 Hz), 3.86 (s, 3H); ^{13}C NMR (75 MHz, DMSO-d_6): δ 153.57, 151.74, 149.93, 145.80, 143.53, 142.76, 138.82, 133.22, 129.62, 129.08, 128.91, 128.68, 128.40, 126.89, 126.22, 120.19, 117.36, 54.87. **5d**: mp 160–162 °C; ESI-MS (*m/z*, %): 331.1 $(M+H)^+$; ^{1}H NMR (300 MHz, DMSO-d_6): δ 11.56 (s, 1H), 8.45 (s, 1H), 8.00 (s, 1H), 7.84 (s, 1H), 7.56 (t, 2H, *J* = 7.3 Hz), 7.48 (t, 2H, *J* = 7.2 Hz), 7.38 (d, 1H, *J* = 7.1 Hz), 7.25 (dd, 1H, *J* = 7.7, 4.7 Hz), 7.02 (s, 1H), 6.54 (s, 1H), 6.19 (s, 1H). **5e**: mp 154–156 °C; ESI-MS (*m/z*, %): 381.1 $(M+H)^+$; ^{1}H NMR (300 MHz, DMSO-d_6): δ 11.64 (s, 1H), 8.80 (s, 1H), 8.47 (d, 1H, *J* = 5.9 Hz), 7.99 (m, 2H), 7.82 (m, 1H), 7.52 (m, 4H), 7.40 (d, 2H, *J* = 7.2 Hz), 7.34 (d, 1H, *J* = 7.1 Hz), 7.25 (m, 3H). **5f**: mp 156–158 °C; ESI-MS (*m/z*, %): 432.1 $(M+H)^+$; ^{1}H NMR (300 MHz, DMSO-d_6): δ 11.41 (s, 1H), 8.53 (s, 1H), 8.06 (dd, 1H, *J* = 4.6, 1.6 Hz), 7.95 (dd, 1H, *J* = 7.9, 1.6 Hz), 7.53 (d, 2H, *J* = 7.7 Hz), 7.48 (t, 1H, *J* = 7.4 Hz), 7.37 (d, 2H, *J* = 7.1 Hz), 7.26 (dd, 1H, *J* = 7.9, 4.7 Hz), 7.00 (s, 2H), 3.84 (s, 6H), 3.69 (s, 3H). **5g**: mp: 169–171 °C; ESI-MS (*m/z*, %): 387.1 $(M+H)^+$; ^{1}H NMR (300 MHz, DMSO-d_6): δ 11.49 (s, 1H), 8.79 (s, 1H), 8.61 (s, 1H), 8.29 (d, 1H, *J* = 8.2 Hz), 8.21 (d, 1H, *J* = 7.8 Hz), 8.12 (d, 1H, *J* = 3.2 Hz), 8.06 (d, 1H, *J* = 7.7 Hz), 7.79 (t, 1H, *J* = 8.0 Hz), 7.58 (t, 2H, *J* = 7.4 Hz), 7.51 (t, 1H, *J* = 7.2 Hz), 7.41 (d, 2H, *J* = 7.4 Hz), 7.31 (dd, 1H, *J* = 7.9, 4.7 Hz). **10a**: mp 179–181 °C; ESI-MS (*m/z*, %): 492.2 $(M+H)^+$; ^{1}H NMR (300 MHz, DMSO-d_6): δ 13.19 (s, 1H), 11.95 (s, 1H), 9.77 (s, 1H), 8.14 (d, 2H, *J* = 8.9 Hz), 7.95 (d, 2H, *J* = 9.0 Hz), 7.91 (d, 1H, *J* = 8.1 Hz), 7.62 (m, 5H), 7.42 (t, 1H, *J* = 7.4 Hz), 7.34 (dd, 1H, *J* = 7.6, 4.6 Hz), 7.27 (d, 1H, *J* = 8.9 Hz); ^{13}C NMR (75 MHz, DMSO): δ 160.57, 151.79, 149.37, 147.98, 145.63, 143.04, 137.10, 134.74, 132.98, 131.52, 131.16, 129.60, 129.00, 124.11, 121.68, 120.27, 113.46, 110.74, 107.66, 102.72. **10b**: mp 169–161 °C; ESI-MS (*m/z*, %): 462.1 $(M+H)^+$; ^{1}H NMR (300 MHz, DMSO-d_6): δ 11.62 (s, 1H), 8.61 (s, 1H), 8.11 (d, 1H, *J* = 4.5 Hz), 8.01 (d, 1H, *J* = 7.8 Hz), 7.80–7.73 (m, 2H), 7.58 (s, 4H), 7.53 (d, 1H, *J* = 4.7 Hz), 7.31 (dd, 1H, *J* = 7.8, 4.7 Hz); ^{13}C NMR (75 MHz, DMSO): δ 167.32, 151.85, 147.98, 146.08, 145.42, 143.41, 142.59, 134.75, 133.25, 131.62, 131.14, 128.84, 128.65, 124.26, 121.68, 120.26, 118.79, 118.19, 118.02, 115.16. **10c**: mp 187–190 °C; ESI-MS (*m/z*, %): 486.1 $(M+H)^+$; ^{1}H NMR (300 MHz, DMSO-d_6): δ 11.40 (s, 1H), 8.87 (s, 1H), 8.07 (s, 1H), 7.95 (m, 3H), 7.57 (m, 4H), 7.28 (s, 1H), 6.67 (d, 2H, *J* = 10.3 Hz), 3.87 (s, 3H), 3.84 (s, 3H). **10d**: mp 150–152 °C; ESI-MS (*m/z*, %): 504.1 $(M+H)^+$; ^{1}H NMR (300 MHz, DMSO-d_6): δ 8.74 (s, 1H), 8.12 (dd, 1H, *J* = 4.7, 1.5 Hz), 8.05 (d, 1H, *J* = 7.9 Hz), 8.03 (s, 4H), 7.59 (s, 4H), 7.32 (dd, 1H, *J* = 7.9, 4.7 Hz), 3.27 (s, 3H). **10e**: mp 161–163 °C ESI-MS (*m/z*, %): 472.1 $(M+H)^+$; ^{1}H NMR (300 MHz, DMSO-d_6): δ 11.29 (s, 1H), 9.35 (s, 1H), 8.49 (s, 1H), 8.08 (d, 1H, *J* = 4.2 Hz), 7.95 (d, 1H, *J* = 7.6 Hz), 7.57 (s, 4H), 7.33 (s, 1H), 7.29 (dd, 1H, *J* = 7.8, 4.7 Hz), 7.04 (d, 1H, *J* = 7.7 Hz), 6.98 (d, 1H, *J* = 8.3 Hz), 3.81 (s, 3H). **10f**: mp 160–162 °C; ESI-MS (*m/z*, %): 466.1 $(M+H)^+$; ^{1}H NMR (300 MHz, DMSO-d_6): δ 9.93 (d, 1H, *J* = 6.8 Hz), 8.95 (s, 1H), 8.22 (s, 1H), 8.09 (d, 1H, *J* = 4.3 Hz), 8.03 (d, 1H, *J* = 8.0 Hz), 7.86 (d, 1H, *J* = 9.0 Hz), 7.68 (t, 1H, *J* = 6.9 Hz), 7.59–7.56 (m, 5H), 7.43 (t, 1H, *J* = 6.8 Hz), 7.32 (dd, 1H, *J* = 7.8, 4.8 Hz). **10g**: mp 160–162 °C; ESI-MS (*m/z*, %): 472.1 $(M+H)^+$; ^{1}H NMR (300 MHz, DMSO-d_6): δ 11.29 (s, 1H), 9.58 (s, 1H), 8.52 (d, 1H, *J* = 7.2 Hz), 8.09 (s, 1H), 7.97 (d, *J* = 7.7 Hz, 1H), 7.58 (s, 4H), 7.32 (m, 2H), 7.15 (d, 1H, *J* = 17.1 Hz), 6.86 (d, 1H, *J* = 7.8 Hz), 3.87 (s, 3H).