Chinese Chemical Letters 24 (2013) 619–621

Contents lists available at SciVerse ScienceDirect

Chinese Chemical Letters

journal homepage: www.elsevier.com/locate/cclet

Original article

Synthesis and biological evaluation of new pyrrolopyrazinone compounds as potential antitumor agents

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ARTICLE INFO

Article history:
Received 6 March 2013
Received in revised form 24 March 2013
Accepted 28 March 2013
Available online 20 May 2013

Keywords:
Pyrrolopyrazinone
Antitumor
Synthesis

ABSTRACT

A series of pyrrolo[1,2-a]pyrazinone compounds (**5a**–**9f**) were synthesized, and their cytotoxic activity against SKOV-3, A549, HeLa cells *in vitro* were evaluated by the MTT method. Some of the compounds showed potential antitumor activity against three tumor cell lines. Among them, compounds **9c** and **9d** showed the most potent cytotoxic activity. The preliminary mechanism of action was discussed.

1. Introduction

Modification of natural product leads is an important approach to identify promising anticancer agents [1]. Pyrrolo[1,2-a]pyrazinone core is exclusively found in the natural products from marine sponges, mainly from the families *Agelasidae*, *Axinellidae* [2]. Longamide B and Palau'amine are two examples (Fig. 1). Longamide B methyl ester [3], which was isolated initially in racemic form from the sponge mentioned above in Japan, exhibits cytotoxic activity against the same leukemia cell line *in vitro* [4]. Palau'amine, isolated from the sponge *Stylotella aurantium* by Scheuer and co-workers [5], also exhibits cytotoxic properties [6].

Encouraged by the above results, recently we simplified the structure of these natural product, carried out the synthesis of a series of novel pyrrolo[1,2-a]pyrazinone compounds. We found several compounds with potent cytotoxicity, which was worthy of further investigation.

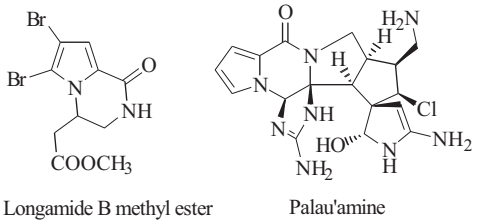
2. Experimental

As depicted in Scheme 1, the starting material pyrrole **1** was firstly converted to 2-(trichloroacetyl)pyrrole **2** by treatment with trichloroacetyl chloride in 62% yield. Then esterification of **2** using CH_3ONa in CH_3OH afforded methyl 2-pyrrolecarboxylate **3**, which was converted to 3-methyl-l*H*-pyrrolo[2,1-c][1,4]oxazin-l-one **4** by reacting with chloroacetone. The overall yield of these two steps from **2** to **4** was 56%. Then compound **4** was converted to **5a**–**5b** by treatment with the corresponding amine, and **5a**–**5b** were converted to **6a**–**6d** by treatment with the corresponding acyl chloride in DCM.

Methyl 2-pyrrolecarboxylate **3** was converted to methyl 1-(2-oxo-2-p-tolylethyl)-1*H*-pyrrole-2-carboxylate **7** by treatment with α-bromo-4-methoxyacetophenone in 76% yield, then compound **7** was converted to **8a**–**8b** by treatment with the corresponding amine, and **8a**–**8b** were converted to **9a**–**9d** by treatment with the corresponding acyl chloride in DCM. The structures of compounds **5a**–**9f** were characterized [7].

3. Results and discussion

All target compounds were evaluated for cytotoxicity against three cells *in vitro* by the MTT method, using 5-Fu as a control. Analysis of cytotoxicity presented in Table 1 for these compounds



Longamide B methyl ester Palau'amine

Fig. 1. The structure of Longamide B methyl ester and Palau'amine.

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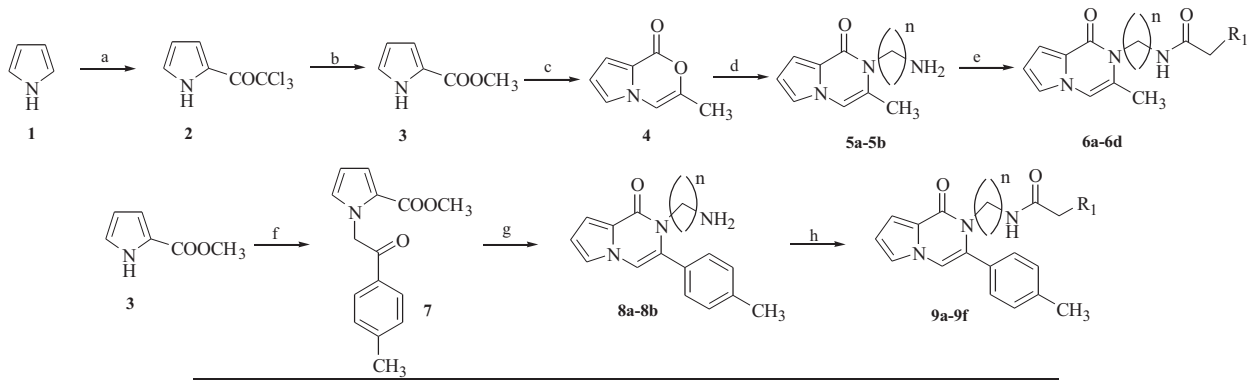
1001-8417/$ – see front matter
http://dx.doi.org/10.1016/j.cclet.2013.04.020



	5a	5b	6a	6b	6c	6d	8a	8b	9a	9b	9c	9d	9e	9f
n	0	2	0	2	0	2	0	2	0	2	0	2	0	2
R_1	-	-	H	H	Cl	Cl	-	-	H	H	Cl	Cl	CH_2Cl	CH_2Cl

Scheme 1. Reagents and conditions: (a) trichloroacetyl chloride, ether, r.t., (b) CH_3ONa, CH_3OH, r.t., (c) chloroacetone, acetone, r.t., (d) corresponding amine, CH_3CH_2OH, r.f., (e) corresponding acyl chloride, DCM, 0 °C, (f) α-bromo-4-methoxyacetophenone, K_2CO_3, DMF, r.t., (g) corresponding amine, CH_3CH_2OH, r.f., and (h) corresponding acyl chloride, DCM, 0 °C.

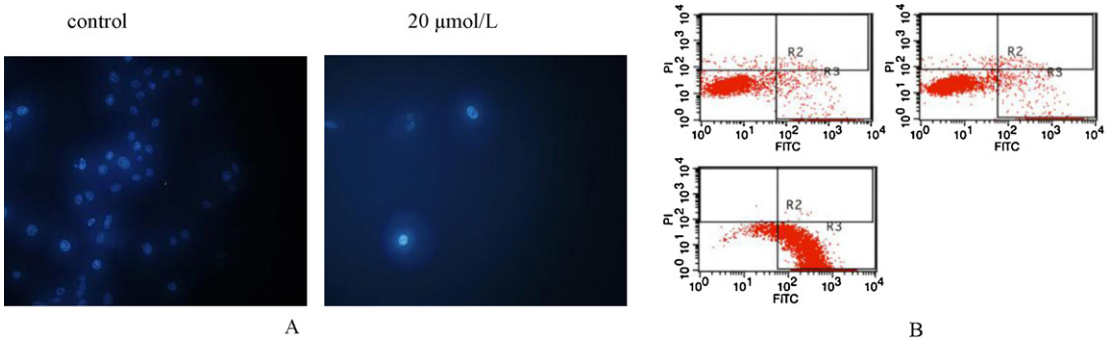


Fig. 2. Effects of compound **9c** on the induction of apoptosis in A549 cells. (A) Karyomorphism altered after treated with compound **9c** as revealed by the DNA-binding AT-specific fluorochrome 4′-6-diamidino-2-phenylindole (DAPI) fluorescence staining. (B) Annexin V-FITC was proceeded and the lower right quadrant represents early apoptosis.

and their structural features had revealed several patterns of structure-activity relationship. (1) Compounds **5a**, **5b** and **6a**–**6d** exhibited weak or no cytotoxicity compared to **8a**, **8b** and **9a**–**9f**, suggesting that the presence of large or hydrophobic groups at C3 enhanced their antitumor activity significantly in all three cancer cell lines. (2) Compounds with R_1 = Cl (**6c**, **6d**, **9c**, and **9d**) showed highest cytotoxicity, The chlorine atom was three atoms away from the nucleus in **6c** and **9c** and five atoms away from the nucleus in **6d** and **9d**, while the chlorine atom in Palau'amine was 4 atoms away from the nucleus, Comparing to the structure of Palau'amine a conclusion could be drawn that a certain length of chain with a chlorine atom might be essential for the antitumor activity. Moreover, it could be seen from Fig. 2 that compound **9c** inhibits cell proliferation by inducing apoptosis in A549 cells.

Table 1
IC_{50}s (μmol/L) of compounds against three tumor cell lines. The IC_{50} values of compounds against SKOV-3, A549, HeLa cells were evaluated by methylthiazoltetrazolium (MTT) assay. ND: not determined.

Compounds	SKOV-3	A549	HeLa
5a	>50	>50	>50
5b	>50	>50	>50
6a	>50	>50	>50
6b	>50	>50	>50
6c	43.803	49.239	44.117
6d	39.118	41.109	40.554
8a	22.107	26.551	29.332
8b	27.314	23.477	30.736
9a	>50	>50	>50
9b	>50	>50	>50
9c	6.908	8.967	5.551
9d	5.438	12.184	10.532
9e	34.157	29.639	35.682
9f	40.126	38.965	43.683
5-Fu	ND	86.934	61.438

4. Conclusion

In summary, a series of pyrrolo[1,2-a]pyrazinone derivatives were synthesized and evaluated for their *in vitro* cytotoxicity against SKOV-3, A549 and HeLa cells. DAPI staining and Annexin V/PI experiments showed that compound **9c** inhibits cell proliferation by inducing apoptosis in A549 cells. The study will provide valuable information for further research on the pyrazinone antitumor analogs.

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[7] Data for new compounds. **5a**: Yield 62%. Mp: 147–150 °C; ESI-MS: *m/z* 163.9 $[M+H]^+$; ^{1}H NMR (300 MHz, DMSO-d_6): δ 6.95 (s, 1H), 6.63 (d, 1H), 6.19–6.12 (m, 1H), 4.83 (s, 1H), 4.67 (s, 2H), 1.13 (s, 3H). **5b**: Yield 67%. Mp: 153–155 °C; ESI-MS: *m/z* 192.2 $[M+H]^+$; ^{1}H NMR (300 MHz, DMSO-d_6): δ 6.99–6.93 (m, 1H), 6.60 (dd, 1H), 6.13 (dd, 1H), 4.24 (d, 1H), 3.89 (d, 1H), 3.51 (dt, 1H), 3.36–3.24 (m, 2H), 3.13 (m, 2H), 1.17 (s, 3H). **6a**: Yield 31%. Mp: 144–146 °C; ESI-MS: *m/z* 205.9 $[M+H]^+$; ^{1}H NMR (300 MHz, DMSO-d_6): δ 7.32 (dd, 1H), 7.24 (s, 1H), 6.83–6.80 (m, 1H), 6.50 (dd, 1H), 5.37 (s, 1H), 2.20 (s, 3H), 1.13 (s, 3H). **6b**: Yield 27%. Mp: 182–185 °C; ESI-MS: *m/z* 234.2 $[M+H]^+$; ^{1}H NMR (300 MHz, DMSO-d_6): δ 7.33 (m, 1H), 7.25 (dd, 1H), 6.84 (dd, 1H), 6.50 (s, 1H), 4.06 (m, 2H), 3.50 (m, 2H), 2.24 (s, 3H), 1.23 (s, 3H). **6c**: Yield 36%. Mp: 176–178 °C; ESI-MS: *m/z* 261.9 $[M+Na]^+$; ^{1}H NMR (300 MHz, DMSO-d_6): δ 7.33 (dd, 1H), 7.24 (s, 1H), 6.84–6.79 (m, 1H), 6.50 (dd, 1H), 5.39 (s, 2H), 2.21 (s, 3H). **6d**: Yield 34%. Mp: 200–202 °C; ESI-MS: *m/z* 267.9 $[M+H]^+$; ^{1}H NMR (300 MHz, DMSO-d_6): δ 7.11–7.05 (m, 1H), 6.66 (dd, 1H), 6.14 (dd, 1H), 4.94 (d, 1H), 4.94 (d, 1H), 4.51–4.29 (m, 1H), 4.07 (dt, 1H), 3.99 (d, 1H), 3.95–3.78 (m, 1H), 3.73 (dd, 1H), 3.45 (dt, 1H), 1.40 (s, 3H). **8a**: Yield 73%. Mp: 139–141 °C; ESI-MS: *m/z* 240 $[M]^+$; ^{1}H NMR (300 MHz, DMSO-d_6): δ 7.44 (d, J = 8.2 Hz, 1H), 7.09 (s, 1H), 7.03 (d, J = 8.2 Hz, 2H), 6.66 (d, 1H), 6.55 (s, 1H), 5.93–5.88 (m, 1H), 5.50 (s, 2H), 2.20 (s, 3H). **8b**: Yield 78%. Mp: 191–193 °C; ESI-MS: *m/z* 268 $[M]^+$, ^{1}H NMR (300 MHz, DMSO-d_6): δ 7.11 (d, 2H, J = 8.2 Hz), 7.03 (d, 2H, J = 8.2 Hz), 6.72–6.67 (m, 1H), 6.56 (dd, 1H), 5.97 (dd, 1H), 4.55 (d, 1H), 4.30 (t, 1H), 4.23 (d, 1H), 3.54 (dt, 1H), 3.47–3.35 (m, 1H), 2.18 (s, 3H), 1.02 (t, 2H). **9a**: Yield 29%. Mp: 111–112 °C; ESI-MS: *m/z* 282 $[M+H]^+$; ^{1}H NMR (300 MHz, DMSO-d_6): δ 10.52 (s, 1H), 7.45 (dd, 1H), 7.37 (s, 1H), 7.27 (d, 2H, J = 8.1 Hz), 7.19 (d, 2H, J = 8.1 Hz), 6.96 (d, 1H, J = 3.9 Hz), 6.57 (dd, 1H, J = 3.9, 2.6 Hz), 2.31 (s, 3H), 1.71 (s, 3H). **9b**: Yield 31%. Mp: 194–197 °C; ESI-MS: *m/z* 310 $[M+H]^+$; ^{1}H NMR (300 MHz, DMSO-d_6): δ 7.18 (d, 2H, J = 8.3 Hz), 7.13–7.09 (m, 1H), 7.06 (d, 2H, J = 8.3 Hz), 6.60 (dd, 1H), 6.09 (dd, 1H), 5.62 (d, 1H, J = 13.0 Hz), 4.34 (d, 1H, J = 13.0 Hz), 4.22–4.10 (m, 1H), 3.94 (dd, 1H), 3.88–3.77 (m, 1H), 3.62 (dt, 1H), 2.21 (s, 3H), 2.04 (s, 3H). **9c**: Yield 23%. Mp: 110–113 °C; ESI-MS: *m/z* 316 $[M+H]^+$; ^{1}H NMR (300 MHz, DMSO-d_6): δ 7.90 (d, 2H, J = 8.2 Hz), 7.35 (d, 2H, J = 8.2 Hz), 7.13–7.08 (m, 1H), 6.87 (dd, 1H), 6.14 (dd, 1H), 5.81 (s, 2H), 3.57 (s, 2H), 2.37 (s, 3H). **9d**: Yield 23%. Mp: 191–193 °C; ESI-MS: *m/z* 343.9 $[M+H]^+$; ^{1}H NMR (300 MHz, DMSO-d_6): δ 7.15 (d, 2H, J = 8.3 Hz), 7.12–7.09 (m, 1H), 7.05 (d, 2H, J = 8.3 Hz), 6.59 (dd, 1H), 6.07 (dd, 1H), 5.60 (d, 1H), 4.40 (dt, 3H), 4.16 (dt, 1H), 4.03–3.80 (m, 2H), 3.62 (dt, 1H), 2.18 (s, 3H). **9e**: Yield 32%. Mp: 123–125 °C; ESI-MS: *m/z* 330 $[M+H]^+$; ^{1}H NMR (300 MHz, DMSO-d_6): δ 10.71 (s, 1H), 7.49–7.33 (m, 2H), 7.25 (d, 2H, J = 7.9 Hz), 7.17 (d, 2H, J = 7.9 Hz), 6.97 (s, 1H), 6.57 (s, 1H), 3.57 (t, 2H), 2.70–2.52 (m, 2H), 2.28 (s, 3H). **9f**: Yield 27%. Mp: 192–194 °C; ESI-MS: *m/z* 357.5 $[M+H]^+$, 380 $[M+Na]^+$; ^{1}H NMR (300 MHz, DMSO-d_6): δ 7.18 (d, 2H, J = 8.3 Hz), 7.12 (d, 1H), 7.04 (d, 2H, J = 8.3 Hz), 6.71–6.51 (m, 2H), 6.26–6.00 (m, 2H), 5.82–5.52 (m, 2H), 4.33 (m, 1H), 4.22–4.09 (m, 1H), 4.04 (m, 1H), 3.99–3.89 (m, 1H), 3.73 (dt, 1H), 3.68–3.54 (m, 1H), 2.18 (s, 3H).