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Synthesis and anti-tumor activity of all-trans retinoic acid derivatives

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Abstract

A series of retinoate and retinamide derivatives were designed, synthesized, and their anti-tumor activities were investigated in NB4 by MTT and flow cytometry assays (FCM). All compounds showed cytotoxicity, especially compounds **1a** and **1d** exhibited a higher cytotoxicity than other derivatives and all-trans retinoic acid (ATRA). Furthermore, compound **1d** could induce NB4 cell lines differentiation efficiently.

Keywords: All-trans retinoic acid; Synthesis; Cell differentiation; NB4 cell lines

In the treatment of oncologic diseases, all-trans retinoic acid (ATRA) has now emerged as efficient therapeutic agent [1]. But it has also been suffered from two main disadvantages: chemoresistance to the drugs can occur easily, and can lead severe toxic side effects, at least in one form of leukemia (acute promyelocytic leukemia, APL) due to reduced serum concentration after prolonged treatment. Therefore, it is necessary to develop other ATRA derivatives with better clinical efficacy. Gander and Gurney [2] reported series of retinoic acid ester and amide derivatives, in which fenretinide [N-(4-hydroxyphenyl)retinamide, 4-HPR] showed better anti-tumor activity, but its low cell toxicity and poor solubility limited the clinical application. Recently, Mershon and coworkers [3] synthesized new 4-HPR derivatives. Among them, N-(2-hydroxy-4-nitrophenyl)-retinamide showed induced apoptosis ability. The nitrophenyl in this compound has an electron withdrawing effect, and nitro-group may be potentially harmful, including increased oxidative stress, endothelial dysfunction, and cardiac autonomic dysfunction [4]. Herein, we designed and synthesized a novel series of ATRA derivatives with another electron withdrawing units—trifluoromethylphenyl or trifluoromethoxyphenyl, hoping to increase their activity and stability [5].

The target compounds **1a**–**1g** (Scheme 1) were prepared using ATAR and trifluoromethylphenyl or trifluoromethoxyphenyl derivatives as the starting materials by condensation with DCC and DMAP [6], conveniently. By the synthetic procedure of retinoic amide derivatives **2a**–**2d**, we have improved the operation method in the Ref.

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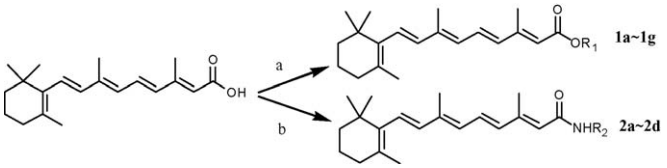
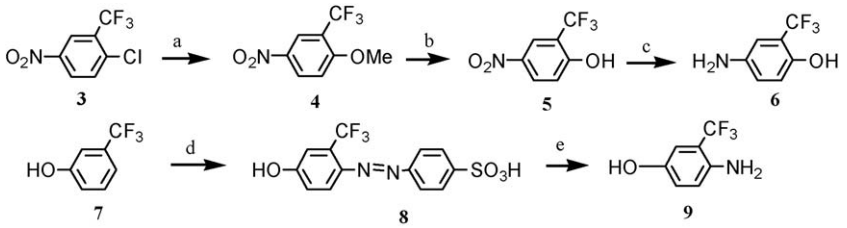


Scheme 1. Synthesis of retinoate and retinamide derivatives: (a) CH_2Cl_2, DCC, DMAP, 25 °C, 6 h; (b) CH_2Cl_2, DCC, DMAP·PTSA, 25 °C, 6 h.



Scheme 2. Synthesis of intermediates **6**, **9**: (a) KOH, MeOH, reflux, 1 h, (b) C_5H_5N·HCl, 210 °C, 25 min, (c) H_2, 5%Pd/C, 95%ethanol, 4 h, (d) i: sulfanilic acid, $NaNO_2$, HCl, 4 °C, 20 min; ii: KOH, 25 °C, 1 h, and (e) $Na_2S_2O_4·2H_2O$, H_2O, 50 °C, 30 min.

[7], and used DMAP·PTSA (para-toluenesulfonic acid, PTSA) as catalyst. To synthesize compounds **1d** and **1e**, we synthesized the key intermediate **6** [8,9] and **9** [10] (Scheme 2). The structure and anti-tumor activity of the obtained target compounds were shown in Table 1 and characterized by ^{1}H NMR, ^{13}C NMR and HR-MS [11].

As shown in Table 1, the anti-tumor activities of these ATRA derivatives against NB4 cell lines were evaluated *in vitro* by MTT assay. NB4 cells were seeded at an initial density of 1000 cells/well in 96-well plates and treated with medium containing various concentrations of ATRA derivatives. The control group was treated with medium. The toxicity activities of these compounds depended on their concentration increased, respectively. At the mean while, compounds **1a** and **1d** exhibited a higher cytotoxicity than other derivatives at each concentration. Furthermore, compounds **1a** and **1d** showed potent anti-tumor activities to tested cell lines. Their inducing differentiation activity in NB4 cells was measured by flow cytometric analysis (Table 2). After treatment with 10 μmol/L of compound **1d** for 72 h, most cells showed to be increased in G_1 phase and reduced in S phases. The results indicated that compound **1d** exhibited superior inducing differentiation activity to that of the two compounds, **1a** and ATRA. The control group showed the normal cell cycle distribution of NB4 cells. The biological data indicated that the amino and hydroxy group of the phenyl might be essential groups to the anti-tumor activities of ATRA derivatives. Studies to identify the mechanism of the action of compound **1d** are in progress.

Table 1
Summary of retinoic acid derivatives and their growth inhibition on NB4 cell lines.

Compound	*R*	Growth inhibition[a] (%)		
		0.1 μmol/L	1 μmol/L	10 μmol/L
1a	2-CF_3-C_6H_4	25.6	34.4	44.3
1b	3-CF_3-C_6H_4	8.3	8.5	10.2
1c	4-CF_3-C_6H_4	6.2	9.4	13.7
1d	2-CF_3-4-NH_2-C_6H_3	22.6	36.1	46.5
1e	3-CF_3-4-NH_2-C_6H_3	6.2	10.9	13.5
1f	3-CF_3O-C_6H_4	15.6	21.9	29.8
1g	4-CF_3O-C_6H_4	12.9	19.2	22.4
2a	3-CF_3-C_6H_4	14.0	23.8	26.2
2b	4-CF_3-C_6H_4	5.0	15.1	30.3
2c	3-CF_3O-C_6H_4	8.2	13.5	20.4
2d	4-CF_3O-C_6H_4	10.5	23.3	28.9
ATRA		17.2	29.3	41.9

[a] Growth inhibition (%) = [(Average OD value for the control group − Average OD value for the experiment)/Average OD value for the control group] × 100%. The cells were incubated for a period of 72 h. Experiments were repeated a minimum of three times.

Table 2
Drug-induced differentiation in NB4 cells as measured by flow cytometric analysis.

Compound	G_1 (%)	S (%)	G_2/M (%)
1a	49.2	43.8	7.0
1d	72.6	19.7	7.7
ATRA	62.3	33.7	4.0
Control	47.0	51.5	1.5

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[11] The date of selected compounds **1a**: Yellow solid, mp: 94–95 °C. ^{1}H NMR (Brucker AV-300, $CDCl_3$, δppm): 1.04(s, 6H), 1.46–1.49(m, 2H), 1.59–1.64(m, 2H), 1.73(s, 3H), 2.03(brs, 5H), 2.40(s, 3H), 6.02(s, 1H), 6.15–6.41(m, 4H), 7.10(dd, 1H, J = 15.01, 11.35), 7.26–7.34(m, 2H), 7.57(t, 1H), 7.68(d, 1H, J = 8.99); ^{13}C NMR ($CDCl_3$, δppm): 13.06, 14.24, 19.31, 21.84, 29.06, 33.23, 34.37, 39.72, 116.28, 124.43, 125.57, 126.88, 129.40, 129.44, 130.37, 132.54, 132.88, 133.06, 134.66, 137.26, 137.77, 140.82, 148.52, 156.75, 164.78; HR-MS: m/z 444.2275 (Calcd. for $C_{27}H_{31}F_3O_2$, 444.2276). **1d**: Yellow solid, mp: 89.7–91.3 °C. ^{1}H NMR (Brucker AV-300, $CDCl_3$, δppm): 1.04(s, 6H), 1.46–1.49(m, 2H), 1.59–1.65(m,2H), 1.72(s, 3H), 2.02(brs, 5H), 2.39(s, 3H), 3.77(s, 2H), 5.99(s, 1H), 6.14–6.39(m, 4H), 6.82(dd, 1H, J = 8.55, 2.72), 6.91(d, 1H, J = 2.76), 7.02(d, 1H, J = 8.7), 7.08(dd, 1H, J = 14.98, 11.37); ^{13}C NMR ($CDCl_3$, δppm): 13.03, 14.16, 19.30, 21.83, 29.05, 33.21, 34.36, 39.71, 112.54, 112.58, 116.69, 118.63, 121.7, 123.34, 123.65, 124.42, 125.45, 129.21, 129.48, 130.29, 132.20, 134.81, 137.28, 137.76, 139.81, 140.52, 144.12, 156.02, 165.55; HR-MS: m/z 459.2378.(Calcd. for $C_{27}H_{32}F_3NO_2$, 459.2385).