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α-TRIFLUOROMETHYLATED ACYLOINS INDUCE APOPTOSIS IN HUMAN ORAL TUMOR CELL LINES

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Abstract: Cytotoxic activity of newly synthesized trifluoromethyl ketones and related compounds was studied using two human oral tumor cell lines (HSG and HSC-2). Among them, α-trifluoromethylacyloins (**1** and **2**) were found to induce apoptotic cell death, as judged by the terminal deoxynucleotidyl transferase (TdT) dUTP nick end-labeling (TUNEL) method which detects DNA nick or fragments. Furthermore, the cytoplasm of **1** or **2** treated HSG cells was stained by M30 monoclonal antibody, which detects the product resulting from the cleavage of cytokeratin 18 by activated caspase.

Apoptosis, or programmed cell death, is a selective process of physiological cell deletion.[1] Failure of cells to undergo appropriate apoptotic cell death is involved in a variety of human diseases including autoimmune disease, viral infections, and cancer.[2] Thus, developing and identifying pharmaceutical agents that can selectively modulate apoptotic pathways may provide an effective tactic for the treatment and the prevention of many deseases. Therefore, there is a great deal of interest in cytotoxic agents that induce apoptotic cell death.[3]

Recently, certain cysteine proteases were discovered as key elements in apoptosis.[4] Biological changes include the limited proteolysis of cellular proteins by the caspase family of cysteine proteases which leads the activation of DNase by specific degradation of its inhibitor by caspase-3.[5]

Polarized carbonyls, such as trifluoromethyl ketones (TFMKs), are putative isosteric analogs of tetrahedral intermediates formed during the hydrolysis of esters and amides.[6] Since these tetrahedral intermediates are thought to be similar to the transition state along the reaction coordinate, TFMKs are strong inhibitors of hydrolytic enzymes such as serine esterases, juvenile hormone esterase, or mammalian carboxylesterases.[6] In this paper, we examined the cytotoxicity of newly synthesized TFMKs and related compounds against two human oral tumor cell lines, such as human squamous cell carcinoma HSC-2 cells and salivary gland tumor HSG cells. The most cytotoxic α-trifluoromethyled acyloins (TFACs) (**1** and **2**) were found to induce apoptotic cell death.

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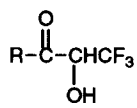
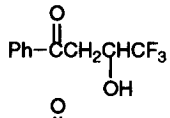
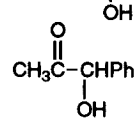
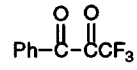
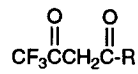
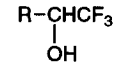
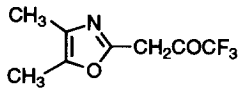
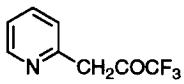
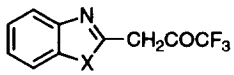
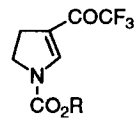
Table Cytotoxic activity of trifluoromethyl ketones and related compounds

Compd	Structure		Cytotoxic activity (CC_{50}: μM)		
			HSC-2	HSG	HGF
1		R=Ph	36	240	1300
2		R=Bn	22	110	310
3		R=C_6H_{13}	90	220	790
4		R=PhCONH$(CH_2)_3$	31	400	420
5			240	1500	1700
6			1700	2900	3200
7			79	390	510
8		R=Ph	150	420	1400
9		R=CH_3	2500	3200	>3200
10		R=OCH_3	1300	1800	>2900
11		R=Ph	1100	2100	2200
12		R=Bn	460	1300	1700
13		R=Ph	>2900	2700	>2900
14		R=Bn	1400	1600	2200
15		R=2-Thienyl	1900	2700	>2800
16		R=2-Pyrrolyl	870	1800	2100
17		R=2-Benzoxazolyl	580	1700	2200
18		R=3-Indolyl	94	1300	2000
19			<68	150	77
20			620	1600	2100
21		X=O	43	79	630
22		X=S	150	390	430
23		X=NH	53	340	200
24		R=Ph	230	270	620
25		R=Allyl	110	240	140
26		R=Fluorenyl-9-yl	140	180	230
	Doxorubicin•HCl		4.1	-	-

To our knowledge, the present study is the first demonstration of the apoptosis induction by TFAC compounds.

Synthesis. TFACs (**1-3**) were synthesized by the reaction of the corresponding α-hydroxy acids with trifluoroacetic anhydride (TFAA).[7] TFAC (**4**) was prepared by treatment of *N*-benzoylproline with TFAA.[8] Compounds **5** and **6** were obtained by the catalytic reduction of the corresponding diketones. Compounds **11** and **12** were synthesized by the reduction of the corresponding ketones with $NaBH_4$ in MeOH. Compound **17** was obtained by the trifluoroacetylation of 2-lithiobenzoxazole, generated by lithiation of benzoxazole with LDA, with ethyl trifluoroacetate. Compounds (**19-23**) were synthesized by treatment of the corresponding methyl-substituted azines with TFAA in the presence of pyridine.[9] Compounds (**24-26**) were prepared by the reaction of *N*-alkoxycarbonylprolines with TFAA.[10] Other TFMKs (**7-10**, **13-16**, and **18**) are commercially available.

Cytotoxic activity *in vitro*. The cytotoxic activity of 26 TFMKs and related compounds against two human oral tumor cell lines (HSC-2 and HSG) was quantified by MTT method (after incubation at 37 ℃ for 24 h with the test compounds in DMEM medium supplemented with 10% FBS in a humidified 5% CO_2 atmosphere).[11] The 50% cytotoxic concentration (CC_{50}) was determined from the dose-response curve (Table). Among them, **1** and **2** were the most active in both cell lines and their cytotoxities were slightly lower than that of doxorubicin. All compounds showed the selective cytotoxicity against human oral tumor cell lines (HSC-2 and HSG), as compared with human gingival fibroblasts (HGF)[12] between fifth and seventh passages of healthy gingival biopsies and were more cytotoxic to HSC-2 cells than HSG cells.

Induction of apoptosis by TFACs (1 and 2). When HSG cells were incubated for 6 h with **1** or **2**, the nuclei of the cells were strongly stained with TUNEL reagent (Figure 1), indicating the production of DNA nicks or fragments.[13]

Apoptosis requires specialized machinary, the central component of which is a proteolytic system involving a family of proteases, called caspases.[14] These enzymes participate in a cascade that is triggered by pro-apoptotic signals and cleave a set of proteins and finally disassemble the cells. Since intercalated and striated duct luminal cells in human salivary glands express cytokeratin (CK 7, 8, 18, and 19),[15] it was of great interest to investigate whether TFACs (**1** and **2**) can activate caspase(s) so as to cleave the substrate cytokeratin(s).[16] For this purpose, M30 monoclonal antibody (mouse IgG_{2b}, Boehringer Mannheim) was used to detect the fragmented cytokeratin 18. M30 monoclonal antibody reacted with the cytoplasm of HSG cells for 6h after treatment with **1**

or **2**, but not with that of control cells (Figure 2).[16] These data, taken together, demonstrate that TFAC induces apoptosis of HSG cells. Activation of a specific proteolytic cascade is essential in most cell types to proceed the apoptotic cell death induced by pharmacological agents.

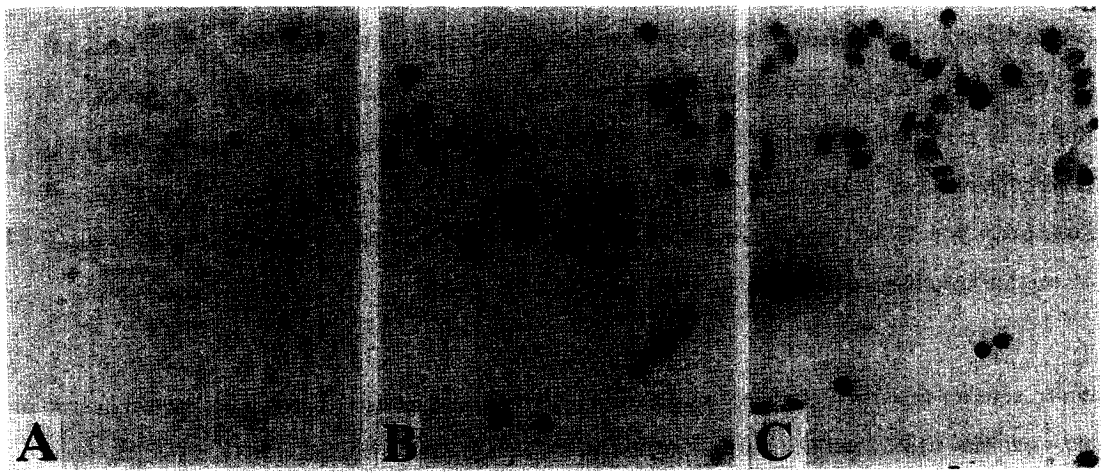


Figure 1. Induction of apoptosis in HSG cells by TFACs (1 and 2). HSG cells were treated for 6 h with none (A), **1** (0.12 mM)(B), or **2** (0.23 mM)(C). The cells were fixed with 10% neutralized buffered formation and stained by TUNEL method.

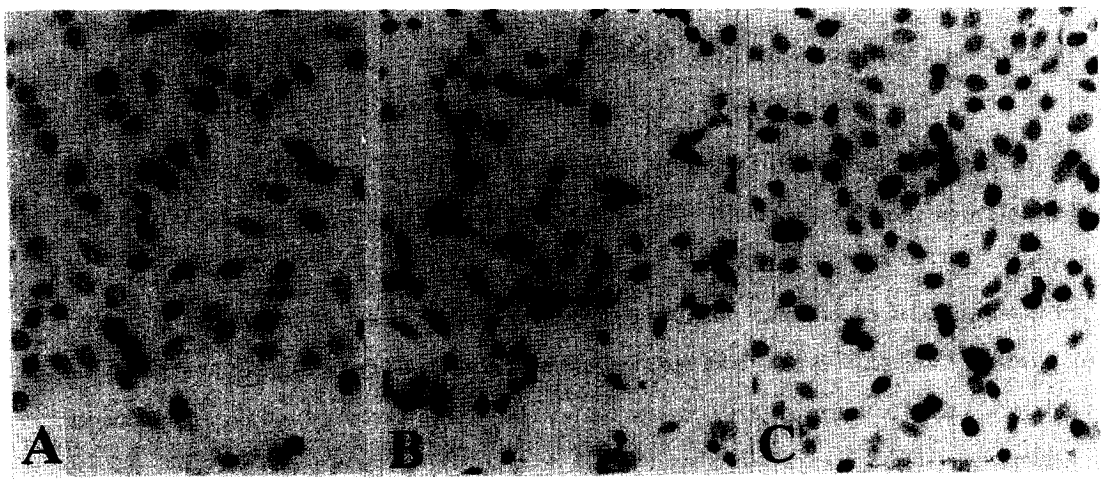


Figure 2. Activation of caspase by TFACs (1 and 2) in HSG cells. HSG cells were treated for 6 h with none (A), **1** (0.12 mM)(B), or **2** (0.23 mM)(C). The cells were fixed with a mixture of 95% ethanol and 5% acetic acid, and then stained by immunocytochemical methods using M30 monoclonal antibody.

Discussion. We screened our in-house library of compounds to obtain possible lead structures bearing a trifluoromethyl group. The compound exhibiting the highest cytotoxity was TFACs (**1**-**4**). A series of structurally related compounds were tested, to further define the importance of TFAC moiety. TFMK compounds tested were classified into four groups: (A) α-hydroxy ketones (acyloins) (**1**-**4**), (B) α- (**7**) and β-diketone (**8**), (C) alcohols (**11** and **12**), and (D) aromatic and heteroaromatic ketones (**13**-**26**). The potency of cytotoxic activity was in the order of A > B > C > D. Non-fluorinated α-hydroxy ketone (**6**) and β-hydroxy ketone (**5**) significantly diminished the cytotoxic activity, whereas α-diketone (**7**) exhibited cytotoxicity comparable to those of TFACs. These data suggest the importance of α-hydroxy ketone moiety for cytotoxicity induction. Among the D group, the heteroaromatic ketones were generally more cytotoxic than aromatic ketones and oxazole derivatives (**19** and **21**) demonstrated significant cytotoxic activity.

Apoptosis was evident in cell cultured with **1** or **2** more frequently than in control cells, determined using TUNEL method (Fig. 1). Furthermore, M30 monoclonal antibody detected the product resulting from the cleavage of cytokeratin 18 by activated caspase (Fig. 2).

Conclusion. The present study demonstrated that newly synthesized TFACs (**1** and **2**) induce apoptosis of human cancer cells. This is the first demonstration that TFMKs induce apoptosis of human cancer cells *in vitro*. Induction of apoptosis by TFACs is mediated by the activation of caspase pathway. The results suggest that TFACs (**1** and **2**) may be attractive lead compounds for further development as a chemotherapeutic agent for cancer. Further investigations of the structure and activity relationship and the mechanism of apoptosis induction are under way.

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