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Looking for a PET tracer for imaging apoptosis.

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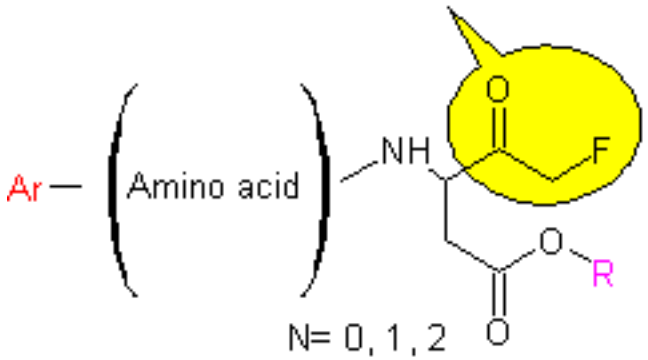
Looking for a PET tracer for imaging apoptosis.

Graphical Abstract



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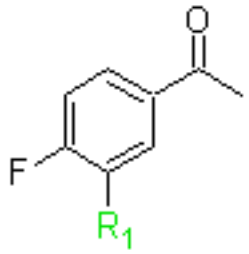
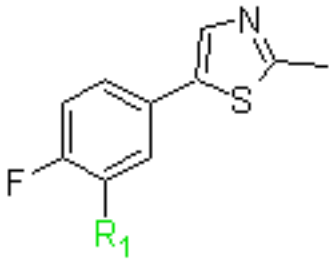
	H	H
	t-But	CN
		CF3

Abstract:

In multicellular organisms, homeostasis is maintained by a balance between cell proliferation and apoptosis (programmed cell death). It is a physiological form of cell death responsible for the deletion of non-repairable damaged, mutated, or cells which have lost their function.
We describe the synthesis of a series of potential inhibitors of caspases from a modified aspartic acid residue (fluoromethylketone, fmk). The addition to the entire series of, 3-cyano-4-fluoro-benzoyl- pattern on one hand or of, 4-fluoro-2-thiazolamino- pattern on the other hand will subsequently allow the introduction of a PET isotope (^{18}F).
In order to determine potential candidates, the inhibitory activity of these compounds was evaluated *in vitro* on a series of human T cells compared to the z-VAD-fmk as a reference.

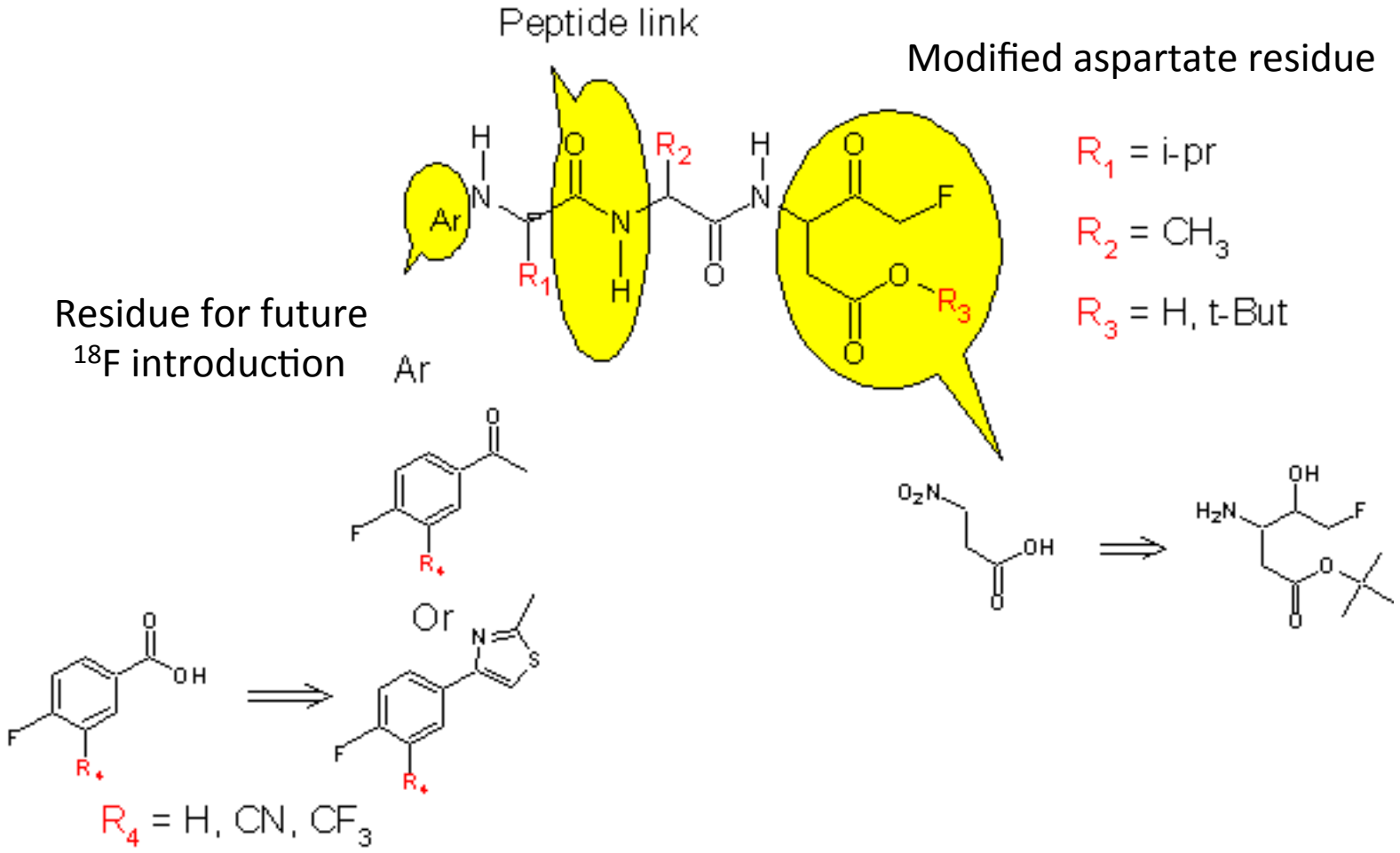
Keywords: Apoptosis; PET; fluoromethylketone

Introduction

Apoptosis is a form of programmed cell death in multicellular organisms.[1] In adult individuals cell homeostasis is achieved when the rate of mitotic cell division is balanced by cell death. However, apoptosis failure can contribute to profound pathologies such as tumor growth and autoimmune diseases whereas unwanted apoptosis occurs in many neurodegenerative disorders.[2] Apoptotic cell death is induced by complex regulated signaling pathways triggered by either activation of death receptors (extrinsic pathway) or mitochondria (intrinsic pathway).[3] Both pathways activate the intracellular enzyme class of cysteinyl aspartate-specific proteases, in short caspases.[4] Among these the executioner caspases-3, -6 and -7, once activated, irrevocably initiate cellular death through cleavage of proteins which are responsible for DNA repair, signaling and cell maintenance. Therefore, these enzymes are suitable *in vivo* biomarkers of living apoptotic cells and tissues. Here, we report on the synthesis and *in vitro* evaluation of the caspase inhibition potencies of fluorinated derivatives aspartate fluoromethylketone.[5]

1.Kerr, J. F.; Wyllie, A. H.; Currie, A. R. Br. J. Cancer 1972, 26, 239.
2.Brunner, T.; Mueller, C. Essays Biochem. 2003, 39, 119.
3.Hengartner, M. O. Nature 2000, 407, 770.
4.Li, J.; Yuan, J. Oncogene 2008, 27, 6194.
5.Cai, S. X., L. Guan, et al. (2004) Bioorg & Med. Chem. Lett. **14**(21): 5295-5300.

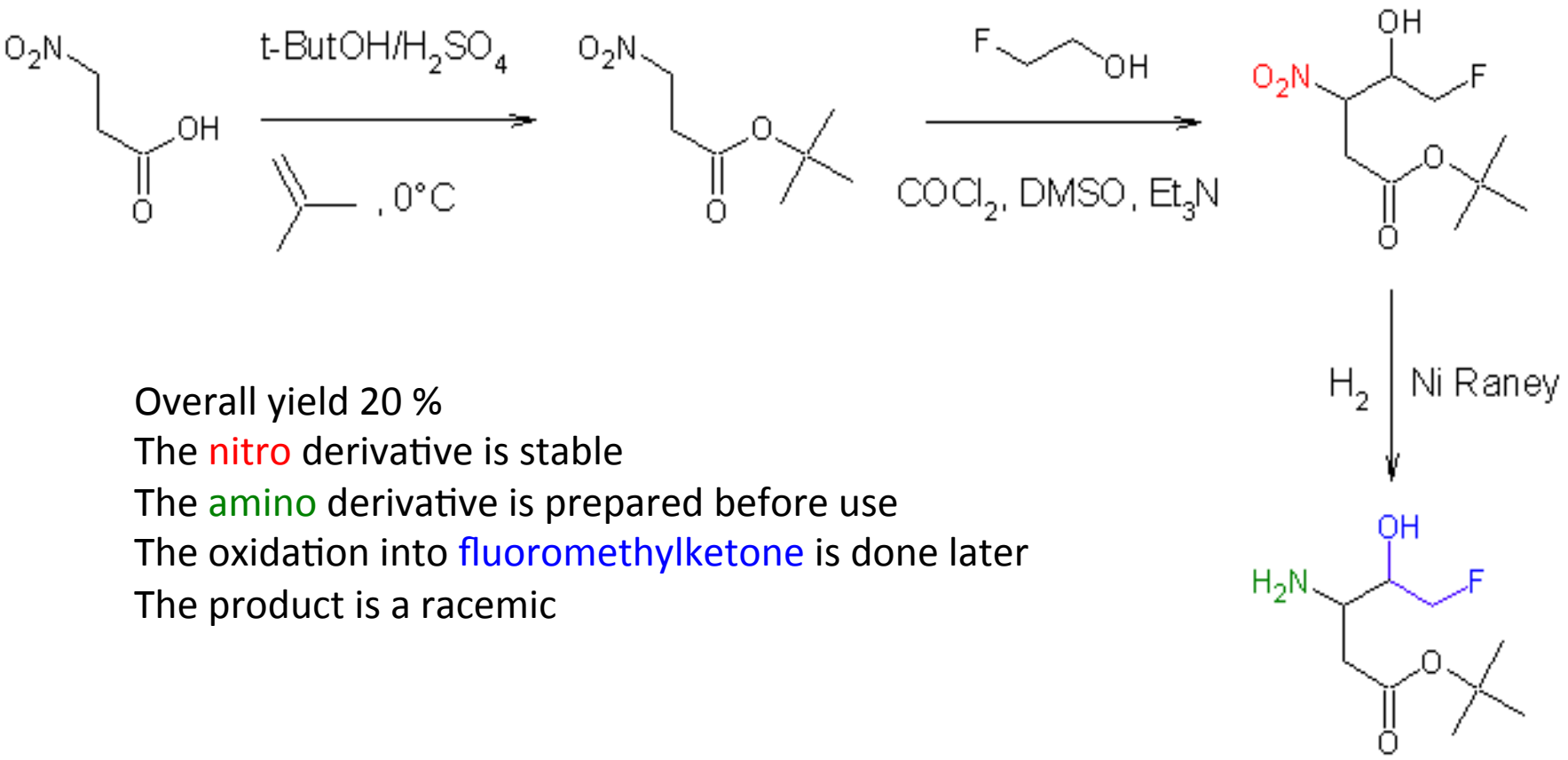
Results and discussion: Synthesis strategy



Results and discussion: Synthesis strategy

1. Aspartate fmk synthesis
2. Bromomethylketone synthesis
3. Thiourea synthesis
4. aminothiazole synthesis
5. Coupling aminoacid using DMTMM
6. Oxidation with Dess Martin
7. Hydrolysis

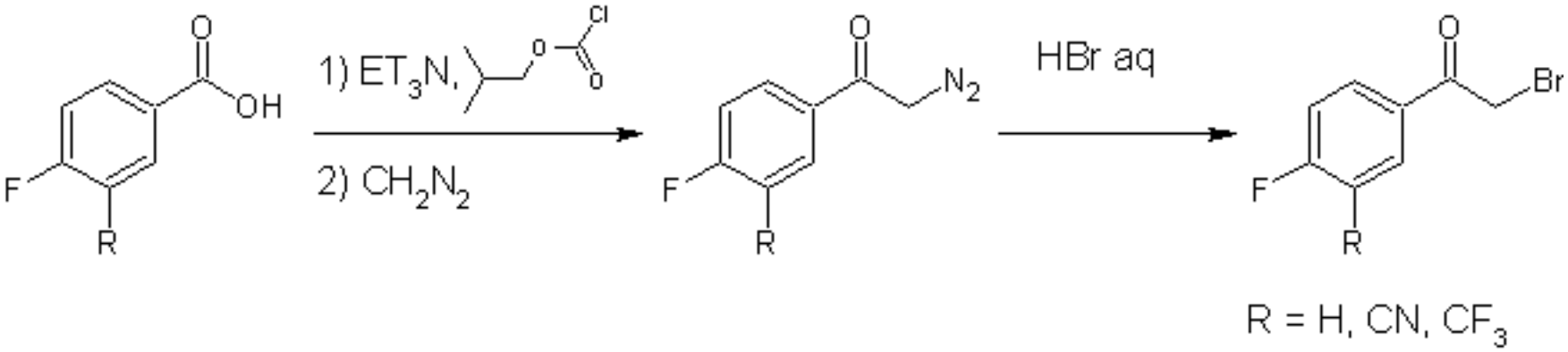
Results and discussion: 1. Aspartate fmk synthesis



Overall yield 20 %
The nitro derivative is stable
The amino derivative is prepared before use
The oxidation into fluoromethylketone is done later
The product is a racemic

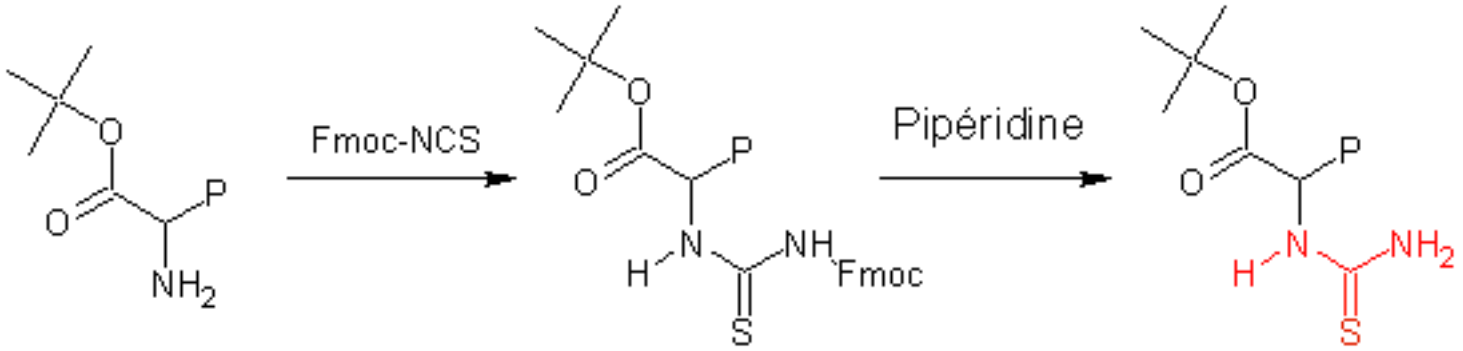
Revesz, L., C. Briswalter, et al. (1994). Tetrahedron Lett. **35**(52): 9693-9696.

Results and discussion: 2. Bromomethylketone synthesis



Overall yield 66-70 %
Stable
Key intermediate to aminothiazoles preparation

Results and discussion: 3. Thiourea synthesis

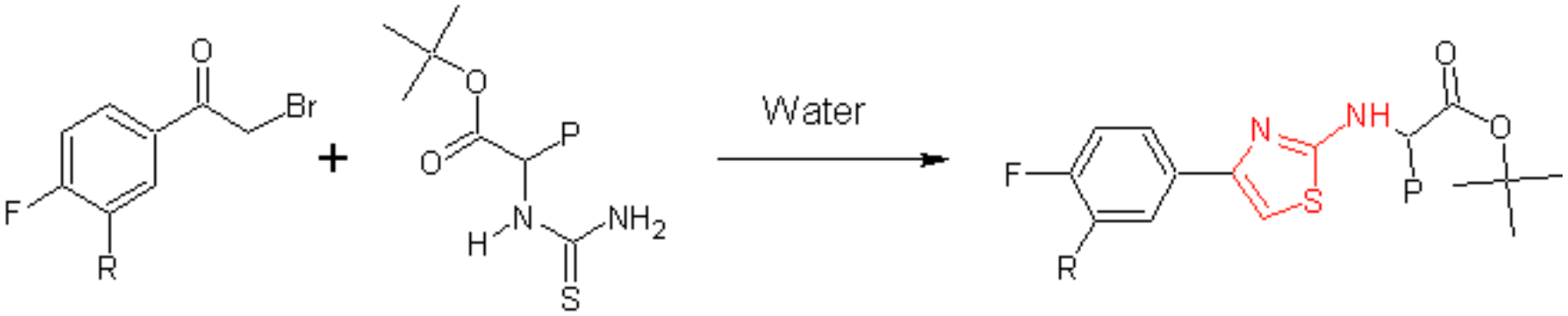


P = aminoacid or peptide radical

One pot synthesis from commercial Fmoc-NCS

Kearney, P. C., M. Fernandez, et al. (1998). The J. Org. Chem. **63**(1): 196-200.

Results and discussion: 4. Aminothiazoles synthesis



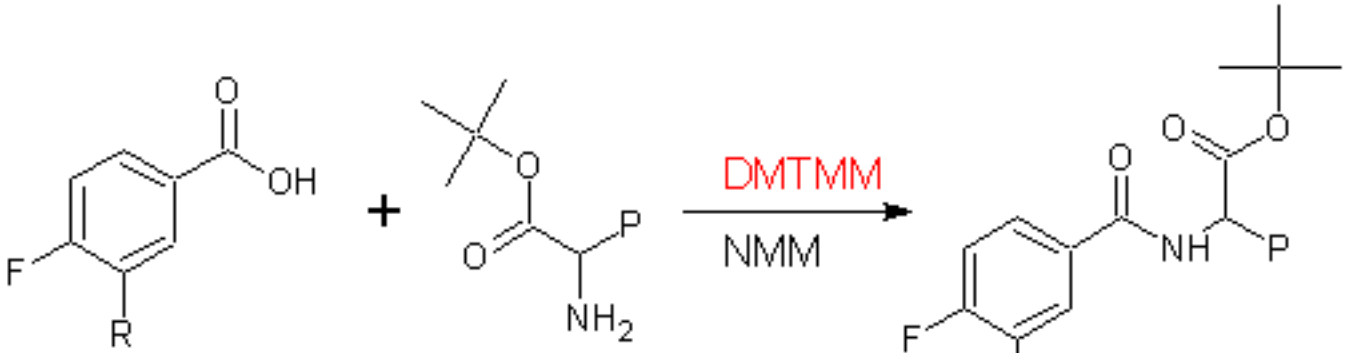
R = H, CN, CF_3

P = aminoacid or peptide radical

Reaction made at ambient temperature
Not water sensible or even made in water
Click like chemistry

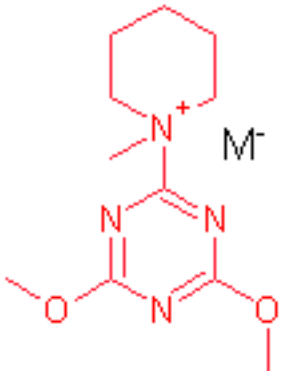
Potewar, T. M., S. A. Ingale, et al. (2008). Tetrahedron **64**(22): 5019-5022.

Results and discussion: 5. Coupling réaction



R = H, CN, CF_3

P = aminoacid or peptide radical



M^- = CL, BF_4

Very easy to use reagent
Not sensible to water
Very good yields
Commercial Cl^- form not stable in solvent
Non commercial BF_4^- form more reliable

Raw, S. A. (2009) Tetrahedron Lett. **50**(8): 946-948.

Results and discussion: final observations

6. Oxidation:
Fluoromethylketones were obtained by oxidation of the corresponding fluorhydrines with Dess Martin reagent in 87-97% yield.
For aminothiazole derivatives a degradation was observed in usual solvent (DCM). The use of dry ethyl acetate avoids his problem.

7. Hydrolysis of t-But ester protection:
Was achieved in 65-95% yield.
To free carboxylic group for subsequent peptide synthesis
Degradation into amide of compounds with nitrile group were observed. This problem is avoided by the use of dry solvent.

Results and discussion

List of compounds synthesized with this strategy

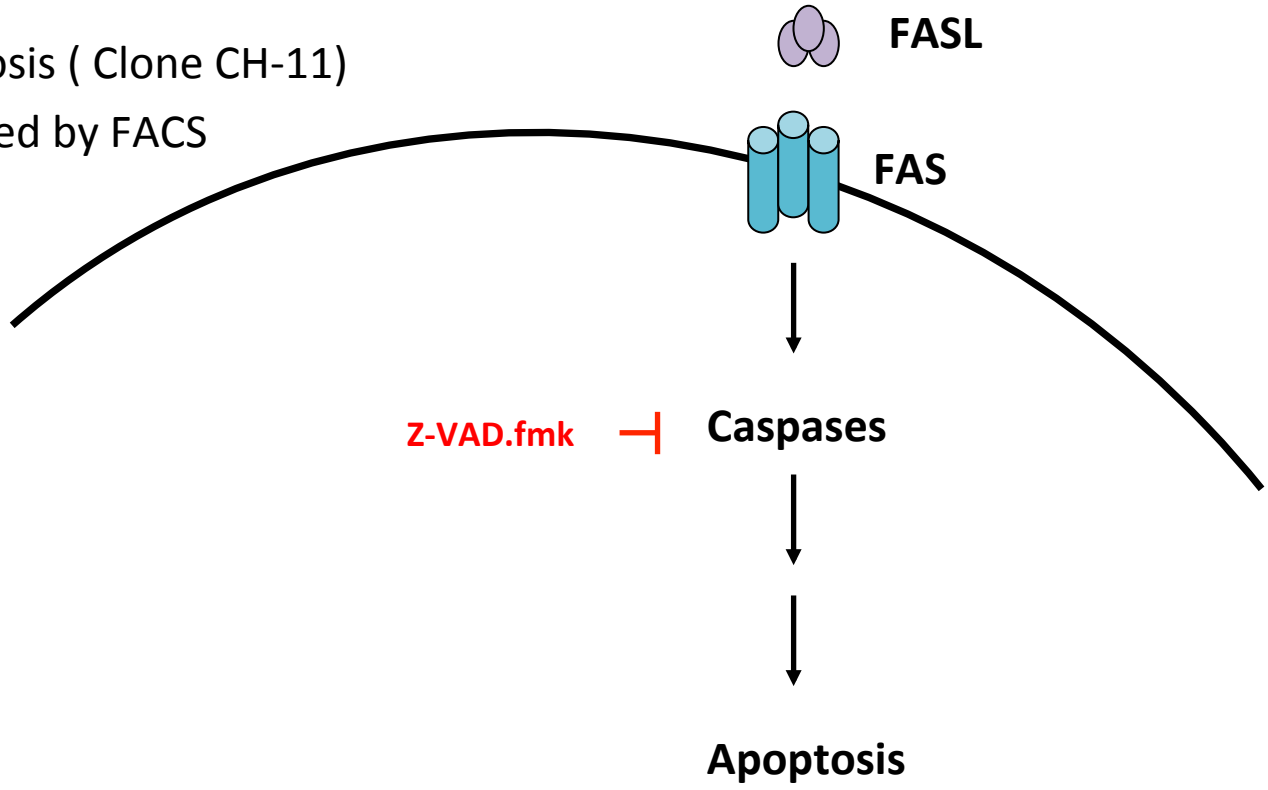
Entry	compound	Cell survival	Entrry	compound	Cell survival
1	3-CN-4-F-Bz-Asp OH fmk	-	11	3-CN-4-F-Bz-Val-Asp O-t-But fmk	-
2	4-F-Ph-Tz-Val-Asp O-t-But fmk	Toxic	12	4-F-Bz-Asp O-t-But fmk	-
3	3-CF3-4-F-Bz-Val-Asp O-t-But fmk	-	13	3-CN-4-F-Bz-Asp O-t-But fmk	Toxic
4	3-CF3-4-F-Bz-Val-Ala-Asp O-t-But fmk	-	14	3-CF3-4-F-Bz-Asp O-t-But fmk	-
5	4-f-Bz-Val-Asp O-t-But fmk	+	15	4-F-Bz-Val-Asp OH fmk	-
6	4-F-Ph-Tz-Val-Ala-Asp O-t-But fmk	-	16	3-CF3-4-F-Bz-Val-Asp OH fmk	+
7	3-CF3-4-F-Bz-Asp OH fmk	+	17	4-F-Bz-Val-Ala-Asp OH fmk	+
8	3-CN-4-F-Bz-Val-Ala-Asp OH fmk	+	18	3-CN-4-F-Bz-Val-Ala-Asp O-t-But fmk	-
9	3-CF3-4-F-Bz-Val-Ala-Asp OH fmk	+	19	3-CN-4-F-Bz-Val-Asp OH fmk	+
10	4-F-Bz-Val-Ala-Asp O-t-But fmk	+	20	4-F-Ph-Tz-Val-Asp OH fmk	-

Results and discussion: Model use for the screening

Jurkat T cell line

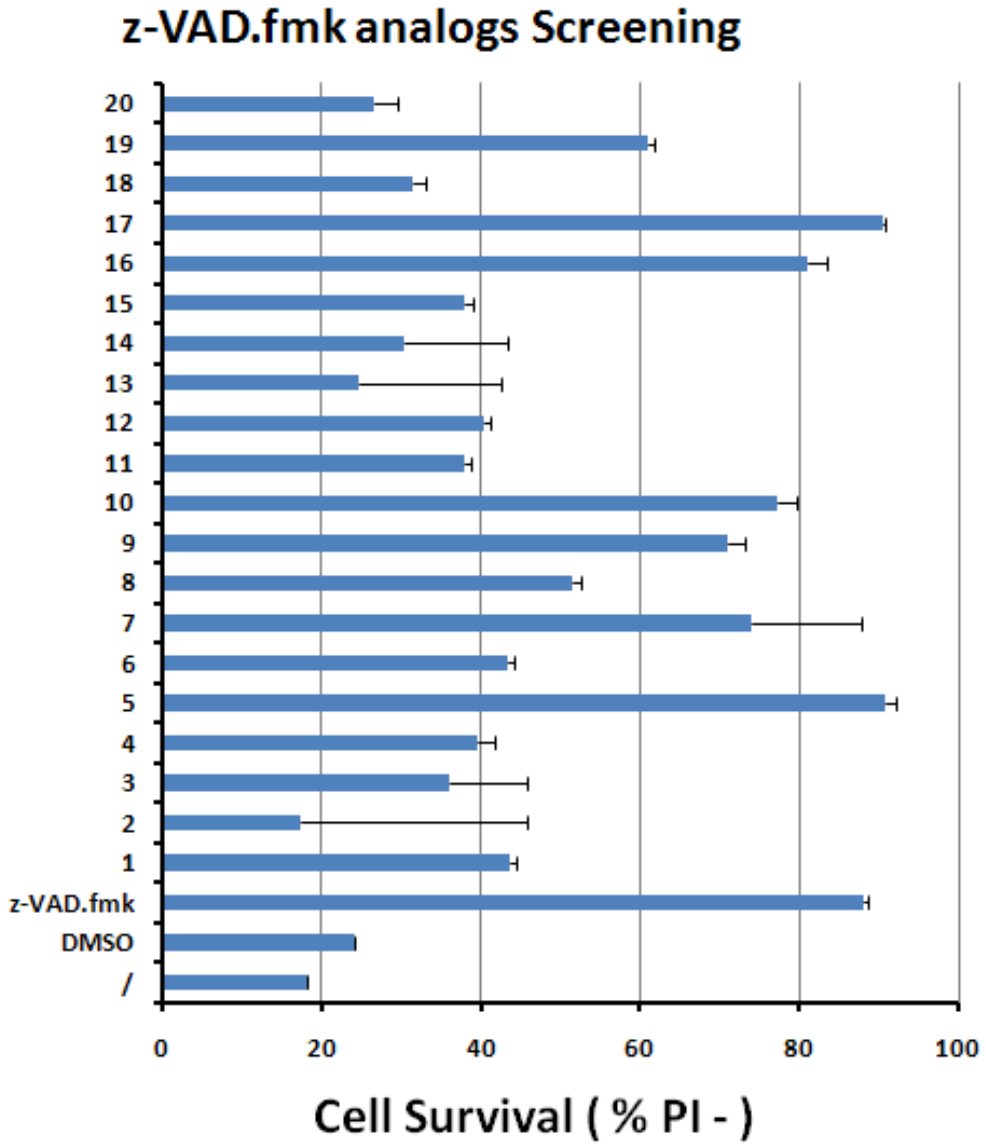
FAS-induced apoptosis (Clone CH-11)

Viability was analyzed by FACS



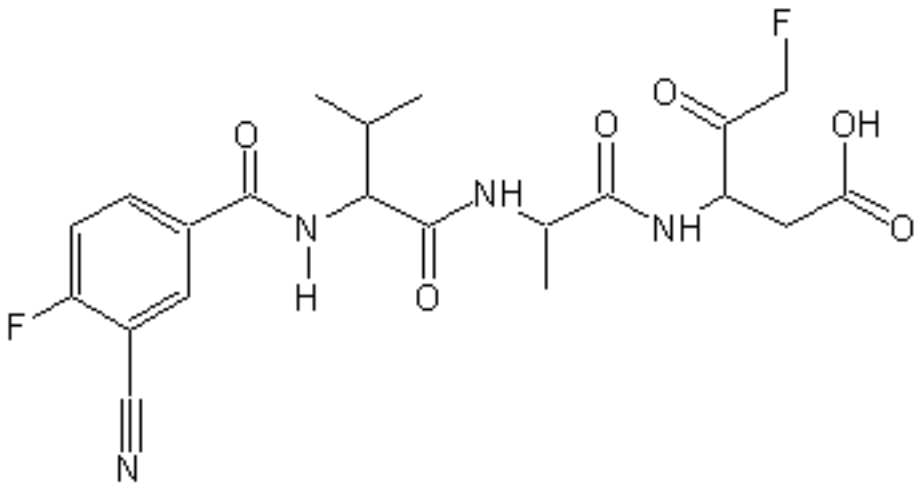
Results and discussion

- Statistic on 3 independent experiments
- Stock solution 10 mM
- Working concentration 25 µM
- #2 and #13 are toxic for untreated cells
- #5, #7, #9, #10, #16 are as effective as z-VAD.fmk
- #17 is about 3 times more effective than z-VAD.fmk (not shown here)



Conclusions

Synthesis of 20 aspartate fluoromethylketone derivatives
Introduction of N-terminal fluorobenzoyl and aminothiazoles
Quaternary ammonium derivatives easy achievable for ^{18}F radiolabelling
1 potential caspase inhibitor upon 7 which is more than 3 times active than z-VAD-fmk
Potential new click like radiochemistry.



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