Looking for a PET tracer for imaging apoptosis.

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Graphical Abstract

Fluoromethylketone

Ar

(Amino acid)

N = 0, 1, 2

<table>
<thead>
<tr>
<th>Ar</th>
<th>R</th>
<th>R1</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>H</td>
<td>H</td>
</tr>
<tr>
<td></td>
<td>t-But</td>
<td>CN</td>
</tr>
<tr>
<td></td>
<td></td>
<td>CF3</td>
</tr>
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</table>
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

Results and discussion: Synthesis strategy

Peptide link

Modified aspartate residue

Residue for future ¹⁸F introduction

\[ R_1 = \text{i-pr} \]
\[ R_2 = \text{CH}_3 \]
\[ R_3 = \text{H, t-But} \]

Or

\[ R_4 = \text{H, CN, CF}_3 \]
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

Results and discussion: 2. Bromomethylketone synthesis

Overall yield 66-70 %
Stable
Key intermediate to aminothiazoles preparation
Results and discussion: 3. Thiourea synthesis

\[ \text{Fmoc-NCS} \rightarrow \text{Fmoc-Thiourea} \rightarrow \text{Piperidine} \rightarrow \text{Thiourea} \]

\( P = \text{aminoacid or peptide radical} \)

One pot synthesis from commercial Fmoc-NCS

Results and discussion: 4. Aminothiazoles synthesis

\[
\begin{align*}
\text{R} & = \text{H, CN, CF}_3 \\
\text{P} & = \text{aminoacid or peptide radical}
\end{align*}
\]

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

Results and discussion: 5. Coupling réaction

Very easy to use reagent
Not sensible to water
Very good yields
Commercial Cl⁻ form not stable in solvent
Non commercial BF₄⁻ form more reliable

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

<table>
<thead>
<tr>
<th>Entry</th>
<th>compound</th>
<th>Cell survival</th>
<th>Entry</th>
<th>compound</th>
<th>Cell survival</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>3-CN-4-F-Bz-Asp OH fmk</td>
<td>-</td>
<td>11</td>
<td>3-CN-4-F-Bz-Val-Asp O-t-But fmk</td>
<td>-</td>
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<tr>
<td>2</td>
<td>4-F-Ph-Tz-Val-Asp O-t-But fmk</td>
<td>Toxic</td>
<td>12</td>
<td>4-F-Bz-Asp O-t-But fmk</td>
<td>-</td>
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<tr>
<td>3</td>
<td>3-CF3-4-F-Bz-Val-Asp O-t-But fmk</td>
<td>-</td>
<td>13</td>
<td>3-CN-4-F-Bz-Asp O-t-But fmk</td>
<td>Toxic</td>
</tr>
<tr>
<td>4</td>
<td>3-CF3-4-F-Bz-Val-Ala-Asp O-t-But fmk</td>
<td>-</td>
<td>14</td>
<td>3-CF3-4-F-Bz-Asp O-t-But fmk</td>
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<tr>
<td>5</td>
<td>4-f-Bz-Val-Asp O-t-But fmk</td>
<td>+</td>
<td>15</td>
<td>4-F-Bz-Val-Asp OH fmk</td>
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<tr>
<td>6</td>
<td>4-F-Ph-Tz-Val-Ala-Asp O-t-But fmk</td>
<td>-</td>
<td>16</td>
<td>3-CF3-4-F-Bz-Val-Asp OH fmk</td>
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<tr>
<td>7</td>
<td>3-CF3-4-F-Bz-Asp OH fmk</td>
<td>+</td>
<td>17</td>
<td>4-F-Bz-Val-Ala-Asp OH fmk</td>
<td>+</td>
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<tr>
<td>8</td>
<td>3-CN-4-F-Bz-Val-Ala-Asp OH fmk</td>
<td>+</td>
<td>18</td>
<td>3-CN-4-F-Bz-Val-Ala-Asp O-t-But fmk</td>
<td>-</td>
</tr>
<tr>
<td>9</td>
<td>3-CF3-4-F-Bz-Val-Ala-Asp OH fmk</td>
<td>+</td>
<td>19</td>
<td>3-CN-4-F-Bz-Val-Asp OH fmk</td>
<td>+</td>
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<tr>
<td>10</td>
<td>4-F-Bz-Val-Ala-Asp O-t-But fmk</td>
<td>+</td>
<td>20</td>
<td>4-F-Ph-Tz-Val-Asp OH fmk</td>
<td>-</td>
</tr>
</tbody>
</table>
Results and discussion: Model use for the screening

Jurkat T cell line
FAS-induced apoptosis (Clone CH-11)
Viability was analyzed by FACS

![Diagram showing FAS, FASL, Z-VAD.fmk, Caspases, and Apoptosis]
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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