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Solid-phase analogue synthesis of caspase-3 inhibitors via palladium-catalyzed amination of 3-bromopyrazinones

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Abstract—Caspase-3 is a cysteinyl protease that mediates apoptotic cell death. Its inhibition may have an important impact on the treatment of several degenerative diseases. Here we report the synthesis of reversible inhibitors via a solid-support palladium-catalyzed amination of 3-bromopyrazinones and the discovery of a pan-caspase reversible inhibitor.
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The human caspases are a family of cysteinyl-aspartate-specific proteinases that are central components in the molecular pathways that result in the apoptosis of cells.[1] These enzymes are divided into three groups. Group I caspases (1, 4, 5, and 14) mediate cytokine maturation and are implicated in the inflammatory response. Group II caspases (2, 3, and 7) are the major effectors of cell death. Group III caspases (6, 8, 9, and 10) are upstream activator enzymes of the group II caspases. There are no human caspases 11 and 13, whereas human caspase 12 was reported only once.[2] Caspase-3 appears to be a critical mediator of apoptosis in neurons. Prototype peptidyl inhibitors of caspase-3 have shown efficacy in models such as stroke, traumatic brain or spinal cord injury, hypoxic brain damage, cardiac ischemia, and reperfusion injury.[3] Ac-DEVD-CHO (Fig. 1) is a tetrapeptide inhibitor based on a natural substrate recognized by caspase-3. The aspartic acid in P1 is an essential element of recognition of the enzyme and was used as the basis to develop new inhibitors. Replacement of the aldehyde warhead by ketones provided reversible, cell-penetrant inhibitors. Previous communications refer to such a strategy.[4–7] Here, we report the use of a palladium-catalyzed amination of 3-bromopyrazinone aspartyl ketones on resin to synthesize potent and reversible inhibitors of caspase-3.

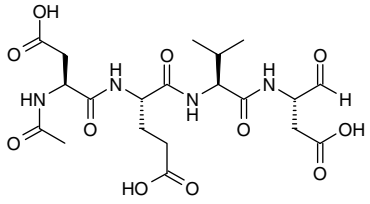

Figure 1. Structure of Ac-DEVD-CHO.

The purpose of this work was to find a breakthrough in the SAR around the amine portion of the furazan lead compound in a timely manner. In order to generate libraries of aspartyl ketones, rapid analogue synthesis was performed using solid-phase chemistry. As shown in Scheme 1, the pyrazinone core was expeditiously built following modified literature procedures.[6,8] Ethyl (*S*)-(+)-2-amino-butyrate hydrochloride **1** was reacted with ethyl oxalyl chloride in CH_2Cl_2 in the presence of a base. The more reactive ethyl ester of **2** was reacted with an amino alcohol to give the oxalyl diamide in good yield. The alcohol was oxidized with Dess–Martin periodinane and the resulting aldehyde **3** was cyclized in the presence of trifluoroacetic anhydride and trifluoroacetic acid in acetic acid at 100 °C to provide **4**. The bromide **5** was

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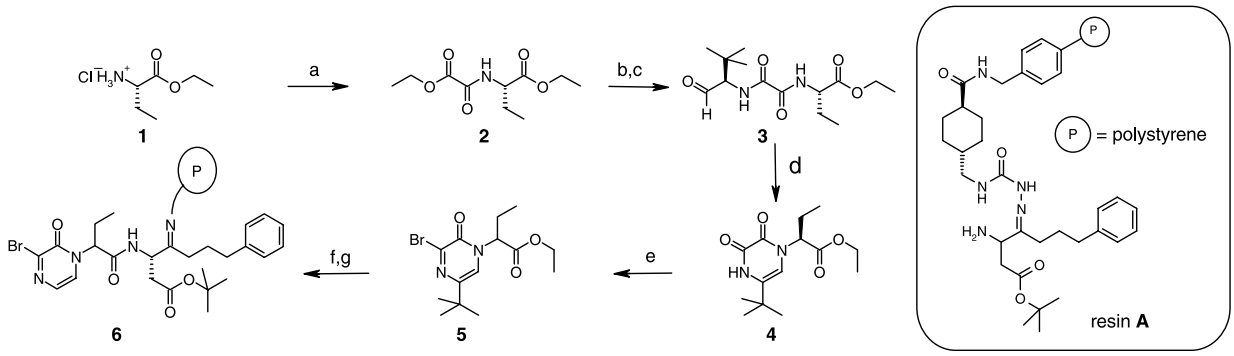
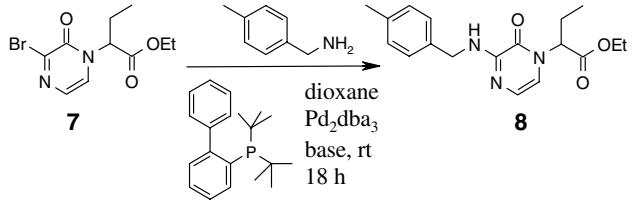


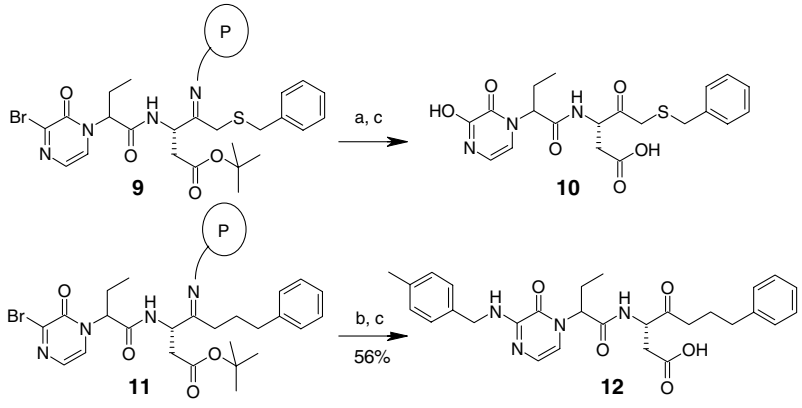
Scheme 1. Reagents and conditions: (a) ethyl oxalyl chloride, Et_3N, CH_2Cl_2, 0 °C, 95%; (b) (*R*)-(−)-*tert*-leucinol, Et_3N, EtOAc, 80 °C; (c) Dess–Martin periodinane, CH_2Cl_2, 0 °C, 95% over 2 steps; (d) TFAA/TFA, AcOH, 100 °C; (e) $POBr_3$, $ClCH_2CH_2Cl$, 60 °C, 41% over 2 steps; (f) $LiOH{\cdot}H_2O$, THF, H_2O, quantitative; (g) resin **A**, HATU, *i*-Pr_2NEt, DMF, rt, quantitative.

formed using $POBr_3$ in dichloroethane at 60 °C. The bromination is specific to this position; however it is hard to push it to completion. Partial racemization (20–25%) occurred at this step, although the *S*-isomer remained the major one. The ethyl ester was hydrolyzed with LiOH in a mixture of THF and H_2O. The resulting acid (mixture of diastereomers) was coupled to the resin according to published procedures to provide the starting material **6** for the current work.[4]

Considerable literature precedent exists for the Pd-mediated solution-based amination using aliphatic amines, benzylic amines, and anilines reacting with aryl and heteroaryl bromides.[9–11] None to our knowledge however ever used bromopyrazinones as a coupling partner. To confirm the relevance of these methods to our substrates, the chemistry was first probed in solution with a model substrate 3-bromopyrazinone **7** (Scheme 2). Hence **7** was reacted with 4-methylbenzylamine in dioxane with base in the presence of 2-(di-*tert*-butylphosphino)biphenyl and palladium.[12] Whereas *t*-BuONa gave only ester hydrolysis, Cs_2CO_3 gave 66% of the expected isolated material **8**. Parameters of the reactions such as stoichiometry, temperature, concentration, and presence of water had no impact on the yield. This robust reaction was then transferred to a substrate bound to the Merrifield resin via a semicarbazone linker.[13] Our first attempts were conducted on substrate **9** bearing a masked α-methylthiobenzyl ketone. After TFA cleavage of the resin, pyrazinone (**10**) resulting from bromide hydrolysis was the major observed product. An explanation for this lack of amination emerged when the solid-phase reaction was conducted using a stoichiometric amount of palladium and showed loss of the SBn moiety. Hypothesizing that the sulfur was the source of the reactivity problem, a slightly less potent carbon analogue (**11**) was used. Under the same conditions, the expected aminopyrazinone **12** could be isolated as well as some hydrolyzed material. Heating the reaction mixture to 90 °C for 18 h afforded the desired



Scheme 2. Reagents: 4-methylbenzylamine (5 equiv), *t*-BuONa or Cs_2CO_3 (5 equiv), 2-(di-*tert*-butylphosphino)biphenyl (0.3 equiv), tris-(dibenzylideneacetone) dipalladium (0.1 equiv), dioxane, 66%.



Scheme 3. Reagents and conditions: (a) 4-methylbenzylamine, Cs_2CO_3, 2-(di-*tert*-butylphosphino)biphenyl, tris(dibenzylideneacetone)dipalladium, dioxane, rt., 18 h; (b) 4-methylbenzylamine, Cs_2CO_3, 2-(di-*tert*-butylphosphino)biphenyl, tris(dibenzylideneacetone)dipalladium, dioxane, 90 °C, 18 h; (c) TFA/H_2O 9:1, 20 min.

pyrazinone in 56% yield with less than 10% of the hydrolyzed bromide (Scheme 3). These conditions were used to construct the library.[14]

Most reagents were prepared as solutions to speed up the setup of the reactions. The resin in dioxane was treated with an excess of amine and base in a reaction vial. The phosphine and palladium were added last. Reaction vials were put in a heated orbital shaker overnight at 90 °C. After this period, the reaction mixtures were transferred to fritted syringes. Following washes with organic solvents, the resin was submitted to a fast cleavage step using a TFA/H_2O solution. *tert*-Butyl ester cleavage was achieved at the same time. The pyrazinones were collected and concentrated. Crude reaction mixtures were purified on an automated semi-preparative LC–MS apparatus. A typical chromatogram from the purification process is shown in Figure 2. 25 mM DMSO solutions of pure compounds were submitted to the enzymatic screen against caspases 1, 3, 7, and 8.[15]

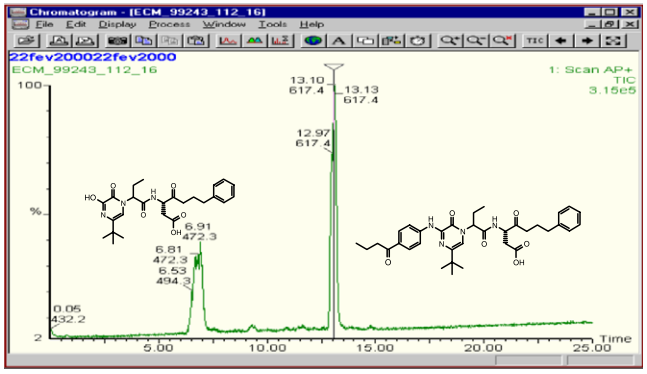
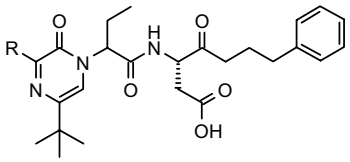


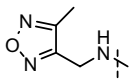
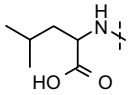
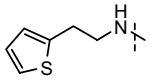
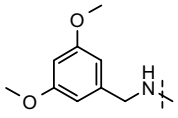
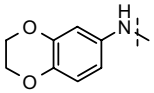
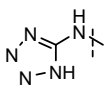
Figure 2. Typical chromatogram from the purification by semi-preparative LC–MS.

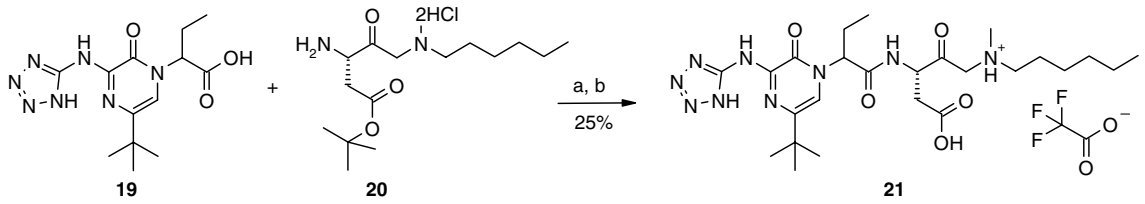
A selection of six (**13**–**18**) of the 80 synthesized inhibitors of caspase-3 is shown in Table 1, representing the most potent molecules for each of the subtypes (aliphatic and benzylic amines, anilines) that were studied. Moderate to high potency was achieved against caspase-3. However, the most striking result was obtained with the 5-aminotetrazole moiety. It appears that **18** is a pan-caspase reversible inhibitor. This inhibitor was then tested in the whole cell assay using NT2 cell line.[16] Introduction of a polar group such as a tetrazole seems to greatly decrease the ability of the inhibitor to penetrate the cells. To improve on this aspect, the tetrazole moiety was combined with an α-amino ketone which we have previously found to have superior whole cell activity (Scheme 4).[6] Compound **21** was still a potent pan-caspase inhibitor (Table 2). Moreover, the shift between the enzyme and the whole cell assay values was reduced below 1000-fold (Table 3).

In summary, palladium-catalyzed amination of bromopyrazinones on solid support was developed and demonstrated with a wide variety of amines. An automated

Table 1. Inhibitory activity (IC_{50}) against human recombinant caspases 1, 3, 7, and 8



Compound	R	Human recombinant enzymes (μM)			
		cas-1	cas-3	cas-7	cas-8
13		5.13	0.014	0.14	5.29
14		5.49	0.10	3.49	>10.0
15		2.31	0.05	0.92	0.30
16		0.32	0.16	0.80	3.05
17		0.33	0.028	0.28	1.000
18		0.009	0.005	0.008	0.014



Scheme 4. Reagents and conditions: (a) HATU, DMF, Et_3N, 0 °C, 1.5 h; (b) TFA/H_2O 9:1, 0.5 h.

Table 2. Inhibitory activity (IC_{50}, nM) of compound **21** against human recombinant caspases 1, 3, 7, and 8

Caspase-1	Caspase-3	Caspase-7	Caspase-8
3.4	6.6	64.7	163.5

Table 3. Inhibitory activity (IC_{50}, nM) in the whole cell assay (NT2)

Compound	Caspase-3	Whole cell	Shift
18	5.4	19390	3579
21	6.6	6068	917

purification step allowed the screening of pure compounds against caspases 1, 3, 7, and 8. The 5-amino-tetrazole was found to be highly potent against the four enzymes tested and provided us with a valuable pan-caspase reversible inhibitor. However, due to the polarity of the tetrazole moiety, this compound suffers from a large shift in the whole cell assay. By combining it with an α-methyldialkylamino ketone, the resulting compound is less than 1000-fold shifted in the cell-based assay.

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14. *Typical procedure:* The resin (200 mg, loading being 4.5 mmol/g of resin) and 2.0 ml of dioxane were mixed together in a 10 ml screw-cap vial. A 1.0 M solution of amine in dioxane (0.45 ml, 5.0 equiv) was added to the reaction vial. A 0.27 M dioxane solution of 2-di-*tert*-butylphosphinobiphenyl (0.10 ml, 0.3 equiv) was then added followed by cesium carbonate (~150 mg, 5.0 equiv). A 0.05 M solution of Pd_2dba_3 (0.20 ml, 0.1 equiv) was added and a flow of N_2 was bubbled into each reaction mixture for ~15 s. Reaction vials were sealed, transferred to a heated orbital shaker, and heated to 90 °C for 20 h. After this period, the reaction mixtures were allowed to cool to ambient temperature and were transferred to fritted syringes. The resin was washed with DMF (2×), 1:1 DMF/H_2O (2×), DMF (2×), MeOH (4×), THF (4×), and CH_2Cl_2 (4×). The resin was dried under house vacuum, then treated with 2 ml of a 9:1 TFA/H_2O solution and rotated for 20 min. Pyrazinones were collected in culture tubes and the resin was washed with 3–5 ml of acetonitrile. Volatiles were evaporated on a GeneVac™. Crude reaction mixtures were dissolved in a minimum volume of DMSO and purified on a semi-preparative LC–MS (CH_3CN/0.1% formic acid) unit equipped with a C8column. Combined fractions were concentrated on the GeneVac™. All compounds were characterized by MS (negative mode) and representative samples were checked by proton NMR.
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