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High-Speed Microwave-Assisted Synthesis of the Trifluoromethylpyrazol-Derived Canonical Transient Receptor Potential (TRPC) Channel Inhibitor Pyr3

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Dedicated to Professor Dr. Peter Stanetty on the occasion of his 65th birthday

Canonical transient receptor potential (TRPC) channels have been implicated in a variety of diverse biological functions.[1] Importantly, they control the influx of Ca^{2+} and other cations, such as Na^{+}, to govern cellular functions in response to stimulation of plasma membrane receptors coupled to phospholipase C.[1] Ca^{2+}-dependent signaling pathways that depend on TRPC function appear essential for the function of a variety of tissues and cell types, including neurons, myocytes, epithelial and immune cells. In B lymphocytes, the TRPC1 or TRPC3 subtypes were suggested to regulate B cell receptor-mediated Ca^{2+} oscillations that activate the nuclear factor of activated T cells (NFAT), a Ca^{2+}-responsive transcription factor.[2] Interestingly, TRPC3 was found to associate with PLCγ2 to control amplification of receptor-mediated signals and T cell receptor-dependent Ca^{2+} entry.[3] Recent investigations have shown that TRPC3, as well as its close relative TRPC6, are key signaling molecules in the heart hypertrophy through activation of calcineurin and its downstream effector NFAT.[4] These results have suggested TRPC channels as new targets for the development of pharmaceutical agents to treat cardiac hypertrophy.

Recently, Mori and co-workers described a pyrazole-based compound (Pyr3, **1**, Figure 1) that selectively inhibits TRPC3 channels.[5] Structure–function relationship studies of Pyr3 and related pyrazoles demonstrated that the rather unusual trichloroacrylic amide group is important for TRPC3 selectivity, and electrophysiological and photoaffinity labeling experiments have confirmed a direct action of Pyr3 on the TRPC3 protein.[5] Moreover, Pyr3 attenuates activation of NFAT and hypertrophic growth in rat neonatal cardiomyocytes, and in vivo pressure overload-induced cardiac hypertrophy in mice.[5] The novel TRPC3-selective inhibitor Pyr3 (**1**), therefore, appears to be not only a useful tool to study the in vivo function of TRPC3, but may also serve as a lead structure for the development of useful drugs for the treatment of TRPC3-mediated diseases, such as pathological cardiac remodeling and heart failure.

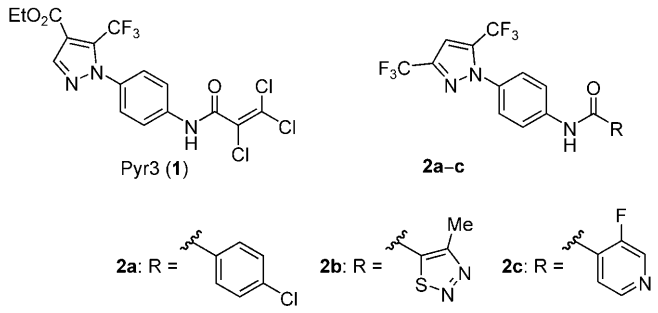
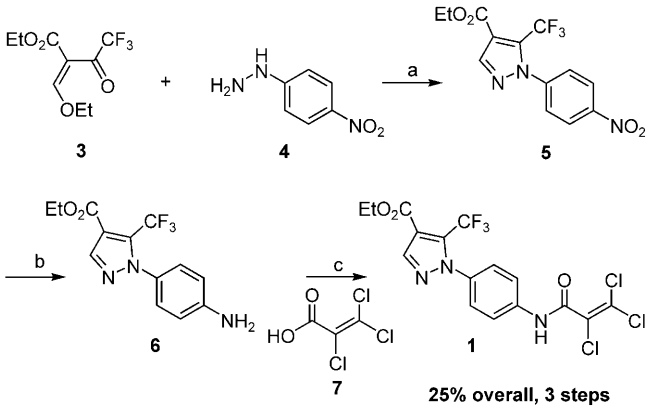


Figure 1. Pyrazole-based TRPC3 inhibitor **1** and NFAT transcription factor regulators **2**.

Herein, we describe an improved synthetic protocol that allows the rapid synthesis of Pyr3 (**1**) and a number of related known pyrazole-based NFAT transcription factor regulators (**2a–c**)[6] by applying controlled microwave heating as enabling technology.[7] Compared to the published three-step method requiring 2 days overall reaction time,[5] our operationally simplified protocol allows the generation of compounds **1** and **2** within less than 40 min.

The details of the literature preparation[5] of Pyr3 (**1**) are shown in Scheme 1, which closely follow the general three-step protocol disclosed by Abbott in 2000 for the generation of related pyrazole structures (e.g. **2a–c**).[8] The desired final compound **1** (Pyr3) was obtained in ~25% overall yield over three steps requiring ~2 days of processing time.

In an attempt to make this protocol more efficient, in particular in the context of a planned high-throughput/combinatorial library approach, we have performed all of the three synthetic steps using sealed vessel controlled microwave heating,



Scheme 1. Published three-step synthesis of TRPC3 inhibitor Pyr3 (**1**).[5] *Reagents and conditions*: a) H_2SO_4, EtOH, Δ, 80 °C (reflux), o/n, 82%; b) H_2, Pd/C, EtOAc, RT, 2 h, 87%; c) BOP, DIEA, DMF, **7**, RT, o/n, 35%.

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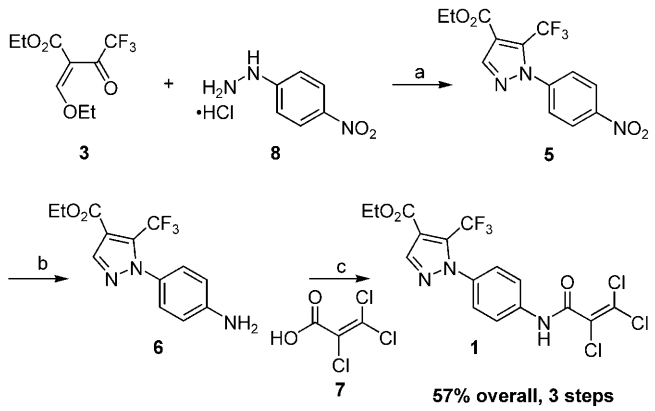
replacing some of the originally chosen synthetic methods with protocols more compatible with microwave chemistry.[7,9]

For the initial condensation step leading to pyrazole **5**[10] we slightly modified the original method in that 4-nitrophenylhydrazine **4** was replaced with the commercially available and more stable HCl salt (**8**) (Scheme 2). This alleviates the need to add H_2SO_4 for the condensation step but otherwise does not present any disadvantages. Various temperature/time regimes



Scheme 2. Microwave-assisted three-step synthesis of TRPC3 inhibitor Pyr3 (**1**). *Reagents and conditions*: a) EtOH, MW, 160 °C, 2 min, 81 %; b) cyclohexene, Pd/C, EtOH, MW, 160 °C, 2 min, 92 % (74 % overall, 2 steps); c) PCl_3, MeCN, **7**, MW, 150 °C, 5 min, 76 %.

were applied in the condensation step (**3**+**8**→**5**), and it was immediately obvious that the reported reaction time of ~16 h under reflux conditions (80 °C) could be substantially shortened by switching to sealed vessel microwave heating in a dedicated single-mode reactor using superheated ethanol as the solvent. Monitoring of the reaction by reversed-phase HPLC demonstrated that full conversion to pyrazole **5** could be obtained, for example, at 140 °C within 20 min, or at 150 °C within 8 min. Careful monitoring of the purity profile of the high-temperature microwave runs showed no negative effect as compared to a control run performed under reflux conditions at 80 °C. Even at a reaction temperature of 160 °C (14 bar internal pressure), the condensation remained clean and no apparent byproducts could be detected by HPLC. Under these conditions, full conversion required only 2 min hold time at the set temperature and led to a 81 % isolated product yield after automated flash column chromatography. The total processing time in the microwave reactor including the ramp time to 160 °C and cooling to ambient conditions for this microwave run was 6 min.

Several options were considered for the reduction of the aromatic nitro group in **5** to the amine functionality. Although it is now possible to perform hydrogenations with molecular hydrogen under pressure using controlled microwave conditions,[11] we considered a catalytic transfer hydrogenation step for safety and convenience reasons, not requiring a specialized microwave apparatus that allows pre-pressurization of the sealed reaction vial with hydrogen gas. For the nitro group reduction step (**5**→**6**), cyclohexene was found to be of appropriate reactivity, not requiring the use of more reactive (and expensive) 1,4-cyclohexadiene or 1-methyl-1-cyclohexene, which have recently been reported by groups from AMRI and GlaxoSmithKline, respectively, for microwave-assisted catalytic transfer hydrogenations.[12] Clean and complete reduction of the nitro group was obtained using 5 mol % of a standard 10 % (*w*/*w*) Pd/C catalyst and five equivalents of cyclohexene and ethanol as the solvent, at 100 °C within 10 min.[12] Running the same reaction at 160 °C (14 bar) using only two equivalents of cyclohexene and 1 mol % Pd/C did not negatively impact reaction purity compared to the 100 °C run, but the hold time could be reduced to 2 min (total processing time 6 min) providing aniline derivative **6** in 92 % yield after filtration from the catalyst and subsequent flash chromatography.

For the final amidation step, again several options were considered. Control experiments showed that the desired target structure **1** could be obtained at room temperature from aniline **6** using standard coupling reagents such as PyBOP/DIEA, or from the preformed acid chloride of acrylic acid **7** (data not shown).[5] Both methods, however, require comparatively long reaction times and provided only moderate yields (see Scheme 1). At this stage, we became interested in a recent literature report by Nikem Research, which described an experimentally very straightforward microwave-assisted amidation protocol that simply involves heating aromatic amines and carboxylic acids (aromatic, heteroaromatic, aliphatic) in acetonitrile in the presence of one equivalent of phosphorus trichloride.[13] High isolated yields for a large variety of amide motifs were obtained by applying a reaction temperature of 150 °C for 5 min.[13] Gratifyingly, the reported procedure using 150 °C (9 bar) reaction temperature and 5 min reaction time worked well without modification for the desired condensation of aniline **6** with acrylic acid **7**, and provided a 76 % isolated yield of target structure **1** (Pyr3) after flash chromatography. No further optimization was attempted at this stage.

In an endeavor to further simplify the synthetic protocol shown in Scheme 2, we considered performing steps 1 and 2 in a one-pot operation. In the event, after the pyrazole condensation step (**3**+**8**→**5**) was completed and the microwave process vial had reached 50 °C by cooling with compressed air, the sealed vial was removed from the microwave cavity and decrimped. Two equivalents of cyclohexene and 1 mol % Pd/C catalyst were added, the vial was resealed and introduced back into the microwave reactor. Processing for an additional 2 min at 160 °C resulted in the formation of the desired reduced aniline **6** in an overall isolated yield of 74 % after flash chromatography. We then wondered if purification of the aniline intermediate **6** resulting from this process was required, and conducted an additional experiment in which the crude reaction mixture containing **9** was simply filtered to remove the Pd/C catalyst, evaporated to dryness to eliminate ethanol (not compatible with the phosphorus trichloride reagent) and taken up in acetonitrile. Subjection of the crude aniline starting material **6** to the phosphorus trichloride/carboxylic acid amide coupling conditions, as described above, gratifyingly provided an overall 57 % isolated of **1** (three steps) after purification by automated flash chromatography. This last experiment was

performed on a ~5 mmol scale in a 20 mL microwave process vial, thus providing the desired inhibitor Pyr3 (**1**) on a >1 g scale. The total processing time to conduct the three steps under microwave conditions, including the filtration/evaporation step (but excluding purification by flash chromatography), was ~40 min, comparing well with the traditional synthesis shown in Scheme 1.[5] Apart from the convenience of speed, the isolated yield using the microwave approach was also significantly higher (57% vs 25%).

Finally, we wanted to explore the scope of this high-speed microwave protocol, and thus have additionally evaluated the synthesis of the related Abbott/Axellas pyrazole-based NFAT transcription factor regulators **2a–c** (Figure 1).[6,8] The microwave protocol could be applied with only minor reoptimization of conditions to the synthesis of analogues **2** derived from 3,5-bis(trifluoromethyl)pyrazole. Specifically, the amount of acid for the pyrazole condensation step needed to be increased, since the initially formed hemiaminal intermediate is difficult to dehydrate due to the trifluoromethyl group. In combination with an increased reaction time of 5 min, a high isolated yield of the corresponding 3,5-bis(trifluoromethyl)pyrazole was obtained (see Supporting Information for further details).

In conclusion, we have presented an improved synthetic protocol for the generation of pyrazole-derived inhibitors of TRPC3 of type **1** and related NFAT transcription factor regulators **2**. Our method relies on the use of high-speed sealed vessel microwave synthesis in automated single-mode reactors that not only resulted in a dramatic reduction of the required reaction and overall processing times, but also provided consistently better product yields than the conventional methods. Therefore, we believe that this new method will be very useful for generating compound libraries of this (and related) scaffolds, in particular considering the translation of the method disclosed herein to a parallel microwave synthesis approach.[14]

Experimental Section

Ethyl 1-[4-(2,3,3-Trichloroacrylamido)phenyl]-5-(trifluoromethyl)-1*H*-pyrazole-4-carboxylate (Pyr3, 1): To a stirred mixture of 4-nitrophenylhydrazine hydrochloride (**8**) (171 mg, 0.9 mmol) and EtOH (2 mL) in a 5 mL Pyrex microwave vial, ethyl 2-(ethoxymethylene)-4,4,4-trifluoro-3-oxobutyrate (**3**) (228 mg, 184 μL, 0.95 mmol) was added. The suspension was stirred for 10 s and sealed with an aluminum crimp and Teflon septa. The reaction mixture was subjected to microwave heating for 2 min (hold time) at 160 °C and then cooled to 50 °C. Cyclohexene (148 mg, 139 μL, 1.8 mmol) was added to the resulting yellow solution, immediately followed by 10% (*w*/*w*) Pd/C (9.6 mg, 0.009 mmol, 1 mol%). The sealed microwave vial was once more exposed to microwave heating for 2 min at 160 °C, filtered and concentrated in vacuo. The crude product (**6**) was dissolved in MeCN (2 mL) and treated with acrylic acid **7** (174 mg, 0.99 mmol) and (dropwise) phosphorus trichloride (137 mg, 88 μL, 0.99 mmol). The suspension was stirred for a further 10 s before being subjected to microwave heating for 5 min (hold time) at 150 °C. The reaction was cooled to room temperature, the solvent was evaporated, and the residue was purified by flash chromatography to yield **1**[5] as a white solid (234 mg, 57%). For further details, see the Supporting Information.

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Keywords: Ca signaling · microwave chemistry · NFAT transcription factor regulators · pyrazole compounds · TRPC channels

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