Microreactor Synthesis

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Integrated One-Flow Synthesis of Heterocyclic Thioquinazolinones through Serial Microreactions with Two Organolithium Intermediates**

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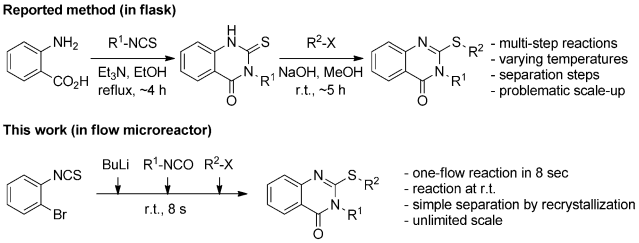
Abstract: *The synthesis of pharmaceutical compounds via short-lived intermediates in a microreactor is attractive, because of the fast flow and high throughput. Additionally, intermediates can be utilized sequentially to efficiently build up a library in a short time. Here we present an integrated microfluidic synthesis of biologically active thioquinazolinone libraries. Generation of o-lithiophenyl isothiocyanate and subsequent reaction with aryl isocyanate is optimized by controlling the residence time in the microreactor to 16 ms at room temperature. Various S-benzylic thioquinazolinone derivatives are synthesized within 10 s in high yields (75–98 %) at room temperature. These three-step reactions involve two organolithium intermediates, an isothiocyanate-functionalized aryllithium intermediate, and a subsequent lithium thiolate intermediate. We also demonstrate the gram-scale synthesis of a multifunctionalized thioquinazolinone in the microfluidic device with a high yield (91 %) and productivity (1.25 g in 5 min).*

Due to their ubiquitous structure as well as biological and pharmacological activity, quinazolinone derivatives are important heterocyclic compounds.[1] They are well-known as a class of drugs with hypnotic and sedative function and contain a 4-quinazolinone core such as afloqualone, cloroqualone, and diproqualone.[2] Moreover, several quinazolinone derivatives have anti-inflammatory[3] and antifungal[4] properties. In addition, S-functionalized thioquinazolinones have recently been reported as a new type of bioactive chemical structure with antiplatelet activity that prohibits platelet aggregation induced by AA (arachidonic acid) and collagen.[5]

In general, S-functionalized thioquinazolinones are synthesized from anthranilic acid and isothiocyanate under harsh reaction conditions and low yield of around 50 % are obtained. Long reaction times of several hours are required and the intermediate after the first step (Scheme 1) needs to be separated for the subsequent reaction.[6] Moreover, for a scale-up of the lab-scale reaction a re-optimization of the reaction conditions is necessary. Therefore, it is desirable to develop a synthetic route toward thioquinazolinones that proceeds under mild conditions to facilitate the development of pharmaceuticals. Furthermore, continuous and integrated synthesis would allow for fast screening and scale-up production.



Scheme 1. Comparison of integrated one-flow synthesis of thioquinazolinones using a microfluidic device compared with conventional flask synthesis.

Flow microreactors based on microfluidics[7] have been shown to be excellent devices for organic chemical synthesis. They are effective in exploring undiscovered synthetic pathways, including in total synthesis, and have recently been utilized for various applications in green chemistry.[8] A unique area, in which microfluidic devices are useful, are syntheses involving short-lived, highly reactive intermediates to produce chemical substances with high efficiency.[9]

In the conventional flask reactor, it is challenging to utilize highly unstable intermediates for the synthesis of chemicals, even at very low temperature. In contrast, the use of a one-flow microreactor enables precise control of the reaction time for vigorously reactive intermediates in the order of milliseconds at room or modest temperatures, resulting in high yields.[10] In particular, it has been reported that short-lived organolithium intermediates such as alkoxycarbonyl-, nitro-, cyano-, or acyl-substituted aryllithium compounds generated in a flow microreactor could be used for reactions with electrophilic reagents.[11] To date, however, there has been no attempt to utilize aryllithiums bearing a strongly electrophilic isothiocyanate (NCS) group in flow chemistry for synthetic chemistry, although the NCS group with two heteroatoms has great potential for the construction of biologically active heterocycles. Therefore, we envisage that biologically active thioquinazolinone libraries can be formed by an intramolecular cyclic reaction of aryllithium-bearing isothiocyanate (NCS) groups with isocyanates (R^1–NCO) from commercially available starting materials in a continuous one-flow microreactor (Scheme 1).

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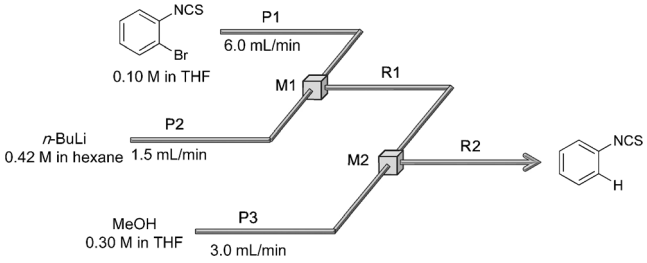
Herein, we present an integrated one-flow microfluidic device for the efficient synthesis of biologically active thioquinazolinone derivatives with sequential reactions involving short-lived NCS-substituted aryllithium species. Multifunctionalized thioquinazolinones could be synthesized within 10 s at room temperature by the three-step integrated microfluidic synthetic process via a subsequently generated lithium thiolate intermediate in high productivity (15 g h^{-1}).

Initially, we focused on the Br–Li exchange reaction of *o*-bromophenyl isothiocyanate **1a** to generate NCS-substituted aryllithium intermediates that react with the electrophilic reagents. In flask, the Br–Li exchange reaction was generally conducted at low temperature, such as −78 °C. The reaction time for the generation of NCS-substituted aryllithium was kept within 1 min to avoid decomposition of the intermediate.[12] However, it was reported that the microfluidic device allowed the reaction of an aryllithium intermediate, generated by Br–Li exchange, with electrophiles at much higher temperature due to excellent control of the reaction time at a time scale that is within the decomposition time scale of reactive intermediates.[11] Therefore, we conducted the Br–Li exchange reaction of *o*-bromophenyl isothiocyanate at mild temperature in a microfluidic device consisting of two T-shaped micromixers (M1 and M2) and two microtube reactors (R1 and R2) as shown in Table 1. Note that the head-on collision of two in-flows through reverse but parallel alignment at M1 and M2 leads to excellent mixing efficiency due to the natural vortex by violent clash.[9a]

Table 1: Br–Li exchange reaction of *o*-bromophenyl isothiocyanate **1a** followed by reaction with MeOH at different temperatures and residence times.



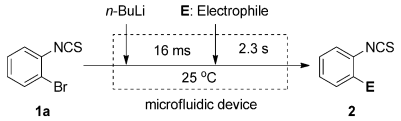
Entry	*T* [°C]	t^R [s][a]	Yield [%][b]
1	0	6.20	72
2	0	1.80	77
3	0	0.25	86
4	0	0.016	91
5	25	6.20	58
6	25	1.80	65
7	25	0.25	73
8	25	0.016	90

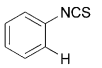
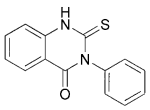
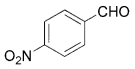
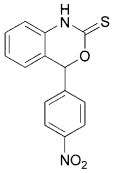
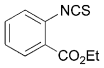
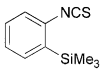
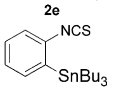
[a] Residence time in the microtube reaction zone R1. [b] Yield of isolated product.

To study the reaction parameters for the generation of *o*-lithiophenyl isothiocyanate, the exchange reaction was followed by protonation with MeOH. The reactions were conducted at two different temperatures (0 °C, 25 °C) with various residence times for the intermediate in the range between 16 ms and 6.2 s (Table 1). In general, the yields of the desired product decreased with increasing reaction time because of the decomposition of unstable *o*-lithiophenyl isothiocyanate. When the reaction time was 16 ms, a good performance was achieved at both temperatures (yields of 90–91 %) by virtue of fast mixing and precise control of residence time in the microfluidic device (entries 4 and 8, Table 1). The result obviously proved that a short reaction time was essential for the generation of the *o*-NCS-substituted aryllithium intermediate through Br–Li exchange and the subsequent reaction could even be performed at room temperature.

With the optimized conditions (16 ms residence time at 25 °C), we further investigated the reactions of *o*-NCS-substituted aryllithium intermediates with various electrophilic reagents. The reaction with phenyl isocyanate (NCO) produced a thioquinazolinone ring compound with high yield (86 %, entry 2, Table 2) by intramolecular cyclization in a two-step integrated microflow synthesis only within 3 s reaction time. In contrast, the reaction in a flask required heating to reflux in ethanol solvent for 2 h to obtain the product **2b** from anthranilic acid.[6] When 4-nitrobenzaldehyde was used as an electrophile, the corresponding benzoxazine was produced in slightly lower yield (75 %), probably due to the strong electron-withdrawing effect of the nitro group (entry 3, Table 2). It is notable that chloroethyl

Table 2: Br–Li exchange reaction of *o*-bromophenyl isothiocyanate **1a** followed by reaction with various electrophilic reagents at 25 °C.



Entry	Electrophile	Product	Yield [%][a]
1	MeOH	 **2a**	90
2		 **2b**	86
3		 **2c**	75
4	EtO_2CCl	 **2d**	96
5	Me_3SiCl	 **2e**	99
6	Bu_3SnCl	 **2f**	90

[a] Yield of isolated product.

formate, chlorotrimethylsilane, and tributyltin chloride could effectively be employed as electrophiles to obtain the desired products in high yields (90–96%, entries 4–6, Table 2).

The formation of thioquinazolinone ring structures by intramolecular cyclic reaction of *o*-NCS-substituted aryllithium with electrophilic isocyanates (NCO) is extended to produce a series of multifunctionalized and biologically active thioquinazolinone derivatives at room temperature by a three-step integrated microfluidic synthetic process.

Specifically, S-benzylic thioquinazolinone derivatives with antiplatelet activity[5] can be synthesized by sequential reactions. In the one-flow reaction, two organolithium intermediates are generated such as 2-isothiocyanatophenyl lithium intermediates **1′** from Br–Li exchange and lithium thiolate intermediate **2′** from serial reactions with two types of electrophiles. As the first example of the S-benzylic thioquinazolinone library, the sequence of the reaction of generated *o*-lithiophenyl isothiocyanate with phenyl isocyanate followed by the reaction with benzyl bromide (Figure 1a) was effectively conducted to produce the desired product **3a** in 80% isolated yield. Moreover, four different phenyl isocyanates with fluoro-, methoxy-, nitro-, and chlorophenyl substituents at the *para* position were used. Additionally, four benzyl bromides substituted with fluoro-, chloro-, trifluoromethoxy-, and trifluoromethylthio groups were employed in the three-step reaction. The S-benzylic thioquinazolinone library was obtained by selecting various pairs of phenyl isocyanates and benzyl bromides as shown in Figure 1b, resulting in good yields (75–98%). Note that all desired final products were readily obtained by a simple recrystallization process.

Finally, a gram-scale synthesis of multifunctionalized S-benzylic thioquinazolinone was performed at room temperature and high yield and high productivity was obtained. By integration of the Br–Li exchange reaction of the starting compound **1b** with 4-methoxyphenyl isocyanate and the sequential reaction with 4-bromobenzyl bromide, multifunctionalized thioquinazolinone could be synthesized within 10 s as shown in Figure 2. An amount of 1.25 g of the product was obtained after only 5 min operation time at the same conditions mentioned before. This excellent productivity is much higher than that of the ordinary microfluidic organic reaction by virtue of the high total flow rate (12 mL min^{-1}), which readily enables pilot scale-up production with no extra investment for the equipment.[12]

In conclusion, we have demonstrated the continuous-flow integrated microfluidic synthesis of a library of biologically active thioquinazolinones. The generation and subsequent sequential reaction of *o*-lithiophenyl isothiocyanate was optimized by controlling the retention time to 16 ms at room temperature. Various S-benzylic thioquinazolinone derivatives were synthesized in yields ranging from 75 to 98% within 10 s at room temperature by three-step integrated flow system. The flash chemistry for short-lived organolithium intermediate in the microreactor also enabled the gram-scale synthesis of a thioquinazolinone in high productivity (1.25 g in 5 min), which bodes well for synthesizing diverse samples for pharmacological screening.

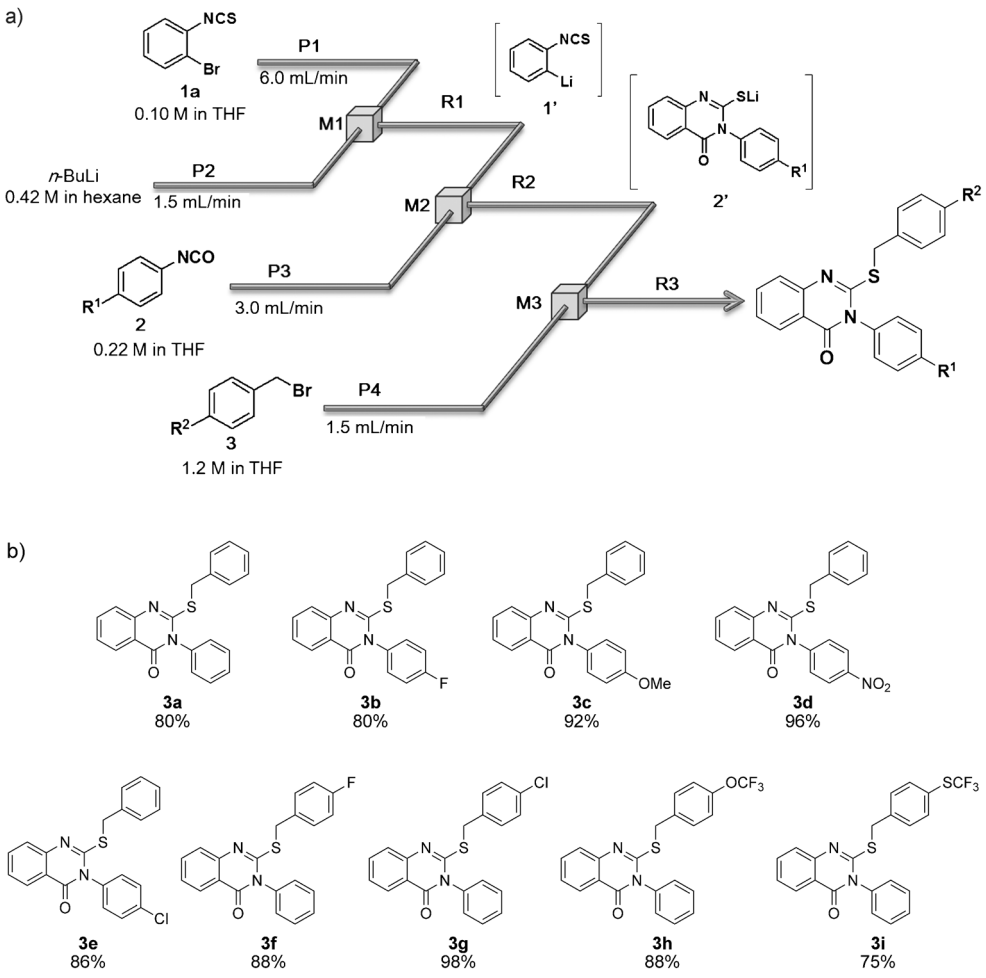


Figure 1. Three-step integrated microflow system for the synthesis of a library of S-benzylic thioquinazolinones through a Br–Li exchange reaction of *o*-bromophenyl isothiocyanate followed by a two-step reaction with various electrophiles at room temperature.

Experimental Section

A microfluidic system consisting of three T-shaped micromixers (M1, M2, and M3), three microtube reactors (R1, R2, and R3)

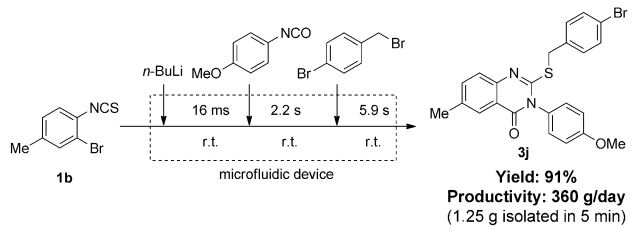


Figure 2. Gram-scale synthesis of multifunctionalized S-benzylic thioqinazolinone **3j** in a microflow system.

and four tube pre-temperature retaining units [P1, P2, P3, and P4 (inner diameter $\varnothing = 1000$ μm, $L = 50$ cm)] were used. A solution of *o*-bromophenyl isothiocyanate (0.10 M in THF) (flow rate: 6.0 mL min^{-1}) and a solution of *n*BuLi (0.42 M in hexane) (flow rate: 1.5 mL min^{-1}) were introduced to M1 ($\varnothing = 250$ μm) by syringe pumps. The resulting solution was passed through R1 and was mixed with a solution of functionalized phenyl isocyanate (0.22 M in THF) (flow rate: 3.0 mL min^{-1}) in M2 ($\varnothing = 500$ μm). The resulting solution was passed through R2 ($\varnothing = 1000$ μm, $L = 50$ cm) and was mixed with a solution of functionalized benzyl bromide (1.2 M in THF; flow rate: 1.5 mL min^{-1}). The resulting solution was passed through R3 ($\varnothing = 1000$ μm, $L = 150$ cm). After a steady state was reached, the product solution was collected for 30 s, while being quenched with sat. NH_4Cl aqueous solution. The reaction mixture was analyzed by GC. The organic phase was separated and the aqueous phase was extracted with EtOAc. Afterwards, the combined organic phase was dried over Na_2SO_4, and solvent was removed. The product was isolated by recrystallization using hexane/methanol and analyzed by ^{1}H and ^{13}C NMR spectroscopy and GC-MS.

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