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ARTICLE

Continuous Flow Photochemistry as an Enabling Synthetic Technology: Synthesis of Substituted-6(5*H*)-Phenanthridinones for use as Poly(ADP-ribose) Polymerase Inhibitors†

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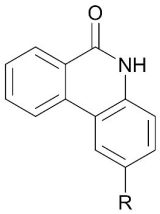
Methods utilizing continuous flow photochemistry, an enabling synthetic technology, have been developed for the generation of phenanthridinones via an intramolecular photochemical cyclization of 2-chloroanilides for the purposes of generating poly(ADP-ribose) polymerase inhibitors. Herein we report 16 examples of a single-step flow photocyclization which produces substituted phenanthridinones in yields up to 99%, while a two-step method leads directly to phenanthridinones from 2-chlorobenzoyl chlorides and anilines via a novel continuous flow amidation/photocyclization protocol. Overall, the flow photocyclization reactions typically progress in good to excellent yields, and in a superior fashion to analogous batch methods, greatly enabling the drug discovery process.

Introduction

Heterocyclic ring systems are an essential constituent core of a large number of bioactive compounds and as a result, serve as important building blocks for modern drug discovery.[1,2] In recent years, new efforts have concentrated upon using the many benefits of flow chemistry to develop improved methods for the synthesis of various heterocycles and apply these advantages within medicinal chemistry programs.[3] Additionally, considerable interest has focused upon the use of flow photochemistry as a tool in organic synthesis,[4,5] and as an enabling synthetic technology in the drug discovery process. In particular, the flow photochemical synthesis of Artemisinin[6] stands out as an excellent example of the many benefits that can be realized when flow chemistry techniques are applied to the synthesis of medicinally relevant compounds. In a direct comparison of batch versus flow photochemical reactions, increased productivity and a demonstrated ability to scale-up the reactions were highlighted as the main benefits of flow photochemistry, and were cited as key advantages over batch processes.[7] A detailed explanation on the myriad of benefits of flow chemistry will not be offered here, as several reviews have been authored on the subject.[8-11] We would like to summarize, however, the benefits derived from flow photochemistry, namely: flow photochemistry is more efficient due to the narrow width of the reactor tubing (Beer-Lambert Law[see 4]) in comparison to batch; the constant elution of product from the flow reaction aids in scalability; control over flow-rates/volume/concentration allows for increased control over reaction conditions and UV irradiation duration, which can control competing reactions or side-products; decreased levels of product decomposition, as the final product spends relatively little time being exposed to photochemical conditions; flow photochemistry can benefit intramolecular reactions, as low concentration reactions avoid intermolecular dimerizations/polymerizations; while continuous processing enhances the production of product from low concentration reactions, a distinct limitation of low concentration batch reactions. Overall, the use of flow photochemistry has experienced increased interest in recent years, due in part to the advent and utility of bespoke, and commercially available flow photochemistry systems.

Poly(ADP-ribose) polymerase (PARP) is a family of nuclear proteins involved in DNA repair and programmed cell death and are responsible for the synthesis of an adenine-containing RNA-like polymer. In general, PARP binds to DNA strand breaks, undergoes automodification, and gives rise to branched poly(ADP-ribose) polymers, enabling the DNA repair process.[12] Several inhibitors of PARP are currently in clinical trials as potential cancer therapies,[13] as inhibitors of PARP are proposed to enhance the effects of cytotoxic alkylating agents.[14] 6(5*H*)-Phenanthridinone and its structural analogs have been known to be inhibitors of PARP family proteins, however, some lack specificity and can elicit promiscuous



(**1**, PJ97A) R = H, unselective
(**2**, PJ34) R = $NHCOCH_2NMe_2$, unselective
(**3**) R = $SO_2NHCH(CH_2)_2$, PARP1-4 selective

Figure 1 6(5*H*)-Phenanthridinone PARP inhibitors

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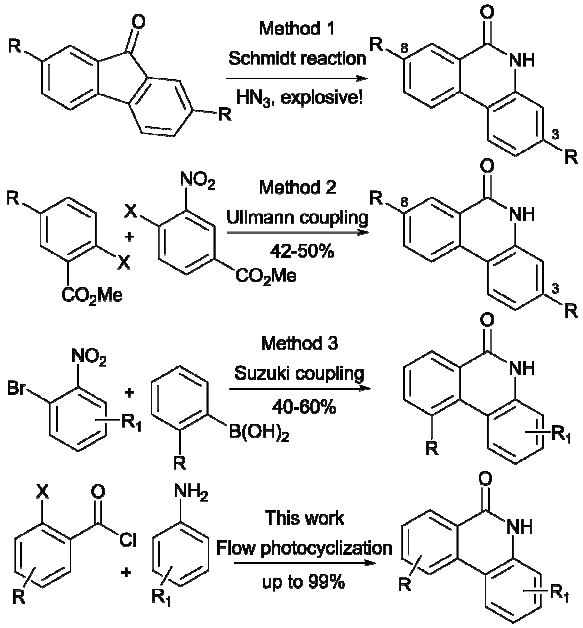
inhibitory behaviour on any number of the family of PARP proteins.[15] For instance, in terms of percent inhibition of PARP activation in macrophages at 1μM concentration, 6(5*H*)-phenanthridinone (PJ97A, **1**) provided 71% inhibition, while PJ34 (**2**) provided 90% inhibition,[16] however, they are both considered to be unselective.[15,17] Interestingly, similar agents containing a sulphonamide residue (**3**) have been found to be PARP1-4 selective.[15]

As part of a medicinal chemistry program, our group needed to develop a robust method for the synthesis of substituted-6(5*H*)-phenanthridinones. An examination of the literature revealed only a single communication which has been published on the synthesis of 6(5*H*)-phenanthridinones for the purposes of PARP1 inhibition.[18] In this communication, three different inefficient methods were explored to construct the characteristic tricyclic framework of phenanthridinone and provide various substitution patterns. Method 1: a Schmidt reaction was used to convert 9-fluorenones to 3,8-disubstituted-6(5*H*)-phenanthridinones using sodium azide in concentrated sulfuric acid, generating hydrazoic acid *in situ* which creates a significant safety risk. Additionally, if the substitution on the 9-fluorenones were not symmetrical, a mixture of regioisomers was observed. Method 2: an Ullmann coupling was used to afford a biphenyl ester, followed by reduction and amidation, however, the key step only progresses in 42-50% yield. Method 3: a Suzuki reaction, followed by intramolecular Friedel-Crafts acylation provided limited access to desired products as the key cross-coupling step only occurs in 40-60% yield. Additionally, other methods have been used to generate phenanthridinone ring systems, such as radical mechanisms[19] or palladium-catalyzed C-H functionalization[20], however, these reactions often lead to complex mixtures of side-products[20] and moderate yields when aryl chlorides are used[19]. As a result of the literature providing poor routes for the synthesis of substituted-6(5*H*)-phenanthridinones, we set out to develop a superior route using alternate methods. We envisioned a simplified two-step process wherein an acyl chloride is reacted with an aniline to provide a 2-halobenzanilide derivative, which in turn, provides a substituted-6(5*H*)-phenanthridinone via a photocyclization reaction. A thorough examination of the literature provided only a single example of the batch photocyclization of 2-halobenzanilides.[21] Unfortunately, the batch method was very limited in scope and synthetic utility, as the communication contained only 6 examples and provided products in low to moderate yield, 23-74%. Additionally, this method was performed in cyclohexane which limits the substrate scope due to solubility issues, while the relatively low auto-ignition temperature of cyclohexane (245˚C) provides for a safety hazard when using hot UV lamps. Our group set out to provide a significant improvement over existing methods by using the many benefits of flow photochemistry to provide a superior route towards substituted-6(5*H*)-phenanthridinones for use in our medicinal chemistry program.



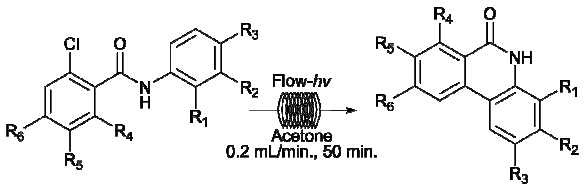
Scheme 1 Methods for 6(5*H*)-phenanthridinone synthesis

Results and discussion

In order to perform our flow photochemical cyclizations, we purchased a Vapourtec R2C/R4 flow chemistry system equipped with a UV-150 photochemical reactor which possesses a medium pressure pure mercury lamp and a 10 mL fluorinated ethylene propylene (FEP) reactor coil. The system contains two HPLC-style pumps which progress material through the UV-150 reactor via sample loops and is mounted to the front of the Vapourtec R4 heating/cooling module. For our purposes, we set the UV power output to 75% (112W) and maintained a constant temperature of 60˚C within the reactor using the R4's forced air cooling feature. This temperature control is one of the many advantages of the Vapourtec flow photochemistry system as the UV-150's integrated temperature probe allows precise knowledge of flow photochemical reaction temperature, which is often problematic (or unknown) for most bespoke flow photochemistry systems. Additionally, the UV lamp is housed within the UV-150 reactor, increasing safety by eliminating user UV-exposure and cooling the system via the R4, reducing the risk of fires caused by the hot UV lamps approaching the auto-ignition temperature of flammable solvents (cyclohexane, 245˚C), a hazard that is often ignored.

Extensive optimization reactions were carried out on the photocyclization of 2-chloranilide (entry 1, Table 1) in order to ascertain the impact of varying solvent, substrate concentration, residence time/flow rate and UV light filter. Protic solvents, such as methanol, did not yield any desired product, however, both acetonitrile and acetone were found to produce the product in significant amounts. Additionally, the photocyclization reaction was also found to proceed if a polar aprotic solvent, such as DMSO, was added to acetone, ratio 1:40 respectively. This result will become useful for future reactions when solubility of potential substrates becomes an issue. Overall, the reactions performed best in acetone, while acetonitrile produced more by-products and showed numerous impurity peaks via HPLC. As for the effect of

concentration on yield, for the parent compound **1**, the reaction performed well in concentration ranges from 0.02M to 5 mM, as all reactions gave yields of 95+%. However, for some of the other substrates, a concentration of 5 mM (0.1mmol/20mL) appeared to give the cleanest reactions, in combination with adequate conversion. At higher concentrations, some substrates gave incomplete conversion, as starting material remained. Obviously, flow rate and concentration are key factors in ensuring that all starting material has been converted to product. For the parent compound **1**, a 5 mM solution could be run at 400 µL/min without any noticeable effect on conversion, however, at higher flow rates >0.5 mL/min, unreacted starting material was observed. For less reactive substrates, an overall flow rate of 200 µL/min was found to give the best results, however, for substrates with unreacted starting material remaining, slower flow rates did give higher rates of conversion. In order to perform a library of reactions, Table 1, we chose to set the flow rate at 0.2 mL/min, as this setting provided for the best balance between substrate throughput and adequate conversion of starting material. However, slower flow rates (~0.15-0.1 mL/min) did give higher yields in some cases where starting material remained, such as entry **2**, **4**, **6**, **11** and **12**. We also investigated different filters for our photochemical cyclization which are available commercially from Vapourtec. Filter 1 is a simple quartz filter which allows irradiation across the full spectrum of a medium pressure pure Hg lamp, however, filter 2 cuts off wavelengths above 400 nm. For the compounds tested, no observable difference in yield was obtained when filter 2 was used, implying that the photochemical reaction was taking place a wavelengths less than 400 nm.



Entry	R_1	R_2	R_3	R_4	R_5	R_6	Yield[a]
1	H	H	H	H	H	H	99
2	H	COMe	H	H	H	H	76
3	OMe	H	H	H	H	H	80
4	Cl	H	H	H	H	H	74
5	H	H	Cl	H	H	H	93
6	H	H	F	H	H	H	80
7	H	H	CF_3	H	H	H	86
8	H	H	H	Cl	H	H	67
9	H	H	H	H	H	Cl	85
10	H	H	Cl	H	H	Cl	83
11	H	H	Cl	Cl	H	H	74
12	H	H	F	Cl	H	H	67
13	H	H	F	H	H	F	84
14	Me	H	H	H	H	H	21
15	H	H	H	H	CF_3	H	26
16	H	Cl	Me	H	H	H	13

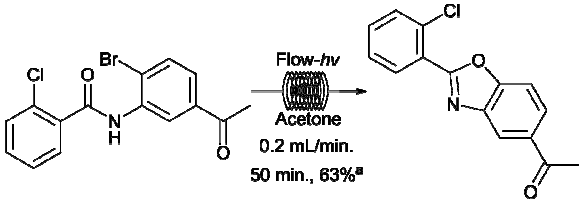
[a] Isolated yields; 10 mL fluorinated ethylene propylene (FEP) coil, 60 °C, Vapourtec UV-150 medium pressure Hg lamp (75%, ~112 W)

Table 1 Flow photochemical synthesis of 6(5*H*)-phenanthridinones from 2-chloroanilides

Using our general reaction conditions of 5 mM of the 2-chloroanilide, 0.2 mL/min (50 minutes flow-*hv* exposure time), in acetone using filter 1 (quartz), we set out to produce a library of compounds which is highlighted in Table 1. The parent compound, entry **1**, produced a product in near quantitative yield, while electron-donating (OMe) or electron withdrawing (Cl) ortho-substituted anilines (R_1) gave similarly high yields (80, 74%, respectively), indicating little electronic effects for this substitution. Para-substituted anilines (R_3) also gave high isolated yields, ranging between 80-93% for electron withdrawing substituents. Substitution was also permitted on the carbonyl component of the cyclization partner, yielding mono- and di-substituted products, typically in good yields (67-85%). We also found three problematic reactions, entries **14-16**, and wish to report that these reactions typically gave multiple unidentified side-products, and some unreacted starting material which contributed to the low yields. We are currently exploring the mechanistic aspects of this reaction, and further exploring the effects of substitution (position and electron-withdrawing/donating nature) on the yield of this photocyclization and will report results in due course.

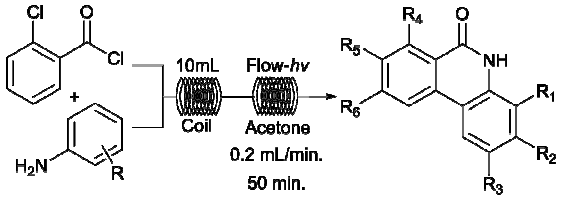
When the Flow-*hv* reaction was performed on a 2-chloro-2'-bromoanilide, an unexpected result was obtained, Scheme 2. The reaction of *N*-(5-acetyl-2-bromophenyl)-2-chlorobenzamide under our generalized flow photochemical conditions produced a benzoxazole ring system in 63% isolated yield, rather than the desired phenanthridinone. A thorough examination of the literature did produce a single communication which describes this reaction[22]. However, the report of this batch photochemical reaction contained only three examples, and occurred in lower yields ranging from 25-60%. Interestingly, the reaction is proposed to proceed via an oxygen radical which participates in the cyclization reaction, however, following the addition of 1.0 equivalent of butylated hydroxytoluene (BHT), a known radical inhibitor, the photochemical reaction, and subsequent yield, appeared to be unaffected, 60+%. An investigation of this reaction mechanism and the development of a protocol for the flow photochemical synthesis of benzoxazoles are proposed for future study.

One of the many benefits of flow chemistry is the ability to combine several separate synthetic steps into a single flow sequence. An early example of this approach is the total synthesis of oxomaritidine[23], which combined seven separate synthetic steps into a single flow sequence. It was our plan to develop a photochemical flow synthesis of phenanthridinones that would take simple commercially available starting materials and produce a tricyclic heterocycle, combining a two-step batch sequence into a novel single flow method. To accomplish this task, a 2-chlorobenzoyl chloride is loaded into a single sample loop (pump A) while a corresponding aniline is



Scheme 2 Flow-*hv* reaction of 2-chloro-2'-bromoanilides

loaded into a second sample loop (pump B). Each of these flow streams are then connected via a T-piece through a 10mL reactor coil to generate an amide which is then directed through the UV-150 photochemical reactor at a total flow rate of 0.2 mL/min. In each case, we found the reaction proceeded as expected and a summary of this single (two-step) flow protocol can be found in Table 2 where a 2-chlorobenzoyl chloride was combined with an aniline to produce the corresponding phenanthridinone in yields which are slightly lower than the single photochemical reaction of the corresponding amide. Traditionally, the amidation reaction is typically performed in cooperation with a base, such as Et_3N or pyridine. We found, however, that the two-step reaction gave higher isolated yields when no additional base was used. We would also like to highlight that by combining two synthetic steps into a continuous flow sequence, there was no need for purification or handling of intermediates, offering greener reaction processing and a more rapid synthesis of drug-like molecules, reducing the cost and cycle-time for development.



Entry	R_1	R_2	R_3	R_4	R_5	R_6	Yield[a]
1	H	H	H	H	H	H	72
2	Cl	H	H	H	H	H	47
3	H	COMe	H	H	H	H	51
4	H	H	Cl	H	H	H	74
5	OMe	H	H	H	H	H	77

[a] Isolated yields; 10 mL fluorinated ethylene propylene (FEP) coil, 60 °C, Vapourtec UV-150 medium pressure Hg lamp (75%, ~112 W)

Table 2 Multi-step phenanthridinone flow-*hv* synthesis via amidation of anilines and 2-chlorobenzoyl chloride

Conclusions

Overall, we have developed a highly efficient method for the synthesis of substituted phenanthridinones using a flow photochemical cyclization reaction. Using a single step flow protocol, we were able to produce 15 examples of the photoflow cyclization of 2-chloroanilides which occurred in yields up to 99%. We were also able to combine two separate synthetic steps into a single flow process which reacted 2-chlorobenzoyl chlorides with anilines, developing a novel flow method for the generation of substituted phenanthridinones in good yields. Currently, mechanistic studies are underway on the photocyclization reaction, and we will shortly initiate testing of our molecules for activity against poly(ADP-ribose) polymerase in a variety of disease-state models. In the near future, we plan to expand the scope of this reaction to include other substrates that will be tested for activity and selectively as PARP inhibitors. The research contained herein represents one of the first examples of the use of continuous flow photochemistry for the synthesis of drug-like molecules and can serve as an example of how the many benefits of flow chemistry can enable the process of drug discovery.

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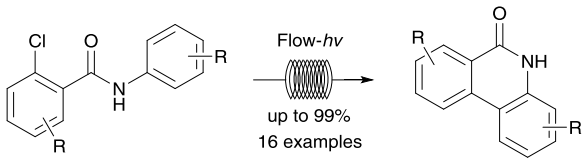
Graphical Abstract



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