Simple, Microwave-Assisted Synthesis of Functionalized *N*-Substituted Quinazolinones

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KEYWORDS. Quinazolinone, microwave-assisted synthesis, organic methodology

BRIEF. A simple, broad method of quinazolinone synthesis was developed.

ABSTRACT. Quinazolinones are a class of compounds that exhibit a diverse range of medicinal properties, such as antitumor activities. As a result, simple methods for their preparation are needed for further pharmacological study. Previous synthetic methods have utilized formamide, orthoformate, various metal catalysts, and microwave technology. In this study, good yields of *N*-substituted quinazolinones were achieved through a novel reaction of anthranilic acid and ethanolamine under optimized microwave conditions. This strategy culminated in a one-step formal total synthesis of the terrestrial alkaloid tryptoquivaline G that avoids flaws in traditional methods such as the necessity for masking and deprotection steps.

INTRODUCTION.

Before the advent of modern chemistry, medicine was sourced from plants. Modern drug development relies far more on laboratory research and synthesis than natural products. In drug discovery, molecules are screened *in vitro* or *in vivo* for biological activities. Methodology research aids the preparation of these molecules. Organic synthesis methods are necessary once a promising compound has been discovered because molecules need to be altered in order to optimize pharmacokinetic characteristics such as efficacy, potency, affinity, and selectivity.

One such promising family of compounds is the quinazolinones. Quinazolinones (2,3-disubstituted 3H-quinazolin-4-ones) arebicyclic heterocycles comprised of pyrimidine fused with a benzene ring. Quinazolinone compounds exhibit medicinal properties such as antitumor [1], anticonvulsant [2], and antiviral [3] activities. The quinazolinone core can be found in naturally occurring alkaloids such as febrifugine [4, 5], an antimalarial compound, and marketed drugs such as methaqualone [6], a sedative-hypnotic. Given their wide range of biological functions, quinazolinones are of pharmacological interest. Thus, simple methods of quinazolinone synthesis are necessary in order to prepare quinazolinone derivatives for further testing.

Past efforts at synthesizing quinazolinones utilized reactants such as formamide [7], orthoformate [8], and metal catalysts [9] in reactions spanning multiple steps, which require intermediate work-ups—labor intensive processes of purifying the crude reaction and isolating the desired product—between each stage of the reaction. However, some efforts have been made at utilizing microwave technology to improve upon these traditional methods. In a two-step, one-pot process [8], Deau *et al.* esterify anthranilic acid using dimethylformamide dimethylacetal (DMFDMA). The instability of the intermediates hampered the purification process, which was required before the amidinoesters could be converted to the desired product through an acid-catalyzed cyclization process. Yield varied dramatically, ranging from 41% to 97%.

In another two-step, one-pot process [6], benzoxazinone and various diamides were prepared from anthranilic acid, acyl chlorides, aminopyrene trisulfonic acid, and the coupling reagent phenyl phospite At first, the diamide intermediates could not cyclize to yield desired products under conventional heating conditions, which led to the observation that microwave irradiation facilitates cyclization. Yield varied dramatically, ranging from 46% to 88%.

Based on a literature survey, it was speculated that anthranilic acids and amines could react to form substituted quinazolinones under microwave conditions in a single-step, one-pot process that avoids some of the flaws of existing processes. Dimethylformamide (DMF) might act as both the solvent and a carbon-source, while microwave irradiation facilitates the ring-closing step. When applied to a variety of substrates, the method was hypothesized to give rise to different yield rates depending on the structure of the substrate.

MATERIALS AND METHODS.

Experimental Procedures.

In a microwave vial, the appropriate anthranilic acid was dissolved in DMF at room temperature. After the appropriate amine was added, the reaction mixture was heated under microwave conditions. The crude reaction was monitored via low resolution mass spectrometry and purified using reverse-phase HPLC to provide the desired product. The percent yield was calculated from the mass of the purified sample. ^{1}H NMR was used to confirm the structure of the product.

Materials.

Commercially available starting materials were purchased from Sigma-Aldrich and were used as received. Microwave reactions were run using a Biotage Initiator Microwave System. Analytical thin layer chromatography (TLC) was performed on Sorbent Technologies HL 0.25 mm silica gel plates with UV indicator. Visualization was accomplished by irradiation under a 254 nm UV lamp. Low resolution mass spectra were obtained on an Agilent 1200 series 6130 mass spectrometer with electrospray ionization. Analytical HPLC was performed on an Agilent 1200 series with UV detection at 215 nm and 254 nm along with ELSD detection. Preparative RP-HPLC purification was performed on a Gilson Inc. preparative UV-based system using a Phenomenex Luna C18 column (50 x 30 mm I.D., 5 μm) with either an acetonitrile (unmodified)-water (0.1% TFA) or an acetonitrile (unmodified)-water (0.1% NH_4OH) custom gradient. The gradient conditions used were either method A) 10% A (water + 0.1% TFA), 90% B (acetonitrile), to 90% A in 5 minutes or method B) 10% A (water + 0.1% NH_4OH), 90% B (acetonitrile), to 90% A in 5 minutes. ^{1}H NMR spectra were recorded on a Bruker AV-II NMR (600 MHz) instrument. ^{13}C NMR spectra were recorded on a Bruker DRX-500 NMR (125 MHz) instrument. All Chemical shifts are reported in ppm from the solvent resonance as an internal standard. Data are reported as follows: chemical shift, multiplicity (s = singlet, d = doublet, t = triplet, m = multiplet), coupling constant (Hz), and number of protons. Statistical analyses were graphed using JMP Pro. Sample purity and identity of select compounds were verified using low resolution mass spectra, ^{1}H NMR, and ^{13}C NMR. The purity and identity of remaining samples were inferred through low resolution mass spectra. The author made all compounds, purified all reactions, and quantified yield.

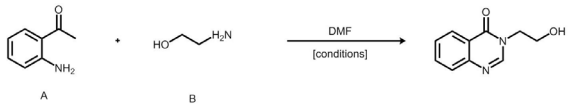
RESULTS.

Optimization.

In order to optimize the process, reaction time, reaction temperature, and stoichiometry of starting materials were adjusted. As a result, yield varied over a range of values from 4% to 71% (Table 1). The optimal reaction conditions were 10 equivalents of ethanolamine at 200 °C over a span of 15 minutes (entry 1, Table 1).

Overall, temperature appeared to have the greatest impact on yield, followed by equivalence and reaction time. Increased temperature, equivalence, and reaction time all led to higher yield rates.

Table 1. Optimization of methodology. Dimethylformamide served as the solvent. Reactions proceeded under specified conditions. Entry 1 (highlighted in brown) specifies optimal conditions.



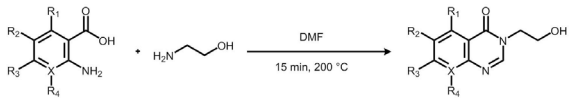
A B

entry	time (min)	temperature (°C)	equiv. (B:A)	yield (%)
1	15	200	10	71
2	15	200	20	67
3	10	200	10	59
4	5	200	10	53
5	15	200	5	43
6	3	200	10	41
7	15	180	5	26
8	15	200	2	22
9	1	200	10	21
10	15	200	1	10
11	15	150	20	7
12	15	150	5	5
13	15	180	1	4

Substrate scope.

In the first stage of the substrate scope study, ten anthranilic acids reacted with ethanolamine (Table 2) under the optimal conditions specified in Table 1, entry 1. Yield varied from 1% to 82% based on electronic properties of the substitution groups. For instance, anthranilic acids that contain highly electronegative substitution groups such as fluorine and chlorine in proximity to COOH or NH2 produced low yields (Table 2, entries 6-10).

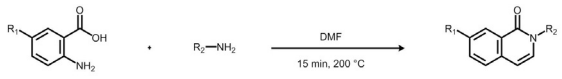
Table 2. Reaction of various anthranilic acids with ethanolamine. Yield decreased with the addition of electronegative R-groups. Me = methyl.



entry	R_1, R_2, R_3, R_4, X	yield
1	H, Me, H, H, C	82
2	Me, H, H, H, C	67
3	H, Cl, H, H, C	67
4	H, F, H, H, C	66
5	H, H, H, Me, C	43
6	H, H, H, CF_3, C	12
7	H, F, H, Br, C	10
8	H, Cl, H, H, N	9
9	H, Br, F, H, C	9
10	F, H, H, H, C	1

To further study the scope of this method, various amines were tested against three anthranilic acids under the optimized conditions of Table 1, entry 1. The yield rates generated ranged from 31% to 81%. Since no anthranilic acids containing strong electron-withdrawing groups were used, yield remained above 30% for all reactions. The electron donating methyl group had a positive effect on yield (Table 3, entries 1, 3, 6).

Table 3. Three different anthranilic acids reacted with four types of amines. Gly = glycine, Benzyl = benzylamine, Furfuryl = furfurylamine, Trp = tryptophan.



entry	R_1, R_2	yield (%)
1	Me, Gly	81
2	H,Gly	62
3	Me, Benzyl	58
4	F, Furfuryl	57
5	H, Trp	54
6	Me, Furfuryl	49
7	F, Benzyl	49
8	H, Benzyl	45
9	H, Furfuryl	40
10	F, Gly	31

In order to demonstrate utility, the method was applied to the formal total synthesis of the natural product tryptoquivaline G (Figure 1a), extracted from the fungal species *Asperlligus clavatus* and first synthesized by Büchi *et al* (Figure 1b). [10] To prepare tryptoquivaline G, Büchi's method required six steps and resulted in 50% yield while the method proposed in this study required only one step at 54% yield.

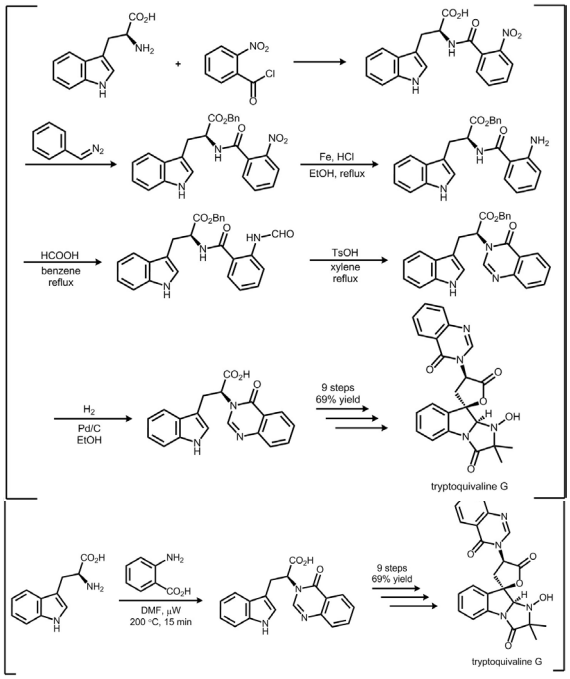


Figure 1. A comparison of the formal total synthesis (**1a**) and Büchi's total synthesis (**1b**).

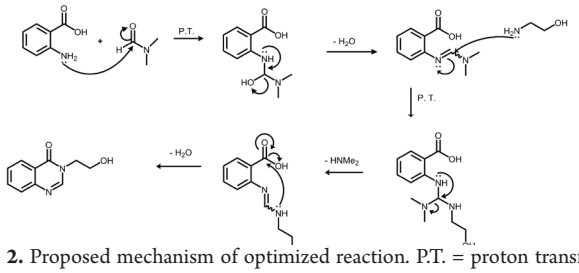


Figure 2. Proposed mechanism of optimized reaction. P.T. = proton transfer. ~ =cis or trans double bond.

DISCUSSION.

A one-pot, one-step method for synthesizing substituted quinazolinones was developed. A proposed mechanism for the optimized reaction is provided in Figure 2. In the first step, the nucleophilic nitrogen of the anthranilic acid attacks the central carbon in DMF, leading to a proton transfer. The nitrogen on the ethanolamine molecule attacks the newly gained carbon after the elimination of a water molecule. The loss of the protonated dimethylamine initiates the ring closing step, and the final product is formed after the loss of another water molecule.

In applying this method to various substrates, the desired product was formed in every instance, which demonstrates the generality of the method. However, this method needs to be optimized for anthranilic acids that contain electron-withdrawing substituents (Table 2, entries 6-10). Electron-withdrawing groups cause an inductive effect that lessens the nucleophilic strength of the amine group. The weakened partial charge of the amine makes the attack on the central carbon atom in DMF more difficult to occur.

In testing various amines, this method demonstrated greater success, with yields ranging from fair (above 50%) to very good (above 80%) in most instances (Table 3, entries 1-9).

The method studied here has certain advantages as well as flaws when compared to previous efforts at quinazolinone synthesis through microwave irradiation. The resulting yield from this method is comparable to yield from previous processes [6, 8] developed by Liu *et al.* and Deau *et al.* Both previous processes are two-step methods that sometimes generate unstable intermediates and require multiple reagents such as APTS, dioxane, pyridine, acid chlorides, and carboxylic acids in addition to anthranilic acids and amines. The method studied here also has a shorter reaction time compared to Liu *et al.*'s 13 to 70 minutes and Deau *et al.*'s 30 minutes. Liu *et al.*'s process requires harsher conditions than this method (250°C compared to 200°C). This method is also advantageous in negating the need for masking the nitro group and deprotecting the amine, which are steps taken by Büchi [10] in his synthesis of tryptoquivaline G (Figure 1b).

Although this method is simpler in execution, the processes developed by Liu *et al.* and Deau *et al.* afford greater chemistry scope. Liu *et al.*'s process remains most efficient when used to synthesize aza-anthranilic acids and anthranilic acids that contain electron-withdrawing groups, while Deau *et al.*'s process remains most efficient when synthesizing aminonicotinic and isonicotinic acids.

In the future, this method of quinazolinone synthesis could be further optimized by running a reaction with optimized stoichiometry while extending the temperature beyond 200°C and lengthening the reaction time past 20 minutes. It would be of interest to observe whether the standard least squares trends will still hold true beyond the conditions that have already been tested. In addition, aminonicotinic acids, aza-anthranilic acids, and other substrates could be tested to further explore the scope of this method.

In conclusion, microwave irradiation facilitated the cyclization step and enables access to scaffolds previously unavailable through conventional heating. The scope of the method indicates potential for the synthesis of diverse analogs. The simplicity of the method gives it appeal for drug discovery purposes.

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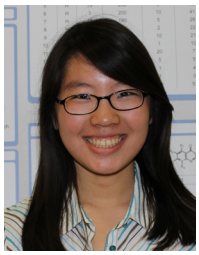

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