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Communications to the Editor

An Efficient Synthesis of Dibenzo[*c*,*f*]-2,7-naphthyridine Ring System through Design of Experiments

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Abstract:

The dibenzo[*c*,*f*]-2,7-naphthyridine ring system was found to be of biological interest, but had limited synthetic accessibility. The initial compound of interest, 10,11-dimethoxy-4-methyldibenzo[*c*,*f*]-2,7-naphthyridine-3,6-diamine, was obtained in <25% yield by reacting 4-chloro-6,7-dimethoxy-quinoline-3-carbonitrile with 2-methyl-benzene-1,3-diamine. A reliable high-yielding procedure was identified through the use of design of experiments (DOE). The effects of stoichiometry of reagents, catalyst, and temperature were explored in this study. The DOE optimization suggested temperatures higher than the boiling point of our solvent. Hence, microwave heating was used, resulting in 80% yield of the desired product.

Introduction

During the course of a high-throughput campaign for a high-priority discovery project, we identified 10,11-dimethoxy-4-methyldibenzo[*c*,*f*]-2,7-naphthyridine-3,6-diamine, **1**, (Figure 1) as a potent compound with favorable biological profile. Further database mining was unable to provide additional analogues with the dibenzo[*c*,*f*]-2,7-naphthyridine scaffold. The high-throughput screening hit **1** had been isolated in a previous project as a by-product in <25% yield. To understand the structure activity relationships of this singleton, and to explore the potential of this series, we needed a reliable high-yielding synthetic procedure.

The original synthetic route that produced **1** as a side product was one that we commonly employ to generate 4-anilino-3-quinolinecarbonitriles[1] such as **8** (Scheme 1). The method involves heating an equimolar mixture of 4-chloro-3-quinolinecarbonitrile, aniline, and pyridine hydrochloride in ethoxyethanol at 130 °C for 8 h. This method has found utility in several instances to generate various other anilino

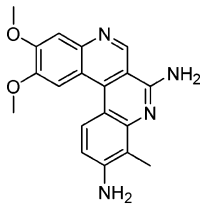

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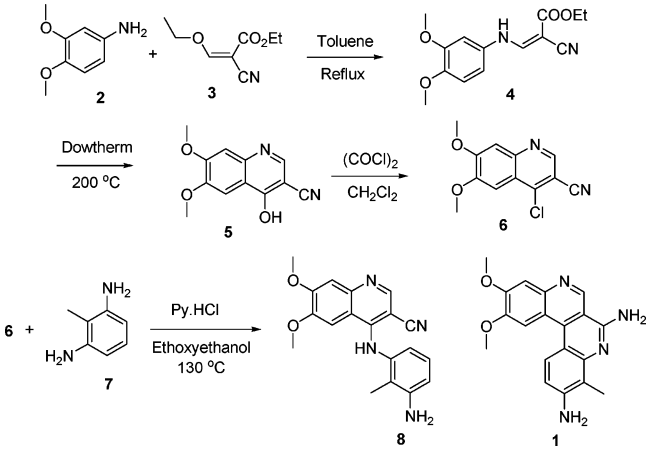
Figure 1. **HTS hit - 10,11-dimethoxy-4-methyldibenzo[*c*,*f*]-2,7-naphthyridine-3,6-diamine.**

Scheme 1. **Original synthetic route for 1**



hetrocyclic carbonitriles such as 7-phenylamino-thieno[3,2-*b*]pyridine-6-carbonitriles,[2a] 4-anilino-benzo[*b*][1,5]-naphthyridine-3-carbonitrile,[2b] 4-anilino-benzo[*b*][1,8]-naphthyridine-3-carbonitrile,[2b] 4-anilino-benzothieno[3,2-*b*]pyridine-3-carbonitrile,[2c] and 4-anilino-benzofuro[3,2-*b*]pyridine-3-carbonitrile.[2c] However, in this instance, the electron-rich nature of the aniline employed led to the isolation of **1** along with the expected product **8**. While the mechanism for formation of **1** can be postulated by invoking a carbon nucleophile of the electron-rich aniline, factors that would favor the isolation of **1** as the major product remained a challenge.

Instead of attempting extensive ad hoc optimization, we chose to use design of experiments (DOE) to evaluate the effects of various synthetic conditions. DOE is used to explore the response surface using a small number of carefully chosen experiments. The experiments are carried out, and a regression equation is fit to the resulting data. The regression model can then be used to find the optimum

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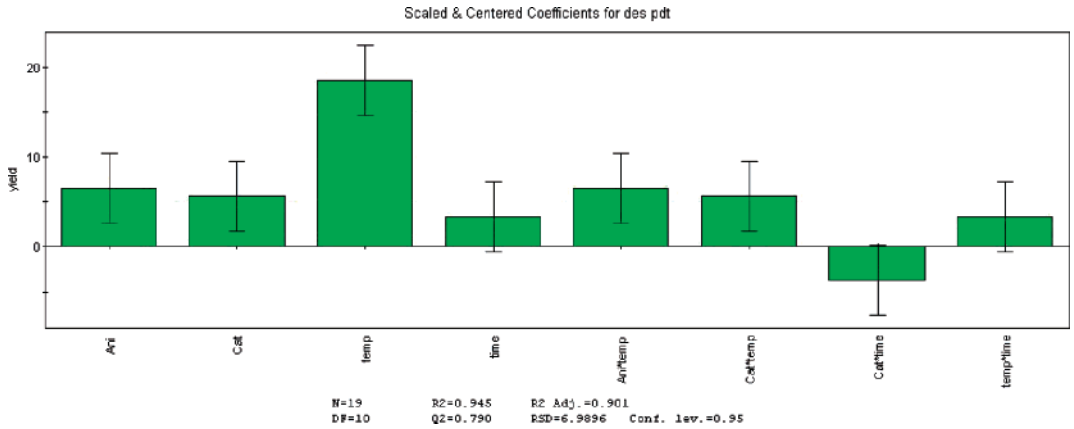

Figure 2. **Plot of the coefficients.**

Table 1. **Factors and ranges**

factor	conc. units	range
aniline	equiv	1−4
catalyst	equiv	0−5
temperature	°C	50−130
time	h	8−24

conditions to maximize the yield. This approach has been extensively used in several disciplines[3] and is now commonly being adopted by synthetic chemists as well.[4]

Results and Discussion

Experimental design, model building, analysis, and optimization of reaction conditions were all performed using the MODDE[5] software application. Four different factors[6] were considered in our study—stoichiometry of aniline **7**, stoichiometry of pyridine hydrochloride (henceforth referred to as catalyst), temperature, and reaction time. The initial ranges of these factors are shown in Table 1. A two-level full factorial design was chosen to screen the factor space. A set of 16 experiments plus three replicates of the center point was carried out to develop a model of the response surface. The design and the yields from these experiments are shown in Table 2. The three center point experiments had a mean value of 26.1 with a standard deviation of 3.5. The data was used to build a predictive model using multiple linear regression. We obtained a good fit, with $R^2 = 0.95$ and $Q^2 = 0.79$. Figure 2 shows a plot of the coefficients of the regression model. It can be seen that temperature, stoichiometry of aniline, stoichiometry of catalyst, and two cross terms are significant.

In order to understand the response surface better, we plotted the predicted yields as a function of two variables in a series of contour plots as seen in Figure 3. In the four plots, yield is plotted as a function of aniline and catalyst stoichiometry. Four combinations of temperature and time are employed in the four different plots. An analysis of the plots shows that the strongest dependency of yield is on temperature, with higher temperatures giving higher yields. Similarly, higher stoichiometries of both aniline and catalyst give higher yields. As per the model, in order to improve our yield beyond 65% (the highest yield we obtained from the initial set of experiments), we needed to increase the stoichiometry of aniline, catalyst, temperature, or various combinations thereof.

Figure 4 shows a contour plot of aniline versus catalyst at 130 °C with an incubation time of 24 h. It can be seen that the model predicts yields in the 90% range with high equivalents of both aniline and catalyst. This prompted us to modify the initial ranges that we set for the factors aniline (1−12 equiv) and catalyst (0−9 equiv) while we maintained the temperature at the boiling point of the solvent (130 °C). The results of these experiments are shown in Table 3. As seen from Table 3, at 9 equiv each of aniline and catalyst we obtained ∼70% yield as predicted by the model. This was an acceptable yield for our purposes, but such high equivalents of aniline and catalyst were not too appealing since it would involve additional work to remove excess reagents at the end of the reaction, particularly during scale-up. We were then left with temperature and incubation time as the only remaining factors. Reaction times of >24 h are inconvenient, thus leaving temperature as the only remaining factor to vary.

The maximum temperature used in the design is indeed the boiling point of the solvent, 2-ethoxyethanol. To achieve higher temperatures under conventional heating, we would need to use a different, higher-boiling solvent. Switching the solvent at this stage of optimization might cause unforeseeable variations and was deemed undesirable. Taking these factors into consideration, we decided to try the reaction under microwave irradiation conditions. Use of microwave irradiation in organic synthesis has gained popularity in recent years, particularly for parallel synthesis. The higher reaction temperatures that can be attained using microwave irradiation—well above the boiling point of the solvent—was especially attractive in our situation. Table 4 shows results

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(5) *MODDE 6.0*; Umetrics: Kennelon, NJ. http://www.umetrics.com.

(6) Preliminary experiments carried out in parallel using the conditions detailed in method A, but varying the concentration (0.025 M, 0.05 M, 0.1 M and 0.2 M) did not show any change. As a result, a detailed study was not carried out using concentration as a factor.

Table 2. **Full factorial design: experimental matrix and measured response**

exp no	exp name	aniline	catalyst	temp (°C)	time (h)	yield (%)	exp no	exp name	aniline	catalyst	temp (°C)	time (h)	yield (%)
1	N1	1	0	50	8	0	11	N11	1	5	50	24	0
2	N2	4	0	50	8	0	12	N12	4	5	50	24	0
3	N3	1	5	50	8	0	13	N13	1	0	130	24	32
4	N4	4	5	50	8	0	14	N14	4	0	130	24	47.3
5	N5	1	0	130	8	0	15	N15	1	5	130	24	31.4
6	N6	4	0	130	8	23	16	N16	4	5	130	24	64
7	N7	1	5	130	8	33	17	N17	2.5	2.5	90	16	22
8	N8	4	5	130	8	65	18	N18	2.5	2.5	90	16	27.8
9	N9	1	0	50	24	0	19	N19	2.5	2.5	90	16	28.4
10	N10	4	0	50	24	0							

Table 3. **Iterative design: experimental matrix and measured response**

exp no	exp name	aniline	catalyst	temp (°C)	time (h)	yield (%)	exp no	exp name	aniline	catalyst	temp (°C)	time (h)	yield (%)
1	N20	1	0	130	8	0	9	N28	1	0	130	22	32
2	N21	4	0	130	8	23	10	N29	4	0	130	22	47
3	N22	1	5	130	8	33	11	N30	1	5	130	22	31
4	N23	4	5	130	8	65	12	N31	4	5	130	22	64
5	N24	12	0	130	8	27	13	N32	12	0	130	22	38
6	N25	12	3	130	8	55	14	N33	12	3	130	22	73
7	N26	2	0	130	8	10	15	N34	2	0	130	22	61
8	N27	9	9	130	8	71	16	N35	9	9	130	22	69

Table 4. **Experiments carried out under microwave irradiation conditions**

exp no	exp name	aniline	catalyst	temp (°C)	time (h)	yield (%)	exp no	exp name	aniline	catalyst	temp (°C)	time (h)	yield (%)
1	N36	1	1	210	1	65.5	3	N38	4	1	210	1	79
2	N37	2	1	210	1	80	4	N39	2	1	130	1	20

of our microwave experiments. As can be seen from the table, we were able to achieve 80% yield by increasing the reaction temperature to 210 °C in a microwave, without the need to use high aniline or catalyst concentrations. Experiment N39 in Table 4 was carried out as a control experiment to judge the microwave effect in the absence of high temperature. It clearly indicates that the high yield obtained can be attributed to the effect of higher temperature.

Conclusion

In conclusion, we have identified a set of practical high-yielding reaction conditions for the synthesis of the dibenzo[*c*,*f*]-2,7-naphthyridine ring system. The effects of stoichiometry of reagents, catalyst, and temperature were explored using DOE techniques. The DOE optimization suggested higher temperatures beyond the boiling point of our solvent. Hence, microwave heating was used, resulting in an 80% yield of the desired product. Although the use of microwave limits the scale-up at present, the method is useful for rapid synthesis of analogues.

Experimental Section

2-Cyano-3-(3,4-dimethoxyphenylamino)acrylic Acid Ethyl Ester (4).[1] A mixture of 3,4-dimethoxyaniline **2** (30.6 g, 200 mmol), ethyl (ethoxymethylene)cyano acetate **3** (33.8 g, 200 mmol), and 80 mL of toluene was stirred at 100 °C for 1 h at 125 °C for 15 min. After evaporation of the toluene, the residue was recrystallized from EtOAc to give 40.0 g (72%) of 2-cyano-3-(3,4-dimethoxyphenylamino)acrylic acid ethyl ester **4** as a tan solid. Mp 166−170 °C; MS (ES^+): *m*/*z* 277.2 [M + H].

6,7-Dimethoxy-4-oxo-1,4-dihydroquinoline-3-carbonitrile (5).[1] A stirred mixture of 2-cyano-3-(3,4-dimethoxyphenylamino)acrylic acid ethyl ester **4** (40 g, 145 mmol) and 1.2 L of Dowtherm A was heated at reflux under N_2 for10 h. After cooling to 50 °C the mixture was diluted with hexane. The product was filtered off, washed with hexane followed by methylene chloride, and dried to give 21.1 g (63%) of 6,7-dimethoxy-4-oxo-1,4-dihydroquinoline-3-carbonitrile **5** as a brown solid. Mp 330−350 °C; ^1H NMR (DMSO-d_6): δ 12.57 (s,1H), 8.59 (s, 1H), 7.44 (s, 1H), 7.03 (s, 1H), 3.89 (s, 3H), 3.87 (s, 3H); MS (ES^+): *m*/*z* 231.0 [M + H].

4-Chloro-6,7-dimethoxyquinoline-3-carbonitrile (6).[1] A stirred mixture of 6,7-dimethoxy-4-oxo-1,4-dihydroquinoline-3-carbonitrile **5** (20 g, 87 mmol) and 87 mL of $POCl_3$ was heated at reflux for 2 h. Volatile materials were removed under vacuum at about 70 °C. The residue was stirred at 0 °C with methylene chloride and H_2O; solid K_2CO_3 was carefully added until the pH was 8−9. After stirring for 30 min at 25 °C the organic layer was separated, washed with H_2O, dried, filtered through Celite, and concentrated to give 19.8 g (92%) of an off-white solid. A sample recrystallized from CH_2Cl_2 gave 4-chloro-6,7-dimethoxyquinoline-3-car-

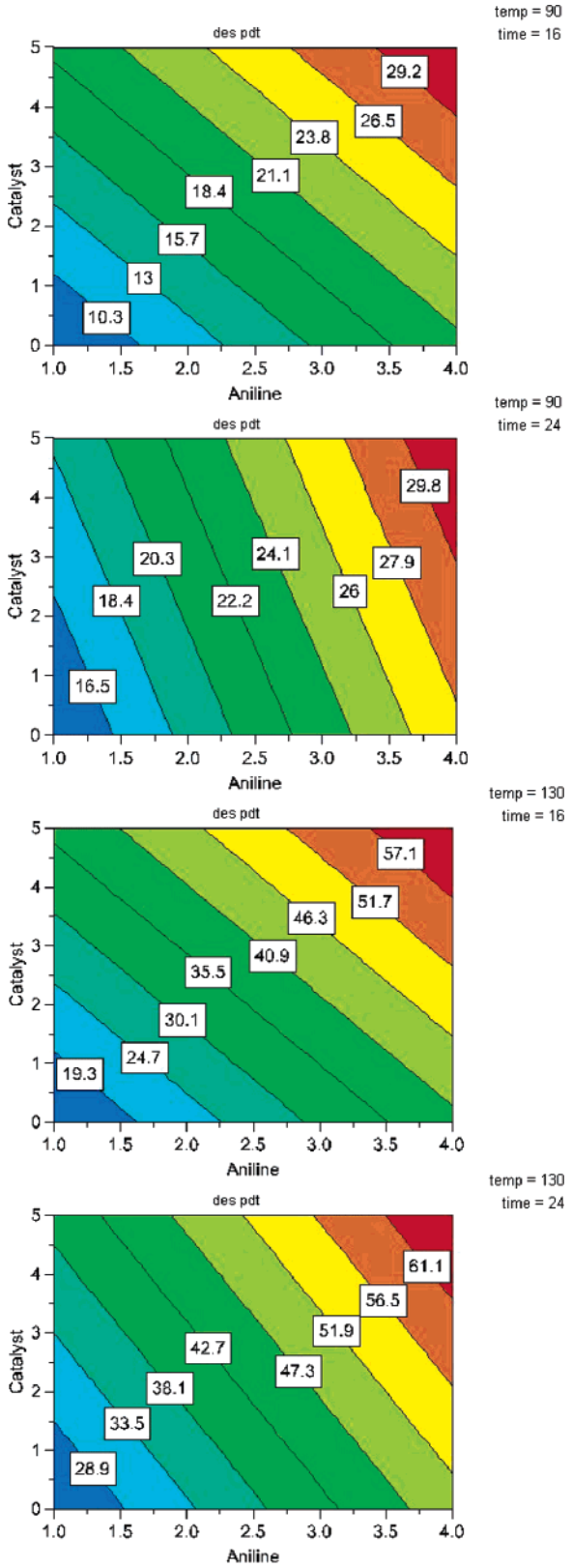

Figure 3. **Contour plots showing dependency on temperature.**

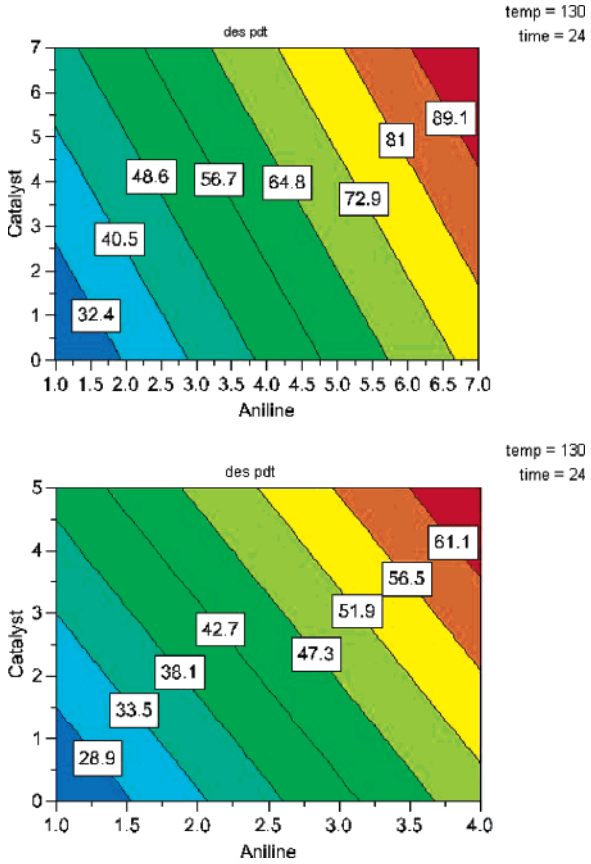

Figure 4. **Contour plot showing dependency on aniline and catalyst at 130 °C for 24 h.**

bonitrile **5** as an off-white solid. Mp 220−223 °C; ^{1}H NMR (DMSO-d_6): δ 8.98 (s, 1H), 7.54 (s, 1H), 7.42 (s, 1H), 4.02 (s, 3H), 4.01 (s, 3H).

10,11-Dimethoxy-4-methyldibenzo[*c*,*f*]-2,7-naphthyridine-3,6-diamine (1). *Method A.* A stirred mixture of 4-chloro-6,7-dimethoxyquinoline-3-carbonitrile **6** (25 mg, 0.10 mmol), 2,6-diaminotoluene **7** (49 mg, 0.40 mmol), and pyridine hydrochloride (58 mg, 0.50 mmol) (catalyst) in 2 mL of 2-ethoxyethanol was heated at 130 °C for 8 h. 10,11-Dimethoxy-4-methyldibenzo[*c*,*f*]-2,7-naphthyridine-3,6-diamine **1** was formed in 65% yield (based on LC/MS analysis).

Experiments listed in Tables 2, 3, and 4 were carried out in parallel. All the experiments listed in a given table were performed on the same day, while the experiments in different tables were performed on different days.

Method B. A stirred mixture of 4-chloro-6,7-dimethoxyquinoline-3-carbonitrile **6** (25 mg, 0.10 mmol), 2,6-diaminotoluene **7** (24 mg, 0.20 mmol), and pyridine hydrochloride (12 mg, 0.10 mmol) in 2 mL of 2-ethoxyethanol was heated in a sealed tube at 210 °C in a microwave oven (Biotage, Initiator) for 1 h using a power limit of 300 W and a pressure inside the reaction vessel of less than 20 psi. 10,11-Dimethoxy-4-methyldibenzo[*c*,*f*]-2,7-naphthyridine-3,6-diamine **1** was formed in 80% yield (based on LC/MS analysis). The reaction mixture was concentrated under reduced pressure, diluted with ethyl acetate, and washed with brine. The organic layer was collected, dried over sodium sulfate, and concentrated. The crude product was purified by flash column chromatography using silica gel. Isolated yield: 72%. HPLC: R_t = 2.13 min; MS (ES$^+$): *m*/*z* 335.1 [M + H]. HRMS: 335.15122 [M + H]; 335.15026 [calculated]; ^{1}H NMR (DMSO-d_6): δ 9.4 (1H, s), 8.4 (1H, d), 8.2 (1H, s), 7.5 (1H, s), 6.9 (1H, br), 6.8 (1H, d), 5.4 (2H, br),

4.0 (3H, s), 3.9 (1H, s), 2.4 (3H, s); IR (cm^{-1}). Cyano group's absorption around 2200 cm^{-1} is not observed.

4-[(3-Amino-2-methylphenyl)amino]-6,7-dimethoxyquinoline-3-carbonitrile (8): ^{1}H NMR (DMSO-d_6): δ 9.3 (1H, s), 8.3 (1H, d), 7.8 (1H, s), 7.3 (1H, s), 6.9 (1H, m), 6.6 (1H, d), 6.5 (1H, d), 5.0 (2H, br), 4.0 (3H, s), 3.9 (1H, s), 1.9 (3H, s), HRMS: 335.14954 [M + H]; 335.15026 [calculated]; IR (cm^{-1}). Cyano group's absorption around 2200 cm^{-1}

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Supporting Information Available

^{1}H NMR, ^{13}C NMR, and IR spectroscopic data for compounds **1** and **8**. This material is available free of charge via the Internet at http://pubs.acs.org.

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