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The accelerated development of an optimized synthesis of 1,2,4-oxadiazoles: application of microwave irradiation and statistical design of experiments

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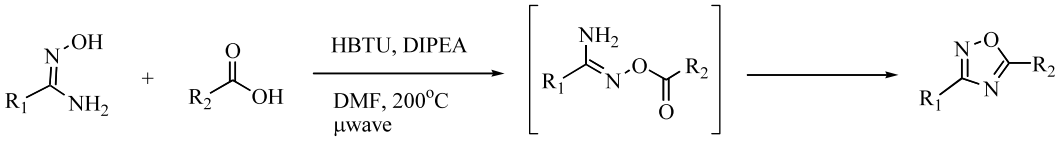
Abstract—Herein, we report the development of an optimized microwave-assisted synthesis of 1,2,4-oxadiazoles. The chemistry development process was significantly accelerated by employing a statistical software package (MODDE 6.0™) to guide in the optimization of the reaction conditions. The resulting optimized reaction conditions were then utilized in the synthesis of a focused library of 1,2,4-oxadiazoles.

The use of microwave irradiation in organic chemistry has exploded over the last few years.[1] Two of the main advantages of this technology are the potential for dramatically shortened reaction times and access to reaction conditions that are not attainable under conventional thermal heating. Combining the speed of microwave-assisted synthesis with the statistical design of experiments affords a powerful tool for the rapid and comprehensive development of optimized reaction conditions. Herein, we report the application of this approach to the development and synthesis of a purified library of 1,2,4-oxadiazoles. 1,2,4-Oxadiazoles are a class of heterocyclic compounds that have been well studied in the literature.[2] This class of heterocycles has been shown to possess a variety of CNS (central nervous system) related activities.[3] 1,2,4-oxadiazoles have also been described as bioisosteres of esters and amides in a number of animal models[4] and have been used in the design of dipeptidomimetics.[5]

An existing synthetic route involves the coupling of amidoximes with carboxylic acids in the presence of *O*-benzotriazol-1-yl-*N*,*N*,*N*′,*N*′-tetramethyluronium tetrafluoroborate (TBTU), 1-hydroxybenzotriazole hydrate (HOBt) and excess *N*,*N*-diisopropylethylamine (DIPEA) followed by in situ thermal cyclization at 110°C.[2] We were intrigued by the possibility of rapidly developing a more efficient process using an approach that couples microwave irradiation[6] with statistical design of experiments. This strategy can be described as follows:

- Step 1. **Synthetic route validation**: probe 5–10 reactions to confirm that the final products can be produced via the synthetic route
- Step 2. **Optimization of reaction conditions**: optimize reaction conditions using a statistical design approach to predict the optimized reaction conditions for one representative final product



Scheme 1. Synthesis of 1,2,4-oxadiazoles.

Keywords: oxadiazole; microwave; design of experiments; amidoxime; optimization.
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- Step 3. **Validation of optimized reaction conditions**: validate the optimized conditions on the step 1 probe set using the predicted optimal conditions along with four additional sets of conditions that vary by 20% from the optimum.
- Step 4. **Test plate**: synthesize a plate of 24 compounds using the best conditions identified in step 3 to confirm the utility of the method for library synthesis

Based on internal results, we chose to substitute TBTU/HOBt with *O*-benzotriazol-1-yl-*N*,*N*,*N*′,*N*′-tetramethyluronium hexafluorophosphate (HBTU) and also to combine both the coupling and cyclization steps for

Table 1. Results from selected experiments generated by MODDE 6.0

Experiment	Time (min)	Temperature (^{O}C)	Equiv. DIPEA	Log (ELSD area)
1	2	110	0	5.08
2	10	110	0	5.43
3	2	200	0	5.21
4	10	200	0	5.13
5	2	110	4.14	5.8
6	10	110	4.14	5.99
7	2	200	4.14	6.03
8	10	200	4.14	6.08
9	2	155	2.07	6.14
10	10	155	2.07	6.21
11	6	110	2.07	6.14
12	6	200	2.07	6.13
13	6	155	0	5.21
14	6	155	4.14	5.99
15	6	155	2.07	6.28
16	6	155	2.07	6.18
17	6	155	2.07	6.21

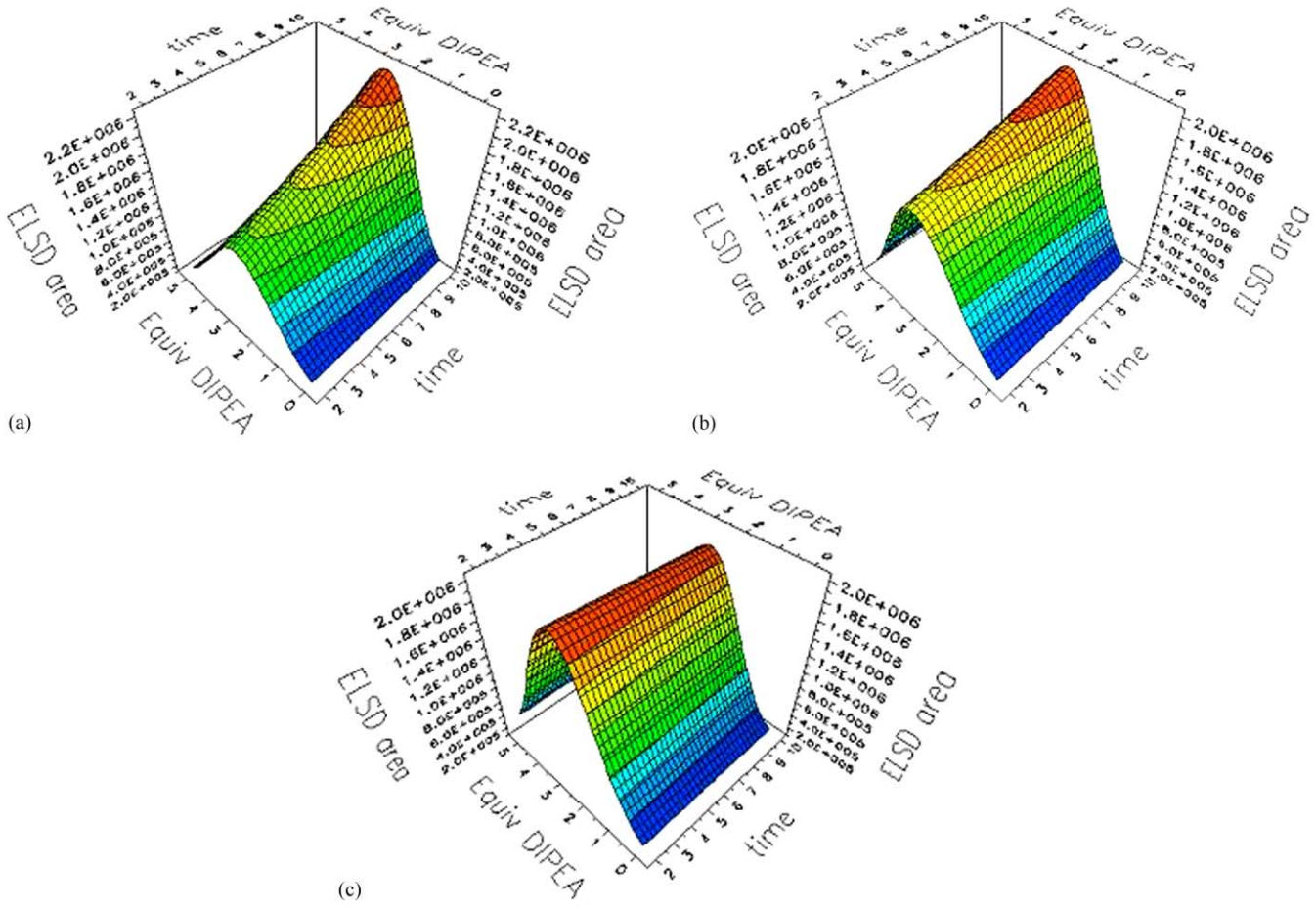


Figure 1. Surface maps of ELSD peak area (reaction yield) as a function of DIPEA equivalents, time, and temperature: (a) 110°C; (b) 155°C; (c) 200°C.

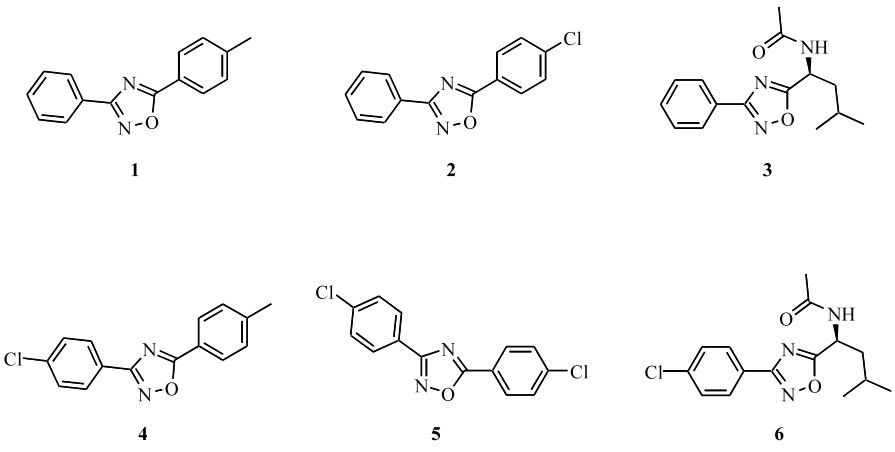


Figure 2. Six compounds synthesized to test the predicted optimal reaction conditions.

Table 2. Five sets of reaction conditions that were used to validate the predicted optimal conditions. ELSD results reported as Log (ELSD)

Conditions	1	2	3	4	5
Temperature (°C)	191	191	200	200	200
Equiv. DIPEA	2.35	3.35	2.35	3.35	2.85
Time (min)	2	2	2	2	2
ELSD area 1	4.95	4.93	4.95	4.79	5.03
ELSD area 2	5.54	5.47	5.42	5.53	5.3
ELSD area 3	6.15	6.1	6.05	6.31	6.2
ELSD area 4	5.38	5.36	5.38	5.28	5.38
ELSD area 5	5.41	5.47	5.39	5.5	5.47
ELSD area 6	6.26	6.1	6.15	6.09	6.01

our initial experiments. A small group of diverse commercially available amidoximes and carboxylic acids were selected and tested under this set of conditions. The results of this one-pot conversion by microwave irradiation at 200°C for 10 min (Scheme 1) were very promising and provided the desired product in all cases (>80% by evaporative light scattering detection (ELSD) purity).

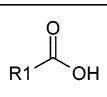
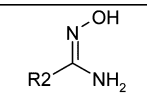
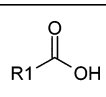
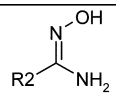
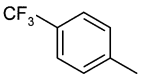
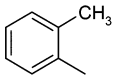
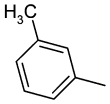
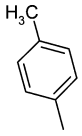
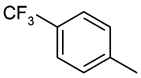
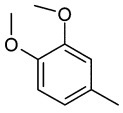
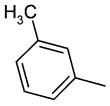
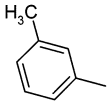
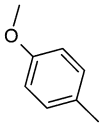
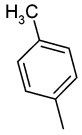
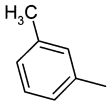
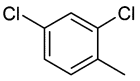
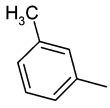
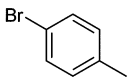
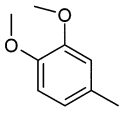
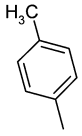
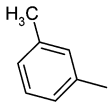
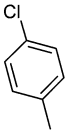
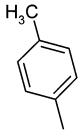
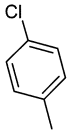
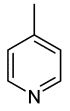
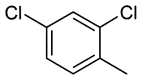
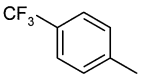
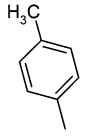
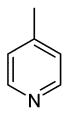
N-Acetyl leucine and benzamidoxime were selected as representative starting materials for optimizing the reaction conditions. We supplied the statistical software package (MODDE 6.0™ from Umetrics, Inc.) with the set of variables we intended to investigate—microwave irradiation time (2–10 min), temperature (110–200°C), and equivalents of DIPEA (0–4.14). Using a central composite face-centered quadratic model, 17 test conditions were generated (Table 1). After each of the reactions conditions were performed, aliquots were removed, diluted to constant concentration and characterized[7] by LC/MS. The log transform of ELSD peak area was used as a measure of crude product formation. In Table 1, the higher values of log ELSD (>6.1) correspond to >95% purity by ELSD and complete conversion based on disappearance of both starting materials.

Surface maps of the logarithm of ELSD peak area as a function of microwave irradiation time, temperature, and equivalents of DIPEA are shown in Figure 1. The surface map identifies that the reaction has a quadratic dependence on the equivalents of DIPEA suggesting that there is an optimal value that can be determined. It also shows that the reaction has an inversely proportional time-temperature relationship requiring longer reaction times at lower temperature and shorter reaction times at higher temperature. Based on the statistical data, the optimal conditions were determined to be 2 min, 200°C, with 2.85 equivalents of DIPEA.

The next step was to validate the optimal conditions by synthesizing the original probe set (Fig. 2). In addition, four other sets of reaction conditions that varied by +/−20% in each variable (except for time, which was held constant at 2 min) were also sampled (Table 2). The results of the validation suggested that there was no significant difference among the five sets of conditions. The following conditions of 2 min, 191°C, and 2.35 equiv. of DIPEA were selected since this minimized the equivalents of base required for the reaction. This corresponded to >90% conversion to the oxadiazole based on ELSD in each of the six cases.

The generality of this method for library synthesis was confirmed by preparing a set of 24 compounds (Table 3)[8] using aromatic acids and both electron-rich and electron-poor amidoximes. The crude ELSD purities ranged from 21–93% by ELSD detection with an aver-

Table 3. Starting materials used in the library synthesis of 1,2,4-oxadiazoles[a]

Entry			Entry		
1			13		
2			14		
3			15		
4			16		
5			17		
6			18		
7			19		
8			20		
9			21		
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[a]Compounds were purified to greater than 98% purity (ELSD) with a general reverse phase HPLC method, which was applied across the whole set and not optimized for individual compounds. All compounds were confirmed by LC/MS; NMR data was collected for representative compounds.[10]

age purity of 68%. For a majority of the samples conversion to the oxadiazole was greater than 90% as measured by disappearance of both starting materials. The crude purities do not reflect the degree of conversion, as the HOBt byproduct was not completely removed by liquid-liquid extraction after the reaction was complete. In all cases, the products were purified by HPLC-MS purification[9] to greater than 98% by ELSD with a typical recovery between 10–20 mg, which corresponds to a recovered yield between 15 and 41%. A general, mass-triggered, reverse-phase method was used to purify the compounds. The method was not optimized for individual compounds and as such recoveries were not fully optimized. However, the milligram quantities met our typical quantity requirements for library synthesis.

It should be pointed out that the total amount of laboratory time spent running reactions and gathering analytical data was approximately 2 days, while the total time spent building the computational models was less than two hours. In total, the process of validating the synthetic route, optimizing reaction conditions, validating the optimized conditions, and synthesizing a small library of compounds took only three days. The quality of the compounds was comparable to compounds synthesized via traditional heating methods.

In summary, the utility of combining microwave irradiation with statistical design of experiments to rapidly develop synthetic methods was demonstrated through the preparation of a 1,2,4-oxadiazole library.

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7. Characterization description:
   Equipment: All RP-HPLC-MS experiments were performed using a Shimadzu HPLC system consisting of two LC-10ADvp pumps, a LEAP Technologies auto-sampler, and an SCL-10A system controller, interfaced with a Waters ZQ mass spectrometer. Relative purity was assessed using an SPD-10A UV detector (@ 254nm) and a SEDEX 75 ELSD detector. The molecular weight of the compounds were confirmed using Electrospray ionization with positive ion detection. A mass spectrometer cone voltage of 20 volts was used for these experiments.
   HPLC columns: The Zorbax SB-C8 columns (4.6×30 mm columns, packed with 3.5 μm particles) were obtained from Mac-MOD.
   Chemicals: Water and acetonitrile were HPLC grade from EM Science. The trifluoroacetic acid was 99+% spectrophotometric grade from Aldrich. The HPLC eluents were prepared by adding 0.1% (vol/vol) trifluoroacetic acid to water (solvent A) and acetonitrile (solvent B).
8. **Production procedure for library of 1,2,4-oxadiazoles**. To a 0.25 M solution of carboxylic acid in DMF (800 μL, 0.2 mmol) was added a 0.25 M solution of HBTU in DMF (800 μL, 0.2 mmol) followed by neat *N*,*N*-diisopropylethylamine (82 μL, 0.47 mmol), and lastly a 0.25 M solution of amidoxime in DMF (800 μL, 0.2 mmol). After the mixture was irradiated in a Personal Chemistry Smith Workstation at 191°C for 2 min, the solvent was evaporated. The resulting oil was dissolved in dichloromethane (2 mL) and washed twice with water (2 mL). The solvent was evaporated to afford the crude product.
9. Purification conditions:
   Equipment: A Maccel semi-prep SH-C18 50×20 mm column was used. All compounds were purified using a Shimadzu HPLC system consisting of two LC-8A prep pumps, LC-10ADvp pump, SPD-10A UV detector, and an SCL-10A system controller. Two Gilson liquid handlers were used for injection and collection and a Micromass Platform LCZ mass spectrometer for detection. The Masslynx version 3.3 platform controlled the injections, tracked all data output, and interfaced with the Gilson Unipoint software to trigger the collection of fractions.
   Experimental conditions: Purification was done by RP-HPLC with MS trigger using a gradient of water (solvent A) and acetonitrile (solvent B) with 0.1% formic acid. Molecular weights were detected using positive electrospray ionization mode on the mass spectrometer with a cone voltage of 20 volts. These general conditions were applied across the set and not optimized for individual compounds.
10. Proton NMR data was collected on a Varian 300 MHz instrument for representative compounds: Entry 8 ($CDCl_3$): δ 2.45 (s, 3H), δ 7.35 (d, 2H, $J=8$ Hz), δ 7.47 (d, 2H, $J=9$ Hz), δ 8.08 (d, 2H, $J=8$ Hz), δ 8.10 (d, 2H, $J=9$ Hz). Entry 21 ($CDCl_3$): δ 2.48 (s, 3H), δ 7.39 (d, 2H, $J=8$ Hz), δ 8.11 (d, 2H, $J=8$ Hz), δ 8.17 (d, 2H, $J=5$ Hz), 8.85 (br, 2H).