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Catalytic one-pot microwave assisted synthesis of 4-azapodophyllotoxin derivatives and rational design of experiment

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A catalytic one-pot microwave assisted synthesis of 4-azapodophyllotoxin has been described. Rational design of experiment has been used to perform reaction yield.

Podophyllotoxin (PT) **1**, a lignin found in herbaceous genus of Podophyllum, has been described to be a strong microtubule destabilizing agent by interaction with the colchicine site of tubulin.[1] The basic structure of PT was modified in order to obtain more potent and less toxic anticancer agents. This research has led to etoposide **2** and teniposide **3**, two glycoside derivatives, used in chemotherapy (Figure 1).[2] However, these compounds present several limitations, such as poor water solubility, development of drug resistance, metabolic inactivation and toxic effects. To overcome such problems, significant effort in the synthesis of 4-azapodophyllotoxins such as **4**, possessing anticancer activity has been carried out by a number of researchers.[3] Particularly, multicomponent reactions starting from substituted anilines, aldehydes and tetronic acid, constitute an ideal tool to access a wide range of these 4-azapodophyllotoxin derivatives.[4] This method approach is particularly efficient when aniline is substituted by electron-donating groups.

Following our interest on synthesis of 4-azapodophyllotoxins,[5] we wish to report a catalytic one-pot synthesis of their derivatives starting from 2-aminobenzyl alcohols combining high speed microwave with design of experiment (DoE) approach. In recent years, catalyzed reactions of 2-aminoaryl alcohol with β-ketoesters via benzylation/propargylation-cyclisation to produce quinoline derivatives have been developed as an alternative method of the Friedländer annulation.[6] Contrarily to this previous reaction, our method presents the advantage to easily access to didehydroquinolines, particularly 4-azapodophyllotoxin derivatives.

Our initial exploration started with the reaction of 4-aminobenzyl alcohol **5a** with dimedone **6** to produce compound **7a** (Scheme 1).[7] The reaction was performed in toluene in the presence of a catalytic amount of *p*-toluenesulfonic acid (PTSA). For the synthesis of **7a**, a study was performed using a face centered composite design[8] with the aim to optimize four variables including reaction temperature (X1), time (X2), amount of dimedone (X3), and amount of catalyst (X4) (Table 1).

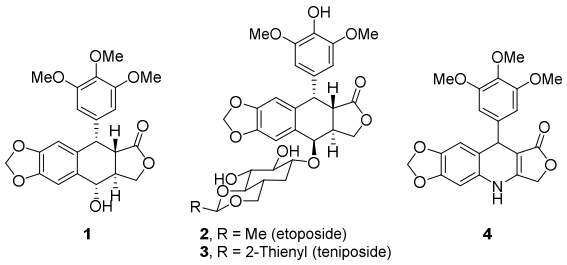
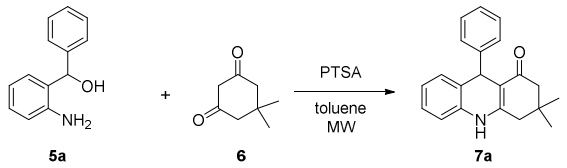


Fig. 1 Podophyllotoxin and their derivatives



Scheme 1 Catalytic addition of dimedone on 4-aminobenzyl alcohol

This experimental design was subjected to a second-order multiple regression analysis to explain the behavior of the system using the least square regression methodology.

This experimental design consisted of 25 trials including 2^4 full factorial design points (Table 1, entries 1-16), 8 axial points (Table 1, entries 17-24)[9] and 2 central points (Table 1, entries 25 and 26).[10]

The low and high factor levels are coded *Xi* = -1 and *Xi* = +1 respectively. The relation between coded and natural variables was given as follows: $ui = ui^0 + \Delta uiXi$, with *ui* the real value, ui^0 the real value at the center point, *Xi* the coded value and $+\Delta ui$ the step change value.

The central composite experimental design was represented by a mathematical model obtained by multiple regressions and fitted with a second order polynomial function represented by the following equation where *y* is the predicted response:

$$y = \beta_0 + \sum_i \beta_i X_i + \sum_i \beta_{ii} X_i^2 + \sum_{ij} \beta_{ij} X_i X_j + \varepsilon$$

Coefficients of the model β are determined by matrix algebra according to the relation: $\beta = (X^t X)^{-1} X^t . y$

X is the experiment matrix in coded variables; X^t is the transposed experiment matrix and $(X^t X)^{-1}$ is the reverse of the matrix product of X^t by *X*, y is the matrix of the answers. β_0, β_i, β_{ii}, β_{ij} are the regression coefficients for intercept, linear, quadratic and interaction terms respectively.

ε represents the experimental error. Real values of each variable as well as the response of each experiment are described on Table 1 and results given were analyzed using the software Modde 5.0 (Umetrics, Sweden).

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Table 1 Design of experiment for screening of the synthesis of **7a**

Exp.[a]	Temp (°C) (X1)	T (min) (X2)	Dimedone (equiv) (X3)	Catalyst[b] (mol%) (X4)	Yield[c] (%)
1	100	20	1.5	5	38
2	140	20	1.5	5	80
3	100	40	1.5	5	38
4	140	40	1.5	5	73
5	100	20	2	5	64
6	140	20	2	5	83
7	100	40	2	5	59
8	140	40	2	5	80
9	100	20	1.5	10	64
10	140	20	1.5	10	67
11	100	40	1.5	10	69
12	140	40	1.5	10	75
13	100	20	2	10	64
14	140	20	2	10	67
15	100	40	2	10	68
16	140	40	2	10	74
17	80	30	1.75	7.5	13
18	160	30	1.75	7.5	74
19	120	10	1.75	7.5	66
20	120	50	1.75	7.5	74
21	120	30	1.25	7.5	69
22	120	30	2.25	7.5	78
23	120	30	1.75	2.5	83
24	120	30	1.75	12.5	68
25	120	30	1.75	7.5	73
26	120	30	1.75	7.5	68

[a] General conditions: **5a** (50 mg, 0.25 mmol), dimedone (x equiv) in toluene (2 mL) with y mol% of PTSA as catalyst under microwave irradiation. [b] *p*-Toluenesulfonic acid. [c]NMR yield with 1,3,5-trimethoxybenzene as internal reference.

Figure 2 summarizes the results of the effects of each factors and possible interactions between them. The models were found to be significant at 95% confidence level by the *F*-test and the coefficients *R*2 of models are above 0.97 indicating that over 97% of the data could be explained by the model.

The results revealed a strong and positive influence of the factor X1 (temperature) on the yield. Furthermore, the interactions between X1 and the X4 (amount of catalyst) and between X3 (amount of dimedone) and X4 were found significant. Only the factor X2 (time) had a negligible effect on the yield indicating that it could be set at its lowest level of 20 minutes.

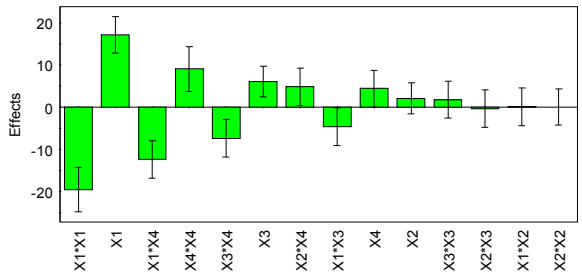


Fig 2 Effects of different factors and their interactions on the yield

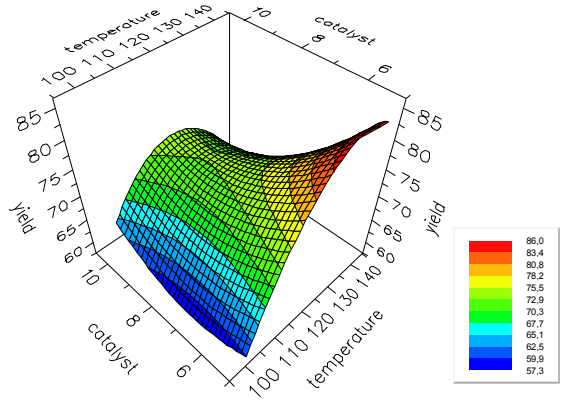


Fig 3. 3D surface contour plots in function of the amount catalyst and temperature factor

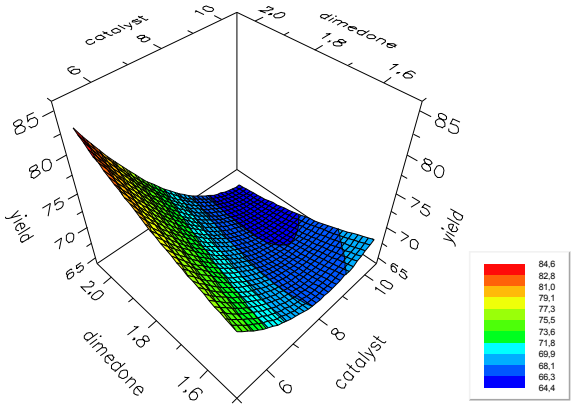


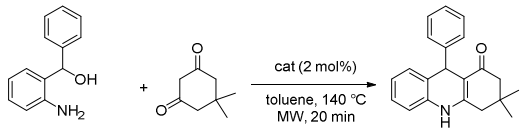
Fig 4. 3D surface contour plots in function of the amount catalyst and dimedone

Figures 3 and 4 illustrate the 3D response surface plots of the second order polynomial model for the studied response (yield) and highlight the interactions X1X4 and X3X4. As observed in Figure 3, the highest yield (85%) was predicted at a high temperature (140 °C) with a small amount of catalyst for a reaction time of 20 min and 2 equivalents of dimedone. In Figure 4, with the reaction conditions set to 20 min at 140 °C, a 85% yield was predicted when a small amount of catalyst and a high quantity of dimedone were combined.

In conclusion, the most favorable conditions to apply were found to be a 20 min reaction time at 140 °C with an amount of 2 equivalents of dimedone and in the presence of 2 mol% of catalyst.

Under these optimized conditions, we explored the reaction using different Brønsted and Lewis acids catalyzed formation of didehydroquinolines (Table 2). As shown in Table 2, in the absence of catalyst a low yield of 42% was obtained (entry 1). Although all catalysts have furnished the product with yields ranging from 67 to 95% (entries 2-7), $ZnCl_2$ was proven to be the most effective.

Table 2 Effect of different catalysts on the reaction of **5a** with dimedone **6**[a]



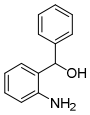
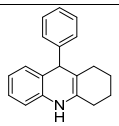
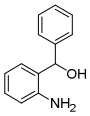
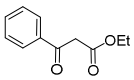
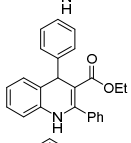
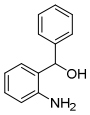
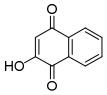
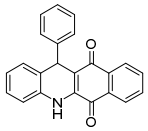
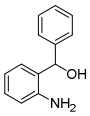
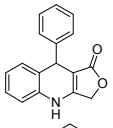
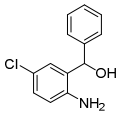
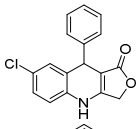
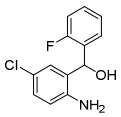
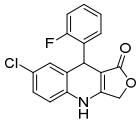
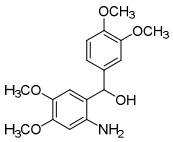
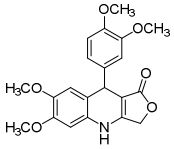
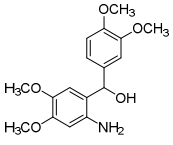
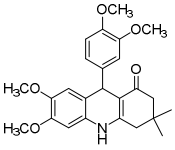
5a **6** **7a**

Entry	Catalyst	Yield[b] (%)
1	none	42
2	PTSA	85
3	H_2SO_4	81
4	TFA[c]	82
5	PA[d]	67
6	$ZnCl_2$	95
7	$CuCl_2$	81

[a]Reaction conditions: **5a** (50 mg, 0.25 mmol), dimedone (2 equiv) in toluene (2 mL) with 2 mol% of catalyst under microwave irradiation for 20 min at 140°C. [b]NMR yield. [c]TFA: Trifluoroacetic acid. [d]PA: Phenylphosphinic acid

With the optimized conditions in hand, the scope of the reaction was explored with various substituted aminobenzylalcohols and nucleophiles (Table 3). In a typical procedure, the reaction was performed with 2 equivalents of ketone and 2 mol% of $ZnCl_2$ at 140 °C for 20 min. Starting from aminoalcohol **5a**, the reaction with ketones such as cyclohexanone, ethyl 3-phenylacetoacetate, tetronic acid and 2-hydroxynaphthoquinone afforded the compounds **7b**-**e** in 60-93% yields (entries 1-4). The reaction was then carried out with tetronic acid and diversely substituted aminoalcohols (entries 5-7). Excellent yields were obtained starting from aminoalcohol **5b** and **5c** (entries 5 and 6). However, the presence of methoxy electron-donating groups on both phenyl groups led a lower 58% yield due to the aromatization of compound **7h** (entry 7). The same reaction with **5e** with dimedone instead of tetronic acid led to **7i** in a better yield of 85% (entry 8).

Table 3. Using optimal condition for some reagents[a]

Entry	Aminoalcohol		Ketone	Product		Yield (%)[b]
1		**5a**			**7b**	93
2		**5a**			**7c**	60
3		**5a**			**7d**	60
4		**5a**			**7e**	68
5		**5b**			**7f**	98
6		**5c**			**7g**	93
7		**5d**			**7h**	58
8		**5e**			**7i**	85

[a]Reaction run employing **5** (0.25 mmol), ketone (2 equiv) in toluene (2 mL) with 2 mol% of $ZnCl_2$ under microwave irradiation for 20 min at 140 °C. [b]Isolated yield.

In summary, we described a catalytic one-pot microwave assisted synthesis of 4-azapodophyllotoxin derivatives. The statistical design of experiments approach has allowed the screening for an optimal system for this synthesis. Further studies on an enantioselective approach are currently underway in our laboratory.

Experimental

Typical experimental procedure (optimized) for synthesis of ***7a****:*[7] a mixture of 4-aminobenzylalcohol 5 (50 mg, 0.25 mmol), dimedone 6 (70 mg, 0.50 mmol) and 2 mol% $ZnCl_2$ (0.9 mg) in toluene (2 mL) were placed in a 10 mL microwave tube equipped with a magnetic stirrer. The sealed tube was placed in the cavity of the microwave reactor and irradiated for 20 min at 150 °C. After evaporation of solvent, the residue was crystallized in a CH_2Cl_2/pentane mixture. M.p. 192-194°C; ^{1}H NMR (DMSO, 300 MHz) δ 9.39 (s, 1H), 7.18 (m, 4H), 7.14-7.01 (m, 3H), 6.93 (dd, *J* = 7.9 and 1.0 Hz, 1H), 6.85 (td, *J* = 7.4 and 1.2 Hz, 1H), 5.06 (s, 1H), 2.51 (d, J = 16.6 Hz, 1H), 2.40 (d, J = 16.8 Hz, 1H), 2.17 (d, J = 16.0 Hz, 1H), 2.00 (d, J = 15.8 Hz, 1H), 1.03 (s, 3H), 0.95 (s, 3H). ^{13}C NMR (DMSO, 75 MHz) δ 192.7, 151.7, 148.7, 136.1, 129.5, 128.0, 126.8, 126.7, 125.7, 125.5, 122.7, 115.2, 106.2, 50.2, 39.7, 32.0, 29.1, 26.7.

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9 Star distance α between the axial points and the center of the domain is generally given by: $\alpha = [nf]^{0.25}$ (nf is equal to 2^k in this study).

10 Central points are repeated and conducted to estimate the experimental error.

A catalytic one-pot microwave assisted synthesis of 4-azapodophyllotoxin has been described. Rational design of experiment has been used to perform reaction yield.