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A novel and practical synthesis of iclaprim

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ABSTRACT

A novel and facile synthesis of iclaprim was reported. Started from Trimethoprim (TMP), the amino-protection and Friedel-Crafts acetylation with acetic anhydride were simultaneously completed in CH_2Cl_2 with $SnCl_4$ as catalyst. The Knoevenagel condensation of 2,4-diamino-5-(2-acetyl-3-hydroxy-4,5-dimethoxybenzyl)pyrimidine with cyclopropyl carboxaldehyde followed by the intramolecular Michael addition in the buffer system (pyrrolidine and acetic acid) installed the key framework (chromanone **13**). The dehydration was catalyzed by H_2SO_4 so that the formation of 5-cyclopropyl-2,3-dimethoxy-4,5,6,6a,7,12-hexahydronaphtho[1,8-bc]pyrimido[5,4-f]azepin-9-amine, an impurity of iclaprim reported at the first time, could be minimized. In the end, iclaprim was obtained in a total yield of 21%.

Keywords

synthesis, iclaprim, acetylation, buffer system, chromanone and sulfuric acid.

Introduction

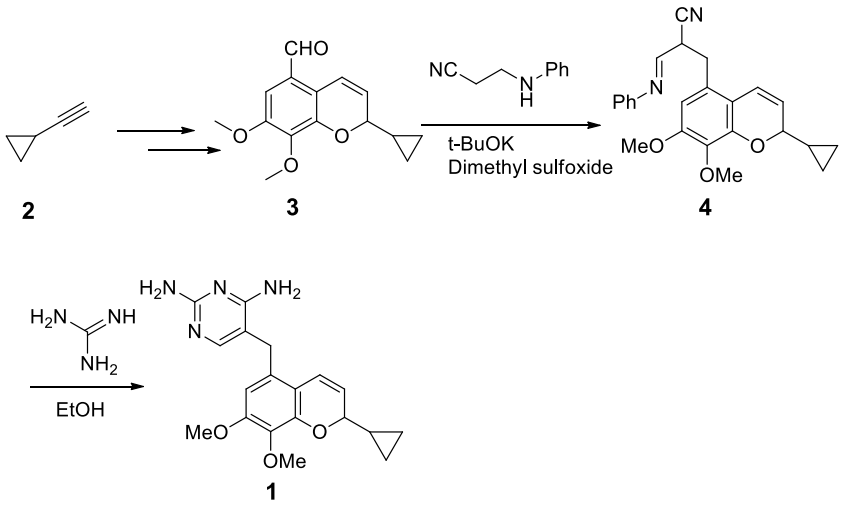
Dihydrofolate reductase (DHFR) is an enzyme essential for bacterial survival that is also an excellent target for antibacterial drug development [1]. TMP is an inhibitor of DHFR used as an antibacterial agent in clinic for many years [2]. Iclaprim (as a racemate), a derivative of TMP was developed by Motif Bio plc. The clinical trial demonstrated that iclaprim was effective for treatment of acute bacterial skin and skin structure infections [3]. Since iclaprim is active against multi-drug resistant Gram-positive pathogens such as methicillin-resistant *Staphylococcus aureus* (MRSA) and vancomycin-resistant *Staphylococcus aureus* [4,5], the synthesis has attracted much attention.

The structure of iclaprim is composed of two heterocycle rings: 2*H*-chromene and pyrimidine. Two kinds of synthetic strategy existed in the related patents [6,7,8], depending on which heterocycle was built at first.

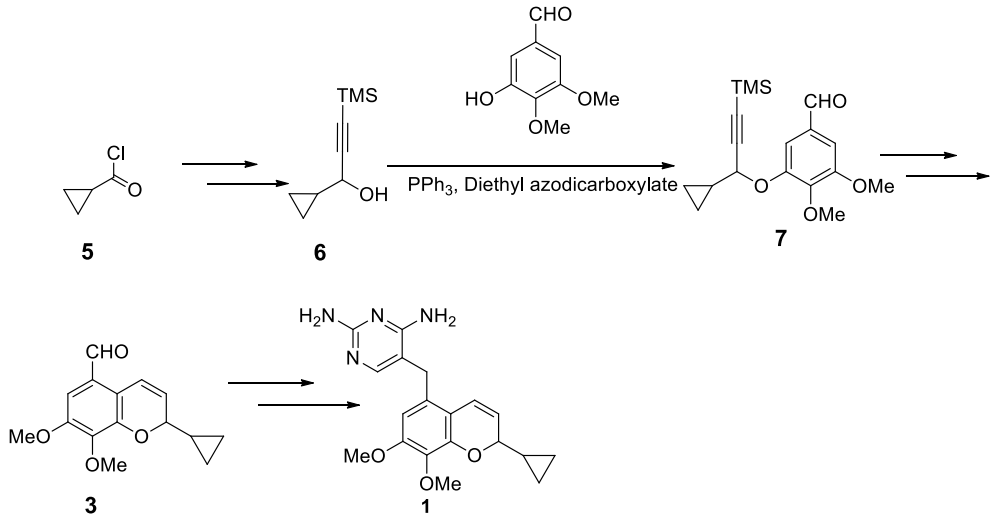
The strategy A (construction of the 2*H*-chromene first), included route 1 (Scheme 1) and route 2 (Scheme 2). In both routes the starting materials (compound **2** or compound **5**) were expensive and there were many key bottleneck steps also. In route 1 [7], three steps were required to establish the 2-cyclopropyl-7,8-dimethoxy-2*H*-chromene-5-carbaldehyde (**3**) from the cyclopropyl acetylene (**2**) in both low yield (<26%) and purity (80%). And the route 2 [6] involved an

essential *Mitsunobu* reaction between 3-hydroxy-4,5-dimethoxybenzaldehyde and 1-cyclopropyl-3-(trimethylsilyl)prop-2-yn-1-ol (**6**) to generate 3-[[1-cyclopropyl-3-(trimethylsilyl)prop-2-yn-1-yl]oxy]-4,5-dimethoxybenzaldehyde (**7**) in a yield of only 31%, and the removal of triphenylphosphine and its oxide in the work-up needed column chromatography. So, these routes mentioned above are difficult to implement in industrial production.

Scheme 1. Route 1:

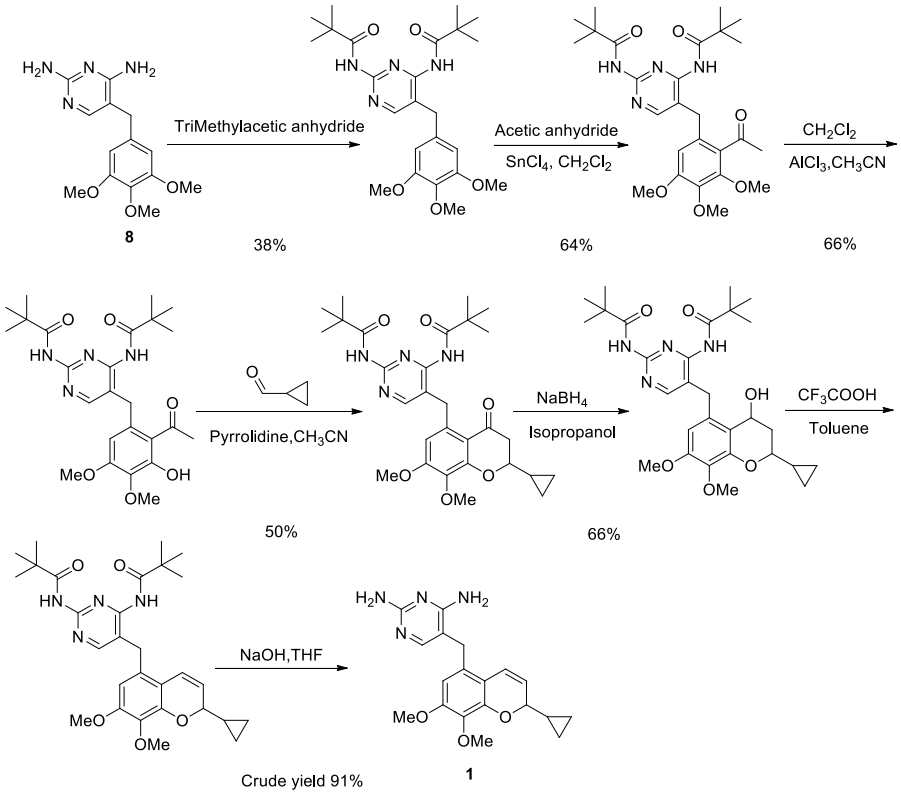


Scheme 2. Route 2:

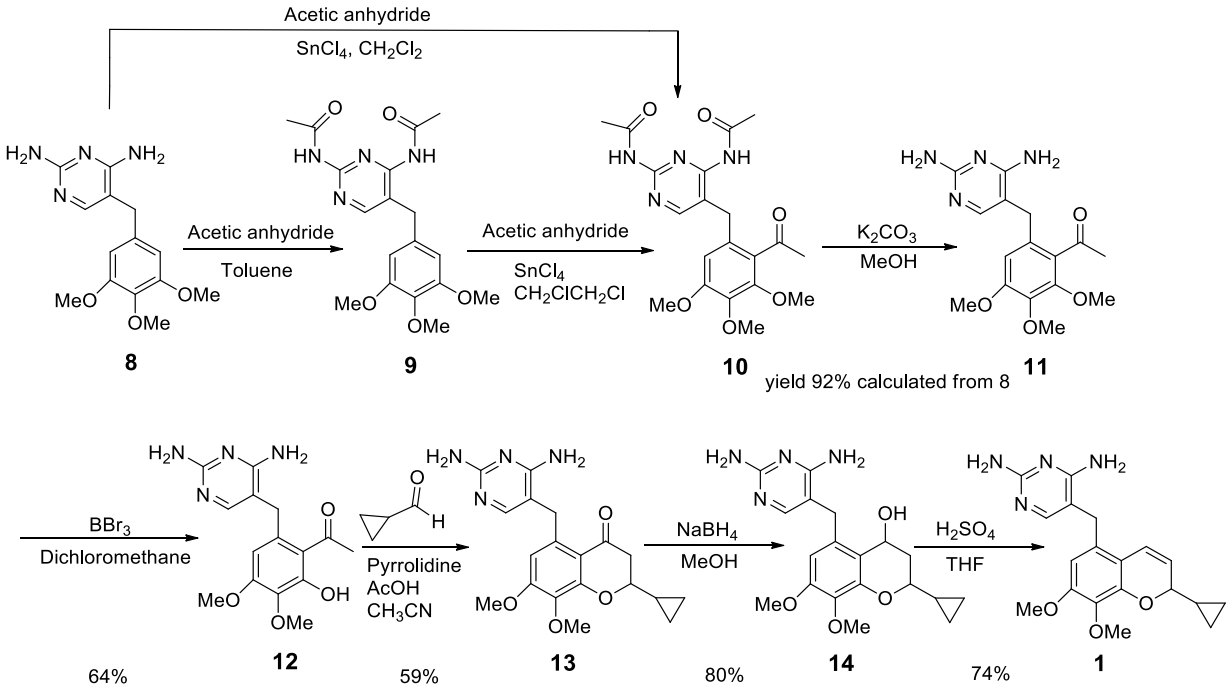


In strategy B (construction of the pyrimidine first), route 3 [8] (Scheme 3) appeared in the patents. It was started with TMP (**8**), a very cheap material. Subsequent protection with trimethylacetic anhydride or iso-butylic anhydride, Friedel-Crafts acetylation, demethylation, cyclization, reduction, dehydration and hydrolysis led to iclaprim (as a crude product) in 4.8% overall yield. According to the patent, almost all the intermediates were subjected to purification by column chromatography. From a scaleup standpoint, it is also an undesirable method. On the basis of these limitations, it is necessary to design a novel method to address the shortcomings. Since TMP is cheap and readily available, we devised a new synthetic route for the iclaprim also with TMP as the starting material, based on strategy B.

Scheme 3. Route 3:



Scheme 4. New route to iclaprim:



Results and discussion

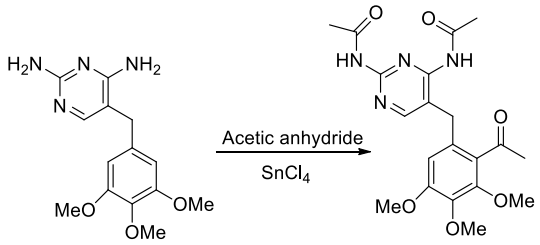
In route 3, the amino groups were protected with the steric hindrance groups, such as pivaloyl or isobutyryl. However, our preliminary experiment found that the following Friedel-Crafts acetylation product was contaminated by the N-acetyl byproducts, i.e., one or two hindrance groups were partially replaced by acetyl group in the Friedel-Crafts reaction. To resolve the problem, we attempted the same reagent both for the amino-protection and for the Friedel-Crafts reaction. So, as the first step in our new route to iclaprim, TMP was reacted with 5 equiv of acetic anhydride in toluene by refluxing to provide 2,4-diacetamido-5-(3,4,5-trimethoxybenzyl)pyrimidine (**9**) in a 86% yield.

To screen the reaction conditions of the Friedel-Crafts acetylation of compound **9** to form 2,4-diacetamido-5-(2-acetyl-3,4,5-trimethoxybenzyl)pyrimidine (**10**), two acetic agents (acetic anhydride and acetic chloride) with different lewis acid ($AlCl_3$, $SnCl_4$, and $TiCl_4$) in CH_2Cl_2 or CH_2ClCH_2Cl were evaluated. To our surprise, in these cases, $AlCl_3$ didn't work at all. Finally, the best result (yield 55%) was obtained by reaction of compound **9** with $SnCl_4$ (2 eq) and acetic anhydride (5 eq) in dichloroethane at refluxing.

After TMP was successfully transformed into compound **10** by two steps, the one-pot synthesis was attempted. Initially, the conversion of TMP to compound **10** was performed by 5 equiv of acetic anhydride and 2 equiv of $SnCl_4$ in dichloroethane under refluxing in a reasonable yield (46%, Table 1, entry 1), that was equivalent to the yields from two steps. However, there was a limitation from the standpoint of largescale preparation. When TMP was charged on a 100-gram scale, a severe emulsification occurred during phase separation in the work-up, probably due to the poor solubility of compound **10** in dichloroethane. Therefore, some solvents that are suitable for the Friedel-Crafts acetylation were tested by solubility experiment. From Tab 2，it was seen that Compound **10** is much more soluble in chloroform than dichloroethane. So, the reaction was carried out in chloroform and give an exciting yield (93%, Table 1, entry 2). The modification on the amount of $SnCl_4$ and acetic anhydride (Table 1, entry 2-5) found that the best result (95%, entry 4) was achieved when acetic anhydride decreased to 4.5eq. Because of the concern of toxicity of chloroform in pharmaceutical industry, the alternative (dichloromethane) was used, and the reaction (entry 6) gave the almost same

result although the amount of solvent was increased.

Table 1. Condition for Amino protection and Friedel-Crafts acetylation of TMP



8 **10**

entry	Acetic anhydride	Lewis acid	Solvent	t (h)	Yield (%)
1	5eq	$SnCl_4$ 2eq	Dichloroethane (10V[a])	10	46%
2	5eq	$SnCl_4$ 2eq	Chloroform (10V)	1	93%
3	5eq	SnCl4 1.5eq	Chloroform (10V)	7.5	85%
4	4.5eq	$SnCl_4$ 2eq	Chloroform (10V)	2.5	95%
5	4eq	SnCl4 2eq	Chloroform (10V)	4	83%
6	**4.5eq**	**$SnCl_4$ 2eq**	**Dichloromethane (15V)**	**7.5**	**96%**

a: volume (ml)/weight (g) of compound **8**

Table 2: The solubility of compound **10**

Solvents	Chloroform	Dichloromethane	Dichloroethane
Solubility (%)	11.2	4.6	0.6

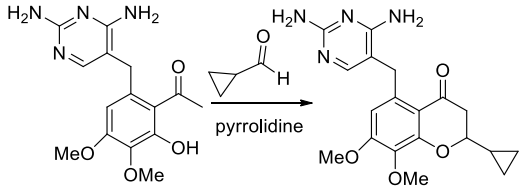
For the demethylation, the preliminary experiments started with the amino-protected compound **10** and lewis acid ($AlCl_3$ and BBr_3) and various solvents (acetonitrile, toluene, dichloroethane, and dichloromethane) were tested, the best result was obtained with BBr_3 in dichloromethane to provide 2,4-diacetamido -5-(2-acetyl-3-hydroxy-4,5-dimethoxybenzyl)pyrimidine in a 21% yield. The reason of a such low yield was due to the formation of some by-products (partial de-protection in

2,4-diacetamido), that made the purification complicated. Therefore, our attention turned to demethylation of 2,4-diamino-5-(2-acetyl-3,4,5-trimethoxybenzyl)pyrimidine (**11**). Compound **11** was prepared by reaction of compound **10** with K_2CO_3 (0.1 eq) in methanol in 97% yield. And then, demethylation of 11 with 1.5 equiv BBr_3 in dichloromethane gave 2,4-diamino-5-(2-acetyl-3-hydroxy-4,5-dimethoxybenzyl)pyrimidine (**12**) in a yield of 65%. When the amount of BBr_3 was increased or decreased (1.2 or 2 equiv), the yields were both declined to 50%.

The key step in the synthesis of iclaprim is the cyclization of compound **12** with cyclopropyl carboxaldehyde to form 2-cyclopropyl-5-[(2,4-diaminopyrimidin-5-yl)methyl]-7,8-dimethoxychroman-4-one (**13)**. The reaction should involve the Knoevenagel condensation of compound **12** with cyclopropyl carboxaldehyde followed by the intramolecular Michael addition by the ortho-phenolic group. In general, the Knoevenagel condensation was catalyzed by organic base such as the secondary amines. So, the reaction was carried out in the presence of pyrrolidine, and it gave a too complicated mixture to purify (Table 3, entry 1), in which beside compound **13**, some impurities formed by the intermolecular addition of the Knoevenagel product with pyrrolidine were found by the ^{1}HNMR data. Therefore, diisopropylamine, the sterically hindered base was employed, but no improvement was observed (entry 2). And then, the buffer system (pyrrolidine1.5eq/acetic acid 1eq) was attempted, a 44% yield of the compound **13** was achieved (entry 3). Addition of 1.5 equiv

acetic acid to the reaction led to a 15% raise in the reaction yield (entry 4). The other modification such as increasing the amount of acetic acid or the aldehyde didn't produce the better result (entries 5-7). Surprisingly, in the tests with piperidine, a very similar base with pyrrolidine (entry 8), the reaction did not take place (most of the starting material **12** was recovered).

Table 3. Condition for cyclization of compound **13**



12 **13**

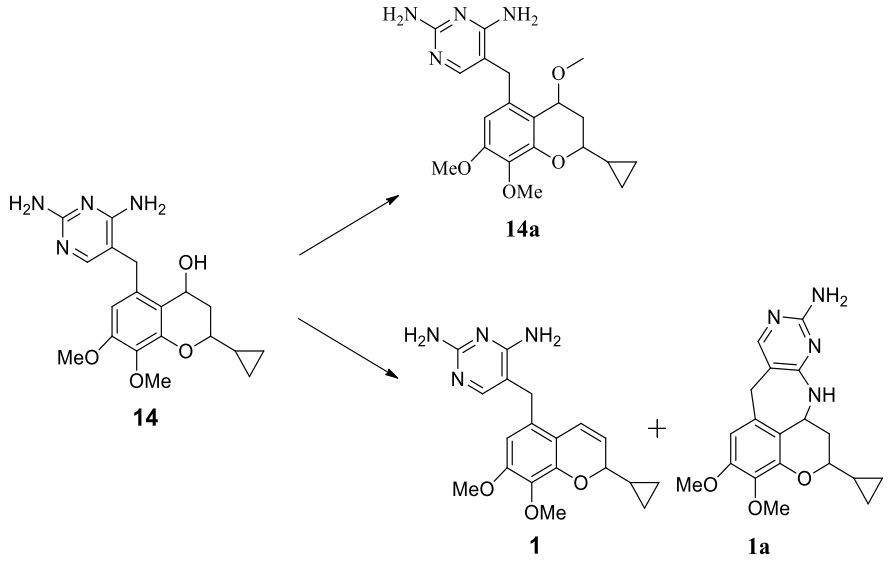
Entry	Cyclopropanal	Base	Solvent	T	t (h)	Yield (%)
1	1.2eq	Pyrrolidine 1.5eq	Acetonitrile	rt	3	mixture
2	1.2eq	Diisopropylamine 2eq	Acetonitrile	reflux	8	mixture
3	1.2eq	Pyrrolidine 1.5eq CH_3COOH 1eq	Acetonitrile	rt	36	44
4	**1.2eq**	**Pyrrolidine 1.5eq CH_3COOH 1.5eq**	**Acetonitrile**	**rt**	**36**	**59**
5	1.2eq	Pyrrolidine 1.5eq CH_3COOH 2eq	Acetonitrile	rt	36	59
6	1.5eq	Pyrrolidine 1.5eq CH_3COOH 1.5eq	Acetonitrile	rt	36	58
7	1.2eq	Piperidine 1.5eq CH_3COOH 1.5eq	Acetonitrile	rt	50	recovery of starting material **12**

With cyclization product **13** in hands, the

2-cyclopropyl-5-[(2,4-diaminopyrimidin-5-yl)methyl]-7,8-dimethoxychroman-4-ol (**14**) was prepared by reduction with 0.5 equiv of $NaBH_4$ in 80% yield.

The final step was the dehydration. In the beginning, compound **13** was heated under refluxing in methanol under the persence of $TsOH{\cdot}H_2O$ (Table 4, entry 1), and 2-cyclopropyl-4-methoxyl-5-[(2,4-diaminopyrimidin-5-yl)methyl]-7,8-dimethoxychroma n (**14a**) instead of iclaprim (entry 1) was obtained as the main product. It was seen that methanol worked as a nucleophilic reagent to attack the benzyl position of chroman in compound **13**. So, the solvent was changed into tetrahydrofuran (THF) and the reaction produced iclaprim in a low yield (34%, entry 2). Different acids as the catalyst were evaluated for this reaction, and the results were listed in Table 4. The best condition was achieved using H_2SO_4 (1.2eq) in THF (entry 6). Although the reactions with CF_3COOH as a catalyst gave disappointing yields, an unknown impurity was isolated in the reaction with toluene as the solvent (entry 4). It was identified as 5-cyclopropyl-2,3-dimethoxy-4,5,6,6a,7,12-hexahydronaphtho[1,8-bc]pyrimido[5,4-f]aze pin-9-amine (**1a**) by ^{1}HNMR, ^{13}CNMR, and MS data. With compound **1a** in hands, an HPLC method was established to monitor the process. Under the presence of H_2SO_4, the best refluxing time was 4 hrs. At this time, although ca 2% of compound **14** was retained, the formation of impurity 1a was controlled under 2%. Finally, iclaprim was obtained with 73% yield and with a satisfactory purity of >99% after recrystallization with 95% ethanol (Table 4 entry 6).

Table 4. Condition for dehydration



Entry	Acid	Solvent	T(℃)	t (h)	Yield (%)	Monitoring the reaction process by HPLC		
						1 (%)	Material 14 (%)	1a (%)
1	$TsOH.H_2O$ 1.5eq	Methanol	reflux	1h	Product 14a	-	-	-
2	$TsOH.H_2O$ 1.5eq	THF	reflux	4h	34%	54.02	2.53	15.50
3	CH_3SO_3H 1.5eq	THF	reflux	7h	43%	77.63	2.30	12.20
4	CF_3COOH 5eq	Toluene	reflux	2h	42%[a]	-	-	-
5	H2SO4 1.2eq	THF	reflux	2h	64%	84.28	10.04	1.92
6	**H_2SO_4 1.2eq**	**THF**	**reflux**	**4h**	**73%**	**92.53**	**2.03**	**1.95**
7	H2SO4 1.2eq	THF	reflux	8h	-	89.98	2.25	2.36
8	H_2SO_4 1.5eq	THF	reflux	4h	69%	-	-	-
9	H_2SO_4 1eq	THF	reflux	4h	67%	-	-	-

a: Impurity **1a** was isolated in the reaction.

Conclusions

In summary, the novel and practical synthesis of iclaprim was accomplished. Beginning with TMP, the very cheap material, our new synthetic routes only included six

reaction steps with an overall yield of 21%, which was almost four times as much as those existing processes. In addition, all purification only involved recrystallization, without column chromatography. This process offers the distinctive advantages over the reported routes to synthesize iclaprim.

Experimental Section

Solvents and reagents from vendors were used as received unless otherwise indicated. Melting points were determined on a capillary melting point apparatus and were uncorrected. NMR spectra were taken on a Bruker Avance III instrument operated at 400 or 600 MHz for ^{1}H-NMR and 100 MHz for ^{13}C-NMR with tetramethylsilane (Me_4Si) as an internal standard. HPLC was performed on a YMC-Pack ODS-AQ column (150 × 3.0 mm, 3 μm silica) with mobile phase A: 0.02 M KH_2PO_4 aqueous solution; mobile phase B: acetonitrile; flow rate: 0.6 mL/ min.

2,4-diacetamido-5-(2-acetyl-3,4,5-trimethoxybenzyl)pyrimidine (10)

A mixture of TMP (201.0 g, 0.69 mol), acetic anhydride (317.0g, 3.11 mol), dichloromethane (3.0 L) and $SnCl_4$ (160 mL, 1.39 mol) was heated at refluxing for 7.5 h. After cooling to rt, the reaction solution was poured into 1500 ml ice water. The organic phase was separated and the aqueous layer was extracted with dichloromethane (3 × 300ml). Combine the organic phase and wash with saturated aqueous Na_2CO_3. The organic layers were dried over anhydrous $NaSO_4$. Evaporation of the dichloromethane to

afford product **10** (277.0 g, 96.1%). It was directly used for the next step without purification. Mp: 204-206℃. ^{1}H-NMR (600 MHz, DMSO-d_6): δ 10.41 (s, 1H), 10.13 (s, 1H), 8.08, (s, 1H), 6.66 (s, 1H), 3.82 (s, 3H), 3.78 (s, 3H), 3.76 (s, 3H), 3.73 (s, 2H), 2.20 (s, 3H), 2.16 (s, 3H), 2.13 (s, 3H); ^{13}C-NMR (100 MHz, DMSO-d_6): δ 203.29, 169.56, 169.06, 159.78, 156.73, 155.62, 153.99, 150.38, 139.69, 131.08, 128.18, 119.86, 109.86, 61.34, 60.41, 55.92, 32.00, 31.14, 24.50, 23.69; MS (ESI^{+}): m/z, 417 ([M+H]$^{+}$).

2,4-diamino-5-(2-acetyl-3,4,5-trimethoxybenzyl)pyrimidine (11)

Compound **10** (200.2 g, 0.48 mol), postassium carbonate (6.6 g, 0.05 mmol) and methanol (2000 mL) were charged into reactor and stirred at refluxing for 2 h. After cooling to room temperature, The solid was isolated by filtration, washed with water, and dried at 80 °C to provide **11** as a yellow solid (154.7 g, 96.8 % yield). Mp: 121-123℃. ^{1}H-NMR (400 MHz, DMSO-d_6): δ 7.28 (s, 1H), 6.66 (s, 1H), 6.12, (brs, 2H), 5.70 (brs, 2H), 3.82 (s, 3H), 3.75 (s, 3H), 3.74 (s, 3H), 3.42 (s, 2H), 2.29 (s, 3H); ^{13}C-NMR (100 MHz, DMSO-d_6): δ 204.23, 162.18, 162.12, 155.78, 153.87, 150.04, 139.36, 131.99, 128.22, 109.35, 104.79, 61.32, 60.40, 55.90, 32.27, 29.93.

2,4-diamino-5-(2-acetyl-3-hydroxy-4,5-dimethoxybenzyl)pyrimidine (12)

To a 3 L round bottom flask charged the solution of Compound **11** (90.2 g, 0.27 mol) and 1.8 L of CH_2Cl_2, cooled to *ca* -10 °C, boron tribromide (410ml, 0.41mol, 17% in dichloromethane, *ca*. 1mol/L) was added dropwise to the reaction mixture. And then, the

mixture was stirred at room temperature for 5 h. The reaction mixture was cooled to 0-5 °C and quenched with 400ml methanol, stirred at rt for 1 h, concentrated in vacuo, water (0.9 L) was added, the mixture was stirred for 8 h at 0-5° C. The crude product **12** was isolated by filtration, and crystallized from isopropanol to give 56.2 g of product **12** as a white solid (Yield: 65.0%). Mp. 217℃. ^{1}H-NMR (400 MHz, DMSO-d_6): δ 9.66 (s, 1H), 8.31 (brs, 1H), 7.91 (brs, 1H), 7.54 (brs, 2H), 7.14 (s, 1H), 6.46 (s, 1H), 3.77 (s, 3H), 3.69 (s, 3H), 3.48 (s, 2H), 2.42 (s, 3H); ^{13}C-NMR (100 MHz, DMSO-d_6): δ 203.89, 163.87, 153.97, 153.91, 149.04, 139.35, 134.94, 130.13, 122.70, 109.01, 105.89, 60.38, 55.91, 32.10, 29.85; MS (ESI$^+$): m/z, 319 ([M+H]$^+$).

2-cyclopropyl-5-[(2,4-diaminopyrimidin-5-yl)methyl]-7,8-dimethoxychroman-4-one (13)

Acetic acid (15.6 g, 0.26 mol) was added dropwise to a mixture of compound **12** (55.0 g, 0.17 mol), cyclopropanecarboxaldehyde (14.5 g, 0.21 mol), pyrrolidine (18.4 g, 0.26 mol) and 550 ml acetonitrile. The reaction mixture was stirred at room temperature for 36 h. The solid was collected by filtration, and washed with Na_2CO_3 aqueous solution and water. The product was dried at 80 ºC to give 37.5 g compound **13** as a white solid (Yield: 58.6%). Mp168-171℃. ^{1}H-NMR (600 MHz, DMSO-d_6): δ 7.18 (s, 1H), 6.47 (s, 1H), 6.11, (brs, 2H), 5.63 (brs, 2H), 3.99-3.91 (m, 2H), 3.85-3.81 (m, 1H), 3.77 (s, 3H), 3.70 (s, 3H), 2.83-2.79 (m, 1H), 2.69-2.65 (m, 1H), 1.20-1.24 (m, 1H), 0.63-0.54 (m, 2H), 0.49-0.46 (m, 1H), 0.42-0.39 (m, 1H); MS (ESI$^+$): m/z, 371 ([M+H]$^+$).

2-cyclopropyl-5-[(2,4-diaminopyrimidin-5-yl)methyl]-7,8-dimethoxychr

oman-4-ol (14)

Compound **13** (32.6 g, 0.088 mmol) in 600 mL of methanol was added to a 1 L round bottom flask. The mixture was cooled to 0 °C, sodium borohydride (1.7g, 0.045 mmol) was added slowly to the reaction mixture. The reaction was stirred at room temperature for 1 h. After concentration, water (300 mL) was added and stirred for 20 mins. The solid was filtered, recrystallized from methanol, and dried at 80 °C to afford **14** as an off-white solid (26.3 g, 80.2%). Mp. 211-213℃. ^{1}H-NMR (400 MHz, DMSO-d_6): δ 7.49 (s, 1H), 6.21 (s, 1H), 6.17 (brs, 2H), 5.64 (brs, 2H), 5.60-5.58 (d, 1H), 4.94-4.93 (d, 1H), 3.82-3.78 (d, 1H), 3.63 (s,3H), 3.62 (s, 3H), 3.57-3.53 (m, 1H), 2.26-2.24 (m, 1H), 2.02-196 (m, 1H), 1.37-1.34 (m, 1H)，0.51-0.49 (d, 2H), 0.36 (s, 1H), 0.31 (s, 1H); ^{13}C-NMR (100 MHz, DMSO-d_6): δ 162.31, 162.29, 155.84, 151.68, 148.61, 135.21, 135.04, 117.58, 105.20, 78.52, 60.82, 59.82, 55.68, 37.02, 29.49, 14.63, 3.78, 1.91; MS (ESI+): m/z, 373 ($[M+H]^+$).

2-cyclopropyl-5-[(2,4-diaminopyrimidin-5-yl)methyl]-7,8-dimethoxychromen (1)

To a suspension of **14** (26.3 g, 0.071 mol) in THF (260 ml) was added concentrated sulfuric acid (8.3 g, 0.085 mol) at room temperature, and the whole was stirred for 4 h at refluxing. After the reaction was completed, the pH of the solution was adjusted to 9-10 with saturated aqueous Na_2CO_3. Evaporation of the THF under vacuum and the crude product **1** (24.0 g) was collected by filtration. The crude was recrystallized from ethanol-water to give **1** (18.3 g, 73.1 %, HPLC purity 99.41%). Mp. 215℃. ^{1}H-NMR (600

MHz, DMSO-d_6): δ 7.07 (s, 1H), 6.46-6.45 (d, 1H), 6.42 (s, 1H), 6.19 (brs, 2H), 5.72-5.70 (m, 1H), 5.68 (brs, 2H), 4.26-4.24 (m, 1H), 3.71 (s, 3H),3.70 (s, 3H), 3.52 (d, 2H), 1.15-1.11 (m, 1H), 0.43-0.51 (m, 2H), 0.35-0.39 (m, 1H), 0.30-0.33 (m, 1H); ^{13}C-NMR (100 MHz, DMSO-d_6): δ 162.06, 161.94, 154.76, 152.51, 146.77, 135.47, 129.98, 122.36, 121.00, 114.83, 106.23, 105.09, 77.54, 60.10, 55.67, 29.43, 14.84, 2.55, 1.09; MS (ESI+): m/z, 355 ($[M+H]^+$).

Supporting information

Supporting Information File 1

Preparation of 14a and 1a

^{1}H-NMR ^{13}C-NMR, HPLC and MS spectra of compound 10-14, 14a, 1 and 1a.

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