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朝格尔碱衍生物的制备及其催化的 4*H*-色烯并[2,3-*b*]吡啶-3-腈的合成

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摘要 由朝格尔碱-氨基合成了一系列席夫碱(**4**), 将其作为高效催化剂催化了取代水杨醛、丙二腈和取代 2-巯基咪唑的多组分反应, 得到一系列 5-(1*H*-咪唑-2-硫代)-2,4-二氨基-5*H*-色烯并[2,3-*b*]吡啶-3-腈衍生物(**8**). 通过 ^{1}H NMR 初步探讨了催化机理. 生物活性结果表明: 大多数产品对人乳腺癌细胞(MCF-7)和腺癌人肺泡基底上皮细胞(A549)表现出高度抑制作用. 5-(1*H*-咪唑-2-基硫基)-2,4-二氨基-7-氟-5*H*-苯并[2,3-*b*]吡啶-3-腈(**8s**)对金黄色葡萄球菌(野生型)和大肠杆菌(野生型)具有抗菌活性. 这些结果扩展了朝格尔碱衍生物在有机催化中的应用, 显示产品在新药开发领域具有潜力.
关键词 朝格尔碱; 有机催化; 色烯[2,3-*b*]吡啶; 生物活性

Preparation and Application in the Synthesis of 4*H*-Chromeno-[2,3-*b*]pyridine-3-carbonitriles of Tröger's Base Derivatives

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Abstract A series of Schiff base catalysts (**4**) derived from Tröger's base-NH_2 were synthesized and used to promote the multi-component reactions of substituted salicylaldehydes, malononitrile and substituted 2-mercaptoimidazole to afford 5-(1*H*-imidazol-2-ylthio)-2,4-diamino-5*H*-chromeno[2,3-*b*]pyridine-3-carbonitrile derivatives (**8**) efficiently. The reaction mechanism was discussed based on the ^{1}H NMR analysis preliminarily. Most of products showed high inhibitory on the human breast adenocarcinoma cell (MCF-7) and adenocarcinomic human alveolarbasal epithelial cell (A549). 5-(1*H*-Imidazol-2-ylthio)-2,4-diamino-7-fluoro-5*H*-chromeno[2,3-*b*]pyridine-3-carbonitrile has antibacterial activity against *Staphylococcus aureus* (wild type) and *Escherichia coli* (wild type). The results expanded the application of Tröger's bases in organocatalysis and showed the potential of products in new drug development.
Keywords Tröger's base; organoatalysis; chromeno[2,3-*b*]pyridine; biological activity

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1 Introduction

The chromenopyridine and its derivatives have characteristic drug-related properties. Compounds which contain the framework have some significant bioactivities including glucocorticoid receptor agonism,[1] anti-tumor,[2] anti-proliferation,[3] anti-myopia,[4] anti-hypertensive,[5] anti-histamine,[6] anti-rheumatic,[7] anti-asthma[8] and so on.

As an important part of the chromenopyridine family, 4*H*-chromenopyridine derivatives have also a variety of bioactivities such as anti-cancer,[9] anti-allergy,[10] anti-inflammatory,[11] resistance proliferation,[12] anti-bacterial[13] and anti-tuberculosis,[14] *etc*. A classic example is ibuprofen, which acts as a non-steroidal anti-inflammatory and analgesic medicine by inhibiting the synthesis of prostaglandins. Ibuprofen has been used to treat conjunctivitis and relieve pain and inflammation.[15,16] Kemnitzer found that many 4-aryl-4*H*-chromenes could inhibit the proliferation of cancer cell by inducing apoptosis.[17] Therefore, it is of theoretical significance and practical value to synthesize more chromeno[2,3-*b*]pyridine derivatives with new structure.

The 4*H*-chromeno[2,3-*b*]pyridine derivatives were usually obtained by the condensation of aromatic aldehydes, malononitrile and phenols in the presence of K_2CO_3,[18] Et_3N[19] or chitosan,[20,21] *etc*. However, the range of catalyst and substrate is still a major problem. Therefore, it is still necessary to develop a new type of catalyst for their preparation.

Tröger's base (TB, Figure 1), which was synthesized in 1887,[22] has caused great attention in molecular recognition,[23,24] bioorganic chemistry,[25~27] supramolecular chemistry[28,29] and so on due to its particular structure,[27] which enables TB and its derivatives to capture appropriate molecules and then to show catalytic activities.[27] However, the weak alkalescence, limited active sites and simple hydrogen bond acceptor limit their application in organocatalysis.

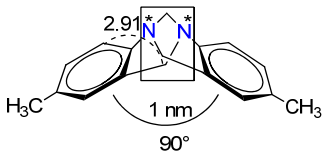
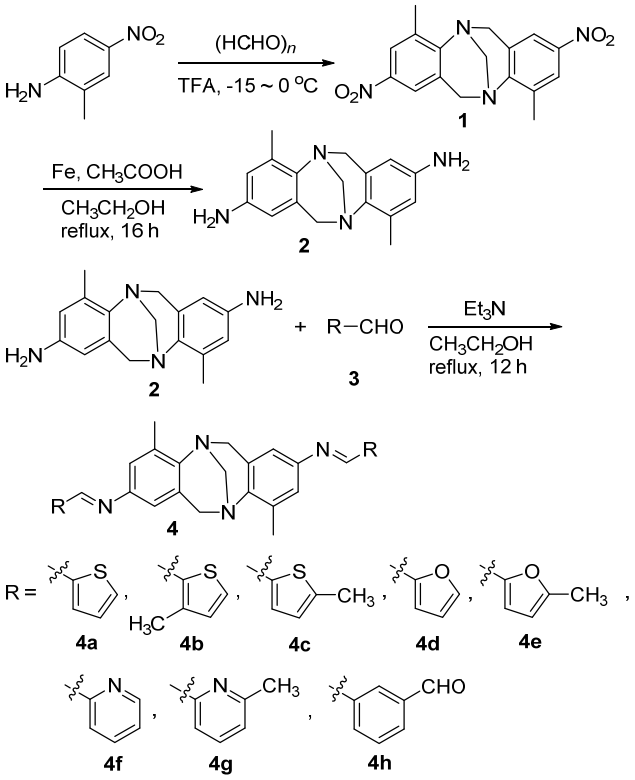


Figure 1 Structure of Tröger's base

To make full use of the structural advantages of TB and explore a new catalyst to synthesize chromeno[2,3-*b*]-pyridine derivatives with potential bioactivity, herein we designed and synthesized a series of Schiff bases by the reaction of TB-NH_2 with heterocyclic aldehydes to increase the number and type of catalytic sites. Then they were used as efficient catalyst for the multicomponent reaction of substituted salicylaldehyde, malononitrile and substituted 2-mercaptoimidazole to afford 5-(1*H*-imidazol-2-ylthio)-2,4-diamino-5*H*-chromeno[2,3-*b*]pyridine-3-carbonitriles in mild condition with high yield.

2 Results and discussion

Firstly, a series of catalysts based on TB derivatives were synthesized (Scheme 1). Because *p*-nitroaniline could not react, 2-methyl-4-nitroaniline was used to obtain corresponding TB derivatives successfully due to the electro-donating effect of methyl.



Scheme 1 Synthesized Schiff bases

Then, the pH and calculated the pK_a value of catakyst **4** were tested. It can be seen from Table 1 that the basicity of **4** has been enhanced compared with that of TB by introducing heterocycles. **4f** had the strongest basicity, which may be come from the pyridine ring. The basicity of **4g** was weaker than that of **4f**, probably because the *ortho*-methyl increased the steric hindrance of the N atom and weakened its ability to bind with hydrogen ions. The data in Table 1 also indicated that the methyl and its position on the heterocyclic

Table 1 pK_a values and yields of **4**[a]

Compd.	pK_a	R	yield/%
TB	9.05	—	
1	9.13	—	
2	9.28	—	
4a	9.26	Thiophene-2-yl	93
4b	9.20	3-Methyl-thiophene-2-yl	91
4c	9.21	5-Methyl-thiophene-2-yl	88
4d	9.11	Furan-2-yl	89
4e	9.13	5-Methyl-furan-2-yl	86
4f	9.35	Pyridine-2-yl	85
4g	9.06	5-Methyl-pyridine-2-yl	81

[a] Calculated from the pH value tested by dissolving 0.01 mmol of compounds in appropriate amount of ethanol and diluting to 10.0 mL with ethanol.

ring had a significant effect on the melting point of **4**.

The catalytic activity of **4** to the synthesis of **8h** was evaluated firstly. As expected, the reaction of salicylaldehyde (1 mmol), malononitrile (2 mmol) and 1-methyl-1*H*-imidazole-2-thiol (1 mmol) could be catalyzed by **4** (20 mol%) successfully by refluxing for 6 h in EtOH to give **8h** in 58%～89% yields.

The reaction conditions were then optimized based on the yield of **8h**. As shown in Table 2, this reaction did not occur without catalyst (Entry 1). Neither the common organic base such as pyridine (Entry 2, pK_a = 5.14[31]) and 2,2'-bipyridyl (Entry 3, pK_a=4.23[31]) nor inorganic base such as $NaHCO_3$ (Entry 4, pK_a=10.25[32]), $CsCO_3$ (Entry 5, pK_a = 10.25[32]), K_3PO_4 (Entry 6, pK_a = 2.12[32]), NaOH (Entry 7) and EtONa (Entry 8) could catalyze this reaction. Schiff base *N*-(pyridin-3-ylmethylene)aniline could also not catalyze the reaction (Entry 9). The intermediate **2** could catalyze this reaction, but the low yield of **8h** was obtained (16%, Entry 10). Heterocyclic aldehydes **3a**～**3g** were also not able to catalyze the reaction (Entries 11～17). The catalyst **4h** which was synthesized by the condensation of intermediate **2** with benzaldehyde **3h** could also catalyze the reaction, but the yield of **8h** was also very low (15%, Entry 18). When seven Schiff base **4a**～**4g** were used as catalyst, **8h** could be obtained with 58%～89% yields (Entries **19**～**25**), among which **4f** gave the best result. The results showed that the electron effect and steric hindrance of the heterocyclic aldehyde have a great influence on the yield of **8h** and were in accordance with the relevant laws. These results also indicated that the high catalytic function of **4** was not from their appropriate basicity or the C=N double bond separately, but from their whole structure.

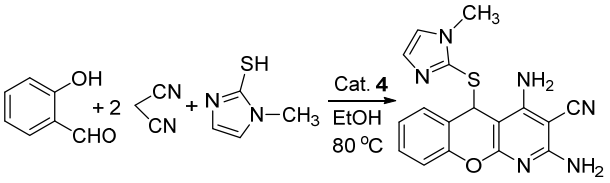
The loading of catalyst was then tested (Entries 24, 26～29). As it was increased, the yield increased obviously. When it was increased to 20 mol%, the yield increased significantly (89%). But the yield did not increase obviously (91%) after the loading of catalyst reached 25 mol%. Therefore, 20 mol% was the optimal one. Subsequently, the optimum solvent (Entries 24, 30～32) and temperature (Entries 24, 33, 34) was studied. To sum up, the optimal condition was using 20 mol% **4f** as catalyst, the reaction system was stirred in ethanol at 80 ℃ for 6 h.

With the optimized conditions in hand, we proceeded to explore the substrate scope of this transformation with a variety of salicylaldehydes **5** and substituted 2-mercaptoimidazoles **7** (Table 3). As can be seen from Table **3**, **4f** has a good catalytic effect on this reaction, the reaction afforded medium to high yields (61%～89%) of the desired products with wide substrate scope. Electronic effects and steric hindrance of the substituents attached to salicylaldehyde and 2-mercaptoimidazole has little effect on the reaction yield.

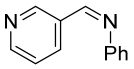
2.1 Study on the reaction mechanism

In order to understand the catalytic function of catalyst **4f**, ^{1}H NMR analysis (400 MHz) was applied to monitor the reaction process. The specific steps were as follows: catalyst **4f** (20 mol%), 2-mercaptobenzimidazole (1 mmol), malononitrile (2 mmol), 3-methyl salicylaldehyde (1 mmol) and ethanol (10 mL) were added in a 25 mL round bottom flask and refluxed. 0.20 mL of the mixture was taken out and the solvent was removed under vaccum. The residue was then dissolved in d_6-DMSO for NMR analysis. The reaction process was monitored by repeating the operation

Table 2 Synthesis of **8h** under different reaction conditions[a]



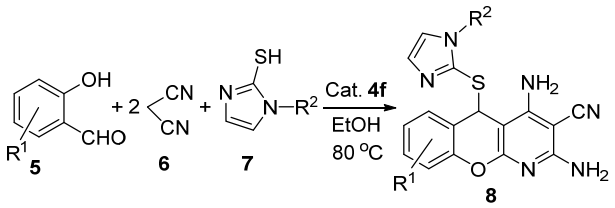
8h

Entry	Cat.	Cat./ mol%	Solvent	*t*/h	*T*/℃	Yield[b]/%
1	None	—	EtOH	6	Reflux	—
2	Pyridine	20	EtOH	6	Reflux	—
3	2,2'-Dipyridyl	20	EtOH	6	reflux	—
4	$NaHCO_3$	20	EtOH	6	Reflux	—
5	$CsCO_3$	20	EtOH	6	Reflux	—
6	K_3PO_4	20	EtOH	6	Reflux	—
7	NaOH	20	EtOH	6	Reflux	—
8	EtONa	20	EtOH	6	Reflux	—
9		20	EtOH	6	Reflux	—
10	**2**	20	EtOH	6	Reflux	16
11	**3a**	20	EtOH	6	Reflux	—
12	**3b**	20	EtOH	6	Reflux	—
13	**3c**	20	EtOH	6	Reflux	—
14	**3d**	20	EtOH	6	Reflux	—
15	**3e**	20	EtOH	6	Reflux	—
16	**3f**	20	EtOH	6	Reflux	—
17	**3g**	20	EtOH	6	Reflux	—
18	**3h**	20	EtOH	6	Reflux	15
19	**4a**	20	EtOH	6	Reflux	71
20	**4b**	20	EtOH	6	Reflux	63
21	**4c**	20	EtOH	6	Reflux	58
22	**4d**	20	EtOH	6	Reflux	78
23	**4e**	20	EtOH	6	Reflux	70
24	**4f**	**20**	**EtOH**	**6**	**Reflux**	**89**
25	**4g**	20	EtOH	6	Reflux	85
26	**4f**	5	EtOH	6	Reflux	35
27	**4f**	10	EtOH	6	Reflux	51
28	**4f**	15	EtOH	6	Reflux	62
29	**4f**	25	EtOH	6	Reflux	91
30	**4f**	20	MeCN	6	Reflux	35
31	**4f**	20	DCM	6	Reflux	38
32	**4f**	20	H_2O	6	Reflux	46
33	**4f**	20	EtOH	6	r.t.	—
34	**4f**	20	EtOH	6	40	53

[a] Unless otherwise noted, the reactions were carried out using salicylaldehyde (1 mmol), malononitrile (2 mmol) and 1-methyl-*1H*-imidazole-2-thiol (1 mmol).

at five-minute intervals.

Table 3 Synthesis of **8** under the optimal conditions



Entry	R^1	R^2	Product	Yield/%
1	3-OH	H	**8a**	86
2	3-OH	CH_3	**8b**	83
3	4-OH	H	**8c**	76
4	5-Cl	H	**8d**	61
5	3-CH_3O	CH_3	**8e**	79
6	5-CH_3	CH_3	**8f**	87
7	5-Cl	CH_3	**8g**	85
8	H	CH_3	**8h**	89
9	3-CH_3	CH_3	**8i**	80
10	3-CH_3O	H	**8j**	77
11	5- CH_3	H	**8k**	80
12	3-F	H	**8l**	81
13	5-OH	CH_3	**8m**	79
14	5-OH	H	**8n**	83
15	5-NO_2	H	**8o**	88
16	5-NO_2	CH_3	**8p**	84
17	5-Br	CH_3	**8q**	87
18	5-Br	H	**8r**	86
19	5-F	H	**8s**	78

As the signals in the aromatic hydrogen area merged together, it is hard to figure out the characteristic peaks in the ^{1}H NMR spectrum. Thus, the research was focus on the signals in δ 4.00～4.65 (Figure 2).

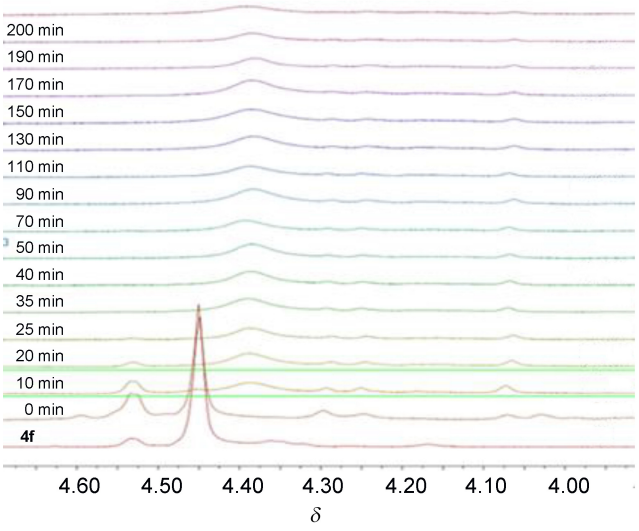


Figure 2 Changes of the signals as the reaction proceeded

From Figure 2, as the reaction proceeded, the signals of the methylene H atoms on TB backbone of **4f** in δ 4.45 and 4.54 decreased gradually and disappeared at 20 and 25 min respectively. And a new signal (δ 4.35) appeared at 10 min. The results indicated that the TB framework participated in the catalytic process. More specifically, it can be speculated that the bridge N atoms react with special substrates or intermediates and then cause the shift of the adjacent H.

Based on the results mentioned above, a possible mechanism was put forward (Scheme 3). In the presence of **4f**, malononitrile changed as carbanion and condensated with the formyl group of the salicylaldehyde to produce the intermediate **I**. Intermediate **I** underwent intramolecular cyclization to form intermediate **II**. The sulfhydryl anion formed by 2-mercapto-1-methylimidazole under basic conditions attacked **II** to give intermediate **III** by 1,4-addition. **III** attacked another malononitrile via cycloaddition to give intermediate **IV** which was then aromatized to give the final product **8**.

As mentioned above, the catalyst promotes the reaction by providing two active sites and alkaline environment.

We then tested their anti-tumor activity on human breast adenocarcinoma cell (MCF-7), adenocarcinomic human alveolarbasal epithelial cell (A549) and cytotoxicity on human bronchial epithelial cell (HBE) with methyl thiazolyl tetrazolium (MTT) method (Table 4).

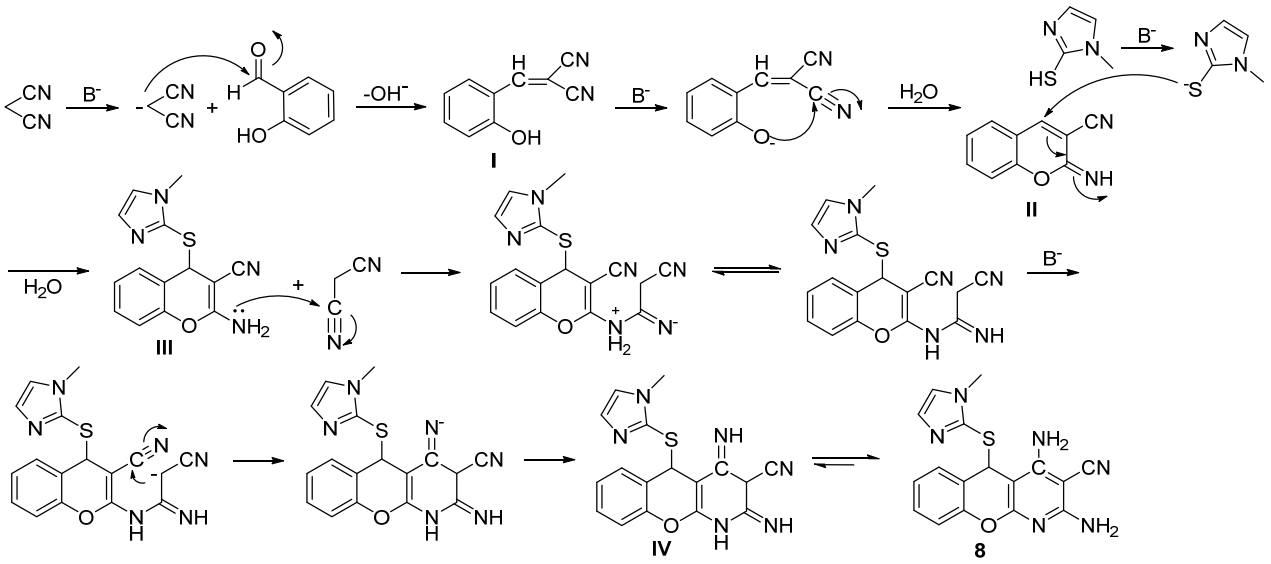
Table 4 IC_{50} value of compound **8** against antitumor and normal cells[a]

Entry	A549	MCF-7	HBE
8a	—	12.2	16.3
8b	—	12.2	15.3
8d	—	17.9	15.7
8f	—	15.1	17.4
8h	39.7	8.4	4.9
8i	—	16.4	11.5
8j	—	15.5	26.7
8m	—	16.2	15.8
8n	—	13.8	15.1
8r	40.9	—	11.3
8s	—	11.1	7.2
Adriamycin	4.05	0.027	7.01
Cisplatin	27.59	66.61	7.77

[a] IC_{50}>64 μg/mL were marked with "—".

From Table 4, compounds **8a**, **8b**, **8d**, **8f**, **8i**, **8j**, **8m**, **8n** and **8s** had inhibitory effects on MCF-7 while **8r** had inhibitory effect on A549. **8h** had better inhibitory effect in lower concentrations on the two tumor cells. In terms of structure, it is the only one no substituents on the phenyl ring. Probably, its spatial structure is the most appropriate one to the two tumor cells. However, it also had cytoxicity on HBE. Therefore, it would be necessary to reduce its toxicity by structure modification. The compound with a fluorine atom was more effective than that bearing a bromine atom, probably due to the special properties of fluorine atom such as inductive effect.

In the last, the anti-bacterial activities of product **8** on *Staphylococcus aureus* (wild type), *Bacillus subtilis* (wild



Scheme 3 A supposed mechanism

type) and *Escherichia coli* (wild type) were also evaluated (Table **5**). Only **8s** was screened out due to its inhibition on *Staphylococcus aureus* and *Escherichia coli*. Further researches are necessary to study the specific reason.

Table 5 Inhibition ratio of **8s** on two bacteria

Bacterium	Inhibition ratio/%			
	16	32	64	128
Staphylococcus aureus	—	—	84.82	90.84
Escherichia coli	—	94.56	100.00	100.00

[a] The concentration (μg/mL) of **8s**; [b] No inhibition.

3 Conclusions

In summary, a new series of Schiff base catalyst derived from Tröger's base and heterocyclic aldehydes was synthesized and used to promote the multi-component reaction of substituted salicylaldehyde, malononitrile and substituted 2-mercaptoimidazole to afford 5-(1*H*-imidazol-2-ylthio)-2,4-diamino-5*H*-chromeno[2,3-*b*]pyridine-3-carbonitrile derivatives. The mechanism study via ^{1}H NMR analysis indicated that the catalyst promotes the reaction by providing two active sites and alkaline environment. Some products were screened out due to their high anti-tumor and anti-bacterial activities *in vitro*. This study extended the application range of Tröger's base derivatives as catalysts.

4 Experimental section

4.1 General

All the reagents were purchased commercially. Tetrahydrofuran (THF) and toluene were dried and distilled from sodium. Ethanol was dried and distilled from magnesium. All reactions involving air- or moisture-sensitive materials were carried out under an argon atmosphere. All cancer cells, normal cell and bacterial were purchased from Shanghai Institute of Cell Biology, Chinese Academy of Sciences (Shanghai, China).

All melting points were determined with an electrothermal digital melting point apparatus and were uncorrected. *p*Hs were measured using a *p*H-3c digital *p*H meter (Shanghai Leici Device Works, Shanghai, China). NMR spectra were measured using a Bruker 400 MHz spectrometer. Mass spectra were recorded on a Micro TOF-Q (ESI) instrument. The MTT assay was performed on a microplate reader (SpectraMax M2).

4.2 Synthesis of compound 1

4-Nitro-2-toluidine (5.52 g, 40 mmol) and paraformaldehyde (7.2 g, 80 mmol) were added to a dry 50 mL two-necked flask, 80 mL of trifluoroacetic acid was then added to the flask dropwise at −15 ℃. After 3 h, the reaction was moved to room temperature for 48 h (TLC) in the dark. The reaction mixture was poured into ice water. When pH was adjusted with sodium hydroxide solution to 9, the yellow solid precipitated out. The filtered solid was dissolved in acetone and refluxed for about 20 min, cooled and recrystallized to give 4,10-dimethyl-2,8-dinitro-6*H*,12*H*-5,11-methanodibenzo[*b*,*f*][1,5]diazocine (**1**), yellow solid, yield 68%. m.p.＞300 ℃; ^{1}H NMR (400 MHz, $CDCl_3$) *δ*: 7.96 (s, 2H, ArH), 7.72 (s, 2H, ArH), 4.71 (d, *J*=17.2 Hz, 2H, CH_2×2), 4.33 (s, 2H, CH_2-Bridge), 4.11 (d, *J*=17.2 Hz, 2H, CH_2*2), 2.50 (s, 6H, CH_3×2); ^{13}C NMR (100 MHz, $CDCl_3$) *δ*: 134.7, 128.3, 124.3, 120.2, 67.0, 54.7, 17.3. HRMS (ESI) calcd for $C_{17}H_{17}N_4O_4$ [M+H]$^+$ 341.1250; found 341.1291.

4.3 Synthesis of compound 2

Compound **1** (0.34 g, 1 mmol), iron powder (0.84 g, 15 mmol) and glacial acetic acid (1.2 g, 20 mmol) were sequentially added to a 50 mL dry round bottom flask containing 10 mL of absolute ethanol. The mixture was refluxed for 16 h. After the reaction completion (TLC), 30 mL of distilled water was added, and the mixture was filtered with suction. The filtrate was extracted with dichloromethane (50 mL×3). The organic layer was washed

with saturated sodium hydrogen carbonate until the pH value was 7. The organic layer was then dried over sodium sulfate and evaporated under reduced pressure. The crude product was purified by column chromatography ($V_{\text{petroleum ether}}$: $V_{\text{ethyl acetate}}$=1 : 9 gradient elution) to give 4,10-dimethyl-2,8-diamine-6*H*,12*H*-5,11-methanodibenzo-[*b*,*f*][1,5]diazocine (**2**), gray solid, yield 83%. m.p. 209.6～211.2 ℃; ^{1}H NMR (400 MHz, DMSO-d_6) δ: 6.28 (s, 2H, ArH), 5.96 (s, 2H, ArH), 4.59 (s, 4H, NH_2×2), 4.27 (d, J=17.2 Hz, 2H, CH_2×2), 4.07 (s, 2H, CH_2- Bridge), 3.62 (d, J=17.2 Hz, 2H, CH_2×2), 2.19 (s, 6H, CH_3×2); ^{13}C NMR (100 MHz, $CDCl_3$) δ: 142.3, 133.7, 129.0, 116.3, 110.4, 68.0, 55.3, 17.0. HRMS (ESI) calcd for $C_{17}H_{17}N_4$ $[M+H]^+$ 281.1766; found 281.1786.

4.4 Synthesis of compound 4

Compound **2** (0.28 g, 1.0 mmol), aldehyde **3** (1.2 mmol), triethylamine (1 mol%) and ethanol (5 mL) were added to a 50 mL dry round bottom flask and refluxed for 12 h. When the reaction finished (TLC), excess solvent was evaporated under reduced pressure, and the crude product was separated by appropriate column chromatography to afford compound **4**.

N,*N*′-(4,10-Dimethyl-6*H*,12*H*-5,11-methanodibenzo[*b*,*f*]-[1,5]diazocine-2,8-diyl)bis(1-(thiophen-2-yl)methanimine) (**4a**): Yellow solid, yield 93%. m.p. 212.5～214.2 ℃; ^{1}H NMR (400 MHz, $CDCl_3$) δ: 8.50 (s, 2H, N=CH), 7.47 (d, J=5.2 Hz, 2H, Thiophene-H), 7.42 (d, J=2.8 Hz, 2H, Thiophene-H), 7.11～7.09 (m, 2H, Thiophene-H), 6.98 (d, J=1.6 Hz, 2H, ArH), 6.69 (d, J=1.6 Hz, 2H, ArH), 4.62 (d, J=16.8 Hz, 2H, CH_2×2), 4.36 (s, 2H, CH_2-Bridge), 4.01 (d, J=16.8 Hz, 2H, CH_2×2), 2.44 (s, 6H, CH_3×2); ^{13}C NMR (100 MHz, $CDCl_3$) δ: 152.3, 145.8, 144.1, 142.7, 133.1, 132.9, 130.6, 128.8, 128.1, 121.6, 116.7, 54.5, 30.6, 16.8. HRMS (ESI) calcd for $C_{27}H_{25}N_4S_2$ $[M+H]^+$ 469.1521; found 469.1506.

N,*N*′-(4,10-Dimethyl-6*H*,12*H*-5,11-methanodibenzo[*b*,*f*]-[1,5]diazocine-2,8-diyl)bis(1-(3-methyl-thiophen-2-yl)methanimine) (**4b**): Yellow solid, yield 91%. m.p. 231.6～233.8 ℃; ^{1}H NMR (400 MHz, $CDCl_3$) δ: 8.58 (s, 2H, N=C-H), 7.39 (d, J=3.2 Hz, 2H, Thiophene-H), 6.98 (d, J=2.0 Hz, 2H, Thiophene-H), 6.89 (dd, J=3.6, 1.2 Hz, 2H, ArH), 6.74 (d, J=3.2 Hz, 2H, ArH), 4.52 (d, J=17.2 Hz, 2H, CH_2×2), 4.26 (s, 2H, CH_2-Bridge), 3.99 (d, J=17.2 Hz, 2H, CH_2×2), 2.48 (s, 2H, $ArCH_3$×2), 2.37 (s, 2H, Thiophene CH_3×2); ^{13}C NMR (100 MHz, DMSO-d_6) δ: 193.1, 170.1, 150.9, 142.3, 136.3, 133.0, 131.3, 129.8, 128.8, 122.0, 116.5, 30.7, 16.8, 13.6. HRMS (ESI) calcd for $C_{29}H_{29}N_4S_2$ $[M+H]^+$ 497.1834; found 497.1829.

N,*N*′-(4,10-Dimethyl-6*H*,12*H*-5,11-methanodibenzo[*b*,*f*]-[1,5]diazocine-2,8-diyl)bis(1-(5-methyl-thiophen-2-yl)methanimine) (**4c**): Yellow solid, yield 88%. m.p. 178.3～179.9 ℃; ^{1}H NMR (400 MHz, DMSO-d_6) δ: 8.55 (s, 2H, N=CH), 7.39 (d, J=4.8 Hz, 2H, Thiophene-H), 6.99 (s, 2H, ArH), 6.91 (d, J=4.8 Hz, 2H, Thiophene-H), 6.68 (s, 2H, ArH), 4.65 (d, J=17.2 Hz, 2H, CH_2×2), 4.39 (s, 2H, CH_2-Bridge), 4.04 (d, J=16.8 Hz, 2H, CH_2×2), 2.46 (s, 2H, $ArCH_3$×2), 2.45(s, 2H, Thiophene CH_3×2); ^{13}C NMR (100 MHz, DMSO-d_6) δ: 152.2, 145.9, 144.8, 143.8, 140.5, 140.5, 133.4, 133.0, 128.7, 126.6, 121.5, 54.5, 30.6, 16.8, 15.5. HRMS (ESI) calcd for $C_{29}H_{29}N_4S_2$ $[M+H]^+$ 497.1834; found 497.1848.

N,*N*′-(4,10-Dimethyl-6*H*,12*H*-5,11-methanodibenzo[*b*,*f*]-[1,5]diazocine-2,8-diyl)bis(1-(furan-2-yl)methanimine) (**4d**): Yellow solid, yield 89%. m.p. 197.3～200.9 ℃; ^{1}H NMR (400 MHz, $CDCl_3$) δ: 8.22 (s, 2H, N=CH), 7.58 (s, 2H, Oxole-H), 7.01 (s, 2H, ArH), 6.88 (d, J=3.2 Hz, 2H, Oxole-H), 6.72 (d, J=1.6 Hz, 2H, ArH), 6.54～6.52 (m, 2H, Oxole-H), 4.62 (d, J=16.8 Hz, 2H, CH_2×2), 4.34 (s, 2H, CH_2-Bridge), 4.01 (d, J=16.8 Hz, 2H, CH_2×2), 2.44 (s, 6H, CH_3×2); ^{13}C NMR (100 MHz, $CDCl_3$) δ: 152.2, 146.8, 146.5, 145.4, 133.9, 128.7, 121.9, 116.6, 115.8, 112.1, 55.2, 17.2. HRMS (ESI) calcd for $C_{27}H_{25}N_4O_2$ $[M+H]^+$ 437.1978; found 437.1967.

N,*N*′-(4,10-Dimethyl-6*H*,12*H*-5,11-methanodibenzo[*b*,*f*]-[1,5]diazocine-2,8-diyl)bis(1-(5-methylfuran-2-yl)methanimine) (**4e**): Yellow solid, yield 86%. m.p. 120.6～124.5 ℃; ^{1}H NMR (400 MHz, DMSO-d_6) δ: 8.24 (s, 2H, N=CH), 6.98 (d, J=1.6 Hz, 2H, Oxole-H), 6.95 (d, J=3.2 Hz, 2H, ArH), 6.73 (s, 2H, Oxole-H), 6.31 (d, J=2.4 Hz, 2H, ArH), 4.53 (d, J=16.8 Hz, 2H, CH_2×2), 4.25 (s, 2H, CH_2-Bridge), 3.98 (d, J=16.8 Hz, 2H, CH_2×2), 2.37 (s, 6H, Ar-CH_3), 2.36 (s, 6H, Oxole-CH_3); ^{13}C NMR (100 MHz, DMSO-d_6) δ: 178.2, 169.8, 155.6, 150.6, 146.4, 144.0, 133.0, 128.8, 118.3, 116.4, 108.9, 67.0, 54.5, 16.8, 13.5. HRMS (ESI) calcd for $C_{29}H_{29}N_4O_2$ $[M+H]^+$ 465.2291; found 465.2260.

N,*N*′-(4,10-Dimethyl-6*H*,12*H*-5,11-methanodibenzo[*b*,*f*]-[1,5]diazocine-2,8-diyl)bis(1-(pyridin-2-yl)methanimine) (**4f**): Yellow solid, yield 85%. m.p. 193.4～195.8 ℃; ^{1}H NMR (400 MHz, $CDCl_3$) δ: 8.69 (d, J=8 Hz, 2H, pyridine-H), 8.54 (s, 2H, N=CH), 8.14 (d, J=8 Hz, 2H, pyridine-H), 7.78 (t, J=7.7, 4.4 Hz, 2H, pyridine-H), 7.35～7.33 (m, 2H, pyridine-H), 7.07 (d, J=1.6 Hz, 2H, ArH), 6.79 (d, J=1.6 Hz, 2H, ArH), 4.65 (d, J=16.8 Hz, 2H, CH_2×2), 4.37 (s, 2H, CH_2-Bridge), 4.05 (d, J=16.8 Hz, 2H, CH_2×2), 2.46 (s, 6H, Ar-CH_3); ^{13}C NMR (100 MHz, $CDCl_3$) δ: 159.3, 154.7, 149.6, 146.4, 145.0, 136.6, 134.0, 128.7, 124.9, 122.0, 121.7, 117.1, 67.6, 55.2, 17.2. HRMS (ESI) calcd for $C_{29}H_{27}N_6$ $[M+H]^+$ 459.2297; found 459.2248.

N,*N*′-(4,10-Dimethyl-6*H*,12*H*-5,11-methanodibenzo[*b*,*f*]-[1,5]diazocine-2,8-diyl)bis(1-(6-methylpyridin-2-yl)methanimine) (**4g**): Yellow solid, yield 81%. m.p. 113.1～115.2 ℃; ^{1}H NMR (400 MHz, $CDCl_3$) δ: 8.54 (s, 2H, C=NH), 7.96 (d, J=7.6 Hz, 2H, pydirine-H), 7.67 (t, J=7.6 Hz, 2H, pydirine-H), 7.20 (d, J=7.2 Hz, 2H, pydirine-H), 7.08 (s, 2H, ArH), 6.79 (s, 2H, ArH), 4.64 (d, J=16.8 Hz, 2H, CH_2×2), 4.36 (s, 2H, CH_2-Bridge), 4.04 (d, J=16.8 Hz, 2H, CH_2×2), 2.62 (s, 3H, pydirine-CH_3), 2.45 (s, 3H, pydirine-CH_3); ^{13}C NMR (100 MHz, $CDCl_3$) δ: 159.6, 158.3, 154.1, 146.4, 144.9, 136.8, 133.9, 128.6, 124.6, 121.9, 118.7, 117.3, 67.6, 55.1, 24.3, 17.2. HRMS (ESI) calcd for $C_{31}H_{31}N_6$ $[M+H]^+$ 487.2610; found 487.2592.

4.5 Synthesis of compound 8

Salicylaldehydes (**5**) (1 mmol), malononitrile (**6**) (0.66 g, 2 mmol), 2-mercaptoimidazole (**7**) (1 mmol), **4f** (20 mol%) and ethanol (5 mL) were added to a 50 mL round bottom flask and reacted to completion at 80 ℃ (TLC). After reaction was cooled to room temperature, the solid was filtered and washed with a small amount of water, then dissolved in *N*,*N*-dimethylformamide. Removed the insoluble substances via filtering under vacuum and distilled. Solid was precipitated out by adding water to the filtrate, then the mixture was filtered and recrystallized in DMSO to give compound **8**.

5-(1*H*-Imidazol-2-ylthio)-2,4-diamino-9-hydroxy-5*H*-chromeno[2,3-*b*]pyridine-3-carbonitrile (**8a**): Yellow solid, yield 86%. m.p. 286.2～289.5 ℃; ^{1}H NMR (400 MHz, DMSO-d_6) δ: 12.43 (s, 1H, NH), 9.96 (s, 1H, OH), 7.10 (s, 1H, imidazole-H), 6.95 (s, 1H, imidazole-H), 6.88～6.87 (m, 2H, ArH), 6.78 (s, 1H, ArH), 6.70～6.68 (m, 2H, NH_2), 6.66～6.64 (m, 2H, NH_2), 6.59 (s, 1H, methine-H); ^{13}C NMR (100 MHz, DMSO-d_6) δ: 160.3, 159.1, 157.2, 145.5, 139.0, 135.9, 124.3, 120.2, 117.8, 116.4, 116.1, 115.6, 115.5, 86.0, 70.3, 47.3. HRMS (ESI) calcd for $C_{16}H_{11}N_6O_2S$ [M－H]$^-$ 351.0664; found 351.0689.

2,4-Diamino-9-hydroxy-5-(1-methyl-1*H*-imidazol-2-ylthio)-5*H*-chromeno[2,3-*b*]pyridine-3-carbonitrile (**8b**): Yellow solid, yield 83%. m.p. 253.6～256.2 ℃; ^{1}H NMR (400 MHz, DMSO-d_6) δ: 9.97 (s, 1H, OH), 7.11 (s, 1H, imidazole-H), 7.08 (d, J=2.4 Hz, 1H, imidazole-H), 6.91 (d, J=7.6 Hz, 1H, ArH), 6.88～6.86 (m, 2H, ArH), 6.71 (s, 2H, NH_2), 6.65 (d, J=2.8 Hz, 2H, NH_2), 6.63 (s, 1H, methine-H), 3.52 (s, 3H, OCH_3); ^{13}C NMR (100 MHz, DMSO-d_6) δ: 167.0, 166.5, 154.7, 153.5, 152.4, 134.6, 134.4, 128.6, 123.4, 122.0, 121.6, 114.6, 114.2, 88.8, 69.0, 43.8, 32.9. HRMS (ESI) calcd for $C_{17}H_{13}N_6O_2S$ [M－H]$^-$ 365.0821; found 365.0845.

5-(1*H*-Imidazol-2-ylthio)-2,4-diamino-8-hydroxy-5*H*-chromeno[2,3-*b*]pyridine-3-carbonitrile (**8c**): Yellow solid, yield 76%. m.p.＞300 ℃; ^{1}H NMR (400 MHz, DMSO-d_6) δ: 9.96 (s, 1H, OH), 8.48 (s, 1H, NH), 7.85 (s, 1H, imidazole-H), 7.31 (d, J=5.6 Hz, 1H, imidazole-H), 7.21 (d, J=8.4 Hz, 1H, ArH), 7.05 (s, 2H, ArH), 6.67 (s, 2H, NH_2), 6.55 (d, J=2.0 Hz, 1H, NH_2), 6.40 (d, J=7.2 Hz, 1H, NH_2), 6.06 (s, 1H, methine-H); ^{13}C NMR (100 MHz, DMSO-d_6) δ: 166.4, 162.5, 160.5, 149.0, 147.6, 144.7, 144.1, 133.7, 129.3, 124.7, 119.5, 117.9, 110.9, 95.4, 71.3, 44.0. HRMS (ESI) calcd for $C_{16}H_{11}N_6O_2S$ [M－H]$^-$ 351.0664; found 351.0684.

5-(1*H*-Imidazol-2-ylthio)-2,4-diamino-7-chloro-5*H*-chromeno[2,3-*b*]pyridine-3-carbonitrile (**8d**): Yellow solid, yield 61%. m.p. 267.6～270.5 ℃; ^{1}H NMR (400 MHz, DMSO-d_6) δ: 12.52 (s, 1H, NH), 7.54 (dd, J=8.8, 2.8 Hz, 1H, imidazole-H), 7.40 (d, J=2.4 Hz, 1H, imidazole-H), 7.29 (dd, J=5.6, 4.0 Hz, 1H, ArH), 7.18 (t, 1H, J=3.0 Hz, ArH), 7.14 (s, 1H, ArH), 6.92 (s, 1H, NH_2), 6.77 (t, J=13.4 Hz, 2H, NH_2), 6.62 (s, 1H, NH_2), 4.92 (s, 1H, methine-H); ^{13}C NMR (100 MHz, DMSO-d_6) δ: 163.1, 162.5, 159.5, 135.7, 134.4, 133.8, 131.9, 131.3, 131.2, 130.2, 129.3, 120.3, 119.8, 97.9, 73.1, 44.4. HRMS (ESI) calcd for $C_{16}H_{10}ClN_6OS$ [M－H]$^-$ 369.0325; found 369.0310.

2,4-Diamino-9-methoxy-5-(1-methyl-1*H*-imidazol-2-ylthio)-5*H*-chromeno[2,3-*b*]pyridine-3-carbonitrile (**8e**): Yellow solid, yield 79%. m.p. 258.6.2～261.9 ℃; ^{1}H NMR (400 MHz, DMSO-d_6) δ: 7.14 (s, 1H, imidazole-H), 7.08 (d, J=2.0 Hz, 2H, ArH), 7.07 (s, 1H, imidazole-H), 6.78～6.76 (m, 1H, ArH), 6.75 (s, 2H, NH_2), 6.69 (d, J=2.4 Hz, 1H, methine-H), 6.62 (s, 2H, NH_2), 3.86 (s, 3H, OCH_3), 3.52 (s, 3H, CH_3); ^{13}C NMR (100 MHz, DMSO-d_6) δ: 162.7, 162.0, 157.2, 144.6, 139.7, 138.8, 126.8, 125.9, 124.3, 123.8, 120.5, 114.8,111.0, 85.8, 73.4, 55.9, 48.5, 34.3. HRMS (ESI) calcd for $C_{18}H_{15}N_6O_2S$ [M－H]$^-$ 379.0977; found 379.0969.

2,4-Diamino-7-methyl-5-(1-methyl-*1H*-imidazol-2-ylthio)-5*H*-chromeno[2,3-*b*]pyridine-3-carbonitrile (**8f**): Yellow solid, yield 87%. m.p. 295.4～296.1 ℃; ^{1}H NMR (400 MHz, DMSO-d_6) δ: 7.22 (d, J=8.0 Hz, 1H, imidazole-H), 7.14 (s, 1H, ArH), 7.11 (s, 1H, ArH),7.09 (d, J=2.4 Hz, 1H, ArH), 7.01 (s, 1H, imidazole-H), 6.74 (s, 2H, NH_2), 6.67 (d, J=2.4 Hz, 1H, methane-H), 6.64 (s, 2H, NH_2), 3.53 (s, 3H, CH_3), 2.23 (s, 3H, CH_3); ^{13}C NMR (100 MHz, DMSO-d_6) δ: 168.5, 163.9, 157.4, 139.3, 138.8, 136.7, 136.1, 134.3, 131.5, 125.6, 124.2, 123.3, 113.9, 99.3, 77.1, 43.5, 35.2, 27.1. HRMS (ESI) calcd for $C_{18}H_{15}N_6OS$ [M－H]$^-$ 363.1028; found 363.1059.

2,4-Diamino-7-chloro-5-(1-methyl-1*H*-imidazol-2-ylthio)-5*H*-chromeno[2,3-*b*]pyridine-3-carbonitrile (**8g**): Yellow solid, yield 85%. m.p. 281.1～283.4 ℃; ^{1}H NMR (400 MHz, DMSO-d_6) δ: 7.48 (dd, J=7.2, 2.4 Hz, 1H, imidazole-H), 7.29 (d, J=4.8 Hz, 1H, ArH), 7.19～7.18 (m, 2H, ArH), 7.12 (d, J=2.4 Hz, 1H, imidazole-H), 6.82 (s, 2H, NH_2), 6.79 (d, J=2.4 Hz, 1H, methine-H), 6.56 (s, 2H, NH_2), 3.54 (s, 3H, CH_3); ^{13}C NMR (100 MHz, DMSO-d_6) δ: 166.9, 160.4, 156.0, 149.7, 148.7, 130.8, 130.6, 129.1, 128.2, 127.6, 126.0, 113.7, 102.9, 96.5, 71.7, 42.8, 33.0. HRMS (ESI) calcd for $C_{17}H_{12}ClN_6OS$ [M－H]$^-$ 383.0482; found 383.0463.

2,4-Diamino-5-(1-methyl-1*H*-imidazol-2-ylthio)-5*H*-chromeno[2,3-*b*]pyridine-3-carbonitrile (**8h**): Orange solid, yield 89%. m.p. 257.6～259.4 ℃; ^{1}H NMR (400 MHz, DMSO) δ: 7.43～7.39 (m, 1H, imidazole-H), 7.23 (d, J=8.4 Hz, 2H, ArH), 7.16 (d, J=4.4 Hz, 2H, ArH), 7.09 (d, J=2.4 Hz, 1H, imidazole-H), 6.77 (s, 2H, NH_2), 6.71 (d, J=2.4 Hz, 1H, methane-H), 6.62 (s, 2H, NH_2), 3.52 (s, 3H, CH_3); ^{13}C NMR (100 MHz, DMSO-d_6) δ: 160.4, 157.2, 149.9, 148.6, 143.1, 138.6,130.2, 127.3, 126.6, 125.9, 119.4, 116.9, 114.8, 105.6, 78.0, 43.5, 34.3. HRMS (ESI) calcd for $C_{17}H_{13}N_6OS$ [M－H]$^-$ 349.0872; found 349.0874.

2,4-Diamino-9-methyl-5-(1-methyl-1*H*-imidazol-2-ylthio)-5*H*-chromeno[2,3-*b*]pyridine-3-carbonitrile (**8i**): Yellow solid, yield 80%. m.p. 266.5～270.3 ℃; ^{1}H NMR (400 MHz, DMSO-d_6) δ: 7.28～7.26 (m, 1H, imidazole-H), 7.15 (s, 1H, ArH), 7.08 (d, J=2.4 Hz, 1H, imidazole-H), 7.05～7.04 (m, 2H, ArH), 6.82 (s, 2H, NH_2), 6.69 (d, J=2.4 Hz, 1H, methane-H), 6.63 (s, 2H, NH_2), 3.52 (s,

3H, CH_3), 2.34 (s, 3H, CH_3); ^{13}C NMR (100 MHz, DMSO-d_6) δ: 160.4, 159.2, 157.2, 152.4, 148.2, 145.9, 125.7, 120.5, 119.4, 119.3, 118.9, 114.8, 112.7, 85.9, 70.3, 43.9, 34.3, 15.6. HRMS (ESI) calcd for $C_{18}H_{15}N_6OS$ [M－H]$^-$ 363.1028; found 363.1063.

5-(1*H*-Imidazol-2-ylthio)-2,4-diamino-9-methoxy-5*H*-chromeno[2,3-*b*]pyridine-3-carbonitrile (**8j**): Yellow solid, yield 77%. m.p. 272.4～274.6 ℃; ^{1}H NMR (400 MHz, DMSO-d_6) δ: 12.45 (s, 1H, NH), 7.14 (s, 1H, imidazole-H), 7.09～7.08 (m, 3H, ArH), 6.87 (t, J=2.2 Hz, 1H, imidazole-H), 6.74 (s, 2H, NH_2), 6.70 (s, 2H, NH_2), 6.64 (t, J=1.6 Hz, 1H, methane-H), 3.87 (s, 3H, OCH_3); ^{13}C NMR (100 MHz, DMSO-d_6) δ: 160.3, 159.2, 157.2, 149.7, 147.4, 139.7, 131.6, 126.6, 125.0, 119.2, 115.9, 115.5, 112.2, 88.2, 70.3, 55.8, 47.0. HRMS (ESI) calcd for $C_{17}H_{13}N_6O_2S$ [M－H]$^-$ 365.0821; found 365.0865.

5-(1*H*-Imidazol-2-ylthio)-2,4-diamino-7-methyl-5*H*-chromeno[2,3-*b*]pyridine-3-carbonitrile (**8k**): Yellow solid, yield 80%. m.p. 295.4～296.1 ℃; ^{1}H NMR (400 MHz, DMSO-d_6) δ: 7.96 (s, 1H, NH), 7.26 (d, J=8.4 Hz, 1H, imidazole-H), 7.20 (s, 1H, ArH), 7.12 (s, 1H, imidazole-H), 7.10 (s, 2H, ArH), 6.82～6.73 (m, 1H, NH_2), 6.69 (s, 2H, NH_2), 6.62 (d, J=1.8 Hz, 1H, methane-H), 2.33 (s, 3H, CH_3); ^{13}C NMR (100MHz, DMSO-d_6) δ: 162.3, 160.4, 156.9, 149.4, 142.7, 133.1, 130.6, 129.0, 126.1, 117.5, 116.5, 112.9, 98.6, 83.6, 70.3, 42.9, 20.3. HRMS (ESI) calcd for $C_{17}H_{13}N_6OS$ [M－H]$^-$ 349.0872; found 349.0841.

5-(*1H*-Imidazol-2-ylthio)-2,4-diamino-9-fluoro-5*H*-chromeno[2,3-*b*]pyridine-3-carbonitrile (**8l**): Yellow solid, yield 81%. m.p. 289.1～291.7 ℃; ^{1}H NMR (400 MHz, DMSO-d_6) δ: 12.49 (s, 1H, NH), 7.37 (s, 1H, imidazole-H), 7.20 (s, 1H, ArH), 7.17～7.13 (s, 1H, ArH), 7.03 (d, J=8.0 Hz, 1H, imidazole-H), 6.90 (s, 1H, ArH), 6.84 (s, 2H, NH_2), 6.81 (s, 1H, methane-H), 6.72 (s, 2H, NH_2); ^{13}C NMR (100 MHz, DMSO-d_6) δ: 162.5, 160.8, 156.1, 155.4 (d, J=240 Hz), 141.9, 129.8, 127.8 (d, J=20 Hz), 127.7, 127.6 (d, J=8 Hz), 127.1, 124.5, 121.8 (d, J=10 Hz), 117.4 (d, J=20 Hz), 93.3, 77.9, 43.1. HRMS (ESI) calcd for $C_{16}H_{10}FN_6OS$ [M－H]$^-$ 353.0621; found 353.0639.

2,4-Diamino-7-hydroxy-5-(1-methyl-1*H*-imidazol-2-ylthio)-5*H*-chromeno[2,3-*b*]pyridine-3-carbonitrile (**8m**): Yellow solid, yield 79%. m.p. 255.6～258.2 ℃; ^{1}H NMR (400 MHz, DMSO-d_6) δ: 9.48 (s, 1H, OH), 7.10 (d, J=2.4 Hz, 1H, imidazole-H), 7.07～7.05 (m, 2H, ArH), 6.92 (dd, J=8.8, 2.8 Hz, 1H, imidazole-H), 6.70 (s, 2H, NH_2), 6.67 (d, J=2.4 Hz, 1H, ArH), 6.66 (d, J=3.2 Hz, 1H, methane-H), 6.58 (s, 2H, NH_2), 3.53 (s, 3H, CH_3); ^{13}C NMR (100 MHz, DMSO-d_6) δ: 169.5, 168.3, 166.4, 153.7, 146.8, 142.6, 141.6, 140.9, 139.4, 135.0, 117.0, 114.0, 112.7, 95.6, 54.7, 44.3, 34.3. HRMS (ESI) calcd for $C_{17}H_{13}N_6O_2S$ [M－H]$^-$ 365.0821; found 365.0838.

5-(1*H*-Imidazol-2-ylthio)-2,4-diamino-7-hydroxy-5*H*-chromeno[2,3-*b*]pyridine-3-carbonitrile (**8n**): Brick red solid, yield 83%. m.p. 260..3～261.5 ℃; ^{1}H NMR (400 MHz, DMSO-d_6) δ: 12.46 (s, 1H, NH), 9.49 (s, 1H, OH), 7.06 (d, J=3.2 Hz, 1H, imidazole-H), 7.05 (s, 1H, ArH), 6.89 (t, 1H, J=2.4 Hz, imidazole-H), 6.80 (d, J=2.8 Hz, 1H, ArH), 6.77 (d, J=2.4 Hz, 1H, ArH), 6.69 (s, 2H, NH_2), 6.65～6.63 (m, 2H, NH_2), 6.62 (t, J=6.0 Hz, 1H, methine-H); ^{13}C NMR (100 MHz, DMSO-d_6) δ: 166.2, 166.0, 156.1, 134.2, 133.2, 133.0, 132.6, 131.8, 131.6, 130.1, 129.2, 128.5, 128.0, 89.8, 72.9, 45.54. HRMS (ESI) calcd for $C_{16}H_{11}N_6O_2S$ [M－H]$^-$ 351.0664; found 351.0680.

5-(1*H*-Imidazol-2-ylthio)-2,4-diamino-7-nitro-5*H*-chromeno[2,3-*b*]pyridine-3-carbonitrile (**8o**): Yellow solid, yield 88%. m.p. 233.5～235.5 ℃; ^{1}H NMR (400 MHz, DMSO-d_6) δ: 12.46 (s, 1H, NH), 7.23 (dd, J=8.8, 2.8 Hz, 1H, imidazole-H), 7.14 (s, 1H, ArH), 7.06 (d, J=3.2 Hz, 1H, imidazole-H), 6.80～6.78 (m, 2H, ArH), 6.69 (s, 2H, NH_2), 6.65～6.64 (m, 1H, methane-H), 6.43 (s, 2H, NH_2); ^{13}C NMR (100 MHz, DMSO-d_6) δ: 162.2, 159.4, 154.3, 153.3, 144.4, 142.8, 141.7, 137.7, 133.6, 130.1, 128.0, 125.9, 124.5, 108.5, 75.7, 43.3. HRMS (ESI) calcd for $C_{16}H_{10}N_7O_3S$ [M－H]$^-$ 380.0566; found 380.0557.

2,4-Diamino-5-(1-methyl-1*H*-imidazol-2-ylthio)-7-nitro-5*H*-chromeno[2,3-*b*]pyridine-3-carbonitrile (**8p**): Orange solid, yield 84%. m.p. 279.9～282.4 ℃; ^{1}H NMR (400 MHz, DMSO-d_6) δ: 8.27 (dd, J=9.2, 2.8 Hz, 1H, imidazole-H), 8.06 (d, J=2.8 Hz, 1H, ArH), 7.49 (d, J=5.2 Hz, 1H, imidazole-H), 7.31 (s, 1H, ArH), 7.14 (d, J=2.4 Hz, 1H, ArH), 6.91～6.90 (m, 2H, NH_2), 6.87 (s, 1H, methine-H), 6.61 (s, 2H, NH_2), 3.54 (s, 3H, CH_3); ^{13}C NMR (100 MHz, DMSO-d_6) δ: 164.8, 162.3, 158.5, 156.6, 135.9, 135.4, 126.1, 124.5, 124.1, 118.5, 109.3, 106.3, 98.3, 93.7, 73.8, 42.0, 34.5. HRMS (ESI) calcd for $C_{17}H_{12}N_7O_3S$ [M－H]$^-$ 394.0722; found 394.0719.

2,4-Diamino-7-bromo-5-(1-methyl-1*H*-imidazol-2-ylthio)-5*H*-chromeno[2,3-*b*]pyridine-3-carbonitrile (**8q**): Yellow solid, yield 87%. m.p. 286.3～288.1 ℃; ^{1}H NMR (400 MHz, DMSO-d_6) δ: 7.59 (dd, J=8.8, 2.0 Hz, 1H, imidazole-H), 7.32 (d, J=2.0 Hz, 1H, ArH), 7.24 (d, J=8.8 Hz, 1H, imidazole-H), 7.18 (s, 1H, ArH), 7.12 (d, J=2.0 Hz, 1H, ArH), 6.81 (s, 2H, NH_2), 6.79 (d, J=2.4 Hz, ,1H, methine-H), 6.57 (s, 2H, NH_2), 3.54 (s, 3H, CH_3); ^{13}C NMR (100 MHz, DMSO-d_6) δ: 162.4, 162.3, 160.4, 157.1, 152.4, 152.1, 148.1, 142.9, 135.5, 124.6, 119.5, 114.2, 112.7, 94.6, 77.3, 41.6, 34.4. HRMS (ESI) calcd for $C_{17}H_{12}BrN_6OS$ [M－H]$^-$ 426.9977; found 426.9993.

5-(1*H*-Imidazol-2-ylthio)-2,4-diamino-7-bromo-5*H*-chromeno[2,3-*b*]pyridine-3-carbonitrile (**8r**): Yellow solid, yield 86%. m.p. 279.4～282.3 ℃; ^{1}H NMR (400 MHz, DMSO-d_6) δ: 12.52 (s, 1H, NH), 7.61～7.57 (m, 1H, imidazole-H), 7.32 (d, J=2.0 Hz, 1H, ArH), 7.23 (d, J=8.8 Hz, 1H, imidazole-H), 7.17 (s, 1H, ArH), 6.92 (s, 1H, ArH), 6.81 (s, 2H, NH_2), 6.74 (s, 1H, methane-H), 6.63 (s, 2H, NH_2); ^{13}C NMR (100 MHz, DMSO-d_6) δ: 164.7, 161.5, 154.9, 151.5, 150.8, 143.7, 137.4, 136.6,136.2, 116.6, 113.1, 112.7, 94.3, 93.7, 75.5, 45.0. HRMS (ESI) calcd for $C_{16}H_{10}BrN_6OS$ [M－H]$^-$: 412.9820; found 412.9862.

5-(1*H*-Imidazol-2-ylthio)-2,4-diamino-7-fluoro-5*H*-chromeno[2,3-*b*]pyridine-3-carbonitrile (**8s**): Yellow solid,

yield 78%. m.p. 276.9～280.1 ℃; ^{1}H NMR (400 MHz, DMSO-d_6) δ: 12.51 (s, 1H, NH), 7.33～7.29 (m, 3H, ArH), 7.19～7.16 (m, 1H, imidazole-H), 7.12 (s, 2H, NH_2), 6.97～6.91 (m, 1H, imidazole-H), 6.75 (s, 1H, NH_2), 6.62 (s, 1H, methine-H); ^{13}C NMR (100 MHz, DMSO-d_6) δ: 160.4, 160.2, 157.0 (d, J=240 Hz), 148.0, 128.7, 119.2 (d, J=7 Hz), 118.6, 116.2, 115.4, 115.1, 114.9, 113.6, 113.3 (d, J=23 Hz), 112.7, 82.8, 70.5. HRMS (ESI) calcd for $C_{16}H_{10}FN_6OS$ $[M-H]^-$ 353.0621; found 353.0612.

4.6 Materials and methods of bioactivity *in vitro*

Human breast adenocarcinoma cell line (MCF-7), adenocarcinomic human alveolarbasal epithelial cells (A549) and human bronchial epithelial cells (HBE) were maintained in RPMI 1640 medium supplemented with 10% fetal bovine serum (FBS), 100 U/mL penicillin and 100 μg/mL streptomycin at 37 ℃ in 5% CO_2 atmosphere. Cell viability was determined using the MTT assay (Sigma). In brief, cancer cells with same number were inoculated into each well in 96-well plates (Costar, Charlotte, NC) in 100 μL culture medium. After an overnight incubation, various concentrations of compounds were added for 48 h, and then MTT (1 mg/mL) was added to each well and incubated for 3 h at 37 ℃. Then, the medium was removed and MTT-formazan complex was dissolved in DMSO. The plates were then placed on a shaking table for 10 min to solubilize crystals adequately. The absorbance was measured using a SpectraMax M2 (Molecular Devices) at λ_{ex}=570 nm and λ_{em}=630 nm. The experiment was repeated 3 times. The 50% inhibition concentration (IC_{50}) was calculated by the following formula:

$$\lg IC_{50}=X_m-I(P-(3-P_m-P_n)/4)$$

X_m is lg(maximum dose), I is lg(maximum dose/adjacent dose), P is sum of the positive response rates, P_m is maximum positive response rate, P_n is minimum positive response rate, determination of antibacterial activities

4.7 Antitumor activity in vitro of the products

In the determination of antibacterial activities, the stock solution (10 mg/mL) of every product was prepared by dissolving relative compound in DMSO. The solution was diluted to 1, 10 or 100 g/mL, respectively, and then the solutions were mixed with bacterial solution at ratio of 1∶99 (the solution was diluted 100 times with the bacterial solution), and the final concentrations of the compound were 0.01, 0.1 and 1 μg/mL, and the content of DMSO was 1%. The same concentrations (1, 10 and 100 g/mL, respectively) of ampicillin (AP), kanamycin (KAN) and meropenem were used as the controls. The experiment was performed in triplicate.

Supporting Information NMR of synthetic compounds. The Supporting Information is available free of charge via the Internet at http://sioc-journal.cn/.

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