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Synthesis and Cytotoxicity Evaluation of Novel Indolylpyrimidines and Indolylpyrazines as Potential Antitumor Agents

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Abstract—Novel indolylpyrimidines and indolylpyrazines have been synthesized as potential antitumor agents. They were screened in a panel of 60 human tumor cell lines in vitro. Compounds **7**, **9**, **10**, **15**, **21** exhibited efficiently cytotoxic activities with GI_{50} values in the low micromolar range against a variety of human cancer cell lines. 2,4-Bis(3′-indolyl)pyrimidine **8** displayed selective cytotoxic activity against IGROV1 tumor cell line with the GI_{50} value below 0.01 μM. © 2001 Elsevier Science Ltd. All rights reserved.

Introduction

Natural bisindole alkaloids have emerged as an important structural class based upon their high degree of biological activity.[1] A very interesting group of this class of compounds incorporates a five- or six-membered heterocyclic ring between the two indole rings.[2–5] Due to the interesting biological activities and unique chemical structures of marine indole alkaloids and its low availability, marine indole alkaloids as lead compounds for discovery of new drugs have been an attractive field in medicinal chemistry. In our efforts to search for novel antitumor agents, we have designed and synthesized a novel type of bisindole alkaloids, including the analogues of the cytotoxic marine bisindole alkaloid, nortopsentines in which imidazole moiety was replaced with a thiazole, pyrazinone or pyrazine ring. Those bisindole analogues were found to possess significant cytotoxic activities against human cancer cell lines with GI_{50} values in the low micro-molar concentration range.[6,7] These results prompted us to further study the SAR of new type of indole alkaloids.

In this paper, we designed and synthesized new series of 4-indolylpyrimidines, 2,4-bis(3′-indolyl)pyrimidines, 5-indolylpyrazines, and 3,5-bis(3′-indolyl)pyrazines, which were evaluated for cytotoxic activity against the diverse human tumor cell lines in vitro by the National Cancer Institute, USA.

Chemistry

Meridianin D, isolated from the marine tunicate *Aplidium meridianum*, showed cytotoxicity toward LMM3 (murine mamarian adenocarcinoma cell line) with an IC_{50} value of 33.9 μM.[8] It has a structure of brominated indole ring linking a 2-aminopyrimidine at the 3-position of indole. We have reported an efficient synthesis of meridianin D (**1**) and its analogue 6-debromomeridianin D (**2**), via the palladium catalyzed cross-coupling reaction.[9] Moreover, a series of novel bis(*N*-tosylindolyl)pyrimidine alkaloids **5**–**7** were synthesized by palladium catalyzed reaction of 2,4-dichloropyrimidines with *N*-tosyl-3-indolylboronic acid.[9,10] Deprotection of the *N*-toluenesulfonyl group in the indole ring with sodium hydroxide in refluxing methanol afforded the corresponding 2,4-bis(3′-indolyl)pyrimidines **8**–**10** (Scheme 1).

In order to further study the SAR of bisindole alkaloids, we planned to synthesize the indole alkaloids bearing a pyrazine ring. In the previous paper, we have reported the synthesis of 2,5-bisindolylpyrazine from 3-(α-azidoacetyl)indole via condensation reaction.[7] However, this method was not suitable to the synthesis of bisindolylpyrazines bearing a functional group in pyrazine ring. Therefore, a new synthetic route starting from 2-aminopyrazine (**11**) via the Suzuki cross-coupling reaction was employed, as depicted in Scheme 2. Thus 2-aminopyrazine (**11**) was converted to 2-amino-3,5-diboromopyrazine (**12**) by bromination with *N*-bromosuccinimide in aqueous dimethyl sulfoxide in 85% yield. Treatment of the dibromopyrazine **12** with sodium methoxide regioselectively afforded 2-amino-3-methoxyl-5-bromopyrazine (**14**) in methanol under

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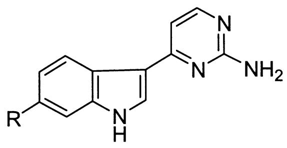
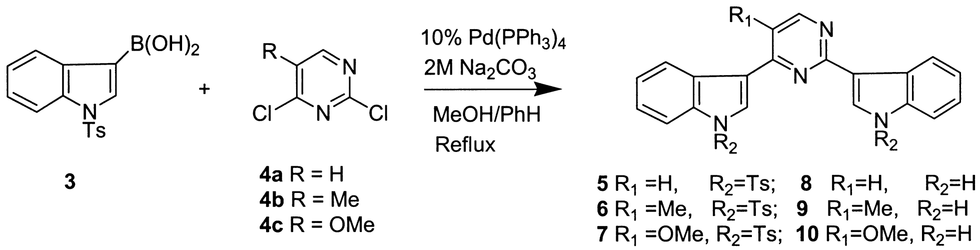
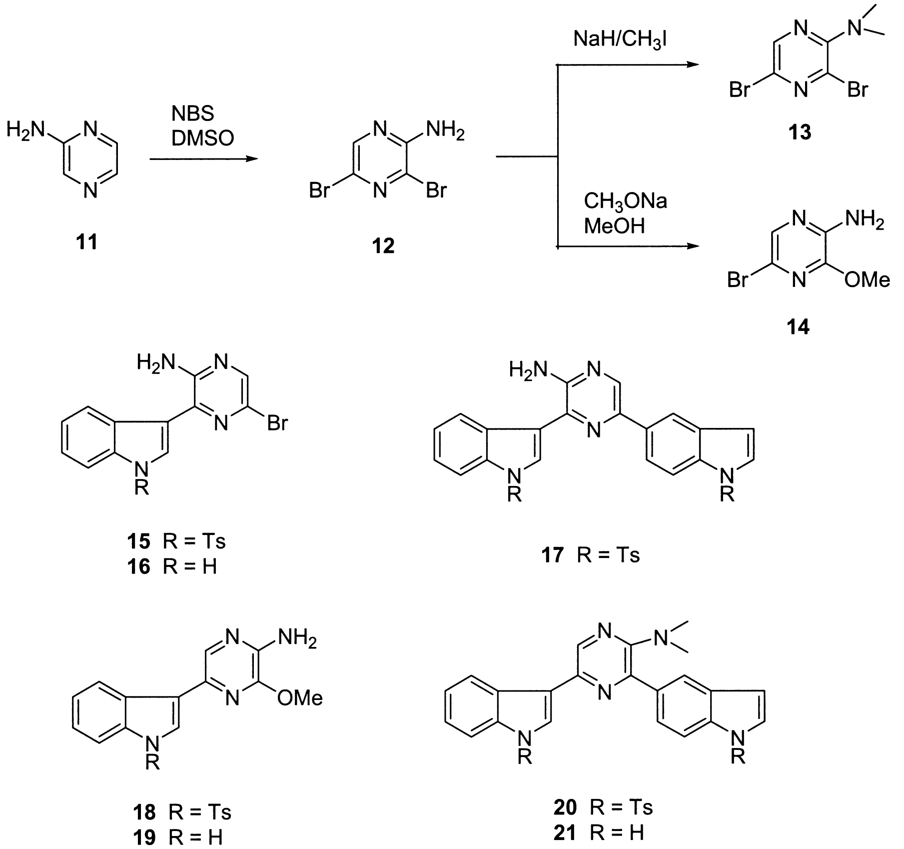


1 R = Br Meridianin D
2 R = H 6-Debromomeridianin D



Scheme 1.



Scheme 2.

refluxing.[11] 2-(*N,N*-Dimethyl)amino-3,5-dibromopyrazine (**13**) was obtained by treatment **12** with sodium hydride and iodomethane in dimethylformamide quantitatively.

Finally, the desired monoindolylpyrazine **15** or bisindolylpyrazine **17** were respectively prepared by Suzuki cross-coupling reaction between 2-amino-3,5-dibromopyrazine **12** and 1.1 equiv or 2.2 molar equiv of *N*-tosyl-3-indolylboronic acid **3** in the presence of 10% tetrakis(triphenylphosphine)palladium and aqueous cesium carbonate in DME under refluxing. The structure of compound **15** was confirmed by X-ray single crystal analysis (Fig. 1). The mild condition and good yields of the cross-coupling reaction between the 3-indolylboronic acid and the brominated pyrazines are considered to be an excellent method for the preparation of indolylpyrazines. Bisindo-lylpyrazine **20** was obtained through cross-coupling reaction between 2-(*N,N*-dimethyl)-amino-3,5-dibromopyrazine **13** and *N*-tosyl-3-indo-

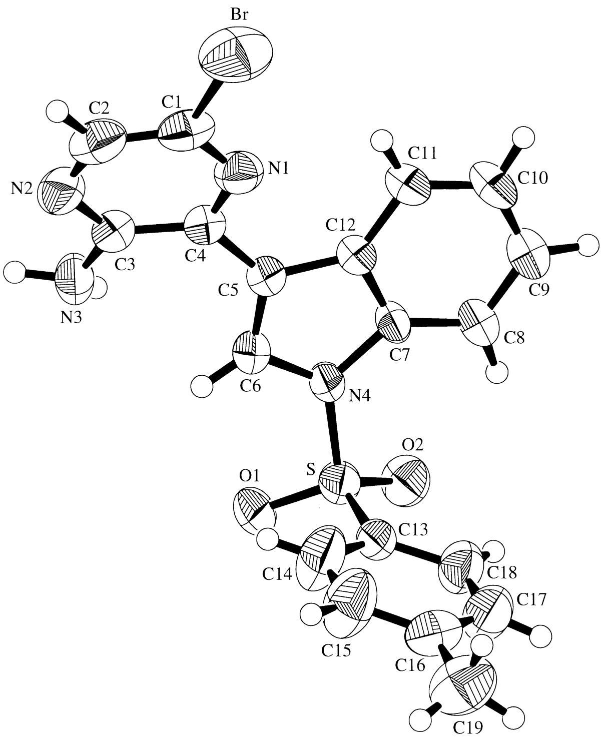


Figure 1. ORTEP drawing of the X-ray crystal structure of compound **15**. Crystal structure data: cell parameters: $a = 15.843(4)$ Å, $b = 8.936(5)$ Å, $c = 17.966(7)$ Å, $\gamma = 104.68(2)°$, $V = 2460(1)$ Å.^{3} Space group: $P2_1/a$ (#14). Z value 4. No. of observations: 2542. No. of variables: 299. Residuals: R; Rw 0.048; 0.053. Goodness of fit indicator 1.84.

lylboronic acid **3** in 79% yield and 2-amino-3-methoxyl-5-(*N*-tosyl-3′-indolyl)-pyrazine **18** was gained by coupling of 2-amino-3-methoxyl-5-bromopyrazine **14** and **3** in 91% yield under the same condition. Deprotection of the *N*-toluenesulfonyl group with sodium hydroxide in refluxing methanol afforded the corresponding mono-indolyl- and bisindolyl-pyrazines **16**, **19** and **21** in high yields.

Pharmacological Results and Discussion

The obtained indole alkaloids were evaluated for cytotoxicity in the National Cancer Institute (USA) in vitro disease-oriented antitumor screening using sulforhodamines B (SRB) assay including a panel of 60 human tumor cell lines representing nine different cancer types.[12–14] The results are presented in Table 1 as GI_{50} values in μM.

The natural product, meridianin D **1** showed weak antitumor activities against a variety of tumor cell lines, and its analogue 6-debromomeridianin D **2** did not show any antitumor activity at all.

Although 2,4-bis(*N*-tosyl-3′-indolyl)pyrimidines **5**–**6** were inactive, their *N*-detosylated derivatives **8**–**9** demonstrated good inhibitory effects against a variety of tumor cell lines with the GI_{50} values less than 10 μM. Among these compounds, bis(*N*-tosylindolyl)pyrimidine **7** exhibited significant inhibitory activities against leukemia SR, CNS Cancer SF-539, and breast cancer MDA-MB-435 cell lines with the GI_{50} value of 0.22, 0.16, and 0.22 μM, respectively. 2,4-Bis(3-indolyl)pyrimidine **8** displayed strong selective cytotoxic activity against IGROV1 tumor cell line with GI_{50} values below 0.01 μM.

One dose primary anticancer assay results indicated that *N*-protected bis(indolyl)pyrazines **17**, **20** were inactive against NCI-H460, MCF7 and SF-268 tumor cells. 3,5-Bis(3′-indolyl)pyrazine **21** demonstrated good inhibitory effects against a variety of tumor cell lines with the GI_{50} values less than 10 μM. However, compared to 2,5-bis(3′-indolyl)pyrazine and its *N*-methylated derivative,[7] their antitumor activity were weaker. To our surprise, 2-amino-5-bromo-3-(*N*-tosyl)pyrazine **15** also showed good inhibitory activity against a variety of tumor cell lines with GI_{50} value below 10 μM. It was noted that 2-amino-3-methoxyl-5-(3′-indolyl)pyrazine **19** exhibited excellent selective inhibition against CCRF-CEM cancer lines ($GI_{50} = 0.81$ μM) and HOP-92 cancer lines ($GI_{50} = 0.03$ μM).

In summary, we have synthesized a series of novel indolylpyrimidines and indolylpyrazines that showed sufficient inhibitory effects in the growth of a wide variety of human tumor cell lines in vitro. 2-Amino-3-methoxyl-5-(3′-indolyl)pyrazine **19** exhibited good selective inhibition against CCRF-CEM and HOP-92 cancer lines and 2,4-bis(3′-indolyl)pyrimidine **8** displayed excellent selective inhibitory activity against the IGROV1 tumor cell line. They are considered to be promising lead compounds for the further development of indole alkaloids as antitumor agents.

Experimental

All melting points were measured with a WRS-1A digital melting point apparatus, without correction. IR spectra were determined with a Shimadzu IR-440 spectrometer. ^1H NMR and ^{13}C NMR spectra were recorded on a Bruker AM-300 instrument. The chemical photo shifts are expressed in ppm and coupling constants are given in Hz. Low-resolution MS spectra were obtained on a VG-Quattro or HP-5969A spectrometer and high-resolution MS spectra were recorded on a Finnigan MAT-95 spectrometer. Elemental analyses were carried out on a Heraeus Rapid-CHNO instrument. Column chromatography was performed on silica gel H (10–40 μm). Reagents purchased commercially from Aldrich were used without further purification. Solvents were dried using standard procedures. 2,4-Bis(*N*-tosyl-3′-indolyl)pyrimidines (**5**), (**6**) and (**7**) were prepared according our previous reported.[9]

General procedure for synthesis of 2,4-bis(3′-indolyl)pyrimidines. 2,4-Bis(*N*-tosyl-3′-indolyl)pyrimidines[9] and sodium hydroxide in methanol were refluxed for 2 h. After cooled to rt, methanol was removed under vacuum and the residue was dispersed into water and ethyl acetate. The organic layer was separated and washed with water and brine successively. Then the organic phase was dried over anhydrous sodium sulfate

Table 1. Cancer cell growth inhibitory activity of compounds **1**, **7**–**10** and **15**, **19**, **21** in vitro[a,b]

Panel/cell lines	**1**	**7**	**8**	**9**	**10**	**15**	**19**	**21**
Leukemia								
CCRF-CEM	41.1	ND[c]	1.51	1.52	1.13	ND	0.81	ND
RPMI-8226	22.6	0.37	1.91	3.90	2.80	2.28	1.01	2.74
SR	17.3	0.22	4.24	11.3	9.53	0.77	15.0	3.24
Non-small cell lung cancer								
A549/ATCC	15.7	0.52	3.28	3.64	3.26	1.81	31.2	3.33
HOP-92	45.6	1.42	1.70	2.03	2.34	0.87	0.03	3.20
NCI-H460	30.0	0.39	2.87	2.65	3.08	2.82	26.3	2.12
Colon cancer								
COLO 205	18.7	0.36	5.44	4.58	8.32	2.61	29.0	2.30
HCT-116	18.6	0.40	4.36	4.56	3.43	0.31	21.7	3.13
KM12	24.2	0.51	3.93	3.79	4.18	2.34	9.19	2.19
CNS cancer								
SF-295	35.3	0.50	2.93	3.29	3.14	2.86	29.4	2.33
SF-539	32.6	0.16	4.37	11.7	7.14	2.87	33.7	4.21
SNB-19	20.7	0.56	4.81	7.88	5.26	0.73	66.5	1.15
U251	30.9	0.36	3.86	6.46	4.19	0.91	31.0	3.37
Melanoma								
LOX IMVI	25.3	0.48	2.82	3.62	4.84	2.66	25.3	2.51
M14	24.6	0.36	1.99	4.32	4.08	1.76	30.1	1.89
Ovarian cancer								
IGROV1	23.7	0.25	<0.01	1.14	1.86	1.37	19.6	3.24
OVCAR-5	17.5	0.38	>100	5.50	8.13	4.15	39.6	4.68
Renal cancer								
SN12C	21.7	0.53	2.78	1.68	ND	0.42	5.88	2.88
UO-31	18.7	0.58	3.98	6.97	5.53	4.36	21.2	3.73
Prostate cancer								
PC-3	30.1	0.39	2.72	3.49	2.84	2.23	26.5	3.69
Breast cancer								
MCF7	19.9	0.39	2.74	3.05	3.55	0.82	2.85	2.30
MDA-MB-435	26.6	0.22	4.52	5.87	5.76	0.29	23.5	3.31

[a] Data obtained from NCI's in vitro disease-oriented tumor cells screen.

[b] GI_{50} is the molar concentration causing 50% growth inhibition of tumor cells. Compounds with $GI_{50} > 100$ μM are considered inactive. GI_{50} values in μM.

[c] Not determined.

overnight. After evaporation under vacuum, the solid residue was purified by chromatography to give 2,4-bis(3′-indolyl)pyrimidines.

2,4-Bis(3′-indolyl)pyrimidine (8). Yield 97%. Mp >300 °C; IR (KBr) ν_{max} 3391, 1615, 1578, 1548, 1455, 1439, 1265, 1171, 1092 cm^{-1}; ^{1}H NMR (DMSO-d_6) δ 7.16–7.27 (m, 4H), 7.50–7.55 (m, 2H), 7.59 (d, J=5.4 Hz, 1H), 8.33 (d, J=1.1 Hz, 1H), 8.41 (d, J=1.8 Hz, 1H), 8.62–8.67 (m, 3H), 11.64 (br s, 1H), 11.83 (br s, 1H); EIMS m/z (%) 310 (M$^+$, 100), 141 (22). Anal. calcd for $C_{20}H_{14}N_4$: C, 77.40; H, 4.55; N, 18.05. Found: C, 77.30; H, 4.47; N, 17.98.

5-Methyl-2,4-bis(3′-indolyl)pyrimidine (9). Yield 97%. Mp 263.2–265.3 °C; IR (KBr) ν_{max} 3397, 1618, 1552, 1538, 1445, 1239, 1114, 1010 cm^{-1}; ^{1}H NMR (DMSO-d_6) δ 2.51 (s, 3H), 7.09–7.28 (m, 4H), 7.86 (dd, J=13.4 and 7.3 Hz, 2H), 8.07 (d, J=2.1 Hz, 1H), 8.24 (d, J=1.5 Hz, 1H), 8.59 (s, 1H), 7.86 (dd, J=14.0 and 7.9 Hz, 2H), 11.61 (br s, 1H), 11.80 (br s, 1H); ^{13}C NMR (DMSO-d_6) δ 18.4, 112.3 113.7, 116.0, 120.5, 120.8, 122.3, 122.6, 122.7, 126.2, 127.0, 128.5, 128.7, 129.6, 129.8, 136.6, 136.8, 137.6, 158.4, 160.6, 161.7; EIMS m/z (%) 324 (M$^+$, 100), 181 (7), 154 (21), 115 (12); HRMS calcd for $C_{21}H_{16}N_4$: 324.1375. Found: 324.1357.

5-Methoxy-2,4-bis(3′-indolyl)pyrimidine (10). Yield 92%. Mp 291.4–292.8 °C; IR (KBr) ν_{max} 3384, 1566, 1532, 1453, 1432, 1297, 1242, 1130, 1035 cm^{-1}; ^{1}H NMR (DMSO-d_6) δ 4.09 (s, 3H), 7.14–7.28 (m, 4H), 7.49–7.56 (m, 2H), 8.19 (d, J=1.5 Hz, 1H), 8.40 (d, J=1.8 Hz, 1H), 8.54 (s, 1H), 8.61 (d, J=7.7 Hz, 1H), 8.89- 8.92 (m, 1H), 11.52 (br s, 1H), 11.80 (br s, 1H); ^{13}C NMR (DMSO-d_6) δ 56.1, 110.2, 111.8, 115.6, 119.9, 120.5, 121.6, 121.8, 122.2, 122.7, 125.6, 126.2, 127.2, 131.5, 131.7, 136.2, 137.0, 138.8, 146.5, 150.8, 156.2; EIMS m/z (%) 340 (M$^+$, 100), 297 (21), 183 (50), 155 (55), 128 (41), 101 (16); HRMS calcd for $C_{21}H_{16}N_4O$: 340.1324. Found: 340.1322.

2-Amino-3,5-dibromopyrazine (12). *N*-Bromosuccinimide (14.95 g, 84 mmol) is added over 50 min to a mixture of 2-aminopyrazine (**11**) (3.80 g, 40 mmol) in DMSO (80 mL) and H_2O (2 mL) below 15 °C. After stirring for 6 h at rt, the mixture is poured into ice-water (250 mL) and extracted with EtOAc (4×50 mL). The extract is filtered, washed with 5% aq Na_2CO_3 (50 mL) and H_2O, and dried with anhydrous sodium sulfate. The solvent is evaporated and the residue is sublimed to recrystallize from EtOH to give 2-amino-3,5-dibromopyrazine (**12**) 8.61 g (85% yield) as white solid. Mp 117.6–118.8 °C (lit.[15] mp 116 °C); IR (KBr) ν_{max} 3451, 3284, 1625, 1508, 1453, 1316, 1099 cm^{-1}; ^1H NMR ($CDCl_3$) δ 5.00 (br s, 2H), 7.97 (s, 1H); EIMS *m*/*z* (%) 253 (M^+, 100), 251:255 (52:53), 172:174 (24:23), 66 (20).

2-(*N*,*N*-Dimethyl)amino-3,5-dibromopyrazine (13). To a solution of 2-amino-3,5-dibromopyrazine (**12**) (253 mg, 1 mmol) in DMF is added 60% NaH (88 mg, 2.2 mmol). Then, iodomethane (568 mg, 4 mmol) is added to the mixture in one port. After stirring for 15 min, the mixture is treated with water (5 mL) and extracted with ether (2×10 mL). The organic layer is washed with water and brine and dried over anhydrous sodium sulfate. The solvent is evaporated and the residue is purified with column chromatography to give a yellow liquid **13** (248 mg, 90%). IR (KBr) ν_{max} 2954, 1543, 1498, 1403, 1160, 1046 cm^{-1}; ^1H NMR ($CDCl_3$) δ 3.09 (s, 6H), 8.10 (s, 1H); EIMS *m*/*z* (%) 281 (M^+, 26), 266 (12), 252 (45), 44 (100).

2-Amino-3-methoxyl-5-bromopyrazine (14). A mixture of 2-amino-3,5-dibromopyrazine (**12**) (5.06 g, 20 mmol) and sodium methoxide (1.06 g, 20 mmol) in methanol is refluxed. After the reaction was completed, the mixture is cooled to rt slowly and the product is precipitated as a colorless crystal weighed 3.43 g, 84% yield. Mp 140.5–141.6 ºC (lit.[11] mp 138 ºC); IR (KBr) ν_{max} 3489, 3287, 3140, 1633, 1544, 1505, 1423, 1304, 1222, 1007 cm^{-1}; ^1H NMR ($CDCl_3$) δ 5.00 (br s, 2H), 7.97 (s, 1H); EIMS *m*/*z* (%) 204 (M^+, 2), 203/205 (5/5), 182 (55), 125 (100), 109 (54), 69 (64).

General procedure for palladium catalyzed cross-coupling reaction of bromopyrazine with *N*-tosyl-3-indolylboronic acid (3). A mixture of *N*-tosyl-3-indolylboronic acid (**3**) (1 or 2 mmol), bromopyrazine (1 mmol), DME (15 mL), aqueous cesium carbonate (1 or 2 mL, 2 M) and tetrakis(triphenylphosphine)palladium (0.1 or 0.2 mmol) was refluxed under an argon atmosphere. The reaction was monitored with TLC. When the reaction completed, anhydrous sodium sulfate was added. The mixture was filtered and the filtrate was evaporated under reduced pressure. The residue was subjected to flash column chromatography (eluted with ethyl acetate/hexane) to give cross-coupling products.

2-Amino-3-(*N*-tosyl-3′-indolyl)-5-bromopyrazine (15). Yield 90%. Mp 188.9–190.6 °C; IR (KBr) ν_{max} 3456, 3297, 3171, 1631, 1448, 1372, 1281, 1172, 1130, 1089, 1014 cm^{-1}; ^1H NMR (DMSO-d_6) δ 2.32 (s, 3H), 6.69 (br s, 2H), 7.32–7.45 (m, 4H), 7.94–8.09 (m, 4H), 8.11 (s, 1H), 8.37 (s, 1H); EIMS *m*/*z* (%) 442:444 (M^+, 52:59), 287:289 (97:100), 208 (65), 91 (32). Anal. calcd for $C_{19}H_{15}N_4BrO_2S$: C, 51.58; H, 3.39; N, 12.67. Found: C, 51.54; H, 3.61; N, 12.60.

2-Amino-3,5-bis(*N*-tosyl-3′-indolyl)pyrazine (17). Yield 51%. Mp 246.5 °C (dec.); IR (KBr) ν_{max} 3494, 3380, 1609, 1478, 1445, 1364, 1279, 1173, 1086 cm^{-1}; ^1H NMR (DMSO-d_6/$CDCl_3$) δ 2.31 (s, 3H), 2.34 (s, 3H), 6.48 (br, 2H), 7.17–7.42 (m, 8H), 7.90–8.05 (m, 6H), 8.12 (d, *J* = 8.0 Hz, 1H), 8.26 (d, *J* = 6.5 Hz, 1H), 8.35 (d, *J* = 2.7 Hz, 2H), 8.65 (s, 1H); ^{13}C NMR (DMSO-d_6) δ 20.9, 21.0, 113.0, 113.2, 117.9, 119.9, 122.3, 122.4, 123.3, 123.5, 123.7, 125.1, 125.2, 125.9, 126.8, 127.2, 128.2, 129.2, 130.3, 131.9, 133.9, 134.0, 134.1, 134.8, 136.0, 138.7, 145.6, 145.7, 152.1; EIMS *m*/*z* (%) 634 (M^+, 14), 478 (44), 323 (42), 262 (100), 183 (87), 108 (52); HRMS calcd for $C_{34}H_{27}N_5O_4S_2$: 633.1504. Found: 633.1505.

2-Amino-3-methoxyl-5-(*N*-tosyl-3′-indolyl)pyrazine (18). Yield 89%. Mp 214.3–215.5 °C; IR (KBr) ν_{max} 3464, 3289, 3152, 1629, 1561, 1486, 1372, 1220, 1174, 1092 cm^{-1}; ^1H NMR (DMSO-d_6) δ 2.34 (s, 3H), 4.08 (s, 3H), 6.12 (br, 2H), 7.27–7.38 (m, 4H), 7.85 (d, *J* = 8.4 Hz, 2H), 7.97–8.06 (m, 3H), 8.23 (dd, *J* = 7.06 and 1.0 Hz, 1H); EIMS *m*/*z* (%) 394 (M^+, 33), 239 (100), 212 (8), 155 (6); HRMS calcd for $C_{20}H_{18}N_4O_3S$: 394.1100. Found: 394.1108. Anal. calcd for $C_{20}H_{18}N_4O_3S$: C, 60.91; H, 4.57; N, 14.21. Found: C, 60.81; H, 4.72; N, 14.09.

2-(*N*,*N*-Dimethyl)amino-3,5-bis(*N*-tosyl-3′-indolyl)pyrazine (20). Yield 94%. Mp 120.9–121.7 °C; IR (KBr) ν_{max} 2924, 1597, 1548, 1493, 1446, 1373, 1270, 1172, 1089 cm^{-1}; ^1H NMR ($CDCl_3$) δ 2.31 (s, 3H), 2.33 (s, 3H), 2.86 (s, 6H), 7.20–7.42 (m, 8H), 7.79–7.87 (m, 4H), 8.02 (s, 1H), 8.02–8.10 (m, 2H), 8.16–8.23 (m, 2H), 8.19 (s, 1H), 8.46 (s, 1H); ^{13}C NMR ($CDCl_3$) δ 21.5, 40.9, 113.5, 113.6, 120.2, 120.9, 122.1, 122.6, 123.5, 123.6, 123.7, 124.9, 125.1, 126.0, 126.9, 128.6, 129.0, 129.8, 129.9, 135.1, 135.6, 136.1, 136.5, 138.6, 145.1, 145.2, 154.9; EIMS *m*/*z* (%) 662 (M^+, 48), 506 (100), 350 (63), 91 (32); HRMS calcd for $C_{36}H_{31}N_5O_4S_2$: 661.1834. Found: 661.1826.

General procedure for removal *N*-tosyl to indolyl-pyrazine. The procedure is same as the synthesis of compounds **8**–**10**.

2-Amino-3-(3′-indolyl)-5-bromopyrazine (16). Yield 96%. Mp 187 °C (dec.); IR (KBr) ν_{max} 3469, 3291, 3175, 1624, 1534, 1514, 1444, 1224, 1130, 987 cm^{-1}; ^1H NMR (DMSO-d_6) δ 6.34 (br s, 2H), 7.13–7.24 (m, 2H), 7.49 (d, *J* = 8.0 Hz, 1H), 7.92 (s, 1H), 8.08 (d, *J* = 1.5 Hz, 1H), 8.22 (d, *J* = 7.7 Hz, 1H), 11.69 (br s, 1H); ^{13}C NMR (DMSO-d_6) δ 110.4, 111.9, 120.3, 121.5, 122.5, 124.1, 126.1, 126.8, 136.5, 137.8, 138.4, 151.7; EIMS *m*/*z* (%) 288/290 (M^+, 100/98), 209 (17), 182 (21), 155 (30), 128 (10); HRMS calcd for $C_{12}H_9N_4Br$: 288.0009. Found: 288.0002.

2-Amino-3-methoxyl-5-(3′-indolyl)pyrazine (19). Yield 95%. Mp 182 °C (dec.); IR (KBr) ν_{max} 3479, 3406, 3140, 1733, 1631, 1558, 1491, 1448, 1314, 1216, 1095 cm^{-1}; ^1H NMR (DMSO-d_6) δ 4.04 (s, 3H), 6.01 (br s, 2H), 7.05–7.16 (m, 2H), 7.42 (dd, *J* = 7.6 and 1.5 Hz, 1H), 7.83 (d, *J* = 1.6 Hz, 1H), 8.00 (s, 1H), 8.25 (d, *J* = 7.8 Hz, 1H), 11.27 (br s, 1H); ^{13}C NMR (DMSO-d_6) δ 53.0, 111.6, 113.2, 119.4, 120.8, 121.3, 122.8, 124.9, 128.8, 134.9, 136.7, 143.2, 146.9; EIMS *m*/*z* (%) 240 (M^+, 100), 198 (20), 156 (16); HRMS calcd for $C_{13}H_{12}N_4O$: 240.1011. Found: 240.1028. Anal. calcd for $C_{13}H_{12}N_4O$ 1/8H_2O:

C, 64.40; H, 5.06; N, 23.12. Found: C, 64.37; H, 5.14; N, 22.93.

2-(*N*,*N*-Dimethyl)amino-3,5-bis(3′-indolyl)pyrazine (21). Yield 99%. Mp 140.8 °C (dec.); IR (KBr) ν_{max} 3396, 2924, 2853, 1723, 1537, 1488, 1455, 1336, 1240, 1170, 1097 cm^{-1}; ^{1}H NMR (DMSO-d_6) δ 2.79 (s, 6H), 7.05–7.22 (m, 4H), 7.46–7.52 (m, 2H), 8.02 (s, 1H), 8.12 (s, 1H), 8.32–8.37 (m, 2H), 8.48 (s, 1H), 11.46 (br s, 1H), 11.54 (br s, 1H); ^{13}C NMR (DMSO-d_6) δ 40.9, 111.7, 111.8, 113.3, 113.5, 119.6, 119.7, 121.0, 121.6, 121.7, 121.8, 124.3, 125.1, 125.9, 126.8, 132.7, 136.4, 136.9, 140.3, 142.0, 152.8; EIMS *m*/*z* (%) 354 (M$^+$, 100), 339 (21), 140 (15); HRMS calcd for $C_{22}H_{19}N_5$: 353.1640. Found: 353.1609.

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References

1. (a) Nishizawa, Y. *Nature* **1984**, *308*, 693. (b) Nishizawa, Y. *Science* **1986**, *233*, 305. (c) Nishizawa, Y. *Nature* **1988**, *334*, 661. (d) Stewart, A. F.; Schultz, G. *Cell* **1987**, *50*, 1109. (e) Wu, H. Y.; Shyy, S. H.; Wang, J. C.; Liu, L. F. *Cell* **1988**, *53*, 433. (f) Merino, A.; Madden, K. R.; Lane, W. S.; Champoux, J. J.; Deinberg, D. *Nature* **1993**, *365*, 227.
2. (a) Alvarez, M.; Salas, M. *Heterocycles* **1991**, *32*, 1391. (b) Wipf, P. *Chem. Rev.* **1995**, *95*, 2115. (c) Faulkner, D. J. *Nat. Prod. Rep.* **1999**, *16*, 155.
3. (a) Wright, A. E.; Pomponi, S. A.; Cross, S. S.; McCarthy, P. *J. Org. Chem.* **1992**, *57*, 4772. (b) Capon, R. J.; Rooney, F.; Murray, L. M.; Collins, E.; Sim, A. T. R.; Rostas, J. A. P.; Butler, M. S.; Carroll, A. R. *J. Nat. Prod.* **1998**, *61*, 660.
4. Kohmoto, S.; Kashman, Y.; McConnell, O. J.; Rinehart, K. L.; Wright, A., Jr.; Koehn, F. *J. Org. Chem.* **1988**, *53*, 3116.
5. (a) Sakemi, S.; Sun, H. H. *J. Org. Chem.* **1991**, *56*, 4304. (b) Kawasaki, I.; Yamashita, M.; Ohata, M. *Chem. Pharm. Bull.* **1996**, *44*, 1831.
6. (a) Gu, X.-H.; Wan, X.-Z.; Jiang, B. *Bioorg. Med. Chem. Lett.* **1999**, *9*, 569. (b) Jiang, B.; Gu, X.-H. *Heterocycle* **2000**, *53*, 1559.
7. Jiang, B.; Gu, X.-H. *Bioorg. Med. Chem.* **2000**, *8*, 363.
8. Franco, L. H.; Joffé, E. B. K.; Puricelli, L.; Tatian, M.; Seldes, A. M.; Palermo, J. A. *J. Nat. Prod.* **1998**, *61*, 1130.
9. Jiang, B.; Yang, C.-G. *Heterocycles* **2000**, *53*, 1489.
10. Zheng, Q.; Yang, Y.; Martin, A. R. *Heterocycles* **1994**, *37*, 1761.
11. Camerino, B.; Palamidessi, G. *Gazz. Chim. Ital.* **1960**, *90*, 1807.
12. Rubinstein, L. V.; Shoemaker, R. H.; Paull, K. D.; Simon, R. M.; Tosini, S.; Skehan, P.; Scudiero, D. A.; Monks, A.; Boyd, M. R. *J. Natl. Cancer Inst.* **1990**, *82*, 1113.
13. Skehan, P.; Storeng, R.; Scudiero, D. A.; Monks, A.; McMahon, J.; Vistica, D.; Warren, J. T.; Bokesch, H.; Kenney, S.; Boyd, M. R. *J. Natl. Cancer Inst.* **1990**, *82*, 1107.
14. Grever, M. R.; Schepatz, S. A.; Chabner, B. A. *Semin. Oncol.* **1992**, *19*, 622.
15. Brachwitz, V. H. *J. Prakt. Chem.* **1969**, *311*, 40.