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Preliminary communication

Synthesis and anti-tumor activity of 2-amino-3-cyano-6-(1*H*-indol-3-yl)-4-phenylpyridine derivatives *in vitro*

Fan Zhang [a], Yanfang Zhao [a], Li Sun [a], Lu Ding [a], Yucheng Gu [b], Ping Gong [a,*]

[a] *Key Laboratory of New Drugs Design and Discovery of Liaoning Province, School of Pharmaceutical Engineering, Shenyang Pharmaceutical University, 103 Wenhua Road, Shenyang 110016, PR China*
[b] *Syngenta, Jealott's Hill International Research Centre, Bracknell, Berkshire RG42 6EY, UK*

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ABSTRACT

A series of novel 2-amino-3-cyano-6-(1*H*-indol-3-yl)-4-phenylpyridine derivatives were synthesized and their cytotoxic activity against A549, H460, HT-29 and SMMC-7721 cell lines was evaluated *in vitro*. Among them, ten compounds (**10**, **11**, **14**, **16**, **17**, **26**, **27**, **29**, **30** and **31**) displayed excellent anti-tumor activity against different cell lines. The most promising compound **27** showed strong anti-tumor activity against A549, H460, HT-29 and SMMC-7721 cell lines with IC_{50} values of 22, 0.23, 0.65 and 0.77 nM, which were 2.6-, 83-, 1.1×10^3- and 2.0×10^3- fold more active than MX-58151 (IC_{50} values of 0.058, 0.019, 0.70 and 1.53 μM), respectively.

1. Introduction

The mortality and morbidity of cancer patients are the second highest among all diseases in the world, after heart disease [1,2]. The demand for new lead compounds in this therapeutic area is higher than ever before. It has encouraged scientists to search for anti-tumor agents with novel chemical eternity and new model of action. 2-Amino-3-cyanopyridine derivatives are known to have multiple biological activities, such as anti-microbial [3], cardiotonic [4], anti-inflammatory [5] and anti-parkinsonism properties [6]. We are much interested in the anti-tumor activity of these compounds owing to their different types of mechanisms of action. As reported, several 4,6-diaryl-substituted and tricyclic 2-amino-3-cyanopyridines exhibited potent anti-tumor activity against human breast cancer cell lines T-47D and ZR-75-1 and significant inhibitory activity of mitogen activated protein kinase-activated protein kinase 2 (MK-2) in μM range [7–10]. Therefore, optimizations of 2-amino-3-cyanopyridines may lead to more active anti-tumor chemotherapeutics. Considerable attention has been focused on modification of 4,6-diaryl-2-amino-3-cyanopyridines. Generally, in these literatures, the aryl group at 6-position of pyridine is always phenyl or other single heteroaromatic ring including furan-2-yl, thiophen-2-yl and pyrrol-2-yl groups, and there are hardly any studies referring to compounds bearing a bicyclic core structure so far. In view of the attractive pharmacological activity present of indole and its analougues in several drugs [11,12], the bicyclic indole core was introduced into the 4,6-diaryl-2-amino-3-cyanopyridines to identify more potent anti-tumor agents.

Herein, we reported the synthesis and cytotoxic activity of 2-amino-3-cyano-6-(1*H*-indol-3-yl)-4-phenylpyridine derivatives (Fig. 1) against A549, H460, HT-29 and SMMC-7721 cell lines *in vitro*.

2. Chemistry

3-Bromo-4,5-dimethoxybenzaldehyde was synthesized *via* the reaction of 5-bromovanillin with dimethyl sulfate (Scheme 1).

Due to the different substituents on the indole core, two methods were used to synthesize the 1-(1*H*-indol-3-yl)ethanones with the starting materials of their corresponding anilines and benzaldehydes. The general synthetic routes of substituted 1-(1*H*-indol-3-yl)ethanones is illustrated in Scheme 2. The substituted phenylhydrazine hydrochlorides (**1a**–**1b**) were prepared *via* a three-step reaction starting from the corresponding aniline. Substituted anilines were diazotized with sodium nitrite under acidic conditions, reduced by sodium bisulfite and acidified with hydrochloric acid to afford intermediates **1a**–**1b**. By a nucleophilic addition reaction of **1a**–**1b** with ethyl pyruvate, Schiff's bases ethyl

* Corresponding author. Tel./fax: +86 24 2398 6429.
E-mail address: gongpinggp@126.com (P. Gong).

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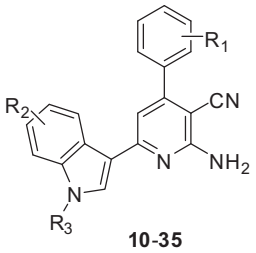


Fig. 1. Structures of target compounds **10**–**35**.

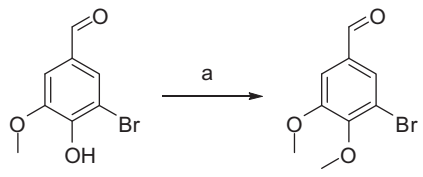
2-(2-phenylhydrazono)propanoates (**2a**–**2b**) were synthesized, which were then cyclized in PPA to afford the key intermediates ethyl 1*H*-indole-2-carboxylates (**3a**–**3b**). In an alternative approach, ethyl bromoacetate and sodium azide were heated to 63 °C in acetone to give ethyl 2-azidoacetate (**7**) as a colorless oil, which was then treated with substituted benzaldehyde using EtONa in ethanol to form the corresponding ethyl 2-azido-3-phenylacrylates (**8c**–**8d**) as yellow solids. Cyclization by heating in xylene then led to the required ethyl 1*H*-indole-2-carboxylates (**3a**–**3b**). Furthermore, hydrolysis of **3a**–**3d** yielded 1*H*-indole-2-carboxylic acids (**4a**–**4d**), which were decarboxylated to give 1*H*-indoles (**5a**–**5d**). Thereafter, substituted 1-(1*H*-indol-3-yl) ethanones (**6a**–**6d**) were successfully obtained *via* the reaction of **5a**–**5d** with glacial acetic acid and acetic anhydride, which were then reacted with iodomethane to afford the corresponding 1-(1-methyl-1*H*-indol-3-yl)ethanones (**9b**–**9e**).

Finally, the desired compounds were prepared in good yields *via* a one-pot reaction between a substituted benzaldehyde, 1-(1*H*-indol-3-yl)ethanone or 1-(1-methyl-1*H*-indol-3-yl)ethanone, malononitrile and ammonium acetate, according to methods previously described in the literature (Scheme 3) [13]. All target compounds were purified by column chromatography on silica gel. The chemical structures of target compounds were confirmed by ^{1}H NMR, ^{13}C NMR, IR and MS spectra (Table 1).

3. Biological evaluation

All compounds, along with the reference compound MX-58151 [14], were tested for their *in vitro* cytotoxic activity against A549 (human non-small-cell lung cancer cell line), H460 (human lung cancer cell line), HT-29 (human colorectal cancer cell line) and SMMC-7721 (human liver cancer cell line) using MTT assay. The results expressed as IC_{50} were summarized in Table 2. The IC_{50} values are the average of three independent experiments.

As shown in Table 2, most of the prepared compounds exhibited moderate to strong cytotoxic activity against all four cancer cell lines. Among them, compounds **17** and **27** were superior to MX-58151 against all four cell lines, while compounds **10**, **11**, **26** and **31** were more active than MX-58151 against H460, HT-29 and SMMC-7721 cells. 2-Amino-4-(3-bromo-4,5-dimethoxyphenyl)-6-(1-methyl-1*H*-indol-3-yl)nicotinonitrile (**27**), the most promising compound, showed strong anti-tumor activity against A549, H460, HT-29 and SMMC-7721 cell lines with IC_{50} values of 22, 0.23, 0.65 and 0.77 nM, which were 2.6-, 83-, 1.1×10^3- and 2.0×10^3- fold more active than MX-58151 (IC_{50} values of 0.058, 0.019, 0.70 and 1.53 μM), respectively.



Scheme 1. Reagents and conditions: (a) Dimethyl sulfate, K_2CO_3, acetone, reflux, 6 h.

Interestingly, the cytotoxic activity of compounds **10**–**35** against H460 and HT-29 cells was much higher than those against A549 and SMMC-7721. On the other hand, compounds **16**, **17**, **29** and **30** exhibited excellent selectivity for H460 cells, while compounds **10**, **11**, **14**, **26**, **27** and **31** followed by compounds **17**, **13** and **30** were the most potent of the tested compounds against HT-29 cells. Furthermore, compound **27** was further far stronger against SMMC-7721 cell line than others.

The present study investigated the effect of several substituents on indole ring and benzene ring. In most cases, compounds with methyl group at the 1-postion of indole (**26**–**35**) displayed slightly higher cytotoxic activity than those of the corresponding compounds without substituent at the same position (**10**–**12**, **15**–**18** and **22**–**24**). Nevertheless, introduction of 5,6,7-trimethoxy group to indole (**21**–**25**, **33**–**35**) led to a dramatic decrease in anti-tumor activity. It can be concluded that halogen or no substituent on indole ring was benefit for cycotoxicity. The results suggested that the anti-tumor activity might be impacted by the size or electronic effects of substituents on indole ring. The position of substituents on indole ring also played an important role on the pharmacological activity. Generally, substituents at the 5-position (**15**–**17**, **29**) were preferred in comparison to those at the 6-position (**18**–**20**, **32**), particularly in terms of their activity against H460 cells. On the other hand, the trisubstituted phenyl groups were chosen to study the effect of substituents on benzene ring, including 2,3,4-trimethoxyphenyl, 3,4,5-trimethoxyphenyl and 3-bromo-4,5-dimethoxyphenyl. It was obvious that the cytotoxic activity was improved by replacement of the methoxy group at the 3-positon with a bromo group (**10** *vs* **11**, **15** *vs* **16**, **18** *vs* **19**, **26** *vs* **27**, **29** *vs* **30**).

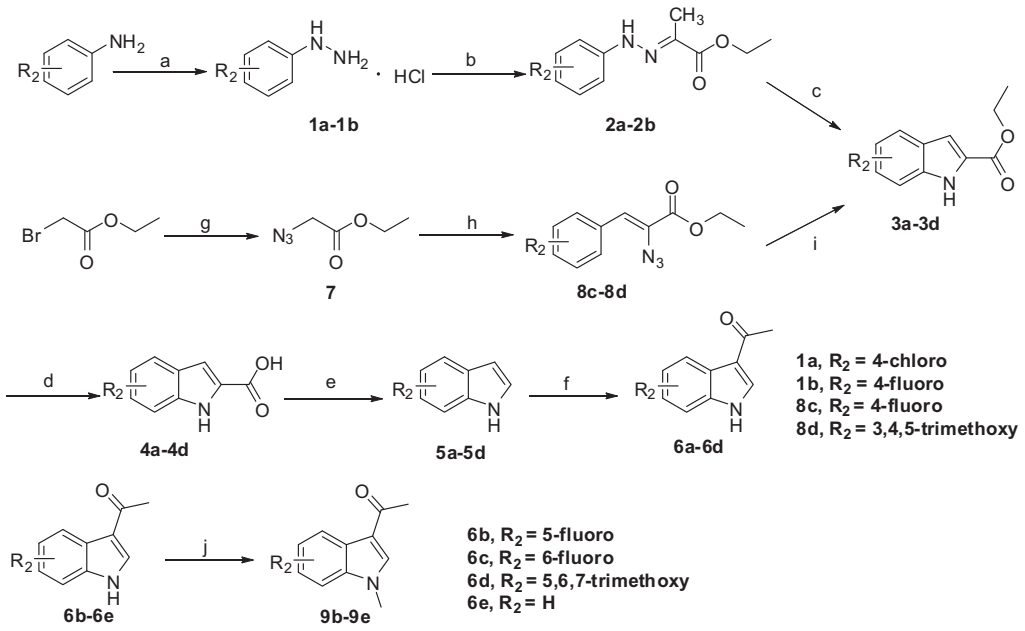
4. Conclusion

In summary, a series of 2-amino-3-cyano-6-(1*H*-indol-3-yl)-4-phenylpyridine derivatives were synthesized and screened for their cytotoxic activity against four human cell lines (A549, H460, HT-29 and SMMC-7721). Among them, ten compounds (**10**, **11**, **14**, **16**, **17**, **26**, **27**, **29**, **30** and **31**) displayed excellent anti-tumor activity against different cell lines. The most promising compound **27** showed potent anti-tumor activity against A549, H460, HT-29 and SMMC-7721 cell lines with IC_{50} values of 22, 0.23, 0.65 and 0.77 nM, which were 2.6-, 83-, 1.1×10^3- and 2.0×10^3- fold more active than MX-58151 (IC_{50} values of 0.058, 0.019, 0.70 and 1.53 μM), respectively. The pharmacological data indicated that introduction of indole core improved the anti-tumor activity of the 4,6-diaryl-2-amino-3-cyanopyridines. From the preliminary structure activity relationships, we may conclude that introduction of methyl group to the 1-postion of indole slightly enhance the cytotoxic activity, while halogen or no substituent on indole ring was favorable in this region. Furthermore, introduction of halogen groups into benzene ring was essential for their cytotoxic activity and the 3-bromo-4,5-dimethoxy group was the best of 3,4,5-trisubstituted groups. Studies on the mechanism of action of these compounds are in progress and will be reported upon in the future.

5. Experimental protocols

5.1. Chemistry

All melting points were obtained on a Büchi Melting Point B-540 apparatus (Büchi Labortechnik, Flawil, Switzerland) and were uncorrected. Mass spectra (MS) were taken in ESI mode on Agilent



Scheme 2. Reagents and conditions: (a) (i) $NaNO_2$, HCl, H_2O, 1 h; (ii) $NaHSO_3$, NaOH, H_2O, 80 °C, 1 h; (iii) HCl, H_2O, reflux, 3 h; (b) Pyruvate, AcOH, EtOH, reflux, 3–5 h; (c) PPA, 100 °C, 4 h; (d) NaOH, EtOH, reflux, 3 h; (e) Cu, Quinoline, reflux, 3 h; (f) Ac_2O, AcOH, reflux, 20 h; (g) NaN_3, MeCN, 63 °C, 4 h; (h) EtONa, EtOH, 0 °C, 4 h; (i) xylene, reflux, 3–5 h; (j) Iodomethane, KOH, DMSO, r. t., 4 h.

1100 LC-MS (Agilent, Palo Alto, CA, USA). Proton (1H) nuclear magnetic resonance spectroscopy was performed using Bruker ARX-300, 300 MHz spectrometers (Bruker Bioscience, Billerica, MA, USA) with TMS as an internal standard. IR spectra (KBr disks) were recorded with a Bruker IFS 55 instrument (Bruker).

All the materials were obtained from commercially available sources and used without further purification, unless otherwise specified. Yields were not optimized.

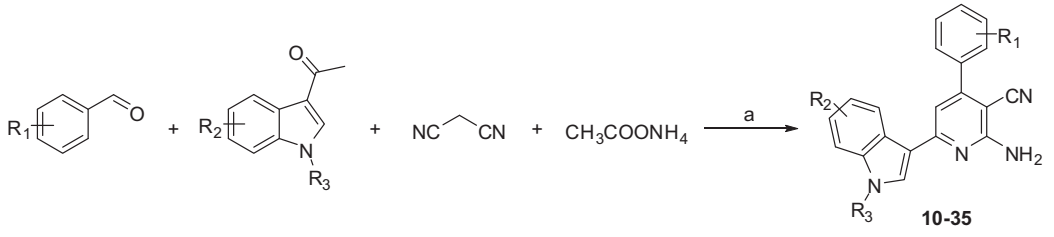
5.1.1. 3-Bromo-4,5-dimethoxybenzaldehyde

To a solution of 3-bromo-4-hydroxy-5-methoxybenzaldehyde (23.1 g, 0.1 mol) and anhydrous K_2CO_3 (27.6 g, 0.2 mol) in acetone (120 mL) was added dimethyl sulfate (10.5 mL, 0.11 mol) dropwise at room temperature during 10 min with stirring. After addition, the mixture was heated to reflux for 6 h and then the solvent was removed under reduced pressure. The residue was extracted with CH_2Cl_2 (3×100 mL), washed with brine (100 mL), dried over Na_2SO_4, and then the solvent was removed under reduced pressure to give 3-bromo-4,5-dimethoxybenzaldehyde as a white solid (16.2 g, 66%). m.p. 61–62 °C. ESI-MS *m/z*: 243.2 (Br = 79), 245.2 (Br = 81) $[M-H]^-$. ^1H NMR (300 MHz, DMSO-d_6) δ: 3.84 (s, 3H), 3.91 (s, 3H), 7.54 (s, 1H), 7.78 (s, 1H), 9.88 (s, 1H).

*5.1.1.1. (4-Chlorophenyl)hydrazine hydrochloride (**1a**).* A solution of 4-chloroaniline (127.6 g, 1 mol) in concentrated hydrochloric acid (293 mL) was stirred for 2 h at room temperature. To a solution of sodium nitrite (72.5 g, 1.05 mol) in water (145 mL) was added dropwise below 5 °C. After stirring 1 h, filtrate was separated by filtration, to which a solution of $NaHSO_3$ (213.2 g, 2.05 mol) in water (500 mL) was added dropwise at 0 ~ 10 °C and pH was kept between 6 to 7 using 25% NaOH solution. The resulted mixture was heated to 80 °C for 1 h. Upon cooling to room temperature, concentrated hydrochloric acid (600 mL) was added dropwise and then the mixture was heated to 90–100 °C for 1 h. The mixture was cooled to 10 °C and the precipitate was collected by filtration. **1a** was obtained as a light red solid (129.3 g, 60%). m.p. 218 °C. ESI-MS *m/z*: 142.6 (Cl = 35), 144.7 (Cl = 37) $[M+H]^+$.

*5.1.1.2. (4-Fluorophenyl)hydrazine hydrochloride (**1b**).* According to the procedure used to prepare **1a**, starting from 4-fluoroaniline, **1b** was obtained as a light red solid. Yield: 55%. m.p. 252–253 °C. ESI-MS *m/z*: 127.1 $[M+H]^+$.

*5.1.1.3. (E)-Ethyl 2-(2-(4-chlorophenyl)hydrazono)propanoate (**2a**).* A mixture of compound **1a** (107.8 g, 0.5 mol), ethyl pyruvate (88 mL, 0.8 mol) and glacial acetic acid (5.7 mL, 0.1 mol) in anhydrous ethanol (539 mL) was heated to reflux for 3–5 h. Upon cooling to room temperature, the precipitate was collected by filtration. **2a** was obtained as a light yellow solid (110.7 g, 92%). m.p. 110–112 °C. ESI-MS *m/z*: 240.9 (Cl = 35), 242.8 (Cl = 37) $[M+H]^+$.



Scheme 3. Reagents and conditions: (a) toluene, reflux, 8 h.

Table 1
Substituents of target compounds.

Compd.	R_1	R_2	R_3
10	3,4,5-trimethoxy	H	H
11	3-bromo-4,5-dimethoxy	H	H
12	2,3,4-trimethoxy	H	H
13	3,4,5-trimethoxy	5-cloro	H
14	2,3,4-trimethoxy	5-cloro	H
15	3,4,5-trimethoxy	5-fluoro	H
16	3-bromo-4,5-dimethoxy	5-fluoro	H
17	2,3,4-trimethoxy	5-fluoro	H
18	3,4,5-trimethoxy	6-fluoro	H
19	3-bromo-4,5-dimethoxy	6-fluoro	H
20	2,3,4-trimethoxy	6-fluoro	H
21	2,3,4-trimethoxy	5,6,7-trimethoxy	H
22	2,4-difluoro	5,6,7-trimethoxy	H
23	3,4-difluoro	5,6,7-trimethoxy	H
24	2-cloro-4-fluoro	5,6,7-trimethoxy	H
25	2,3-dicloro	5,6,7-trimethoxy	H
26	3,4,5-trimethoxy	H	Methyl
27	3-bromo-4,5-dimethoxy	H	Methyl
28	2,3,4-trimethoxy	H	Methyl
29	3,4,5-trimethoxy	5-fluoro	Methyl
30	3-bromo-4,5-dimethoxy	5-fluoro	Methyl
31	2,3,4-trimethoxy	5-fluoro	Methyl
32	3,4,5-trimethoxy	6-fluoro	Methyl
33	2,4-difluoro	5,6,7-trimethoxy	Methyl
34	3,4-difluoro	5,6,7-trimethoxy	Methyl
35	2-cloro-4-fluoro	5,6,7-trimethoxy	Methyl

*5.1.1.4. (E)-Ethyl 2-(2-(4-fluorophenyl)hydrazono)propanoate (**2b**).* According to the procedure used to prepare **2a**, starting from **1b**, **2b** was obtained as a light orange solid. Yield: 88%. m.p. 83–87 °C. ESI-MS *m/z*: 246.9 $[M + Na]^+$. ^{1}H NMR (300 MHz, DMSO-d_6) δ: 1.26 (t, $J = 7.2$ Hz, 3H), 2.04 (s, 3H), 4.22–4.15 (q, $J_1 = 6.9$ Hz, $J_2 = 14.1$ Hz, 2H), 7.14–7.09 (m, 2H), 7.28–7.23 (m, 2H), 9.84 (s, 1H).

*5.1.1.5. Ethyl 5-chloro-1H-indole-2-carboxylate (**3a**).* A mixture of compound **2a** (96 g, 0.4 mol) and polyphosphoric acid (960 g) was heated to 100 °C for 4 h and then poured into crush ice with acutely stirring. The precipitate was collected by filtration, washed with water and dried. The crude product was purified by recrystallization from ethanol. **3a** was obtained as a white solid (39.4 g, 44%). m.p. 175 °C. ESI-MS *m/z*: 222.6 (Cl = 35), 224.6 (Cl = 37) $[M - H]^-$. ^{1}H NMR (300 MHz, DMSO-d_6) δ: 1.34 (t, $J = 7.5$ Hz, 3H), 4.38–4.31 (q, $J_1 = 7.5$ Hz, $J_2 = 14.4$ Hz, 2H), 7.12 (s, 1H), 7.26 (dd, $J_1 = 8.7$ Hz, $J_2 = 2.7$ Hz, 1H), 7.46 (d, $J = 8.7$ Hz, 1H), 7.72 (d, $J = 2.1$ Hz, 1H), 12.08 (s, 1H).

*5.1.1.6. Ethyl 5-fluoro-1H-indole-2-carboxylate (**3b**).* According to the procedure used to prepare **3a**, starting from **2b**, **3b** was obtained as a light yellow solid. Yield: 48%. m.p. 115 °C. ESI-MS *m/z*: 206.2 $[M - H]^-$. ^{1}H NMR (300 MHz, DMSO-d_6) δ: 1.34 (t, $J = 6.9$ Hz, 3H), 4.37–4.30 (q, $J_1 = 6.9$ Hz, $J_2 = 14.4$ Hz, 2H), 7.16–7.09 (m, 2H), 7.48–7.39 (m, 2H), 11.99 (s, 1H).

*5.1.1.7. 5-Chloro-1H-indole-2-carboxylic acid (**4a**).* A mixture of compound **3a** (33.5 g, 0.15 mol), 4% NaOH solution (335 mL) and ethanol (335 mL) was heated to reflux for 3 h. Upon cooling to room temperature, the mixture was poured into ice-water, adjusted to pH 5 with glacial acetic acid, and then the precipitate was collected by filtration. **4a** was obtained as a white solid (26.4 g, 90%). m.p. 295–298 °C. ESI-MS *m/z*: 193.4 (Cl = 35), 195.4 (Cl = 37) $[M - H]^-$. ^{1}H NMR (300 MHz, DMSO-d_6) δ: 7.06 (s, 1H), 7.24 (s, 1H), 7.43 (s, 1H), 7.70 (s, 1H), 11.95 (s, 1H), 13.10 (s, 1H).

*5.1.1.8. 5-Fluoro-1H-indole-2-carboxylic acid (**4b**).* According to the procedure used to prepare **4a**, starting from **3b**, **4b** was obtained as a light yellow solid. Yield: 85%. m.p. 265–266 °C. ESI-MS *m/z*: 177.9 $[M - H]^-$. ^{1}H NMR (300 MHz, DMSO-d_6) δ: 7.14–7.06 (m, 2H), 7.45–7.38 (m, 2H), 11.86 (s, 1H), 13.03 (s, 1H).

Table 2
Cytotoxicity of target compounds against A549, H460, HT-29 and SMMC-7721 cell lines *in vitro*.

Compd.	IC_{50}(μM)[a]			
	A549[b]	H460[b]	HT-29[b]	SMMC-7721[b]
10	0.21 ± 0.03	0.00073 ± 0.00005	0.00084 ± 0.00009	0.49 ± 0.01
11	0.12 ± 0.06	0.00070 ± 0.00004	0.00076 ± 0.00002	0.12 ± 0.05
12	1.58 ± 0.39	0.28 ± 0.01	0.19 ± 0.07	2.64 ± 0.21
13	12.20 ± 1.51	0.90 ± 0.03	0.069 ± 0.002	3.68 ± 0.28
14	3.91 ± 0.45	0.097 ± 0.001	0.00056 ± 0.00011	2.99 ± 0.31
15	3.82 ± 0.72	1.60 ± 0.24	1.86 ± 0.53	6.93 ± 0.43
16	1.84 ± 0.12	0.00093 ± 0.00021	0.69 ± 0.08	4.49 ± 0.53
17	0.048 ± 0.003	0.00088 ± 0.00002	0.019 ± 0.005	0.24 ± 0.07
18	1.05 ± 0.55	5.02 ± 0.37	0.45 ± 0.11	2.14 ± 0.48
19	0.96 ± 0.04	1.43 ± 0.28	0.30 ± 0.06	0.64 ± 0.15
20	1.31 ± 0.85	1.77 ± 0.29	0.76 ± 0.02	2.05 ± 0.31
21	26.50 ± 8.54	7.54 ± 0.32	4.89 ± 0.57	50.97 ± 4.55
22	27.5 ± 2.77	0.23 ± 0.01	0.69 ± 0.11	38.9 ± 5.36
23	>100	0.50 ± 0.04	1.83 ± 0.74	>100
24	>100	10.38 ± 0.69	99.4 ± 4.23	>100
25	3.60 ± 0.56	0.26 ± 0.09	0.19 ± 0.03	7.00 ± 0.48
26	0.28 ± 0.03	0.00050 ± 0.00021	0.00068 ± 0.00005	0.87 ± 0.04
27	0.022 ± 0.011	0.00023 ± 0.00021	0.00065 ± 0.00005	0.00077 ± 0.00006
28	5.79 ± 0.35	2.65 ± 0.51	2.66 ± 0. 26	1.5 ± 0.31
29	1.69 ± 0.45	0.012 ± 0.005	1.14 ± 0.12	4.32 ± 0.35
30	0.12 ± 0.02	0.0069 ± 0.0013	0.042 ± 0.003	3.47 ± 0.24
31	0.324 ± 0.011	0.023 ± 0.009	0.00046 ± 0.00012	0.30 ± 0.10
32	10.40 ± 2.34	10.41 ± 3.41	12.30 ± 4.35	32.40 ± 5.54
33	17.54 ± 2.86	5.77 ± 0.23	10.20 ± 3.11	6.40 ± 0.29
34	22.20 ± 5.63	10.21 ± 3.45	21.98 ± 1.11	9.90 ± 0.84
35	15.60 ± 4.82	9.42 ± 0.35	11.57 ± 1.69	>100
MX-58151[c]	0.058 ± 0.012	0.019 ± 0.005	0.70 ± 0.06	1.53 ± 0.49

[a] IC_{50} is 50% inhibitory concentration, and the results are presented as average values ± standard deviation.
[b] A549, non-small-cell lung cancer cell line; H460, human lung cancer cell line; HT-29, human colorectal cancer cell line; SMMC-7721, human liver cancer cell line.
[c] Used as a positive control.

*5.1.1.9. 6-Fluoro-1H-indole-2-carboxylic acid (**4c**).* According to the procedure used to prepare **4a**, starting from **3c**, **4c** was obtained as a white solid. Yield: 89%. m.p. 247 °C. ESI-MS *m/z*: 177.9 $[M-H]^-$. ^{1}H NMR (300 MHz, DMSO-d_6) δ: 6.97–6.90 (m, 1H), 7.15–7.10 (m, 2H), 7.66 (dd, $J_1 = 5.4$ Hz, $J_2 = 8.7$ Hz, 1H), 11.83 (s, 1H).

*5.1.1.10. 5,6,7-Trimethoxy-1H-indole-2-carboxylic acid (**4d**).* According to the procedure used to prepare **4a**, starting from **3d**, **4d** was obtained as a light yellow solid. Yield: 93%. m.p. 216–217 °C. ESI-MS *m/z*: 250.3 $[M-H]^-$. ^{1}H NMR (300 MHz, DMSO-d_6) δ: 3.80 (s, 3H), 3.91 (s, 3H), 3.99 (s, 3H), 6.99 (s, 1H), 7.06 (d, $J = 1.8$ Hz, 1H), 11.61 (s, 1H), 12.76 (s, 1H).

*5.1.1.11. 5-Chloro-1H-indole (**5a**).* A mixture of compound **4a** (23.5 g, 0.12 mol), copper powder (5.4 g, 0.084 mol) and quinoline (235 mL) was heated to reflux for 3 h. Upon cooling to room temperature, the filtrate was collected by filtration, diluted with ice-water and adjusted to pH 4 with concentrated hydrochloric acid, extracted with CH_2Cl_2 (3 × 100 mL), washed with 2 N hydrochloric acid (3 × 50 mL), saturated $NaHCO_3$ solution (3 × 50 mL) and brine (2 × 50 mL), and dried over Na_2SO_4. The solvent was removed under reduced pressure and the residue was purified by silica gel column chromatography. **5a** was obtained as a white solid (9.6 g, 53%). m.p. 71–72 °C. ESI-MS *m/z*: 151.6 (Cl = 35), 153.6 (Cl = 37) $[M+H]^+$. ^{1}H NMR (300 MHz, DMSO-d_6) δ: 6.42–6.40 (m, 1H), 7.06 (dd, $J_1 = 8.7$ Hz, $J_2 = 2.1$ Hz, 1H), 7.42–7.38 (m, 2H), 7.57 (d, $J = 2.1$ Hz, 1H), 11.27 (d, $J = 1.8$ Hz, 1H).

*5.1.1.12. 5-Fluoro-1H-indole (**5b**).* According to the procedure used to prepare **5a**, starting from **4b**, **5b** was obtained as a white solid. Yield: 46%. m.p. 47–48 °C. ESI-MS *m/z*: 136.2 $[M+H]^+$. ^{1}H NMR (300 MHz, DMSO-d_6) δ: 6.41 (t, $J = 2.4$ Hz, 1H), 6.94–6.87 (m, 1H), 7.30–7.26 (m, 1H), 7.41–7.35 (m, 2H), 11.16 (s, 1H).

*5.1.1.13. 6-Fluoro-1H-indole (**5c**).* According to the procedure used to prepare **5a**, starting from **4c**, **5c** was obtained as a white solid. Yield: 50%. m.p. 75–76 °C. ESI-MS *m/z*: 136.2 $[M+H]^+$. ^{1}H NMR (300 MHz, DMSO-d_6) δ: 6.42 (s, 1H), 6.87–6.81 (m, 1H), 7.17–7.10 (m, 1H), 7.32 (s, 1H), 7.52 (s, 1H), 11.12 (s, 1H).

*5.1.1.14. 5,6,7-Trimethoxy-1H-indole (**5d**).* According to the procedure used to prepare **5a**, starting from **4d**, **5d** was obtained as a white solid. Yield: 49%. m.p. 92–94 °C. ESI-MS *m/z*: 208.4 $[M+H]^+$. ^{1}H NMR (300 MHz, DMSO-d_6) δ: 3.78 (s, 3H), 3.86 (s, 3H), 3.95 (s, 3H), 6.39–6.38 (m, 1H), 7.07 (s, 1H), 7.12–7.11 (m, 1H), 10.87 (s, 1H).

*5.1.1.15. 1-(5-Chloro-1H-indol-3-yl)ethanone (**6a**).* A mixture of compound **5a** (9.1 g, 0.06 mol), glacial acetic acid (9.1 mL) and acetic anhydride (91 mL) was heated to reflux for 20 h. Upon cooling to room temperature, the solid was collected by filtration and purified by silica gel column chromatography. **6a** was obtained as a white solid (5.2 g, 45%). m.p. 246–247 °C. ESI-MS *m/z*: 194.1 (Cl = 35), 196.0 (Cl = 37) $[M+H]^+$. ^{1}H NMR (300 MHz, DMSO-d_6) δ: 2.44 (s, 3H), 7.23–7.19 (m, 1H), 7.49 (d, $J = 9.0$ Hz, 1H), 8.15 (d, $J = 1.8$ Hz, 1H), 8.37 (s, 1H).

*5.1.1.16. 1-(5-Fluoro-1H-indol-3-yl)ethanone (**6b**).* According to the procedure used to prepare **6a**, starting from **5b**, **6b** was obtained as a white solid. Yield: 32%. m.p. 157–159 °C. ESI-MS *m/z*: 178.2 $[M+H]^+$. ^{1}H NMR (300 MHz, DMSO-d_6) δ: 2.57 (s, 3H), 7.30–7.23 (m, 1H), 7.90 (dd, $J_1 = 9.6$ Hz, $J_2 = 2.7$ Hz, 1H), 8.38–8.33 (m, 1H), 8.85 (s, 1H).

*5.1.1.17. 1-(6-Fluoro-1H-indol-3-yl)ethanone (**6c**).* According to the procedure used to prepare **6a**, starting from **5c**, **6c** was obtained as a light yellow solid. Yield: 41%. m.p. 236 °C. ESI-MS *m/z*: 178.1 $[M+H]^+$. ^{1}H NMR (300 MHz, DMSO-d_6) δ: 2.44 (s, 3H), 7.06–6.99 (m, 1H), 7.27–7.23 (m, 1H), 8.14 (dd, $J_1 = 8.7$ Hz, $J_2 = 5.4$ Hz, 1H), 8.31 (s, 1H), 11.96 (s, 1H).

*5.1.1.18. 1-(5,6,7-Trimethoxy-1H-indol-3-yl)ethanone (**6d**).* According to the procedure used to prepare **6a**, starting from **5d**, **6d** was obtained as a white solid. Yield: 48%. m.p. 240–241 °C. ESI-MS *m/z*: 250.3 $[M+H]^+$. ^{1}H NMR (300 MHz, DMSO-d_6) δ: 2.44 (s, 3H), 3.80 (s, 3H), 3.89 (s, 3H), 3.94 (s, 3H), 7.86 (s, 1H), 8.16 (s, 1H), 11.69 (s, 1H).

*5.1.2. Ethyl 2-azidoacetate (**7**)*

To a solution of ethyl bromoacetate (80 g, 0.48 mol) in acetone (500 mL) at 0–5 °C, was added a solution of sodium azid (77.8 g, 1.2 mol) in water (400 mL) dropwise. After addition, the mixture was heated to 63 °C for 4 h. Then, the reaction mixture was cooled to room temperature and extracted with CH_2Cl_2 (7 × 140 mL). The combined extracts were washed with saturated $NaHCO_3$ (3 × 80 mL) and water (3 × 80 mL) and dried over Na_2SO_4. The solvent was removed under reduced pressure and **7** was obtained as a colorless oil (61.9 g, 100%) without purification. ESI-MS *m/z*: 130.1 $[M+H]^+$. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 1.29 (t, $J = 7.2$ Hz, 3H), 3.84 (s, 2H), 4.27–4.20 (q, $J_1 = 7.2$ Hz, $J_2 = 14.1$ Hz, 2H).

*5.1.2.1. (Z)-Ethyl 2-azido-3-(4-fluorophenyl)acrylate (**8c**).* Na (11.0 g, 0.48 mol) was dissolved in ethanol (150 mL), followed by addition of a solution of 4-fluorobenzaldehyde (31 g, 0.25 mol) and compound **7** (61.9 g, 0.48 mol) in ethanol at −5 ∼ −10 °C. The reaction was stirred at 0 °C for 4 h and then poured into ice-water. The precipitated was collected by filtration. **8c** was obtained as a yellow solid (37.0 g, 63%). m.p. 37–39 °C. ^{1}H NMR (300 MHz, DMSO-d_6) δ: 1.32 (t, $J = 6.9$ Hz, 3H), 4.35–4.28 (q, $J_1 = 7.2$ Hz, $J_2 = 14.1$ Hz, 2H), 6.94 (s, 1H), 7.29–7.23 (m, 2H), 7.97–7.92 (m, 2H).

*5.1.2.2. (Z)-Ethyl 2-azido-3-(3,4,5-trimethoxyphenyl)acrylate (**8d**).* According to the procedure used to prepare **8c**, starting from 3,4,5-trimethoxybenzaldehyde, **8d** was obtained as a yellow solid. Yield: 68%. m.p. 60–61 °C. ^{1}H NMR (300 MHz, DMSO-d_6) δ: 1.31 (t, $J = 6.9$ Hz, 3H), 3.75 (s, 3H), 3.87 (s, 6H), 4.35–4.28 (q, $J_1 = 6.9$ Hz, $J_2 = 14.4$ Hz, 2H), 6.90 (s, 2H), 7.12 (s, 1H)).

*5.1.2.3. Ethyl 6-fluoro-1H-indole-2-carboxylate (**3c**).* A solution of compound **8c** (35.3 g, 0.15 mol) in xylene (350 mL) was heated to reflux for 3–5 h and then the solvent was removed under reduced pressure to form a light yellow solid. The crude product was purified by recrystallization from toluene and **3c** was obtained as a yellow solid (16.1 g, 52%). m.p. 147–148 °C. ESI-MS *m/z*: 206.1 $[M-H]^-$. ^{1}H NMR (300 MHz, DMSO-d_6) δ: 1.33 (t, $J = 6.9$ Hz, 3H), 4.37–4.30 (q, $J_1 = 6.9$ Hz, $J_2 = 14.1$ Hz, 2H), 6.99–6.92 (m, 1H), 7.17–7.14 (m, 2H), 7.71–7.66 (m, 1H), 11.95 (s, 1H).

*5.1.2.4. Ethyl 5,6,7-trimethoxy-1H-indole-2-carboxylate (**3d**).* According to the procedure used to prepare **3c**, starting from **8d**, **3d** was obtained as a light yellow solid. Yield: 57%. m.p. 158–159 °C. ESI-MS *m/z*: 278.0 $[M-H]^-$. ^{1}H NMR (300 MHz, DMSO-d_6) δ: 1.32 (t, $J = 7.2$ Hz, 3H), 3.81 (s, 3H), 3.91 (s, 3H), 3.99 (s, 3H), 4.34–4.27 (q, $J_1 = 7.2$ Hz, $J_2 = 14.4$ Hz, 2H), 7.01 (s, 1H), 7.10 (d, $J = 1.5$ Hz, 1H), 11.70 (s, 1H).

*5.1.2.5. 1-(5-Fluoro-1-methyl-1H-indol-3-yl)ethanone (**9b**).* A mixture of compound **6b** (2 g, 11.3 mmol) and KOH (2.5 g, 45.2 mmol) in DMSO (20 mL) was stirring at room temperature for 1 h, followed by addition of iodomethane (1.1 mL, 22.6 mmol). The mixture was allowed to stirring at this temperature for another 4 h and then poured into water, the precipitate was collected by

filtration. **9b** was obtained as a light yellow solid (1.6 g, 76%). m.p. 155–158 °C. ESI-MS *m/z*: 192.3 $[M+H]^+$, 214.3 $[M+Na]^+$. ^{1}H NMR (300 MHz, DMSO-d_6) δ: 2.42 (s, 3H), 3.86 (s, 3H), 7.17–7.10 (m, 1H), 7.59–7.54 (m, 1H), 7.86–7.82 (m, 1H), 8.38 (s, 1H).

*5.1.2.6. 1-(6-Fluoro-1-methyl-1H-indol-3-yl)ethanone (**9c**).* According to the procedure used to prepare **9b**, starting from **6c**, **9c** was obtained as a white solid. Yield: 69%. m.p. 95–96 °C. ESI-MS *m/z*: 192.1 $[M+H]^+$, 214.1 $[M+Na]^+$. ^{1}H NMR (300 MHz, DMSO-d_6) δ: 2.42 (s, 3H), 3.83 (s, 3H), 7.11–7.04 (m, 1H), 7.46–7.42 (m, 1H), 8.17–8.12 (m, 1H), 8.34 (s, 1H).

*5.1.2.7. 1-(5,6,7-Trimethoxy-1-methyl-1H-indol-3-yl)ethanone (**9d**).* According to the procedure used to prepare **9b**, starting from **6d**, **9d** was obtained as a yellow solid. Yield: 69%. m.p. 135–137 °C. ESI-MS *m/z*: 264.3 $[M+H]^+$, 286.4 $[M+Na]^+$. ^{1}H NMR (300 MHz, DMSO-d_6) δ: 2.44 (s, 3H), 3.79 (s, 3H), 3.90 (s, 3H), 3.93 (s, 6H), 7.86 (s, 1H), 8.09 (s, 1H).

*5.1.2.8. 1-(1-Methyl-1H-indol-3-yl)ethanone (**9e**).* According to the procedure used to prepare **9b**, starting from 1-(1*H*-indol-3-yl) ethanone, **9e** was obtained as a white solid. Yield: 80%. m.p. 104–105 °C. ESI-MS *m/z*: 195.9 $[M+Na]^+$. ^{1}H NMR (300 MHz, DMSO-d_6) δ: 2.43 (s, 3H), 3,86 (s, 3H), 7.30–7.19 (m, 2H), 7.52 (d, $J=8.1$ Hz, 1H), 8.18 (d, $J=7.5$ Hz, 1H), 8.31 (s, 1H).

*5.1.3. General procedure for the preparation of compounds **10**–**35***

The substituted 1-(1*H*-indol-3-yl)ethanone (1 mmol), together with the respective benzaldehyde (1 mmol), malononitrile (1 mmol) and ammonium acetate (8 mmol) were dissolved in toluene (30 mL) and heated to reflux for 8 h. The solvent was removed under reduced pressure and absolute ethanol was added to the residue. The precipitate was collected by filtration and purified by silica gel column chromatography to give the desired product.

*5.1.3.1. 2-Amino-6-(1H-indol-3-yl)-4-(3,4,5-trimethoxyphenyl)nicotinonitrile (**10**).* Yield: 36%. m.p. 278–279 °C. ESI-MS *m/z*: 401.2 $[M+H]^+$. IR (KBr) cm^{-1}: 3458.6, 3334.5, 3001.3, 2935.2, 2838.9, 2196.5, 1630.5, 1575.4, 1536.4, 1508.1, 1439.9, 1413.4, 1396.1, 1336.9, 1264.0, 1243.3, 1126.7, 991.8, 811.4, 741.9. ^{1}H NMR (300 MHz, DMSO-d_6) δ: 3.74 (s, 3H), 3.88 (s, 6H), 6.81 (s, 2H), 6.96 (s, 2H), 7.21–7.11 (m, 2H), 7.26 (s, 1H), 7.44 (d, $J=7.2$ Hz, 1H), 8.32 (d, $J=2.7$ Hz, 1H), 8.71 (d, $J=7.2$ Hz, 1H), 11.73 (d, $J=2.1$ Hz, 1H). ^{13}C NMR (DMSO-d_6) δ: 161.90, 158.92, 153.71, 153.59 (2C), 139.02, 137.81, 133.56, 129.53, 126.28, 123.67, 122.82, 121.15, 118.86, 115.35, 112.50, 109.05, 106.66 (2C), 83.20, 60.86, 56.82 (2C). Anal. Calcd. For $C_{23}H_{20}N_4O_3$: C, 68.99; H, 5.03; N, 13.99; O, 11.99. Found: C, 68.88; H, 5.06; N, 13.98.

*5.1.3.2. 2-Amino-4-(3-bromo-4,5-dimethoxyphenyl)-6-(1H-indol-3-yl)nicotinonitrile (**11**).* Yield: 33%. m.p. 251–253 °C. ESI-MS *m/z*: 449.1 (Br = 79), 451.1 (Br = 81) $[M+H]^+$. IR (KBr) cm^{-1}: 3375.2, 3209.9, 2951.5, 2202.4, 1635.4, 1580.8, 1537.1, 1491.2, 1433.9, 1395.4, 1263.4, 1243.8, 1131.1, 1046.0, 988.4, 819.4, 742.7. ^{1}H NMR (300 MHz, DMSO-d_6) δ: 3.82 (s, 3H), 3.93 (s, 3H), 6.89 (s, 2H), 7.20–7.12 (m, 2H), 7.26 (s, 1H), 7.35 (d, $J=2.1$ Hz, 1H), 7.45–7.42 (m, 2H), 8.36 (s, 1H), 8.72 (d, $J=7.8$ Hz, 1H), 11.76 (s, 1H). ^{13}C NMR (DMSO-d_6) δ: 161.19, 158.46, 153.29, 151.30, 146.45, 137.15, 134.76, 129.07, 125.60, 123.91, 123.03, 122.19, 120.53, 117.92, 116.83, 114.63, 113.03, 111.83, 108.40, 82.27, 60.29, 56.42. Anal. Calcd. For $C_{22}H_{17}BrN_4O_2$: C, 58.81; H, 3.81; Br, 17.78; N, 12.47; O, 7.12. Found: C, 58.77; H, 3.84; N, 12.43.

*5.1.3.3. 2-Amino-6-(1H-indol-3-yl)-4-(2,3,4-trimethoxyphenyl)nicotinonitrile (**12**).* Yield: 25%. m.p. 249–250 °C. ESI-MS *m/z*: 401.3 $[M+H]^+$. IR (KBr) cm^{-1}: 3382.2, 2925.9, 2852.7, 2205.3, 1601.3, 1579.5, 1533.0, 1496.0, 1456.1, 1409.6, 1385.1, 1296.5, 1264.7, 1098.2, 1009.5, 747.0. ^{1}H NMR (300 MHz, DMSO-d_6) δ: 3.73 (s, 3H), 3.81 (s, 3H), 3.87 (s, 3H), 6.73 (s, 2H), 6.93 (d, $J=8.7$ Hz, 1H), 7.05 (d, $J=9.0$ Hz, 1H), 7.18–7.09 (m, 3H), 7.42 (d, $J=8.4$ Hz, 1H), 8.22 (d, $J=2.7$ Hz, 1H), 8.67 (d, $J=7.8$ Hz, 1H), 11.68 (s, 1H). ^{13}C NMR (DMSO-d_6) δ: 161.13, 158.44, 154.92, 151.56, 151.40, 142.40, 137.80, 129.14, 126.22, 125.26, 125.13, 123.48, 122.77, 121.12, 118.39, 115.35, 112.50, 110.23, 108.51, 85.74, 61.85, 61.27, 56.72. Anal. Calcd. For $C_{23}H_{20}N_4O_3$: C, 68.99; H, 5.03; N, 13.99; O, 11.99. Found: C, 68.96; H, 5.01; N, 14.00.

*5.1.3.4. 2-Amino-6-(5-chloro-1H-indol-3-yl)-4-(3,4,5-trimethoxyphenyl) nicotinonitrile (**13**).* Yield: 33%. m.p. 244–245 °C. ESI-MS *m/z*: 435.3 (Cl = 35), 437.3 (Cl = 37) $[M+H]^+$. IR (KBr) cm^{-1}: 3327.8, 2941.4, 2203.1, 1635.9, 1603.1, 1578.3, 1506.9, 1452.0, 1406.9, 1383.3, 1319.2, 1250.5, 1125.7, 992.4, 800.6. ^{1}H NMR (300 MHz, DMSO-d_6) δ: 3.74 (s, 3H), 3.87 (s, 6H), 6.96 (s, 2H), 7.26–7.17 (m, 4H), 7.45 (d, $J=9.0$ Hz, 1H), 8.42 (s, 1H), 8.74 (s, 1H), 11.92 (s, 1H). ^{13}C NMR (DMSO-d_6) δ: 161.96, 158.37, 153.83, 153.57 (2C), 139.03, 136.25, 133.44, 130.93, 127.29, 126.06, 122.90, 122.77, 118.77, 115.06, 113.99, 108.91, 106.66 (2C), 83.44, 60.86, 56.81 (2C). Anal. Calcd. For $C_{23}H_{19}ClN_4O_3$: C, 63.52; H, 4.40; Cl, 8.15; N, 12.88; O, 11.04. Found: C, 63.55; H, 4.35; N, 12.94.

*5.1.3.5. 2-Amino-6-(5-chloro-1H-indol-3-yl)-4-(2,3,4-trimethoxyphenyl) nicotinonitrile (**14**).* Yield: 21%. m.p. 247–249 °C. ESI-MS *m/z*: 434.9 (Cl = 35), 437.0 (Cl = 37) $[M+H]^+$. IR (KBr) cm^{-1}: 3348.8, 3177.1, 2211.0, 1662.7, 1597.8, 1500.6, 1448.3, 1405.3, 1290.5, 1242.3, 1106.9, 796.1. ^{1}H NMR (300 MHz, DMSO-d_6) δ: 3.73 (s, 3H), 3.80 (s, 3H), 3.87 (s, 3H), 6.72 (s, 2H), 6.94 (d, $J=8.4$ Hz, 1H), 7.07 (d, $J=8.7$ Hz, 1H), 7.20–7.17 (m, 1H), 7.27 (s, 1H), 7.47 (s, 1H), 8.37 (d, $J=3.3$ Hz, 1H), 8.66 (d, $J=2.1$ Hz, 1H), 12.02 (d, $J=1.8$ Hz, 1H). ^{13}C NMR (DMSO-d_6) δ: 161.86, 159.45, 155.51, 154.25, 151.41, 142.34, 136.36, 131.64, 126.62, 126.57, 125.31, 124.22, 123.34, 121.58, 117.09, 114.54, 112.32, 110.50, 108.58, 85.46, 62.02, 61.32, 56.78. Anal. Calcd. For $C_{23}H_{19}ClN_4O_3$: C, 63.52; H, 4.40; Cl, 8.15; N, 12.88; O, 11.04. Found: C, 63.48; H, 4.41; N, 12.86.

*5.1.3.6. 2-Amino-6-(5-fluoro-1H-indol-3-yl)-4-(3,4,5-trimethoxyphenyl)nicotinonitrile (**15**).* Yield: 37%. m.p. 272–275 °C. ESI-MS *m/z*: 416.6 $[M-H]^-$. IR (KBr) cm^{-1}: 3333.9, 2997.7, 2205.5, 1707.5, 1634.8, 1577.1, 1414.1, 1384.9, 1320.7, 1261.8, 1171.1, 1117.4, 1067.5, 1015.2, 828.8. ^{1}H NMR (300 MHz, DMSO-d_6) δ: 3.73 (s, 3H), 3.87 (s, 6H), 6.89 (s, 2H), 6.95 (s, 2H), 7.05–6.98 (m, 1H), 7.26 (s, 1H), 7.46–7.42 (m, 1H), 8.43 (s, 1H), 8.51–8.47 (m, 1H), 12.08 (s, 1H). ^{13}C NMR (DMSO-d_6) δ: 161.96, 158.67, 158.55, 153.77, 153.58 (2C), 139.05, 134.43, 133.48, 131.17, 126.71, 118.82, 115.39, 113.42, 111.00, 108.77, 108.45, 106.69 (2C), 83.22, 60.85, 56.81 (2C). Anal. Calcd. For $C_{23}H_{19}FN_4O_3$: C, 66.02; H, 4.58; F, 4.54; N, 13.39; O, 11.47. Found: C, 66.00; H, 4.55; N, 13.41.

*5.1.3.7. 2-Amino-4-(3-bromo-4,5-dimethoxyphenyl)-6-(5-fluoro-1H-indol-3-yl)nicotinonitrile (**16**).* Yield: 31%. m.p. 236–238 °C. ESI-MS *m/z*: 467.1 (Br = 79), 469.2 (Br = 81) $[M+H]^+$. IR (KBr) cm^{-1}: 3335.4, 2940.2, 2206.4, 1606.1, 1580.8, 1527.2, 1485.4, 1465.1, 1404.5, 1263.3, 1233.0, 1134.8, 1046.6, 994.1, 828.3, 799.1. ^{1}H NMR (300 MHz, DMSO-d_6) δ: 3.82 (s, 3H), 3.93 (s, 3H), 6.96 (s, 2H), 7.02–6.98 (m, 1H), 7.26 (s, 1H), 7.35 (d, $J=1.8$ Hz, 1H), 7.46–7.42 (m, 2H), 8.52–8.44 (m, 2H), 12.02 (s, 1H). ^{13}C NMR (DMSO-d_6) δ: 161.91, 158.81, 158.67, 153.93, 151.98, 147.11, 135.34, 134.47, 131.45, 126.67, 124.58, 118.57, 117.46, 115.25, 113.69, 113.50, 110.94, 108.77, 108.39, 82.88, 60.94, 57.09. Anal. Calcd. For $C_{22}H_{16}BrFN_4O_2$: C, 56.55; H, 3.45; Br, 17.10; F, 4.07; N, 11.99; O, 6.85. Found: C, 56.47; H, 3.46; N, 12.01.

*5.1.3.8. 2-Amino-6-(5-fluoro-1H-indol-3-yl)-4-(2,3,4-trimethoxyphenyl) nicotinonitrile (**17**).* Yield: 23%. m.p. 245–247 °C. ESI-MS *m/z*: 419.0 $[M+H]^+$. IR (KBr) cm^{-1}: 3418.7, 3158.6, 2939.8, 2220.1, 1645.1, 1594.8, 1506.7, 1485.3, 1463.6, 1410.0, 1296.4, 1236.8, 1178.8, 1100.0, 806.4. ^1H NMR (300 MHz, DMSO-d_6) δ: 3.75 (s, 3H), 3.81 (s, 3H), 3.88 (s, 3H), 6.46 (s, 2H), 6.96 (d, $J = 8.7$ Hz, 1H), 7.10–7.03 (m, 1H), 7.14–7.10 (m, 2H), 7.50–7.45 (m, 1H), 8.28 (d, $J = 10.5$ Hz, 1H), 8.49 (d, $J = 3.0$ Hz, 1H), 12.10 (s, 1H). ^{13}C NMR (DMSO-d_6) δ: 161.36, 159.02, 158.63, 155.74, 152.18, 151.45, 142.34, 134.54, 132.45, 125.75, 125.38, 123.90, 116.56, 114.34, 111.73, 111.40, 110.44, 108.60, 107.18, 88.42, 62.08, 61.35, 56.78. Anal. Calcd. For $C_{23}H_{19}FN_4O_3$: C, 66.02; H, 4.58; F, 4.54; N, 13.39; O, 11.47. Found: C, 66.07; H, 4.55; N, 13.31.

*5.1.3.9. 2-Amino-6-(6-fluoro-1H-indol-3-yl)-4-(3,4,5-trimethoxyphenyl) nicotinonitrile (**18**).* Yield: 32%. m.p. 237–238 °C. ESI-MS *m/z*: 419.1 $[M+H]^+$. IR (KBr) cm^{-1}: 3445.0, 3287.7, 3167.6, 2933.7, 2835.4, 2215.1, 1625.6, 1578.5, 1529.0, 1497.0, 1451.4, 1407.2, 1382.0, 1296.9, 1267.2, 1236.4, 1220.4, 1141.2, 1097.7, 1011.9, 832.8, 812.1. ^1H NMR (300 MHz, DMSO-d_6) δ: 3.74 (s, 3H), 3.88 (s, 6H), 6.83 (s, 2H), 7.01–6.87 (m, 3H), 7.24–7.20 (m, 1H), 7.27 (s, 1H), 8.34 (d, $J = 3.0$ Hz, 1H), 8.77–8.72 (m, 1H), 11.80 (s, 1H). ^{13}C NMR (DMSO-d_6) δ: 161.91, 159.87, 158.47, 153.82, 153.58 (2C), 139.02, 137.74, 133.47, 130.01, 124.96, 123.08, 118.77, 115.43, 109.38, 108.96, 106.66 (2C), 98.50, 83.43, 60.85, 56.80 (2C). Anal. Calcd. For $C_{23}H_{19}FN_4O_3$: C, 66.02; H, 4.58; F, 4.54; N, 13.39; O, 11.47. Found: C, 66.01; H, 4.63; N, 13.35.

*5.1.3.10. 2-Amino-4-(3-bromo-4,5-dimethoxyphenyl)-6-(6-fluoro-1H-indol-3-yl)nicotinonitrile (**19**).* Yield: 36%. m.p. 263–265 °C. ESI-MS *m/z*: 467.3 (Br = 79), 469.2 (Br = 81) $[M+H]^+$. IR (KBr) cm^{-1}: 3339.0, 2201.6, 1633.7, 1580.0, 1537.7, 1491.8, 1425.5, 1262.2, 1237.9, 1141.1, 1109.5, 1046.7, 980.3, 822.7. ^1H NMR (300 MHz, DMSO-d_6) δ: 3.81 (s, 3H), 3.93 (s, 3H), 6.90 (s, 2H), 7.00–6.92 (m, 1H), 7.25-7.22 (m, 2H), 7.34 (d, $J = 1.8$ Hz, 1H), 7.44 (d, $J = 1.8$ Hz, 1H), 8.36 (s, 1H), 8.74–8.69 (m, 1H). ^{13}C NMR (DMSO-d_6) δ: 161.84, 159.80, 158.76, 153.94, 152.00, 147.10, 137.87, 135.32, 130.38, 124.85, 124.55, 123.06, 118.53, 117.47, 115.20, 113.67, 109.32, 108.93, 98.63, 83.04, 60.95, 57.08. Anal. Calcd. For $C_{22}H_{16}BrFN_4O_2$: C, 56.55; H, 3.45; Br, 17.10; F, 4.07; N, 11.99; O, 6.85. Found: C, 56.43; H, 3.46; N, 11.95.

*5.1.3.11. 2-Amino-6-(6-fluoro-1H-indol-3-yl)-4-(2,3,4-trimethoxyphenyl)nicotinonitrile (**20**).* Yield: 28%. m.p. 238–239 °C. ESI-MS *m/z*: 419.3 $[M+H]^+$. IR (KBr) cm^{-1}: 3472.6, 3378.6, 3297.3, 2930.1, 2836.3, 2211.7, 1608.0, 1581.8, 1533.7, 1498.4, 1445.9, 1410.3, 1384.9, 1294.8, 1258.1, 1216.2, 1144.9, 1098.0, 1011.0, 949.7, 837.2, 815.1. ^1H NMR (300 MHz, DMSO-d_6) δ: 3.72 (s, 3H), 3.80 (s, 3H), 3.86 (s, 3H), 6.77 (s, 2H), 7.08–6.93 (m, 4H), 7.20-7.17 (m, 1H), 8.24 (s, 1H), 8.71 (s, 1H), 11.72 (s, 1H). Anal. Calcd. For $C_{23}H_{19}FN_4O_3$: C, 66.02; H, 4.58; F, 4.54; N, 13.39; O, 11.47. Found: C, 66.04; H, 4.53; N, 13.35.

*5.1.3.12. 2-Amino-6-(5,6,7-trimethoxy-1H-indol-3-yl)-4-(2,3,4-trimethoxyphenyl)nicotinonitrile (**21**).* Yield: 41%. m.p. 197–200 °C. ESI-MS *m/z*: 491.7 $[M+H]^+$. IR (KBr) cm^{-1}: 3315.7, 3171.4, 2939.4, 2219.8, 1648.6, 1599.5, 1507.3, 1464.1, 1419.5, 1278.9, 1235.4, 1204.2, 1160.9, 1122.1, 1097.2, 817.9. ^1H NMR (300 MHz, DMSO-d_6) δ: 3.75 (s, 3H), 3.79 (s, 3H), 3.81 (s, 3H), 3.87 (s, 3H), 3.89 (s, 3H), 3.95 (s, 3H), 6.89 (s, 2H), 6.96 (d, $J = 8.7$ Hz, 1H), 7.12 (d, $J = 7.5$ Hz, 2H), 7.73 (s, 1H), 8.24 (d, $J = 2.7$ Hz, 1H), 11.90 (s, 1H). Anal. Calcd. For $C_{26}H_{26}N_4O_6$: C, 63.66; H, 5.34; N, 11.42; O, 19.57. Found: C, 63.69; H, 5.33; N, 11.47.

*5.1.3.13. 2-Amino-4-(2,4-difluorophenyl)-6-(5,6,7-trimethoxy-1H-indol-3-yl)nicotinonitrile (**22**).* Yield: 47%. m.p. 230–231 °C. ESI-MS *m/z*: 437.7 $[M+H]^+$. IR (KBr) cm^{-1}: 3454.8, 3371.8, 3251.3, 2953.9, 2835.1, 2205.6, 1653.3, 1584.8, 1535.6, 1508.1, 1467.1, 1290.8, 1127.1, 1055.5, 972.2. ^1H NMR (300 MHz, DMSO-d_6) δ: 3.78 (s, 3H), 3.91 (s, 3H), 3.94 (s, 3H), 6.97 (s, 2H), 7.19 (s, 1H), 7.31–7.26 (m, 1H), 7.52–7.45 (m, 1H), 7.66–7.58 (m, 1H), 7.97 (s, 1H), 8.16 (d, $J = 3.0$ Hz, 1H), 11.70 (s, 1H). ^{13}C NMR (DMSO-d_6) δ: 161.43, 161.26, 159.34, 158.13, 150.21, 147.42, 139.39, 138.70, 133.10, 129.25, 125.91, 122.73, 122.34, 118.05, 115.51, 112.76, 109.90, 105.26, 101.04, 84.24, 61.78, 61.65, 57.16. Anal. Calcd. For $C_{23}H_{18}F_2N_4O_3$: C, 63.30; H, 4.16; F, 8.71; N, 12.84; O, 11.00. Found: C, 63.28; H, 4.19; N, 12.79.

*5.1.3.14. 2-Amino-4-(3,4-difluorophenyl)-6-(5,6,7-trimethoxy-1H-indol-3-yl)nicotinonitrile (**23**).* Yield: 39%. m.p. 230–231 °C. ESI-MS *m/z*: 436.7 $[M+H]^+$. IR (KBr) cm^{-1}: 3448.6, 3368.0, 3253.5, 2999.1, 2953.4, 2836.5, 2208.1, 1656.0, 1580.7, 1535.3, 1518.3, 1466.6, 1431.7, 1409.9, 1289.7, 1266.9, 1200.5, 1128.5, 1054.9, 959.0, 873.8, 767.8. ^1H NMR (300 MHz, DMSO-d_6) δ: 3.79 (s, 3H), 3.91 (s, 3H), 3.95 (s, 3H), 6.96 (s, 2H), 7.24 (s, 1H), 7.65–7.52 (m, 2H), 7.83–7.75 (m, 1H), 7.98 (s, 1H), 8.22 (d, $J = 3.0$ Hz, 1H), 11.70 (s, 1H). Anal. Calcd. For $C_{23}H_{18}F_2N_4O_3$: C, 63.30; H, 4.16; F, 8.71; N, 12.84; O, 11.00. Found: C, 63.33; H, 4.12; N, 12.75.

*5.1.3.15. 2-Amino-4-(2-chloro-4-fluorophenyl)-6-(5,6,7-trimethoxy-1H-indol-3-yl)nicotinonitrile (**24**).* Yield: 45%. m.p. 230–233 °C. ESI-MS *m/z*: 452.1 (Cl = 35), 454.2 (Cl = 37) $[M+H]^+$. IR (KBr) cm^{-1}: 3449.1, 3363.0, 3247.8, 2942.8, 2204.6, 1649.5, 1605.8, 1580.5, 1531.3, 1497.4, 1466.2, 1433.9, 1289.5, 1206.1, 1119.0, 1046.6, 877.0. ^1H NMR (300 MHz, DMSO-d_6) δ: 3.78 (s, 3H), 3.90 (s, 3H), 3.94 (s, 3H), 6.98 (s, 2H), 7.13 (s, 1H), 7.43–7.36 (m, 1H), 7.59–7.54 (m, 1H), 7.69–7.65 (m, 1H), 7.98 (s, 1H), 8.14 (s, 1H). ^{13}C NMR (DMSO-d_6) δ: 164.45, 161.07, 159.25, 150.85, 150.20, 139.37, 138.70, 134.10, 133.38, 133.02, 129.23, 125.92, 122.33, 117.84, 117.54, 115.53, 115.47, 109.69, 101.04, 84.56, 61.78, 61.65, 57.18. Anal. Calcd. For $C_{23}H_{18}ClFN_4O_3$: C, 61.00; H, 4.01; Cl, 7.83; F, 4.20; N, 12.37; O, 10.60. Found: C, 61.08; H, 4.00; N, 12.34.

*5.1.3.16. 2-Amino-4-(2,3-dichlorophenyl)-6-(5,6,7-trimethoxy-1H-indol-3-yl)nicotinonitrile (**25**).* Yield: 44%. m.p. 216–218 °C. ESI-MS *m/z*: 470.7 $[M+H]^+$. IR (KBr) cm^{-1}: 3473.6, 3451.1, 3340.9, 2969.6, 2939.2, 2833.0, 2200.8, 1623.1, 1571.9, 1535.7, 1464.1, 1425.6, 1388.1, 1319.1, 1291.1, 1264.8, 1206.2, 1145.7, 1109.1, 1041.6, 889.5, 794.2, 727.7. ^1H NMR (300 MHz, DMSO-d_6) δ: 3.79 (s, 3H), 3.92 (s, 3H), 3.94 (s, 3H), 7.01 (s, 2H), 7.16 (s, 1H), 7.52–7.48 (m, 2H), 7.80–7.77 (m, 1H), 7.99 (s, 1H), 8.15 (d, $J = 2.7$ Hz, 1H), 11.70 (d, $J = 2.4$ Hz, 1H). ^{13}C NMR (DMSO-d_6) δ: 161.07, 159.41, 151.30, 150.22, 139.87, 139.38, 138.71, 132.92, 131.68, 130.53, 130.09, 129.31 (2C), 125.93, 122.34, 117.74, 115.53, 109.24, 101.07, 83.98, 61.78, 61.66, 57.18. Anal. Calcd. For $C_{23}H_{18}Cl_2N_4O_3$: C, 58.86; H, 3.87; Cl, 15.11; N, 11.94; O, 10.23. Found: C, 58.85; H, 3.86; N, 12.01.

*5.1.3.17. 2-Amino-6-(1-methyl-1H-indol-3-yl)-4-(3,4,5-trimethoxyphenyl)nicotinonitrile (**26**).* Yield: 22%. m.p. 197–200 °C. ESI-MS *m/z*: 415.1 $[M+H]^+$. IR (KBr) cm^{-1}: 3476.5, 3361.3, 3148.6, 2935.6, 2835.0, 2200.9, 1641.1, 1580.1, 1530.3, 1506.6, 1466.4, 1399.4, 1253.4, 1128.4, 1000.7, 812.7, 729.3. ^1H NMR (300 MHz, DMSO-d_6) δ: 3.74 (s, 3H), 3.86 (s, 3H), 3.87 (s, 6H), 6.85 (s, 2H), 6.95 (s, 2H), 7.28–7.16 (m, 3H), 7.50 (d, $J = 8.4$ Hz, 1H), 8.32 (s, 1H), 8.72 (d, $J = 7.5$ Hz, 1H). ^{13}C NMR (DMSO-d_6) δ: 161.92, 158.46, 153.77, 153.60 (2C), 139.06, 138.29, 133.53, 133.37, 126.65, 123.79, 122.92, 121.44, 118.79, 114.33, 110.88, 108.92, 106.64 (2C), 83.27, 60.87, 56.84 (2C), 33.73. Anal. Calcd. For $C_{24}H_{22}N_4O_3$: C, 69.55; H, 5.35; N, 13.52; O, 11.58. Found: C, 69.49; H, 5.37; N, 13.56.

*5.1.3.18. 2-Amino-4-(3-bromo-4,5-dimethoxyphenyl)-6-(1-methyl-1H-indol-3-yl)nicotinonitrile (**27**).* Yield: 29%. m.p. 235 °C. ESI-MS *m/z*: 462.5 (Br = 79), 464.6 (Br = 81) $[M+H]^+$. IR (KBr) cm^{-1}: 3451.2, 3345.2, 2943.3, 2198.3, 1628.6, 1579.3, 1533.7, 1493.8, 1470.5, 1265.1, 1222.4, 1132.4, 1113.4, 1045.2, 999.2, 823.2, 745.4. ^1H

NMR (300 MHz, DMSO-d_6) δ: 3.82 (s, 3H), 3.86 (s, 3H), 3.93(s, 3H), 6.89 (s, 2H), 7.28–7.16 (m, 3H), 7.36 (s, 1H), 7.45 (s, 1H), 7.50 (d, $J = 8.1$ Hz, 1H), 8.35 (s, 1H), 8.73 (d, $J = 8.1$ Hz, 1H). ^{13}C NMR (DMSO-d_6) δ: 161.87, 158.67, 153.94, 151.99, 147.14, 138.30, 135.37, 133.60, 126.65, 124.54, 123.82, 122.95, 121.49, 118.54, 117.51, 114.25, 113.65, 110.88, 108.92, 82.96, 60.96, 57.09, 33.75. Anal. Calcd. For $C_{23}H_{19}BrN_4O_2$: C, 59.62; H, 4.13; Br, 17.25; N, 12.09; O, 6.91. Found: C, 59.65; H, 4.16; N, 12.02.

*5.1.3.19. 2-Amino-6-(1-methyl-1H-indol-3-yl)-4-(2,3,4-trimethoxyphenyl)nicotinonitrile (**28**).* Yield: 26%. m.p. 238–239 °C. ESI-MS *m/z*: 415.6 $[M + H]^+$. IR (KBr) cm^{-1}: 3426.6, 3338.8, 3231.7, 3040.4, 2963.2, 2932.8, 2214.8, 1635.8, 1604.0, 1574.5, 1535.2, 1499.5, 1464.8, 1414.0, 1369.3, 1296.8, 1266.3, 1228.8, 1101.2, 1042.1, 1015.5, 993.2, 812.2, 734.3. ^{1}H NMR (300 MHz, DMSO-d_6) δ: 3.72 (s, 3H), 3.80 (s, 3H), 3.83 (s, 3H), 3.86 (s, 3H), 6.76 (s, 2H), 6.92 (d, $J = 8.1$ Hz, 1H), 7.06–6.94 (m, 2H), 7.26–7.14 (m, 2H), 7.49 (d, $J = 8.1$ Hz, 1H), 8.24 (s, 1H), 8.68 (d, $J = 7.8$ Hz, 1H). ^{13}C NMR (DMSO-d_6) δ: 161.18, 158.00, 154.95, 151.56, 151.38, 142.39, 138.28, 133.10, 126.60, 125.14 (2C), 123.61, 122.87, 121.42, 118.36, 114.30, 110.88, 110.10, 108.51, 85.72, 61.87, 61.30, 56.71, 33.68. Anal. Calcd. For $C_{24}H_{22}N_4O_3$: C, 69.55; H, 5.35; N, 13.52; O, 11.58. Found: C, 69.51; H, 5.38; N, 13.49.

*5.1.3.20. 2-Amino-6-(5-fluoro-1-methyl-1H-indol-3-yl)-4-(3,4,5-trimethoxyphenyl)nicotinonitrile (**29**).* Yield: 28%. m.p. 267–269 °C. ESI-MS *m/z*: 432.9 $[M + H]^+$. IR (KBr) cm^{-1}: 3490.6, 3371.4, 2991.4, 2936.4, 2832.5, 2206.2, 1622.7, 1579.7, 1535.4, 1495.3, 1480.7, 1463.1, 1415.7, 1372.9, 1271.3, 1242.7, 1224.9, 1100.3, 892.2, 802.8. ^{1}H NMR (300 MHz, DMSO-d_6) δ: 3.72 (s, 3H), 3.81 (s, 3H), 3.83 (s, 3H), 3.86 (s, 3H), 6.86 (s, 2H), 6.93 (d, $J = 9.0$ Hz, 1H), 7.12–7.01 (m, 3H), 7.50 (dd, $J_1 = 8.7$ Hz, $J_2 = 4.2$ Hz, 1H), 8.33 (s, 1H), 8.51 (dd, $J_1 = 10.5$ Hz, $J_2 = 2.4$ Hz, 1H). ^{13}C NMR (DMSO-d_6) δ: 161.92, 158.93, 158.10, 153.76, 153.56 (2C), 139.04, 134.96, 134.85, 133.42, 127.00, 118.74, 114.21, 112.06, 111.00, 108.96, 108.57, 106.60 (2C), 83.20, 60.83, 56.80 (2C), 34.01. Anal. Calcd. For $C_{24}H_{21}FN_4O_3$: C, 66.66; H, 4.89; F, 4.39; N, 12.96; O, 11.10. Found: C, 66.65; H, 4.87; N, 12.93.

*5.1.3.21. 2-Amino-4-(3-bromo-4,5-dimethoxyphenyl)-6-(5-fluoro-1-methyl-1H-indol-3-yl)nicotinonitrile (**30**).* Yield: 31%. m.p. 266–268 °C. ESI-MS *m/z*: 481.3 (Br = 79), 483.2 (Br = 81) $[M + H]^+$. IR (KBr) cm^{-1}: 3482.0, 3372.7, 3003.7, 2932.6, 2203.4, 1623.2, 1578.7, 1561.3, 1533.6, 1485.2, 1260.9, 1233.0, 1132.2, 1106.4, 1045.2, 999.1, 836.4, 792.5. ^{1}H NMR (300 MHz, DMSO-d_6) δ: 3.82 (s, 3H), 3.85 (s, 3H), 3.93 (s, 3H), 6.97 (s, 2H), 7.12–7.04 (m, 1H), 7.17 (s, 1H), 7.35 (s, 1H), 7.44 (s, 1H), 7.51 (s, 1H), 8.43 (s, 1H), 8.53–8.49 (m, 1H). ^{13}C NMR (DMSO-d_6) δ: 161.93, 158.99, 158.31, 153.93, 152.04, 147.16, 135.31, 135.09, 135.03, 127.04, 124.54, 118.50, 117.50, 114.17, 113.66, 112.07, 111.05, 108.98, 108.60, 82.98, 60.97, 57.09, 34.06. Anal. Calcd. For $C_{23}H_{18}BrFN_4O_2$: C, 57.39; H, 3.77; Br, 16.60; F, 3.95; N, 11.64; O, 6.65. Found: C, 57.41; H, 3.73; N, 11.59.

*5.1.3.22. 2-Amino-6-(5-fluoro-1-methyl-1H-indol-3-yl)-4-(2,3,4-trimethoxyphenyl)nicotinonitrile (**31**).* Yield: 24%. m.p. 253–255 °C. ESI-MS *m/z*: 433.0 $[M + H]^+$. IR (KBr) cm^{-1}: 3481.4, 3378.8, 2970.8, 2939.2, 2835.6, 2199.8, 1625.1, 1571.3, 1533.2, 1505.2, 1484.2, 1466.2, 1425.7, 1373.4, 1349.1, 1261.6, 1229.9, 1112.5, 1000.8, 836.0, 787.9. ^{1}H NMR (300 MHz, DMSO-d_6) δ: 3.74 (s, 3H), 3.86 (s, 3H), 3.87 (s, 6H), 6.95 (s, 4H), 7.13–7.06 (m, 1H), 7.18 (s, 1H), 7.52 (dd, $J_1 = 8.7$ Hz, $J_2 = 4.5$ Hz, 1H), 8.41 (s, 1H), 8.52 (dd, $J_1 = 10.8$ Hz, $J_2 = 3.0$ Hz, 1H). ^{13}C NMR (DMSO-d_6) δ: 161.23, 158.94, 157.68, 154.95, 151.60, 151.38, 142.40, 135.02, 134.63, 127.02, 125.13 (2C), 118.31, 114.14, 112.03, 110.99, 109.77, 108.86, 108.51, 85.72, 61.86, 61.29, 56.72, 33.99. Anal. Calcd. For $C_{24}H_{21}FN_4O_3$: C, 66.66; H, 4.89; F, 4.39; N, 12.96; O, 11.10. Found: C, 66.69; H, 4.82; N, 13.05.

*5.1.3.23. 2-Amino-6-(6-fluoro-1-methyl-1H-indol-3-yl)-4-(3,4,5-trimethoxyphenyl)nicotinonitrile (**32**).* Yield: 30%. m.p. 237–239 °C. ESI-MS *m/z*: 433.4 $[M + H]^+$. IR (KBr) cm^{-1}: 3371.9, 3165.3, 2934.9, 2201.0, 1627.7, 1577.3, 1533.0, 1507.3, 1470.9, 1402.0, 1241.0, 1130.2, 1072.6, 1000.6, 930.7, 825.7. ^{1}H NMR (300 MHz, DMSO-d_6) δ: 3.74 (s, 3H), 3.83 (s, 3H), 3.86 (s, 6H), 6.86 (s, 2H), 7.04–6.94 (m, 3H), 7.18 (s, 1H), 7.39 (d, $J = 8.7$ Hz,1H), 8.32 (s, 1H), 8.74 (s, 1H). Anal. Calcd. For $C_{24}H_{21}FN_4O_3$: C, 66.66; H, 4.89; F, 4.39; N, 12.96; O, 11.10. Found: C, 66.69; H, 4.73; N, 13.01.

*5.1.3.24. 2-Amino-4-(2,4-difluorophenyl)-6-(5,6,7-trimethoxy-1-methyl-1H-indol-3-yl)nicotinonitrile (**33**).* Yield: 34%. m.p. 185–187 °C. ESI-MS *m/z*: 451.3 $[M + H]^+$. IR (KBr) cm^{-1}: 3140.2, 2219.1, 1646.8, 1602.2, 1513.5, 1468.9, 1408.5, 1336.2, 1283.1, 1259.5, 1144.3, 1111.9, 1032.3, 967.8, 852.0, 813.0. ^{1}H NMR (300 MHz, DMSO-d_6) δ: 3.79 (s, 3H), 3.90 (s, 3H), 3.93 (s, 6H), 6.98 (s, 2H), 7.05 (s, 1H), 7.31–7.25 (m, 1H), 7.52–7.45 (m, 1H), 7.66–7.58 (m, 1H), 8.01 (s, 1H), 8.12 (s, 1H). ^{13}C NMR (DMSO-d_6) δ: 161.40, 160.72, 159.18, 157.80, 150.44, 148.17, 140.65, 139.64, 134.78, 132.99, 125.60, 123.31, 122.40, 117.58, 112.99, 112.76, 109.87, 105.37, 100.89, 84.84, 62.68, 61.51, 57.07, 36.68. Anal. Calcd. For $C_{24}H_{20}F_2N_4O_3$: C, 63.99; H, 4.48; F, 8.44; N, 12.44; O, 10.66. Found: C, 63.87; H, 4.49; N, 12.41.

*5.1.3.25. 2-Amino-4-(3,4-difluorophenyl)-6-(5,6,7-trimethoxy-1-methyl-1H-indol-3-yl)nicotinonitrile (**34**).* Yield: 37%. m.p. 190–193 °C. ESI-MS *m/z*: 450.7 $[M + H]^+$. IR (KBr) cm^{-1}: 3336.6, 3116.4, 3002.4, 2223.9, 1653.9, 1598.4, 1522.3, 1463.7, 1414.2, 1338.8, 1276.6, 1237.0, 1112.3, 1029.1, 881.3, 826.8. ^{1}H NMR (300 MHz, DMSO-d_6) δ: 3.79 (s, 3H), 3.91 (s, 3H), 3.93 (s, 3H), 3.95 (s, 3H), 7.12 (s, 2H), 7.25 (s, 1H), 7.54 (s, 1H), 7.66-7.60 (m, 1H), 7.82–7.78 (m, 1H), 7.97 (s, 1H), 8.20 (s, 1H). Anal. Calcad. For $C_{24}H_{20}F_2N_4O_3$: C, 63.99; H, 4.48; F, 8.44; N, 12.44; O, 10.66. Found: C, 63.94; H, 4.50; N, 12.39.

*5.1.3.26. 2-Amino-4-(2-chloro-4-fluorophenyl)-6-(5,6,7-trimethoxy-1-methyl-1H-indol-3-yl)nicotinonitrile (**35**).* Yield: 38%. m.p. 175–177 °C. ESI-MS *m/z*: 466.9 (Cl = 35), 468.7 (Cl = 37) $[M + H]^+$. IR (KBr) cm^{-1}: 3420.1, 3095.3, 2933.3, 2218.5, 1644.5, 1604.8, 1510.3, 1466.6, 1414.5, 1333.8, 1283.3, 1252.0, 1210.2, 1113.1, 1033.2, 879.3, 859.5, 828.9, 790.7. ^{1}H NMR (300 MHz, DMSO-d_6) δ: 3.79 (s, 3H), 3.90 (s, 3H), 3.93 (s, 6H), 7.00 (s, 1H), 7.29 (s, 2H), 7.44–7.37 (m, 1H), 7.60–7.55 (m, 1H), 7.70–7.66 (m, 1H), 7.96 (s, 1H), 8.14 (s, 1H). Anal. Calcd. For $C_{24}H_{20}ClFN_4O_3$: C, 61.74; H, 4.32; Cl, 7.59; F, 4.07; N, 12.00; O, 10.28. Found: C, 61.72; H, 4.35; N, 12.03.

5.2. Cytotoxicity assay in vitro

The cytotoxic activity of compounds **10**–**35** was evaluated with A549, H460, HT-29 and SMMC-7721 cell lines by the standard MTT assay *in vitro*. The cancer cell lines were cultured in minimum essential medium (MEM) supplement with 10% fetal bovine serum (FBS).

Approximately 4×10^3 cells, suspended in MEM medium, were plated onto each well of a 96-well plate and incubated in 5% CO_2 at 37 °C for 24 h. The test compounds at indicated final concentrations were added to the culture medium and the cell cultures were continued for 72 h. Fresh MTT was added to each well at a terminal concentration of 5 μg/mL and incubated with cells at 37 °C for 4 h. The formazan crystals were dissolved in 100 μL DMSO each well, and the absorbency at 492 nm (for absorbance of MTT formazan) and 630 nm (for the reference wavelength) was measured with the ELISA reader. All of the compounds were tested three times in each of the cell lines. The results expressed as IC_{50} (inhibitory concentration 50%) were the averages of three determinations and calculated by using the Bacus Laboratories Incorporated Slide Scanner (Bliss) software.

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