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Synthesis and Biological Evaluation of Novel 5,7-Diphenylimidazo[1,2-a]pyridine Derivatives

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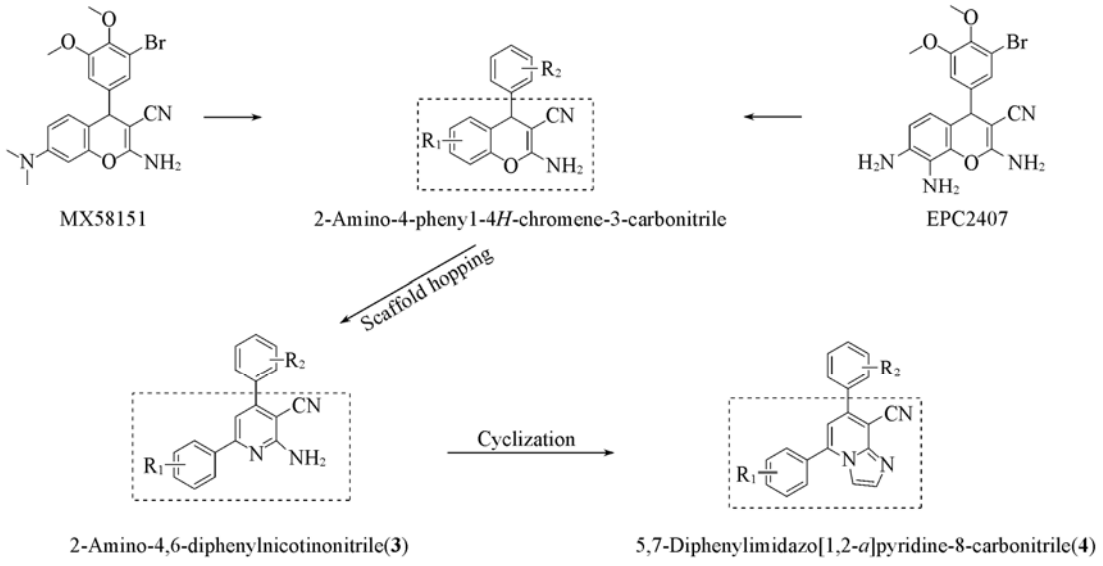
Abstract A series of novel 5,7-diphenylimidazo[1,2-a]pyridine derivatives was designed and synthesized. The *in vitro* cytotoxic activities of all the target compounds against human colorectal cancer(HT-29), human lung cancer(H460), human gastric cancer(MKN45) and human breast cancer(MDA-MB-231) cell lines were evaluated. The pharmacological results indicated that most of the target compounds showed moderate to excellent activities against the tested cell lines. The most promising compound **4**h(0.20, 0.006, 0.08, 0.021 μmol/L) was 2.6, 5.1, 3.6 and 21.9 times more active than EPC2407(0.52, 0.031, 0.29, 0.46 μmol/L) against HT-29, H460, MKN45 and MDA-MB-231 cell lines, respectively.

Keywords Imidazo[1,2-a]pyridine; Biological evaluation; Antitumor activity

1 Introduction

Cancer is a major devastating disease and one of the leading causes of death worldwide[1]. Although significant progress has been made in treating cancer, cancer mortality rate remains high because of drug resistance and adverse side effects[2]. Therefore, searching for highly efficient and safe chemotherapeutic agents with novel structural frameworks has become more urgent than ever before.

In the last decade, considerable attentions have been paid to chromenes on account of their strong cytotoxicity against human cancer cells through various pathways, including tubulin depolymerization, G2/M cell-cycle arrest and caspase-dependent apoptotic cell death[3—8]. MX58151(Scheme 1) is a chromene-based tubulin inhibitor, which displays potent cytotoxicity against human ductal breast cancer(T47D) cell line with an EC_{50}(concentration for 50% of maximal effect) of 0.019 μmol/L[9]. Further optimization of MX58151 led to EPC2407(Scheme 1), which exerts excellent antiproliferative effects against an array of human cancer cell lines and is currently undergoing phase I/II clinical trials[10,11]. The structure-activity relationship(SAR) investigations revealed that a cyano group at 3-position played an important role in their antitumor activity and the introduction of a phenyl moiety at 4-position remarkably enhanced the potency[12—15].



Scheme 1 Design of 5,7-diphenylimidazo[1,2-a]pyridine-8-carbonitriles

R_1 and R_2 are listed in Table 1.

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In our previous studies, the chromene ring was replaced by a phenyl-substituted pyridine moiety on the basis of scaffold hopping. The resulting 2-amino-4,6-diphenylnicotinonitrile derivatives exhibited antitumor activity to some extent against some cancer cell lines. According to related reports, imidazo [1,2-a]pyridine structural moieties have remarkable biological properties similar to those of indoles and azaindoles[16], which are of great chemical and pharmacological interest[17]. For further research, the 2-amino-4,6-diphenylnicotinonitrile moiety was converted into an imidazo[1,2-a]pyridine skeleton by cyclization with chloroacetaldehyde, while the cyano group was remained in attempt to identify potential antitumor agents with a novel structural framework. Accordingly, a series of 5,7-diphenylimidazo[1,2-a]pyridine-8-carbonitrile derivatives (**4**a—**4**n) was designed and synthesized. Their cytotoxic activities against HT-29, H460, MKN45 and MDA-MB-231 cell lines were evaluated *in vitro* by standard 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide(MTT) assay.

2 Experimental

2.1 Reagents and Apparatuses

Unless otherwise noted, all the materials were obtained from commercial available sources and were used without further purification.

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 electrospray ionization(ESI) mode on an Agilent 1100 LC-MS(Agilent, Palo Alto, CA, USA). Proton nuclear magnetic resonance(^{1}H NMR) spectroscopy was performed using a Bruker ARX-300(300 MHz) spectrometer(Bruker Bioscience, Billerica, MA, USA) with tetramethylsilane(TMS) as an internal standard. Thin layer chromatography(TLC) was carried out on plate silica gel F254(Qindao Haiyang Chemical, China). All chemical yields were not optimized, and they were generally the result of a single experiment.

2.2 General Procedure for Preparation of Compounds 3a—3n

To a mixture of substituted benzaldehydes(0.03 mol), substituted acetophenones(0.03 mol) and malononitrile(1.98 g, 0.03 mol) in toluene(90 mL) was added ammonium acetate(18.48 g, 0.24 mol). The mixture was refluxed for 12 h. After cooling, the supernatant liquid was concentrated under reduced pressure and the residue obtained was triturated with ethanol. The solid product obtained was collected by filtration and recrystallized from ethanol to give compounds **3**a—**3**n.

2-Amino-6-(4-methoxyphenyl)-4-(3,4,5-trimethoxyphenyl)nicotinonitrile(**3**a): a pale yellow solid, yield 56%. MS(ESI), *m/z*: 392.4(M+H).

2-Amino-6-(4-fluorophenyl)-4-(3,4,5-trimethoxyphenyl)-nicotinonitrile(**3**b): a yellow solid, yield 55%. MS(ESI), *m/z*: 380.4(M+H).

2-Amino-6-[4-(trifluoromethyl)phenyl]-4-(3,4,5-trimethoxyphenyl)nicotinonitrile(**3**c): a yellow solid, yield 51%. MS(ESI), *m/z*: 380.4(M+H).

2-Amino-6-(2,4-dichlorophenyl)-4-(3,4,5-trimethoxyphenyl)nicotinonitrile(**3**d): a pale yellow solid, yield 70%. MS(ESI), *m/z*: 431.3(M+H).

2-Amino-6-(4-methoxyphenyl)-4-[4-(trifluoromethyl)-phenyl]nicotinonitrile(**3**e): a white solid, yield 66%. MS(ESI), *m/z*: 370.4(M+H).

2-Amino-4-(2-chlorophenyl)-6-(4-methoxyphenyl)nicotinonitrile(**3**f): a yellow solid, yield 65%. MS(ESI), *m/z*: 336.8(M+H).

2-Amino-4-(2,3-dichlorophenyl)-6-(4-methoxyphenyl)-nicotinonitrile(**3**g): a yellow solid, yield 64%. MS(ESI), *m/z*: 371.3(M+H).

2-Amino-4-(2,4-dichlorophenyl)-6-(4-methoxyphenyl)-nicotinonitrile(**3**h): a yellow solid, yield 62%. MS(ESI), *m/z*: 371.3(M+H).

2-Amino-6-(3-methoxyphenyl)-4-[4-(trifluoromethyl)-phenyl]nicotinonitrile(**3**i): a yellow solid, yield 67%. MS(ESI), *m/z*: 370.3(M+H).

2-Amino-4-(2,3-dichlorophenyl)-6-(3-methoxyphenyl)-nicotinonitrile(**3**j): a golden yellow solid, yield 50%. MS(ESI), *m/z*: 371.2(M+H).

2-Amino-4-(2,4-difluorophenyl)-6-(3-methoxyphenyl)-nicotinonitrile(**3**k): a white solid, yield 64%. MS(ESI), *m/z*: 338.3(M+H).

2-Amino-4-(2-chloro-4-fluorophenyl)-6-(3-methoxyphenyl)nicotinonitrile(**3**l): a light yellow solid, yield 58%. MS(ESI), *m/z*: 354.8(M+H).

2-Amino-6-(3-methoxyphenyl)-4-(2,3,4-trimethoxyphenyl)nicotinonitrile(**3**m): a yellow solid, yield 68%. MS(ESI), *m/z*: 392.4(M+H).

2-Amino-6-(3-methoxyphenyl)-4-(3,4,5-trimethoxyphenyl)nicotinonitrile(**3**n): a yellow solid, yield 65%. MS(ESI), *m/z*: 392.4(M+H).

2.3 General Procedure for Preparation of Compounds 4a—4n

To a solution of intermediate **3**(0.01 mol) in absolute ethanol(30 mL) was added chloroacetaldehyde(4.71 g, 0.06 mol). The mixture was refluxed for 12 h and then most of the solvent was removed under reduced pressure and the residue obtained was cooled in an ice bath for 2 h. The solid product obtained was collected by filtration and dissolved in heating acetone(10 mL). Saturated aq. $NaHCO_3$ solution was added to adjust the pH value to 8.0, and the resulted mixture was poured into ice-water(100 mL). The precipitate was filtered to afford the corresponding products **4**a—**4**n.

5-(4-Methoxyphenyl)-7-(3,4,5-trimethoxyphenyl)imidazo-[1,2-a]pyridine-8-carbonitrile(**4**a): a light yellow solid, yield 64%. m. p. 176—177 °C. MS(ESI), *m/z*: 415.4(M+H). ^{1}H NMR(300 MHz, DMSO-d_6), δ: 3.75(s, 3H, OCH_3), 3.88 (s, 9H, OCH_3), 7.12(s, 2H, 7-aryl-2H,6H), 7.20(d, 2H, *J*=8.8 Hz, 5-aryl-3H,5H), 7.24(s, 1H, imidazo[1,2-a]pyridine-6H), 7.78(d, 1H, *J*=1.1 Hz, imidazo[1,2-a]pyridine-3H), 7.83(d, 2H, *J*=8.8 Hz, 5-aryl-2H,6H), 8.00(d, 1H, *J*=1.2 Hz,

imidazo[1,2-a]pyridine-2H).

5-(4-Fluorophenyl)-7-(3,4,5-trimethoxyphenyl)imidazo-[1,2-a]pyridine-8-carbonitrile(**4**b): a yellow solid, yield 60%. m. p. 225—227 °C. MS(ESI), *m/z*: 403.4(M+H). ^{1}H NMR(300 MHz, DMSO-d_6), δ: 3.74—3.76(m, 3H, OCH_3), 3.87(s, 6H, OCH_3), 7.13(s, 2H, 7-aryl-2H, 6H), 7.33(s, 1H, imidazo-[1,2-a]pyridine-6H), 7.50(t, 2H, *J*=8.8 Hz, 5-aryl-3H,5H), 7.81(d, 1H, *J*=1.1 Hz, imidazo[1,2-a]pyridine-3H), 7.89—8.00 (m, 3H, 5-aryl-2H, 6H, imidazo[1,2-a]pyridine-2H).

5-[(4-(Trifluoromethyl)phenyl)-7-(3,4,5-trimethoxyphenyl)imidazo[1,2-a]pyridine-8-carbonitrile(**4c**): a yellow green solid, yield 63%. m. p. 203—204 °C. MS(ESI), *m/z*: 453.4(M+H). ^{1}H NMR(300 MHz, DMSO-d_6), δ: 3.75(s, 3H, OCH_3), 3.87(s, 6H, OCH_3), 7.14(s, 2H, 7-aryl-2H, 6H), 7.41(s, 1H, imidazo[1,2-a]pyridine-6H), 7.80(s, 1H, imidazo-[1,2-a]pyridine-3H), 8.00—8.05(m, 3H, 5-aryl-3H,5H,imidazo-[1,2-a]pyridine-2H), 8.11(d, 2H, *J*=8.2 Hz, 5-aryl-2H,6H).

5-(2,4-Dichlorophenyl)-7-(3,4,5-trimethoxyphenyl)-imidazo[1,2-a]pyridine-8-carbonitrile(**4**d): a yellow solid, yield 65%. m. p. 125—126 °C. MS(ESI), *m/z*: 454.3(M+H). ^{1}H NMR(300 MHz, DMSO-d_6), δ: 3.75(s, 3H, OCH_3), 3.87(s, 6H, OCH_3), 7.13(s, 2H, 7-aryl-2H, 6H), 7.43(s, 1H, imidazo-[1,2-a]pyridine-6H), 7.63(s, 1H, 5-aryl-3H), 7.73(dd, 1H, *J*=8.3, 1.8 Hz, 5-aryl-5H), 7.79(t, 2H, *J*=4.9 Hz, 5-aryl-6H, imidazo[1,2-a]pyridine-3H), 7.97(d, 1H, *J*=1.7 Hz, imidazo[1,2-a]pyridine-2H).

5-(4-Methoxyphenyl)-7-[4-(trifluoromethyl)phenyl]-imidazo[1,2-a]pyridine-8-carbonitrile(**4**e): a yellow solid, yield 61%. m. p. 147—148 °C. MS(ESI), *m/z*: 393.4(M+H). ^{1}H NMR(300 MHz, DMSO-d_6), δ: 3.82(s, 3H, OCH_3), 7.12(s, 1H, imidazo[1,2-a]pyridine-6H), 7.15(d, 2H, *J*=1.8 Hz, 5-aryl-3H,5H), 7.77(d, 2H, *J*=1.2 Hz, 5-aryl-2H,6H), 7.80(s, 1H, imidazo[1,2-a]pyridine-3H), 7.91(d, 2H, *J*=8.4 Hz, 7-aryl-2H, 6H), 7.99(d, 2H, *J*=8.6 Hz, 7-aryl-3H, 5H), 8.01(d, 1H, *J*=1.2 Hz, imidazo[1,2-a]pyridine-2H).

7-(2-Chlorophenyl)-5-(4-methoxyphenyl)imidazo[1,2-a]-pyridine-8-carbonitrile(**4**f): a white solid, yield 61%. m. p. 181—182 °C. MS(ESI), *m/z*: 359.8(M+H). ^{1}H NMR(300 MHz, DMSO-d_6), δ: 3.87(s, 3H, OCH_3), 7.07(s, 1H, imidazo-[1,2-a]pyridine-6H), 7.13—7.21(m, 2H, 5-aryl-3H,5H), 7.50—7.62(m, 2H, 7-aryl-3H, 5H), 7.63—7.73(m, 2H, 7-aryl-4H, 6H), 7.77—7.85(m, 3H, imidazo[1,2-a]pyridine-3H, 5-aryl-2H,6H), 8.09(d, 1H, *J*=1.4 Hz, imidazo[1,2-a]-pyridine-2H).

7-(2,3-Dichlorophenyl)-5-(4-methoxyphenyl)imidazo[1,2-a]pyridine-8-carbonitrile(**4**g): a white solid, yield 58%. m. p. 148—150 °C. MS(ESI), *m/z*: 394.2(M+H). ^{1}H NMR(300 MHz, DMSO-d_6), δ: 3.86(s, 3H, OCH_3), 7.13(s, 1H, imidazo[1,2-a]pyridine-6H), 7.17(d, 2H, *J*=8.8 Hz, 5-aryl-3H,5H), 7.57(t, 1H, *J*=7.8 Hz, 7-aryl-5H), 7.65(dd, 1H, *J*=7.7, 1.7 Hz, 7-aryl-6H), 7.79(dd, 2H, *J*=9.2, 2.2 Hz, 5-aryl-2H,6H), 7.84(td, 2H, *J*=4.1, 1.7 Hz, imidazo[1,2-a]pyridine-3H, 7-aryl-4H), 8.11(d, 1H, *J*=1.3 Hz, imidazo[1,2-a]pyridine-2H).

7-(2,4-Dichlorophenyl)-5-(4-methoxyphenyl)imidazo[1,2-a]pyridine-8-carbonitrile(**4**h): a yellow green solid, yield 27%. m. p. 174—175 °C. MS(ESI), *m/z*: 394.2(M+H). ^{1}H NMR(300 MHz, DMSO-d_6), δ: 3.84—3.89(m, 3H, OCH_3), 7.10(s, 1H, imidazo[1,2-a]pyridine-6H), 7.18(d, 2H, *J*=8.8 Hz, 5-aryl-3H,5H), 7.66(dd, 1H, *J*=8.3, 1.9 Hz, 7-aryl-5H), 7.71(d, 1H, *J*=8.3 Hz, 7-aryl-6H), 7.80(d, 2H, *J*=8.8 Hz, 5-aryl-2H,6H), 7.84(s, 1H, imidazo[1,2-a]pyridine-3H), 7.91(d, 1H, *J*=1.8 Hz, 7-aryl-3H), 8.10(d, 1H, *J*=0.5 Hz, imidazo[1,2-a]pyridine-2H).

5-(3-Methoxyphenyl)-7-[4-(trifluoromethyl)phenyl]-imidazo[1,2-a]pyridine-8-carbonitrile(**4**i): a yellow solid, yield 27%. m. p. 145—147 °C. MS(ESI), *m/z*: 393.4(M+H). ^{1}H NMR(300 MHz, DMSO-d_6), δ: 3.85(s, 3H, OCH_3), 7.21(d, 1H, *J*=7.9 Hz, 5-aryl-4H), 7.29(s, 1H, imidazo[1,2-a]-pyridine-6H), 7.43(d, 2H, *J*=8.0 Hz, 5-aryl-2H,6H), 7.56(t, 1H, *J*=7.8 Hz, 5-aryl-5H), 7.83(s, 1H, imidazo- [1,2-a]pyridine-3H), 7.97(d, 2H, *J*=8.0 Hz, 7-aryl-2H,6H), 8.01—8.10(m, 3H, imidazo[1,2-a]pyridine-2H, 7-aryl-3H,5H).

7-(2,3-Dichlorophenyl)-5-(3-methoxyphenyl)imidazo[1,2-a]pyridine-8-carbonitrile(**4**j): a white solid, yield 55%. m. p. 152—153 °C. MS(ESI), *m/z*: 394.2(M+H). ^{1}H NMR(300 MHz, DMSO-d_6), δ: 3.85(s, 3H, OCH_3), 7.20(dd, 1H, *J*=8.5, 2.4 Hz, 5-aryl-4H), 7.23(s, 1H, 5-aryl-2H), 7.34—7.39(s, 1H, imidazo-[1,2-a]pyridine-6H), 7.40(d, 1H, *J*=7.8 Hz, 5-aryl-6H), 7.54(t, 1H, *J*=8.3 Hz, 5-aryl-5H), 7.59(d, 1H, *J*=7.9 Hz, 7-aryl-5H), 7.66(dd, 1H, *J*=7.7, 1.7 Hz, 7-aryl-6H), 7.85(td, 2H, *J*=4.1, 1.7 Hz, imidazo[1,2-a]pyridine-3H, 7-aryl-4H), 8.13(d, 1H, *J*=0.7 Hz, imidazo[1,2-a]pyridine-2H).

7-(2,4-Difluorophenyl)-5-(3-methoxyphenyl)imidazo[1,2-a]pyridine-8-carbonitrile(**4**k): a yellow solid, yield 60%. m. p. 135—137 °C. MS(ESI), *m/z*: 361.3(M+H). ^{1}H NMR(300 MHz, DMSO-d_6), δ: 3.84(s, 3H, OCH_3), 7.22—7.17(m, 2H, imidazo-[1,2-a]pyridine-6H, 5-aryl-4H), 7.37(d, 2H, *J*=12.5 Hz, 5-aryl-2H,6H), 7.55(s, 1H, 5-aryl-5H), 7.71(d, 2H, *J*=2.8 Hz, 7-aryl-5H,6H), 7.83(s, 2H, imidazo[1,2-a]pyridine-3H, 7-aryl-3H), 8.08(d, 1H, *J*=1.1 Hz, imidazo[1,2-a]pyridine-2H).

7-(2-Chloro-4-fluorophenyl)-5-(3-methoxyphenyl)imidazo [1,2-a]pyridine-8-carbonitrile(**4**l): a yellow solid, yield 23%. m. p. 205—207 °C. MS(ESI), *m/z*: 377.8(M+H). ^{1}H NMR(300 MHz, DMSO-d_6), δ: 3.84(s, 3H, OCH_3), 7.18(s, 1H, 5-aryl-2H), 7.19—7.23(m, 1H, 5-aryl-4H), 7.37(s, 1H, imidazo[1,2-a]-pyridine-6H), 7.40(d, 1H, *J*=8.6 Hz, 5-aryl-5H), 7.48(dd, 1H, *J*=8.5, 2.2 Hz, 5-aryl-6H), 7.55(t, 1H, *J*=7.8 Hz, 7-aryl-5H), 7.70—7.80(m, 2H, 7-aryl-3H,6H), 7.84(s, 1H, imidazo-[1,2-a]pyridine-3H), 8.12(s, 1H, imidazo[1,2-a]pyridine-2H).

5-(3-Methoxyphenyl)-7-(2,3,4-trimethoxyphenyl)imidazo-[1,2-a]pyridine-8-carbonitrile(**4**m): a yellow solid, yield 22%. m. p. 148—150 °C. MS(ESI), *m/z*: 415.4(M+H). ^{1}H NMR(300 MHz, DMSO-d_6), δ: 3.73—3.76(m, 3H, OCH_3), 3.82(s, 3H, OCH_3), 3.84(s, 3H, OCH_3), 3.88(s, 3H, OCH_3), 6.99(d, 1H, *J*=8.6 Hz, 7-aryl-5H), 7.06(s, 1H, 7-aryl-2H), 7.19(dd, 1H, *J*=9.3, 1.4 Hz, 5-aryl-4H), 7.26(d, 1H, *J*=8.6 Hz, 7-aryl-6H), 7.38(d, 2H, *J*=8.4 Hz, 5-aryl-6H, imidazo[1,2-a]pyridine-6H), 7.55(t, 1H, *J*=7.8 Hz, 5-aryl-5H), 7.77(s, 1H, imidazo-[1,2-a]pyridine-3H), 8.02(s, 1H, imidazo[1,2-a]pyridine-2H).

5-(3-Methoxyphenyl)-7-(3,4,5-trimethoxyphenyl)imidazo-[1,2-a]pyridine-8-carbonitrile(**4**n): a yellow solid, yield 66%. m. p. 176—177 °C. MS(ESI), *m/z*: 415.4(M+H). ^{1}H NMR(300 MHz, DMSO-d_6), δ: 3.75(s, 3H, OCH_3), 3.85(s, 3H, OCH_3), 3.87(s, 6H, OCH_3), 7.12(s, 2H, 7-aryl-2H,6H), 7.21(dd, 1H, *J*=7.6, 1.9 Hz, 5-aryl-4H), 7.32(s, 1H, 5-aryl-2H), 7.41(d, 2H,

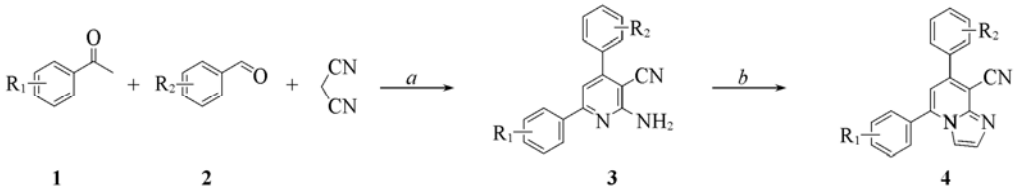
J=7.4 Hz, 5-aryl-6H, imidazo[1,2-a]pyridine-6H), 7.56(t, 1H, *J*=7.9 Hz, 5-aryl-5H), 7.78(d, 1H, *J*=1.2 Hz, imidazo-[1,2-a]pyridine-3H), 8.00(d, 1H, *J*=1.3 Hz, imidazo-[1,2-a]pyridine-2H).

3 Results and Discussion

3.1 Chemistry

As illustrated in Scheme 2, target compounds **4**a—**4**n(Table 1) were synthesized by a convenient two-step procedure. Intermediates **3**a—**3**n were prepared by a one-pot reaction of the commercially available substituted acetophenones **1** and benzaldehydes **2** with malononitrile in the presence of ammonium acetate in yields of 50%—70%. Cyclization of compounds **3**a—**3**n with chloroacetaldehyde in absolute ethanol afforded the corresponding **4**a—**4**n in yields of 22%—66%.

In our previous synthetic route, aldol condensation of acetophenones with benzaldehydes produced the corresponding chalcones, which were cyclized with malononitrile to afford intermediate **3**. After repeated attempts, we found that a one-pot reaction of acetophenones and benzaldehydes with malononitrile in the presence of ammonium acetate in toluene was available for the preparation of intermediate **3**. This multi-component reaction not only simplified the separation and purification processes, but also improved the atom economy.



Scheme 2 Synthesis of target compounds 4a—4n

Reagents and conditions: *a*. ammonium acetate, toluene, reflux 12 h, yield 50%—70%; *b*. chloroacetaldehyde, absolute ethanol, reflux 12 h, yield 22%—66%. R_1 and R_2 are listed in Table 1.

Table 1 Substituents and cytotoxic activity of target compounds against a panel of human cancer cell lines

Compd.	R_1	R_2	IC_{50}[a]/(μmol·L^{-1})			
			HT-29	H460	MKN45	MDA-MB-231
4a	4-Methoxy	3,4,5-Trimethoxy	32.12±0.94	1.08±0.12	**0.11**±0.01	**0.388**±0.021
4b	4-Fluoro	3,4,5-Trimethoxy	6.14±0.21	2.25±0.19	1.62±0.26	ND[b]
4c	4-Trifluoromethyl	3,4,5-Trimethoxy	12.37±0.29	8.75±0.43	9.32±0.48	ND[b]
4d	2,4-Dichloro	3,4,5-Trimethoxy	6.07±0.23	0.92±0.04	18.12±0.47	54.24±0.68
4e	4-Methoxy	4-Trifluoromethyl	2.41±0.17	1.24±0.10	2.73±0.36	5.65±0.43
4f	4-Methoxy	2-Chloro	1.33±0.11	0.035±0.009	4.60±0.21	12.17±0.74
4g	4-Methoxy	2,3-Dichloro	2.74±0.31	**0.021**±0.005	1.91±0.23	3.52±0.38
4h	4-Methoxy	2,4-Dichloro	**0.20**±0.02	**0.006**±0.001	**0.080**±0.012	**0.021**±0.003
4i	3-Methoxy	4-Trifluoromethyl	3.12±0.42	1.55±0.12	ND[b]	ND[b]
4j	3-Methoxy	2,3-Dichloro	2.27±0.24	0.069±0.015	3.57±0.43	20.61±0.57
4k	3-Methoxy	2,4-Difluoro	0.81±0.03	**0.018**±0.004	**0.162**±0.013	**0.071**±0.007
4l	3-Methoxy	2-Chloro-4-Fluoro	1.23±0.11	**0.010**±0.003	0.940±0.016	16.16±0.72
4m	3-Methoxy	2,3,4-Trimethoxy	3.68±0.27	0.42±0.03	0.347±0.020	0.925±0.015
4n	3-Methoxy	3,4,5-Trimethoxy	9.85±0.54	1.15±0.11	**0.270**±0.015	**0.434**±0.052
EPC2407[c]			0.52±0.17	0.031±0.002	0.290±0.011	0.460±0.013

a. IC_{50}: concentration for 50% of maximal inhibitory; *b*. ND: not determined; *c*. used as a positive control.

3.2 Biological Evaluation

Taking EPC2407 as the positive control, all the target compounds were evaluated for their *in vitro* cytotoxic activities against HT-29, H460, MKN45 and MDA-MB-231 cell lines by the MTT assay, and the results expressed as IC_{50} are summarized in Table 1.

As illustrated in Table 1, most of the synthesized compounds showed potent cytotoxic activities against one or more cancer cell lines. Compounds **4**g, **4**h, **4**k and **4**l exhibited enhanced cytotoxic activities than EPC2407 against H460 cell line. The most promising compound **4**h(0.20, 0.006, 0.08, 0.021 μmol/L) was 2.6, 5.1, 3.6 and 21.9 times more active than EPC2407(0.52, 0.031, 0.29, 0.46 μmol/L) against HT-29, H460, MKN45 and MDA-MB-231 cell lines, respectively.

The pharmacological data indicated that the electric effects of R_1 appeared to have some relationship with the cytotoxicity. Compound **4**b with a weak electron-withdrawing group(fluoro) in the benzene ring exhibited lower activity than compound **4**a with an electron-donating group(methoxy), and the introduction of a strong electron-withdrawing group (4-trifluoromethyl) resulted in a more significant drop of activity, such as compound **4**c. Furthermore, it was found that the electron-donating group was preferred at the *para*-position of the benzene ring. Most of the derivatives with methoxy group at *para*-position, such as compounds **4**a, **4**e and **4**g showed moderately higher cytotoxicity than those with methoxy group at *meta*-position, such as compounds **4**n, **4**i and **4**j. In addition, the nature of R_2 in the benzene ring had an important influence on antitumor activity against H460 cell line. In most cases, compounds(**4**f—**4**h, **4**j—**4**l) with halogen groups(such as 2-Cl, 4-Cl, 2,4-di-Cl in this study) in the benzene ring showed

enhanced cytotoxicity against H460 cell line than those(**4**e, **4**i) with other groups(such as 4-CF_3, 3,4,5-tri-OCH_3). Besides, it was worth to mention that the halogen groups substituted at 2,4-position of the benzene ring improved the cytotoxic activity significantly, as compounds **4**h and **4**k were 4—300 times more active than compounds **4**g and **4**j whose halogen groups were substituted at 2,3-position.

4 Conclusions

A series of novel 5,7-diphenylimidazo[1,2-a]pyridine derivatives was synthesized and their *in vitro* antitumor activities were evaluated. Compound **4**h exhibited more potent activity against all the tested cancer cell lines as compared with EPC2407 and could be served as a candidate for further development. From preliminary SARs, we may conclude that electron-donating groups(R_1) and halogen groups(R_2) are required for optimal potency. Most noteworthy was the steric effect of R_2, such as 2,4-difluoro(**4**k), 2,4-dichloro(**4**h), which led to a significant improvement in activity. This study may provide valuable information for future design and development of 5,7-diaryl-imidazo[1,2-a]pyridine derivatives with more potent activity.

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