SCIENCE CHINA
Chemistry

• ARTICLES •

June 2013 Vol.56 No.6: 702–715
doi: 10.1007/s11426-013-4840-x

Novel *2H*-pyrazolo[4,3-c]hexahydropyridine derivatives: Synthesis, crystal structure, fluorescence properties and cytotoxicity evaluation against human breast cancer cells

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Received September 13, 2012; accepted November 7, 2012; published online March 13, 2013

A series of novel *2H*-pyrazolo[4,3-c]hexahydropyridine derivatives (**II**) have been designed and synthesized. The target compounds have been identified by elemental analysis and spectral (^{1}H NMR, IR, and MS) data and the absolute configuration of compound ($\mathbf{II_1}$) was confirmed by single crystal X-ray diffraction. The cytotoxicity of the target compounds have been evaluated *in vitro* against two human breast cancer cell lines MCF-7 and MDA-MB-231 by MTT assay. Most compounds exhibited good inhibition, and compounds $\mathbf{II_{21}}$ (IC_{50} = 4.7 μM for MCF-7 and IC_{50} = 9.3 μM for MDA-MB-231), $\mathbf{II_{33}}$ (IC_{50} = 2.4 μM for MCF-7 and IC_{50} = 4.2 μM for MDA-MB-231) and $\mathbf{II_{40}}$ (IC_{50} = 3.3 μM for MCF-7 and IC_{50} =8.6 μM for MDA-MB-231) displayed better inhibitory activity than 5-fluorouracil (IC_{50} = 4.8 μM for MCF-7 and IC_{50} = 9.6 μM for MDA-MB-231, respectively). Flow cytometric analysis and DNA fragmentation suggest that $\mathbf{II_{33}}$ is cytotoxic and able to induce the apoptosis of MCF-7 cells. The fluorescence properties of compounds $\mathbf{II_1}$, $\mathbf{II_6}$, $\mathbf{II_{11}}$, $\mathbf{II_{16}}$, $\mathbf{II_{23}}$, $\mathbf{II_{28}}$, and $\mathbf{II_{35}}$ were also studied and compound $\mathbf{II_{28}}$ afforded the highest photoluminescence quantum yield (38%).

pyrazolopyridine, synthesis, crystal structure, breast cancer cells, cytotoxicity evaluation, apoptosis, fluorescence properties

1 Introduction

Among diseases cancer is considered as the deadliest and a substantial number of new antineoplastic agents have been discovered [1, 2]. Cancer cells differ from their normal counterparts in a number of biochemical processes, particularly during the control of cell growth and division. One characteristic of cancer cells, that distinguishes them from most normal cells, is their high proliferative index [3]. As a result, targeting of proliferative pathways resulting in cell death via apoptosis is considered as an effective strategy for fighting this disease.

Breast cancer is the most prevalent form of cancer diagnosed in women, and its incidence is steadily rising [4, 5]. In hormone dependent breast cancer, estrogens play a critical role, stimulating cancer cell proliferation [6, 7]. Some studies have showed that the human breast cancer cell lines ER (estrogen receptor)-positive MCF-7 and ER (estrogen receptor)-negative MDA-MB-231 are considerably more resistant to apoptosis than a number of other human breast cancer cell lines [8]. Thus, there is a strong impetus to identify new anti-breast cancer agents with improved cytotoxicity for ER-positive MCF-7 and ER-negative MDA-MB-231 human breast cancer cell lines.

Five and six-membered nitrogen-containing heterocycles are abundant in nature and exhibit diverse and important biological properties [9, 10]. Pyrazolopyridine derivatives are an important class of heterocyclic compounds, which exhibit a variety of biological activities including anticancer, anxiolytic, antiviral, antileishmanial and anti-inflammatory activities [11–16]. Because of their biological activities these compounds have distinguished themselves as hetero-

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cycles of profound chemical and biological significance [17]. As a result, the design of new pyrazolopyridine derivatives has attracted considerable attention.

In our previous publication we described synthesis and *in vitro* antiproliferative activity against leukemia K562 cell lines of *N*-(substituted benzyl)-3,5-bis(benzylidene)-4-piperidones (Scheme 1, **I**). Significant antiproliferative activity of compounds **I** were found and some of these compounds have better inhibitory activities than the anticancer drug 5-fluorouracil (5-FU, IC_{50} = 8.56), however, no anti-breast cancer activity was observed with these compounds [18]. Therefore, further structural modification of **I** with the goal of discovering novel compounds with potent anti-breast cancer activity is necessary.

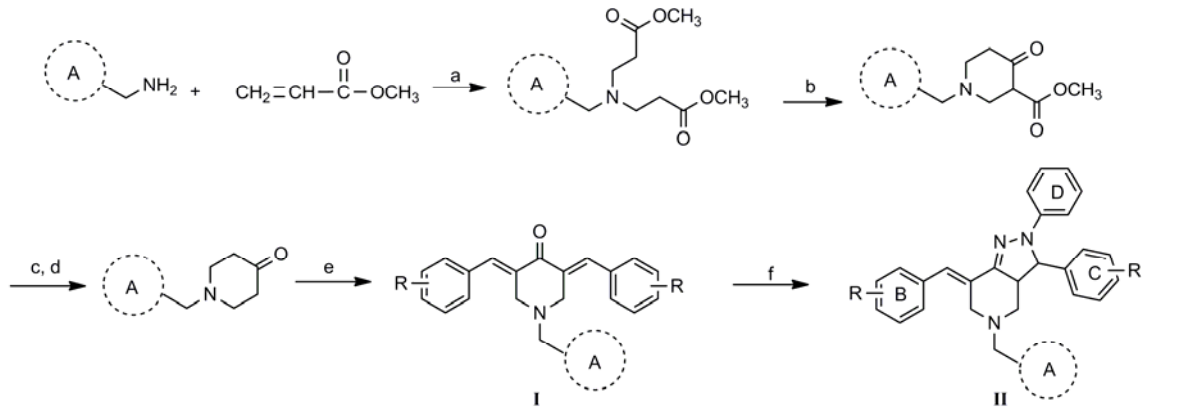
Pyrazolopyridine derivatives have mostly been synthesized through the reaction of α,β-unsaturated ketones with substituted hydrazine [17]. Motivated by this method, in the search for effective anti-breast cancer agents, we have directed our efforts toward the derivatization of *N*-(substituted benzyl)-3,5-bis(benzylidene)-4-piperidones (**I**) to afford *2H*-pyrazolo[4,3-c]hexahydropyridine derivatives (**II**). The derivatives (**II**) can be easily obtained by reacting (**I**) and phenylhydrazine as raw materials catalyzed by NaOEt in HOEt.

Herein, in a continuation of our research on anti-breast cancer compounds, we report the synthesis of a series of *2H*-pyrazolo[4,3-c]hexahydropyridine derivatives (**II_1–II_{41}**) as anti-breast cancer agents. The structures of all new compounds have been confirmed by ^{1}H NMR, IR, MS and elemental analysis, and the absolute configuration of the compound **II_1** was confirmed by single crystal X-ray diffraction. Photoluminescence spectra were studied for compounds **II_1**, **II_6**, **II_{11}**, **II_{16}**, **II_{23}**, **II_{28}**, and **II_{35}** and these compounds each gave an intense emission. Biological evaluation indicated that some of the synthesized compounds exhibited good inhibition, among them compound **II_{33}** was found to be the most potent compound with IC_{50} values of 2.4 and 4.2 μM against MCF-7 and MDA-MB-231, respectively. Moreover, the compound **II_{33}** induced apoptosis of MCF-7 cells, which was detected by DNA fragmentation and flow cytometric analysis using annexin V and PI double staining.

2 Experimental

2.1 Materials and apparatus

All the chemical reagents purchased were of analytical grade and used without further purification, except for the toluene, which was dried by refluxing in the presence of sodium and distilled prior to use. ^{1}H NMR spectra were recorded on a Bruker Avance 400 (400 MHz) spectrometer, using $CDCl_3$ or DMSO-*d6* as solvents and tetramethylsilane (TMS) as internal standard. Melting points were determined by an RK1 microscopic melting apparatus (uncorrected).



II_1: A = 4-$CH_3C_6H_4$, R = H;
II_2: A = 4-$CH_3C_6H_4$, R = 4-CH_3;
II_3: A = 4-$CH_3C_6H_4$, R = 4-CH_3O;
II_4: A = 4-$CH_3C_6H_4$, R = 4-F;
II_5: A = 4-$CH_3C_6H_4$, R = 4-Cl;
II_6: A = 4-$CH_3OC_6H_4$, R = H;
II_7: A = 4-$CH_3OC_6H_4$, R = 4-CH_3;
II_8: A = 4-$CH_3OC_6H_4$, R = 4-CH_3O;
II_9: A = 4-$CH_3OC_6H_4$, R = 4-F;
II_{10}: A = 4-$CH_3OC_6H_4$, R = 4-Cl;
II_{11}: A =2-F-C_6H_4, R = H;
II_{12}: A = 2-F-C_6H_4, R = 4-CH_3;
II_{13}: A = 2-F-C_6H_4, R = 4-CH_3O;
II_{14}: A = 2-F-$CH_3C_6H_4$, R = 4-F;
II_{15}: A = 2-F-C_6H_4, R = 4-Cl;
II_{16}: A = 4-F-C_6H_4, R = H;
II_{17}: A = 4-F-C_6H_4, R = 4-CH_3;
II_{18}: A = 4-F-C_6H_4, R = 4-$CH(CH_3)_2$;
II_{19}: A = 4-F-$CH_3C_6H_4$, R = 4-CH_3O;
II_{20}: A = 4-F-C_6H_4, R = 3-F;
II_{21}: A = 4-F-C_6H_4, R = 4-F;
II_{22}: A = 4-F-C_6H_4, R = 4-Cl;
II_{23}: A = 4-Br-C_6H_4, R = H;
II_{24}: A = 4-Br-C_6H_4, R = 4-CH_3;
II_{25}: A = 4-Br-C_6H_4, R = 4-CH_3O;
II_{26}: A = 4-Br-$CH_3C_6H_4$, R = 4-F;
II_{27}: A = 4-Br-C_6H_4, R = 4-Cl;
II_{28}: A = 3-pyridyl, R = H;
II_{29}: A = 3-pyridyl, R = 4-CH_3;
II_{30}: A = 3-pyridyl, R = 4-$CH(CH_3)_2$;
II_{31}: A = 3-pyridyl, R = 4-CH_3O;
II_{32}: A = 3-pyridyl, R = 3-F;
II_{33}: A = 3-pyridyl, R = 4-F;
II_{34}: A = 3-pyridyl, R = 4-Cl;
II_{35}: A = 3-furyl, R = H;
II_{36}: A = 3-furyl, R = 4-CH_3;
II_{37}: A = 3-furyl, R = 4-$CH(CH_3)_2$;
II_{38}: A = 3-pyridyl, R = 4-CH_3O;
II_{39}: A = 3-pyridyl, R = 3-F;
II_{40}: A = 3-pyridyl, R = 4-F;
II_{41}: A = 3-pyridyl, R = 4-Cl;

Scheme 1 General synthetic route to 2*H*-pyrazolo[4,3-c]hexahydropyridine derivatives (**II**). Reagents and conditions: (a) absolute methanol, refluxing; (b) sodium/absolute methanol, toluene, refluxing; (c) 25% HCl, refluxing; (d) 35% sodium hydroxide, r.t.; (e) aromatic aldehyde, EtOH, 10% NaOH, r.t.; (f) phenylhydrazine, EtONa, refluxing.

Elemental analysis was performed with a PerkinElmer 2400 instrument. MS spectra were recorded in ESI mode on a Bruker microTOF-Q II spectrometer. IR spectra were obtained on a Nicolet 5DX FT-IR spectrophotometer in the region 4000–400 cm^{-1} using KBr discs. X-ray diffraction data were recorded on a Bruker Smart CCD diffractometer. The single-photon fluorescence spectra were collected on a PerkinElmer LS55 luminescence spectrometer. Visible absorption spectra were determined on PerkinElmer Lambda 35 spectrophotometer.

2.2 General procedure for the preparation of compounds II

N-(substituted benzyl)-3,5- bis(ben- zylidene)-4-piperidones (**I**) were prepared according to reported procedures [16]. A mixture of (**I**) (0.001 mol), phenylhydrazine (0.004 mol) and Na metal (0.05 g) in ethanol (4 mL) was heated under reflux for 10–24 h. After this time, the separated solid was filtered off and recrystallized from CH_2Cl_2:EtOH = 1:2 to obtain title compounds **II**.

*7-Benzylidene-5-(4-methylbenzyl)-3,3a,4,5,6,7-hexahydro-2,3-diphenyl-2H-pyrazolo[4,3-c]pyridine (**II$_1$**)*

Yield, 87%; chartreuse solid; mp 168–169 °C; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 2.30 (s, 3H), 2.47 (t, J = 10.4Hz, 1H), 3.14–3.18 (m, 1H), 3.22–3.36 (m, 2H), 3.52–3.56 (m, 2H), 4.02 (d, J = 12.1Hz, 1H), 4.61 (d, J = 12.4Hz, 1H), 6.80 (t, J = 7.2Hz, 1H), 7.04–7.37 (m,19H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 20.11, 53.54, 54.08, 54.46, 56.12, 70.66, 114.09, 114.24, 114.82, 119.62, 122.46, 125.05, 125.51, 125.67, 127.69, 128.09, 128.38, 128.91, 132.09, 135.54, 136.44, 136.49, 137.46, 145.72, 147.79, 148.96, 150.16. IR (KBr, cm^{-1}) 3001, 2819, 1705, 1581, 1278, 1207, 1090; ESI-MS: 492.3 ($C_{33}H_{31}N_3Na$, $[M+Na]^+$); Anal. Calcd. for $C_{33}H_{31}N_3$: C, 84.47; H, 6.69; N, 8.94. Found: C, 84.40; H, 6.65; N, 8.95.

*5-(4-Methylbenzyl)-7-(4-methylbenzylidene)-3,3a,4,5,6,7-hexahydro-3-(4-methylphenyl)-2-phenyl-2H-pyrazolo[4,3-c]pyridine (**II$_2$**)*

Yield, 81%; chartreuse solid; mp 208–209 °C; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 2.30 (s, 3H), 2.34 (s, 6H), 2.43 (t, J = 10.2Hz, 1H), 3.14–3.20 (m, 1H), 3.21–3.26 (m, 1H), 3.28–3.30 (m, 1H), 3.54 (d, J = 13.1Hz, 1H), 3.65 (d, J = 13.1Hz, 1H), 4.04 (d, J = 13.9Hz, 1H), 4.55 (d, J = 12.4Hz, 1H), 6.81 (t, J = 7.2Hz, 1H), 7.03–7.27 (m, 17H). ^{13}C NMR ($CDCl_3$, 100 MHz) δ 20.10, 20.22, 20.28, 53.31, 54.09, 54.42, 57.37, 70.44, 113.56, 114.05, 114.21, 114.22, 119.26, 125.03, 125.06, 126.29, 127.64, 127.73, 129.32, 129.39, 129.89, 132.32, 132.36, 132.51, 145.87, 150.72, 157.94, 158.07. IR (KBr, cm^{-1}) 3022, 2918, 1596, 1509, 1042, 1030, 997; ESI-MS: 520.6 ($C_{35}H_{35}N_3Na$, $[M+ Na]^+$); Anal. Calcd. for $C_{35}H_{35}N_3$: C, 84.47; H, 7.09; N, 8.44. Found: C, 84.48; H, 7.08; N, 8.48.

*5-(4-Methylbenzyl)-7-(4-methoxylbenzylidene)-3,3a,4,5,6,7-hexahydro-3-(4-methoxylphenyl)-2-phenyl-2H-pyrazolo[4,3-c]pyridine (**II$_3$**)*

Yield, 78%; chartreuse solid; mp 186–187 °C; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 2.30 (s, 3H), 2.43 (t, J = 10.2 Hz, 1H), 3.16–3.23 (m, 2H), 3.26–3.52 (m, 1H), 3.61 (dd, J = 13.1, 38.3 Hz, 2H), 3.81 (s, 3H), 3.82 (s, 3H), 4.03 (d, J = 13.9 Hz, 1H), 4.52 (d, J = 12.3 Hz, 1H), 6.79–6.85(m,1H), 6.90–7.30 (m,17H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 20.19, 52.68, 52.71, 53.74, 54.25, 54.73, 70.58, 112.70, 114.08, 114.24, 114.28, 119.40, 125.08, 125.39, 125.99, 127.65, 127.92, 128.67, 128.88, 132.27, 136.29, 136.32, 137.56, 141.29, 145.81, 150.11, 152.38. IR (KBr, cm^{-1}) 2948, 2831, 1596, 1509, 1497, 1250, 1030, 829; ESI-MS: 552.2 ($C_{35}H_{35}N_3NaO_2$, $[M+Na]^+$). Anal. Calcd. for $C_{35}H_{35}N_3O_2$: C, 79.32; H, 6.67; N, 7.92. Found: C, 79.37; H,6.66; N, 7.93.

*7-(4-Fluorobenzylidene)-5-(4-methylbenzyl)-3,3a,4,5,6,7-hexahydro-3-(4-fluorophenyl)-2-phenyl-2H-pyrazolo[4,3-c]pyridine (**II$_4$**)*

Yield, 70%; chartreuse solid; mp 183–185 °C; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 2.34 (s, 3H), 2.52(t, J = 10.1 Hz, 1H), 3.14–3.28 (m, 3H), 3.54 (d, J = 13.0Hz, 1H), 3.65 (d, J = 13.1Hz, 1H), 4.04 (d, J = 13.5Hz, 1H), 4.55 (d, J = 12.6Hz, 1H), 6.81 (t, J = 7.2Hz, 1H), 6.83–6.88 (m, 8H), 7.03–7.06 (m, 4H), 7.09–7.16 (m, 2H), 7.25–7.27 (m, 3H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 20.21, 53.02, 53.08, 54.18, 57.38, 71.12, 112.69, 114.13, 114.19, 114.39, 115.04, 115.25, 119.52, 124.25, 126.67, 126.76, 127.74, 129.06, 130.06, 130.21, 131.09, 131.13, 136.23, 136.26, 145.50, 150.46, 157.86, 159.80, 162.3. IR (KBr, cm^{-1}) 3022, 2918, 1584, 1485, 1273, 1207, 1092; ESI-MS: 528.3 ($C_{33}H_{29}F_2N_3Na$, $[M+Na]^+$). Anal. Calcd. for $C_{33}H_{29}F_2N_3$: C, 78.37; H, 5.79; N, 8.34. Found: C, 78.39; H, 5.78; N, 8.31.

*7-(4-Chlorobenzylidene)-5-(4-methylbenzyl)-3,3a,4,5,6,7-hexahydro-3-(4-chlorophenyl)-2-phenyl-2H-pyrazolo[4,3-c]pyridine (**II$_5$**)*

Yield, 81%; chartreuse solid; mp 210–212 °C; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 2.30 (s, 3H), 2.40–2.85 (m, 1H), 3.02–3.33 (m, 3H), 3.54(d, J = 12.4Hz, 1H), 3.65(d, J = 12.6 Hz, 1H), 4.04 (d, J = 13.4Hz, 1H), 4.55 (d, J = 12.0Hz, 1H), 6.81 (t, J = 6.8Hz, 1H), 7.08–7.16 (m, 4H), 7.16–7.28 (m, 10H), 7.30–7.37 (m, 3H). ^{13}C NMR ($CDCl_3$, 100 MHz) δ 20.30, 52.86, 53.85, 54.21, 58.02, 70.11, 112.71, 114.08, 119.61, 124.17, 126.47, 127.48, 127.78, 128.24, 128.42, 128.79, 129.03, 129.64, 132.21, 132.42, 133.43, 139.04, 145.31, 150.29, 157.87. IR (KBr, cm^{-1}) 3022, 2918, 1592, 1584, 1273, 1107, 1023; ESI-MS: 560.1($C_{33}H_{29}Cl_2N_3Na$, $[M+Na]^+$). Anal. Calcd. for $C_{33}H_{29}Cl_2N_3$: C, 73.62; H, 5.40; N, 13.19. Found: C, 73.60; H, 5.43; N, 13.17.

*7-Benzylidene-5-(4-methoxylbenzyl)-3,3a,4,5,6,7-hexahydro-2,3-diphenyl-2H-pyrazolo[4,3-c]pyridine (**II$_6$**)*

Yield, 88%; chartreuse solid; mp 180-181 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 2.38–2.43 (m, 1H), 3.13–3.25 (m, 3H), 3.55–3.64 (m, 2H), 3.76 (s, 3H), 4.04 (d, J = 12.5 Hz, 1H), 4.55 (d, J = 12.8 Hz, 1H), 6.83 (t, J = 7.2 Hz, 1H), 6.92-7.23 (m, 19H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 53.05, 54.10, 54.19, 54.48, 60.15, 71.12, 114.08, 114.24, 114.46, 119.66, 122.62, 122.78, 123.06, 124.16, 126.47, 127.23, 127.49, 127.77, 128.20, 128.43, 129.67, 130.32, 132.21, 132.43, 133.38, 139.02, 145.29, 149.94, 159.03, 161.50. IR (KBr, cm^{-1}) 2972, 2850, 1568, 1552, 1266, 1112, 1042; ESI-MS: 508.2 ($C_{33}H_{31}N_3NaO$, $[M+Na]^+$). Anal. Calcd. for $C_{33}H_{31}N_3O$: C, 81.64; H, 6.47; N, 8.62. Found: C, 81.62; H, 6.43; N, 8.65.

*5-(4-Methoxylbenzyl)-7-(4-methylbenzylidene)-3,3a,4,5,6,7-hexahydro-3-(4-methylphenyl)-2-phenyl-2H-pyrazolo[4,3-c]pyridine (**II$_7$**)*

Yield, 83%; chartreuse solid; mp 170–172 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 2.30 (s, 6H), 2.43 (t, J = 10.3 Hz, 1H), 3.12–3.34 (m, 3H), 3.55–3.61 (m, 2H), 3.78 (s, 3H), 4.14 (d, J = 11.5 Hz, 1H), 4.52 (d, J = 12.3 Hz, 1H), 6.81 (t, J = 7.2 Hz, 1H), 6.92–7.23 (m, 17H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 20.11, 20.29, 52.69, 52.78, 53.75, 54.25, 54.75, 70.57, 112.71, 114.08, 114.23, 114.26, 119.42, 125.06, 125.36, 125.94, 127.63, 127.99, 128.61, 128.83, 132.27, 136.29, 136.32, 137.58, 141.29, 145.81, 150.12, 152.36. IR (KBr, cm^{-1}) 2972, 2889, 1590, 1513, 1280, 1096, 1043; ESI-MS: 536.2($C_{35}H_{35}N_3NaO$, $[M+Na]^+$). Anal. Calcd. for $C_{35}H_{35}N_3O$: C, 81.82; H, 6.87; N, 8.12. Found: C, 81.84; H, 6.87; N, 8.18.

*5-(4-Methoxylbenzyl)-7-(4-methoxylbenzylidene)-3,3a,4,5,6,7-hexahydro-3-(4-methoxylphenyl)-2-phenyl-2H-pyrazolo[4,3-c]pyridine (**II$_8$**)*

Yield, 83%; chartreuse solid; mp 161–163 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 2.45 (t, J = 12.6 Hz, 1H), 3.16–3.34 (m, 3H), 3.56–3.61 (m, 2H), 3.77(s, 3H), 3.81 (s, 6H), 3.98 (d, J = 13.5 Hz, 1H), 4.52 (d, J = 12.3 Hz, 1H), 6.84 (t, J = 7.1 Hz, 1H), 6.92-7.23 (m, 17H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 52.25, 53.29, 54.26, 54.44, 57.38, 60.03, 70.42, 113.54, 114.01, 114.17, 114.22, 119.24, 125.01, 125.02, 126.27, 126.64, 127.72, 129.30, 129.38, 129.87, 132.32, 132.35, 132.51, 145.86, 150.71, 157.93, 158.06. IR (KBr, cm^{-1}) 2981, 2868, 1546, 1520, 1268, 1162, 1062; ESI-MS: 568.6($C_{33}H_{35}N_3NaO_3$, $[M+Na]^+$). Anal. Calcd. for $C_{35}H_{35}N_3O_3$: C, 77.02; H, 6.47; N, 7.72. Found: C, 77.04; H, 6.47; N, 7.70.

*7-(4-Fluorobenzylidene)-5-(4-methoxylbenzyl)-3,3a,4,5,6,7-hexahydro-3-(4-fluorophenyl)-2-phenyl-2H-pyrazolo[4,3-c]pyridine (**II$_9$**)*

Yield, 63%; chartreuse solid; mp 183–184 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 2.40 (t, J = 10.8 Hz, 1H), 3.12–3.34 (m, 3H), 3.56–3.72 (m, 2H), 3.79 (s, 3H), 4.07 (d, J = 12.8 Hz, 1H), 4.51 (d, J = 11.4 Hz, 1H), 6.81 (t, J = 7.2 Hz, 1H), 6.92–7.23 (m, 17H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 53.01, 53.09, 54.20, 54.28, 54.48, 60.15, 70.11, 112.68, 114.10, 114.17, 114.38, 115.03, 115.25, 119.51, 124.23, 126.67, 126.74, 127.73, 129.05, 130.06, 130.14, 131.09, 131.13, 136.22, 136.25, 145.50, 150.45, 157.85, 159.69, 162.15. IR (KBr, cm^{-1}) 2985, 2876, 1608, 1554, 1273, 1147, 1068; ESI-MS: 544.2($C_{33}H_{29}F_2N_3NaO$, $[M+Na]^+$). Anal. Calcd. for $C_{33}H_{29}F_2N_3O$: C, 75.92; H, 5.67; N, 8.02. Found: C, 75.99; H, 5.60; N, 8.06.

*7-(4-Chlorobenzylidene)-5-(4-methoxylbenzyl)-3,3a,4,5,6,7-hexahydro-3-(4-chlorophenyl)-2-phenyl-2H-pyrazolo[4,3-c]pyridine (**II$_{10}$**)*

Yield, 73%; chartreuse solid; mp 200–201 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 2.43 (t, J = 10.3 Hz, 1H), 3.12–3.34 (m, 3H), 3.55–3.61 (m, 2H), 3.78 (s, 3H), 4.14 (d, J = 11.5 Hz, 1H), 4.52 (d, J = 12.3 Hz, 1H), 6.81 (t, J = 7.2 Hz, 1H), 6.92–7.23 (m, 17H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 52.99, 53.84, 54.20, 54.39, 60.12, 70.11, 112.70, 114.07, 119.61, 124.07, 126.46, 127.47, 127.76, 128.24, 128.41, 128.79, 129.03, 129.64, 132.20, 132.42, 133.42, 139.04, 145.31, 150.28, 157.87. IR (KBr, cm^{-1}) 2982, 2861, 1566, 1554, 1273, 1147, 1082; ESI-MS:576.1 ($C_{33}H_{29}Cl_2N_3NaO$, $[M+Na]^+$). Anal. Calcd. for $C_{33}H_{29}Cl_2N_3O$: C, 71.42; H, 5.27; N, 7.52. Found: C, 71.48; H, 5.27; N, 7.58.

*7-Benzylidene-5-(2-fluorobenzyl)-3,3a,4,5,6,7-hexahydro-2,3-diphenyl-2H-pyrazolo[4,3-c]pyridine (**II$_{11}$**)*

Yield, 88%; chartreuse solid; mp 165–167 °C; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 2.57 (s, 1H), 2.93–3.38 (m, 3H), 3.75(s, 2H), 4.08 (d, J = 14.1Hz, 1H), 4.63 (d, J = 12.4Hz, 1H), 6.77 (t, J = 7.2Hz, 1H), 6.95–7.54 (m, 19H). ^{13}C NMR ($CDCl_3$, 100 MHz) δ 50.06, 53.38, 53.98, 54.56, 71.32, 112.68, 113.52, 114.18, 114.37, 119.21, 122.95, 122.99, 124.98, 126.29, 127.62, 127.77, 128.01, 128.09, 129.92, 130.33, 132.54, 145.82, 150.53, 158.02, 159.05, 161.49. IR (KBr, cm^{-1}) 3447, 1643, 1491, 1420, 1083; ESI-MS: 498.1($C_{32}H_{28}FN_3Na$, $[M+ Na]^+$). Anal. Calcd. for $C_{32}H_{28}FN_3$: C, 81.15; H, 5.93; N, 8.86. Found: C, 81.16; H, 5.96; N, 8.87.

*5-(2-Fluorobenzyl)-7-(4-methylbenzylidene)-3,3a,4,5,6,7-hexahydro-3-(4-methylphenyl)-2-phenyl-2H-pyrazolo[4,3-c]pyridine (**II$_{12}$**)*

Yield, 85%; chartreuse solid; mp 193–195 °C; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 2.06(s,1H), 2.37 (s, 6H), 2.46–3.33 (m, 3H), 3.74 (td, J = 12.0, 7.0 Hz, 2H), 4.14 (dd, J = 14.3,7.2 Hz,1H), 4.58 (d, J = 11.0Hz, 1H), 6.83 (t, J = 7.3Hz, 1H), 7.07–7.54 (m,17H). ^{13}C NMR ($CDCl_3$, 100 MHz) δ 20.11, 20.27, 53.06, 53.33, 54.04, 54.57, 70.63, 114.09, 114.17, 114.39, 119.21, 122.96, 122.99, 125.06, 125.29, 127.64, 127.96, 128.08, 128.43, 128.85, 130.35, 130.41, 132.22,

136.36, 137.57, 145.78, 159.06, 161.50. IR (KBr, cm^{-1}) 3448, 1643, 1485, 1419, 1084, 1022, 1175, 753; ESI-MS: 524.3 ($C_{34}H_{32}FN_3Na$, $[M+Na]^+$). Anal. Calcd. for $C_{34}H_{32}FN_3$: C, 81.42; H, 6.41; N, 8.36. Found: C, 81.41; H, 6.43; N, 8.38.

*5-(2-Fluorobenzyl)-7-(4-methoxylbenzylidene)-3,3a,4,5,6,7-hexahydro-3-(4-methoxylphenyl)-2-phenyl-2H-pyrazolo [4,3-c]pyridine (**II**$_{13}$)*

Yield, 73%; chartreuse solid; mp 176–178 °C; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 2.55 (s,1H), 3.27 (m, 3H), 3.61–3.68 (m, 2H), 3.74 (s, 3H), 3.75 (s, 3H), 3.93 (d, J = 13.9 Hz, 1H), 4.56 (d, J = 12.4 Hz, 1H), 6.74 (dd, J = 4.2, 10.4 Hz, 1H), 6.80–7.26 (m, 17H). ^{13}C NMR ($CDCl_3$, 100 MHz) δ 50.06, 53.36, 53.99, 54.23, 54.53, 70.34, 112.72, 113.53, 114.17, 114.37, 119.20, 122.95, 124.98, 126.29, 127.62, 127.77, 128.01, 128.09, 129.93, 130.32, 132.53, 145.87, 150.52, 157.90, 158.06, 159.04, 161.49. IR (KBr, cm^{-1}) 3438, 1597, 1501, 1424, 1247, 1087, 1028, 759; ESI-MS: 556.1($C_{34}H_{32}FN_3NaO_2$, $[M+Na]^+$). Anal. Calcd. for $C_{34}H_{32}FN_3O_2$: C, 76.52; H, 6.07; N, 7.86. Found: C, 76.51; H, 6.04; N, 7.87.

*5-(2-Fluorobenzyl)-7-(4-fluorobenzylidene)-3,3a,4,5,6,7-hexahydro-3-(4-fluorophenyl)-2-phenyl-2H-pyrazolo[4,3-c]pyridine (**II**$_{14}$)*

Yield, 75%; chartreuse solid; mp 172–174 °C; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 2.57 (s, 1H), 3.14–3.41 (m, 3H), 3.76 (s, 2H), 4.19 (d, J = 13.9 Hz, 1H), 4.61 (d, J = 12.3 Hz, 1H), 6.88 (t, J = 7.2 Hz, 1H), 7.05–7.41 (m, 17H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 53.11, 54.05, 54.46, 60.06, 70.14, 114.11, 114.23, 114.46, 115.03, 115.31, 119.55, 120.11, 124.36, 126.40, 126.69, 126.71, 127.70, 129.34, 130.08, 130.09, 130.43, 131.21, 131.97, 135.45, 136.11, 145.42, 150.10, 159.73, 162.20. IR (KBr, cm^{-1}) 3448, 1598, 1498, 1088, 1027, 835; ESI-MS: 532.2($C_{32}H_{26}F_3N_3Na$,$[M+Na]^+$). Anal. Calcd. for $C_{32}H_{26}F_3N_3$: C,75.41; H, 5.15; N, 8.28. Found: C, 75.43; H, 5.14; N, 8.25.

*7-(4-Chlorobenzylidene)-5-(2-fluorobenzyl)-3,3a,4,5,6,7-hexahydro-3-(4-chlorophenyl)-2-phenyl-2H-pyrazolo[4,3-c]pyridine (**II**$_{15}$)*

Yield, 82%; chartreuse solid; mp 187–189 °C; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 2.56 (s, 1H), 3.12–3.36 (m, 3H), 3.76(s, 2H), 4.01(d, J = 13.9Hz, 1H), 4.61 (d, J = 12.4Hz, 1H), 6.83 (t, J = 7.3Hz, 1H), 6.99–7.41 (m, 17H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 52.78, 53.32, 53.88, 54.46, 70.07, 114.09, 114.24, 114.46, 119.66, 122.61, 122.76, 123.03, 124.16, 126.46, 127.22, 127.49, 127.76, 128.19, 128.43, 129.66, 130.24, 132.28, 132.48, 133.38, 139.01, 145.28, 149.93, 159.03, 161.47. IR (KBr, cm^{-1}) 3449, 1643, 1593, 1488, 1088, 1021, 829; ESI-MS: 564.1 ($C_{32}H_{26}Cl_2FN_3Na$, $[M+Na]^+$). Anal. Calcd. for $C_{32}H_{26}Cl_2FN_3$: C, 70.84; H, 4.84; N, 7.76. Found: C, 70.85; H, 4.83; N, 7.75.

*7-Benzylidene-5-(4-fluorobenzyl)-3,3a,4,5,6,7-hexahydro-2,3-diphenyl-2H-pyrazolo[4,3-c]pyridine (**II**$_{16}$)*

Yield, 78%; chartreuse solid; mp 174–175 °C; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 2.39–2.48 (m, 1H), 3.14–3.33 (m, 3H), 3.54 (d, J = 12.8Hz, 1H), 3.65 (d, J = 13.1Hz, 1H), 4.04 (d, J = 12.1Hz, 1H), 4.55 (d, J = 13.1Hz, 1H), 6.81 (t, J = 7.2Hz, 1H), 6.86–7.00 (m, 4H), 7.04–7.12 (m, 11H), 7.15–7.21 (m, 4H). ^{13}C NMR ($CDCl_3$, 100 MHz) δ 50.12, 53.42, 53.98, 54.34, 71.02, 112.48, 113.58, 114.16, 114.38, 119.26, 122.92, 122.99, 124.98, 126.35, 127.68, 127.77, 128.01, 128.09, 129.98, 130.38, 132.53, 145.88, 150.55, 158.01, 159.05, 161.22. IR (KBr, cm^{-1}) 3002, 2819, 1605, 1581, 1278, 1187, 1090; ESI-MS: 498.2($C_{32}H_{28}FN_3Na$, $[M+Na]^+$). Anal. Calcd. for $C_{32}H_{28}FN_3$: C, 81.17; H, 5.99; N, 8.84. Found: C, 81.16; H, 5.96; N, 8.87.

*5-(4-Fluorobenzyl)-7-(4-methylbenzylidene)-3,3a,4,5,6,7-hexahydro-3-(4-methylphenyl)-2-phenyl-2H-pyrazolo[4,3-c]pyridine (**II**$_{17}$)*

Yield, 67%; chartreuse solid; mp 190–191 °C; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 2.35 (s, 6H), 2.46–3.33 (m, 4H), 3.58 (dd, J = 28.3, 13.2 Hz, 2H), 4.01 (d, J = 13.9 Hz,1H), 4.52 (d, J = 12.5Hz, 1H), 6.84 (t, J = 7.3Hz, 1H), 6.92 (dd, J = 12.1, 5.3 Hz, 2H), 7.04 (dd, J = 8.7, 1.0 Hz, 2H), 7.11–7.21 (m, 10H), 7.25 (s, 1H), 7.26–7.30 (m, 2H). ^{13}C NMR ($CDCl_3$, 100 MHz) δ 20.10, 20.26, 53.36, 53.58, 54.28, 54.87, 70.03, 114.09, 114.16, 114.62, 119.11, 122.46, 122.87, 125.09, 125.31, 127.68, 127.85, 128.11, 128.46, 128.85, 130.32, 130.46, 132.28, 136.36, 137.57, 148.32, 156.02, 159.54. IR (KBr, cm^{-1}) 2998, 2918, 1588, 1482, 1175, 1111, 998; ESI-MS: 524.2 ($C_{34}H_{32}FN_3Na$, $[M+Na]^+$). Anal. Calcd. for $C_{34}H_{32}FN_3$: C, 81.47; H, 6.49; N, 8.34. Found: C, 81.41; H, 6.43; N, 8.38.

*5-(4-Fluorobenzyl)-7-(4-isopropylbenzylidene)-3,3a,4,5,6,7-hexahydro-3-(4-isopropylphenyl)-2-phenyl-2H-pyrazolo [4,3-c]pyridine (**II**$_{18}$)*

Yield, 75%; chartreuse solid; mp 148–150 °C; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 1.16 (d, J = 8.2Hz, 12H), 2.47 (t, J = 10.3 Hz, 1H), 2.76–2.83 (m, 2H), 3.12–3.34 (m, 3H), 3.61 (dd, J = 13.1, 38.3 Hz, 2H), 4.03 (d, J = 13.9 Hz, 1H), 4.52 (d, J = 12.3 Hz, 1H), 6.81 (t, J = 7.2 Hz, 1H), 6.83– 6.92 (m, 4H), 7.05 (t, J = 8.1 Hz, 4H), 7.08–7.23 (m, 7H), 7.29 (d, J = 8.7 Hz, 2H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 18.26, 19.03, 30.07, 30.12, 50.28, 53.46, 54.22, 59.48, 71.26, 112.52, 112.68, 114.26, 114.38, 119.64, 122.88, 122.94, 126.26, 127.37, 127.68, 128.22, 128.43, 130.23, 130.59, 133.34, 141.82, 146.52, 151.95, 154.02, 159.12, 160.03. IR (KBr, cm^{-1}) 2996, 2901, 1620, 1579, 1273, 1207, 1004; ESI-MS: 580.3($C_{38}H_{40}FN_3Na$, $[M+Na]^+$). Anal. Calcd. for $C_{38}H_{40}FN_3$: C, 81.82; H, 7.27; N, 7.52. Found: C, 81.83; H,7.23; N, 7.53.

*5-(4-Fluorobenzyl)-7-(4-methoxylbenzylidene)-3,3a,4,5,6,7-hexahydro-3-(4-methoxylphenyl)-2-phenyl-2H-pyrazolo [4,3-c]pyridine (**II$_{19}$**)*

Yield, 73%; chartreuse solid; mp 159–161 °C; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 2.39 (t, J = 10.5 Hz,1H), 3.01–3.20 (m, 3H), 3.53 (dd, J = 13.2, 28.0 Hz, 2H), 3.75 (s, 3H), 3.76 (s, 3H), 3.93 (d, J = 13.9 Hz, 1H), 4.46 (d, J = 12.5 Hz, 1H), 6.74 (dd, J = 4.2, 10.4 Hz, 1H), 6.80 (dd, J = 5.4, 9.7 Hz, 3H), 6.89–6.82 (m, 2H), 6.97 (dd, J = 1.0, 8.8 Hz, 2H), 7.14–7.26 (m, 10H). ^{13}C NMR ($CDCl_3$, 100 MHz) δ 50.21, 53.46, 53.92, 54.23, 59.42, 71.30, 112.52, 113.55, 114.23, 114.38, 119.35, 122.88, 124.94, 126.36, 127.48, 127.72, 128.01, 128.46, 129.93, 130.54, 132.31, 148.87, 151.52, 152.90, 156.06, 158.04, 160.42. IR (KBr, cm^{-1}) 2994, 2877, 1683, 1584, 1224, 1207, 1082; ESI-MS: 556.2 ($C_{34}H_{32}FN_3NaO_2$, [M+Na]$^+$). Anal. Calcd. for $C_{34}H_{32}FN_3O_2$: C, 76.52; H, 6.07; N, 7.86. Found: C, 76.51; H, 6.04; N, 7.87.

*5-(4-Fluorobenzyl)-7-(3-fluorobenzylidene)-3,3a,4,5,6,7-hexahydro-3-(3-fluorophenyl)-2-phenyl-2H-pyrazolo[4,3-c]pyridine (**II$_{20}$**)*

Yield, 80%; chartreuse solid; mp 153–155 °C; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 2.40 (t, J = 10.8 Hz, 1H), 3.12-3.26 (m, 3H), 3.51 (d, J =13.2Hz, 1H), 3.69 (d, J =13.2Hz, 1H), 4.02 (d, J =12.5Hz, 1H), 4.61 (d, J =13.6Hz, 1H), 6.80 (t, J =7.3Hz, 1H), 6.82–6.88 (m, 8H), 7.03–7.06 (m, 4H), 7.09–7.16 (m, 2H), 7.21–7.25 (m, 3H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 50.11, 53.05, 57.46, 58.06, 71.14, 112.11, 112.23, 113.46, 114.88, 114.91, 119.50, 122.15, 124.56, 126.42, 126.66, 126.70, 127.55, 129.34, 130.34, 130.49, 130.83, 131.21, 131.97, 135.45, 136.11, 141.42, 155.10, 157.23, 161.20. IR (KBr, cm^{-1}) 3020, 2917, 1633, 1577, 1265, 1207, 1012; ESI-MS: 532.2($C_{32}H_{26}F_3N_3Na$, [M+Na]$^+$). Anal. Calcd. for $C_{32}H_{26}F_3N_3$: C,75.42; H, 5.14; N, 8.26. Found: C, 75.43; H, 5.14; N, 8.25.

*5-(4-Fluorobenzyl)-7-(4-fluorobenzylidene)-3,3a,4,5,6,7-hexahydro-3-(4-fluorophenyl)-2-phenyl-2H-pyrazolo[4,3-c]pyridine (**II$_{21}$**)*

Yield, 78%; chartreuse solid; mp 196–197 °C; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 2.44 (t, J = 11.3 Hz, 1H), 3.14–3.28 (m, 3H), 3.54 (d, J = 13.0Hz, 1H), 3.68 (d, J = 13.4Hz, 1H), 4.04 (d, J = 13.5Hz, 1H), 4.55 (d, J = 12.6Hz, 1H), 6.83 (t, J = 7.5Hz, 1H), 6.92–7.33 (m, 17H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 53.12, 54.14, 54.46, 60.01, 70.12, 114.20, 114.28, 114.46, 115.03, 115.38, 119.42, 120.11, 124.36, 126.38, 126.69, 126.71, 127.70, 129.39, 131.08, 131.09, 131.43, 135.21, 135.97, 136.45, 138.16, 149.45, 151.13, 157.62, 159.98. IR (KBr, cm^{-1}) 3022, 2918, 1603, 1584, 1273, 1200, 1082; ESI-MS: 532.3($C_{32}H_{26}F_3N_3Na$, [M+Na]$^+$). Anal. Calcd. for $C_{32}H_{26}F_3N_3$: C,75.42; H, 5.14; N, 8.26. Found: C, 75.43; H, 5.14; N, 8.25.

*7-(4-Chlorobenzylidene)-5-(4-fluorobenzyl)-3,3a,4,5,6,7-hexahydro-3-(4-chlorophenyl)-2-phenyl-2H-pyrazolo[4,3-c]pyridine (**II$_{22}$**)*

Yield, 69%; chartreuse solid; mp 210–212 °C; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 2.40–2.85 (m, 1H), 3.02–3.33 (m, 3H), 3.54 (d, J = 12.4Hz, 1H), 3.65 (d, J = 12.6Hz, 1H), 4.04 (d, J = 13.4Hz, 1H), 4.55 (d, J = 12.0Hz, 1H), 6.81 (t, J = 6.82Hz, 1H), 7.08–7.37 (m, 17H). ^{13}C NMR ($CDCl_3$, 100 MHz) δ 52.89, 53.38, 53.96, 54.38, 70.11, 114.15, 114.24, 114.86, 119.68, 122.67, 122.78, 123.23, 124.16, 126.46, 127.28, 127.41, 127.78, 128.19, 128.46, 129.68, 130.24, 132.28, 132.48, 133.64, 138.55, 145.20, 149.90, 159.55, 162.49. IR (KBr, cm^{-1}) 3025, 2911, 1610, 1584, 1273, 1201, 1003; ESI-MS: 564.1($C_{32}H_{26}Cl_2FN_3Na$, [M+Na]$^+$). Anal. Calcd. for $C_{32}H_{26}Cl_2FN_3$: C, 70.82; H, 4.85; N, 7.73. Found: C, 70.85; H, 4.83; N, 7.75.

*5-(4-Bromobenzyl)-7-benzylidene-3,3a,4,5,6,7-hexahydro-2,3-diphenyl-2H-pyrazolo[4,3-c]pyridine (**II$_{23}$**)*

Yield, 78%; chartreuse solid; mp 178–179 °C; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 2.43 (t, J = 10.9Hz, 1H), 3.14–3.36 (m, 3H), 3.54 (d, J = 12.4Hz, 1H), 3.65 (d, J = 12.8Hz, 1H), 4.04 (d, J = 12.4Hz, 1H), 4.55 (d, J = 13.1Hz, 1H), 6.89 (t, J = 7.41Hz, 1H), 6.98–7.28 (m, 19H). ^{13}C NMR ($CDCl_3$, 100 MHz) δ 51.16, 53.40, 54.01, 54.34, 70.12, 112.48, 112.58, 114.12, 114.38, 119.28, 122.90, 122.97, 126.08, 126.35, 127.68, 127.72, 128.35, 128.76, 129.23, 131.32, 132.53, 145.80, 150.55, 158.01, 159.05, 160.38. IR (KBr, cm^{-1}) 2998, 2919, 1633, 1581, 1223, 1186, 1065; ESI-MS: 555.1 ($C_{32}H_{28}BrN_3Na$, [M+Na]$^+$). Anal. Calcd. for $C_{32}H_{28}BrN_3$: C, 71.97; H, 5.28; N, 7.84; Found: C, 71.91; H, 5.28; N, 7.86.

*5-(4-Bromobenzyl)-7-(4-methylbenzylidene)-3,3a,4,5,6,7-hexahydro-3-(4-methylphenyl)-2-phenyl-2H-pyrazolo[4,3-c]pyridine (**II$_{24}$**)*

Yield, 76%; chartreuse solid; mp 200–202 °C; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 2.33 (s, 6H), 2.46 (t, J = 10.3 Hz, 1H), 3.15–3.36 (m, 3H), 3.50–3.54 (m, 2H), 4.03 (d, J = 12.9 Hz,1H), 4.65 (d, J = 12.5Hz, 1H), 6.81 (t, J = 7.3Hz, 1H), 6.92–7.30 (m, 17H). ^{13}C NMR ($CDCl_3$, 100 MHz) δ 20.16, 21.09, 53.26, 53.73, 54.18, 54.89, 71.25, 112.09, 112.16, 114.62, 119.13, 122.46, 122.87, 125.09, 125.31, 127.68, 127.85, 128.11, 128.46, 128.89, 130.32, 130.48, 132.18, 137.38, 138.54, 148.63, 158.68, 160.09. IR (KBr, cm^{-1}) 3022, 2918, 1644, 1584, 1273, 1207, 1000; ESI-MS: 584.1 ($C_{34}H_{32}BrN_3$ Na, [M+Na]$^+$). Anal. Calcd. for $C_{34}H_{32}BrN_3$: C, 72.57; H, 5.79; N, 7.44. Found: C, 72.59; H, 5.73; N, 7.47.

*5-(4-Bromobenzyl)-7-(4-methoxylbenzylidene)-3,3a,4,5,6,7-hexahydro-3-(4-methoxylphenyl)-2-phenyl-2H-pyrazolo 4,3-c]pyridine (**II$_{25}$**)*

Yield, 85%; chartreuse solid; mp 203-205 °C; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 2.38 (t, J = 10.8 Hz, 1H), 3.14-3.37 (m,

3H), 3.50-3.74 (m, 2H), 3.88(s, 6H), 4.00 (d, J = 13.9 Hz,1H), 4.58 (d, J =12.5Hz, 1H), 6.81 (t, J =7.3Hz, 1H), 6.96-7.38 (m, 17H). ^{13}C NMR ($CDCl_3$, 100 MHz) δ 53.50, 54.02, 54.23, 54.40, 58.13, 70.48, 112.81, 113.52, 114.15, 119.31, 122.48, 124.61, 125.17, 126.29, 127.61, 127.63, 129.86, 132.01, 132.31, 135.57, 138.47, 145.82, 147.85, 149.01, 150.23, 157.98, 158.18. IR (KBr, cm^{-1}) 3448, 1596, 1501, 1424, 1247, 1087, 1027; ESI-MS: 616.1 ($C_{34}H_{32}BrN_3NaO_2$, [M+Na]$^+$). Anal. Calcd. for $C_{34}H_{32}BrN_3O_2$: C, 68.67; H, 5.49; N, 7.04. Found: C, 68.69; H, 5.43; N, 7.07.

*5-(4-Bromobenzyl)-7-(4-fluorobenzylidene)-3,3a,4,5,6,7-hexahydro-3-(4-fluorophenyl)-2-phenyl-2H-pyrazolo 4,3-c]pyridine (**II$_{26}$**)*

Yield, 81%; chartreuse solid; mp 197–199 °C; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 2.46 (t, J = 10.3 Hz, 1H), 3.15-3.36 (m, 3H), 3.50–3.54 (m, 2H), 4.03 (d, J = 12.9 Hz, 1H), 4.65 (d, J = 12.8Hz, 1H), 6.81 (t, J = 7.3Hz, 1H), 6.92–7.30 (m, 17H). ^{13}C NMR ($CDCl_3$, 100 MHz) δ 53.23, 54.08, 54.47, 60.06, 70.10, 114.12, 114.25, 114.46, 115.09, 115.30, 119.58, 120.14, 124.34, 126.47, 126.64, 126.72, 127.75, 129.35, 130.01, 130.09, 130.45, 130.97, 131.00, 135.55, 136.12, 136.15, 145.42, 150.10, 159.73, 162.20. IR (KBr, cm^{-1}) 3447, 1597, 1496, 1088, 1027, 835, 753; ESI-MS: 592.1 ($C_{32}H_{26}BrF_2N_3Na$, [M+Na]$^+$). Anal. Calcd. for $C_{32}H_{26}BrF_2N_3$: C, 67.37; H, 4.59; N, 7.34. Found: C, 67.37; H, 4.59; N, 7.37.

*5-(4-Bromobenzyl)-7-(4-chlorobenzylidene)-3,3a,4,5,6,7-hexahydro-3-(4-chlorophenyl)-2-phenyl-2H-pyrazolo 4,3-c] pyridine (**II$_{27}$**)*

Yield, 79%; chartreuse solid; mp 206–207 °C; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 2.37 (t, J = 11.3 Hz, 1H), 3.17–3.46 (m, 3H), 3.55–3.62 (m, 2H), 4.17 (d, J = 12.9 Hz, 1H), 4.75 (d, J = 12.5Hz, 1H), 6.87 (t, J = 7.1Hz, 1H), 6.90–7.34 (m, 17H). ^{13}C NMR ($CDCl_3$, 100 MHz) δ 53.37, 53.87, 54.33, 58.12, 70.16, 114.11, 119.77, 122.45, 124.31, 126.43, 126.93, 127.51, 127.70, 128.45, 129.56, 131.84, 132.40, 132.50, 133.50, 135.30, 138.63, 138.81, 145.28, 147.93, 149.07, 149.72. IR (KBr, cm^{-1}) 3449, 1643, 1594, 1487, 1088, 1027, 829; ESI-MS: 624.0 ($C_{32}H_{26}BrCl_2N_3Na$, [M+Na]$^+$). Anal. Calcd. for $C_{32}H_{26}BrCl_2N_3$: C, 63.68; H, 4.30; N, 6.94. Found: C, 63.70; H, 4.34; N, 6.96.

*7-Benzylidene-5-(3-pyridylmethyl)-3,3a,4,5,6,7-hexahydro-2,3-diphenyl-2H-pyrazolo[4,3-c]pyridine (**II$_{28}$**)*

Yield, 71%; chartreuse solid; mp 213–215 °C; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 2.63–2.68 (m, 1H), 3.37 (s, 3H), 3.71–3.83 (m, 2H), 4.06 (d, J = 14.3 Hz,1H), 4.64 (d, J = 12.6 Hz, 1H), 6.86 (t, J = 7.2Hz, 1H), 7.02–7.45 (m, 17H), 8.50(d, J = 3.3 Hz ,2H). ^{13}C NMR ($CDCl_3$, 100 MHz) δ 53.52, 54.09, 54.43, 57.21, 70.16, 114.09, 119.29, 122.35, 125.02, 125.50, 125.66, 127.67, 128.04, 128.38, 128.89, 132.07, 132.58, 133.24, 135.44, 138.85, 145.21, 147.95, 149.09, 149.75. IR (KBr, cm^{-1}) 3444, 1643, 1419, 1082, 1022, 843, 751; ESI-MS: 479.2 ($C_{31}H_{28}N_4Na$, [M+Na]$^+$); Anal. Calcd. for $C_{31}H_{28}N_4$: C, 81.57; H, 6.19; N, 12.28. Found: C, 81.55; H, 6.18; N, 12.27.

*7-(4-Methylbenzylidene)-5-(3-pyridylmethyl)-3,3a,4,5,6,7-hexahydro-3-(4-methylphenyl)-2-phenyl-2H-pyrazolo 4,3-c]pyridine (**II$_{29}$**)*

Yield, 79%; chartreuse solid; mp 217–219 °C; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 2.13–2.26 (m, 1H), 2.36(s, 6H), 3.17–3.31 (m, 3H), 3.43–3.62 (m, 2H), 4.07 (d, J = 14.2 Hz,1H), 4.66 (d, J = 12.6Hz, 1H), 6.76 (t, J = 7.2Hz, 1H), 7.07–7.52 (m, 15H), 8.41 (d, J = 3.4Hz, 2H). ^{13}C NMR ($CDCl_3$, 100 MHz) δ 20.11, 20.26, 53.56, 54.10, 54.43, 56.09, 70.65, 114.09, 119.28, 122.44, 125.02, 125.50, 125.66, 127.67, 128.04, 128.38, 128.89, 132.07, 135.53, 136.28, 136.44, 137.44, 145.71, 147.75, 148.99, 150.15. IR (KBr, cm^{-1}) 3451, 1643, 1602, 1419, 1084, 1026, 750; ESI-MS: 507.2($C_{33}H_{32}N_4Na$, [M+Na]$^+$); Anal. Calcd. for $C_{33}H_{32}N_4$: C, 81.77; H, 6.69; N, 11.58. Found: C, 81.78; H, 6.66; N, 11.56.

*7-(4-Isopropylbenzylidene)-5-(3-pyridylmethyl)3,3a,4,5,6,7-hexahydro-3-(4-isopropylphenyl)-2-phenyl-2H-pyrazolo 4,3-c]pyridine (**II$_{30}$**)*

Yield, 80%; chartreuse solid; mp 215–216 °C; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 1.46 (m,12H), 2.52–2.54 (m, 1H), 3.12 (m, 2H), 3.16–3.30 (m, 3H), 3.63–3.71 (m, 2H), 4.04 (d, J = 14.2Hz,1H), 4.66 (d, J = 12.6Hz,1H), 6.87 (t, J = 7.2Hz,1H), 6.97–7.75 (m,15H), 8.51(d, J = 3.2Hz, 2H). ^{13}C NMR ($CDCl_3$, 100 MHz) δ 18.28, 19.42, 30.11, 30.26, 51.55, 53.92, 54.43, 58.59, 70.65, 112.23, 119.26, 122.45, 125.02, 125.58, 125.83, 127.69, 128.14, 128.28, 129.21, 131.27, 133.53, 135.41, 135.82, 138.49, 145.71, 147.75, 148.99, 151.18. IR (KBr, cm^{-1}) 3451, 1644, 1602, 1495, 1419, 1384, 1293, 1084; ESI-MS: 563.3($C_{37}H_{40}NaN_4$, [M+Na]$^+$); Anal. Calcd. for $C_{37}H_{40}N_4$: C, 82.17; H, 7.49; N, 10.38. Found: C, 82.18; H, 7.46; N, 10.36.

*7-(4-Methoxylbenzylidene)-5-(3-pyridylmethyl)-3,3a,4,5,6,7-hexahydro-3-(4-methoxylphenyl)-2-phenyl-2H-pyrazolo[4,3-c]pyridine (**II$_{31}$**)*

Yield, 77%; chartreuse solid; mp 187–189 ℃; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 2.53–2.56 (m, 1H), 3.16–3.30 (m, 3H), 3.56–3.72 (m, 2H), 3.78 (s, 6H), 3.97 (d, J = 14.2 Hz,1H), 4.56 (d, J = 12.6Hz, 1H), 6.77 (t, J = 7.2Hz, 1H), 6.97–7.68 (m, 15H), 8.41 (d, J = 3.1Hz, 2H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 53.55, 54.03, 54.25, 54.41, 58.12, 70.44, 112.80, 113.59, 114.18, 119.30, 122.45, 124.61, 125.19, 126.27, 127.60, 127.65, 129.88, 132.07, 132.36, 135.51, 145.80, 147.80, 149.02, 150.29, 157.99, 158.12. IR (KBr, cm^{-1})

3452, 1644, 1496, 1419, 1083, 1027, 750; ESI-MS: 539.2($C_{33}H_{32}N_4NaO_2$, $[M+Na]^+$); Anal. Calcd. for $C_{33}H_{32}N_4O_2$: C, 76.77; H, 6.29; N, 10.88. Found: C, 76.72; H, 6.24; N, 10.84.

*7-(3-Fluorobenzylidene)-5-(3-pyridylmethyl)-3,3a,4,5,6,7-hexahydro-3-(3-fluorophenyl)-2-phenyl-2H-pyrazolo [4,3-c]pyridine (**II$_{32}$**)*

Yield, 73%; chartreuse solid; mp 205–206 °C; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 2.57–2.59 (m, 1H), 3.17–3.31 (m, 3H), 3.57–3.73 (m, 2H), 4.07 (d, J = 14.2 Hz,1H), 4.59 (d, J = 12.6Hz, 1H), 6.85 (t, J = 7.2Hz, 1H), 6.96–7.61 (m, 15H), 8.45 (d, J = 3.2 Hz, 2H). ^{13}C NMR ($CDCl_3$, 100 MHz) δ 53.01, 53.38, 54.28, 58.28, 70.11, 112.72, 114.10, 114.17, 114.38, 115.17, 115.25, 119.51, 124.23, 126.67, 126.74, 127.73, 129.05, 130.06, 130.14, 131.09, 131.13, 136.22, 136.25, 145.73, 151.78, 159.69, 162.10. IR (KBr, cm^{-1}) 3451, 1643, 1495, 1419, 1024, 750; ESI-MS: 515.2 ($C_{31}H_{26}F_2NaN_4$, $[M+Na]^+$); Anal. Calcd. for $C_{31}H_{26}F_2N_4$: C, 75.57; H, 5.33; N, 11.38. Found: C, 75.59; H, 5.32; N, 11.37.

*7-(4-Fluorobenzylidene)-5-(3-pyridylmethyl)-3,3a,4,5,6,7-hexahydro-3-(4-fluorophenyl)-2-phenyl-2H-pyrazolo [4,3-c]pyridine (**II$_{33}$**)*

Yield, 79%; chartreuse solid; mp 163–164 °C; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 2.53–2.56 (m, 1H), 3.16–3.30 (m, 3H), 3.56–3.72 (m, 2H), 4.07 (d, J = 14.2 Hz,1H), 4.56 (d, J = 12.6 Hz, 1H), 6.77 (t, J = 7.2Hz, 1H), 6.97–7.68 (m, 15H), 8.41 (d, J = 3.1Hz, 2H). ^{13}C NMR ($CDCl_3$, 100 MHz) δ 52.01, 54.07, 56.11, 57.59, 71.07, 114.35, 119.86, 122.32, 124.64, 126.28, 126.95, 127.58, 128.19, 128.55, 129.44, 131.46, 132.74, 132.98, 133.68, 135.50, 138.83, 146.20, 148.12, 151.36, 159.65. IR (KBr, cm^{-1}) 3453, 1644, 1484, 1417, 1085, 1022, 833; ESI-MS: 515.2($C_{31}H_{26}F_2N_4Na$, $[M+Na]^+$); Anal. Calcd. for $C_{31}H_{26}F_2N_4$: C, 81.57; H, 6.19; N, 12.28. Found: C, 81.55; H, 6.18; N, 12.27.

*7-(4-Chlorobenzylidene)-5-(3-pyridylmethyl)-3,3a,4,5,6,7-hexahydro-3-(4-chlorophenyl)-2-phenyl-2H-pyrazolo[4,3-c]pyridine (**II$_{34}$**)*

Yield, 86%; chartreuse solid; mp 156–157 °C; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 2.47–2.51 (m, 1H), 3.12–3.29 (m, 3H), 3.53–3.73 (m, 2H), 4.11 (d, J = 14.2 Hz,1H), 4.56 (d, J = 12.6 Hz, 1H), 6.87 (t, J = 7.2Hz, 1H), 7.01–7.59 (m, 15H), 8.41(d, J = 3.3Hz, 2H). ^{13}C NMR ($CDCl_3$, 100 MHz) δ 53.35, 53.90, 54.38, 58.10, 70.12, 114.11, 119.74, 122.44, 124.30, 126.44, 126.93, 127.57, 127.79, 128.49, 129.60, 131.86, 132.41, 132.56, 133.56, 135.34, 138.85, 145.21, 147.96, 149.04, 149.74. IR (KBr, cm^{-1}) 3451, 1643, 1486, 1416, 1085, 1021, 833; ESI-MS: 547.1($C_{31}H_{26}Cl_2N_4Na$, $[M+Na]^+$); Anal. Calcd. for $C_{31}H_{26}Cl_2N_4$: C, 70.83; H, 4.95; N, 10.69. Found: C, 70.86; H, 4.99; N, 10.66.

*7-Benzylidene-5-(2-furfuryl)-3,3a,4,5,6,7-hexahydro-2,3-diphenyl-2H-pyrazolo[4,3-c]pyridine (**II$_{35}$**)*

Yield, 77%; chartreuse solid; mp 223–225 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 2.52 (s, 1H), 3.21–3.43 (m, 3H), 3.66–3.98 (m, 2H), 4.06 (d, J = 14.1Hz, 1H), 4.63 (d, J = 12.4 Hz, 1H), 6.17（s，1H）, 6.31（s，1H）, 6.80 (t, J = 7.2Hz，1H), 7.07–7.51 (m, 16H). ^{13}C NMR ($CDCl_3$, 100 MHz) δ 52.37, 52.81, 53.26, 54.25, 70.59, 107.97, 109.59, 114.28, 119.21, 125.21, 125.47, 125.86, 127.32, 127.87, 128.35, 128.85, 132.55, 136.29, 136.33, 137.63, 141.69, 145.86, 150.18, 150.42. IR (KBr, cm^{-1}) 3444, 1641,1599, 1494, 1436,1081,749; ESI-MS:468.2 ($C_{30}H_{27}N_3NaO$, $[M+Na]^+$); Anal. Calcd. for $C_{30}H_{27}N_3O$: C, 80.83; H, 6.15; N, 9.49. Found: C, 80.87; H, 6.11; N, 9.43.

*5-(2-Furfuryl)-7-(4-methylbenzylidene)-3,3a,4,5,6,7-hexahydro-3-(4-methylphenyl)-2-phenyl-2H-pyrazolo[4,3-c]pyridine (**II$_{36}$**)*

Yield, 79%; chartreuse solid; mp 227–228 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 2.21 (d, J = 13.0 Hz, 1H), 2.38 (s, 6H) , 3.21–3.31 (m, 3H), 3.65–3.75 (m, 2H), 4.05 (d, J = 14.2 Hz,1H), 4.59 (d, J = 11.2 Hz, 1H), 6.14 (s, 1H), 6.32 (s, 1H), 6.83 (t, J = 7.1 Hz, 1H), 7.03–7.38 (m,14H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 20.11, 20.28, 52.68, 52.76, 53.77, 54.65, 70.57, 107.97, 109.09, 114.07, 119.18, 125.04, 125.33, 125.93, 127.62, 127.97, 128.48, 128.82, 132.26, 136.28, 136.31, 137.57, 141.28, 145.80, 150.12, 150.35. IR (KBr, cm^{-1}) 3445,2357, 1643, 1495, 1420, 1292, 1082, 1022; ESI-MS: 496.2($C_{32}H_{31}N_3NaO$, $[M+Na]^+$); Anal. Calcd. for $C_{32}H_{31}N_3O$: C, 81.17; H, 6.69; N, 8.88. Found: C, 81.15; H, 6.60; N, 8.87.

*5-(2-Furfuryl)-7-(4-isopropylbenzylidene)-3,3a,4,5,6,7-hexahydro-3-(4-isopropylphenyl)-2-phenyl-2H-pyrazolo [4,3-c]pyridine (**II$_{37}$**)*

Yield, 71%; chartreuse solid; mp 222–223 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.55 (m,12H) , 2.52–2.54 (m, 1H), 3.12 (m, 2H) 3.16–3.30 (m, 3H), 3.62–3.69 (m, 2H), 4.03 (d, J=14.2 Hz, 1H),4.65 (d, J = 12.6Hz, 1H), 6.13 (s, 1H), 6.34 (s,1H), 6.87 (t, J = 7.2Hz, 1H), 7.11–7.72 (m, 14H). ^{13}C NMR ($CDCl_3$, 100 MHz) δ 18.01, 18.48, 30.22, 31.04, 51.08, 52.76, 55.43, 58.86, 70.57, 107.03, 109.21, 112.15, 119.35, 122.43, 122.64, 124.86, 126.42, 127.84, 128.49, 128.96, 131.88, 135.30, 135.78, 138.52, 141.28, 146.80, 152.25, 158.48. IR (KBr, cm^{-1}) 3444, 2356, 1642, 1494, 1421, 1291, 1081, 1032; ESI-MS: 552.3($C_{36}H_{39}N_3NaO$, $[M+Na]^+$); Anal. Calcd. for $C_{36}H_{39}N_3O$: C, 81.67; H, 7.49; N, 7.92. Found: C, 81.63; H, 7.42; N, 7.93.

*5-(2-Furfuryl)-7-(4-methoxylbenzylidene)-3,3a,4,5,6,7-hexahydro-3-(4-methoxylphenyl)-2-phenyl-2H-pyrazolo[4,3-c]pyridine (**II$_{38}$**)*

Yield, 81%; chartreuse solid; mp 225–226 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 2.27–2.34 (m, 1H), 3.12–3.21 (m, 3H),

3.63–3.74 (m, 2H), 3.77 (s, 3H), 3.78 (s, 3H), 4.07 (d, J = 13.9 Hz, 1H), 4.59 (d, J = 11.2 Hz, 1H), 6.18 (s, 1H), 6.37 (s, 1H), 6.81 (t, J = 7.2Hz, 1H), 7.03–7.47 (m, 14H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 50.08, 52.73, 53.97, 54.53, 54.68, 70.35, 107.62, 109.22, 114.82, 119.15, 122.94, 124.33, 125.27, 127.30, 127.47, 128.27, 128.82, 132.53, 136.28, 136.31, 137.57, 141.28, 145.87, 150.52, 158.05. IR (KBr, cm^{-1}) 3444, 1643, 1594, 1420, 1082, 1021, 811, 548; ESI-MS: 528.2($C_{32}H_{31}N_3NaO_3$, [M+Na]$^+$); Anal. Calcd. for $C_{32}H_{31}N_3O_3$: C, 76.07; H, 6.19; N, 8.32. Found: C, 76.02; H, 6.18; N, 8.31.

*5-(2-Furfuryl)-7-(3-fluorobenzylidene)-3,3a,4,5,6,7-hexahydro-3-(3-fluorophenyl)-2-phenyl-2H-pyrazolo[4,3-c]pyridine (**II$_{39}$**)*

Yield, 82%; chartreuse solid; mp 231–232 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 2.53–2.56 (m, 1H), 3.17–3.31 (m, 3H), 3.57–3.73 (m, 2H), 4.07 (d, J = 14.2 Hz, 1H), 4.59 (d, J = 12.6 Hz, 1H), 6.15 (s, 1H), 6.32 (s, 1H), 6.85 (t, J = 7.2 Hz, 1H), 7.04–7.59 (m, 14H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 53.02, 54.53, 54.97, 60.03, 70.15, 108.12, 109.66, 114.15, 118.96, 120.94, 124.36, 126.46, 127.62, 127.79, 128.47, 128.62, 130.53, 135.28, 135.31, 137.57, 145.08, 150.07, 150.52, 154.05. IR (KBr, cm^{-1}) 3448, 1642, 1599, 1493, 1438, 1083, 749; ESI-MS: 504.1($C_{30}H_{25}F_2N_3NaO$, [M+Na]$^+$). Anal. Calcd. for $C_{30}H_{25}F_2N_3O$: C, 74.81; H, 5.24; N, 8.74. Found: C, 74.83; H, 5.23; N, 8.73.

*5-(2-Furfuryl)-7-(4-fluorobenzylidene)-3,3a,4,5,6,7-hexahydro-3-(4-fluorophenyl)-2-phenyl-2H-pyrazolo[4,3-c]pyridine (**II$_{40}$**)*

Yield, 79%; chartreuse solid; mp 237–238 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 2.43–2.49 (m, 1H), 3.15–3.32 (m, 3H), 3.47–3.63 (m, 2H), 4.07 (d, J = 14.1 Hz, 1H), 4.49 (d, J = 12.7Hz, 1H), 6.11 (s, 1H), ,6.37 (s, 1H), 6.81 (t, J = 7.2Hz, 1H), 7.01–7.72 (m, 14H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 53.01, 53.09, 54.19, 60.13, 71.11, 107.57, 109.04, 112.65, 119.56, 122.24, 124.36, 126.73, 127.52, 127.82, 129.17, 129.82, 132.33, 134.56, 135.31, 138.68, 145.74, 151.26, 152.56, 155.47. IR (KBr, cm^{-1}) 3445, 1640, 1597, 1495, 1436, 1081, 749; ESI-MS: 504.2 ($C_{30}H_{25}F_2N_3NaO$, [M+Na]$^+$); Anal. Calcd. for $C_{30}H_{25}F_2N_3O$: C, 74.80; H, 5.23; N, 8.75. Found: C, 74.83; H, 5.23; N, 8.73.

*5-(2-Furfuryl)-7-(4-chlorobenzylidene)-3,3a,4,5,6,7-hexahydro-3-(4-chlorophenyl)-2-phenyl-2H-pyrazolo[4,3-c]pyridine (**II$_{41}$**)*

Yield, 89%; chartreuse solid; mp 234–235 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 2.23–2.46 (m, 1H), 3.12–3.31 (m, 3H), 3.54–3.71 (m, 2H), 4.03 (d, J = 14.1 Hz, 1H), 4.61 (d, J = 12.6Hz, 1H), 6.25 (s, 1H), 6.42 (s, 1H), 6.95 (t, J = 7.2Hz, 1H), 7.14–7.74 (m, 14H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 52.92, 53.32, 53.88, 58.20, 70.08, 107.60, 109.25, 114.21, 119.56, 122.24, 122.86, 124.13, 126.52, 127.24, 128.43, 129.82, 130.33, 130.46, 132.47, 139.68, 145.33, 149.26, 159.56, 161.49. IR (KBr, cm^{-1}) 3446, 1641, 1601, 1495, 1435, 1082, 889; ESI-MS: 536.1 ($C_{30}H_{25}Cl_2N_3NaO$, [M+Na]$^+$); Anal. Calcd. for $C_{30}H_{25}Cl_2N_3O$: C, 70.03; H, 4.95; N, 8.19. Found: C, 70.04; H, 4.90; N, 8.17.

2.3 X-ray crystallography

Compound (**II$_1$**) was recrystallized by slow evaporation from a mixed solution of methylene dichloride/ethanol (v:v = 1:5). A chartreuse crystal of (**II$_1$**) with dimensions of 0.16 mm x 0.15 mm x 0.10 mm was mounted on a glass fiber for data collection which was made on a Bruker SMART APEX 1000 CCD diffractometer equipped with a graphite-monochromatic Mo Kα radiation (λ = 0.71073 Å) by using a φ–ω scan mode in the range $2.35 \leqslant \theta \leqslant 20.55°$ ($-7 \leqslant h \leqslant 7$, $-11 \leqslant k \leqslant 11$, $-69 \leqslant l \leqslant 69$) at 298(2) K. A total of 30742 reflections were collected with 6366 unique ones (R_{int} = 0.0545), of which 3804 with $I > 2\sigma(I)$ were considered as observed and used in the succeeding refinements. The intensity data were corrected for Lp factors and empirical absorption. All of the non-hydrogen atoms were refined anisotropically, and hydrogen atoms were located at their idealized positions. The structure was solved by direct methods and refined by full-matrix least-squares techniques on F^2 using the SHELXTL program package. All of the non-hydrogen atoms were refined anisotropically, and hydrogen atoms were located at their idealized positions. The final R = 0.0545, wR = 0.1211 ($w = 1/[\sigma^2(Fo^2) + (0.0568P)^2 + 0.2960P]$, where $P = (Fo^2 + 2Fc^2)/3$), S = 1.025, $(\Delta/\sigma)_{max}$ = 0.093, $(\Delta\rho)_{max}$ = 0.186 and $(\Delta\rho)_{min}$ = –0.189 e/Å^3. The structural graphics were drawn with the SHELXTL-97 software package. Other details of the structure have been deposited with the Cambridge Crystallographic Data Centre, CCDC 845735.

2.4 Fluorescence measurements

The single-photon fluorescence spectra were collected on a PerkinElmer LS55 luminescence spectrometer. Visible absorption spectra were determined on PerkinElmer Lambda 35 spectrophotometer.

The solution concentration of the compounds in CH_2Cl_2 was 1.0×10^{-5} mol L^{-1} and the samples were excited at a wavelength of 360 nm. The photoluminescence quantum efficiencies of the compounds and their derivatives were calculated using 9,10-diphenylanthracene as the standard [19].

2.5 Biology

2.5.1 Cell lines and culture

The anti-breast cancer activity tests of the *2H*-pyrazolo [4,3-c]hexahydropyridine derivatives (**II**) were evaluated

with human breast cancer MCF-7 and MDA-MB-231 cell lines by the standard MTT assay in vitro [20]. Human breast cancer cells MCF-7 and MDA-MB-231 cell lines were cultured in RPMI 1640 medium at 37 °C with 5% CO_2, and 95% air, supplemented with 10% (*v*/*v*) bovine calf serum and 80 U mL^{-1} gentamicin. The cells were seeded onto 96-well plates at the density of 6250 cm^{-2}. About 10 mg of the title compounds were dissolved in 100 μL of dimethylsulfoxide (DMSO), then concentrations of 1.0, 10.0, and 100.0 μg mL^{-1} were adjusted stepwise in each 96-well plates, and the concentration of DMSO was 1‰. One hundred thousand cells, 20 μL of FBS, RPMI-1640 and different concentrations of the title compounds were seeded in each 100 μL well of a 96-well plate. Each group had eight wells. The control well of 5-fluorouracil (5-FU) was established and the wells (containing cells) were incubated. After 44 h in a humidified atmosphere with 5% CO_2 at 37 °C, 10 μL of MTT (5 mg/mL) was added to every well, and 100 μL of 10% sodium dodecyl sulfate (SDS) solution was added to stop the reaction after 4 h. After leaving overnight at 37 °C, the number of absorbency (A) of every well was measured by enzyme immunity at 570 nm.

2.5.2 Apoptosis detection

Fluorescein isothiocyanate (FITC)-conjugated annexin V was utilized to detect the externalization of phosphatidylserine that occurs at an early stage of apoptosis. Propidium iodide (PI) was used as a marker of necrosis due to cell membrane destruction. To elucidate whether compound-induced cell death involved apoptosis or necrosis, we performed a flow cytometric analysis using annexin V and PI double-staining.

2.5.3 Electrophoretic analysis of DNA fragmentation

Human breast cancer cells MCF-7 were lysed in 200.0 mL lysis buffer (10.0 mM EDTA:50.0 mM Tris–HCl, pH 8.0; 0.5% sodium lauryl sulfate; 100.0 mg mL^{-1} proteinase K) at 37 °C for 12 h, then incubated with RNase (50.0 mg mL^{-1}) at 37 °C for an additional 1 h. After incubation, DNA in the lysate was extracted with an equal volume of phenol/chloroform/isoamyl alcohol (25:24:1), then with chloroform. DNA was precipitated with two volumes of ethanol in the presence of 0.3 M sodium acetate. After centrifugation at 12,000 g for 15 min, the DNA pellets were washed with 70% ethanol, air-dried, and resuspended in 20.0 mL of TE (10.0 mM Tris–HCl and 1.0 mM EDTA, pH 8.0). DNA was separated on 1.5% agarose gels containing 0.5 mg mL^{-1} ethidium bromide and photographed by Bio-Rad GD2000 (Bio-Rad, Hercules, CA, USA).

3 Results and discussion

3.1 Crystal structure analysis

The structure of compound (**II_1**) was unambiguously confirmed by single crystal X-ray diffraction. Figure 1 shows that the structure is composed of six rings: four benzene rings, one hexahydropyridine ring and one dihydropyrazolo ring. The benzene rings are marked A, B, C, and D. The dihedral angle between the mean planes of the benzene A and benzene B rings is 60.06 (0.05)°, between benzene C and benzene D rings is 79.16 (0.06)°. In the structure of compound **II_1**, the hexahydropyridine ring adopts a skew boat conformation and the dihedral angle between the benzene A and hexahydropyridine (N1–C9–C11–C12) rings is 86.46 (0.06)°. The carbon–carbon double bonds (C10–C14) in **II_1** clearly adopt an (*E*)-configuration. The steric repulsion is reduced by the expansion of the bond angle C(15)–C(14)–C(10) (132.16°) relative to the normal angle of 120°.

The relative stereochemistry of the remaining compounds **II** was determined by NMR analysis. The *anti* and *syn* isomers were identified by the coupling constants (*J*) of the vicinal protons adjacent to N–Ph and CH_2 groups in their ^{1}H NMR spectra. The coupling constants (*J*) of the *anti* isomer are higher than those of the *syn* isomer. The ^{1}H NMR spectrum of (**II_1**) showed two doubles at δ = 4.02, 4.61 from CH protons, and the corresponding coupling constant is *J* = 12.4; this indicates that compounds (**II**) have the *anti* configuration. We suggest that the *anti* configuration of structure (**II_1**) is the thermodynamically more stable configuration because of the exclusion between crowded aryl systems adjacent to N=C and CH moieties (Figure 2).

3.2 Bioactivity

The anti-breast cancer activity tests *in vitro* of the *2H*-pyrazolo[4,3-c]hexahydropyridine derivatives (**II**) along with 5-fluorouracil for comparison were carried out on human breast cancer MCF-7 and MDA-MB-231 cell lines. The screening results expressed as IC_{50} are summarized in Table 1. Most of the compounds showed good inhibitory activity against breast cancer cells, among them, compound **II_{33}** found to be the most potent compound with IC_{50} values of 2.4 μM, 4.2 μM against MCF-7 and MDA-MB-231, respectively, an improvement of more than two-fold compared with 5-fluorouracil (IC_{50} = 4.8 μM for MCF-7 and IC_{50} =9.6 μM for MDA-MB-231, respectively) and compounds **II_{21}** and **II_{40}** also displayed better inhibitory activity than 5-fluorouracil.

The activity of the tested compounds can be correlated with the structure modifications. By investigating the tested compounds with the two cell lines, it was found that different A rings led to different anti-breast cancer activity. When the substituents on B and C rings were constant, compounds (**II_{28}–II_{41}**) with a heteroaromatic A ring showed better activities than when A was a benzene ring (**II_1–II_{27}**). When A was a benzene ring, change of substituents on the ring also affects the activities of the compounds. A fluoro substituent

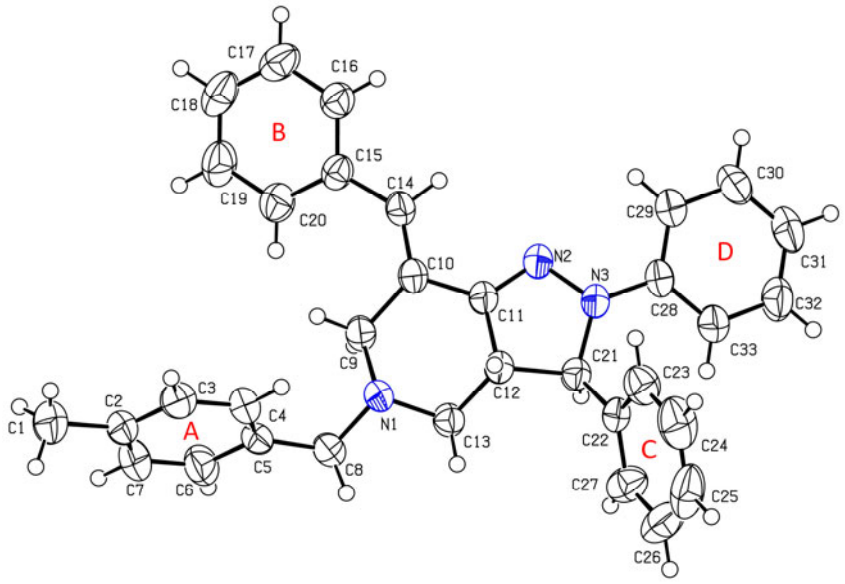


Figure 1 Molecular structure of compound $\mathbf{II}_1$ (Number CCDC 845735) with atom-labeling.

Figure 2 The structures of the *anti* and *syn* isomers of $\mathbf{II}_1$.

on the benzene ring leads to increased activity, and a *para*-fluoro substituent on the A ring gave better anticancer activities than the corresponding compound with an *ortho*-fluoro substituent. For example, the potency order decreased in the order 3-pyridyl ($\mathbf{II}_{33}$) > 2-furyl ($\mathbf{II}_{40}$) > 4- fluorophenyl ($\mathbf{II}_{21}$) > 2-fluorophenyl ($\mathbf{II}_{14}$) > 4-bromophenyl ($\mathbf{II}_{26}$) > 4-methoxylphenyl ($\mathbf{II}_{9}$) > 4-methylphenyl ($\mathbf{II}_{4}$).

Regarding the compounds ($\mathbf{II}_{28}$–$\mathbf{II}_{34}$) which all have a 3-pyridyl A ring, compounds with electron-withdrawing substituents ($\mathbf{II}_{32}$ –$\mathbf{II}_{34}$) in the B and C rings showed more potent activities than those with electron-donating substituents ($\mathbf{II}_{29}$ –$\mathbf{II}_{31}$) in these rings. A comparison of the substituents on the

B and C rings demonstrated that electron-withdrawing groups $\mathbf{II}_{32}$, $\mathbf{II}_{33}$, and $\mathbf{II}_{34}$ gave significantly improved antiproliferative activity, whereas Me ($\mathbf{II}_{29}$), $CH(CH_3)_2$ ($\mathbf{II}_{30}$) and OCH_3 ($\mathbf{II}_{31}$) substituents gave slightly reduced antiproliferative activity compared with $\mathbf{II}_{28}$, which has no substituent. Moreover, compounds with a *para*-fluoro substituent on the B and C rings showed better anticancer activity than compounds with a *meta*-fluoro substituent. Our results therefore show that the anti-breast cancer activities of our compounds were largely influenced by the A ring and the anti-breast cancer activities of these compounds could be increased by the introduction of a 3-pyridyl ring or 2-furyl ring as the A ring.

3.3 Apoptosis in MCF-7 cells and DNA fragmentation induced by $\mathbf{II}_{33}$

Owing to the notable cytotoxic effect of $\mathbf{II}_{33}$, we performed further tests to elucidate whether its proliferation inhibitory of MCF-7 cells was related to apoptosis. MCF-7 cells which treated with $\mathbf{II}_{33}$ at concentrations of 1, 10 and 100 μg mL^{-1} double stained with annexin V-FITC and propidium iodide (PI) were analyzed by flow cytometry for 24 h. Annexin V staining in cells make it possible to detect the expression of phosphatidylserine on the plasma membrane surface, which is a characteristic related to apoptosis. As shown in Figure 3(a), the proportion of cells stained with annexin V was increased with $\mathbf{II}_{33}$ treatment, analyzed by flow cytometry. These results suggested that $\mathbf{II}_{33}$ induced cytotoxicity of MCF-7 cells by the induction of apoptosis.

The parameter which was selected to assess the DNA damage upon treatment with $\mathbf{II}_{33}$ was chromosomal DNA fragmentation. The chromosomal DNA extracted from the MCF-7 cells treated with increasing concentrations (10, 50 and 100 μg mL^{-1}) of $\mathbf{II}_{33}$ after 72 h, was used for agarose gel electrophoresis. The results showed fragmentation of the DNA leading to a smear in the lanes in which cells were treated with $\mathbf{II}_{33}$ (Figure 3(b)). The observed smear is the result of DNA breakage at multiple positions across the

Table 1 Inhibition (IC_{50}) of MCF-7 and MDB-MB-231 cells proliferation by compounds ($\mathbf{II_1}$–$\mathbf{II_{41}}$)

Compound	IC_{50} (μM)		Compound	IC_{50} (μM)	
	MCF-7	MDB-MB-231		MCF-7	MDB-MB-231
$\mathbf{II_1}$	>50	>50	$\mathbf{II_{22}}$	14.9	12.7
$\mathbf{II_2}$	>50	>50	$\mathbf{II_{23}}$	41.8	>50
$\mathbf{II_3}$	>50	>50	$\mathbf{II_{24}}$	46.4	>50
$\mathbf{II_4}$	48.3	>50	$\mathbf{II_{25}}$	48.2	>50
$\mathbf{II_5}$	>50	>50	$\mathbf{II_{26}}$	27.6	33.5
$\mathbf{II_6}$	>50	>50	$\mathbf{II_{27}}$	33.2	39.2
$\mathbf{II_7}$	>50	>50	$\mathbf{II_{28}}$	10.6	14.7
$\mathbf{II_8}$	>50	>50	$\mathbf{II_{29}}$	13.8	19.4
$\mathbf{II_9}$	42.8	38.6	$\mathbf{II_{30}}$	16.2	23.4
$\mathbf{II_{10}}$	44.2	41.5	$\mathbf{II_{31}}$	21.6	32.4
$\mathbf{II_{11}}$	40.1	34.4	$\mathbf{II_{32}}$	17.6	26.5
$\mathbf{II_{12}}$	40.3	36.2	$\mathbf{II_{33}}$	2.4	4.2
$\mathbf{II_{13}}$	40.3	38.8	$\mathbf{II_{34}}$	8.6	14.2
$\mathbf{II_{14}}$	9.6	20.3	$\mathbf{II_{35}}$	23.6	26.8
$\mathbf{II_{15}}$	16.4	24.5	$\mathbf{II_{36}}$	29.6	29.3
$\mathbf{II_{16}}$	32.4	36.3	$\mathbf{II_{37}}$	30.5	33.4
$\mathbf{II_{17}}$	35.4	37.2	$\mathbf{II_{38}}$	32.8	40.6
$\mathbf{II_{18}}$	38.4	42.9	$\mathbf{II_{39}}$	16.8	21.2
$\mathbf{II_{19}}$	38.6	44.8	$\mathbf{II_{40}}$	3.3	8.6
$\mathbf{II_{20}}$	23.5	28.6	$\mathbf{II_{41}}$	7.9	17.5
$\mathbf{II_{21}}$	4.7	9.3	5-FU	4.8	9.6

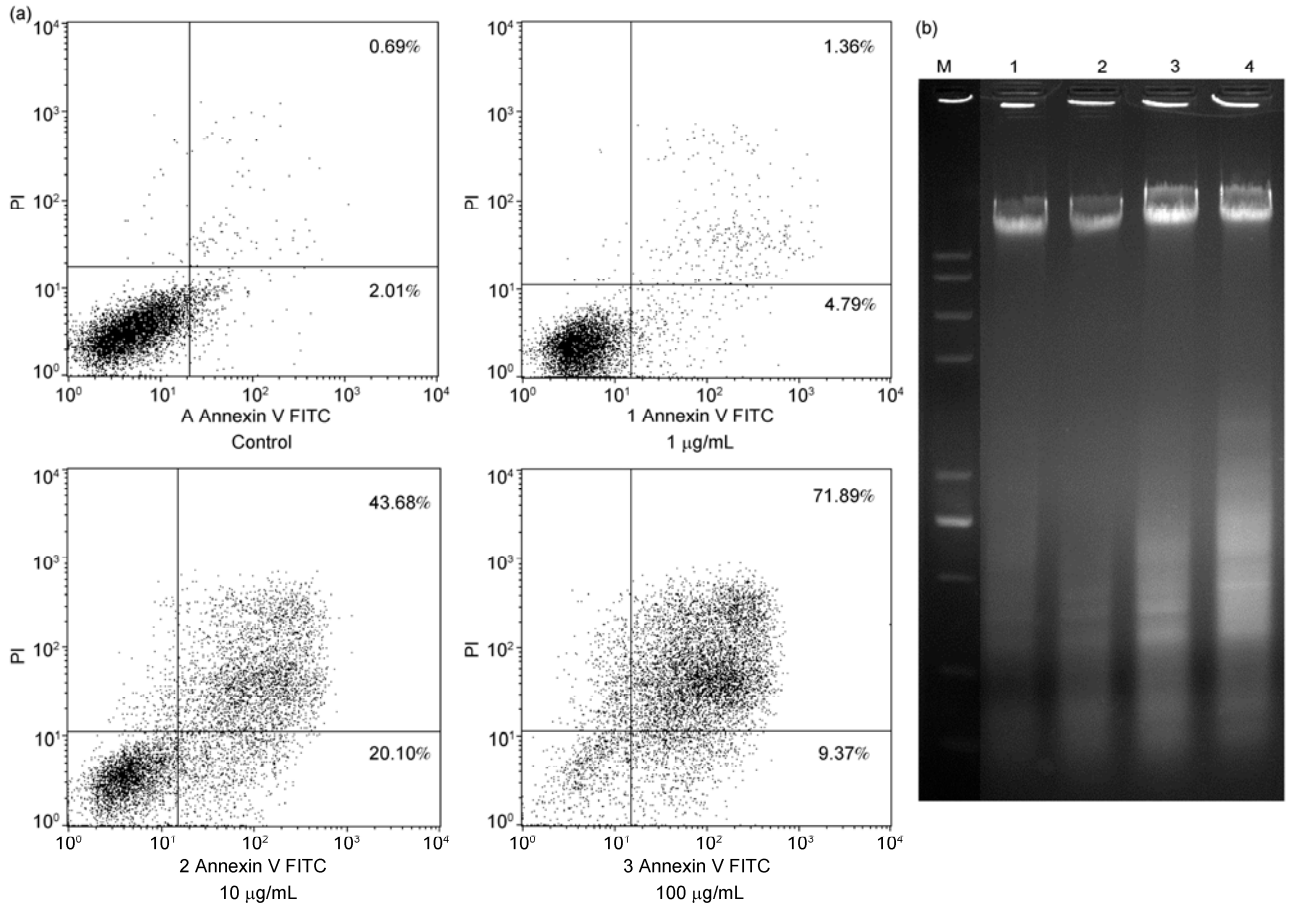


Figure 3 Effects of compound $\mathbf{II_{33}}$ on MCF-7 cells. (a) Flow cytometric analysis of phosphatidylserine externalization and propidium iodide at 1, 10 and 100 μg mL^{-1} for 24 h; (b) DNA ladder assay. Lane 1: DMSO; Lane 2–4: MCF-7 cells treated with 10, 50 and 100 μg mL^{-1}, respectively. 'M' is a Marker.

chromosomal DNA. The intensity of the smear increased with the dose. In case of **II$_{33}$**, a dose of 50 μg mL^{-1} showed moderate smearing and a dose of 100 μg mL^{-1} showed maximum smearing.

3.4 Fluorescence measurements

Photoluminescence spectra were studied for the compounds **II$_1$**, **II$_6$**, **II$_{11}$**, **II$_{16}$**, **II$_{23}$**, **II$_{28}$**, and **II$_{35}$** with excitation at 360 nm. The most striking feature was that all six compounds gave an intense emission and the photoluminescence spectra of these compounds in dichloromethane are shown in Figure 4. The maximum luminescence intensity was observed at 773 nm for compound **II$_{28}$** with a photoluminescence quantum yield of 39%. When A ring was 3-pyridyl (**II$_{28}$**) or 2-furfuryl (**II$_{35}$**), this led an increase in the intensity of the main peaks and a shift to higher emission wavelength. When **A** was a benzene ring, different substituents on the benzene ring lead to different intensities of the main peaks and the intensity decreased in the order: 2-F (**II$_{11}$**) > 4-F (**II$_{16}$**) > 4-OCH$_3$ (**II$_6$**)> 4-CH$_3$ (**II$_1$**) > 4-Br (**II$_{23}$**).

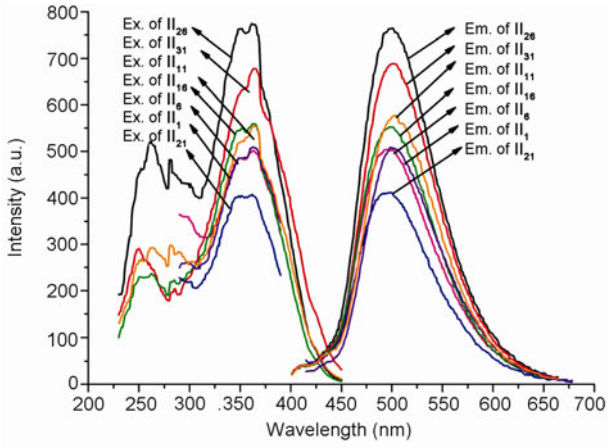


Figure 4 Photoluminescence spectra of compounds in CH_2Cl_2. samples were excited at 360 nm.

The photoluminescence data are summarized in Table 2. The photoluminescent properties of these compounds may indicate their great potential for numerous optical applications and for use as medicinal biomarkers.

4 Conclusion

In an attempt to develop more potent anti-breast cancer agents, a series of previously unreported *2H*-pyrazolo [4,3-c]hexahydropyridine derivatives (**II**) have been synthesized. The cytotoxicity of the target compounds have been evaluated *in vitro* against two human breast cancer cell lines MCF-7 and MDA-MB-231 by MTT assay. Most compounds exhibited good inhibition, among them compounds **II$_{21}$** (IC_{50} = 4.7 μM for MCF-7 and IC_{50} = 9.3 μM for MDA-MB-231), **II$_{33}$** (IC_{50} = 2.4 μM for MCF-7 and IC_{50} = 4.2 μM for MDA-MB-231) and **II$_{40}$** (IC_{50} = 3.3 μM for MCF-7 and IC_{50} =8.6 μM for MDA-MB-231) displayed better inhibitory activity than 5-fluorouracil (IC_{50} = 4.8 μM for MCF-7 and IC_{50} =9.6 μM for MDA-MB-231). Structure-activity relationships further indicated that the anti-breast cancer activities of our compounds were largely influenced by the nature of the A ring and the anti-breast cancer activities could be increased by the introduction of a 3-pyridyl ring or a 2-furyl ring as the **A** ring. The fluorescence properties of compounds **II$_1$**, **II$_6$**, **II$_{11}$**, **II$_{16}$**, **II$_{23}$**, **II$_{28}$**, and **II$_{35}$** were studied and the photoluminescent properties of these compounds may indicate their great potential for numerous optical applications and for use as medicinal biomarkers. Moreover, the compound **II$_{33}$** induced apoptosis of MCF-7 cells and was studied by DNA fragmentation and flow cytometric analysis, and much more studies of the biological mechanism of compound **II$_{33}$** are currently ongoing.

Table 2 Photoluminescence data for selected compounds **II**

Compound	$\lambda_{max\ (Ex)}$ (nm) [a]	$I_{(Ex)}$ [b]	$\lambda_{max(Em)}$ (nm) [c]	$I_{(Em)}$ [d]	φ_f (%) [e]
II$_1$	363	500	494	503	31
II$_6$	362	510	495	503	33
II$_{11}$	363	557	504	576	34
II$_{16}$	363	557	498	552	33
II$_{23}$	362	409	496	412	30
II$_{28}$	363	773	499	765	39
II$_{35}$	364	676	502	690	37

a) $\lambda_{max(Ex)}$: maximum excitation wavelength; b) $I_{(Ex)}$: maximum excitation intensity; c) $\lambda_{max(Em)}$: maximum emission wavelength; d) $I_{(Em)}$: maximum emission intensity; e) φ_f: quantum yield.

The bioassay was supported by Zooblast-molecular Biology Laboratory of Shanghai Normal University. This work was supported by the National Natural Science Foundation of China (21042010, 21102092 and 30870560), the Key Scientific "Twelfth Five-Year" National Technology Support Program (2011BAE06B01-17), the Innovation Project of Shanghai Education Commission (12YZ078), the Key Project of the Science and Technology Commission of Shanghai 105405503400), the Leading Academic Discipline Project of Shanghai Normal University (DZL808), and Shanghai Key Laboratory of Rare Earth Functional Materials, Shanghai Normal University (07dz22303).

1 Verweij J, Jonge MJA. Achievements and future of chemotherapy. *Eur J Cancer*, 2000, 36: 1479–1487
2 Verdecchia A, Mariotto A, Capocaccia R, Gatta G, Micheli A, Sant M, Berrino F. Incidence and prevalence of all cancerous diseases in Italy: Trends and implications. *Eur J Cancer*, 2001, 37: 1149–1157
3 Chandrappa S, Kavitha CV, Shahabuddin MS, Vinaya K, Ananda Kumar CS, Ranganatha SR. Synthesis of 2-(5-((5-(4-chlorophenyl)furan-2-yl)methylene)-4-oxo-2-thioxothiazolidin-3-yl)acetic acid derivatives and evaluation of their cytotoxicity and induction of apoptosis in human leukemia cells. *Bioorg Med Chem*, 2009, 17: 2576–2584
4 Jemal A, Siegel R, Ward E, Murray T, Xu J, Smigal C, Thun MJ. Cancer statistics 2006. *CA Cancer J Clin, 2006*, 56: 106–130
5 Baselga J, Norton L. Focus on breast cancer. *Cancer Cell*, 2002, 1: 319–322
6 Brueggemeier RW, Richards JA, Joomprabutra S, Bhat AS, Whetstone JL. Molecular pharmacology of aromatase and its regulation by endogenous and exogenous agents. *J Steroid Biochem Mol Biol*, 2001, 79: 75–84
7 Miller WR. Aromatase inhibitors in the treatment of advanced breast cancer. *Cancer Treat Rev*, 1989, 16: 83–93
8 Yadav B, Taurin S, Rosengren RJ, Schumacher M, Diederich M, Larsen L. Synthesis and cytotoxic potential of heterocyclic cyclohexanone analogues. *Bioorg Med Chem*, 2010, 18: 6701–6707
9 Sashidhara KV, Rosaiah JN, Kumar M, Gara RK, Nayak LV, Srivastava K, Konwar R. Neo-tanshinlactone inspired synthesis, in vitro evaluation of novel substituted benzocoumarin derivatives as potent anti-breast cancer agents. *Bioorg Med Chem Lett*, 2010, 20: 7127–7131
10 Chen YX, Sun CW, Wen XX, Zhang WG. Design, synthesis, insecticidal evaluation and molecular docking studies of cis-nitenpyram analogues bearing diglycine esters. *Sci China Chem*, 2012, 55, 159-168
11 Bare TM, McLarem CD, Campbell DJB, Firor JW, Resch JF, Walters CP, Salama AI, Meiners BA, Patel JB. Synthesis and structure–activity relationships of a series of anxioselective pyrazolopyridine ester and amide anxiolytic agents. *J Med Chem*, 1989, 32: 2561–2573
12 Bernardino AMR, Castro HC, Frugulhetti ICPP, Loureiro NIV, Azevedo AR, Pinheiro LCS, Souza TML, Giongo V, Passamani F, Magalhães UO, Albuquerque MG, Cabral LM, Rodrigues CR. SAR of a series of anti-HSV-1 acridone derivatives, and a rational acridone-based design of a new anti-HSV-1 3H-benzo[b]pyrazolo [3,4-h]-1,6-naphthyridine series. *Bioorg Med Chem*, 2008, 16: 313–321
13 Mello H, Echevarria A, Bernardino AMR, Canto-Cavalheiro M, Leon LL. Antileishmanial pyrazolopyridine derivatives: Synthesis and structure–activity relationship analysis. *J Med Chem*, 2004, 47: 5427–5432
14 Lu Z, Ott GR, Anand R, Liu R, Covington MB, Vaddi K, Qian M, Newton RC, Christ DD, Trzaskos J, Duan JJJ. Potent, selective, orally bioavailable inhibitors of tumor necrosis factor-α converting enzyme (TACE): Discovery of indole, benzofuran, imidazopyridine and pyrazolopyridine P1′ substituents. *Bioorg Med Chem Lett*, 2008, 18: 1958–1962
15 Fucini RV, Hanan EJ, Romanowski MJ, Elling RA, Lew W, Barr KJ, Zhu J, Yoburn JC, Liu Y, Fahr, BT. Design and synthesis of 2-amino-pyrazolopyridines as Polo-like kinase 1 inhibitors. *Bioorg Med Chem Lett*, 2008, 18: 5648–5652
16 Warshakoon NC, Wu S, Boyer A, Kawamoto R, Renock S, Xu K. Design and synthesis of a series of novel pyrazolopyridines as HIF 1-α prolyl hydroxylase inhibitors. *Bioorg Med Chem Lett*, 2006, 16: 5687–5690
17 Peng JH, Hao WJ, Tu SJ. An efficient and stereoselective synthesis of pyrazolo[4,3-c]pyridine derivatives under microwave heating. *J Heterocyclic Chem*, 2009, 45: 849–853
18 Wang J, Meng W, Ni ZJ, Xue SJ. *N*-(substituted benzyl)-3,5-bis(benzylidene)-4-piperidones:synthesis and preliminary anti-leukemia activity (I). *Chin J Chem*, 2011, 29: 2109–2113
19 Xiao HB, Mei C, Wang YC, Li H, Yin HY. Novel triphenylamine-cored two-photon absorbing dyes for labeling of biomolecules. *Mater Chem Phys*, 2011, 130: 897–902
20 Feng Y, Ding X, Chen T. Design, synthesis, and interaction study of quinazoline-(1H)-thione derivatives as novel potential Bcl-x_L inhibitors. *J Med Chem*, 2010, 53: 3465–3479