

## Synthesis and Cytotoxicity of Dibenzo[( $\gamma$ -aryl)pyridino]aza-17-crown-5 Ethers

Le Tuan Anh,<sup>@a</sup> Nguyen T. Thanh Phuong,<sup>a</sup> Truong Hong Hieu,<sup>b</sup>  
Anatoly T. Soldatenkov,<sup>c</sup> Bui T. Van,<sup>a</sup> Tran T. Thanh Van,<sup>a</sup> Dao T. Nhung,<sup>a</sup>  
Leonid G. Voskressensky,<sup>c</sup> To Hai Tung,<sup>a</sup> and Victor N. Khrustalev<sup>c,d</sup>

<sup>a</sup>Department of Pharmaceutical Chemistry, Faculty of Chemistry, VNU University of Science, 100000 Hanoi, Vietnam

<sup>b</sup>Department of Biotechnology, Vietnam-Russia Tropical Centre, 100000 Hanoi, Vietnam

<sup>c</sup>Faculty of Science, Peoples' Friendship University of Russia, 117198 Moscow, Russian Federation

<sup>d</sup>X-Ray Structural Centre, A.N. Nesmeyanov Institute of Organoelement Compounds, Russian Academy of Sciences, 119991 Moscow, Russian Federation

<sup>@</sup>Corresponding author E-mail: [huschemical.lab@gmail.com](mailto:huschemical.lab@gmail.com)

*For the development of new anticancer agents, dibenzo[( $\gamma$ -aryl)pyridino]aza-17-crown-5 ethers containing 2,4,6-triarylpyridine were synthesized successfully by one-step domino-condensation of 1,8-bis(2-acetylphenoxy)-3,6-dioxaoctane, arylaldehydes and ammonium acetate according to the conditions of Hantzsch reactions. The synthesized  $\gamma$ -arylpyridine derivatives show high cytotoxic activity against human cancer cell lines: Hep-G2, RD, FL, Lu1. Compounds (**3b,c**) showed significant cytotoxicity against all four human cell lines whereas the similar synthesized compound (**3d**) possessed cytotoxicity against HepG2, Lu1 and RD cell lines. Meantime, both compounds (**3f,g**) containing  $\gamma$ -heteroaryl only exhibited cytotoxicity against RD and FL cell lines. Azacrown ethers (**3b-d**) exhibited low activity on the Vero cell line, meaning that they can be evaluated for their potential as promising anticancer agents. X-Ray structure study was performed to determine the structure of the representative compound **3a**.*

**Keywords:** Arylpyridine, azacrown ethers, cytotoxicity, anticancer agent, Hantzsch reaction, X-ray analysis.

## Синтез и цитотоксичность дибензо[( $\gamma$ -арил)пиридино]аза-17-краун-5-эфиров

Л. Т. Ань,<sup>@a</sup> Н. Т. Т. Фьонг,<sup>a</sup> Т. Х. Хиеу,<sup>b</sup> А. Т. Солдатенков,<sup>c</sup> Б. Т. Ван,<sup>a</sup>  
Т. Т. Т. Ван,<sup>a</sup> Д. Т. Нунг,<sup>a</sup> Л. Г. Воскресенский,<sup>c</sup> Т. Х. Тунг,<sup>a</sup> В. Н. Хрусталеv<sup>c,d</sup>

<sup>a</sup>Кафедра фармацевтической химии, Факультет химии, Университет науки, Вьетнамский национальный университет, 100000 Ханой, Вьетнам

<sup>b</sup>Факультет биотехнологии, Российско-Вьетнамский Тропический центр, 100000 Ханой, Вьетнам

<sup>c</sup>Российский университет дружбы народов, 117198 Москва, Российская Федерация

<sup>d</sup>Центр рентгеноструктурных исследований, Институт элементоорганических соединений им. А.Н. Несмеянова РАН, 119991 Москва, Российская Федерация

<sup>@</sup>E-mail: [huschemical.lab@gmail.com](mailto:huschemical.lab@gmail.com)

*Для разработки новых противоопухолевых агентов одностадийной доминоконденсацией 1,8-бис(2-ацетилфенокси)-3,6-диоксооктана, ариальдегидов и ацетата аммония по реакции Ганча были успешно синтезированы дибензо[( $\gamma$ -арил)пиридино]аза-17-краун-5-эфиры, содержащие 2,4,6-триарилпиридин. Синтезированные производные  $\gamma$ -арилпиридина показали высокую цитотоксическую активность против линий раковых клеток человека: Hep-G2, RD, FL, Lu1. Соединения (**3b,c**) показали значительную цитотоксическую активность против всех четырех линий раковых клеток, а соединение (**3d**) – против линий HepG2, Lu1 и RD. Между тем, оба соединения (**3f,g**), содержащие  $\gamma$ -гетероарил, проявили цитотоксичность только против линий RD и FL. Азакраун-эфиры (**3b-d**) проявили низкую активность на клеточной линии Vero, что позволяет оценить*

их потенциал для использования в качестве перспективных противораковых агентов. Для определения структуры соединения **3a** были проведены рентгеноструктурные исследования.

**Ключевые слова:** Арилпиридин, азакраун-эфиры, цитотоксичность, противораковый агент, реакция Ганча, рентгеноструктурный анализ.

## Introduction

Multi-substituted pyridine derivatives occupy a central position in modern heterocyclic chemistry, particularly in pharmaceutical, bioorganic and medicinal chemistry.<sup>[1-5]</sup> Due to these interesting biological properties, the synthesis of functionalized heterocycles containing  $\gamma$ -arylpyridine fragment is one of the major objectives for various laboratories.<sup>[6-9]</sup> In addition, several prepared ( $\gamma$ -aryl)pyridinoaza-14-crown-4 ethers (**1a-d**) were evaluated as to their cytotoxicity to human cancer cell lines: Hepatocellular carcinoma (HepG2), Rhabdomyosarcoma (RD), Human Uterine (FL), Human Breast adenocarcinoma (MCF7), Human Prostate Cancer (PC3).<sup>[10-12]</sup> Based on these results of previous studies, it would be very interesting to evaluate the cytotoxicity of system aza-17-crown-5 ether. Due to its ability to form metal complexes and its status as an anticancer agent, azacrown ether has valuable applications.

In the past, a similar system containing a polyether bridge was also reported.<sup>[13,14]</sup> The main drawbacks of these methods were multi-step synthesis, the amount of time spent for reactions, separation and purification. Here, in connection with published studies,<sup>[10,11]</sup> we present a simple, one-pot domino Hantzsch-type reaction to obtain the desired compounds, namely dibenzo[( $\gamma$ -aryl)pyridino]aza-17-crown-5 ethers from arylaldehyde, 1,8-bis(2-acetylphenoxy)-3,6-dioxaoctane and ammonium acetate. The combination of  $\gamma$ -arylpyridines and crown ether promises to bring a novel class of bioactive compounds.

## Experimental

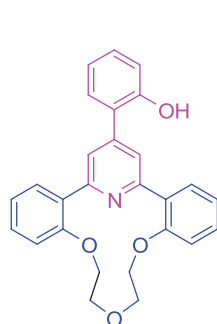
Reagents were purchased from commercial sources (Sigma-Aldrich) and were used without any additional purification. <sup>1</sup>H and <sup>13</sup>C NMR spectra were recorded in CDCl<sub>3</sub> solutions at 25 °C, using a 500 MHz NMR spectrometer; chemical shifts are given

in parts per million ( $\delta$ ) and referenced to the appropriate solvent residual peak. Mass spectra were recorded on LTQ Orbitrap XL using electrospray ionization source. IR spectra were recorded in FTIR Affinity – 1S SHIMADZU.

**General method for synthesis of bis(benzo)( $\gamma$ -arylpyridino)aza-17-crown-5 ethers (**3a-g**).** Equimolar amounts of 1,8-bis(2-acetylphenoxy)-3,6-dioxaoctane **2** (1.71 g, 5.00 mmol), aromatic aldehyde (5.00 mmol) and ammonium acetate (10 g, 13 mmol) were refluxed in acetic acid (50 ml). The reaction was monitored by TLC and was complete in 3 h. The mixture was allowed to cool to room temperature and neutralized with sodium carbonate solution; then, the product was extracted with ethyl acetate (3×50 ml), drying over Na<sub>2</sub>SO<sub>4</sub>. After filtration, the solvent was evaporated *in vacuo*; the residue was first purified by column chromatography with a gradient elution of ethyl acetate/*n*-hexane affording the white solid which was then recrystallized from ethanol to obtain the pure azacrown ether product.

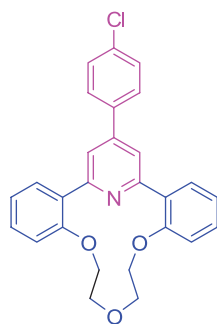
**26-(4-Methoxyphenyl)-8,11,14,17-tetraoxa-28-azatetracyclo[22.3.1.0<sup>2,7</sup>.0<sup>18,23</sup>]pentacosa-2,4,6,18(23),20,22,24,27,1(28)-nonaene (**3a**).** Yield: 0.72 g (30 %), white crystals. M.p. 198–199 °C (from EtOH). *R<sub>f</sub>*=0.43 (*n*-hexane:ethylacetate=1:1). *m/z* (HRMS) Calc. for C<sub>30</sub>H<sub>30</sub>NO<sub>4</sub><sup>+</sup>: 484.2118, found 484.2105 [M+H]<sup>+</sup>. IR (KBr)  $\nu_{\max}$  cm<sup>-1</sup>: 1606 (CN<sub>pyridine</sub>), 1545, 1514, 1492 (aromatic), 1182, 1120, 1058, 1029 (COC). <sup>1</sup>H NMR (500 MHz, CDCl<sub>3</sub>, Me<sub>4</sub>Si)  $\delta_{\text{H}}$  ppm: 3.89 (s, 3H, Me), 3.19 (s, 4H, OCH<sub>2</sub>CH<sub>2</sub>), 3.62 (m, 4H, OCH<sub>2</sub>CH<sub>2</sub>), 4.10 (m, 4H, OCH<sub>2</sub>CH<sub>2</sub>), 6.98–7.02 (m, 4H<sub>Ar</sub>), 7.12 (t, 2H<sub>Ar</sub>, *J*=7.5 Hz), 7.35 (td, 2H<sub>Ar</sub>, *J*=7.5 Hz, 2 Hz), 7.64–7.68 (m, 4H<sub>Ar</sub>), 7.72 (s, 2H<sub>pyridine</sub>). <sup>13</sup>C NMR (125 MHz, CDCl<sub>3</sub>)  $\delta_{\text{C}}$  ppm: 55.51, 69.56, 70.58, 71.16, 114.52, 114.62, 121.63, 121.95, 128.47, 129.67, 130.76, 131.35, 132.60, 146.77, 156.40, 156.94, 160.43.

**26-(4-Methylphenyl)-8,11,14,17-tetraoxa-28-azatetracyclo[22.3.1.0<sup>2,7</sup>.0<sup>18,23</sup>]pentacosa-2,4,6,18(23),20,22,24,27,1(28)-nonaene (**3b**).** Yield: 0.9 g (40 %), white crystals. M.p. 156–156 °C (from EtOH). *R<sub>f</sub>*=0.62 (*n*-hexane:ethylacetate=1:1). *m/z* (HRMS) Calc. for C<sub>30</sub>H<sub>30</sub>NO<sub>4</sub><sup>+</sup>: 468.2175, found 468.2166 [M+H]<sup>+</sup>. IR (KBr)  $\nu_{\max}$  cm<sup>-1</sup>: 1606 (CN<sub>pyridine</sub>), 1543, 1514, 1493 (aromatic), 1159, 1122, 1056 (COC). <sup>1</sup>H NMR (500 MHz, CDCl<sub>3</sub>, Me<sub>4</sub>Si)  $\delta_{\text{H}}$  ppm: 2.42 (s, 3H, Me), 3.18 (s, 4H, OCH<sub>2</sub>CH<sub>2</sub>), 3.62 (m, 4H, OCH<sub>2</sub>CH<sub>2</sub>), 4.10 (m, 4H, OCH<sub>2</sub>CH<sub>2</sub>), 6.99 (d, 2H<sub>Ar</sub>, *J*=8.5 Hz), 7.11 (td, 2H<sub>Ar</sub>, *J*=7.5 Hz, 1 Hz), 7.29 (d, 2H<sub>Ar</sub>, *J*=8.5 Hz), 7.35 (td, 2H<sub>Ar</sub>, *J*=8.0 Hz, 2 Hz), 7.62 (d, 2H<sub>Ar</sub>, *J*=8.5 Hz), 7.65 (dd, 2H<sub>Ar</sub>, *J*=7.5 Hz, 1.5 Hz),



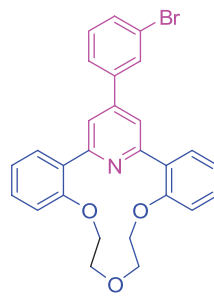
(**1a**)

RD (IC<sub>50</sub> 1.89  $\mu$ g/ml)



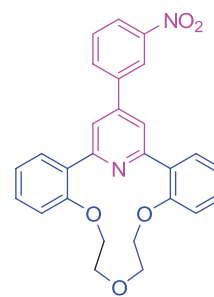
(**1b**)

HepG2 (IC<sub>50</sub> 2.79  $\mu$ g/ml)  
MCF7 (IC<sub>50</sub> 4.56  $\mu$ g/ml)  
RD (IC<sub>50</sub> 1.46  $\mu$ g/ml)  
FL (IC<sub>50</sub> 1.51  $\mu$ g/ml)



(**1c**)

HepG2 (IC<sub>50</sub> 4.367  $\mu$ g/ml)  
MCF7 (IC<sub>50</sub> 1.977  $\mu$ g/ml)  
PC3 (IC<sub>50</sub> 4.063  $\mu$ g/ml)  
RD (IC<sub>50</sub> 4.113  $\mu$ g/ml)



(**1d**)

MCF7 (IC<sub>50</sub> 4.78  $\mu$ g/ml)

7.75 (s, 2H<sub>pyridine</sub>). <sup>13</sup>C NMR (125 MHz, CDCl<sub>3</sub>) δ<sub>c</sub> ppm: 14.4, 19.4, 33.7, 55.8, 101.6, 113.0, 115.9, 116.5, 119.0, 119.6, 128.0, 145.0, 146.3, 156.0.

**26-(4-Hydroxyphenyl)-8,11,14,17-tetraoxa-28-azatetracyclo[22.3.1.0<sup>2,7</sup>.0<sup>18,23</sup>]pentacosa-2,4,6,18(23),20,22,24,27,1(28)-nonaene (3c).** Yield: 0.58 g (25 %), white crystals. M.p. 140–141 °C (from EtOH). R<sub>f</sub>=0.40 (hexane:ethylacetate=1:1). *m/z* (HRMS) Calc. for C<sub>29</sub>H<sub>28</sub>NO<sub>5</sub><sup>+</sup>: 470.1967, found 470.1950 [M+H]<sup>+</sup>. IR (KBr) ν<sub>max</sub> cm<sup>-1</sup>: 3240 (OH), 1608 (CN<sub>pyridine</sub>), 1593, 1547, 1493 (aromatic), 1159, 1122, 1056 (COC). <sup>1</sup>H NMR (500 MHz, CDCl<sub>3</sub>, Me<sub>4</sub>Si) δ<sub>H</sub> ppm: 3.19 (s, 4H, OCH<sub>2</sub>CH<sub>2</sub>), 3.62 (m, 4H, OCH<sub>2</sub>CH<sub>2</sub>), 4.10 (m, 4H, OCH<sub>2</sub>CH<sub>2</sub>), 6.93 (d, 2H<sub>Ar</sub>, *J*=8.5 Hz), 6.98 (d, 2H<sub>Ar</sub>, *J*=8.0 Hz), 7.11 (t, 2H<sub>Ar</sub>, *J*=8.5 Hz), 7.35 (td, 2H<sub>Ar</sub>, *J*=8.5 Hz, 1.5 Hz), 7.62 (d, 2H<sub>Ar</sub>, *J*=8.5 Hz), 7.64 (dd, 2H<sub>Ar</sub>, *J*=8.5 Hz, 1.5 Hz), 7.71 (s, 2H<sub>pyridine</sub>). <sup>13</sup>C NMR (125 MHz, CDCl<sub>3</sub>) δ<sub>c</sub> ppm: 14.4, 19.4, 33.7, 55.8, 101.6, 113.0, 115.9, 116.5, 119.0, 119.6, 128.0, 145.0, 146.3, 156.0.

**26-(2-Methoxyphenyl)-8,11,14,17-tetraoxa-28-azatetracyclo[22.3.1.0<sup>2,7</sup>.0<sup>18,23</sup>]pentacosa-2,4,6,18(23),20,22,24,27,1(28)-nonaene (3d).** Yield: 0.65 g (27 %), white crystals. M.p. 180–182 °C (from EtOH). R<sub>f</sub>=0.48 (hexane:ethylacetate=1:1). *m/z* (HRMS) Calc. for C<sub>30</sub>H<sub>30</sub>NO<sub>5</sub><sup>+</sup>: 484.2118, found 484.2100 [M+H]<sup>+</sup>. IR (KBr) ν<sub>max</sub> cm<sup>-1</sup>: 1610 (CN<sub>pyridine</sub>), 1581, 1547, 1491 (aromatic), 1168, 1126, 1047, 1026 (COC). <sup>1</sup>H NMR (500 MHz, CDCl<sub>3</sub>, Me<sub>4</sub>Si) δ<sub>H</sub> ppm: 3.85 (s, 3H, Me), 3.25 (s, 4H, OCH<sub>2</sub>CH<sub>2</sub>), 3.65 (m, 4H, OCH<sub>2</sub>CH<sub>2</sub>), 4.12 (m, 4H, OCH<sub>2</sub>CH<sub>2</sub>), 6.98 (d, 2H<sub>Ar</sub>, *J*=8.0 Hz), 7.02 (d, 1H<sub>Ar</sub>, *J*=8.5 Hz), 7.11 (m, 3H<sub>Ar</sub>), 7.35 (td, 2H<sub>Ar</sub>, *J*=8.5 Hz, 2 Hz), 7.40 (td, 2H<sub>Ar</sub>, *J*=8.0 Hz, 1.5 Hz), 7.47 (dd, 1H<sub>Ar</sub>, *J*=7.5 Hz, 1.5 Hz), 7.66 (dd, 2H<sub>Ar</sub>, *J*=7.5 Hz, 1.5 Hz), 7.71 (s, 2H<sub>pyridine</sub>). <sup>13</sup>C NMR (125 MHz, CDCl<sub>3</sub>) δ<sub>c</sub> ppm: 14.4, 19.4, 33.7, 55.8, 101.6, 113.0, 115.9, 116.5, 119.0, 119.6, 128.0, 145.0, 146.3, 156.0.

**26-(2-Thienyl)-8,11,14,17-tetraoxa-28-azatetracyclo[22.3.1.0<sup>2,7</sup>.0<sup>18,23</sup>]pentacosa-2,4,6,18(23),20,22,24,27,1(28)-nonaene (3e).** Yield: 0.96 g (42 %), white crystals. M.p. 147–148 °C (from EtOH). R<sub>f</sub>=0.50 (hexane:ethylacetate=1:1). *m/z* (HRMS) Calc. for C<sub>27</sub>H<sub>26</sub>NO<sub>4</sub>S<sup>+</sup>: 460.1577, found 460.1606 [M+H]<sup>+</sup>. IR (KBr) ν<sub>max</sub> cm<sup>-1</sup>: 1604 (CN<sub>pyridine</sub>), 839 (β-thiophen), 1548, 1493 (aromatic), 1132, 1057 (COC). <sup>1</sup>H NMR (500 MHz, CDCl<sub>3</sub>, Me<sub>4</sub>Si) δ<sub>H</sub> ppm: 3.22 (s, 4H, OCH<sub>2</sub>CH<sub>2</sub>), 3.63 (m, 4H, OCH<sub>2</sub>CH<sub>2</sub>), 4.12 (m, 4H, OCH<sub>2</sub>CH<sub>2</sub>), 6.99 (d, 2H<sub>Ar</sub>, *J*=8.0 Hz), 7.11 (t, 2H<sub>Ar</sub>, *J*=7.5 Hz), 7.14 (t, 1H<sub>Ar</sub>, *J*=5.0 Hz), 7.36 (td, 2H<sub>Ar</sub>, *J*=8.0 Hz, 1.5 Hz), 7.40 (d, 2H<sub>Ar</sub>, *J*=5.5 Hz), 7.53 (d, 1H<sub>Ar</sub>, *J*=3.5 Hz), 7.63 (dd, 2H<sub>Ar</sub>, *J*=7.5 Hz, 1.5 Hz), 7.73 (s, 2H<sub>pyridine</sub>). <sup>13</sup>C NMR (125 MHz, CDCl<sub>3</sub>) δ<sub>c</sub> ppm: 14.4, 19.4, 33.7, 55.8, 101.6, 113.0, 115.9, 116.5, 119.0, 119.6, 128.0, 145.0, 146.3, 156.0.

**26-(2-Furanyl)-8,11,14,17-tetraoxa-28-azatetracyclo[22.3.1.0<sup>2,7</sup>.0<sup>18,23</sup>]pentacosa-2,4,6,18(23),20,22,24,27,1(28)-nonaene (3f).** Yield: 0.89 g (40 %), white crystals. M.p. 182–183 °C (from EtOH). R<sub>f</sub>=0.63 (hexane:ethylacetate=1:1). *m/z* (HRMS) Calc. for C<sub>27</sub>H<sub>26</sub>NO<sub>5</sub><sup>+</sup>: 444.1805, found 444.1805 [M+H]<sup>+</sup>. IR (KBr) ν<sub>max</sub> cm<sup>-1</sup>: 1610 (CN<sub>pyridine</sub>), 750 (β-furan), 1577, 1546, 1489, 1446 (aromatic), 1114, 1058 (COC). <sup>1</sup>H NMR (500 MHz, CDCl<sub>3</sub>, Me<sub>4</sub>Si) δ<sub>H</sub> ppm: 3.22 (br s, 4H, OCH<sub>2</sub>CH<sub>2</sub>), 3.63 (m, 4H, OCH<sub>2</sub>CH<sub>2</sub>), 4.11 (m, 4H, OCH<sub>2</sub>CH<sub>2</sub>), 6.53 (m, 1H<sub>Ar</sub>), 6.89 (br s, 1H<sub>Ar</sub>), 6.98 (d, 2H<sub>Ar</sub>, *J*=8 Hz), 7.10 (td, 3H<sub>Ar</sub>, *J*=7.5 Hz, 1.0 Hz), 7.35 (td, 2H<sub>Ar</sub>, *J*=8.5 Hz, 1.5 Hz), 7.55 (br s, 1H<sub>Ar</sub>), 7.61 (dd, 2H<sub>Ar</sub>, *J*=7.5 Hz, 2.0 Hz), 7.76 (s, 2H<sub>pyridine</sub>). <sup>13</sup>C NMR (125 MHz, CDCl<sub>3</sub>) δ<sub>c</sub> ppm: 69.41, 70.55, 70.88, 108.33, 111.99, 114.14, 118.11, 121.70, 129.63, 130.56, 132.10, 136.55, 143.55, 152.08, 156.22, 157.05.

**26-(2-Pyrrolyl)-8,11,14,17-tetraoxa-28-azatetracyclo[22.3.1.0<sup>2,7</sup>.0<sup>18,23</sup>]pentacosa-2,4,6,18(23),20,22,24,27,1(28)-nonaene (3g).** Yield: 1.06 g (48 %), white crystals. M.p. 185–186 °C (from EtOH). R<sub>f</sub>=0.40 (hexane:ethylacetate=1:1). *m/z* (HRMS) Calc. for C<sub>27</sub>H<sub>27</sub>N<sub>2</sub>O<sub>4</sub><sup>+</sup>: 443.1965, found 443.1947 [M+H]<sup>+</sup>. IR (KBr) ν<sub>max</sub> cm<sup>-1</sup>: 1608 (CN<sub>pyridine</sub>), 747 (β-pyrrol), 1548, 1493 (aromatic), 1128, 1043 (COC). <sup>1</sup>H NMR (500 MHz, CDCl<sub>3</sub>, Me<sub>4</sub>Si) δ<sub>H</sub> ppm: 3.20 (br s, 4H, OCH<sub>2</sub>CH<sub>2</sub>), 3.62 (m, 4H, OCH<sub>2</sub>CH<sub>2</sub>), 4.10 (m, 4H, OCH<sub>2</sub>CH<sub>2</sub>), 6.33 (dt, 1H<sub>Ar</sub>, *J*=6.0 Hz, 2.0 Hz), 6.73 (d,

1H<sub>Ar</sub>, *J*=3.5 Hz), 6.94 (d, 1H<sub>Ar</sub>, *J*=1.5 Hz), 6.98 (d, 2H<sub>Ar</sub>, *J*=8.0 Hz), 7.11 (t, 2H<sub>Ar</sub>, *J*=7.0 Hz), 7.34 (td, 2H<sub>Ar</sub>, *J*=8.0 Hz, 1.5 Hz), 7.59 (s, 2H<sub>pyridine</sub>), 7.63 (dd, 2H<sub>Ar</sub>, *J*=7.5 Hz, 1.5 Hz), 8.88 (br s, 1H, NH). <sup>13</sup>C NMR (125 MHz, CDCl<sub>3</sub>) δ<sub>c</sub> ppm: 14.20, 21.05, 60.41, 60.43, 70.37, 71.05, 108.48, 110.60, 114.58, 118.40, 120.53, 121.92, 129.61, 129.98, 130.61, 132.37, 138.55, 156.20, 156.86.

### X-Ray diffraction experiment

The crystal of **3a** (C<sub>30</sub>H<sub>29</sub>NO<sub>5</sub>, *M*=483.54) is monoclinic, space group *P*2<sub>1</sub>/*c*, at *T*=120 K: *a*=10.239(4) Å, *b*=10.344(4) Å, *c*=23.413(8) Å, *b*=98.648(6)°, *V*=2451.5(15) Å<sup>3</sup>, *Z*=4, *d*<sub>calc</sub>=1.310 g/cm<sup>3</sup>, *F*(000)=1024, *μ*=0.089 mm<sup>-1</sup>. 21314 total reflections (5116 unique reflections, *R*<sub>int</sub>=0.082) were measured on a three-circle Bruker APEX-II CCD diffractometer (*I*(MoK<sub>α</sub>)-radiation, graphite monochromator, *j* and *w* scan mode, 2θ<sub>max</sub>=54.0°) and corrected for absorption (*T*<sub>min</sub>=0.975; *T*<sub>max</sub>=0.980).<sup>[15]</sup> The structure was determined by direct methods and refined by full-matrix least squares technique on *F*<sup>2</sup> with anisotropic displacement parameters for non-hydrogen atoms. The hydrogen atoms were placed in calculated positions and refined within riding model with fixed isotropic displacement parameters [*U*<sub>iso</sub>(H)=1.5*U*<sub>eq</sub>(C) for the methyl group and 1.2*U*<sub>eq</sub>(C) for the other groups]. The final divergence factors were *R*<sub>1</sub>=0.116 for 3809 independent reflections with *I* > 2*s*(*I*) and *wR*<sub>2</sub>=0.242 for all independent reflections, *S*=1.031. All calculations were carried out using the SHELXTL program.<sup>[16]</sup>

Crystallographic data for **3a** have been deposited with the Cambridge Crystallographic Data Center, CCDC 1532287. Copies of this information may be obtained free of charge from the Director, CCDC, 12 Union Road, Cambridge CB2 1EZ, UK (fax: +44 1223 336033; e-mail: deposit@ccdc.cam.ac.uk).

### Cytotoxicity assay

Four human cancer cell lines were obtained from the American Type Culture Collection (Manassas, VA) ATCC as FL (human cervix carcinoma), RD (human rhabdomyosarcoma), Lu1 (human lung adenocarcinoma), HepG2 (human hepatocellular carcinoma). The Vero cell line was initiated from the kidney of a normal adult African green monkey. They were grown in DMEM (Dulbecco's Modified Eagle Medium) (Thermo Fisher Scientific, Mÿ) supplemented with L-glutamine, Sodium piruvat, Na, PSF (Penicillin-Streptomycin sulfate – Fungizone), NAA (Non-Essential Amino Acids), 10 % BCS (Bovine Calf Serum). All incubations were performed at 37 °C for 72 hours in a CO<sub>2</sub> (5 %) incubator with the plates capped in the normal fashion.

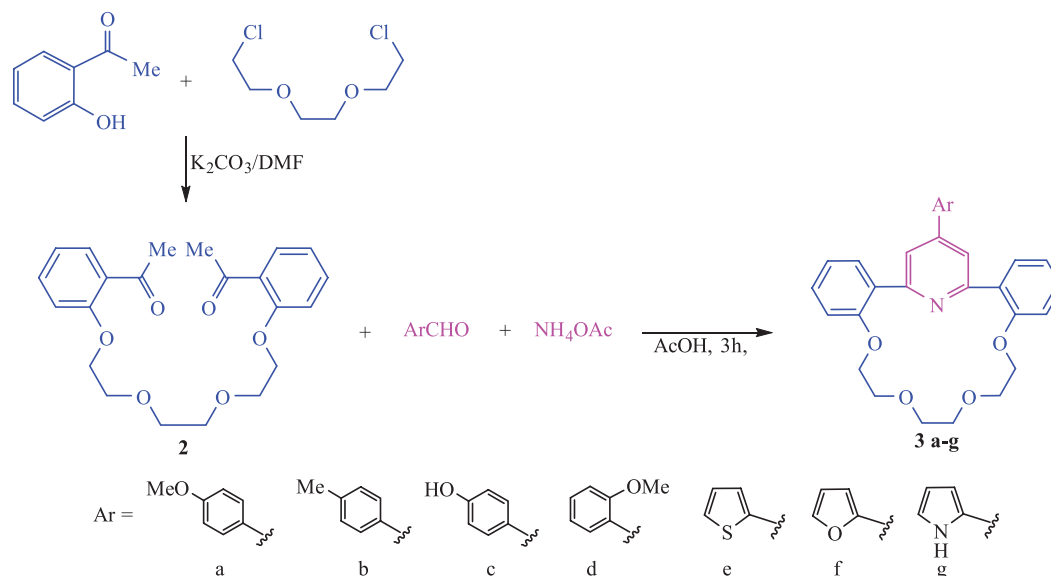
The MTT is based on the protocol described by Skehan & CS (1990)<sup>[17]</sup> and Likhiwitayawuid & CS (1993).<sup>[18]</sup> This method has worldwide application and is recommended by National Cancer Institute (NCI) and College of Medicine, University of Illinois at Chicago for routine drug screening.

### Results and Discussion

The starting compound (**2**) was prepared in a similar way using the reported method.<sup>[19]</sup> The expected dibenzo[(γ-aryl)pyridino]aza-17-crown-5 ethers (**3**) were prepared by condensation of diketone (**2**) with arylaldehyde derivatives and ammonium acetate in acetic acid (Scheme 1).

The structures of compounds (**3a–g**) were determined by <sup>1</sup>H NMR, <sup>13</sup>C NMR, IR, HRMS and X-ray analyses. For example, the <sup>1</sup>H NMR spectrum of the product (**3a**) showed a singlet signal at δ=3.87 ppm which is assigned to the three protons of methoxy group (–OCH<sub>3</sub>). There are three signals at δ=3.19 ppm (s, 4H), 3.17 ppm (t, 4H), 4.10 ppm (t, 4H) for twelve methylene protons of the polyether moiety –

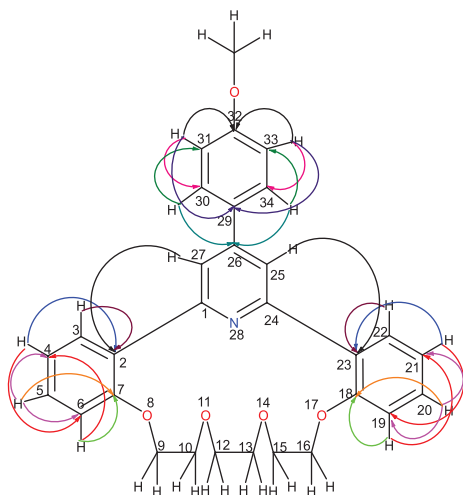




**Scheme 1.** Synthetic pathway for the dibenzo[( $\gamma$ -aryl)pyridino]aza-17-crown-5 ethers.

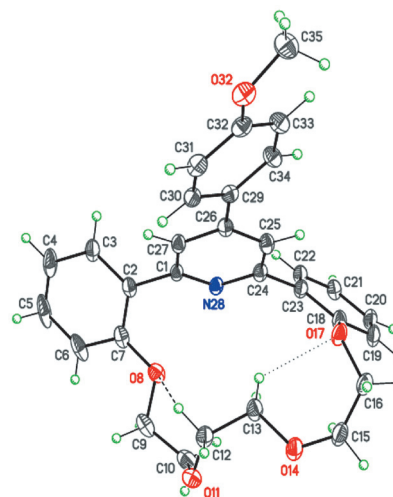
( $\text{OCH}_2\text{CH}_2\text{O}$ )-. Twelve protons of three benzene rings showed four signals at 7.64 (4H, m), 7.33 (2H, m), 7.10 (2H, q) and 6.98 (4H, m). Two  $\beta$ -protons belonging to the pyridine subunit (H-25, H-27) gave a singlet signal at 7.72 ppm.

To further understand of the structure of these compounds and also to deeper study of the 3D structure-activity relationship, a substance (**3a**) was chosen as a model for acquiring 2D-spectra (HSQC and HMBC) and X-ray structure analysis, which gave valuable information to establish its structure.

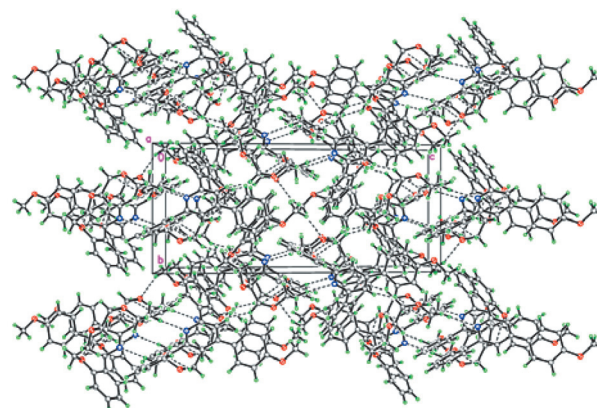


**Figure 1.**  $^1\text{H}$ - $^{13}\text{C}$  HMBC connectivity of **3a**.

The structure of dibenzo[( $\gamma$ -aryl)pyridino]aza-17-crown-5 ether (**3a**) was exactly confirmed by X-ray crystallography. The general shape of the molecule (**3a**) and the packing of its molecules in the crystal are shown in Figures 2, 3. The two intramolecular  $\text{C}-\text{H}\cdots\text{O}$  hydrogen bonds at positions 12 and 13 explained the lowest chemical shift of protons of  $-(\text{OCH}_2\text{CH}_2\text{O})$ - group in the  $^1\text{H}$  NMR spectrum.



**Figure 2.** Molecular structure of **3a**. The dashed and dotted lines indicate the intramolecular  $\text{C}-\text{H}\cdots\text{O}$  hydrogen bonds.



**Figure 3.** Crystal packing of **3a**. The dashed and dotted lines indicate the intramolecular  $\text{C}-\text{H}\cdots\text{O}$  and intermolecular  $\text{C}-\text{H}\cdots\text{N}$  and  $\text{C}-\text{H}\cdots\text{O}$  hydrogen bonds.

The molecule of (**3a**) comprises a fused tetracyclic system containing the aza-17-crown-5 ether macrocycle, pyridine and two benzene rings (Figure 2). The aza-17-crown-5 ether ring adopts a bowl conformation which is stabilized by two intramolecular C–H···O hydrogen bonds (Table 1, Figure 2). The configuration of the C7–O8–C9–C10–O11–C12–C13–O14–C15–C16–O17–C18 polyether chain is *t*-g<sup>+</sup>-g<sup>+</sup>-g<sup>+</sup>-g<sup>+</sup>-t-g<sup>+</sup>-g<sup>+</sup>-g<sup>+</sup> (*t*=*trans*, 180°, *g*=*gauche*, ±60°). The central pyridine ring is twisted by 45.99(16), 56.32(16) and 29.06(18)° relative to the two benzene rings, fused to the aza-17-crown-5 ether moiety, and phenyl substituent, respectively. The methoxy group lies almost within the phenyl ring plane (the C33–C32–O32–35 dihedral angle is 8.9(7)°). In the crystal, the molecules of (**3a**) form centrosymmetrical dimers by two weak intermolecular C–H···N hydrogen bonds (Table 1). Further, the dimers are linked by weak intermolecular C–H···O hydrogen bonds into a three-dimensional framework (Table 1, Figure 3).

**Table 1.** Hydrogen bonds for **3a** [Å and °].

D*–H···A*	d(D–H)	d(H···A)	d(D···A)	∠DHA
C9–H9a···N28 <sup>a</sup>	0.99	2.59	3.491(7)	151.9
C12–H12a···O17 <sup>b</sup>	0.99	2.56	3.335(7)	135.1
C12–H12b···O8	0.99	2.39	3.013(6)	119.9
C13–H13b···O17	0.99	2.55	2.958(6)	104.8
C35–H35a···O11 <sup>c</sup>	0.98	2.55	3.465(7)	156.1

\* D – proton donor; A – proton acceptor;

Symmetry transformations used to generate equivalent atoms:

<sup>a</sup> –x, –y+1, –z; <sup>b</sup> –x, y+0.5, –z+0.5; <sup>c</sup> x+1, –y+1.5, z+0.5

Deep understanding the structure of dibenzo[γ-aryl]pyridino]aza-17-crown-5 ethers is very useful for further study of the structure-activity relationship. All factors – steric, electrostatic, the intramolecular C–H···O and intermolecular C–H···N and C–H···O hydrogen bonds influence the cytotoxicity of these compounds.

All of the synthesized compounds (**3a–g**) were evaluated *in vitro* for their biological activity against four human tumor cell lines: FL (human cervix carcinoma), RD (human rhabdomyosarcoma), Lu1 (human lung adenocarcinoma), HepG2 (human hepatocellular carcinoma). The bioactivities of synthesized compounds toward human cancer cell lines were shown in Table 2. Azacrown ethers (**3b,c**) showed significant activity against all four cell lines with cell survival (CS) value as 0 (%). Compound (**3d**) had positive results on cytotoxicity in tests against the HepG2, Lu1, RD cell lines. In addition, analogues (**3f,g**) have inhibited RD and FL cell lines. Based on these results, dibenzo[γ-aryl]pyridino]aza-17-crown-5 ethers (**3b–d,f,g**) were selected for further evaluation.

To continuously investigate the effectiveness of (**3b–d,f,g**) in inhibiting cancer cell lines, an inhibitory test (IC<sub>50</sub>) was conducted. Azacrown ether (**3b**) showed the highest activity against all of four human cell lines with an IC<sub>50</sub> value below 3.0 μg/ml whereas the similar synthesized compound (**3c**) exhibited potent cytotoxicity against those cell lines with an IC<sub>50</sub> value in the 6.7–8.0 μg/ml. Compound (**3d**) possessed cytotoxicity against HepG2, Lu1 and RD cell lines. In contrast, both compounds (**3f,g**) containing γ-heteroaryl only exhibited cytotoxicity against RD and FL cell lines with an IC<sub>50</sub> value in the 8.3–10.0 μg/ml. These results were shown in Table 3.

**Table 2.** Cytotoxicity tests performed on compounds **3a–g** in human cancer cell lines.

№	Entry	Concentration (μg/ml)	Cell line, cell survival (%)				Conclusion
			HepG2	Lu1	RD	FL	
	DMSO	–	100	100	100	100	
	Taxol (+)	5	1.34±0.80	3.66±0.90	0.68±0.30	3.87±1.10	+
1	<b>3a</b>	10	100	99.06±1.50	100	100	–
2	<b>3b</b>	10	<b>0</b>	<b>0</b>	<b>0</b>	<b>0</b>	+ (04 cell lines)
3	<b>3c</b>	10	<b>0</b>	<b>0</b>	<b>0</b>	<b>0</b>	+ (04 cell lines)
4	<b>3d</b>	10	<b>20.07±0.80</b>	<b>38.25±0.40</b>	<b>39.26±0.70</b>	64.09±0.8	+ (03 cell lines)
5	<b>3e</b>	10	67.61±1.40	64.44±1.50	83.27±1.20	59.3±2.30	–
6	<b>3f</b>	10	58.45±0.80	72.89±2.80	<b>44.83±1.10</b>	<b>13.46±1.60</b>	+ (RD, FL)
7	<b>3g</b>	10	60.85±1.40	69.47±2.60	<b>50.09±2.80</b>	<b>41.71±3.10</b>	+ (RD, FL)

**Table 3.** Results of IC<sub>50</sub> test.

№	Entry	Cell lines IC <sub>50</sub> (μg/ml)				Conclusion
		HepG2	Lu1	RD	FL	
	Taxol (+)	0.290	0.310	0.250	0.330	+
1	<b>3b</b>	2.607	2.664	2.560	1.392	+ (04 cell lines)
2	<b>3c</b>	7.957	6.946	6.887	6.742	+ (04 cell lines)
3	<b>3d</b>	6.586	7.904	7.528	–	+ (03 cell lines)
4	<b>3f</b>	–	–	8.881	8.346	+ (RD, FL)
5	<b>3g</b>	–	–	10.00	9.505	+ (RD, FL)

**Table 4.** Cytotoxicity tests performed on compounds **3b-d** in Vero cell line.

Entry	Entry	Concentration ( $\mu\text{g/ml}$ )	Vero cell line, cell survival (%)	Conclusion
	DMSO	–	100	
	Taxol (+)	5	32.24 $\pm$ 1.70	
1	<b>3b</b>	10	71.60 $\pm$ 2.30	–
		5	86.55 $\pm$ 0.70	–
2	<b>3c</b>	10	65.86 $\pm$ 0.80	–
		5	92.22 $\pm$ 1.50	–
3	<b>3d</b>	10	80.92 $\pm$ 1.30	–

In the continuing evaluation of potential anticancer agents, compounds (**3b-d**) were tested for cytotoxicity on the normal African green monkey kidney cell line (Vero cell line). The results are shown in Table 4, and these compounds are not active on the Vero cell line.

None of the new azacrown ethers (**3b-d**) had significant activity against the Vero cell line achieving an CS value in the 65.86 to 92.22 (%). Therefore, compounds (**3b-d**) could be considered as promising anticancer agents due to their low cytotoxicity on the Vero cell line and their high cytotoxic effect on the cancer cells. The results are shown in Table 4.

Thus, according to the results of the *in vitro* tests, compounds (**3b,c**) containing [ $\gamma$ -(4-R-aryl)] have an outstanding effect in treating HepG2, Lu1, RD and FL cell lines. Compound (**3d**) showed high cytotoxic effect on HepG2, Lu1 and RD cell lines. At the same time, both azacrown ethers (**3f,g**) containing ( $\gamma$ -heteroaryl) had weaker cytotoxicity against the RD and FL cell lines.

## Conclusions

We have demonstrated good prospects for synthesis and discovery of new potential anticancer agents in the group of azacrown ethers. Seven new dibenzo[( $\gamma$ -aryl)pyridino]aza-17-crown-5 ethers containing 2,4,6-triarylpyridine were synthesized successfully using Hantzsch type multicomponent reaction. Some of the obtained compounds showed high cytotoxic activity against human tumor cells (HepG2, Lu1, RD, FL), and they were not active on the Vero cell line. These findings are interesting for further studies on active azacrown ethers (**3b-d**) for the proper assessment of their chemotherapeutic properties as well as for the development of promising anticancer drugs.

**Acknowledgements.** This research was funded by the Vietnam National Foundation for Science and Technology Development (NAFOSTED) under grant number 104.01-2015.27 and supported by the Ministry of Education and Science of the Russian Federation (the Agreement number 02.a03.21.0008).

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Received 24.04.2017

Accepted 22.09.2017