Full Paper

Design, Synthesis and Anticancer Activity Evaluation of Some Novel Pyrrolo[1,2-*a*]azepine Derivatives

Amany Belal[1,2]

[1] Pharmaceutical Chemistry Department, College of Pharmacy, Taif University, Taif, Kingdom of Saudi Arabia
[2] Faculty of Pharmacy, Medicinal Chemistry Department, Beni-Suef University, Beni-Suef, Egypt

A novel series of pyrrolo[1,2-*a*]azepine derivatives **3**–**7** were synthesized and their structures were confirmed by spectral and elemental analyses. Antitumor activity evaluation of these compounds was carried out against liver (HepG2), breast (MCF7), and colon (HCT116) cancer cell lines using the sulforhodamine-B (SRB) assay method and doxorubicin as reference standard. Compounds **3** and **6** were found to be more potent than doxorubicin against HepG2 cells, with IC_{50} values of 4, 1.6 and 10.8 nM, respectively. Moreover, compounds **3** and **7** showed broad-spectrum anticancer activity against all the tested cell lines, and their IC_{50} values were in the nanomolar range (4–44.2 nM and 20.7–45.4 nM, respectively). The 2-benzoylamino derivative of pyrrolo[1,2-*a*]azepine **5b** was the most potent one against MCF7 cells (IC_{50} of 10.7 nM); however, the 2-(2-chloro-acetylamino)-pyrroloazepine derivative **6** was the most potent against the HCT116 cell line, with an IC_{50} value of 21.1 nM. The novel compounds were docked into the active site of cyclin-dependent kinase 2 (CDK2) to explore the ability of these compounds to interact with these kinases. All compounds showed a lower binding score energy than the reference ligand.

Keywords: Antitumor / Pyrimidopyrroloazepine / Pyrrolo[1,2-*a*]azepine / Pyrroloazepine derivatives

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Introduction

Cancer is a malignant neoplasm characterized by unregulated cell growth, in which cell division and growth are uncontrollable and cancer cells can invade nearby tissues of the body and even can spread to more distant organs and tissues through blood stream or the lymphatic system [1]. Cancer is a major health problem threatening people worldwide. More than 200 types of cancers can affect human beings, with 25% of deaths in the United States because of cancer. Moreover, the expected number of new cancer cases and cancer death cases in the United States in 2013 is 1,660,290 and 580,350, respectively [2]. The aforementioned facts call for continuous search for new antitumor active agents.

Pyrrolo[1,2-*a*]azepine represents a core for over 80 alkaloids known as stemona alkaloids separated from Stemonaceae family plants. Herbal extracts of these plants have been used for thousands of years in folk medicine in East Asia as cough suppressant, antibacterial, antifungal, antitubercular and antiparasitic [3–5]. Lindsay and Pyne [6] reported the synthesis of 5,7-heterocyclic ring system (1*H*-pyrrolo[1,2-*a*]azepine) as an analogue for 5,5- and 5,6-heterocyclic systems to be explored as a potential glycosidase inhibitor. Croomine alkaloid that has a pyrrolo[1,2-*a*]azepine scaffold showed antitussive activity with an ID_{50} value of 0.18 mmol/kg using citric acid-induced guinea pig cough model [7]. Muchowski et al. [8] reported the synthesis and anti-inflammatory activity of some 3*H*-pyrrolo[1,2-*a*]azepine-1-carboxylic acid derivatives as an analog for pyrrolo[1,2-*a*]pyrrole-1-carboxylic acid system. All these findings proved the importance of the pyrrolo[1,2-*a*]-azepine in medicinal chemistry. It proved to be a useful scaffold for the design of several analogs of pharmacological interest.

It is observed also from the literature that pyrrolo[1,2-*a*]-azepine is the main scaffold of some antitumor active agents. The most important examples for compounds bearing the pyrrolo[1,2-*a*]azepine skeleton are esters of cephalotaxine,

Correspondence: Dr. Amany Belal, Faculty of Pharmacy, Medicinal Chemistry Department, Beni-Suef University, Beni-Suef 62514, Egypt.
E-mail: abilalmoh1@yahoo.com
Fax: +20 82 2317958

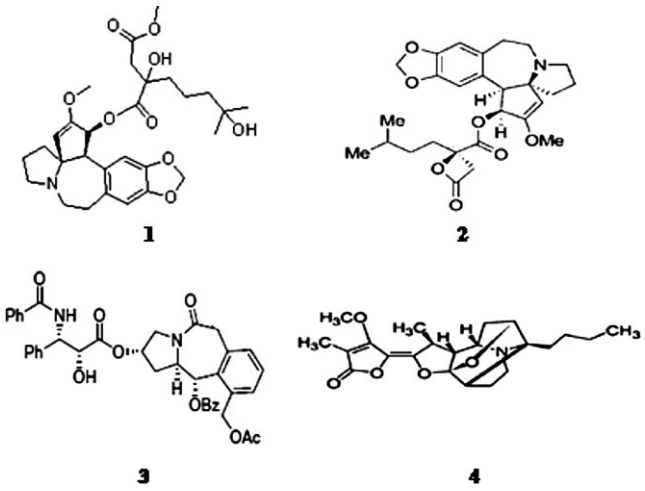


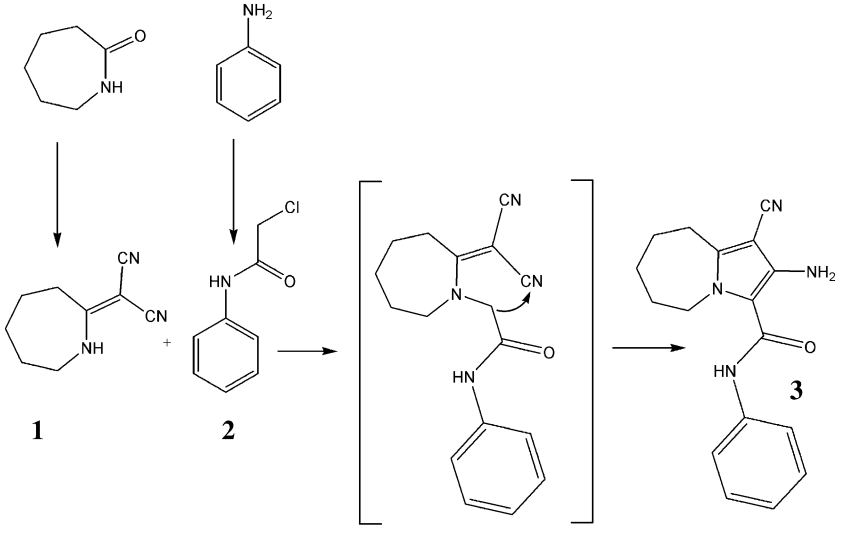
Figure 1. Antitumor active agents with pyrrolo[1,2-*a*]azepine scaffold.

which showed significant activity as anti-leukemic agents [9]. Homoharringtonine **1**, the antitumor alkaloid (Fig. 1), has reached phase I/II in the United States in clinical investigation against myeloid leukemia, its side effects still a major limitation [10–12]. Acylating 3-OH group of cephalotaxine revealed compound **2** (Fig. 1) that showed significant cytotoxicity against several human hematopoietic and solid tumor cell lines [13]. The alkaloid **3** (Fig. 1) bearing a pyrrolo[1,2-*a*]azepine scaffold was designed and synthesized as a paclitaxel mimic and proved to be a potent compound against several drug-resistant and drug-sensitive human cancer cell lines [14]. Moreover, stemofoline **4** (Fig. 1) proved to have the ability of increasing the intracellular P-glycoprotein, decrease vinblastine efflux in multidrug-resistant human cervical carcinoma KB-V1, and increase their sensitivity to paclitaxel, vinblastine, and doxorubicin [15, 16]. Based on these findings, we report in this work the synthesis of novel compounds containing the pyrrolo[1,2-*a*]azepine scaffold **3**–**7** to evaluate their antitumor activity, hoping to obtain a new series of antitumor active agents. Docking studies against cyclin-dependent kinase 2 (CDK2) are also performed using a PDB file (2VTO) and MOE program for a better understanding of the antitumor results and to investigate the ability of these compounds to act as inhibitors for this receptor.

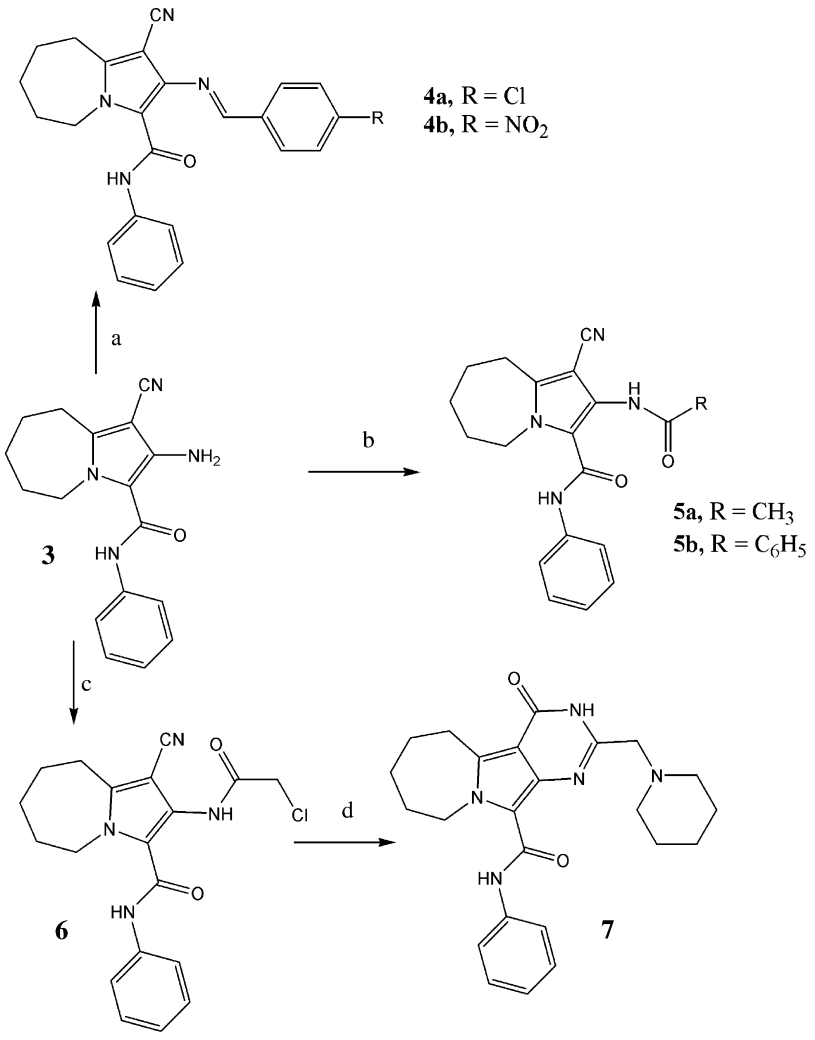
Results and discussion

Chemistry

The aim of this work was to develop novel derivatives having a pyrrolo[1,2-*a*]azepine scaffold to evaluate their anticancer activity. Compounds **1** and **2** were obtained from azepan-2-one and aniline, respectively, as the previously reported methods [17, 18]; heating both compounds under reflux for 24 h in dry acetone and in presence of dry potassium carbonate afforded compound **3** directly, as shown in Scheme 1. The IR spectrum of compound **3** showed absorption bands for cyano, NH_2, NH, and carbonyl group; in addition, the ^{1}H NMR spectrum revealed characteristic signals for $5CH_2$ of azepan, aromatic protons signal at 7.11–7.55 ppm, and two singlets at 8.3 and 9.3 corresponding to NH_2 and NH, respectively. Reaction of the obtained pyrrolo[1,2-*a*]azepine derivative **3** (Scheme 2) with equimolar amount of *p*-chloro or *p*-nitro benzaldehyde in absolute ethanol in presence of glacial acetic acid afforded the benzylidene derivatives of pyrrolo[1,2-*a*]azepine **4a** and **4b**. The ^{1}H NMR spectrum of **4a**



Scheme 1. Synthesis of compound **3**.

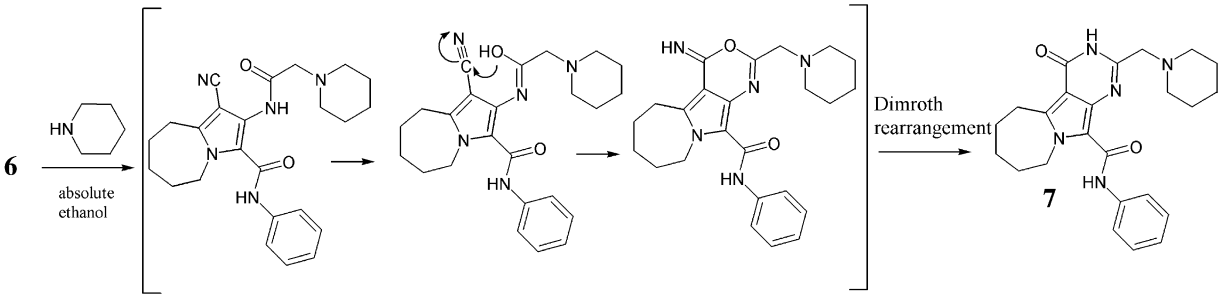


Scheme 2. Reagents and conditions: (a) *p*-Substituted benzaldehyde, five drops of glacial acetic acid, absolute ethanol, reflux 10 h. (b) Acid chloride, dry benzene, stirring at RT for 24 h. (c) Chloroacetylchloride, dioxan, stirred at RT for 12 h. (d) Piperidine, absolute ethanol, reflux 24 h.

and **4b** showed the appearance of additional aromatic protons and two singlets at 9.9 and 10.1 ppm corresponding to N=CH of **4a** and **4b**, respectively. Acylated derivatives of pyrrolo[1,2-*a*]azepine **5a** and **5b** were obtained through reaction of compound **3** with acetyl or benzoyl chloride. IR spectrum of both compounds showed an additional absorption band for carbonyl group, ^{1}H NMR spectrum of **5a** showed a singlet of 3H at 2.2 corresponding to CH_3 and that of **5b** showed additional aromatic protons that prove benzoylation of compound **3** amino group. 2-(2-Chloro-acetylamino)-pyrrolo[1,2-*a*]azepine derivative **6** was obtained by reacting compound **3** with chloroacetylchloride in dioxan at room temperature, ^{1}H NMR spectrum of compound **6** showed the appearance of a singlet signal at 4 ppm for CH_2Cl in addition to $5CH_2$ of azepan and aromatic proton signals. Compound **6** was refluxed in absolute ethanol with excess piperidine to give pyrimido pyrroloazepine derivative **7**. The IR spectrum revealed disappearance of the cyano group, and ^{1}H NMR spectrum showed the appearance of two multiplets at 2.1 and 3.1 ppm for piperidine protons in addition to a singlet signal at 4 ppm corresponding to CH_2-piperidine. The proposed mechanism for prymidine derivative **7** formation is shown in Scheme 3 [19].

Pharmacological screening

In this study, cytotoxic activity of the novel compounds was evaluated *in vitro* against liver (HepG2), breast (MCF7), and colon (HCT116) cancer cell lines, using doxorubicin as a reference drug. The survival curve was obtained by plotting surviving fraction of cancer cells against drug concentration. The concentration of the tested compounds that causes 50% inhibition of viable cells (IC_{50}) was calculated. Data represented in Table 1 show the IC_{50} values for the tested compounds and doxorubicin in μg and μM. All the tested



Scheme 3. Mechanism of formation of compound **7**.

compounds exhibited good activity against all three tested cell lines. The most potent activity against HepG2 cell line was shown by compounds **3** and **6**. They were more potent than doxorubicin itself, followed by pyrimido pyrroloazepine derivative **7** with an IC_{50} value of 20.7 nM. Compounds **5b**, **5a**, **7**, **4b**, and **3** were shown to be the most potent against MCF7 cell line in a sequence mode with IC_{50} range (10.7–44.2 nM). The most active compounds against HCT116 cell line were **6** > **3** > **7** > **5b** according to their IC_{50} values that were expressed also in nanomoles. From these observed results, it can be concluded that:

(1) Compounds based on pyrroloazepine as a main nucleus showed a very good and potent activity against HepG2, MCF7, and HCT116 cell lines and this represents a promising scaffold in cancer chemotherapy.
(2) The unsubstituted amino group of pyrroloazepine derivative **3** and pyrimidopyrroloazepine **7** showed a broad-spectrum anticancer activity against all the three tested cell lines, their IC_{50} expressed in a nanomolar range. Moreover, compound **3** was more potent than doxorubicin against HepG2 cancer cells.
(3) Acylating the free amino group of pyrroloazepine **3** with chloro acetyl group afforded the most potent compound **6** against both HepG2 and HCT116 cell lines; its IC_{50} was 1.6 and 21.1 nM, respectively, and showed a more potent activity than doxorubicin against HepG2 cell line.
(4) Benzoylation of the 2-amino group of compound **3** revealed a compound **5b** with high potency against two cancer cell lines MCF7 and HCT116, and showed to be the most potent one against MCF7 cell line with IC_{50} value of 10.7 nM. However, acylating this amino group with acetyl moiety afforded compound **5a** with high selectivity against MCF7 cancer cells; its IC_{50} is 15.7 nM.
(5) *p*-Nitro benzylidene derivative of pyrroloazepine **4b** showed to be more potent than the *p*-chloro derivative **4a** with high selectivity against MCF7 cell line (IC_{50} 34 nM); the *p*-chloro derivative **4a** was the least active one of the tested compounds.
(6) In addition to docking results against CDK2 receptor that revealed the ability of all the synthesized compounds to quick fit this receptor with lower score energy than the ligand itself (Table 2), the best fitting score energy was shown by the broad-spectrum compound **7** followed by the most active compound against HepG2 cell line **6**, then compounds **4b** and **5b** that showed great activity specially against MCF7 cell line. Compound **4a** showed a better score energy than both **3** and **5a**; however, its activity was the least one of them and this could be explained by

Table 1. *In vitro* anticancer activity of the synthesized pyrroloazepines.

Compound no.	HepG2 (liver cancer cell line)	MCF7 (breast cancer cell line)	HCT116 (colon cancer cell line)
IC_{50} μg (μM)			
3	1.17 (0.0040)	13.0 (0.0442)	8.025 (0.0273)
4a	87.25 (0.2093)	106.33 (0.2551)	108.2 (0.2595)
4b	101.44 (0.2373)	14.54 (0.0340)	116.2 (0.2718)
5a	47.07 (0.1399)	5.27 (0.0157)	38.58 (0.1147)
5b	106.44 (0.2671)	4.25 (0.0107)	22.51 (0.0565)
6	0.61 (0.0016)	51.06 (0.1377)	7.81 (0.0211)
7	8.671 (0.0207)	11.54 (0.0275)	19.06 (0.0454)
Doxorubicin	6.26 (0.0108)	4.92 (0.0085)	5.88 (0.0101)

Table 2. Binding energy scores, amino acid interactions, and hydrogen bond length of the docked compounds on the active site of cyclin-dependent kinase 2 (CDK2).

Compound no.	*S* (kcal/mol)	Amino acid residue	H bond length *A*
3	−15.6194	lIe10	2.78
4a	−17.3536	Lys89	2.82
4b	−18.1355	Lys89	3.25
5a	−13.9417	Leu83	2.74
5b	−18.0423	Lys89, lIe10	2.71, 2.67
6	−18.7142	Lys89, lIe10	3.17, 2.81
7	−19.2410	Lys89, Asp86	2.37, 1.82
LZ8	−6.4785	Leu83, Glu81	2.77, 3.11, 2.61

amino acid interactions as compounds **3** and **5a** form a hydrogen bonding with lle10 and Leu83, respectively, but compound **4a** formed a hydrogen bonding with Lys89.

Molecular docking

Cyclin-dependent kinases (CDKs) are key regulatory protein kinases in cell cycle progression. Inhibition of these cell cycle proteins has proved to be an effective strategy in the development of new anticancer active agents [20]. The newly synthesized compounds **3–7** were docked into ATP binding site of CDK2 in a trial to suggest their mechanism of action and explore their ability to act as inhibitors for these kinases. The PDB file 2VTO was used to perform docking simulation. This file contains CDK2 co-crystallized with LZ8 [21]. Molecular docking was achieved by Molecular Operating Environment software 10.2008 (MOE) provided by the Chemical Computing Group, Canada. Verification step was performed by redocking of the co-crystallized ligand LZ8 into the active site of CDK2 with root mean standard deviation (RMDS) = 1.2854 and the score energy was (*S*) = −6.4785 kcal/mol. LZ8 showed three interactions with the active site of CDK2: two hydrogen bonds with Leu83 of 2.77 and 3.11 Å and one hydrogen bond with Glu81 of 2.61 Å (Fig. 2). The obtained data for all the novel docked compounds, energy score (*S*), amino acid interactions, and hydrogen bond length, are presented in Table 2. All compounds **3–7** were fit to the active site of CDK2 and showed a better score energy than the reference ligand, it suggests the idea of being able to act as inhibitors for this receptor. The best score energy was shown by compound **7** (*S*) = −19.2410 kcal/mol that interacted with two amino acids (Fig. 3) Lys89 and Asp86 with hydrogen bonding of 2.37 and 2.7 Å, respectively, followed by compound **6** with score energy (*S*) = −18.7142 kcal/mol and interactions with two amino acids, Lys89 with hydrogen bond of 3.17 Å and Ile10 with hydrogen bond of 2.81 Å (Fig. 4).

Conclusion

Pyrroloazepines proved to be a promising scaffold for designing new anticancer active agents as noticed during this work. All the synthesized pyrroloazepines **3–7** showed a potent inhibitory activity against liver (HepG2), breast (MCF7), and colon (HCT116) cancer cell lines. Compound **3** and the pyrimidopyrroloazepine **7** showed a broad-spectrum anticancer activity against all the tested cell lines and their IC_{50} values were expressed in nanomoles. Moreover, compound **6**, which was the most potent against HepG2 and HCT116, and compound **3** were found to be more potent than doxorubicin against HepG2 cells; their IC_{50} was 1.6 and 4 nM, respectively. The benzoylated derivative of pyrroloazepine **5b** showed to be the most active one against MCF7 cells with an IC_{50} value of 10.7 nM. All the synthesized compounds when docked into the active site of CDK2 showed to quick fit this site with less

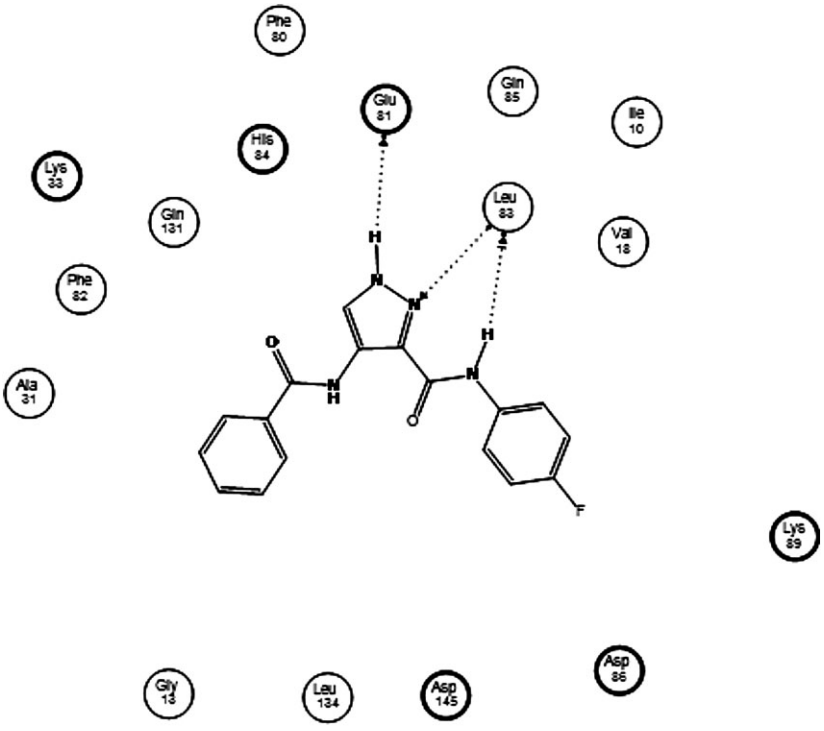


Figure 2. LZ8 ligand co-crystallized with CDK2.

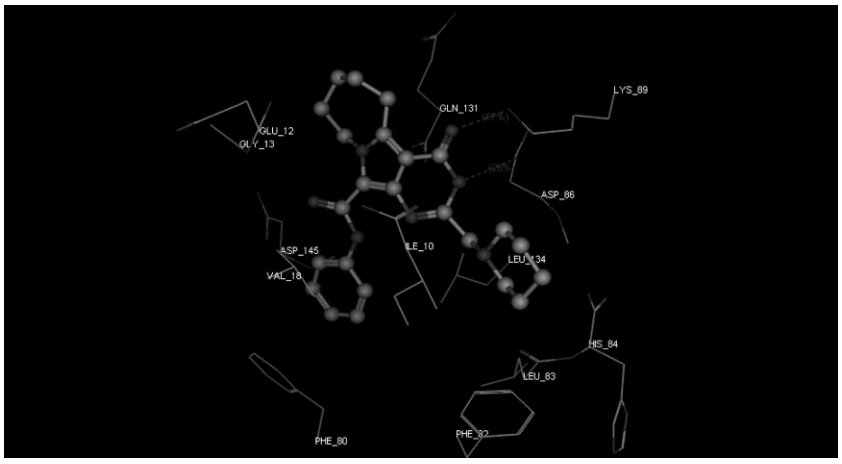


Figure 3. 3D interactions of compound **7** with CDK2.

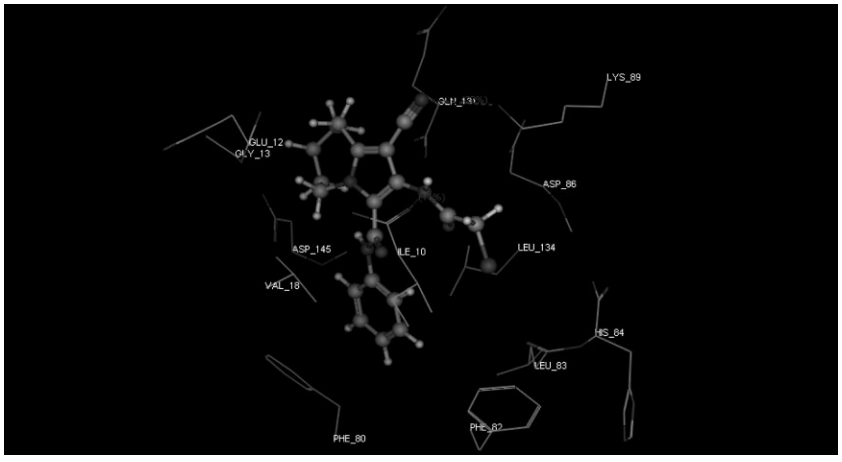


Figure 4. 3D interactions of compound **6** with CDK2.

energy score than the reference ligand LZ8 and this may help suggesting their mechanism as CDK2 inhibitors. All the obtained information from this work might be helpful in design and synthesis of new chemotherapeutic active agents based on pyrroloazepine scaffold.

Experimental

Chemistry

Compounds **1** and **2** were prepared according to the previously reported procedures [17, 18]. Melting points were detected using Electrothermal Stuart $5MP_3$ digital melting point apparatus and were uncorrected. Kieselgel 0.25 mm, 60 G F 254, Merck Silica gel plates were used for thin layer chromatography (TLC). The running solvent system was chloroform/methanol (9:1) and ultraviolet light was used to detect the spots. Infrared (IR) spectra (KBr disc) were performed on FT-IR spectrophotometer. IR and elemental microanalyses were performed at the Microanalytical Center, Faculty of Science, Cairo University; the ^{1}H NMR spectra were recorded in $CDCl_3$ and on a Varian Mercury spectrometer (300 and 400 MHz), and ^{13}C NMR (DMSO-d_6) spectra were recorded at 100.62 MHz at the aforementioned research center in Cairo University and the magnetic resonance unit at Beni-Suef University. Chemical shifts are expressed in values (ppm) and tetramethylsilane (TMS) is the internal standard. Addition of D_2O was used to confirm the exchangeable protons.

*Amino-1-cyano-6,7,8,9-tetrahydro-5H-pyrrolo[1,2-a]-azepine-3-carboxylic acid phenylamide **3***

To a mixture of **1** (0.16 g, 1 mmol) and **2** (0.17 g, 1 mmol) in dry acetone (20 mL), dry potassium carbonate (0.5 g) was added and the reaction mixture was refluxed for 24 h. The solvent was removed under vacuum; the obtained residue was washed with water, filtered, and crystallized from ethanol to give white solid, 0.2 g, 68% yield, m.p. 148–150°C. IR: $\nu_{max.}/cm^{-1}$ 3066 (CH aromatic), 3266, 3163 (NH_2, NH), 2202 (CN), 1591 (C=O). ^{1}H NMR ($CDCl_3$, 400 MHz): δ 1.47–1.83 (3m, 6H, azepane), 2.69 (t, 2H, azepane), 3.40 (t, 2H, azepane), 7.11–7.55 (m, 5H, aromatic protons), 8.32 (s, 2H, NH_2 exchangeable with D_2O), 9.34 (s, 1H, NH

exchangeable with D_2O). ^{13}C NMR (DMSO-d_6): 24.5, 28.1, 29.5, 31.2, 45.1, 47.2, 116.2, 117.7, 120.2, 125.6, 128.9, 129.3, 177.4. Anal. calcd. for $C_{17}H_{18}N_4O$(294.35): C, 69.37; H, 6.16; N, 19.03. Found: C, 69.81; H, 6.10; N, 19.43.

*General procedure for the preparation of **4a** and **4b***

To a solution of **3** (0.3 g, 2 mmol) in absolute ethanol (20 mL), the appropriate aldehyde (2 mmol) and five drops of glacial acetic acid were added; the reaction mixture was heated under reflux for 10 h. Solvent was removed under vacuum and the obtained solid was crystallized from ethanol/water.

*2-[(4-Chloro-benzylidene)-amino]-1-cyano-6,7,8,9-tetrahydro-5H-pyrrolo[1,2-a]azepine-3-carboxylic acid phenylamide **4a***

Compound **4a** was prepared from **3** and 4-chlorobenzaldehyde. Yellowish green crystals, 0.35 g, 41% yield, m.p. 153–155°C. IR: $\nu_{max.}/cm^{-1}$ 3163 (NH), 3062 (CH aromatic), 2202 (CN), 1649 (C=O). ^1H NMR ($CDCl_3$, 400 MHz): δ 1.63–1.78 (3m, 6H, azepane), 2.70 (t, 2H, azepane), 3.41 (t, 2H, azepane), 7.28–7.47 (m, 9H, aromatic protons), 9.10 (s, 1H, NH exchangeable with D_2O), 9.98 (s, 1H, N=CH). ^{13}C NMR (DMSO-d_6): 24.5, 28.1, 29.5, 31.2, 45.1, 47.2, 53.2, 116.2, 117.7, 125.6, 127.1, 129.3, 129.8, 177.4. Anal. calcd. for $C_{24}H_{21}ClN_4O$ (416.90): C, 69.14; H, 5.08; N, 13.44. Found: C, 69.63; H, 5.45; N, 13.55.

*1-Cyano-2-[(4-nitro-benzylidene)-amino]-6,7,8,9-tetrahydro-5H-pyrrolo[1,2-a]azepine-3-carboxylic acid phenylamide **4b***

Compound **4b** was obtained from compound **3** and 4-nitro-benzaldehyde. yellow crystals, 0.40 g, 46% yield, m.p. 163–165°C. IR: $\nu_{max.}/cm^{-1}$ 3163 (NH), 3068 (CH aromatic), 2202 (CN), 1706 (C=O). ^1H NMR ($CDCl_3$, 400 MHz): δ 1.62–1.90 (3m, 6H, azepane), 2.72 (t, 2H, azepane), 3.41 (t, 2H, azepane), 7.16–7.62 (m, 5H, aromatic protons), 8.08–8.42 (m, 4H, aromatic protons), 9.26 (s, 1H, NH exchangeable with D_2O), 10.17 (s, 1H, N=CH). ^{13}C NMR (DMSO-d_6): 24.5, 26.5, 28.1, 29.5, 45.1, 116.2, 117.7, 123.8, 124.7, 125.6, 126, 126.1, 127.1, 128.5, 129.1, 129.3, 131, 140.3, 141.2, 164.5, 177.4. Anal. calcd. for $C_{24}H_{21}N_5O_3$ (427.46): C, 67.44; H, 4.95; N, 16.38. Found: C, 67.24; H, 5.09; N, 16.79.

*General procedure for the preparation of **5a** and **5b***

Compound **3** (0.3 g, 2 mmol) was dissolved in dry benzene (20 mL) and the appropriate acid chloride (2 mmol) was added; the reaction mixture was stirred at room temperature for 24 h, and the solvent was evaporated under vacuum. The obtained product was crystallized from methanol.

*2-Acetylamino-1-cyano-6,7,8,9-tetrahydro-5H-pyrrolo-[1,2-a]azepine-3-carboxylic acid phenylamide **5a***

Compound **5a** was prepared from **3** and acetyl chloride. White solid, 0.34 g, 50% yield. IR: $\nu_{max.}/cm^{-1}$ 3265, 3163 (2NH), 2202 (CN), 1591 (br. 2C=O). ^1H NMR ($CDCl_3$, 400 MHz): δ 1.60–1.90 (3m, 6H, azepane), 2.23 (s, 3H, CH_3), 2.86 (t, 2H, azepane), 3.60 (t, 2H, azepane), 4.55 (s, 1H, NH exchangeable with D_2O), 7.10–7.64 (m, 5H, aromatic protons), 9.81 (s, 1H, NH exchangeable with D_2O). ^{13}C NMR (DMSO-d_6): 23.1, 26.4, 28.1, 31.2, 45.1, 47.2, 116.2, 117.7, 119.9, 124.3, 125.6, 127.1, 129.2, 129.3, 177.4. Anal. calcd. for $C_{19}H_{20}N_4O_2$ (336.39): C, 67.84; H, 5.99; N, 16.66. Found: C, 67.89; H, 6.04; N, 16.44.

*Benzoylamino-1-cyano-6,7,8,9-tetrahydro-5H-pyrrolo-[1,2-a]azepine-3-carboxylic acid phenylamide **5b***

Compound **5b** was prepared from compound **3** and benzoyl chloride. White crystals, 0.36 g, 45% yield, m.p. 139–141°C. IR: $\nu_{max.}/cm^{-1}$ 3265, 3162 (2NH), 3066 (CH aromatic), 2202 (CN), 1695, 1591 (2C=O). ^1H NMR ($CDCl_3$, 400 MHz): δ 1.66–1.97 (3m, 6H, azepane), 2.72 (t, 2H, azepane), 3.42 (t, 2H, azepane), 7.28–7.62 (m, 8H, aromatic protons), 8.09 (m, 2H, aromatic protons), 8.60, 10.01 (2s, 2H, 2NH exchangeable with D_2O). ^{13}C NMR (DMSO-d_6): 24.5, 28.1, 29.5, 31.2, 45.1, 47.2, 116.2, 117.7, 125.6, 128, 128.9, 129.2, 129.3, 129.7, 131.2, 133.2, 167.7, 177.4. Anal. calcd. for $C_{24}H_{22}N_4O_2$ (398.46): C, 72.34; H, 5.57; N, 14.06. Found: C, 72.47; H, 5.63; N, 14.56.

*2-(2-Chloro-acetylamino)-1-cyano-6,7,8,9-tetrahydro-5H-pyrrolo[1,2-a]azepine-3-carboxylic acid phenylamide **6***

To a solution of compound **3** (0.3 g, 2 mmol) in dioxan (20 mL) chloro acetyl chloride (0.22 g, 2 mmol) was added dropwise; the reaction mixture was stirred at room temperature for 12 h, then evaporated under vacuum. The obtained white precipitate was recrystallized from acetone/ethanol mixture, 0.30 g, 40% yield, m.p. 143–145°C. IR: $\nu_{max.}/cm^{-1}$ 3265, 3158 (2NH), 2202 (CN), 1592 (br. 2C=O), 1629 (C=N). ^1H NMR ($CDCl_3$, 400 MHz): δ 1.64–1.79 (3m, 6H, azepane), 2.70 (t, 2H, azepane), 3.41 (t, 2H, azepane), 4.09 (s, 2H, CH_2Cl), 7.15–7.56 (m, 5H, aromatic protons), 8.32 (s, 2H, 2NH exchangeable with D_2O). ^{13}C NMR (DMSO-d_6): 24.5, 28.1, 29.5, 31.2, 44, 45.1, 47.2, 116.2, 117.7, 119.8, 124.2, 125.6, 127.1, 129.2, 129.3, 138.9, 140.6, 165, 177.4. Anal. calcd. for $C_{19}H_{19}ClN_4O_2$ (370.83): C, 61.54; H, 5.16; N, 15.11. Found: C, 61.18; H, 5.63; N, 15.35.

*4-Oxo-2-piperidin-1-ylmethyl-4,5,6,7,8,9-hexahydro-3H-1,3,9a-triaza-benzo[a]azulene-10-carboxylic acid phenylamide **7***

Compound **6** (0.37 g, 1 mmol) was refluxed with piperidine (0.426 g, 5 mmol) in absolute ethanol for 24 h; the reaction mixture was cooled to room temperature, and the obtained white precipitate was filtered and recrystallized from ethanol. 0.30 g, 71% yield, m.p. 155–157°C. IR: $\nu_{max.}/cm^{-1}$ 3433, 3266 (2NH), 3056 (CH aromatic), 1651, 1591 (2C=O). ^1H NMR ($CDCl_3$, 300 MHz): δ 1.66–1.86 (3m, 6H, azepane), 2.10 (m, 4H, piperidine), 2.21 (t, 2H, azepane), 3.10 (m, 4H, piperidine), 3.65 (t, 2H, azepane), 4.03 (s, 2H, CH_2-piperidine), 7.27–7.10 (m, 5H, aromatic protons), 9.02, 10.90 (2s, 2H, 2NH exchangeable with D_2O). ^{13}C NMR (DMSO-d_6): 22.1, 22.5, 24.5, 28.1, 29.5, 31.2, 44, 45.1, 47.2, 53.1, 53.2, 116.2, 117.7, 119.8, 125.6, 127.1, 129.2, 129.3, 164.5, 177.4. Anal. calcd. for $C_{24}H_{29}N_5O_2$ (419.52): C, 68.71; H, 6.97; N, 16.69. Found: C, 68.48; H, 6.63; N, 16.35.

Pharmacological studies

Cytotoxicity of the newly synthesized compounds was evaluated using sulforhodamine-B (SRB) assay method that was previously reported by Skehan et al. [22]. HepG2, MCF7, and HCT116 cancer cell lines were obtained from the American Type Culture Collection (ATCC, Minisota, USA) through the Tissue Culture Unit, The Egyptian Organization for Biological Products and

Vaccines (Vacsera, Egypt). Anticancer activity evaluation was performed at the Center for Genetic Engineering, Al-Azhar University, Cairo, Egypt. Reagents and chemicals were purchased from Sigma–Aldrich Chemical Company (St. Louis, MO, USA).

Cells were seeded for 24 h in 96-well microtiter plates at a concentration of 1000–2000 cells/well, 100 μL/well; then cells were incubated for 48 h with various concentrations (0, 6.25, 12.5, 25, 50, and 100 μg/mL) of the tested compounds and doxorubicin. Three wells were used for each concentration. After incubation for 48 h, the cells were fixed with 10% trichloroacetic acid 150 μL/well for 1 h at 4°C and washed with distilled water three times. Wells were stained for 10–30 min at room temperature with 0.4% SRB and dissolved in 1% acetic acid 70 μL/well. They were then washed with acetic acid 1% to remove unbound dye till colorless drainage was obtained. The plates were subjected to air drying for 24 h not exposed to UV. The dye was solubilized with 150 μL/well of 10 mM Tris–EDTA (pH 7.4) for 5 min on a shaker at 1600 rpm. The optical density (OD) of each well was measured spectrophotometrically at 545 nm with an ELISA microplate reader. The percent of surviving cells was calculated and plotted against different concentrations of the tested compounds to obtain the survival curve. The IC_{50} values were calculated using sigmoidal concentration–response curve fitting models (Sigmaplot software).

Molecular docking

All the molecular docking studies were performed using an Intel Core i5 processor 2.5 GHz, memory (RAM) 4 GB Windows XP operating system and Molecular Operating Environment (MOE, 10.2008) software. 3D structure of the newly synthesized compounds was generated from their 2D structures; then energy minimization was performed using MOE with an RMSD gradient of 0.05 kcal/mol Å; MMFF94X force field and partial charges were automatically calculated. Co-crystallized structure for CDK2 with LZ8 (2VTO) [23] was obtained from protein data bank (http://www.rcsb.org/pdb/home/home.do) and was refined for docking. Ligand was removed from the active site, hydrogens were added, and MOE Alpha Site Finder was used to detect the active site. Then the obtained model was saved as MOE file and docking process was performed.

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References

[1] M. Nishanthi, P. Prasanthi, K. M. Teja, K. M. K. Reddy, P. Nishanthi, M. Nagendramma, B. Vijayakumar, *Asian J. Pharm. Res.* **2013**, *3* (1), 47–52.

[2] R. Siegel, D. Naishadham, A. Jemal, *CA Cancer J. Clin.* **2013**, *63*, 11–30. DOI: 10.3322/caac.21166

[3] M. L. Schulte, M. L. Turlington, S. S. Phatak, J. M. Harp, S. R. Staufferand, C. W. Lindsley, *Chem. Eur. J.* **2013**, *19*, 11847–11852. DOI: 10.1002/chem.201302669

[4] H. Greger, *Planta Med.* **2006**, *72*, 99–113. DOI: 10.1055/s-2005-916258

[5] R. A. Pilli, G. B. Rosso, M. D. C. F. D. Oliveira, *Nat. Prod. Rep.* **2010**, *27*, 1908–1937. DOI: 10.1039/c005018k

[6] K. B. Lindsay, S. G. Pyne, *Tetrahedron* **2004**, *60* (19), 4173–4176. DOI: 10.1016/j.tet.2004.03.050

[7] L. Lin, H. P. Leung, J. Zhu, C. Tang, C. Ke, J. A. Rudd, G. Lin, Y. Ye, *Tetrahedron* **2008**, *64*, 10155–10161. DOI: 10.1016/j.tet.2008.08.046

[8] J. M. Muchowski, S. H. Unger, J. Ackrell, P. Cheung, G. F. Cooper, J. Cook, P. Gallegra, O. Halpern, R. Koehler, A. F. Kluge, A. R. V. Horn, Y. Antonio, H. Carpio, F. Franco, E. Galeazzi, I. Garcia, R. Greenhouse, A. Guzmb, J. Iriarte, A. Leon, A. Pefia, V. Perz, D. Valdez, N. Ackerman, S. A. Ballaron, D. V. K. Murthy, J. R. Rovito, A. J. Tomolonis, J. M. Young, W. H. Rooks, *J. Med. Chem.* **1985**, *28* (8), 1037–1049.

[9] R. G. Powell, D. Weisleder, C. R. Smith, *J. Pharm. Sci.* **1972**, *61*, 1227–1230.

[10] C. Luo, J. Tang, Y. Wang, *Hematology* **2004**, *9*, 259–270.

[11] H. Kantsrjian, J. Cortes, *Int. J. Hematol.* **2006**, *83*, 289–293.

[12] H. Itokawa, S. L. Morris-Natschke, T. Akiyama, K. Lee, *J. Nat. Med.* **2008**, *62*, 263–280. DOI: 10.1007/s11418-008-0246-z

[13] J. D. Eckelbarger, J. T. Wilmot, M. T. Epperson, C. S. Thakur, D. Shum, C. Antczak, L. Tarassishin, H. Djaballah, D. Y. Gin, *Chem. Eur. J.* **2008**, *14*, 4293–4306. DOI: 10.1002/chem.200701998

[14] L. Sun, J. M. Veith, P. Pera, R. J. Bernacki, I. Ojima, *Bioorg. Med. Chem.* **2010**, *18* (19), 7101–7112. DOI: 10.1016/j.bmc.2010.07.069

[15] W. Chanmahasathien, S. Ohnuma, S. Ambudkar, P. Limtrakul, *Planta Med.* **2011**, *77* (18), 1990–1995. DOI: 10.1055/s-0031-1280054

[16] W. Chanmahasathien, C. Ampasavate, H. Greger, P. Limtrakul, *Phytomedicine* **2011**, *18*, 199–204. DOI: 10.1016/j.phymed.2010.07.014

[17] M. Y. Ebeid, I. Bitter, *Egypt. J. Pharm. Sci.* **1978**, *19*, 349.

[18] W. A. Jacobs, M. Heidelberger, *J. Am. Chem. Soc.* **1917**, *39*, 1435–1439. DOI: 10.1021/ja02252a017

[19] O. K. Al-Duaij, *J. Chem. Chem. Eng.* **2013**, *7*, 111–119.

[20] X. F. Huang, X. Lu, Y. Zhang, G. Q. Song, Q. L. He, Q. S. Li, X. H. Yang, Y. Wei, H. L. Zhu, *Bioorg. Med. Chem.* **2012**, *20*, 4895–4900. DOI: 10.1016/j.bmc.2012.06.056

[21] P. G. Wyatt, A. J. Woodhead, V. Berdini, J. A. Boulstridge, M. G. Carr, D. M. Cross, D. J. Davis, L. A. Devine, T. R. Early, R. E. Feltell, E. J. Lewis, R. L. McMenamin, E. F. Navarro, M. A. O'Brien, M. O'Reilly, M. Reule, G. Saxty, L. C. Seavers, D. M. Smith, M. S. Squires, G. Trewartha, M. T. Walker, A. J. Woolford, *J. Med. Chem.* **2008**, *51*, 4986–4999. DOI: 10.1021/jm800382h

[22] P. Skehan, R. Storeng, D. Scudiero, A. Monks, J. McMahon, D. Vistica, J. T. Warren, H. Bokesch, S. Kenney, M. R. Boyd, *J. Nat. Cancer Inst.* **1990**, *82*, 1107–1112. DOI: 10.1093/jnci/82.13.1107

[23] http://www.rcsb.org/pdb/explore.do?structureId=2VTO (last accessed at December 2, **2013**).

