Available online www.jocpr.com

Journal of Chemical and Pharmaceutical Research, 2016, 8(5):273-282

Research Article

ISSN : 0975-7384
CODEN(USA) : JCPRC5

Design, synthesis and biological evaluation of some novel Schiff base derivatives as potential anticancer agents

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ABSTRACT

*Novel series of pyrrolizine Schiff bases has been synthesized then biologically evaluated as potential anticancer agents. The starting compounds,7-cyano-6-amino-N-(4-(un)substituted-phenyl)-2,3-dihydro-1H-pyrrolizine-5-carboxamides **17a–c**, were reacted with different aldehydes to give the target compounds **18-20**. Structural characterizations of the novel compounds were performed using spectral and elemental analysis. The anticancer activity of these compounds was evaluated using Sulforhodamine-B (SRB) assay method .All of these compounds showed anticancer activity against bothHEPG2 and MCF7 cancer cell lines comparable to that of the standard Doxorubicin (HEPG2 IC_{50} =0.00699µM/ml). Most of compounds are more active against (MCF7) than (HEPG2) cell lines. Compound **18c** showed the highest anticancer activity with IC50 value 0.250µM/ml against (MCF7).While, Compound**18b** was the most potent one against liver (HEPG2) with IC_{50} value 0.784 µM/ml. Modeling studies into ATP binding site of EGFR tyrosine kinase were done to predict their scores and mode of interaction with amino acid residues. Furthermore selectivity of the prototypes(**18-20a**) on normal Wish cell was evaluated and showed IC_{50} of 0.946, 1.322 and 1.122 respectively.*

Keywords: Pyrrolizine; Schiff base; Anticancer; EGFR tyrosine kinase; EGFR-TK inhibitor

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INTRODUCTION

Cancers represent one of the most complicated health problems in the world [1].The higher rate of mortality due to cancers cancer, in addition to of multidrug resistance to some of the currently used anticancer agents [2-5] present an urgent need for development of effective and safe anticancer agents. Pyrrolizines were recently reported as promising scaffold for the design of potent anticancer agents. The dual COX/LOX inhibitor Licofelone **1** showed potent anticancer activities against several cell lines [6-8]. Exploring the mechanism of action of Licofelone**1** revealed its ability to induce apoptosis [9-10]. The tripentone (MR22388) **2** showed strong anticancer activity against leukemia L1210 with IC_{50} of 15 nM. In addition to its ability to act as tubulin polymerization inhibitor [11-12]; MR22388 was found also to acts as a very strong inhibitor for several kinases [13]. Recently, we have reported compound**3** as potent anticancer agents with IC_{50} in the range of 0.98 and 1.12 µM against [14] . The ureido derivative **3** was able to activate caspase 3/7, resulting in apoptosis in MCF-7 cells. Since caspase 3/7 are terminal enzymes in process of programmed cell death.

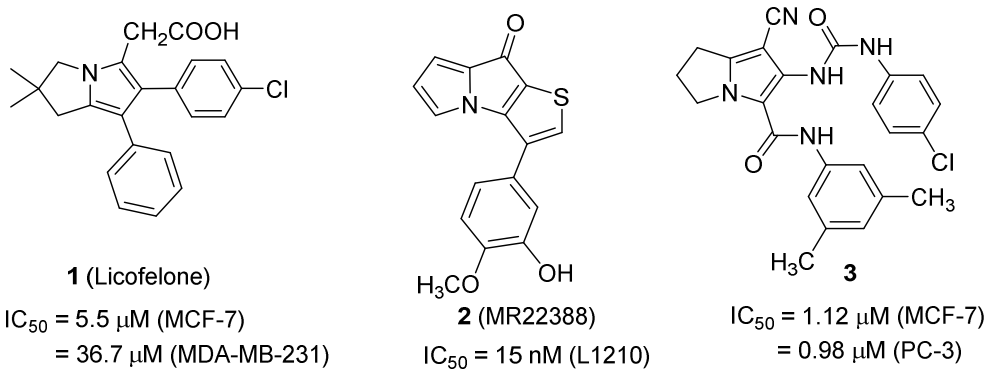


Fig.1.pyrrolizine derivatives with potent anticancer activities

Identification of molecular targets involved in proliferation, malignancy and cell death were helpful in the rational based design of new anticancer agents. Protein kinases are one of these targets which play an important role in regulation of cellular proliferation, differentiation and survival [15]. Several kinases have become relevant therapeutic targets for development of new anticancer agent. The epidermal growth factor receptor (EGFR-TK) play an important role in promoting cell division and survival [16], and it is frequently over-expressed in tumors and it is associated with progression and resistance of cancer cell to anticancer drugs[17]. Several EGFR inhibitors erlotinib **4**and Gefitinib **5**were approved for treatment of cancer displaying high rate of response and high efficacy in treatment of cancer [18,19].But recently, resistance to EGFR inhibitors was developed [20], and development of new EGFR-TK inhibitors became a must to overcome this problem.

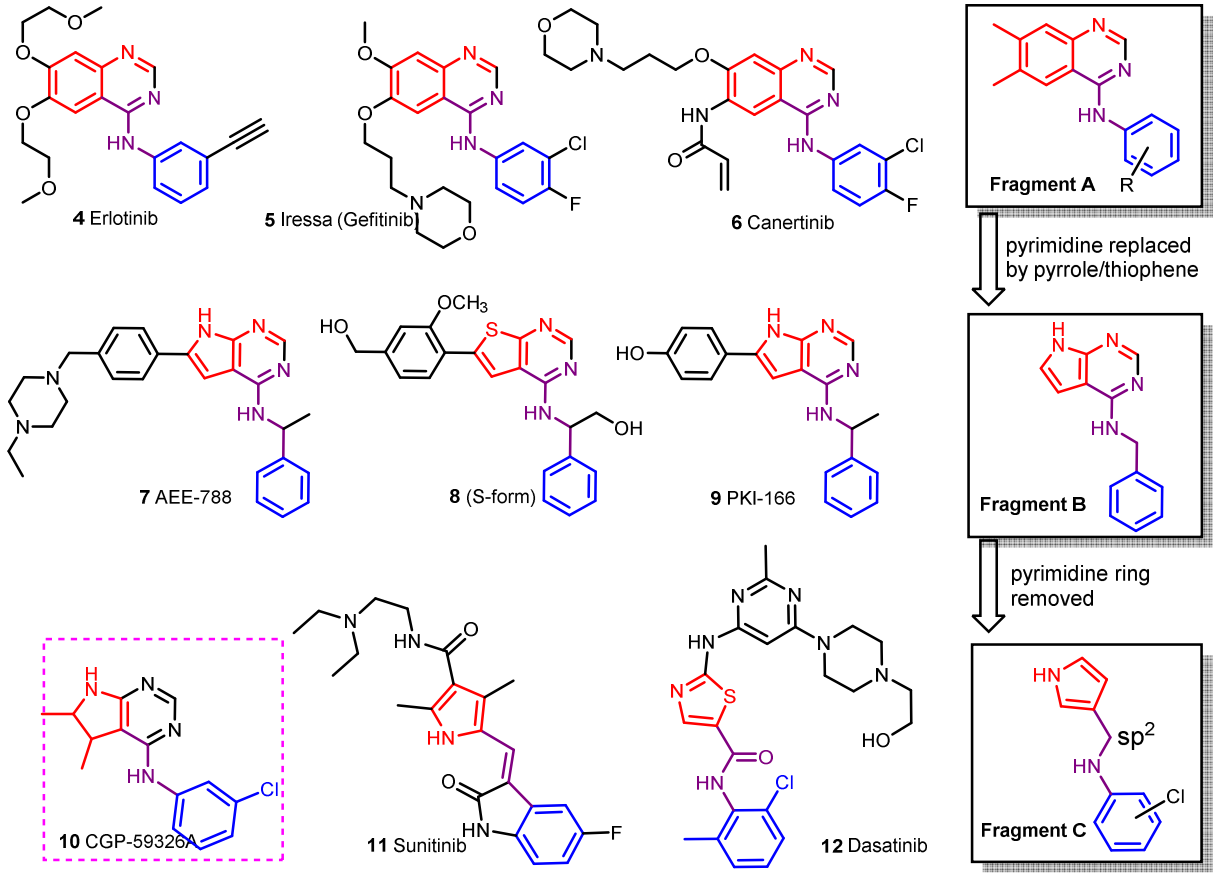


Fig.2.representative EGFR-TK inhibitors

Representative quinazoline, pyrrolopyrimidine, thienopyrimidine, thiazole and pyrrole-based EGFR-TK inhibitors [21-25] sharing some pharmacophoric groups indicated in red and blue colors, **Fig. 2**.Threefragments (A, B and C) were identified in the squares.

Benzene ring replacement in the quinazoline-based EGFR-TK inhibitory compounds with the isosteric pyrrole/thiophene afforded new compounds retaining EGFR-TK inhibitory activity. Additionally, no loss of activity observed on removal of the pyrimidine ring as indicated by sunitinib**11** and dasatinib**12Fig. 2**.

In the present work we aimed to design a new pyrrolizine derivatives bearing some of the three fragments. It was of interest to develop compound **18a** by combining fragments A, B and C in one scaffold with some modification, **Fig. 3**. Several derivatives of compound **18a**were prepared through replacement of the 2-chloro group with electron withdrawing (4-bromo) and electron donating (4-dimethyamino) groups. Moreover, substitution the phenyl ring at C-5 was done using the electron donating (4-CH_3), and electron withdrawing (4-Cl) substituents in order to explore the electronic effects of these substituents on activity of the produced compounds.

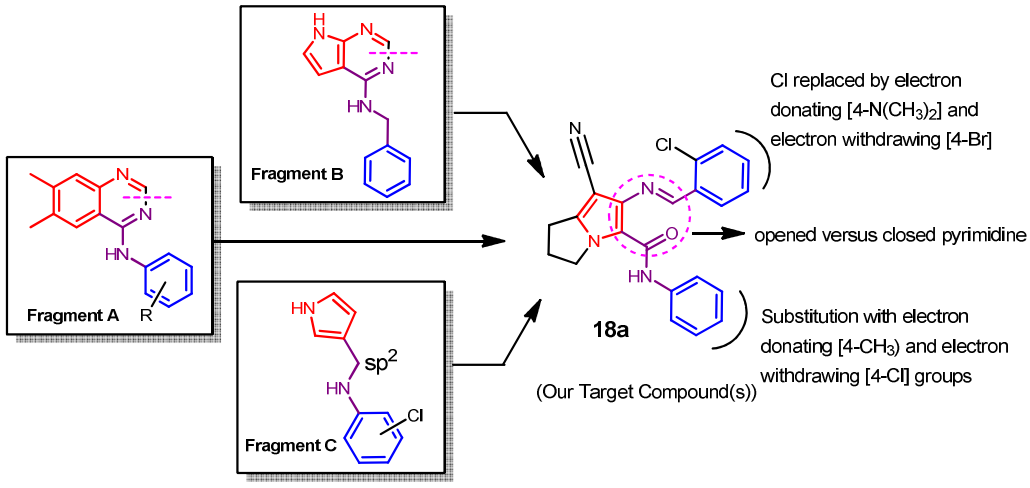
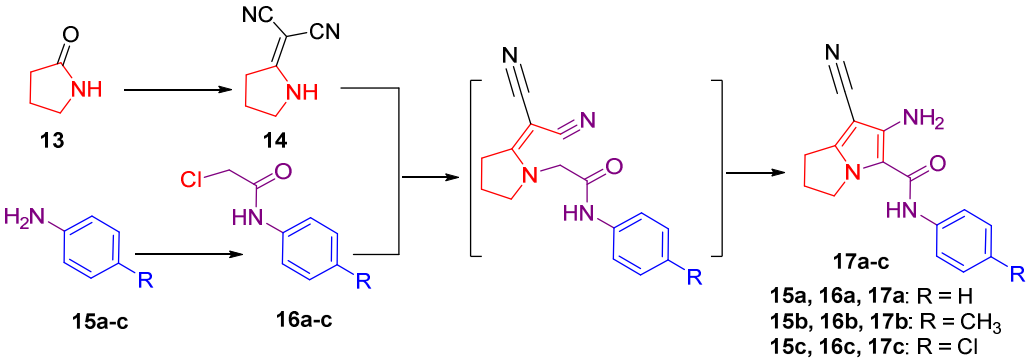


Fig.3. Design strategy and structural modification of compound 18a

RESULTS AND DISCUSSION

2.1. Chemistry

As shown in **Scheme 1**, preparation of the intermediates **14** and **16a-c**was done according to previously reported procedures [26,27]Compounds **17a-c** were synthesized from the reaction of 2-pyrrolidin-2-ylidine malononitrile **14** with the corresponding acetanilide **16a-c** in dry acetone according to previously reported procedures [28].



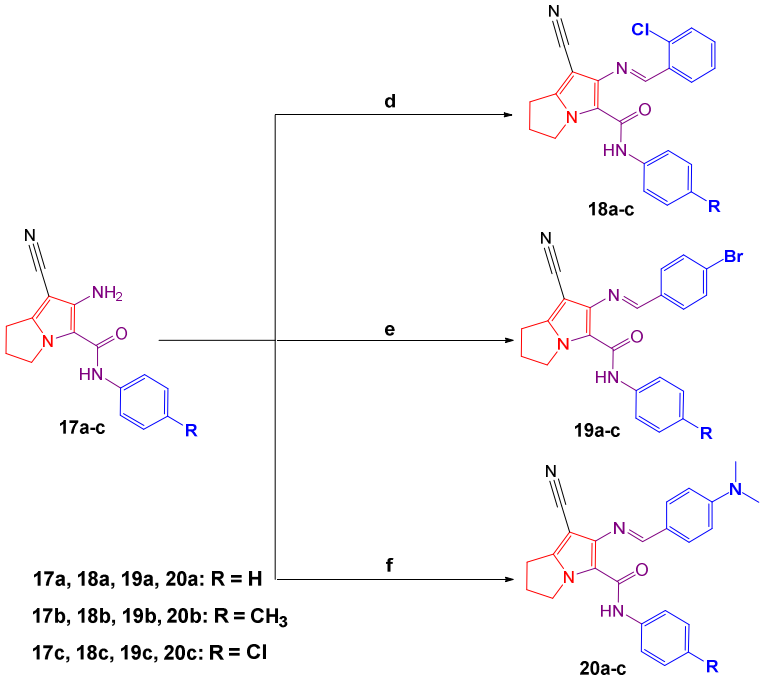
Scheme 1

Reagents and conditions: ***(a)*** *$ClCH_2COCl$, gl. acetic acid, CH_3COONa;* ***(b)*** *$(CH_3)_2SO_4$, benzene, malononitrile;* ***(c)*** *K_2CO_3, acetone, reflux, 24 h*

Synthesis of the Schiff base derivatives **18-20**was done by refluxing the starting materials **17a-c** with the appropriate aldehydes in absolute ethanol in the presence of glacial acetic acid as catalyst.

Preparation of compounds**18a-c** was obtained by refluxing the starting material **17a-c** with 2-chlorobenzaldehyde in absolute ethanol in the presence of glacial acetic acid. Structural elucidation of compounds **18a-c** was done using spectral and elemental analysis. The IR spectra of **18a-c** revealed absorption bands at 3230-3274 cm^{-1} attributed to the NH groups, a sharp band at2212-2217 cm^{-1} due to the cyano groups and absorption bands at1662-1667 cm^{-1} for the carbonyl groups. The 1H-NMR spectra of **18a-c** showed singlet signal at δ2.25 ppm due to the CH_3 protons in compound **18b**, multiplet, and two triplets at the range ofδ 2.50-4.59 ppm assigned for the aliphatic protons of the three methylene groups of the pyrrolizine nucleus. Multiplet at the range of δ 7.16-8.19 ppm due to the aromatic protons, two singlet signals at the δ 9.32-10.71 ppm due to N=CH and NH protons.^{13}C-NMR spectra of compounds **18a-c** revealed two signals at δ156.42-158.35 ppm due to N=CH and C=O carbons. Mass spectra of compounds **18a-c** revealed the molecular ions at 388, 402 and 422 respectively.

Compounds **19a-c** was prepared from the reaction of the starting material **17a-c** with 4-bromobenzaldehyde. The IR spectra of **19a-c** revealed absorption bands at 3231-3282 cm^{-1} attributed to the NH groups, a sharp band at 2212-2214 cm^{-1} due to the cyano groups and absorption bands at1661-1665 cm^{-1} for the carbonyl groups. The $_1$H-NMR spectra of **19a-c**showed two singlet signals at the δ 9.00-10.60 ppm due to N=CH and NH protons. ^{13}C-NMR spectra of compounds **19a-c** revealed two signals at δ157.82-158.52 ppm due to N=CH and C=O carbons. Mass spectra of compounds **19a-c** revealed the molecular ions at 432, 446 and 466 respectively.



Scheme 2

Reagents and conditions: *(d) 2-Chlorobenzaldehyde, absolute ethanol, glacial acetic acid, reflux, 4 h; (e) 4-bromobenzaldehyde, absolute ethanol, glacial acetic acid, reflux, 4 h; (f) 4-dimethylaminobenzaldehyde, absolute ethanol, glacial acetic acid, reflux, 4 h.*

Compounds **20a-c** was prepared from the reaction of the starting material **17a-c** with 4-dimethylaminobenzaldehyde. The IR spectra of **20a-c** revealed absorption bands at 3432-3433cm^{-1} attributed to the NH groups, a sharp band at 2208-2210 cm^{-1} due to the cyano groups and absorption bands at1665-1666 cm^{-1} for the carbonyl groups. The $_1$H-NMR spectra of**20a-c** showed two singlet signals at the δ8.98-11.00 ppm due to N=CH and NH protons. ^{13}C-NMR spectra of compounds **20a-c** revealed two signals at δ158.87-159.59 ppm due to N=CH and C=O carbons. Mass spectra of compounds **20a-c** revealed the molecular ions at 397, 411 and 431 respectively.

2.2. Pharmacological screening

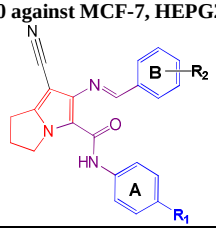
2.2.1. Anticancer activity

Cytotoxic activity of the novel pyrrolizines **18-20** were evaluated against HEPG2 and MCF7 cancer cell lines using Sulforhodamine-B (SRB) assay method [29]. $IC_{50}$was calculated and represented in µM/mlin **Table 1**.The tested compounds showed potent anticancer activity against both HEPG2 and MCF7 cell lines in micromolar range. Compounds**18c** and **18b** are the most active ones against (MCF7) and (HEPG2) cell lines with IC50 values of 0.250 and 0.784µM/ml respectively.

2.2.2. Inhibitory activity against normal cells

Cytotoxicty of the prototypes(**18-20a**) on normal Wish cells (non-tumorous cell line) was evaluated using Sulforhodamine-B (SRB) assay method [29] showed IC_{50} of 0.946, 1.322 and 1.122 respectively **Table 1.**

Table 1 IC_{50} values of compounds 18-20 against MCF-7, HEPG2 cancer cell lines and normal Wish cell



Comp. No.	R_1	R_2	HEPG2 IC_{50}µM	MCF-7 IC_{50} µM	Wish cell IC_{50} µM
18a	H	2-Cl	7.839	0.856	0.946
18b	CH_3	2-Cl	0.784	0.843	-
18c	Cl	2-Cl	1.668	0.250	-
19a	H	4-Br	5.195	0.897	1.322
19b	CH_3	4-Br	3.27	0.323	-
19c	Cl	4-Br	1.852	3.921	-
20a	H	4-$(CH_3)_2$N	3.586	0.422	1.122
20b	CH_3	4-$(CH_3)_2$N	9.446	0.394	-
20c	Cl	4-$(CH_3)_2$N	1.737	5.458	-
Doxorubicin	-	-	0.0069	-	-

2.2.3. Docking study

In this work, a docking study was performed between the new pyrrolizines with EGFR-TK. This study aimed to understand the binding mode of the new pyrrolizines **18-20** with the active site of the EGFR-TK. Molecular docking studies were performed using MOE 2008.01, **Table 2** and **Fig. 4-6**.

Table 2: Docking scores, interacting groups, amino acid interactions, and distances of the docked compounds into the active site of EGFR-TK

Comp	S (Kcal/mol)	Interacting moieties	Amino acid	Distance
18a	-16.0343	N of CN pyrrolizine	Lys860 Tyr764	3.2 4.35
18b	-18.257	N of CN pyrrolizine	Lys860 Tyr764	2.23 4.31
18c	-18.929	N of CN 2-chloro-benzylidene	Lys860 Tyr764	2.77 4.68
19a	-13.998	N of CN 4-bromo-benzylidene	Lys860 Lys757	2.88 3.79
19b	-17.191	N of CN	Lys860	3.2
19c	-15.080	N of CN pyrrolizine	Lys860 Tyr764	4.043.2
20a	-15.477	N of CN	Lys860	2.87
20b	-17.869	N of CN	Lys860	2.94
20c	-16.865	4-dimethylamino-benzylidene	Lys860	4.38
AEE	-21.442	NH of piperidine N of pyrimidine	Glu758 Lys860	1.23 3.27

The binding affinity of compounds **18-20**with essential amino acids in the active site was evaluated and compared to the co-crystallized ligand AEE788**,** which was redocked into EGFR and revealed score energy (S) of-

21.442kcal/mol and hydrogen bonding with Glu758 andLys860 through NH of piperidine moiety and N of pyrimidine respectively **Fig. 4**. All the compounds were docked into ATP binding site of EGFR kinase (PDB: 2J6M).[30]

All compounds were nicely and in a comparable manner of AEE **7** bound to the EGFR binding domain and form a hydrogen bond through the nitrogen atom of the cyano group with amino acid Lys860 which is an important binding site of EGFR inhibitor AEE **7**as shown in **Fig 4.** As shown in **Fig5-6**, compounds**18b** and**18c** have a good interactions with EGFR, with the highest scores and less distance and this result was reliable with anticancer activity of these compounds which are the most active compounds against MCF-7 and HEPG2 cell lines.

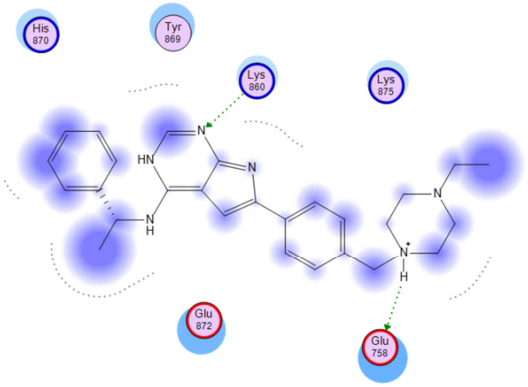


Fig 4. 2D interactions of AEE ligand with EGFR

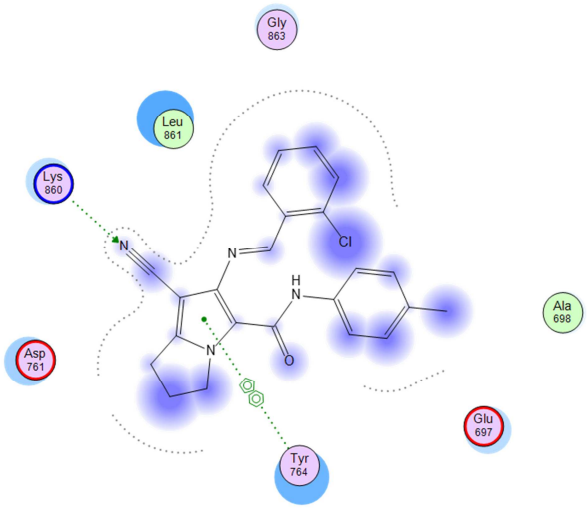


Fig 5. 2D interactions of comp 18b with EGFR

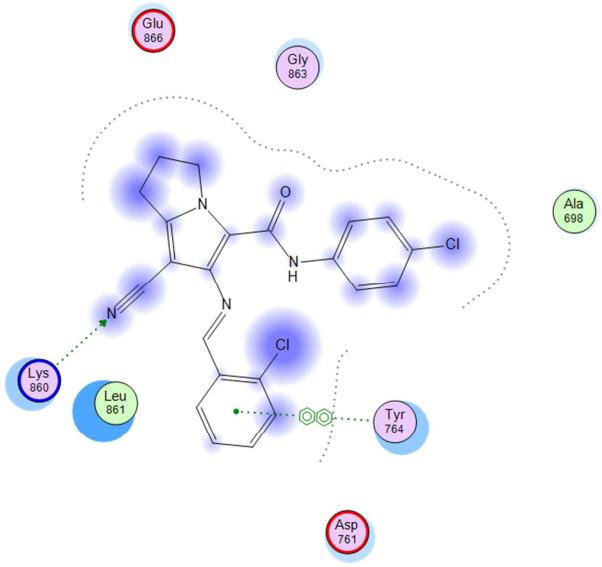


Fig 6. 2D interactions of comp 18c with EGFR

EXPERIMENTAL SECTION

4.1. Chemistry

Chemical reagents and solvents were obtained from commercial sources. Solvents are dried by standard methods when necessary. Melting points (m.p.) were uncorrected and were carried out by open capillary tube method using IA 9100MK-Digital Melting Point Apparatus. Microanalyses were carried out at the microanalytical Center, Faculty of Science, Cairo University. Infrared spectra were made on BRUKER Vector 22 (Japan), infrared spectrophotometer and were expressed in wavenumber (cm^{-1}) using potassium bromide disc. The proton magnetic resonance^{1}H NMR spectra were recorded on a Varian Mercury VX-300 NMR spectrometer at 400 MHz and BRUKER APX400 spectrometer at 400 MHz in the specified solvent, chemical shifts were reported on the δ scale and were related to that of the solvent and J values are given in Hz.^{13}C NMR spectra were obtained on a Bruker APX400 at 100 MHz at the faculty of pharmacy, Beni-Suef University. Mass spectra were recorded on Fennigan MAT, SSQ 7000, Mass spectrometer, at 70 eV (EI) at themicroanalytical Center, Faculty of Science, Cairo University. All mass spectra were recorded in the EI mode. Thin layer chromatography, was done using Macherey Nagel Alugram Sil G/UV254 silica gel plates and benzene–ethanol (9.5:0.5) as the eluting system.

Compounds **14**[26],**16a-c**[27],**17a-c**[28]were prepared according previously reported procedures.

4.2. General procedure for the preparation of compounds (18-20)

A mixture of the carboxamide derivatives **17**(3.75 mmol) and the appropriate aldehyde (3.75 mmol) was refluxed in absolute ethanol (20 ml) in the presence of glacial acetic acid (0.5 ml) for 4 h. The reaction mixture was concentrated, set aside to cool, the formed crystals was collected and recrystallized from ethanol.

7-Cyano-6-[(2-chloro-benzylidene)-amino]-*N*-phenyl-2,3-dihydro-1*H*-pyrrolizine-5-carboxamide (18a):

Compound **18a** was prepared by refluxing compound **17a** with 2-chlorobenzaldehyde. The product obtained yellow crystals, m.p. 245-7 0C, yield 84%. IRυmax/cm-1, 3230 (NH) 3065 (C-H aromatic, 2907 (CH2), 2212 (CN) ,1665 (C=O) ,1593 (C=C) ,1542 (C=N) ,1462, 1433, 1314(C-N) ,754 (C-Cl),1H-NMR (CDCl3-400 MHz): δ 2.58 (m, 2H, CH2-2), 3.08 (t, 2H, J = 7.2Hz, CH2-1), 4.58 (t, 2H, J = 6.8Hz, CH2-3), 7.16-8.19 (m, 9H, aromatic protons), 9.62 (s, 1H, N=CH), 10.65 (s,1H,NHC=O).13C-NMR (DMSOd6): 24.55, 25.45, 50.19, 115.87, 118.35, 119.60, 119.78, 124.02, 127.25, 127.42, 129.06, 129.14, 130.66, 132.81, 133.06, 136.97, 138.23, 139.28, 148.44, 156.42, 158.35, MS (EI): m/z (%): 389.95 (M+2,11.84), 388.95 (M+1,10.19), 388 (M+,34.95), 295.9 (82.68), 296.9 (17.69), 297.9 (28.31), 277.95 (19.92), 277 (100), 269.95 (1.8), 92 (3.69), 77 (13.8), 65 (16.22). Anal. Calcd. for C22H17ClN4O (388.85). C, 67.95; H, 4.41; N, 14.41. Found: C, 68.14; H, 4.47; N, 14.62.

7-Cyano-6-[(2-chloro-benzylidene)-amino]-*N*-p-tolyl-2,3-dihydro-1*H*-pyrrolizine-5-carboxamide (18b):
Compound **18b** was obtained by refluxing compound **17b** with 2-chlorobenzaldehyde. The product obtained yellow crystals, m.p. 265-6 ^{0}C, yield 87%. IRυmax/cm^{-1}, 3231 (NH) 3093,3066 (C-H aromatic), 2856 (CH_2), 2213 (CN),1662 (C=O) ,1595 (C=C) ,1546 (C=N) ,1462, 1403, 1257(C-N) ,828,802 (C-Cl),^{1}H-NMR ($CDCl_3$-400 MHz): δ2.25(s,3H,CH_3Ph)2.50 (m, 2H, CH_2-2), 2.99 (t, 2H, *J* = 7.2Hz, CH_2-1), 4.55 (t, 2H, *J* = 6.8Hz, CH_2-3), 7.12-7.38 (m, 8H, aromatic protons), 9.32 (s, 1H, N=CH), 10.14 (s,1H,NHC=O).^{13}C-NMR ($DMSOd_6$): 19.7, 20.9, 24.5, 40.58, 116.27, 118.19, 126.95, 129.67, 132.41, 133.06, 136.55, 148.39, 157.82, 158.07. Anal. Calcd. for $C_{23}H_{19}ClN_4O$ (402.88). C, 68.57; H, 4.75; N, 13.91 Found: C, 68.71; H, 4.78; N, 14.08.

7-Cyano-6-[(2-chloro-benzylidene)-amino]-*N*-(4-chlorophenyl)-2,3-dihydro-1*H*-pyrrolizine-5-carboxamide (18c):
Compound **18c** was obtained by refluxing compound **17c** with 2-chlorobenzaldehyde. The product obtained yellow crystals, m.p. 273-6 ^{0}C, yield 81%. IRυmax/cm^{-1}, 3274 (NH) 3097,3056 (C-H aromatic),2986, 2801 (CH_2), 2217 (CN) ,1667 (C=O) ,1592 (C=C) ,1544 (C=N) ,1491, 1309, 1257(C-N) ,829 (C-Cl),^{1}H-NMR ($CDCl_3$-400 MHz): δ 2.60 (m, 2H, CH_2-2), 3.09 (t, 2H, *J* = 7.2Hz, CH_2-1), 4.59 (t, 2H, *J* = 6.8Hz, CH_2-3), 7.30-8.18 (m, 8H, aromatic protons), 9.67 (s, 1H, N=CH), 10.71 (s,1H,NHC=O).^{13}C-NMR ($DMSOd_6$):24.60, 25.47, 50.18, 115.57, 118.11, 120.76, 127.24, 127.39, 128.87, 129.14, 130.76, 132.80, 133.18, 136.84, 137.02, 139.46, 148.56, 156.72, 158.34. Anal. Calcd. for $C_{22}H_{16}Cl_2N_4O$ (423.29). C, 62.42; H, 3.81; N, 13.24 Found: C, 62.53; H, 3.79; N, 13.40.

7-Cyano-6-[(4-bromo-benzylidene)-amino]-*N*-phenyl-2,3-dihydro-1*H*-pyrrolizine-5-carboxamide (19a):
Compound**19a** was obtained by refluxing compound **17a** with 4-bromobenzaldehyde. The product obtained yellow crystals, m.p. 223-5 ^{0}C, yield 82%. IRυmax/cm^{-1}, 3231 (NH) 3062 (C-H aromatic, 2958,2848 (CH_2), 2213 (CN),1665 (C=O) ,1596 (C=C) ,1551 (C=N) ,1474, 1413, 1315C-N) ,770 (C-Br),^{1}H-NMR ($CDCl_3$-400 MHz): *δ* 2.51 (m, 2H, CH_2-2), 2.92 (t, 2H, *J* = 7.2Hz, CH_2-1), 4.74 (t, 2H, *J* = 6.8Hz, CH_2-3), 7.16-7.60 (m, 9H, aromatic protons), 9.00 (s, 1H, N=CH), 10.41 (s,1H,NHC=O).^{13}C-NMR ($DMSOd_6$): 24.38, 25.29, 50.18, 116.20, 118.05, 119.32, 123.99, 127.06, 129.17, 129.78, 132.43, 134.12, 138.18, 138.50, 148.54, 157.09, 158.13, MS (EI): m/z (%): 434.85 (M^{+2},13.15), 433.85(M^{+1},49.06), 432.85 (M^+,18.88), 431.9 (50.87), 341.85 (95.42), 340.85 (21.92), 339.85 (99.06),313.8 (5.69), 312.85 (4.09), 311.85 (7.03), 276.95(100), 92 (3.94), 91(4.9), 77(15.88), 65(19.83). Anal. Calcd. for $C_{22}H_{17}BrN_4O$ (433.3). C, 60.98; H, 3.95; N, 12.93. Found: C, 61.07; H, 3.99; N, 13.02.

7-Cyano-6-[(4-bromo-benzylidene)-amino]-*N*-*p*-tolyl-2,3-dihydro-1*H*-pyrrolizine-5-carboxamide (19b):
Compound**19b** was obtained by refluxing compound **17b** with 4-bromobenzaldehyde. The product obtained yellow crystals, m.p. 240-2 ^{0}C, yield 80%. IRυmax/cm^{-1}, 3280 (NH) 3070(C-H aromatic), 2968 (CH_2), 2212 (CN) ,1661 (C=O) ,1611 (C=C) ,1596 (C=N) ,1418, 1315, 1292(C-N) ,830,809 (C-Br),^{1}H-NMR ($CDCl_3$-400 MHz): δ2.35(s,3H,CH_3Ph)2.55 (m, 2H, CH_2-2), 2.95 (t, 2H, *J* = 7.6Hz, CH_2-1), 4.51 (t, 2H, *J* = 7.2Hz, CH_2-3), 7.15-7.68 (m, 8H, aromatic protons), 9.01 (s, 1H, N=CH), 10.42 (s,1H,NHC=O).^{13}C-NMR ($DMSOd_6$):20.93, 24.43, 25.34, 50.17, 116.27, 118.19, 119.36, 126.99, 129.81, 132.41, 133.61, 134.21, 135.62, 138.38, 148.39 , 157.82, 158.07. Anal. Calcd. for $C_{23}H_{19}BrN_4O$ (447.33). C, 61.75; H, 4.28; N, 12.52 Found: C, 61.87; H, 4.32; N, 12.64.

7-Cyano-6-[(4-bromo-benzylidene)-amino]-*N*-(4-chlorophenyl)-2,3-dihydro-1*H*-pyrrolizine-5-carboxamide (19c):
Compound**19c** was obtained by refluxing compound **17c** with 4-bromobenzaldehyde. The product obtained yellow crystals, m.p. 272-3 ^{0}C, yield 87%. IRυmax/cm^{-1}, 3282 (NH) 3073(C-H aromatic),2968 (CH_2), 2214 (CN) ,1661 (C=O) ,1611 (C=C) ,1586 (C=N) ,1418, 1315, 1298(C-N) ,830,805 (C-Br),^{1}H-NMR ($CDCl_3$-400 MHz): δ 2.62 (m, 2H, CH_2-2), 3.08 (t, 2H, *J* = 7.2Hz, CH_2-1), 4.56 (t, 2H, *J* = 7Hz, CH_2-3), 7.31-7.79 (m, 8H, aromatic protons), 9.16 (s, 1H, N=CH), 10.60 (s,1H,NHC=O).^{13}C-NMR ($DMSOd_6$):24.57, 25.44, 50.20, 116.08, 117.84, 120.70, 127.28, 128.93, 129.19, 129.85, 132.56, 134.22, 136.78, 138.95, 148.54, 158.31, 158.52. Anal. Calcd. for $C_{22}H_{16}BrClN_4O$ (467.75). C, 56.49; H, 3.45; N, 11.98 Found: C, 56.64; H, 3.47; N, 12.13.

7-Cyano-6-[(4-dimethylamino-benzylidene)-amino]-*N*-phenyl-2,3-dihydro-1*H*-pyrrolizine-5-carboxamide (20a)
Compound**20a** was obtained by refluxing compound **17a** with 4-dimethylaminobenzaldehyde. The product obtained yellow crystals, m.p. 250-1 ^{0}C, yield 79%. IRυmax/cm^{-1}, 3432 (NH) 3065 (C-H aromatic, 2910 (CH_2), 2209 (CN),1665 (C=O) ,1588 (C=C) ,1537 (C=N) ,1432, 1369, 1308(C-N) ,^{1}H-NMR ($CDCl_3$-400 MHz): *δ* 2.52 (m, 2H, CH_2-2), 2.99 (t, 2H, *J* = 7.4Hz, CH_2-1), 3.11(s,6H,N(CH_3)$_2$), 4.48 (t, 2H, *J* = 7Hz, CH_2-3), 6.76-7.2 (m, 9H, aromatic protons), 9.00 (s, 1H, N=CH), 10.91 (s,1H,NHC=O).^{13}C-NMR ($DMSOd_6$): 24.54, 25.36, 40.16,50.00, 50.87, 116.67, 111.95, 116.52, 116.75, 119.60, 123.10,123.53,129.03, 130.73, 138.70, 141.14, 147.85, 153.15,

158.96,159.58. MS (EI): m/z (%): 398 (M^{+1},28.46), 397 (M^{+},100), 306 (19.05), 305(92.42), 277.95(3.28), 277(13.6), 148.05(94.3), 92(2.9), 77(7.59), 65(5.55).Anal. Calcd. for $C_{24}H_{23}N_5O$ (397.47). C, 72.52; H, 5.83; N, 17.62. Found: C, 72.68; H, 5.89; N, 17.84.

7-Cyano-6-[(4-dimethylamino-benzylidene)-amino]-*N*-p-tolyl-2,3-dihydro-1*H*-pyrrolizine-5-carboxamide (20b):
Compound**20b** was obtained by refluxing compound **17b** with 4-dimethylaminobenzaldehyde. The product obtained yellow crystals, m.p. 258-9 ^{0}C, yield 81%. IRυmax/cm^{-1}, 3433 (NH) 2914 (CH_2), 2210 (CN) ,1665 (C=O) ,1581 (C=C) ,1535 (C=N) ,1475, 1370, 1307(C-N),^{1}H-NMR ($CDCl_3$-400 MHz): δ2.35(s,3H,CH_3Ph)2.50(m, 2H, CH_2-2), 3.01 (t, 2H, *J* = 7Hz, CH_2-1), 3.11(s,6H,N(CH_3)$_2$) ,4.51 (t, 2H, *J* = 6.4Hz, CH_2-3), 6.76-7.81 (m, 8H, aromatic protons), 9.01 (s, 1H, N=CH), 10.86 (s,1H,NHC=O).^{13}C-NMR ($DMSOd_6$): 19.76, 20,91, 24.55,25.39, 40.16, 49.99, 111.66, 116.65, 16.81, 119.61, 120.19, 123.16, 129.53, 130.70, 133.07, 136.11, 140.95, 147.72, 153.12, 158.87, 159.45. Anal. Calcd. for $C_{25}H_{25}N_5O$ (411.5). C, 72.97; H, 6.12; N, 17.02 Found: C, 73.18; H, 6.19; N, 17.28.

7-Cyano-6-[(4-dimethylamino-benzylidene)-amino]-*N*-(4-chlorophenyl)-2,3-dihydro-1*H*-pyrrolizine-5-carboxamide (20c):
Compound**20c** was obtained by refluxing compound **17c** with 4-dimethylaminobenzaldehyde. The product obtained yellow crystals, m.p. 262-5 ^{0}C, yield 85%. IRυmax/cm^{-1}, 3433 (NH) 3045(C-H aromatic),2899 (CH_2), 2208 (CN),1666 (C=O) ,1590 (C=C) ,1536 (C=N) ,1482, 1365, 1308(C-N) ,^{1}H-NMR ($CDCl_3$-400 MHz): δ 2.58 (m, 2H, CH_2-2), 3.01 (t, 2H, *J* = 7.2Hz, CH_2-1), 3.12(s,6H,N(CH_3)$_2$), 4.51 (t, 2H, *J* = 6.6Hz, CH_2-3), 6.75-7.75 (m, 8H, aromatic protons), 8.98 (s, 1H, N=CH), 11.00 (s,1H,NHC=O).^{13}C-NMR ($DMSOd_6$): 24.56, 25.33, 40.16, 49.97, 111.64, 116.28, 116.64, 120.63, 122.93, 128.21, 128.96, 130.70, 137.33, 141.24, 148.00, 153.19, 158.88, 159.59. Anal. Calcd. for $C_{24}H_{22}ClN_5O$ (431.92). C, 66.74; H, 5.13; N, 16.21 Found: C, 66.91; H, 5.16; N, 16.42.24,148.00,153.19,158.88,159.59. Anal. Calcd. for $C_{24}H_{22}ClN_5O$ (431.92).C, 66.74; H , 5.13 ; N, 16.21 Found C ,66.91 ; H, 5.16 ; N,16.42

4.2. Pharmacological screening

4.2.1. In vitro cytotoxic activity evaluation by SRB assay.

Cytotoxicity of the novel pyrrolizines (**18-20**) was evaluated against HEPG2 and MCF7 cancer cell lines using Sulforhodamine-B (SRB) assay method as previously reported by Skehan *et al.*[29]. Antitumor activity evaluation was completed at the Center for Genetic Engineering, Al-Azhar University, Cairo, Egypt. Reagents and chemicals were obtained from Sigma Aldrich Chemical Company (St. Louis, Mo, U.S.A.).The tested cell lines were obtained from the American Type Culture Collection (ATCC, Minnesota, USA) through the Tissue Culture Unit, The Egyptian Organization for Biological Products and Vaccines (Vacsera, Egypt).Cells were seeded for 24h in a 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, 100 µg/ml) of the tested compounds, 3 wells were used for each concentration, after incubation for 48h the cells were fixed with 10% trichloroacetic acid 150 µl/well for 1 hr at 40C, washed by distilled water for 3 times. Wells were stained for 10-30 min at r.t. with 0.4% SRB, dissolved in 1% acetic acid 70 µl/well. Washed with acetic acid 1% to eliminate unbound dye till colorless drainage obtained. The plates were subjected to air drying, 24 hr not exposed to UV. The dye was solubilized with 150 µl/well of 10 mMTrise-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. Survival curve was obtained by plotting the percent of surviving cells against different concentrations of the tested compounds. The IC50 values were calculated using sigmoidal concentration– response curve fitting models (Sigmaplot software).

4.3. Molecular docking

Docking analysis was carried using the Molecular Operating Environment (MOE 2008.10) on the basis of high resolution crystal structures of EGFR complexes with Ligand (PDB 2J6M). Target proteins and ligands were energy-minimized using MOE, all water molecules were removed and hydrogen atoms were added and the protonation states of the amino acid residues were assigned using the Protonate3D algorithm. The following parameters were usedfor energy minimization; gradient: 0.01, force field: MMFF94X, chiral constraint: current geometry; total runs = 30.We First, crystallographic inhibitor AEE**7** was redocked to reproduce their structure and adjust docking parameters accordingly then compared with the newly synthesized active compounds docking results**tab.2**. **Fig. 4-6**showing docking of inhibitorAEE**7**and compounds **18b** and **18c.**

CONCLUSION

According to the results obtained during this work, we can conclude that:
(1) compounds with substituted phenyl ring are more active than the unsubstituted compounds.
(2) Chlorosubstitued compounds are more active than methylsubstitued ones.
(3) compounds**18b**and **18c**are the most active compounds against (HEPG2) and (MCF7) cancer cell lines respectively.
(4)Most of compounds are more active against (MCF7) than (HEPG2) cell lines.
Furthermore studies will be carried out to investigate the most probable mechanism of action of these compounds.

REFERENCES

[1] Jemal, F. Bray, M.M. Center, J. Ferlay, E. Ward, D. Forman, *CA. Cancer J. Clin.*, **2011**, 61, 69–90.
[2] A.-M. Florea, D. Büsselberg, *Cancers*, **2011**, 3, 1351–71.
[3] E.P. Simard, L.A. Torre, A. Jemal, *Oral Oncol.*, **2014**, 50, 387–403.
[4] P. Li, A. Znaor, I. Holcatova, E. Fabianova, D. Mates, M.B. Wozniak, et al., *Eur. Urol.*, **2015**, 67, 1134–1141.
[5] M.M. Center, A. Jemal, J. Lortet-Tieulent, E. Ward, J. Ferlay, O. Brawley, et al., *Eur. Urol.*, **2012**, 61, 1079–1092..
[6] S. Ghatak, A. Vyas, S. Misra, P. O'Brien, A. Zambre, V.M. Fresco, et al., *Bioorganic. Med. Chem. Lett.*, **2014**, 24, 317–324.
[7] W. Liu, J. Zhou, K. Bensdorf, H. Zhang, H. Liu, Y. Wang, et al., *Eur. J. Med. Chem.*, **2011**, 46, 907–913.
[8] N.K. Narayanan, D. Nargi, M. Attur, S.B. Abramson, B. a. Narayanan, *Anticancer Res.*, **2007**, 27, 2393–2402.
[9] S. Tavolari, M. Bonafè, M. Marini, C. Ferreri, G. Bartolini, E. Brighenti, et al., Carcinogenesis, **2008**, 29, 371–380.
[10] G. Kus, P. Oztopcu-Vatan, R. Uyar, S. Kabadere, *Acta Biol. Hung.*, **2013**, 64, 438–52.
[11] V. Lisowski, C. Enguehard, J.C. Lancelot, D.H. Caignard, S. Lambel, S. Leonce, et al., *Bioorganic Med. Chem. Lett.*, **2001**, 11, 2205–2208.
[12] V. Lisowski, S. Léonce, L. Kraus-Berthier, J.S.D.O. Santos, A. Pierré, G. Atassi, et al., *J. Med. Chem.*, **2004**, 47, 1448–1464.
[13] C. Rochais, T. Cresteil, V. Perri, M. Jouanne, A. Lesnard, S. Rault, et al., *Cancer Lett.*, **2013**, 331, 92–98.
[14] A.M. Gouda, A.H. Abdelazeem, E.-S. a Arafa, K.R. a Abdellatif, *Bioorg. Chem.*, **2014**, 53, 1–7.
[15] T. Ishikawa, M. Seto, H. Banno, Y. Kawakita, M. Oorui, T. Taniguchi, et al., *J. Med. Chem.*, **2011**, 54, 8030–8050.
[16] N.E. Hynes, H.A. Lane, *Nat Rev Cancer.*, **2005**, 5, 341–354..
[17] P.Z. Gatzeva-Topalova, L.R. Warner, A. Pardi, M.C. Sousa, *Structure*,**2010**, 18, 1492–1501.
[18] B.A. Chabner, *Oncologist.*, **2004**, 9, 245–246.
[19] C. Gridelli, F. De Marinis, M. Di Maio, D. Cortinovis, F. Cappuzzo, T. Mok, *Lung Cancer*, **2011**, 71, 249–57.
[20] L. Huang, L. Fu, *Acta Pharm. Sin. B.*, **2015**, 5, 390–401.
[21] M.J. Lavecchia, R. Puig de la Bellacasa, J.I. Borrell, C.N. Cavasotto, *Bioorg. Med. Chem.*, **2015**, 7, 768-778.
[22] S. Mowafy, N.A. Farag, K.A.M. Abouzid, *Eur. J. Med. Chem.*, **2013**, 61, 132–145.
[23] A. Tarozzi, C. Marchetti, B. Nicolini, M. D'Amico, N. Ticchi, L. Pruccoli, et al., *Eur. J. Med. Chem.*, **2016**, (2016).
[24] S. Yin, L. Zhou, J. Lin, L. Xue, C. Zhang, *Eur. J. Med. Chem.*, **2015**, 101, 462–475.
[25] H.-Q. Zhang, F.-H. Gong, C.-G. Li, C. Zhang, Y.-J. Wang, Y.-G. Xu, et al., *Eur. J. Med. Chem.*, **2016**, 109, 371–379.
[26] A.Etienne, Y. Correia,*Bull. Soc. Chem.*, **1969**, 10, 3704-3712.
[27] W.A.Jacobs, M. Heidelberger, *J. Am. Chem. Soc.***1917**, 39, 1435-1439.
[28] [1] A. Gouda, H. Ali, W. Almalki, M. Azim, M. Abourehab, A. Abdelazeem, *Molecules*, **2016**, 21, 201.
[29] P. Skehan, R. Storeng, D. Scudiero, A. Monks, J. McMahon, D. Vistica, et al., *J. Natl. Cancer Inst.*, **1990**, 82, 1107–1112.
[30] C.-H.Yun,T.-J.Baggon,y.-Li,M.-S.Woo,H.-Greeulich, et al,*Cancer cell* ,**2007**,11,217-227.