JPR INFO
Journal of Pharma Research

Journal of Pharma Research
Available online through
www.jprinfo.com

Research Article
ISSN: 2319-5622

Synthesis, characterization, antitumor evaluation and molecular docking of some triazolotriazine derivatives

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Received on: 05-05-2014; Revised and Accepted on: 19-05-2014

ABSTRACT

*Synthesis of 4-amino-[1,2,4]triazolo[5,1-c][1,2,4]triazine (**6a-c**)e derivatives using readily available starting materials were described. Heptaazacyclopenta[a]naphthalene derivatives **7, 8** were prepared upon heating of **6a** with formic acid or formamide. Compound **6a** underwent cyclocondensation reaction upon treatment with carbon donor reagent like triethyl orthoacetate to furnish (z)-ethyl-N-3-cyano[1,2,4]triazolo[5,1-c][1,2,4]triazin-4-ylacetimidate **12**. Compounds **13a, c**, **15a-b, 14a-b** and **16a-c,** respectively were formed when **12** treated with acetohydrazide , pyridyl carbohydrazide or primary amines under different reaction conditions. In this study, six selected derivatives (compounds **6a**, **7**, **8**, **9a, b** and **11a** derivatives) were subjected to a screening system for investigation of their antitumor potency against liver (HEPG2) cell line in comparison to known anticancer drugs: 5-Flurouracil and Doxorubicin. The antitumor activity results indicated that the selected compounds **6a, 7, 8, 9a,b** and **11a** derivatives showed growth inhibition activity against the tested cell line but with varying intensities extents in comparison to 5-Flurouracil and Doxorubicin. The molecular docking is performed and analyzed with the molecular modeling environment (MOE) program. The synthesized compounds **7, 8** are investigated for the binding affinity of c-Kit tyrosine kinases receptor (pdb 1t46). This purpose of lead optimization and to find out the interaction between compounds **7, 8** and the c-Kit protein tyrosine receptor.*

Key words: *[1,2,4]triazine, Heptaazacyclopenta[a]naphthalene , Cytotoxic activities, MOE program.*

INTRODUCTION

1,2,4-Triazole system is a structural unit of many drugs that have antimycotic activity, e.g., *Fluconazole*, *Itraconazole*, *Voriconzole, Tricyclazole*, *Furconazole*, *Hexaconazole, Tetraconazole*, *Quinconazole*, *Penconazole* [1, 2], herbicidal activity, e.g., *Lucarbazone*, *Bencarbazone* e.g., and Virucidal activity, e.g., *Ribavirin*.

Further, the importance of triazolopyrimidines and triazolotriazines is well recognized in the field of medicinal chemistry because these heterocycles have a structure similar to that of purine and adenine [3-5]. Triazolopyrimidines triazolotriazines are useful building blocks in the synthesis of herbicidal drugs, e.g. *Metosulam*, *Flumetsulam*, *Azafenidin, Diclosulam*, *Penoxsulam*, *Floransulan*, and *Cloransulam* etc. Recently, C.M. Richardson *et al*. described the triazolopyrimidines as novel CDK2 inhibitors [6]. Beside these compounds are also useful as potential anticancer [7], antibronchoconstrictor [8], and antiviral [9], diuretic [10], antibacterial [11] and antifungal [12] agents.

Based on these findings, the present work aimed to synthesize a new group of triazolotriazine derivatives and incorporated with different heterocycles as a trial that the resulting compounds would have better biological activity as antiproliferative agents in the field of liver cancer. Since all the selected triazine derivatives were soluble in DMSO at concentrations high enough to allow cell experiments, the in vitro biological activity of these compounds was evaluated by their growth-inhibitory potency in liver HEPG2 cancer cell lines. The cytotoxic potency of (compounds 1-10) was studied in comparison to the known anticancer drugs 5-Flurouracil (5-FU) and Doxorubicin (DOX).

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METHODS AND MATERIALS

Chemistry:

All melting points are uncorrected and measured using Electro thermal A 9100 apparatus, (Shimadzu, Tokyo, Japan). IR spectra were recorded as potassium bromide pellets on a Perkin-Elmer 1650 spectrophotometer (Perkin Elmer, Norwalk, CT, USA), National Research Centre, Cairo, Egypt. ^{1}H-NMR and ^{13}C-NMRspectra were determined on a Jeol-Ex-300 NMR spectrometer (JEOL, Tokyo, Japan) and chemical shifts were expressed as part per million; (ppm, γ values) against TMS as internal reference (Faculty of Science, Cairo University, Cairo, Egypt). Mass spectra were recorded on EI$^+$ Q1 MSLMR UPLR, National Research Centre, Cairo, Egypt. Microanalyses were operated using Mario El Mentar apparatus, Organic Microanalysis Unit, National Research Centre, Cairo, Egypt and the results were within the accepted range (±0.40) of the calculated values.

4-Amino-[1,2,4]triazolo[5.1-c][1,2,4]triazine derivatives (6a-c):

Amine hydrochloride salt solution of compound **1** prepared from (0.16gm, 2mmol of **1**in 5 mL Conc. HCl) and the solution was kept in an ice bath at 0-5 ^{0}C for10mins. Sodium nitrite solution prepared from (0.145 gm, 2.1mmol, and 5ml water) was added drop wise with stirring to the amine hydrochloride salt solution over a period of 20- 25 mins at 0^{0}C.where a yellow precipitate of diazonium hydrochloride salt was formed. The reaction mixture was stirred for additional 15 mins while maintaining the temperature at 0 ^{o}C. To a well cold and stirred solution of amine hydrochloride salt **3** and sodium acetate anhydrous (5gm) in ethanol (100ml), an equimolecular amounts of malononitrile , ethyl cyanoacetate and ethyl cyanoacetamaide was added respectively. The stirring was continued for additional 12 hrs. It left overnight in the refrigerator. Water (250 ml) added to the reaction mixture and the solid product formed and collected by filtration. It was crystallized from Ethanol-.

6a: Molecular formula $C_5H_3N_7$, molecular weight 161.13, yield % =75, m.p= 221- 3 ^{o}C., IR spectrum IR(KBr, υcm^{-1}) 3469-3398 (NH_2),

2220(CN). ^{1}H-NMR (DMSO-d_6) 8.21 (s, 1H, triazole) 5.99 (d, 2H, NH_2, D_2O exchangeable), MS:m/z(%)= 161(60), 63(100%).

6b: Molecular formula $C_7H_8N_6O_2$, molecular weight 208.18, yield % =62, m.p= 182-4 ^oC., IR spectrum (KBr,υ cm^{-1}) 3460-3368 (NH_2), 1653(CO). ^{1}H-NMR (DMSO-d_6) 8.21 (s, 1H, triazole) 5.90 (d, 2H, NH_2, D_2O exchangeable), 4.01(m, 2H, j=12.3Hz, CH_2), 1.45(t, 3H, j=11.10Hz, CH_3) MS:m/z(%)= 208(54), 63(100%).

6c: Molecular formula $C_5H_5N_7O$, molecular weight 179.15, yield % =43, m.p= 250-2 ^oC., IR spectrum (KBr,υ cm^{-1}) 3362-3210 (NH_2), 1630(CO). ^{1}H-NMR (DMSO-d_6) 8.00 (s, 1H, triazole) 6.23 (d, 2H, NH_2, D_2O exchangeable),4.23(d, 2H, NH_2, D_2O exchangeable). MS:m/z(%)= 179(41), 63(100%).

7H-1, 3, 4, 5, 7, 9, 9b-Heptaazacyclopenta[a]naphthalen-6-one (7):

Compound 6a (1.61 g, 10 mmol) was heated under reflux temperature in formic acid (40 mL, 85%) for 10 hrs. The reaction mixture was cooled and poured into water. The formed solid was filtered off, dried, and recrystallized from dioxane to give a product **7**.Yield 77%, mp. 357–359^{o}C.

Molecular formula $C_6H_3N_7O$, molecular weight 189.14, yield % =51, IR (KBr, υcm^{-1}) 3169 (NH), 1662(CO). ^{1}H-NMR (DMSO-d_6) 9.00 (bs, H, NH, D_2O exchangeable), 8.54(s, 1H, pyrimidine). 7.94 (s, 1H, triazole), MS:m/z(%)= 189(60), 77(100%).

1, 3, 4, 5, 7, 9, 9b-Heptaazacyclopenta[a]naphthalen-6-onylamine (8):

Compound 6a (1.61 g, 10 mmol) was heated under reflux temperature in formamid (40 mL, 85%) for 10 hrs. The reaction mixture was cooled and poured into water. The formed solid was filtered off, dried, and recrystallized from dioxane to give a product **8**.Yield 48%, mp. 310–312^{o}C.

Molecular formula $C_6H_4N_8$, molecular weight 188.15, yield % =51, IR (KBr, υcm^{-1}) 3416-3352 (NH_2). ^{1}H-NMR (DMSO-d_6) 8.54(s, 1H, pyrimidine). 7.94 (s, 1H, triazole), 6.21 (bs, 2 H, NH_2, D_2O exchangeable), MS:m/z(%)= 188(60), 77(100%).

(E)-4-(arylidenamino)-[1,2,4]triazolo[1,5-c][1,2,4]triazine-3-carbonitril (9a,b and 10a,b):

An equimolecular amounts of **6a** and appropriate aromatic aldehyde(namely, *p*-chlorobenzaldehyde and 2-chlorocalicaldehyde)or apprppriate aromatic ketone (namely, acetophenon, acetylindole)in dioxane (30ml) in presence of pipridine (1ml) as catalyst was refluxed for 15hrs. The reaction mixture was cooled and the solid so formed was filtered off, washed with petroleum ether 60-80 and finally crystallized from dioxane affording compounds **9a, b** and **10a, b**. 8.01, 8.59(s, 1H, N==CH).

8-(aryl)-8-methyl-8,9-dihydro-7H-1, 3, 4, 5, 7, 9, 9b-Heptaazacyclopenta[a]naphthalen-6-one (11a,b):

Method A:

An equimolecular amounts of **6a** and appropriate aromatic ketone namely, acetophenon, 2-acetylindole in acetic acid (30ml) in presence of sulphuoric acid (1ml) was refluxed for 20hrs. The reaction mixture was cooled and poured onto ice water (100ml), the solid so formed was filtered off and finally crystallized from dioxane affording compounds **11a, b**.

Method B:

A solution of 1g of **10a, b** in acetic acid (30ml) in presence of sulphuoric acid (1ml) was refluxed for 5hrs. The reaction mixture was cooled and poured onto ice water (100ml), the solid so formed was filtered off and finally crystallized from dioxane.

11a: Molecular formula $C_{13}H_{11}N_7O$, molecular weight 281.30, yield % =51, mp. 260-2^{o}C.

IR (KBr, υcm^{-1}) 3052 (NH). ^{1}H-NMR (DMSO-d_6) 9.01, 8.92 (2bs, 2 H, NH, D_2O exchangeable), 7.43(d, 2H, j=8.12 Hz , Ar-H), 7.31 (s, 1H, triazole), 7.13(d, 2H, j=8.51 Hz , Ar-H), 6.99(d, 1H, Ar-H), 6.96 (d, 2H, j=8.51 Hz , Ar-H), 1.65(s,3H, CH_3), MS:m/z(%)= 281(54), 77(100%).

11b: Molecular formula $C_{15}H_{12}N_8O$, molecular weight 320.32, yield % =42, mp. 290-2^{o}C. IR (KBr, υcm^{-1}) 3052 (NH). ^{1}H-NMR (DMSO-d_6) 11.01 (s, H, NH, D_2O exchangeable, indole), 8.01 (s, H, NH, D_2O exchangeable), 7.58(d, 2H, j=8.12 Hz , Ar-H), 7.07 (s, 1H, triazole), 7.00(s, 1H, indole), 6.89(d,2 H, j= Hz , Ar-H),4.25 (s, 1H, NH, D_2O exchangeable) , 1.82(s,3H, CH_3), MS:m/z(%)= 320(60), 65(100%).

(Z)-Ethyl-N-3-cyano[1,2,4]triazolo[5,1-c][1,2,4]triazin-4-ylacetimidate(12):

A mixture of compound **1** (1.61 g, 10 mmol) in triethyl orthoacetate (5 mL) and acetic anhydride (5 mL) was refluxed for 5 hrs. The reaction mixture was evaporated under reduced pressure and the residue was filtered off, dried, and recrystallized from absolute ethanol to give compound **12**. Yield 85%.

Molecular formula $C_9H_9N_7O$, molecular weight 233.23, yield % =85, IR (KBr, υcm^{-1}) 2215(CN). ^{1}H-NMR (DMSO-d_6) 6.99(s, 1H, triazole), 4.01(m, 2H, CH_2), 2.14,1.94(2s,6H, $2CH_3$), MS:m/z(%)= 157(60), 63(100%).

6-Imino(or aryl amino)-8-methylpyrimido[4,5-e][1,2,4]triazolo[5,1-c][1,2,4]triazin-7(6H)-amino(13a-c):

A solution of an equimolecular amounts of compound **12** and hydrazine hydrate or appropriate primary amine (*p*-floroaniline , *p*- methoxyaniline) in absolute ethanol (20 ml) was stirred at 0^{0}C for 8 hrs. the solid so formed was filtered off and finally crystallized from ethanol to give **13a- c**.

13a: Molecular formula $C_7H_7N_9$, molecular weight 217.19, yield % =64, mp. 210 -2^{o}C, IR (KBr, υcm^{-1}) 3412-3125 (NH_2+ NH). ^{1}H-NMR (DMSO-d_6) 8.54 (bs, 1H, NH, D_2O exchangeable), 7.28(s, 1H, triazole), 5.01(bs, 2H, NH_2 , D_2O exchangeable), 2.22(s,3H, CH_3), MS:m/z(%)= 217 (72), 63(100%).

13b: Molecular formula $C_{13}H_{10}FN_9$, molecular weight 311.28, yield % =56, IR (KBr, υcm^{-1}) 3154 (NH). ^{1}H-NMR (DMSO-d_6) 8.78 (bs, 1H, NH, D_2O exchangeable),7 .00(d, 2H, j=8.51 Hz , Ar-H), 6.73(d, 2H, Ar-H, j=8.51 Hz , Ar-H) 7.28(s, 1H, triazole), , MS:m/z(%)= 311(72), 313(36), 63(100%).

13c: Molecular formula $C_{14}H_{13}N_9O$, molecular weight 323.32, yield % =74, IR (KBr, υcm^{-1}) 3154 (NH). ^{1}H-NMR (DMSO-d_6) 8.78 (bs, 1H, NH, D_2O exchangeable),7 .33(d, 2H, j=8.51 Hz , Ar-H), 7.03(d, 2H, Ar-H, j=8.51 Hz , Ar-H) 7.28(s, 1H, triazole), 4.021(s,3H, OCH_3),2.02(s, 3H, CH_3), MS:m/z(%)= 323 (79), 63(100%).

(8-Methyl-1, 3, 4, 5, 7, 9, 9b-heptaeptaaza-cyclopenta[a]naphthalen-6-yl)-hydrazine derivatives (14a-c):

Method A:

A solution of an equimolecular amounts of compound **12** and hydrazine hydrate or appropriate primary amine (p-floroaniline , *p*- methoxyaniline) in absolute ethanol (20 ml) was heated at refluxing temperature for 8 hrs. The reaction mixture was cooled and The solid product collected by filtration and crystallized from ethanol to give **14a-c**.

Method B:

A solution of compound **13a-c (1g)** in absolute ethanol (20 ml) was heated at refluxing temperature for 4 hrs. The reaction mixture was cooled and the solid product was formed collected by filtration and crystallized from ethanol to give **14a-c.**

14a: Molecular formula $C_7H_7N_9$, molecular weight 217.19, yield % =82, mp. 230-2^{o}C. IR (KBr, υcm^{-1})3342-3321(NH_2) 3152 (NH). ^{1}H-NMR (DMSO-d_6) 9.10 (bs, 1H, NH, D_2O exchangeable), 7.56 (s, 1H, triazole), 2.22 (s, 2H, NH_2, D_2O exchangeable), 1.05(s,3H, CH_3), MS:m/z(%)= 217(85), 65(100%).

14b: Molecular formula $C_{13}H_{10}FN_9$, molecular weight 311.28, yield % =76, mp. 189^{o}C, IR (KBr, υcm^{-1}) 3154 (NH). ^{1}H-NMR (DMSO-d_6) 7.88, 6.76 (2bs, 1H, NH, D_2O exchange- able),7 .00(d, 2H, j=8.51 Hz, Ar-H), 6.73(d, 2H, Ar-H, j=8.51 Hz , Ar-H) 7.28(s, 1H, triazole), MS:m/z(%)= 311(72), 313(36), 63(100%).

14c: Molecular formula $C_{14}H_{13}N_9O$, molecular weight 323.32, yield % =74, IR (KBr, υcm^{-1}) 3154 (NH). ^{1}H-NMR (DMSO-d_6) 8.78, 5.54 (2bs, 2H, NH, D_2O exchangeable),7 .33(d, 2H, j=8.51 Hz , Ar-H), 7.03(d, 2H, Ar-H, j=8.51 Hz , Ar-H) 7.28(s, 1H, triazole), 4.021(s,3H, OCH_3), 2.02(s, 3H, CH_3), MS:m/z(%)= 217 (72), 63(100%).

5,7-Dimethyl-1,3,3a,4,5a,6,8,9,10-nonaaza-dicyclopenta[a,f] naphthalene (15a);

5-Methyl-7-pyridin-4-yl-1,3,3a,4,5a,6,8,9,10-nonaaza-dicyclopenta[a,f]naphthalene (15b):

A solution of an equimolecular amounts of compound **12** and acetohydrazide , pyridine carbohydrazide in phosphorus oxychloride (25ml) was heated on water bath at 80° C. for 8 hrs. The reaction mixture was cooled and poured onto ice water (100ml), the solid so formed was filtered off and finally crystallized from isopropanol affording compounds**15a, b.**

15a: Molecular formula $C_9H_7N_9$, molecular weight 241.22, yield % =51, mp. 245– 7°C, ^{1}H-NMR (DMSO-d_6) 7.56 (s, 1H, triazole), 1.65, 1.05(2s,6H, $2CH_3$), MS: m/z(%)= 241(85), 65(100%).

15b: Molecular formula $C_{13}H_8N_{10}$, molecular weight 304.28, yield % =46, mp. 288- 10°C, ^{1}H-NMR (DMSO-d_6) 7.71 (s, 1H, triazole), 8.13(d, 2H, *j*=9.12 Hz, pyridyl), 7.63(d, 2H, *j*=9.12. Hz , pyridyl), 2.05(s,3H, CH_3). MS:m/z(%)= 304(45), 65(100%).

2- Measurement of potential cytotoxicity by SRB assay:

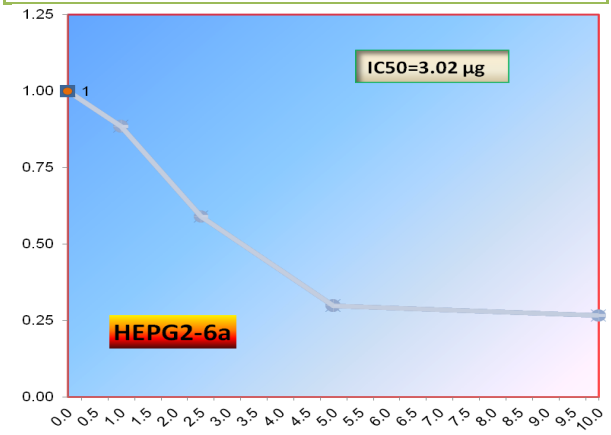
The selected triazole derivatives (6a, 7, 8, 9a,b, 11a) were subjected to a screening system for evaluation of their antitumor activity against liver HEPG2 cancer cell lines in comparison to the known anticancer drugs: 5-FU and DOX.

Potential cytotoxicity of the selected triazine derivatives was tested using the method of Skehan *et al.*[1] as follows:

Cells were plated in 96-multiwell plate (10^4 cells/well) for 24 h before treatment with the compound(s) to allow attachment of cells to the wall of the plate. Different concentrations of the compound under test (0, 1, 2.5, 5, 10 µg/ml) were added to the cell monolayer. Triplicate wells were prepared for each individual dose. Monolayer cells were incubated with the compound(s) for 48 h at 37°C and in an atmosphere of 5% CO_2. Cultures were then fixed with trichloroacetic acid and stained for 30 minutes with 0.4% (wt/vol) sulforhodamine B (SRB) dissolved in 1% acetic acid. Unbound dye was removed by four washes with 1% acetic acid, and protein-bound dye was extracted with 10 mM unbuffered Tris base [tris (hydroxymethyl) aminomethane] for determination of optical density in a computer-interfaced, 96-well microtiter plate reader. The SRB assay results were linear with the number of cells and with values for cellular protein measured by both the Lowry and Bradford assays at densities ranging from sparse subconfluence to multilayered supraconfluence. The signal-to-noise ratio at 564 nm was approximately 1.5 with 1,000 cells per well. The relation between surviving fraction and drug concentration is plotted to get the survival curve of both cancer cell lines after the specified compound.

Table No. 1: Cytotoxic activity of the drug

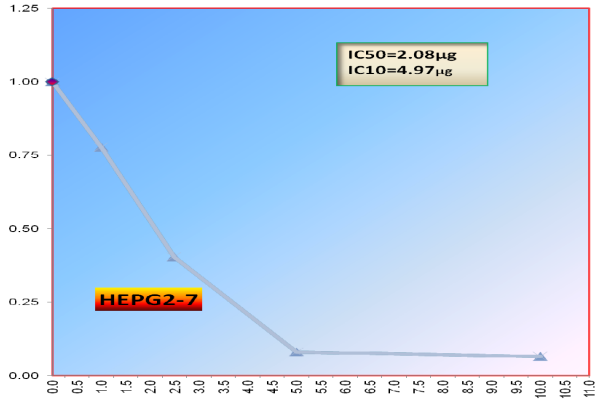
Conc. µg	HEPG2-6a
0.0	1.00000
1.0	0.88390
2.5	0.59050
5.0	0.29724
10.0	0.26726
HEPG2	



Conc; µg

Table No. 2: Cytotoxic activity of the drug

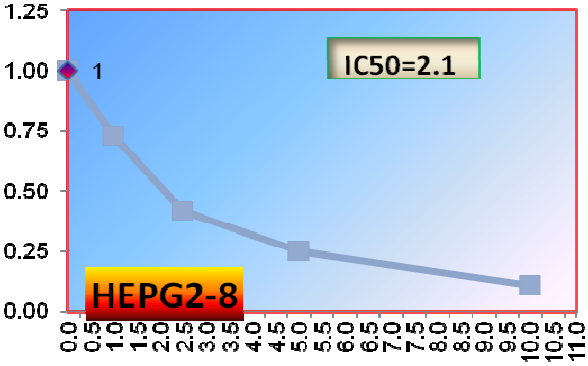
Conc. µg	HEPG2-7
0.0	1.00000
1.0	0.77686
2.5	0.40327
5.0	0.08093
10.0	0.06541
HEPG2	



Conc; µg

Table No. 3: Cytotoxic activity of the drug

Conc. µg	HEPG2-8
0.0	1.00000
1.0	0.72699
2.5	0.41834
5.0	0.24639
10.0	0.10355
HEPG2	



Conc; µg

Table No. 4: Cytotoxic activity of the drug

Conc. µg	HEPG2-9a
0.0	1.00000
1.0	0.74366
2.5	0.48942
5.0	0.40726
10.0	0.14929
HEPG2	

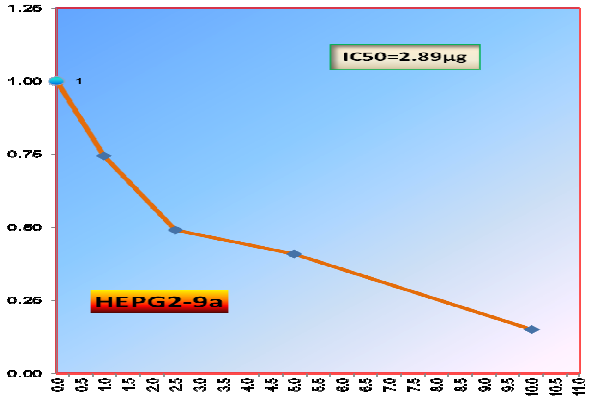


Table No. 5: Cytotoxic activity of the drug

Conc. µg	HEPG2-9b
0.0	1.00000
1.0	0.81636
2.5	0.80206
5.0	0.53780
10.0	0.37793
HEPG2	

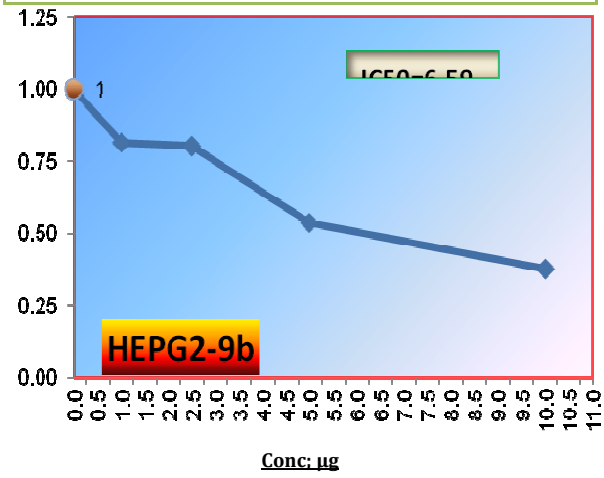
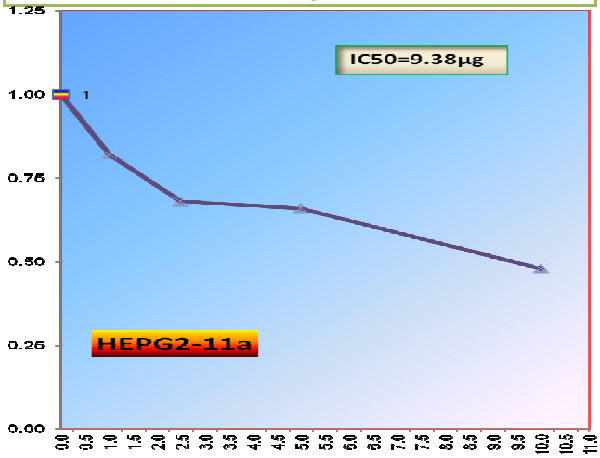


Table No. 6: Cytotoxic activity of the drug

Conc. µg	HEPG2-11a
0.0	1.000000
1.0	0.820616
2.5	0.679432
5.0	0.657549
10.0	0.477877
HEPG2	

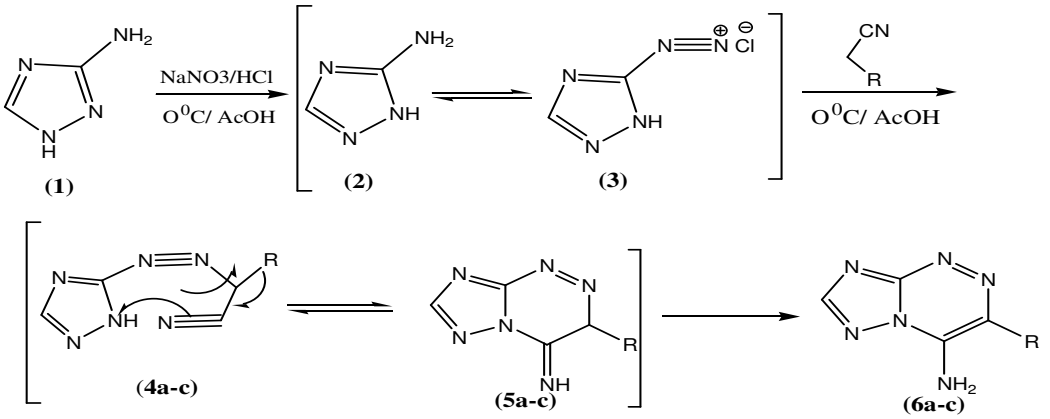


RESULTS AND DISCUSSION

Chemistry:

Heterocyclic diazonium salts represent an interesting class of reactive substrates and their synthetic potentialities have received recent attention. Moreover, several heterocyclic diazo compounds possess biological activities as herbicides [13], fungicidal [13], antiepileptic agents [14] and antioxidant [15] agents. In addition to its wide usage as disperse dye for dying polyamide, polyester and acrylic fibers [16]. In view of these facts and in continuation of our previous work [17-21], we decided to investigate the synthetic potentiality of triazine based on 3-amino, 1, 2, 4, triazole. Thus, we prepared 4-amino-[1,2,4]triazolo[5,1-*c*][1,2,4]triazine derivatives (**6a-c**)through the diazotization of aminotriazol (1) followed by condensation of the formed diazonium salt with active methylene compounds namely malononitriles, ethyl cyanoacetate and ethyl cyanoacetamide in acidic medium at 0^{0}C(Scheme 1, exp.)

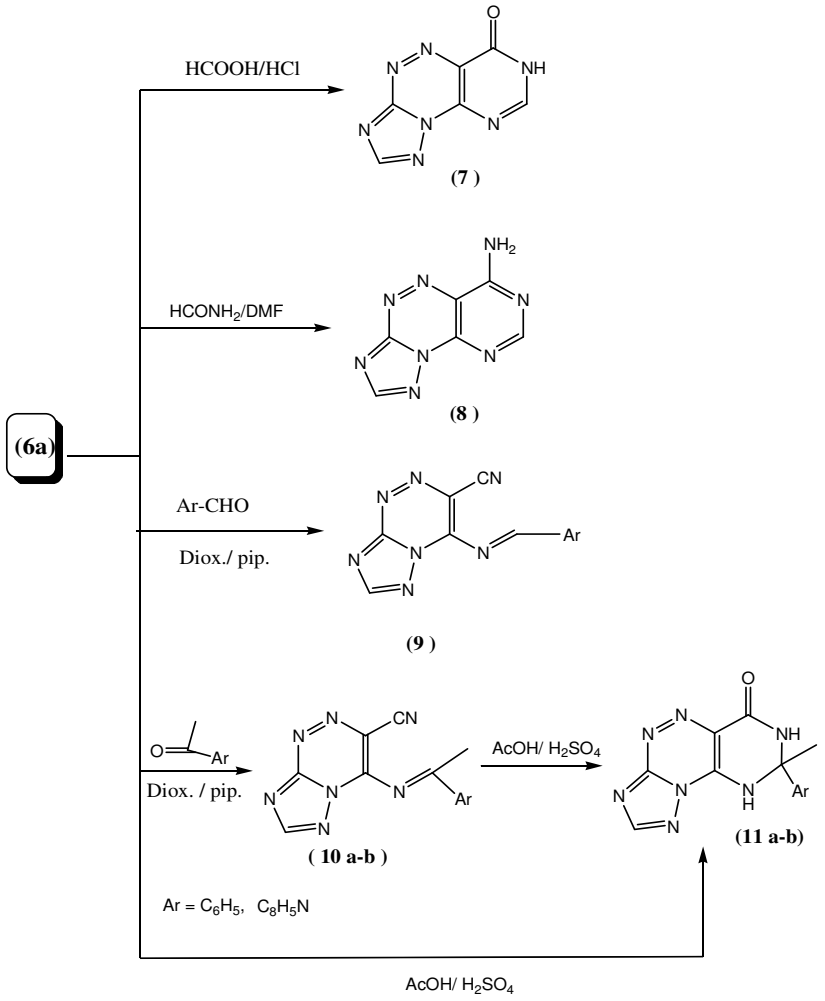
The structure of compounds **6a-c** was established based on the elemental analysis and the spectral data. The IR (KBr, υ cm^{-1}) spectrum of compound **6a** revealed the presence of absorption bands at 3469-3398 cm^{-1} (NH_2) and 2220 cm^{-1} (CN) group. (Scheme 1, exp.). The ^{1}H-NMR (DMSO-d_6, δ ppm) spectrum of compound **6a-c** showed characteristic signals at δ9.02(s, 1H, triazol) (Scheme 1, exp.).Mass spectrum for 6a showed M$^+$ (m/z %) at 161(59%). In addition some chemical conformational reactions.



Scheme 1

Cyclization reactions of **6a** with formic acid and formamide have been formed. These reactions afforded the heptaazacyclopenta[a] naphthalene derivatives **7, 8**. The structure of the obtained products was confirmed from their correct values in elemental analysis and their agreeable spectral data (Scheme 2, exp.).

The shief base of compound 6a was formed upon heating of it with appropriate aldehyde in presesnce of base. The assigenment of the structure of the formed products was based on their correct values inelemntal analysis and agrrable spectral data. ^{1}H-NMR (DMSO-d_6, δ ppm) for compound 9a,b showed signals at 8.01, 8.59(s, 1H, N==CH) (Scheme2, exp.).

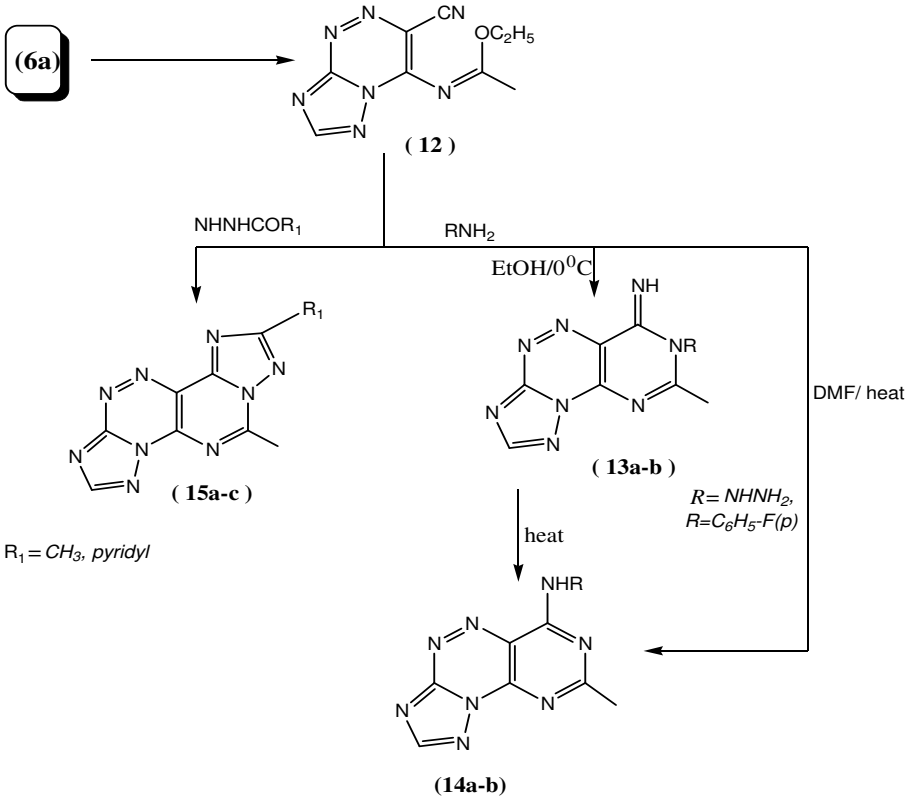


Scheme 2

Our goal is to develop new general and convenient procedures for the preparation of fused triazine derivatives. Therefore, compound **6a** underwent cyclocondensation reaction upon treatment with carbon donor reagent like triethyl orthoacetate to furnish (z)-ethyl-N-3-cyano[1,2,4]triazolo[5,1-c][1,2,4]triazin-4-ylacetimidate derivative **12**. The structure of the formed compound was confirmed chemically when it was stirred with hydrazine hydrate or primary amin (p-Faniline) at 0^0C afforded **13a, c**. respectively. In addition, the agreement of the proposed structure of **13a, b**. with the spectral and analytical data obtained (Scheme 3). These products underwent Dimroth-type rearrangement [22-27] when heated under refluxing temperature to afford **14a-b,** respectively.

To prove this assumption, compound **12** was converted into its corresponding 6-hydrazino or arylamino-8-methylpyrimido[4,5-e][1,2,4]triazine derivatives **14a-c** by heating **12** with hydrazine hydrate or primary amines in presence of DMF which presumably involves a sequence of ring opening and ring closure reactions as depicted in scheme 3.

Dicyclopenta[a,f]naphthalene derivatives**15a,b** was obtained via two different ways. The first way through the reaction of compound **12** with acetohydrazide or pyridine carbohydrazide in presence of phosphorus oxychloride. The second way was through the reaction of imino derivatives (**13a**) with acetohydrazide or pyridine carbohydrazide in dioxane (Scheme 3, exp.).



Scheme 3

Cytotoxic and Biological Effects:

Preliminary screening of the selected triazole derivatives showed that all selected compounds exhibited a moderate to strong growth inhibition activity on the tested cell line between 1-10 µg/ml concentrations in comparison to the known anticancer drugs: 5-Flurouracil and Doxorubicin. Table (1) indicated the cytotoxic activity of the selected synthesized derivatives (compounds 6a, 7, 8, 9a,b and 11a) against liver HEPG2 cancer cell line in comparison to the traditional anticancer drugs: 5-FU and DOX. It can be deduced from our results that compounds 6 and 5 were the most active and induced a reasonable growth inhibition, in a dose-dependent manner against HEPG2 when compared to 5-FU and DOX (IC_{50} equals 3.56, and 5.23 µg/ml, while 5-FU and DOX were 5 and 3.56 µg/ml).

Cytotoxic drugs remain the main stay of cancer chemotherapy and are being administered with novel ways of therapy such as inhibitors of signals [28]. It is therefore important to discover novel cytotoxic agents with spectra of activity and toxicity that differ from those current agents [29]. It is well known that chemotherapy aims to destroy the cancer cells with various types of chemicals [30]. The substances used are supposed to target mainly the cancer cells and doses are calculated to minimize the collateral damage to surrounding tissues, which nevertheless occurs [31]. This kind of treatment increases the entropy of the organism, suppresses the immune system, and forms a toxic cell environment which may destroy surrounding healthy cells. So it is important to minimize curing doses to the least amount possible as well as trying to minimize the side effects of these drugs. For this novel derivatives of triazole possessing a broader spectrum of antitumor activity and fewer toxic side effects than 5-Fu and DOX have been sought. The antitumor activities of such compounds were assessed against HEPG2 cancer cell line in comparison to the traditional anticancer drugs: 5-Fu and DOX. Regarding the antitumor activity study, some of the selected compounds showed reasonable antitumor activity in comparison to 5-FU and DOX. Comparable to 5-FU and DOX, a dose augmentation of compounds 6 and 5, searching for possible higher potency, seems, consequently, realizable without undesirable implications. These results are in agreement with Espinosa *et al.,* [32] and Kamalakannan and Venkappayya [33], who reported that novel derivatives of 5-FU possessing a broader spectrum of antitumor activity and fewer toxic side effects than 5-FU.

Molecular docking Study:

Tyrosine phosphorylation has a key role in intracellular signaling. Inappropriate proliferation and survival cues in tumor cells often occur as a consequence of unregulated tyrosine kinase activity. Much of the current development of anti-cancer therapies tries to target causative proteins in a specific manner to minimize side-effects. One attractive group of target proteins is the kinases. c-Kit is a receptor tyrosine kinase that normally controls the function of primitive hematopoietic cells, melanocytes and germ cells. It has become clear that uncontrolled activity of c-Kit contributes to formation of an array of human tumors. The unregulated activity of c-Kit may be due to overexpression, autocrine loops or mutational activation. This makes c-Kit an excellent target for cancer therapies in these tumors.

The molecular docking is performed and analyzed with the MOE program. The synthesized compounds **7, 8** are investigated for the binding affinity of c-Kit tyrosine kinases receptor (pdb 1t46) [1]. This purpose of lead optimization and to find out the interaction between compounds **7, 8** and the c-Kit protein tyrosine receptor.

Molecular modeling calculations and local docking are done by using MOE (molecular modeling environment) to evaluate the binding free energies of these inhibitors into the target c-Kit tyrosine kinase receptor.

Validation of the docking performance and accuracy:

To validate the docking accuracy of the program used, docking of the native co-crystallized STI-571 ligand (Imatinib or Gleevec ligand) is done into its binding site of c-Kit tyrosine kinase receptor. The docked ligand is exactly superimposed on the native co-crystallized one with RMSD being 0.35Å and binding free energies of (-26.32 kcal/mol).

Molecular docking study:

The docking studies are carried out using Molecular Operating Environment (MOE) 2008.10 (Moe source: Chemical Computing Group Inc., Quebec, Canada, 2008). First, of all a Gaussian Contact surface around the binding site is drawn. The surface surrounds the van der Waals surface of a molecule (filling in solvent inaccessible gaps). Then docking studies are carried out to evaluate the binding free energy of the inhibitors within the macromolecules. The Dock scoring in MOE software is done using London dG scoring

function and has been enhanced by using two different refinement methods, the Force-field and Grid-Min pose have been updated to ensure that refined poses satisfy the specified conformations. We allowed rotatable bonds; the best 10 poses are retained and analyzed for the binding poses best score.

***Preparation of ligands and target c-Kit receptor*:**

The compounds involved in this study as ligands are **7, 8** which are studied for their binding affinity into c-Kit receptor . The Molecule Builder tool in MOE was used to construct a three-dimensional model of the structures. Energy minimization is done through Force-field MMFF94x Optimization using gradient of 0.0001 for determining the lower energy conformations with the most favorable (lowest energy) geometry. The crystal structures of c-Kit receptor in complex with STI-571 ligand (Imatinib or Gleevec ligand) were obtained from the Protein Data Bank (PDB) http://www.rcsb.org/pdb/explore/explore.do?structureId=1T46 (PDB code: 1t46). Hydrogen atoms and partial charges were added to the protein with the protonation 3D application in MOE. This application is performed to assign ionization states and position hydrogen atoms in the macromolecular structure.

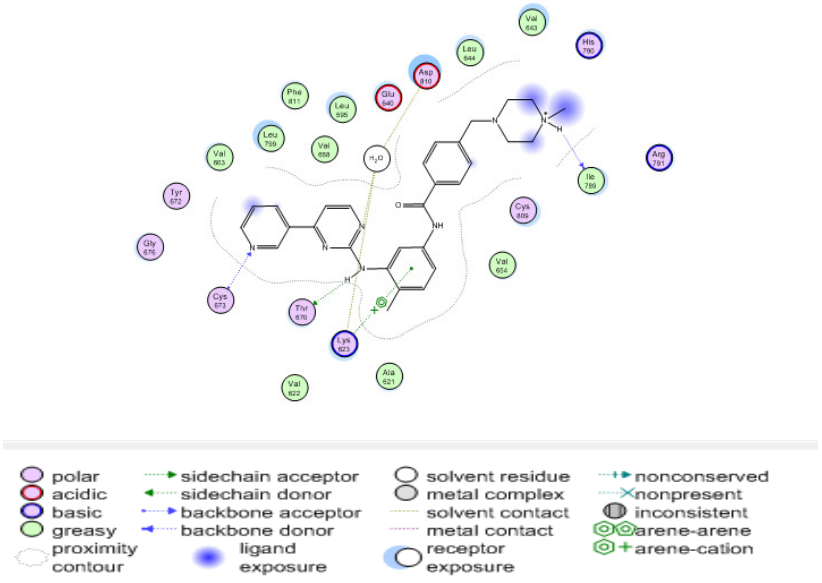


Fig. 1: The ligand interaction and the binding mode of the native ligand STI-571 ligand 4-(4-methyl-piperazin-1-ylmethyl)-N-[4-methyl-3-(4-pyridin-3-yl-pyrimidin-2-yl amino)-phenyl]-Benzamide and exhibited one H-bond donor with THR 670 and at distance 2.95 A° and one H-bond donor with ILE 798 at distance 2.98 A°and one H-bond acceptor with CYS 673 at distance 2.85 A° and one H-bond acceptor with CYS 673 at distance 2.85 A° and it is gave score -26.32 kcal/mol.

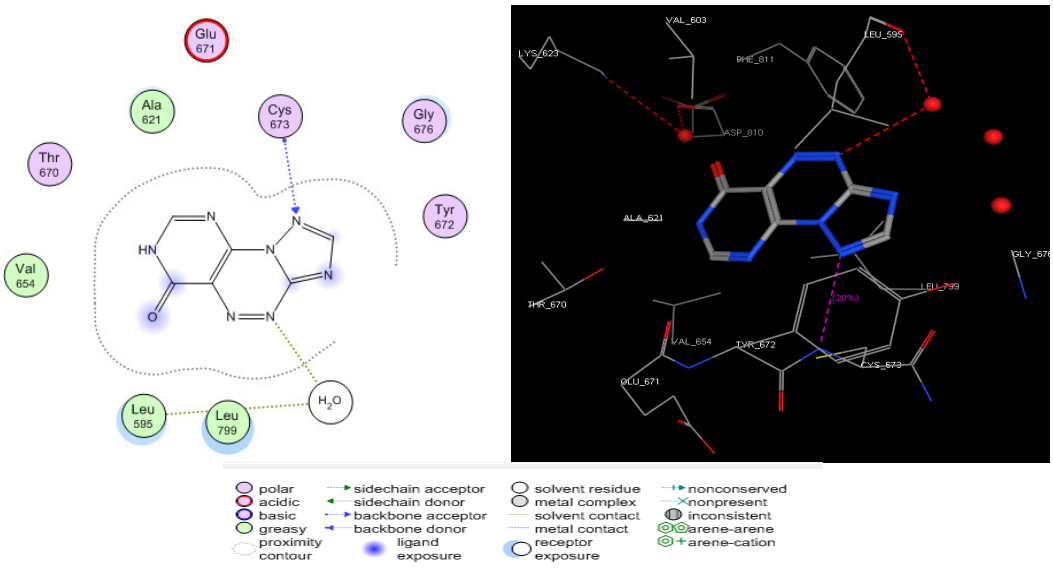


Fig. 2: Ligand interaction and the binding mode of compound 7 with c-Kit receptor , exhibited one H-bond acceptor with CYS 673 at distance 3.15 A° and A water mediated H-bond acceptor with Leu 595 at distance 3.10 A° and it is gave score -11.68 kcal/mol.

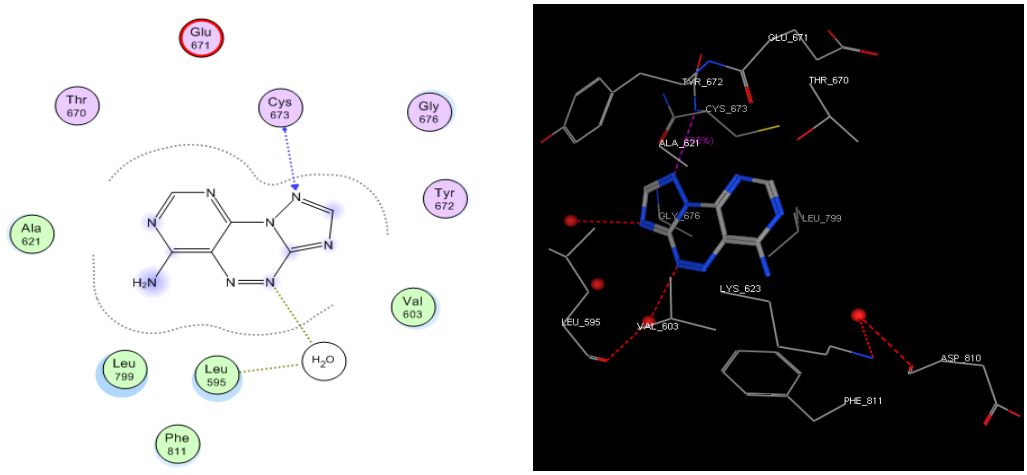


Fig. 3: Ligand interaction and the binding mode of compound 8 with c-Kit receptor , exhibited one H-bond donor with CYS 673 at distance 2.83 A° and A water mediated H-bond acceptor with Leu 595 at distance 2.99 A° and it is gave score -12.60 kcal/mol.

REFERANCE:

1. Mol, C. D., Dougan, D. R., Schneider, T. R., Skene, R. J., Kraus, M. L., Scheibe, D. N., Snell, G. P.; Zou, H., Sang, B. C. and Wilson, K.P., Structural basis for the auto inhibition and STI-571 inhibition of c-Kit tyrosine kinase, J. Biol. Chem., **2004**; 279: 31655-31663.
2. The Merck index, (**1996**) 12th ed, Merck Co. Inc. Whitehouse station,
3. Haber, J. *Present status and perspective on antimycotics with systematic effects*, *Casopis Lekaru Ceskych* (Praha), **2001**; *140*, 596.
4. Fischer, G.*Z. Chem.,* **1990**; *30*, 305.
5. Fischer G. *Adv. Heterocycl. Chem.,* **1993**; *57*, 81.
6. Elashry, E.S.H., Rashed, N. *Adv. Heterocycl. Chem.,* **1999**; *73*, 127.
7. Richardson, C.M., Williamson, D.S., Parratt, M.J., Borgognoni, J., Cansfield, A.D., Dokurno, P., Francis, G.L., Howes, R., Moore, J.D., Murray, J.B., Robertson, A, Surgenor A.E., Torrance, C.J. *Bioorg. Med. Chem. Lett.*, **2006**; *16*(5): 1353; (b) Zaki, Y. H.; Ahmad, S.A; Hussein, A.M.; Abdelhamid, A.O. *Phosphorus SulfurSilicon*, **2006**; *181*(4): 825.
8. Richardson, C.M., Williamson, D.S., Parratt, M.J., Borgognoni, J., Cansfield, A.D., Dokurno, P., Francis, G.L., Howes, R., Moore, J.D., Murray, J.B., Robertson, A, Surgenor A.E., Torrance, C.*J. Bioorg. Med. Chem. Lett.*, **2006**; *16*(5): 1353.
9. Davies, G.E. *J. Pharm. Pharmacol.,* **1973**; *25*(9): 681.
10. Brdar, B., Japelj, M., Kobe. *J. Biochem. Pharmacol.,* **1979**; *28*(10): 1683.
11. Eisa, H.M., El-Ashmawy, M.B., Tayel, M.M., El-Magd, S.A., El. Kashef. *Boll. Chim. Farm.*, **1996**; *135*(10): 585.
12. Prakash, O., Bhardwaj, V. Kumar, R. Tyagi, P. Aneja, K.R. *Eur. J. Med. Chem.,* **2004**; *39*(12): 1073.
13. Moawad, E.B., Yousif, M.Y., Metwally, M.A. *Pharmazie*, **1989**; *44*(12): 820.
14. Cao,J., Zhang, Q., Zhou, Ye. X., Lou, J., Zhu, Y., Hu, Y., He.Q Yang, J., *J. Biomed. Biotech*: **2009**; (1).
15. Attia, A., Michael, M.; *pharnazie*, **1982**; (551).
16. Litvnov, V. P., Krivokolysko, S. G., Dyaschenko, V. D., *Chem. Heterocycl. Comp.*, **1999**; 135(5): 509.
17. Girgis.A. S., Mishriky, N., Farag, A. M., El-Eraky, W.I., Earag, H., *Eur. J. Med. Chem.,* **2008**; 43(9): 1818.
18. Swelam, S. A., El-Said, N. S1, Aly, A. S1, Abdel-Fatth, A. M. Facile and Simple Syntheses of Heterocyclic Compounds Based on Pyridine and Pyrazolopyridine Derivatives. *International Journal of PharmTech Research,* **2009**; (1): 445-453.
19. Swelam, S. A. Abo-Bakr, Sh. M.; Fawzy, N. M.; El-Maghraby, S.; Taha.H.; Synthesis, Toxicological and Biochemical Studies of New Heterocyclic Compounds Derived From Acetanilide and Pyrrole Derivativesaccepted for publication in *Der Pharma Chemica*, **2010**; 2(1): 46-59.
20. Fathalla O. A., Zaki M. E. A., Swelam S. A., El- Eraky W. I. *Acta Polonia Pharmaceutica DrugResearch*, **2003**; *60*(1): 51-60.
21. Zaki M. E. A., Fawzy N. M., Swelam S. A., *Molecules*, **1999***; 3: 1.*
22. Samira. A. Swelam, Omar. A. Fathalla, Magdi. E. A. Zaki, "Syntheses and Reactions of Some Pyrazolopyrimidine and 3H-2, 3,4,5a,6,10-hexazacyclohepta [e] -indene Derivatives", *Afindad,* **2008**; 66(537).
23. Nair V., Lyons A. G., Purdy D. F., *Tetrahedron*, **1991**; 47: 8949-8968.
24. Danagulyan G. G., Saakyan L. G., Zalinyan M. G., *Khim. Geterotsikl. Soedin,* 1992, 2, 225–227; Chem. Abstr., **1992**; 117: 233226c.
25. Swelam S. A., Abdel-Salam O. I., Zaki M. E. A., *J.Serb. Chem. Soc*., **1999**; 64: 655–662.
26. Swelam S.A., Zaki M.E.A., El-Gazzar A.B. *Heterocyclic Communication*, **2003**; 9(2).
27. Taylor E.C., Warrener R.N., McKillop A. *Angew. Chem*., 78, 333, 1966; Intern. Ed. (English): **1966**; 5: 309.
28. Taylor E.C., Warrener R.N. *Tetrahedron*, 1987,23, 891.
29. Skehan P., Storeng R., Scudiero D., Anne Monks A., McMahon J., Vistica D., Warren J., Bokesch H., Kenney S. and Boyd M. *J.* Natl Cancer Inst., **1990**; 82: 1107.
30. Guilbaud N. L., Kraus-Berthier F., Meyer-Losic V., Malivet C., Chacun M., Jan F., Tillequin S., Michel M. and Koch B. Marked Antitumor Activity of a New Potent Acronycine Derivative in Orthotopic Models of Human Solid Tumors., *Clin. Cancer Res.*, **2001**; 7: 2573.
31. Sathish N.K., Rajendra Prasad V.V.S., Raghavendra N.M., Shanta Kumar S.M. and Mayur Y.C. *Sci. Pharm*., **2009**; 77: 19.
32. Hayakawa I., Shioya R., Agatsuma T., Furukawa H., Naruto S. and Sugano Y. A library synthesis of 4-hydroxy-3-methyl-6-phenylbenzofuran-2-carboxylic acid ethyl ester derivatives as anti-tumor agents. Bioorg. Med. Chem. Lett., **2004**; 14: 4383.
33. Kamalakannan P. and Venkappayya D. Synthesis and characterization of cobalt and nickel chelates of 5-dimethylaminomethyl-2-thiouracil and their evaluation as antimicrobial and anticancer agents. J. Inorg. Biochem., **2002**; 90: 22.

How to cite this article:

Samira A. Swelam et al.,: Synthesis, characterization, antitumor evaluation and molecular docking of some triazolotriazine derivatives. J. Pharm. Res., 2014; 3(5): 79-87.

Conflict of interest: The authors have declared that no conflict of interest exists.

Source of support: Nil