TRIAZOLOPHTHALAZINE INCORPORATING PIPERAZINE DERIVATIVES: SYNTHESIS AND *IN VITRO* ANTICANCER EVALUATION STUDY

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ABSTRACT

Cancer remains as one of the top leading causes of death worldwide in the last decade. In an attempt to develop a potent anticancer agent, herein we are reporting the synthesis of two novel series of piperazinyltriazolophthalazines as potential anticancer agents with potential inhibitory activity against PARP-1. All the newly synthesized compounds were

evaluated for their anti-proliferative activity against four human cancer cell lines namely; Hepatocellular carcinoma (HePG-2), Mammary gland breast cancer (MCF-7), Human prostate cancer (PC3) and Colorectal carcinoma (HCT-116)). The results of cytotoxicity evaluation showed that most of the synthesized compounds displayed moderate cytotoxic activity against the selected cell lines. Compound **23** showed the highest inhibitory effect followed by compound **24** against hepatocellular carcinoma (HePG2) with IC_{50} values of 15.05 and 17.23 µM respectively. The same two compounds also showed moderate activity against colorectal carcinoma cell line (HCT-116) with IC_{50} values of 21.93 and 24.06 µM respectively.

Keywords: anticancer, piperazinyltriazolophthalazines, Hepatocellular carcinoma, Mammary gland breast cancer, Human prostate cancer and Colorectal carcinoma.

Introduction

Phthalazine derivatives are biologically important heterocycles and are known to possess variety of biological activities such as antitumor (Fedorov *et al*. 2014), anticonvulsant *(Sun et al*. 2011; Sun, Wei, *et al*. 2010), anti-inflammatory (Abd alla *et al*. 2010; Liu *et al*. 2016; Sun, Hu, et al. 2010), antidiabetic (Awadallah, El-Eraky, and Saleh 2012), antihypertensive (Olmo *et al*. 2006), muscle relaxant (Haack *et al*. 2005) and antimicrobial (Holló *et al*. 2014; Khalil, Berghot, and Gouda 2009; Salvi *et al*. 2006). Phthalazine scaffold is in the core structure of many commercially available drugs (Asif 2012) like, zopolrestat, azelastine, hydralazine and budralazine, the first is antidiabetic, the second is antihistaminic while the third and the last are vasodilating. A promising class of anticancer agents with micromolar range of IC_{50}'s is the 1,2,4-triazoles (Lu *et al*. 2018). Triazolopthalazine rings presented in many anticancer agents with potent bromodomain inhibitory effects (Almahli *et al*. 2018; Boraei *et al*. 2019; Moustakim, Peter G. K. Clark, *et al*. 2017). Few years ago, compound **1** (*L*-45) was discovered as the first potent [1,2,4]triazolo[3,4-a]phthalazine as anticancer with PCAF bromodomain inhibitory effect (Moustakim, Peter G. K. Clark, *et al*. 2017). Additionally, [1,2,4]triazolo[3,4-a]phthalazine is the pharmacophore ring system of certain anticancer agents (El-Helby *et al*. 2018; Moustakim, Peter G.K. Clark, *et al*. 2017; Xue *et al*. 2014). In the last decade, many synthetic [1,2,4]triazolo[3,4-a]phthalazines **(Figure 1)** have been reported to exhibit potent anticancer activities against hepatocellular carcinoma with IC_{50} values range of 1.60-6.04 (El-Helby *et al*. 2017, 2018; Xue *et al*. 2014).

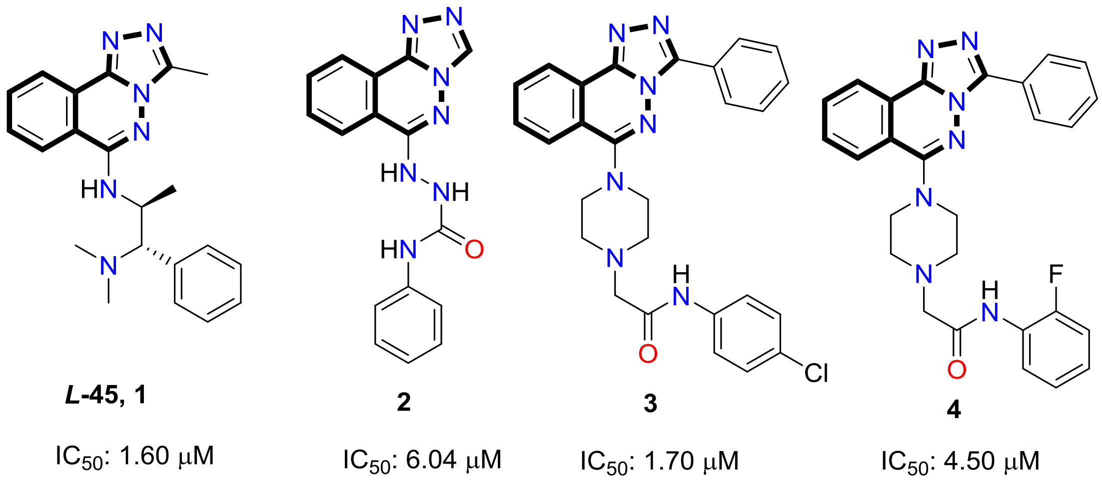
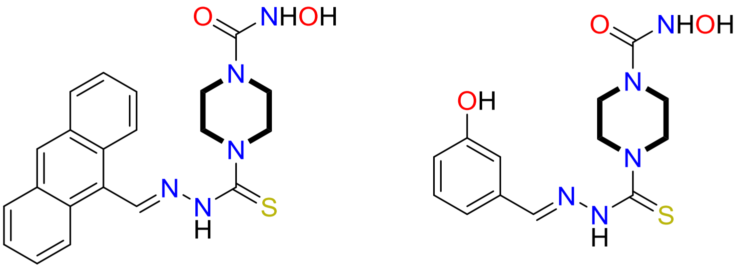


Figure 1: Structures of selected triazolophthalazines with potent anticancer activity against hepatocellular carcinoma.

On the other hand, piperazines are important class of compounds with diverse biological activities, particularly as anticancer (Chetan *et al*. 2010; Gurdal *et al*. 2015). Piperazine derivatives **5** & **6** **(Figure 2)** showed potent cytotoxic activity against NCIH460, HCT116 and U251 cell lines. Considering the above-mentioned facts and as an attempt to develop a new molecule with potent anticancer activity, molecular hybridization between triazolophthalazine scaffold and piperazine moiety was carried out. Here, inspired by the versatility of the[1,2,4]triazolo[3,4-a]phthalazine pharmacophoric ring skeleton of ***L*-45** and the piperazine moiety mentioned above, nine novel compounds were synthesized to evaluate their anticancer activities. Different substitution patterns were introduced to both the C-3 of triazolophthalazine scaffold and the terminal pierazine ring to investigate the effect on cytotoxicity of the synthesized compounds. All the synthesized compounds were evaluated for their *in vitro* anticancer activity against four cancer cell lines namely; hepatocellular carcinoma (HePG-2), mammary gland breast cancer (MCF-7), human prostate cancer (PC3) and colorectal carcinoma (HCT-116).

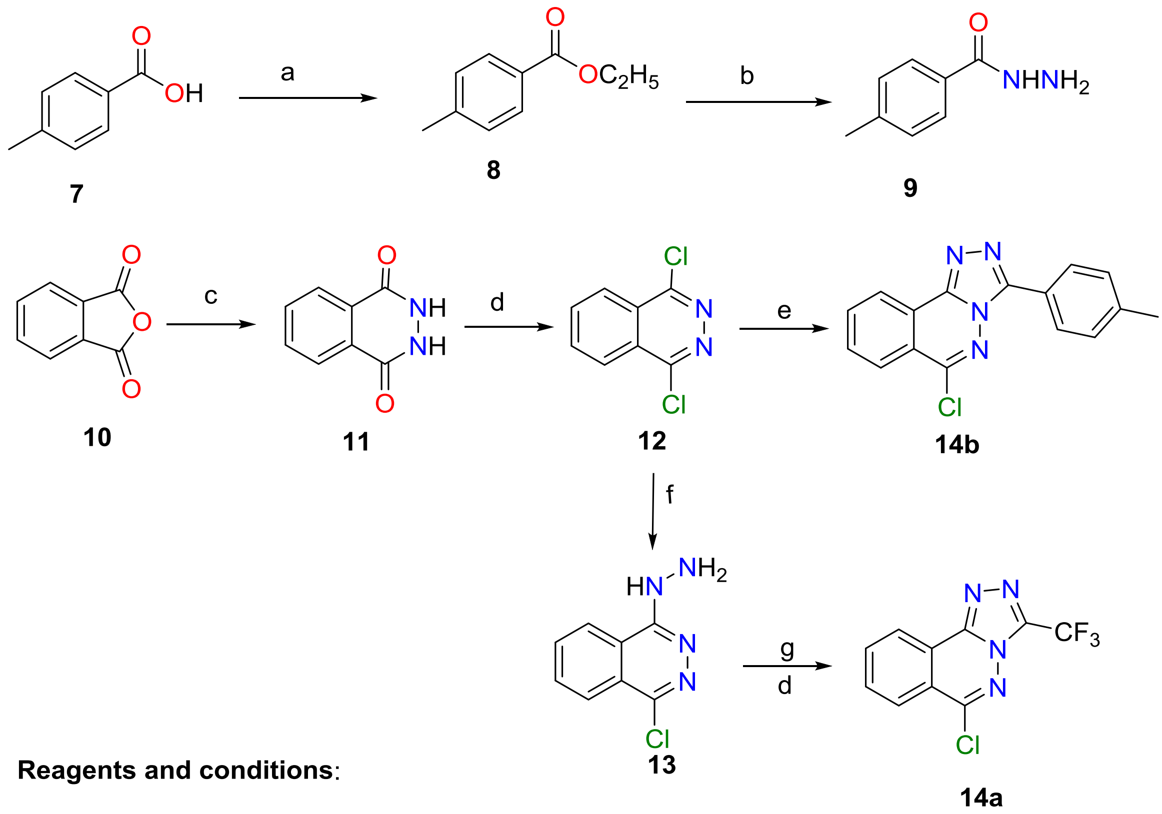


5 **6**

Figure 2: Structures of some reported anticancer agents incorporating piperazine ring.

Results and discussion

Chemistry: The general route for the synthesis of starting triazolophthalazines is illustrated in **Scheme 1**. 4-Methylbenzoic acid converted into the ethyl ester by the action of ethanol in the presence of catalytic amount of sulfuric acid. The resulting ester allowed to react with hydrazine hydrate in refluxing ethanol to get 4-methylbenzohydrazide (**9**). In a parallel pathway, phthalic anhydride reacted with hydrazine hydrate in acetic acid to yield 2,3-dihydrophthalazine-1,4-dione (**11**). The latter reacted under reflux with phosphorus oxychloride to yield 1,4-dichlorophthalazine (**12**). Treating compound with 12 hydrazine hydrate at room temperature in ethanol to produce 1-chloro-4-hydrazinylphthalazine (**13**) (Abou-Seri *et al*. 2016). Reaction of the hydrazinyl derivative **13** with trifluoroacetic acid followed by chlorination with phosphorus oxychloride yielded 6-chloro-3-(trifluoromethyl)-[1,2,4]triazolo[3,4-*a*]phthalazine (**14a**). Alternatively, treating the dichlorophthalazine derivative **12** with 4-methylbenzohydrazide in refluxing ethanol produced the triazolophthalazine derivatives **14b**.



Reagents and conditions:

a) C_2H_5OH (absolute)/H_2SO_4, 100 °C 77%. **b)** H_2N-NH_2.H_2O, C_2H_5oH, 100 °C, 72%.

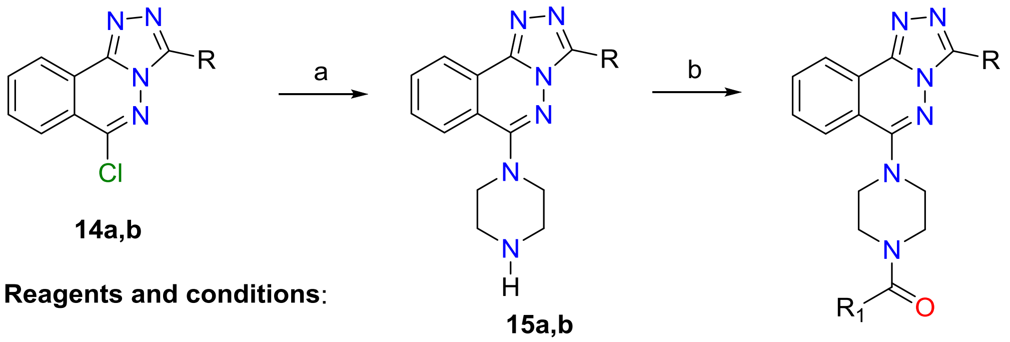
c) H_2N-NH_2.H_2O, CH_3COOH, 120 °C, 85%; **d)** $POCl_3$, 110 °C, 5 h,90%.

e) 4-$CH_3C_6H_4CONHNH_2$ (**9**), dioxane, 140 °C, 6 h, 80%

f) H_2N-NH_2.H_2O, C_2H_5OH, rt, 3 h, 70% ; **g)** CF_3COOH, 140 ° 10 h,, 85%.

Scheme 1: Synthetic route for preparation of starting triazolophthalazines

Reaction of the triazolophthalazine derivatives **14a,b** with piperazine in ethanol according to reported procedures (Tarzia *et al*. 1988) afforded the piperazinyl derivatives **15a,b** (**Scheme 2**). Our final target compounds were readily obtained in good yields and reasonable purities by treating the latter with the appropriate acid chloride.



14a,b **15a,b** **16-24**

Reagents and conditions:

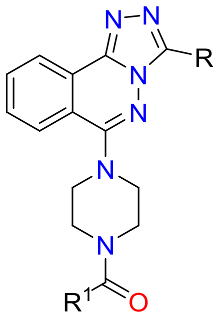
a) Piperazine, K_2CO_3/ethanol, rt °C, 6-10 h, 80%.

b) RCOCl, DMF, 80 °C, 4 h, 66-80 %.

Scheme 2: Synthetic route for preparation of target piperazinyl triazolophthalazines

Anticancer evaluation: Anticancer activities of the synthesized compounds were assessed on four cancer cell lines namely; hepatocellular carcinoma (HePG-2), mammary gland breast cancer (MCF-7), human prostate cancer (PC3) and colorectal carcinoma (HCT-116) using MTT assay (Mosmann 1983). Doxorubicin was used as a reference anticancer agent. The results of preliminary anticancer evaluation are shown in **Table 1**. The results of cytotoxicity evaluation showed that the majority of the synthesized compounds displayed moderate cytotoxic activities against the selected cell lines. Compound **23** showed the highest inhibitory effect followed by compound **24** against hepatocellular carcinoma (HePG2) with IC_{50} values of 15.05 and 17.23 µM respectively. The same two compounds also showed moderate activity against colorectal carcinoma cell line (HCT-116) with IC_{50} values of 21.93 and 24.06 µM respectively. Compounds with trifluoromethy group attached to C-3 of the triazolophthalazine system (**16-19**) displayed relatively lower inhibitory potencies than all other compounds.

Table 1: *In vitro* anticancer activity of the new piperazinyl triazolophthalazines

In vitro* Cytotoxicity IC_{50} (μM)						
						
Cpd.	**R**	**R^1**	**HePG2**	**MCF-7**	**PC3**	**HCT-116**
16	CF_3	CH_3	93.26±5.3	87.10±4.9	>100	94.71±5.6
17	CF_3	C_6H_5	>100	91.17±5.0	>100	>100
18	CF_3	4-ClC_6H_4	75.49±3.8	69.11±3.6	81.10±4.5	78.37±4.1
19	CF_3	$CH=CHC_6H_5$	33.89±2.0	30.49±2.1	40.83±2.3	43.01±2.2
20	4-$CH_3C_6H_4$	CH_3	55.60±3.3	46.29±2.9	67.27±3.5	61.48±3.4
21	4-$CH_3C_6H_4$	C_6H_5	29.18±1.9	23.81±1.8	33.37±2.0	37.82±1.9
22	4-$CH_3C_6H_4$	4-ClC_6H_4	32.75±2.0	29.72±2.1	36.48±2.2	41.38±2.1
23	4-$CH_3C_6H_4$	$CH=CHC_6H_5$	15.05±1.2	77.33±4.1	>100	21.93±1.5
24	4-$CH_3C_6H_4$	4-$CH_3C_6H_4SO_2$	17.23±1.2	22.39±1.6	25.26±1.8	24.06±1.5
Doxorubicin			4.50±0.2	4.17±0.2	8.87±0.6	5.23±0.3

* **IC_{50} (μM)**: 1 – 10 (very strong). 11 – 20 (strong). 21 – 50 (moderate). 51 – 100 (weak) and above 100 (non-cytotoxic).

Experimental

General:

All melting points were determined using capillary tubes with a Stuart SMP30 apparatus and are uncorrected. ^{1}H NMR spectra were recorded on Bruker-400-MHz spectrophotometer using DMSO-d_6 as a solvent and TMS as internal reference. ^{13}C NMR spectra were recorded on Bruker-100-MHz spectrophotometer using DMSO-d_6 as a solvent and TMS as internal reference. Chemical shifts were recorded in δ ppm downfield the TMS signal. Mass spectra were recorded on Hewlett Packard 5988 spectrometer. Elemental analyses were performed on CHN analyzer. All spectral measurements have been performed at the Regional Center for Mycology & Biotechnology, Al-Azhar University, Cairo, Egypt. Anticancer evaluation was carried out at the Holding Company for Biological Products and Vaccines, Egypt (Vacsera).

General procedure for preparation of (4-(3-substituted-[1,2,4]triazolo[3,4-*a*]phthalazin-6-yl)piperazin-1-yl)(acyl)methanones (16-24):

Compounds **15** (0.001 mol) was dissolved in dimethyl formamide (20 ml) and acetyl chloride (0.01 mol) or aryl chloride (0.008 mol) was added and the mixture was refluxed at 80°C for 3h. After the end of the reaction (monitored by TLC), the mixture was allowed to cool and poured on crushed ice. On standing, the precipitate was formed and collected by filtration. This solid was recrystallized from ethanol to afford the corresponding compounds.

1-(4-(3-(Trifluoromethyl)-[1,2,4]triazolo[3,4-a]phthalazin-6-yl)piperazin-1-yl)ethan-1-one (16):

White solid; Yield: 76%; m.p.255-257°C. IR (KBr) cm^{-1}: 3016 (CH aromatic), 2974 (CH aliphatic), 1643 (C=O), 1589 (C=C aromatic). ^{1}H NMR (DMSO-d_6) δ ppm: 8.54 (d, 1H, *J* = 7.8), 8.21 (d, 1H, *J* = 8.1), 8.06 (t, 1H, *J* = 7.5), 7.97 (t, 1H, *J* = 7.6), 3.74 (s, 4H), 3.74 (s, 2H), 3.42 (s, 2H), 2.08 (s, 3H). MS (*m/z*): 364 ($C_{16}H_{15}F_3N_6O$, 18.78%, M^+) 295 ($C_{15}H_{15}N_6O$, 21.33%), 255 ($C_{13}H_{15}N_6$, 16%), 127 ($C_6H_{11}N_2O$, 100%), 71 (C_4H_9N, 24.3%).^{13}C NMR (DMSO-d_6) δ ppm: 169.10, 158.80, 144.66, 139.26, 138.86, 134.22, 132.15, 127.40, 123.83, 123.48, 120.40, 120.23, 117.55, 51.42, 51.01, 45.47, 40.77 and 21.62. Anal. Calc. for: ($C_{16}H_{15}F_3N_6O$) (M.W. = 364): C, 52.75; H, 4.15; F, 15.64; N, 23.07%; Found: C, 53.02; H, 54.41; N, 22.89%.

Phenyl(4-(3-(trifluoromethyl)-[1,2,4]triazolo[3,4-a]phthalazin-6-yl)piperazin-1-yl)methanone (17):

White. Yield: 67%; m.p.263-265°C. IR (KBr) cm^{-1}: 3028 (CH aromatic), 2997 (CH aliphatic), 1624 (C=O), 1589 (C=C aromatic). $_{1}$H NMR (DMSO-d_6) δ ppm: 8.59 (d, 1H, *J* = 7.76), 8.25 (d, 1H, *J* = 8.08), 8.08 (t, 1H, *J* = 7.5), 7.99 (t, 1H, *J* = 7.6), 7.49 (m, 5H), 3.92 (s, 2H), 3.65 (s, 2H), 3.46 (s, 4H). MS (*m/z*): 426 ($C_{21}H_{17}F_3N_6O$, 100%, M^+) 321 ($C_{14}H_{12}F_3N_6$, 4.74%), 292 ($C_{13}H_9F_3N_5$, 2.74%), 104 (C_7H_6N, 1.42%), 77 (C_6H_5, 26.84%),. ^{13}C NMR (DMSO-d_6) δ ppm: 169.89, 158.83, 144.82, 139.33, 138.94, 136.04, 134.39, 132.29, 130.15, 128.95, 127.5, 123.96, 123.58, 120.5, 117.56, 51.14

and 40.45. Anal. Calc. for: ($C_{21}H_{17}F_3N_6O$) (M.W. = 426): C, 59.15; H, 4.02; F, 13.37; N, 19.71%; Found: C, 59.38; H, 4.23; N, 19.87%.

(4-Chlorophenyl)(4-(3-(trifluoromethyl)-[1,2,4]triazolo[3,4-a]phthalazin-6-yl)piperazin-1-yl)methanone (18):

White solid; Yield: 77%; m.p.267-269°C. IR (KBr) cm^{-1}: 3032 (CH aromatic), 2997 (CH aliphatic), 1624 (C=O), 1593 (C=C aromatic). ^1H NMR (DMSO-d_6) δ ppm: 8.59 (d, 1H, *J* = 7.4), 8.24 (d, 1H, *J* = 8.1), 8.09 (t, 1H, *J* = 7.3), 7.99 (t, 1H, *J* = 8.2), 7.56-7.51 (m, 4H), 3.92 (s, 2H), 3.64 (s, 2H), 3.46 (s, 4H). MS (*m/z*): 462 ($C_{21}H_{16}ClF_3N_6O$, 31.81%, M^++2) 460 ($C_{21}H_{16}ClF_3N_6O$, 100%, M^+) 321 ($C_{14}H_{12}F_3N_6$, 1.69%), 279 ($C_{12}H_8F_3N_5$, 1.31%), 139 (C_7H_4ClO, 1.8%), ^{13}C NMR (DMSO-d6) δ ppm: 168.81, 158.84, 144.87, 139.35, 138.95, 134.86, 134.82, 134.43, 132.31, 129.58, 129.02, 127.55, 124.01, 123.66, 120.56, 120.26, 117.58, 51.09 and 40.51. Anal. Calc. for: ($C_{21}H_{16}ClF_3N_6O$) (M.W. = 460): C, 54.73; H, 3.5; Cl, 7.69; F, 12.37; N, 18.24%; Found: C, 54.91; H, 3.67; N, 18.07%.

3-Phenyl-1-(4-(3-(trifluoromethyl)-[1,2,4]triazolo[3,4-a]phthalazin-6-yl)piperazin-1-yl)prop-2-en-1-one (19):

White solid; Yield: 79%; m.p.261-263°C. IR (KBr) cm^{-1}: 3001 (CH aromatic), 2893 (CH aliphatic), 1647 (C=O), 1608 (C=C aromatic). ^1H NMR (DMSO-d_6) δ ppm: 8.6 (d, 1H, *J* = 7.8), 8.27 (d, 1H, *J* = 8.1), 8.09 (t, 1H, *J* = 7.1), 8 (t,1H, *J* = 7.4), 7.74 (d, 2H, *J* = 6.7), 7.55 (d,1H, J= 15.4), 7.44 – 7.34 (m, 4H), 4.03 (s, 2H), 3.89 (s, 2H), 3.47 (s, 4H). MS (*m/z*): 452 ($C_{23}H_{19}F_3N_6O$, 100%, M^+) 375 ($C_{17}H_{14}F_3N_6O$, 1.96%), 280 ($C_{12}H_9F_3N_5$, 2.63%), 131 ($C_8H_7N_2$, 23.52%), 77 (C_6H_5, 36.23%). ^{13}C NMR (DMSO-d_6) δ ppm: 165.24, 158.84, 144.83, 142.29, 139.33, 138.93, 135.52, 134.37, 132.27, 130.05, 129.21, 128.48, 127.54, 123.99, 123.65, 120.56, 120.28, 118.51, 117.6, 51.6 and 41.9. Anal. Calc. for: ($C_{23}H_{19}F_3N_6O$) (M.W. = 452): C, 61.06; H, 4.23; F, 12.6; N, 18.58%; Found: C, 60.89; H, 4.42; N, 18.7%.

1-(4-(3-(*p*-Tolyl)-[1,2,4]triazolo[3,4-a]phthalazin-6-yl)piperazin-1-yl)ethan-1-one (20):

White solid; Yield: 69%; m.p.259-261°C. IR (KBr) cm^{-1}: 3024 (CH aromatic), 2951 (CH aliphatic), 1643 (C=O), 1589 (C=C aromatic). ^1H NMR (DMSO-d_6) δ ppm: 8.53 (d, 1H, *J* = 7.7), 8.27 (d, 2H, *J* = 7.8), 8.19 (d, 1H, *J* = 8.0), 8.01 (t,1H, *J* = 7.4), 7.9 (t, 1H, *J* = 7.5), 7.41 (d, 2H, *J* = 7.7), 3.76 (s, 4H), 3.41 (s, 4H), 2.4 (s, 3H, CH_3), 2.09 (s, 3H,$COCH_3$). MS (*m/z*): 386 ($C_{22}H_{22}N_6O$, 43.57%, M^+) 330 ($C_{20}H_{20}N_5$, 54.39%), 261 ($C_{16}H_{13}N_4$, 26.63%), 214 ($C_{12}H_{14}N_4$, 100%), 124 ($C_6H_{10}N_3$, 15.89%). ^{13}C NMR (DMSO-d_6) δ ppm: 169.06, 157.66, 148.1, 143.18, 140.05, 133.97, 131.15, 129.78, 127.58, 127.19, 124.63, 124.12, 123.49, 119.8, 51.35, 45.74, 21.69 and 21.48. Anal. Calc. for: ($C_{22}H_{22}N_6O$) (M.W. = 386): C, 68.38; H, 5.74; N, 21.75%; Found: C, 68.59; H, 5.81; N, 21.98%.

Phenyl(4-(3-(p-tolyl)-[1,2,4]triazolo[3,4-a]phthalazin-6-yl)piperazin-1-yl)methanone (21):

White solid; Yield: 67%; m.p.261-263°C. IR (KBr) cm^{-1}: 3028 (CH aromatic), 2993 (CH aliphatic), 1635 (C=O), 1589 (C=C aromatic). ^{1}H NMR (DMSO-d_6) δ ppm: 8.55 (d, 1H, *J* = 7.9), 8.27 (d, 2H, *J* = 8.04), 8.2 (d, 1H, *J* = 8.1), 8.02 (t,1H, *J* = 7.6), 7.9 (t, 1H, *J* = 7.5), 7.49 (m, 5H), 7.42 (d, 2H, *J* = 7.9), 3.96 (s, 2H), 3.69 (s, 2H), 3.44(s, 4H), 2.41 (s, 3H). MS (*m/z*): 448 ($C_{27}H_{24}N_6O$, 7.06%, M^+) 301 ($C_{18}H_{15}N_5$, 12.57%), 259 ($C_{16}H_{11}N_4$, 6.33%), 105 (C_7H_5O, 100%). ^{13}C NMR (DMSO-d_6) δ ppm: 169.79, 157.61, 148.16, 143.21, 140.14, 136.11, 134.02, 131.22, 130.16, 129.83, 128.98, 127.63, 127.51, 127.23, 124.58, 124.03, 123.51, 119.77, 51.27, 40.45 and 21.47. Anal. Calc. for: ($C_{27}H_{24}N_6O$) (M.W. = 448): C, 72.3; H, 5.39; N, 18.74%; Found: C, 72.52; H, 5.61; N, 18.92%.

(4-Chlorophenyl)(4-(3-(p-tolyl)-[1,2,4]triazolo[3,4-a]phthalazin-6-yl)piperazin-1-yl)methanone (22): White solid; Yield: 74%; m.p.263-265°C. IR (KBr) cm^{-1}: 3082 (CH aromatic), 2993 (CH aliphatic), 1631 (C=O), 1593 (C=C aromatic). ^{1}H NMR (DMSO-d_6) δ ppm: 8.52 (d, 1H, *J* = 7.6), 8.25 (d, 2H, *J* = 7.6), 8.17 (d, 1H, *J* = 7.9), 8 (t,1H, *J* = 7.4), 7.88 (t, 1H, *J* = 7.2), 7.54 (m, 4H), 7.39 (d, 2H, *J* = 7.5), 3.94 (s, 2H), 3.66 (s, 2H), 3.43 (s, 4H), 2.39 (s, 3H). MS (*m/z*): 484 ($C_{27}H_{23}ClN_6O$, 33.56%, M^{+}+2) 482 ($C_{27}H_{23}ClN_6O$, 64.67%, M^+) 343 ($C_{20}H_{19}N_6$, 12.31%), 259 ($C_{16}H_{11}N_4$, 58.37%), 144 ($C_8H_6N_3$, 100%). ^{13}C NMR (DMSO-d_6) δ ppm: 148.05, 134.18, 130.71, 129.15, 127.76, 124.64, 123.78, 93.79, 51.14, 48.71 and 16.64. Anal. Calc. for: ($C_{27}H_{23}ClN_6O$) (M.W. = 482): C, 67.15; H, 4.8; Cl, 7.34; N, 17.4%; Found: C, 67.42; H, 4.93; N, 17.28%.

3-Phenyl-1-(4-(3-(p-tolyl)-[1,2,4]triazolo[3,4-a]phthalazin-6-yl)piperazin-1-yl)prop-2-en-1-one (23): White solid; Yield: 71%; m.p.266-268°C. IR (KBr) cm^{-1}: 3020 (CH aromatic), 2978 (CH aliphatic), 1647 (C=O), 1604 (C=C aromatic). ^{1}H NMR (DMSO-d_6) δ ppm: 8.55 (d, 1H, *J* = 7.8), 8.28 (d, 2H, *J* = 7.8), 8.23 (d, 1H, *J* = 8.1), 8.03 (t,1H, *J* = 7.4), 7.92 (t, 1H, *J* = 7.4), 7.76 (d, 2H, *J* = 6.7), 7.56 (d, 1H, *J* = 15.4), 7.41-7.35 (m, 6H), 4.05 (s, 2H), 3.92 (s, 2H), 3.44 (s, 4H), 2.4 (s, 3H). MS (*m/z*): 474 ($C_{29}H_{26}N_6O$, 100%, M^+) 329 ($C_{20}H_{19}N_5$, 17.15%), 260 ($C_{16}H_{12}N_4$, 26.66%), 113 ($C_5H_9N_2O$, 32.29%). ^{13}C NMR (DMSO-d_6) δ ppm: 165.23, 157.67, 148.17, 143.24,142.35, 140.13, 135.51, 134.03, 131.23, 130.13, 129.82, 129.27, 128.53, 127.64, 127.25, 124.63, 124.09, 123.54, 119.85, 118.51, 51.56, 45.17, 41.83 and 21.48. Anal. Calc. for: ($C_{29}H_{26}N_6O$) (M.W. = 474): C, 73.4; H, 5.52; N, 17.71%; Found: C, 73.28; H, 5.68; N, 17.89%.

3-(*p*-Tolyl)-6-(4-tosylpiperazin-1-yl)-[1,2,4]triazolo[3,4-a]phthalazine (24): White solid; Yield: 71%; m.p.253-255°C. IR (KBr) cm^{-1}: 3028 (CH aromatic), 2981 (CH aliphatic), 1674 (C=O), 1589 (C=C aromatic). ^{1}H NMR (DMSO-d_6) δ ppm: 8.50 (d, 1H, *J* = 7.9), 8.24 (d, 2H, *J* = 7.9), 8.06 (d, 2H, *J* = 8.1), 7.98 (t, 1H, *J* = 7.9), 7.82 (t, 1H, *J* = 8.0 H), 7.72 (d, 1H, *J* = 7.9), 7.51 (d, 2H, *J* = 7.8), 7.42 (d, 2H, *J* = 7.8), 3.47 (s, 4H), 3.24 (s, 4H), 2.43 (s, 3H). MS (*m/z*): 498 ($C_{27}H_{26}N_6O_2S$, 13.71%, M^+) 407 ($C_{20}H_{19}N_6O_2S$, 57.81%), 381 ($C_{19}H_{19}N_5O_2S$, 100%), 343 ($C_{20}H_{19}N_6$, 29.51%), 254 ($C_{13}H_{14}N_6$, 26.04%), 123 ($C_5H_7N_4$, 12.66%). ^{13}C NMR (DMSO-d_6) δ ppm: 130.46, 129.84, 128.14, 127.61, 50.58, 45.9 and 21.49. Anal. Calc. for: ($C_{27}H_{26}N_6O_2S$) (M.W. = 498): C, 65.04; H, 5.26; N, 16.86%; Found: C, 65.31; H, 5.39; N, 16.81; S, 6.57%.

Anticancer activity: All the synthesized compounds were subjected to MTT proliferation assay to investigate their *in-vitro* cytotoxic activity. Hepatocellular carcinoma (HePG-2), mammary gland breast cancer (MCF-7), Human prostate cancer (PC3) and colorectal carcinoma (HCT-116) cell lines was chosen for investigation.

Conclusion

a series of triazolophthalazine incorporating piperazine derivatives was synthesized and evaluated for their anticancer activity against four human cancer cell lines (Hepatocellular carcinoma (HePG-2), mammary gland breast cancer (MCF-7), Human prostate cancer (PC3) and Colorectal carcinoma (HCT-116)). The results of anticancer evaluation showed that most of the synthesized compounds displayed moderate cytotoxic activities against the selected cell lines. Compound **23** showed the highest inhibitory effect followed by compound **24** against hepatocellular carcinoma and colorectal carcinoma cell lines.

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مشتقات الترياز ولوفثالازين المتصلة ببيبرازين: التشييد و دراسة النشاط المضاد للسرطان

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الملخص :

يُعدُّ السَّرطان أحدُ الأسبابِ الرئيسيَّةُ للوفاةِ في جميع أنحاء العالم خلال العقد الماضي. في محاولة لتطوير عامل مضاد للسرطان، تم تشييد سلسلة جديدة من مشتقات الترياز ولوفثالازين المتصلة ببيبرازين وتقييم نشاطها المضاد للسرطان ضد أربعة أنواع لخلايا السرطان البشرية وهي: سرطان خلايا الكبد وسرطان الثدي وسرطان البروستاتا وسرطان القولون والمستقيم. أظهرت نتائج تقييم السمِّية الخلوية أن معظم المركبات أنتجت أنشطة سامة للخلايا متوسطة ضد أنواع الخلايا السرطانية المُختارة. أظهر المركب **٢٣** أعلى تأثير مثبِّط للخلايا السرطانية يليه المركب **٢٤** ضد كل من سرطان الكبد و سرطان القولون والمستقيم.

الكلمات المفتاحية: المضاد للسرطان، سرطان خلايا الكبد ،سرطان الثدي ،سرطان البروستاتا ،سرطان القولون والمستقيم