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Synthesis of phthalazine-based derivatives as selective anti-breast cancer agents through EGFR-mediated apoptosis: in vitro and in silico studies

Sara M. Emam¹, Samir M. El Rayes^{1*}, Ibrahim A. I. Ali¹, Hamdy A. Soliman¹ and Mohamed S. Nafie^{1*}

Abstract

The parent 2-(4-benzyl-1-oxophthalazin-2(1*H*)-yl)-acetohydrazide (**4**) has twenty-nine compounds. The starting material for their corresponding mono, dipeptides and reactions with active methylene compounds were produced by chemoselective *N*-alkylation of 4-Benzyl-2*H*-phthalazin-1-one (**2**) with ethyl chloroacetate to afford (4-benzyl-1-oxo-1*H*-phthalazin-2-yl) methyl acetate (**3**). The ester **3** was hydrazinolyzed to give hydrazide **4**, then azide **5** coupled with amino acid ester hydrochloride and/or amines to produce several mono-peptides, then the methyl (2-(4-benzyl-1-oxophthalazin-2(1*H*)-yl) acetyl) glycinate (**7a**) was hydrazinolyzed to produce corresponding hydrazide 2-(4-benzyl-1-oxophthalazin-2(1*H*)-yl)-*N*-(2-hydrazineyl-2-oxo ethyl) acetamide (**8a**). The hydrazide **8a** under azide coupling method was coupled with amino acid ester hydrochloride and/or amines to produce several dipeptides, and the hydrazide **8a** was also condensed and/or cyclized with several carbonyl compounds. The cytotoxicity of the synthesized compounds was tested using MTT assay, as well as apoptosis-induction through EGFR inhibition. Compounds **11d**, **12c** and **12d** exhibited potent cytotoxic activities with IC₅₀ values of 0.92, 1.89 and 0.57 μM against MDA-MB-231 cells compared to Erlotinib (IC₅₀ = 1.02 μM). Interestingly compound **12d** exhibited promising potent EGFR inhibition with an IC₅₀ value 21.4 nM compared to Erlotinib (IC₅₀ = 80 nM). For apoptosis, compound **12d** induced apoptosis in MDA-MB-231 cells by 64.4-fold (42.5% compared to 0.66 for the control); hence, this compound may serve as a potential target-oriented anti-breast cancer agent. These results agreed with the molecular docking studies that highlighted the binding disposition of compound **12d** towards EGFR protein. Hence, compound **12d** may serve as a potential and selective anti-breast cancer agent.

Keywords Phthalazine-based, Selective anti-breast, EGFR-mediated, Apoptosis

Introduction

Cancer has been considered one of the major issues of concern, most especially for the public health system globally, which has been a leading cause of death worldwide in the last decade [1]. It is an abnormal development of cells that promulgates through the splitting of unrestricted cells, which shifts the controlled mechanisms of cell proliferation and differentiation associated with a high mortality rate [2]. Epidemiological studies revealed that cancer accounts for one of every five deaths, and it is

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estimated that the annual number of deaths due to cancers will increase from 7.6 million in 2008 to 13 million in 2030 [3]. Chemotherapy is one of the most effective approaches used to treat solid as well as hematological tumors [4, 5]. Cancer chemotherapy has been developed for molecular therapeutics, which are more selective and not associated with the toxicity of conventional cytotoxic drugs [6].

After over half a century of chemotherapy research and despite the advancement in the knowledge of biochemical processes associated with carcinogenesis, the successful treatment of cancer remains a significant challenge because of the general toxicity associated with the clinical use of traditional cancer chemotherapeutic agents and because of some factors that include limitations of animal models, tumor diversity, drug resistance and the side effects assigned for therapy [7]. Therefore, anticancer drug research is never ending with obtaining lower toxicity and more selectivity products for tumor cells. There is an urgent need to give much attention to researchers in pharmaceuticals, medicine, and medicinal chemistry to design and modify the drug to fulfill more potent and effective therapies.

Epidermal growth factor receptor (EGFR) is a type of membrane-bound tyrosine kinase receptor which addicted to the treatment of cancer [8]. EGFR plays a vital role in numerous processes that affect tumor growth and progression, including proliferation, differentiation, angiogenesis, inhibition of apoptosis, and invasiveness [9]. The expression of a specific receptor tyrosine kinase on the cell surface increases the incidence of receptor dimerization. It leads to uncontrolled cell proliferation and tumor formation, which has been shown for EGFR to occur in breast, colon, ovarian, and pancreatic cancer cells [10]. Currently, large numbers of epidermal growth factor receptor inhibitors are approved, including gefitinib, erlotinib, lapatinib, vandetanib, etc. Amin [11] has reported a series of phthalazine derivatives as epidermal growth factor receptors.

Some phthalazine derivatives have significant applications in clinical medicine due to their pronounced activities as antitumor agents [12–15]. Hydrazides constitute an important class of compounds for new drug development as they contain H-bond donor/acceptors that can form H-bonds with their recipients inside the target-protein active sites [16]. Previous literature [17–20] showed that phthalazine-based hydrazide derivatives represented a promising scaffold for kinase-targeted anticancer agents, e.g. EGFR.

In Fig. 1, the phthalazine derivative azelastine (I) is an antihistamine used in the treatment of allergic rhinitis [21]. Potent agents are more selective inhibitors of the cGMP-inhibited phosphodiesterase (PDE) and can be

represented by phthalazine derivatives like MY5445 (II) [22–25]. Zopolrestat (III) is a phthalazinone derivative that has been in clinical trials; it inhibits aldose reductase and has potential use in the prevention of retinopathy, neuropathy, and cataract formation in diabetes [26]. The chemiluminescence reactions of luminol (IV) and related phthalazines have found analytical applications, particularly in biological systems where the inherent signal strength and low signal–noise ratio contribute to sensitivity [27, 28].

Potent antitumor activity was added by many phthalazine-based compounds such as the anilino phthalazines, Vatalanib PTK787 (V) [12, 13, 29]. Vatalanib (V) inhibits VEGFR-2 with IC_{50} value = 20 nM [30], and it is well absorbed orally and shows an in vivo antitumor activity against a panel of human tumor xenograft models; however, Vatalanib (V) is currently in phase III clinical trials for metastatic of colorectal cancer [31, 32]. In addition, some anilino phthalazines have been reported as potent inhibitors of VEGFR-2, such as AAC789 (VI) and IM-023911 (VII) with IC_{50} = 20 and 48 nM, respectively [33–38], as shown in Fig. 2.

Hence, the EGFR & VEGFR-2 inhibitory signaling pathway has become a crucial strategy for the identification and development of novel therapeutics for a variety of human malignancies for the treatment of cancer trauma [39]. So, we herein report the synthesis of new series of phthalazine derivatives aiming to obtain potent EGFR inhibitors with good anticancer activity.

Results and discussion

Chemistry

Most recently Samir El-Rayes et al. [40–44] reported early that, how can control on chemoselective alkylation in both amides and thioamides. As extension of these studies, we decided to apply these findings to structure modification of 4-benzyl-2*H*-phthalazin-1-one (2) as our model heterocyclic amide. The alkylation reaction of the model ambident nucleophile 2 with ethyl chloroacetate in Acetone/DMF mixture solution (1:1) in the presence of anhydrous K_2CO_3 under reflux condition for 20 h afforded (4-benzyl-1-oxo-1*H*-phthalazin-2-yl) methyl acetate (3) as a single *N*-substituted product.

The alkylation reaction proceeds depending on the behavior towards electrophiles according to reaction control points such as basicity and nucleophilicity of both N and O atoms. This reaction occurs selectively on N atom rather than on O atom or even in competition reaction at both atoms. The obtained chemoselective *N*-alkylation reaction may be well explained by counting on the interaction between HOMO at the nitrogen atom of the ambident nucleophile with high energy and the LUMO of the electrophile with low energy, leading to a narrow energy

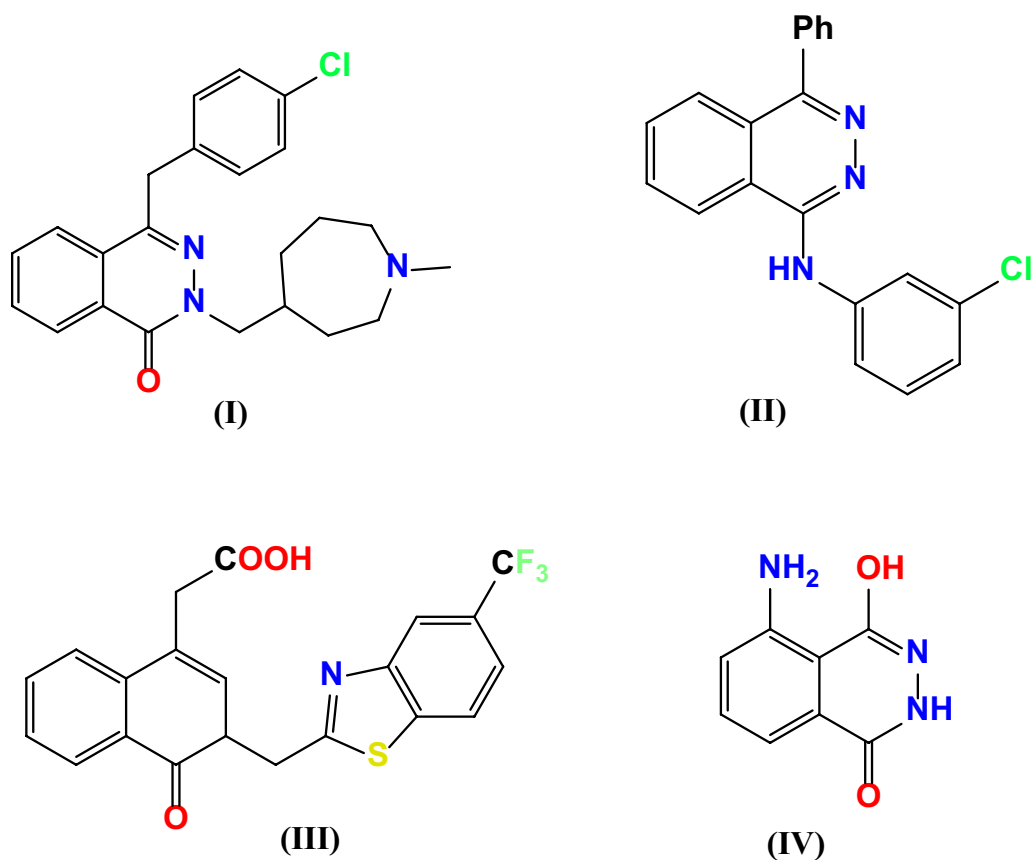


Fig. 1 Some phthalazine-based derivatives

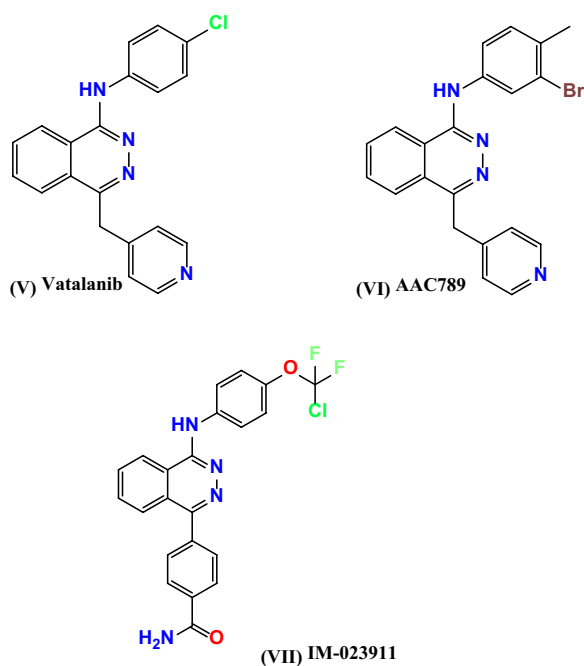


Fig. 2 Phthalazine-based antitumor agents

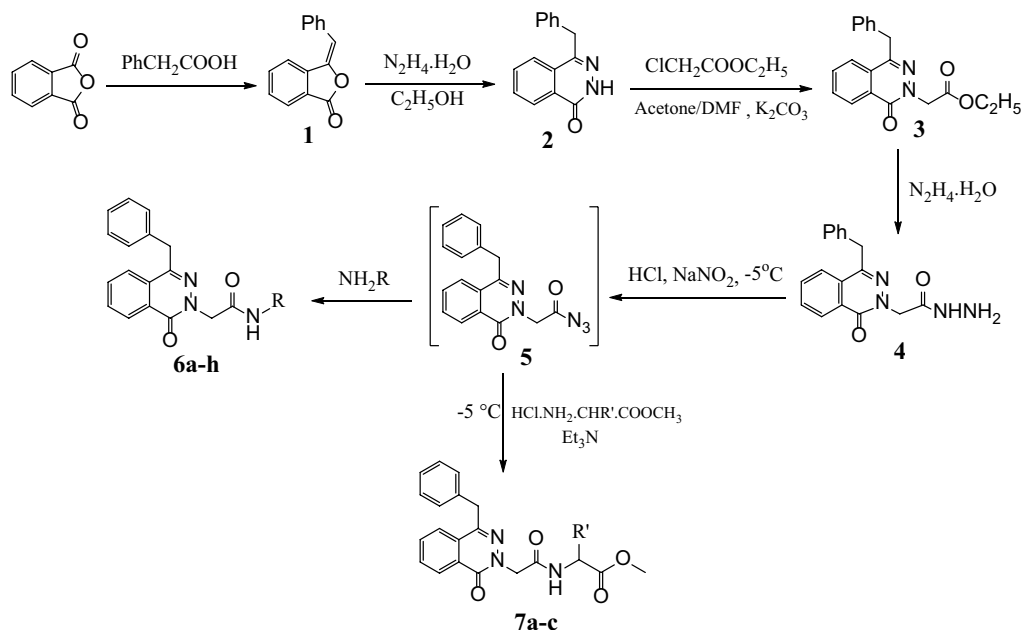
gap and high reactivity to finally give *N*-alkylation. This result was deduced on the premise of Pearson's hard soft-acid base principle.

Hydrazinolysis of ester **3** in ethanol via reaction with hydrazine hydrate under reflux for 6 h to produce the 2-(4-benzyl-1-oxophthalazin-2(1*H*)-yl)-acetohydrazide (**4**) in 90% yield which used as a precursor for the preparation of novel phthalazinone derivatives with potent importance in biological activity (Scheme 1).

The chemoselectivity alkylation occurs on the N-atom not the O isomer which prove that the N atom in present system is stronger nucleophile more than Oxygen, so this reaction is N-regioselective and this can be dedicated by the structure identification using ^1H and ^{13}C -NMR spectroscopy.

The characteristic ^1H -NMR spectral peaks for the hydrazide **4** gave signals at δ 3.60 for NH_2 , 4.32 for CH_2ph , 4.82 for NHCH_2 , 9.02 for NH and (7.18–8.43) for nine aromatic protons [45].

The hydrazide **4** is a superb forerunner for the structural adjustment of phthalazine subordinates by a connection of another amino acid through a peptide bond via azide coupling strategy, which is a well-known strategy in



Scheme 1 Preparation of 2-(4-benzyl-1-oxophthalazin-2(1*H*)-yl)-*N*-substituted derivatives **6a–h** and **7a–c**

6a–h	R	6a–h	R	7a–c	R'
a		b	CH ₃ -CH ₂ -CH ₂ ⁻	a	H
c	CH ₃ -CH ₂ -CH ₂ -CH ₂ ⁻	d		b	-CH ₂ CH ₂ -S-CH ₃
e	CH ₃ -(CH ₂) ₁₃ ⁻	f		c	CH ₃ ⁻ CH-CH ₃
g		h			

peptide synthesis having the advantage of diminishing the degree of racemization beside absence of any interferometer side products.

The azide **5** was prepared from the reaction between hydrazide **4** with NaNO₂/HCl mixture in water for 1 h at - 5 °C that was extracted with ethyl acetate. The produced azide was progressively added to amines to give amide derivatives **6a–h** (Scheme 1).

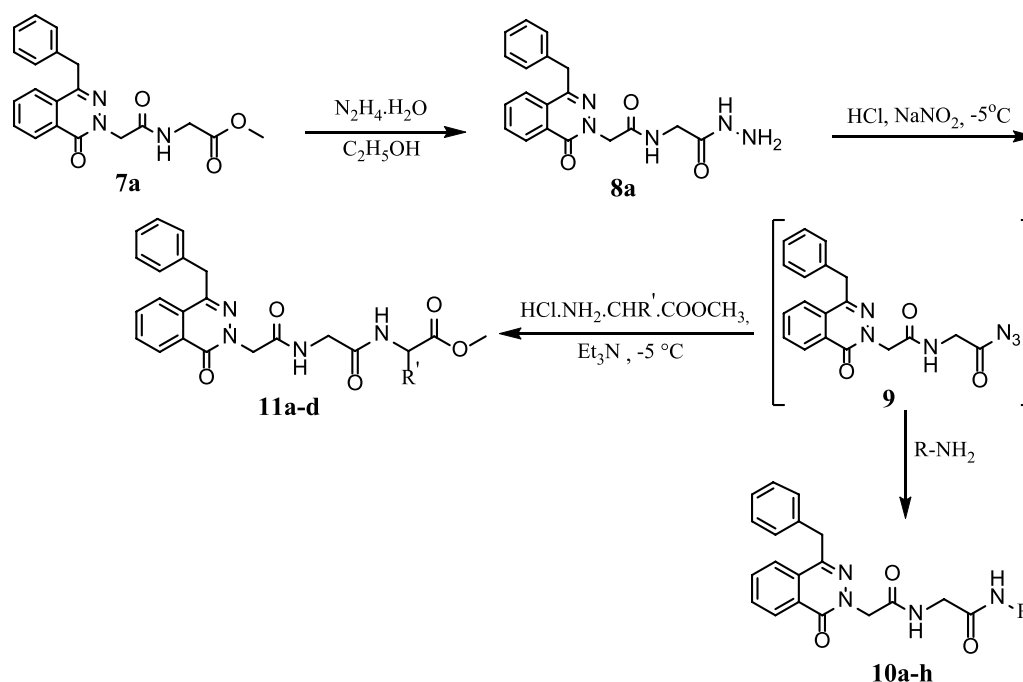
The chemical structure of the synthesized 2-(4-benzyl-1-oxophthalazin-2(1*H*)-yl)-*N*-propyl acetamide (**6b**) was elucidated via different analysis methods for example the ¹H-NMR that give characteristic protons at δ (7.15–8.38) nine protons of Ar–H, 6.16 broad signal for NH, 4.83 singlet peak for CH₂CO, 4.23 singlet peak for CH₂ph, 3.14–3.19 quartet peak of CH₂NH, sextet and triplet peaks at 1.41–1.46 & 0.79–0.83 for CH₂ and CH₃ of propyl molecule respectively and the ¹³C-NMR spectrum has signals at 167.46 and 159.73 for two C=O groups and peaks at 55.33, 41.30, 38.83, 22.70 and 11.17 ppm for CH₂CO,

CH₂NH, CH₂ph, CH₂, CH₃ groups respectively by the addition to 13 aromatic carbons at (125.38–146.32) ppm.

The reaction of amino acid methyl ester hydrochloride such as glycine, methionine, and valine in the presence of triethyl amine at - 5 °C for 1 h to give the methyl-3-[2-(1,4-dioxo-3-phenyl-3,4-dihydro-1*H*-phthalazine-2-yl)-acetylamino]alkanoate **7a–c** (Scheme 1).

The glycine methyl ester of 4-benzyl-1(2*H*)-phthalazinone **7a** has the ¹H-NMR spectrum of characteristic signals at δ 6.71 broad signal for NH molecule, 3.64 singlet peak of OCH₃, 4.00–4.01 doublet peak of CH₂NH, 4.23 and 4.90 ppm singlet peaks for CH₂ph & CH₂CO respectively and the ¹³C-NMR spectrum has signals at 169.99, 167.72 and 159.78 for three C=O groups, 54.80, 52.27, 41.29 and 38.91 ppm for CH₂CO, OCH₃, CH₂NH and CH₂ph groups respectively.

The ester **7a** was considered a key intermediate for chemical structure modification of phthalazinone nucleus. The ester **7a** underwent hydrazinolysis via reflux



10a-h	R	10a-h	R	11a-d	R'
a		b	CH ₃ -CH ₂ -CH ₂ ⁻	a	H
c	CH ₃ -CH ₂ -CH ₂ -CH ₂ ⁻	d		b	CH ₂
e	CH ₃ -(CH ₂) ₁₃ ⁻	f		c	-CH ₂ CH ₂ -S-CH ₃
g		h		d	CH ₃ ⁻ CH-CH ₃
				(Gly)	
				(β-ala)	
				(Met)	
				(Val)	

Scheme 2 Synthesis of (4-benzyl-1-oxophthalazin-2(1H)-yl)-N-(2-oxo-(alkyl amino) ethyl) acetamides **10a-h** & alkanooates **11a-d**

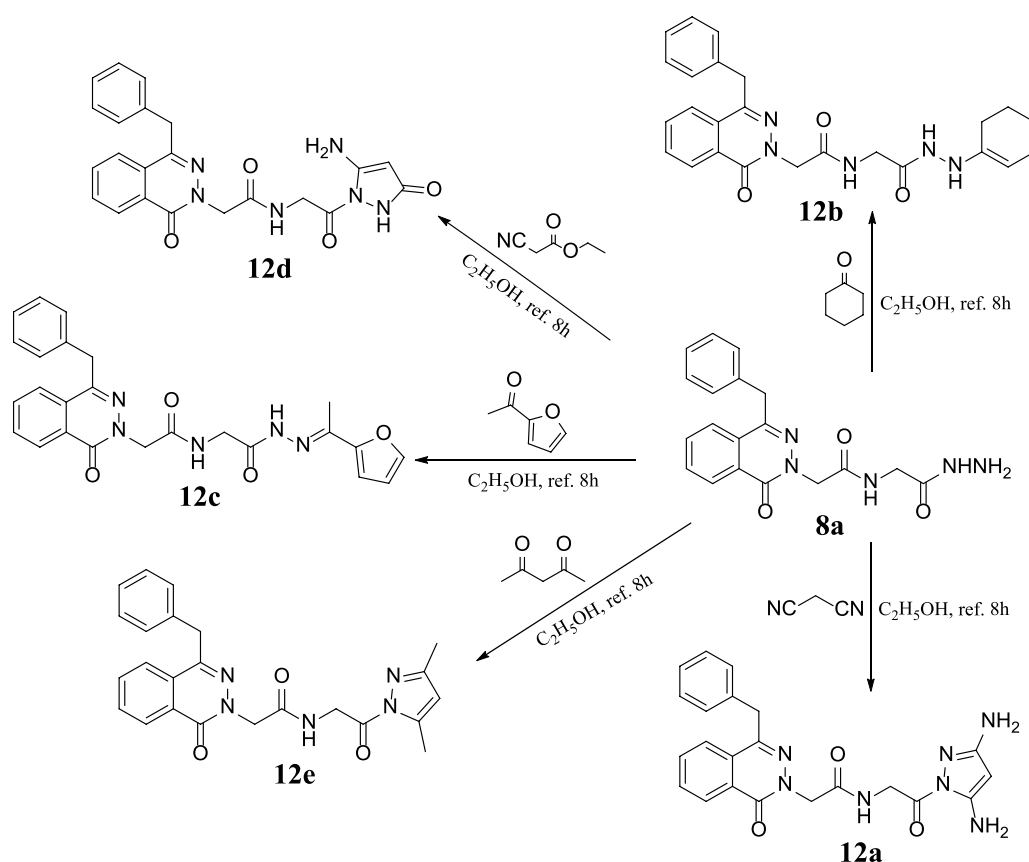
with hydrazine hydrate in ethanol to produce the corresponding hydrazide **8a** as in Scheme 2.

The structure of starting hydrazide 2-(4-benzyl-1-oxophthalazin-2(1H)-yl)-N-(2-hydrazineyl-2-oxo ethyl)acetamide (**8a**) was elucidated by various analysis like ¹H-NMR which give characteristic peaks for protons at δ 9.02 & 7.91–7.93 broad signals for 2 NH, 4.87 singlet peak for CH₂CO, 4.32 singlet peak for CH₂ph, 4.23 doublet peak of CH₂NH and doublet peak at 3.74–3.75 for NH₂ and the ¹³C-NMR spectrum has signals at 168.38, 167.73, 159.08 for three C=O groups and peaks at 53.98, 41.43, 38.13 ppm for CH₂CO, CH₂NH and CH₂ph respectively.

Under azide coupling condition, 2-(4-benzyl-1-oxophthalazin-2(1H)-yl)-N-(2-hydrazineyl-2-oxoethyl)acetamide (**8a**) was treated with a mixture of sodium nitrite and HCl solution in water to give its corresponding azide solution which further reacted with different amines like

benzyl, n-propyl, n-butyl, cyclohexyl, tetra decyl, allyl, piperidine and morpholine amines to obtain N-substituted-2-(4-benzyl-1-oxophthalazin-2(1H)-yl)-2-oxoethyl acetamides **10a-h**, as in Scheme 2.

The chemical structure of 2-(4-benzyl-1-oxophthalazin-2(1H)-yl)-N-(2-(butyl amino)-2-oxoethyl) acetamide (**10c**) was interpreted by ¹H-NMR analysis including two broad singlet peaks at δ 6.76 and 6.50 for NH, 4.94 singlet peak of NCH₂CO, 4.34 singlet peak of CH₂ph, 3.99 doublet peak of NHCH₂CO, 3.24–3.29 quartet peak for NHCH₂CH₂, 1.48–1.54 quintet peak for CH₂CH₂CH₂, 1.32–1.37 sextet peak of CH₂CH₂CH₃ and 0.90–0.93 triplet peak of CH₃ and the ¹³C-NMR spectrum has signals at 168.34, 167.85 and 159.94 for three C=O groups, 55.80, 43.43, 39.41, 38.92, 31.41, 20.01 and 13.67 ppm for NCH₂CO, NHCH₂CO, NHCH₂CH₂, CH₂ph, CH₂CH₂CH₂, CH₂CH₂CH₃ and CH₃ groups respectively.



Scheme 3 Synthesis of some derivatives of phthalazinone **12a-e**

The azide was coupled with different amino acid methyl esters such as glycine, β -alanine, methionine and valine in the presence of triethyl amine affording dipeptides methyl-[2-(4-benzyl-1-oxo-1*H*-phthalazin-2-yl)-acetyl-amino]alkanoates **11a-d** in reasonable yield (Scheme 2).

The structure of methyl 3-(2-(2-(4-benzyl-1-oxophthalazin-2(1*H*)-yl) acetamido acetamido) propanoate (**11b**) was interpreted by various analysis method including ¹H-NMR analysis that noticed characteristic peaks: two broad singlet peaks at δ 6.88 for NH, 4.95 singlet peak of NCH₂CO, 4.34 singlet peak of CH₂ph, 3.97–3.98 doublet peak of NHCH₂CO, 3.67 singlet peak of OCH₃, 3.54–3.55 quartet peak for NHCH₂CH₂CO and 2.56–2.59 triplet peak for NHCH₂CH₂CO and the ¹³C-NMR spectrum has peaks at 172.50, 168.57, 167.88 and 159.93 of four C=O, 55.59, 51.73, 43.25, 38.91, 35.18 and 33.71 ppm for NCH₂CO, OCH₃, NHCH₂CO, CH₂ph, NHCH₂CH₂CO and NHCH₂CH₂CO respectively.

Condensation of the hydrazide 2-(4-benzyl-1-oxophthalazin-2(1*H*)-yl)-*N*-(2-hydrazineyl-2-oxoethyl)acetamide (**8a**) with active methylene compounds such as malononitrile, ethyl cyano acetate and acetyl acetone

in ethanol under reflux to obtain novel derivatives of phthalazinone **12a**, **12d** and **12e** respectively in reasonable yield. Similarly, reaction of hydrazide **8a** with ketones such as cyclohexanone and 2-furyl methyl ketone gave the corresponding hydrazones **12b** and **12c** respectively as shown in Scheme 3.

The chemical structure of 2-(4-benzyl-1-oxophthalazin-2(1*H*)-yl)-*N*-(2-(3,5-diamino-1*H*-pyrazol-1-yl)-2-oxoethyl)acetamide (**12a**) was elucidated using various analysis methods for example ¹H-NMR which gave signals at δ 9.02 broad signal for NHCH₂CO, 7.10–7.12 singlet peak for the proton of pyrazole ring NH₂-C=CH-C-NH₂, 5.46–5.47 doublet peak for NHCH₂CO, 4.86 singlet peak of NCH₂CO, 4.26 singlet peak of CH₂ph and 4.32 & 3.52 two broad singlet peaks for 2 NH₂ molecules on pyrazole ring and the ¹³C-NMR spectrum has peaks at 171.42, 168.34 and 159.21 of three C=O, 148.14 & 148.36 peaks of carbons of pyrazole ring 2 C-NH₂, 110.35 characteristic peak for carbon atom of NH₂-C=CH-C-NH₂, 55.28, 42.82 and 38.64 ppm for NCH₂CO, NHCH₂CO and CH₂ph respectively.

Table 1 Cytotoxicity of the synthesized derivatives against MCF-7, MDA-MB-231 and MCF-10A cells

Compound	IC ₅₀ (μM) ± SD*		
	MCF-7	MDA-MB-231	MCF-10A
6f	23.1 ± 0.94	≥ 50	NT
6a	18.6 ± 0.69	19.8 ± 0.74	NT
6h	17.3 ± 0.31	21.3 ± 0.64	NT
7a	≥ 50	26.5 ± 0.79	NT
7c	12.4 ± 0.34	≥ 50	NT
8a	31.2 ± 1.01	1.34 ± 0.13	NT
10a	≥ 50	6.4 ± 0.18	NT
10f	15.4 ± 0.28	8.6 ± 0.1	NT
10h	16.4 ± 0.31	2.3 ± 0.1	NT
11a	11.3 ± 0.29	7.2 ± 0.3	NT
11d	2.1 ± 0.01	0.92 ± 0.01	39.4 ± 1.8
12c	1.4 ± 0.05	1.89 ± 0.04	43.6 ± 1.9
12d	1.9 ± 0.01	0.57 ± 0.09	41.6 ± 1.8
Erlotinib	1.32 ± 0.04	1.02 ± 0.1	30.9 ± 1.8

*IC₅₀ values were calculated as the average of three independent trials using a dose–response curve in GraphPad prism. NT not tested

Biological investigation

Cytotoxicity against breast cancer cells

The synthesized compounds were investigated for their cytotoxicity against breast MCF-7 and MDA-MB-231 cancer cell lines; IC₅₀ values were summarized in Table 1. As seen in the results, interestingly, compounds **11d**, **12c**, and **12d** exhibited potent cytotoxic activities against MCF-7 cells with IC₅₀ values of 2.1, 1.4, and 1.9 μM, and against MDA-MB-231 cells with potent IC₅₀ values of 0.92, 1.89 and 0.57 μM, respectively, compared to erlotinib as the reference drug with IC₅₀ values of 1.32 and 1.0 μM. As seen in Fig. 3, compound **12d** caused cell MDA-MB-231 cell growth inhibition by 98.2% at the highest concentration. Additionally, compounds **11d**, **12c**, and **12d** exhibited safe cytotoxicity against normal breast cells MCF-10A, having a percentage of cell viability of 11%, 9.6%, and 3%, respectively, at the highest concentration with IC₅₀ values of 39.4, 43.6, and 41.6 μM. Based on these results, compound **12d** was worthy of further testing against EGFR enzymatic targets and the mechanism of cell death in MDA-MB-231 cells.

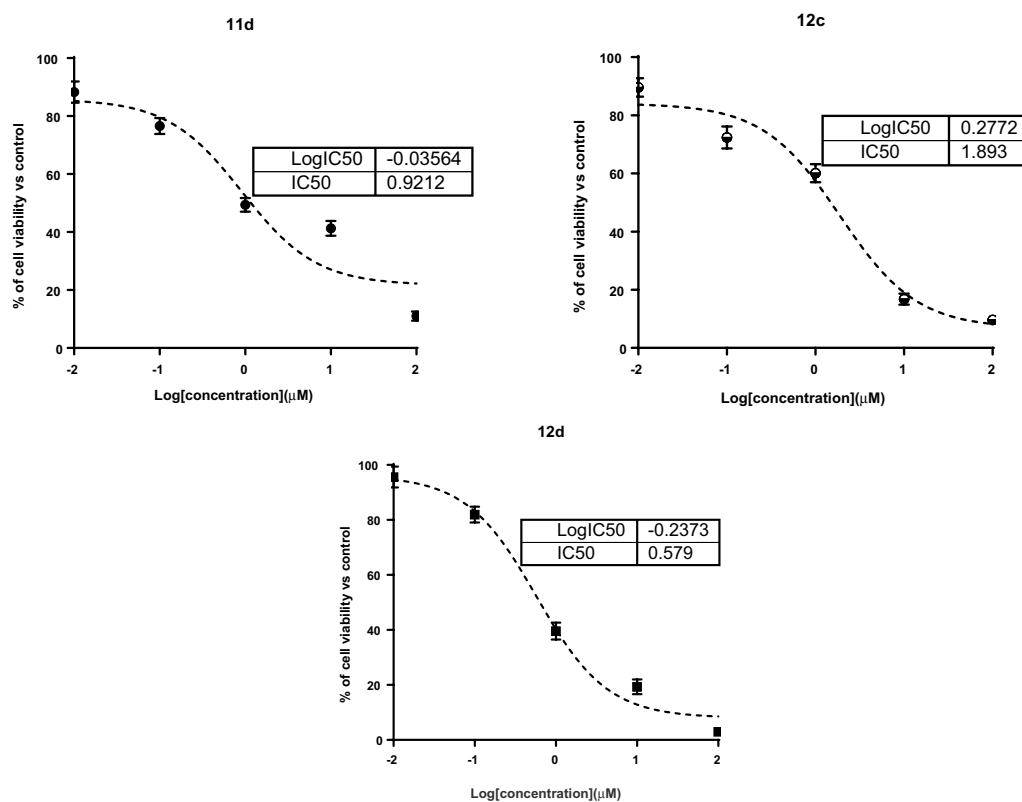


Fig. 3 Percentage of cell growth inhibition versus concentrations of compounds **11d**, **12c** and **12d** against cancer MCF-7 and MDA-MB-231 cells using MTT assay using serial concentration range of 100 μM to 0.01 μM at incubation time of 48 h. Values are expressed as Mean ± SD of three independent values

Structure–activity relationship (SAR)

Based on the cytotoxicity results of the investigated compounds as summarized previously in Table 1, compounds **12d** and **12c** were the first order activity of potent cytotoxicity ($IC_{50} \leq 2.5 \mu\text{M}$), and compounds **11a**, **7c**, **10f**, and **10h** with second order activity of moderate cytotoxicity ($IC_{50} \leq 20 \mu\text{M}$), while compounds **6h**, **6a** and **6f** with poor cytotoxicity ($IC_{50} \geq 20 \mu\text{M}$). As summarized in Fig. 4, highlighted substituents caused variance in activity. Hence, compound **12d** was worthy to be further investigated for the effective target and cell death mechanism.

EGFR enzyme inhibition

Three compounds with potent cytotoxicity **11d**, **12c**, and **12d** were tested for their inhibition against VEGFR2; interestingly, as seen in Table 2, compound **12d** exhibited promising EGFR enzyme inhibition with IC_{50} values of 21.4 nM with 97.6% inhibition compared to erlotinib with standard EGFR inhibition with IC_{50} value of 80 nM (inhibition 93.9%). Additionally, compounds **11d** and **12c** exhibited promising EGFR inhibitory activities with

Table 2 EGFR enzyme activity of compounds **11d**, **12c** and **12d**

Compound	EGFR	
	% of inhibition at [10 μM]	IC_{50} [nM] \pm SD*
11d	92.9 \pm 2.08	79.6 \pm 1.35
12c	96.2 \pm 2.04	65.4 \pm 1.12
12d	97.6 \pm 2.49	21.4 \pm 0.67
Erlotinib	93.9 \pm 2.68	80.1 \pm 1.21

*Values are expressed as an average of three independent replicates. IC_{50} values were calculated using sigmoidal non-linear regression curve fit of percentage inhibition against five concentrations of each compound"

IC_{50} values 79.6 and 65.4 nM, with enzyme inhibition by 92.9% and 96.2%, respectively.

Apoptosis-induction activity

Deregulation of apoptosis is a hallmark of all cancer cells, and the agents that activate apoptosis in cancer cells could be valuable anticancer therapeutics; breast cancer cell lines that hyper express the EGFR have been documented to undergo receptor-mediated apoptosis.

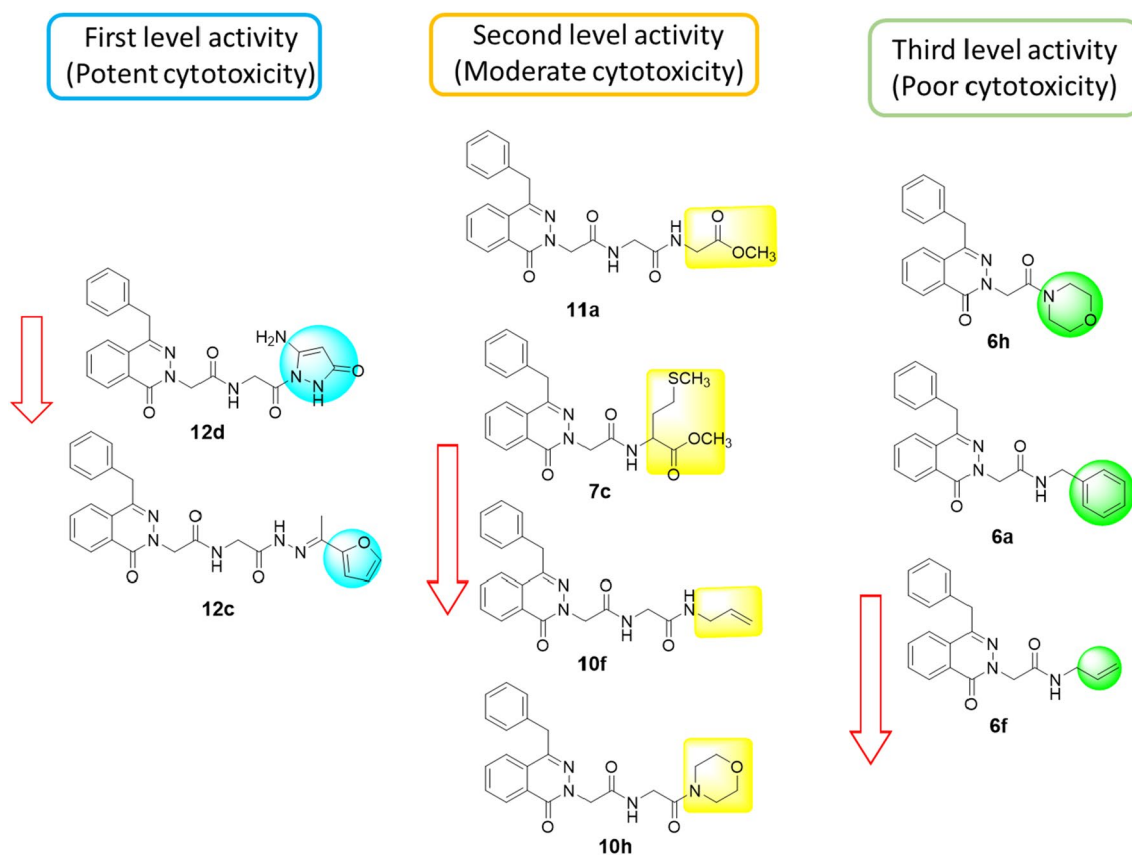


Fig. 4 Highlighted substituents anchored on the pharmacophore with promising cytotoxic activities for investigated compounds

MDA-MB-231 cancer cells were treated with compound **12d** ($IC_{50}=0.57 \mu\text{M}$, 48 h) and were investigated for their apoptosis-inducing activity using Annexin V/PI staining. As seen in Fig. 5, compound **12d** significantly stimulated total apoptotic breast cancer cell death by 64.4-fold (42.5% compared to 0.66% for the control). It induced early apoptosis by 24.2% and late apoptosis by 18.3% compared to 0.66% and 0.15%, respectively, for the control. Moreover, it stimulated cell death by necrosis by 9.25-fold (6.2%, compared to 0.67% for the control).

These results of apoptosis-induction of phthalazine-based derivatives agreed with previous studies that

exhibited promising cytotoxic activities as apoptotic agents through EGFR inhibition.

Molecular docking studies

Based on the promising EGFR inhibition activity of compound **12d**, it was screened for virtual binding towards EGFR protein using the molecular docking approach. As shown in Fig. 6, compound **12d** was docked inside EGFR protein with a binding energy of -18.4 kcal/mol and formed one H-bond with Met 769, one H-bond with Lys 721, besides it formed the lipophilic interactions through phenyl groups with the lipophilic amino

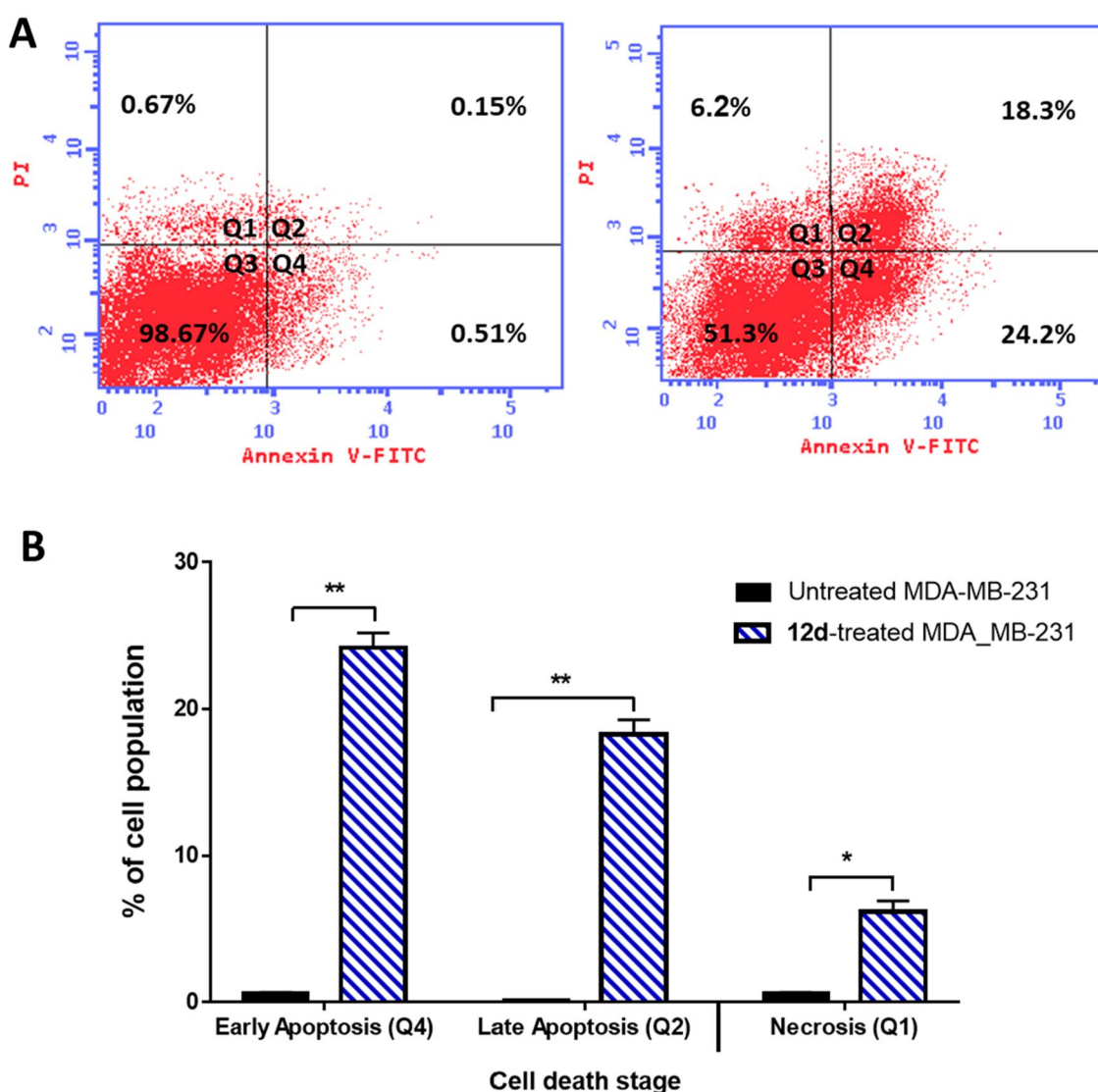


Fig. 5 Flow cytometry analysis for apoptosis/necrosis assessment in the untreated and **12d**-treated MDA-MB-231 cells with the IC_{50} value of $0.57 \mu\text{M}$ for 48 h **A** Cytogram for Annexin V/PI staining. Quadrant charts show Q1 (necrotic cells, AV-/PI+), Q2 (late apoptotic cells, AV+/PI+), Q3 (normal cells, AV-/PI-), Q4 (early apoptotic cells, AV+/PI-). **B** Bar representation with cell percentage at each stage. Values are expressed as Mean \pm SD of three independent trials (**($P \leq 0.05$), and ***($P \leq 0.001$) are significantly different using the un-paired test in GraphPad prism"

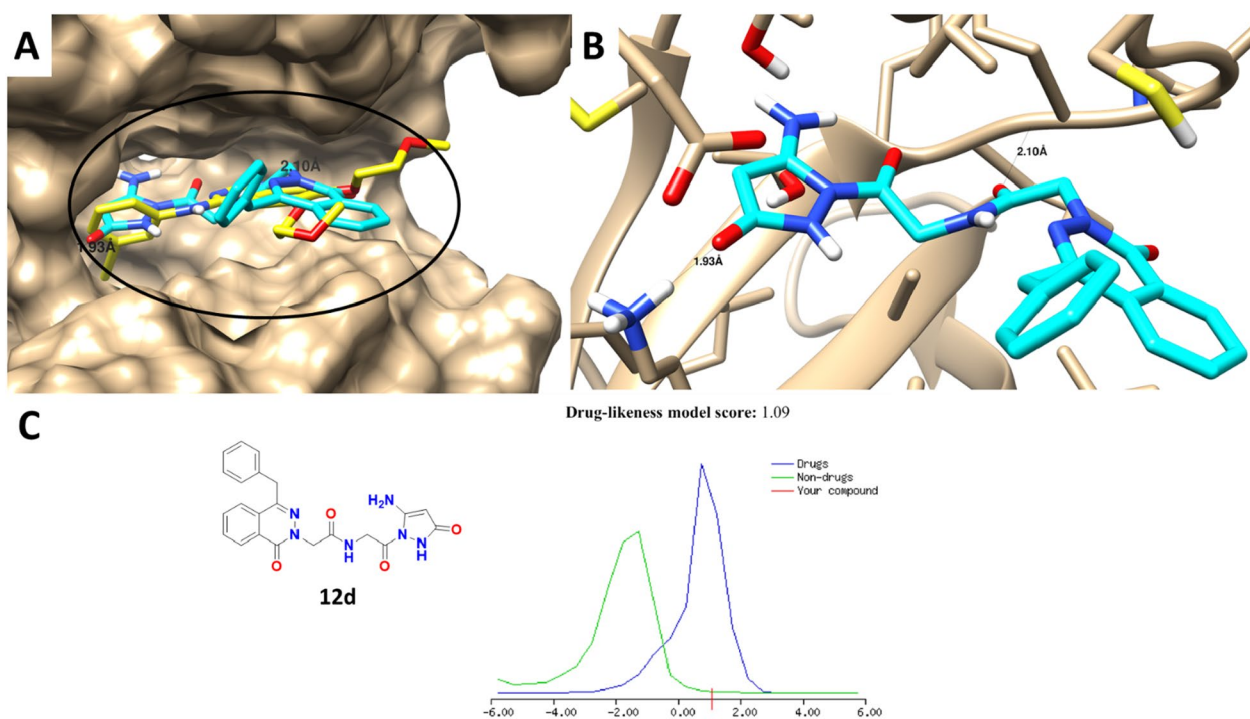


Fig. 6 Binding disposition and molecular docking interactions of the docked compound **12d** (Cyan-colored) and the co-crystallized ligand (Yellow-colored). **A** Surface view and **B** Interactive view with ribbon presentation. **C** Drug-likeness properties of compound **12d** using MolSoft “The green color means non-drug like behavior and those fall under blue color area are considered as drug-like

acids of Ala 719 and Leu 694. Hence, docking results indicated highlighted the virtual mechanism of binding of compound **12d** through the phthalazine moiety for interactions toward EGFR protein, which agreed with its promising experimental activity. Physicochemical properties and ADME pharmacokinetics revealed the drug-likeness score of 1.09, which obeys the Lipinski’s rule of five, having molecular weight = 432 g/mol, topological polar surface area (TPSA) = 144.8 Å², log (P) = 1.56, H-bond donor = 4, and H-bond acceptor = 5.

Experimental

Chemistry

General procedures

The purity of the synthesized compounds was checked by thin layer chromatography (TLC) technique was carried out on silica gel 60 F₂₅₄ aluminum sheets (E. Merck, layer thickness 0.2 mm) in the following solvent system ethyl acetate/ petroleum ether (1:1) and ethyl acetate/ petroleum ether (2:1), the spots on thin layer plates were detected by UV lamp. The melting points were determined using a Buchi 510 melting-point system and are uncorrected. At the Micro Analytical Laboratory, Faculty of Science, Cairo University, Cairo, Egypt, element analyses were performed on a Flash EA-1112 apparatus. The nuclear magnetic resonance laboratory, Faculty of

Science, Sohag University, Egypt, used a Bruker spectrometer running at 400 MHz to estimate ¹H-NMR spectra.

The precursor (4-benzyl-1-oxo-1*H*-phthalazin-2-yl) methyl acetate (**3**) was prepared from 4-Benzyl-2*H*-phthalazin-1-one (**2**) according to the method described in Marzouk et al. [45] that was converted to the hydrazide molecule 2-(4-benzyl-1-oxophthalazin-2(1*H*)-yl)-acetohydrazide (**4**) [13].

*General procedure for preparation of 2-(4-benzyl-1-oxophthalazin-2(1*H*)-yl)-*N*-alkyl acetamide 6a–h* A cold solution at (− 5 °C) of acetohydrazide **4** (3.08 g, 10 mmol) in acetic acid (60 mL) and hydrochloric acid (5*N*, 30 mL) was added portion wise under stirring to a cold solution (0 °C) of sodium nitrite (0.7 g, 0.01 mol) in water (30 mL). After stirring at the same temperature for 30 min, the in situ generated azide was extracted with cold ethyl acetate and washed successively with cold water and 5% Na₂CO₃.

After drying over anhydrous sodium sulphate, the azide was used without further purification in the next step. Amines (12 mmol) were added to the previously prepared cold dried solution of the azide. Afterwards, the mixture was kept 12 h in the refrigerator and then at room temperature for another 12 h. The reaction mixture

was filtered and the filtrated solution washed with 0.1N HCl, 5% Na₂CO₃ and water then dried over anhydrous sodium sulphate, the solvent was evaporated in vacuum and the residue was crystallized from ethyl acetate-petroleum ether to give products **6a–h**.

Synthesis of 2-(4-benzyl-1-oxophthalazin-2(1H)-yl)-N-benzyl acetamide (6a) White crystals (84%), m.p. 174–176 °C, ¹H-NMR (400 MHz, CDCl₃), (δ, ppm), (J, Hz): 8.42–8.44 (m, 1H, ArH); 7.70–7.76 (m, 3H, ArH); 7.27–7.30 (m, 9H, ArH); 7.22–7.25 (m, 1H, ArH); 6.62 (brs, 1H, NH); 4.99 (s, 2H, CH₂CO); 4.50–4.51 (d, J=5.6, 2H, CH₂NH); 4.32 (s, 2H, CH₂ph). ¹³C-NMR: 167.47 (C=O); 159.75 (C=O); 146.39 (C-Ar); 138.02 (C-Ar); 137.51 (C-Ar); 133.22 (CH-Ar); 131.45 (CH-Ar); 129.45 (C-Ar); 128.74 (2 CH-Ar); 128.61 (2 CH-Ar); 128.39 (2 CH-Ar); 128.08 (C-Ar); 127.57 (2 CH-Ar); 127.37 (2 CH-Ar); 126.78 (CH-Ar); 125.39 (CH-Ar); 55.16 (CH₂CO); 43.56 (CH₂NH); 38.88 (CH₂ph).

MS (MALDI, positive mode, matrix DHB) m/z: 406.48 (M+Na)⁺. Elemental analysis: calculated for C₂₄H₂₁N₃O₂ (383.45): % C, 75.18; % H, 5.52; % N, 10.96. Found: % C, 75.20; % H, 5.53; % N, 10.92.

Synthesis of 2-(4-benzyl-1-oxophthalazin-2(1H)-yl)-N-propyl acetamide (6b) White crystals (82%), m.p. 162–164 °C, ¹H-NMR (400 MHz, CDCl₃), (δ, ppm), (J, Hz): 8.36–8.38 (m, 1H, ArH); 7.63–7.68 (m, 3H, ArH); 7.19–7.21 (m, 4H, ArH); 7.12–7.15 (m, 1H, ArH); 6.16 (brs, 1H, NH); 4.83 (s, 2H, CH₂CO); 4.23 (s, 2H, CH₂ph); 3.14–3.19 (q, 2H, CH₂NH); 1.41–1.46 (sextet, 2H, CH₂); 0.79–0.83 (t, J=7.2, 3H, CH₃). ¹³C-NMR: 167.46 (C=O); 159.73 (C=O); 146.32 (C-Ar); 137.56 (C-Ar); 133.21 (CH-Ar); 131.44 (CH-Ar); 129.44 (C-Ar); 128.72 (2 CH-Ar); 128.39 (2 CH-Ar); 128.11 (C-Ar); 127.38 (CH-Ar); 126.79 (CH-Ar); 125.38 (CH-Ar); 55.33 (CH₂CO); 41.30 (CH₂NH); 38.83 (CH₂ph); 22.70 (CH₂); 11.17 (CH₃).

MS (MALDI, positive mode, matrix DHB) m/z: 358.43 (M+Na)⁺. Elemental analysis: calculated for C₂₀H₂₁N₃O₂ (335.41): % C, 71.62; % H, 6.31; % N, 12.53. Found: % C, 71.63; % H, 6.33; % N, 12.51.

Synthesis of 2-(4-benzyl-1-oxophthalazin-2(1H)-yl)-N-butyl acetamide (6c) White crystals (85%), m.p. 170–172 °C, ¹H-NMR (400 MHz, CDCl₃), (δ, ppm), (J, Hz): 8.34–8.37 (m, 1H, ArH); 7.61–7.68 (m, 3H, ArH); 7.19–7.20 (m, 4H, ArH); 7.11–7.14 (m, 1H, ArH); 6.17 (brs, 1H, NH); 4.82 (s, 2H, CH₂CO); 4.22 (s, 2H, CH₂ph); 3.17–3.21 (q, 2H, CH₂NH); 1.35–1.42 (qn, 2H, CH₂); 1.20–1.28 (sextet, 2H, CH₂); 0.78–0.82 (t, J=7.2, 3H, CH₃). ¹³C-NMR: 167.49 (C=O); 159.79 (C=O); 146.43 (C-Ar); 137.55 (C-Ar); 133.34 (CH-Ar); 131.54 (CH-Ar); 129.39 (C-Ar); 128.78 (2 CH-Ar); 128.40 (2 CH-Ar); 128.02 (C-Ar); 127.36 (CH-Ar); 126.84 (CH-Ar); 125.47 (CH-Ar); 55.33

(CH₂CO); 39.39 (CH₂NH); 38.89 (CH₂ph); 31.54 (CH₂); 20.02 (CH₂); 13.73 (CH₃).

MS (MALDI, positive mode, matrix DHB) m/z: 372.46 (M+Na)⁺. Elemental analysis: calculated for C₂₁H₂₃N₃O₂ (349.43): % C, 72.18; % H, 6.63; % N, 12.03. Found: % C, 72.21; % H, 6.61; % N, 12.07.

Synthesis of 2-(4-benzyl-1-oxophthalazin-2(1H)-yl)-N-cyclohexyl acetamide (6d) White crystals (86%), m.p. 158–160 °C, ¹H-NMR (400 MHz, CDCl₃), (δ, ppm), (J, Hz): 8.44–8.47 (m, 1H, ArH); 7.71–7.76 (m, 3H, ArH); 7.27–7.30 (m, 4H, ArH); 7.21–7.24 (m, 1H, ArH); 6.12 (brs, 1H, NH); 4.90 (s, 2H, CH₂CO); 4.32 (s, 2H, CH₂ph); 3.82 (m, 1H, CHNH); 1.11–1.89 (m, 10H, 5 CH₂). ¹³C-NMR: 166.58 (C=O); 159.73 (C=O); 146.35 (C-Ar); 137.58 (C-Ar); 133.31 (CH-Ar); 131.52 (CH-Ar); 129.37 (C-Ar); 128.78 (2 CH-Ar); 128.39 (2 CH-Ar); 128.04 (C-Ar); 127.41 (CH-Ar); 126.83 (CH-Ar); 125.45 (CH-Ar); 55.25 (CH₂CO); 48.40 (CHNH); 38.87 (CH₂ph); 32.92 (2 CH₂); 25.49 (CH₂); 24.72 (2 CH₂).

MS (MALDI, positive mode, matrix DHB) m/z: 398.50 (M+Na)⁺. Elemental analysis: calculated for C₂₃H₂₅N₃O₂ (375.47): % C, 73.57; % H, 6.71; % N, 11.19. Found: % C, 73.55; % H, 6.75; % N, 11.24.

Synthesis of 2-(4-benzyl-1-oxophthalazin-2(1H)-yl)-N-tetradecyl acetamide (6e) White crystals (82%), m.p. 126–128 °C, ¹H-NMR (400 MHz, CDCl₃), (δ, ppm), (J, Hz): 8.45–8.47 (m, 1H, ArH); 7.71–7.76 (m, 3H, ArH); 7.26–7.30 (m, 4H, ArH); 7.22–7.24 (m, 1H, ArH); 6.22 (brs, 1H, NH); 4.92 (s, 2H, CH₂CO); 4.32 (s, 2H, CH₂ph); 3.27 (q, 2H, CH₂NH); 1.22–1.66 (m, 24H, 12 CH₂); 0.87–0.91 (t, J=7.2, 3H, CH₃). ¹³C-NMR: 167.50 (C=O); 159.78 (C=O); 146.42 (C-Ar); 137.54 (C-Ar); 133.33 (CH-Ar); 131.53 (CH-Ar); 129.38 (C-Ar); 128.78 (2 CH-Ar); 128.40 (2 CH-Ar); 128.02 (C-Ar); 127.35 (CH-Ar); 126.84 (CH-Ar); 125.46 (CH-Ar); 55.32 (CH₂CO); 39.71 (CH₂NH); 38.90 (CH₂ph); 31.93 (CH₂); 29.66 (2 CH₂); 29.56 (2 CH₂); 29.46 (2 CH₂); 29.36 (2 CH₂); 29.26 (CH₂); 26.86 (CH₂); 22.70 (CH₂); 14.13 (CH₃).

MS (MALDI, positive mode, matrix DHB) m/z: 512.68 (M+Na)⁺. Elemental analysis: calculated for C₃₁H₄₃N₃O₂ (489.70): % C, 76.03; % H, 8.85; % N, 8.58. Found: % C, 76.09; % H, 8.80; % N, 8.60.

Synthesis of 2-(4-benzyl-1-oxophthalazin-2(1H)-yl)-N-allyl acetamide (6f) White crystals (87%), m.p. 164–166 °C, ¹H-NMR (400 MHz, CDCl₃), (δ, ppm), (J, Hz): 8.36–8.39 (m, 1H, ArH); 7.63–7.68 (m, 3H, ArH); 7.18–7.21 (m, 4H, ArH); 7.13–7.16 (m, 1H, ArH); 6.21 (brs, 1H, NH); 5.70–5.79 (m, 1H, CH=CH₂); 5.01–5.12 (dd, J=17.2, J=13.2, J=10.4, 2H, CH₂=CH); 4.87 (s, 2H, CH₂CO); 4.24 (s, 2H, CH₂ph); 3.83–3.85 (t, J=5.6, 2H, CH₂NH).

^{13}C -NMR: 167.47 (C=O); 159.82 (C=O); 146.57 (C-Ar); 137.49 (C-Ar); 133.78 (CH-Ar); 133.40 ($\text{CH}=\text{CH}_2$); 131.60 (CH-Ar); 129.40 (C-Ar); 128.79 (2 CH-Ar); 128.42 (2 CH-Ar); 127.99 (C-Ar); 127.38 (CH-Ar); 126.86 (CH-Ar); 125.49 (CH-Ar); 116.43 ($\text{CH}=\text{CH}_2$); 55.25 (CH_2CO); 41.92 (CH_2NH); 38.90 (CH_2ph).

MS (MALDI, positive mode, matrix DHB) m/z : 356.41 ($\text{M} + \text{Na}$)⁺. Elemental analysis: calculated for $\text{C}_{20}\text{H}_{19}\text{N}_3\text{O}_2$ (333.39): % C, 72.05; % H, 5.74; % N, 12.60. Found: % C, 72.01; % H, 5.77; % N, 12.54.

Synthesis of 4-benzyl-2-(2-oxo-2-(piperidin-1-yl) ethyl) phthalazin-1(2H)-one (6g) White crystals (80%), m.p. 192–194 °C, ^1H -NMR (400 MHz, CDCl_3), (δ , ppm), (J , Hz): 8.36–8.37 (m, 1H, ArH); 7.60 (m, 3H, ArH); 7.20–7.21 (m, 4H, ArH); 7.12 (m, 1H, ArH); 5.01 (s, 2H, CH_2CO); 4.23 (s, 2H, CH_2ph); 3.53 (m, 2H, CH_2N); 3.41 (m, 2H, CH_2N); 1.53–1.60 (m, 6H, 3 CH_2). ^{13}C -NMR: 164.92 (C=O); 159.70 (C=O); 145.24 (C-Ar); 138.00 (C-Ar); 132.79 (CH-Ar); 130.98 (CH-Ar); 129.68 (C-Ar); 128.66 (2 CH-Ar); 128.38 (2 CH-Ar); 128.38 (C-Ar); 127.37 (CH-Ar); 126.58 (CH-Ar); 125.24 (CH-Ar); 52.54 (CH_2CO); 45.96 (CH_2N); 43.29 (CH_2N); 39.00 (CH_2ph); 26.25 ($\text{CH}_2\text{CH}_2\text{N}$); 25.38 ($\text{CH}_2\text{CH}_2\text{N}$); 24.47 ($\text{CH}_2\text{CH}_2\text{CH}_2\text{N}$).

MS (MALDI, positive mode, matrix DHB) m/z : 384.44 ($\text{M} + \text{Na}$)⁺. Elemental analysis: calculated for $\text{C}_{22}\text{H}_{23}\text{N}_3\text{O}_2$ (361.45): % C, 73.11; % H, 6.41; % N, 11.63. Found: % C, 73.08; % H, 6.39; % N, 11.60.

Synthesis of 4-benzyl-2-(2-morpholino-2-oxoethyl) phthalazin-1(2H)-one (6h) Off-white crystals (81%), m.p. 210–212 °C, ^1H -NMR (400 MHz, CDCl_3), (δ , ppm), (J , Hz): 8.44–8.46 (m, 1H, ArH); 7.68–7.73 (m, 3H, ArH); 7.29–7.30 (m, 4H, ArH); 7.20–7.23 (m, 1H, ArH); 5.10 (s, 2H, CH_2CO); 4.32 (s, 2H, CH_2ph); 3.59–3.75 (m, 8H, 4 CH_2). ^{13}C -NMR: 165.45 (C=O); 159.71 (C=O); 145.60 (C-Ar); 137.83 (C-Ar); 133.01 (CH-Ar); 131.19 (CH-Ar); 129.62 (C-Ar); 128.71 (2 CH-Ar); 128.36 (2 CH-Ar); 128.10 (C-Ar); 127.33 (CH-Ar); 126.68 (CH-Ar); 125.36 (CH-Ar); 66.78 (CH_2O); 66.40 (CH_2O); 52.31 (CH_2CO); 45.37 (CH_2N); 42.41 (CH_2N); 38.97 (CH_2ph).

MS (MALDI, positive mode, matrix DHB) m/z : 386.46 ($\text{M} + \text{Na}$)⁺. Elemental analysis: calculated for $\text{C}_{21}\text{H}_{21}\text{N}_3\text{O}_3$ (363.42): % C, 69.41; % H, 5.82; % N, 11.56. Found: % C, 69.46; % H, 5.88; % N, 11.59.

General procedure for preparation of methyl-3-[2-(1,4-dioxo-3-phenyl-3,4-dihydro-1H-phthalazine-2-yl)-acetyl amino] alkanoate 7a–c A cold solution at (– 5 °C) of acetohydrazide **4** (3.08 g, 10 mmol) in acetic acid (60 mL) and hydrochloric acid (5N, 30 mL) was added portion wise under stirring to a cold solution (0 °C) of sodium nitrite (0.7 g, 10 mmol) in water (30 mL). After stirring at the same temperature for 30 min, the in situ generated azide was

extracted with cold ethyl acetate and washed successively with cold water and 5% Na_2CO_3 .

After drying over anhydrous sodium sulphate, the azide was used without further purification in the next step. Amino acids methyl ester hydrochloride (15 mmol); glycine, methionine and valine which were placed with triethyl amine (1 g, 10 mmol) in ethyl acetate solution at (– 5 °C) for 20 min. Then the amino acid methyl ester hydrochloride solution was added to the previously prepared cold dried solution of the azide. Afterwards, the mixture was kept 12 h in the refrigerator and then at room temperature for another 12 h. The reaction mixture was filtered and the filtrated solution washed with 0.1N HCl, 5% Na_2CO_3 and water then dried over anhydrous sodium sulphate, the solvent was evaporated in vacuum and the residue was crystallized from ethyl acetate-petroleum ether to give products **7a–c**.

Synthesis of methyl (2-(4-benzyl-1-oxophthalazin-2(1H)-yl)acetyl) glycinate (7a) White crystals (85%), m.p. 166–168 °C, ^1H -NMR (400 MHz, CDCl_3), (δ , ppm), (J , Hz): 8.35–8.38 (m, 1H, ArH); 7.60–7.68 (m, 3H, ArH); 7.18–7.21 (m, 4H, ArH); 7.11–7.15 (m, 1H, ArH); 6.71 (brs, 1H, NH); 4.90 (s, 2H, CH_2CO); 4.23 (s, 2H, CH_2ph); 4.00–4.01 (d, $J=5.2$, 2H, CH_2NH); 3.64 (s, 3H, OCH_3). ^{13}C -NMR: 169.99 (C=O); 167.72 (C=O); 159.78 (C=O); 146.45 (C-Ar); 137.55 (C-Ar); 133.28 (CH-Ar); 131.50 (CH-Ar); 129.44 (C-Ar); 128.75 (2 CH-Ar); 128.44 (2 CH-Ar); 128.03 (C-Ar); 127.37 (CH-Ar); 126.78 (CH-Ar); 125.46 (CH-Ar); 54.80 (CH_2CO); 52.27 (OCH_3); 41.29 (CH_2NH); 38.91 (CH_2ph).

MS (MALDI, positive mode, matrix DHB) m/z : 388.41 ($\text{M} + \text{Na}$)⁺. Elemental analysis: calculated for $\text{C}_{20}\text{H}_{19}\text{N}_3\text{O}_4$ (365.39): % C, 65.74; % H, 5.24; % N, 11.50. Found: % C, 65.66; % H, 5.18; % N, 11.43.

Synthesis of methyl (2-(4-benzyl-1-oxophthalazin-2(1H)-yl)acetyl) methioninate (7b) White crystals (81%), m.p. 232–234 °C, ^1H -NMR (400 MHz, CDCl_3), (δ , ppm), (J , Hz): 8.37–8.39 (m, 1H, ArH); 7.62–7.68 (m, 3H, ArH); 7.19–7.22 (m, 4H, ArH); 7.12–7.15 (m, 1H, ArH); 6.80–6.82 (d, $J=7.2$, 1H, NH); 4.84–4.94 (m, 2H, CH_2CO); 4.66–4.71 (q, 1H, CHNH); 4.24 (s, 2H, CH_2ph); 3.64 (s, 3H, OCH_3); 2.43 (m, 2H, CH_2S); 2.08–2.13 (m, 1H, $\text{CH}_2\text{CH}_2\text{S}$); 1.88–1.97 (m, 1H, $\text{CH}_2\text{CH}_2\text{S}$); 1.55 (s, 3H, SCH_3). ^{13}C -NMR: 172.00 (C=O); 167.34 (C=O); 159.72 (C=O); 146.45 (C-Ar); 137.53 (C-Ar); 133.28 (CH-Ar); 131.51 (CH-Ar); 129.44 (C-Ar); 128.76 (2 CH-Ar); 128.40 (2 CH-Ar); 128.07 (C-Ar); 127.39 (CH-Ar); 126.79 (CH-Ar); 125.49 (CH-Ar); 54.90 (CH_2CO); 52.46 (OCH_3); 51.75 (CHNH); 38.94 (CH_2ph); 31.53 ($\text{CH}_2\text{CH}_2\text{S}$); 29.90 (CH_2S); 15.35 (SCH_3).

MS (MALDI, positive mode, matrix DHB) m/z : 462.56 ($\text{M} + \text{Na}$)⁺. Elemental analysis: calculated for $\text{C}_{23}\text{H}_{25}\text{N}_3\text{O}_4\text{S}$ (439.53): % C, 62.85; % H, 5.73; % N, 9.56;

% S, 7.29. Found: % C, 62.81; % H, 5.70; % N, 9.52; % S, 7.23.

Synthesis of methyl (2-(4-benzyl-1-oxophthalazin-2(1H)-yl) acetyl) valinate (7c) White crystals (86%), m.p. 150–152 °C, ¹H-NMR (400 MHz, CDCl₃), (δ, ppm), (J, Hz): 8.47–8.49 (m, 1H, ArH); 7.70–7.75 (m, 3H, ArH); 7.27–7.30 (m, 4H, ArH); 7.20–7.25 (m, 1H, ArH); 6.71–6.74 (d, J=8.1, 1H, NH); 4.98 (s, 2H, CH₂CO); 4.59–4.63 (dd, J=6.4, J=6.4, 1H, CHNH); 4.32 (s, 2H, CH₂ph); 3.70 (s, 3H, OCH₃); 2.16–2.21 (m, 1H, CH₃CHCH₃); 0.87–0.94 (d, J=6.9, 6H, 2 CH₃CH). ¹³C-NMR: 172.12 (C=O); 167.45 (C=O); 159.79 (C=O); 146.42 (C-Ar); 137.56 (C-Ar); 133.33 (CH-Ar); 131.55 (CH-Ar); 129.40 (C-Ar); 128.77 (2 CH-Ar); 128.39 (2 CH-Ar); 128.02 (C-Ar); 127.41 (CH-Ar); 126.80 (CH-Ar); 125.54 (CH-Ar); 57.20 (CHNH); 55.00 (CH₂CO); 52.15 (OCH₃); 38.97 (CH₂ph); 31.38 (CH₃CHCH₃); 18.90 (CH₃CH); 17.74 (CH₃CH).

MS (MALDI, positive mode, matrix DHB) m/z: 430.49 (M+Na)⁺. Elemental analysis: calculated for C₂₃H₂₅N₃O₄ (407.47): % C, 67.80; % H, 6.18; % N, 10.31. Found: % C, 67.86; % H, 6.26; % N, 10.34.

Synthesis of hydrazide 8a To a solution of ester **7a** (3.65 g, 0.01 mol) in ethyl alcohol (30 mL) was added hydrazine hydrate (1.6 mL, 0.05 mol). The reaction mixture was refluxed for 6 h, cooled and the white precipitate filtered and recrystallized from ethanol to obtain the corresponding hydrazide 2-(4-benzyl-1-oxophthalazin-2(1H)-yl)-N-(2-hydrazineyl-2-oxoethyl) acetamide **8a**.

Synthesis of 2-(4-benzyl-1-oxophthalazin-2(1H)-yl)-N-(2-hydrazineyl-2-oxo ethyl) acetamide (8a) White crystals (84%), m.p. 202–204 °C, ¹H-NMR (400 MHz, DMSO), (δ, ppm), (J, Hz): 9.02 (brs, 1H, NH); 8.38–8.41 (m, 1H, ArH); 8.27–8.29 (m, 1H, ArH); 7.91–7.93 (brs, 1H, NH); 7.80–7.88 (m, 2H, ArH); 7.34–7.36 (m, 2H, ArH); 7.27–7.31 (m, 2H, ArH); 7.18–7.21 (m, 1H, ArH); 4.87 (s, 2H, CH₂CO); 4.32 (s, 2H, CH₂ph); 4.23 (d, J=5.2, 2H, CH₂NH); 3.74–3.75 (d, J=5.6, 2H, NH₂). ¹³C-NMR: 168.38 (C=O); 167.73 (C=O); 159.08 (C=O); 145.48 (C-Ar); 138.56 (C-Ar); 133.82 (CH-Ar); 132.14 (CH-Ar); 129.38 (C-Ar); 129.01 (2 CH-Ar); 128.84 (2 CH-Ar); 128.08 (C-Ar); 126.95 (CH-Ar); 126.85 (CH-Ar); 126.27 (CH-Ar); 53.98 (CH₂CO); 41.43 (CH₂NH); 38.13 (CH₂ph).

MS (MALDI, positive mode, matrix DHB) m/z: 388.42 (M+Na)⁺. Elemental analysis: calculated for C₁₉H₁₉N₅O₃ (365.39): % C, 62.46; % H, 5.24; % N, 19.17. Found: % C, 62.44; % H, 5.21; % N, 19.12.

General procedure for synthesis of 2-(4-benzyl-1-oxophthalazin-2(1H)-yl)-N-(2-oxo-2-(alkyl amino) ethyl) acetamide 10a–h Under azide coupling method as previewed

before, A cold solution at (– 5 °C) of 2-(4-benzyl-1-oxophthalazin-2(1H)-yl)-N-(2-hydrazineyl-2-oxoethyl) acetamide (**8a**) (3.65 g, 10 mmol) in acetic acid (60 mL) and hydrochloric acid (5N, 30 mL) was added portion wise under stirring to a cold solution (0 °C) of sodium nitrite (0.7 g, 0.01 mol) in water (30 mL). After stirring at the same temperature for 30 min, the in situ generated azide was extracted with cold ethyl acetate and washed successively with cold water and 5% Na₂CO₃.

After drying over anhydrous sodium sulphate, the azide was used without further purification in the next step. Amines (12 mmol) were added to the previously prepared cold dried solution of the azide. Afterwards, the mixture was kept 12 h in the refrigerator and then at room temperature for another 12 h. The reaction mixture was filtered and the filtrated solution washed with 0.1N HCl, 5% Na₂CO₃ and water then dried over anhydrous sodium sulphate, the solvent was evaporated in vacuum and the residue was crystallized from ethyl acetate-petroleum ether to give products **10a–h**.

Synthesis of N-benzyl-2-(2-(4-benzyl-1-oxophthalazin-2(1H)-yl)acetamido) acetamide (10a) Off-white crystals (83%), m.p. 186–188 °C, ¹H-NMR (400 MHz, CDCl₃), (δ, ppm), (J, Hz): 8.23–8.25 (m, 1H, ArH); 7.68–7.76 (m, 3H, ArH); 7.27–7.33 (m, 9H, ArH); 7.22–7.25 (m, 1H, ArH); 7.04 (brs, 2H, 2 NH); 4.92 (s, 2H, NCH₂CO); 4.43–4.45 (d, J=5.6, 2H, NHCH₂ph); 4.31 (s, 2H, CH₂ph); 4.01–4.03 (d, J=5.6, 2H, NHCH₂CO). ¹³C-NMR: 168.65 (C=O); 168.02 (C=O); 159.93 (C=O); 146.66 (C-Ar); 138.00 (C-Ar); 137.44 (C-Ar); 133.37 (CH-Ar); 131.51 (CH-Ar); 129.52 (C-Ar); 128.78 (2 CH-Ar); 128.57 (2 CH-Ar); 128.41 (2 CH-Ar); 127.83 (C-Ar); 127.72 (2 CH-Ar); 127.32 (CH-Ar); 127.19 (CH-Ar); 126.84 (CH-Ar); 125.47 (CH-Ar); 55.76 (NCH₂CO); 43.51 (NHCH₂CO); 43.35 (NHCH₂ph); 38.88 (CH₂ph).

MS (MALDI, positive mode, matrix DHB) m/z: 463.52 (M+Na)⁺. Elemental analysis: calculated for C₂₆H₂₄N₄O₃ (440.50): % C, 70.89; % H, 5.49; % N, 12.72. Found: % C, 70.93; % H, 5.56; % N, 12.77.

Synthesis of 2-(4-benzyl-1-oxophthalazin-2(1H)-yl)-N-(2-oxo-2-(propyl amino) ethyl) acetamide (10b) White crystals (86%), m.p. 152–154 °C, ¹H-NMR (400 MHz, CDCl₃), (δ, ppm), (J, Hz): 8.41–8.44 (m, 1H, ArH); 7.72–7.79 (m, 3H, ArH); 7.28–7.31 (m, 4H, ArH); 7.22–7.25 (m, 1H, ArH); 6.88 (brs, 1H, NH); 6.62 (brs, 1H, NH); 4.94 (s, 2H, NCH₂CO); 4.34 (s, 2H, CH₂ph); 3.98–3.99 (d, J=4.8, 2H, NHCH₂CO); 3.19–3.24 (q, 2H, NHCH₂CH₂); 1.52–1.57 (sextet, 2H, CH₂CH₂CH₃); 0.89–0.92 (t, J=7.2, 3H, CH₃). ¹³C-NMR: 168.46 (C=O); 167.89 (C=O); 159.93 (C=O); 146.62 (C-Ar); 137.44 (C-Ar); 133.41 (CH-Ar); 131.57 (CH-

Ar); 129.57 (C-Ar); 128.78 (2 CH-Ar); 128.41 (2 CH-Ar); 127.94 (C-Ar); 127.17 (CH-Ar); 126.84 (CH-Ar); 125.55 (CH-Ar); 55.71 (NCH₂CO); 43.42 (NHCH₂CO); 41.37 (NHCH₂CH₂); 38.91 (CH₂ph); 22.57 (CH₂CH₂CH₃); 11.31 (CH₃).

MS (MALDI, positive mode, matrix DHB) m/z: 415.47 (M + Na)⁺. Elemental analysis: calculated for C₂₂H₂₄N₄O₃ (392.46): % C, 67.33; % H, 6.16; % N, 14.28. Found: % C, 67.36; % H, 6.22; % N, 14.32.

Synthesis of 2-(4-benzyl-1-oxophthalazin-2(1H)-yl)-N-(2-butyl amino)-2-oxoethyl acetamide (10c) Off-white crystals (85%), m.p. 156–158 °C, ¹H-NMR (400 MHz, CDCl₃), (δ, ppm), (J, Hz): 8.43–8.45 (m, 1H, ArH); 7.73–7.80 (m, 3H, ArH); 7.29–7.32 (m, 4H, ArH); 7.23–7.27 (m, 1H, ArH); 6.76 (brs, 1H, NH); 6.50 (brs, 1H, NH); 4.94 (s, 2H, NCH₂CO); 4.34 (s, 2H, CH₂ph); 3.99 (d, J = 5.2, 2H, NHCH₂CO); 3.24–3.29 (q, 2H, NHCH₂CH₂); 1.48–1.54 (qn, 2H, CH₂CH₂CH₂); 1.32–1.37 (sextet, 2H, CH₂CH₂CH₃); 0.90–0.93 (t, J = 7.2, 3H, CH₃). ¹³C-NMR: 168.34 (C=O); 167.85 (C=O); 159.94 (C=O); 146.67 (C-Ar); 137.42 (C-Ar); 133.44 (CH-Ar); 131.59 (CH-Ar); 129.58 (C-Ar); 128.79 (2 CH-Ar); 128.42 (2 CH-Ar); 127.93 (C-Ar); 127.21 (CH-Ar); 126.86 (CH-Ar); 125.56 (CH-Ar); 55.80 (NCH₂CO); 43.43 (NHCH₂CO); 39.41 (NHCH₂CH₂); 38.92 (CH₂ph); 31.41 (CH₂CH₂CH₂); 20.01 (CH₂CH₂CH₃); 13.67 (CH₃).

MS (MALDI, positive mode, matrix DHB) m/z: 429.53 (M + Na)⁺. Elemental analysis: calculated for C₂₃H₂₆N₄O₃ (406.49): % C, 67.96; % H, 6.45; % N, 13.78. Found: % C, 67.99; % H, 6.55; % N, 13.86.

Synthesis of 2-(4-benzyl-1-oxophthalazin-2(1H)-yl)-N-(2-cyclohexylamino)-2-oxoethyl acetamide (10d) White crystals (87%), m.p. 157–158 °C, ¹H-NMR (400 MHz, CDCl₃), (δ, ppm), (J, Hz): 8.43–8.45 (m, 1H, ArH); 7.73–7.79 (m, 3H, ArH); 7.28–7.32 (m, 4H, ArH); 7.22–7.25 (m, 1H, ArH); 6.81 (brs, 1H, NH); 6.37–6.38 (d, J = 7.2, 1H, NH); 4.94 (s, 2H, NCH₂CO); 4.34 (s, 2H, CH₂ph); 3.97–3.98 (d, J = 5.2, 2H, NHCH₂CO); 3.75–3.76 (sextet, 1H, NHCH₂CH₂); 1.86–1.89 (m, 2H, CH₂); 1.69–1.72 (m, 2H, CH₂); 1.60–1.63 (m, 1H, CH); 1.25–1.36 (m, 2H, CH₂); 1.15–1.22 (m, 3H, CH₂ / CH). ¹³C-NMR: 167.75 (C=O); 167.40 (C=O); 159.87 (C=O); 146.59 (C-Ar); 137.44 (C-Ar); 133.40 (CH-Ar); 131.56 (CH-Ar); 129.59 (C-Ar); 128.78 (2 CH-Ar); 128.42 (2 CH-Ar); 127.95 (C-Ar); 127.21 (CH-Ar); 126.84 (CH-Ar); 125.55 (CH-Ar); 55.73 (NCH₂CO); 48.44 (NHCH₂CO); 43.45 (NHCH₂CH₂); 38.93 (CH₂ph); 32.83 (2 CH₂); 25.53 (CH₂); 24.81 (2 CH₂).

MS (MALDI, positive mode, matrix DHB) m/z: 455.54 (M + Na)⁺. Elemental analysis: calculated for C₂₅H₂₈N₄O₃ (432.52): % C, 69.42; % H, 6.53; % N, 12.95. Found: % C, 69.48; % H, 6.61; % N, 12.94.

Synthesis of 2-(4-benzyl-1-oxophthalazin-2(1H)-yl)-N-(2-oxo-2-(tetradecyl amino) ethyl) acetamide (10e) Off-white crystals (82%), m.p. 149–150 °C, ¹H-NMR (400 MHz, CDCl₃), (δ, ppm), (J, Hz): 8.40–8.42 (m, 1H, ArH); 7.71–7.77 (m, 3H, ArH); 7.29–7.30 (m, 4H, ArH); 7.21–7.24 (m, 1H, ArH); 6.70 (brs, 1H, NH); 6.62 (brs, 1H, NH); 4.94 (s, 2H, NCH₂CO); 4.33 (s, 2H, CH₂ph); 3.96–3.97 (d, J = 3.2, 2H, NHCH₂CO); 3.20–3.25 (q, 2H, NHCH₂CH₂); 2.70–2.74 (t, J = 6.8, 1H, NH); 2.35 (m, 2H, CH₂); 1.48 (sextet, 2H, CH₂CH₂CH₃); 1.28 (m, 20H, 10 CH₂); 0.88–0.91 (t, J = 6.8, 3H, CH₃). ¹³C-NMR: 168.64 (C=O); 167.96 (C=O); 159.88 (C=O); 146.49 (C-Ar); 137.47 (C-Ar); 133.33 (CH-Ar); 131.47 (CH-Ar); 129.57 (C-Ar); 128.76 (2 CH-Ar); 128.39 (2 CH-Ar); 127.93 (C-Ar); 127.14 (CH-Ar); 126.81 (CH-Ar); 125.52 (CH-Ar); 55.64 (NCH₂CO); 43.34 (NHCH₂CO); 42.06 (CH₂); 39.74 (NHCH₂CH₂); 38.91 (CH₂ph); 33.38 (CH₂); 31.89 (2 CH₂); 29.31–29.63 (4 CH₂); 26.85 (2 CH₂); 22.64 (2 CH₂); 14.04 (CH₃).

MS (MALDI, positive mode, matrix DHB) m/z: 569.80 (M + Na)⁺. Elemental analysis: calculated for C₃₃H₄₆N₄O₃ (546.76): % C, 72.49; % H, 8.48; % N, 10.25. Found: % C, 72.53; % H, 8.45; % N, 10.36.

Synthesis of N-allyl-2-(2-(4-benzyl-1-oxophthalazin-2(1H)-yl)acetamido) acetamide (10f) White crystals (88%), m.p. 188–190 °C, ¹H-NMR (400 MHz, CDCl₃), (δ, ppm), (J, Hz): 8.42–8.44 (m, 1H, ArH); 7.73–7.77 (m, 3H, ArH); 7.28–7.32 (m, 4H, ArH); 7.24–7.25 (m, 1H, ArH); 6.83 (brs, 1H, NH); 6.63 (brs, 1H, NH); 5.81–5.88 (m, 1H, CH=CH₂); 5.11–5.22 (dd, J = 17.2, J = 10.4, 2H, CH₂=CH); 4.95 (s, 2H, NCH₂CO); 4.34 (s, 2H, CH₂ph); 4.02 (d, J = 5.2, 2H, NHCH₂CO); 3.90 (t, J = 5.6, 2H, NHCH₂CH). ¹³C-NMR: 168.39 (C=O); 167.98 (C=O); 159.97 (C=O); 146.73 (C-Ar); 137.41 (C-Ar); 133.77 (CH=CH₂); 133.46 (CH-Ar); 131.60 (CH-Ar); 129.57 (C-Ar); 128.79 (2 CH-Ar); 128.41 (2 CH-Ar); 127.90 (C-Ar); 127.26 (CH-Ar); 126.86 (CH-Ar); 125.54 (CH-Ar); 116.50 (CH₂=CH); 55.80 (NCH₂CO); 43.43 (NHCH₂CO); 41.98 (NHCH₂CH); 38.92 (CH₂ph).

MS (MALDI, positive mode, matrix DHB) m/z: 413.47 (M + Na)⁺. Elemental analysis: calculated for C₂₂H₂₂N₄O₃ (390.44): % C, 67.68; % H, 5.68; % N, 14.35. Found: % C, 67.66; % H, 5.61; % N, 14.23.

Synthesis of 2-(4-benzyl-1-oxophthalazin-2(1H)-yl)-N-(2-oxo-2-(piperidin-1-yl) ethyl) acetamide (10g) White crystals (79%), m.p. 138–140 °C, ¹H-NMR (400 MHz, CDCl₃), (δ, ppm), (J, Hz): 8.44–8.46 (m, 1H, ArH); 7.70–7.71 (m, 3H, ArH); 7.30–7.32 (m, 4H, ArH); 7.22–7.23 (m, 1H, ArH); 7.07 (brs, 1H, NH); 5.00 (s, 2H, NCH₂CO); 4.34 (s, 2H, CH₂ph); 4.10–4.11 (d, J = 5.2, 2H, NHCH₂CO); 3.56 (m, 2H, CH₂N); 3.40 (m, 2H, CH₂N); 1.56–1.65 (m, 6H, 3 CH₂). ¹³C-NMR: 168.34 (C=O); 167.46 (C=O); 159.89

(C=O); 146.43 (C-Ar); 137.56 (C-Ar); 133.34 (CH-Ar); 131.56 (CH-Ar); 129.57 (C-Ar); 128.76 (2 CH-Ar); 128.42 (2 CH-Ar); 127.92 (C-Ar); 127.21 (CH-Ar); 126.67 (CH-Ar); 125.53 (CH-Ar); 55.74 (NCH₂CO); 45.86 (CH₂N); 43.64 (CH₂N); 43.34 (NHCH₂CO); 39.08 (CH₂ph); 26.55 (CH₂CH₂N); 25.41 (CH₂CH₂N); 23.94 (CH₂CH₂CH₂N).

MS (MALDI, positive mode, matrix DHB) m/z: 441.54 (M+Na)⁺. Elemental analysis: calculated for C₂₄H₂₆N₄O₃ (418.50): % C, 68.88; % H, 6.26; % N, 13.39. Found: % C, 68.92; % H, 6.31; % N, 13.48.

Synthesis of 2-(4-benzyl-1-oxophthalazin-2(1H)-yl)-N-(2-morpholino-2-oxoethyl)acetamide (10h) White crystals (81%), m.p. 148–150 °C, ¹H-NMR (400 MHz, CDCl₃), (δ, ppm), (J, Hz): 8.47–8.48 (m, 1H, ArH); 7.72–7.73 (m, 3H, ArH); 7.28–7.32 (m, 4H, ArH); 7.23–7.24 (m, 1H, ArH); 7.10 (brs, 1H, NH); 5.01 (s, 2H, NCH₂CO); 4.34 (s, 2H, CH₂ph); 4.13 (d, J=5.2, 2H, NHCH₂CO); 3.64–3.69 (m, 6H, 2 CH₂O /CH₂N); 3.43 (m, 2H, CH₂N). ¹³C-NMR: 167.44 (C=O); 166.34 (C=O); 159.67 (C=O); 146.34 (C-Ar); 137.62 (C-Ar); 133.20 (CH-Ar); 131.47 (CH-Ar); 129.49 (C-Ar); 128.74 (2 CH-Ar); 128.44 (2 CH-Ar); 128.11 (C-Ar); 127.46 (CH-Ar); 126.74 (CH-Ar); 125.50 (CH-Ar); 66.67 (CH₂O); 66.33 (CH₂O); 54.60 (NCH₂CO); 44.86 (NHCH₂CO); 42.36 (CH₂N); 41.25 (CH₂N); 39.01 (CH₂ph).

MS (MALDI, positive mode, matrix DHB) m/z: 443.50 (M+Na)⁺. Elemental analysis: calculated for C₂₃H₂₄N₄O₄ (420.47): % C, 65.70; % H, 5.75; % N, 13.33. Found: % C, 65.64; % H, 5.65; % N, 13.26.

General procedure for preparation of methyl 2-(4-benzyl-1-oxophthalazin-2(1H)-yl) acetyl glycyll alkanoate 11a–d A cold solution at (– 5 °C) of 2-(4-benzyl-1-oxophthalazin-2(1H)-yl)-N-(2-hydrazineyl-2-oxoethyl)acetamide (**8a**) (3.65 g, 10 mmol) in acetic acid (60 mL) and hydrochloric acid (5N, 30 mL) was added portion wise under stirring to a cold solution (0 °C) of sodium nitrite (0.7 g, 10 mmol) in water (30 mL). After stirring at the same temperature for 30 min, the in situ generated azide was extracted with cold ethyl acetate and washed successively with cold water and 5% Na₂CO₃.

After drying over anhydrous sodium sulphate, the azide was used without further purification in the next step. Amino acids methyl ester hydrochloride (15 mmol); glycine, β-alanine, methionine and valine which were placed with triethyl amine (1 g, 10 mmol) in ethyl acetate solution at (– 5 °C) for 20 min. Then the amino acid methyl ester hydrochloride solution was added to the previously prepared cold dried solution of the azide. Afterwards, the mixture was kept 12 h in the refrigerator and then at room temperature for another 12 h. The reaction mixture was filtered, and the filtrated

solution washed with 0.1N HCl, 5% Na₂CO₃ and water then dried over anhydrous sodium sulphate, the solvent was evaporated in vacuum and the residue was crystallized from ethyl acetate-petroleum ether to give products **11a–d**.

Synthesis of methyl 2-(4-benzyl-1-oxophthalazin-2(1H)-yl) acetyl glycyll glycinate (11a) White crystals (88%), m.p. 162–164 °C, ¹H-NMR (400 MHz, CDCl₃), (δ, ppm), (J, Hz): 8.42 (m, 1H, ArH); 7.73–7.75 (m, 3H, ArH); 7.29–7.31 (m, 4H, ArH); 7.23–7.24 (m, 1H, ArH); 7.08 (brs, 2H, 2 NH); 4.97 (s, 2H, NCH₂CO); 4.33 (s, 2H, CH₂ph); 4.04–4.05 (d, J=4.8, 4H, 2 NHCH₂CO); 3.72 (s, 3H, OCH₃).

¹³C-NMR: 172.11 (C=O); 168.08 (C=O); 167.63 (C=O); 159.94 (C=O); 146.70 (C-Ar); 137.46 (C-Ar); 133.39 (CH-Ar); 131.56 (CH-Ar); 129.55 (C-Ar); 128.78 (2 CH-Ar); 128.42 (2 CH-Ar); 127.93 (C-Ar); 127.26 (CH-Ar); 126.83 (CH-Ar); 125.49 (CH-Ar); 55.63 (NCH₂CO); 52.27 (OCH₃); 43.13 (NHCH₂COO); 41.17 (NHCH₂CO); 38.89 (CH₂ph).

MS (MALDI, positive mode, matrix DHB) m/z: 445.46 (M+Na)⁺. Elemental analysis: calculated for C₂₂H₂₂N₄O₅ (422.44): % C, 62.55; % H, 5.25; % N, 13.26. Found: % C, 62.49; % H, 5.22; % N, 13.30.

Synthesis of methyl 3-(2-(4-benzyl-1-oxophthalazin-2(1H)-yl) acetamido acetamido) propanoate (11b) White crystals (85%), m.p. 108–110 °C, ¹H-NMR (400 MHz, CDCl₃), (δ, ppm), (J, Hz): 8.44–8.46 (m, 1H, ArH); 7.72–7.78 (m, 3H, ArH); 7.28–7.31 (m, 4H, ArH); 7.22–7.25 (m, 1H, ArH); 6.88 (brs, 2H, 2 NH); 4.95 (s, 2H, NCH₂CO); 4.34 (s, 2H, CH₂ph); 3.97–3.98 (d, J=4.4, 2H, NHCH₂CO); 3.67 (s, 3H, OCH₃); 3.54–3.55 (q, 2H, NHCH₂CH₂CO); 2.56–2.59 (t, J=6, 2H, NHCH₂CH₂CO). ¹³C-NMR: 172.50 (C=O); 168.57 (C=O); 167.88 (C=O); 159.93 (C=O); 146.62 (C-Ar); 137.47 (C-Ar); 133.38 (CH-Ar); 131.58 (CH-Ar); 129.55 (C-Ar); 128.77 (2 CH-Ar); 128.42 (2 CH-Ar); 127.98 (C-Ar); 127.28 (CH-Ar); 126.83 (CH-Ar); 125.51 (CH-Ar); 55.59 (NCH₂CO); 51.73 (OCH₃); 43.25 (NHCH₂CO); 38.91 (CH₂ph); 35.18 (NHCH₂CH₂CO); 33.71 (NHCH₂CH₂CO).

MS (MALDI, positive mode, matrix DHB) m/z: 459.48 (M+Na)⁺. Elemental analysis: calculated for C₂₃H₂₄N₄O₅ (436.47): % C, 63.29; % H, 5.54; % N, 12.84. Found: % C, 63.35; % H, 5.63; % N, 12.82.

Synthesis of methyl 2-(4-benzyl-1-oxophthalazin-2(1H)-yl) acetyl glycyll methioninate (11c) Off-white crystals (84%), m.p. 130–132 °C, ¹H-NMR (400 MHz, CDCl₃), (δ, ppm), (J, Hz): 8.48 (m, 1H, ArH); 7.73–7.74 (m, 3H, ArH); 7.55–7.56 (brs, 1H, NH); 7.28 (m, 4H, ArH); 7.19–7.21 (brs, 1H, NH); 7.14–7.17 (m, 1H, ArH); 4.99 (q, 1H, NHCHCO); 4.32 (s, 2H, NCH₂CO); 4.23–4.26 (d, J=6, 2H, NHCH₂CO); 4.18

(s, 2H, CH₂ph); 3.76 (s, 3H, OCH₃); 3.03–3.07 (t, *J*=7.2, 2H, CH₂S); 2.25 (q, 2H, CH₂CH₂S); 1.71 (s, 3H, SCH₃). ¹³C-NMR: 172.18 (C=O); 168.60 (C=O); 167.79 (C=O); 159.84 (C=O); 146.45 (C-Ar); 137.47 (C-Ar); 133.34 (CH-Ar); 131.54 (CH-Ar); 130.80 (C-Ar); 128.78 (2 CH-Ar); 128.42 (2 CH-Ar); 127.86 (C-Ar); 127.43 (CH-Ar); 126.81 (CH-Ar); 125.53 (CH-Ar); 57.41 (NHCHCO); 55.56 (NCH₂CO); 52.08 (OCH₃); 51.67 (NHCH₂CO); 43.34 (CH₂CH₂S); 38.92 (CH₂ph); 31.49 (CH₂S); 16.85 (SCH₃).

MS (MALDI, positive mode, matrix DHB) *m/z*: 519.61 (M+Na)⁺. Elemental analysis: calculated for C₂₅H₂₈N₄O₅S (496.58): % C, 60.47; % H, 5.68; % N, 11.28; % S, 6.46. Found: % C, 60.33; % H, 5.57; % N, 11.15; % S, 6.40.

Synthesis of methyl (2-(4-benzyl-1-oxophthalazin-2(1H)-yl) acetyl) glycyl valinate (11d) White crystals (82%), m.p. 190–192 °C, ¹H-NMR (400 MHz, CDCl₃), (δ, ppm), (*J*, Hz): 8.45 (m, 1H, ArH); 7.72–7.76 (m, 3H, ArH); 7.28–7.32 (m, 4H, ArH); 7.22–7.24 (m, 1H, ArH); 6.88 (brs, 1H, NH); 6.65 (brs, 1H, NH); 4.98 (s, 2H, NCH₂CO); 4.34 (s, 2H, CH₂ph); 4.25 (t, *J*=5.2, 1H, NHCHCO); 3.71 (d, *J*=5.2, 2H, NHCH₂CO); 3.51 (s, 3H, OCH₃); 1.45 (m, 1H, CH₃CHCH₃); 0.92–0.95 (d, *J*=6.4, 6H, 2 CH₃CH). ¹³C-NMR: 172.48 (C=O); 168.52 (C=O); 167.82 (C=O); 159.91 (C=O); 146.44 (C-Ar); 137.36 (C-Ar); 133.38 (CH-Ar); 131.56 (CH-Ar); 130.82 (C-Ar); 128.79 (2 CH-Ar); 128.45 (2 CH-Ar); 127.87 (C-Ar); 127.40 (CH-Ar); 126.84 (CH-Ar); 125.54 (CH-Ar); 57.43 (NHCHCO); 55.51 (NCH₂CO); 52.08 (OCH₃); 43.30 (NHCH₂CO); 38.94 (CH₂ph); 29.68 (CH₃CHCH₃); 18.93 (CH₃CH); 17.88 (CH₃CH).

MS (MALDI, positive mode, matrix DHB) *m/z*: 487.56 (M+Na)⁺. Elemental analysis: calculated for C₂₅H₂₈N₄O₅ (464.52): % C, 64.64; % H, 6.08; % N, 12.06. Found: % C, 64.76; % H, 6.15; % N, 12.16.

Synthesis of 2-(4-benzyl-1-oxophthalazin-2(1H)-yl)-N-(3,5-diamino-1H-pyrazol-1-yl)-2-oxoethyl acetamide (12a) A mixture of hydrazide **8a** (3.65 g, 0.01 mol) and malononitrile (1.32 g, 0.02 mol) in ethanol (30 mL) was refluxed for 8 h. By cooling the solid product formed, filtered off and recrystallized from ethanol solvent gave compound **12a**.

White crystals (85%), m.p. 236–238 °C, ¹H-NMR (400 MHz, DMSO), (δ, ppm), (*J*, Hz): 9.02 (brs, 1H, NHCH₂CO); 8.27–8.28 (m, 1H, ArH); 7.90–7.92 (m, 1H, ArH); 7.81–7.85 (m, 2H, ArH); 7.31–7.32 (m, 3H, ArH); 7.18 (m, 2H, ArH); 7.10–7.12 (s, 1H, NH₂-C=CH-C-NH₂); 5.46–5.47 (d, *J*=5.2, 2H, NHCH₂CO); 4.86 (s, 2H, NCH₂CO); 4.32 (brs, 2H, NH₂); 4.26 (s, 2H, CH₂ph); 3.52 (brs, 2H, NH₂). ¹³C-NMR: 171.42 (C=O); 168.34 (C=O); 159.21 (C=O); 148.14 (C-NH₂); 148.36 (C-NH₂); 146.38 (C-Ar);

137.32 (C-Ar); 133.30 (CH-Ar); 131.64 (CH-Ar); 130.85 (C-Ar); 128.78 (2 CH-Ar); 128.43 (2 CH-Ar); 128.09 (C-Ar); 127.49 (CH-Ar); 126.76 (CH-Ar); 125.34 (CH-Ar); 110.35 (NH₂-C=CH-C-NH₂); 55.28 (NCH₂CO); 42.82 (NHCH₂CO); 38.64 (CH₂ph).

MS (MALDI, positive mode, matrix DHB) *m/z*: 454.49 (M+Na)⁺. Elemental analysis: calculated for C₂₂H₂₁N₇O₃ (431.46): % C, 61.24; % H, 4.91; % N, 22.73. Found: % C, 61.32; % H, 5.02; % N, 22.80.

Synthesis of 2-(4-benzyl-1-oxophthalazin-2(1H)-yl)-N-(2-(2-cyclohexylidene hydrazineyl)-2-oxoethyl) acetamide (12b) A mixture of hydrazide molecule **8a** (3.65 g, 0.01 mol) and cyclohexanone (1.96 g, 0.02 mol) in ethanol (30 mL) was refluxed for 8 h. By cooling the solid product formed, filtered off and recrystallized from ethanol solvent gave compound **12b**.

White crystals (89%), m.p. 233–234 °C, ¹H-NMR (400 MHz, DMSO), (δ, ppm), (*J*, Hz): 9.01 (brs, 1H, CONHNH); 8.40 (m, 1H, ArH); 8.26–8.28 (m, 1H, ArH); 7.90–7.91 (m, 1H, ArH); 7.78–7.86 (m, 2H, ArH); 7.30–7.34 (m, 3H, ArH); 7.16–7.20 (m, 1H, ArH); 7.16–7.20 (t, *J*=5.2, 1H, NHCH₂CO); 7.08–7.12 (m, 1H, NH-C=CH-CH₂); 5.46–5.47 (d, *J*=5.2, 2H, NHCH₂CO); 4.85 (s, 2H, NCH₂CO); 4.32 (brs, 1H, NHNH-C=CH-CH₂); 4.25 (s, 2H, CH₂ph); 2.37 (t, *J*=6, 2H, NH-C-CH₂); 1.65 (q, 2H, NH-C=CH-CH₂); 1.14 (qn, 4H, 2 CH₂). ¹³C-NMR: 170.62 (C=O); 168.32 (C=O); 159.18 (C=O); 150.11 (NH-C=CH-CH₂); 145.83 (C-Ar); 138.34 (C-Ar); 133.77 (CH-Ar); 131.93 (CH-Ar); 129.52 (C-Ar); 129.06 (2 CH-Ar); 128.78 (2 CH-Ar); 128.07 (C-Ar); 127.04 (CH-Ar); 126.87 (CH-Ar); 125.96 (CH-Ar); 102.45 (NH-C=CH-CH₂); 54.86 (NCH₂CO); 41.03 (NHCH₂CO); 38.25 (CH₂ph); 35.19 (NH-C-CH₂); 34.32 (NH-C=CH-CH₂); 26.50 (CH₂); 22.71 (CH₂).

MS (MALDI, positive mode, matrix DHB) *m/z*: 468.55 (M+Na)⁺. Elemental analysis: calculated for C₂₅H₂₇N₅O₃ (445.52): % C, 67.40; % H, 6.11; % N, 15.72. Found: % C, 67.33; % H, 6.01; % N, 15.63.

Synthesis of (E)-2-(4-benzyl-1-oxophthalazin-2(1H)-yl)-N-(2-(2-(1-(furan-2-yl) ethylidene) hydrazineyl)-2-oxoethyl) acetamide (12c) A mixture of hydrazide molecule **8a** (3.65 g, 0.01 mol) and 2-furyl methyl ketone (2.2 g, 0.02 mol) in ethanol (30 mL) was refluxed for 8 h. By cooling the solid product formed, filtered off and recrystallized from ethanol solvent gave compound **12c**.

Faint brown crystals (91%), m.p. 209–210 °C, ¹H-NMR (400 MHz, DMSO), (δ, ppm), (*J*, Hz): 10.64 (m, 1H, CH=CH-O); 8.50 (m, 1H, ArH); 8.27–8.29 (m, 1H, ArH); 7.91–7.93 (m, 1H, ArH); 7.81–7.88 (m, 2H, ArH); 7.75 (brs, 1H, CONHN=C); 7.35–7.37 (m, 2H, ArH); 7.27–7.31 (m, 2H, ArH); 7.17–7.21 (t, *J*=5.2, 1H, NHCH₂CO);

6.89 (m, 1H, $\underline{\text{CH}}=\text{C}-\text{O}$); 6.58 (m, 1H, $\text{CH}-\underline{\text{CH}}=\text{CH}-\text{O}$); 4.89 (s, 2H, NCH_2CO); 4.32 (d, $J=5.2$, 2H, $\text{NH}\underline{\text{CH}}_2\text{CO}$); 4.32 (s, 2H, CH_2ph); 2.18 (s, 3H, CH_3). ^{13}C -NMR: 172.73 ($\text{C}=\text{O}$); 168.82 ($\text{C}=\text{O}$); 159.30 ($\text{C}=\text{O}$); 152.64 ($\text{N}=\underline{\text{C}}-\text{CH}_3$); 146.11 ($\text{C}-\text{Ar}$); 144.58 ($\text{CH}=\underline{\text{C}}-\text{O}$); 144.18 ($\text{CH}=\underline{\text{CH}}-\text{O}$); 140.32 ($\text{C}-\text{Ar}$); 138.39 ($\text{CH}-\text{Ar}$); 133.81 ($\text{CH}-\text{Ar}$); 131.92 ($\text{CH}-\text{Ar}$); 129.75 ($\text{C}-\text{Ar}$); 128.98 (2 $\text{CH}-\text{Ar}$); 128.83 (2 $\text{CH}-\text{Ar}$); 128.07 ($\text{C}-\text{Ar}$); 127.12 ($\text{CH}-\text{Ar}$); 126.40 ($\text{CH}-\text{Ar}$); 112.19 ($\underline{\text{CH}}=\text{CH}-\text{O}$); 110.85 ($\underline{\text{CH}}=\text{C}-\text{O}$); 55.16 (NCH_2CO); 42.88 ($\text{NH}\underline{\text{CH}}_2\text{CO}$); 38.54 (CH_2ph); 13.32 (CH_3).

MS (MALDI, positive mode, matrix DHB) m/z : 480.50 ($\text{M}+\text{Na}$)⁺. Elemental analysis: calculated for $\text{C}_{25}\text{H}_{23}\text{N}_5\text{O}_4$ (457.49): % C, 65.64; % H, 5.07; % N, 15.31. Found: % C, 65.67; % H, 5.11; % N, 15.25.

Synthesis of *N*-(2-(5-amino-3-oxo-2,3-dihydro-1H-pyrazol-1-yl)-2-oxoethyl)-2-(4-benzyl-1-oxophthalazin-2(1H)-yl) acetamide (12d) A mixture of hydrazide molecule **8a** (3.65 g, 0.01 mol) and ethyl cyano acetate (2.26 g, 0.02 mol) in ethanol (30 mL) was refluxed for 8 h. By cooling the solid product formed, filtered off and recrystallized from ethanol solvent gave compound **12d**.

White crystals (84%), m.p. 237–238 °C, ^1H -NMR (400 MHz, DMSO), (δ , ppm), (J , Hz): 9.02 (brs, 1H, $\text{N}-\underline{\text{NH}}-\text{CO}$); 8.39 (m, 1H, ArH); 8.26–8.28 (m, 1H, ArH); 7.90–7.92 (m, 1H, ArH); 7.78–7.86 (m, 2H, ArH); 7.30–7.34 (m, 3H, ArH); 7.17–7.20 (m, 1H, ArH); 7.17–7.20 (t, $J=5.2$, 1H, $\text{NH}\underline{\text{CH}}_2\text{CO}$); 7.08–7.12 (s, 1H, $\text{NH}_2-\underline{\text{C}}=\text{CH}-\text{CO}$); 5.46–5.47 (d, $J=5.2$, 2H, $\text{NH}\underline{\text{CH}}_2\text{CO}$); 4.86 (s, 2H, NCH_2CO); 4.32 (s, 2H, $\text{NH}_2-\underline{\text{C}}=\text{CH}-\text{CO}$); 4.25 (s, 2H, CH_2ph). ^{13}C -NMR: 171.78 ($\text{C}=\text{O}$); 167.36 ($\text{C}=\text{O}$); 166.29 ($\text{C}=\text{O}$); 158.89 ($\text{C}=\text{O}$); 151.93 ($\text{NH}_2-\underline{\text{C}}=\text{CH}-\text{CO}$); 145.33 ($\text{C}-\text{Ar}$); 138.45 ($\text{C}-\text{Ar}$); 133.97 ($\text{CH}-\text{Ar}$); 132.18 ($\text{CH}-\text{Ar}$); 129.18 ($\text{C}-\text{Ar}$); 128.91 (2 $\text{CH}-\text{Ar}$); 128.76 (2 $\text{CH}-\text{Ar}$); 128.09 ($\text{C}-\text{Ar}$); 126.88 ($\text{CH}-\text{Ar}$); 126.21 ($\text{CH}-\text{Ar}$); 125.32 ($\text{CH}-\text{Ar}$); 112.19 ($\text{NH}_2-\underline{\text{C}}=\text{CH}-\text{CO}$); 56.34 (NCH_2CO); 43.24 ($\text{NH}\underline{\text{CH}}_2\text{CO}$); 38.11 (CH_2ph).

MS (MALDI, positive mode, matrix DHB) m/z : 455.48 ($\text{M}+\text{Na}$)⁺. Elemental analysis: calculated for $\text{C}_{22}\text{H}_{20}\text{N}_6\text{O}_4$ (432.44): % C, 61.10; % H, 4.66; % N, 19.43. Found: % C, 61.04; % H, 4.61; % N, 19.50.

Synthesis of 2-(4-benzyl-1-oxophthalazin-2(1H)-yl)-*N*-(2-(3,5-dimethyl-1H-pyrazol-1-yl)-2-oxoethyl) acetamide (12e) A mixture of hydrazide molecule **8a** (3.65 g, 0.01 mol) and acetyl acetone (2 g, 0.02 mol) in ethanol (30 mL) was refluxed for 8 h. By cooling the solid product formed, filtered off and recrystallized from ethanol solvent gave compound **12e**.

White crystals (87%), m.p. 237–238 °C, ^1H -NMR (400 MHz, DMSO), (δ , ppm), (J , Hz): 9.01 (brs, 1H,

$\underline{\text{NH}}\underline{\text{CH}}_2\text{CO}$); 8.26–8.28 (m, 1H, ArH); 7.89–7.91 (m, 1H, ArH); 7.78–7.86 (m, 2H, ArH); 7.30–7.35 (m, 3H, ArH); 7.16–7.20 (m, 2H, ArH); 7.08–7.12 (s, 1H, $\text{CH}_3-\underline{\text{C}}=\underline{\text{CH}}-\text{C}-\text{CH}_3$); 5.46–5.48 (d, $J=5.2$, 2H, $\text{NH}\underline{\text{CH}}_2\text{CO}$); 4.89 (s, 2H, NCH_2CO); 4.25 (s, 2H, CH_2ph); 2.46 (s, 3H, CH_3); 2.43 (s, 3H, CH_3). ^{13}C -NMR: 171.28 ($\text{C}=\text{O}$); 168.37 ($\text{C}=\text{O}$); 159.72 ($\text{C}=\text{O}$); 148.84 ($\underline{\text{C}}-\text{CH}_3$); 146.35 ($\text{C}-\text{Ar}$); 144.33 ($\underline{\text{C}}-\text{CH}_3$); 137.22 ($\text{C}-\text{Ar}$); 133.29 ($\text{CH}-\text{Ar}$); 131.67 ($\text{CH}-\text{Ar}$); 130.45 ($\text{C}-\text{Ar}$); 128.75 (2 $\text{CH}-\text{Ar}$); 128.40 (2 $\text{CH}-\text{Ar}$); 128.07 ($\text{C}-\text{Ar}$); 127.46 ($\text{CH}-\text{Ar}$); 126.74 ($\text{CH}-\text{Ar}$); 125.31 ($\text{CH}-\text{Ar}$); 116.25 ($\text{CH}_3-\underline{\text{C}}=\underline{\text{CH}}-\text{C}-\text{CH}_3$); 55.25 (NCH_2CO); 42.65 ($\text{NH}\underline{\text{CH}}_2\text{CO}$); 38.41 (CH_2ph); 16.21 (CH_3); 13.74 (CH_3).

MS (MALDI, positive mode, matrix DHB) m/z : 452.50 ($\text{M}+\text{Na}$)⁺. Elemental analysis: calculated for $\text{C}_{24}\text{H}_{23}\text{N}_5\text{O}_3$ (429.48): % C, 67.12; % H, 5.40; % N, 16.31. Found: % C, 67.07; % H, 5.47; % N, 16.34.

Biological assays

Cytotoxicity of the synthesized compounds using MTT assay

MCF-7, HepG2 cancer cells and WISH normal cells were obtained from the National Cancer Institute in Cairo, Egypt, they were cultured in complete media of RPMI and DMEM, respectively at 5% carbon dioxide and 37 °C following standard tissue culture work. The cells were grown in “10% fetal bovine serum (FBS) and 1% penicillin–streptomycin” in 96-multiwell plate. All the synthesized compounds were screened for their cytotoxicity using 20 μL of MTT solution (Promega, USA) for 48 hours [46] using untreated and treated cells with concentrations of (0.01, 0.1, 1, 10, and 100 μM) for 48 h. The plate was cultured for 3 h. Percentage of cell viability was calculated following this equation: $100 - (\text{A}_{\text{Sample}}/\text{A}_{\text{Control}}) \times 100$. An ELISA microplate reader was used to measure the absorbance at 690 nm to calculate the viability versus concentration, and the IC_{50} value using GraphPad prism software [47].

EGFR inhibition

The most promising cytotoxic compounds were subjected to EGFR enzyme assay (BPS Bioscience Corporation catalog#40321) using ELISA kit (Enzyme-Linked Immunosorbent Assay) following manufacturer information [48]. The luminescence was measured with a microplate reader at 450 nm by ELISA Reader (PerkinElmer). Inhibition percentage was calculated following this equation: $100 - [\frac{\text{A}_{\text{control}}}{\text{A}_{\text{treated}}} - \text{Control}]$, IC_{50} was determined using GraphPad prism7 using inhibition curves at five different concentrations of each compound.

Flow cytometry using annexin V/PI staining

MDA-MB-231 cells were incubated overnight in 6-well culture plates ($3-5 \times 10^5$ cells/well) and then treated with the IC_{50} values for 48 h with compound **12d**. After that, the cells were incubated in a 100 μ L solution of Annexin binding buffer "25 mM $CaCl_2$, 1.4 M NaCl, and 0.1 M HEPES/NaOH, pH 7.4" in the dark for 30 min with "Annexin V-FITC solution (1:100) and propidium iodide (PI) at a concentration equivalent to 10 g/mL." The labeled cells were then extracted using the CytExpert FACS machine. CytExpert software was used to analyze the data [47, 49, 50].

Molecular docking study

Molecular modeling studies were carried out using Chimera-UCSF and AutoDock Vina on Linux-based systems at the laboratory of Drug Design and Discovery, Suez Canal University. Proteins and compounds structures were prepared and optimized using Maestro, then binding sites inside proteins were determined using grid-box dimensions around the co-crystallized ligands. The investigated compounds were docked against the protein structures of EGFR (PDB=1M17) using AutoDock Vina software following routine work [51, 52]. Vina was used to improve protein and ligand structures and to favor them energetically. Binding activities interpreted molecular docking results in terms of binding energy and ligand-receptor interactions. The visualization was then done with Chimera. ADME pharmacokinetics study was carried out using web-based software "Molsoft" as previously utilized in Youssef et al. [53].

Conclusion

In this study, we synthesized twenty-nine new phthalazinone derivatives starting from 4-Benzyl-2H-phthalazin-1-one (**2**) and their chemical structure were elucidated via different analytical and spectroscopic methods. The cytotoxicity of the synthesized compounds was tested using MTT assay, as well as apoptosis-induction through EGFR inhibition. Compounds **11d**, **12c** and **12d** exhibited potent cytotoxic activities with IC_{50} values of 0.92, 1.89 and 0.57 μ M against MDA-MB-231 cells compared to Erlotinib ($IC_{50}=1.02 \mu$ M). Interestingly compound **12d** exhibited promising potent EGFR inhibition with an IC_{50} value 21.4 nM compared to Erlotinib ($IC_{50}=80$ nM). For apoptosis, compounds **12d** induced apoptosis in MDA-MB-231 cells by 64.4-fold (42.5% compared to 0.66 for the control), Hence, this compound may serve as a potential target-oriented anti-breast cancer agent (Additional file 1).

Abbreviations

EGFR	Epidermal growth factor receptor
RT-PCR	Reverse transcription polymerase
MTT	3-(4,5-Dimethylthiazolyl-2)-2,5-diphenyltetrazolium bromide
SD	Standard deviation
IC_{50}	Half-maximal inhibitory concentration
MCF-7 and MDA-MB-231	Breast cancer cell lines
WISH	Normal cells
G2/M, S, G1, G0	Cell cycle phases

Supplementary Information

The online version contains supplementary material available at <https://doi.org/10.1186/s13065-023-00995-2>.

Additional file 1. Characterization analyses for the synthesized compounds are provided as a Additional file. **Figure S1.** The 1H -NMR spectrum of compound 6a. **Figure S2.** The ^{13}C -NMR spectrum of compound 6a. **Figure S3.** The 1H -NMR spectrum of compound 6b. **Figure S4.** The ^{13}C -NMR spectrum of compound 6b. **Figure S5.** The 1H -NMR spectrum of compound 6c. **Figure S6.** The ^{13}C -NMR spectrum of compound 6c. **Figure S7.** The 1H -NMR spectrum of compound 6d. **Figure S8.** The ^{13}C -NMR spectrum of compound 6d. **Figure S9.** The 1H -NMR spectrum of compound 6e. **Figure S10.** The ^{13}C -NMR spectrum of compound 6e. **Figure S11.** The 1H -NMR spectrum of compound 6f. **Figure S12.** The ^{13}C -NMR spectrum of compound 6f. **Figure S13.** The 1H -NMR spectrum of compound 6g. **Figure S14.** The ^{13}C -NMR spectrum of compound 6g. **Figure S15.** The 1H -NMR spectrum of compound 6h. **Figure S16.** The ^{13}C -NMR spectrum of compound 6h. **Figure S17.** The 1H -NMR spectrum of compound 7a. **Figure S18.** The ^{13}C -NMR spectrum of compound 7a. **Figure S19.** The 1H -NMR spectrum of compound 7c. **Figure S20.** The ^{13}C -NMR spectrum of compound 7c. **Figure S21.** The 1H -NMR spectrum of compound 7d. **Figure S22.** The ^{13}C -NMR spectrum of compound 7d. **Figure S23.** The 1H -NMR spectrum of compound 8a. **Figure S24.** The ^{13}C -NMR spectrum of compound 8a. **Figure S25.** The 1H -NMR spectrum of compound 10a. **Figure S26.** The ^{13}C -NMR spectrum of compound 10a. **Figure S27.** The 1H -NMR spectrum of compound 10b. **Figure S28.** The ^{13}C -NMR spectrum of compound 10b. **Figure S29.** The 1H -NMR spectrum of compound 10c. **Figure S30.** The ^{13}C -NMR spectrum of compound 10d. **Figure S31.** The 1H -NMR spectrum of compound 10d. **Figure S32.** The ^{13}C -NMR spectrum of compound 10d. **Figure S33.** The 1H -NMR spectrum of compound 10e. **Figure S34.** The ^{13}C -NMR spectrum of compound 10e. **Figure S35.** The 1H -NMR spectrum of compound 10f. **Figure S36.** The ^{13}C -NMR spectrum of compound 10f. **Figure S37.** The 1H -NMR spectrum of compound 10h. **Figure S38.** The ^{13}C -NMR spectrum of compound 10h. **Figure S39.** The 1H -NMR spectrum of compound 11a. **Figure S40.** The ^{13}C -NMR spectrum of compound 11a. **Figure S41.** The 1H -NMR spectrum of compound 11b. **Figure S42.** The ^{13}C -NMR spectrum of compound 11b. **Figure S43.** The 1H -NMR spectrum of compound 12a. **Figure S44.** The ^{13}C -NMR spectrum of compound 12a. **Figure S45.** The 1H -NMR spectrum of compound 12c. **Figure S46.** The ^{13}C -NMR spectrum of compound 12d. **Figure S47.** The 1H -NMR spectrum of compound 12d. **Figure S48.** The ^{13}C -NMR spectrum of compound 12e.

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Author contributions

I.A.I., S.M.E-2, H.A.S. designed the idea of synthetic organic chemistry, and made formal analyses of characterization charts, and revision of chemistry part. S.M.E-1 synthesized the compounds under the supervision of I.A.I., S.M.E-2 and H.A.S. While M.S.N. designed the study rational, idea and carried out the biological analyses with molecular docking studies. All authors contributed to writing the manuscript with their corresponding parts and agreed to the final manuscript form.

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Availability of data and materials

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Declarations**Ethics approval and consent to participate**

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Consent for publication

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Competing interests

The authors declare that they have no competing interests.

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