

# Synthesis of Novel Phthalazinedione-Based Derivatives with Promising Cytotoxic, Anti-bacterial, and Molecular Docking Studies as VEGFR2 Inhibitors

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Cite This: *ACS Omega* 2022, 7, 26800–26811



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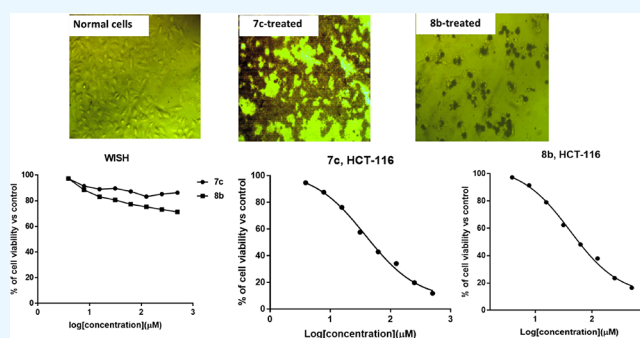


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**ABSTRACT:** The parent ester methyl-3-[2-(4-oxo-3-phenyl-3,4-dihydro-phthalazin-1-yloxy)-acetyl-amino] has 18 compounds. The starting material for alkanooates, their corresponding hydrazides, hydrazones, and dipeptides were produced by chemoselective O-alkylation of 2-phenyl-2,3-dihydrophthalazine-1,4-dione with ethyl chloroacetate(4-oxo-3-phenyl-3,4-dihydro-phthalazin-1-yloxy) acetic acid methyl ester. The starting ester was hydrazinolized, then azide coupled with amino acid ester hydrochloride to produce several parent esters, and then hydrazinolized to produce parent hydrazides. These hydrazides were used to make a series of dipeptides by reacting them with amino acid ester hydrochloride under azide coupling conditions, and they were also condensed with a number of aldehydes to make the hydrazones. These derivatives were subjected to cytotoxicity against HCT-116 and MDA-MB-231 cells and anti-bacterial and molecular docking studies. Results indicated that the tested compounds, especially **7c** and **8b** with the phenyl phthalazinone moieties, had promising cytotoxicity against the HCT-116 cells with IC<sub>50</sub> values of 1.36 and 2.34 μM, respectively. Additionally, the promising compounds **7c** and **8b** exhibited poor cytotoxicity against WISH cells with much higher IC<sub>50</sub> values, so they were safe against normal cells. Compound **8c** exhibited potent anti-bacterial activity with inhibition zones of 12 and 11 mm against *Staphylococcus aureus* and *Escherichia coli*, respectively. The molecular docking results of compounds **7c** and **8b** revealed a good binding disposition and the ligand–receptor interactions like the co-crystallized ligand of the VEGFR2 protein, which may be the proposed mode of action. Finally, compounds **7c** and **8b** exhibited good ADME pharmacokinetics with good drug-likeness parameters. Hence, detailed studies for the mechanism of action of such compounds are highly recommended for the development of new potent anti-cancer and anti-bacterial agents.



## INTRODUCTION

Cancer is a leading cause of death and a major determinant of life expectancy worldwide. Liver, lung, and gastric cancer for males and breast, lung, and colorectal cancer for females are the leading causes of cancer death.<sup>1</sup> Our research group has recently directed our attention on finding new anti-cancer drugs,<sup>2,3</sup> where anti-cancer drug research is never-ending, to develop products with a lower toxicity and greater selectivity for tumor cells. Phthalazinedione and its derivatives have been studied by chemists and pharmacologists for their broad-spectrum natural exercises and applications, which include antitumor,<sup>4</sup> cytotoxic,<sup>5</sup> anticonvulsant, cardiotoxic, vasorelaxant, antimicrobial, and anti-inflammatory properties.<sup>6</sup>

Several phthalazine precursors have emerged as promising and appealing candidates in the development of novel anti-cancer agents against breast, liver, and colon cancer (Figure 1A).<sup>7–11</sup> Because VEGFR2 is highly expressed in several solid tumors and plays an important role in the apoptosis process, VEGFR-2 inhibition has emerged as a promising approach for developing new therapies for many apoptosis-dependent

cancers. Some promising phthalazine derivatives, such as PTK 787, ZD 6474, and Vandetanib, were investigated as inhibitors of vascular endothelial growth factor receptor II (VEGFR-2) (Figure 1B).

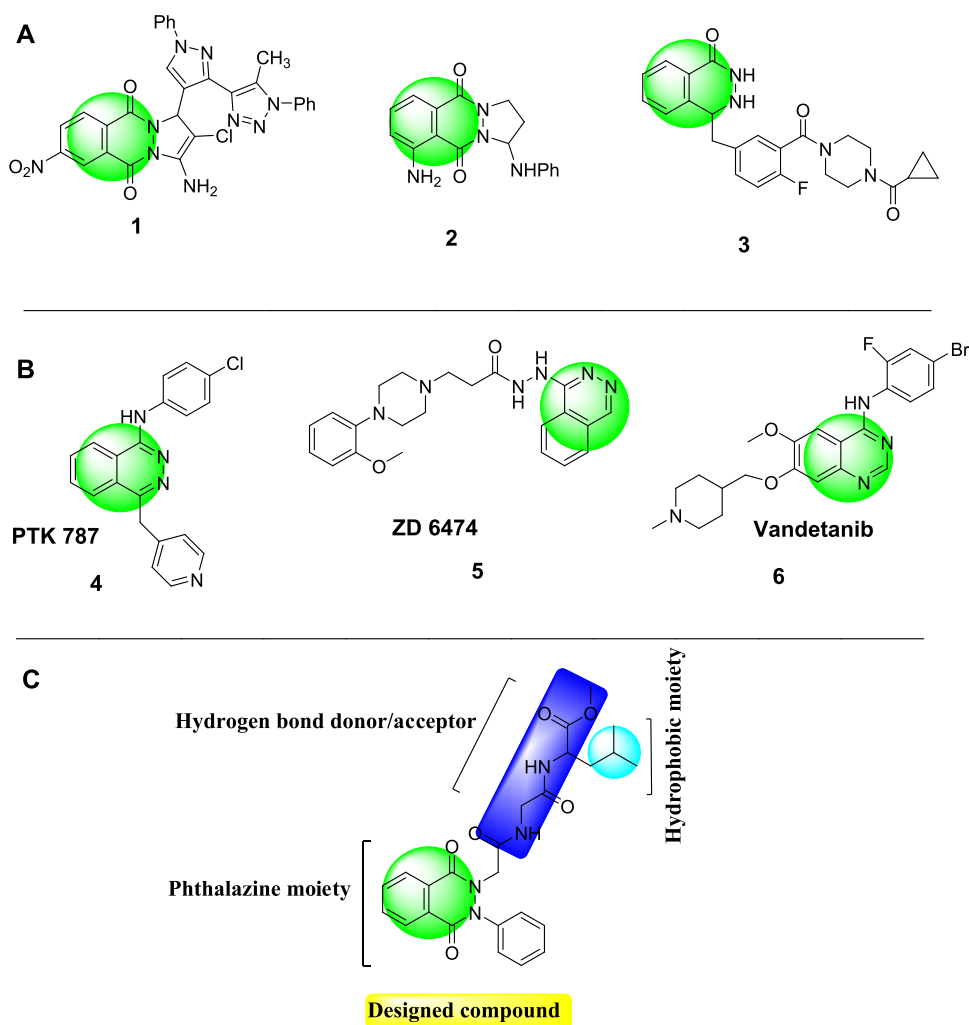
So, the aim of this study is to design and synthesize novel phthalazinedione derivatives, maintaining the essential pharmacophoric regions of phthalazine, hydrogen bond donors/acceptors, and the lipophilic moieties targeting VEGFR2 inhibition, against breast and colon cancer.

Received: May 22, 2022

Accepted: June 10, 2022

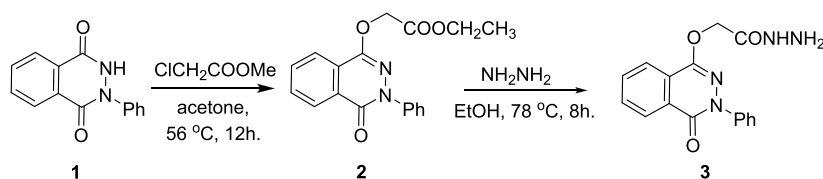
Published: June 27, 2022





**Figure 1.** (A) Some common phthalazine-based compounds are used as anti-cancer drugs. (B) Some common phthalazine-based compounds as VEGFR2 inhibitors. (C) Design strategy for the synthesized compounds.

### Scheme 1. Synthesis of Ester 2 and Corresponding Hydrazide 3 from Phthalazinedione



## RESULTS AND DISCUSSION

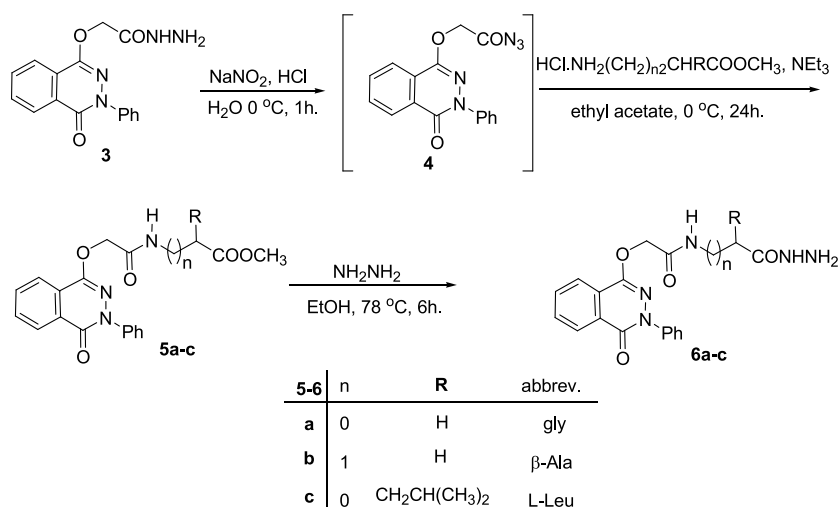
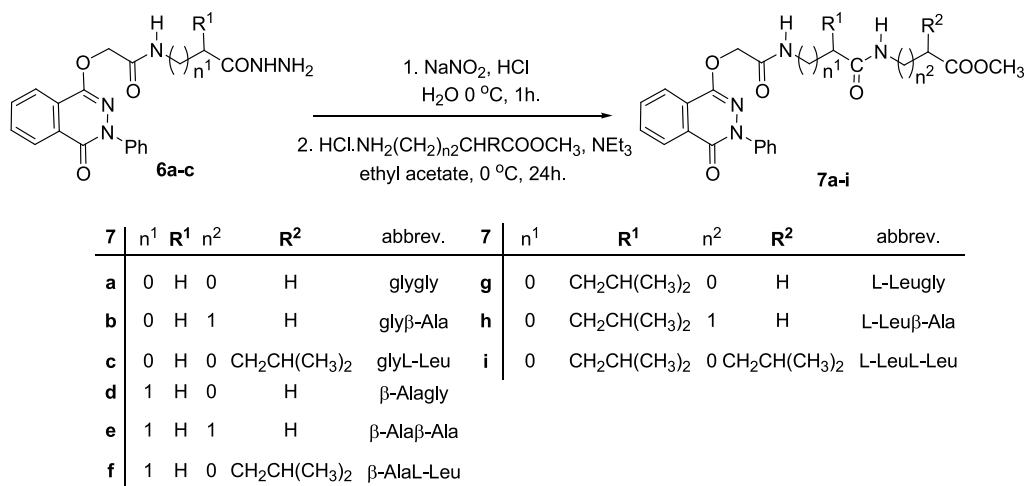
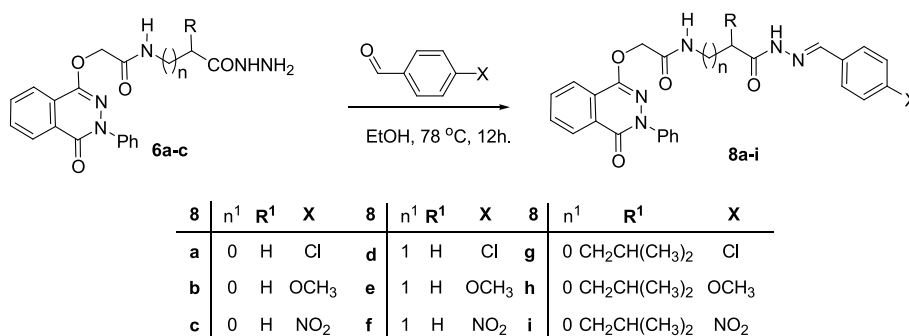
**Chemistry.** Our research team quickly discovered that chemoselective alkylation of amides and thioamides could be regulated.<sup>12–18</sup> We agreed to extend our results to the structure modification of 2-phenyl-2,3-dihydrophthalazine-1,4-dione (1), our model heterocyclic amide, as a follow-up to these studies. The single O-substituted result of the reaction of model ambient nucleophile 1 with ethyl chloroacetate in acetone in the presence of  $\text{K}_2\text{CO}_3$  under reflux for 12 h was (4-oxo-3-phenyl-3,4-dihydrophthalazin-1-yloxy)acetic acid ethyl ester (2). The reaction was selectively carried out on the O atom rather than the N atom or even in a competitive reaction involving both atoms.

Calculating the interaction between HOMO on the oxygen atom of the surrounding high-energy nucleophile and the low-energy LUMO electrophile, which leads to a narrow energy gap

and high reactivity, may explain the obtained chemoselective O-alkylation reaction. Pearson's theory of hard soft acid base was used to arrive at this conclusion (HSAB). In ethanol, hydrazinolysis of ester 2 by refluxing with hydrazine hydrate for 8 h yields (4-oxo-3-phenyl-3,4-dihydrophthalazin-1-yloxy)acetic acid hydrazide (3) in 72% yield, as shown in Scheme 1.

The characteristic  $^1\text{H}$  NMR spectral  $\text{NCH}_2$ , NH ( $\text{D}_2\text{O}$  exchangeable), and 9 aromatic protons gave signals at 4.32, 11.50, and 6.89–8.32 ppm for hydrazide 3. Hydrazide 3 is an excellent precursor for modifying the structure of subordinate phthalazines by using an azide strategy to attach another amino acid through a peptide bond. The azide strategy is a well-known peptide synthesis strategy that has the benefit of minimizing racemization while still avoiding any interferometer byproducts.

The azide 4 was formed by reacting hydrazide 3 with a  $\text{NaNO}_2/\text{HCl}$  mixture in water for 1 h at  $0\text{ }^\circ\text{C}$  and then extracting

**Scheme 2. Synthesis of Methyl-3-[2-(4-oxo-3-phenyl-3,4-dihydro-phthalazin-1-yloxy)-acetylamino]alkanoate 5a–c and Their Corresponding Hydrazides 6a–c**

**Scheme 3. Synthesis of Dipeptides 7a–i from Corresponding Hydrazides 6a–c**

**Scheme 4. Synthesis of Schiff's Bases 8a–i from Corresponding Hydrazides 6a–c**


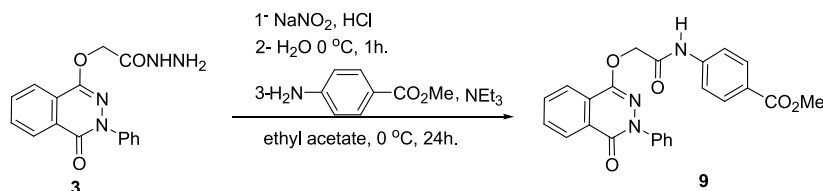
with ethyl acetate. To produce methyl-3-[2-(4-oxo-3-phenyl-3,4-dihydro-phthalazin-1-yloxy)-acetylamino]alkanoates **5a–c**, the *in situ* conveyed azide **4** solution was dynamically applied to amino acid methyl ester hydrochloride, glycine,  $\beta$ -alanine, and L-leucine within the sight of triethyl amine at 40 °C.

Hydrazinolysis of amino acid ester derivatives **5a–c** in ethanol under reflux for 8 h with fabulous hydrazine hydrate. **Scheme 2** shows how to obtain the hydrazides **6a–c** in high yield.

<sup>1</sup>H NMR was used to elucidate the synthetic construction of methyl-3-[2-(4-oxo-3-phenyl-3,4-dihydro-phthalazin-1-yloxy)-acetylamino]acetate (**5a**), which yielded the following signals: a broad signal at 6.91 ppm of NHCH<sub>2</sub>, a doublet signal at 4.07 ppm of NHCH<sub>2</sub>, and a singlet signal at 3.69 ppm of OCH<sub>3</sub>.

The phthalazines containing dipeptide moieties were also obtained using azide coupling methodology by reacting hydrazides **6a–c** with the NaNO<sub>2</sub>/HCl blend and addition of amino acid methyl ester hydrochloride, glycine,  $\beta$ -alanine, and L-

## Scheme 5. Synthesis of Methyl-4-[2-(4-oxo-3-phenyl-3,4-dihydro-phthalazin-1-yloxy)-acetylamino]-benzoate (9)



leucine in the presence of triethyl amine at 0 °C for 24 h to produce methyl-3-3-[2-(1,4-dioxo-3-phenyl-3,4-dihydro-1H-phthalazin-2-yl)-acetylamino]propionyl-aminoalkanoates **7a–i**, Scheme 3.

Different analysis methods were used to elucidate the structure of methyl 2-[2-(4-oxo-3-phenyl-3,4-dihydro-phthalazin-1-yloxy)-acetylamino]-acetylamino-acetate (**7a**). For example, the <sup>1</sup>H NMR spectra of **7a** revealed three singlet signals at 4.83, 4.00, and 3.87 ppm of three (–CH<sub>2</sub>) and a singlet signal at 3.65 ppm of OCH<sub>3</sub>.

Condensations of hydrazides **6a–c** with various aldehydes in ethanol for 12 h under reflux conditions provide the corresponding Schiff's bases **8a–i** (Scheme 4).

Various investigation methods were used to clarify the structure, such as the <sup>1</sup>H NMR spectra of *N*-[2-(4-chlorobenzylidene-hydrazinocarbonyl)-ethyl]-2-(4-oxo-3-phenyl-3,4-dihydro-phthalazin-1-yloxy)-acetamide (**8a**). The following signals appeared in –acetamide (**8a**): a broad signal of NH at 11.47 ppm, multiplet signal of 4H of the aromatic ring of chlorobenzaldehyde at 7.37–7.52 ppm, and singlet signal of CH at 10.11 ppm.

The phthalazine-containing mono-peptide of 4-amino methylbenzoate moieties was also obtained using an azide coupling methodology by reacting hydrazide **3** with the NaNO<sub>2</sub>/HCl blend and adding 4-amino methylbenzoate at 0 °C for 24 h to produce methyl-4-[2-(4-oxo-3-phenyl-3,4-dihydro-phthalazin-1-yloxy)-acetylamino]-benzoate (**9**) Scheme 5.

**Biology. Cytotoxicity against HCT-116 and MDA-MB-231 Cell Lines.** As VEGFR2 is one of the proteins that regulate the proliferation of HCT-116 and MDA-MB-231 cells, some of the synthesized compounds were screened for their cytotoxicity against HCT-116 and MDA-MB-231 cell lines using the MTT assay. Values of IC<sub>50</sub> values were calculated, as shown in Table 1.

Table 1. IC<sub>50</sub> (μM) of the Phenyl Phthalazinone Derivatives against HCT-116 and MDA-MB-231 Cells

compound	IC <sub>50</sub> (μM) <sup>a</sup>	
	HCT-116	MDA-MB-231
<b>2</b>	23.9	36.2
<b>9</b>	34.3	50≤
<b>5c</b>	26.39	12.36
<b>6b</b>	13.69	6.89
<b>7c</b>	1.36	7.67
<b>7d</b>	26.39	34.6
<b>7e</b>	16.39	36.9
<b>7i</b>	50≤	16.3
<b>8a</b>	6.34	27.3
<b>8b</b>	2.34	16.03
<b>8i</b>	13.5	24.3
cisplatin	3.67	5.71

<sup>a</sup>Values are calculated using the GraphPad prism software using the non-linear regression curve fit; ND = not determined.

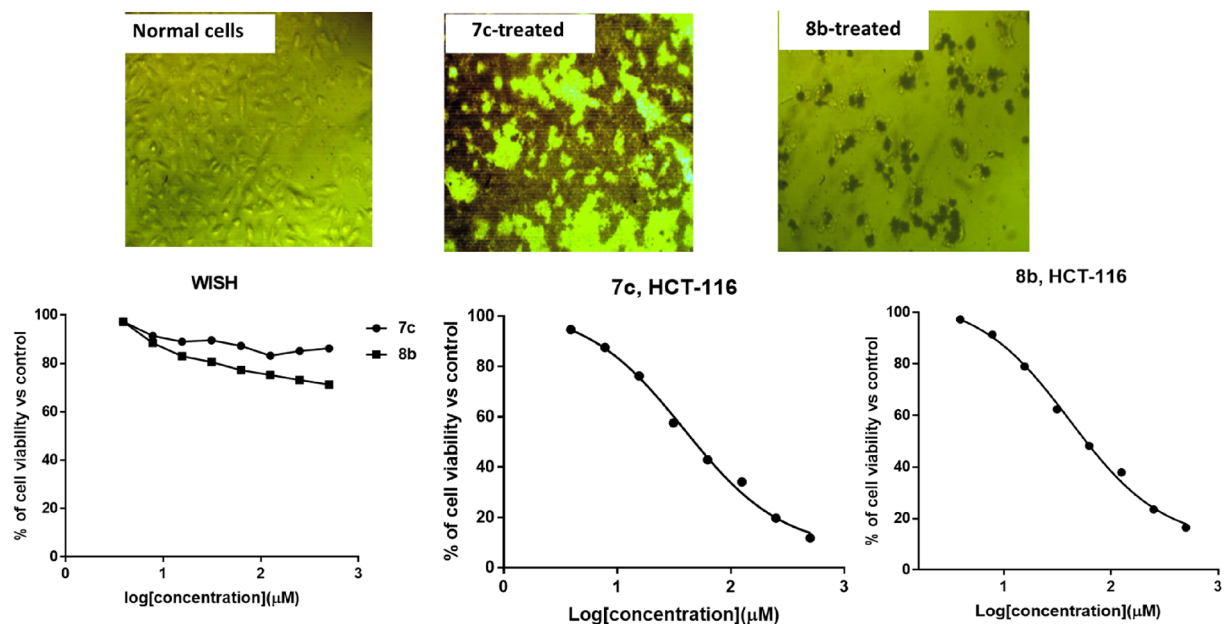
Results indicated that the tested compounds, especially **7c** and **8b** with the phenyl phthalazinone moieties, had promising cytotoxicity against the HCT-116 cells with IC<sub>50</sub> values of 1.36 and 2.34 μM, respectively. Compound **7c** inhibited the proliferation of MDA-MB-231 cells with an IC<sub>50</sub> value of 6.67 μM, while compound **8b** exhibited moderate cytotoxicity with an IC<sub>50</sub> value of 16.03 μM. Other tested compounds exhibited poor cytotoxic activities against the two tested cell lines. As seen in Figure 2, compounds **7c** and **8b** showed promising cytotoxicity, having cell viability by 10.36 and 11.69% at a concentration of 100 μM. Additionally, the promising compounds **7c** and **8b** exhibited poor cytotoxicity against WISH cells with much higher IC<sub>50</sub> values, so they were safe against normal cells.

Our results agreed with previous studies<sup>9,10,19–22</sup> that investigated novel phthalazine-based compounds against a panel of cancer cells; the studies proved the promising cytotoxic activities of such compounds with near IC<sub>50</sub> values in a selective way. Additionally, they investigated the apoptotic cell death as the mechanism of action.

**Anti-bacterial Activity.** Results of anti-bacterial study are reported as the zone of inhibition and are summarized in Table 2, indicating that all tested compounds possess promising anti-bacterial activity against *Staphylococcus aureus* and *Escherichia coli* compared to the starting material 2-phenyl-2,3-dihydrophthalazine-1,4-dione **2**. Compound **8c** exhibited a potent anti-bacterial activity with inhibition zones of 12 and 11 mm against *S. aureus* and *E. coli*, respectively. Other compounds exhibited promising anti-bacterial activity with an inhibition zone of 6–10 mm. These findings supported the main aim of our work, which is to develop new potent anti-bacterial derivatives than the starting compound.

**In Silico Study. Molecular Docking.** A molecular docking study was performed to confirm the binding interactions of the most active cytotoxic compounds **7c** and **8b** against the HCT-116 cells and explore their binding modes in conjunction with the co-crystallized ligands inside the active site VEGFR2 protein (1YWN). By visualization of the ligand–receptor interactions of the co-crystallized ligand (LIF), it was found that Glu 883, Asp 1044, and Cys 917 are the key amino acids with which it interacts. A molecular docking study revealed that both compounds **7c** and **8b** were effectively docked inside the protein active site with a good binding interactive mode like the co-crystallized ligand, as shown in the surface representation (Figure 3). As seen in Table 3, with the summarized ligand–receptor interactions, compound **7c** docked within the 1YWN with a high binding energy of –25.12 Kcal/mol, forming 3HB with Glu 883, Asp 1044, and Cys 917 as the main key amino acids with which the co-crystallized ligand (LIF) binds and one arene–arene with Lys 866. Meanwhile, compound **8b** was docked with a good binding energy of –12.93 Kcal/mol, but it only formed 1HB with Glu 883.

Thus, from the molecular docking results of compounds **7c** and **8b**, a good binding disposition and the ligand–receptor



**Figure 2.** Cytotoxic activity of compounds **7c** and **8b** against HCT-116 and normal WISH cells. Upper panel: microscopy investigation after 48 h of incubation (40X magnification); lower panel: percentages of cell viabilities with increasing concentrations against the three tested cells.

**Table 2.** Anti-bacterial Activity of the Compounds against *S. aureus* and *E. coli*<sup>a</sup>

compound	Gram +ve	Gram -ve
	<i>S. aureus</i> NCMB6571	<i>E. coli</i> ATCC 25922
7a	7 ± 0.1	7 ± 0
7e	8 ± 0.2	7 ± 1.4
7h	9.5 ± 0.7	8 ± 1.4
chloroform	NA	NA
5a	8 ± 1.4	9 ± 0.1
8c	12 ± 1.4	11 ± 1.4
8d	8 ± 0.1	9 ± 0.1
8h	6 ± 0.1	10 ± 1.4
DEMSEO	NA	NA
2-phenyl-2,3-dihydrophthalazine-1,4-dione <b>2</b>	3 ± 0.1	NA

<sup>a</sup>Values are Mean ± SD of measured inhibition zone diameter (mm) of two replicate. NA: Not Active.

interactions like the co-crystallized ligand were maintained, and we may link their cytotoxic activities toward the VEGFR2 protein as the proposed mode of action.

**ADME Pharmacokinetics.** A bioinformatics study was conducted to establish the physicochemical properties and drug-like properties of the lead compounds **7c** and **8b** toward the tested proteins. The compounds examined were all well-permeable and absorbed. As shown in Table 4, compounds had two donors and six acceptors for hydrogen bonding. In addition, they exhibited log *P* values between 2.25 and 2.27, so they were well tolerated by cell membranes. For controlling conformational changes and oral bioavailability, the rotatable bond number (nrotb) should be ≤10. Additionally, compounds exhibited good gastrointestinal tract absorption according to the BOILED-Egg model, as shown in Figure 4.

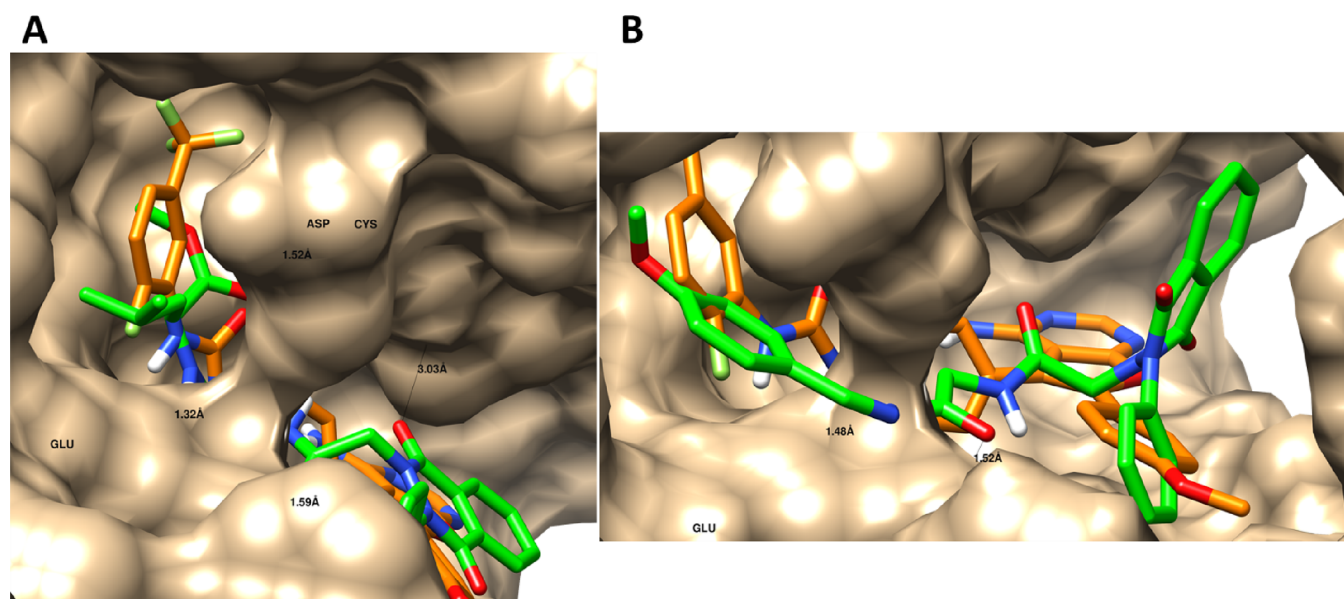
## EXPERIMENTAL PART

**Chemistry. General Procedures.** TLC was performed on silica gel 60 F254 aluminum sheets (E. Merck, layer thickness:

0.2 mm) in the preparation of dissolvable frameworks (S1: petroleum ether/ethyl acetate (2:1); S2: petroleum ether/ethyl acetic acid derivation (1:1)). The UV lamp recognized the spots on thin layer surfaces. 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, elemental 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 the <sup>1</sup>H NMR spectra. The phthalazinediones were made from the precursor 2-phenyl-2,3-dihydrophthalazine-1,4-dione (**1**) according to the method described by Gildeh *et al.*<sup>23</sup>

**Synthesis of Ethyl (1,4-Dioxo-3-phenyl-3,4-dihydro-1H-phthalazin-2-yl)acetate (2).** Ethyl chloroacetate (12.25 mL, 0.1 mol) and 1.2 equiv of K<sub>2</sub>CO<sub>3</sub> were applied to a solution of 2-phenyl-2,3-dihydrophthalazine-1,4-dione (**1**) (23.8 g, 0.1 mol) in 50 mL of dry acetone and 10 mL of DMF mixture. Overnight, the reaction mixture was refluxed. The clear reaction mixture solution was poured over crushed ice, and the solid product obtained was filtered and washed with water several times and was crystallized from aq. EtOH to obtain pure ethyl-3-(1,4-dioxo-3-phenyl-3,4-dihydrophthalazin-2(1H)-yl)-2-oxopropanoate(**2**). Off-white, crystals yield (78%), m.p.166–164 °C. <sup>1</sup>H NMR spectrum, (400 MHz, CDCl<sub>3</sub>), δ, ppm (*J*, Hz):8.38–8.41 (2H, m, ArH);8.00–8.02 (2H, m, ArH);7.71–7.77 (2H, m, ArH);7.61 (1H, d, *J* = 8, ArH);7.17–7.38 (2H, m, ArH);4.82 (2H, s, CH<sub>2</sub>); 4.17 (2H, q, *J* = 7.1, CH<sub>2</sub>CH<sub>3</sub>);1.16 (3H, t, *J* = 7.1, CH<sub>2</sub>CH<sub>3</sub>).MS. (MALDI, positive mode, matrix DHB) *m/z*: 347 (M + Na)<sup>+</sup>. Anal. Calcd. for C<sub>18</sub>H<sub>16</sub>N<sub>2</sub>O<sub>4</sub>(324.3): C, 66.66; H, 4.97; N, 8.64. Found: C, 66.74; H, 5.06; N, 8.73.

**Synthesis of (4-Oxo-3-phenyl-3,4-dihydro-phthalazin-1-yloxy)-acetic Acid Hydrazide (3).** In 25 mL of ethanol, a mixture of ester **2** (3.24 g, 0.01 mol) and hydrazine hydrate (5 mL, 99%) was heated under reflux for 8 h. The resulting precipitate was cooled, then purified, and crystallized from MeOH to yield the hydrazide **3**. White fine powder (72%) with a



**Figure 3.** Binding disposition of the co-crystallized ligand (orange-colored) and the docked compound (green-colored) of (A) **7c** and (B) **8b** toward the VEGFR2 protein (1YWN).

melting point of 262–264 °C. (400 MHz,  $\text{CDCl}_3$ ),  $\delta$ , ppm (J, Hz): 9.4 (1H, s, NH); 8.21–8.34 (2H, m, ArH); 7.95–8.1 (2H, m, ArH); 7.70–7.73 (2H, m, ArH); 7.48–7.52 (1H, m, ArH); 7.35–7.39 (2H, m, ArH); 4.81 (2H, s,  $\text{CH}_2$ ); 4.35 (2H, s,  $\text{NH}_2$ ).  $^{13}\text{C}$  NMR (100.0 MHz,  $\text{CDCl}_3$ ),  $\delta$ , ppm: 65.0 ( $\text{OCH}_2$ ), 124.3, 124.5, 125.6 (2C), 127.38, 127.41 (2C), 128.9, 129.4, 133.3, 134.1, 142.1, 149.1, 157.9 (C=O), 166.6 (C=O). MS. (MALDI, positive mode, matrix DHB)  $m/z$ : 334 ( $\text{M} + \text{Na}$ )<sup>+</sup>. Anal. Calcd. for  $\text{C}_{16}\text{H}_{14}\text{N}_4\text{O}_3$  (310.3) C, 61.93; H, 4.55; N, 18.06. Found: C, 62.01; H, 4.63; N, 18.14.

**General Procedure for Preparation of Methyl-3-[2-(4-oxo-3-phenyl-3,4-dihydro-phthalazin-1-yloxy)-acetylamino]-alkanoate **5a–c**.** Hydrochloric acid (5 N, 30 mL), included parcel shrewd under blending a cold solution (0 °C) of sodium nitrite (0.7 g, 10.0 mmol) in water (30 mL) to a cold solution (–5 °C) of hydrazide **3** (8.0 mmol) in acetic acid (60 mL) at the same temperature for 30 min. The *in situ* created azide **4a–c** was extricated with cold ethyl acetate and washed gradually with cold water, 5%  $\text{NaHCO}_3$ , and water. The azide was used without further purification in the next step after drying over anhydrous sodium sulfate. Amino acid methyl ester hydrochloride (9.0 mmol), glycine,  $\beta$ -alanine, and L-leucine were added to a cold dried solution of azide **4a–c** that had already been prepared. After that, the mixture was kept in the fridge for 12 h and then at room temperature for another 12 h. The reaction mixture was washed with 0.1N HCl, water, 5%  $\text{NaHCO}_3$ , and water and then dried over anhydrous sodium sulfate, the solvent was evaporated in vacuum, and the buildup was crystallized from ethyl acetate-petroleum ether to yield the products **5a–c**.

**Methyl [2-(4-Oxo-3-phenyl-3,4-dihydro-phthalazin-1-yloxy)-acetylamino]-acetate (**5a**).** Off-white crystals (77%), m.p. 118–119 °C (400 MHz,  $\text{CDCl}_3$ ),  $\delta$ , ppm (J, Hz): 8.42–8.39 (1H, m, ArH); 7.98–7.96 (1H, m, ArH); 7.80–7.75 (2H, m, ArH); 7.63 (2H, d,  $J = 8.1$ , ArH); 7.37–7.35 (2H, m, ArH); 7.24–7.22 (1H, m, ArH); 6.91 (1H, bs, NH); 4.84 (2H, s,  $\text{CH}_2$ ); 4.06 (2H, d,  $J = 6.9$ ,  $\text{NHCH}_2$ ); 3.69 (3H, s,  $\text{OCH}_3$ ). MS. (MALDI, positive mode, matrix DHB)  $m/z$ : 390 ( $\text{M} + \text{Na}$ )<sup>+</sup>. Anal. Calcd. for  $\text{C}_{19}\text{H}_{17}\text{N}_3\text{O}_5$  (367.3) C, 62.12; H, 4.66; N, 11.44. Found: C, 62.30; H, 4.84; N, 11.63.

**Methyl-3-[2-(4-oxo-3-phenyl-3,4-dihydro-phthalazin-1-yloxy)-acetylamino]-propionate (**5b**).** Off-white crystals (81%), m.p. 114–115 °C.  $^1\text{H}$  NMR (400 MHz,  $\text{CDCl}_3$ ),  $\delta$ , ppm (J, Hz): 8.42 (2H, d,  $J = 8.0$ , ArH); 7.9 (2H, d,  $J = 8.1$ , ArH); 7.63–7.83 (2H, m, ArH); 7.19–7.40 (3H, m, ArH); 6.87 (1H, bs, NH); 4.78 (2H, s,  $\text{CH}_2$ ); 3.57 (3H, s,  $\text{OCH}_3$ ); 3.31–3.29 (2H, m,  $\text{NHCH}_2$ ); 2.20–2.24 (2H, m,  $\text{CH}_2\text{CH}_2$ ). MS. (MALDI, positive mode, matrix DHB)  $m/z$ : 404 ( $\text{M} + \text{Na}$ )<sup>+</sup>. Anal. Calcd. for  $\text{C}_{20}\text{H}_{19}\text{N}_3\text{O}_5$  (381.3): C, 62.99; H, 5.02; N, 11.02. Found: C, 63.07; H, 5.14; N, 11.11.

**Methyl-4-methyl-2-[2-(4-oxo-3-phenyl-3,4-dihydro-phthalazin-1-yloxy)-acetylamino]-pentanoate (**5c**).** Off-white crystals (72%), m.p. 122–123 °C.  $^1\text{H}$  NMR (400 MHz,  $\text{CDCl}_3$ ),  $\delta$ , ppm (J, Hz): 8.42–8.45 (2H, m, ArH); 7.96–7.98 (2H, m, ArH); 7.75–7.83 (2H, m, ArH); 7.63–7.65 (1H, m, ArH); 7.19–7.39 (2H, m, ArH); 6.87 (1H, bs, NH); 4.85 (2H, s,  $\text{CH}_2$ ); 4.67 (1H, q, CH); 3.65 (3H, s,  $\text{OCH}_3$ ); 1.74–1.72 (2H, m,  $\text{CH}_2$ ); 1.55 (1H, m,  $(\text{CH}_3)_2\text{CH}$ ); 0.83 (6H, d,  $(\text{CH}_3)_2\text{CH}$ ). MS. (MALDI, positive mode, matrix DHB)  $m/z$ : 446 ( $\text{M} + \text{Na}$ )<sup>+</sup>. Anal. Calcd. for  $\text{C}_{23}\text{H}_{25}\text{N}_3\text{O}_5$  (423.46): C, 65.24; H, 5.95; N, 9.92. Found: C, 65.43; H, 6.14; N, 10.10.

**Synthesis of Hydrazides **6a–c**.** Hydrazine hydrate was added to a solution of ester **5a–c** (3.67 g, 0.01 mol) in methyl alcohol (30 mL) (2.5 mL, 0.05 mol). To obtain the corresponding hydrazide, the reaction mixture was refluxed for 4 h and cooled, and the white precipitate was purified and crystallized from MeOH.

**Synthesis of N-Hydrazinocarbonylmethyl-2-(4-oxo-3-phenyl-3,4-dihydro-phthalazin-1-yloxy)-acetamide (**6a**).** Off-white crystals (87%), m.p. 193–195 °C.  $^1\text{H}$  NMR (400 MHz, DMSO),  $\delta$ , ppm (J, Hz): 9.4 (1H, bs, NH); 8.34–8.37 (2H, m, ArH); 8.21–8.23 (2H, m, ArH); 7.97–8.04 (2H, m, ArH); 7.71–7.73 (1H, m, ArH); 7.35–7.52 (2H, m, ArH); 6.32 (1H, bs, NH); 4.79 (2H, s,  $\text{CH}_2$ ); 4.02 (2H, d,  $J = 6.9$ ,  $\text{NHCH}_2$ ); 4.47 (2H, bs,  $\text{NH}_2$ ). MS. (MALDI, positive mode, matrix DHB)  $m/z$ : 390 ( $\text{M} + \text{Na}$ )<sup>+</sup>. Anal. Calcd. for  $\text{C}_{18}\text{H}_{17}\text{N}_5\text{O}_4$  (367.4): C, 58.85; H, 4.66; N, 19.06. Found: C, 59.06; H, 4.84; N, 19.12.

**Synthesis of N-(2-Hydrazinocarbonyl-ethyl)-2-(4-oxo-3-phenyl-3,4-dihydro-phthalazin-1-yloxy)-acetamide (**6b**).**

**Table 3. Summarized Ligand–Receptor Interactions and 3D Visualization of the Two Docked Compounds 7c and 8b inside the VEGFR2 Protein (1YWN)<sup>a</sup>**

Compound	VEGFR2 (1YWN)		
		-25.12 Kcal/mol	4 HB with Glu 883, Asp 1044, Cys 917 and Lys 866
7c			
	-12.93 Kcal/mol	2 HB with Glu 883, Lys 866	-
8b			

<sup>a</sup>Docking calculation using AutoDock4 software was validated by the self-docking step that fulfills RMSD lower than 2.

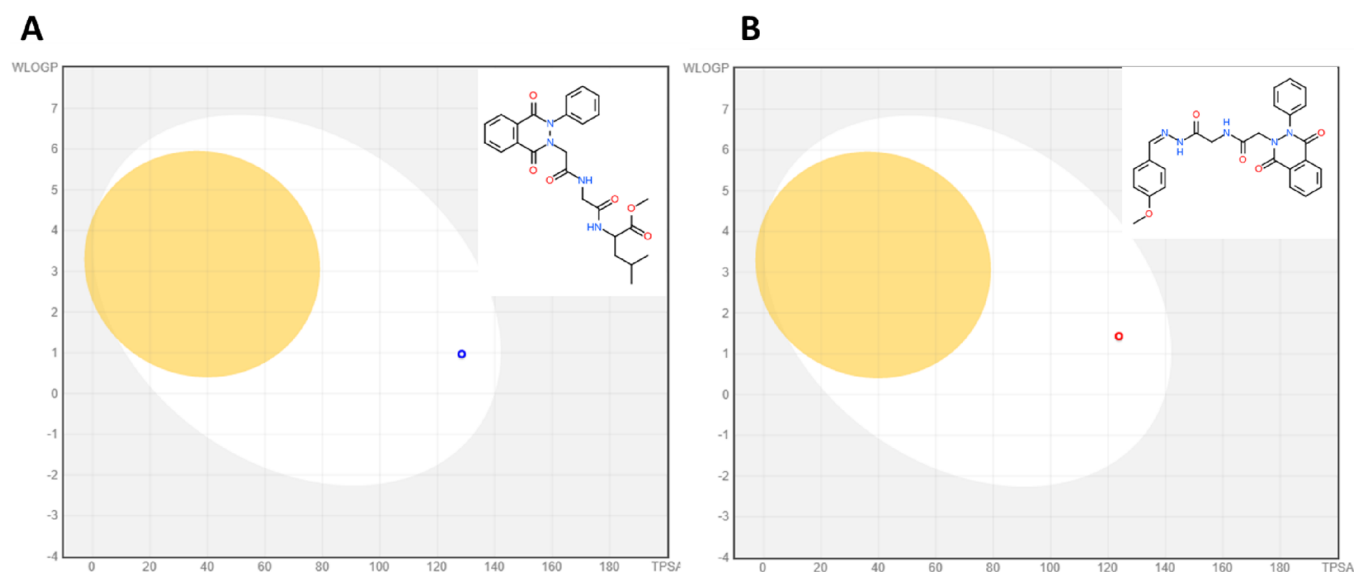
**Table 4. Molecular Properties and Drug-Likeness<sup>a</sup>**

website	ADME	compound	
		7c	8b
molinspiration 2018.10	Mwt (D)	480.5	485.5
	MV (Å <sup>3</sup> )	432.63	424.7
	PSA (Å <sup>2</sup> )	128.5	123.8
	log <i>p</i>	2.25	2.27
	nrotb	10	8
MolSoft	nviolations	0	0
	HBA	6	6
	HBD	2	2
	solubility (mg/L)	2000	1125
SwissADME	drug-likeness score	0.27	0.48
	drug-likeness (Lipinski Pfizer filter)	yes, drug like, MW ≤ 500, log <i>p</i> ≤ 4.15, HBA ≤ 10, and HDD ≤ 5	

<sup>a</sup>Mwt: molecular weight, MV: molecular volume, PAS: polar surface area, log *p*: octanol–water partition coefficient, nrotb: number of rotatable bond, nviolations: number of violations, HBA: hydrogen bond acceptor, HBD: hydrogen bond donor.

Off-white crystals (84%), m.p. 186–188 °C. <sup>1</sup>H NMR (400 MHz, DMSO), δ, ppm (*J*, Hz): 9.02 (1H, bs, NH); 8.34–8.36 (2H, d, *J* = 8.2, ArH); 8.21 (2H, d, *J* = 8.0, ArH); 7.95–8.05 (2H, m, ArH); 7.69–7.71 (2H, m, ArH); 7.45–7.52 (1H, m, ArH); 6.02 (1H, bs, NH); 4.77 (2H, s, CH<sub>2</sub>); 4.51 (2H, bs, NH<sub>2</sub>); 3.4 (2H, m, CH<sub>2</sub>); 2.26 (2H, m, CH<sub>2</sub>). <sup>13</sup>C NMR (100.0 MHz, CDCl<sub>3</sub>), δ, ppm: 33.3 (CH<sub>2</sub>), 35.7 (CH<sub>2</sub>), 65.7 (OCH<sub>2</sub>), 124.3, 125.6, 127.4, 128.9, 129.4, 133.3, 134.2, 142.1, 149.1, 157.9 (C=O), 167.3 (C=O), 170.2 (C=O). MS. (MALDI, positive mode, matrix DHB) *m/z*: 404 (M + Na)<sup>+</sup>. Anal. Calcd. for C<sub>19</sub>H<sub>19</sub>N<sub>5</sub>O<sub>4</sub> (381.4): C; 59.84, H; 5.02, N; 18.36. Found: C, 59.93; H, 5.11; N, 18.44.

**Synthesis of *N*-(1-Hydrazinocarbonyl-3-methyl-butyl)-2-(4-oxo-3-phenyl-3,4-dihydro-phthalazin-1-yloxy)-acetamide (6c).** Off-white crystals (88%), m.p. 198 °C. <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>): 9.19 (1H, bs, NH); 8.35 (2H, d, *J* = 8.1, ArH); 8.19–8.11 (2H, m, ArH); 8.04–7.97 (2H, m, ArH); 7.71 (2H, d, *J* = 8.0, ArH); 7.36–7.50 (1H, m, ArH); 6.22 (1H, bs, NH); 4.88–4.86 (1H, m, CH); 4.51–4.49 (2H, m, CH<sub>2</sub>); 4.29 (2H, bs, NH<sub>2</sub>); 1.94–1.91 (2H, m, CH<sub>2</sub>); 1.45–1.43 (1H, m, (CH<sub>3</sub>)<sub>2</sub>CH); 0.80 (6H, d, *J* = 7.0, (CH<sub>3</sub>)<sub>2</sub>CH). <sup>13</sup>C NMR (100.0 MHz, CDCl<sub>3</sub>), δ, ppm: 22.0, 23.3, 24.6, 65.5 (OCH<sub>2</sub>),



**Figure 4.** BOILED-Egg model for compounds (A) **7c** and (B) **8b** using the SwissADME. “Points located in the BOILED-Egg’s yolk are molecules predicted to passively permeate through the blood–brain barrier, while points located in the BOILED-Egg’s white are molecules predicted to be passively absorbed by gastrointestinal tract”.

124.2, 124.3, 125.5, 127.3, 127.5, 128.8, 129.4, 133.3, 134.3, 142.1, 149.2, 157.9 (C=O), 167.1 (C=O), 171.3 (C=O). MS. (MALDI, positive mode, matrix DHB)  $m/z$ : 446 (M + Na)<sup>+</sup>. Anal. Calcd. for C<sub>22</sub>H<sub>25</sub>N<sub>5</sub>O<sub>4</sub> (423.5): C, 62.40; H, 5.95; N, 16.54. Found: C, 62.49; H, 6.04; N, 16.63.

**General Procedures for Preparation of Dipeptide 7a–i.** A solution of NaNO<sub>2</sub> (0.7 g, 0.01 mol) in cold water (15 mL) was added to a cold solution (0 °C) of hydrazide (**6**) (3.67 g, 0.01 mol) in acetic acid (15 mL), 1N HCl (15 mL), and water (25 mL). The reaction mixture was blended for 15 min at 0 °C. Cold ethyl acetate (30 mL) was used to extract the yellow syrup, which was then washed with cold 5% Na<sub>2</sub>CO<sub>3</sub> (30 mL) and dried over Na<sub>2</sub>SO<sub>4</sub>. The amino acid ester NH<sub>2</sub>(CHR)COOMe·HCl (0.01 mol) in ethyl acetate (20 mL) with 2 mL of Et<sub>3</sub>N was added to this solution. The reaction mixture was held at 0 °C for 24 h before being moved to room temperature for another 24 h. The solution evaporated into nothingness. To achieve the desired result, the buildup was crystallized from petroleum ether/ethyl acetate.

**Methyl {2-[2-(4-Oxo-3-phenyl-3,4-dihydro-phthalazin-1-yloxy)-acetylamino]-acetylamino}-acetate (7a).** From hydrazide **6a** (3.67 g, 0.01 mol) and glyOCH<sub>3</sub>·HCl (1.25 g, 0.01 mol)

Off-white crystals (76%), m.p. 144–143 °C. <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>), δ, ppm (J, Hz): 10.64 (1H, bs, NH); 8.40 (2H, d, J = 8.10, ArH); 7.62–7.79 (2H, m, ArH); 6.68–6.91 (2H, m, ArH); 6.88 (3H, m, ArH); 6.21 (1H, bs, NH); 4.83 (2H, s, CH<sub>2</sub>); 4.00 (2H, s, CH<sub>2</sub>); 3.87 (2H, s, CH<sub>2</sub>); 3.65 (3H, s, OCH<sub>3</sub>). <sup>13</sup>C NMR (100.0 MHz, CDCl<sub>3</sub>), δ, ppm: 41.1, 42.6, 52.4, 65.6 (OCH<sub>2</sub>), 123.2, 123.8, 124.9, 127.2, 128.0, 128.6, 129.9, 132.6, 133.5, 141.6, 148.5, 158.3 (C=O), 168.1 (C=O), 168.6 (C=O), 169.9 (C=O). MS. (MALDI, positive mode, matrix DHB)  $m/z$ : 447 (M + Na)<sup>+</sup>. Anal. Calcd. for C<sub>21</sub>H<sub>20</sub>N<sub>4</sub>O<sub>6</sub> (424.4): C, 59.43; H, 4.75; N, 13.20. Found: C, 59.62; H, 4.90; N, 13.38.

**Methyl-3-{2-[2-(4-oxo-3-phenyl-3,4-dihydro-phthalazin-1-yloxy)-acetylamino]-acetylamino}-propionate (7b).** From hydrazide **6a** (3.67 g, 0.01 mol) and β-AlaOCH<sub>3</sub>·HCl (1.4 g, 0.01 mol)

Off-white crystals (79%), m.p. 137–136 °C. <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>), δ, ppm (J, Hz): 10.64 (1H, bs, NH); 8.40 (2H, d, J = 8.1, ArH); 8.0 (2H, d, J = 8.0, ArH); 7.62–7.09 (2H, m, ArH); 7.19–7.39 (1H, m, ArH); 6.72–6.90 (2H, m, ArH); 6.69 (1H, bs, NH); 4.82 (2H, s, CH<sub>2</sub>); 4.12 (2H, s, CH<sub>2</sub>); 3.92 (2H, m, CH<sub>2</sub>); 3.58 (3H, s, OCH<sub>3</sub>); 2.64 (2H, m, CH<sub>2</sub>). MS. (MALDI, positive mode, matrix DHB)  $m/z$ : 461 (M + Na)<sup>+</sup>. Anal. Calcd. for C<sub>22</sub>H<sub>22</sub>N<sub>4</sub>O<sub>6</sub> (438.4): C, 60.27; H, 5.06; N, 12.78. Found: C, 60.44; H, 5.20; N, 12.91.

**Methyl-4-methyl-2-{2-[2-(4-oxo-3-phenyl-3,4-dihydro-phthalazin-1-yloxy)-acetylamino]-acetylamino}-pentanoate (7c).** From hydrazide (**6a**) (3.67 g, 0.01 mol) and L-leuOCH<sub>3</sub>·HCl (1.81 g, 0.01 mol)

Off-white crystals (73%), m.p. 129–130 °C. <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>), δ, ppm (J, Hz): 10.55 (1H, bs, NH); 8.42 (2H, d, J = 8.0, ArH); 8.1 (2H, d, J = 7.9, ArH); 7.62–7.81 (2H, m, ArH); 7.29–7.39 (1H, m, ArH); 6.64–6.91 (2H, m, ArH); 6.25 (1H, bs, NH); 4.83–4.81 (1H, m, CH); 4.79 (2H, s, CH<sub>2</sub>); 3.87 (2H, s, CH<sub>2</sub>); 3.65–3.64 (2H, m, CH<sub>2</sub>); 3.52 (3H, s, OCH<sub>3</sub>); 1.57 (1H, m, CH-(CH<sub>3</sub>)<sub>2</sub>); 0.85 (6H, d, CH-(CH<sub>3</sub>)<sub>2</sub>). MS. (MALDI, positive mode, matrix DHB)  $m/z$ : 461 (M + Na)<sup>+</sup>. Anal. Calcd. for C<sub>25</sub>H<sub>28</sub>N<sub>4</sub>O<sub>6</sub> (480.5): C, 62.49; H, 5.87; N, 11.66. Found: C, 62.55; H, 6.13; N, 11.75.

**Methyl-2{3-[2-(4-oxo-3-phenyl-3,4-dihydro-phthalazin-1-yloxy)-acetylamino]-propionylamino}-acetate (7d).** From hydrazide **6b** (3.81 g, 0.01 mol) and glyOCH<sub>3</sub>·HCl (1.25 g, 0.01 mol)

Off-white crystals (77%), m.p. 134–135 °C. <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>), δ, ppm (J, Hz): 8.89 (1H, bs, NH); 8.51 (2H, d, J = 8.0, ArH); 8.15 (2H, d, J = 7.9, ArH); 7.84–7.92 (2H, m, ArH); 7.74–7.76 (1H, m, ArH); 7.28–7.50 (2H, m, ArH); 6.25 (1H, bs, NH); 4.88 (2H, s, CH<sub>2</sub>); 3.97–3.95 (2H, m, CH<sub>2</sub>); 3.70 (3H, s, OCH<sub>3</sub>); 3.44–3.42 (2H, m, CH<sub>2</sub>); 2.37–2.35 (2H, m, CH<sub>2</sub>). MS. (MALDI, positive mode, matrix DHB)  $m/z$ : 461 (M + Na)<sup>+</sup>. Anal. Calcd. for C<sub>22</sub>H<sub>22</sub>N<sub>4</sub>O<sub>6</sub> (438.4): C, 60.27; H, 5.06; N, 12.78. Found: C, 60.36; H, 5.21; N, 12.84.

**Methyl-3-{3-[2-(4-oxo-3-phenyl-3,4-dihydro-phthalazin-1-yloxy)-acetylamino]-propionylamino}-propionate (7e).**



From hydrazide **6b** (3.81 g, 0.01 mol) and  $\beta$ -alaOCH<sub>3</sub>·HCl (1.4 g, 0.01 mol)

Off-white crystals (81%), m.p. 129–130 °C. <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>): 9.09 (1H, bs, NH); 8.49 (2H, d, *J* = 8.0, ArH); 8.16 (2H, d, *J* = 8.2, ArH); 7.83–7.91 (2H, m, ArH); 7.72–7.74 (2H, m, ArH); 7.29–7.48 (1H, m, ArH); 6.19 (1H, bs, NH); 4.85 (2H, s, CH<sub>2</sub>); 3.76 (3H, s, OCH<sub>3</sub>); 3.63–3.61 (2H, m, CH<sub>2</sub>); 3.43–3.41 (2H, m, CH<sub>2</sub>); 2.50–2.51 (2H, m, CH<sub>2</sub>); 2.40–2.38 (2H, m, CH<sub>2</sub>). MS. (MALDI, positive mode, matrix DHB) *m/z*: 475 (M + Na)<sup>+</sup>. Anal. Calcd. for C<sub>23</sub>H<sub>24</sub>N<sub>4</sub>O<sub>6</sub> (452.5): C, 61.05; H, 5.35; N, 12.38. Found: C, 61.14; H, 5.45; N, 12.42.

**Methyl {4-Methyl-2-[2-(4-oxo-3-phenyl-3,4-dihydro-phthalazin-1-yloxy)-acetylaminol-pentanoyl] Acetate (7f)}**. From hydrazide **6b** (3.81 g, 0.01 mol) and L-leuOCH<sub>3</sub>·HCl (1.81 g, 0.01 mol)

Off-white crystals (83%), m.p. 124–125 °C. <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>),  $\delta$ , ppm (*J*, Hz): 9.12 (1H, bs, NH); 8.41 (2H, d, *J* = 8.0, ArH); 8.08 (2H, d, *J* = 8.1, ArH); 7.74–7.81 (2H, m, ArH); 7.64–7.66 (2H, m, ArH), 7.29–7.48 (1H, m, ArH), 6.19 (1H, bs, NH); 4.78 (2H, s, CH<sub>2</sub>); 4.51–4.49 (1H, m, CH); 3.32–3.31 (2H, m, CH<sub>2</sub>); 2.65–2.64 (2H, m, CH<sub>2</sub>); 3.58 (3H, s, OCH<sub>3</sub>); 2.42–2.40 (2H, m, CH<sub>2</sub>); 1.55–1.54 (1H, m, (CH<sub>3</sub>)<sub>2</sub>CH); 0.85–0.83 (6H, m, (CH<sub>3</sub>)<sub>2</sub>CH). <sup>13</sup>C NMR (100.0 MHz, CDCl<sub>3</sub>),  $\delta$ , ppm: 21.8, 22.0, 22.7, 24.9, 35.1, 35.2, 41.4, 50.9, 52.3, 65.5 (OCH<sub>2</sub>), 123.5, 124.0, 125.0, 127.2, 127.9, 128.6, 129.8, 132.4, 133.4, 141.7, 148.6, 158.4 (C=O), 167.7 (C=O), 171.7 (C=O), 173.3 (C=O). MS. (MALDI, positive mode, matrix DHB) *m/z*: 517 (M + Na)<sup>+</sup>. Anal. Calcd. for C<sub>26</sub>H<sub>30</sub>N<sub>4</sub>O<sub>6</sub> (494.5): C, 63.15; H, 6.11; N, 11.33. Found: C, 63.22; H, 6.20; N, 11.40.

**Methyl {4-Methyl-2-[2-(4-oxo-3-phenyl-3,4-dihydro-phthalazin-1-yloxy)-acetylaminol-pentanoylamino]-acetate (7g)}**. From hydrazide **6c** (4.23 g, 0.01 mol) and glyOCH<sub>3</sub>·HCl (1.25 g, 0.01 mol)

Off-white crystals (75%), m.p. 127 °C. <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>),  $\delta$ , ppm (*J*, Hz): 8.50 (2H, d, *J* = 8.0, ArH); 8.05 (2H, d, *J* = 8.1, ArH); 7.83–7.90 (2H, m, ArH); 7.71–7.73 (2H, m, ArH); 7.28–7.48 (2H, m, ArH & NH); 6.20 (1H, bs, NH); 4.88–4.86 (1H, m, CH); 4.24 (2H, s, CH<sub>2</sub>); 3.94 (2H, d, *J* = 7.0, CH<sub>2</sub>); 3.72 (3H, s, OCH<sub>3</sub>); 1.94–1.92 (2H, m, CH<sub>2</sub>); 1.63 (1H, m, (CH<sub>3</sub>)<sub>2</sub>CH); 0.09 (6H, d, *J* = 6.9, (CH<sub>3</sub>)<sub>2</sub>CH). MS. (MALDI, positive mode, matrix DHB) *m/z*: 503 (M + Na)<sup>+</sup>. Anal. Calcd. for C<sub>25</sub>H<sub>28</sub>N<sub>4</sub>O<sub>6</sub> (480.5): C, 62.49; H, 5.87; N, 11.66. Found: C, 62.58; H, 6.00; N, 11.72.

**Methyl-3-[4-Methyl-2-[2-(4-oxo-3-phenyl-3,4-dihydro-phthalazin-1-yloxy)-acetylaminol-pentanoylamino]-propionate (7h)}**. From hydrazide **6c** (4.23 g, 0.01 mol) and  $\beta$ -alaninOCH<sub>3</sub>·HCl (1.4 g, 0.01 mol)

Off-white crystals (81%), m.p. 123–122 °C. <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>),  $\delta$ , ppm (*J*, Hz): 8.51 (2H, d, *J* = 8.0, ArH); 8.06 (2H, d, *J* = 8.2, ArH); 7.83–7.91 (2H, m, ArH); 7.71–7.73 (2H, m, ArH & NH); 7.28–7.47 (2H, m, ArH); 6.11 (1H, bs, NH); 4.90 (1H, m, CHCH<sub>2</sub>); 3.55–3.53 (2H, m, CH<sub>2</sub>); 3.68 (3H, s, OCH<sub>3</sub>); 3.54–3.52 (2H, m, CH<sub>2</sub>); 2.56–2.54 (2H, m, CH<sub>2</sub>); 2.52 (2H, m, CH<sub>2</sub>); 1.60 (1H, m, (CH<sub>3</sub>)<sub>2</sub>CH); 0.89 (6H, d, *J* = 6.9, (CH<sub>3</sub>)<sub>2</sub>CH). MS. (MALDI, positive mode, matrix DHB) *m/z*: 517 (M + Na)<sup>+</sup>. Anal. Calcd. for C<sub>26</sub>H<sub>30</sub>N<sub>4</sub>O<sub>6</sub> (494.5): C, 63.15; H, 6.11; N, 11.33. Found: C, 63.34; H, 6.30; N, 11.52.

**Methyl-4-methyl-2-[4-methyl-2-[2-(4-oxo-3-phenyl-3,4-dihydro-phthalazin-1-yloxy)-acetylaminol-pentanoylamino]-acetate (7i)}**. From hydrazide **6c** (4.23 g, 0.01 mol) and L-leuOCH<sub>3</sub>·HCl (1.81 g, 0.01 mol)

Off-white crystals (78%), m.p. 119–118 °C. <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>),  $\delta$ , ppm (*J*, Hz): 8.51 (2H, d, *J* = 8.0, ArH); 8.05 (2H, d, *J* = 7.9, ArH); 7.83–7.90 (2H, m, ArH); 7.71–7.73 (2H, m, ArH & NH); 7.28–7.47 (2H, m, ArH); 6.18 (1H, bs, NH); 4.90–4.88 (1H, m, CHCH<sub>2</sub>); 4.65–4.64 (1H, m, CHCH<sub>2</sub>); 4.20 (2H, s, CH<sub>2</sub>); 3.73 (3H, s, OCH<sub>3</sub>); 1.97–1.95 (2H, m, CH<sub>2</sub>); 1.91–1.92 (1H, m, (CH<sub>3</sub>)<sub>2</sub>CH); 1.77–1.75 (2H, m, CH<sub>2</sub>); 1.62–1.60 (1H, m, (CH<sub>3</sub>)<sub>2</sub>CH); 0.93 (6H, d, *J* = 7.0, (CH<sub>3</sub>)<sub>2</sub>CH); 0.88 (6H, d, *J* = 6.9, (CH<sub>3</sub>)<sub>2</sub>CH). MS. (MALDI, positive mode, matrix DHB) *m/z*: 559 (M + Na)<sup>+</sup>. Anal. Calcd. for C<sub>29</sub>H<sub>36</sub>N<sub>4</sub>O<sub>6</sub> (536.6): C, 64.91; H, 6.76; N, 10.44. Found: C, 65.02; H, 6.85; N, 10.49.

**General Procedures for Preparation of Hydrazones 8a–i**. A mixture of one of the hydrazides **6a–c** (0.01 mol) and an effective aromatic aldehyde (0.01 mol), such as 4-chloro benzaldehyde, anisaldehyde, and/or 4-nitro benzaldehyde, was refluxed in ethanol (25 mL) for 12 h and then crystallized from the ethanol.

**Synthesis of N-(4-Chloro-benzylidene-hydrazinocarbonyl-methyl)-2-(4-oxo-3-phenyl-3,4-dihydro-phthalazin-1-yloxy)-acetamide (8a)**. From 4-chloro-benzaldehyde and hydrazide **6a**

Off-white crystals (84%), m.p. 187–188 °C. <sup>1</sup>H NMR (400 MHz, DMSO),  $\delta$ , ppm (*J*, Hz): 11.47 (1H, bs, NH); 8.38 (2H, d, *J* = 8.0, ArH); 8.23 (2H, d, *J* = 8.2, ArH); 7.98–8.03 (2H, m, ArH); 7.69–7.77 (3H, m, ArH); 7.37–7.52 (4H, m, ArH); 6.69 (1H, s, =CH); 6.04 (1H, bs, NH); 4.90 (2H, s, CH<sub>2</sub>); 4.35 (2H, d, *J* = 7.0, CH<sub>2</sub>). MS. (MALDI, positive mode, matrix DHB) *m/z*: 513 (M + Na)<sup>+</sup>. Anal. Calcd. for C<sub>25</sub>H<sub>20</sub>ClN<sub>5</sub>O<sub>4</sub> (489.9): C, 61.29; H, 4.11; Cl, 7.24; N, 14.30. Found: C, 61.38; H, 4.20; Cl, 7.33; N, 14.39.

**Synthesis of N-(4-Methoxy-benzylidene-hydrazinocarbonylmethyl)-2-(4-oxo-3-phenyl-3,4-dihydro-phthalazin-1-yloxy)-acetamide (8b)**. From 4-methoxy-benzaldehyde and hydrazide **6a**

Off-white crystals (78%), m.p. 181–182 °C. <sup>1</sup>H NMR (400 MHz, DMSO),  $\delta$ , ppm (*J*, Hz): 11.35 (1H, bs, NH); 8.36 (2H, d, *J* = 8.0, ArH); 8.24 (2H, d, ArH, *J* = 8.1); 7.95–8.04 (2H, m, ArH); 7.87–7.89 (3H, m, ArH); 7.48–7.77 (2H, m, ArH); 7.12–7.14 (2H, m, ArH); 6.58 (1H, s, =CH); 6.12 (1H, bs, NH); 4.90 (2H, s, CH<sub>2</sub>); 4.44 (2H, d, CH<sub>2</sub>); 3.88 (3H, s, OCH<sub>3</sub>). MS. (MALDI, positive mode, matrix DHB) *m/z*: 508 (M + Na)<sup>+</sup>. Anal. Calcd. for C<sub>26</sub>H<sub>23</sub>N<sub>5</sub>O<sub>5</sub> (485.5): C, 64.32; H, 4.78; N, 14.43. Found: C, 64.41; H, 4.86; N, 14.52.

**Synthesis of N-(4-Nitro-benzylidene-hydrazinocarbonyl-methyl)-2-(4-oxo-3-phenyl-3,4-dihydro-phthalazin-1-yloxy)-acetamide (8c)**. From 4-nitro-benzaldehyde and hydrazide **6a**

Off-white crystals (78%), m.p. 191–192 °C. <sup>1</sup>H NMR (400 MHz, DMSO),  $\delta$ , ppm (*J*, Hz): 11.82 (1H, bs, NH); 8.35 (2H, d, *J* = 8.0, ArH); 8.25 (2H, d, *J* = 8.1, ArH); 7.93–8.03 (2H, m, ArH); 7.74–7.76 (3H, m, ArH); 7.37–7.52 (4H, m, ArH); 6.81 (1H, s, =CH); 6.11 (1H, bs, NH); 4.91 (2H, s, CH<sub>2</sub>); 4.39 (2H, d, *J* = 7.0, CH<sub>2</sub>). MS. (MALDI, positive mode, matrix DHB) *m/z*: 523 (M + Na)<sup>+</sup>. Anal. Calcd. for C<sub>25</sub>H<sub>20</sub>N<sub>6</sub>O<sub>6</sub> (500.5): C, 60.00; H, 4.03; N, 16.79. Found: C, 60.12; H, 4.08; N, 16.88.

**Synthesis of N-[2-(4-Chloro-benzylidene-hydrazinocarbonyl)-ethyl]-2-(4-oxo-3-phenyl-3,4-dihydro-phthalazin-1-yloxy)-acetamide (8d)**. From 4-chloro-benzaldehyde and hydrazide **6b**

Off-white crystals (80%), m.p. 194–195 °C. <sup>1</sup>H NMR (400 MHz, DMSO): 11.42 (1H, bs, NH); 8.34 (2H, d, *J* = 8.2, ArH); 8.16–8.23 (2H, m, ArH); 7.95–8.04 (4H, m, ArH); 7.63–7.70 (2H, m, ArH); 7.41–7.51 (1H, m, ArH); 7.33 (2H, d, *J* = 8.2, ArH); 6.77 (1H, s, =CH); 6.08 (1H, bs, NH); 4.78 (2H, s, CH<sub>2</sub>);

3.45–3.44 (1H, m, CH); 2.22 (3H, d,  $J = 7.0$ , CH<sub>3</sub>). <sup>13</sup>C NMR (100.0 MHz, CDCl<sub>3</sub>),  $\delta$ , ppm: 32.8, 34.9, 65.7 (OCH<sub>2</sub>), 124.3, 125.5, 127.4, 127.5, 128.7, 129.1, 129.4, 133.3, 133.6, 134.6, 142.1, 145.4, 149.0, 157.9 (C=O), 167.4 (C=O), 167.6 (C=O), 173.3 (C=O). MS. (MALDI, positive mode, matrix DHB)  $m/z$ : 527 (M + Na)<sup>+</sup>. Anal. Calcd. for C<sub>26</sub>H<sub>22</sub>ClN<sub>5</sub>O<sub>4</sub> (503.9): C, 61.97; H, 4.40; Cl, 7.04; N, 13.90. Found: C, 62.06; H, 4.48; Cl, 7.13; N, 14.01.

**Synthesis of *N*-[2-(4-Methoxy-benzylidene-hydrazinocarbonyl)-ethyl]-2-(4-oxo-3-phenyl-3,4-dihydro-phthalazin-1-yloxy)-acetamide (8e).** From 4-methoxy-benzaldehyde and hydrazide 6b

Off-white crystals (82%), m.p. 190–191 °C. <sup>1</sup>H NMR (400 MHz, DMSO),  $\delta$ , ppm ( $J$ , Hz): 9.92 (1H, bs, NH); 8.56 (2H, d,  $J = 8.2$ , ArH); 7.86–7.92 (2H, m, ArH); 7.72 (2H, d,  $J = 8.2$ , ArH); 7.42–7.59 (3H, m, ArH); 7.02–7.04 (2H, d,  $J = 8.2$ , ArH); 7.92–7.94 (2H, d,  $J = 8.2$ , ArH); 6.68 (1H, s, =CH); 6.06 (1H, bs, NH); 4.92 (2H, s, CH<sub>2</sub>); 3.92 (1H, s, CH); 3.87 (3H, s, OCH<sub>3</sub>); 1.54 (3H, d, CH<sub>3</sub>). MS. (MALDI, positive mode, matrix DHB)  $m/z$ : 522 (M + Na)<sup>+</sup>. Anal. Calcd. for C<sub>27</sub>H<sub>25</sub>N<sub>5</sub>O<sub>5</sub> (499.5): C, 64.92; H, 5.04; N, 14.02. Found: C, 65.00; H, 5.11; N, 14.14.

**Synthesis of *N*-[2-(4-Nitro-benzylidene-hydrazinocarbonyl)-ethyl]-2-(4-oxo-3-phenyl-3,4-dihydro-phthalazin-1-yloxy)-acetamide (8f).** From 4-nitro-benzaldehyde and hydrazide 6b

Off-white crystals (88%), m.p. 201–202 °C. <sup>1</sup>H NMR (400 MHz, DMSO),  $\delta$ , ppm ( $J$ , Hz): 11.63 (1H, bs, NH); 8.32–8.34 (2H, m, ArH); 8.18–8.28 (2H, m, ArH); 7.85–8.07 (4H, m, ArH); 7.70 (2H, d,  $J = 8.0$ , ArH); 7.77–7.48 (1H, m, ArH); 7.29–7.37 (2H, m, ArH); 6.57 (1H, s, =CH); 6.10 (1H, bs, NH); 4.77 (2H, s, CH<sub>2</sub>); 3.26 (1H, m, CH); 2.89 (3H, d,  $J = 7.0$ , CH<sub>3</sub>). MS. MALDI, positive mode, matrix DHB)  $m/z$ : 537 (M + Na)<sup>+</sup>. Anal. Calcd. for C<sub>26</sub>H<sub>22</sub>N<sub>6</sub>O<sub>6</sub> (514.5): C, 60.70; H, 4.31; N, 16.33. Found: C, 60.78; H, 4.40; N, 16.42.

**Synthesis of *N*-[1-(4-Chloro-benzylidene-hydrazinocarbonyl)-3-methyl-butyl]-2-(4-oxo-3-phenyl-3,4-dihydro-phthalazin-1-yloxy)-acetamide (8g).** From 4-chloro-benzaldehyde and hydrazide 6c

Off-white crystals (81%), m.p. 190–191 °C. <sup>1</sup>H NMR (400 MHz, DMSO),  $\delta$ , ppm ( $J$ , Hz): 11.55 (1H, bs, NH); 8.40 (2H, d,  $J = 8.1$ , ArH); 8.25 (2H, d,  $J = 8.0$ , ArH); 8.12 (2H, d,  $J = 8.2$ , ArH); 7.95–8.01 (2H, m, ArH); 7.64–7.72 (2H, m, ArH); 7.46 (1H, d,  $J = 8.2$ , ArH); 7.32–7.37 (2H, m, ArH); 6.66 (1H, s, =CH); 6.12 (1H, bs, NH); 4.89–4.88 (1H, m, CH); 4.48–4.47 (2H, m, CH<sub>2</sub>); 1.94–1.91 (2H, m, CH<sub>2</sub>); 1.41–1.39 (1H, m, (CH<sub>3</sub>)<sub>2</sub>CH); 0.83 (6H, d,  $J = 7.0$ , (CH<sub>3</sub>)<sub>2</sub>CH). MS. (MALDI, positive mode, matrix DHB)  $m/z$ : 569 (M + Na)<sup>+</sup>. Anal. Calcd. for C<sub>29</sub>H<sub>28</sub>ClN<sub>5</sub>O<sub>4</sub> (546.0): C, 63.79; H, 5.17; Cl, 6.49; N, 12.83. Found: C, 63.98; H, 5.36; Cl, 6.68; N, 13.00.

**Synthesis of *N*-[1-(4-Methoxy-benzylidene-hydrazinocarbonyl)-3-methyl-butyl]-2-(4-oxo-3-phenyl-3,4-dihydro-phthalazin-1-yloxy)-acetamide (8h).** From 4-methoxy-benzaldehyde and hydrazide 6c

Off-white crystals (82%), m.p. 200–201 °C. <sup>1</sup>H NMR (400 MHz, DMSO),  $\delta$ , ppm ( $J$ , Hz): 10.44 (1H, bs, NH); 8.50 (2H, d,  $J = 8.0$ , ArH); 8.07–8.11 (2H, m, ArH); 7.41–7.88 (2H, m, ArH); 7.28–7.34 (2H, m, ArH); 7.18 (1H, d,  $J = 8.0$ , ArH); 6.86–6.91 (4H, m, ArH); 6.66 (1H, s, =CH); 6.12 (1H, bs, NH); 4.89–4.87 (1H, m, CH); 4.52–4.50 (2H, m, CH<sub>2</sub>); 3.84 (3H, s, OCH<sub>3</sub>); 1.90–1.89 (2H, m, CH<sub>2</sub>); 1.70–1.69 (1H, m, (CH<sub>3</sub>)<sub>2</sub>CH); 1.06 (6H, d,  $J = 7.0$ , (CH<sub>3</sub>)<sub>2</sub>CH). MS. (MALDI, positive mode, matrix DHB)  $m/z$ : 564.8 (M + Na)<sup>+</sup>. Anal.

Calcd. for C<sub>30</sub>H<sub>31</sub>N<sub>5</sub>O<sub>5</sub> (541.6): C, 66.53; H, 5.77; N, 12.93. Found: C, 66.62; H, 5.86; N, 12.98.

**Synthesis of *N*-[3-Methyl-1-(4-nitro-benzylidene-hydrazinocarbonyl)-butyl]-2-(4-oxo-3-phenyl-3,4-dihydro-phthalazin-1-yloxy)-acetamide (8i).** From 4-nitro-benzaldehyde and hydrazide 6c

Off-white crystals (79%), m.p. 205–206 °C. <sup>1</sup>H NMR (400 MHz, DMSO),  $\delta$ , ppm ( $J$ , Hz): 11.79 (1H, bs, NH); 8.22–8.41 (4H, m, ArH); 7.87–8.14 (4H, m, ArH); 7.71 (2H, d,  $J = 8.1$ , ArH); 7.45–7.49 (2H, m, ArH); 7.33–7.38 (1H, m, ArH); 6.71 (1H, s, =CH); 6.09 (1H, bs, NH); 4.90–4.88 (1H, m, CH); 4.55–4.53 (2H, m, CH<sub>2</sub>); 1.89–1.87 (2H, m, CH<sub>2</sub>); 1.41–1.39 (1H, m, (CH<sub>3</sub>)<sub>2</sub>CH); 0.86 (6H, d,  $J = 7.0$ , (CH<sub>3</sub>)<sub>2</sub>CH). MS. (MALDI, positive mode, matrix DHB)  $m/z$ : 579.9 (M + Na)<sup>+</sup>. Anal. Calcd. for C<sub>29</sub>H<sub>28</sub>N<sub>6</sub>O<sub>6</sub> (556.6): C, 62.58; H, 5.07; N, 15.10. Found: C, 62.66; H, 5.15; N, 15.19.

**Synthesis of Methyl-4-[2-(4-oxo-3-phenyl-3,4-dihydro-phthalazin-1-yloxy)-acetyl-amino]-benzoate (9).** A solution of NaNO<sub>2</sub> (0.7 g, 0.01 mol) in cold water (15 mL) was added to a cold solution (0 °C) of hydrazide (6) (3.67 g, 0.01 mol) in acetic acid (15 mL), 1N HCl (15 mL), and water (25 mL). The reaction mixture was blended for 15 min at 0 °C. Cold ethyl acetate (30 mL) was used to extract the yellow syrup, which was then washed with cold 5% Na<sub>2</sub>CO<sub>3</sub> (30 mL) and dried over Na<sub>2</sub>SO<sub>4</sub>. The methyl-4-aminobenzoate (0.01 mol) in ethyl acetate (20 mL) with 2 mL of Et<sub>3</sub>N was added to this solution. The reaction mixture was held at 0 °C for 24 h before being moved to room temperature for another 24 h. The solution evaporated into nothingness. To achieve the desired result, the buildup was crystallized from petroleum ether/ethyl acetate.

Off-white crystals (68%), m.p. 115–116 °C. <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>),  $\delta$ , ppm ( $J$ , Hz): 8.47 (2H, d,  $J = 8.10$ , ArH); 7.95–8.09 (4H, m, ArH); 7.83 (2H, d,  $J = 8.0$ , ArH); 7.54–7.62 (2H, m, ArH); 7.27–7.39 (1H, m, ArH); 7.17 (2H, d,  $J = 8.20$ , ArH); 6.82 (1H, bs, NH); 4.95 (2H, s, CH<sub>2</sub>); 3.80 (3H, s, OCH<sub>3</sub>). <sup>13</sup>C NMR (100.0 MHz, CDCl<sub>3</sub>),  $\delta$ , ppm: 52.0, 66.2 (OCH<sub>2</sub>), 125.0, 126.5, 127.4, 128.3, 128.7, 130.1, 130.9, 132.8, 133.5, 140.9, 141.5, 148.3, 158.2, (C=O), 165.7 (C=O), 166.4 (C=O). MS. (MALDI, positive mode, matrix DHB)  $m/z$ : 452 (M + Na)<sup>+</sup>. Anal. Calcd. for C<sub>24</sub>H<sub>19</sub>N<sub>3</sub>O<sub>5</sub> (429.4): C, 67.13; H, 4.46; N, 9.79. Found: C, 67.22; H, 4.45; N, 9.87.

## ■ BIOLOGICAL INVESTIGATION

**Cytotoxic Activity against HCT-116 and MDA-MB-231 Cell Lines.** The MTT assay was used to examine the cytotoxic activity of the tested compounds against two cancer cell lines: colon (HCT-116) and breast (MDA-MB-231). The cells grew after being cultivated in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% heat-inactivated fetal bovine serum, 1% L-glutamine, HEPES buffer, and 50 g/mL gentamycin. Cells were subcultured and kept at 37 °C in a humidified atmosphere with 5% CO<sub>2</sub> according to routine tissue culture work. The association between surviving cells and drug concentration is used to plot the survival curve of each tumor cell line after treatment with the relevant substance. The 50% inhibitory concentration (IC<sub>50</sub>), or the concentration required to cause cytotoxicity effects in 50% of cells, was derived using practical dose response curve graphs for each concentration using the GraphPad Prism software (San Diego, CA, USA).<sup>24–26</sup>

**Anti-bacterial Activity.** Tested compounds were evaluated for their anti-bacterial activities using “disc diffusion method”<sup>1</sup> against the indicator strains *E. coli* (ATCC 25922) and *S. aureus* (NCMB6571) at two concentrations of samples (0.25 mg/disc

& 0.75 mg/disc and 30  $\mu\text{g}$  each). DMSO was used as a solvent and negative control. After the incubation period, the growth inhibition zones diameter were carefully measured in millimeters.<sup>27</sup>

## MOLECULAR DOCKING STUDIES

A molecular docking study was performed on a computational software basis using the (AutoDock4 2016-08 Chemical Computing Group, Canada) toward the Protein Data Bank's X-ray crystal structure of VEGFR2 in complex with a novel 4-amino-furo[2,3-*d*]pyrimidine (PDB ID: 1YWN). Principles of modeling regarding receptor and ligand preparation and molecular docking were carried out according to Nafie et al.<sup>28</sup> Each ligand–receptor complex was tested for binding energy (Kcal/mol), interaction analysis, and 3D images, which were taken by Chimera as a visualizing software.

**ADME Pharmacokinetics.** In silico ADME pharmacokinetics parameters of the lead compounds were calculated using a set of software including “MolSoft”, “Molinspiration”, and “SwissADME” websites as previously described.<sup>29,30</sup>

## CONCLUSIONS

Searching for novel target-oriented anti-cancer agents is still a continuous research process to investigate effective and selective chemotherapeutic agents. Here, in the study, there are 18 new phthalazinediones beginning with methyl-1,4-dioxo-3-phenyl-3,4-dihydro-1*H*-phthalazin-2-yl-acetate (**2**) synthesized via O-alkylation of 2-phenyl-2,3-dihydrophthalazine-1,4-dione (**1**) with ethyl chloroacetate, yielding the starting material 2,3-[2-(1,4-dioxo-3-phenyl-3,4-dihydro-1*H*-phthalazin-2-yl)-acetylamino] monoamide methyl-3-[2-(1,4-dioxo-3-phenyl-3,4-dihydro-1*H*-phthalazin-2-yl)acetylamino]alkanoate **5a–c** and dipeptides methyl-3-[2-(1,4-Dioxo-3-phenyl-3,4-dihydro-1*H*-phthalazin-2-yl)-acetylamino]propionylamino-alkanoates **7a–i** via the azide coupling technique. Condensation of hydrazides **6a–c** with various aldehydes yielded Schiff's base hydrazones **8a–i**. Upon biological screening, results indicated that the tested compounds, especially **7c** and **8b** with the phenyl phthalazinone moieties, had promising cytotoxicity against the HCT-116 cells with  $\text{IC}_{50}$  values of 1.36 and 2.34  $\mu\text{M}$ , respectively. Additionally, the promising compounds **7c** and **8b** exhibited poor cytotoxicity against WISH cells with much higher  $\text{IC}_{50}$  values, so they were safe against normal cells. Compound **8c** exhibited potent anti-bacterial activity with inhibition zones of 12 and 11 mm against *S. aureus* and *E. coli*, respectively. Molecular docking results of compounds **7c** and **8b** revealed a good binding disposition and the ligand–receptor interactions like the co-crystallized of the VEGFR2, which may be the proposed mode of action. Finally, both compounds **7c** and **8b** exhibited good ADME pharmacokinetics with good drug-likeness parameters. Hence, detailed studies for the mechanism of action of such compounds are highly recommended for development of new potent anti-cancer and anti-bacterial agents.

## ASSOCIATED CONTENT

### Supporting Information

The Supporting Information is available free of charge at <https://pubs.acs.org/doi/10.1021/acsomega.2c03182>.

Spectral data for compounds (PDF)

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<https://pubs.acs.org/doi/10.1021/acsomega.2c03182>

### Notes

The authors declare no competing financial interest.

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## NOTE ADDED AFTER ASAP PUBLICATION

This paper was published on June 27, 2022, with an error in the footnote of Table 3 and also in the first paragraph of Molecular Docking Studies. The corrected version was reposted July 20, 2022.