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EXPLORATION OF PHTHALAZINE BEARING OXADIAZOLYL–TRIAZOLE HYBRIDS AS SELECTIVE BREAST CANCER AGENTS: COMPUTATIONAL DOCKING INTERACTIONS

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ABSTRACT. In this study, a series of novel phthalazine derivatives bearing 1,3,4–oxadiazolyl–1,2,3–triazole moiety were designed, synthesized, and evaluated for anticancer activity. The formation targeted compounds were confirmed for their structure by means of various spectral–analytical techniques like ^{1}H-NMR, ^{13}C-NMR, FT-IR, elemental analysis, and mass spectrum. All synthesized compounds were screened for anticancer activity against three different human breast cancer cell lines MCF-7, T-47D, and MDA-MB-231. From screening results, compound **5f** exhibited the most potent anticancer activity (IC_{50} = 10.21 ± 2.2, 7.53 ± 0.1 µM) towards T-47D, MCF-7 cell lines and **4b, 5b** demonstrated the highest % growth of inhibition (61.25 ± 0.52, 62.48 ± 0.20 µg/mL) against T-47D, and MCF-7 cell lines, respectively, which is equivalent to that reported by the standard cisplatin. The docking study strongly favours compound **4d**, **5a**, **5e**, and **5f** to be a dehydrogenase type 1 complexed in breast cancer inhibitor as it displayed a similar interaction to cisplatin (3HB4). Ligand **5f** exhibited amino acid interactions and having docking score –11.53 kcal/mol, respectively. The in-silico pharmacokinetics studies support the results obtained from docking and biological evaluation and displayed favourable pharmacokinetic profile for a drug to be orally available.

KEY WORDS: Phthalazine, 1,3,4–Oxadiazole, 1,2,3–Triazole, Breast cancer, Docking study, SwissADME

INTRODUCTION

Cancer is very dangerous disease characterized by the uncontrolled multiplication of cells and is able to invade other tissues; which is a leading cause of mortality and morbidity worldwide. Most of the currently used chemotherapeutic drugs are ineffective because of the development of drug resistance during treatment, in spite of advances in the understanding of the molecular biology of cancer and the ensuing rise in the development of anticancer compounds [1]. According to GLOBOCAN 2018, approximately 18.1 million new cases of cancer have recognized and among them, lung cancer (18.4%), followed by breast (11.6%), prostate (7.1%), colorectal (6.1%), stomach and liver cancer are the most common [2]. Breast cancer is the most frequently diagnosed cancer and the leading cause of death among females, accounting for 23% of the total cancer cases and 14% of the cancer deaths; thus, research in this field is important to overcome both economical and psychological burden [3]. Human 17β-hydroxysteroid

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dehydrogenase type 1 is a steroid converting enzyme that has long been known to play crucial roles in estradiol synthesis and more recently in dihydrotestosterone (DHT) inactivation, showing a dual function that promotes breast cancer cell proliferation [4]. Analyses of 17β-hydroxysteroid dehydrogenase type 1 (17β-HSD1) mRNA expression in breast carcinoma specimens from patients revealed that high expression of the enzyme correlates with a weak prognosis for breast cancer [5, 6]. Despite these observations, the relationship between 17β-hydroxysteroid dehydrogenase type 1 expression and that of genes and proteins involved in breast cancer cell growth has not been established. Multiple interactions between E2B and the enzyme include hydrogen bonds and hydrophobic interactions, as well as Pi–Pi interactions. Such strong inhibition is in agreement with our prepared oxadiazole and triazoles are extensive interaction with 3HB4 enzyme, suggesting its potential as a lead compound for breast cancer therapy [7].

Phthalazine moiety is of interest because they are part of many natural products, fine chemicals, and pharmaceuticals [8-10]. They also have attracted considerable attentions in recent years because of their wide range of pharmaceutical activities such as anticonvulsant [11], cardiotonic [12], and vasorelaxant activities [13]. Therefore, a number of methods have been reported for the synthesis of phthalazine derivatives using p–TSA, Me_3SiCl, silica sulphuric acid, H_2SO_4, $Mg(HSO_4)_2$, and silica supported polyphosphoric acid and so forth as catalysts [14-17]. Most of all, these syntheses could only afford non substituted phthalazine framework because of only two substrates, phthalhydrazide, and phthalimide were used as raw materials. It means that other valuable starting materials must be used if we want to obtain diverse structures and phthalazine derivatives bearing substituent on the phthalazine framework. Moreover, 1,3,4-oxadiazoles containing are well known for their different biological activities like anti-inflammatory and analgesic [18], antimicrobial [19], anticancer [20], anticonvulsant [21], anti-spasmolytic and hypotensive [22], anti-allergic [23], anti–proliferative [24], hypoglycemic [25], and ability to bind to DNA [26]. 1,3,4-Oxadiazoles are also known as surrogates for carboxamides, esters and carboxylic acids to improve the biological activities of consequent molecules [27]. Raltegravir, furamizole and nesapidil are well known examples of antiretroviral, antibacterial and anti-arrhythmic drugs which are available in the market. Beside the biological activities, 1,3,4-oxadiazoles have also many industrial applications in material sciences [28-30] (Chart 1).

1,2,3-Triazoles also owes a huge importance in medicinal chemistry due to their numerous biological activities such as antimicrobial [31], antimalarial [32], anti-HIV [33], anti-tuberculosis [34], anticancer and herbicidal [35]. Some 1,2,3-triazoles have also shown application as potassium channel activators [36], cannabinoid CB1 receptor antagonists [37], organo-catalyst and chemo sensors [38, 39]. This heterocycle possesses high dipole moment and is able to participate actively in the hydrogen bond formation as well as in dipole–dipole and Pi–Pi stacking interactions which may be helpful for its binding with bimolecular targets [40]. In continuation of our present work, we prepared new heterocyclic compounds via different procurers such as phthalazine, 1,3,4-oxadiazoles, 1,2,3-triazoles, and these scaffolds are a very important wide range of their antitumor activity (Chart 1).

Molecular docking is a primary and initial approach for evaluating novel therapeutic agents. This method is a promising and emergent field for the reduction of complexities that occur during the drug discovery process. In the process of drug development, the assessment of lead molecules having drug-likeness and good pharmacological activities is a tedious job. The in-silico studies are an easy approach for investigating bioactive compounds with promising druglikeness and desirable ADME-T (adsorption, distribution, metabolism, excretion, and toxicity) properties. The fundamental concept of rational drug designing is the analysis of ligand receptor interaction, and such interactions can be predicted by molecular docking and have gained a lot of importance in the area of structure-based drug design and discovery. Further the docking interactions of our prepared ligands were performed using the crystal structure of 17β-

hydroxysteroid dehydrogenase type 1 complexed in breast cancer (PDB: 3HB4). Additionally, the target compounds were exposed to in silico molecular properties prediction and drug resemblance by employing online web server called SwissADME (http://www.swissadme.ch) [41]. In silico absorption, distribution, absorption, excretion, toxicity is currently used widely to determine whether it is possible for a drug candidate to reach its site of action.

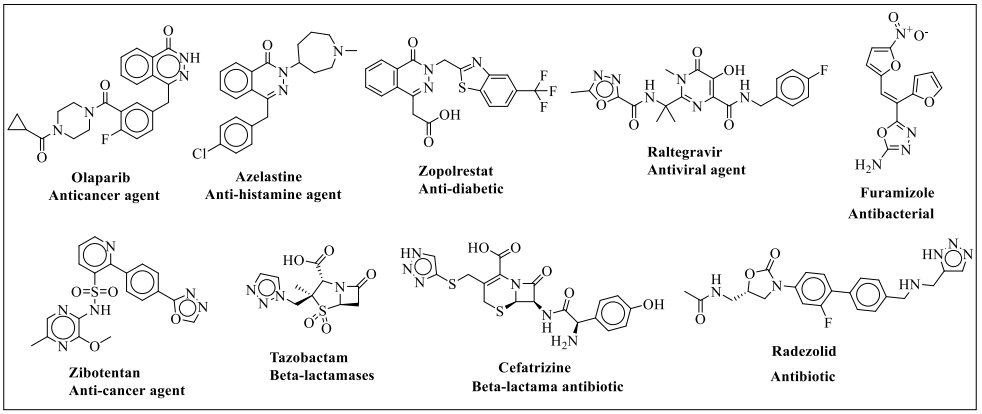


Chart 1. Naturally occurring phthalazine, 1,3,4-oxadiazole, and 1,2,3-triazole derivatives.

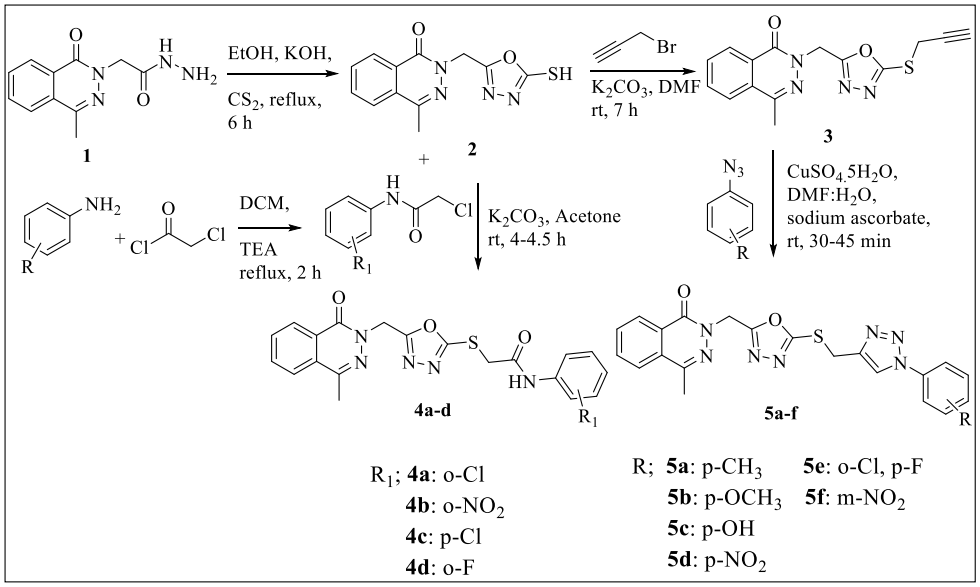
RESULTS AND DISCUSSION

Chemistry

Our initial strategy focused on the modification of 2-(4-methyl-1-oxophthalazin-2(1H)-yl) acetohydrazide **1** by incorporating the 1,3,4–oxadiazole moiety and 1,2,3–triazole linkers using two different synthetic routes (Scheme 1) The related product, 4-methylphthalazin-1(2H)-one was commercially available or synthesised from 2-acetylbenzoic acid and hydrazine hydrate in the presence of ethanol for 5 h at reflux conditions. The key intermediate 2-(4-methyl-1-oxophthalazin-2(1H)-yl)acetohydrazide **1** was afforded via the mixture of ethyl 2-(4-methyl-1-oxophthalazin-2(1H)-yl)acetate in ethanol, hydrazine hydrate was added.

The key intermediate 2-((5-mercapto-1,3,4-oxadiazol-2-yl)methyl)-4-methylphthalazin-1 (2H)-one **2** was prepared by the reaction of 2-(4-methyl-1-oxophthalazin-2(1H)-yl) acetohydrazide **1**, potassium hydroxide and carbon disulphide in ethanol until no H_2S was produced and the reaction was stirred under reflux for 6 h to obtain oxadiazole [42]. The crude product was filtered off, washed with water, dried, and recrystallized from ethanol to obtain 92% of compound **2** as a white solid. The crucial intermediate 2-chloro-N-phenylsubstituted acetamide was prepared via the acetamide coupling reaction of substituted anilines by treatment with 2-chloroacetyl chloride in dichloromethane and triethyl amine under refluxing for 2 h. The phthalazine based 2,5-disubstituted-1,3,4-oxadiazole derivatives (**4a-d**) were synthesized by carrying out the reaction starting from 2-((5-mercapto-1,3,4-oxadiazol-2-yl)methyl)-4-methylphthalazin-1(2H)-one **2** with appropriate acetamides in presence of anhydrous potassium carbonate in acetone solution at room temperature for 4-4. 5 h checking TLC at regular intervals. The solid separated was filtered, dried and purified by column chromatography on silica gel (100-200 mesh) using n-hexane and ethyl acetate (8:2) mixture as eluent to isolate pure desired products in excellent yields (76-86%). To a solution of 2-((5-mercapto-1,3,4-oxadiazol-2-yl)methyl)-4-methylphthalazin-1(2H)-one **2** in DMF, propargyl bromide were added followed by K_2CO_3 and stirred at room temperature for 7 h. After completion of the

reaction the mixture was poured into crushed ice water and extracted with EtOAc. Next, we designed and synthesized the 1,3,4-oxadiazolyl-1,2,3-triazole analogues **5a-f** have been prepared by adding of 4-methyl-2-((5-(prop-2-yn-1-ylthio)-1,3,4-oxadiazol-2-yl)methyl) phthalazin-1(2H)-one **3** with substituted azides in DMF: water, sodium ascorbate, Cu-catalyst and the obtained mixture was stirred at ambient temperature for 30-45 min to obtain in excellent yields (70-89%). Unfortunately, the conversion of the starting material to **5a** was also poor at room temperature for overnight (entry 3). Although the starting material **1** disappeared when the reaction was stirred at ambient temperature for overnight under conventional heating (entry 4), large amounts of different by products were produced and the conversion to product **5a** was minor. Whereas, the model reaction produced the required product **5a** in 50%, 45% yields, under neat reaction conditions at ambient temperature (Table 1, entry 1 and 2). However, adding 1 mol% copper sulphate to the reaction under room temperature conditions in ethanol after 30 min yielded the required product in 49% yield (Table 1, entry 5). In order to reduce reaction time, an attempt was made to implement the framework reaction conditions in DMF at room temperature, which resulted in a 72% increase in product yield (Table 1, entry 6). However, a further increment of mol% from 1.30 to 1.50 led to increasing in yields (Table 1, entries 7). Despite the superior yield, product **5a** was prepared with 2 mol% $CuSO_4.5H_2O$ in DMF to obtained 89% yield. After the finalization of the reaction, DCM was added to the reaction composition, and the organic layer was dried, filtered off, and washed with ethanol to obtained the pure 4-methyl-2-((5-(((1-(p-tolyl)-1H-1,2,3-triazol-4-yl)methyl)thio)-1,3,4-oxadiazol-2-yl)methyl)phthalazin-1 (2H)-one (**5a**).



Scheme 1. Synthesis of novel 1,3,4-oxadiazole and 1,2,3-triazole precursors.

The structures for all the derived molecules were obtained by spectroscopic analysis methods like IR, ^{1}H NMR, ^{13}C NMR, mass and elemental analysis. The results obtained from the spectral data were all appropriate to the suggested structures. FT-IR analysis of new phthalazin-1(2H)-one compound (**4a**) showed that –C=O stretching bands were recorded from 1675 cm^{-1}, –NH band was recorded at ν 3355 cm^{-1}. Moreover, –C=N band was recorded at 1422 cm^{-1}, and COC stretching band exhibited at 1139 cm^{-1}. In the ^{1}H-NMR spectra of **4a**, the signal

symbolizing most of the −NH amide proton found at δ 10.12 ppm singlet and phthalazine protons was recorded doublets at δ 7.67-7.80 ppm. The ^{1}H NMR spectra manifested a singlet around δ 4.61, 3.82 ppm, attributed to $–NCH_2$ and $–SCH_2$ protons situated between the phthalazine and the chloroacetamide ring. A multiplet in the range of δ 6.84-7.35 ppm corresponds to protons that are aromatic, while $–CH_3$ protons of phthalazine appeared around δ 2.60 ppm as a singlet. ^{13}C-NMR spectrum of compound **4a**, the signals belonging to the −C=O groups have been detected at δ 167.27, 161.18 ppm for the amide and phthalazine respectively. Other important oxadiazole carbon signals were detected at δ 163.42 and 140.03 ppm. However, the ^{13}C-NMR spectra exhibited a signal around δ 52.82, 34.89 ppm, corresponding to the carbon of the methylene group located at $–NCH_2$ and $–SCH_2$. The aromatic carbons relating to this moiety resonated in the range between δ 151.94 and 115.67 ppm. Mass results showed the molecular weight and empirical formulae of this compound ($C_{20}H_{16}ClN_5O_3S$) and experimental m/z 442.11 $(M + 1)^+$ value match with N-(2-chlorophenyl)-2-((5-((4-methyl-1-oxophthalazin-2(1H)-yl)methyl)-1,3,4-oxadiazol-2-yl)thio) acetamide (**4a**). All compounds gave the [M + 1] peak at their molecular weight because of catching a hydrogen atom from a medium according to the used method with ESI method.

Table 1. Optimization conditions for the preparation of 1,3,4-oxadiazole-1,2,3-triazole derivatives **5a**.

Entry	Solvent	Catalyst	Catalyst (mol %)	Reaction time	Yield (%)[a]
1	TEA neat	$CuSO_4.5H_2O$	1	50 min	40
2	DCM neat	$CuSO_4.5H_2O$	1	45 min	51
3	ACN	$CuSO_4.5H_2O$	1	Overnight	28
4	DMSO	$CuSO_4.5H_2O$	1	Overnight	17
5	EtOH	$CuSO_4.5H_2O$	1	30 min	49
6	DMF	$CuSO_4.5H_2O$	1.25	25 min	72
7	DMF	$CuSO_4.5H_2O$	1.5	25 min	78
8	DMF	$CuSO_4.5H_2O$	2	30 min	89
9	t–BuOH	$CuSO_4.5H_2O$	1	25 min	75

[a]isolated yield.

For instance, the IR spectrum of compound **5a** showed the characteristics bands for aromatic =CH at 3085 cm^{-1} and aliphatic –CH stretching frequency at 2972 cm^{-1}, –C=O at 1683 cm^{-1}. The appearance of the absorption band of –C=N at 1430 cm^{-1}, and strong absorption band of –COC at 1138 cm^{-1} confirmed the formation of our expected oxadiazole core. Its ^{1}H-NMR spectrum displayed a pair of doublet signals of phthalazine protons at δ 8.17, 7.70 ppm and a singlet at δ 7.84 ppm belongs to triazole ring. Formation of this compound was confirmed by the presence of two characteristic doublet protons for aromatic proton linkers at δ 7.58, 7.33 ppm and another two singlet peaks appeared at δ 5.14, 4.61 ppm for two methyl groups presented in n–linker, s–linker respectively. Coming to the ^{13}C NMR spectrum, the distinctive chemical shift values of the oxadiazole, triazole ring carbons appears at δ, 163.56, 161.56, 159.81 ppm and δ 166.59 ppm (–C=O) in phthalazine, whereas aromatic carbons shows in the ranging between δ 152.98–113.13 ppm which gives the characteristic features for the formation of the corresponding 1,3,4-oxadiazole substituted triazole product **5a**. ESI mass spectrum of compound **5a** showed $(M + 1)^+$ peaks at m/z = 446.09 which are in agreement with their molecular formula $C_{22}H_{19}N_7O_2S$.

Cytotoxicity

All the synthesized compounds **4(a–d)**, **5(a–f)** were evaluated for their in vitro anti-proliferative activity against selected Human breast cancer cell lines (T-47D, MCF-7) and Human breast adenocarcinoma (MDA-MB-231) cells by using 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl

tetrazolium bromide (MTT) assay [43, 44]. Cisplatin was used as reference standard and the screening results are summarized in Table 2. The results designated that the compounds **4b**, **4d**, **5b**, **5e**, and **5f** displayed significant cell growth inhibition with IC_{50} < 20 µM which are comparable to reference standard cisplatin against T–47D, MCF–7 and MDA-MB-231 cell lines.

Table 2. Cytotoxic activity profiles against different cancer cell lines with their % of inhibition and IC_{50} values.

S. No.	% Growth inhibition (20 µg/mL)			IC_{50} values at 20 µM		
	T-47D	MCF-7	MDA-MB-231	T-47D	MCF-7	MDA-MB-231
4a	36.81 ± 0.15	48.57 ± 0.22	40.28 ± 0.13	44.21 ± 0.2	32.49 ± 1.3	18.17 ± 2.1
4b	31.87 ± 0.30	62.48 ± 0.20	58.14 ± 0.52	7.30 ± 1.4	8.86 ± 0.4	9.73 ± 0.2
4c	35.87 ± 0.12	38.40 ± 0.31	53.47 ± 0.26	16.80 ± 1.05	22.43 ± 0.5	> 100
4d	29.48 ± 0.24	28.37 ± 0.22	26.39 ± 0.34	20.77 ± 0.4	12.29 ± 1.9	15.30 ± 1.0
5a	28.67 ± 0.30	38.56 ± 0.23	41.29 ± 0.11	> 100	16.21 ± 1.3	11.37 ± 2.3
5b	61.25 ± 0.52	54.18 ± 0.41	23.49 ± 0.32	10.15 ± 3.2	7.90 ± 1.7	7.11 ± 2.1
5c	20.18 ± 0.11	19.43 ± 0.10	25.60 ± 0.12	57.30 ± 2.0	79.31 ± 0.2	> 100
5d	21.08 ± 0.32	26.74 ± 0.40	32.15 ± 0.41	13.47 ± 3.1	> 100	15.90 ± 2.7
5e	55.03 ± 0.28	57.42 ± 0.33	67.29 ± 0.25	9.50 ± 1.5	7.53 ± 0.1	7.88 ± 1.1
5f	57.21 ± 0.33	44.91 ± 0.22	43.28 ± 0.10	10.21 ± 2.2	12.46 ± 0.2	13.20 ± 0.3
Cisplatin	61.27± 0.12	67.13 ± 0.12	69.84 ± 0.12	8.30 ± 1.9	9.24 ± 1.3	9.16 ± 2.0

IC_{50} (µM): 1-10 (very strong); 11-20 (strong); 21-50 (moderate); > 100 (not active).

Precisely, compound **4b** exhibited potent cytotoxic activity against T-47D [IC_{50} = 7.30 ± 1.4, 20.77 ± 0.4 µM] cell line and **4d** active against MCF-7 cell line with IC_{50} values [8.86 ± 0.4, 12.29 ± 1.9 µM], respectively, whereas the compound **4a** exhibited moderate to good cytotoxic activity against all the cell lines with IC_{50} values range 18.17-44.21 µM. Interestingly, the dual core compound **5e** was the most active compound in this series which is revealed most potent growth of inhibition [57.42 ± 0.33 µg/mL] on MCF-7 cell line with IC_{50} 7.53 ± 0.1 µM and % of inhibition 67.29 ± 0.25 µg/mL on MDA-MB-231 cell line with IC_{50} value 7.88 ± 1.1 µM, respectively. The final prepared compounds **5b**, **5f** were found to be most efficient anticancer activity towards T-47D breast cancer cell line with IC_{50} 10.15 ± 3.2, 10.21 ± 2.2, and against MCF-7 cell line with IC_{50} 7.90 ± 1.7, 7.53 ± 0.1 µM, respectively. The IC_{50} values depicted in Figure 1 for compound **4c**, and **5b** against T-47D, MDA-MB-231 breast cancer cells line were found 16.80 ± 1.05, 7.11 ± 2.1 µM, respectively. The compound **4c** have not identified to more efficient against MDA-MB-231 cell line whereas, **5a**, **5b** were not active against T-47D, MCF-7 cell line, respectively. The compounds **4b**, **5e** were found more potent % of inhibition 62.48 ± 0.20 µg/mL, 57.42 ± 0.33 µg/mL towards MCF-7 cell line and derived triazoles **5b**, **5f** are active against T-47D cell line with % of inhibition 61.25 ± 0.52, 57.21 ± 0.33 µg/mL, respectively. The % of inhibition of compounds **4b**, **5e** were found to be 58.14 ± 0.52 µg/mL, 67.29 ± 0.25 µg/mL against MDA-MB-231 cell line when tested for the standard drug cisplatin [69.84 ± 0.12 µg/mL]. The final scaffolds **4a**, **4c**, **5a**, and **5f** showed moderate to good % growth of inhibition values of 40.28 ± 0.13, 53.47 ± 0.26, 41.29 ± 0.11 and 43.28 ± 0.10 µg/mL, respectively. The compounds **4a** [48.57 ± 0.22 µg/mL], **5b** [54.18 ± 0.41 µg/mL], **5f** [44.91 ± 0.22 µg/mL] exhibits better activity against MCF-7 cell line whereas **4d**, **5a**, **5d** were found to be non-potent anticancer activity against T-47D cell line with growth of inhibition values 29.48 ± 0.24, 28.67 ± 0.30, 21.08 ± 0.32 µg/mL, respectively. Structural activity relationship (SAR) profiles for these derivatives demonstrated that electron-withdrawing groups such as 2-nitrophenyl oxadiazole (**4b**), 2-chloro-4-fluoro phenyl triazole (**5e**) and electron-donating groups like 4-methoxyphenyl oxadiazolyl triazole (**5b**) exhibited significant anticancer activity compared to other prepared heterocyclic compounds.

Docking studies

To validate the accuracy of Autodock 4.2 as an appropriate docking tool for the present purpose, the most active co-crystallized ligands were docked with 17β-hydroxysteroid dehydrogenase type 1 complexed in breast cancer (PDB: 3HB4) (Table 3, Figure 2) [45-47]. According to this method validation the successful scoring function is one in which the RMSD of the best docked conformation is ≤ 2.0 Å from the experimental one. The novel ligand 5e has the highest docking score ΔG = –13.76 kcal/mol and it forms hydrophobic amino acids like PheX:226, ValX:143 (Pi-alkyl), ProX:187, LeuX:149 (Pi-alkyl, Pi-Pi stackings), PheX:192 (Pi-sulphur and Pi-Pi stackings), and conventional hydrogen bondings with LysX;159 (2.543 Å), ValX:188 (2.864 Å), ValX:143 (2.130 Å), and GlyX:144 (1.948 Å). Compound **4d** having a binding score of –11.79 kcal/mol makes hydrogen bonding with the nearby amino acid residues AsnX:90 (H…O), LysX:159 (O…H), GlyX:92 (F…H) with their bond distance 2.341 Å, 2.927 Å, 1.840 Å, respectively and remaining interacting residues like Pi-alkyl amino acids IleX:14, MetX:193, LeuX:96, ValX:196, PheX:192 (Pi-Pi, van der Waals), TyrX:155 (Pi-sulphur, Pi-Pi stackings), GlyX:94 (van der Waals). Further the ligand **5a** having a binding score of –11.59 kcal/mol makes hydrogen bonding with the nearby amino acid residue TyrX:155, CysX:185 (Pi-Pi), ValX:196, ValX:188, LeuX:96, ProX:187 (Pi-alkyl and alkyl-alkyl), PheX:192 (Pi-sigma and Pi-Pi), MetX:193 (Pi-sigma), GlyX:186 (van der Waals), LysX:159 (hydrogen bonding) of E2B protein. Another most active ligand **5f** having a binding score ΔG = –11.53 kcal/mol makes a different bonding interactions like Pi-sigma bonding with ThrX:140, and Pi-alkyl interactions with AlaX:191, MetX:193, ValX:196 amino acids and Pi-sulphur, Pi-Pi stackings, carbon hydrogen bonding with these amino acids PheX:192, TyrX:155, and finally hydrogen bondings with three amino acids LysX:159 (3.018 Å), LeuX:95 (2.942 Å), IleX:14 (1.876 Å), respectively. From the Table 3 compounds **4d**, **5a**, **5e**, and **5f** having a dissociation constant of 2.28 nM, 3.21 nM, 82.76 µM and 3.54 nM makes good binging energy within the active site this protein. From this evident the binding interaction of the reference compound (cisplatin) shows different substantial attractions between the ligand and 17β-hydroxysteroid dehydrogenase type 1 complexed with E2B inhibitor. From the results of docking studies, compound **3c**, **5b**, **5d** have the lowest binding scores and out of the ten 1,3,4-oxadiazolyl-1,2,3-triazole derivatives analysed, compounds **4d**, **5a**, **5e**, and **5f** forms the best interaction patterns with 3HB4 protein.

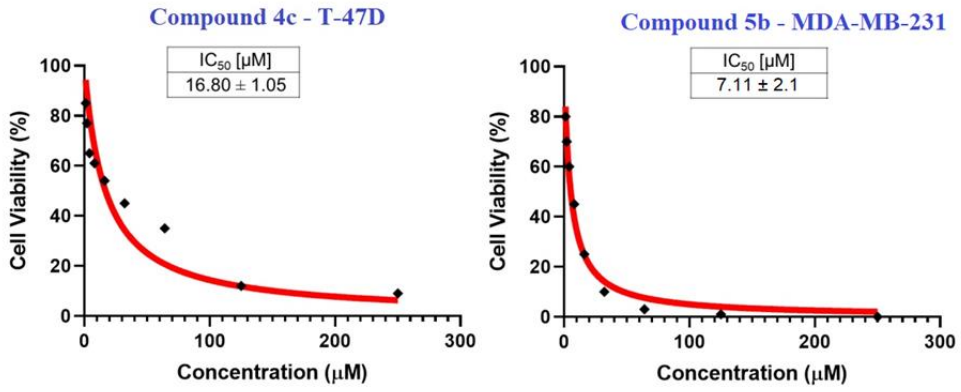


Figure 1. The inhibition effects of compound **4c** and **5b** on cell viability. The IC_{50} values depicted for compound **4c**, and **5b** against T-47D, MDA-MB-231 breast cancer cells line was found in IC_{50} values 16.80 ± 1.05, 7.11 ± 2.1 µM, respectively.

Table 3. Docking analysis of the most active compounds against breast cancer therapy.

Entry	ΔG	Kl	2D–Interacting amino acids	3D–Interacting amino acids
4d	–11.79 kcal/mol	2.28 nM	IleX:14, MetX:193, LeuX:96, ValX:196, PheX:192, TyrX:155, GlyX:94, AsnX:90, LysX:159, GlyX:92	Val143, Gly144, Lys159, Tyr155, Val196, Met193, Val188, Phe226, Phe269, gly166, Cys194
5a	–11.59 kcal/mol	3.21 nM	TyrX:155, CysX:185, ValX:196, ValX:188, LeuX:96, ProX:187, PheX:192, MetX:193, GlyX:186, LysX:159	Asn152, Leu95, Val168, Gly94, Lys159, Thr140, Ile14, Ser142, Cys185, Pro187, Phe126, Val188
5e	–13.76 kcal/mol	82.76 µM	PheX:226, ProX:187, LeuX:149, PheX:192, LysX;159, ValX:188, ValX:143, GlyX:144,	Thr14, Gly92, Lys159, Ser142, Tyr155, Gly94, Ile14, Ser12, Gly13, Phe192, Leu95, Val196, Phe192, Ala191, Thr190
5f	–11.53 kcal/mol	3.54 nM	AlaX:191, MetX:193, ValX:196, ThrX:140, PheX:192, TyrX:155, AsnX:90, LysX:159, LeuX:95, IleX:14	Leu96, Val196, Gly94, Gly92, Lys159, Asn152, Phe192, Gly13, Ile14, Asn90
Cisplatin	–10.24 kcal/mol	3.34 nM	CysX:185, ValX:188, LeuX:96, MetX:193, PheX:192, AsnX:90, LysX:159, LeuX:95, AsnX:90, GlyX:92	Gly94, Ile14, Thr14, Lys159, Ser12, Gly13, Cys194 Val196, Val188, Phe226, Gly166

ADMET studies

All the synthesized compounds (**4** and **5**) were analyzed for toxicity and other ADMET properties by using an online web server called SwissADME (http://www.swissadme.ch) to identify the leads as bestowed molecules for clinical trials and other management. The compound toxicity was also established by ProTox-II chemical toxicity predictions (http://tox.charite.de/protox_II/). After the performance of synthesized compounds with final conventional biological actions, the 10th series of compounds were then assessed for the physicochemical parameters along with the pharmacokinetic properties and drug–likeness with an aid of freely available SwissADME web source [48-50]. As acknowledged in the model study, the compounds were stand alone as a potent satisfactory with their performance in bioavailability (oral). Furthermore, in combination of topological surface polarity (TPSA), lipophilic nature, molecular weight and flexibility, solubility, saturation shown better drug likeness. The synthesized all compounds have showed an acceptable range of the above listed parameters and are graphically displayed in radar plots as mentioned in Figure 3. The pink area in radar figure represents the appropriate range of physicochemical space for optimal oral bioavailability (SIZE: MWt, LIPO: Lipophilicity, INSOLU: Solubility, POLAR: TPSA, INSATU: Insaturation, FLEX: Flexivbility). Here in Figure 3, the red line represents the ability range of compounds tested. Ability of compounds for drug–likeness was determined by number of free rotatable bonds and Lipinski's rule along with Eagan's, Veber's rules. Thus, these compounds were fulfilled with good pharmacokinetic profiles with satisfied criterion of drug–likeness (Table 4). In comparison with above all synthesized compounds, the specific molecules like **5a–f** were identified as prominent antibacterial agents than **4a–d**. Hence, they were also characterized for further pharmacokinetic analysis for future drug development. The Table 5 shows the outcome of listed properties and suggested with no significant violations of Lipinski's rule and the calculated physicochemical and pharmacokinetic descriptors are found to be within the projected thresholds (Drug–likeness acceptance, No. of violations.

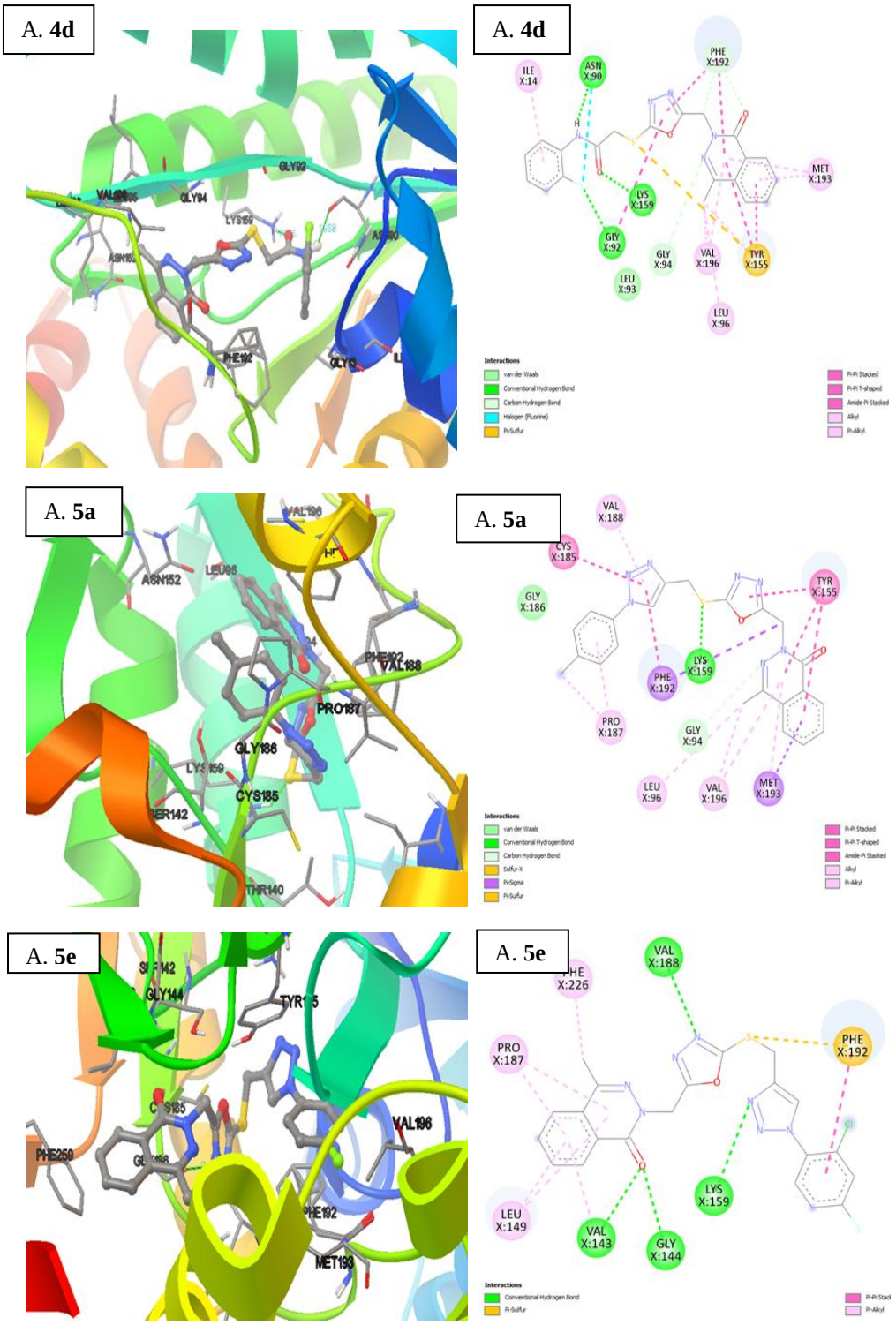

A. 4d
A. 4d
A. 5a
A. 5a
A. 5e
A. 5e

Figure 2. A). 3D Surface flexibility docking images of **4d**, **5a**, and **5e** in the possible PDF binding pocket, performed by Autodock within the active site of 17β-hydroxysteroid dehydrogenase type 1 complexed in breast cancer (3HB4). B). 2D–structures; Superposition of the docked conformations of **4**, **5** (stick model with atom type color: carbon-lime, nitrogen-blue, oxygen-red, hydrogen-gray, fluorine-light green). The backbone of 17β-hydroxysteroid dehydrogenase is shown as ribbon representation (red, sandy brown blue, light green color).

Table 4. List of calculated physicochemical properties using SwissADME web server.

S.No.	MW	nRB	nHBA	nHBD	TPSA	mLogp	cLogp	Logs	Synthetic accessibility
4a	441.89	7	6	1	128.21	2.93	3.21	-5.29	3.45
4b	452.44	8	8	1	174.03	1.62	2.14	-6.00	3.65
4c	441.89	7	6	1	128.21	2.93	3.21	-5.29	3.43
4d	425.44	7	7	1	128.21	2.82	3.02	-4.74	3.45
5a	445.50	6	7	0	129.82	3.22	3.24	-5.10	3.85
5b	461.50	7	8	0	139.05	2.72	2.90	-4.89	3.86
5c	447.47	6	8	1	150.05	2.50	2.50	-4.78	3.79
5d	476.47	7	9	0	175.64	2.20	2.19	-5.51	3.84
5e	483.91	6	8	0	129.82	3.87	3.65	-5.48	3.73
5f	476.47	7	9	0	175.64	2.20	2.14	-5.51	3.96

Table 5. Determination of drug–likeness acceptance.

S.No.	Bioavailability score	GI absorption	BBB permeant	CYP1A2 inhibitor	CYP2C9 inhibitor	CYP2D6 inhibitor	CYP3A4 inhibitor	Lipinski	Veber/ muegge
4a	0.55	High	No	Yes	Yes	No	Yes	Yes	Yes/Yes
4b	0.55	Low	No	No	Yes	No	Yes	Yes	No/No
4c	0.55	High	No	Yes	Yes	No	Yes	Yes	Yes/Yes
4d	0.55	High	No	Yes	Yes	No	Yes	Yes	Yes/Yes
5a	0.55	High	No	Yes	Yes	No	Yes	Yes	Yes/Yes
5b	0.55	Low	No	Yes	Yes	No	Yes	Yes	Yes/Yes
5c	0.55	Low	No	No	Yes	No	Yes	Yes	No/No
5d	0.55	Low	No	Yes	No	No	Yes	Yes	No/No
5e	0.55	Low	No	No	Yes	No	Yes	Yes	Yes/Yes
5f	0.55	Low	No	Yes	No	No	Yes	Yes	No/No

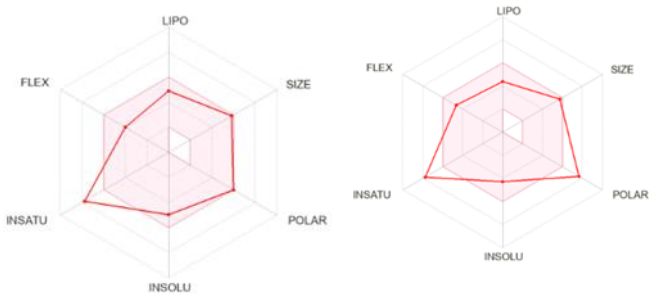


Figure 3. Bioavalibity radar of the most active compounds [**4b**, **4d**, **5b**, **5e**, and **5f**].

EXPERIMENTAL

Chemistry

All commercially available chemicals, reagents and solvents were used as received. Thin layer chromatography (TLC) was used with Merck silica gel 60F-254 plates and ethyl acetate: hexane to monitor the reactions (1:9) and compounds were visualized by UV light. All melting points were determined in open capillaries on an IKON melting point apparatus and are uncorrected. Silica gel (60-120 mesh) was used for column chromatography. Purity of all the synthesized anthracenyl pyrazole products was confirmed by binary gradient HPLC-3000 system. IR spectra were recorded on Perkin-Elmer spectrophotometer (Spectrum-Two) using KBr disk and values are expressed in cm^{-1}. The ^{1}H-NMR and ^{13}C-NMR spectra were recorded at Bruker 300-400 MHz, 100-125 MHz, respectively. The chemical shifts (δ) are reported in parts per million (ppm) downfield to TMS (δ = 0) and coupling constants (J) are expressed in Hertz (Hz). Mass spectra for the compounds were performed on Advion Expression-S CMS system.

*Synthesis of 2-(4-methyl-1-oxophthalazin-2(1H)-yl)acetohydrazide **1***

The related product, 4-methylphthalazin-1(2H)-one was commercially available or synthesised from 2-acetylbenzoic acid and hydrazine hydrate in ethanol for 5 h under reflux conditions. Then followed by N-alkylation with ethyl chloroacetate in the presence of potassium carbonate in acetone at rt for 4 h. The key intermediate 2-(4-methyl-1-oxophthalazin-2(1H)-yl) acetohydrazide **1** was afforded via the mixture of ethyl 2-(4-methyl-1-oxophthalazin-2(1H)-yl)acetate (0.5 g, 2.0 mmol) in ethanol (10 mL), hydrazine hydrate (0.17 g, 3.0 mmol) was added, and the reaction mixture was refluxed for 4 h. Excess ethanol was distilled off, the residue was poured into ice water, and the resulting solution was extracted with ethyl acetate (2 x 50 mL). The combined organic extracts were dried over Na_2SO_4 and evaporated under a vacuum. The crude product was purified by recrystallization from ethanol to obtain 2-(4-methyl-1-oxophthalazin-2(1H)-yl)acetohydrazide as light-yellow solid (85%), mp 98-100 °C.

*Synthesis of 2-((5-mercapto-1,3,4-oxadiazol-2-yl)methyl)-4-methylphthalazin-1(2H)-one **2***

To a solution of 2-(4-methyl-1-oxophthalazin-2(1H)-yl)acetohydrazide 1 (0.5 g, 2.1 mmol), KOH (0.15 g, 2.7 mmol), and CS_2 (0.24 g, 3.0 mmol) in ethanol (10 mL) was stirred under reflux for 6 h until H_2S no longer evolved. The solvent was then distilled off, and the residue was poured into crushed ice, and the solution was acidified with dil. HCl until pH 5. The crude product was filtered off, washed with water, dried, and recrystallized from ethanol to obtain 78% of compound 2 as a white solid, mp 123-125 °C.

*Synthesis of 4-methyl-2-((5-(prop-2-yn-1-ylthio)-1,3,4-oxadiazol-2-yl)methyl)phthalazin-1(2H)-one **3***

To a solution of 2-((5-mercapto-1,3,4-oxadiazol-2-yl)methyl)-4-methylphthalazin-1(2H)-one **2** (0.5 g, 1.8 mmol) in DMF (12 mL), propargyl bromide (0.25 g, 0.0028 mol) were added followed by K_2CO_3 (0.20 g, 1.4 mmol) and stirred for 7 h at rt and checking TLC at regular intervals. After completion of the reaction the mixture was poured into crushed ice water and extracted with EtOAc (2 x 25 mL). Purification of the crude 1,3,4–oxadiazole intermediate **3** has been done in 80% yields.

General procedure 4: Preparation of 2-((5-((4-methyl-1-oxophthalazin-2(1H)-yl) methyl)-1,3,4-oxadiazol-2-yl)thio)-N-acetamide

The crucial intermediate 2-chloro-N-phenylsubstituted acetamide was prepared via the amination reaction of substituted anilines by treatment with 2-chloroacetyl chloride in dichloromethane and triethyl amine under refluxing for 2 h. Finally, the target compounds 4 was synthesized by the condensation reaction between 2-((5-mercapto-1,3,4-oxadiazol-2-yl) methyl)-4-methyl phthalazin-1(2H)-one **2** (0.5 g, 1.8 mmol), an appropriate acetamides (0.37-0.42 g, 2.1 mmol), potassium carbonate (0.199 g, 1.4 mmol) in acetone (12 mL) was stirred vigorously at rt. Then the progress of the reaction was monitored under refluxing for 4 h-4.5 h and checking TLC at regular intervals. After completion of the reaction the mixture was poured into crushed ice (100 g). The solid separated was filtered, dried and purified by column chromatography on silica gel (100-200 mesh) using n-hexane and ethyl acetate (8:2) mixture as eluent to isolate pure desired products in excellent yields (76–86%).

General procedure 5: Preparation of 4-methyl-2-((5-(((1-phenylsubstituted-1H-1,2,3-triazol-4-yl)methyl)thio)-1,3,4-oxadiazol-2-yl)methyl)phthalazin-1(2H)-one

In 15 mL of dry N,N–dimethylformamide, a mixture of 4-methyl-2-((5-(prop-2-yn-1-ylthio)-1,3,4-oxadiazol-2-yl)methyl)phthalazin-1(2H)-one 3 (0.5 g, 1.6 mmol), an appropriate azides (0.22-0.35 g, 1.9 mmol), sodium ascorbate (0.158 g, 0.8 mmol), and copper sulphate pentahydrate (0.11 g, 0.4 mmol) were agitated for 30-45 min at ambient temperature. TLC was used to track the progress of the reaction at regular intervals. After the completion, the reaction mass was poured on to crushed ice (50 g). The solid obtained was filtered, dried and purified by column chromatography using ethyl acetate and hexane mixture as eluent to afford compound 5 (70-89% yield).

Spectral characterization of final compounds

*N-(2-Chlorophenyl)-2-((5-((4-methyl-1-oxophthalazin-2(1H)-yl)methyl)-1,3,4-oxadiazol-2-yl) thio)acetamide **4a***. White crystalline solid, 86% yield, mp: 184-186 °C, IR (KBr, cm^{-1}): ν 3355 (NH), 3064 (=CH), 2931 (–CH), 1675 (C=O), 1527 (C=C), 1422 (C=N), 1139 (COC), 850 (CCl); ^{1}H NMR (400 MHz, DMSO-d6): δ ppm 10.12 (s, 1H, NH), 7.80 (d, J = 7.6 Hz, 1H, Ar-H), 7.67 (d, J = 7.6 Hz, 1H, Ar-H), 7.35 (d, J = 7.6 Hz, 1H, Ar-H), 7.23 (m, 2H, Ar-H), 7.12 (t, J = 7.9 Hz, 2H, Ar-H), 6.84 (t, J = 7.9 Hz, 1H, Ar-H), 4.61 (s, 2H, –NCH_2), 3.82 (s, 2H, –SCH_2), 2.60 (s, 3H, CH_3); ^{13}C NMR (100 MHz, DMSO-d6): δ ppm 167.27, 163.42, 161.18, 151.94, 140.03, 139.34, 134.93, 133.47, 132.40, 130.88, 130.01, 128.75, 126.09, 120.77, 115.67, 52.82, 34.89, 20.24; m/z 442.11 $(M + 1)^+$. Found, %: C, 54.32; H, 3.60; Cl, 8.04; N, 15.24; S, 7.19. $C_{20}H_{16}ClN_5O_3S$. Calculated, %: C, 55.13; H, 3.27; Cl, 8.29; N, 15.63; S, 7.86.

*2-((5-((4-Methyl-1-oxophthalazin-2(1H)-yl)methyl)-1,3,4-oxadiazol-2-yl)thio)-N-(2-nitrophenyl) acetamide **4b***. Yellow crystalline solid, 81% yield, mp: 170-172 °C, IR (KBr, cm^{-1}): ν 3364

(NH), 3076 (=CH), 2951 (–CH), 1688 (C=O), 1531 (C=C), 1410 (C=N), 1145 (COC); ^{1}H NMR (400 MHz, DMSO-d6): δ ppm 10.05 (s, 1H, NH), 8.13 (d, J = 7.6 Hz, 1H, Ar-H), 7.67 (d, J = 7.5 Hz, 2H, Ar-H), 7.31 (m, 2H, Ar-H), 7.20 (dd, J = 8.1, 2.8 Hz, 1H, Ar-H), 6.93 (t, J = 7.9 Hz, 2H, Ar-H), 4.82 (s, 2H, –NCH_2), 3.71 (s, 2H, –SCH_2), 2.90 (s, 3H, CH_3); ^{13}C NMR (100 MHz, DMSO-d6): δ ppm 166.82, 163.31, 160.20, 149.73, 140.29, 139.15, 134.37, 133.13, 132.25, 130.49, 129.73, 128.55, 126.21, 121.11, 116.46, 53.27, 33.19, 22.62; m/z 453.06 $(M + 1)^{+}$. Found, %: C, 53.10; H, 3.58; N, 18.64; S, 7.04. $C_{20}H_{16}N_6O_5S$. Calculated, %: C, 54.21; H, 3.94; N, 19.13; S, 7.55.

N-(4-Chlorophenyl)-2-((5-((4-methyl-1-oxophthalazin-2(1H)-yl)methyl)-1,3,4-oxadiazol-2-yl) thio)acetamide ***4c***. White crystalline solid, 80% yield, mp: 190-192 °C, IR (KBr, cm^{-1}): ν 3348 (NH), 3044 (=CH), 2923 (–CH), 1676 (C=O), 1525 (C=C), 1430 (C=N), 1158 (COC), 864 (CCl); ^{1}H NMR (400 MHz, DMSO-d6): δ ppm 10.06 (s, 1H, NH), 7.76 (d, J = 7.6 Hz, 1H, Ar-H), 7.49 (d, J = 7.7 Hz, 2H, Ar-H), 7.21 (d, J = 7.7 Hz, 2H, Ar-H), 7.14 (dd, J = 8.0, 3.0 Hz, 1H, Ar-H), 6.84 (t, J = 7.9 Hz, 2H, Ar-H), 4.65 (s, 2H, –NCH_2), 3.89 (s, 2H, –SCH_2), 2.80 (s, 3H, CH_3); ^{13}C NMR (100 MHz, DMSO-d6): δ ppm 167.33, 163.70, 161.47, 151.20, 140.30, 139.96, 139.34, 133.99, 130.85, 129.97, 128.74, 128.27, 126.75, 120.77, 118.77, 51.42, 35.31, 21.43; m/z 442.09 $(M + 1)^{+}$. Found, %: C, 54.20; H, 3.51; Cl, 8.37; N, 15.66; S, 7.01. $C_{20}H_{16}ClN_5O_3S$. Calculated, %: C, 54.87; H, 3.84; Cl, 8.34; N, 16.13; S, 7.64.

N-(2-Fluorophenyl)-2-((5-((4-methyl-1-oxophthalazin-2(1H)-yl)methyl)-1,3,4-oxadiazol-2-yl) thio)acetamide ***4d***. Brownish color solid, mp. 142-144 °C, yield: 76%; IR (ν, cm^{-1}): 3324 (NH), 3085 (=CH), 2972 (CH), 1683 (C=O), 1561 (C=C), 1430 (C=N), 1225 (C–S–C), 1138 (C–O–C), 953 (C–F). 1H NMR (400 MHz, DMSO-d6): δ ppm 10.03 (s, 1H, NH), 7.82 (d, J = 7.6 Hz, 1H, Ar-H), 7.70 (d, J = 7.7 Hz, 2H, Ar-H), 7.40 (m, 2H, Ar-H), 7.35 (t, J = 7.9 Hz, 2H, Ar-H), 7.09 (d, J = 7.4 Hz, 1H, Ar-H), 6.88 (t, J = 7.9 Hz, 2H, Ar-H), 4.68 (s, 2H, –NCH_2), 4.08 (s, 2H, –SCH_2), 2.02 (s, 3H, CH_3); ^{13}C NMR (100 MHz, DMSO-d6): δ ppm 165.00, 160.50, 156.35, 151.39, 150.72, 147.87, 138.92, 136.85, 134.55, 130.21, 128.79, 128.19, 126.76, 126.03, 124.77, 110.28, 51.44, 38.18, 21.33; m/z: 426.15 $(M + 1)^{+}$. Anal. calcd. $C_{20}H_{16}FN_5O_3S$: C, 56.24; H, 3.71; F, 4.43; N, 16.41; S, 7.30. Found: C, 56.88; H, 3.30; F, 4.27; N, 16.76; S, 7.11.

4-Methyl-2-((5-(((1-(p-tolyl)-1H-1,2,3-triazol-4-yl)methyl)thio)-1,3,4-oxadiazol-2-yl) methyl) phthalazin-1(2H)-one ***5a***. Light brown solid, mp. 201-203 °C, yield: 89%; IR (ν, cm^{-1}): 3085 (=CH), 2972 (CH), 1683 (C=O), 1561 (C=C), 1430 (C=N), 1225 (C–S–C), 1138 (C–O–C), 953 (C–F). ^{1}H NMR (400 MHz, DMSO-d6): δ ppm 8.17 (d, J = 7.5 Hz, 1H, Ar-H), 7.84 (s, 1H, triazole-H), 7.70 (d, J = 7.5 Hz, 1H, Ar-H), 7.58 (d, J = 7.4 Hz, 2H, Ar-H), 7.33 (d, J = 7.4 Hz, 2H, Ar-H), 7.23 (dd, J = 8.1, 2.8 Hz, 2H, Ar-H), 5.14 (s, 2H, –NCH2), 4.61 (s, 2H, –SCH_2), 2.91 (s, 3H, CH_3), 2.68 (s, 3H, CH_3); ^{13}C NMR (100 MHz, DMSO-d6): δ ppm 166.59, 163.56, 161.56, 159.81, 152.98, 147.15, 140.42, 138.14, 134.03, 130.55, 128.33, 125.00, 124.28, 121.70, 118.70, 115.47, 113.13, 55.48, 36.47, 20.86, 19.46; m/z: 446.09 $(M + 1)^{+}$. Anal. calcd. $C_{22}H_{19}N_7O_2S$: C, 59.31; H, 4.30; N, 22.01; S, 7.20. Found: C, 59.75; H, 4.94; N, 22.87; S, 7.44.

2-((5-(((1-(4-Methoxyphenyl)-1H-1,2,3-triazol-4-yl)methyl)thio)-1,3,4-oxadiazol-2-yl) methyl)-4-methylphthalazin-1(2H)-one ***5b***. Light brown solid, mp. 215-217 °C, yield: 74%; IR (ν, cm^{-1}): 3094 (=CH), 2966 (CH), 1702 (C=O), 1555 (C=C), 1424 (C=N), 1230 (C–S–C), 1120 (C–O–C). ^{1}H NMR (400 MHz, DMSO-d6): δ ppm 8.06 (d, J = 7.6 Hz, 1H, Ar-H), 7.70 (s, 1H, triazole-H), 7.54 (d, J = 7.6 Hz, 1H, Ar-H), 7.43 (d, J = 7.2 Hz, 2H, Ar-H), 7.30 (d, J = 7.2 Hz, 2H, Ar-H), 7.16 (dd, J = 8.0, 2.9 Hz, 1H, Ar-H), 6.97 (dd, J = 8.0, 2.9 Hz, 1H, Ar-H), 5.12 (s, 2H, –NCH_2), 4.75 (s, 2H, –SCH_2), 3.26 (s, 3H, OCH_3), 2.88 (s, 3H, CH_3); ^{13}C NMR (100 MHz, DMSO-d6): δ ppm 165.20, 163.44, 161.11, 150.74, 145.33, 140.46, 137.17, 134.19, 131.35, 129.10, 125.73, 124.22, 122.58, 119.00, 115.44, 113.30, 56.88, 37.10, 22.47, 20.55; m/z: 462.10

$(M + 1)^+$. Anal. calcd. $C_{22}H_{19}N_7O_3S$: C, 57.41; H, 4.19; N, 21.32; S, 6.84. Found: C, 57.01; H, 4.64; N, 21.77; S, 7.23.

2-((5-(((1-(4-Hydroxyphenyl)-1H-1,2,3-triazol-4-yl)methyl)thio)-1,3,4-oxadiazol-2-yl) methyl)-4-methylphthalazin-1(2H)-one ***5c***. Brown color solid, mp. 222-224 °C, yield: 70%; IR (ν, cm^{-1}): 3422 (OH), 3073 (=CH), 2915 (CH), 1710 (C=O), 1536 (C=C), 1416 (C=N), 1253 (C–S–C), 1130 (C–O–C). ^{1}H NMR (400 MHz, DMSO-d6): δ ppm 7.86 (d, J = 7.2 Hz, 1H, Ar-H), 7.72 (d, J = 7.0 Hz, 2H, Ar-H), 7.58 (d, J = 7.0 Hz, 2H, Ar-H), 7.30 (s, 1H, triazole-H), 7.18 (d, J = 7.2 Hz, 1H, Ar-H), 7.03 (t, J = 8.0 Hz, 2H, Ar-H), 5.50 (s, 1H, OH), 5.10 (s, 2H, $–NCH_2$), 4.80 (s, 2H, $–SCH_2$), 3.52 (s, 3H, CH_3); ^{13}C NMR (100 MHz, DMSO-d6): δ ppm 167.51, 163.68, 161.84, 151.44, 140.54, 139.31, 137.34, 134.21, 131.50, 130.36, 128.48, 126.96, 120.68, 118.46, 115.56, 114.39, 50.04, 35.90, 19.03; m/z: 448.12 $(M + 1)^+$. Anal. calcd. $C_{21}H_{17}N_7O_3S$: C, 56.37; H, 3.83; N, 21.91; S, 7.17. Found: C, 56.85; H, 3.91; N, 22.34; S, 7.46.

4-Methyl-2-((5-(((1-(4-nitrophenyl)-1H-1,2,3-triazol-4-yl)methyl)thio)-1,3,4-oxadiazol-2-yl) methyl)phthalazin-1(2H)-one ***5d***. Yellow color solid, mp. 185-187 °C, yield: 87%; IR (ν, cm^{-1}): 3102 (=CH), 2939 (CH), 1677 (C=O), 1510 (C=C), 1425 (C=N), 1213 (C–S–C), 1129 (C–O–C). ^{1}H NMR (400 MHz, DMSO-d6): δ ppm 7.90 (d, J = 7.5 Hz, 1H, Ar-H), 7.76 (d, J = 7.5 Hz, 1H, Ar-H), 7.57 (d, J = 7.4 Hz, 2H, Ar-H), 7.36 (s, 1H, triazole-H), 7.24 (d, J = 7.4 Hz, 2H, Ar-H), 7.16 (d, J = 7.5 Hz, 1H, Ar-H), 7.02 (d, J = 7.5 Hz, 1H, Ar-H), 4.82 (s, 2H, $–NCH_2$), 4.26 (s, 2H, $–SCH_2$), 2.81 (s, 3H, CH_3); ^{13}C NMR (100 MHz, DMSO-d6): δ ppm 166.79, 163.73, 161.64, 152.27, 147.76, 140.13, 136.32, 134.63, 132.93, 131.73, 131.51, 129.04, 128.91, 128.63, 125.67, 124.97, 116.50, 115.89, 51.49, 35.31, 21.40; m/z: 477.04 $(M + 1)^+$. Anal. calcd. $C_{21}H_{16}N_8O_4S$: C, 52.94; H, 3.38; N, 23.52; S, 6.73. Found: C, 52.31; H, 3.73; N, 23.88; S, 6.10.

2-((5-(((1-(2-Chloro-4-fluorophenyl)-1H-1,2,3-triazol-4-yl)methyl)thio)-1,3,4-oxadiazol-2-yl) methyl)-4-methylphthalazin-1(2H)-one ***5e***. Brown solid, mp. 164-166 °C, yield: 81%; IR (ν, cm^{-1}): 3110 (=CH), 2942 (CH), 1710 (C=O), 1556 (C=C), 1423 (C=N), 1211 (C–S–C), 1142 (C–O–C), 967 (CF), 859 (CCl). ^{1}H NMR (400 MHz, DMSO-d6): δ ppm 8.13 (d, J = 7.5 Hz, 1H, Ar-H), 7.87 (s, 1H, triazole-H), 7.80 (d, J = 7.5 Hz, 1H, Ar-H), 7.60 (m, 2H, Ar-H), 7.50 (d, J = 7.5 Hz, 1H, Ar-H), 7.19 (d, J = 7.5 Hz, 1H, Ar-H), 7.03 (t, J = 7.9 Hz, 1H, Ar-H), 4.72 (s, 2H, $–NCH_2$), 4.17 (s, 2H, $–SCH_2$), 2.26 (s, 3H, CH_3); ^{13}C NMR (100 MHz, DMSO-d6): δ ppm 164.02, 161.41, 159.71, 146.33, 140.44, 139.12, 138.36, 131.03, 130.25, 126.67, 125.18, 121.70, 121.57, 120.58, 118.33, 115.22, 112.50, 55.48, 36.47, 22.15; m/z: 484.16 $(M + 1)^+$. Anal. calcd. $C_{21}H_{15}ClFN_7O_2S$: C, 52.38; H, 3.61; Cl, 7.10; F, 3.22; N, 19.31; S, 6.20. Found: C, 52.76; H, 3.98; Cl, 7.24; F, 3.84; N, 20.86; S, 6.55.

4-Methyl-2-((5-(((1-(3-nitrophenyl)-1H-1,2,3-triazol-4-yl)methyl)thio)-1,3,4-oxadiazol-2-yl) methyl)phthalazin-1(2H)-one ***5f***. Yellow crystalline solid, mp. 170-172 °C, yield: 87 %; IR (ν, cm^{-1}): 3075 (=CH), 2963 (CH), 1704 (C=O), 1534 (C=C), 1418 (C=N), 1216 (C–S–C), 1134 (C–O–C). ^{1}H NMR (400 MHz, DMSO-d6): δ ppm 8.10 (d, J = 7.5 Hz, 1H, Ar-H), 7.84 (d, J = 7.5 Hz, 1H, Ar-H), 7.55 (s, 1H, triazole-H), 7.43 (s, 1H, Ar-H), 7.35 (d, J = 7.2 Hz, 2H, Ar-H), 7.26 (m, 1H, Ar-H), 7.13 (d, J = 7.5 Hz, 1H, Ar-H), 6.98 (t, J = 7.9 Hz, 1H, Ar-H), 4.87 (s, 2H, $–NCH_2$), 4.24 (s, 2H, $–SCH_2$), 3.10 (s, 3H, CH_3); ^{13}C NMR (100 MHz, DMSO-d6): δ ppm 162.15, 160.30, 153.18, 147.37, 141.21, 135.33, 134.55, 132.46, 131.28, 130.21, 129.22, 128.15, 127.24, 125.10, 124.42, 116.81, 115.23, 53.37, 33.99, 22.43; m/z: 477.12 $(M + 1)^+$. Anal. calcd. $C_{21}H_{16}N_8O_4S$: C, 53.06; H, 3.52; N, 23.34; S, 6.67. Found: C, 53.24; H, 3.67; N, 23.99; S, 6.13.

CONCLUSION

In summary, we have developed phthalazine with 1,3,4-oxadiazole substituents and 1,2,3-triazoles displayed excellent anticancer activity and examined evidencing some interesting relationship between the synthesised structures, their anticancer activity and in silico docking. All the results demonstrated that phthalazin-1(2H)-one-1,3,4-oxadiazoles **4a–d**, phthalazin-1(2H)-one-1,3,4-oxadiazolyl-1,2,3-triazoles **5a–f** possess promising and wonderful in vitro antitumor activity versus breast cancer cell lines when compared to the cisplatin. Cytotoxic results concluded that compound **5e** bearing *o*-chloro-*p*-fluoro phenyl ring was found to be the most active which blocks proliferation of breast cancer cells with IC_{50} 9.50 ± 1.5, 7.53 ± 0.1, and 7.88 ± 1.1 μM, respectively. Additionally, the conducted molecular docking studies assured the binding affinities of the afforded compounds to the breast cancer, particularly compound 4d and **5e**, which displayed a binding score exceeding that shown by cisplatin. Furthermore, the established ADMET studies have put eyes on the eligible pharmacokinetics of the afforded compounds, particularly their oral bioavailability. The dedicated SAR elicited the usefulness of the 1,3,4-oxadiazole and 1,2,3-triazole moiety for activity and can pave the way for future structural modifications with anticipated activity.

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Conflict of interest

The authors declare that they have no competing interests.

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