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Targeting CDK2 Kinase in Breast Cancer Employing Novel Oxindole-Quinazoline Conjugates: Design, Synthesis, Biological Assessments and Molecular Docking Study

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Abstract

This study involved the design and synthesis of a novel series of oxindole-quinazoline hybrids targeting CDK2 in breast cancer. MTT assay investigated the cytotoxic activity of all the synthesized compounds **8a-e** towards MCF-7 breast adenocarcinoma with IC_{50} ranging from 0.007 to 0.104 µM. The most active compound **8a** was also evaluated for its cytotoxic activity on normal breast epithelial cell line MCF-10a cell line. The hybrid **8a** demonstrated a high safety margin with SI = 10.57. Further assessment of **8a** on its inhibitory activity on CDK2 proved its optimum potency with IC_{50} = 0.011 nM. Molecular docking of **8a** in the binding pocket of CDK2 proved the expected binding mode.

Keywords: oxindole-quinazoline hybrids, breast cancer; CDK-2 inhibitors; molecular docking

1. Introduction

Cancer continues to be the primary cause of morbidity and death despite substantial research and clinical trials on potential new treatments [1]. In 2020, there were approximately 19.3 million new cancer cases and 10.0 million cancer-related fatalities [2]. By 2040, the number of cancer cases is expected to rise from 19.3 to 28.4 million [3], necessitating scientists in the academic and pharmaceutical communities to actively search for preventive and therapeutic measures. Till date, the most available three options for treatments are surgery, radiotherapy and chemotherapy that provide systemic cancer therapy [4]. Despite the wide accessibility of potentchemotherapeutic agents, a number of factors, including multidrug resistance, low patient compliance, narrow therapeutic index and the serious side effects of the anticancer drugs currently in use, provide a significant challenge for the ongoing search for effective and targeted antitumor agents. Many extensive researches is being conducted to innovate new strategies targeting signaling pathways. Compared to chemotherapy, targeted therapy that targets signaling pathways has demonstrated more selectivity, less harmful side effects and highly effective anticancer agents [5].

Since protein kinases alter growth factor signaling, they are regarded as a crucial target in cancer treatment [6]. The common structural features of typical kinases are conserved ATP binding site that is targeted by most protein kinases inhibitors to prevent phosphorylation of substrate protein, substrate interaction site, activation and allosteric site [7-10]. Serine/thereonine kinases are considered as an important subclass ofprotein kinases, that includes 385 kinases [11]. The CDK constitutes a family of serine/threonine protein kinases plays a vital role in maintaining cancer cell proliferation in addition to being engaged in many physiological processes such as cell division, apoptosis, and gene transcription [12]. Out of all the CDK subtypes, CDK2 attracted the most attention owing to its implication in variousimportant cellular function when complexed with its activating proteins cyclin A or E [13]. CDK2 is essential for regulating several aspects of the cell cycle division, including transcription of genes, repair of DNA, the G1-S progression, and modulation of G2 transition [14]. CDK2 is required for the proliferation of tumor cells and the overactivation of its cyclins A and/or E is crucial mechanism in several forms of cancer [15]. It is commonly over expressed in various human malignancies as breast [16], lung [17], pancreatic [18], colon cancers [19], glioblastoma[20], melanoma [21] and osteosarcoma [22]. Numerous clinical studies have examined CDK2 inhibitors with different scaffolds including Dinaciclib (**I**), Milciclib (**II**), Roniciclib (**III**), BMS-387032 (**IV**), PHA-793887 (**V**), Roscovitine (**VI**) and its updated analogFadraciclib (**VII**)[23]. The ongoing clinical trials have yielded encouraging results about the possible effectiveness of these CDK2 inhibitors, though many of them revealed some side effects like toxicity and limited efficacy [24]. Consequently, the development of novel CDK2 inhibitors continues to be an intriguing area of study for medicinal chemists seeking to create new anticancer drugs (**Figure 1**).

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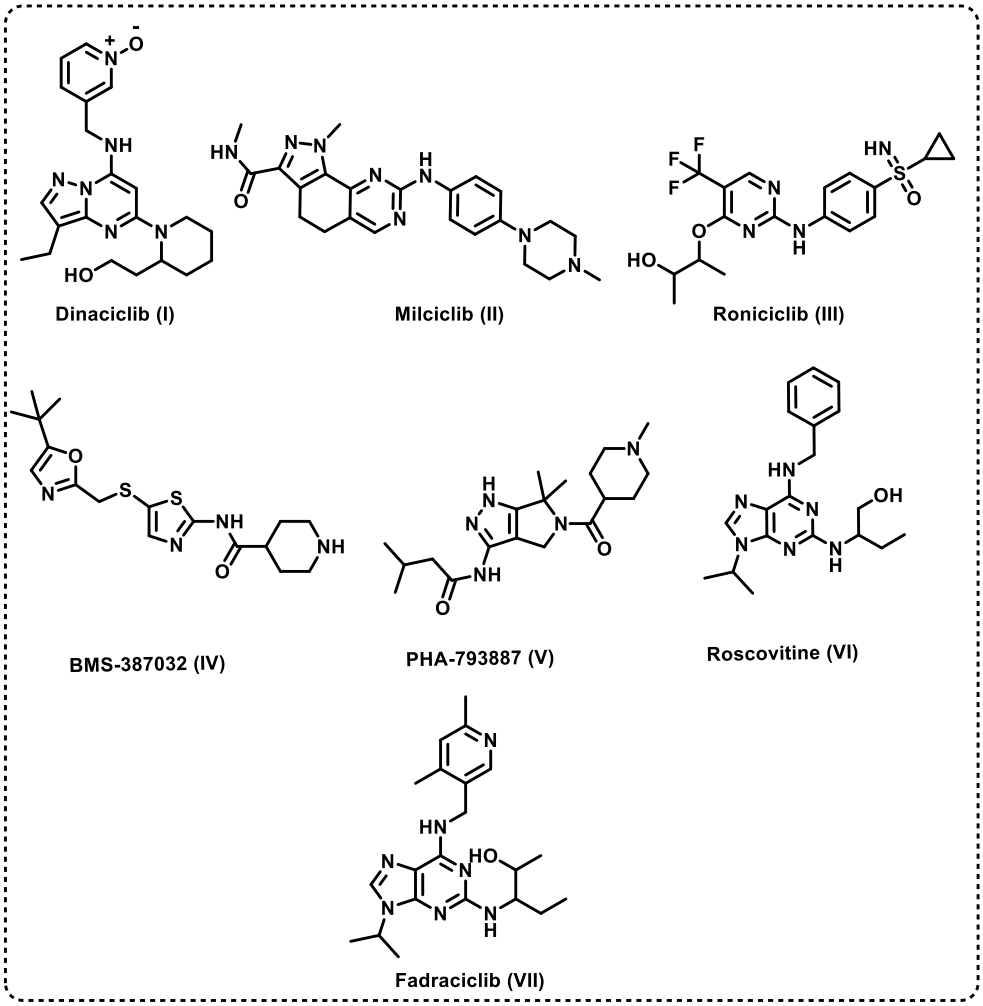


Figure 1: Structures of different CDK2 inhibitors under clinical trials

As a versatile molecule, isatin (1*H*-indol-2,3-dione) that is a natural alkaloid, is the precursor of a huge number of synthetic derivatives and possessing a wide range of pharmacological properties particularly as antitumor agents[25-28]. Few isatin derivatives have been approved as anticancer agents while others are undergoing clinical studies[29]. For example, Sunitinib (**VIII**), and Toceranib (**IX**) have been clinically approved by FDA in 2006 and 2009, respectively as selective inhibitors for different protein kinases as platelet-derived growth factor receptors PDGFRα/β and the vascular endothelial one VEGFR2/3 [30, 31]. Nintedanib (**X**), Orantinib (**XI**) and Semaxinib (**XII**) are currently undergoing clinical tests as antiangiogenic agents against different types of cancers including colorectal, renal, ovarian and hepatocellular carcinoma. They act as potent triple angiokinase inhibitors preventing the proangiogenic pathways mediated by VEGFR1/2/3, PDGFRα/β and fibroblast growth factor receptor FGFR1/2/3 [32-34]. In addition, 6-BIO (6-bromo-induribin-3'-oxime) (**XIII**) that is under clinical trials for chronic myelocyticleukaemia (CML) treatment exerting GSK-3β and CDK-2 inhibition activity by IC_{50} = 5 and 300 nM, respectively [35]. While, the indirubin-5-sulphonic acid (**XIV**) demonstrated strong CDK2 inhibitory action (IC_{50} = 35 nM) [36], with less significant effects against GSK-3β (IC_{50} = 280 nM) [37] (**Figure 2**).

On the other hand, quinazolines are considered as an important scaffold in medicinal chemistry[38-45]. Diverse quinazoline derivatives were reported as CDK2 inhibitors. The quinazoline derivatives **XV** and **XVI**, both are acting as potential CDK2 inhibitors with IC_{50} = 1.5 and 1.0 uM, respectively [46, 47]. In addition, many literatures confirmed the CDK2 inhibiting activity of different quinazoline derivatives as the 2-phenoxy methyl quinazolinone derivatives **XVII** and X**VIII** exhibited potential CDK2 inhibitory activity with IC_{50} = 0.63 and 1.74 uM respectively compared to roscovitine (IC_{50}= 1.28 uM) [48], in addition to their excellent cytotoxic activity towards melanoma (MDA-MB-435) cell line with GI% 94.53 and 94.15%, respectively. The pyrimido[4,5-*f*]quinazolineanalogs**XIX** potentially inhibited CDK2 enzyme with IC_{50} = 0.11 and 0.09 uM in comparison to BMS-387032 (IC_{50}= 0.052 uM) besides to their anticancer activity towards MCF-7 and HCT116 cell lines with IC_{50} ranging from 0.9-1.4 uM equipotent to the reference standard BMS-387032 that displayed IC_{50} = 1.0 and 0.7 uM, respectively [23] (**Figure 3**).

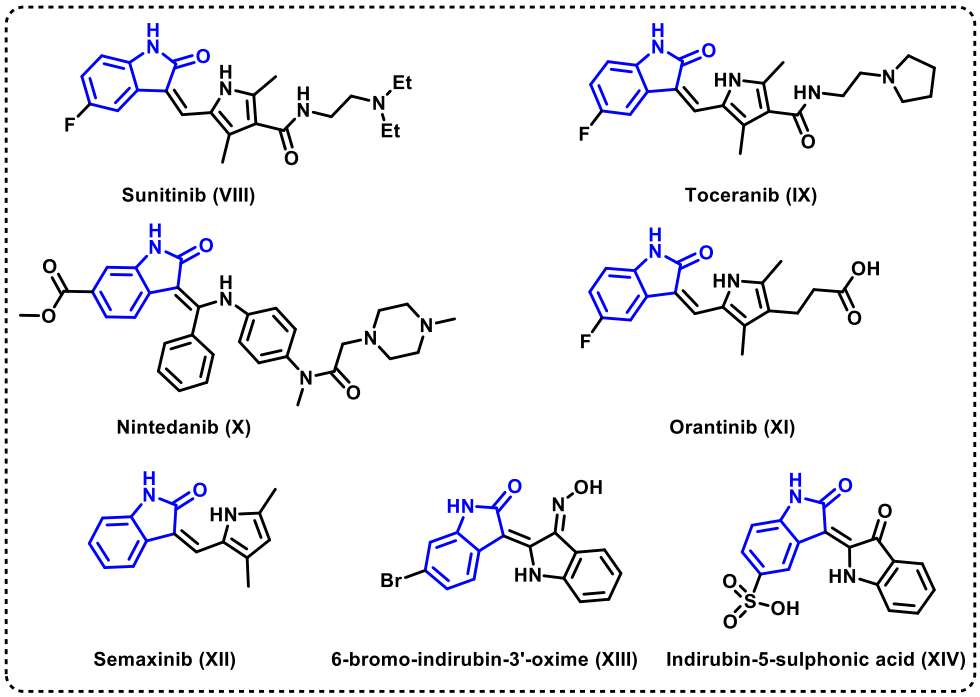


Figure 2: Structure of different oxindole as protein kinase inhibitors

Molecular hybridization has gained popularity as a powerful technique for discovering novel medications [49]. In this regards, pharmacophoreoxindole-containing molecular hybrids with other bioactive molecule such as quinazolines are of special interest to medicinal chemists.

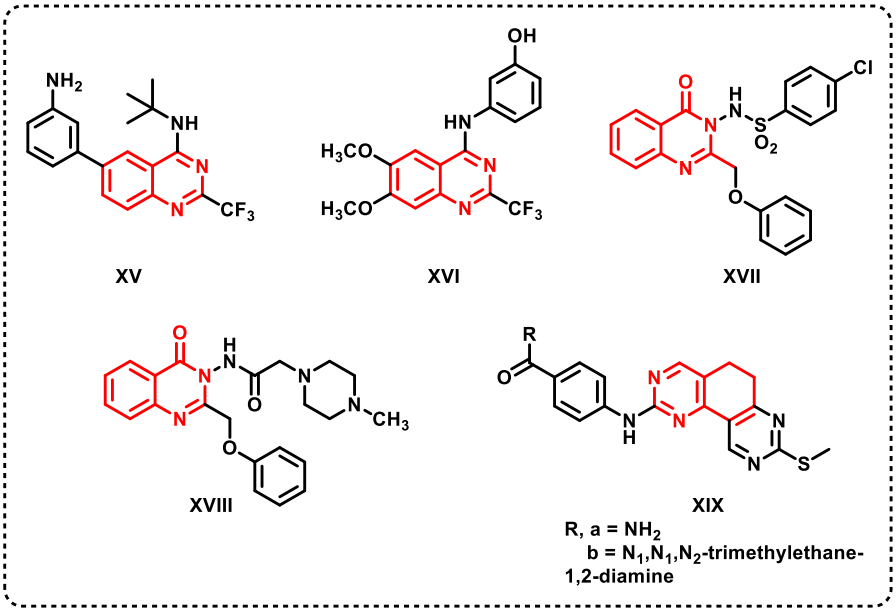


Figure 3: Structure of different reported quinazolines with CDK2 inhibition activity

In an attempt to create single compact hybrid out of two bioactive moieties as isatin and quinazoline, the current study plot the design and synthesize of oxindole-quinazoline new hybrids of the general structure **XX**connected through the acetohydrazide linkage (**Figure 4**) in order to generate novel CDK2 inhibitors of potential anticancer activity based on all of the aforementioned facts and in continuation of our earlier efforts[25]. The design approach relies on the fitting of the oxindole moiety in the ATP binding site of the target enzyme formingtwohydrogen bonds with the essential amino acids Glu81 and Leu83 through CONH moiety, while the quinazoline nucleus is directed to the solvent region. The cytotoxic activity of all the designed and synthesized compounds were assessed towards breast cancer MCF-7 cell line. The safety profile of the most active compound was determined through investigating its effect on the normal epithelial breast cell lineMCF-10a. *In-vitro* CDK2 inhibition assay was performed for the most active derivative **8a** to examine the molecular mechanism of its anticancer effect.*In silico*, docking simulation study was also performed for the same compound so as to prove the anticipated binding interactions with the target enzyme's active site.

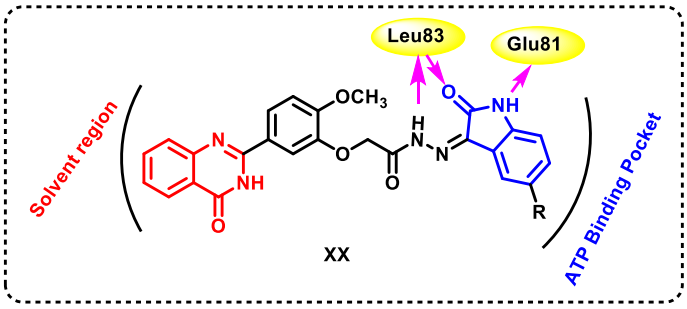


Figure 4: Structure of the design target compounds and their predicted interactions with CDK2 enzyme active site

2. Experimental

2.1. Chemistry

2.1.1. General remarks

Melting points were detected with the Electrothermal equipment 9100 in open capillary tubes and were uncorrected. VarioElementar was used to conduct elemental microanalyses at the Microanalytical Unit, Central Services Laboratory, National Research Centre, Dokki, Cairo, Egypt. The results were determined to be within ± 0.4% of the theoretical values. The Schimadzu FT/IR Affinity-1 spectrometer was used at the Faculty of Pharmacy, Cairo University, Cairo, Egypt, to record infrared spectra at the cm^{-1} scale using the KBr disc technique. Bruker High Performance Digital FT-NMR Spectrometer (300 or 400 or 500 and 75 or 100 or 125 MHz) was used to determine the ^{1}H and ^{13}C NMR spectra at the Faculty of Pharmacy, Cairo University, Cairo, Egypt, and National Research Center, Cairo, Egypt. Due to the low solubility of the target derivatives, all ^{13}C NMR spectra were measured overnight. Using TMS as an internal reference, chemical shifts were represented as δ (ppm) downfield.

2.1.2. Synthesis of the starting compounds

ethyl 2-(2-methoxy-5-(4-oxo-3,4-dihydroquinazolin-2-yl)phenoxy)acetate (5)

A mixture of ethyl 2-(5-formyl-2-methoxyphenoxy)acetate(**3**) (10 mmol, 2.38 g), 2-aminobenzamide (**4**) (10 mmol, 1.36 g) and copper (II) chloride (20 mmol, 2.69 g) was refluxed in absolute ethanol (20 mL) for 3 h. After cooling the formed precipitate was filtered, washed several times with ethanol followed by water, dried and recrystallized from ethanol to give the title compound **5** in yield 87%, m.p183-185 °C; IR: 3190 (NH), 3090 (CH-aromatic), 2953 (CH-aliphatic), 1700 (CO, Ester) and 1665 (CO, amide), 1640 (C=N); ^{1}H NMR (400 MHz; DMSO-d_6) δ_H: 1.25 (t, ^{3}J = 7.00 Hz, 3H), 3.88 (s, 3H), 4.21 (q, ^{3}J = 7.00 Hz, 2H), 4.90 (s, 2H), 7.17 (d, ^{3}J = 8.50 Hz, 1H), 7.49 (t, ^{3}J = 7.30 Hz, 1H), 7.69 (d, ^{3}J = 8.00 Hz, 1H), 7.81 (dd, ^{3}J = 18.70, 11.20 Hz, 2H), 7.93 (d, ^{3}J = 8.10 Hz, 1H), 8.13 (d, ^{3}J = 7.70 Hz, 1H), 12.39 (s, 1H); Anal. Calcd for $C_{19}H_{18}N_2O_5$ (M.wt: 354.36): C, 64.40; H, 5.12; N, 7.91; Found: C, 64.67; H, 5.35; N, 8.09.

2-(2-Methoxy-5-(4-oxo-3,4-dihydroquinazolin-2-yl)phenoxy)acetohydrazide (6)

A mixture of compound **5** (10 mmol, 3.54 g) and hydrazine hydrate 98% (50 mmol, 3.75 mL) in ethanol (20 mL) was refluxed for 1h. The formed precipitated was filtered, dried and crystallized from ethanol to give the corresponding product **6** in yield 75%, m.p 237-239°C; IR: 3330, 3275, 3190 and 3135 (NH, NH2), 3086 (CH-aromatic), 2950 (CH-aliphatic), 1664 (CO, amide). ^{1}H NMR (400 MHz; DMSO-d_6) δ_H: 3.83 (s, 3H), 4.91 (s, 2H), 7.16 (d, ^{3}J = 7.30 Hz ,1H), 7.49 (s, 1H), 7.69-7.73 (m, 1H), 7.77 (d, ^{3}J = 7.00 Hz ,1H), 7.83 (d, ^{3}J = 8.30 Hz 1H), 7.90-7.94 (m, 1H), 8.13 (d, ^{3}J = 8.50 Hz, 1H), 8.59, 9.36 (2s, 2H), 12.41 (br s, 2H); Anal. Calcd for $C_{17}H_{16}N_4O_4$ (M.wt: 340.34): C, 60.00; H, 4.74; N, 16.46; Found: C, 59.85; H, 4.92; N, 16.73.

2.1.3. General procedure for the synthesis of *N'*-(5-substituted-2-oxoindolin-3-ylidene)-2-(2-methoxy-5-(4-oxo-3,4-dihydroquinazolin-2-yl)phenoxy)acetohydrazide 8a–e

A suspension of the hydrazide derivative **6** (0.5 mmol, 0.17 g) and isatin derivatives **7a–e** (5 mmol) in a mixture of ethanol (15 mL) and glacial acetic acid (1.0 mL) was refluxed for 4h. The precipitated solid was collected by filtration, and purified by crystallization using ethanol as a solvent to give the target derivatives **8a–e**.

2-(2-Methoxy-5-(4-oxo-3,4-dihydroquinazolin-2-yl)phenoxy)-*N*'-(2-oxoindolin-3-ylidene)acetohydrazide (8a)
Yield: 75%; mp: 285-287 °C; IR: 3329, 3190, and 3124 (NH), 3074 (CH-aromatic), 2966 (CH-aliphatic), 1701, and 1670 (CO), 1650 (C=N). ^{1}H NMR (400 MHz; DMSO-d_6) δ_H: 3.91 (s, 3H), 4.97 (s, 2H), 6.89 (s, 1H), 7.07-7.10 (m, 1H), 7.19 (d, ^3J = 7.20 Hz, 1H), 7.36-7.40 (m, 1H), 7.45-7.49 (m, 1H), 7.57 (s,1H), 7.67-7.69, 7.77-7.79, 7.89-7.94 (3m, 4H), 8.13-8.21 (dd, ^3J = 15.70 Hz, ^3J = 4.00 Hz, 1H), 11.02 (s, 1H), 11.25 (s, 1H), 12.40 (s, 1H); ^{13}C NMR (100 MHz; DMSO-d_6) δ_C: 56.36, 74.96, 111.55, 112.59, 120.52, 121.04, 122.16, 123.21, 125.71, 126.72, 132.47, 135.08, 139.02, 146.40, 146.70, 149.11, 149.41, 151.82, 152.12, 162.93, 179.38 ppm; Anal. Calcd for $C_{25}H_{19}N_5O_5$ (M.wt: 469.46): C, 63.96; H, 4.08; N, 14.92; Found: C, 64.07; H, 4.18; N, 15.07.

2-(2-Methoxy-5-(4-oxo-3,4-dihydroquinazolin-2-yl)phenoxy)-*N*'-(5-methyl-2-oxoindolin-3-ylidene)acetohydrazide (8b)
Yield: 77%; mp: 295-297 °C; IR: 3336, 3182, and 3132 (NH), 3086 (CH-aromatic), 2931 (CH-aliphatic), 1710, and 1678 (CO), 1600 (C=N). ^{1}H NMR (400 MHz; DMSO-d_6) δ_H: 2.29 (s, 3H), 3.87 (s. 3H), 4.97 (s,2H), 6.82 (d, ^3J = 7.80 Hz, 1H), 7.18 (d, ^3J = 7.60 Hz, 1H), 7.40 (s, 1H), 7.46-7.50 (m, 1H), 7.68 (d,^3J = 8.64 Hz, 1H), 7.76-7.80 (m, 1H), 7.91 (d, J = 15.6 Hz, 2H), 7.95 (s, 1H), 8.12 (d, ^3J = 7.30 Hz, 1H), 11.13, 11.91, 12.41 (3s, 3H). ^{13}C NMR (100 MHz; DMSO-d_6) δ_C: 20.51, 55.89, 67.96, 110.98, 112.13, 119.74, 120.69, 121.39, 122.73, 124.59, 125.84, 126.23, 127.31, 131.73, 132.37, 134.58, 138.79, 140.48, 148.84, 151.53, 162.33, 172.41 ppm; Anal. Calcd for $C_{26}H_{21}N_5O_5$ (M.wt: 483.48): C, 64.59; H, 4.38; N, 14.49; Found: C, 64.72; H, 4.52; N, 14.39.

***N*'-(5-Methoxy-2-oxoindolin-3-ylidene)-2-(2-methoxy-5-(4-oxo-3,4-dihydroquinazolin-2-yl)phenoxy)acetohydrazide (8c)**
Yield: 77%; mp: > 300 °C; IR: 3325, 3182, and 3132 (NH), 3086 (CH-aromatic), 2931 (CH-aliphatic), 1678 (CO), 1600 (C=N). ^{1}H NMR (400 MHz; DMSO-d_6) δ_H: 3.77, 3.92 (2s, 6H), 4.99 (s, 2H), 6.88 (s, 1H), 6.98 (d,^3J= 2.5 hz, 1H), 7.13-7.20 (m, 2H), 7.49-7.50 (m, 1H), 7.70 (s, 1H), 7.81 (d, ^3J= 5.3 hz, 1H), 7.92-7.96 (m, 2H), 8.14 (d, ^3J=20hz, 1H), 11.10 (s, 1H), 12.42 (s,1H), 13.63 (s, 1H); Anal. Calcd for $C_{26}H_{21}N_5O_6$ (M.wt: 499.48): C, 62.52; H, 4.24; N, 14.02; Found: C, 62.37; H, 4.39; N, 13.86.

2-(2-Methoxy-5-(4-oxo-3,4-dihydroquinazolin-2-yl)phenoxy)-*N*'-(5-nitro-2-oxoindolin-3-ylidene)acetohydrazide (8d)

Yield 73%; mp: > 300 °C; IR: 3333, 3184, and 3134 (NH), 3067 (CH-aromatic), 2989(CH-aliphatic), 1700, 1680 (CO), 1600 (C=N). ^{1}H NMR (400 MHz; DMSO-d6) δ_H: 3.91 (s, 3H), 4.90 (s, 2H), 7.15 (s, 1H), 7.18-7.23 (m,1H), 7.47-7.51 (m, 1H), 7.68-7.71 (m, 1H), 7.77 (s,1H), 7.79-7.83 (m, 1H),7.90 (d, ^3J = 6.7 Hz,, 1H), 7.94-7.97 (m, 1H), 8.12 (d, ^3J = 7.7 Hz,, 1H), 8.31-8.33 (m,1H),11.91 (s, 1H), 12.41 (s, 1H), 12.56 (s, 1H); Anal. Calcd for $C_{25}H_{18}N_6O_7$ (M.wt: 514.45): C, 58.37; H, 3.53; N, 16.34; Found: C, 58.40; H, 3.71; N, 16.50.

***N*'-(5-Bromo-2-oxoindolin-3-ylidene)-2-(2-methoxy-5-(4-oxo-3,4-dihydroquinazolin-2-yl)phenoxy)acetohydrazide (8e)**
Yield: 75%; mp: 298-300 °C; IR: 3313, 3190, and 3132 (NH), 3066 (CH-aromatic), 2966 (CH-aliphatic), 1710, and 1670 (CO), 1604 (C=N). ^{1}H NMR (400 MHz; DMSO-d_6) δ_H: 3.87 (s, 3H, OCH_3), 5.00 (s, 2H), 6.86 (s, 1H), 6.88-6.92 (m, 1H), 7.20 (d, ^3J =6.40 Hz, 1H), 7.47-7.50 (m, 1H), 7.55 (d, ^3J = 7.70Hz, 1H), 7.65-7.75 (m, 2H), 7.89 (d, , ^3J = 7.8 Hz, 1H), 7.95 (s, 1H), 8.12 (d, ^3J = 7.0 Hz, 1H), 11.13 (s, 1H), 11.39 (s, 1H), 12.41 (s, 1H);Anal. Calcd for $C_{25}H_{18}BrN_5O_5$ (M.wt: 548.35): C, 54.76; H, 3.31; N, 12.77; Found: C, 54.85; H, 3.43; N, 13.00.

2. Biological Evaluation

2.2. Cytotoxic evaluation

2.2.1. Cell culture:

Antiproliferative activities of the tested compounds in breast cancer cell line MCF-7 and normal cell line MCF-10a was determined using MTT assay [50]. Data presented are the results of at least three independent experiments. The results of these studies are presented as mean IC_{50} (μM) ± standard deviation (SD).

2.2.2. CDK2 inhibition assay

CDK2 enzyme inhibition activity was measured for the most active compound **8a** using CDK2 assay kit (BPS Bioscience) (catalog no. 79599), according to the manufacturer's protocol.

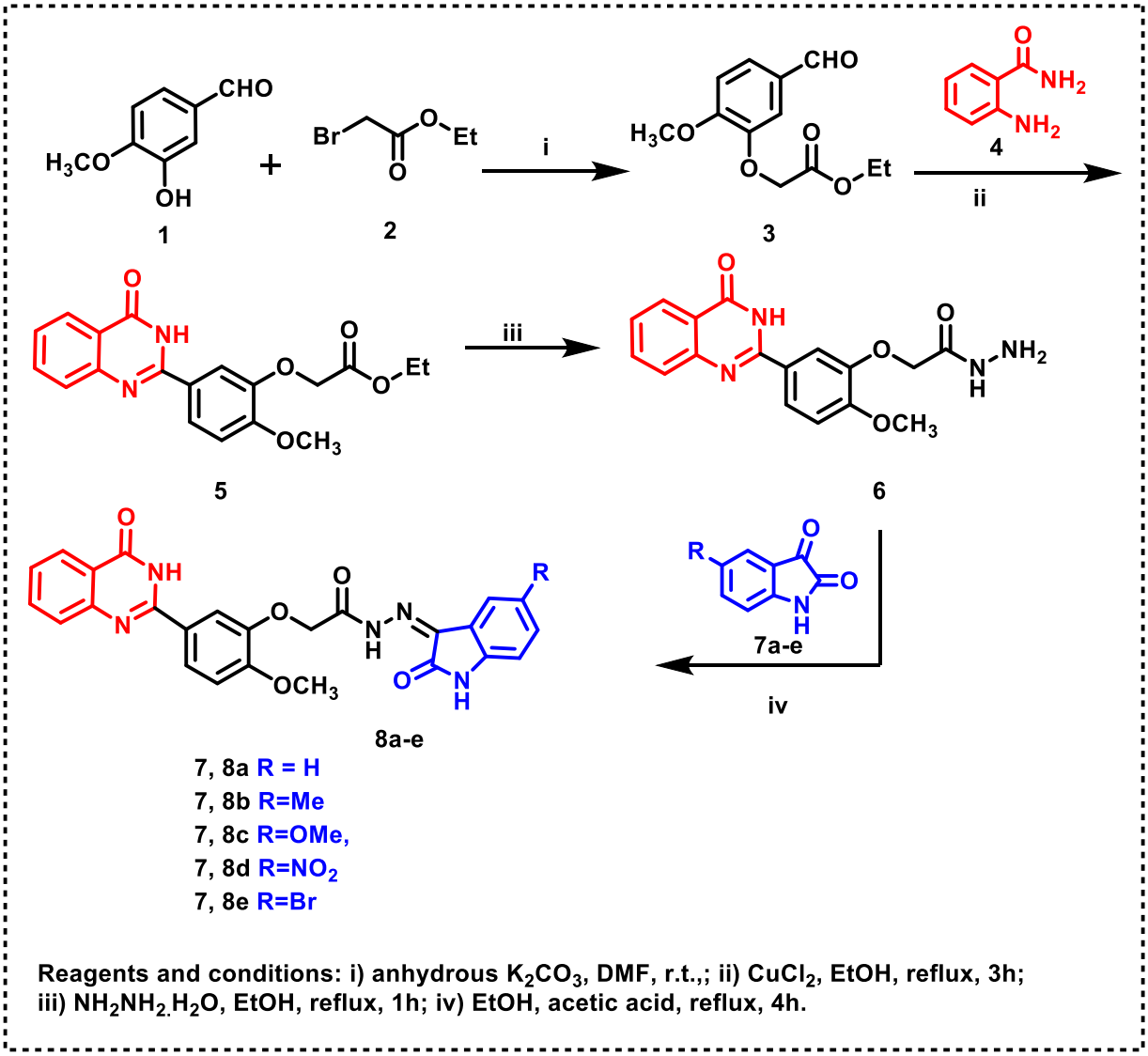
2.3. Molecular Modeling

Molecular docking simulations were achieved utilizing AutodockVina software [50] and the results were visualized by BIOVIA Discovery Studio Visualizer as previously reported.

3. Results and discussion

3.3.1 Chemistry

The synthetic pathway for the new oxindole-quinazoline based candidates **8a-e** was started from the nucleophilic substitution reaction of hydroxybenzaldehyde derivative **1** with the ethyl bromoacetate (**2**) to yield ethyl 2-(5-formyl-2-methoxyphenoxy)acetate (**3**) which underwent a copper-catalyzed condensation reaction with 2-aminobenzamide (**4**) to produce 3,4 dihydroquinazolin-2-acetate derivative **5**. The quinazolinone-acetohydrazide derivative **6** was obtained through the reaction of the former acetate analog**5** with hydrazine hydrate that was finally condensed with various isatinanalogs**7a-e** to get the target compounds **8a-e (Scheme 1)**. The obtained new analogs chemical structure was approved through elemental analyses and spectral data (IR, ^{1}H NMR, and ^{13}C NMR). IR spectrum of the target oxindole-quinazoline candidate **8a** showed three absorption stretching band at 3329, 3190 and 3124 cm^{-1} related to the three NH groups besides tow absorption bands at 1710 and 1670 cm^{-1} corresponding to the two carbonyl moieties. Also, the ^{1}H NMR spectrum of the same derivative **8a**revealed that, besides the original protons of the quinazoline ring three additional multiplets at δ 7.67-7.69, 7.77-7.79 and 7.89-7.94 ppm for the four protons of the isatin ring and one singlet signal at δ 11.02 ppm related to the NH group of the isatin moiety. Also, its ^{13}C NMR spectrum showed two signals at δ 56.36 and 74.96 ppm due to both the methoxy and methylene carbons, respectively, as well as other seventeen signals at the range δ 111.55 to 152.12 related to the aromatic carbons, while the two carbonyl carbons of the quinazoline and hydrazide moieties were presented at δ 162.93 and 179.38 ppm, respectively.



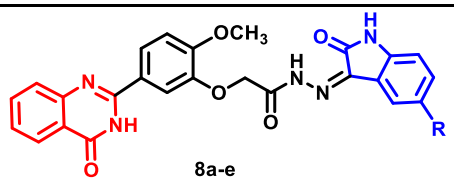
Scheme 1: Synthesis of target oxindole-quinazoline hybrids 8a–e

3.2. Biological Evaluation

3.2.1. *In vitro* anti-proliferative activity

The cytotoxic impact of the target compounds **8a-e** was evaluated against human breast adenocarcinoma (MCF-7) utilizing Doxorubicin as positive control through via the MTT colorimetric assay [50] and the resulted data were summarized in **Table 1.** Inspection of the results confirmed the potent antiproliferative activity of most of the tested compounds and the varying effect of the substitution on the oxindole ring on the biological potency. The unsubstitutedoxoindole congener **8a** elicited the most promising cytotoxic activity to be four-fold more powerful than the reference drug Doxorubicin with IC_{50} = 0.007 uM in reference to 0.028 uM, respectively. Also, the substitution with either methoxy or bromo groups on the 5-position of the oxindole moiety as in compounds **8c** and **8e** conserve the potency to be approximately from 1.75 to 1.5 fold more powerful than the reference standard with IC_{50} = 0.016 and 0.018 uM, respectively. On the other hand, slight reduction in activity was observed in the 5-methyl-2-oxoindole analog**8b** with IC_{50} = 0.041 uM, while significant reduction resulted from the substitution with electronegative NO_2 group on the oxoindole ring as in compound **8d** to produce IC_{50}= 0.104 uM (**Table 1).**

Table 1: The cytotoxicity (IC_{50}; µM) of the newly synthesized target compounds 8a-e towards breast adenocarcinoma MCF-7 cell line



8a-e

Compound No.	R	IC_{50} (uM)[a]
8a	**H**	0.007 ± 0.18
8b	**Me**	0.041 ± 1.03
8c	**OMe**	0.016 ± 0.42
8d	**NO_2**	0.104 ± 2.84
8e	**Br**	0.018 ± 0.51
Doxorubicin	**R**	0.028 ± 0.81

[a]Data are expressed as mean of 3 independent experiments ± SD

The cytotoxic activity of the most potent analog**8a** was evaluated using normal epithelial breast cells MCF-10a employing MTT assay in order to detect its safety profile. It is important to note that **8a** has IC_{50} = 0.074 µM on the normal cell line with SI = 10.57 surpassing the used reference standard Doxorubicin (**Table 2**)

Table 2: Cytotoxic activity of compound 8a and Doxorubicin against normal epithelial breast cells MCF-10a and their selectivity index

Compound No.	IC_{50} (µM)	Selectivity index[a]
8a	0.074 ± 1.49	10.57
Doxorubicin	0.029 ± 0.83	1.04

[a]Selectivity index was attained by dividing the activity of the tested compound (IC_{50}) in normal cell line (MCF-10a) by its activity (IC_{50}) in cancer cell line

3.2.2. *In vitro* CDK2 Enzyme assay

Based on the over expression of CDK2 in malignant human breast epithelial cells, besides its inhibition efficiently prevents the proliferation of the breast cancer cells even the resistant types to endocrino-therapy [51, 52]. The compound displayed the most powerful antiproliferative potency **8a** was further assessed for its inhibiting activity towards human cyclin dependent kinase enzyme using Human CDK2 ELISA Assay Kit. The tested compound displayed very potent activity with IC_{50} in nanomolar range (IC_{50}= 0.011 nM), but unfortunately less than the reference standard Roscovitine (IC_{50}= 0.006 nM) (**Table 3)**.

Table 3: *In vitro* inhibitory activity of the tested compound against CDK2 enzyme

Compound	IC_{50} (nM)[a]
8a	0.011 ± 0.19
Roscovitine	0.006 ± 0.08

[a]IC_{50} was represented of two independent experiments ± SD

From the obtained results, it could be concluded that quinazoline-oxindole conjugated compounds displayed potential activity as anticancer agents possessing promising CDK2 inhibition activity. The unsubstitutedoxoindole compound **8a** elicited potential activity also the substitution with electron donating groups maintained the potential anticancer activity, in contrast to the substitution with electronegative groups that decreased the biological activity. The possible structure activity relationship (SAR) for the quinazoline-oxoindole conjugated compounds is displayed in **Figure 5**.

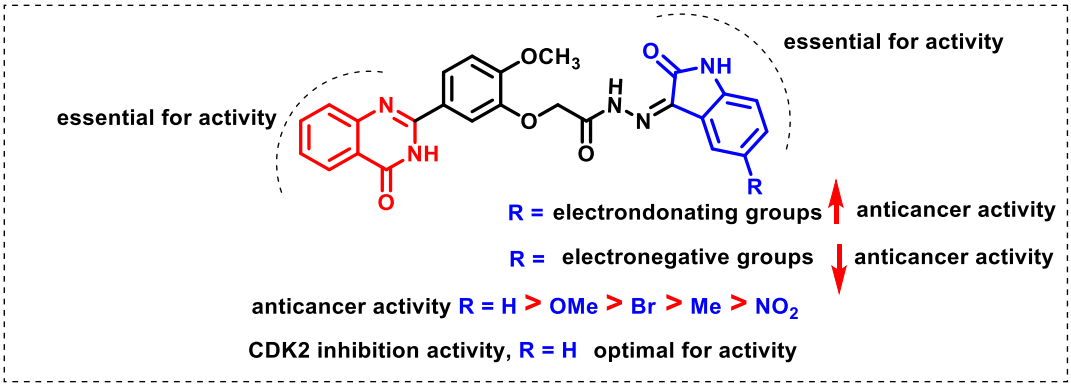


Figure 5: SAR for the quinazoline-oxoindole conjugated compounds

3.3. Molecular docking simulation

In order to validate the anticipated interactions of the oxindole-quinazoline derivatives **8a-e** to CDK2 active site, **8a** was chosen to be docked into the binding pocket of the target enzyme through AutodockVina[53]. The outcomes were visualized using BIOVIA Discovery Studio Visualizer https://discover.3ds.com/discovery-studio visualizer. The crystal structure of CDK2 (PDB ID: 1FVT) was firstly obtained [54] from the protein data bank, the protein was prepared and the native ligand was re-docked according to the reported procedure[25, 55]. After docking of the oxindole-quinazoline hybrid **8a** into CDK2's binding pocket, the outcomes were examined. Compared to the docking energy score (*S*) of the native ligand (–9.1 kcal/mol), the synthesized oxindole-quinazoline conjugate **8a** demonstrated a greater affinity towards the CDK2 active site with docking energy scores (S) of –10.9 kcal/mol. The oxindole part of the oxindole-quinazoline conjugate **8a**, as depicted in **Fig.5** is positioned in the binding pocket of ATP where the CONH forms hydrogen bonds with Glu81 and Leu83 amino acids residues, the NH group of the acetohydrazide interacts with the active site through hydrogen bond with Leu83. The oxindole fragment forms hydrophobic interactions with the amino acid residues Val18, Ala31, Phe80, Leu134, and Ala144. In the meantime, the 2-phenylquinazoline moiety is oriented towards the solvent region, where it is forming a hydrogen bond through the CO-NH group of the quinazoline with His84. Additionally, hydrophobic interactions between the quinazoline scaffold with Ile10, Lys20, Lys89, and Leu298 amino acid residues were observed (**Figure6**).

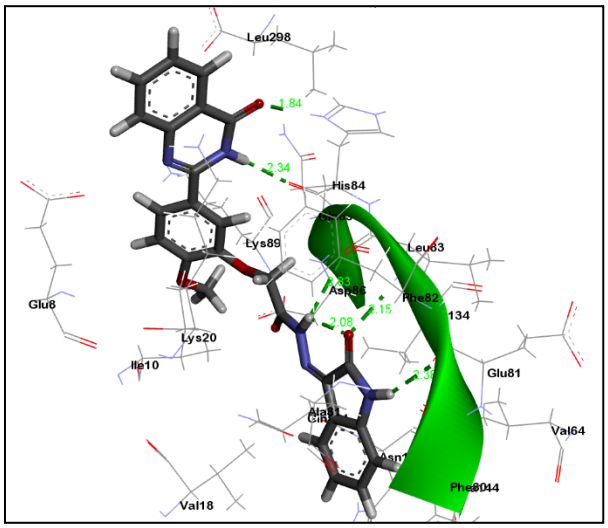


Figure 6: 3D diagram of **8a** showing its interaction with CDK2 binding pocket

4. Conclusions

In summary, this study is concerned with the synthesis of new series of oxindole-quinazoline hybrids **8a-e** in order to be evaluated as cytotoxic agents against breast cancer cell line (MCF-7). All the evaluated compounds displayed promising anticancer potency with IC_{50} range 0.007-0.104 uM in comparison to Doxorubicin (IC_{50} = 0.028 uM). The unsubstitutedoxindole congener **8a** revealed the most promising antiproliferative activity with IC_{50} = 0.007 uM in addition to high safety profile towards human normal epithelial breast cells MCF-10a with selectivity index = 10.57. Moreover compound **8a** was further subjected to CDK2 inhibitory activity and elicited potential activity with IC_{50} = 0.011 nM in comparison to Roscvitine as reference standard. Molecular docking study was also performed for compound **8a** that demonstrated high binding interaction with the active site of CDK2 enzyme with docking energy scores (*S*) of –10.9 kcal/molas the oxindole ring occupied the ATP binding pocket performing hydrogen bonding and hydrophopic interaction with the active site while the 2-phenylquinazoline moiety formed hydrogen bonding through the CONH of the quinazoline ring that is also involved in hydrophobic interactions with four amino acids of the active site of the target enzyme

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Conflicts of interest
There are no conflicts of interest.

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