Design and synthesis of thiazolidine-2,4-diones hybrids with 1,2-dihydroquinolones and 2-oxindoles as potential VEGFR-2 inhibitors: in-vitro anticancer evaluation and in-silico studies

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ABSTRACT
A thiazolidine-2,4-dione nucleus was molecularly hybridised with the effective antitumor moieties; 2-oxo-1,2-dihydroquinoline and 2-oxindole to obtain new hybrids with potential activity against VEGFR-2. The cytotoxic effects of the synthesised derivatives against Caco-2, HepG-2, and MDA-MB-231 cell lines were investigated. Compound 12a was found to be the most potent candidate against the investigated cell lines with IC\textsubscript{50} values of 2, 10, and 40\,\mu M, respectively. Furthermore, the synthesised derivatives were tested in vitro for their VEGFR-2 inhibitory activity showing strong inhibition. Moreover, an in vitro viability study against Vero non-cancerous cell line was investigated and the results reflected a high safety profile of all tested compounds. Compound 12a was further investigated for its apoptotic behaviour by assessing the gene expression of four genes (Bcl2, Bcl-xL, TGF, and Survivin). Molecular dynamic simulations authenticated the high affinity, accurate binding, and perfect dynamics of compound 12a against VEGFR-2.

1. Introduction
Cancer is the second health burden around the world after cardiovascular diseases, in accordance with estimates of the world health organisation (WHO)\textsuperscript{1}. It is forecasted to become the major cause of death in the impending years\textsuperscript{2}. Cancer therapy is a challenging area for medicinal chemists, in which they need to discover safe and effective targeted chemotherapeutic agents\textsuperscript{3}. Such agents are designed for inhibiting cancer cell growth by interacting with specific molecular targets resulting in significant damage to the cancerous cells\textsuperscript{4}.

Among the most efficient targets in cancer management, the vascular endothelial growth factor receptor-2 (VEGFR-2) is a vital transmembrane tyrosine kinase receptor\textsuperscript{5}. VEGFR-2 orchestrates important steps in cell proliferation, division, motility, adhesion, and angiogenesis\textsuperscript{6}. Accordingly, stopping the VEGFR-2 signalling cascade reduces the proliferation of different types of cancer cells\textsuperscript{7}.

Over the past few decades, many anti-angiogenic drugs targeting VEGFR-2 were approved by FDA as anticancer drugs e.g. sunitinib, cabozantinib, and sorafenib\textsuperscript{8}. VEGF-R2 has an advantage of the weak expression in the healthy tissue and overexpression is reported in several types of cancer namely, breast cancer, prostate cancer, colon cancer, cervical cancer, NSCLC, kidney clear cell cancer, brain glioma, bladder carcinoma, pancreatic cancer, oral cancer, skin melanoma oesophageal cancer, and ovarian cancer\textsuperscript{9,10}. In addition, it was reported that the selective blockage of VEGFR-2 rather than of both receptors (VEGFR-1 and VEGFR-2), ideally overcomes the disadvantageous haematologic consequences that occurred in malignancy due to the elevated VEGF levels\textsuperscript{11}.

Studying the pharmacophoric features of these drugs showed four essential features that should be included. First, a heteroaromatic moiety binds to Cys919 in the hinge part of the ATP binding site of VEGFR-2. Secondly, a spacer group to occupy the gatekeeper region\textsuperscript{12}. Third, a pharmacophore moiety that can be incorporated in hydrogen-bonds with Glu885 and Asp1046 in the DFG domain of the receptor\textsuperscript{13}. Finally, a terminal hydrophobic moiety to be incorporated with the allosteric hydrophobic pocket of the ATP binding site by some hydrophobic interactions (Figure 1)\textsuperscript{14-17}.

Indoline scaffold, a special class in the drug design and discovery\textsuperscript{18-22}, was among the first kinase inhibitors that reached the clinic and their inhibition of VEGFR-2 has been extensively
investigated\textsuperscript{23,24}. Furthermore, different compounds carrying the quinoline skeleton were reported as tyrosine kinase inhibitors \textsuperscript{25}. Among the quinoline derivatives, 2-oxoquinoline scaffolds attracted great attention because of their interesting pharmacological activities, such as antioxidant\textsuperscript{26}, antimicrobial\textsuperscript{27}, anti-inflammatory\textsuperscript{28}, and virtuous anticancer effects \textsuperscript{29,30}. Additionally, thiazolidine-2,4-dione moiety was a fascinating heterocyclic core in medicinal chemistry for the design and discovery of active compounds particularly anticancer agents\textsuperscript{31–33}.

Keeping in mind the aforementioned findings and continuing our trip in the discovery of novel VEGFR-2 inhibitors \textsuperscript{34–41}, we hybridised 2-oxoindoline and 2-oxoquinolin with thiazolidine-2,4-dione moiety to display kinase inhibitory and anti-proliferative activities introducing a new class of VEGFR-2 inhibitors (Figure 1).

1.1. Design concept

The basic idea of molecular design’s rationale is the modification of lead VEGFR-2 inhibitors keeping the same essential pharmacophoric features of sunitinib, cabozantinib, and sorafenib. Also, employing ligand-based drug design, in particular the molecular hybridisation strategy\textsuperscript{42–46}, a molecular hybridisation of thiazolidine-2,4-dione with the effective antitumor moieties 2-oxoindoline and 2-oxoquinolin was conducted to get new hybrids of prospective VEGFR-2 inhibitory effects. The approach utilised for designing our target candidates is illustrated in Figure 1.

In detail, for the hinge regions, 2-oxo-1,2-dihydroquinolin (\textit{scaffold I}) and 2-oxoindoline (\textit{scaffold II}), were used as hetero-aromatic moieties. The bicyclic structures of such moieties are suitable for the big space of the ATP binding region\textsuperscript{47}. The gatekeeper region was targeted to be occupied by thiazolidine-2,4-dione moiety as a spacer group. The many hydrogen bond acceptor atoms in thiazolidine-2,4-dione moiety may give a good chance for efficient binding to the targeted receptor. With regards to the DFG-motif region, we selected an amide group as a pharmacophore moiety to be buried into it. Additionally, several aromatic derivatives were selected to occupy the allosteric hydrophobic region to investigate the structure-activity relationship (Figure 1).

This kind of work—pharmacophoric features based synthesis—was a goal for our team to achieve rather than the usual SAR-
based synthesis. It enables getting promising results through the syntheses of fewer compounds. saves time, effort, and costs as well as protects the environment from the drawbacks of chemical synthesis and the use of excessive organic solvents.

All tested products were screened for their in vitro antiproliferative activity against three cancer cell lines and one non-cancerous cell line. Besides, the VEGFR-2 inhibitory potentialities of the tested compounds were assessed. In addition, deep biological testing was investigated for the most promising candidates to have a good insight into the mechanism of action against cancerous cell lines. Furthermore, an in silico docking, MD, MM-PBSA, ADMET, and toxicity studies were also performed to predict the binding mode of the synthesised compounds within the binding site of VEGFR-2 kinase as well as their drug-likeness degrees.

2. Results and discussion

2.1. Chemistry

The sequence of chemical synthesis reactions that clarified in Schemes 1 and 2 was utilised to synthesise the hybrid compounds. Initially, 2-chloroquinoline-3-carbaldehyde 2 was obtained via the Vilsmeier–Haack–Arnold reaction, which included the condensation of N-phenylacetamide 1 with N,N-dimethylformamide (DMF) in the presence of phosphorus oxychloride. Moreover, thiazolidine-2,4-dione (TZD) 5 was obtained following the reported procedures through the reaction of thiourea 3 with chloroacetic acid 4 under reflux in concentrated HCl. Compounds 6 was obtained from the condensation of 2-chloroquinoline-3-carbaldehyde 2 with thiazolidine-2,4-dione 5 in dry toluene in the presence of piperidine as a base. Subsequent heating of compound 6 with 2-chloro-N-substitutedacetamide derivatives (7a–c) in DMF in the presence of potassium carbonate produced the corresponding final compounds 8a–c, respectively (Scheme 1).

The chemical structures of compounds 8a–c were authenticated by IR, 1H NMR, and 13C NMR spectroscopic techniques. IR spectra showed strong bands ranging from 3449 to 3142 cm⁻¹ due to the presence of the NHs. Moreover, 1H NMR spectral data displayed singlet signals around δ 12.18 and 10.25 ppm of the two amidic NHs. Following such results, the 13C NMR spectral data also confirmed the validity of the suggested chemical structures, where characteristic signals were appeared at their fingerprint region.

Condensation of isatin 9 with thiazolidine-2,4-dione (TZD) 5 was better with sodium acetate in acetic acid, to provide the isatin derivative 10 in a satisfactory yield. Treatment of compounds 10 with the alcoholic solution of potassium hydroxide resulted in its potassium salt 11. Then, heating a mixture of compound 11 with the 2-chloro-N-substituted acetamide derivatives (7a, c) in dry DMF, in the presence of KI, afforded the corresponding compounds 12a, b, respectively (Scheme 2).
The generated spectral data confirmed the chemical structures of the synthesised derivatives. In detail, the $^1$H NMR spectra of compounds 12a and b showed typical downfield singlet signals around $\delta$ 11.32 and 10.74 ppm, respectively, corresponding to NHs. Considering compound 12a as an example, the IR spectrum exhibited stretching bands at 2992 and 2936 cm$^{-1}$ due to the aliphatic CH bonds. Furthermore, the $^1$H NMR spectrum displayed an up-field singlet signal at $\delta$ 2.26 ppm of the aliphatic protons of the CH$_3$. Likewise, the $^{13}$C NMR spectrum exhibited two peaks at $\delta$ 44.06 and 20.91 of the CH$_2$ and CH$_3$, respectively.

### 2.2. Biological evaluation

#### 2.2.1. In-vitro anticancer effects

Target quinolines 8a–c, indolines 12a and 12b were assessed for their potential anti-proliferative effects against a panel of three different cancer cell lines; colon (Caco-2), hepatocellular (HepG2), and breast (MDA-MB-231) cancer cell lines using standard MTT assay protocol$^{52}$–$^{54}$. The obtained results confirmed the anti-cancer effects of the tested compounds against the three types of cancerous cell lines, expressed as the median growth inhibitory concentration ($IC_{50}$), and were presented in Table 1. The obtained results indicated that compound 12a was the most potent candidate against Caco-2, HepG-2 and MDA-MB-231 cell lines exhibiting $IC_{50}$ values of 2, 10, and 40 nM, respectively. This indicated that hybridisation of 2-oxoindolin with terminal p-tolyl moiety improved the in vitro anti-proliferative activities of the synthesised derivatives.

With respect to the 2-oxo-1,2-dihydroquinolin based compounds, compound 8a incorporating p-tolyl moiety showed the best cytotoxic activity against Caco-2 cell line with $IC_{50}$ value of 9 nM. Meanwhile, 2-oxo-1,2-dihydroquinolin-based derivatives, the phenethyl containing compound, 8b was the most potent member against HepG-2 and MDA-MB-231 cells and displayed equipotent cytotoxic activities against the two cell lines ($IC_{50} = 60 \mu$M). The remaining substituents, on the other hand, showed moderate $IC_{50}$ values against the tested cell lines.

#### 2.2.2. In vitro VEGFR-2 enzyme assay inhibition

To explore the molecular mechanism of the tested compounds as anticancer agents, the compounds were further evaluated to assess their enzymatic inhibitory potential against VEGFR-2. For the derivative of scaffold I (compounds 8a–c), compound 8a had the highest activity with an $IC_{50}$ value of 87.37 nM. Regarding scaffold II (compounds 12a, b), compound 12b exhibited the highest enzymatic inhibitory potential with an $IC_{50}$ value of 84.05 nM. The reference drug, sorafenib, exhibited an $IC_{50}$ value of 53.65 nM (Table 2).

An in-vitro viability test was used to investigate the safety patterns of the tested compounds at different concentrations (1.0 mM/mL to 50 nM/mL). The Vero cell line was used as a non-cancerous cell line model to investigate the safety of the synthesised compounds. Using MTT assay, the tested compounds recorded $IC_{50}$ values ranging from 390 to 1590 nM. Such values were very high in comparison to the corresponding values on cancer cell lines, which reflect the high in vitro safety profile of the tested members towards non-cancerous cell lines (Table 2).

The selectivity index (SI) of a particular compound is the ratio of its toxic concentration against the effective concentration (SI = $IC_{50}$ on non-cancer cell/$IC_{50}$ on cancer cell)$^{55}$. The ideal compound should have an SI value $\geq 10^{56}$. Relatively, low SI (<1) means that the tested compound is toxic and cannot be used as a safe drug. If the SI value is between 1 and 10, further evaluation using other biological systems is encouraged for confirming the findings.$^{57}$

### Table 1. In vitro anti-proliferative activities of 8a–c and 12a,b against Caco-2, HepG2, and MDA-MB-231 cell lines.

| Compounds | Caco-2 ($IC_{50}$ nM) | HepG2 ($IC_{50}$ nM) | MDA-MB-231 ($IC_{50}$ nM) |
|-----------|-------------------------|------------------------|---------------------------|
| 8a        | 9 ± 0.001               | 86 ± 0.001             | 150 ± 0.01                |
| 8b        | 150 ± 0.013             | 60 ± 0.008             | 60 ± 0.003               |
| 8c        | 90 ± 0.004              | 156 ± 0.004            | 70 ± 0.009               |
| 12a       | 2 ± 0.005               | 10 ± 0.001             | 40 ± 0.002               |
| 12b       | 120 ± 0.001             | 49 ± 0.008             | 37 ± 0.002               |
| Doxorubicin| 3.46 ± 0.003            | 1.15 ± 0.02            | 0.98 ± 0.01              |

$^a$The results were the mean of three replicates.
Fascinatingly, as aforementioned, all the tested hybrids showed decreased potency against Vero cell lines. This finding encouraged us to investigate the selectivity profile of the synthesised compounds. The selectivity index values of the synthesised compounds against cancer cells were indicated in Figure 2. In general, all compounds showed SI of more than 2.5 indicating high selectivity of the tested compounds against cancer cell lines. Interestingly, compound 12a showed the highest SI values of 365, 73, and 18 against the Caco-2, HepG2, and MDA-MB-231 cell lines, respectively (Figure 2). Therefore, we selected compound 12a for further biological testing.

### 2.2.3. Effect of compound 12a on Caco-2 cells migration

The potential of compound 12a to inhibit the ability of Caco-2 cells to migrate and heal was investigated through the in-vitro scratch assay which is a low-cost easy and well-developed method\(^58\). The basic concept of this method involves the generation of a scratch in a cancer cell line monolayer, recording the diameter at the beginning, and at regular intervals during cell migration to close that scratch. The obtained results of the treated cell line are then compared to the untreated cell line. Images of scratched areas from the time points; 0, 24 are illustrated in Figure 3. Regarding the representative control, the scratch was completely closed within 24 h (Figure 3(C)). While the wound diameter of the treated Caco-2 cells with 12a (sub IC\textsubscript{50} value) slightly decreased from 0.348 (Figure 3(B)) to 0.335 mm (Figure 3(A)). These results confirmed that compound 12a significantly inhibited the scratch closure and cancer cell migration at a low concentration of 1 \(\mu\text{M}\). Interestingly, compound 12a changed the Caco-2 cell shape from an irregular into the normalised round form within 24 h. This significant morphological change could be linked to the incidence of apoptosis in the treated cancer cells\(^59\).

### 2.2.4. Alternation of cancer cells gene expression after Caco-2 treatment with 12a using RT-qPCR

Apoptosis is a homeostatic mechanism that keeps the number of cells in an organism constant and eliminates unwanted cells which are damaged or unmanageable during different developmental stages. Different gene families such as the p53 gene, caspases, tumour necrosis factor (TNF) receptor gene superfamily, or B cell lymphoma (Bcl)-2 family of genes are involved and/or collaborate in the apoptosis process.

The balance between both the proapoptotic and anti-apoptotic molecules keeps stabilising the cellular homeostasis and defines cell destiny, either to apoptosis or growth and proliferation. Mitochondria have an essential role in releasing several vital apoptosis molecules such as SMAC, cytochrome c, apoptosis-inducing factor, and endonuclease G as a result of mitochondrial membrane permeabilization, which is activated by proapoptotic B cell lymphoma (Bcl)-2 family proteins. Meanwhile, the anti-apoptotic members of the Bcl-2 family (Bcl2 and Bcl-xl) maintains the integrity of the mitochondrial membrane.

Overexpression of the Bcl2 gene can inhibit the apoptotic cell death and partially the nonapoptotic cell death, which has a role in arrest of cell cycle. Meanwhile, overexpression of Bcl-xL enhances autophagic cell death\(^60\). Moreover, Survivin is a pro-survival protein that is overexpressed in many cancer cells in the G2-M phase. This protein has been linked to tumour progression control and resistance to cancer chemotherapeutics. Furthermore, the transforming growth factor (TGF) is one of various proteins that secreted by transformed cells and stimulate the growth of non-cancerous cells in addition to its role as initiators of the signalling pathway that suppresses the early development of cancer cells\(^61\).

Disregulation of TGF-\(\beta\) activation and signalling may result in apoptosis.

In this study, the Caco-2 cell line was treated with 2 \(\mu\text{M}\) (IC\textsubscript{50} value) of compound 12a. The results showed noticeable variations in the expression levels of the four cancer correlated genes (Bcl2, Bcl-xL, TGF, and Survivin). In detail, compared to control cells, compound 12a caused significant down-regulation of Bcl2, Bcl-xL, and Survivin genes, meanwhile, it showed an upregulation effect of the TGF gene. These findings indicate the efficiency of compound 12a in the induction of apoptosis (Figure 4).

### Table 2. IC\textsubscript{50} values of the tested compounds against VEGFR-2 and Vero cell line and their selectivity index (SI) against different cancer cell lines.

| Compounds | IC\textsubscript{50} (nM) | Cytotoxicity against Vero (IC\textsubscript{50} \(\mu\text{M}\)) | Selectivity index (SI) |
|-----------|-----------------|-----------------|-------------------------|
| 8a        | 96.64           | 1590 ± 0.068    | 176.67                  |
| 8b        | 87.37           | 890 ± 0.015     | 14.83                   |
| 8c        | 317.7           | 390 ± 0.20      | 2.50                    |
| 12a       | 116.3           | 730 ± 0.015     | 18.25                   |
| 12b       | 84.65           | 480 ± 0.040     | 12.97                   |
| Sorafenib | 53.65           |                  |                         |

\(^a\)SI = Cytotoxicity against Vero cells / Cytotoxicity against Caco-2 cell line. 
\(^b\)SI = Cytotoxicity against Vero cells / Cytotoxicity against HepG2 cell line. 
\(^c\)SI = Cytotoxicity against Vero cells / Cytotoxicity against MDA-MB-231 cell line.
Figure 3. Effect of compound 12a on cells migration and healing efficacy of Caco-2 cells.

Figure 4. Relative gene expression levels of 4 different genes (BCL2, BCLXL, Survivin, and TGF) in Caco-2 cell line treated with 12a using RT-qPCR.
2.3. In silico studies

2.3.1. Molecular docking

In terms of binding energy, molecular docking analysis can assist in predicting the most energetically favourable binding pose of a ligand to its receptor. Docking simulations of compounds 8a–c and 12a, b were adopted against VEGFR-2 protein (PDB: 4ASD) using the co-crystallised ligand (sorafenib) as a reference molecule. To ensure the accuracy of the docking process, a re-docking validation step was successfully regenerated. The valid binding of the sorafenib was authenticated by the low RMSD value (1.11 Å). Table 3 outlines the computed ΔG (binding free energies) of both synthesised candidates and sorafenib against VEGFR-2.

The docking pose accomplished by sorafenib involved the H-bonding of Cys919 with the nitrogen of the pyridine ring, as well as the NH of the amide group. Additional H-bonding interaction was observed between Glu885 and NHs of the urea moiety from one side and the carbonyl oxygen of urea moiety and Asp1046 on the other side. Also, sorafenib exhibited several hydrophobic interactions with the hydrophobic pocket that formed by Leu889, Leu1019, and Ile892 (Figure 5).

Docking studies of compound 8a with VEGFR-2 revealed that it has an affinity value of –26.60 kcal/mol and formed four H-bonds. The 2-oxoquinoline arm occupied the hinge region by incorporating in two H-bonds with Cys919 and five hydrophobic interactions with Leu840, Leu1035, Ala866, and Phe918. Next, thiazolidine-2,4-

| Compounds | ΔG (kcal/mol) |
|-----------|---------------|
| 8a        | –26.60        |
| 8b        | –23.97        |
| 8c        | –23.96        |
| 12a       | –27.44        |
| 12b       | –26.44        |
| Sorafenib | –26.30        |

Figure 5. 3D and 2D binding mode of sorafenib into VEGFR-2 active site.

Figure 6. 3D and 2D binding mode of 8a with the active site of VEGFR-2.

Figure 7. 3D and 2D binding mode of 12a with the active site of VEGFR-2.
dione moiety occupied the linker region by the formation of seven hydrophobic interactions with Val916, Val848, Val899, Cys1045, Leu1035, Ala866, and Phe1047. Moreover, the acetamide (pharmacophore) moiety interacted with the DFG motif region and formed two H-bonds with the vital amino acid residues Asp1046 (2.43 Å) and Glu885 (2.22 Å). Finally, the p-tolyl arm was buried in the hydrophobic back pocket making two hydrophobic interactions with Leu889 (Figure 6).

The docking findings of compound 12a revealed that it can interact with the essential amino acids in the VEGFR-2 active site. As displayed in Figure 7, both thiazolidine-2,4-dione and 2-oxindole moieties were oriented towards the gate keeper area and the hinge region, respectively. Also, compounds 12a incorporated in two H-bonds with the key amino acids; Glu885 and Asp1046 in the DFG motif. Additionally, the of the later moieties’ orientation allowed the hydrophobic substituents in the docked compounds to fit inside the hydrophobic allosteric pocket. Such a binding pattern of compound 12a encouraged us to study its MD simulations.

2.3.2. Molecular dynamics (MD) simulations

The methods of Molecular dynamics (MD) simulations are nearly utilised as routine in silico work in the field of drug discovery. The main two advantages of these experiments can be described as firstly, an extremely accurate ability to analyse every structural and entropic change in the examined ligand-protein system. Additionally, this analysis occurred through a decided time at an extraordinary resolution of atomic level. Correspondingly, MD simulations can exactly compute the resulting changes after the ligand-protein binding in both thermodynamics and kinetics levels. The previous advantages made the MD a flourishing tool to understand the structure-function relationship of the tested ligand-protein complex. It declares essential factors as the stability of the tested complex, the binding free energy, and the kinetics of the tested ligand.

To compute the conformational variations that occurred in VEGFR-2 compound 12a complex due to binding, RMSD values were calculated before and after 12a bonding with VEGFR-2. Figure 8(A) shows that VEGFR-2, compound 12a, and the VEGFR-2-compound 12a complex had low values of RMSD and didn’t show any major fluctuations through the examination time (100 ns). At first 20 ns, both of VEGFR-2 and the complex were stable. Then, starting from 21 ns till 38 ns, comparatively higher RMSD has been demonstratedremained in the stable state. The RMSD values were normalised again starting from the39 ns till the end of the experiment. The achieved results reveal a great degree of stability. The flexibility of VEGFR-2 protein was examined in terms of RMSF to explore the fluctuated regions through 100 ns. It was observed that compound 12a binding makes the VEGFR-2 slightly flexible in 1050–1100 residue areas (Figure 8(B)). The radius of gyration (Rg) of VEGFR-2 was estimated to figure out the compactness of the VEGFR-2-compound 12a complex. Although a slight fluctuation occurred at

![Figure 8. M D simulations experiment: (A) RMSD values of compound VEGFR-2-compound 12a complex before and after binding, (B) RMS of VEGFR-2-compound 12a complex, (C) Rg of VEGFR-2-compound 12a complex, (D) SASA of VEGFR-2-compound 12a complex, (E) H- bonding between VEGFR-2-compound 12a complex.](image-url)
30 ns, the Rg of the VEGFR-2-compound 12a complex reduced again and remained stable till the end of the experiment (Figure 8(C)). VEGFR-2-compound 12a complex interaction with the surrounding solvents was calculated by solvent accessible surface area (SASA) over 100 ns. Interestingly, VEGFR-2 protein did not show a marked reduction nor expansion of its surface area showing almost similar SASA values at 0 and 100 ns (Figure 8(D)). Such results show that there are no dramatic conformational changes occurred due to the binding of compound 12a. Hydrogen bonding among the VEGFR-2-compound 12a complex was estimated and the maximum number of incorporated H-bonds was four (Figure 8(E)).

2.3.3. MM-Pbsa

The Molecular Mechanics Poisson–Boltzmann Surface Area (MM-PBSA) is a popular in silico method for accurate calculation of the binding free energy of an examined compound (ligand) in the active site of a specific protein. Because MM-PBSA is more precise than usual molecular docking methods and less computationally complicated than other MD free energy prediction methods, it has been widely employed in different biomolecular experiments such as protein-ligand-binding, protein-protein-interaction as well as protein folding.

The main difference between MM/PBSA and MM/GBSA is the first experiment calculates the energies coupled with Poisson–Boltzmann, and surface area continuum solvation. While the second computes the energies coupled with the generalised Born, and surface area continuum solvation. Both methods have been employed favourably to reproduce, rationalise and improve the results obtained by virtual screening and molecular docking. However, Hou et al reported a better performance for MM/PBSA over MM/GBSA in the calculation of absolute binding free energies in a comprehensive study of different 59 ligands with six proteins. In this research, MM/PBSA was employed to investigate the binding free energy of the VEGFR-2-compound 12a complex over the final 20 ns of the MD trajectories with an interval of 100 ps. Compound 12a demonstrated a low binding free energy of −92 KJ/mol with VEGFR-2 (Figure 9(A)). Following, the participation of every amino acid residue of VEGFR-2 in the obtained binding free energy was computed by breaking down the total binding energy of the VEGFR-2–compound 12a complex into per amino acid residue share energy. The fulfilled findings indicate the fundamental residues that contribute auspiciously to the binding of the VEGFR-2–compound 12a complex. The obtained binding declared that VAL-848, CYS-919, CYS-1045 and PHE-1047 residues of VEGFR-2 contributed higher than 5 KJ/mol binding energy (Figure 9(B)). Consequently, the mentioned amino acids are considered fundamental residues in the binding with compound 12a.

2.3.4. Admet profiling study

The ADMET parameters presented in Table 4 and Figure 10 were determined computationally for compounds 8a–c and 12a, b using Discovery studio 4.0. Sorafenib and sunitinib were used as reference molecules.

The tested compounds exhibited low to very low BBB penetration levels. So, undesirable CNS adverse effects were expected to be absent after the administration of such molecules. For the aqueous solubility, the experimented compounds were expected to have poor solubility levels. Furthermore, compounds 8a, 8b, and 12a displayed good predicted absorption levels, while compounds 8c and 12b demonstrated poor range. Further, all compounds didn’t display inhibitory potential against theCYP2D6. Finally, all experimented compounds expressed in silico bind plasma protein level of more than 90%.

2.3.5. In silico toxicity studies

In this study, seven toxicity parameters were computed based on the toxicity models created in Discovery studio 4.0. The constructed models were presented in Table 5. Sorafenib and sunitinib were used as reference molecules.

All investigated compounds were predicted to be non-carcinogenic showing carcinogenic potency TD50 values ranging from 18.26 to 812.63 mg/kg/day. Such values were higher than that of sorafenib and sunitinib (14.24, 4.13 mg/kg/day, respectively). All tested members had rat maximum tolerated doses (MTD) lower than that of sorafenib and sunitinib. Compound 12a displayed an oral LD50 value of 4.07 g/kg. Such value is far more than that of

Table 4. ADMET parameters for the synthesised compounds and reference molecules.

| Compound | BBB | Sol | Ab | CYP2D6 | PPB |
|----------|-----|-----|----|--------|-----|
| 8a       | 3   | 2   | 0  | NI     | M   |
| 8b       | 3   | 2   | 0  | NI     | M   |
| 8c       | 4   | 2   | 2  | NI     | M   |
| 12a      | 3   | 2   | 0  | NI     | M   |
| 12b      | 4   | 2   | 2  | NI     | M   |
| Sorafenib| 4   | 1   | 0  | NI     | M   |
| Sunitinib| 2   | 2   | 0  | NI     | L   |

*BBB, blood brain barrier penetration level, 0 = very high, 1 = high, 2 = medium, 3 = low, 4 = very low.*
*Sol, Solubility level, 1 = very low, 2 = low, 3 = good, 4 = optimal.*
*Ab, Absorption level, 0 = good, 1 = moderate, 2 = poor, 3 = very poor.*
*CYP2D6, cytochrome P2D6 inhibition, I = inhibitor, NI = non-inhibitor.*
*PPB, plasma protein binding, L = less than 90%, M = more than 90%.*
sorafenib (0.82 g/kg) and sunitinib (2.88 g/kg). Also, the same compound showed rat chronic LOAEL of 0.046 g/kg which was higher than that of sorafenib and sunitinib Moreover, all the examined compounds expressed mild to moderate predicted irritation against the eye and skin.

3. Conclusion

A new series of 2-oxo-1,2-dihydroquinolin and 2-oxoindoline derivatives were designed and synthesised as potential anticancer agents and VEGFR-2 inhibitors. The anticancer activities of these compounds was evaluated against Caco-2, HepG-2 and MDA-MB-231 cell lines. Compound 12a (IC50 = 2, 10, and 40 μM) was found to be the most active antiproliferative member against Caco-2, HepG-2 and MDA-MB-23, respectively. In addition, kinase inhibition assay results showed that all compounds had good inhibitory activity against VEGFR-2, compared to the reference drug, sorafenib. Going further, derivative 12a was selected for further evaluation owing to its notable high selectivity index to determine whether it can also exert anticancer effects against Caco-2 cell line (chosen as the most sensitive cancer cell line). Cell migration assay confirmed that 12a significantly inhibited the ability of cancer cells to migrate and heal at a low concentration of 1 μM. Additionally, 12a changed the cancer cell shape from irregular to a normalised round form. The further biological assay revealed the ability of 12a to downregulate Bcl2, Bcl-xl, and Survivin expression levels, and upregulate the TGF expression level. Molecular docking demonstrated the ability of 12a to recognise the ATP binding pocket of VEGFR-2 and to elicit significant interactions with its key amino acids in a fashion comparable to that of the well-known VEGFR-2 inhibitor sorafenib. Molecular dynamic (MD) simulation experiments revealed that 12a has a high potential and optimal dynamics to fit inside VEGFR-2 active site. The MM-PBSA studies determined the binding free energy against VEGFR-2 precisely to be-92 KJ/mol. Finally, the in silico ADMET and toxicity assessment of the synthesised candidates indicated the favourable properties as well as the satisfactory drug-like profiles. To conclude, the above results demonstrated that compound 12a had emerged as a promising candidate and can be further adapted for hit optimisation and/or drug lead discovery.

4. Experimental

4.1. Chemistry

All the reagents, chemicals, and apparatus were described in Supplementary data. Compounds 2, 3, 5, 6, and 10 were furnished following the reported methods. A mixture of potassium 5-((2-oxo-1,2-dihydroquinolin-3-yl)methylene)thiazolidine-2,4-dione 6 (0.001 mol) and the appropriate 2-chloro-N-substitutedacetamide derivatives 7a–c (0.001 mol), K2CO3 (0.001 mol) and KI (0.001 mol) in DMF (10 mL) was refluxed on a water bath for 6 h. The reaction mixture was then poured on
a crushed ice. The precipitate was filtered, dried, and crystallised from ethanol to give compounds 8a-c.

4.1.2.1. 2-(2,4-Dioxo-5-((2-oxo-1,2-dihydroquinolin-3-yl)methylene)thiazolidin-3-yl)-N-phenethylacetamide 8a.

Yellowish white powder (yield, 71%); m. p. = 255–257 °C; IR (KBr, cm\(^{-1}\)): 3447, 3273 (NH), 2984, 2920 (CH aliphatic), 1672 (C=O); \(^1\)H NMR (400 MHz, DMSO-d\(_6\)) \(\delta\) 12.10 (s, 1H), 10.25 (s, 1H), 8.45 (s, 1H), 7.47 (s, 1H), 7.43 (d, \(J = 2.7\) Hz, 2H), 7.33 (s, 1H), 7.26 (d, \(J = 6.8\) Hz, 2H), 7.21 (t, \(J = 5.4\) Hz, 3H), 4.60 (s, 2H), 2.35 (s, 3H); \(^13\)C NMR (101 MHz, DMSO-d\(_6\)) \(\delta\) 171.63, 161.56, 160.73, 157.08, 155.01, 154.98, 142.25, 137.60, 136.51, 130.14, 130.08, 129.64, 127.02, 126.19, 124.16, 120.06, 119.61, 119.16, 117.30, 111.56, 56.03, 21.16; C\(_{23}\)H\(_{19}\)N\(_3\)O\(_4\)S (433.48).

4.1.2.2. 2-(2,4-Dioxo-5-((2-oxo-1,2-dihydroquinolin-3-yl)methylene)thiazolidin-3-yl)-N-phenethylacetamide 8b.

Reddish White powder (yield, 77%); m. p. = 271–272 °C; IR (KBr, cm\(^{-1}\)): 3185, 3142 (NH), 3062 (CH aromatic) 2992, 2936 (CH aliphatic), 1745, 1690 (C=O); \(^1\)H NMR (400 MHz, DMSO-d\(_6\)) \(\delta\) 11.31 (s, 1H), 10.34 (s, 1H), 8.77 (d, \(J = 7.9\) Hz, 1H), 7.45 (t, \(J = 6.2\) Hz, 3H), 7.14 (d, \(J = 8.1\) Hz, 2H), 7.09 (t, \(J = 7.8\) Hz, 1H), 6.98 (d, \(J = 7.8\) Hz, 1H), 4.56 (s, 2H), 2.26 (s, 3H); \(^13\)C NMR (101 MHz, DMSO-d\(_6\)) \(\delta\) 170.26, 168.72, 165.70, 163.97, 144.60, 136.31, 133.56, 133.24, 129.74 (2 C), 129.40, 128.42, 128.17, 120.21, 119.71 (2 C), 111.15, 44.06, 20.91; C\(_{20}\)H\(_{15}\)N\(_3\)O\(_4\)S (419.46).

4.1.2.3. 2-(2,4-Dioxo-5-((2-oxo-1,2-dihydroquinolin-3-yl)methylene)thiazolidin-3-yl)-N-(2-nitrophenyl)acetamide 8c.

Yellow powder (yield, 76%); m. p. = 263–265 °C; IR (KBr, cm\(^{-1}\)): 3175, 3142 (NH), 3062 (CH aromatic) 2996, 2953 (CH aliphatic), 1744, 1692 (C=O); \(^1\)H NMR (400 MHz, DMSO-d\(_6\)) \(\delta\) 11.32 (s, 1H), 10.74 (s, 1H), 8.78 (d, \(J = 7.9\) Hz, 1H), 7.99 (dd, \(J = 8.2, 1.5\) Hz, 1H), 7.77 – 7.72 (m, 1H), 7.67 (dd, \(J = 8.2, 1.4\) Hz, 1H), 7.48 – 7.40 (m, 2H), 7.10 (t, \(J = 7.7\) Hz, 1H), 6.99 (d, \(J = 7.8\) Hz, 1H), 4.61 (s, 2H); \(^13\)C NMR (101 MHz, DMSO-d\(_6\)) \(\delta\) 170.12, 168.70, 165.52, 165.01, 144.63, 134.08, 134.59, 133.60, 130.64, 129.30, 128.43, 128.24, 126.46, 126.13, 125.53, 122.68, 120.20, 111.16, 43.91, 40.10, 35.32. C\(_{23}\)H\(_{19}\)N\(_3\)O\(_4\)S (433.48).

4.2. Biological testing

4.2.1. In vitro anti-proliferative activities

Anti-proliferative activities of the experimented compounds were assessed against Caco-2, HepG-2, and MDA-MB-231 cell lines using MTT assay protocol\(^{54,74}\) as described in Supplementary data.

4.2.2. In vitro VEGFR-2 kinase assay

VEGFR-2 inhibitory activity of the experimented compounds was preceded using a VEGFR-2 ELISA kit as reported and described in Supplementary data\(^75\).

4.2.3. Safety assay

The safety tests of the profiled compounds were checked on one non-cancerous cell line (Vero) to determine the treatment concentrations that do not depict toxic effects against the tested cells as described in Supplementary data\(^76\).
4.2.4. Selectivity index (SI)
The selectivity index values of the tested compounds on cancer cells were calculated as described (Supplementary data)\(^77\).

4.2.5. Cell migration assay
Cell migration assay was fulfilled according to the reported protocol as described\(^89\) in Supplementary data.

4.2.6. Gene expression pattern
The molecular anticancer mode of action of 12a was investigated by screening their ability to control the gene expression levels of Bcl2, Bcl-xl, TGF and Survivin genes as reported\(^79\) in Supplementary data.

4.3. In silico studies

4.3.1. Docking studies
Docking studies were fulfilled using MOE software against the crystal structure of VEGFR-2 [PDB ID: 4ASD] and the results were visualised using Discovery studio 4.0\(^80\)–\(^84\) as described in Supplementary data.

4.3.2. Molecular dynamics simulation
The system preparation used the web-based CHARMM-GUI\(^85\)–\(^87\) interface utilising the CHARMM36 force field\(^88\) and the TIP3P explicit solvation model was used (Supplementary data).

4.3.3. MM-Pbsa studies
The G\(_{\text{MM-PBSA}}\) package of GROMACS was utilised to calculate the MM/PBSA (Supplementary data).

4.3.4. Admet studies
ADMET description was fulfilled using Discovery studio 4.0 according to the reported method\(^90\)–\(^94\) in Supplementary data.

4.3.5. Toxicity studies
The toxicity of the investigated compounds was fulfilled using Discovery studio 4.0 as described\(^82\)–\(^84\) in Supplementary data.

Disclosure statement
No potential conflict of interest was reported by the author(s).

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