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RESEARCH PAPER

Discovery of sulfonamide-tethered isatin derivatives as novel anticancer agents and VEGFR-2 inhibitors

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ABSTRACT

In this work, new isatin-based sulphonamides (**6a-i**, **11a-c**, **12a-c**) were designed and synthesised as potential dual VEGFR-2 and carbonic anhydrase inhibitors with anticancer activities. Firstly, all target isatins were examined for *in vitro* antitumor action on NCI-USA panel (58 tumour cell lines). Then, the most potent derivatives were examined for the potential CA inhibitory action towards the physiologically relevant hCA isoforms I, II, and tumour-linked *h*CA IX isoform, in addition, the VEGFR-2 inhibitory activity was evaluated. The target sulphonamides failed to inhibit the CA isoforms that could be attributable to the steric effect of the neighbouring methoxy group, whereas they displayed potent VEGFR-2 inhibitory effect. Following that, isatins **11b** and **12b** were tested for their influence on the cell cycle disturbance, and towards the apoptotic potential. Finally, detailed molecular modelling analyses, including docking and molecular dynamics, were carried out to assess the binding mode and stability of target isatins.

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KEYWORDS
Synthesis; antitumor agents; biological activities; VEGFR-2 inhibitors; molecular dynamics

Introduction

Cancer, a condition of uncontrolled cell growth, remains the most difficult life-threatening disease to treat, despite advances in understanding of its biochemistry and progression[1]. The rising prevalence of cancer treatment failure derives mainly from antitumor drug resistance in cancer cell, posing new challenges to the healthcare system[2]. Many targets have been identified that are involved in one or more steps in regulating tumour cell growth or death[3]. Combining anticancer drugs is becoming a widely accepted strategy and treatment standard for avoiding drug resistance and treatment failure[4].

Carbonic anhydrase (CA) is an effector enzyme in the tumour cell survival mechanism that regulates the pH of the tumour microenvironment[5]. CAs are zinc metalloenzymes that catalyse the reversible interconversion of CO_2 and bicarbonate ions. Several CA isoforms have been identified, with CA IX and CA XII isoforms being upregulated in nearly all hypoxic tumours, promoting tumour growth and metastasis. CA IX isoform overexpression is associated with a poor prognosis in many cancers and is involved in cell proliferation and communication[6]. The primary sulfonamide-based small molecules were discovered to be the most potent chemotype of the identified CA inhibitors (CAIs)[7]. Among those sulfonamide-based inhibitors, **SLC-0111** (Figure 1) displayed effective CA IX and XII inhibitory action, and is currently being examined in the clinical trials for the hypoxic malignancies management[8].

The clinically validated anticancer drug target, vascular endothelial growth factor receptor-2 (VEGFR-2) is one of the receptor tyrosine kinases that have critical role in vasculogenesis and angiogenesis in many solid cancers. VEGFR-2 mediates the phosphorylation of many proteins in the downstream signalling pathways promoting tumour angiogenesis[9]. Clinical antiangiogenic drugs that are VEGFR-2 inhibitors, such as Sunitinib (Figure 1) showed to achieve normal tumour vasculature and, consequently, contribute in improving chemotherapy treatment. These treatments are planned to hit both antiapoptotic functions and pro-angiogenic activities of VEGF.

Isatin, an endogenic molecule in mammalaian tissues including human, stands for valuable privileged scaffold in drug design and pharmaceutical chemistry[10]. Isatin-tethered compounds have been found to display various pharmacological effects, in particular

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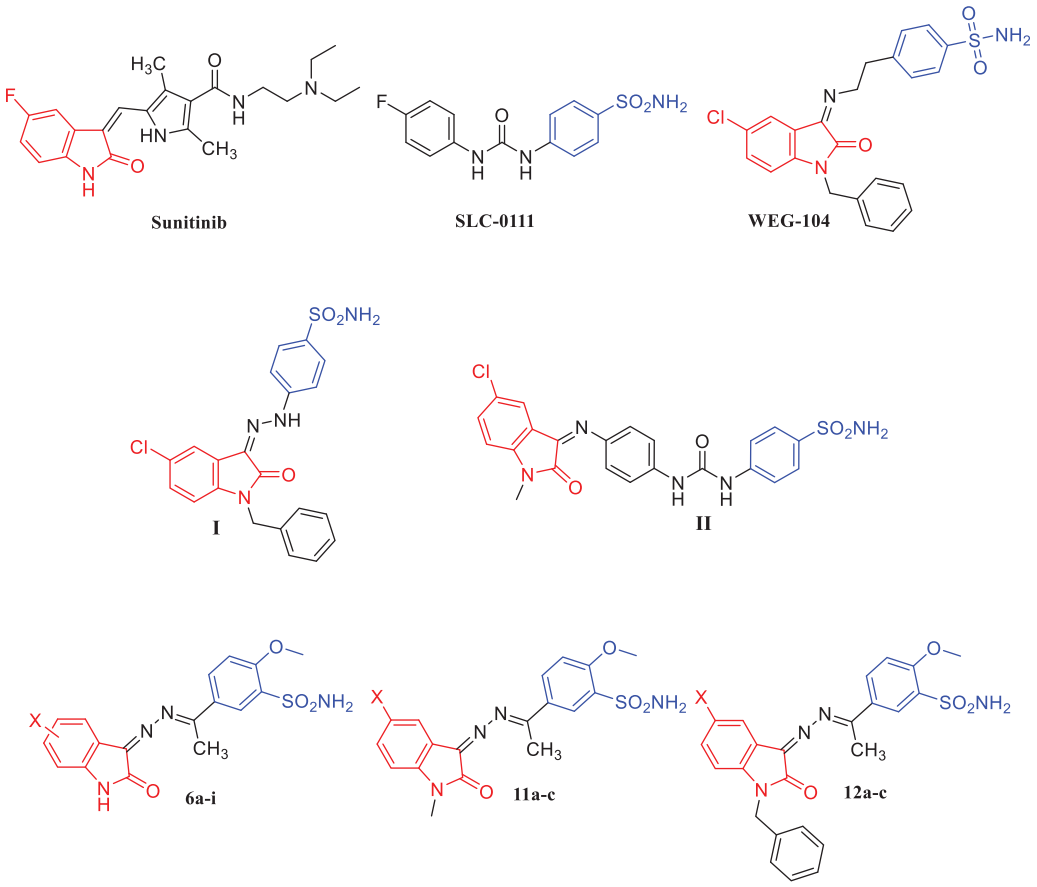


Figure 1. Structure of Sunitinib, SLC-0111, WEG-104, compounds **I-II**, and target compounds **6a-i**, **11a-c** and **12a-c**.

carbonic anhydrase[11–14] and VEGFR-2[15–17] inhibitory activities. In the last few years, isatin motif has been exploited to develop several CAIs with effective *in vitro* and *in vivo* antitumor activities, such as **WEG-104**[18,19] and compound **I**[20] (Figure 1). In 2019, we reported a new set of *N*-substituted isatin derivatives as promising CAIs. Among this set, compound **II** (Figure 1) displayed excellent activity against cancer-related CA IX and XII isoforms; K_I = 5.2 and 6.3 nM, respectively. Also, it exerted good VEGFR-2 inhibitory action (IC_{50} = 260.64 nM), as well as effective cell growth inhibitory action on breast cancer cell lines[21].

Herein, we decided to develop new isatin-based sulphonamides (**6a-i**, **11a-c** and **12a-c**) as potential dual VEGFR-2 and carbonic anhydrase activities. All target synthesised isatin derivatives **6a-i**, **11a-c** and **12a-c** will be evaluated for *in vitro* antitumor action on NCI-USA panel covering 58 distinct human tumour cell lines. Thereafter, the most potent derivatives will be examined for the potential CA inhibitory action towards physiologically relevant hCA isoforms I, II, and cancer-related hCA IX isoform, in addition, the inhibitory activity against VEGFR-2 will be evaluated.

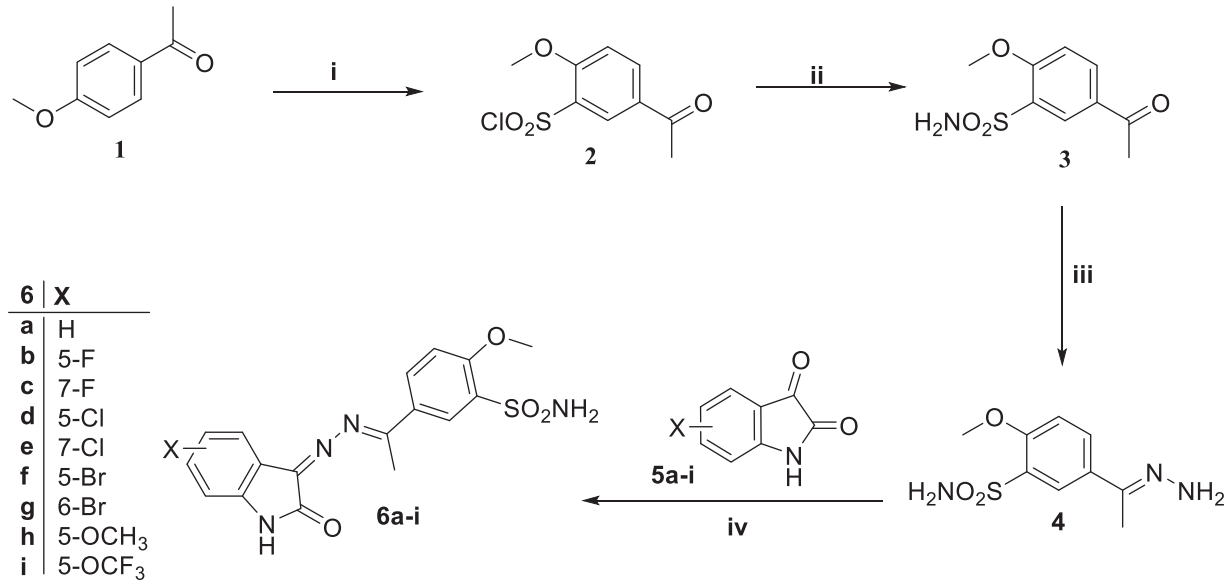
Results and discussion

Chemistry

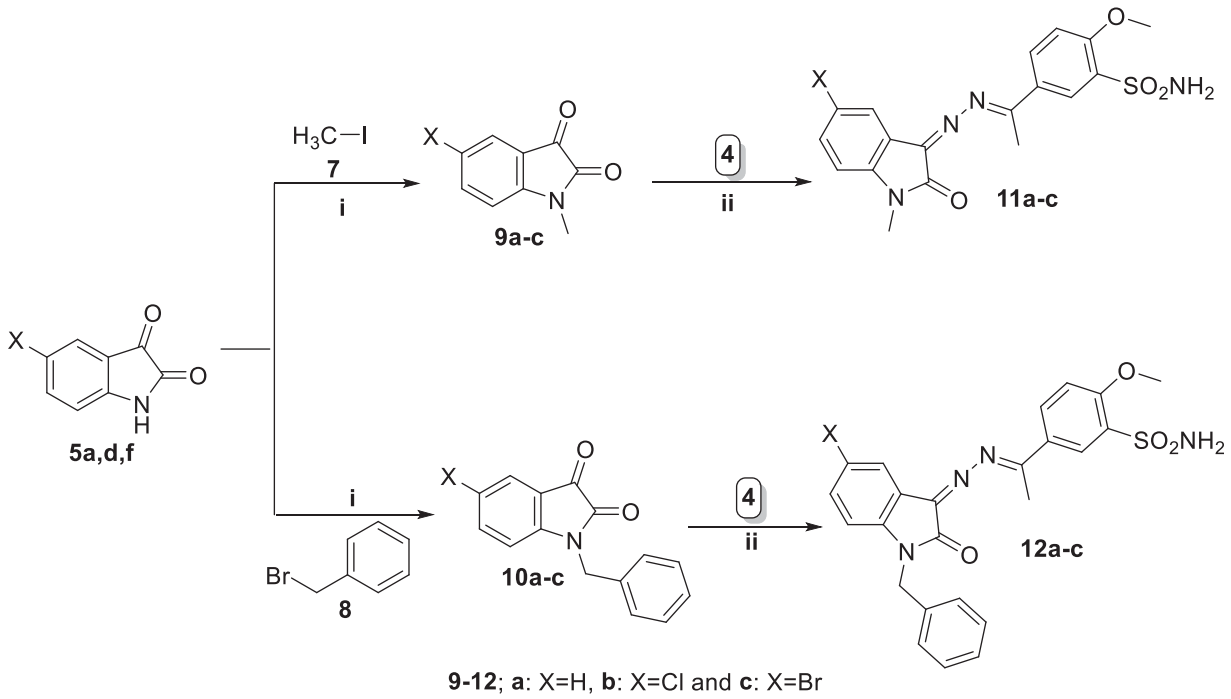
General preparation procedures used in synthesising the designed compounds **6a-i** are shown in Scheme 1. The first step in synthesis was the preparation of benzenesulfonyl chloride **2** using thionyl chloride and chlorosulfonic acid in performing chlorosulfonation of compound **1**. An excess chlorinating agent 2:1 was used to enhance the yield after 26 h. The formation of benzenesulfonamide intermediate **3** was accomplished by reacting compound **2** with ammonia using ethanol as solvent[22]. The synthesis of hydrazone intermediate **4** was carried out by refluxing compound **3** with hydrazine hydrate for 4 h in the presence of glacial acetic acid as catalyst and ethanol as solvent[23]. The target compounds **6a-i** were prepared by reacting them with various isatin derivatives **5a-i** in the presence of a catalytic amount of glacial acetic acid and under reflux conditions[24–26].

The general procedures for synthesising target *N*-methylated/-benzylated isatin derivatives are illustrated in Scheme 2. The *N*-methylation step of isatins **5a**, **5d**, and **5f** was carried out by their reaction with methyl iodide **7** in the presence of potassium carbonate and acetonitrile to afford desired *N*-methylated isatins **9a-c**, which undergo a further condensation reaction with compound **4** in glacial acetic acid to obtain the final compounds **11a-c**. Similarly, benzylated isatin derivatives **10a-c** were synthesised by reacting isatins **5a**, **5d**, and **5f** with benzyl bromide **8** in the presence of acetonitrile and potassium carbonate under refluxing conditions[24]. Thereafter, *N*-benzyl derivatives **10a-c** were condensed with hydrazone intermediate **4** using glacial acetic acid as a catalyst to produce target 2-indolinones **12a-c**.

All the newly prepared molecules were confirmed with ^{1}H and ^{13}C NMR, mass spectroscopy, and elemental analysis. The protons and carbons signals emerged in the expected chemical shifts (experimental section). According to the ^{1}H NMR spectra, the target isatin derivatives exist as E- and Z-isomers, but they interconvert fast in solution at room temperature and cannot be separated. As previously described, the ratio of isomers for isatin



Scheme 1. General procedure for the synthesis of target compounds **6a-i**. Reagents and conditions: i) $HOSO_2Cl$, $SOCl_2$ at 0 °C and then rt, 26 h ii) EtOH, ammonia, rt iii) $NH_2NH_2.H_2O$, AcOH (cat.), and EtOH, reflux, 4 h. iv) EtOH, AcOH (cat.), reflux, 6–8 h.



Scheme 2. General procedure for the synthesis of target compounds **11a-c** and **12a-c**. Reagents and conditions: **i**) K_2CO_3, acetonitrile, reflux, 5 h; **ii**) EtOH, AcOH, reflux, 4 h.

hydrazones is solvent and temperature dependent[27–29], we have reported several studies with such E/Z mixture for different isatin hydrazones[24,30–32].

Biological activity

In vitro *single-dose cellular antiproliferative assay*

The designed isatin derivatives **6a-i**, **11a-c** and **12a-c** were examined for *in vitro* antitumor activity on NCI panel involving 58 distinct human tumour cell lines covering nine distinct types of cancer, at single concentration of 10 μM (supplementary file). For analysing the activity of the new isatin series on 58-cancer cell line, GI (mean % growth inhibition) displayed by **6a-i**, **11a-c** and **12a-c** on different cell lines were calculated, and results were presented in Figure 2. Based on the findings, we concluded that none of compounds **6a**, **6c**, or **11a** were able to increase cell inhibition by more than 20%. And by looking at the mean growth inhibition of the other compounds on each cancer, we discovered that, in addition to CNS cancer in compound **11c** and leukaemia in compounds **12b** and **12c**, all the compounds had the strongest mean growth inhibition on breast cancer cell lines. We also found that the most sensitive cells in breast cancer subpanel is the T47D cells, and it responded best GI% to compounds **6f**, **6i**, **11b**, **11c**, **12a**, **12b**, and **12c** with GI%; 54, 32, 55, 52, 57, 28 and 39, respectively (Figure 2).

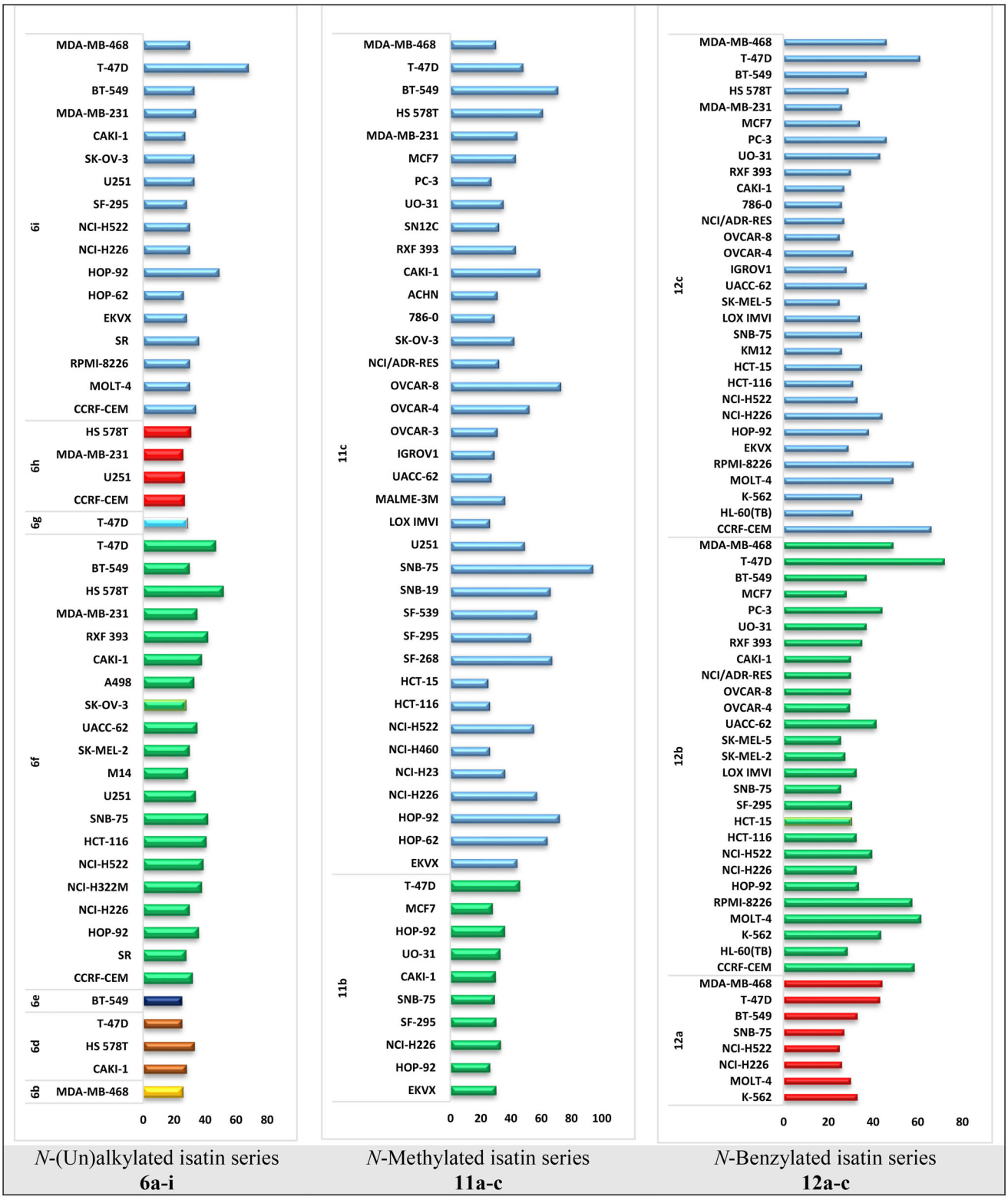


Figure 2. % GI for the highly affected cell lines on treatment by the target isatin derivatives using single dose of 10 μM.

In vitro *cytotoxicity against T47D*

For further exploration of possible anti-proliferative properties of isatin derivatives, dose-response evaluation was carried out on the T47D breast cancer cells (the most sensitive cancer cell line according to % GI *in vitro* single-dose cellular antiproliferative assay), employing the sulforhodamine B colorimetric test. Doxorubicin was set as reference in this assay. IC_{50} values were ascertained and are displayed in Table 1. Derivatives **6f, 11b-c,** and **12b** revealed good cytotoxic action demonstrating IC_{50} from 1.83 to 10.40 μM in comparison to doxorubicin (IC_{50} of 2.26 μM). As earlier noted in single-dose assay, a bromo-isatin analogue of C-5-functionalized isatins was preferred in the *N*-(un)alkylated

derivatives of isatin series. Compound **6f** (IC_{50} = 5.45 ± 0.24 μM) was found to be the most potent among the studied series. Regarding the *N*-alkylated/arylated analogs of isatin series with methyl or benzyl moiety, the presence of a chloro substitution at position 5 led to the best potency (*N*-methyl analogue **11b**, IC_{50} of 1.83 ± 0.08 μM and *N*-benzyl analogue **12b**, IC_{50} of 3.59 ± 0.16 μM), followed by the C-5-bromo substituted derivatives (compounds **11c** and **12c**, IC_{50} of 10.40 ± 0.47 and 16.52 ± 0.74 μM respectively) as shown in Table 1.

In vitro *VEGFR-2 and carbonic anhydrase inhibition activity*

Isatins **6f**, **11b-c**, and **12b**, which revealed the most cytotoxic activity on the T47D cells that characterised by the overexpression of VEGFR-2 and carbonic anhydrase[34–36], were selected for further analysis of their *in vitro* VEGFR-2 and CA inhibition properties (Table 2).

All derivatives under test demonstrated good VEGFR-2 inhibition with an IC_{50} of 23.10 to 63.40 nM, with compound **12b** being the most potent (IC_{50} = 23.10 nM) and compound **11c** being equivalent to the commonly used kinase inhibitor sorafenib (IC_{50} = 30.10 nM), which had an IC_{50} value of 29.70 nM and the remaining two being marginally less potent.

On the other hand, all the evaluated isatin derivatives failed to inhibit the CA isoforms (K_I > 100 μM), contrary to predictions, that could be attributable to the steric hindrance by the neighbouring methoxy group[37].

Cell cycle distribution analysis

To learn more about their cellular mechanisms of action, the most potent compounds (**11b** and **12b**) were selected. DMSO (control) or compounds **11b** and **12b** were applied to T47D cells, and flow cytometry was used to determine the DNA concentration. Figure 3 presents the findings. Both compounds showed different cell-cycle arrest patterns.

Cells treated with **11b** revealed an increase in sub-G1 and G0/G1 phases (45.88% and 68.42%, respectively), compared to 61.39% and 2.41%, respectively, in control. Additionally, compound **12b** increased the proportion of cells in the S and sub-G1 phases of the cell cycle from 28.55% and 2.41% for untreated T47D cells to 41.05% and 39.15%, respectively (Figure 3).

Table 1. Cytotoxicity of selected compounds **6f**, **6i**, **11b-c**, and **12a-c** (IC_{50}) against T47D cells.

Compound	IC_{50} ± S.D. (μM) T47D[a]
6f	5.45 ± 0.24
6i	24.13 ± 1.08
11b	1.83 ± 0.08
11c	10.40 ± 0.47
12a	11.58 ± 0.52
12b	3.59 ± 0.16
12c	16.52 ± 0.74
Doxorubicin[33]	2.26 ± 0.10

[a]IC_{50} values are mean ± SD of triplicate experiments.

Apoptosis assay

To explore link between growth suppression activity of compounds **11b** and **12b** with initiation of apoptosis indicated by rising population of sub-G1 in treated T47D cell lines, the Annexin V-FITC/PI double labelling (AV/PI) apoptosis assay was done. The outcomes of the experiment are shown in Figure 4, showing that compounds **11b** and **12b** caused T47D cell lines to undergo early and late apoptosis. In fact, % of apoptotic cells amplified from 0.41% at early and 0.22% at late apoptosis in T47D cells that had not been treated to 31.0% of early and 12.07% of late apoptosis in T47D cell that had been treated with compound **11b** and to 23.5% at early and 9.4% at late apoptosis of T47D cells that had been treated with compound **12b**. According to these findings, compounds **11b** and **12b** increased the overall apoptosis of T47D cells by roughly 19 and 16.2 times, respectively, in comparison to the control.

In silico *studies*

Molecular docking

The VEGFR-2 crystal structure (code 4ASD) was used in the molecular docking investigation that was done for predicting binding mode of **12b** as most active compound presented in this study. As predicted, **12b** fits deeply into the active site region with nearly the same orientation as the co-crystallized ligand (sorafenib, Figure S64).

In addition, the H-bonds were formed by the sulfamoyl amino group with Ile1025 and His1026 and between the isatin carbonyl group with Asp1046 (Figure 5). The heterocyclic rings of **12b** were

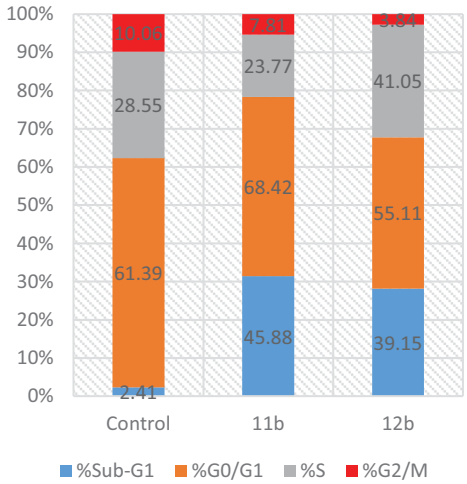


Figure 3. Phases distribution of T47D cells upon incubation with **11b** and **12b**.

Table 2. IC_{50} values of selected compounds **6f**, **11b-c**, and **12b** against VEGFR-2 and CA inhibition activity.

	VEGFR-2[a]		Carbonic anhydrase activity K_I (μM)[b]		
Compound	% Inhibition at 10 μM	IC_{50} ± S.D. (nM)	CA I	CA II	CA IX
6f	86.82	56.70 ± 0.72[a]	>100	>100	>100
11b	72.67	63.40 ± 0.72[a]	>100	>100	>100
11c	92.03	30.10 ± 0.31[a]	>100	>100	>100
12b	97.18	23.10 ± 0.41[a]	>100	>100	>100
Sorafenib	96.40	29.70 ± 0.17[a]	–	–	–
Acetazolamide (AAZ)	–	–	0.25	0.012	0.026

[a]IC_{50} value is mean ± SD of triplicate experiments, [b]Mean of triplicate different assays, *via* stopped flow assay.

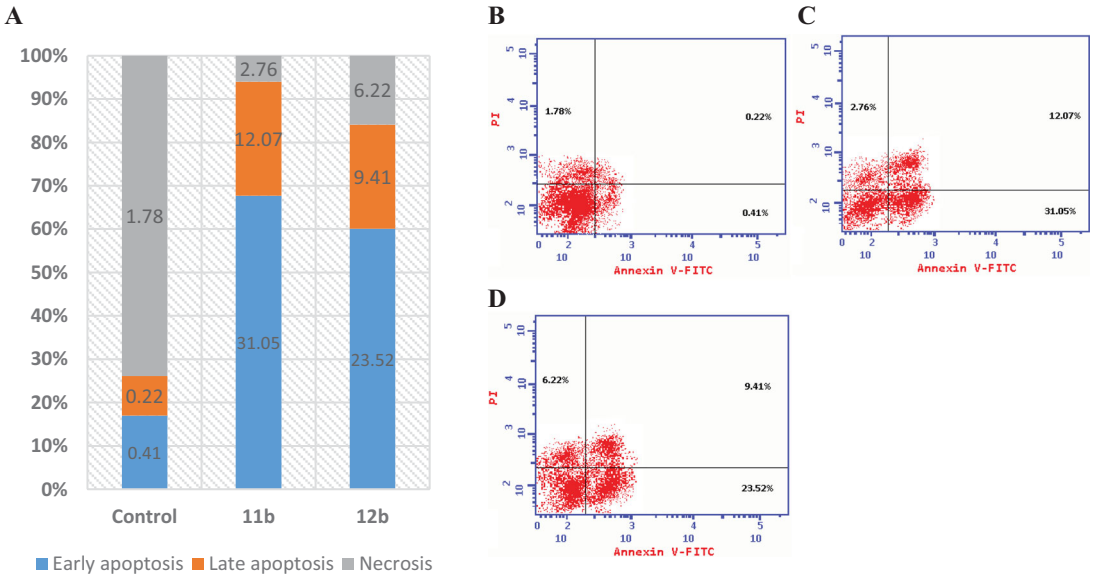


Figure 4. Apoptosis rate quantification (%) and necrosis in (**A**) flow cytometry, effect of (**B**) control, (**C**) **11b** and (**D**) **12b** on annexin V-FITC-positive staining (%) in T47D cell line.

involved in π-ion interactions with Asp1046, Lys868 and Glu885 while Cys1045 interacted with the five membered ring of isatin via π-sulfur interaction. Additionally, the benzyl ring and other hydrophobic components of **12b** were shown to interact with several hydrophobic residues found in the active site of the VEGFR-2 receptor, including Val848, Ala866, Leu889, Val899, Leu901, Val914, Val916, and Leu1035. All the aforementioned interactions were responsible for the good affinity of **12b** to the studied receptor as indicated by the docking score of −8.5 kcal/mol.

Molecular dynamic simulation

After the docking process of **12b** into the VEGFR-2 receptor, the stability of the complex structure needed to be examined. To test the durability of the best pose for the **12b**-VEGFR-2 complex at room temperature circumstances, a 100 ns MD simulation was used. Over this simulation period, the complex system's temperature, pressure and potential energy, are shown in Figure 6 showing converged system throughout whole simulation period.

Analysing trajectories after simulation showing **12b** ligands stayed attached to active site in protein pocket as indicated by the calculated RMSD, radius of gyration and average of distance at mass centre between ligand and protein Figure 7.

Furthermore, SASA and RMSF of protein over the simulation period showed no significate change in absence and presence of **12b** as shown in Figure 8.

Evaluating interactions of **12b** to the nearby residues within the active site of VEGFR-2 showed various stable interactions over the simulation period (Figure 9). Different forces contributing to the affinity of **12b** to the receptor were observed during 100 ns of the simulation run including π-stacking, H-bonding and hydrophobic, Figure S65–67. Figure 10 depicts the total number of H-bonds formed by the protein and ligand during the simulation period. On average, there were two to three H-bonds to the VEGFR-2 receptor remained stable throughout the entire simulation. Finally, all these interactions were reflected in the binding free energy (MMPBSA) that were carried out to assess the stability of the formed complex structure (Table 3).

Conclusions

In the current study, different sets of isatin-based sulphonamides (**6a-i**, **11a-c** and **12a-c**) were reported with the prime goal of developing new anticancer candidates with dual VEGFR-2 and carbonic anhydrase inhibitory actions. *In vitro* anticancer activities of target isatins were first explored towards 58 tumour cell lines (NCI-USA panel). The findings revealed that the breast cancer subpanel was the most susceptible to the influence of target isatins. In particular, the T47D cells growth was effectively inhibited by compounds **6f**, **6i**, **11b-c** and **12a-c**. Then, the IC_{50} values of these isatins towards T47D cells were determined (IC_{50} range: 1.83 − 24.13 μM). Thereafter, the VEGFR-2 and carbonic anhydrase inhibitory actions of isatins **6f**, **11b-c**, and **12b** were evaluated. While the target isatin sulphonamides potently inhibited VEGFR-2 (IC_{50} range: 23.10 − 63.40 nM), they failed to inhibit the CA isoforms (K_I > 100 μM), contrary to predictions, that could be attributable to the steric hindrance by the neighbouring methoxy group. Moreover, isatins **11b** and **12b** were tested for their effect on cell cycle, and towards apoptotic potential.

Experimental

Chemistry

General

Reactions progress was checked by TLC sheets (Silica 60 F_{254}, Fastman Kodak Co.) using methanol and chloroform: (10: 90) as elution system: and visualisation was done at 254 nm. Melting point was measured on Stuart SMP10 using the open capillary technique and was reported uncorrected. ^{13}C NMR (101–126 MHz) and ^{1}H (400–500 MHz) spectra were obtained using Bruker FTNMR spectrometer using DMSO-d_6 as a solvent. Spectra of high-resolution mass were recorded on Bruker MicroTOF spectrometer. Elemental analysis (% C, H, N, and S) was done using CHNS analyser (2400 Perkin–Elmer). The spectral data and their interpretation were deposited in the supplementary file.

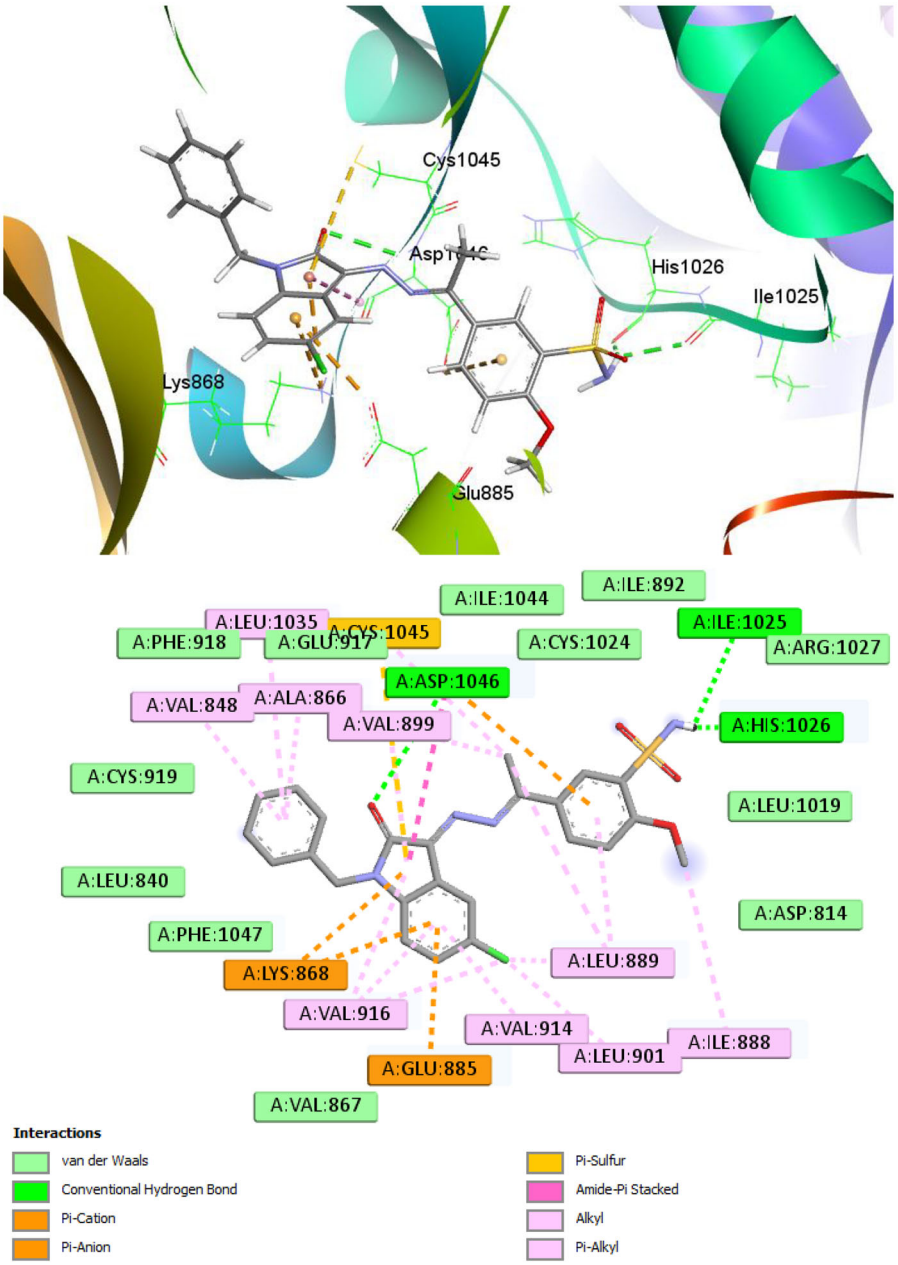


Figure 5. Docking of **12b** inside the active site of VEGFR-2 receptor (code: 4ASD); active site view (top) and 2D schematic view of the interactions (bottom).

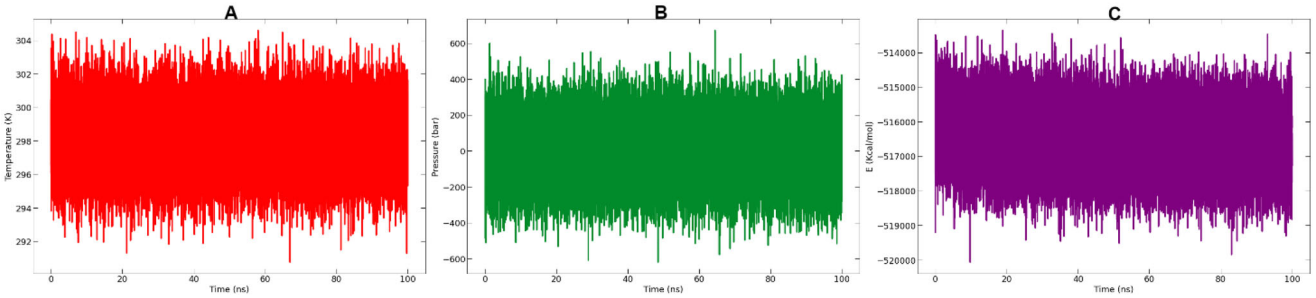


Figure 6. From left to right: **(A)** Temperature, **(B)** pressure and **(C)** potential energy during the 100 ns MD simulations.

General steps for preparation of 5-acetyl-2-methoxybenzene-1-sulfonyl chloride (2)

Chlorosulfonic acid (90 mmol) was stirred with thionyl chloride (30 mmol) in an ice bath for 30 min. After that, 4-methoxyacetophenone **1** (15 mmol) was added to the mixture dropwise, followed by stirring the reaction mixture for 26 h at room temperature. Subsequently, ice water was added, and the

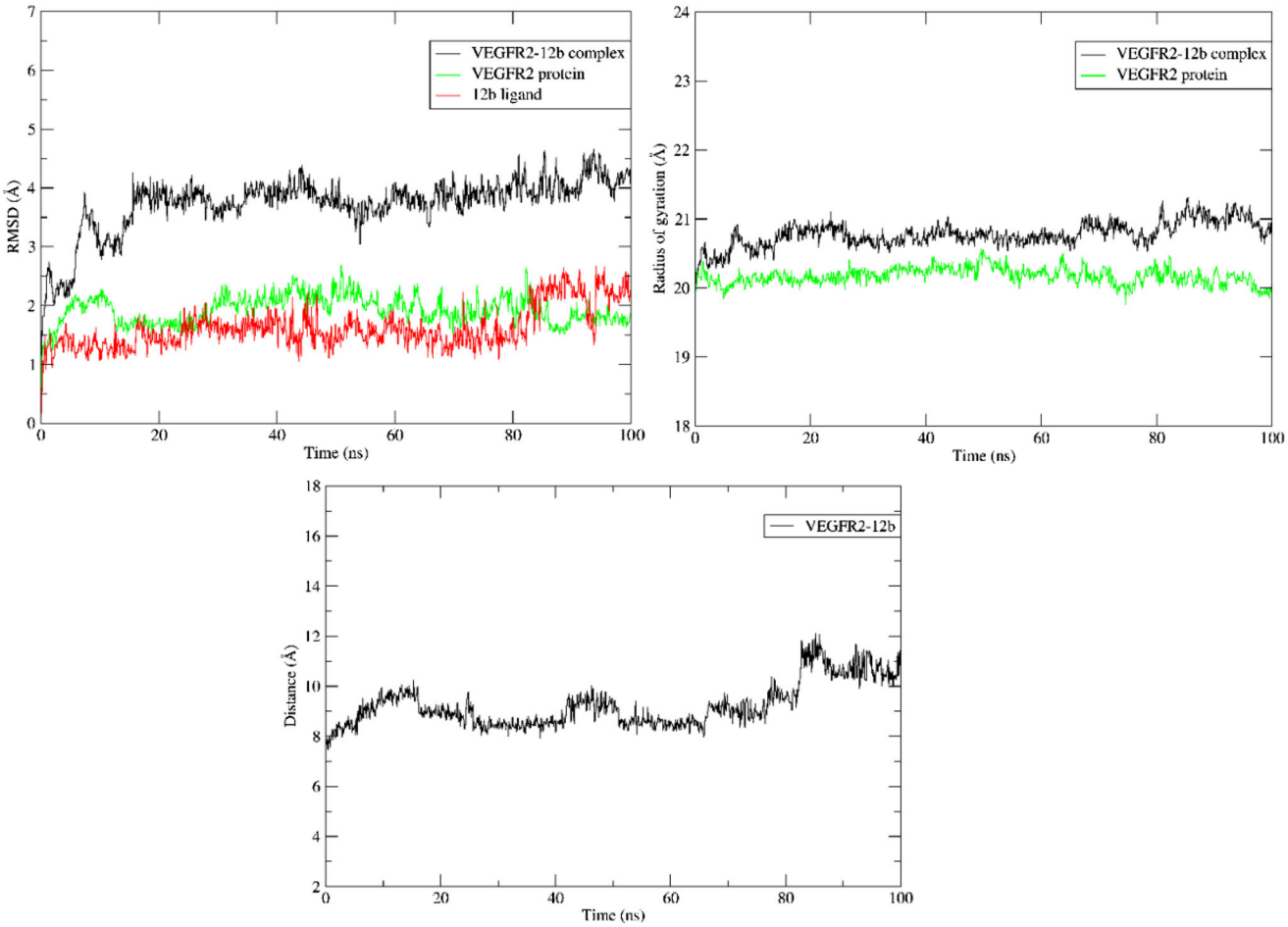


Figure 7. RMSD, Radius of gyration and average centre of mass distance of heavy atoms of **12b** during 100 ns MD simulation.

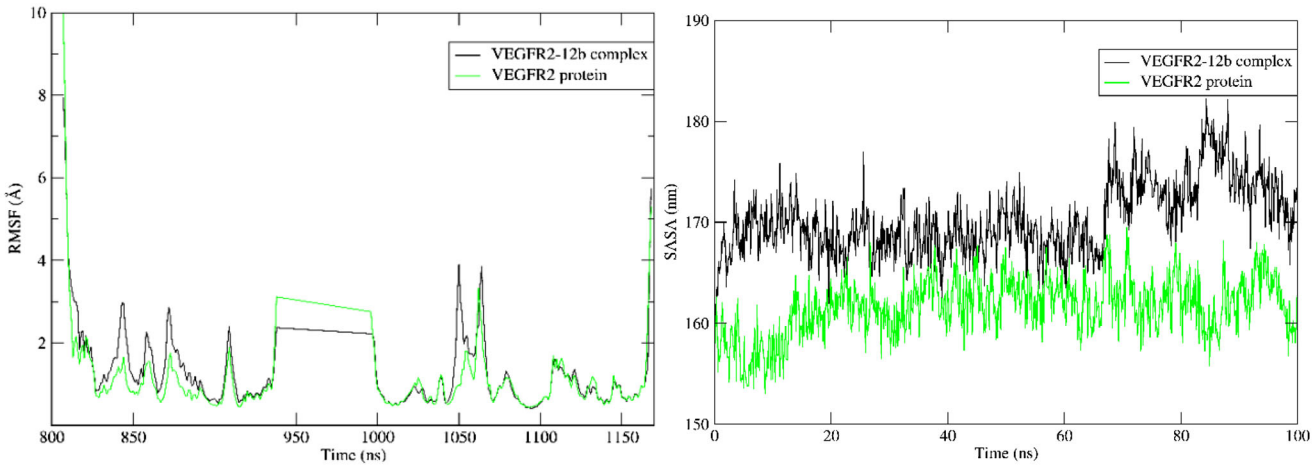


Figure 8. RMSF and SASA of VEGFR-2 in presence and absence of **12b** over 100 ns MD simulation.

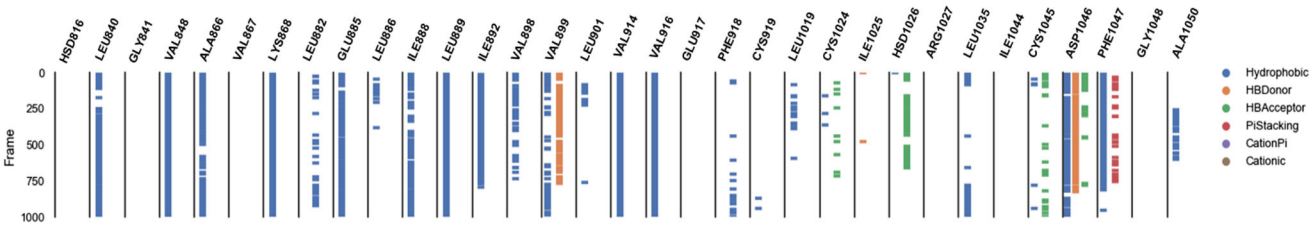


Figure 9. Different types of interaction exhibited by **12b** with the amino acids within the active site of VEGFR-2 during the whole MD simulation frames.

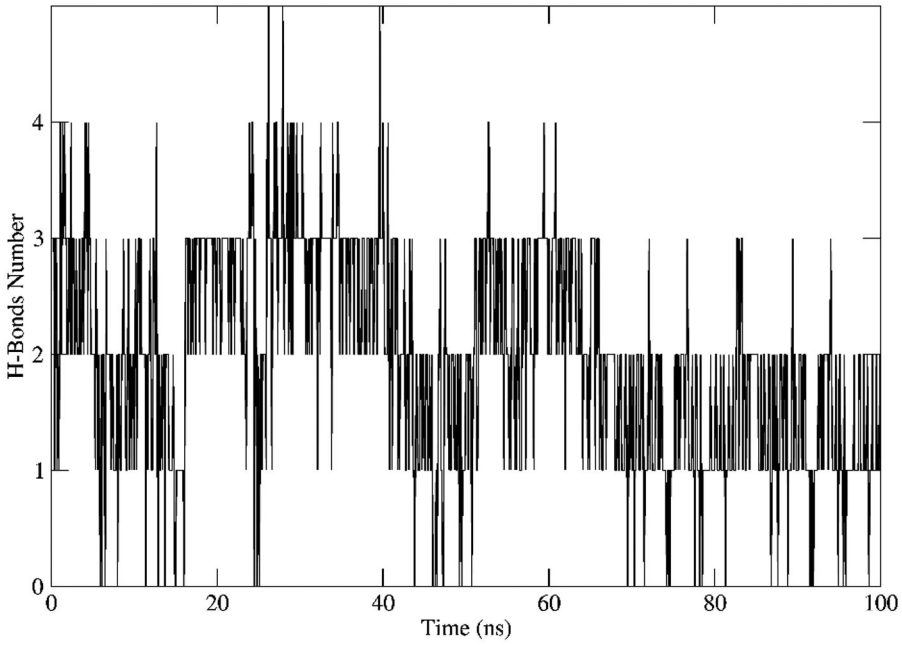


Figure 10. Hydrogen bonds between VEGFR-2 and **12b** during 100 ns MD simulation.

Table 3. Free binding energies of **12b** with VEGFR-2 in kJ/mol.

Binding energies	kJ/mol
Electrostatic energy	−84.723 ± 46.032
Polar solvation energy	189.645 ± 48.831
SASA energy	−24.848 ± 1.635
van der Waal energy	−195.211 ± 25.865
ΔG	−115.137 ± 28.682

resulting pale orange precipitate **2** was taken after being filtered and washed using H_2O (3.35 g, 90%), Mp: 102–103 °C[22,37].

General steps for preparation of 5-acetyl-2-methoxybenzenesulfonamide (3)

Compound **2** (10 mmol) was stirred in ethanol (30 ml) as solvent at room temperature while 30 ml ammonia was added to the mixture. Completion of the reaction detected *via* TLC. The pink precipitated compound **3** was isolated and purified using ethanol and water (1.81 g, 79%), Mp: 202–203 °C[22,38].

General procedure for preparation of 5-[(1E)-ethanehydrazonoyl]-2-methoxybenzene-1-sulfonamide (4)

Compound **3** (7.9 mmol) was refluxed with hydrazine hydrate (10 mmol), glacial acetic acid (2.5 ml) and ethanol (50 ml) for 4 h. Next, the mixture brought to room temperature, then its extraction with DCM (50 ml × 3). The obtained product **4** was dried over Na_2SO_4, concentrated under vacuum, and used in the next step without further purification.

General steps for preparation of 2-methoxy-6-[1–(2-[2-oxo-2,3-dihydro-1H-indol-3-ylidene]hydrazin-1-ylidene)ethyl]benzene-1-sulfonamide derivatives (6a-i)

Compound **4** (0.4 mmol) was stirred with different derivatives of isatin **5a-i** (0.4 mmol) using absolute ethanol as solvent under reflux conditions for 6–8 h with the addition of glacial acetic acid using catalytic amount. The formed precipitate was filtered and washed with ethanol before recrystallizing it from DMF to afford desired compounds **6a-i**.

General steps for preparation of N-methylated derivatives of 2-methoxy-6-[(1E)-1–(2-[(3E)-2-oxo-2,3-dihydro-1H-indol-3-ylidene]-hydrazin-1-ylidene)ethyl]benzene-1-sulphonamides (11a-c)

Isatin derivatives **5a**, **5d** and **5f** (2 mmol) were refluxed with (2.8 mmol) of methyl iodide **7** in acetonitrile (20 ml) using a catalytic amount of potassium iodide and dry potassium carbonate (10 mmol). TLC was used in monitoring the reaction progression. After reaction completion, it was added over ice water; the resulting solid was collected, washed with water, and recrystallized from ethanol and water to produce the intermediate compounds **9a–c**. Subsequently, isatin derivatives **9a-c** reacted with compound **4** in the same conditions described previously in the preparation of compounds **6a-i**.

General procedure for preparation of N-benzylated derivatives of 2-methoxy-6-[(1E)-1–(2-[(3E)-2-oxo-2,3-dihydro-1H-indol-3-ylidene]-hydrazin-1-ylidene)ethyl]benzene-1-sulphonamides (12a-c)

Isatin derivatives **5a**, **5d** and **5f** (2 mmol) were refluxed with (2.8 mmol) of benzyl bromide **8** in acetonitrile (20 ml) using a catalytic amount of potassium iodide and dry potassium carbonate (4 mmol). TLC was used in monitoring the reaction progression. After reaction completion, it was added over ice water; the resulting solid was collected, washed with water, and recrystallized from ethanol and water to produce the intermediate compounds **10a-c**. Subsequently, isatin derivatives **10a-c** reacted with compound **4** in the same conditions described previously in the synthesis of compounds **6a-i**.

Biological evaluation

The NCI-USA antitumor assessment[39], MTT viability[40,41], VEGFR-2 inhibition[42,43], CA I, II, and IX inhibition studies[44–47], cell cycle analysis and annexin V-FITC apoptosis assay[48,49] were all carried out

as previously published in the current study. The supplementary data included descriptions of every experimental technique.

Molecular modeling

Molecular docking studies

The crystal structure of VEGFR-2 (Code: 4ASD)[50] was retrieved from protein data bank. The docking study was carried out on the co-crystalized ligand (sorafenib) and **12b** as a promising inhibitor. Ligands were drawn into Marvin Sketch V19.12[51]. The docking was performed using AutoDock Vina[52] in accordance with our previous report[5]. The active site was defined by the grid box of (x= −23.3, y = 0.1 and z= −10.1) coordinates with size (x = 23.2, y = 18.3, z = 21.6). Using the Discovery studio client, the 3D visualisation and the 2D schematic presentation were produced[53].

MD Simulation

The best docking pose was subjected for molecular dynamics (MD) using GROMACS 2021 over 100 ns[54]. The details of applied parameters and procedures were mentioned in the supplementary file.

Disclosure statement

CT Supuran is Editor-in-Chief of the Journal of Enzyme Inhibition and Medicinal Chemistry. He was not involved in the assessment, peer review, or decision-making process of this paper. The authors have no relevant affiliations of financial involvement with any organisation or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript. This includes employment, consultancies, honoraria, stock ownership or options, expert testimony, grants or patents received or pending, or royalties.

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References

1. Kamboj P, Anjali, Husain A, Shaquiquzzaman M, Alam MM, Amir M. A review on the synthesis and anticancer potentials of imidazothiazole derivatives. Min Rev Med Chem. 2023;23:1–27.
2. Mansoori B, Mohammadi A, Davudian S, Shirjang S, Baradaran B. The different mechanisms of cancer drug resistance: a brief review. Adv Pharm Bull. 2017;7(3):339–348.
3. Zhong L, Li Y, Xiong L, Wang W, Wu M, Yuan T, Yang W, Tian C, Miao Z, Wang T, et al. Small molecules in targeted cancer therapy: advances, challenges, and future perspectives. Signal Transduct Target Ther. 2021;6(1):201.
4. Kalinin S, Malkova A, Sharonova T, Sharoyko V, Bunev A, Supuran CT, Krasavin M. Carbonic anhydrase IX inhibitors as candidates for combination therapy of solid tumors. Int J Mol Sci. 2021;22(24):13405.
5. Supuran CT. Structure and function of carbonic anhydrases. Biochem J. 2016;473(14):2023–2032.
6. Angeli A, Carta F, Nocentini A, Winum JY, Zalubovskis R, Akdemir A, Onnis V, Eldehna WM, Capasso C, Simone G, et al. Carbonic anhydrase inhibitors targeting metabolism and tumor microenvironment. Metabolites. 2020;10(10):412.
7. Mishra CB, Tiwari M, Supuran CT. Progress in the development of human carbonic anhydrase inhibitors and their pharmacological applications: where are we today? Med Res Rev. 2020;40(6):2485–2565.
8. Shaldam M, Eldehna WM, Nocentini A, Elsayed ZM, Ibrahim TM, Salem R, El-Domany RA, Capasso C, Abdel-Aziz HA, Supuran CT. Development of novel benzofuran-based SLC-0111 analogs as selective cancer-associated carbonic anhydrase isoform IX inhibitors. Eur J Med Chem. 2021;216:113283.
9. Wang X, Bove AM, Simone G, Ma B. Molecular bases of VEGFR-2-mediated physiological function and pathological role. Front Cell Dev Biol. 2020;8:599281.
10. Cheke RS, Patil VM, Firke SD, Ambhore JP, Ansari IA, Patel HM, Shinde SD, Pasupuleti VR, Hassan MI, Adnan M, et al. Therapeutic outcomes of isatin and its derivatives against multiple diseases: recent developments in drug discovery. Pharmaceuticals (Basel. 2022;15(3):272.
11. Eldehna WM, Fares M, Ceruso M, Ghabbour HA, Abou-Seri SM, Abdel-Aziz HA, Abou El Ella DA, Supuran CT. Amido/ureidosubstituted benzenesulfonamides-isatin conjugates as low nanomolar/subnanomolar inhibitors of the tumor-associated carbonic anhydrase isoform XII. Eur J Med Chem. 2016;110:259–266.
12. Eldehna WM, Al-Ansary GH, Bua S, Nocentini A, Gratteri P, Altoukhy A, Ghabbour H, Ahmed HY, Supuran CT. Novel indolin-2-one-based sulfonamides as carbonic anhydrase inhibitors: Synthesis, in vitro biological evaluation against carbonic anhydrases isoforms I, II, IV and VII and molecular docking studies. Eur J Med Chem. 2017;127:521–530.
13. Abo-Ashour MF, Eldehna WM, Nocentini A, Ibrahim HS, Bua S, Abou-Seri SM, Supuran CT. Novel hydrazido benzenesulfonamides-isatin conjugates: Synthesis, carbonic anhydrase inhibitory activity and molecular modeling studies. Eur J Med Chem. 2018;157:28–36.
14. Eldehna WM, Abo-Ashour MF, Nocentini A, Gratteri P, Eissa IH, Fares M, Ismael OE, Ghabbour HA, Elaasser MM, Abdel-Aziz HA, et al. Novel 4/3-((4-oxo-5-(2-oxoindolin-3-ylidene)-thiazolidin-2-ylidene)amino) benzenesulfonamides: Synthesis, carbonic anhydrase inhibitory activity, anticancer activity and molecular modelling studies. Eur J Med Chem. 2017;139:250–262.
15. Dhokne P, Sakla AP, Shankaraiah N. Structural insights of oxindole based kinase inhibitors as anticancer agents: Recent advances. Eur J Med Chem. 2021;216:113334.
16. Elkaeed EB, Taghour MS, Mahdy HA, Eldehna WM, El-Deeb NM, Kenawy AM, A Alsfouk B, Dahab MA, Metwaly AM, Eissa IH, et al. New quinoline and isatin derivatives as apoptotic VEGFR-2 inhibitors: design, synthesis, anti-proliferative

activity, docking, ADMET, toxicity, and MD simulation studies. J Enzyme Inhib Med Chem. 2022;37(1):2191–2205.,
17. Taghour MS, Elkady H, Eldehna WM, El-Deeb NM, Kenawy AM, Elkaeed EB, Alsfouk AA, Alesawy MS, Metwaly AM, Eissa IH. 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. J Enzyme Inhib Med Chem. 2022;37(1):1903–1917.
18. Eldehna WM, El Hassab MA, Abdelshafi NA, Eissa RA, Diab NH, Mohamed EH, Oraby MA, Al-Rashood ST, Eissa RG, Elsayed ZM, et al. Development of potent nanosized carbonic anhydrase inhibitor for targeted therapy of hypoxic solid tumors. Int J Pharm. 2023;631:122537.
19. Eldehna WM, Nocentini A, Al-Rashood ST, Hassan GS, Alkahtani HM, Almehizia AA, Reda AM, Abdel-Aziz HA, Supuran CT. Tumor-associated carbonic anhydrase isoform IX and XII inhibitory properties of certain isatin-bearing sulfonamides endowed with in vitro antitumor activity towards colon cancer. Bioorg Chem. 2018;81:425–432.
20. Abo-Ashour MF, Eldehna WM, Nocentini A, Bonardi A, Bua S, Ibrahim HS, Elaasser MM, Kryštof V, Jorda R, Gratteri P, et al. 3-Hydrazinoisatin-based benzenesulfonamides as novel carbonic anhydrase inhibitors endowed with anticancer activity: Synthesis, in vitro biological evaluation and in silico insights. Eur J Med Chem. 2019;184:111768.
21. Eldehna WM, Abo-Ashour MF, Nocentini A, El-Haggar RS, Bua S, Bonardi A, Al-Rashood ST, Hassan GS, Gratteri P, Abdel-Aziz HA, et al. Enhancement of the tail hydrophobic interactions within the carbonic anhydrase IX active site via structural extension: Design and synthesis of novel N-substituted isatins-SLC-0111 hybrids as carbonic anhydrase inhibitors and antitumor agents. Eur J Med Chem. 2019;162:147–160.
22. Castaño LF, Cuartas V, Bernal A, Insuasty A, Guzman J, Vidal O, Rubio V, Puerto G, Lukáč P, Vimberg V, et al. New chalcone-sulfonamide hybrids exhibiting anticancer and antituberculosis activity. Eur J Med Chem. 2019;176:50–60.,
23. Yan L, Ding W, Wang L, Dou Q, Luo Q. A new synthesis protocol for photochromic triarylethenes and their multifunctional derivatives. Synth Commun. 2020;50(20):3099–3112.
24. Sabt A, Eldehna WM, Ibrahim TM, Bekhit AA, Batran RZ. New antileishmanial quinoline linked isatin derivatives targeting DHFR-TS and PTR1: Design, synthesis, and molecular modeling studies. Eur J Med Chem. 2023;246:114959.
25. Tawfik HO, Belal A, Abourehab MA, Angeli A, Bonardi A, Supuran CT, El-Hamamsy MH. Dependence on linkers' flexibility designed for benzenesulfonamides targeting discovery of novel hCA IX inhibitors as potent anticancer agents. J Enzyme Inhib Med Chem. 2022;37(1):2765–2785.
26. Khalil AF, El-Moselhy TF, El-Bastawissy EA, Abdelhady R, Younis NS, El-Hamamsy MH. Discovery of novel enasidenib analogues targeting inhibition of mutant isocitrate dehydrogenase 2 as antileukaemic agents. J Enzyme Inhib Med Chem. 2023;38(1):2157411.
27. Konkel MJ, Lagu B, Boteju LW, Jimenez H, Noble S, Walker MW, Chandrasena G, Blackburn TP, Nikam SS, Wright JL, et al. 3-arylimino-2-indolones are potent and selective galanin GAL3 receptor antagonists. J Med Chem. 2006;49(13):3757–3758.
28. Wegermann CA, Monzani E, Casella L, Ribeiro MA, Bruzeguini CET, Vilcachagua JD, Costa LAS, Ferreira AMDC. Unveiling geometrical isomers and tautomers of isatin-hydrazones by NMR spectroscopy. J Mol Struct. 2022;1250:131633.
29. Jakusová K, Donovalová J, Gáplovský M, Cigáň M, Stankovičová H, Gáplovský A. Self-association, tautomerism and E–Z isomerization of isatin–phenylsemicarbazones – spectral study and theoretical calculations. J Phys Org Chem. 2013;26(10):805–813.
30. Eldehna WM, Salem R, Elsayed ZM, Al-Warhi T, Knany HR, Ayyad RR, Traiki TB, Abdulla M-H, Ahmad R, Abdel-Aziz HA, et al. Development of novel benzofuran-isatin conjugates as potential antiproliferative agents with apoptosis inducing mechanism in Colon cancer. J Enzyme Inhib Med Chem. 2021;36(1):1423–1434.
31. Abdel-Aziz HA, Ghabbour HA, Eldehna WM, Qabeel MM, Fun H-K. Fun, synthesis, crystal structure, and biological activity of *cis/trans* amide rotomers of (*Z*)-*N*′-(2-oxoindolin-3-ylidene)formohydrazide. J Chem. 2014;2014:1–7.
32. Abdelrahman MA, Almahli H, Al-Warhi T, Majrashi TA, Abdel-Aziz MM, Eldehna WM, Said MA. Development of novel isatin-tethered quinolines as anti-tubercular agents against multi and extensively drug-resistant mycobacterium tuberculosis. Molecules. 2022;27(24):8807.
33. Mullin A, Jean-Claude B. HER2/neu oncogene and sensitivity to the DNA-interactive drug doxorubicin. MJM. 2020;4:9–15.
34. Li X, Gao Y, Li J, Zhang K, Han J, Li W, Hao Q, Zhang W, Wang S, Zeng C, et al. FOXP3 inhibits angiogenesis by downregulating VEGF in breast cancer. Cell Death Dis. 2018;9(7):744.
35. Weigand M, Hantel P, Kreienberg R, Waltenberger J. Autocrine vascular endothelial growth factor signalling in breast cancer. Evidence from cell lines and primary breast cancer cultures in vitro. Angiogenesis. 2005;8(3):197–204.
36. Syed Khaja AS, Dizeyi N, Kopparapu PK, Anagnostaki L, Härkönen P, Persson JL. Cyclin A1 modulates the expression of vascular endothelial growth factor and promotes hormone-dependent growth and angiogenesis of breast cancer. PLOS One. 2013;8(8):e72210.
37. Aboukhatwa SM, Sidhom PA, Angeli A, Supuran CT, Tawfik HO. Terminators or guardians? design, synthesis, and cytotoxicity profiling of chalcone-sulfonamide hybrids. ACS Omega. 2023;8(8):7666–7683.
38. Shaldam M, Tawfik H, Elmansi H, Belal F, Yamaguchi K, Sugiura M, Magdy G. Synthesis, crystallographic, DNA binding, and molecular docking/dynamic studies of a privileged chalcone-sulfonamide hybrid scaffold as a promising anticancer agent. J Biomol Struct Dyn. 2022;40:1–15.
39. Saad MH, El-Moselhy TF, Nabaweya E-DS, Mehany AB, Belal A, Abourehab MA, Tawfik HO, El-Hamamsy MH. Discovery of new symmetrical and asymmetrical nitrile-containing 1, 4-dihydropyridine derivatives as dual kinases and P-glycoprotein inhibitors: synthesis, in vitro assays, and in silico studies. J Enzyme Inhib Med Chem. 2022;37(1):2489–2511.
40. Hassan A, Badr M, Abdelhamid D, Hassan HA, Abourehab MA, Abuo-Rahma GEDA. Design, synthesis, in vitro antiproliferative evaluation and in silico studies of new VEGFR-2 inhibitors based on 4-piperazinylquinolin-2 (1H)-one scaffold. Bioorg Chem. 2022;120:105631.
41. Hassan A, Badr M, Hassan HA, Abdelhamid D, Abuo-Rahma GEDA. Novel 4-(piperazin-1-yl) quinolin-2 (1H)-one bearing thiazoles with antiproliferative activity through VEGFR-2-TK inhibition. Bioorg Med Chem. 2021;40:116168.

42. Al-Sanea MM, Hamdi A, Mohamed AAB, El-Shafey HW, Moustafa M, Elgazar AA, Eldehna WM, Ur Rahman H, Parambi DGT, Elbargisy RM, et al. New benzothiazole hybrids as potential VEGFR-2 inhibitors: design, synthesis, anticancer evaluation, and in silico study. J Enzyme Inhib Med Chem. 2023;38(1):2166036.,
43. Zahran SS, Ragab FA, El-Gazzar MG, Soliman AM, Mahmoud WR, Ghorab MM. Antiproliferative, antiangiogenic and apoptotic effect of new hybrids of quinazoline-4 (3H)-ones and sulfachloropyridazine. Eur J Med Chem. 2023;245(Pt 1): 114912.
44. Tawfik HO, Petreni A, Supuran CT, El-Hamamsy MH. Discovery of new carbonic anhydrase IX inhibitors as anticancer agents by toning the hydrophobic and hydrophilic rims of the active site to encounter the dual-tail approach. Eur J Med Chem. 2022;232:114190.
45. Zain-Alabdeen AI, El-Moselhy TF, Sharafeldin N, Angeli A, Supuran CT, El-Hamamsy MH. Synthesis and anticancer activity of new benzensulfonamides incorporating s-triazines as cyclic linkers for inhibition of carbonic anhydrase IX. Sci Rep. 2022;12(1):16756.
46. Petreni A, Bonardi A, Lomelino C, Osman SM, ALOthman ZA, Eldehna WM, El-Haggar R, McKenna R, Nocentini A, Supuran CT. Inclusion of a 5-fluorouracil moiety in nitrogenous bases derivatives as human carbonic anhydrase IX and XII inhibitors produced a targeted action against MDA-MB-231 and T47D breast cancer cells. Eur J Med Chem. 2020;190:112112.
47. Said MA, Eldehna WM, Nocentini A, Bonardi A, Fahim SH, Bua S, Soliman DH, Abdel-Aziz HA, Gratteri P, Abou-Seri SM, et al. Synthesis, biological and molecular dynamics investigations with a series of triazolopyrimidine/triazole-based benzenesulfonamides as novel carbonic anhydrase inhibitors. Eur J Med Chem. 2020;185:111843.
48. Chen R, Wang Z, Sima L, Cheng H, Luo B, Wang J, Guo B, Mao S, Zhou Z, Peng J, et al. Design, synthesis and evaluation of 2, 6, 8-substituted Imidazopyridine derivatives as potent PI3K α inhibitors. J Enzyme Inhib Med Chem. 2023; 38(1):2155638.
49. Kaur H, Singh A, Kaur K, Kumar A, Attri S, Rashid F, Singh S, Bedi N, Tuli HS, Haque S, et al. 4-methylthiobutyl isothiocyanate synergize the antiproliferative and pro-apoptotic effects of paclitaxel in human breast cancer cells. Biotechnol Genetic Engineer Rev. 2023;39:1–25.
50. McTigue M, Murray BW, Chen JH, Deng Y-L, Solowiej J, Kania RS. Molecular conformations, interactions, and properties associated with drug efficiency and clinical performance among VEGFR TK inhibitors. Proc Natl Acad Sci U S A. 2012; 109:18281–18289.
51. Guo Y, Song G, Sun M, Wang J, Wang Y. Prevalence and Therapies of Antibiotic-Resistance in Staphylococcus aureus. Front Cell Infect Microbiol. 2020;10:107.
52. Trott O, Olson AJ. AutoDock Vina: improving the speed and accuracy of docking with a new scoring function, efficient optimization, and multithreading. J Comput Chem. 2010; 31(2):455–461.
53. Biovia D. Systèmes discovery studio visualizer, V16.1.0, San Diego: Dassault Systèmes; 2016,
54. Abraham MJ, Murtola T, Schulz R, Páll S, Smith JC, Hess B, Lindahl E. GROMACS: High performance molecular simulations through multi-level parallelism from laptops to supercomputers. SoftwareX. 2015;1-2:19–25.