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# A comprehensive review and recent advances on isatin-based compounds as a versatile framework for anticancer therapeutics (2020–2025)

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Isatin (1*H*-indole-2,3-dione) is a privileged nitrogen-containing heterocyclic framework that has received considerable attention in anticancer drug discovery owing to its general biological behavior and structural diversity. This review focuses on isatin–heterocyclic hybrids as a valuable model in the development of new anti-cancer drugs that may reduce side effects and help overcome drug resistance, discussing their synthetic approaches and mechanism of action as apoptosis induction through kinase inhibition. With various chemical modifications, isatin had an excellent ability to build powerful isatin hybrids and conjugates targeting multiple oncogenic pathways. It is worth mentioning that isatin-hybrids exhibited anticancer activity against various cancer cell lines, such as breast, liver, colon, lung, and multidrug-resistant carcinomas. Their mechanisms include mitochondrial-mediated apoptosis, caspase activation, tubulin polymerization inhibition, and kinase modulation, particularly VEGFR-2, EGFR, CDK2, and STAT-3. Numerous synthesized isatin-based compounds have shown superior cytotoxicity compared to established chemotherapeutics, with favorable IC<sub>50</sub> values and minimal toxicity toward normal cells. In addition, this review summarizes more recent synthetic innovations, e.g., microwave-assisted and multi-component techniques, which offer improved pharmacological profiles of these isatin–heterocyclic hybrids with improved cytotoxicity and target signaling pathways. Overall, these results underscore the value of isatin as a flexible scaffold for the rational design of new anticancer agents. To increase bioavailability and targeted delivery, especially in solid tumors, and to lead to the creation of novel, potent anticancer therapies, nano-formulation drug delivery systems with revolutionary drug signaling pathways will be further advocated in the future.

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## 1. Introduction

Cancer remains one of the most formidable challenges in modern medicine, not only for its complexity and adaptability but also for the collateral damage inflicted by its treatment. Chemotherapy, a cornerstone of cancer therapy, is designed to eradicate rapidly dividing cancer cells. However, its lack of

absolute specificity often results in significant toxicity to normal, healthy cells, particularly those in the bone marrow, digestive tract, and hair follicles, which limits the therapeutic window and diminishes patients' quality of life.<sup>1,2</sup> This dual-edged nature of chemotherapy underscores a central dilemma: maximizing the destruction of malignant cells while preserving the integrity and function of normal tissues. Therefore, it is essential to create novel anti-cancer medications that are highly selective and effective against drug-sensitive and drug-resistant tumors.<sup>3,4</sup>

In medicinal chemistry, heterocyclic compounds are beneficial molecules.<sup>5</sup> They display various pharmacological and biological activities.<sup>6,7</sup> As a fundamental building block of a vast heterocyclic library, nitrogen-containing heterocyclic congeners are widely employed in many scientific fields.<sup>8,9</sup> Moreover, nitrogen-containing heterocycles have remarkable structural properties and are commonly found in a variety of herbal components, including alkaloids and vitamins.<sup>10</sup> Isatin is a nitrogen-containing single scaffold. Many bioactive natural compounds have the isatin skeleton, e.g., chitosenine,

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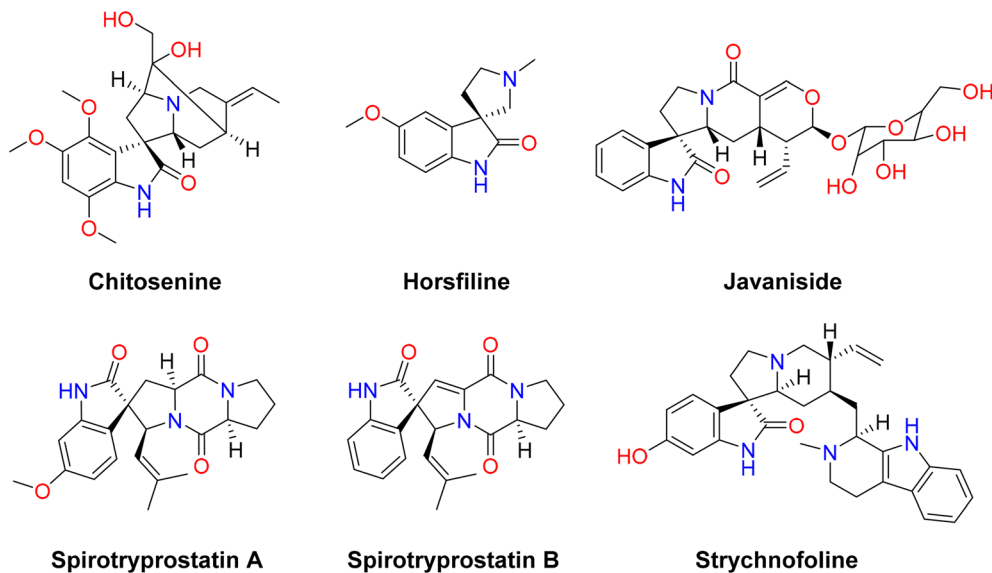



Fig. 1 Bioactive natural products containing isatin.

horsfiline, javaniside, spirotryprostatin A, spirotryprostatin B, and strychnofoline (Fig. 1).<sup>11</sup>

Isatin's structure allows the insertion of various substituents into almost any position of the moiety. Numerous derivatives with enhanced biological activity have been produced as a result of structural modifications to the isatin ring.<sup>12,13</sup> Additionally, numerous cancer forms, even those that are resistant to treatment, may be treated by specific anti-cancer treatments that contain isatin, indicating the possibility of creating new anti-cancer drugs.<sup>14</sup> Fig. 2 represents currently marketed isatin-based anti-cancer drugs.<sup>15</sup>

Isatin is an indole derivative that is produced by oxidizing indigo dye. Its chemical name is 1*H*-indole-2,3-dione. First synthesized by Erdman and Laurent in 1841,<sup>16</sup> isatin has since become a pivotal entity in organic synthesis, attributable to its structural intricacy and chemical reactivity. Isatin is a naturally occurring substance that can be found in plants like *Calanthe discolor* and *Couroupita guianensis*. It has also been found within the secretion of the parotid gland of Bufo frogs and

human metabolic pathways stemming from adrenaline. Additionally, various plant species include substituted isatins, such as the melastatin alkaloids obtained from the Caribbean tumorigenic plant *Melochia tomentosa*.<sup>14,15</sup>

## 2. Chemical properties

Isatin is an endogenous compound with the molecular formula  $C_6H_5NO_2$ . According to Fig. 3, it contains two carbonyl groups at positions two and three and a nitrogen atom at position one. It consists of two cyclic rings, one with five members and the other with six. The two rings are flat. Whereas the 5-membered ring has an anti-aromatic property, the 6-membered ring has an aromatic one.<sup>17</sup>

Isatin can undergo chemical reactions in three different places: *N*-alkylation, aromatic ring substitution, and carbonyl reaction at its C2 and/or C3 carbonyl functionalities (Fig. 4).<sup>18</sup>

Derivatives of isatin exhibit improved anticancer properties through strategic hybridization. Imidazole–isatin hybrids

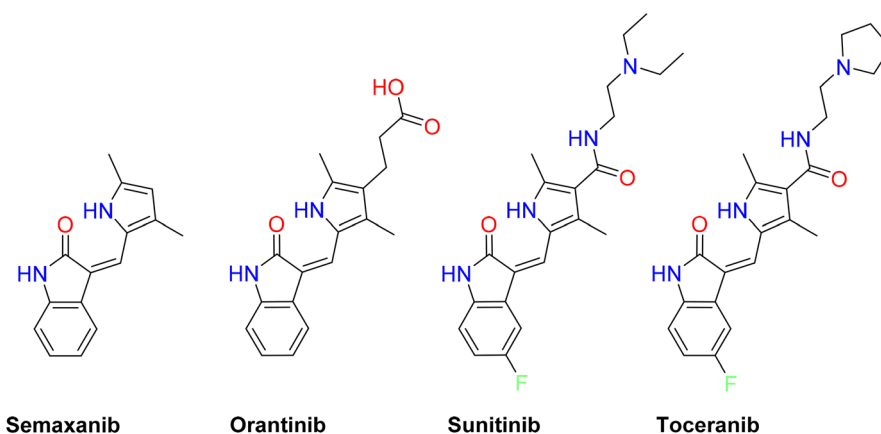


Fig. 2 Isatin-based marketed anti-cancer drugs.



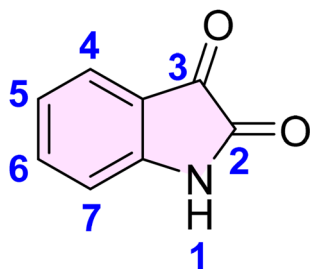


Fig. 3 Structure of isatin.

inhibit COX-2 and PI3K enzymes, key targets in inflammation and breast cancer. Isatin-hydrazones suppress Bcl-2, activate caspases, and induce ROS-mediated apoptosis in breast cancer cells. Triazole-tethered isatin-coumarin hybrids inhibit tubulin polymerization ( $IC_{50} \approx 1-5 \mu M$ ) and overcome multidrug resistance in prostate and breast cancers. Brominated isatin derivatives from marine organisms target CDK2, a kinase critical for cell cycle progression.<sup>20,21</sup>

Due to these remarkable and fantastic properties and conversions (Fig. 5), the generation of macrocyclic complexes with isatin or its derivatives is being explored more.<sup>22</sup>

### 3. Molecular targeting of isatin-based derivatives as anti-cancer agents

The apoptotic (programmed cell death) effects of isatin-based derivatives on several types of cancer cells make them

promising candidates for use as anticancer agents. The apoptotic (programmed cell death) effects of isatin-based derivatives on several types of cancer cells make them promising contenders for anticancer drug development. Drugs that inhibit CDK2, receptor tyrosine kinases, and histone deacetylases can be designed using the isatin scaffold. These drugs affect cell cycle progression, mitosis, and epigenetic control in tumor cells, as summarized in Fig. 6.

#### 3.1. Molecular mechanisms of isatin-induced apoptosis

**3.1.1. Kinase-mediated apoptosis.** Reducing tumor angiogenesis and metastasis, isatin-based derivatives inhibit vascular endothelial growth factor receptor 2 (VEGFR2) and epidermal growth factor receptor (EGFR). By inhibiting receptors for the MAPK and PI3K/AKT signaling pathways, which are frequently dysregulated in cancers, the compounds reduce the availability of survival signals for cancer cells.<sup>23</sup> This promotes cell survival and proliferation. Anticancer treatments can be more effective if specific pathways are targeted. Many malignancies have dysregulated signaling pathways that allow cells to survive and proliferate, such as RAS/RAF/MEK/ERK (MAPK) and PI3K/AKT/mTOR.<sup>24</sup>

Also, isatin-based derivatives have a significant impact on cell cycle regulation. By blocking the G1-S phase transition and consequently inhibiting cell growth, these compounds have shown promise as cyclin-dependent kinase 2 (CDK2) inhibitors. Moreover, these chemicals cause cell cycle arrest by inhibiting

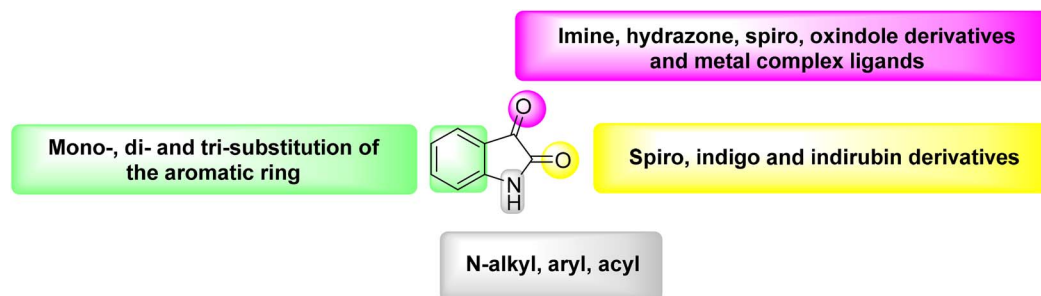


Fig. 4 Possible substitution on the isatin scaffold.<sup>19</sup>

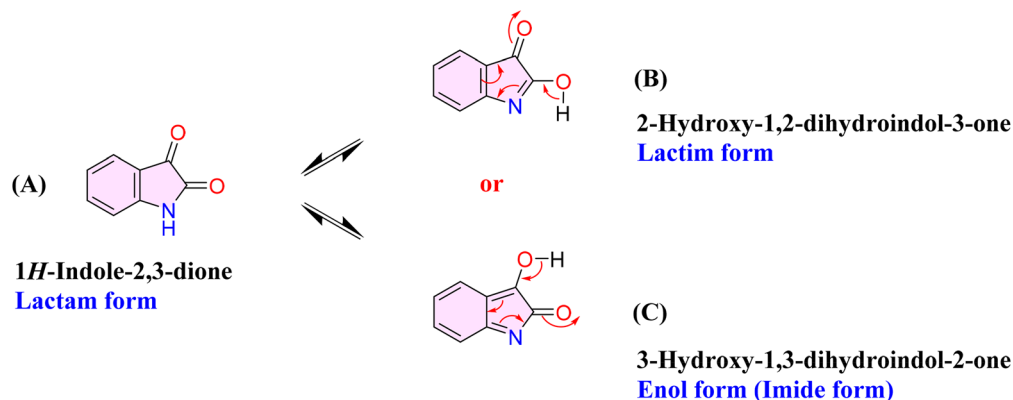


Fig. 5 Tautomerism in isatin. (A) Lactam form, (B) lactim form and (C) imide form.



histone deacetylase (HDAC), which in turn induces chromatin remodeling and suppresses oncogenic transcription.<sup>25</sup>

There have been other investigations into isatin-based compounds that target HER-2. On the other hand, they are known to suppress the RAS/RAF/MEK/ERK (MAPK) and PI3K/AKT/mTOR signaling pathways, which are involved in cancer cell survival and metastasis. Thus, blocking these pathways may slow cancer progression and improve therapy options.<sup>26</sup>

**3.1.2. Mitochondrial pathway activation.** Isatin derivatives effectively decrease the expression of anti-apoptotic Bcl-2 protein while maintaining Bax (pro-apoptotic) expression levels, significantly reducing the Bcl-2/Bax ratio. This altered ratio is a critical regulatory step that sets the threshold for apoptosis susceptibility in the mitochondrial pathway. The disruption of mitochondrial integrity represents one of the earliest events in isatin-induced apoptosis. Treatment with isatin markedly decreases the mitochondrial transmembrane potential in cancer cells, indicating mitochondrial dysfunction. This destabilization leads to elevated cytochrome c release into the cytosol, a universal feature of the apoptotic process.<sup>27–31</sup>

**3.1.3. Caspase cascade activation.** Upon cytochrome c release, isatin initiates the apoptotic program by triggering a caspase cascade. Isatin stimulates caspase-9 and caspase-3, according to studies. The dicarbonyl functionality is crucial to isatin's activity because it activates caspases. By interacting with the nucleophilic cysteine thiolate functionality and the electrophilic C-3 carbonyl carbon of isatin, this group forms a thiohemiketal that binds to the cysteine residue at the active site of activated caspases. In the next step, caspase-3 cleaves the ICAD inhibitor, which activates caspase-activated DNase (CAD) and causes DNA fragmentation within nuclear internucleosomes. The hallmarks of cell death, such as DNA fragmentation and chromatin condensation, have been seen in cancer cells treated with isatin.<sup>32,33</sup>

## 4. Isatin–hybrids as anti-cancer agents

Drug hybridization is a beneficial strategy in pharmaceutical development, as such compounds can enhance efficacy,

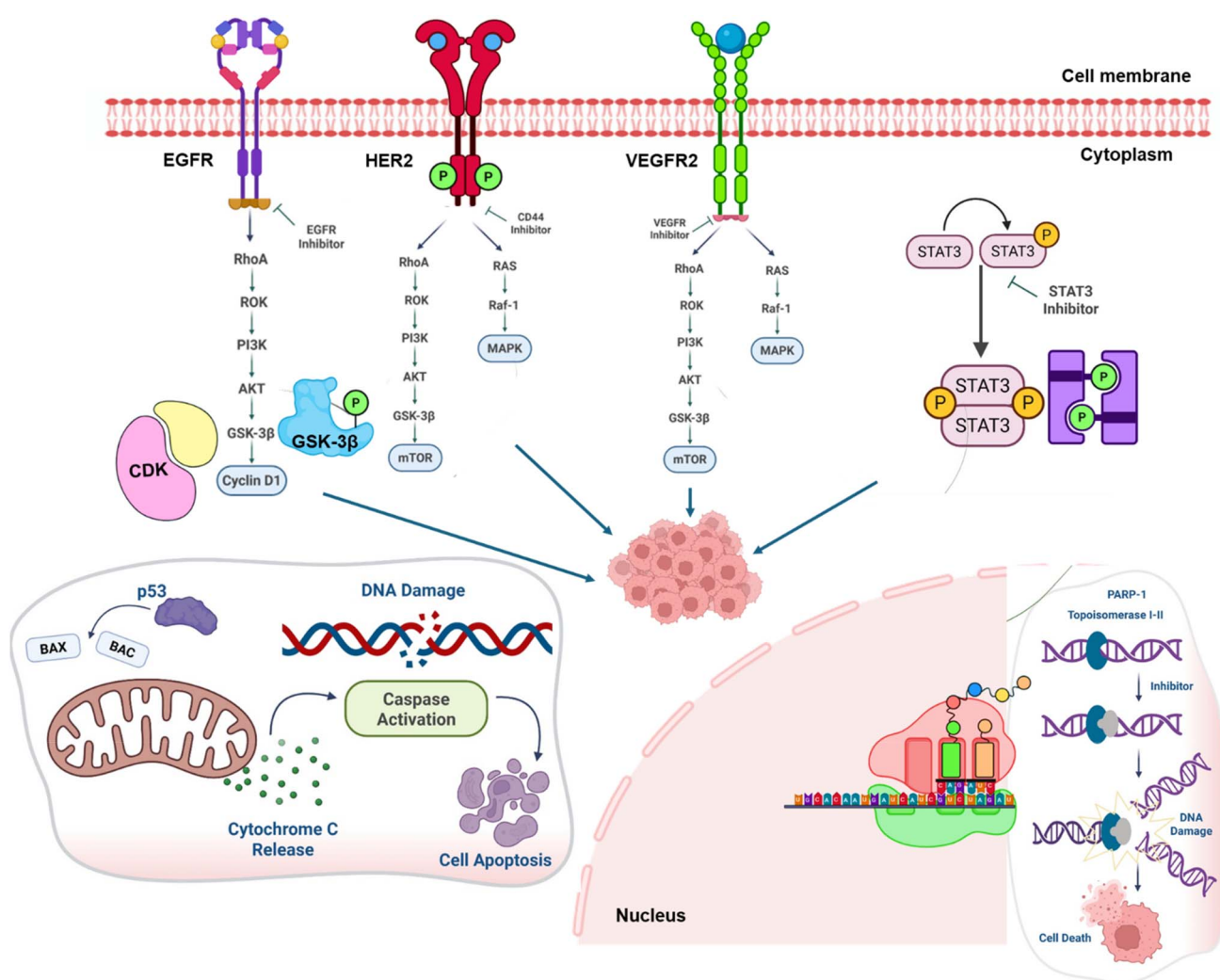


Fig. 6 The signaling therapeutic pathways of isatin-based derivatives as anti-cancer agents. This figure is partially generated by Biorender and reproduced from our previously published work.<sup>34</sup>



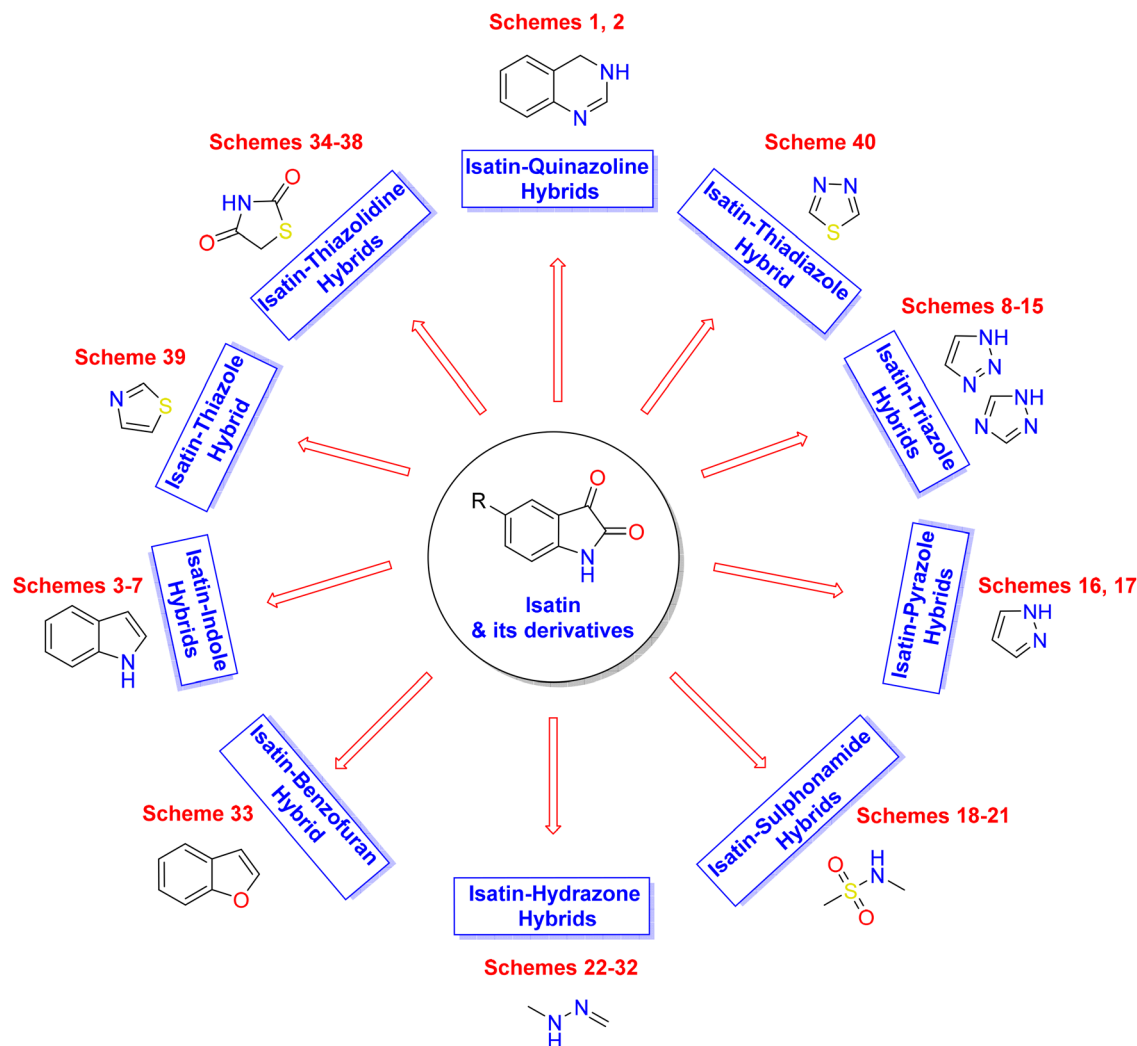
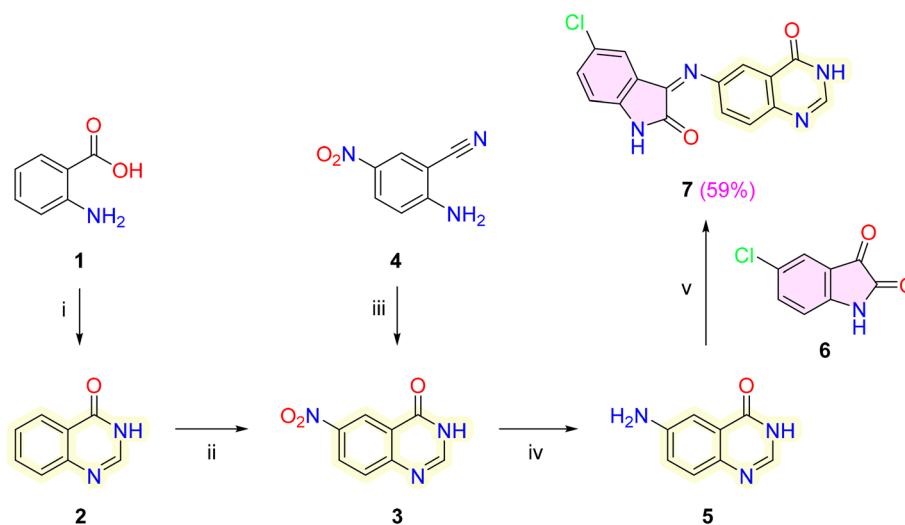


Fig. 7 Hybridization of isatin core with other anti-cancer pharmacophores.



**Scheme 1** Synthesis of isatin–quinazoline **7**. Reagents and conditions: (i)  $\text{HCONH}_2$ , reflux, 6 h; (ii)  $\text{HNO}_3$ ,  $\text{H}_2\text{SO}_4$ ,  $90^\circ\text{C}$ , 30 min; (iii)  $\text{HCOOH}$ ,  $\text{H}_2\text{SO}_4$ , reflux, 1 h; (iv)  $\text{Fe}$ ,  $\text{NH}_4\text{Cl}$ ,  $i\text{PrOH}$ , reflux, 1.5 h; (v)  $\text{EtOH}$ ,  $gl.$   $\text{AcOH}$  (cat.), reflux, 4–6 h.



improve target specificity, and combat resistance.<sup>35</sup> The isatin scaffold serves as a valuable model in the development of new anti-cancer drugs. By merging the isatin core with other anti-cancer pharmacophores (Fig. 7), it is possible to design hybrid molecules that may reduce side effects and help overcome drug resistance. These isatin-based hybrids represent promising candidates for novel cancer therapies.<sup>36</sup> This section displays recent advances in isatin-based hybrid compounds as potential anti-cancer agents. It includes isatin scaffold with quinazoline, indole, 1,2,3-triazole, 1,2,4-triazole, pyrazole, sulphonamide, hydrazone, benzofuran, thiazolidine, thiazole, and thiadiazole hybrids, respectively.

#### 4.1. Isatin–quinazoline hybrids

Kandeel *et al.* reported synthesizing indolinone-based derivatives as cytotoxic kinase inhibitors.<sup>37</sup> The synthetic routes employed to synthesize the target derivatives (**7** and **12**) are represented in Schemes 1 and 2, respectively. In Scheme 1, anthranilic acid **1** was heated with formamide to afford 4-quinazolinone **2**, which was nitrated with a nitrating mixture to give 6-nitroquinazolinone **3**. Also, 6-nitroquinazolinone **3** can be obtained through the cyclization of 2-amino-5-nitrobenzonitrile **4** with formic acid. Furthermore, 6-nitroquinazolinone **3** was then reduced using iron and ammonium chloride in isopropanol, producing 6-aminoquinazolinone **5**. Finally, 6-aminoquinazolinone **5** was allowed to react with 4-chloroisatin **6** in EtOH and in the presence of catalytic acetic acid under reflux to furnish the target 6-(indolyldona-amino)quinazolinone **7** (yield: 59%).

On the other hand, Scheme 2, synthesis was started *via* heating a mixture of anthranilamide **8** with *p*-nitrobenzaldehyde **9** and copper(ii) chloride in EtOH, which yielded the 2-(nitro-phenyl)quinazolinone **10**. The latter compound **10** was reduced with iron and hydrochloric acid afforded 2-(4-amino-phenyl)quinazolinone **11**. Finally, compound **11** was condensed with 4-chloroisatin **6** in EtOH and in the presence of catalytic

acetic acid under reflux for 6 h to furnish the target **12** (yield: 61%).

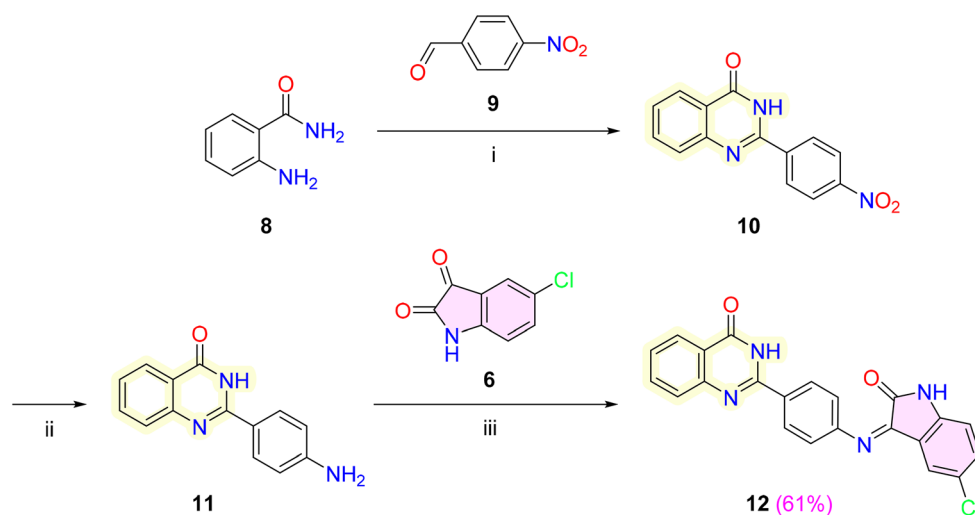
Compounds **7** and **12** were examined against two human tumor cancer cell lines (HepG-2 and MCF-7), using indirubin as the positive control. They displayed the highest cytotoxicity against the HepG2 ( $IC_{50} = 2.53$  and  $3.08 \mu\text{M}$ ) and MCF-7 ( $IC_{50} = 7.54$  and  $5.28 \mu\text{M}$ ) cell lines, respectively, compared to indirubin ( $IC_{50} = 6.92$  and  $6.12 \mu\text{M}$ ). Compound **7** demonstrated potent inhibition against VEGFR2 and CDK-2 ( $IC_{50} = 56.74$  and  $9.39 \text{ nM}$ ), respectively. Compound **7** was around five times more potent than indirubin when inhibiting CDK-2. On the other hand, compound **12** demonstrated potent inhibition of both EGFR and VEGFR-2 with  $IC_{50}$  values of  $14.31 \text{ nM}$  and  $32.65 \text{ nM}$ , respectively. Molecular docking studies supported the potential binding modes and interactions **7** with CDK-2 and **12** with VEGFR-2.<sup>37</sup>

#### 4.2. Isatin–indole hybrids

Al-Wabli *et al.* synthesized a new isatin–indole conjugate.<sup>38</sup> The synthesis started with the esterification of indole-2-carboxylic acid **13** to give the methyl ester **14**. Next, compound **14** was allowed to react with  $\text{N}_2\text{H}_4 \cdot \text{H}_2\text{O}$ , forming the acid hydrazide **15**. The target conjugate **17** was prepared by reacting the acid hydrazide **15** with isatin derivative **16** in EtOH-containing drops of acetic acid (yield: 54%), Scheme 3.

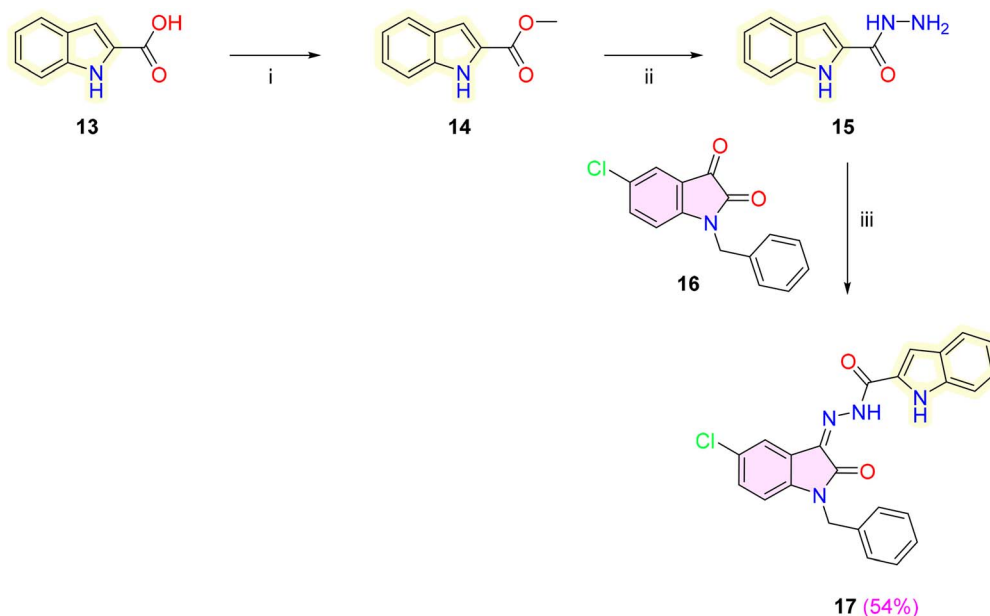
Using human breast (ZR-75), colon (HT-29), and lung (A-549) carcinoma cells, the antiproliferative efficacy of compound **17** was assessed. With  $IC_{50}$  values of  $0.74$ ,  $2.02$ , and  $0.76 \mu\text{M}$ , respectively, it exhibited the most potent anticancer activity when contrasted with the standard drug sunitinib, which had  $IC_{50}$  values of  $8.31$ ,  $10.14$ , and  $5.87 \mu\text{M}$ , respectively.<sup>38</sup>

A novel isatin–indole conjugate was synthesized by Eldehna *et al.*<sup>39</sup> The synthetic strategy used for the preparation of the target compound **23** was illustrated in Scheme 4. First, 1*H*-indole **18** was formylated *via* the Vilsmeier–Haack reaction to produce 1*H*-indole-3-carbaldehyde **19**, in which the CHO



Scheme 2 Synthesis of isatin–quinazoline hybrid **12**. Reagents and conditions: (i)  $\text{CuCl}_2 \cdot 2\text{H}_2\text{O}$ , EtOH, reflux, 16 h; (ii) Fe, HCl; (iii) EtOH, *gl.* AcOH (cat.) reflux, 6 h.





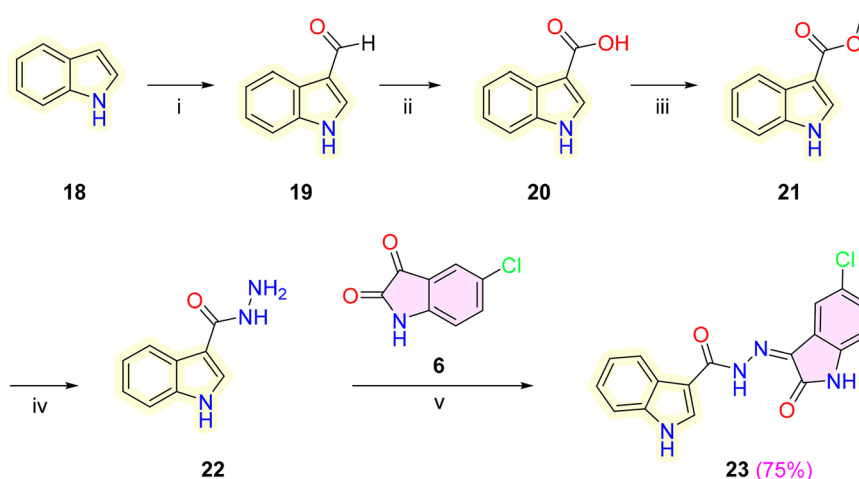
**Scheme 3** Synthesis of isatin–indole hybrid **17**. Reagents and conditions: (i) MeOH, H<sub>2</sub>SO<sub>4</sub> (cat.), reflux, 4 h; (ii) N<sub>2</sub>H<sub>4</sub>·H<sub>2</sub>O, MeOH, reflux, 2 h; (iii) EtOH, *gl. AcOH* (cat.), reflux, 4 h.

functionality was then oxidized by KMnO<sub>4</sub> in acetone to furnish 1*H*-indole-3-carboxylic acid **20**. Furthermore, the acid **20** was esterified through refluxing in dry methanol (MeOH) containing a catalytic dehydrating agent, H<sub>2</sub>SO<sub>4</sub>, to get carboxylate **21**, where the ester group reacted with N<sub>2</sub>H<sub>4</sub>·H<sub>2</sub>O in MeOH to produce the intermediate 1*H*-indole-3-carbohydrazide **22**. Finally, the intermediate **22** was condensed with 5-chloroisatin **6** in glacial acetic acid to give the targeted compound **23** (yield: 75%).

Compound **23** was examined against two human cell lines, colorectal cancer HT-29 and SW-620. In comparison to standard 5-FU, which had IC<sub>50</sub> values of 4600 and 1500 μM, respectively, it exhibited effective and selective cytotoxicity at 206 and 188 nM, respectively.<sup>39</sup>

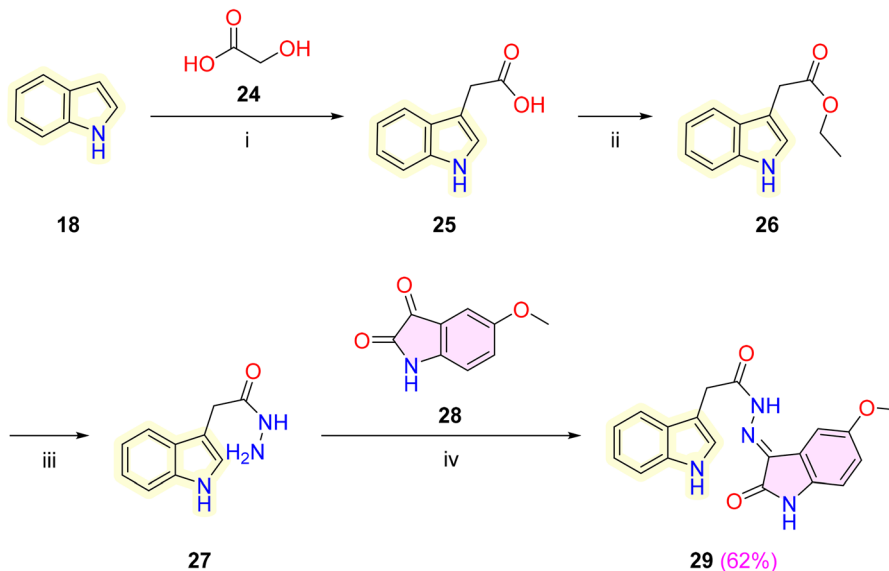
Based on the structural analysis of the reported CDK2 inhibitor, a new compound with 3-hydrazoneindolin-2-one scaffold **29** was developed by Al-Sanea *et al.*<sup>40</sup> The target compound **29** was prepared through a four-step reaction. First is the coupling dehydration of glycolic acid **24** with indole **18** to give indol-3-yl-acetic acid **25**, which is esterified with EtOH in an acidic medium to afford indole acetic acid ester **26**. The ester **26** reacted with N<sub>2</sub>H<sub>4</sub>·H<sub>2</sub>O to give hydrazide **27**. 5-Methoxyindole 2,3-dione **28** was condensed with **27** in EtOH and in the presence of catalytic glacial acetic acid under reflux for 4 h to produce the target **29** (yield: 62%), Scheme 5.

Metastatic cancer (MCF-7, MDA-MB-231) and ovarian cancer (NCI-ADR) cell lines were used to test compound **29**'s anti-proliferative activity. Compared to Dox, which inhibits the



**Scheme 4** Synthesis of isatin–indole hybrid **23**. Reagents and conditions: (i) DMF, POC<sub>3</sub>, reflux 8 h, (ii) KMnO<sub>4</sub>, acetone, stirring, r.t., 12 h, (iii) MeOH, H<sub>2</sub>SO<sub>4</sub> (cat.), reflux, 7 h, (iv) N<sub>2</sub>H<sub>4</sub>·H<sub>2</sub>O, MeOH, reflux, 4 h, (v) *gl. AcOH*, reflux, 5–7 h.





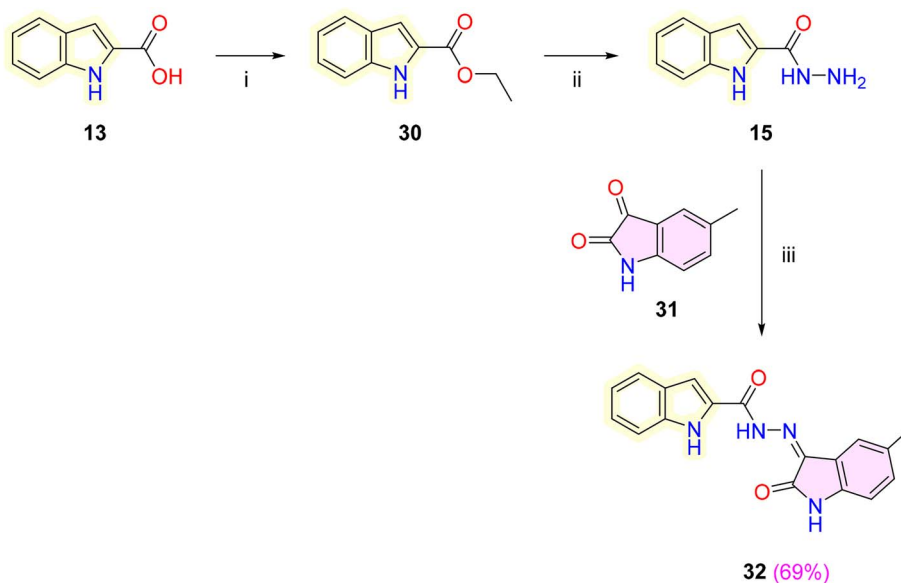
**Scheme 5** Synthesis of isatin–indole hybrid **29**. Reagents and conditions: (i) KOH, HCl, H<sub>2</sub>O; (ii) EtOH, H<sup>+</sup>, reflux, 10 h; (iii) N<sub>2</sub>H<sub>4</sub>·H<sub>2</sub>O, EtOH, reflux, 2 h; (iv) EtOH, *gl.* AcOH (cat.), reflux, 4 h.

proliferation of the breast cancer cell line MCF-7 with an IC<sub>50</sub> value of 6.81 ± 0.22 μM, compound **29**'s antiproliferative action is four times more potent, with a value of 1.15 ± 0.04 μM, while it was found to be equipotent with Dox in inhibiting the proliferation of the breast cancer cell line MDA-MB-231. Furthermore, it has demonstrated antiproliferative activity against ovarian cancer cells. NCI-ADR. **29** exhibited pronounced CDK2 inhibitory activity with IC<sub>50</sub> value of 6.32 μM. Additionally, docking studies have shown that it can interact with CDK2.<sup>40</sup>

Al-Warhi *et al.* reported synthesizing and biologically evaluating certain oxindole–indole conjugates as anticancer CDK

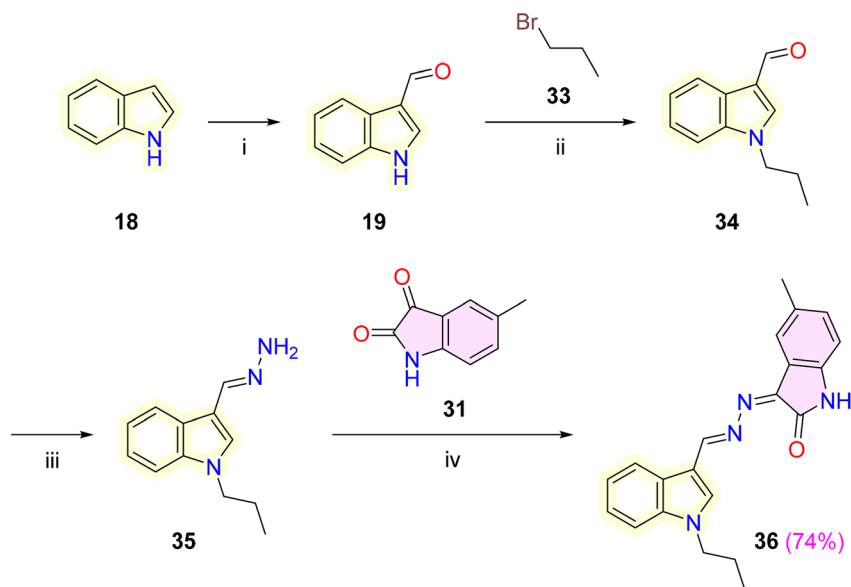
inhibitors.<sup>41</sup> The targeted conjugate **32** was prepared, as shown in Scheme 6. The first step involved the esterification of indole 2-carboxylic acid **13** through refluxing in EtOH in the presence of thionyl chloride to get ethyl-1*H*-indole-2-carboxylate **30**. Subsequently, the ester **30** was treated with N<sub>2</sub>H<sub>4</sub>·H<sub>2</sub>O in boiling EtOH to get intermediate **15**. Final target **32** was prepared by condensing hydrazide **15** with 5-methylisatin **31** in refluxing glacial acetic acid (yield: 69%).

The antiproliferative activity of compound **32** was evaluated *in vitro* against breast cancer MCF-7 and MDA-MB-231 cell lines. It showed more cytotoxic activity with (IC<sub>50</sub> = 0.39 μM) against MCF-7 than MDA-MB-231 cell line compared to the reference



**Scheme 6** Synthesis of isatin–indole hybrid **32**. Reagents and conditions: (i) EtOH, SOCl<sub>2</sub>, reflux, 6 h; (ii) N<sub>2</sub>H<sub>4</sub>·H<sub>2</sub>O, EtOH, reflux, 2 h; (iii) *gl.* AcOH, reflux, 4 h.



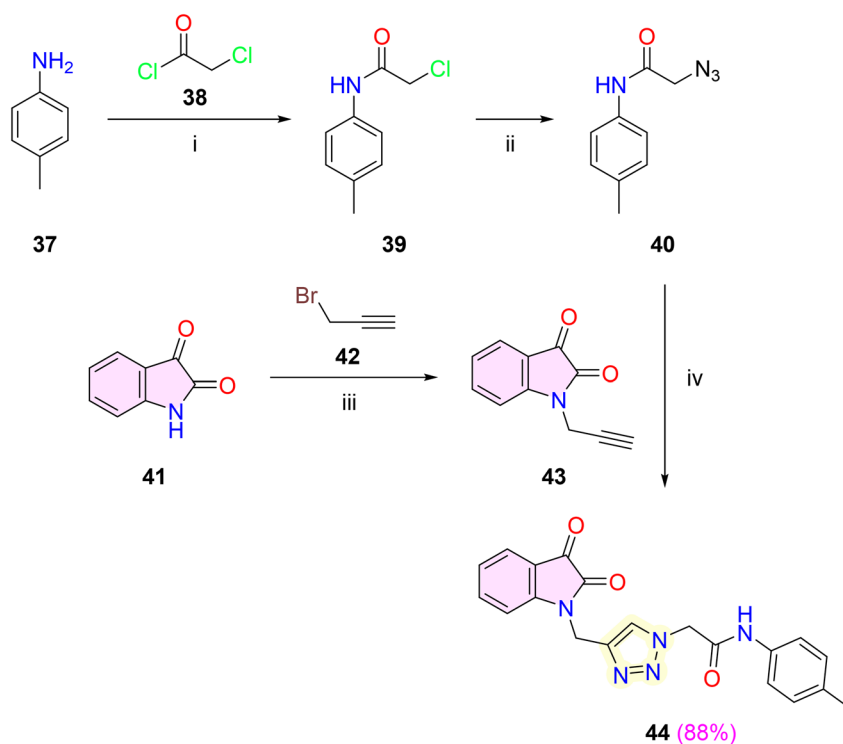


Scheme 7 Synthesis of isatin–indole hybrid **36**. Reagents and conditions: (i) DMF,  $\text{POCl}_3$ , reflux, 8 h; (ii) DMF, NaH, stirring, r.t., 24 h; (iii)  $\text{N}_2\text{H}_4 \cdot \text{H}_2\text{O}$ , EtOH, reflux, 2 h; (iv) EtOH, *gl.* AcOH (cat.), reflux, 3 h.

drug staurosporine with ( $\text{IC}_{50} = 6.81 \mu\text{M}$ ). It displayed good CDK4 inhibitory activity with an  $\text{IC}_{50}$  equal to  $1.26 \mu\text{M}$ . The ability of **32** to interact with CDK4 was also confirmed by a docking study.<sup>41</sup>

A novel set of *N*-alkylindole-isatin conjugates is developed by Al-Warhi *et al.* to prepare more efficient isatin-based anticancer

candidates.<sup>42</sup> Synthetic routes proposed to get the targeted conjugate **36** have been illustrated in Scheme 7. In the first step, Vilsmeier formylates indole **18** using DMF and phosphorus oxychloride to form *1H*-indole-3-carbaldehyde **19**. Then, aldehyde **19** underwent *N*-alkylation with propyl bromide **33** in DMF and sodium hydride base to get intermediate **34**. The latter



Scheme 8 Synthesis of isatin–1,2,3-triazole hybrid **44**. Reagents and conditions: (i) acetone, r.t., 2–3 h; (ii)  $\text{NaN}_3$ , DMF, r.t., 24 h; (iii)  $\text{K}_2\text{CO}_3$ , DMF, r.t., 24 h; (iv)  $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ , sodium ascorbate, *n*-BuOH/ $\text{H}_2\text{O}$  (1 : 1 v/v), DMF, 24 h.



compound **34** was condensed with  $N_2H_4 \cdot H_2O$  under reflux in EtOH to furnish hydrazide **35**. Finally, the key intermediate **35** was reacted with 5-methylisatin **31** in EtOH in the presence of catalytic glacial acetic acid to afford the target product **36** (yield: 74%).

A-549, MDA-MB-231, and HCT-116 cell lines were all significantly inhibited by compound **36**, with  $IC_{50}$  values of 7.3, 4.7, and 2.6  $\mu M$ , respectively, indicating its potent antiproliferative effect. With an  $IC_{50}$  value of  $2.6 \pm 0.17 \mu M$ , it was found to be the most powerful analog, surpassing the reference drug DOX ( $IC_{50} = 3.7 \pm 0.24 \mu M$ ). It exhibited good inhibitory action against CDK2 with  $IC_{50}$  values equal to  $0.85 \pm 0.03 \mu M$ . Results from docking experiments showed that **36** bound firmly to the active sites of CDK-2 and formed a stable complex.<sup>42</sup>

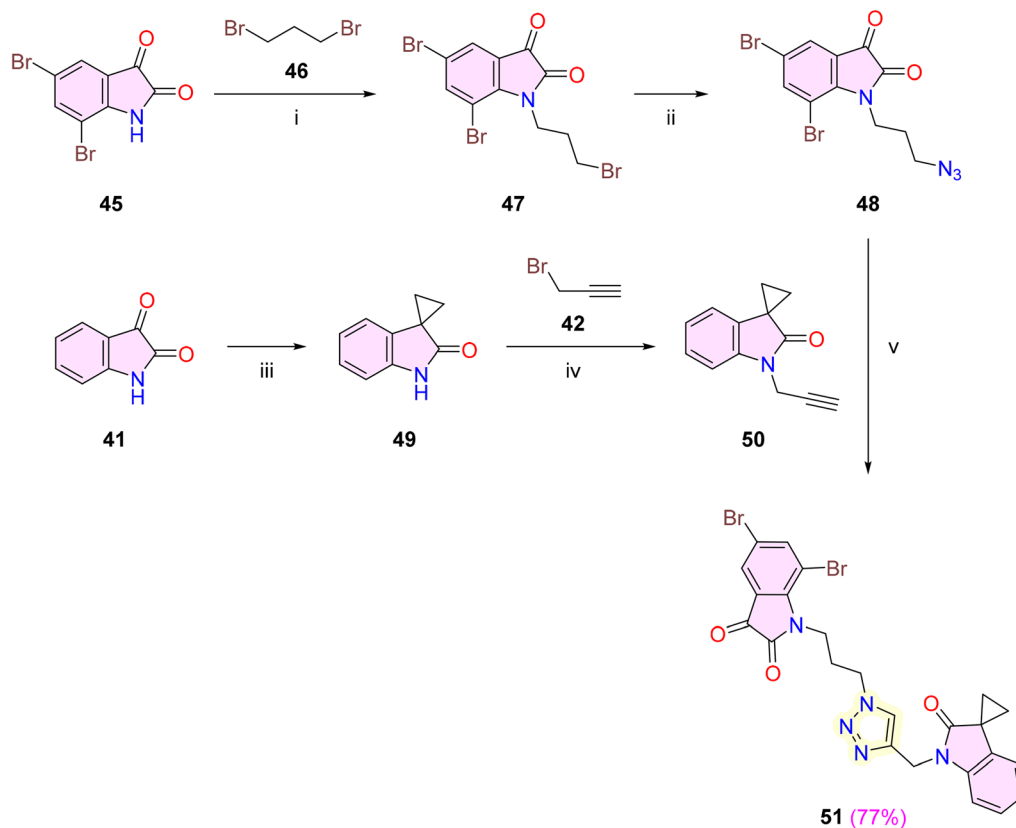
### 4.3. Isatin-1,2,3-triazole hybrids

Mohite *et al.* reported the synthesis of isatin-1,2,3-triazole hybrids as anticancer agents.<sup>43</sup> The synthetic strategy for preparing the target compound **44** is outlined in Scheme 8. The 2-azido-*N*-(*p*-tolyl)acetamide **40** was produced by the reaction of 4-methyl aniline **37** with chloroacetyl chloride **38** in acetone, followed by the reaction of compound **39** with sodium azide in DMF. The *N*-alkylation of isatin **41** with propargyl bromide **42** was performed using  $K_2CO_3$  to give the *N*-terminal alkyne **43**. Lastly, the synthesis of a triazole–isatin hybrid **44** by combining

azide **40** with the terminal alkyne **43** in a solution of  $CuSO_4 \cdot 5H_2O$  and *L*-sodium ascorbate in *n*-butanol/ $H_2O$  (1 : 1 v/v) at room temperature afforded the target **44** (yield: 88%).

Compound **44** demonstrated potent activity, displaying a submicromolar  $IC_{50}$  value against MCF-7 ( $IC_{50} = 0.67 \pm 0.12 \mu M$ ) and HCC1937 ( $IC_{50} = 0.53 \pm 0.11 \mu M$ ) cell lines. It was evaluated for its potential PARP-1 inhibitory activity, utilizing olaparib as a reference PARP-1 inhibitor. **44** as the most effective PARP-1 inhibitors showed  $IC_{50}$  value of  $13.65 \pm 1.42 nM$ . A molecular docking study revealed excellent binding strength in the active site vicinity of PARP-1.<sup>43</sup>

Preeti *et al.* have reported the synthesis and apoptotic assessment of triazole–isatin hybrids.<sup>44</sup> The synthetic methodology for the synthesis of the target hybrid **51** is outlined in Scheme 9. Base-promoted alkylation of 5,7-dibromoisatin **45** with dibromopropane **46** yielded **47**, which was followed by subsequent treatment with sodium azide, resulting in the desired azide **48**. The preparation of spirocyclopropyl oxindole **49** from isatin **41** was done by treating it with trimethylsulfonium iodide (TMSI) in the presence of base NaH in dry DMF through Domino Corey–Chaykovsky reaction. Subsequent treatment of **49** with propargyl bromide **42** resulted in the formation of alkyne **50**. By applying Cu-promoted azide–alkyne cycloaddition, the precursors **48** and **50** were used to synthesize the desired isatin hybrid **51** in DMSO in a microwave reactor at 80 °C for 10 min (yield: 77%).



**Scheme 9** Synthesis of isatin-1,2,3-triazole hybrid **51**. Reagents and conditions: (i)  $K_2CO_3$ , DMF, 100 °C, 10 min, MW; (ii)  $NaN_3$ , DMF, 120 °C, 20 min, MW; (iii) TMSI, NaH, DMSO, r.t.; (iv)  $K_2CO_3$ , DMF, 60 °C, 2 h; (v) DMSO, CuI, DIPEA, 80 °C, 10 min, MW.



Compound **51** was screened for its anticancer activity against the MDAMB-231 cell line. It displayed the best  $IC_{50}$  value of 0.73  $\mu\text{M}$  compared to tamoxifen citrate, with the best  $IC_{50}$  value of 12.88  $\mu\text{M}$ . Docking studies revealed a stable complex and strong binding affinity of **51** to the active sites of EGFR.<sup>44</sup>

Utilizing the molecular hybridization approach, Seliem *et al.* reported the synthesis of sets of triazole–isatin hybrids.<sup>45</sup> The synthetic protocol developed for the preparation of the targeted hybrid **56** is adopted in Scheme 10. 5-Methylisatin **31** was treated with propargyl bromide **42** in the presence of  $K_2CO_3$  in DMF at room temperature to obtain alkyne **52**. The synthesized alkyne **52** was coupled with 1-azido-2-methoxybenzene **53** using a click chemistry approach in the presence of  $CuSO_4 \cdot 5H_2O$  and sodium D-isoascorbate in *t*-butanol/water mixture under microwave irradiation for 2 h at 100 °C to furnish the desired triazole **54**. Finally, the reaction of triazole **54** with compound **55** in EtOH at room temperature for 2 h gave the desired isatin **56** (yield: 84%).

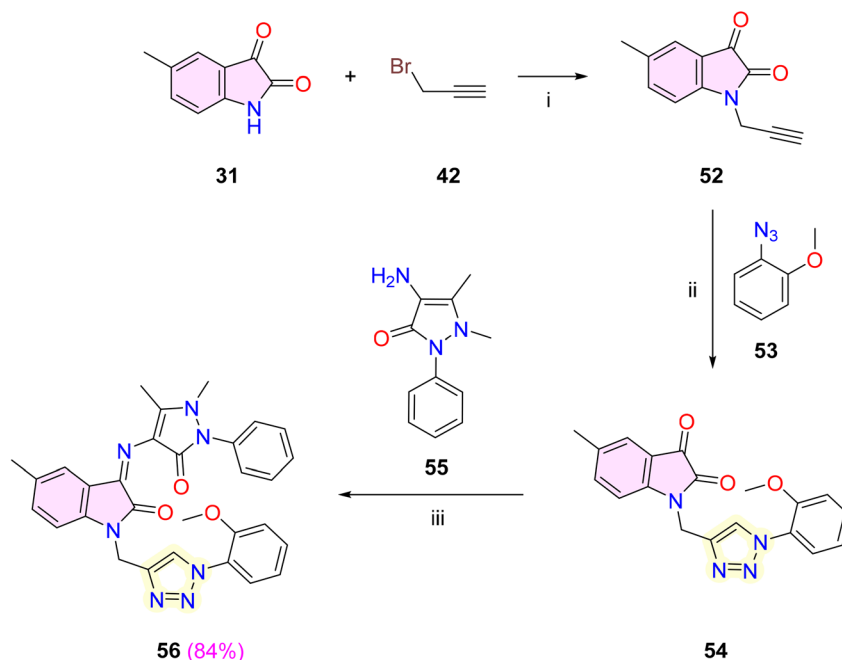
Compound **56** was screened for antiproliferation properties against breast cancer MCF7, HCT116 (colon), and PaCa2 (pancreatic) cell lines. It showed a higher potency of cytotoxicity against MCF7 ( $IC_{50} = 5.361 \mu\text{M}$ ) than the standard reference sunitinib ( $IC_{50} = 11.304 \mu\text{M}$ ). It also displayed higher antiproliferation properties against HCT116 ( $IC_{50} = 12.50 \mu\text{M}$ ) than 5-FU ( $IC_{50} = 20.43 \mu\text{M}$ ). It revealed high VEGFR2 inhibition properties (% inhibition = 77.6) comparable to that of sunitinib (% inhibition = 67.1).<sup>45</sup>

As a potential dual VEGFR2/STAT-3 inhibitor, Elsebaie *et al.* reported the synthesis of isatin-incorporated phenyl-1,2,3-triazole derivatives.<sup>46</sup> Scheme 11 shows the synthesis route of the target isatin **63**. First, 1-azido-4-methoxybenzene **57** was

allowed to react with ethyl acetoacetate **58** at 90 °C in diethylamine and dimethyl sulfoxide to afford triazole intermediate **59**. Then, the synthesis of carbohydrazide **60** was achieved by the reaction of compound **59** with  $N_2H_4 \cdot H_2O$  (99%) in absolute EtOH under reflux. Isatin **41** underwent an *N*-alkylation *via* reacting with benzyl bromide **61** in the presence of KI and  $K_2CO_3$  using acetonitrile to afford compound **62**. Target compound **63** has been synthesized by reacting hydrazide **60** with *N*-alkylated isatin **62** in absolute EtOH and catalytic glacial acetic acid under reflux (yield: 81%).

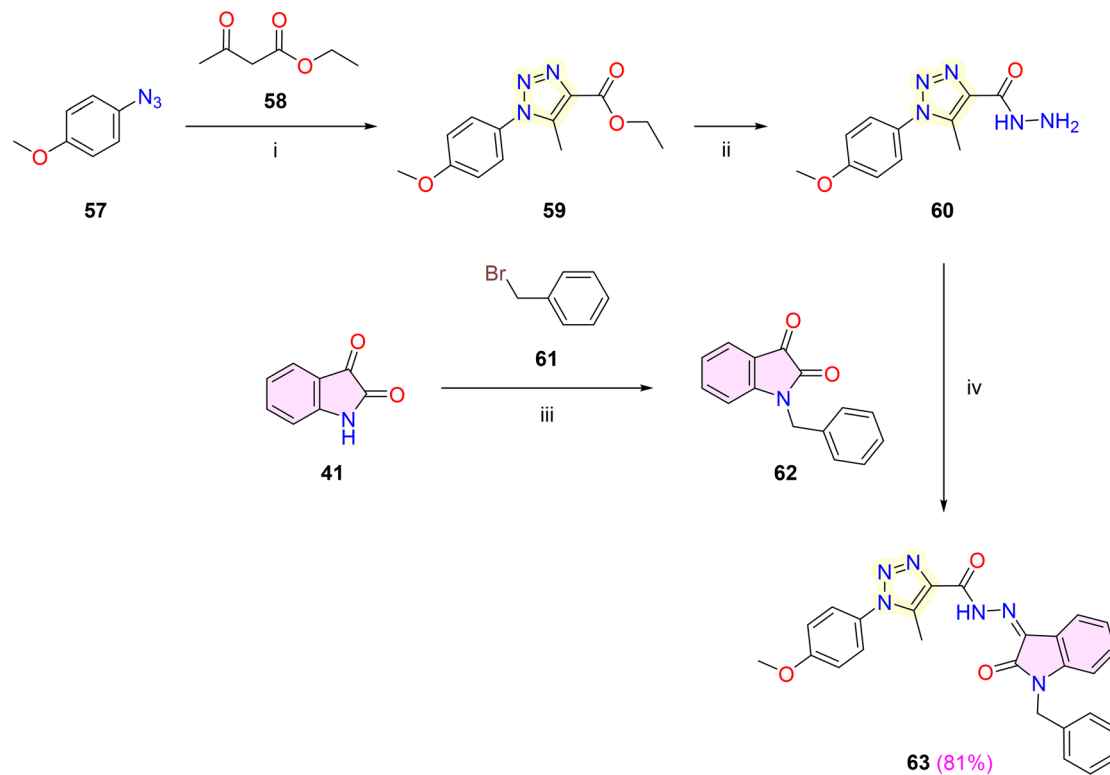
Prostate cancer (PC3) and pancreatic cancer (PANC1) cells were used to study compound **63**'s anti-proliferative activity. It showed more effective cytotoxicity against PANC1 ( $IC_{50} = 0.13 \mu\text{M}$ ) and PC3 ( $IC_{50} = 0.10 \mu\text{M}$ ) compared to DOX ( $IC_{50} = 0.45 \mu\text{M}$  and 0.24  $\mu\text{M}$ , respectively) and sunitinib ( $IC_{50} = 1.49 \mu\text{M}$  and 0.60  $\mu\text{M}$ , respectively). Its  $IC_{50}$  value of 26.3 nM showed an effective suppression of VEGFR2, in contrast to sunitinib's  $IC_{50}$  value of 30.7 nM. The STAT-3 inhibitory potential of compound **63** was also investigated. Its  $IC_{50}$  value of 5.63 nM demonstrated its efficacy in inhibiting STAT-3. Molecular docking analyses showed that the powerful **63** binds to the VEGFR2 and STAT-3 active sites in a significant way.<sup>46</sup>

A series of novel indole-2-one derivatives based on 1,2,3-triazole scaffolds were synthesized by Wang *et al.*<sup>47</sup> The synthetic route adopted to synthesize the target indole-2-one-1,2,3-triazole derivative **70** is depicted in Scheme 12. First, the preparation of 1-azido-4-methylbenzene **67** involved diazo-reaction and displacement reaction with sodium azide. Then, click chemistry *via* Cu(I)-catalyzed azide–alkyne-type cycloaddition between aryl-azide **67** and 4-ethynylbenzaldehyde **68** in the presence of sodium ascorbate and  $CuSO_4 \cdot 5H_2O$  as a catalyst in



Scheme 10 Synthesis of isatin-1,2,3-triazole hybrid **56**. Reagents and conditions: (i)  $K_2CO_3$ , DMF, r.t., 4 h; (ii) sodium D-isoascorbate,  $CuSO_4 \cdot 5H_2O$ , *t*-butanol/ $H_2O$ , MW, 100 °C, 2 h; (iii) EtOH, r.t., 2 h.

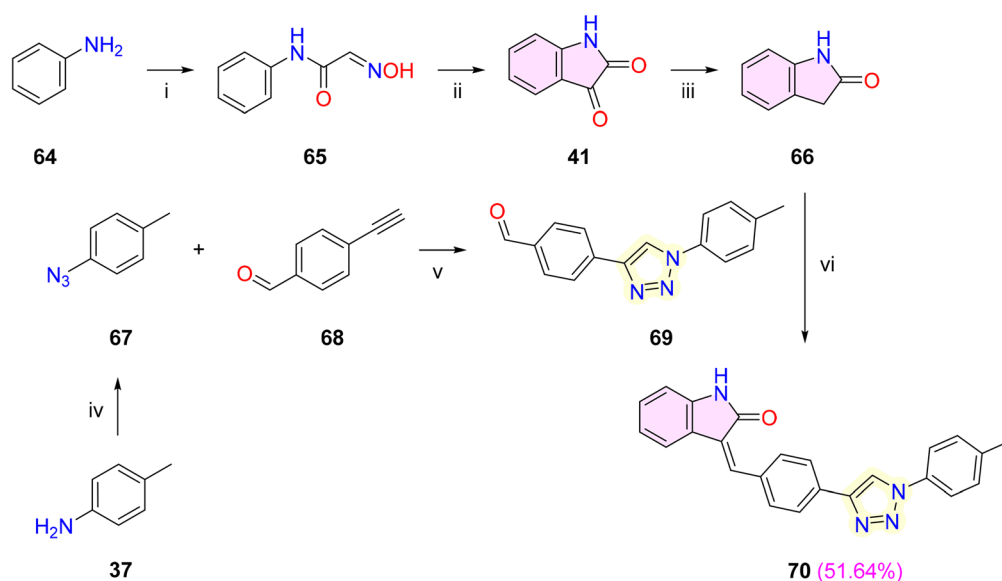




**Scheme 11** Synthesis of isatin-1,2,3-triazole hybrid **63**. Reagents and conditions: (i) Et<sub>2</sub>NH, DMSO, reflux, 6 h; (ii) N<sub>2</sub>H<sub>4</sub>·H<sub>2</sub>O, EtOH, reflux, 4 h; (iii) K<sub>2</sub>CO<sub>3</sub>, KI, MeCN, reflux, 6 h; (iv) EtOH, *gl. AcOH* (cat.), reflux, 12 h.

DMF and water mixture afforded a 1,2,3-triazole **69**. Isatin **41** was prepared from aniline **64** *via* Sandmeyer's method. Moreover, compound **66** was prepared from isatin **41** by reacting with N<sub>2</sub>H<sub>4</sub>·H<sub>2</sub>O. Finally, the preparation of the title compound **70**

was accomplished by employing Claisen–Schmidt condensation between indolin-2-one **66** and 1,2,3-triazole aromatic aldehyde **69** with a catalytic amount of piperidine as a base (yield: 51.64%).



**Scheme 12** Synthesis of isatin-1,2,3-triazole hybrid **70**. Reagents and conditions: (i) chloral hydrate, Na<sub>2</sub>SO<sub>4</sub>, NH<sub>2</sub>OH·HCL, HCl, H<sub>2</sub>O, 85 °C, 3 h; (ii) conc. H<sub>2</sub>SO<sub>4</sub>, 60 °C, 0.5 h, 90 °C, 1.5 h; (iii) N<sub>2</sub>H<sub>4</sub>·H<sub>2</sub>O, EtOH, H<sub>2</sub>O, 100 °C, 10 h; (iv) NaNO<sub>2</sub>, HCl, NaN<sub>3</sub>, DCM, H<sub>2</sub>O, 0–5 °C, 3–5 h; (v) CuSO<sub>4</sub>·5H<sub>2</sub>O, ascorbic acid, KI, DMF, H<sub>2</sub>O, 50 °C, 6–10 h; (vi) EtOH, piperidine, 80 °C, 4–8 h.



Human colon cancer (HT-29), gastric cancer (MKN-45), and umbilical vein endothelial cells (HUVECs) cell lines were used to test compound 70's antiproliferative activities. In comparison to the positive control, sunitinib, which had  $IC_{50}$  values of 10.34 and 9.25  $\mu\text{M}$ , respectively, it effectively inhibited cell viability for HT-29 and MKN-45 cells, with  $IC_{50}$  values of 1.61 and 1.92  $\mu\text{M}$ , respectively. Furthermore, it had lower toxicity to HUVECs than of sunitinib. It exhibited excellent inhibitory activity against VEGFR2 with an  $IC_{50}$  value of 26.3 nM compared to sunitinib with an  $IC_{50}$  value of 83.2 nM. The results of docking experiments and molecular dynamics simulations showed that 70 bound firmly to the active sites of VEGFR2, forming a stable complex.<sup>47</sup>

Nazari *et al.* reported the synthesis of a distinctive family of isatin derivatives. The synthesis of the most active compound 74 is depicted in Scheme 13. First, the nucleophilic reaction of isatin 41 and propargyl bromide 42 in DMF in the presence of anhydrous  $K_2CO_3$  gave *N*-propargyl isatin 71. Second, Cu-catalyzed click reaction of 71 and azide 43 under ultrasonic irradiation in *t*-BuOH- $H_2O$  for 1 h was used to give the target triazole 72. Finally, the 1,2,3-triazol-linked oxindole-thiosemicarbazone conjugate 74 was prepared by the reaction of 72 and thiosemicarbazide 73 in isopropyl alcohol under ultrasonic irradiation (yield: 80%).<sup>48</sup>

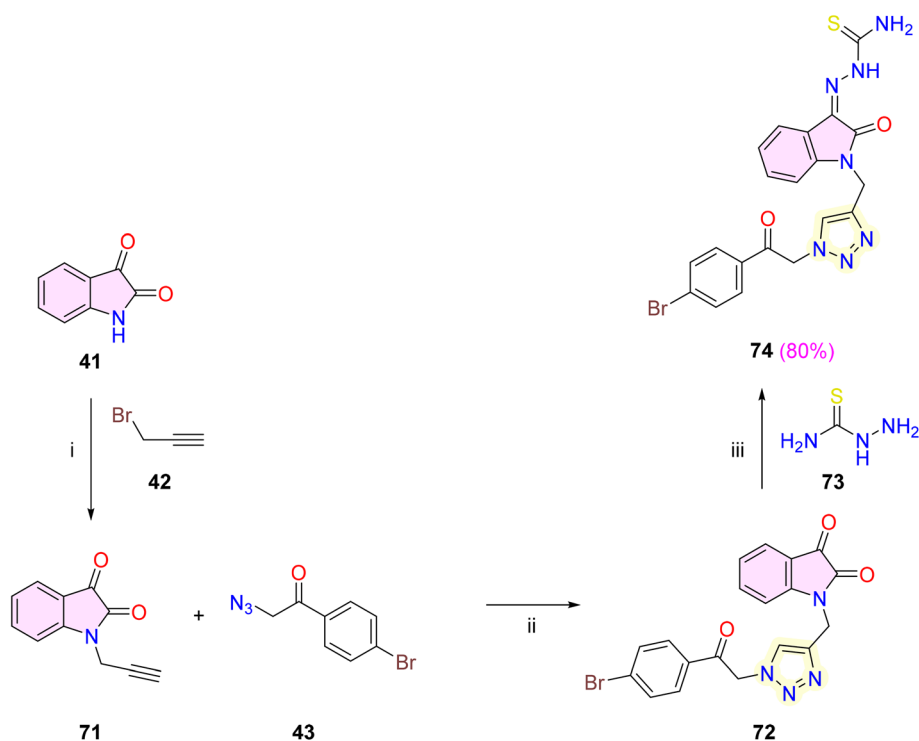
Compound 74 showed encouraging cytotoxicity against a variety of cell types, including A375, MDA-MB-231, PC3, and LNCaP. In terms of cytotoxic activity, it was most effective against the A375, MDA-MB-231, PC3, and LNCaP cell lines, with  $IC_{50}$  values of 25.91  $\mu\text{M}$ , 18.42  $\mu\text{M}$ , 15.32  $\mu\text{M}$ , and 29.23  $\mu\text{M}$ ,

respectively, compared to etoposide ( $IC_{50}$  = 24.46, 31.02, 30, and 31.21  $\mu\text{M}$ ).<sup>48</sup>

#### 4.4. Isatin-1,2,4-triazole hybrids

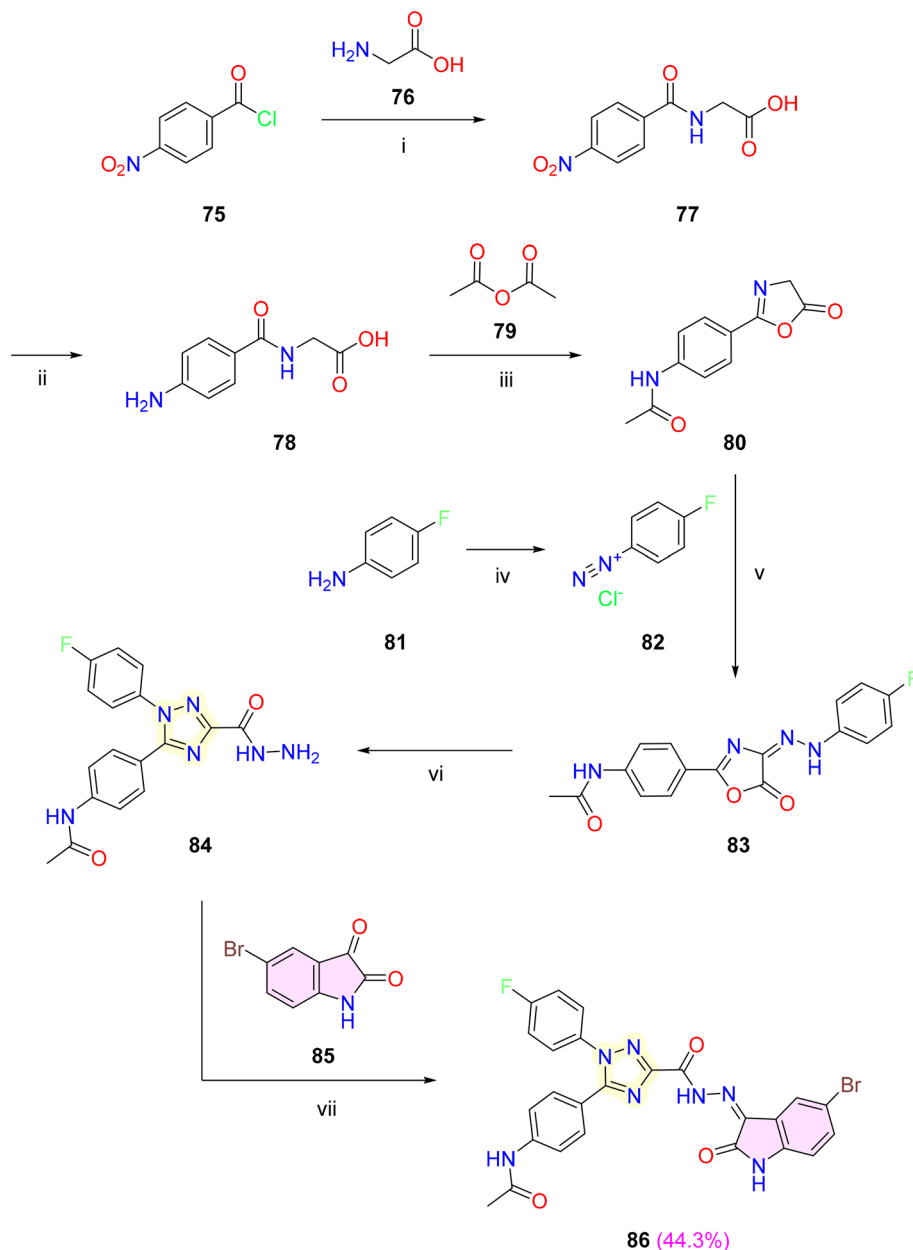
Elsawi *et al.* developed 1,2,4-triazole-tethered indolinones as new cancer-fighting small molecules targeting VEGFR2.<sup>49</sup> The preparation of the targeted hybrid 86 is illustrated in Scheme 14. 4-Aminohippuric acid 78 was synthesized by acylating the amino group of glycine amino acid 76 using *p*-nitrobenzoyl chloride 75 in an aqueous NaOH solution. Subsequently, the nitro group was reduced to the required amino group using Pd/C. To obtain compound 80, 4-aminohippuric acid 78 was heated with acetic anhydride 79, resulting in the acylation of two distinct functional groups. The active methylene of compound 80 was then coupled through the Kuskov-like reaction with freshly prepared diazonium salt 82 derived from 4-fluoroaniline 81 using sodium acetate salt to give hydrazone linker-tethered compound 83. Next, the azalactone ring of compound 83 was opened and underwent Sawdey rearrangement *via* refluxing in EtOH with  $N_2H_4 \cdot H_2O$ , ultimately forming hydrazide 84. Finally, hydrazide 84 underwent condensation with 5-bromoisatin 85 under reflux in absolute EtOH in the presence of glacial acetic acid as a catalyst to furnish hybrid 86 (yield: 44.3%).

Two cell lines, PANC1 and HepG2, were used to evaluate compound 86. In comparison to the reference medication DOX, which had  $IC_{50}$  values of 0.19 and 0.43  $\mu\text{M}$ , respectively, it demonstrated cytotoxic activity with an  $IC_{50}$  value of 1.16 and 0.73  $\mu\text{M}$ . With  $IC_{50}$  values of  $8.35 \pm 0.62 \mu\text{M}$ , it exhibited



Scheme 13 Synthesis of isatin-1,2,3-triazole hybrid 74. Reagents and conditions: (i)  $K_2CO_3$ , DMF, r.t., 3 h; (ii)  $CuSO_4 \cdot 5H_2O$ , sodium ascorbate, *t*-BuOH,  $H_2O$ , r.t., sonication, 1 h; (iii) *i*PrOH, 65  $^{\circ}C$ , sonication, 1 h.





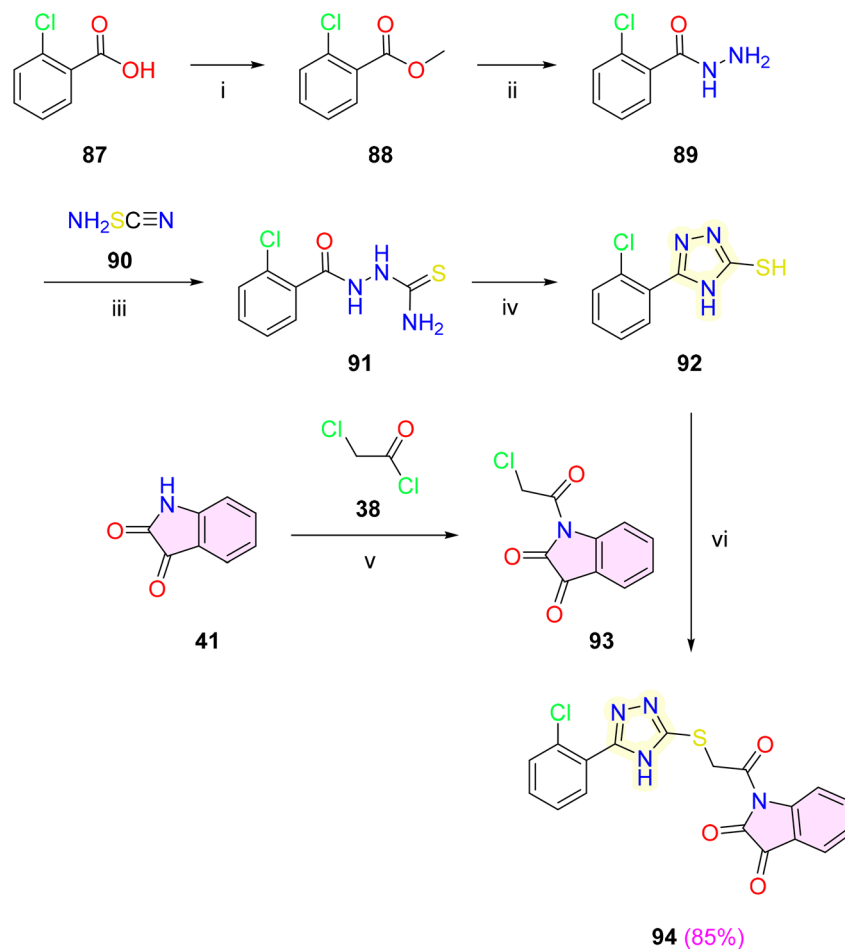
**Scheme 14** Synthesis of isatin-1,2,4-triazole hybrid **86**. Reagents and conditions: (i) NaOH (aq.), r.t., 1 h; (ii) MeOH, Pd/C, r.t., 3 h; (iii) heating, 75 °C, 40 min; (iv) HCl, NaNO<sub>2</sub>, 0–5 °C, 20 min; (v) AcONa, 0–10 °C, 3 h; (vi) EtOH, N<sub>2</sub>H<sub>4</sub>·H<sub>2</sub>O, reflux, 1 h; (vii) EtOH, AcOH, reflux, 2 h.

minimal toxicity to normal vero cells as well. In contrast to Sorafenib, which exhibited weak VEGFR2 inhibitory action, it exhibited robust activity, with an IC<sub>50</sub> value of 16.3 nM. The most potent inhibitor of VEGFR2 thus far, **86**, was simulated using molecular docking, and the results showed a robust binding to the essential amino acid residues of the VEGFR2 ATP binding site.<sup>49</sup>

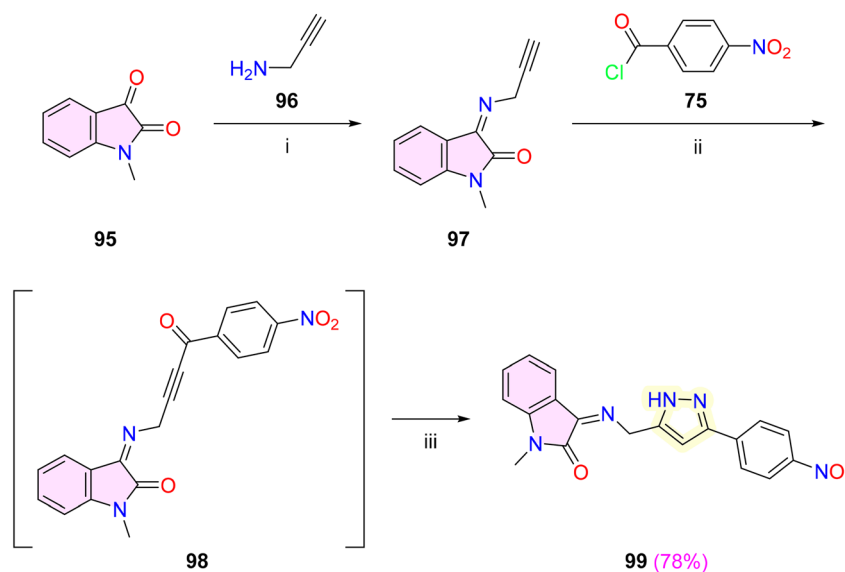
Utilizing a hybrid pharmacophore approach, Rasgania *et al.* reported the synthesis of triazole-functionalized isatin hybrids with potent antiproliferative activity.<sup>50</sup> The synthetic procedures adopted for the synthesis of target **94** is outlined in Scheme 15. First, reactive chloroacetyl isatin **93** was obtained by refluxing

isatin **41** with chloroacetyl chloride **38**. Secondly, the triazole derivative **92** was synthesized by a four-step reaction starting with the esterification of *o*-chlorobenzoic acid **87** with H<sub>2</sub>SO<sub>4</sub> in MeOH. Subsequently, **88** reacted with N<sub>2</sub>H<sub>4</sub>·H<sub>2</sub>O to generate **89**. Compound **91** was obtained by heating **89** with ammonium thiocyanate **90** in the presence of conc. HCl as a catalyst. Finally, triazole **92** was efficiently synthesized by refluxing compound **91** with sodium hydroxide in H<sub>2</sub>O.<sup>51</sup> The target product **94** has been synthesized by condensation of chloroacetyl isatin **93** and 5-(2-chlorophenyl)-4*H*-1,2,4-triazole-3-thiol **92** in EtOH under reflux and in the presence of sodium carbonate. The nucleophilic





**Scheme 15** Synthesis of isatin-1,2,4-triazole hybrid **94**. Reagents and conditions: (i) MeOH, H<sub>2</sub>SO<sub>4</sub>, reflux, 8 h; (ii) N<sub>2</sub>H<sub>4</sub>·H<sub>2</sub>O, MeOH, r.t., 4 h; (iii) EtOH, conc. HCl, reflux, 6 h; (iv) NaOH, H<sub>2</sub>O, reflux, 4 h; (v) reflux, 140 °C, 5 h, r.t., overnight; (vi) EtOH, K<sub>2</sub>CO<sub>3</sub>, reflux, 4 h.



**Scheme 16** Synthesis of isatin-pyrazole hybrid **99**. Reagents and conditions: (i) MeOH, 60 °C, 9 h; (ii) PdCl<sub>2</sub>(PPh<sub>3</sub>)<sub>2</sub>, CuI, sodium laurylsulfate, K<sub>2</sub>CO<sub>3</sub>, water, 65 °C, 7 h; (iii) N<sub>2</sub>H<sub>4</sub>·H<sub>2</sub>O, 65 °C, 12 h.



attack of the thiol of the triazole moiety on the carbonyl carbon of chloroacetyl isatin leads to the desired novel **94** (yield: 85%).

Compound **94** was screened for its anticancer activity against the MDAMB-231 and MCF-7 cell lines. It has shown the inhibition of MDAMB-231 and MCF-7 with  $GI_{50}$  values of 0.003 and  $2.00 \times 10^{-4}$ , respectively, compared to adriamycin with  $GI_{50}$  values of  $2.00 \times 10^{-7}$  and  $2.00 \times 10^{-8}$ . Molecular docking studies supported the potential binding modes and interactions of compound **94** with the VEGFR-2 active site.<sup>50</sup>

#### 4.5. Isatin-pyrazole hybrids

Shreedhar Reddy *et al.* developed a one-pot synthesis of isatin-pyrazole hybrids as VEGFR2 inhibitors.<sup>52</sup> The synthesis of targeted isatin-pyrazole hybrid **99** was achieved in two main steps, Scheme 16. The first step involved the condensation reaction between 1-methylisatin **95** and propargyl amine **96** in MeOH at 60 °C for 9 h. Later, the acyl-Sonogashira coupling of 1-methyl-3-(prop-2-yn-1-ylimino)isatin **97** with 4-nitrobenzoyl chloride **75** in the presence of sodium lauryl sulphate and  $K_2CO_3$  in water at 65 °C for 7 h gave the corresponding *in situ*  $\alpha,\beta$ -unsaturated ynone **98**, that subsequently treated with  $N_2H_4 \cdot H_2O$  at 65 °C for 12 h to provide the desired product **99** (yield: 78%).

The synthesized compound **99** was evaluated for its potential to inhibit the proliferation of TNBC cell lines MDA-MB-231 and MDA-MB-468. It showed the most potent cytotoxicity with  $IC_{50}$  values of  $10.24 \pm 1.27$  and  $8.23 \pm 1.87$   $\mu M$  against MDA-MB-468 and MDA-MB-231 cancer cells, respectively, compared to both TAM and 5-fluorouracil with  $IC_{50}$  values of 15.29  $\mu M$  and  $12.4 \pm 1.3$   $\mu M$  against MDA-MB-468, respectively, and 23.05  $\mu M$  and  $10.5 \pm 1.2$   $\mu M$  against MDA-MB-231 cancer cells, respectively.

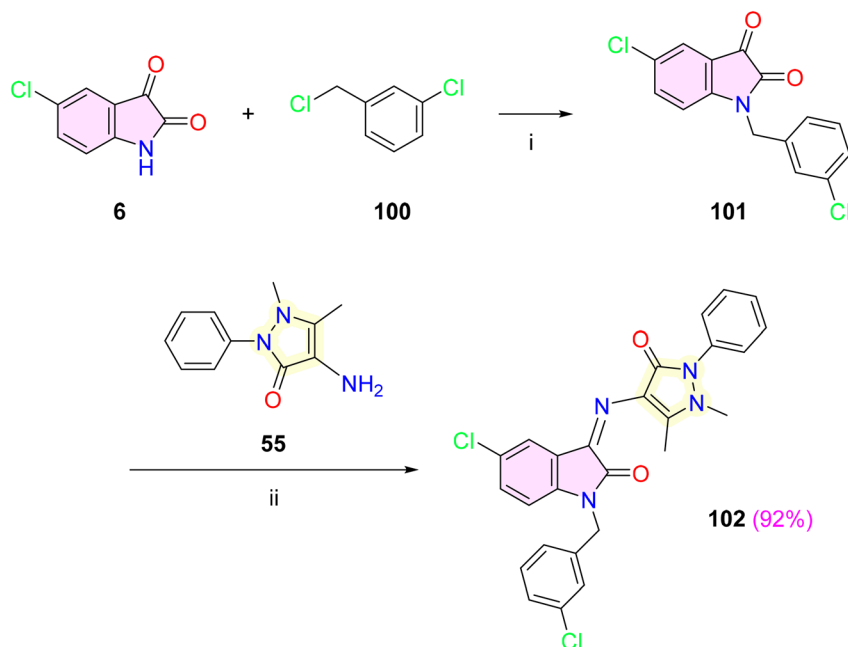
Docking studies revealed a stable complex and strong binding affinity of **99** to the active sites of EGFR.<sup>52</sup>

Emami *et al.* reported the preparation of novel isatin-pyrazole hybrids as a new class of antiproliferative agents.<sup>53</sup> The synthetic route of target compound **102** is described in Scheme 17. The preparation was achieved by the reaction of 5-chloroisatin **6** with  $K_2CO_3$  as a base in acetonitrile, followed by *N*-benzylation with 3-chlorobenzyl chloride **100** at 80 °C, which afforded the intermediate **101**. Then, condensation of **101** with 4-aminoantipyrine (ampyrone) **55** in absolute EtOH in the presence of a catalytic amount of glacial acetic acid under reflux for 24 h gave the desired product **102** (yield: 92%).

In comparison to cisplatin, which served as a positive control, compound **102** exhibited superior activity against MCF-7, A549, and SCO3, with  $IC_{50}$  values of 5.12, 25.5, and 12.9  $\mu M$ , respectively. Evidence from docking and MD simulations suggests that **102** binds most strongly to the VEGFR and JNK3 MAP kinase receptors.<sup>53</sup>

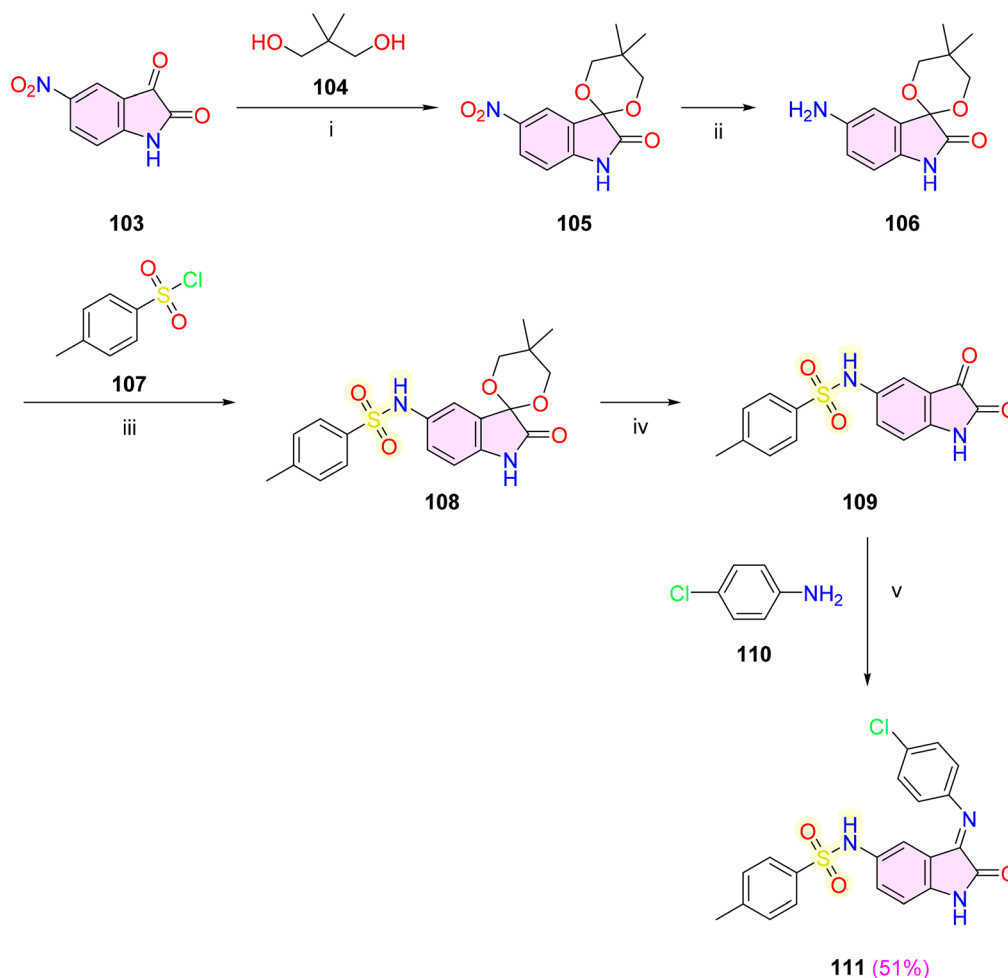
#### 4.6. Isatin-sulphonamide hybrids

As potential anti-cancer agents, Demirel *et al.* reported the synthesis of novel sulfonamide derivatives of isatin Schiff bases.<sup>54</sup> The synthesis of target compound **111** is shown in Scheme 18. The starting material, 5-nitroisatin **103** was treated with 2,2-dimethylpropane-1,3 diol **104** with catalytic PTSA. Then, the nitro group in compound **105** was reduced by using 1 atm  $H_2/Pd-C$  (10%) in MeOH to yield the amine **106**. The resulting amine **106** was allowed to react with *p*-toluene sulphonyl chloride **107** in DCM in the presence of pyridine to afford sulfonamide **108**. Finally, after deprotection of the third position with a mixture of glacial acetic acid and HCl, Schiff



Scheme 17 Synthesis of isatin-pyrazole hybrid **102**. Reagents and conditions: (i)  $K_2CO_3$ , TBAB, MeCN, reflux, 24 h; (ii) EtOH, AcOH (cat.), 50–60 °C, 24 h.





**Scheme 18** Synthesis of isatin-sulphonamide hybrid **111**. Reagents and conditions: (i) PTSA, cyclohexane, reflux, 24 h; (ii) Pd/C, H<sub>2</sub>, MeOH, r.t., 24 h; (iii) pyridine, DCM, r.t., 24 h; (iv) *gl.* AcOH, HCl, 30 °C, overnight; (v) PTSA, MeOH, 80 °C, 8 h.

base of sulfonamide **111** was obtained by reaction of sulfonamide **109** with 4-chloroaniline **110** in MeOH with catalytic PTSA (yield: 51%).

The novel synthesized compound **111** was investigated *in vitro* to determine its cytotoxicity against four cancer cell lines (PC-3, HepG2, SH-SY5Y, and A549) by using an MTT assay. HepG2 cell were more sensitive to cytotoxic activity among the other studied cell lines. **111** induced the potential inhibition of cellular proliferation activity against HepG2 cells with IC<sub>50</sub> value of 37.81 μM, which was more potent than a standard drug, DOX with IC<sub>50</sub> value of 51.15 μM. A selectivity index of **111** was found to be 8.57, so it might be safe for treatment.<sup>54</sup>

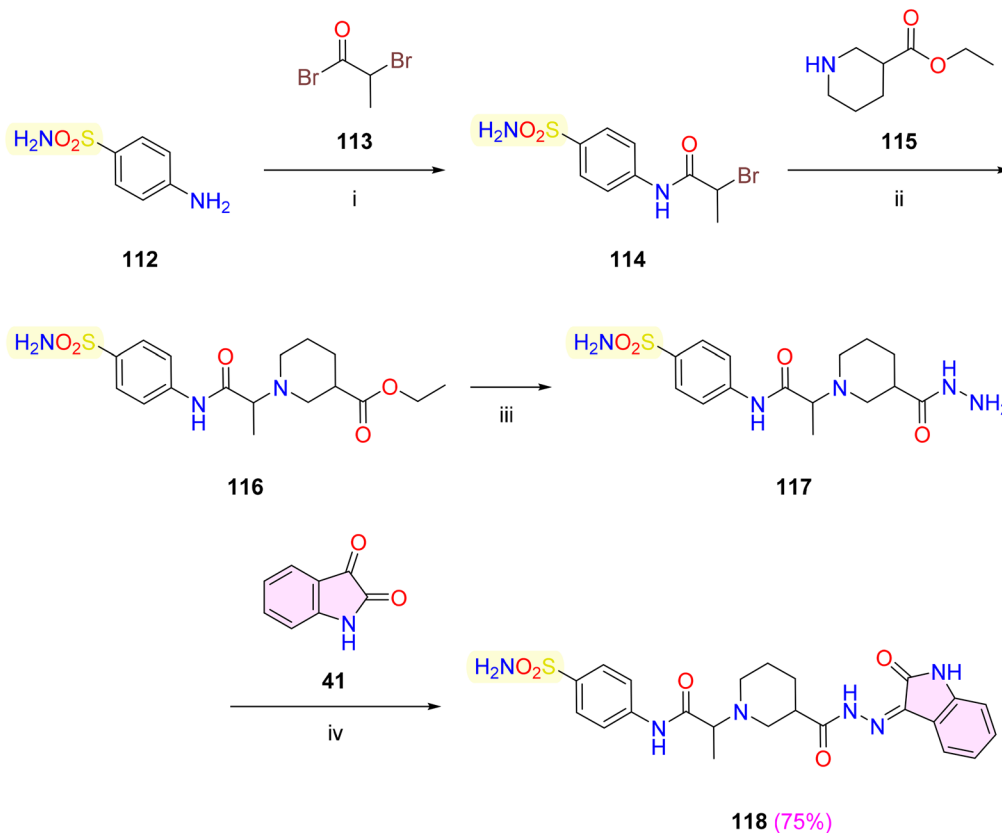
Saied *et al.* reported the synthesis and biological assessment of a series of novel indolinone-based benzenesulfonamides.<sup>55</sup> The synthesis of the target isatin-based benzenesulfonamide **118** is described in Scheme 19. The synthesis started with acetylating 4-aminobenzenesulfonamide **112** with 2-bromopropionyl bromide **113** in dioxane and TEA, which afforded compound **114**. Then, the produced amide **114** was alkylated with ethyl nipecotate **115** in refluxing acetone with dry K<sub>2</sub>CO<sub>3</sub> and catalytic KI, which afforded compound **116**. Moreover,

hydrazinolysis of **116** under reflux with N<sub>2</sub>H<sub>4</sub>·H<sub>2</sub>O in EtOH afforded hydrazide; compound **117**. Finally, hydrazide **117** was condensed with isatin **41** in EtOH and glacial acetic acid to afford the target compound **118** (yield: 75%).

The *in vitro* antiproliferative effect of compound **118** against the MDA-MB-231 and MCF-7 breast cancer cell lines was investigated. When tested against MDA-MB-231 cell lines, it showed a more substantial growth inhibitory effect with an IC<sub>50</sub> value of 4.083 μM, compared to the standard drug (5-FU; IC<sub>50</sub> = 8.704 μM). When tested against MCF-7 cell lines, it showed an IC<sub>50</sub> value of 9.997 μM, which was similar to the standard drug (5-FU; IC<sub>50</sub> = 5.167 μM). It seems to be the most effective VEGFR2 inhibitor, with an IC<sub>50</sub> of 204 nM, which was on par with the gold standard (sorafenib; IC<sub>50</sub> = 41 nM). Analysis of molecular docking data showed that the potent molecule **118** bound to the VEGFR2 active site in a significant way.<sup>55</sup>

The development of sulfonamide-tethered isatin derivatives as novel anticancer agents and VEGFR2 inhibitors was discovered by Shaldam *et al.*<sup>56</sup> Preparation procedures used in synthesizing the designed compound **123** are shown in Scheme 20. The first step in synthesis was performing chlorosulfonation





**Scheme 19** Synthesis of isatin–sulphonamide hybrid **118**. Reagents and conditions: (i) dioxane, Et<sub>3</sub>N, stirring, r.t., 20 h; (ii) acetone, K<sub>2</sub>CO<sub>3</sub>, KI, stirring, r.t., 2 h; (iii) N<sub>2</sub>H<sub>4</sub>·H<sub>2</sub>O, EtOH, reflux, 4 h; (iv) EtOH, *gl.* AcOH (cat.), reflux, 6 h.

of compound **119** using thionyl chloride and chlorosulfonic acid, which afforded benzenesulfonyl chloride **120**. Then, the reaction of compound **120** with ammonia using EtOH as solvent afforded benzenesulfonamide **121**. Moreover, the synthesis of hydrazone **122** was accomplished by refluxing compound **121** with N<sub>2</sub>H<sub>4</sub>·H<sub>2</sub>O for 4 h in EtOH and in the presence of catalytic glacial acetic acid. *N*-benzylated isatin **16** was synthesized by reacting 5-chloroisatin **6** with benzyl bromide **61** in acetonitrile and K<sub>2</sub>CO<sub>3</sub> under reflux. Finally, compound **16** was allowed to react with hydrazone **122** in the presence of a catalytic amount of glacial acetic acid and under reflux (yield: 82%).

Compound **123** was evaluated *in vitro* against T47D breast cancer cell line. It demonstrated cytotoxic activity (IC<sub>50</sub> = 3.59 ± 0.16 μM) compared to DOX (IC<sub>50</sub> of 2.26 μM). It demonstrated good VEGFR2 inhibition with an IC<sub>50</sub> of 23.10 nM, compared to sorafenib (IC<sub>50</sub> = 29.70 nM). Docking studies and molecular dynamic simulations revealed a stable complex and strong binding affinity of **123** to the active sites of VEGFR2.<sup>56</sup>

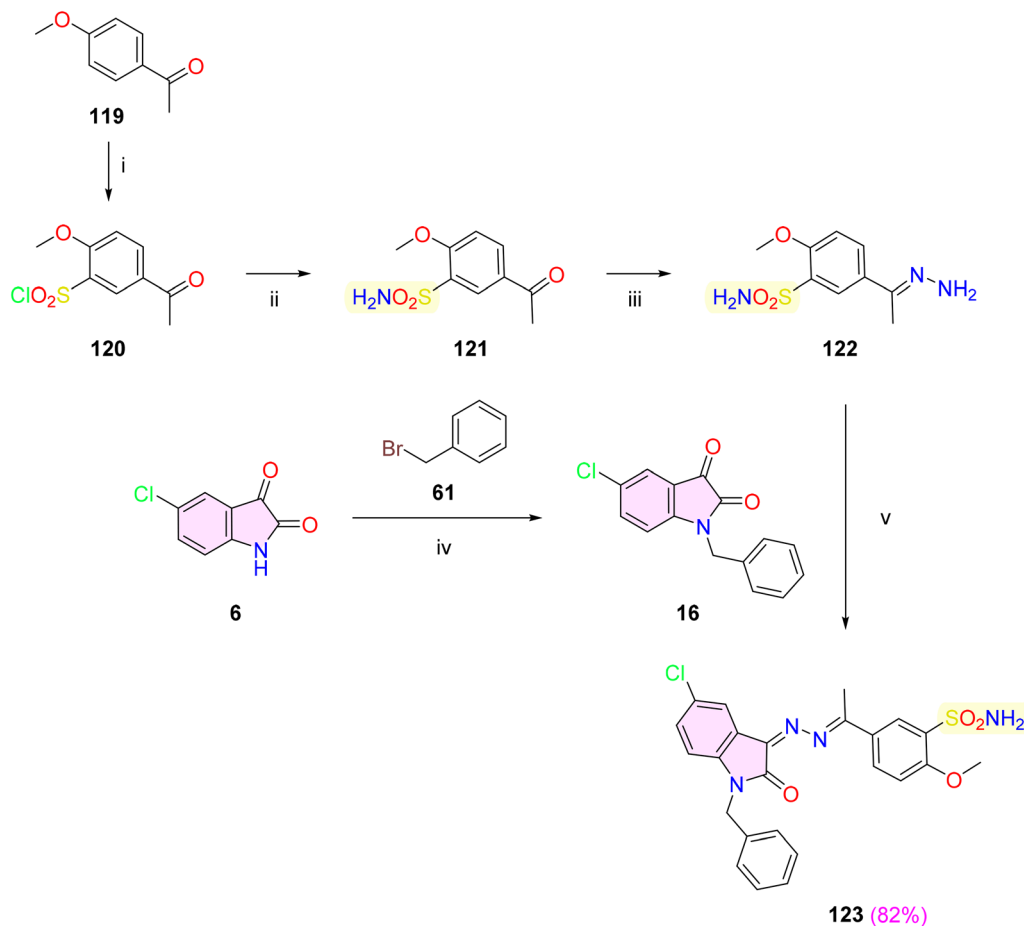
The cytotoxic effect of novel synthesized isatin sulfonamide-molecular hybrid derivatives targeting EGFRs have been investigated by Eldeeb *et al.*<sup>57</sup> The synthesis of the target compound **126** was done by the reaction of the parent molecule 5-(piperidin-1-ylsulfonyl) indoline-2,3-dione **124** with 1-(*p*-tolyl) ethenone **125** in the presence of ethylamine in MeOH, Scheme 21.

The antiproliferative effects of compound **126** were tested against two human hepatocellular carcinoma cell lines, HepG2 and Huh7. The IC<sub>50</sub> value of 16.80 ± 1.44 μM, which was lower than the DOX IC<sub>50</sub> value of 21.60 ± 0.81 μM, demonstrated a higher selectivity to HepG2 than Huh7. Additionally, it demonstrated a lack of cytotoxicity when tested on the RPE1 cell line, which is not malignant, indicating a promising safety profile as a selective anticancer drug. With EGFR levels reduced to 42 ± 2.3 pmol per mg protein, it demonstrated a notable decrease. Results from docking experiments showed a stable compound **126** with an affinity for the **126** active sites on EGFR.<sup>57</sup>

#### 4.7. Isatin–hydrazone hybrids

A novel poly(ADP-ribose) polymerase inhibitor, El Hassab *et al.* have reported the synthesis of a novel series of isatin–hydrazone hybrids.<sup>58</sup> The general strategy for preparing the targeted molecule **133** is presented in Scheme 22. First, ethyl 4-aminobenzoate **127** was heated under reflux with N<sub>2</sub>H<sub>4</sub>·H<sub>2</sub>O in EtOH to afford 4-aminobenzohydrazide **128**. Furthermore, 5-chloroisatin **6** was allowed to react with 1-iodo-2-methylpropane **129** in DMF at 100 °C to furnish 5-chloro-1-isobutylisatin **130**. Through condensation of 4-aminobenzohydrazide **128** with 5-chloro-1-isobutylisatin **130** in refluxing absolute EtOH in the presence





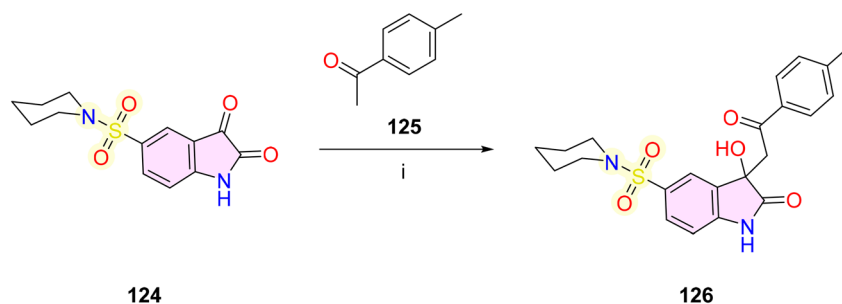
Scheme 20 Synthesis of isatin–sulphonamide hybrid **123**. Reagents and conditions: (i)  $\text{HOSO}_2\text{Cl}$ ,  $\text{SOCl}_2$ , 0 °C, 30 min, r.t., 26 h; (ii) EtOH, ammonia, r.t.; (iii)  $\text{N}_2\text{H}_4 \cdot \text{H}_2\text{O}$ , EtOH, *gl.* AcOH (cat.) reflux, 4 h; (iv)  $\text{K}_2\text{CO}_3$ , MeCN, reflux, 5 h; (v) EtOH, *gl.* AcOH (cat.), reflux, 4 h.

of catalytic glacial acetic acid, hydrazone **131** was formed. Thereafter, the latter compound **131** reacted with phthalic anhydride **132** *via* heating in glacial acetic acid using anhydrous sodium acetate to afford the target molecule **133** (yield: 89%).

Compound **133** was evaluated for its *in vitro* cytotoxicity against three human cancer cell lines, A549, PC3, and MCF-7. It displayed the highest cytotoxic activity with  $\text{IC}_{50}$  values of 5.32, 35.1, and 4.86  $\mu\text{M}$  against A549, PC3, and MCF-7 cells, respectively, compared to 5-FU with  $\text{IC}_{50}$  values of 12.3, 68.4, and 13.15

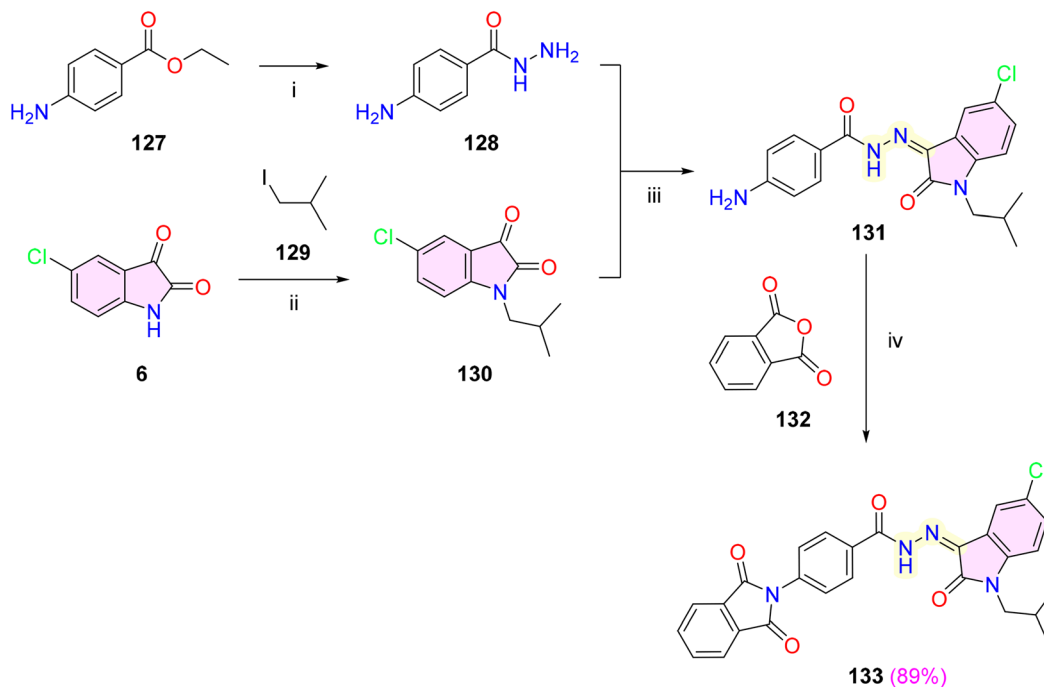
$\mu\text{M}$ . It displayed double inhibitory activity with an  $\text{IC}_{50}$  value of  $16.28 \pm 1.21$  nM compared to sorafenib, which has shown inhibitory activity with an  $\text{IC}_{50}$  value of  $35.62 \pm 1.52$  nM. Molecular docking studies of compound **133** towards human VEGFR2 kinase have shown good binding interactions with the target protein.<sup>58</sup>

Al-Rasheed *et al.* demonstrated an efficient strategy for merging *s*-triazine and isatin *via* a hydrazone linkage as new potential anticancer derivatives.<sup>59</sup> The new target *s*-triazine-



Scheme 21 Synthesis of isatin–sulphonamide hybrid **126**. Reagents and conditions: (i)  $\text{EtNH}_2$ , MeOH.



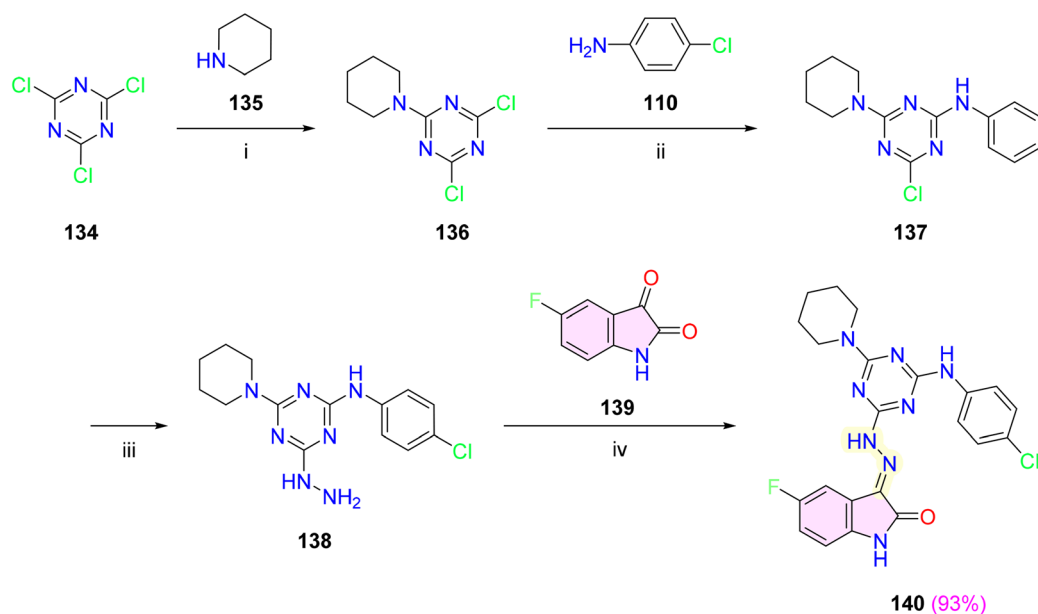


**Scheme 22** Synthesis of isatin–hydrazone hybrid **133**. Reagents and conditions: (i)  $\text{N}_2\text{H}_4 \cdot \text{H}_2\text{O}$ , EtOH, reflux, 6 h; (ii)  $\text{K}_2\text{CO}_3$ , DMF, 100 °C, 9 h; (iii) EtOH, *gl. AcOH* (cat.), reflux, 7 h; (iv) *gl. AcOH*, AcONa, reflux, 6 h.

isatin hydrazone **140** was synthesized following the procedures in Scheme 23. The initial step involved the nucleophilic substitution of the chlorine atom of cyanuric chloride **134** by piperidine **135** at 0–5 °C to afford the 2,4-dichloro-6-(piperidin-1-yl)-1,3,5-triazine **136**. The second step involved the replacement of the second chlorine atom by 4-chloroaniline **110** at room temperature, yielding compound **137**. The compound **137**

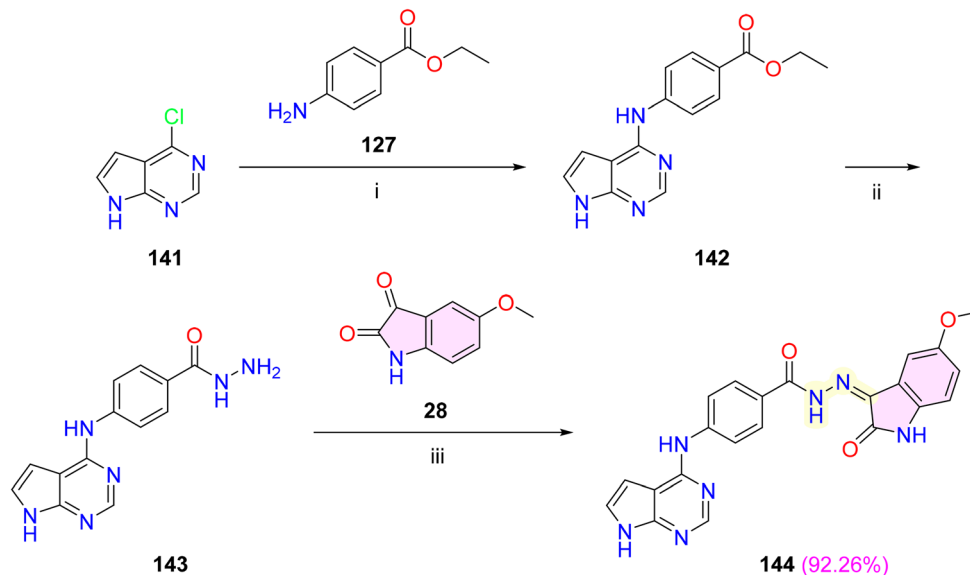
was reacted with  $\text{N}_2\text{H}_4 \cdot \text{H}_2\text{O}$  (80%) under reflux in EtOH for 8–12 h to afford **138**. Finally, compound **138** was condensed with 5-fluoroisatin **139** in EtOH and in the presence of catalytic acetic acid to afford the target product **140** (yield: 93%).

Compound **140** was evaluated for its antiproliferative activity against the lung cancer cell line (A549). It showed cytotoxicity with an  $\text{IC}_{50}$  value of 0.114  $\mu\text{M}$  compared to sorafenib  $\text{IC}_{50}$  value



**Scheme 23** Synthesis of isatin–hydrazone hybrid **140**. Reagents and conditions: (i)  $\text{NaHCO}_3$ , 0 °C, 1–2 h; (ii) acetone,  $\text{H}_2\text{O}$ ,  $\text{NaHCO}_3$ , 0 °C to r.t., overnight; (iii)  $\text{N}_2\text{H}_4 \cdot \text{H}_2\text{O}$ , EtOH, reflux, 6–12 h; (iv) EtOH, AcOH (cat.), reflux, 6–8 h.





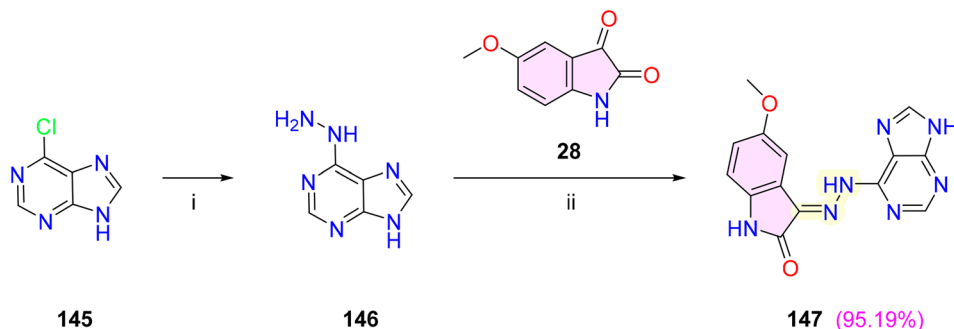
**Scheme 24** Synthesis of isatin–hydrazone hybrid **144**. Reagents and conditions: (i) EtOH, reflux, 7 h; (ii)  $\text{N}_2\text{H}_4 \cdot \text{H}_2\text{O}$ , EtOH, reflux, 5 h; (iii) EtOH, AcOH, reflux, 6–9 h.

of 0.195  $\mu\text{M}$ . Compound **140** exhibited promising anti-trypsin effects at its anticancer  $\text{IC}_{50}$  value ( $75.123 \pm 4.32 \mu\text{M}$ ) when compared to the inhibitory effect of rivaroxaban ( $53.223 \pm 0.98 \mu\text{M}$ ). It exhibited potentially greater potency as EGF inhibitors ( $65.34 \pm 5.42 \mu\text{M}$ ) compared to the  $\text{IC}_{50}$  of sorafenib ( $68.25 \pm 5.93 \mu\text{M}$ ). Docking studies supported the obtained results and demonstrated the ability of these derivatives to interact with EGFR active sites, as well as broad-spectrum anti-trypsin activity.<sup>59</sup>

M. M. Alanazi and A. S. Alanazi have reported the synthesis of novel isatin–hydrazone hybrid compounds as protein kinase inhibitors.<sup>60</sup> Initially, the ethyl 4-aminobenzoate **127** was added to a 4-chloro-7H-pyrrolo[2,3-d]pyrimidine **141** solution in absolute EtOH under reflux for 7 h. Then, ethyl-4-((7H-pyrrolo[2,3-d]pyrimidin-4-yl)amino)benzoate **142** was refluxed in excess of  $\text{N}_2\text{H}_4 \cdot \text{H}_2\text{O}$  for 5 h afforded hydrazide **143**. In the final step, the hydrazide **143** and 5-methoxyisatin **28** were mixed in absolute EtOH and glacial acetic acid under reflux to furnish the final target **144** (yield: 92.26%), Scheme 24.

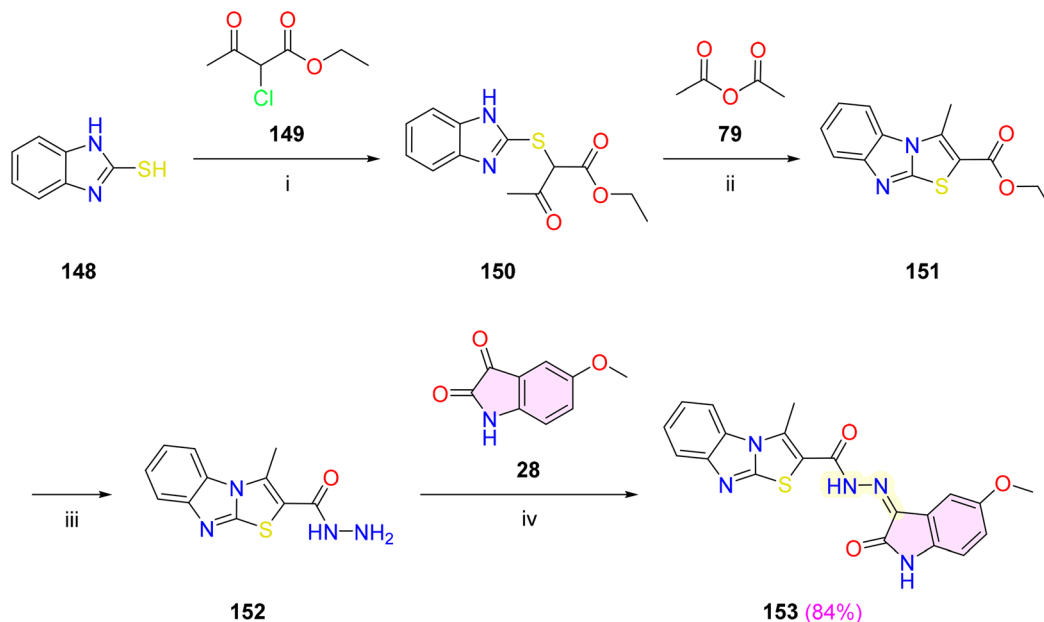
The antiproliferative activity of compound **144** was evaluated *in vitro* against four human cancer cell lines: hepatocellular carcinoma (HepG2), mammary gland cancer (MCF-7), breast cancer (MDA-MB-231), and epithelioid cervix carcinoma (HeLa), using DOX and sunitinib as reference drugs. It had a potent antiproliferative activity with  $\text{IC}_{50}$  values of 6.11, 5.93, 2.48, and 1.98  $\mu\text{M}$  against HepG2, MCF-7, MDA-MB-231, and HeLa cell lines, respectively. It exhibited multi-kinase inhibition and exhibited inhibitory activities against EGFR, HER2, VEGFR2, and CDK2 with  $\text{IC}_{50}$  values of 0.103, 0.081, 0.178, and 0.131  $\mu\text{M}$ , respectively, comparable to reference drugs, ribociclib, erlotinib, lapatinib, and sorafenib. A molecular docking study revealed a stable binding interaction in the active site of the selected protein kinase enzymes.<sup>60</sup>

Based on a molecular hybridization strategy, Alanazi *et al.* reported the synthesis of novel isatin–hydrazone hybrids.<sup>61</sup> The synthesis started with refluxing 6-chloro-9H-purine **145** in excess of  $\text{N}_2\text{H}_4 \cdot \text{H}_2\text{O}$  for 1 h to furnish 6-hydrazinyl-9H-purine **146**. Synthesis of the final compound **147** started with mixing 6-



**Scheme 25** Synthesis of isatin–hydrazone hybrid **147**. Reagents and conditions: (i)  $\text{N}_2\text{H}_4 \cdot \text{H}_2\text{O}$ , reflux, 1 h; (ii) EtOH, *gl.* AcOH (cat.), reflux, 3–7 h.





**Scheme 26** Synthesis of isatin–hydrazone hybrid **153**. Reagents and conditions: (i) EtOH, TEA, reflux, 6 h; (ii) reflux, 5 h; (iii)  $\text{N}_2\text{H}_4 \cdot \text{H}_2\text{O}$ , iPrOH, reflux, 6 h; (iv) *gl.* AcOH, reflux, 3–5 h.

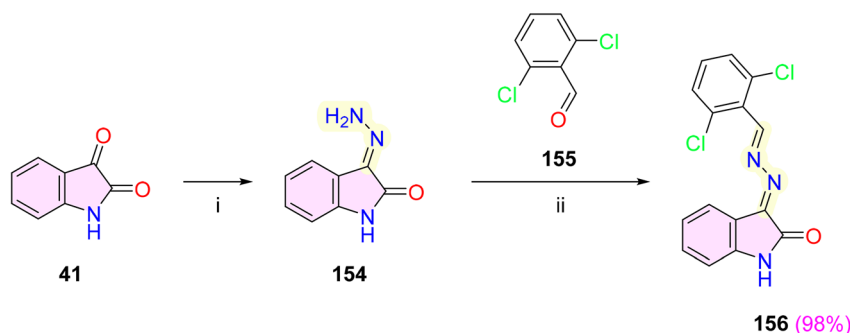
hydrazinyl-9H-purine **146**, 5-methoxyisatin **28**, and glacial acetic acid in absolute EtOH under reflux (yield: 95.19%, Scheme 25).

The antiproliferative activity of compound **147** was evaluated *in vitro* against four human cancer cell lines: hepatocellular carcinoma (HepG2), mammary gland cancer (MCF-7), breast cancer (MDA-MB-231), and epithelioid cervix carcinoma (HeLa), using sunitinib as a reference drug. It demonstrated cytotoxic activity comparable to the reference drug sunitinib against the HepG2, MCF-7, MDA-MB-231, and HeLa cell lines, with  $\text{IC}_{50}$  values of 9.61, 10.78, 14.89, and 8.93  $\mu\text{M}$ , respectively. It exhibited inhibitory activities against EGFR, HER2, VEGFR2, and CDK2 with  $\text{IC}_{50}$  values of 0.143, 0.15, 0.192, and 0.534  $\mu\text{M}$ , respectively. A molecular docking study revealed a stable binding interaction in the active site of the investigated protein kinase enzymes.<sup>61</sup>

The discovery of new isatin hybrids as novel CDK2 inhibitors with potent *in vitro* antiproliferative activity was reported by Eldehna *et al.*<sup>62</sup> The synthetic strategy deliberated for the

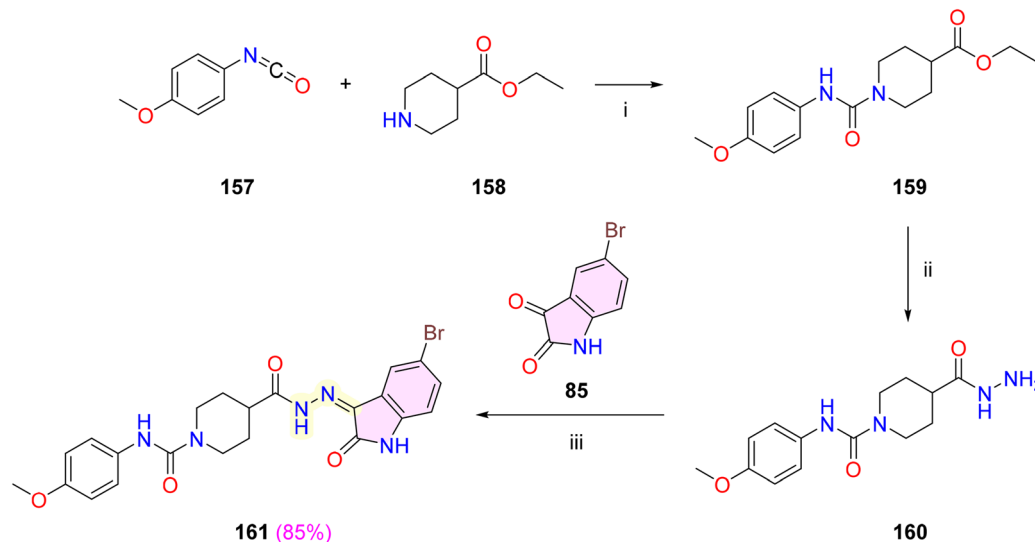
synthesis of the target hybrid **153** is illustrated in Scheme 26. 1H-benzimidazole-2-thiol **148** was reacted with ethyl 2-chloro-3-oxobutanoate **149** in absolute EtOH to furnish intermediate **150**, which then heterocyclized to compound **151** *via* heating in acetic anhydride **79**. The ester analog **151** was subjected to hydrazinolysis to produce hydrazide **152**, condensed with 5-methoxyisatin **28** in glacial acetic acid to give the targeted hybrid **153** (yield: 84%).

The antiproliferative activity of compound **153** was screened *in vitro* towards MDA-MB-231 and MCF-7 breast cancer cell lines; staurosporine, an anticancer agent, was used as a positive control drug. It was the most active derivative, with an  $\text{IC}_{50}$  value of  $3.30 \pm 0.21$   $\mu\text{M}$  against MDA-MB-231 compared to staurosporine, which displayed an  $\text{IC}_{50}$  value equal to  $4.29 \pm 0.72$   $\mu\text{M}$ . It showed inhibitory activity against the cell cycle regulator CDK2 protein kinase with an  $\text{IC}_{50}$  value of 26.24 nM, superior to staurosporine, which exhibited an  $\text{IC}_{50}$  value of 38.5 nM. Molecular docking revealed that the compound achieved the



**Scheme 27** Synthesis of isatin–hydrazone hybrid **156**. Reagents and conditions: (i)  $\text{N}_2\text{H}_4 \cdot \text{H}_2\text{O}$ , MeOH, reflux, 1 h; (ii) EtOH, *gl.* AcOH, reflux, 4 h.





**Scheme 28** Synthesis of isatin–hydrazone hybrid **161**. Reagents and conditions: (i) toluene, stirring, 90 °C, 2 h; (ii)  $\text{N}_2\text{H}_4 \cdot \text{H}_2\text{O}$ , EtOH, reflux, 4 h; (iii) *gl.* AcOH, reflux, 4 h.

best binding score (−11.2 kcal per mole) and formed the most stable complex with the CDK2 enzyme.<sup>62</sup>

Al-Salem *et al.* synthesized a series of novel isatin-hydrazones in excellent yields.<sup>63,64</sup> The synthesis of target compound **156** was straightforward, as illustrated in Scheme 27. First, isatin **41** was refluxed with  $\text{N}_2\text{H}_4 \cdot \text{H}_2\text{O}$  in EtOH to afford isatin monohydrazone **154**. Next, the isatin monohydrazone **154** was refluxed with 2,6-dichlorobenzaldehyde **155** in EtOH and in the presence of a catalytic glacial acetic acid to afford the target compound **156** (yield: 98%).

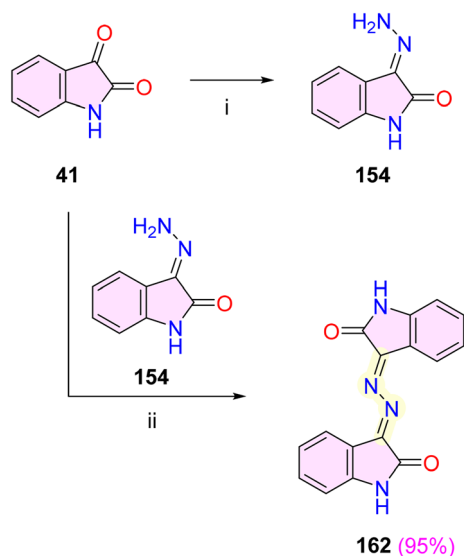
Compound **156** was tested for its cytotoxicity against human breast adenocarcinoma (MCF7) and human ovarian

adenocarcinoma (A2780) cell lines. It ( $\text{IC}_{50} = 1.51 \pm 0.09 \mu\text{M}$ ) showed excellent activity against MCF-7 compared to A2780 ( $\text{IC}_{50} = 26 \pm 2.24 \mu\text{M}$ ) and to DOX ( $\text{IC}_{50} = 3.10 \pm 0.29 \mu\text{M}$  and  $0.20 \pm 0.03 \mu\text{M}$ , respectively). Compound **156** ( $\text{IC}_{50} = 0.245 \mu\text{M}$ ) exhibited good inhibitory activity against the cell cycle regulator CDK2 protein kinase compared to imatinib ( $\text{IC}_{50} = 0.131 \mu\text{M}$ ). Its ability to interact with CDK2 was also confirmed by a docking study.<sup>63</sup>

As potential VEGFR2 inhibitors, Eldehna *et al.* reported the preparation of novel *N'*-(2-oxoindolin-3-ylidene) piperidine-4-carbohydrazide derivatives.<sup>65</sup> The targeted molecule **161** was synthesized *via* straightforward methodologies outlined in Scheme 28. Firstly, piperidine carboxylate ester **158** was allowed to react with 4-methoxyphenyl isocyanate **157** in toluene at 90 °C for 2 h to give compound **159**. In the next step, the ester **159** reacted with an excess of  $\text{N}_2\text{H}_4 \cdot \text{H}_2\text{O}$  to give the desired hydrazide **160**. After that, the hydrazide **160** was condensed with 5-bromoindolin-3-one **85** in glacial acetic acid under reflux for 4 h to afford the target compound **161** (yield: 85%).

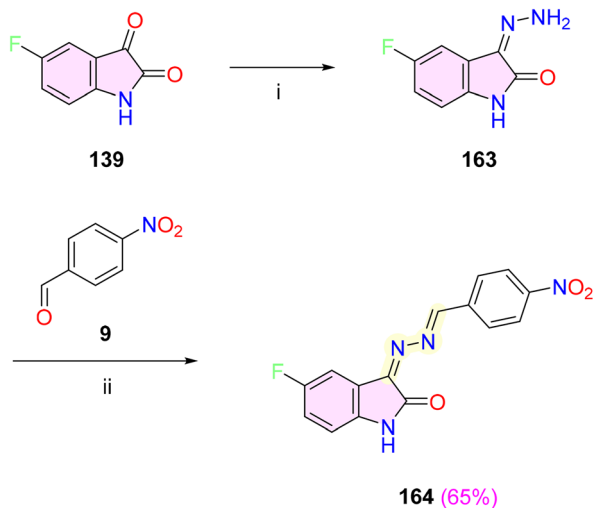
Compound **161** was tested for its cytotoxicity using the MDA-MB-231 and MCF-7 breast cancer cell lines. Cytotoxic activity was shown with  $\text{IC}_{50}$  values of 1.03 and 8.00  $\mu\text{M}$ , respectively. It was investigated for its inhibitory effects on VEGFR2 using sorafenib as the reference medication. It displayed the most promising inhibitory efficacy with an  $\text{IC}_{50}$  value of 45.9 nM, compared to sorafenib, with an  $\text{IC}_{50}$  value of 48.6 nM. Within the VEGFR2 active region, molecular docking and dynamic simulations uncovered **161** important binding interactions.<sup>65</sup>

The design and synthesis of CDK2 inhibitors using an isatin-based scaffold have been developed by Espinosa-Rodriguez *et al.*<sup>66</sup> The synthesis began with the reaction of isatin **41** with  $\text{N}_2\text{H}_4 \cdot \text{H}_2\text{O}$  in MeOH under reflux, which afforded hydrazone **154**. The latter hydrazone **154** was allowed to react with isatin **41** in MeOH under reflux, which yielded the desired product **162** (yield: 95%), Scheme 29.



**Scheme 29** Synthesis of isatin–hydrazone hybrid **162**. Reagents and conditions: (i)  $\text{N}_2\text{H}_4 \cdot \text{H}_2\text{O}$ , EtOH, reflux, 1 h; (ii) EtOH, *gl.* AcOH (cat.), reflux, 3 h.





**Scheme 30** Synthesis of isatin–hydrazone hybrid **164**. Reagents and conditions: (i)  $\text{N}_2\text{H}_4 \cdot \text{H}_2\text{O}$ , EtOH, reflux, 1 h; (ii) EtOH, *gl.* AcOH (cat.), reflux, 3 h.

The cytotoxicity of compound **162** was examined by subjecting it to tests on MCF-7 and PC-3 cell lines. In comparison to the positive control lapatinib, which exhibited cytotoxic activity with  $\text{IC}_{50}$  values of  $50.61 \mu\text{M}$  and  $32.4 \mu\text{M}$  in MCF-7 and PC-3 cells, respectively, it exhibited cytotoxic activity with  $\text{IC}_{50}$  values of  $19.07 \mu\text{M}$  and  $41.17 \mu\text{M}$ . Additionally, docking tests demonstrated that it might bind to CDK2 active sites and so suppress their activity.<sup>66</sup>

The *in vitro* antiproliferative activities of novel synthesized fluorinated isatin–hydrazones were investigated by Başaran *et al.*<sup>67</sup> As shown in Scheme 30, a mixture of 5-fluoroisatin **139** and  $\text{N}_2\text{H}_4 \cdot \text{H}_2\text{O}$  in EtOH was refluxed for 1 h to give compound **163**. Then, compound **163** was allowed to react with 4-

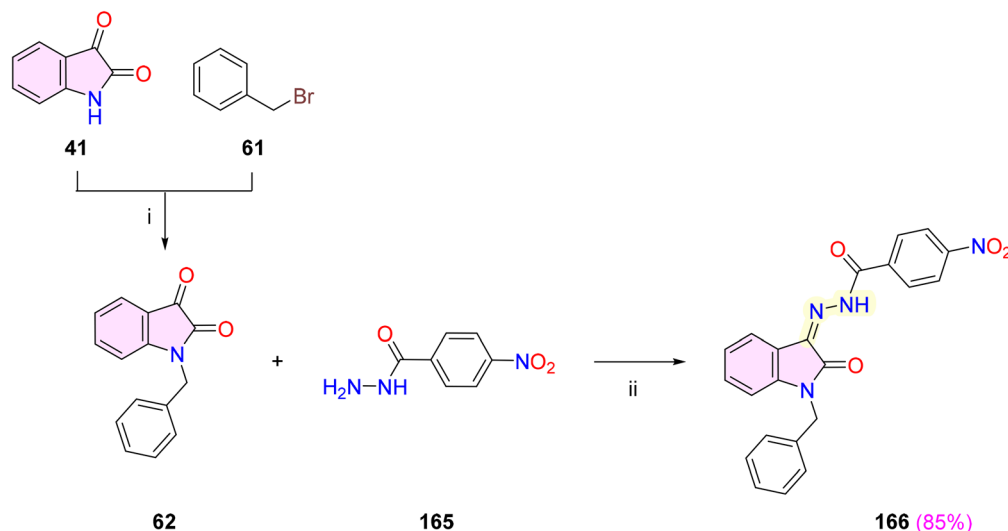
nitrobenzaldehyde **9** in absolute EtOH using catalytic drops of glacial acetic acid under reflux for 3 h, affording the desired hydrazone **164** (yield: 65%).

*In vitro* tests were conducted on Compound **164** using the lung cancer cell line and the liver cancer cell line. The  $\text{IC}_{50}$  value was  $42.43 \mu\text{M}$ , indicating a significant suppression of lung cell development, while the  $\text{IC}_{50}$  value was  $48.43 \mu\text{M}$ , indicating cytotoxicity, when tested on the HepG2 cell line. Further evidence of its capacity to bind to and inhibit the function of EGFR and VEGFR2 was provided by docking studies.<sup>67</sup>

Munir *et al.* reported the synthesis of novel *N*-benzylisatin-based hydrazones.<sup>68</sup> *N*-Benzylation of isatin was done *via* a reaction of isatin **41** with benzyl bromide **61** in acetonitrile and in the presence of  $\text{K}_2\text{CO}_3$  and KI to yield **62**. Then, the desired hydrazone **166** was synthesized by refluxing the equimolar ratio of *N*-benzylisatin **62** and 4-nitrobenzohydrazide **165** in EtOH and a catalytic amount of acetic acid (yield: 85%), Scheme 31.

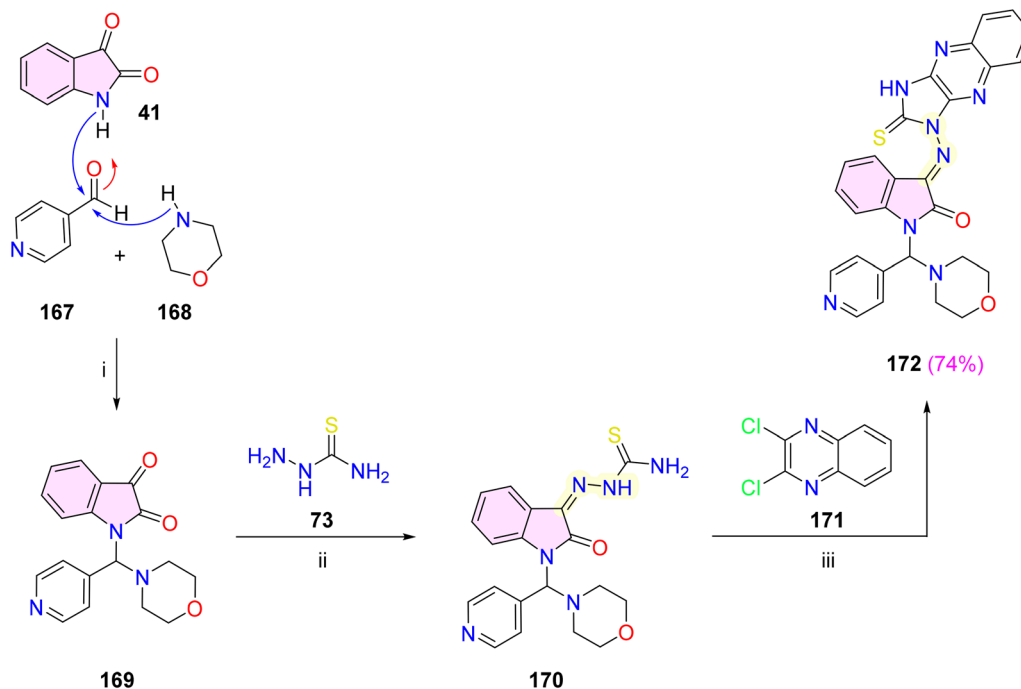
Both the MDA-MB-231 breast cancer cell line and the MCF-10A breast epithelial cell line were tested *in vitro* to determine the potential of compound **166**. When examined *in vitro* using the triple-negative MDA-MB-231 breast cancer cell line. The antiproliferative activities against the MDA-MB-231 were encouraging, with an  $\text{IC}_{50}$  value of  $15.8 \pm 0.6 \mu\text{M}$ . Furthermore, docking studies confirmed that it suppressed EGFR activity by interacting with its active regions.<sup>68</sup>

Abu-Hashem and Al-Hussain reported the design and synthesis of compound **172**.<sup>69</sup> The synthesis started with a one-pot synthesis using the Mannich reaction by stirring a mixture of isatin **41** with freshly distilled isonicotinaldehyde **167** and morpholine **168** in sodium ethoxide solution to compound **169**. Moreover, the latter compound **169** was refluxed with thiosemicarbazide **73** in glacial acetic acid to produce the target **170**. Finally, the nucleophilic aromatic substitution reaction of



**Scheme 31** Synthesis of isatin–hydrazone hybrid **166**. Reagents and conditions: (i) KI,  $\text{K}_2\text{CO}_3$ , MeCN, reflux, 4 h; (ii) EtOH, AcOH (cat.), reflux, 4–6 h.



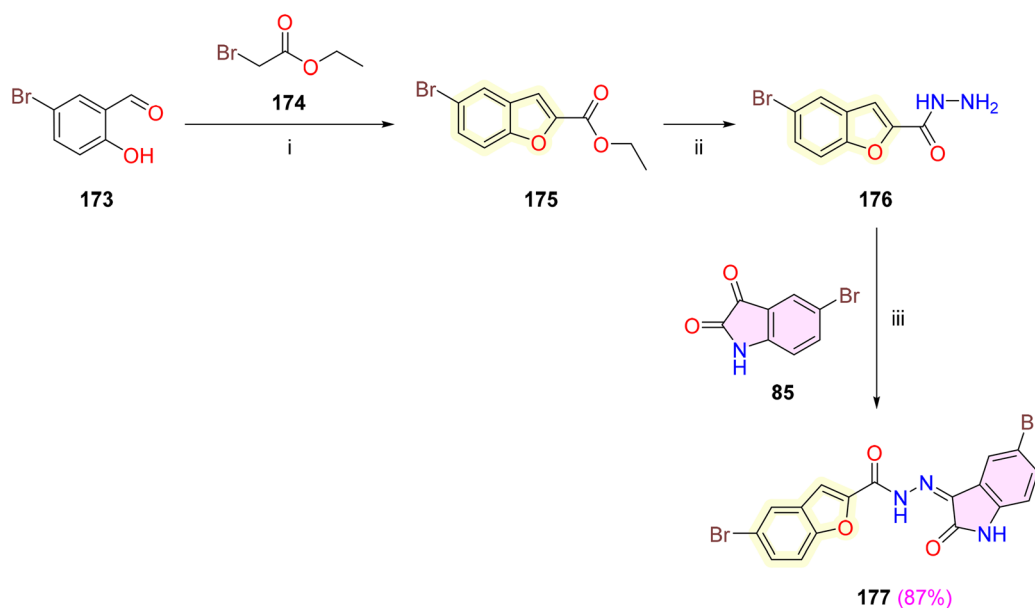


Scheme 32 Synthesis of isatin–hydrazone hybrid **172**. Reagents and conditions: (i) EtONa, stirring, r.t., 8–10 h; (ii) *gl.* AcOH, reflux, 5–7 h; (iii) EtOH, TEA, reflux, 22–25 h.

compound **170** with 2,3-dichloro-quinoxaline **171** under reflux in absolute EtOH and in the presence of catalytic triethylamine (TEA) gave 1-(morpholino(pyridin-4-yl)methyl)-3-((2-thioxo-2,3-dihydro-1*H*-imidazo[4,5-*b*]quinoxalin-1-yl)imino)indolin-2-one **172** in 74% yield, Scheme 32.

Using 5-FU as a reference medication, compound **172**'s antiproliferative activity was tested *in vitro* against four human

cancer cell lines: gastric carcinoma cells (MGC-803), breast adenocarcinoma cells (MCF-7), nasopharyngeal carcinoma cells (CNE2), and oral carcinoma cells (KB). Compared to 5-FU, the cytotoxic action was demonstrated with IC<sub>50</sub> values of 9.7, 9.6, 9.5, and 9.4 μM, respectively, compared to 10.7, 10.5, 10.3, and 10.1 μM for 5-FU.<sup>69</sup>

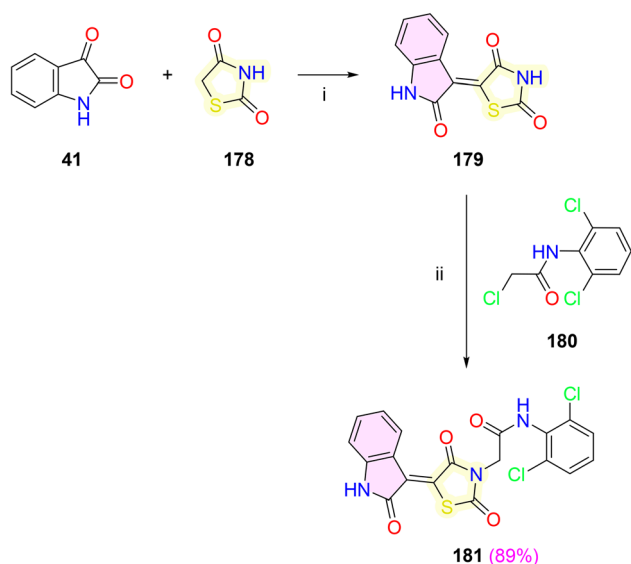


Scheme 33 Synthesis of isatin–benzofuran hybrid **177**. Reagents and conditions: (i) MeCN, K<sub>2</sub>CO<sub>3</sub>, reflux, 4 h; (ii) N<sub>2</sub>H<sub>4</sub>·H<sub>2</sub>O, MeOH, reflux, 3 h; (iii) EtOH, *gl.* AcOH (cat.), reflux, 4–7 h.

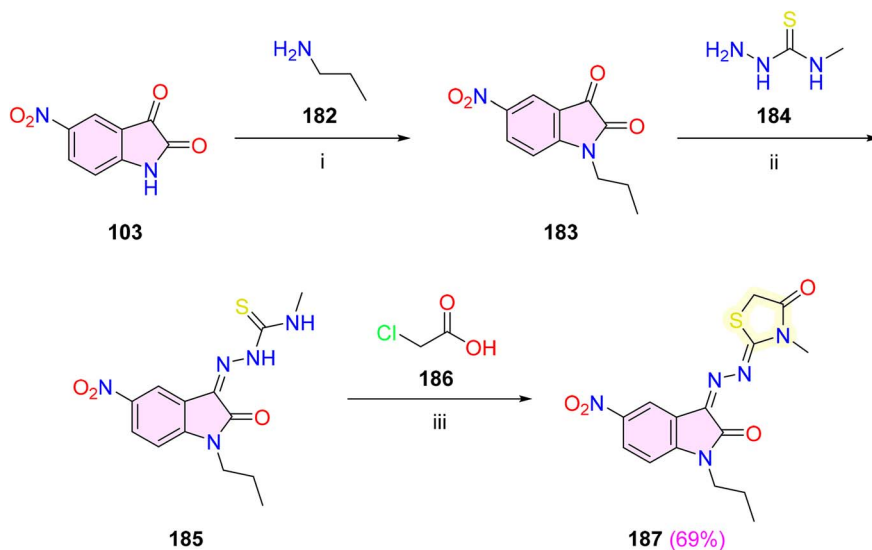


#### 4.8. Isatin–benzofuran hybrid

Eldehna *et al.* reported the preparation of novel isatin–benzofuran hybrids.<sup>70</sup> The strategy designed for the preparation of the target compound **177** is illustrated in Scheme 33. First, the reaction of ethyl bromoacetate **174** with 5-bromosalicylaldehyde **173** in acetonitrile to furnish ethyl 5-bromobenzofuran-2-carboxylate **175**. Thereafter, hydrazinolysis of the ester **175** *via* refluxing with  $N_2H_4 \cdot H_2O$  gave the intermediate hydrazide **176**. Finally, key intermediate **176** was condensed with 5-bromo-isatin **85** in absolute EtOH with catalytic drops of glacial acetic acid to get the final compound **177** (yield: 87%).



**Scheme 34** Synthesis of isatin–thiazolidine hybrid **181**. Reagents and conditions: (i) *gl.* AcOH, AcONa, reflux, 5 h; (ii) DMF,  $K_2CO_3$ , KI, reflux, 6 h.



**Scheme 35** Synthesis of isatin–thiazolidine hybrid **187**. Reagents and conditions: (i)  $K_2CO_3$ , DMF, 80 °C, 45 min; (ii) EtOH, *gl.* AcOH (cat.), reflux, 2 h; (iii) EtOH, AcONa (cat.), reflux, 24 h.

T-47D and MCF-7 breast cancer cell lines were used to study compound **177**'s antiproliferative effects. Relative to the standard staurosporine, which exhibited  $IC_{50}$  values of 4.34 and 4.81  $\mu M$ , respectively, it had a potent cytotoxic effect at concentrations of 3.82 and 3.41  $\mu M$ . It showed the most potent inhibitory activity on CDK-2 and GSK-3 $\beta$  with  $IC_{50}$  of 37.77 and 32.09 nM, comparable to staurosporine  $IC_{50}$  of 38.5 and 43.38 nM. Molecular docking studies revealed important binding interactions of potent compound **177** with the CDK-2 and GSK-3 $\beta$  active sites.<sup>70</sup>

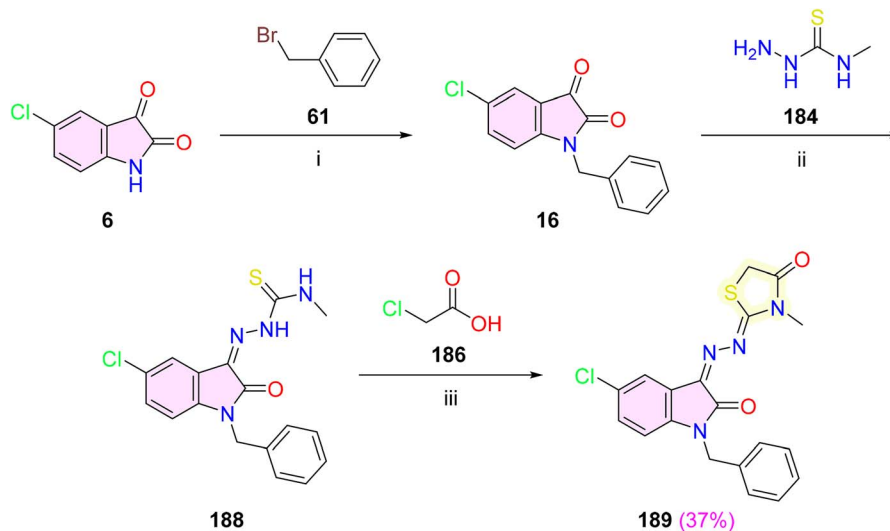
#### 4.9. Isatin–thiazolidine hybrid

As potential VEGFR2 inhibitors, Taghour *et al.* developed new thiazolidine–isatin hybrids.<sup>71</sup> For preparing the target hybrid **181**, the synthetic route is clarified in Scheme 34. Primarily, compound **179** was prepared by the reaction of isatin **41** with thiazolidine-2,4-dione **178** under reflux in glacial acetic acid with catalytic sodium acetate for 5 h. Heating a mixture of compound **179** with 2-chloro-*N*-(2,6-dichlorophenyl)acetamide **180** in dry DMF/KI yielded the corresponding target compound **181** (yield: 89%).

Compound **181** was tested for its cytotoxic activity against the MCF-7 cell line. It exhibited cytotoxicity with an  $IC_{50}$  value of 12.47  $\mu M$  compared to 5-FU as a reference drug. A molecular docking study was conducted against the Hsp90 protein and obtained crucial molecular interactions.<sup>71</sup>

Yousef *et al.* reported the synthesis of novel isatin-based derivatives as potential anticancer agents.<sup>72</sup> The target molecule **187** was synthesized utilizing a three-step reaction, Scheme 35. The first step involved the reaction of 5-nitroisatin **103** with propylamine **182** to furnish *N*-propyl intermediate **183**. In the following step, refluxing 5-nitro-*N*-propylisatin **183** with 4-methyl-3-thiosemicarbazide **184** in EtOH in the presence of a catalytic amount of glacial acetic acid afforded isatin-3-(*Z*)-thiosemicarbazone **185**. The final step was the cyclization of **185**



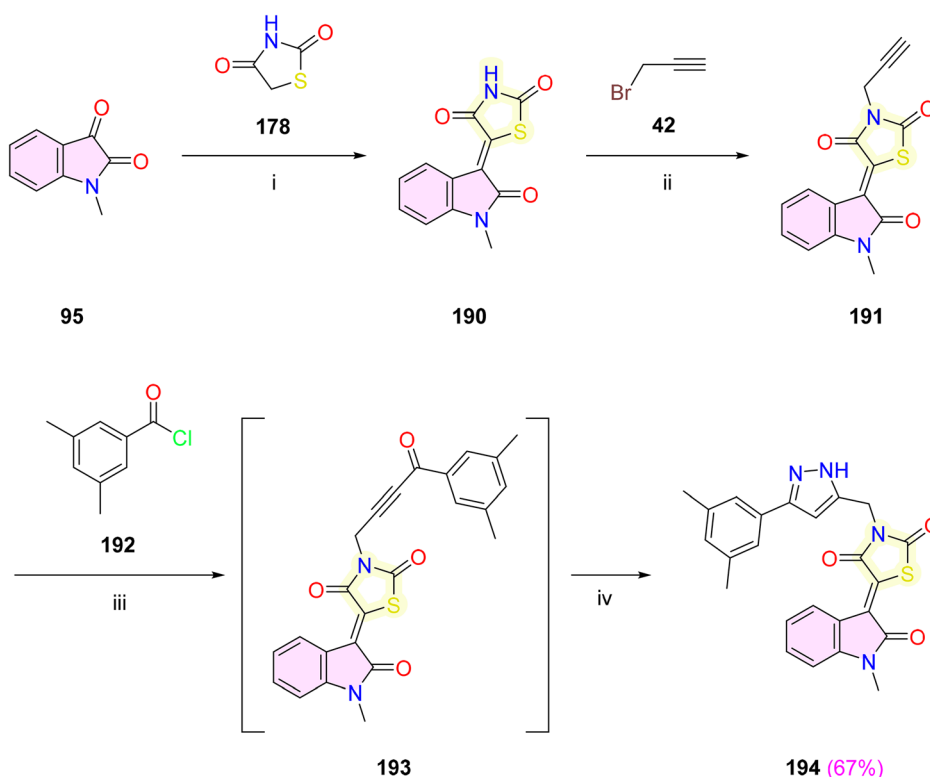


**Scheme 36** Synthesis of isatin–thiazolidine hybrid **189**. Reagents and conditions: (i)  $K_2CO_3$ , DMF, KI, 80 °C, 45 min; (ii) EtOH, *gl.* AcOH (cat.), reflux, 3 h; (iii) EtOH, AcONa (cat.), reflux, 24 h.

with chloroacetic acid **186** in refluxing EtOH in the presence of catalytic anhydrous sodium acetate, which gave the target compound **187** (yield: 69%).

The *in vitro* cytotoxicity of compound **187** was evaluated against three cell lines: human liver cancer cells (HepG2), breast cancer cells (MCF-7), and human colon cancer cells (HT-29), using DOX as a reference. It exhibited cytotoxic activity with

$IC_{50}$  values of 4.97, 5.33, and 3.29  $\mu M$ , respectively, compared to DOX ( $IC_{50}$  = 4.50, 4.17, and 4.01  $\mu M$ , respectively). The inhibitory effect of **187** against CDK1 was also significant, with  $IC_{50}$  = 0.38  $\mu M$ , compared to reference DOX ( $IC_{50}$  = 0.42  $\mu M$ ). A docking study also confirmed the ability of **187** to interact with CDK1.<sup>72</sup>



**Scheme 37** Synthesis of isatin–thiazolidine hybrid **194**. Reagents and conditions: (i) EtOH, piperidine, reflux, 24 h; (ii)  $Cs_2CO_3$ , MeCN, 80 °C, 10 h; (iii)  $PdCl_2(PPh_3)_2$ , CuI,  $K_2CO_3$ , sodium lauryl sulfate,  $H_2O$ , 65 °C, 8 h; (iv)  $N_2H_4 \cdot H_2O$ , 65 °C, 12 h.



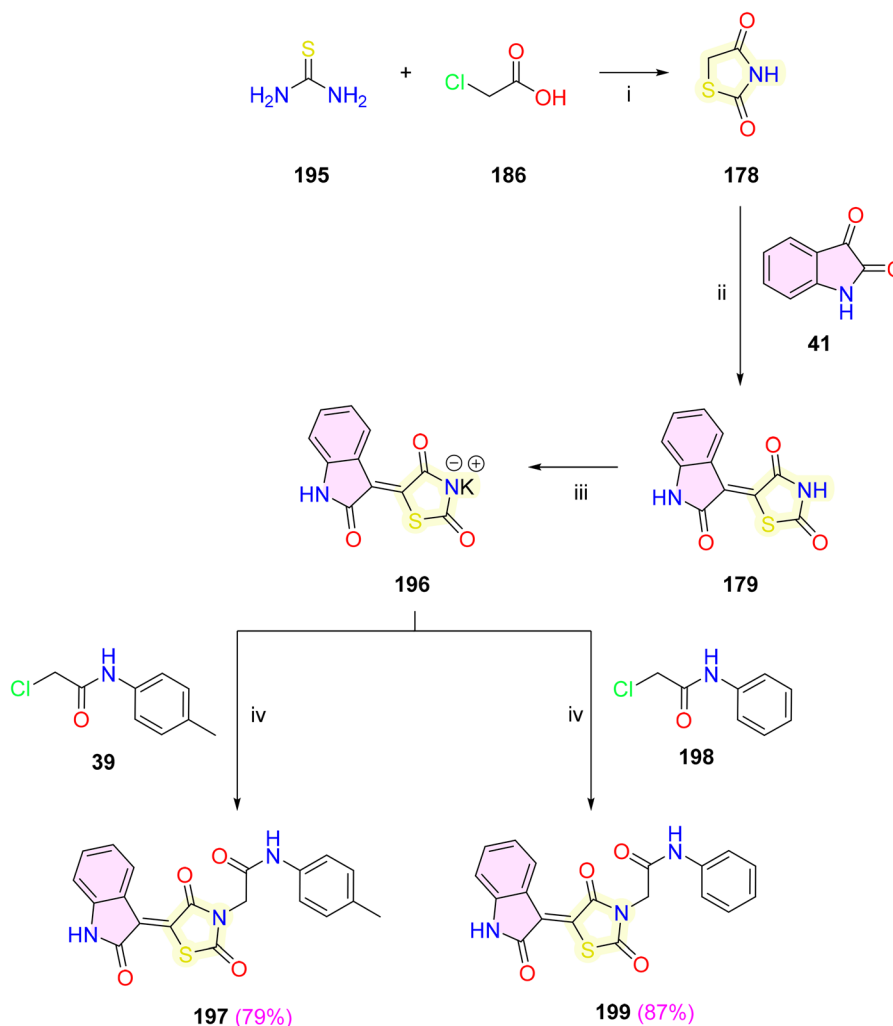
Novel azine-linked hybrids of isatin and thiazolidinone scaffolds as CDK2 inhibitors were reported by Qayed *et al.*<sup>73</sup> The designed compound **189** was synthesized as illustrated in Scheme 36. The route started with the synthesis of *N*-benzyl-5-chloroisatin **16** via the reaction of 5-chloroisatin **6** with benzyl bromide **61** in DMF in the presence of  $K_2CO_3$  and KI at 80 °C. Next, refluxing *N*-benzyl-5-chloroisatin **16** with 4-methyl-3-thiosemicarbazide **184** in EtOH in the presence of catalytic glacial acetic acid afforded (*Z*)-thiosemicarbazone **188**. Finally, the target hybrid **189** was produced from the cyclization of compound **188** by reacting with chloroacetic acid **186** in EtOH in the presence of a catalytic amount of anhydrous sodium acetate (yield: 37%).

Using Dox as a reference medicine, compound **189** showed antiproliferative efficacy against HepG2, MCF7, and HCT-29 cell lines, which are human liver, breast, and colon, respectively. It showed cytotoxic activity with  $IC_{50}$  values of 3.0, 5.19, and 3.10  $\mu M$ , respectively, compared to Dox with  $IC_{50}$  values of 4.15, 4.61, and 4.65  $\mu M$ . The inhibitory effect of **189** against CDK2 was also significant, with  $IC_{50} = 27.42$  nM, compared to the reference

drug sunitinib ( $IC_{50} = 23.8$  nM). The molecular dynamics simulations and docking studies showed that there is a stable complex with a strong binding affinity of **189** to the active regions of CDK2.<sup>73</sup>

As possible VEGFR2 inhibitors, Mallikarjuna Rao *et al.* reported the synthesis of some isatin-thiazolidine-2,4-dione-pyrazoles.<sup>74</sup> The synthetic path followed to get the designed compound **194** is shown in Scheme 37. Initially, Knoevenagel condensation between 1-methylindoline-2,3-dione **95** and thiazolidine-2,4-dione **178** under piperidine catalyst in EtOH under reflux for 24 h afforded compound **190**. Later, treatment of compound **190** with propargyl bromide **42** in acetonitrile at 80 °C for 10 h gave the terminal alkyne **194** (yield: 67%).

Human cancer cell lines HepG2, Caco-2, and MDA-MB231 were used to study compound **194**'s antiproliferative properties. It displayed remarkable activity (HepG2;  $IC_{50} = 2.4$   $\mu M$ , Caco-2;  $IC_{50} = 6.2$   $\mu M$  and MDA-MB231;  $IC_{50} = 7.5$   $\mu M$ ) against all cancer cell lines and this was higher than the standard drug DOX (HepG2;  $IC_{50} = 2.9$   $\mu M$ , Caco-2;  $IC_{50} = 8.3$   $\mu M$  and MDA-MB231;  $IC_{50} = 9.2$   $\mu M$ ). The results showed that **194** inhibited



**Scheme 38** Synthesis of isatin–thiazolidine hybrids **197** and **199**. Reagents and conditions: (i) (a)  $H_2O$ , 0–5 °C, stirring, 15 min; (b) conc. HCl, reflux, 10 h; (ii) AcOH, AcONa, reflux, 6 h; (iii) KOH, EtOH, reflux; (iv) DMF,  $K_2CO_3$ , KI, reflux, 6 h.



VEGFR2 more effectively than sorafenib ( $IC_{50} = 51.3 \text{ nM}$  vs.  $53.8 \text{ nM}$ ). Through molecular docking studies, it was found to bind extensively to the VEGFR2 active site.<sup>74</sup>

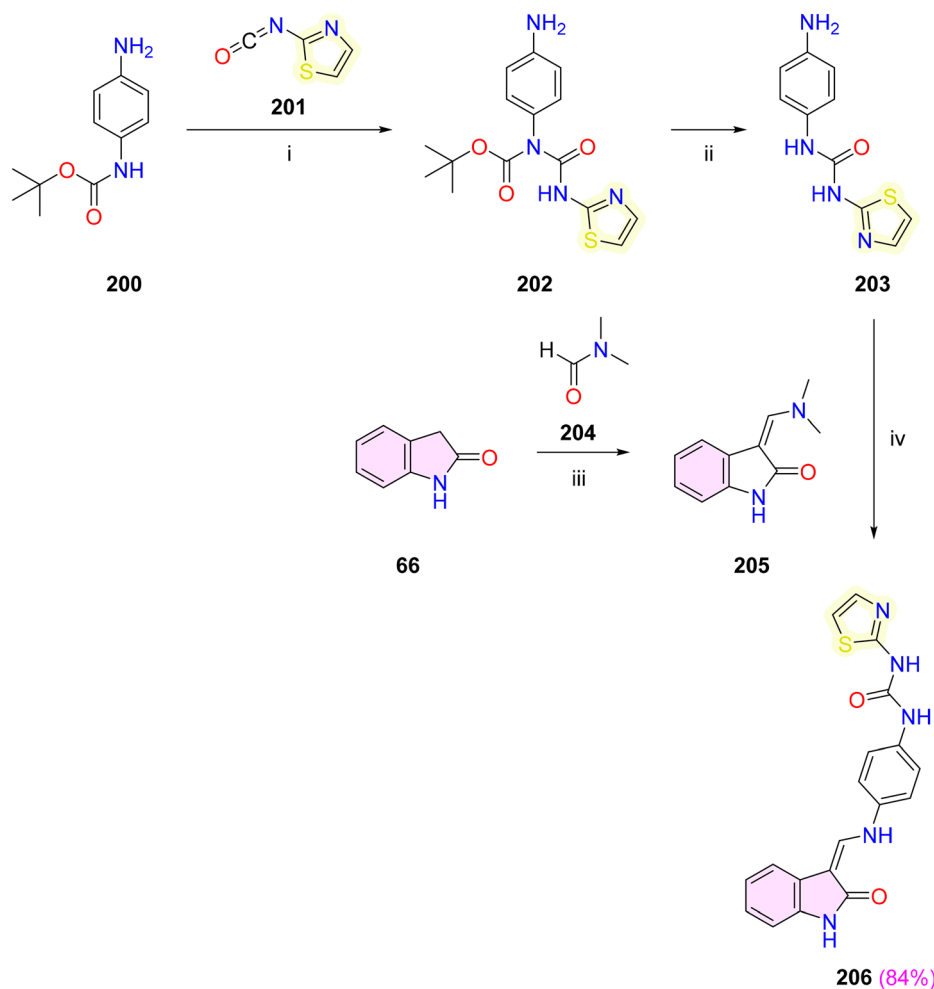
Taghour *et al.* reported the synthesis of thiazolidine-2,4-diones hybrids as potential VEGFR-2 inhibitors.<sup>75</sup> The sequence of chemical synthesis is clarified in Scheme 38. The synthesis started with the preparation of thiazolidine-2,4-dione **178** through the reaction of thiourea **195** with chloroacetic acid **186** under reflux in conc. HCl. Moreover, condensation of isatin **41** with thiazolidine-2,4-dione **178** was done with sodium acetate in acetic acid to give the isatin derivative **179**. The treatment of compound **179** with the alcoholic solution of KOH provided its potassium salt **196**. Then, heating a mixture of compound **196** with 2-chloro-*N*-(*p*-tolyl)acetamide **39** in dry DMF and KI afforded the target compound **197** (yield: 79%).

A panel of three cancer cell lines, namely colon (Caco-2), hepatocellular (HepG2), and breast (MDA-MB-231) cancer cell lines, was used to evaluate the potential anti-proliferative effects of compound **197**. It displayed  $IC_{50}$  values of 2.0, 10, and  $40 \mu\text{M}$  in comparison to DOX, which had  $IC_{50}$  values of 3.46, 1.15, and

$0.98 \mu\text{M}$ , respectively. It showed  $IC_{50}$  values of 2.0, 10, and  $40 \mu\text{M}$ , in contrast to DOX's  $IC_{50}$  values of 3.46, 1.15, and  $0.98 \mu\text{M}$ , respectively. The molecular dynamics and docking studies have revealed the presence of a stable complex that binds to the active areas of VEGFR2 with a high affinity of 197.<sup>75</sup>

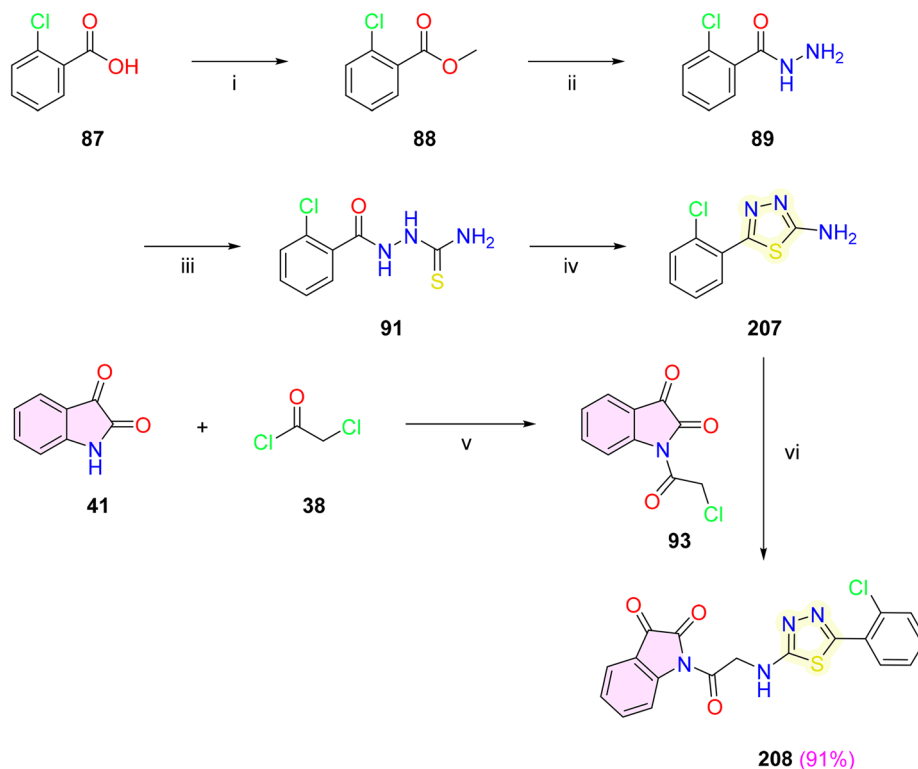
Elkaeed *et al.* also reported the synthesis of thiazolidine-2,4-diones hybrid as an apoptotic VEGFR2 inhibitor. The synthetic procedure was the same as adopted for the synthesis of **197** in Scheme 38, except in the final step. 2-Chloro-*N*-phenylacetamide **198** was heated with compound **196** in dry DMF, and KI gave the target compound **199** (yield: 87%).<sup>76</sup>

Four cancer cell lines were tested for compound **199**'s anti-proliferative effects: A549, Caco-2 (colon cancer), HepG2 (hepatocellular cancer), and MDA-MB-231 (breast cancer). The compound demonstrated superior cytotoxic effects compared to DOX, with  $IC_{50}$  values of 49.5, 9.3, and  $28 \mu\text{M}$  against A549, Caco-2, and MDA-MB-231, respectively. The  $IC_{50}$  value was  $69.11 \text{ nM}$ , indicating a substantial inhibitory action against VEGFR2. This compound's molecular docking investigations on the target VEGFR2 protein demonstrated its binding capabilities.<sup>76</sup>



Scheme 39 Synthesis of isatin-thiazole hybrid **206**. Reagents and conditions: (i) DCM,  $-25 \text{ }^\circ\text{C}$  to r.t.; (ii) TFA, DCM,  $0 \text{ }^\circ\text{C}$ ; (iii) toluene, reflux, 2 h; (iv) AcOH, reflux, 4 h.





Scheme 40 Synthesis of isatin–thiadiazole hybrid **208**. Reagents and conditions: (i)  $\text{H}_2\text{SO}_4$ , MeOH, reflux, 16 h; (ii)  $\text{N}_2\text{H}_4 \cdot \text{H}_2\text{O}$ , reflux, 4 h; (iii) KSCN, HCl,  $\text{H}_2\text{O}$ , reflux, 5 h; (iv)  $\text{H}_2\text{SO}_4$ , stirring, r.t., 6 h; (v) (a) reflux, 5 h; (b) stirring, r.t., 24 h; (vi) EtOH,  $\text{K}_2\text{CO}_3$ , reflux, 4 h.

#### 4.10. Isatin–thiazole hybrid

An optimization strategy was adopted for synthesizing a new series of 2-oxindole conjugates by Ismail *et al.*<sup>77</sup> The synthetic route adopted for the preparation of the target 3-(methylene)-indol-2-one **206** is depicted in Scheme 39. The synthesis started with the preparation of urea intermediate **202** by the reaction of mono-BOC protected phenylenediamine **200** with 2-isocyanatothiazole **201**. Then, BOC removal with trifluoroacetic acid (TFA) gave the required compound **203**.<sup>77</sup> Furthermore, condensation of the active methylene group of 2-oxindole **66** with *N,N*-dimethylformamide dimethyl acetal (DMF-DMA) **204** to afford the *N*-methylene intermediate **205**. Finally, the latter compound **205** reacted with the prepared amine **203** in glacial acetic acid to give the target compound **206** (yield: 84%).

Compound **206** was screened *in vitro* for its cytotoxicity towards human MCT-7 (Breast), DU-145 (Prostate), and HCT-116 (Colon) cancer cell lines. It showed distinct potent and broad antiproliferative activity with  $\text{IC}_{50}$  values of 4.39, 1.06, and 0.34 nM, respectively, against MCT-7, DU 145, and HCT-116 cell lines. It showed the most active inhibition activity against FGFR, VEGFR2, and RET kinases, showing  $\text{IC}_{50}$  values of 1.28, 0.117, and 1.18  $\mu\text{M}$ , respectively. Molecular docking studies, which demonstrated its ability to achieve essential interactions, are crucial for inhibiting FGFR, VEGFR-2, and RET kinases.<sup>77</sup>

#### 4.11. Isatin–thiadiazole hybrid

A series of 1-(2-((aryl-1,3,4-thiadiazol-2-yl)amino)acetyl)indoline-2,3-diones as anti-breast cancer leads was produced

by Rasgania *et al.*<sup>78</sup> The synthesis of the target compound **208** is outlined in Scheme 40. First, the synthesis of compound **207** *via a* four-step method. *o*-Chlorobenzoic acid **87** was refluxed in  $\text{H}_2\text{SO}_4$  containing MeOH for 16 h to get the benzoate **88**. By refluxing the benzoate with  $\text{N}_2\text{H}_4 \cdot \text{H}_2\text{O}$  for 4 h, the hydrazide **89** was obtained, which was further converted into its respective thiosemicarbazide **91** by refluxing with KSCN in acidic media ( $\text{HCl}/\text{H}_2\text{O}$ ) for 5 h. 2-Amino-5-(2-chlorophenyl)-1,3,4-thiadiazole **207** was obtained by stirring thiosemicarbazides in  $\text{H}_2\text{SO}_4$  for 6 h, followed by neutralization with an ammonia solution. Second, isatin **41** was vigorously refluxed with chloroacetyl chloride for 5 h, followed by an overnight stir at room temperature, affording 1-(2-chloroacetyl)indoline-2,3-dione **93** as yellow crystals. The target molecule **208** was efficiently synthesized by the thermal integration of the synthesized thiadiazole **207** with chloroacetyl isatin **93** under reflux in EtOH for 4 h (yield: 91%).

The antiproliferative activity of compound **208** was evaluated *in vitro* against a triple-negative breast cancer (MDA-MB-231) cell line. It showed the highest level of potency when tested *in vitro*, with an  $\text{IC}_{50}$  value of 57.79  $\mu\text{g ml}^{-1}$ , when compared to tamoxifen citrate as the positive control. Additionally, docking tests verified that it could bind to EGFR active sites and so decrease its activity, suggesting that it acted as an EGFR inhibitor.<sup>78</sup>

Table 1 summarizes the biological findings of some recently synthesized isatin hybrids as anti-cancer agents, highlighting the effective molecular targets, melting points, and cytotoxicity values.





Table 1 Cytotoxicity of isatin-based derivatives with highlighted molecular targets

Feature	Scheme	Structure	m.p. (°C)	Kinase inhibition activity	Anticancer activity	Ref.		
Isatin-quinazoline hybrids	1		292–294	Enzymes VEGFR2 EGFR CDK-2 CDK-4 IC <sub>50</sub> [nM] Cpd 7 56.74 ± 4.3 87.48 ± 6.71 9.39 ± 0.51 36.39 ± 4.52	Cell lines HepG2 MCF-7 IC <sub>50</sub> [μM] Cpd 7 2.53 ± 0.11 7.54 ± 0.71	Indirubin 126.42 ± 20 175.46 ± 18.33 45.60 ± 2.24 23.64 ± 2.15	Indirubin 6.92 ± 0.65 6.12 ± 0.35	37
	2		>300	Enzymes VEGFR2 EGFR CDK-2 CDK-4 IC <sub>50</sub> [nM] Cpd 12 14.31 ± 2.70 32.65 ± 1.61 225.32 ± 12.56 244.32 ± 12.21	Cell lines HepG2 MCF-7 IC <sub>50</sub> [μM] Cpd 12 3.08 ± 0.35 5.28 ± 0.22	Indirubin 126.42 ± 20 175.46 ± 18.33 45.60 ± 2.24 23.64 ± 2.15	Indirubin 6.92 ± 0.65 6.12 ± 0.35	37
	3		268–270	Enzymes —	Cell lines HT-29 ZR-75 A-549 IC <sub>50</sub> [μM] Cpd 17 2.02 ± 0.36 0.74 ± 0.88 0.76 ± 0.12	Sunitinib 10.14 ± 0.8 8.31 ± 2.4 5.87 ± 0.3	38	
	4		>300	Enzymes —	Cell lines HT-29 SW-620 IC <sub>50</sub> [μM] Cpd 23 206 188	5-FU 4600 1500	39	
	5		232.5–234	Enzymes CDK2 IC <sub>50</sub> [μM] Cpd 29 6.32	Cell lines MCF-7 MDA-MB-231 NCI-ADR IC <sub>50</sub> [μM] Cpd 29 1.15 ± 0.04 10.54 ± 0.43 9.17	DOX 6.81 ± 0.22 10.29 ± 0.72 —	40	
Isatin-indole hybrids								

Table 1 (Contd.)

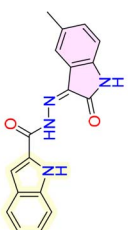
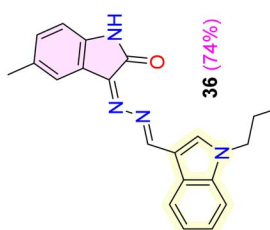
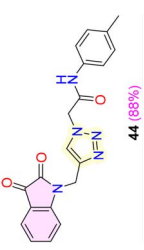
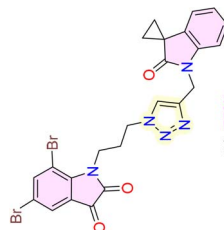
6		> 300	Enzymes CDK4	% inhibition Cpd 32 1.26	Staurosporine 0.017	Cell lines MCF-7 MDA-MB-231	IC <sub>50</sub> [μM] Cpd 32 0.39 ± 0.05 22.54 ± 1.67	Staurosporine 6.81 ± 0.22 10.29 ± 0.72	41	
7		195–197	Enzymes CDK2	IC <sub>50</sub> [nM] Cpd 36 0.85 ± 0.03	Roscovitine 0.1 ± 0.01	Cell lines A-549 MDA-MB-231 HCT-116	IC <sub>50</sub> [μM] Cpd 36 7.3 ± 0.42 4.7 ± 0.28 2.6 ± 0.17	DOX 2.3 ± 0.17 4.5 ± 0.29 3.7 ± 0.24	42	
8		8	Enzymes CDK2	ND	Compound 44 displayed the best docking score of -8.89	Cell lines Panel of cell lines	%Growth inhibition (PGI)/lethality Cpd 44 Ranging from 3%–98%	43		
9		110–113	Enzymes EGFR	110–113	Compound 51 displayed the best docking score of -7.33	Cell lines MDA-MB-231 MDA-MB-468	IC <sub>50</sub> [μM] Cpd 51 8.23 ± 1.87 10.24 ± 1.27	5-FU 10.5 ± 1.2 12.4 ± 1.3	Tamoxifen 23.05 15.29	44





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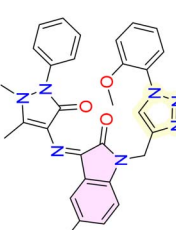
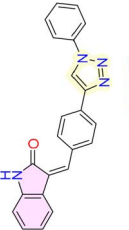
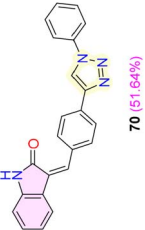
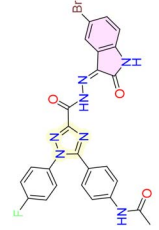
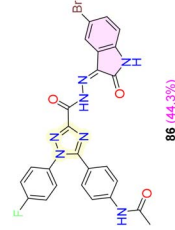
Isatin- 1,2,3- triazole hybrids	 56 (84%)	Enzymes	% Inhibition	Sunitinib	Cell lines	IC <sub>50</sub> [μM]	Sunitinib	5-FU	45
		VEGFR-2	77.6%	67.1%	MCF7 HCT116 PaCa-2	5.361 ± 0.31 12.50 ± 0.88 12.128 ± 0.79	11.304 ± 0.28 9.67 ± 0.84 6.596 ± 0.43	—	
Isatin- 1,2,3- triazole hybrids	 63 (61%)	Enzymes	IC <sub>50</sub> [nM]	Sunitinib	Cell lines	IC <sub>50</sub> [μM]	Sunitinib	Dox	46
		VEGFR2	26.3 ± 0.38	30.70 ± 0.17	PANC1 PC3 WPMY-1 (normal cell)	0.13 ± 0.01 0.10 ± 0.01 >10.0	1.49 ± 0.04 0.60 ± 0.02 >10.0	0.45 ± 0.01 0.24 ± 0.02 >3.0	
Isatin- 1,2,3- triazole hybrids	 70 (51.64%)	Enzymes	IC <sub>50</sub> [nM]	Sunitinib	Cell lines	IC <sub>50</sub> [μM]	Sunitinib	Sunitinib	47
		VEGFR2	26.38 ± 1.09	83.20 ± 1.36	HT-29 MKN-45 HUVECs	1.61 ± 0.45 1.92 ± 0.37 7.94 ± 0.36	10.34 ± 0.96 9.25 ± 0.77 6.37 ± 0.59	—	
Isatin- 1,2,3- triazole hybrids	 74 (80%)	Enzymes	IC <sub>50</sub> [nM]	Sunitinib	Cell lines	IC <sub>50</sub> [μM]	Etoposide	Etoposide	48
		190-192	16.3 ± 0.42	29.7 ± 0.39	A375 MDA-MB231 PC-3 LNCaP HDF (normal cell)	25.91 ± 0.005 18.42 ± 0.002 15.32 ± 0.002 29.23 ± 0.003 >100	24.46 ± 0.019 31.02 ± 0.051 30 ± 0.037 31.21 ± 0.005 >100	—	
Isatin- 1,2,4- triazole hybrids	 86 (44.3%)	Enzymes	IC <sub>50</sub> [nM]	Sorafenib	Cell lines	IC <sub>50</sub> [μM]	DOX	DOX	49
		VEGFR2	16.3 ± 0.42	29.7 ± 0.39	PANC1 HepG2	1.16 ± 0.02 0.73 ± 0.02	0.19 ± 0.01 0.43 ± 0.02	—	



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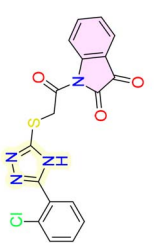
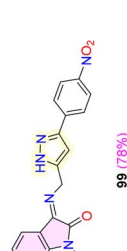
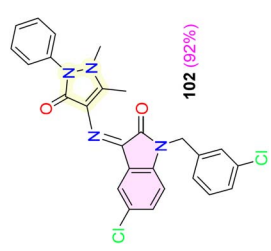
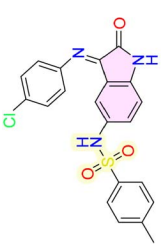
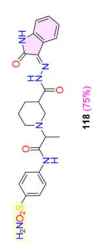
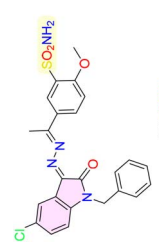
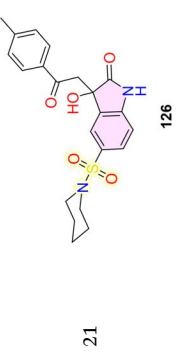
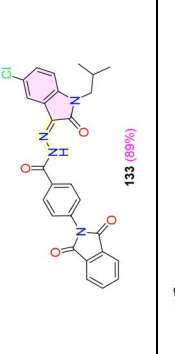
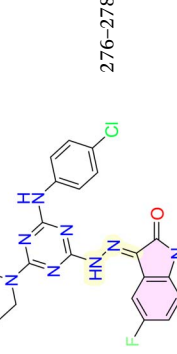
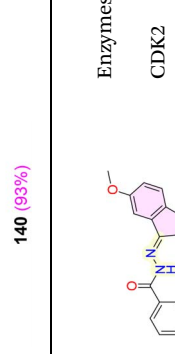

Isatin-1,2,4-triazole hybrids	 <b>94 (85%)</b>	101–102	Enzymes EGFR	Docking study Cpd <b>94</b> Compound <b>94</b> displayed the best docking score of $-9.2$	Cell lines MDA-MB-231	IC <sub>50</sub> [ $\mu$ M] Cpd <b>94</b> 0.73	Tamoxifen citrate 12.88	50,51
Isatin-pyrazole hybrids	 <b>99 (78%)</b>	ND	Enzymes VEGFR-2	IC <sub>50</sub> [nM] Cpd <b>99</b> $16.28 \pm 1.21$	Sorafenib $35.62 \pm 1.52$	Cell lines A549 PC3 MCF-7	5-FU $12.30 \pm 0.48$ $68.48 \pm 1.42$ $13.15 \pm 1.02$	52
Isatin-pyrazole hybrids	 <b>102 (92%)</b>	205–209	Enzymes VEGFR  JNK3	Docking study Cpd <b>102</b> Compound <b>102</b> displayed the best docking score of $-9.9$ Compound <b>102</b> displayed the best docking score of $-9.4$	Cell lines MCF-7 A549  SCOV3 MCF-10A (normal cell)	IC <sub>50</sub> [ $\mu$ M] Cpd <b>102</b> $5.12 \pm 1.34$ $25.5 \pm 1.8$  $12.9 \pm 2.9$ $32.6 \pm 3.1$	Cisplatin $14.9 \pm 2.1$ $12 \pm 1.9$  $18.7 \pm 0.58$ $42.5 \pm 1.7$	53
Isatin-sulphonamide hybrids	 <b>111 (51%)</b>	125–127	Enzymes —	—	Cell lines HepG2 NIH/3T3 (normal cell)	IC <sub>50</sub> [ $\mu$ M] Cpd <b>111</b> $37.81 \pm 5.05$ $324.2 \pm 20.51$	DOX $51.15 \pm 9.9$ $53.8 \pm 6.8$	54
Isatin-sulphonamide hybrids	 <b>118 (75%)</b>	228–230	Enzymes VEGFR2	IC <sub>50</sub> [ $\mu$ M] Cpd <b>118</b> $204 \pm 9$	Sorafenib $41 \pm 2$	Cell lines MDA-MB-231 MCF-7	5-FU $8.704 \pm 0.372$ $5.167 \pm 0.188$	55
Isatin-sulphonamide hybrids	 <b>123 (82%)</b>	245–247	Enzymes VEGFR2	IC <sub>50</sub> [ $\mu$ M] Cpd <b>123</b> $23.10 \pm 0.41$	Sorafenib $29.70 \pm 0.17$	Cell lines T47D	DOX $2.26 \pm 0.10$	56

Table 1 (Contd.)

Isatin-sulphonamide hybrids	21		238-240	Enzymes EGFR	Docking study Cpd 126 Compound 126 displayed the best docking score of -21.74	Cell lines HepG2 Huh7 RPE1 (normal cell)	IC <sub>50</sub> [μM] Cpd 126 16.80 ± 1.44 40.00 ± 2.20 >100	DOX 21.60 ± 0.81 11.60 ± 0.90 —	57
Isatin-hydrazone hybrids	22		270	Enzymes PARP-1	IC <sub>50</sub> [nM] Cpd 133 13.65 ± 1.42	Cell lines MCF-7 HCC1937	IC <sub>50</sub> [μM] Cpd 133 0.67 ± 0.12 0.53 ± 0.11	Olaparib 32.81 ± 2.26 >100	58
Isatin-hydrazone hybrids	23		276-278	Enzymes EGFR Trypsin	IC <sub>50</sub> [μM] Cpd 140 65.34 ± 5.42 75.12 ± 4.32	Cell lines A549 WI-38 (normal cell) Selectivity index	IC <sub>50</sub> [μM] Cpd 140 0.114 ± 0.01 1.458 ± 0.09	Sorafenib 0.195 ± 0.02 2.15 ± 0.01	59
Isatin-hydrazone hybrids	24		>300	Enzymes CDK2 EGFR Her2 VEGFR2	IC <sub>50</sub> [μM] Cpd 144 0.131 ± 0.007 0.103 ± 0.006 0.081 ± 0.002 0.178 ± 0.009	Cell lines HepG2 MCF-7 MDA-MB-231 HeLa	IC <sub>50</sub> [μM] Cpd 144 6.11 ± 0.4 5.93 ± 0.3 2.48 ± 0.1 1.98 ± 0.1	DOX 4.50 ± 0.2 4.17 ± 0.2 3.18 ± 0.1 5.57 ± 0.4	60
Isatin-hydrazone hybrids	25		>300	Enzymes CDK2 EGFR Her2 VEGFR2	IC <sub>50</sub> [μM] Cpd 147 0.534 0.143 0.15 0.192	Cell lines HepG2 MCF-7 MDA-MB-231 HeLa	IC <sub>50</sub> [μM] Cpd 147 9.61 ± 0.8 10.78 ± 0.9 14.89 ± 1.2 8.93 ± 0.8	Sunitinib 6.82 ± 0.5 5.19 ± 0.4 8.41 ± 0.7 7.48 ± 0.6	61

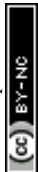




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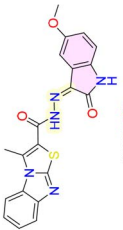
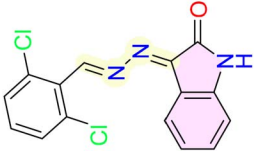
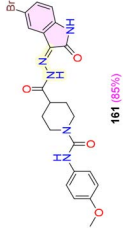
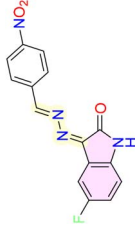
 <b>153 (84%)</b>	Enzymes CDK2	IC <sub>50</sub> [nM] Cpd <b>153</b> 26.24 ± 1.4	Staurosporine 38.5 ± 2.1	Cell lines MDA-MB-231 MCF-7	IC <sub>50</sub> [μM] Cpd <b>153</b> 3.30 ± 0.21 5.82 ± 0.32	Staurosporine 4.29 ± 0.72 3.81 ± 0.22	62		
	Enzymes CDK2	IC <sub>50</sub> [μM] Cpd <b>156</b> 0.2456	Imatinib 0.1312	Cell lines MCF7 A2780	IC <sub>50</sub> [μM] Cpd <b>156</b> 1.51 ± 0.09 26 ± 2.24	DOX 3.10 ± 0.29 0.20 ± 0.03	63 and 64		
 <b>156 (98%)</b>	Enzymes VEGFR2	IC <sub>50</sub> [nM] Cpd <b>161</b> 45.9	MCF-7 MDA-MB-468	IC <sub>50</sub> [μM] Cpd <b>161</b> 8.00 ± 0.76 1.03 ± 0.03	Tamoxifen 8.36 ± 0.90	Sorafenib 4.75 ± 0.56	Staurosporine — 5.93 ± 0.16	65	
	Enzymes CDK2	Enzymes VEGFR2	Enzymes VEGFR2	Cell lines MCF-7 MDA-MB-468	Sorafenib 48.6	—	—	—	66
 <b>162 (95%)</b>	Enzymes VEGFR2	Enzymes VEGFR2	Enzymes VEGFR2	Enzymes VEGFR2	Enzymes VEGFR2	Enzymes VEGFR2	Enzymes VEGFR2	Enzymes VEGFR2	Enzymes VEGFR2
	Enzymes EGFR	Enzymes EGFR	Enzymes EGFR	Enzymes EGFR	Enzymes EGFR	Enzymes EGFR	Enzymes EGFR	Enzymes EGFR	Enzymes EGFR
 <b>164 (85%)</b>	Enzymes EGFR	Enzymes EGFR	Enzymes EGFR	Enzymes EGFR	Enzymes EGFR	Enzymes EGFR	Enzymes EGFR	Enzymes EGFR	Enzymes EGFR
	Enzymes EGFR	Enzymes EGFR	Enzymes EGFR	Enzymes EGFR	Enzymes EGFR	Enzymes EGFR	Enzymes EGFR	Enzymes EGFR	Enzymes EGFR

Table 1 (Contd.)

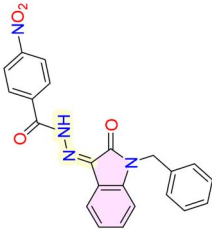
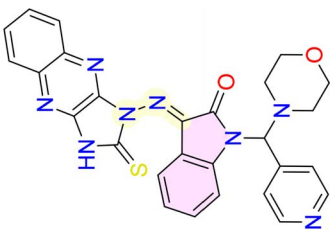
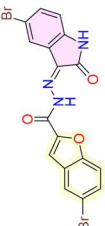
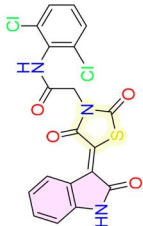
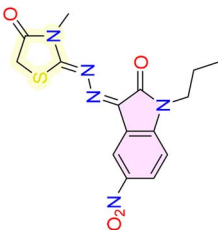
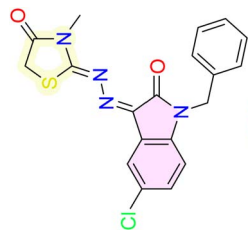
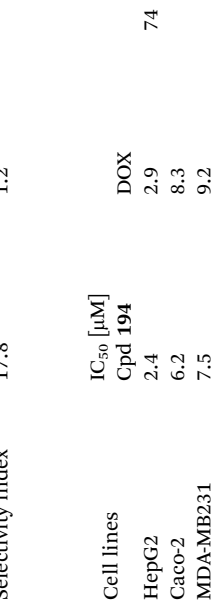
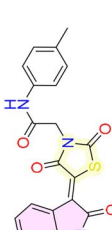
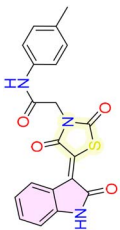
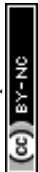
Isatin-hydrazone hybrids	 <b>166 (85%)</b>	174–176	Enzymes EGFR	Docking study Cpd <b>166</b> Compound <b>166</b> displayed the best docking score of –7.561	Cell lines MDA-MB231 MCF-10A	IC <sub>50</sub> [μM] Cpd <b>166</b> 15.8 ± 0.6 >50	68	
		31						
Isatin-hydrazone hybrids	 <b>172 (74%)</b>	>350	Enzymes —	Cell lines MGC-803 MCF-7 CNE2 KB	IC <sub>50</sub> [μM] Cpd <b>172</b> 9.7 ± 1.1 9.6 ± 1.2 9.5 ± 1.1 10.1 ± 1.2	5-FU 10.7 ± 1.2 10.5 ± 1.1 10.3 ± 1.3 10.1 ± 1.1	69	
		32						
Isatin-benzofuran hybrid	 <b>177 (87%)</b>	>300	Enzymes CDK2 GSK-3β	Stauroporine 38.5 ± 2.1 43.38 ± 2.4	IC <sub>50</sub> [nM] Cpd <b>177</b> 37.77 ± 2.1 32.09 ± 1.7	IC <sub>50</sub> [μM] Cpd <b>177</b> 3.41 ± 0.10 3.82 ± 0.12	Stauroporine 4.81 ± 0.14 4.34 ± 0.14	70
		33						
Isatin-thiazolidine hybrids	 <b>181 (89%)</b>	166–168	Enzymes VEGFR2	Sorafenib 53.65	IC <sub>50</sub> [nM] Cpd <b>181</b> 76.64	IC <sub>50</sub> [μM] Cpd <b>181</b> 5.40 ± 0.14 0.58 ± 0.01 14.45 ± 0.07 0.94 ± 0.05 0.38 ± 0.03	Stauroporine 4.81 ± 0.14 4.34 ± 0.14	71
		34						



Table 1 (Contd.)

 <b>35</b>	193–196	Enzymes CDK1	IC <sub>50</sub> [μM] Cpd <b>187</b> 0.04 ± 0.38	DOX 0.07 ± 0.42	Cell lines HepG2 MCF7 HCT-29	IC <sub>50</sub> [μM] Cpd <b>187</b> 4.97 ± 0.3 5.33 ± 0.4 3.29 ± 0.2	DOX 4.50 ± 0.2 4.17 ± 0.2 4.01 ± 0.4	72	
	 <b>187 (69%)</b>		Enzymes CDK2	IC <sub>50</sub> [nM] Cpd <b>189</b> 27.42	Sunitinib 23.8	Cell lines HepG2 MCF7 HCT-29 WI-38 (normal cell)	IC <sub>50</sub> [μM] Cpd <b>189</b> 3.0 ± 0.92 5.19 ± 1.15 3.10 ± 0.96 67.01 ± 5.78	DOX 4.15 ± 0.5 4.61 ± 1.1 4.65 ± 0.6 5.05 ± 0.7	73
		 <b>189 (37%)</b>	258–260	Enzymes VEGFR2	IC <sub>50</sub> [nM] Cpd <b>194</b> 51.3	Sorafenib 53.8	Cell lines HepG2 Caco-2 MDA-MB231 Vero (normal cell)	IC <sub>50</sub> [μM] Cpd <b>194</b> 2.4 6.2 7.5 27.5	DOX 2.9 8.3 9.2 25.2
 <b>194 (67%)</b>			222–224	Enzymes VEGFR2	IC <sub>50</sub> [nM] Cpd <b>197</b> 116.3	Sorafenib 53.65	Cell lines Caco-2 HepG2 MDA-MB-231 Vero (normal cell)	IC <sub>50</sub> [μM] Cpd <b>197</b> 2.0 ± 0.005 10 ± 0.001 40 ± 0.002 730 ± 0.015	DOX 3.46 ± 0.003 1.15 ± 0.02 0.98 ± 0.01 —
	 <b>197 (79%)</b>		271–272	Enzymes VEGFR2	IC <sub>50</sub> [nM] Cpd <b>197</b> 116.3	Sorafenib 53.65	Cell lines Caco-2 HepG2 MDA-MB-231 Vero (normal cell)	IC <sub>50</sub> [μM] Cpd <b>197</b> 2.0 ± 0.005 10 ± 0.001 40 ± 0.002 730 ± 0.015	DOX 3.46 ± 0.003 1.15 ± 0.02 0.98 ± 0.01 —





## 5. Conclusion and expert opinion

In medicinal chemistry, isatin has recently come to light as an incredibly adaptable scaffold that provides a one-of-a-kind platform for the design of novel bioactive agents. It is highly favored in the field of drug development due to its structural simplicity, ease of functionalization, and wide range of biological activities. Hybrid compounds, such as isatin–heterocycles, are routinely generated using this scaffold. These molecules frequently exhibit improved selectivity and efficacy through specific target signaling pathways. Furthermore, it is critical to reveal innovative methods for producing this scaffold, to analyze the different strengths of that heterocycle, and to investigate potent applications for isatin.

Isatin can promote cell death in different types of cells and alter the expression of genes associated with cell death since it is a potent inhibitor of many enzymes and receptors. Because of the advantages of hybrid compounds in terms of efficiency, selectivity, and resistance to drug resistance, hybridization is an attractive strategy for drug discovery. The chemical and pharmacological characteristics of isatin–heterocycle hybrids are attracting attention towards novel chemotherapeutics. Various isatin analogs have been synthesized, and their bioactivities have been evaluated as lead drugs. As a future perspective, nano-formulations, drug delivery systems with innovative drug signaling pathways, will be further recommended to improve bioavailability and targeted delivery, particularly in solid tumors, to lead to the development of novel, potent anticancer medicines.

## Conflicts of interest

The authors declare that they have no financial or personal interests.

## Data availability

No primary research results, and no new data were generated or analysed as part of this review.

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