SYNTHETIC APPROACHES OF ISATIN DERIVATIVES AS AN ANTI-CANCER AGENT

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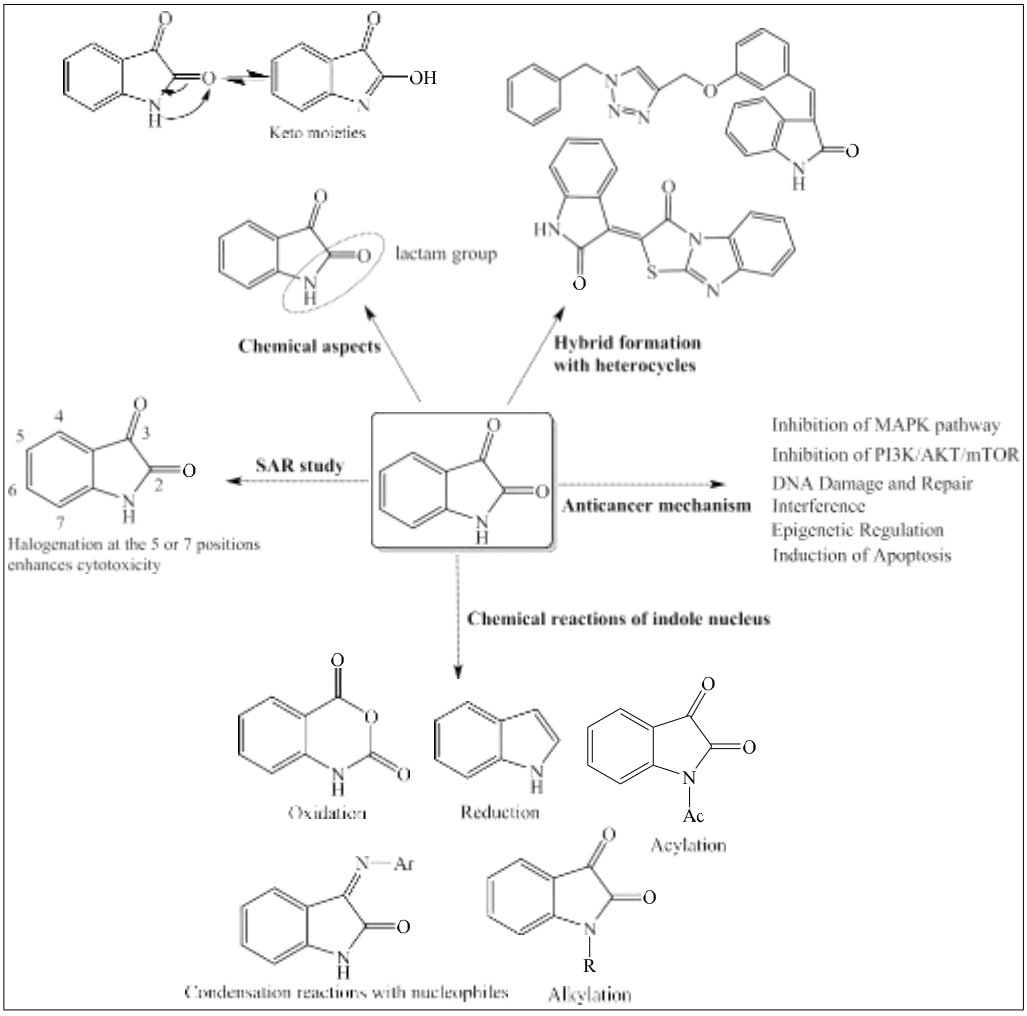
ABSTRACT
Isatin (1H-Indole-2,3-dione) and its derivatives are an important class of Heterocyclic compounds and are majorly used as a Precursor for drug Synthesis. Since its discovery, so many research work has been done for synthesis, chemical & biological activity, and also its industrial applications. In this, we have reported several novel derivatives of N-, C-2 & C-3 substituted isatin For pharmacological activity. Here, in this study modification at C-2 and/ or C-3 carbonyl functionalities, leads to novel isatin derivatives as an anticancer activity. N- substituted novel derivatives are effective inhibitors of Carbonic anhydrase isoform IX, the form which is found to be over-expressed in a large number of solid tumors. Here also A wide range of C3-substituted isatins, like thiosemicarbazones, oxindoles, and their derivatives, imines, and hydrazones, have been synthesized. We have also summarized some recently reported biological activities exhibited by isatin derivatives, anticancer, anti-bacterial, anti-diabetic, and others. Special attention has been paid to their anti-cancer activity, and various anti-cancer targets such as histone deacetylase, carbonic anhydrase, tyrosine kinase, and tubulin against a variety of human cancer cell lines have been discussed in detail.

Graphical Abstract



Introduction:

Heterocyclic entities are denoted as a vital class of organic compounds with wide biological and pharmacological potency [1,2]. In view of wide-ranging biological activities, these heterocyclic analogues, either individually or as fused forms, are consistently utilized by scientists and research groups for the design of the novel candidates [3]. Among them, isatin, or (1*H*-indole-2,3-dione), has a wide spectrum of pharmacological potential and is thus appraised as a fortunate bioactive heterocyclic moiety. The isatin molecule is comprised of six- and five-membered cyclic planar rings; in contrast, the six-member ring has an aromatic character while the five-member ring possesses antiaromatic properties. Isatin was first brought to light by L. Erdman and A. Laurent in 1841 from the oxidation of indigo dye by nitric and chromic acids furnished as orange monoclinic crystals [4]. This isatin was considered a synthetic moiety for almost 140 years until it was isolated from the plants of *Isatis genus* [5], *Calanthe discolor* LINDL [6], the fruit of the cannon ball tree *Couroupita guianensis* Aubl [7], as a constituent of the secretion from the parotid gland of Bufo frogs [8], and as metabolic derivatives of adrenalin in humans [9]. Moreover, isatin was found to be a component of coal tar while its derivatives fall out in

variable dye, pharmaceuticals, and agriculture chemicals [7]. Nowadays, isatin has achieved a position in the design and development of medicinally active analogues because of its fortunate electronic nature. The possible substitutions for isatin hybrids are depicted in Figure 1.

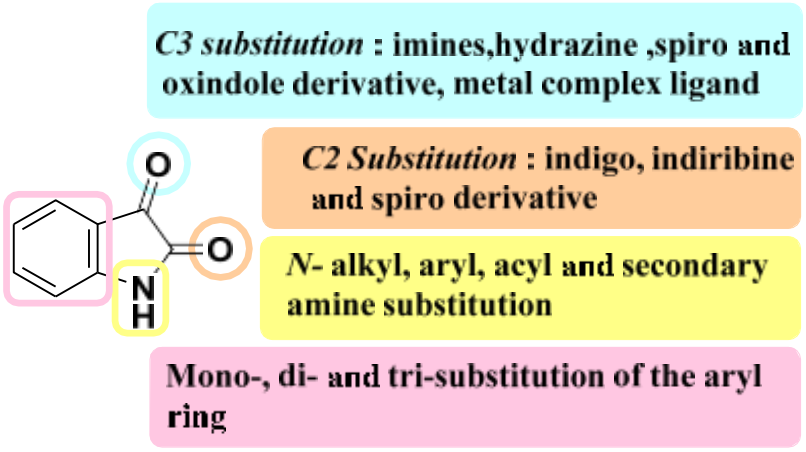


Figure 1. Probable substitution possible on isatin nucleus.

Isatin (1*H*-indole-2,3-dione) and its derivatives represent an important class of heterocyclic compounds that can be used as precursors for drug synthesis. Since its discovery, a lot of research work has been done regarding the synthesis, chemical properties, and biological and industrial applications of isatin. In this review, we have reported several novel methods for the synthesis of *N*-C2- and C3-substituted and spiro derivatives of isatin. The isatin moiety also shows important chemical reactions such as oxidation, ring expansion, Friedel–Crafts reaction and aldol condensation. These reactions, in turn, produce several biologically viable compounds like 2-oxindoles, tryptanthrin, indirubins, and many more. We have also summarized some recently reported biological activities exhibited by isatin derivatives, like anti-cancer, anti-bacterial, anti-diabetic and others. Special attention has been paid to their anti-cancer activity, and various anti-cancer targets such as histone deacetylase, carbonic anhydrase, tyrosine kinase, and tubulin have been discussed in detail. Other applications of isatin derivatives, such as in the dye industry and in corrosion prevention, have also been discussed[9,10].

Synthesis outlines Of Isatin Scaffolds:

1) Sandmeyer Synthesis

The Sandmeyer methodology is the oldest and straightforward way for the synthesis of isatin. The method involves the condensation between chloral hydrate and a primary arylamine (e.g. aniline), in the presence of hydroxylamine hydrochloride, in aqueous sodium sulfate to form an α-isonitrosoacetanilide. Isolation of this intermediate and

subsequent electrophilic cyclization promoted by strong acids (e.g. sulfuric acid) furnishes isatin in >75% yield.[5]

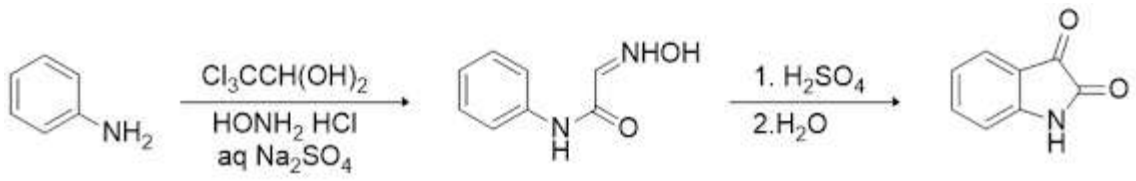


Figure 2. Sandmayer Synthesis.

2) Stolle methodology

The Stolle procedure is considered the best alternative to Sandmeyer methodology for the synthesis of both substituted and unsubstituted isatins. In this case primary or secondary arylamines are condensed with oxalyl chloride to form a chlorooxalylanilide intermediate which can then cyclize in the presence of a Lewis acid (e.g. aluminium trichloride, titanium tetrachloride, boron trifluoride, *etc*.).[5]

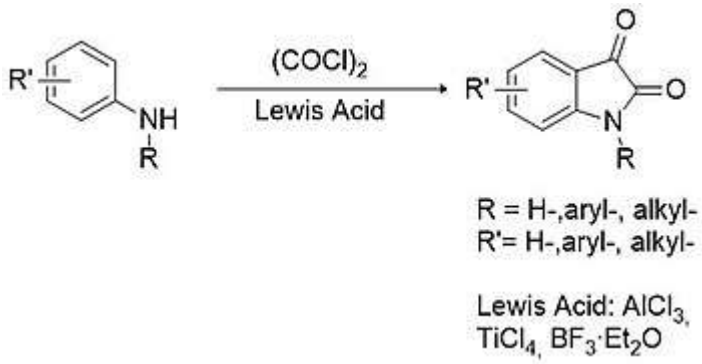


Figure 3. Stolle Synthesis

Other procedures

More recent approaches to the synthesis of N-substituted isatins involves the direct oxidation of commercially available, substituted indoles or oxindoles with different oxidizing agents such as TBHP, IBX-SO3K, tBuONO etc.[11]

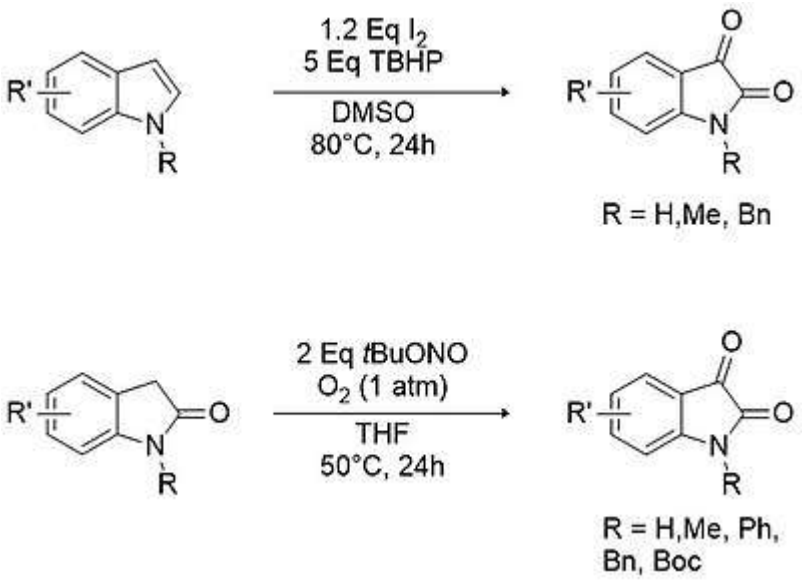


Figure 4. N- Substituted Isatin Synthesis

C3-Substituted Isatins Synthesis:

C3-substituted isatins like thiosemicarbazone, oxindole and their derivatives, imines and hydrazones, have been synthesized. Among these derivatives, the 3-ylideneoxindole constitutes an important part of many pharmaceutical essential compounds.

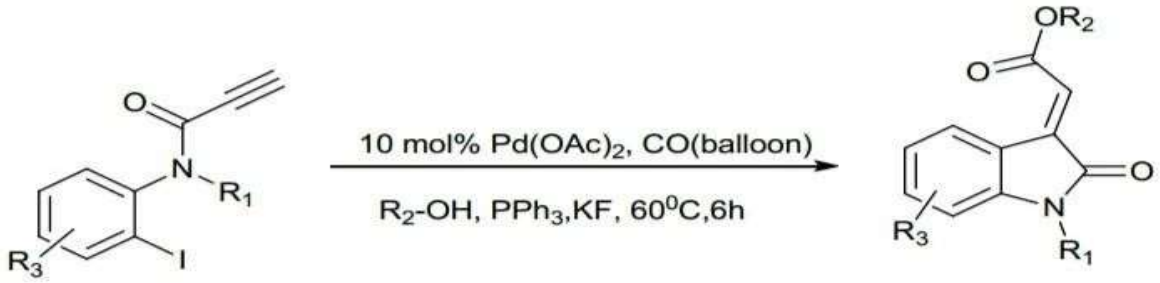


Figure 5. C-3 Substituted Isatin Derivatives

Reactivity

The presence of an aromatic ring, a ketone and a γ-lactam moiety, gives to isatin the rare potential to be used as both an electrophile and a nucleophile: indeed, it undergoes an enormous number of reactions, such as N-substitutions, electrophilic aromatic substitution at positions C-5 and C-7 of the phenyl ring, nucleophilic additions onto the C-3 carbonyl

group, chemoselective reductions, oxidations, ring-expansions and spiro-annulations. Because of this unique reactivity, isatin is considered one of the most valuable building blocks in organic synthesis.

N-Substitution

The N-functionalization of the isatin core can be readily obtained by the deprotonation of the amino moiety, forming the corresponding sodium or potassium salt, and subsequent addition of an electrophile (e.g. alkyl or acyl halides).[11]

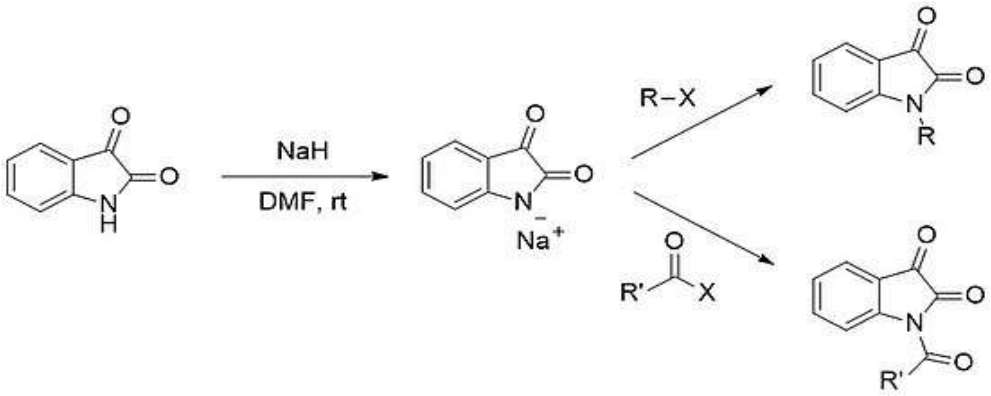


Figure 6. N-Substitution Reaction

On the other hand, N-arylation is usually achieved by cross-coupling reactions with aryl halides using copper al palladium catalysts.

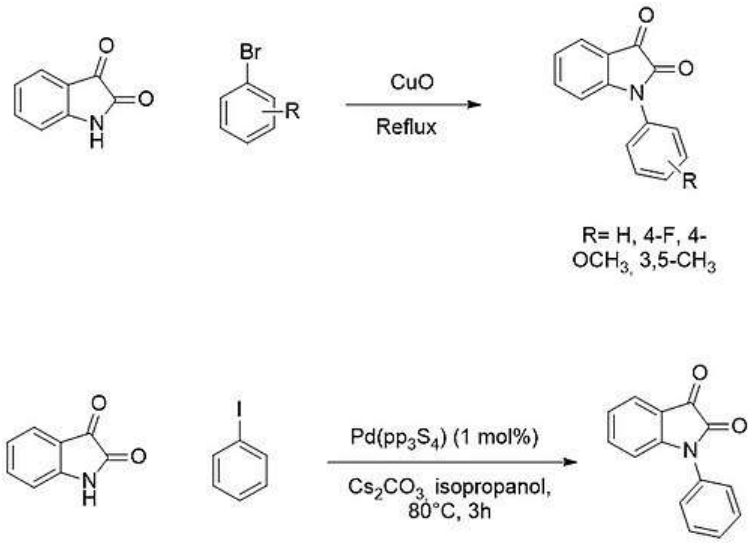


Figure 7. N-Arylation Reaction

Ring expansion

In the field of organic synthesis, ring expansions are considered valuable reactions since they allow the obtainment medium-size ring (7-9 atoms) which are difficult to synthesize through "classical" methods.

To date, only few articles concerning the ring expansion of isatin derivatives has been reported. The first one is an acid-catalyzed one-pot multicomponent reaction involving isatins, aminouracils, and isooxazolones to form isoxazoquinolines, important scaffolds in medicinal chemistry.

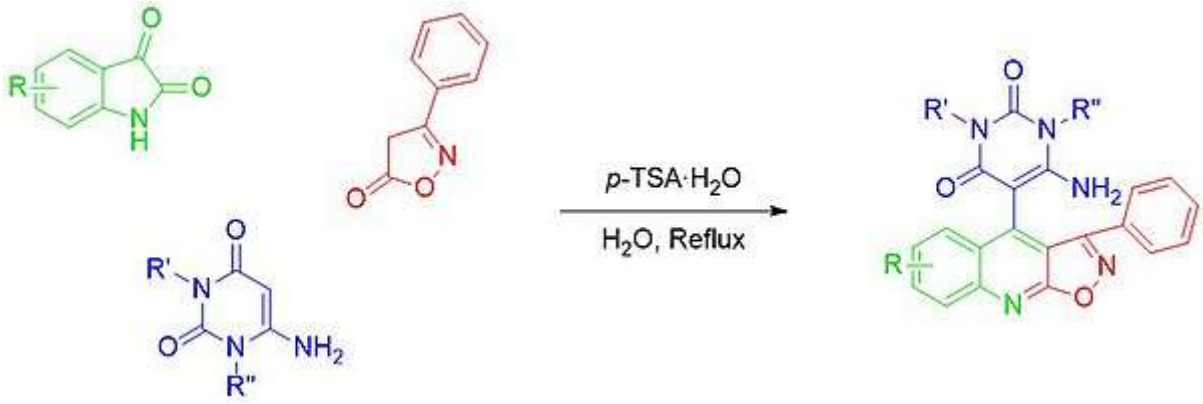


Figure 8. Ring Expansion Of Isatin Derivatives

In another one-pot multicomponent reaction, a unique two-carbon expansion has been achieved by reacting isatin with indene-1,3-dione and N-substituted pyridinium bromide to form dibenzo[b,d]azepin-6-ones.

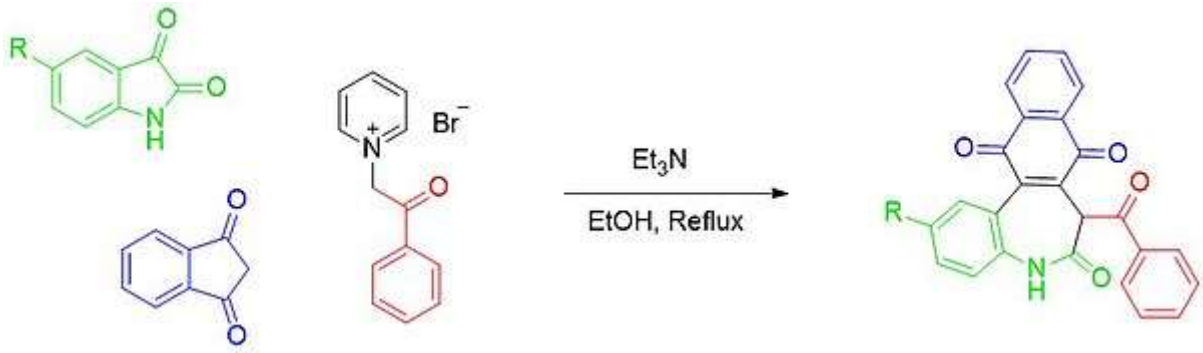


Figure 9. One Pot multicomponent reaction

C-2/C-3 nucleophilic addition

Isatin suffers nucleophilic addition on carbonyls at *C-2* and *C-3* positions.
The regioselectivity of the process strongly depends both on the substrate (properties of the substituents on the isatin core, especially those bonded to the nitrogen atom) and the reaction conditions (solvent, temperature etc.). In some cases the nucleophilic addition could be followed by secondary reactions (*e.g.* cyclization, ring expansion, ring opening *etc.*)

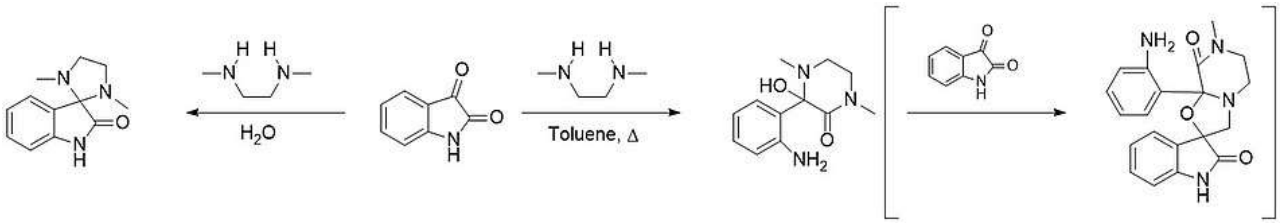


Figure 10. C-2 & C-3 Nucleophilic addition Reaction

Oxidation

The oxidation of isatin using hydrogen peroxide or chromic anhydride yields isatoic anhydride, a compound widely used either in herbicide products and in medicinal chemistry.

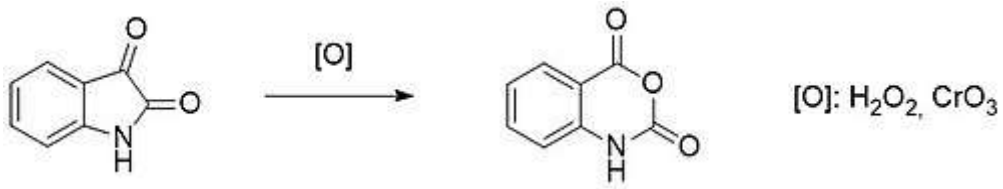


Figure 11. Oxidation Reaction of Isatin

Dimerization

Dimerization of isatin with KBH_4 in methanol yield Indirubin. This represent the indigo pigment's red component and a highly effective cytotoxic compound.

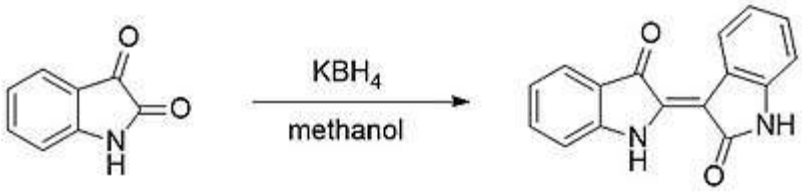


Figure 12. Dimerization of Isatin

Reduction

Reduction of the non-amide carbonyl group obviously occurs to give oxindole, respectively.[12]

Mechanism of anti-cancer action of isatin

Since it has so many different biological actions, isatin, a multifunctional indole scaffold, has attracted a lot of interest in cancer research. Isatin's potential as an anti-cancer drug was realized after it was first

shown to have broad-spectrum antibacterial and antiviral qualities. The cytotoxic effects of isatin and its derivatives on different cancer cell lines have been emphasized by a number of researches over the years [20].

Given their varied structural changes and biological activity, investigating the anti-cancer processes of isatin derivatives necessitates a multipurpose approach. Isatin produces its antineoplastic actions through numerous mechanisms, including the induction of apoptosis, cell cycle arrest, inhibition of angiogenesis, modulation of key signalling paths, inhibition of topoisomerases, and generation of ROS. This multi-targeted approach makes isatin and its scaffolds promising candidates for cancer treatment. Isatin scaffolds also induce cell cycle arrest at specific checkpoints. It prevents cancer cell proliferation. The possible mechanisms at play are broken down in detail here (Fig. 13) [21].

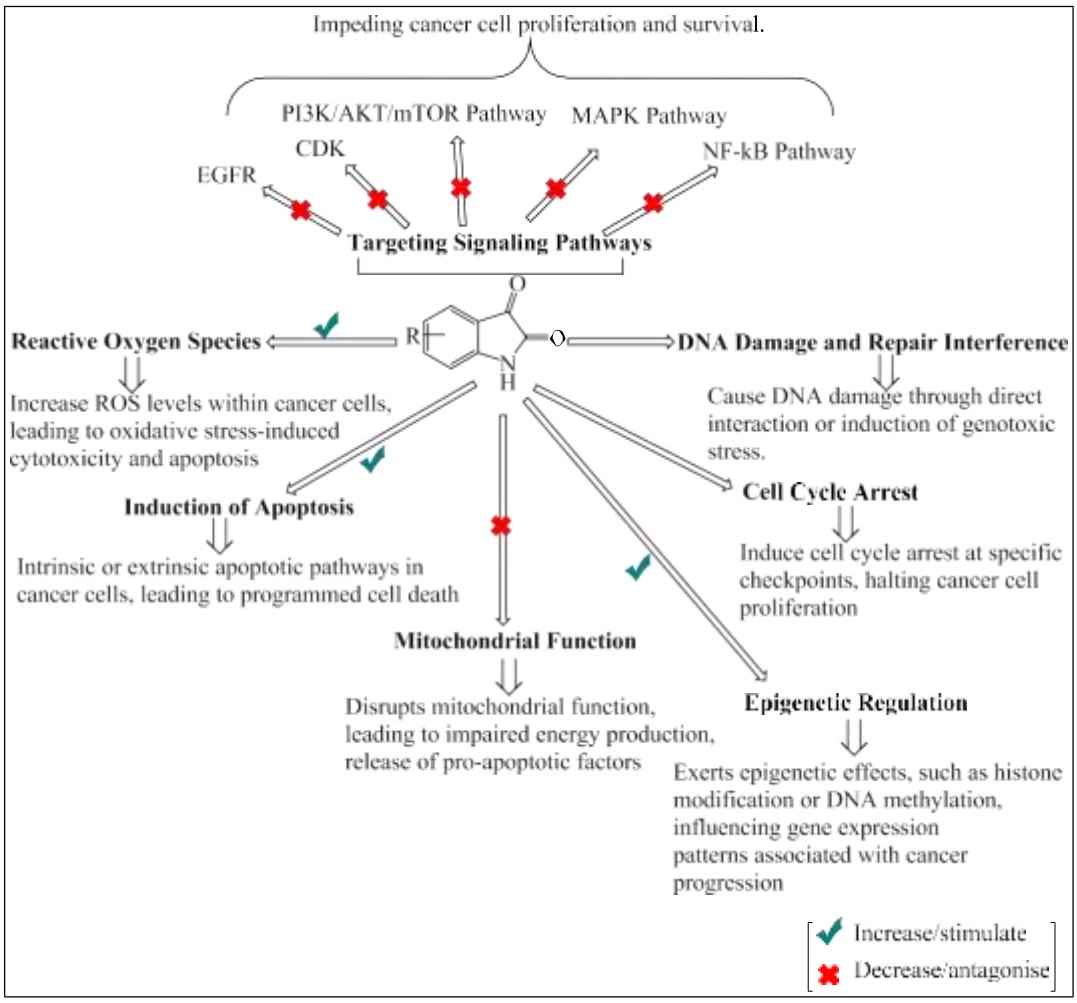


Fig. 13 Isatin's anti-cancer mechanism

Isatin as antineoplastic agents:

Several isatin hybrids were produced, and their anti-cancer efficacy was evaluated by Altamimi M et al. [26]. When treated with a concentration of 10 mM as given, some of the produced compounds demonstrated excellent anti-proliferative action. The series' chemicals' inhibitory activity suggests a weak form of inhibition with the enzyme. Additionally, the action must be mostly limited to molecules with favoured groups linked to the isatin moiety. **Compounds 1, 2, 3, 4, and 5 (**Fig. 14**)** for instance, showed exceptional efficacy against a kidney cancer cell line UO-31.

Mushtaq A and co researchers have generated isatin mono- and bis-thiosemicarbazones with good yield [27]. The spectral data of each synthesized chemical is used to describe it. Using docking

experiments, the inhibitory potential of the chosen drugs against the phosphoinositide 3-kinase (PI3K) anti-cancer protein's active sites was investigated. **Compound 6** exhibited the highest binding energy of − 10.3 kcal mol − 1 among the produced derivatives. These results showed that mode of inhibition against PI3K signalling pathways could be employed as a preferred chemotherapeutic scaffold.In their study, Oguz A et al. [28] investigated the anti-cancer potential of newly developed lower rim-functionalized calixarenes integrated with isatin, as well as the p-position of calixarenes with 1,4-dimethylpyridinium iodine against a range of human cancer cell lines, including the PNT1A healthy epithelial cell line and the MCF-7 and MDA-MB-231 cell lines.
According to their outcomes the **compound 7** had the lowest values in MDA-MB-231 (3.32 µM) and MCF-7 (8.83 µM). Using confocal microscopy and flow cytometry, respectively, examinations of apoptotic activity and cell imag- ing were conducted. To identify chemicals in cells that have damaged DNA, the Comet assay test was employed. Compar- ing treated and untreated cells, it was discovered that the former had broken DNA structures and aberrant tail nuclei. **Compound 7** demonstrated a notable inhibitory impact on aromatase, as demonstrated by in vitro human aromatase enzyme inhibition profiles. As seen by its IC_{50} value of 0.104 ± 0.004 µM. Thus, it has been demonstrated that the novel fluorescent compounds that are the focus of this investigation exhibit both aromatase inhibitory characteristics and anti-cancer potential.

Farshid H et al. created N-alkyl-isatin-3-imino aromatic amine derivatives by alkylation and condensation processes [29]. The final compounds' cytotoxic examination showed that the type of substitution in isatin's N1 region appears to have an impact on the cytotoxic activity. Electron-withdrawing group substitutions are essential for the cytotoxic effects they provide. The MCF-7 is more vulnerable to **compounds 8 and 9**, according to the IC_{50} values of the examined compounds.

Raju and his colleagues produced a distinctive family of isatin hybrids [30]. By using the in vitro MTT test, the antineo- plastic action of the synthesized scaffolds (**compound 10 and 11**) was assessed against the human breast cancer cell line (MCF-7). The most active of the examined compounds was determined to be **compound 10** (Fig. 14), which had a benzyl moiety at N4 piperazine and a promising IC_{50} (12.47 mM). Chalcone is the organic compound which is an α,β-unsaturated ketone. Certain chalcones may offer medicinal promise for treating a variety of disorders, according to studies. Chalcones are aromatic ketones and enones with well-established anti-cancer properties [31]. Chalcone containing isatin nucleus was described as anti-cancer scaffolds by Cahyana AH et al. [32]. Testing of the synthesized compounds' efficacy against MCF-7 cancer cells has been done. **Compounds 12 and 13** have considerable activity against MCF-7 cancer cells when compared to another product, as indicated by the measured IC_{50} value.

Shaldama MA et al. synthesized new isatin-based sulphonamides as possible dual vascular endothelial growth factor receptor-2 (VEGFR-2) and carbonic anhydrase (CA) antagonists with antineoplastic activities [33]. Target isat- ins' in vitro anti-cancer effects were initially investigated against 58 tumor cell lines (NCI-USA panel). The results showed that target isatins had the greatest impact on the breast cancer subpanel. In particular, **compounds 14, 15, 16, 17, 18, 19, and 20** efficiently suppressed the development of T47D cells. Next, the isatins' IC_{50} values (IC_{50} range: 1.83–24.13 mM) for T47D cells were ascertained. After that, the inhibitory effects of isatins 14, 16, 17, and 19 on VEGFR-2 and carbonic anhydrase were assessed. The target isatin sulphonamides were able to inhibit VEGFR-2 (IC_{50} range: 23.10–63.40 nM) potently. However, in contrast to expectations, they were unable to antagonise the CA isoforms (KI > 100 mM). This could be attributed to steric hindrance by the adjacent methoxy group.

Using *Annona muricata* L leaf extract and the sol–gel method, Cahyana AH et al. [32] created Cu/NiO nanoparticles that were then utilized in the manufacture of isatin-chalcone congeners. Taking 5% mmol of these nanoparticles, the Claisen-Schmidt condensation process is used in the reflux technique to synthesize isatin based on chalcone, yielding good yields for each product. It was noted how well the six molecules worked against MCF-7 cancer cells. **Compounds 21** (0.00157 µg/ml) and **22** (25.4521

µg/ml) exhibit potent action against MCF-7 cancer cells in comparison to another product, as indicated by the measured IC_{50} value.

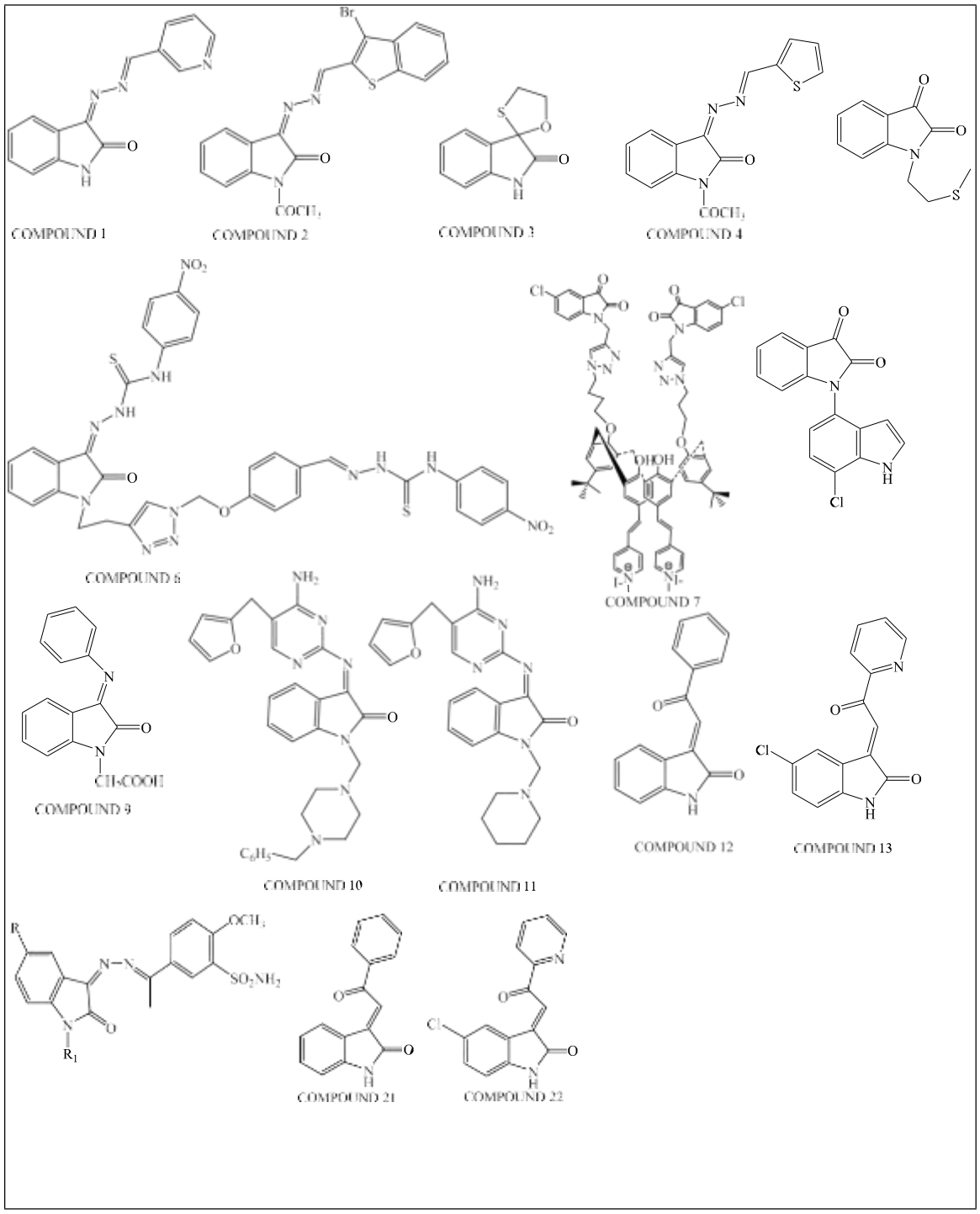


Fig. 14 Synthesized isatin scaffolds as anti-cancer agents (compound 1–22).

Conclusion

As a promising new avenue for the development of anti-cancer scaffolds, isatin, and its hybrid congeners provide a variety of structural frameworks that can be optimized for increased biological activity. The present explanatory review has elucidated the many properties of isatin derivatives, demonstrating their capacity to engage with multiple biological targets, consequently impeding the growth of cancer cells, triggering programmed cell death, and preventing their spread.

Isatin-derived compounds are highly versatile due to their structural simplicity, which permits considerable chemi- cal modification. Their overall efficacy against a variety of cancer types can be improved by these alterations, as well as their pharmacokinetic qualities and target specificity. The therapeutic potential is further increased by hybrid scaffolds, which combine isatin moieties with other bioactive substances. This often produces synergistic effects that are more effective than single-agent therapies.

Understanding the processes by which isatin derivatives exert their anti-cancer properties has advanced significantly. Among them include interactions with vital enzymes, receptors, and signalling pathways that facilitate the advancement of cancer. Furthermore, research on the structure–activity relationship (SAR) has yielded important information to design more efficient isatin-based antineoplastic scaffolds.

Despite these developments, a number of obstacles still exist. Key areas that need more research are the emergence of resistance, possible toxicity, and the requirement for targeted delivery systems. Subsequent research endeavours ought to concentrate on refining the pharmacological characteristics of isatin hybrids, thereby enhancing their specificity towards cancer cells and reducing any unfavourable impacts on healthy tissues.

In summary, isatin provides a strong platform for the creation of anti-cancer drugs, as do its hybrid scaffolds. Prolonged investigation and creativity in this area may yield new anti-cancer scaffolds that will be useful in the fight against cancer, enhance patient outcomes, and possibly even result in the treatment of other cancers. Moving these chemicals from the laboratory to the bedside will need the integration of interdisciplinary techniques, such as medicinal chemistry, molecular biology, and pharmacology.

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