1.1 Introduction

Isatin (1H-indole-2,3-dione) (1) was first discovered in 1841 by Erdmann and Laurent from nitric and chromic acid promoted oxidation of indigo. For almost 140 years, the compound was considered synthetic until it was found in plants belonging to genus Isatis, Calanthe discolor LINDL., Couroupita guianensis Aubl., fruits of the cannon ball tree and in secretions from the parotid gland of Bufo frog. It is also found in humans as the adrenaline metabolic derivative. Substituted isatins have also been identified in plants for example, the Caribbean tumorigenic plant known as Melochia tomentosa containing methoxy phenylpentyl isatin has been obtained, 6-(3'-methylbuten-2'-yl) isatin obtained from Streptomyces albus and 5-(3'-methylbuten-2'-yl) isatin from Chaetomium globosum, some from symbiotic bacteria and marine molluscs, where they play a defensive role against pathogenic organisms.

![Figure 1. General structure of Isatin](image)

In medicinal chemistry, isatin is considered as a privileged scaffold with broad spectrum of medicinal properties and vast possibility to undergo chemical transformation. It is one of the most promising heterocyclic scaffolds, with anti-HIV, anti-tumor, anti-fungal, anti-angiogenic, anti-convulsant, and anti-Parkinson’s activities. It is an effective SARS coronavirus 3CL protease inhibitor with well tolerance in human subjects. Isatin conjugates have also been testified for their anti-viral potencies especially for their activities against vaccinia virus, pox virus, rhino virus, sars virus and moloney leukemia virus. The synthetic versatility of isatin is due to the presence of an extremely reactive C-3 carbonyl functionality which upon spiroannulation or nucleophilic addition allows easy transformation into 2-oxoindole derivatives. A plethora of biologically active C3-substituted indole-2,3-dione have been reported in the literature, due to the susceptibility of isatin to undergo nucleophilic addition by various nucleophiles at C-3 position. The C3-substituted indole-2,3-diones such as indirubin and its derivatives such as 5-bromoindirubin, indirubin-3'-oxime and 5-bromoindirubin-3-oxime were
found to have potent anticancer activity with excellent inhibition of tyrosin kinases CDK2, CDK5 and GSK. Analysis of the structure activity relationships (SAR) in isatin derivatives disclosed that 5-halogenation, N-alkylation, 3-thiosemicarbazone formation and N-Mannich base were effective in improving potency against various fungi, virus and bacteria. Moreover, cyclization of 1H-indole-2,3-diones to the 4-thiazolidinone, 4-thiazoline, and pyridazinoindole was efficient in improving its antimicrobial activity. Thus, a large number of structurally diverse isatin-based compounds have been reported derived either from mono-, di- and tri-substitution of the aryl ring, and/or those obtained by derivatization of the nitrogen and C-2/C-3 carbonyl moieties (Figure 2).

**Figure 2.** Various possible substitution types and patterns

1H-indole-2,3-diones containing arylidene functionality at C-3 position such as SU5402, SU6668 and SU14813 showed significant cytotoxic activity. The 2-oxoindoles compounds such as SU-5416 (Semaxanib 2, Figure 3) and SU-11248 (Sunitinib 3, Figure 3) exhibited tyrosine kinase inhibitory activity and considered as the first-line treatment for gastrointestinal stromal cancers and renal cell carcinoma. Moreover, the structurally relevant SU9516 (4, Figure 3) was also reported to inhibit cyclin-dependent kinases (CDKs) which induces apoptosis in the cells of colon carcinoma.

**Figure 3.** Pharmacologically active 2-oxindole-containing compounds.

Molecular hybridization is an emerging approach in drug discovery that involves the synthesis of innovative chemical scaffolds by the fusion of either two
drugs, pharmacophoric units obtained from known bioactive compounds with different mechanisms of action and/or both active compounds. Pharmacophore hybridization is supposed to be equivalent to the well-known combination therapy. The choice of two moieties in dual drug is generally based on their anticipated synergistic or additive pharmacological properties to assist the recognition of extremely active and innovative chemical scaffolds. In recent years, the growing efforts to discover hybrid drugs resulting from the combination of pharmacophoric moieties of different known lead compounds have brought a new hope for the treatment of multi-factorial diseases. Recently, this approach has been used in the discovery of new anti-alzheimer, anti-cancer, anti-viral and anti-malarial agents.

The initial step in drug designing via molecular hybridization involves identification of the molecular recognition pattern of pharmacophoric subunits present in the two or more biologically active molecules, followed by their fusion in the molecular architecture of the hybrid compound combining pre-selected characteristics of the original templates. A significant application of molecular hybridization is the discovery of active frameworks which can prevent drug resistance by connecting bioactive moieties into molecules that are recognized and then transported into target cells. In addition molecular hybridization also allows the selective modification of the more reactive unit within the hybrid molecules, providing an easy access to innovative functionalized scaffolds with biological interest.

Molecular hybridization, as shown in Figure 4, is explained by lock and key method in which two different compounds for example Drug A and Drug B, known to interact with different receptors viz. Receptor A and B respectively, are combined either directly or through a spacer to form a new hybrid compound which can interact with the receptor synergistically.
Figure 4. Hybridization approach explained by Lock-Key method

The hybrid molecules can be obtained by linking, fusing, or merging the two pharmacophores of the two selective molecules. Depending upon the type of linker connecting the two pharmacophores, hybrid molecules can be classified as following:

1. *Conjugate hybrids:* In these hybrids, the pharmacophores are joined through a linker which is not part of either of the selective pharmacophores.
2. *Cleavage conjugate hybrids:* Metabolically stable linker is used in these hybrids which get metabolized inside the biological system to release the ligands that interact independently with each target.
3. *Fused hybrids:* No linker is required and the hybrid is regarded as a fused molecule.
4. *Merged hybrids:* The two selected molecules are merged to each other through common pharmacophore present in the starting compounds.

Keeping the importance of molecular hybridization in the present drug discovery paradigm, the present review of literature explicates the importance of various isatin-derivatives as well as isatin-based molecular conjugates along with their anti-cancer, anti-malarial, anti-tubercular and anti-microbial potential.

1.2 *Anticancer potential of isatin analogues:*
Cancer, the rapid pathological multiplication and out-of-controlled growth of abnormal cells, is considered as one of the most challenging infirmities in the world. Despite vast approaches in the area of medicinal research, which have led to expensive treatment costs for a number of afflictions, cancer remains a leading cause of deaths in the world. Approximately seven million people die from cancer every year resulting in 12.5% of death worldwide. Chemotherapy has been the mainstay for treatment of various types of cancers. Most of the anticancer drugs
presently available are incapable to discriminate between neoplastic and normal cells or to combat resistance mechanisms developed in the cancer cells. Hence there is an essential requirement for the development of new anticancer drugs with unique targets of action, high effectiveness and less toxic to host cells. Presently, cancer therapy involving either a sole biological scaffold or pathway has been effectively employed. The lack of selectivity in most of the anti-cancer drugs and the development of resistance in tumors to chemotherapy is one of the major hindrances in the treatment of cancer. However, it is believed that agents acting on more than one target could have higher efficacy as compared to the single target drugs. Therefore, regulating multiple targets simultaneously can be accomplished by the combination of multiple drugs with different mechanisms of action or by single chemical moiety that could modulate various targets of a multi-factorial disease. Hence there is increasing interest in the development of new anticancer agents that can act simultaneously on more than one biological target for cancer treatment.

Dweedar and co-workers reported the synthesis of a series of indoline-2,3-dione hydrazones along with their anticancer evaluation. The methodology involved an initial treatment of indoline-2,3-diones with hydrazine hydrate in absolute methanol to afford the corresponding hydrazones. The treatment of with variedly substituted aldehyde resulted in the isolation of (Scheme). The synthesized hybrids were evaluated against human breast cancer cell line MCF-7 at different concentration and compared with reference drug doxorubicin. Biological assay results indicated that all the compounds showed significant activity with the conjugate 3-(((1H-pyrrol-2-yl)methylene)hydrazono)indolin-2-one being the most potent with an IC\textsubscript{50} value of 6.25 μM; similar to that of doxorubicin (IC\textsubscript{50} = 6.10 μM).
Scheme 1. Synthesis of indoline-2,3-dione hydrazones 6

Sirisoma et al. synthesized a series of substituted N-(2-oxoindolin-3-ylidene)-benzohydrazides conjugates which act as inducers of apoptosis via proprietary cell-and caspase-based ASAP HTS assay. Substituted oxoindolinylmethoxybenzohydrazides 8 were synthesized via condensation of substituted isatins 1 with 3,4,5-trimethoxybenzohydrazide 7 according to reported procedure. N-substituted analogues 9 were prepared by Mannich condensation of 8 with an amine and formaldehyde following literary protocol (Scheme 2). All the synthesized hybrids were evaluated against a panel of cancer cell lines viz. hepato-cellular carcinoma cancer SNU398 cells, HCT116 carcinoma cells of human colorectal and human colon cancer RKO cells. The most potent compound of the series viz. 8a act as apoptosis inducer showing an EC50 value of 0.24 µM in carcinoma cell line HCT116. The highly active compound 8a of the series displayed GI50 value of 0.056 µM against HCT116 cells. The activity data suggested that the presence of strong electron withdrawing, hydrophilic, or bulky groups at C-5 position of the isatin ring are not suitable for good activity profiles. The synthesized 4-chloro analogue was two folds more potent than the bromo-substituted analogue indicating the preference of a smaller electron withdrawing substituent for good activity profiles. Further, a group of water soluble N-alkylated analogues 9 were analysed against a series of human cancer cell lines. Among them, the compound 9a, having methyl piperazine moiety exhibited EC50 values of 0.14, 0.17 and 0.088 µM against human colon RKO cancer cell lines, HCT116 carcinoma cells lines and SNU398 hepatocellular carcinoma, respectively.
The mechanism of action for the active compounds was observed to be the tubulin polymerization inhibition.

Scheme 2. Synthesis of substituted N-(2-oxoindolin-3-ylidene)benzohydrazides

Kamal et al.\textsuperscript{82} have reported an efficient and high yielding synthesis of di-indolyl oxyindoles 11, by the reaction of isatin 1 with substituted indoles 10 using catalytic amount (5 mol \%) of FeCl\textsubscript{3} at room temperature as shown in Scheme 3. Evaluation of all the synthesized compounds against five human cancer lines comprising liver (Hep-2), lung (A-549), prostate (DU-145), CNS (SK-N-SH) and breast (MCF-7) by using MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) assay. Results indicated that good anticancer activity has been shown by most of the synthesized compounds against the tested cell lines. The most active compound 11a of the series exhibited IC\textsubscript{50} of 5.0 \(\mu\)M and 4.7 \(\mu\)M against DU-145 and SK-N-SH cell lines respectively. In most cases, the presence of methoxy substituent at the 4\textsuperscript{th}, 5\textsuperscript{th} and 6\textsuperscript{th} position of the isatin ring showed significant increase in the activity. Compounds possessing methoxy substituent on C-5 and C-6 position exhibited higher potency with IC\textsubscript{50} values of 2.2 and 1.2 \(\mu\)M against DU-145 cell line.
Scheme 3. Synthesis of diindolyl oxyindole derivatives 11

A series of novel oxoimidazolidin-isatin conjugates 13 have been synthesized by Penthala and coworkers. The synthetic protocol involved the condensation of the appropriately substituted N-alkyl isatins 12 with creatinine, in presence of the acetic acid and sodium acetate utilizing both the conventional heating and microwave irradiation methodologies (Scheme 4). Microwave irradiation method was preferred because of good yields and faster reaction times as compared to the conventional heating. The *in vitro* cytotoxicity of these analogues were evaluated against series of 57 human tumor cell lines. The most active compound 13b displayed GI50 values of 750 nM and 190 nM against LOX IMVI melanoma cell lines and A549/ATTC non-small cell lung cancer, respectively, while both 13a and 13b showed GI50 values in the range of 2 to 5 µM against MOLT-4, RPMI-8226, CCRF-CEM, K-562, and HL60(TB) leukemia cell lines. The two most active compounds viz. 13a and 13b were successively analysed in five dose–response studies to determine their *in vitro* cytotoxic effects on proliferation of the 57 human tumor cell lines.
Scheme 4. Synthesis of oxoimidazolidin isatin derivatives 13

Havrylyuk et al. and co-workers have reported the synthesis and antitumor screening of isatin-pyrazoline and isatin-thiazolidine conjugates. The precursor 3,5-diaryl-4,5-dihydropyrazoles were synthesized by the reaction of appropriate chalcones with chloroacetyl chloride. Alkylation of isatin and 5-bromoisatin with appropriate 2-chloro-1-(3,5-diaryl-4,5-dihydropyrazol-1-yl)-ethanones in DMF at room temperature resulted in the synthesis of corresponding diaryl-dihydropyrazol-isatin derivatives 17 (Scheme 5). The conjugates 17 were further reacted with 2-thioxo-4-thiazolidinone, 2,4-thiazolidinedione and 2-amino-4-thiazolone in accordance to the typical Knoevenagel condensation protocol to yield the desired isatin-4-thiazolidinone and isatin-pyrazoline conjugates 19 and 21 respectively. The synthesized compounds were evaluated against NCI60 cell lines for their anticancer activity. Compound 17a was found to be the most potent candidate among all the tested compounds with GI₅₀ values ranging from 0.69–3.35 µM against leukemia subpanel tumor cell lines. SAR studies revealed that the hybridization of isatin with pyrazoline could result antitumor activity while the presence of bromo substituent on C-5 position of isatin ring further increased the potency of these hybrids. Condensation of 4-thiazolidinones with the highly active isatin-pyrazoline conjugate 17a resulted in complete activity loss.
Scheme 5. Synthesis of isatin-pyrazoline and isatin-thiazolidine conjugates

In an extension, Havrylyuk et al. reported the synthesis and antitumor screening of novel pyrazoline-thiazolidinone-isatin conjugates as depicted in Scheme 6. The target conjugates were synthesized by the reaction of 3,5-diaryl-1-thiocarbamoyl-2-pyrazolines with appropriate isatins and chloroacetic acid in the presence of fused sodium acetate via one-pot methodology in refluxing acetic acid. Most of the synthesized conjugates displayed anticancer activity against renal, leukemia, CNS, melanoma, lung, prostate, colon, breast, and ovarian cancers cell lines. The most effective conjugate was found to exhibit TGI and GI values of 0.76 μM and 0.071 μM respectively and showed a very high anti-proliferative effect on non-small-cell CNS cancer cell line SNB-75 (GI = 0.0159 μM), lung cancer cell line HOP-92 (GI < 0.01 μM), ovarian cancer cell line NCI/ADR-RES (GI = 0.0169 μM), renal cancer cell line RXF 393 (GI = 0.0197 μM) and colon cancer line HCT-116 (GI = 0.018 μM). The SAR study of tested compounds showed that the anticancer potential depends on the presence of three heterocycles in a single
molecule; thus the pyrazoline-thiazolidinone-isatin hybrids were more potent as compared to the pyrazole–indoline-2-ones or pyrazoline–thiazolidinone hybrids. The introduction of a halogen group at the C-5 position of isatin ring improved the activity by one log unit (GI₅₀ level), in comparison to the C-5 un-substituted isatin analogues. The presence of substituent on the 5-aryl moiety also showed significant effect on the antitumor activity with the improvement in antiproliferative profile observed with the introduction of electron withdrawing chloro substituent.

**Scheme 6. Synthesis of pyrazoline-thiazolidinone-isatin conjugates 24**

Krishnegowda⁹⁰ and co-workers synthesized a panel of 5,7-dibromoisatin analogues and analysed against four human cancer cell lines for their cytotoxicities comprising breast MCF-7, colon HT29, melanoma UACC903 and lung A549. The synthetic protocol involved an initial alkylation of 5,7-dibromoisatin by using K₂CO₃ in DMF to result in the formation of 26 and 27.⁹¹ Iodide-catalyzed nucleophilic substitution of 26 and 27 with KSeCN and KSCN in dry acetonitrile at room temperature afforded the corresponding thiocyanates 30, 31, 34 and selenocyanates 32, 33, 35 respectively (Scheme 7). The isothiocyanate derivatives were prepared by the reaction of 5,7-dibromoisatin with tert-butyl 3-bromopropylcarbamate or tert-butyl(4-bromomethyl benzyl)-carbamate in the presence of K₂CO₃ in DMF, to afford the corresponding Boc-protected intermediates 28 and 29, respectively. The protective group in 28 and 29 was removed by the treatment with trifluoroacetic acid, further its reaction with thiophosgene in dry methylene chloride yielded 36 and 37. Analogues 34, 35 and 36 exhibited good *in vitro* anticancer activity against HT29 cell line in 1
µM range. Analogue having selenocyanate functionality in the alkyl chain displayed most promising activity against MCF-7 cell line of breast cancer. Compounds’ 31 and 36 were found to suppress tubulin polymerization to the similar amount as the vinblastine sulfate, the known anticancer drug, while compounds 34 and 37 exhibited better inhibition than vinblastine. Compounds 34 and 37 were emerged as dual inhibitors of the Akt signaling pathway and tubulin polymerization and were considered as the most suitable candidates for in vivo investigation as antitumor agents for colon cancer.

Scheme 7. Synthesis of derivatives of 5,7-dibromoisatin.

Matesic et al.\textsuperscript{92} reported an encouraging strategy for anticancer drug delivery involving the conjugation of a cytotoxin with a tumor targeting protein via an acid-labile linker stable at physiological pH. The synthetic protocol involved the reaction of isatin-derived cytotoxins 38 with anilino carboxylic acids, 39 and 40 to afford the corresponding imine linked cytotoxins 41 and 42 as shown in Scheme 8. The cytotoxins 41 and 42, prepared by using standard carbodiimide coupling procedures\textsuperscript{93,94} were then selectively coupled to the protected lysine derivative Ac-Lys-OCH\textsubscript{3} affording the desired imine–lysine conjugates 43 and 44, respectively. The
synthesized derivatives functionalised at the keto-carbonyl group of \( N \)-substituted isatins were showed stability at physiological pH but readily cleaved at pH 4.5. The observed rates of hydrolysis were in order \( p \)-phenylpropionic acid > phenylacetic acid \((p>m)\) > benzoic acid \((m>p)\) for the inserted imine-acid moiety.

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\begin{align*}
\text{Scheme 8. Synthesis of aryl imine-lysine conjugates} \\
\text{Klock and co-workers}^{95} \text{ synthesized a class of 3-acylidene-2-oxoindoles via a two-step condensation reaction as elucidated in Scheme 9. The synthetic methodology involved the base promoted condensation reaction of the isatin with arylmethyl ketone afforded the corresponding \( \beta \)-hydroxy ketones 47 which upon dehydration under either acidic conditions or by the aid of methane sulfonyl chloride in pyridine resulted in the formation of acylidene oxoindoles 48. The introduction of phenyl substituent at \( N \)-1 position of acylidene oxoindole was carried out via copper-mediated \( N \)-arylation as elucidated in the synthesis of 49. The most potent reversible inhibitor of human TG2, compound 48b was considered to be useful in the treatment of a variety of diseases including celiac sprue as well as cancers and certain CNS}
\end{align*}
\]
disorders. Significant non-competitive character was observed in the synthesized conjugates, suggesting that the synthesized analogues could bind at one or more allosteric regulatory sites on the multidomain enzyme. The 4-chloro substituted analog 48b exhibited the highest potency among the synthesized compounds with an IC50 value of 1.5 µM and a Ki value of 0.7 µM.

Hung and coworkers synthesized a series of the pyridinyl and quinolinyl conjugates of isatin and evaluated for their cytotoxic activity toward HL-60 cells. The synthetic procedure for the target compounds 53 involved an initial alkylation of isatin 1 with various aryl/alkyl halides 50 to give the corresponding 1-substituted isatins 51, the reduction of which with hydrazine hydrate resulted in the corresponding 1-substituted indolin-2-ones 52 as shown in Scheme 10. Further condensation of 52 with various quinolinyl or pyridinyl aldehydes, in the presence of piperidine yielded the target compounds 53. Most of the tested compounds enhanced all trans retinoic acid (ATRA) induced differentiation; with the compound 53a being the most promising one with 33.9% cell differentiation at 2.5 µM, and 94.5% cell differentiation when combined with 5 nM ATRA. The two isomers, 53a(Z) and 53a(E) were displayed similar differentiation activity. The combination of 53 with all trans retinoic acid (ATRA) resulted into complete differentiation of HL-60 cells and arrest the cells in the G0/G1 phase of the cell cycle with relatively low cytotoxicity.
towards normal cells. The SAR studies revealed that the pyridin-4-yl derivatives showed more differentiation activity than both the pyridin-2-yl or pyridin-3-yl derivatives.

Scheme 10. Synthesis of pyridinyl and quinolinyl analogs of isatin

Solomon and co-workers designed and synthesized a series of isatin-Mannich bases 54 and isatin-linked benzothiazole Schiff bases 57. The isatin-Mannich bases 54 were prepared via one pot multi-component reaction of isatin, formaldehyde and an amine (Scheme 11) in refluxing ethanol for 4 h. Isatin-benzothiazole Schiff bases 57, on the other hand were synthesized via condensation reaction of Mannich base 54 with 6-methyl-benzothiazol-2-yl-amine\(^9\) 56 in the presence of glacial acetic acid. The cytotoxicity of these compounds was evaluated against different human breast tumor cell lines viz. MCF7, MDA-MB468, MDA-MB231 and two non-cancerous breast epithelial cell lines, MCF10A and 184B5. The SAR studies suggested that the presence of hydrophobic substituent e.g chloro and bromo at the 4th position of isatin ring resulted into improved cytotoxic activity as compared to un-substituted analogues. Among all the tested compounds, compound 54a emerged as the most active with GI\(_{50}\) values of 20.2 \(\mu\)M, 20.22 and 11.68 against MCF7 cells, MDA-MB231 and MDAMB468 respectively and exhibited cytotoxic effect 10–15 times higher on cancer than the non-cancer cells. The differential killing
of cancer and non-cancer cells observed in this case might be because of its variance effects on cancer and non-cancer cell cycle progression. Among the Schiff base analogues, the compound 57a was the most effective and exhibited GI50 values of 14.56, 17.61 and 19.76 µM against MCF7 cells, MDA-MB231 and MDA-MB468 respectively.

Further Solomen et al. synthesized a series of novel 4-piperazinylquinoline-isatin conjugates via Mannich addition and evaluated against two human breast tumor cell lines viz. MCF7 and MDA-MB468, and two non-cancer breast epithelial cell lines viz. MCF10A and 184B5. The synthetic strategy involved an initial preparation of 7-substituted-4-piperazin-1-yl-quinoline 59 via aromatic nucleophilic substitution of 58 with excess of triethylamine and piperazine. The desired conjugates were synthesized via Mannich addition using isatin, formaldehyde and 59 in refluxing ethanol with subsequent treatment with thiosemicarbazide. All the synthesized compounds were effective against breast cancer cell lines. The conjugate viz. 60a exhibited GI50 values of 15.12 and 15.88 µM on MCF7 and MDA-MB468 cells, respectively while the conjugate 61a exhibited GI50 values of 21.56 and 23.04 µM against MCF7 and MDA-MB468 cells respectively.
Scheme 12. Synthesis of 4-piperazinylquinoline analogs 61 via Mannich reaction

Bedi et al. synthesized a series of 3,5-diaryl N-acetylpyrazoline-isatin conjugates via click chemistry approach as elucidated in Scheme 13 and evaluated against a panel of cell lines viz. HeLa (cervix cancer), CAKI-I (Renal cancer), PC-3 (Prostate cancer) and Miapaca-2 (pancreatic cancer). The hybrids were classified as right-handed and left-handed conjugates on the basis of the placement of the isatin ring. The length of the alkyl armed triazole linker was varied from 2 to 6. Out of the synthesized conjugates, two right handed conjugates viz. 64a and 64b and two left-handed conjugates 66a and 66b displayed significant cytotoxic potential against HeLa cell line with IC50 values ranging from 1.3 to 3.5 μM.
1.3 Isatin as antimalarial agents:

With 207 million cases and 627 thousand deaths in 2012, malaria is one of the world’s deadliest diseases, affecting 64% of the global population.\textsuperscript{100} \textit{Plasmodium falciparum} is the most virulent human malaria parasite, and is responsible for most of the malaria-related deaths.\textsuperscript{101,102} Since the discovery of the natural product quinine, many compounds with a quinoline scaffold have displayed good antimalarial activity, leading to the development of effective antimalarials, including chloroquine, amodiaquine, piperaquine and mefloquine.\textsuperscript{103-105} Chloroquine has been the mainstay
for decades because of its efficacy, safety and low cost. However, the widespread resistance of \textit{P. falciparum} to chloroquine\textsuperscript{101,106} has hampered efforts to combat malaria and led to the development of the natural endoperoxide artemisinin and its semisynthetic derivatives (artemether, artesunate, and dihydroartemisinin) as potent and fast acting antimalarials.\textsuperscript{107} However, the worldwide deployment of artemisinin based combination therapy is limited by relatively high cost of treatment, safety in pregnancy and early signs of resistance to artemisinin derivatives in southeast Asia.\textsuperscript{108,109,110}

Chibale \textit{et al.}\textsuperscript{111} in a communication reported the synthesis of 1\textit{H}-1,2,3-triazole tethered isatin-chalcone conjugates along with their antimalarial evaluation. Synthetic protocol involved an initial protection of keto-carbonyl group of isatin with trimethyl orthoformate in the presence of \textit{p}-toluene sulfonic acid to yield 67. Treatment of 67 with epichlorohydrin in the presence of KF/\textit{Al}_2\text{O}_3 afforded the corresponding ketal 68 which upon reaction with sodium azide opened up the oxirane ring to yield the corresponding azide 69. Deprotection of 69 with 10\% HCl regenerated the keto-carbonyl of the isatin to yield 70 which was explored in Cu-promoted azide-alkyne cycloaddition reactions with \textit{O}-propargylated chalcones 71 and 73 resulting in the isolation of corresponding isatin-chalcone conjugates 72 and 74 (\textbf{Scheme 14}). The results revealed that the synthesized isatin-chalcone conjugates inhibited falcipain-2 activity, although 100-folds less compared to cysteine protease inhibitor E64. The results further showed the preference of \textit{meta}- substitution for falcipain-2 inhibitory activity while increase in methoxylation did not enhance the activity.
demonstrated that the tetracycles falciparum well as n = 5 as linker, exhibited an IC₅₀ improvement in potency was observed with an increase alkyl chain length. The most study suggested the dependence of activity on C₇ displayed superior antiplasmodial activity than the precursors. An using the MABA, LORA and BACTEC assays. The tetracyclic derivatives strain of 79 bromo isatin. All the synthesized compounds were evaluat

Scheme 15

R₁= 4-methoxy, 2,4-dimethoxy, 2,3,4-trimethoxy

The above work was further extended towards the synthesis of novel thiolactone-isatin conjugates 78 prepared via sequence of synthetic steps as depicted in Scheme 15 involving the reaction of potassium salt of thiolactone with N-alkyl bromo isatin. The reaction also resulted in the formation of a tetracyclic byproduct 79. All the synthesized compounds were evaluated against the CQ-resistant (W2) strain of P. falciparum, falcipain-2 inhibitory activity and anti-tubercular activity using the MABA, LORA and BACTEC assays. The tetracyclic derivatives 79 displayed superior antiplasmodial activity than the precursors. Antimalarial SAR study suggested the dependence of activity on C-5 substituent of the isatin ring and an improvement in potency was observed with an increase alkyl chain length. The most potent tetracyclic analogue 79a having iodo substituent at the C-5 position of isatin as well as n = 5 as linker, exhibited an IC₅₀ value of 6.92 μM against W2 strain of P. falciparum. In contrast to the antimalarial results, the anti-tubercular results obtained demonstrated that the tetracycles 79 were devoid of any activity whereas
intermediates showed growth inhibitory activity against the H$_{37}$Rv strain of *M. tuberculosis* as discovered by MABA, BACTEC and LORA assays.

![Chemical structure](image)

**Scheme 15.** Synthesis of thiolactone-isatin conjugates

Wang et al. synthesized a series of indolo[3,2-c]quinolines by modifying the side chains of the ω-aminoalkylamines. The synthetic protocol involved an initial heating of isatin 1 with 2-aminomethyl-phenylamine in acetic acid, followed by its conversion to 6-chloro-11H-indolo[3,2-c]quinoline 82 using POCl$_3$. Various amines were introduced at C-6 position of 82 by aromatic nucleophilic substitution reaction leading to the formation of 83 (Scheme 16). The terminal amino group of 88 was coupled with phenyl isocyanate to give 89 as shown in Scheme 18. The synthesized compounds were evaluated for their *in vitro* antiplasmodial activities against two different strains (CQS: NF54 and CQR: K1) while the cytotoxicity was assessed against L6 cell line. The results showed that the compounds 84a and 84b containing the branched methyl groups at C-6 along with a chloro-substituent at C-2 position exhibited high antimalarial efficacy with IC$_{50}$ values of about 11 nM for NF54 (CQ-sensitive) and 17 nM for K1 (CQ-resistant) strains along with resistance indices (RI) of 1.6 and low cytotoxicity (IC$_{50}$ ≥ 4000 nM). The compounds were also tested for β-haematin inhibition, and QSAR revealed an interesting linear correlation between the biological activity and three contributing factors, namely solubility, hydrophilic surface area, and β-haematin inhibition. Compound 84b with the lowest cytotoxicity (IC$_{50}$ above 4004 nM), low resistant index of 1.6 and good *in vitro* antiplasmodial activity (11 nM) was selected for an *in vivo* drug testing model against *Plasmodium berghei* in mice and showed a reduction in parasitaemia on day 4 with an activity of 38%.
Scheme 16. Synthesis of indolo[3,2-c]quinolines with amino group at the C-6 position

Scheme 17. Synthesis of indolo[3,2-c]quinolines by further modifications of the terminal amino group
**Scheme 18.** Synthesis of indolo[3,2-c]quinolines by N-11 methylation and amination at the C6 position.

1.4 Isatin as Anti-Tuberculosis agent:

Globally, tuberculosis (TB) is one of the major challenging health problems, caused by *Mycobacterium tuberculosis* (MTB) and is the leading cause of infectious disease mortality in the world. Despite the availability of a cheap and effective treatment, tuberculosis still accounts for millions of cases and deaths worldwide. Approximately 8.6 million people developed TB in 2012, and 1.3 million died from TB (including 3,200,000 deaths among HIV-infected people).\(^{114}\) TB has many manifestations distressing central nervous system, bone, and other organ systems, but primarily it is a pulmonary disease, developed by the deposition of *M. Tuberculosis* present on lung alveolar surfaces enclosed in form of aerosol droplets. The progression of this disease can have several outcomes, determined largely by the response of the host immune system. HIV infected patients with weakened immune system are prone to be infected with TB because of which the significance of the disease has been increased dramatically. In patients infected with HIV, opportunistic infection with *M. tuberculosi*s commonly occurs as a result of exogenous infection.\(^{115}\) There is almost 30% risk of developing progressive primary tuberculosis in HIV-infected persons within the first year in contrast with the 3% risk in non-HIV-infected persons.\(^{116}\) A further complicating factor is the occurrence of drug resistant tuberculosis. Many of
the TB cases are multidrug-resistant (MDR) *i.e.* resistant to isoniazid (INH) and rifampin (RIF), the first-line anti-TB drugs. At the same time, the emergence of extensively drug-resistant tuberculosis (XDR TB); which is MDR TB with additional resistance to fluoroquinolones (FLQ) [moxifloxacin (MOX), ofloxacin (OFL), and levofloxacin] and at least one of the three injectable second-line drugs [capreomycin (CM), amikacin (AM) and kanamycin (KM)], has also become an important global health problem. Therefore, treatment of tuberculosis is a complex process due to several factors including resistance to existing drugs, the emergence of multi drug-resistant TB (MDRTB) strains and the association with human immunodeficiency virus (HIV). Thus, there is pressing need to discover new anti-TB drugs for the treatment of TB, particularly in its hard-to-kill multidrug-resistant, persistent and latent forms.

Kumar *et al.*\(^{117}\) described the one-pot three-component domino reaction of cyclic ketones, sarcosine and isatin yielding highly functionalised dispiropyrrrolidines in adequate yields along with their *in vitro* screening against H\(_{37}\)Rv (MTB) (*Scheme 19*). The work was further extended using substituted amino acids leading to the synthesis of dispiropyrrrolidines as depicted in *Scheme 20*. The reaction involved domino reactions of cyclic ketones with substituted isatins and amino acids *viz.* phenylglycine, proline, thia-proline and pipecolic acid in a 1:1:2 molar ratios in refluxing methanol for 6-24 h. Among all the tested compounds, 94a was found to be most potent with MIC of 1.98 μM against MTB and was 25.64 and 3.86 times more active than the standard first line TB drugs *viz.* pyrazinamide and ethambutol respectively. Structure activity relationship showed that the introduction of electron withdrawing substituent at the C-5 position of isatin enhanced the activity with a preference for bromo substitution.
Aboul-Fadl\textsuperscript{118} and co-workers designed and synthesized a series of Schiff bases of isatin and nalidixic acid carbohydrazide derivatives. The Schiff bases were obtained through 2 steps reaction, involving base promoted $N$-substitution of isatin 1 to yield
the corresponding 1-benzyl or 1-alkyl derivatives,\textsuperscript{119} 1-hydroxymethyl isatin conjugates were synthesized in by the reaction of suitable isatin with 40% formaldehyde in water in good yields.\textsuperscript{120} The second step involved the condensation of substituted isatins with nalidixic acid carbohydrazide 100 leading towards the synthesis of isatin-nalidixic acid conjugates 101 (Scheme 21). Mannich bases of isatin-nalidixic acid were synthesized \textit{via} treatment of 102 or 103 with formaldehyde and appropriate secondary amines as shown in Scheme 22. The synthesized compounds were evaluated for their antimycobacterial activity against four \textit{Mycobacterium} strains \textit{viz.} \textit{Mycobacterium smegmatis} (ATCC 35797), \textit{Mycobacterium intercellulari} (ATCC35743), \textit{Mycobacterium cheleneo} (ATCC 35751) and \textit{Mycobacterium xenopi} (ATCC 14470). The antitubercular activity data showed that with the exception of compound 101a, no considerable activity was shown by other synthesized compounds. Potent anti-TB activity was shown by conjugate 101a exhibited MIC value of 0.625 \(\mu\)g/mL, which is 20 folds higher than the reference drug isoniazid, INH, (MIC = 12.5 \(\mu\)g/mL). The preliminary antimycobacterial evaluation results further showed that unlike N-alkyl derivatives of isatin, Mannich bases of isatin are completely devoid of anti-TB activity, most probably due to solubility issues of these derivatives in DMSO.

\textbf{Scheme 21. Synthesis of Schiff’s bases of isatin}
Scheme 22. Synthesis of Mannich bases

Prasanna et al. synthesized a series of novel spiro-pyrrolothiazolyloxindoles by 1,3-dipolar cycloaddition of azomethine ylides derived in situ from 1,3-thiazolane-4-carboxylic acid and substituted isatins with 2-(arylmethylene)-2,3-dihydro-1H-inden-1-ones 105. The target compounds were obtained by reacting an equimolar mixture of 105, 1,3-thiazolane-4-carboxylic acid 106 and substituted isatins 1 in methanol for 5-8 h. All the synthesized compounds were screened for their in vitro activity against Mycobacterium tuberculosis H37Rv (MTB). Among 29 compounds screened, compound 107a, was found to be the most potent conjugate exhibited MIC value of 2.8 μM against MTB, being 2.70 and 1.67 times more active than the standard drugs ethambutol and ciprofloxacin respectively.

Scheme 23. Synthesis of spiro-pyrrolothiazolyloxindoles 107

Feng and co-workers synthesized a series of novel 8-OCH₃ ciprofloxacin (8-OCH₃-CPFX) isatin conjugates linked via methyl or ethyl linker and evaluated their in vitro activity against M. smegmatis CMCC 93202, ATCC 27294, MTB H₃₇Rv and MDR-MTB 09710, respectively. Condensation of isatin 1 with substituted amine
hydrochloride in the presence of NaHCO$_3$ afforded Schiff’s bases 108. Mannich reaction of the compound 1 or 108 with 8-OCH$_3$-CPFX and paraformaldehyde [(CH$_2$O)$_n$] in refluxing ethanol under an atmosphere of nitrogen afforded the corresponding 8-OCH$_3$-CPFX methylene isatin derivatives 109 (Scheme 24).$^{123,124}$ For the synthesis of ethyl-linked conjugates, $N$-alkylated isatins 77 were subjected to nucleophilic substitution reactions with 8-OCH$_3$-CPFX in DMF at 40 °C to yield 8-OCH$_3$-CPFX derivatives 110. Subsequent condensation of 110 with substituted amine hydrochlorides in the catalytic amount of NaHCO$_3$ formed the corresponding Schiff’s bases (Scheme 25). The anti-TB evaluation studies showed that most of the tested methylene isatin derivatives were more potent than isoniazid, 8-OCH$_3$ ciprofloxacin and rifampin against ATCC 27294 and MTB H$_37$Rv. Results revealed that the compound 109a (MIC: 0.074 μM) was 2-13 folds more active than the reference drugs against MTB H$_37$Rv and ATCC 27294 while compounds 109b and 109c-d (MIC: 6.72-7.05 μM) were around 1.6 folds more active than the parent 8-OCH$_3$ ciprofloxacin and 3.5 folds more active than ciprofloxacin against MDR-MTB 09710.

Scheme 24. Synthesis of 8-OCH$_3$-CPFX methylene isatin derivatives 109
Scheme 25. Synthetic route of 8-OCH₃CPFX ethylene isatin derivatives 111

Banerjee et al.¹²⁵ synthesized a series of novel thiosemicarbazones of isatins and their Mannich bases analogues and evaluated for anti-tubercular and anti-HIV activity in both log phase and starved cultures. The title conjugates were synthesized by the condensation reaction of thiosemicarbazide hydrochloride salts 114 with variedly substituted isatins 1 in the presence of sodium acetate (Scheme 26). The N-Mannich bases were prepared by the condensation reaction of the acidic imino group of isatin derivatives with various secondary amines and formaldehyde under microwave conditions. The compound 116a was found to be the most active in inhibiting the replication of HIV-1 cells with an EC₅₀ of 1.69 μM. Anti-mycobacterial evaluation results revealed that the compound 116a was effective in growth inhibition of both starved (MIC 12.11 μM) MTB and log phase (MIC 3.30 μM) cultures. The evaluation of the synthesized compounds showed activity in the range of 0.15 to 81.34 μM against the replication of MTB in the logarithmic growth phase. The methoxy thiosemicarbazones exhibited superior activity as compared to the hydroxy thiosemicarbazone analogues against MTB growth. Compound 116b was found to be the most active compound among the hydroxyl thiosemicarbazone analogues with an MIC of 0.16 μM.
1.5. Isatin as Antimicrobial agent:

Microscopic organisms caused fungal infections that can invade the epithelial tissue. Factors that contribute to infection include necrotic tissue, moist environment, and immunosuppression. Fungal infections can be of three types (i) superficial and irritating (eg., dermatophytosis) (ii) systemic and (iii) life threatening (eg., blastomycosis, cryptococcosis, histoplasmosis, coccidioidomycosis). Over the past several years, microbial infections pose a serious and continuous threat to human life and health. Infections caused by fungi and multidrug-resistant Gram-positive bacteria represent major public health burden, not only in terms of mortality and morbidity, but also due to the increased costs on patient management and execution of infection control measures. For many years, the only available agents were amphotericin B and the azole antifungals. Due to increasing infections in the complex patient populations, there is an immense requirement for the development of new
antimicrobial drugs in the clinical therapy.\textsuperscript{127} In recent years, Mannich and Schiff bases of isatins have reported to exhibit broad-spectrum chemotherapeutic properties such as anti-TB,\textsuperscript{42} antiviral,\textsuperscript{21(a),81,128} antifungal,\textsuperscript{129} and antibacterial activities.\textsuperscript{43}

Akhaja and Raval\textsuperscript{130} synthesized a series of tetrahydropyrimidine-isatin hybrids and evaluated their \textit{in vitro} antibacterial, antifungal and anti-tubercular activities. Tetrahydropyrimidine carbohydrazide \textsuperscript{118} was synthesized \textit{via} refluxing a solution of $\beta$-ketoester, urea/thiourea and aldehyde in ethanol in the presence of catalytic amount of CaCl$_2$ to give tetrahydropyrimidine carboxylate \textsuperscript{117}, followed by its reaction with hydrazine hydrate. Treatment of tetrahydropyrimidine carbohydrazide \textsuperscript{118} with ammonium thiocyanate in acidic medium yielded the tetrahydropyrimidine carbonyl hydrazine-carbothioamide, which in presence of conc. H$_2$SO$_4$ undergo heterocyclization gave \textsuperscript{119}. Compound \textsuperscript{119} on condensation with various 5-substituted isatin \textsuperscript{1} in acidic medium yielded the conjugates \textsuperscript{120}. \textit{In vitro} antibacterial activity data revealed that the compounds containing strong electron withdrawing fluorine substituent on the isatin ring exhibited brilliant activity against all microbial strains, while the conjugates with bromo- and nitro-substituents were found to exhibit comparable activities against gram positive and gram negative strains to the standard antibiotic ampicillin. \textit{In vitro} antifungal activity data showed that the conjugates \textsuperscript{120a} and \textsuperscript{120b} displayed highest activity against all the fungal strains, while compounds having nitro-substituent on isatin showed reduction in activity. The order of antibacterial activity with respect to substituents at the C-5 position of isatin was F$>$NO$_2$$>$Br$>$Cl$>$H$=$I. While the introduction of sulphur at position-2 displayed better activity as compared to oxygen counterparts. The synthesized compounds were also tested against \textit{M. tuberculosis} H$_{37}$Rv. Compounds containing a 5-flouro substituent on the isatin ring with oxygen atom on tetrahydropyrimidine nucleus showed better activity (50 $\mu$g/mL) while compounds having nitro and chloro substituents at C-5 position on 2-thioxo-tetrahydropyrimidine indolone exhibited activity in the range of 50-62.5 $\mu$g/mL.
Further, Raval\(^{131}\) and co-workers synthesized a series of novel isatin-carbodithioate derivatives and evaluated for their \textit{in vitro} antibacterial, antifungal, anti-tubercular and antimalarial activities. The synthetic methodology involved the preparation of 4-(3,4-dichlorophenyl)piperazine-1-carbodithioate 122 by the reaction of 1-(2,3-dichlorophenyl)piperazine 121 with sodium hydroxide and carbon disulphide as shown in 

**Scheme 28.** Condensation of 77, prepared via K\(_2\)CO\(_3\) promoted alkykation of 5-sustituted isatins with dibromo-alkanes and 122 in refluxing ethanol afforded the target compounds 123. The evaluation results showed that the compounds 123a, 123b, and 123c exhibited excellent activity against all the tested microbial and fungal strains. Compounds 123a, 123b, and 123c also displayed the highest inhibition (99\%) in the range of 3.10–6.25 µg/mL against \textit{M. tuberculosis} H\(_{37}\)Rv.
Scheme 28. Synthesis of isatin-carbodithioate conjugates 123

Pervez and co-workers synthesized a series of \( N^4 \)-aryl-substituted 5-fluoroisatin-3-thiosemicarbazones and evaluated for selected biological activities. 5-fluoroisatin-3-thiosemicarbazones were synthesized by reacting 5-fluoroisatin 1 with appropriate \( N \)-substituted thiosemicarbazides 124 in aqueous ethanol containing a few drops of glacial acetic acid as depicted in Scheme 29. Antifungal activity of the synthesized compounds were evaluated against six fungal systems viz. Candida albicans, Trichophyton longifusus, Microsporum canis, Candida glabrata, Aspergillus flavus and Fusarium solani and their phytotoxic, brine shrimp lethality and urease inhibitory effects were also investigated. All the title compounds have shown good activity against one or more fungi and exhibited inhibition in the range of 10–40%. Compound 125a having fluoro group on the ortho-position of the phenyl ring was observed to be active amongst all the mono-halogenated compounds against two fungal strains i.e. \( F. \) solani and \( M. \) canis, demonstrating 20 and 30% inhibition, respectively. Similarly, compound containing fluoro groups at ortho and para positions observed to be the most potent one in case of di-halogenated compounds,
showing inhibitory activity against *A. flavus*, *F. solani* and *M. canis*. Further confirming the effect of various substituents on the phenyl ring attached to $N^4$ position.

$$\text{RNHCSNHNH}_2 \xrightarrow{\text{50% EtOH(aq.)-AcOH reflux, 2hr}} \text{NNHCNHR}$$

$R = \text{C}_6\text{H}_5$, 2-F$_3$C$_6$H$_4$, 3-F$_3$C$_6$H$_4$, 4-F$_3$C$_6$H$_4$, 2-F$_3$COC$_6$H$_4$, 2-FC$_6$H$_4$, 3-FC$_6$H$_4$, 4-FC$_6$H$_4$, 2,4-(F)$_2$C$_6$H$_3$, 2,6-(F)$_2$C$_6$H$_3$, 3,5-(F)$_2$C$_6$H$_3$, 2-F-4-BrC$_6$H$_3$

![Scheme 29. Synthesis of $N^4$-aryl-substituted 5-fluoroisatin-3-thiosemicarbazones 125](image)

Perumal and co-workers$^{133}$ synthesized a series of spirooxindoles through 1,3-dipolar cycloaddition of an azomethine ylide synthesized from sarcosine or L-proline and isatin with 1,4-naphthoquinone acting as dipolarophile, followed by spontaneous dehydrogenation. The synthetic protocol involved the refluxing of an equimolar mixture of substituted isatins 1, sarcosine 126 and 1,4-naphthoquinone 127 in ethanol for 1.5 h (Scheme 30). Synthesized compounds were evaluated for their antimicrobial activities against three fungi and eight bacteria. All the spirooxindol conjugates exhibited significant antibacterial activity and anti-fungal activity, with compound 128a being 1.6 times more active than streptomycin and ciprofloxacin against *S. Aureus* (MRSA) and 6.4 times more active than ciprofloxacin against *M. luteus* and *S. Typhimurium*. 
Scheme 30. Synthesis of spirooxindoles 128

Agrawal et al.\textsuperscript{134} synthesized a series of indophenazine-chalcone hybrids derived from benzofuran chalcones 136 and indophenazine-6-acetic acid hydrazide 135 using microwave-assisted synthesis. Indophenazine 133 was prepared either by reaction of isatin 1 with equivalent amount of ortho-phenylene diamines 129 in presence of 2-Iodoxybenzoic acid (IBX) at room temperature (Scheme 31) or by the microwave assisted synthesis using equivalent amount of ortho-phenylene diamine and isatin in glacial acetic acid in microwave. Further microwave irradiation of indophenazine 133 and ethyl chloro acetate in dry acetone in the presence of anhydrous potassium carbonate led to the formation of 6-carbethoxymethyl indophenazine 134 which was reacted with hydrazine hydrate in absolute ethanol under microwave irradiation to yield indophenazine-6-acetic acid hydrazide 135. The target compounds 137 were prepared by the microwave irradiation of benzofuran chalcones 136 and indophenazine-6-acetic acid hydrazide 135 in glacial acetic acid. Synthesized compounds were found to exhibit good activity profiles and compared with standard fluoroquinolones drugs. SAR studies revealed that 4,5-dihydro pyrazole having substituted phenyl ring at 5\textsuperscript{th} position resulted in antibacterial activity against gram negative bacteria and gram positive. The \textit{para} substitution with -OCH\textsubscript{3} and \textit{ortho} substitution with –OH in phenyl ring present at 5\textsuperscript{th} position of pyrazole resulted in excellent antibacterial activity against gram negative bacteria while the presence of unsubstituted phenyl ring reduced the antibacterial activity. The replacement of
phenyl ring by five membered rings at 5th position of pyrazole ring resulted in reduction in antibacterial activity.

Scheme 31. Synthesis of Indophenazine-chalcone hybrids 137

Singh et al. synthesized a series of spiro-compounds having 2-azetidinones and 1-methylindolin-2-one rings. Synthetic strategy involved an initial Wolff-rearrangement of 2-diazo-1,2-diphenylethanone 138 to generate the ketene which underwent Ketene-Imine cycloaddition with N-substituted imino-indolin-2-ones 139 in dry benzene to afford the corresponding spiro(azetidin-2,3'-indoline)-2',4-diones 140 as shown in Scheme 32. All the synthesized compounds were evaluated for their antibacterial activity against Gram-(+) Staphylococcus aureus, Bacillus subtilis and Gram-(-) Pseudomonas aeruginosa and Escherichia coli strains while antifungal activities were investigated against Candida albicans and Saccharomyces cerevisiae. Out of all the compounds screened, compound 140a with two 4-methylphenyl
substituents and one isopropyl substituent at the 2-azetidinone ring showed activity with MIC= 50 µg mL⁻¹ against S. cerevisiae, MIC= 10 µg mL⁻¹ against E. coli, and MIC= 50 µg mL⁻¹ against P. aeruginosa.

\[\text{Scheme 32. Synthesis of spiro(azetidin-2,3'-indoline)-2',4-diones 140}\]

1.6 References:


