Synthetic Indole Alkaloids in Cancer: An Overview

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ABSTRACT:

Cancer is genetic irregularity of immortal cells which aggravates various metabolic disorders that can be fatal and also untreatable. Every year it causes millions of death and therefore notoriously ranked on the second place in the category of deadly diseases. Present circumstances pose a serious threat to the current anticancer chemotherapy and are mainly governed by poor selectivity, high toxicity and increased incidences of resistance. Therefore there is pre-requisite need of the new alternative scaffold which can become a lead molecule to eradicate the cancer anomalies. Moreover the plant derived secondary metabolites have proved to be an important natural source of the lead molecule in anti-cancer drug discovery. In recent year's synthetics of indole alkaloids disclosed broad spectrum of anticancer features and broadly can be characterized into 5 different categories: 2-Phenyl indole derivatives, Indomethacin derivatives, Indoline-sulphonamides, Carbazoles, Hydrazones of isatin. However this paper unfolds the mechanistic intervention, structure activity relationship and molecular modeling of synthetics derived from indole alkaloids based scaffolds.

INTRODUCTION:

Synthetics derived from natural origin been shown diverse biological activities since the medieval period of human civilization. Usually the most active compounds of natural origin belong to glycosides, terpenoids, flavonoids and alkaloids. Alkaloids can be categorised into different sub-categories based on the possessed scaffold in their structure. Moreover indole alkaloids emerge as anticancer agents and also show commendable activities against different targets in cancer. But these well-characterise anticancer alkaloids (especially vinca alkaloid, taxanes and other antimitotic agents) have certain limitations as they are difficult to extract and also higher cost of productivity. Along with these limitations, alkaloids also have ADMET problems.

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So, venture for the development of synthetic derivative to overcome the concerning issues urge a call for better designing of indole alkaloids based synthetics [1]. In recent year's synthetics derived from the indole alkaloids, disclosed broad spectrum of anticancer features[2] and can be broadly characterized into 5 different categories: 2-Phenyl indole derivatives, Indomethacin derivatives, Indoline-sulphonamides, Carbazoles, Hydrazones of isatin.

2-Phenyl indole derivatives

The indole nucleus has emerged as a versatile scaffold for the development of compounds with promising anti-proliferative activity**.** Arrays of 2-phenyl indoles were found to inhibit the growth of human breast cancer cells by various mechanisms depending on the type and position of the substituents attached to the main nucleus[3].

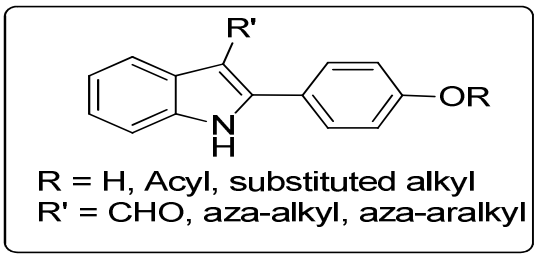
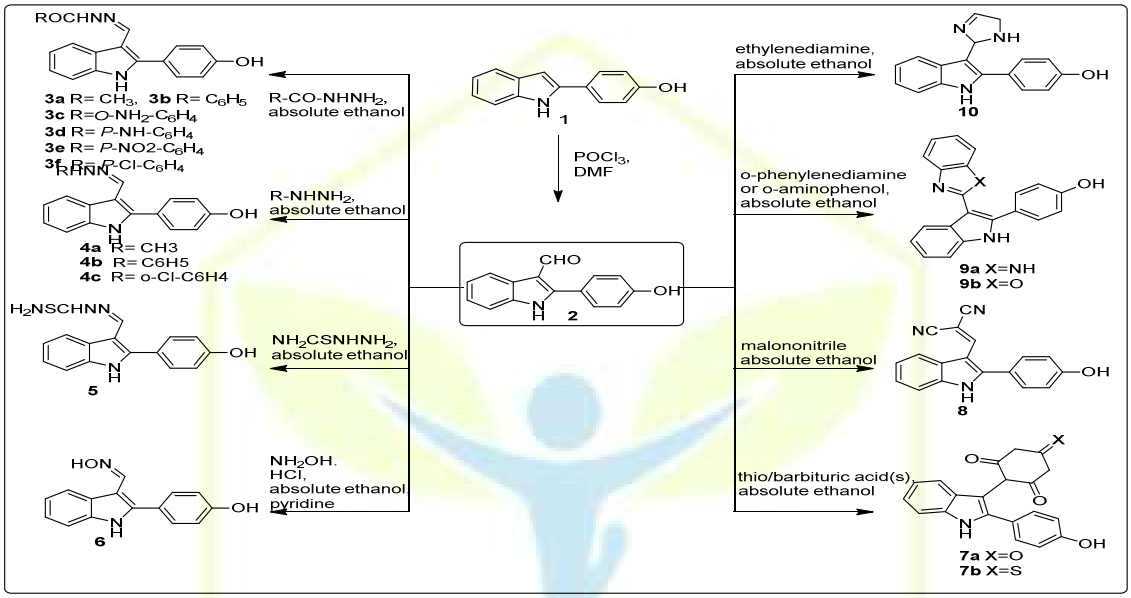
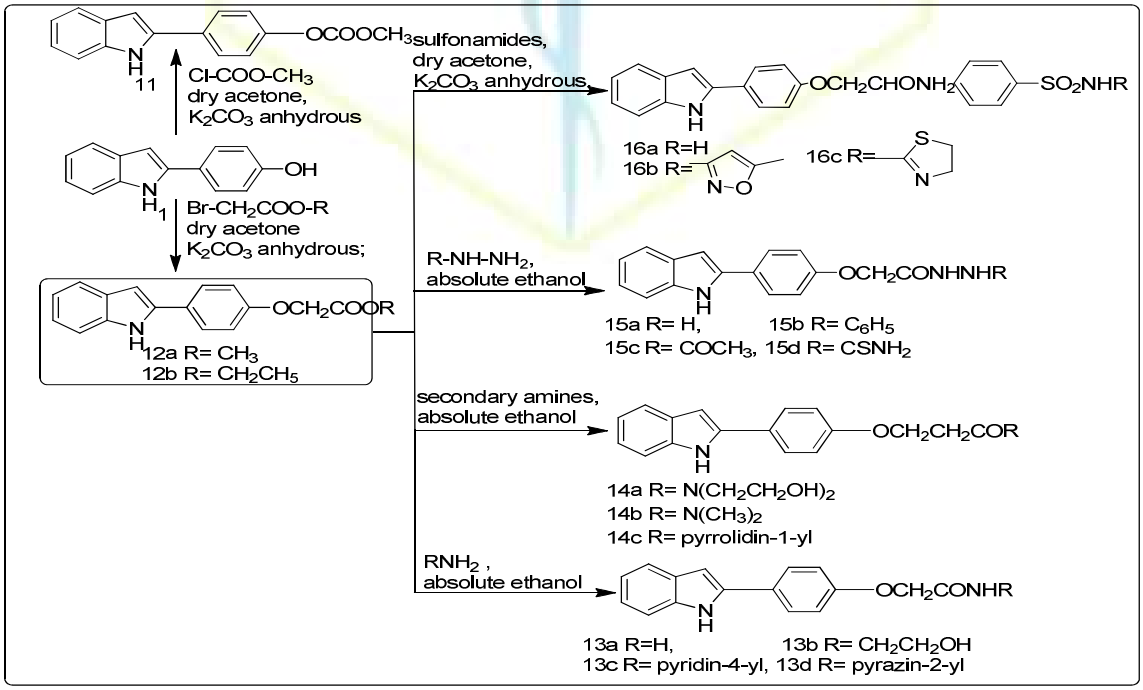


Figure 1: General formula of 2-Phenyl indole nucleus.

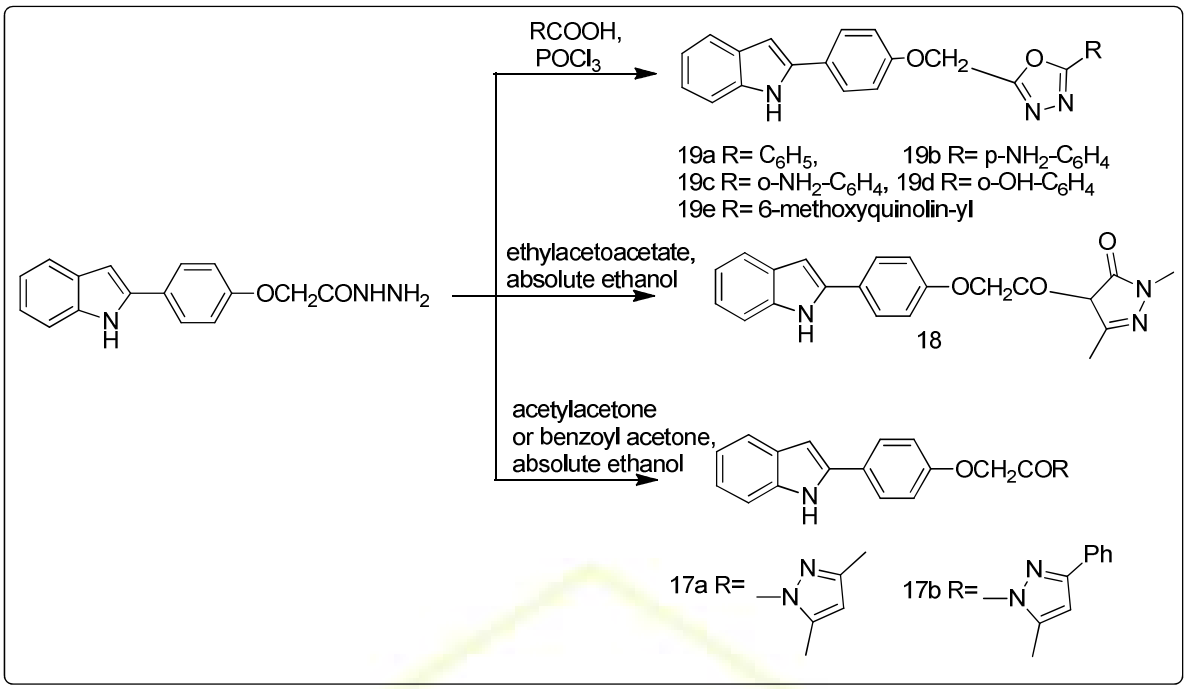
Several modifications were carried out by El-Nakkady *et al*. on the 3-formylof 2-phenyl indole (**Figure 1**) to overcome the *in-vivo* instability of the aldehyde functional group. The major one included the formation of oximes, methylamine, propanedinitriles, and hydrazones (**Scheme 1, 2, 3**). These derivatives proved to possess high stability and good antimitotic activity. Also these indole derivatives were structurally optimized by use of 3D-QSAR and docking studies on tubulin (**Figure 2, 3, 4, 5**).



Scheme 1: Synthetic diversifications achieved at 3-formyl of 2-phenyl indole derivative



Scheme 2: Modifications installed in the 2-phenyl ring of indole derivative



Scheme 3: Derivatization in the 2-phenyl ring of indole derivative

Antitumor activity

The synthesized compounds and the precursor scaffold **1** were screened for their cytotoxic activity against MCF-7 (human breast cancer cell line)taking vincristine as reference drug. The most active compounds **2**, **3b**, **3c**, **3d**, **3e**, **3f**, **4a**, **4b**, **4c**, **9b**, **14c**, **16b**, **16c** and **19a**, revealed IC_{50}< 20 nM against MCF-7 and MDA-MB- 231 cell line. All compounds showed reasonable cytotoxic activity. The 3-formyl derivative **2** was found to be more potent than the **1** against MCF-7 cell line.

Structure activity relationship

- **3a-f** synthetics revealed appreciable activity. However compound **3e** exhibited IC_{50}=1.60 nM which is more active even than vincristine (IC_{50}=2.00 nM).
- The 4-amino and 4-chloro substituted benzohydrazides **3d** and **3f** revealed similar IC_{50} values, signifying that the activity is not co-related in terms of the electronic nature of the substituent.
- Aliphatic substitution in **3a** decreased the activity, inferred that free rotation of aliphatic substitution because of the stereochemistry clashes with the adjacent neighbouring amino acid.
- Among the hydrazone-based indole synthetics **4a-c** compounds, **4b** and **4c** were found to be the most potent in this series suggesting the role of pie-pie interaction.
- **5** (hydrazinecarbothioamide), **6** (carboxaldehydeoxime), **7a-b** (methylene hybrids) and **8** (propanedinitrile) derivatives have decreased activity advocate the unsuitability of the electron withdrawing group functionality as a substituent at *C*3 of indole alkaloid.
- Introduction of a heterocyclic moiety at position 3 displayed good activity, the 3-benzoxazole derivative **9b** revealed better activity than the benzimidazole isostere **9a**, while the imidazole derivative disclosing the compared feasibility of oxygen over nitrogen in the ring structure.
- **12a** was found to be more potent than the methyl carbonate analogue **11** which may be attributed to the presence of the methylene

spacer in **12a**. Furthermore, compound **12a** exhibited better activity than its ethyl ester analogue **12b** that showed a marked decrease in activity suggesting an optimum chain length in the ester moiety.

- The amide derivatives **13a-d** and **14a-c** showed mild to moderate activity except the hydroxyethylacetamide derivative **13b** that exhibited a marked drop in activity. However, the pyrido derivative **13c** and the pyrrolidino derivative **14c** were the most active of these series, suggesting the accommodation of aromatic substituent for the activity.
- The hydrazide derivative **15a** revealed higher activity than the other substituted hydrazide **15b, c** and thiosemicarbazide **15d** indicating the triviality of the substituent.
- Sulfonamide derivatives **16a-c**, in general showed moderate activity. The *N*-thiazolesulfonamido derivative **16c** was the most potent, while the unsubstituted sulfanilamido derivative **16a** was the least, inferring the unique substituent pattern required for the activity.
- The pyrazolone derivative **18** exhibited better activity than the pyrazolo derivatives (**17a, b**). Indolyloxadiazole derivatives **19a-e** were found to show good anticancer agents. However, changing the position of amino group in the aniline moiety from the *ortho*-position **19c** to the *para*-position **19b** decreased the activity inferred the 5-membered hetrocyclics and pattern of heteroatoms must be critical for activity.

Molecular docking

Later on, molecular modelling further suggested mechanistic binding pattern of these heterocyclics. These studies was done *via* Molecular Operating Environment (MOE) on starting compound **1**, the synthesized compounds **2**, **3b**, **3c**, **3d**, **3e**, **3f**, **4a**, **4b**, **4c**, **9a**, **9b**, **13c**, **14c**, **15a**, **16a**, **16b**, **16c**, **19a**, **19e** (which are having IC_{50}<25nM (MCF-7 cancer cell line)). Moreover the structure were retrieved from the protein data bank (tubulin-colchicine co-crystallized structure (PDB ID code- 1SA0) (see in **Figure 3, 4, 5, 6**)[4].

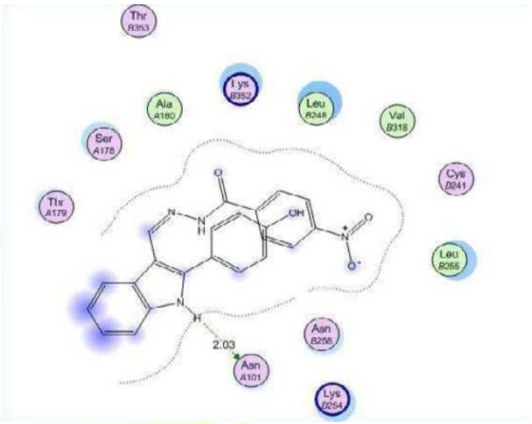


Figure 2: Docked structures of compound 3e

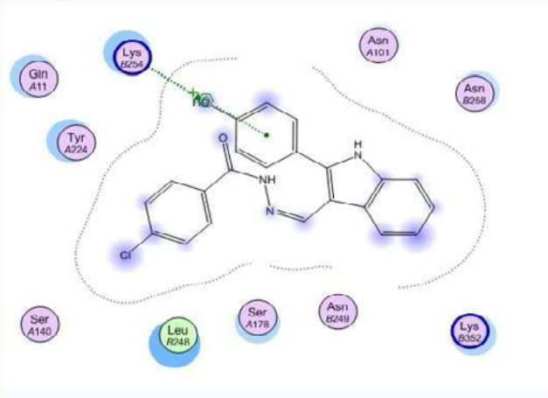


Figure 3: Docked structures of compound 3f

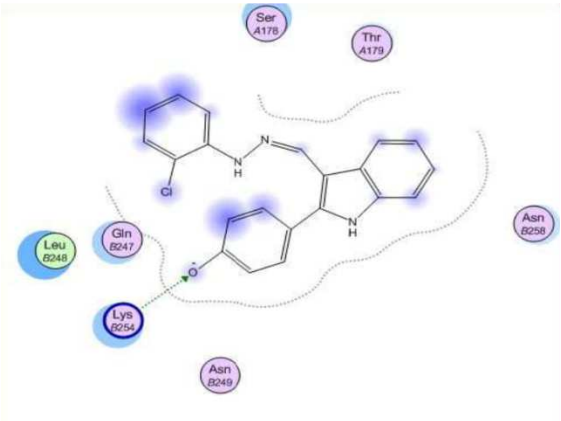


Figure 4: Docked structures of compound 4c

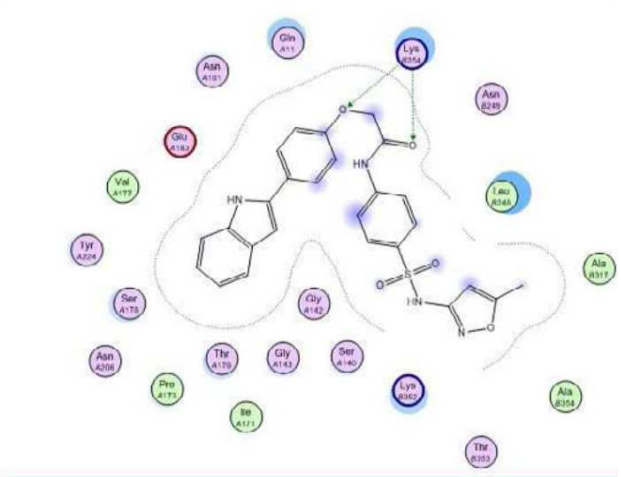


Figure 5: Docked structures of compound 16b

These docking studies revealed some important points.

- The most general interaction encountered in all docked structures was between the phenolic OH, which acted as *H*-bond acceptor, and Lys*β*-254 as shown in figure 3.
- Additional hydrophobic interaction through the 2-phenyl ring was also observed as shown in figure 4.
- Compounds substituted at the phenolic OH were also capable of forming this type of interaction since the OH group is not *H*-bond donor but *H*-bond acceptor group as shown in figure 4 and 5.
- The most active compound **3e** showed *H*-bond between indole-NH and Asn*α*101.
- From these studies it was concluded that the binding mode of synthesised compounds is the colchicine binding site of tubulin and also suggesting that the mode of action might be possibly *via* tubulin inhibition.

Indomethacin

The indomethacin (**20**) was developed as a non-steroidal anti-inflammatory drug (NSAID) (non-selective COX-2 inhibitor). Its basic scaffold has been further explored *via* substitution at different positions, especially at *C*3, in concern to increase its efficacy and potency. In this context, Singh *et al.* introduced a *γ*-butyrolactone moiety at *C*3 and a tolylsulfonyl group at *N*1 of the indole nucleus which finally resulted in the synthesis of **21** and later on screened for COX-1, COX-2 inhibitory activities and for its anti-cancer properties against 59 human cancer cell lines (Figure6, scheme 4)[5].

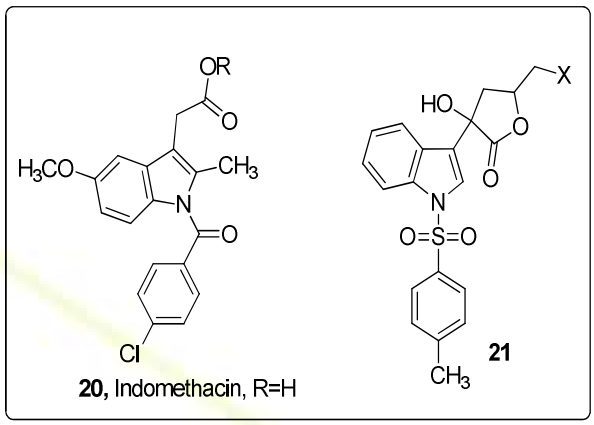
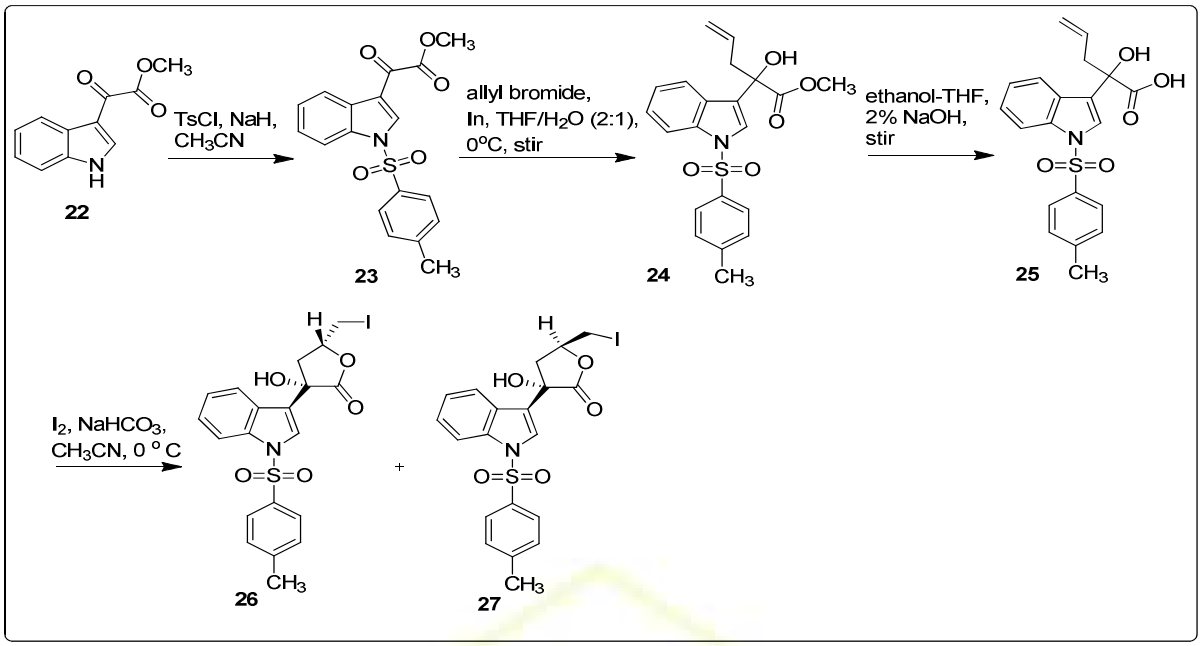


Figure 6: Indomethacin-based molecules designed for COX-1, COX-2 and anti-cancer properties

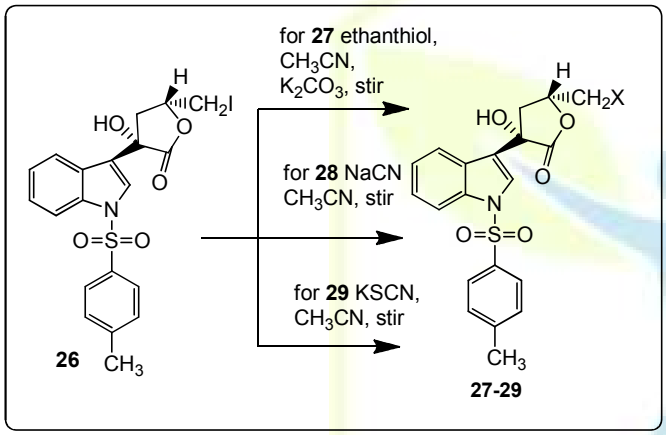
On the basis of previous results, docking studies and the literature reports, these researchers further introduced various groups like $CH_2SC_2H_5$, CH_2SCN, and CH_2CN at C-s5' of butyrolactone ring (**scheme 5**). Nucleophilic substitutions of **26** with ethanthiol, NaCN and KSCN in CH_3CN gave compounds **27, 28** and **29** possessing, respectively, SC_2H_5, CN and SCN groups in place of iodine in **26**.

Biological activity

It is established that COX-2 has the inflammatory role in the propagation of cancer. Screening for anti-cancer properties on 59 human cancer cell lines led that out of the four compounds (**26-29**), compounds **28** and **29** with CH_2CN and CH_2SCN group at C-5'showed appreciable anti-cancer activities with average GI_{50} (the concentration required to achieve 50 % growth inhibition) over all the cell lines as 1.9 and 9.1 µM, respectively, which was better than the average GI_{50} value of their parent compound indomethacin (64.3 µM) (**Table 1**). Additionally **28** was found to be highly specific for most leukaemia cell lines, MALME-3M



Scheme 4: Synthesis of indomethacin derivatives



Scheme 5: Synthesis of indomethacin derivatives

and M14 of melanoma, RXF393 of renal cancer, PC-3 of prostate cancer and MCF-7, MDA-MB-435 of breast cancer where it exhibited GI_{50} in sub-micromolar concentration (0.1–0.8 µM). Moreover, the relatively high LC_{50} values of **28** and **29** disclosed their low toxic profile.

Table 1: Anticancer activities of compound 28 and 29 (average GI_{50})

Compound	GI_{50} (µM)	LC_{50} (µM)
28	1.9	95
29	9.1	74
Indomethacin	64.3	100

Indoline-sulfonamide

Liou *et al* synthesized a novel oral indoline-sulfonamide agent**, j 30** (N-[1-(4-Methoxy-benzenesulfonyl)-2,3-dihydro-1*H*-indol-7-yl] Isonicotinamide) (**30**) showing potent *in vitro* and *in-vivo* activities against human cancer cells *via* teasing the microtubule physiology [6].

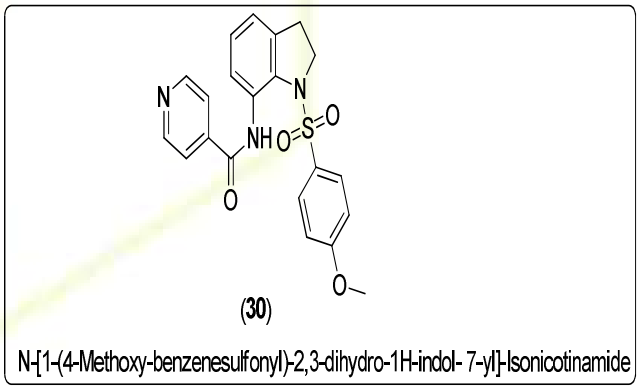


Figure 7: Structure of novel oral indoline-sulfonamide agent

These studies revealed that **J30** has a broad spectrum capability, at nM range, to inhibit proliferation in various cancer cell lines. However drug resistance pose a serious threat to the use of microtubule-interfering drugs for clinical therapy. Moreover **J30**, reported cases of drug resistance were lesser so it has an edge over the clinical agents of its category. Inspite **J30** exerts a similar potency,

regardless of the cell's MDR (multi drug resistance) or MRP (multidrug-resistance associated protein) status. **J30** was also effective against cell lines which were resistant to other chemotherapeutics, such as CPT (campothecin) and oxaliplatin. It was found that **J30** strongly depolymerises microtubules by binding to the colchicine-binding site with a high affinity than colchicine itself. **J30** also exerts a concentration and time dependent G2/M blockade. Along with the effect of **J30** on the cell cycle, it also provokes apoptosis, as indicated by the increase in subG1 population and Annexin V positivity. Apoptotic signalling involves activation of intracellular caspases. **J30** increases the activity of caspase-9 and caspase-3. Caspases are known to be activated during apoptosis by two important pathways:

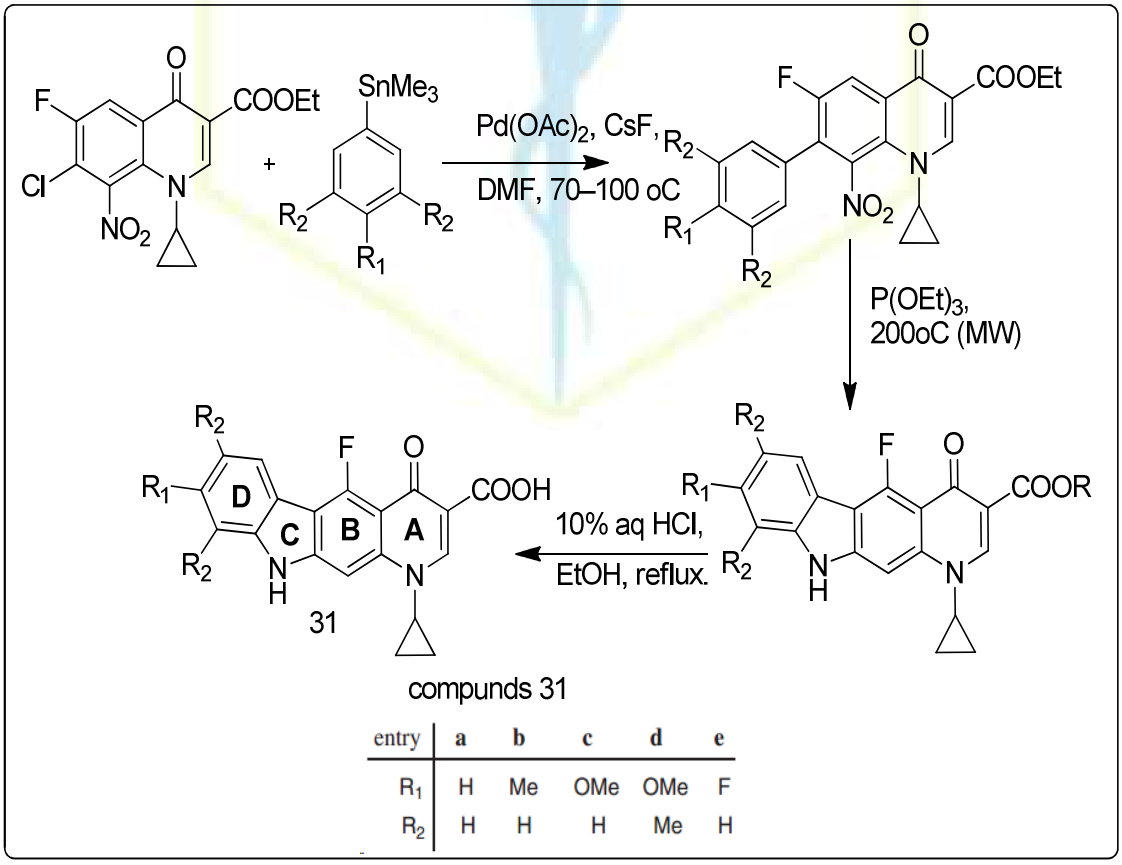
(1) Cross-linking of "death receptors" subsequent extrinsic triggering.

(2) Release of apoptogenic factors from mitochondria subsequent intrinsic signals.

Further, it was hypothetically concluded that **J30** triggers apoptosis *via* the intrinsic mitochondrial pathway. It was also shown that by the oral administration of **J30** has significant therapeutic efficacy against human cervical (KB), gastric (MKN45), and drug resistant (KB-Vin10) tumour xenografts in mice.

Carbazoles

The topoisomerase I and II have an important role in cell proliferation; these enzymes often over express in the metastatic active cancer cells and therefore can be suited as major targets for anticancer agents. The various carbazole derivatives synthesized by Al-Trawneh *et al.* were designed to form a ternary complex with DNA and the concerning enzymes. Ellipticine *N*-glycosides, a carbazole, showed potent antitumor activity[7]. It act as inhibitors of the topoisomerase II, since the complexes of those derivatives halt the DNA dynamics. Moreover similar sort of ternary complex formation could be observed with elliptinium[8-9].



entry	a	b	c	d	e
R_1	H	Me	OMe	OMe	F
R_2	H	H	H	Me	H

Scheme 6: Synthesis for carbazole derivatives

Different carbazole derivatives **31a–e** were prepared and assayed for the anticancer activity with respect to ellipticine as control drug against MCF-7 breast cancer and A549 non-small cell lung cancer (NSCLC) cell lines. The structure–activity relationships (SAR) analysis concluded

Among carbazole analogues **31a–e**, lipophilic and electron-donor substituent, such as a methyl group (**31b**) at the *para*-position of the D-ring, drastically decreased the antiproliferative activity on both cancer cell lines.

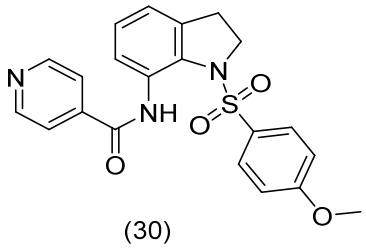
Substitution of the parent compound **31a** with a hydrophilic and electron-donor methoxy group (**31c**), resulted in increased potency as compared to **31a**.

Compound **31e**, bearing a fluoro-substituent with electron-withdrawing properties, had a four times increase in potency as compared to the unsubstituted analogue **31a**.

It was observed that the 4-methoxy-3, 5-di-methyl derivative **31d** was more potent than the 4-methoxy substituted carbazole **31c**, showing that the presence of two small lipophilic and electron-donor methyl groups in the *C*3 and *C*5 positions is beneficial for activity and potency.

Hydrazones of isatin

Pajouhesh *et al.* synthesized 3-*o*-nitrophenyl hydrazones of isatin (**32**) by condensation of isatin with o-nitrophenyl hydrazine[10]. These compounds were found to be active intramuscularly against Walker carcinoma-256 and inactive againstL-1210 lymphoid leukaemia[11-12].



(30)
N-[1-(4-Methoxy-benzenesulfonyl)-2,3-dihydro -1H-indol- 7-yl]-Isonicotinamide

Figure 8: Structure of novel oral indoline-sulfonamide agent

These studies found position and electron richness of R group altered the activity and selectivity whereas C3 position substitution is critical for anti proliferative activities.

CONCLUSION:

In recent years numerous natural occurring and synthetics indole alkaloids were isolated or prepared and shown commendable\appreciable anticancer activity. Their potential can be estimated from the propensity of addressing and beneficial abilityto interact with more than one cancer target *via* multimodal mechanism of actions. The most significant attribute of indole alkaloids is in their scaffold which on substitutions switches the activity from one target to other targets, so in near future it can be possible to eradicate the multi drug resistance of cancer by designing the semi-synthetics of indole alkaloids. This will boost the rational drug designing of anticancer drug discovery research into a new level.

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