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ITRACONAZOLE: Potent Anti-Cancer Agent

Gulshan Nath,
D.I.P.S.A.R.(Delhi Institute of Pharmaceutical Sciences and Research) Master's in Pharmaceutical Chemistry | Class of 2020
Dr. Sharad Wakode
Associate Professor D.I.P.S.A.R

Abstract: Itraconazole has been used for years as a potent board spectrum anti-fungal agent in treating fungal infections like blastomycosis, Histoplasmosis, aspergillosis.
With continuing research on this molecule, it's use is being repurposed in many clinical trials as a possible promising anti-cancer agent. Many studies have shown that ITZ can effectively reduce the activity of the Hedgehog pathway via angiogenesis, autophagy and other possible mechanisms at a concentration with minimal side-effects on other cells of the body.

Key words- Hedgehog pathway, angiogenesis, anti-fungal, azoles.

1.Introduction:

Itraconazole also known as Sproanox, is an antifungal agent and belongs to azole family of antifungals[1,23].

Chemical structure- Itraconazole is a racemic mixture (1:1:1:1) of four diastereomers, in which each have three chiral centers[1]. The structural formula and nomenclature are given as follows-

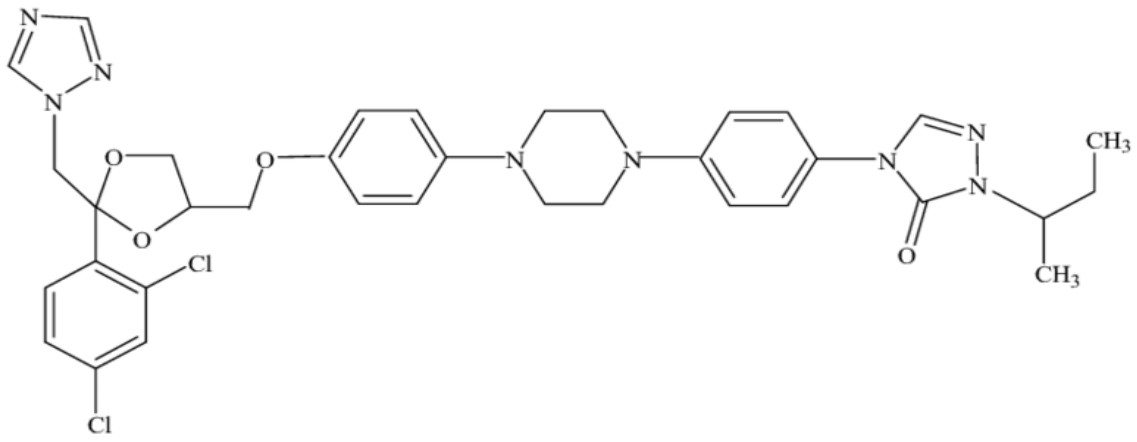

Figure 1- Structure of Itraconazole[1]

Nomenclature- (±)-1-[(RS)-sec-butyl]-4-[p-[4-[p-[[(2R,4S)-2-(2,4-dichlorophenyl)-2-(1H-1,2,4-triazol-1-ylmethyl)-1,3-dioxolan-4-yl]methoxy]phenyl]-1-piperazinyl]phenyl]-Δ -1,2,4-triazolin-5-one[1].

Itraconazole has a molecular formula of $C_{35}H_{38}Cl_2N_8O_4$ and a molecular weight of 705.64[1].

1.2 Pharmacokinetics- It is white to yellowish powder, which is insoluble in water, slightly soluble in alcohols, and freely soluble in dichloromethane[2,23].

pKa	**3.70**
Partition coefficient (at pH 8.1)	5.66
Peak plasma concentration	2-5 hours
C_{max} (for 100 mg dose)	0.5 $\mu g/ml$
Half-life	16-28 hours (with single dose) and 34-42 hours (with repeated dosing)
Bioavailability	55%
Protein binding	99.8%
Volume of Distribution	>700L

Table 1- Pharmacokinetics properties of Itraconazole[1]

It is seen that absorption of itraconazole increases when administered with meals or acidic beverage. Itraconazole is mainly metabolized by the CYP3A4 enzyme. Thus, there is lot of plasma level variation seen among individuals. The main metabolite is hydroxy-itraconazole which in vitro has greater antifungal activity than itraconazole[1].

1.3 Mechanism of Action- Studies suggest that itraconazole inhibits the synthesis of Ergosterol by inhibiting 14α demethylase which is the main component of the fungal cell membranes[2]. Hence, it is able to inhibit the growth of the fungus in the host[2,24].

1.4 Activity of Itraconazole in Clinical infections- The following table shows the activity of itraconazole against Different species[2].

Blastomyces dermatidis	**Active**
Histoplasma capsulatum	Active
Histoplasma duboisii	Active
***Zygomycetes (Rhizopus* spp, Rhizomucor spp, *Mucor* spp and *Absidia* spp)**	Not Active
***Fusarium* spp.**	Not Active
Aspergillus flavus	Active
Aspergillus fumigatus	Active
***Scedosporium* spp.**	Not Active
***Scopulariopsis* spp.**	Not Active
***Trichophyton* spp.**	Active

Table 2- Activity of Itraconazole in Clinical Infections[2]

1.5 Traditional Indications and Dosages:
SPORANOX ® (itraconazole) Capsules are recommended for the treatment of the following fun gal infections in both immunocompromised and non-immunocompromised patients:
1. Pulmonary, and extrapulmonary blastomycosis

2. Histoplasmosis, including chronic pulmonary cavitary disease and dispersed non-meningeal histoplasmosis, and
3. Pulmonary and extrapulmonary aspergillosis in patients with no tolerance to amphotericin B therapy.[3]

2.0 Itraconazole as Potent Anti-cancer agent: Besides ITZ traditional use as an effective antifungal agent, it is now been considered as a potent anti-cancer agent and seems effective in various types of cancer including prostate, lung and basal cell carcinoma[4]. There are additional cases of its efficacy in leukemia, ovarian, breast and pancreatic cancer[4].

2.1 Preclinical studies in cancer: ITZ is effective in reversing the drug resistance in murine leukemia P388/ADR cell lines to daunorubicin in a dose-dependent manner[5]. It works by Correcting the accumulation of the daunorubicin in the cells and thus reversing the drug resistance in the cells[5]. This can be shown by the following graph in which accumulation of daunorubicin is compare in P388/ADR vs P388- sensitive cells in the absence and presence of various amount of ITZ[5]. It was seen that in presence of ITZ, P388/ADR cells showed less accumulation as compare to the P388 cells[5].

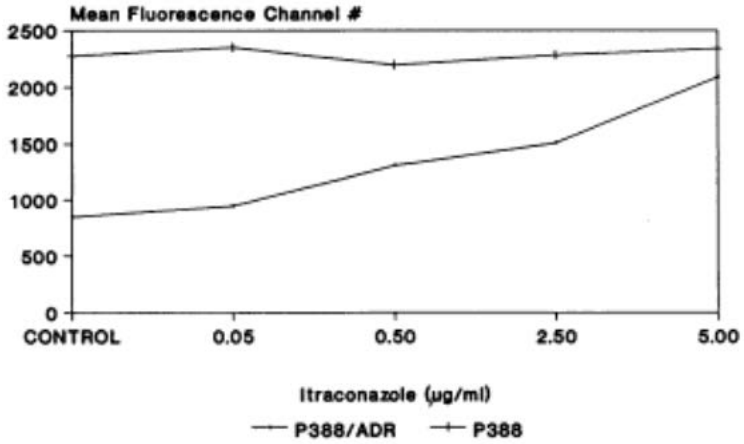

Figure-2: Effect of ITZ on accumulation of P388/ADR vs P388 cells in absence or presence of ITZ[5].

In addition to that, ITZ also corrected the plasma membrane potential of the P388/ADR cells[5]. All these activities were seen at the same concentrations of the ITZ as it is used for treating antifungal infections[5].

It is also seen that ITZ is involved in inhibition of Angiogenesis which involves formation of new blood vessels that are usually been reported in diseases like cancer, diabetic neuropathy and rheumatoid arthritis[6]. ITZ acts in specific and dose-dependent manner to inhibit the endothelial proliferation, migration and tube formation due to the growth factors like Vascular endothelial growth factor (VEGF) and basic fibroblast growth factor (bFGF) that helps in stimulating angiogenesis[7].

2.3 Clinical Trials in Human supporting the pre-clinical studies of ITZ: (i) As mentioned above in pre-clinical studies, ITZ can effectively reverse the drug resistance to

daunorubicin in a murine leukemia cell lines[5]. Vreugdenhil et al conducted a double-blinded randomized clinical trial of ITZ an anti-fungal prophylactic in neutropenic leukemia patients that were being treated for daunorubicin[5]. This trial included 23 patients with acute lymphoblastic leukaemia (ALL), 11 of which received ITZ, and 42 patients with acute myeloid leukaemia (AML), 17 of which received ITZ[5].

Results showed that in patients with ALL, disease-free survival (DFS) tended to be longer in the ITZ group as compare to control and no significant difference in remission rate were observed whereas in AML patients, DFS and remission rates showed no difference in ITZ and group groups[5].

2.4 Mechanism of Action: There are main four types of mechanism being researched for action of ITZ on cancer cells. These mechanisms include:

2.4.1. Anti- angiogenic: As ITZ has been identified as inhibitor of 14 α- demethylase which is responsible for converting lanosterol to ergosterol in fungi and cholesterol in humans[6]. It has been observed that ITZ can interrupt the function of endothelial cells by the same mechanism[6]. It arrests the cell at the G1 phase. Thus, inhibiting the endothelial cell proliferation and angiogenesis[6]. ITZ can inhibit endothelial cells at normal dosing of ITZ with an ic50 of 0.16 μM and minimal or no effects on non-endothelial cells.

2.4.2. Inhibition of Hedgehog pathway: The Hh pathway is crucial for proper development and growth in embryo[8]. It also functions post embryonically in maintaining of tissue homeostasis through its effects on stem or progenitor cells[8]. Any defect in the Hh pathway signaling can leads to tumor growth and development[8]. This pathway plays a great role in maintenance of cancer stem cells which are small cells within the tumor that have the capability of self- growth, self- differentiation, tumorigenicity when transplanted into another host[9,10]. It has been seen that the ITZ can inhibit the Hh pathway very effectively than other members of anti-fungal like Ketoconazole. ITZ has an IC50 of 800nm while that of Ketoconazole is IC50 of 9 μM[9]. The primary metabolite of ITZ is hydroxyitraconazole which also inhibits the pathway with IC50 of 1.2 μM[9].

It has been seen that smoothened (SMO) play a critical role in Hh signaling[11]. It is a seven-pass transmembrane protein that is present in primary cilium[11]. When a Hh protein ligand binds to PTCH which is a twelve-pass transmembrane protein and responsible for suppression of SMO activation[11]. On attachment of ligand, the suppression is reduced, and SMO is activated which then start accumulating in the primary cilium and activate several transcription factors and genes like GLI2, GLI1 and PTCH1[11].Hence many smoothened antagonists have been used to inhibit the Hh pathway[11,19]. ITZ have been very effective in those cells which have been resistant to smoothened antagonists[10].

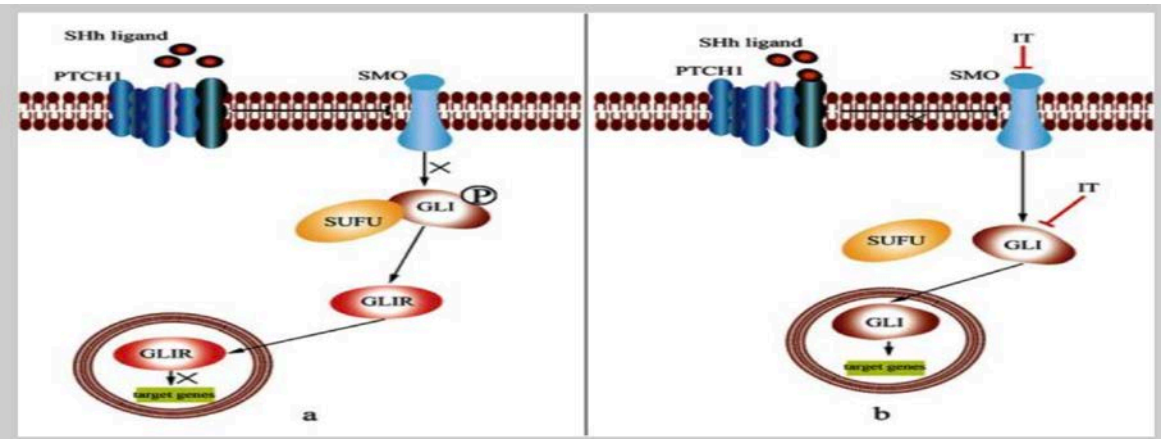

Figure 3 (a): This figure describes when Hh ligand binds to PTCH it activates the SMO, which then activates the GLI2 which activate the transcription gene GLI1 and PTCH1[11]. (b) ITZ inhibits the activation of SMO and GLI thus inhibit the target genes to treat cancer[11].

It has been also seen that ITZ can directly act on SMO, GLI and inhibit their functions by down streaming their targets through various mechanisms like cell cycle arrest, induce apoptosis and autophagy[11,19].

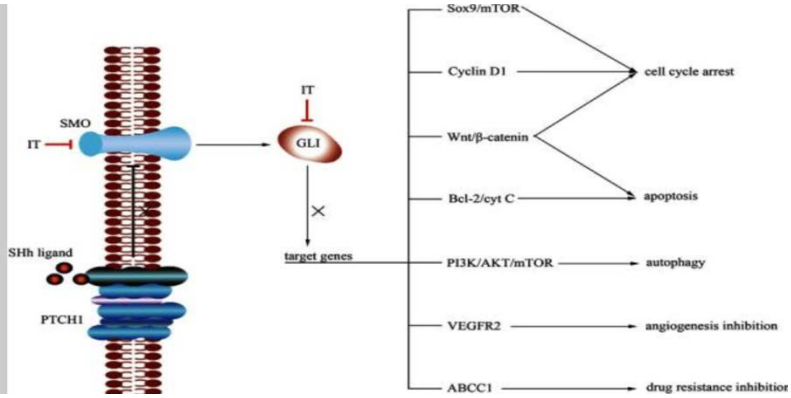

Figure 4: Down streaming of SMO and GLI targets by ITZ through various mechanisms[11].

ITZ modulates expression of genes in Hh pathways in melanoma cells: To understand the effects of the ITZ on proliferation of melanoma cells, experiments on A375 and SK-MEL-28 melanoma cells were done[12,27]. They found that when these cells are treated with 0,1,2 or 4 μM of ITZ for 48 h, then the protein molecules are isolated from the cells and verified at translation level by western blotting in order to understand the effect of ITZ on cell melanoma proliferation[12]. This treatment shows significant reduction of Gli-2 and Gli-1 but increase of Gli-

3 in both cells[12,13]. Gli-3 is the repressor which modulates gene transcription and expression of Hh pathway12. Therefore, an increase in Gli-3 shows significant reduction in signaling of Hh pathway[12,13].

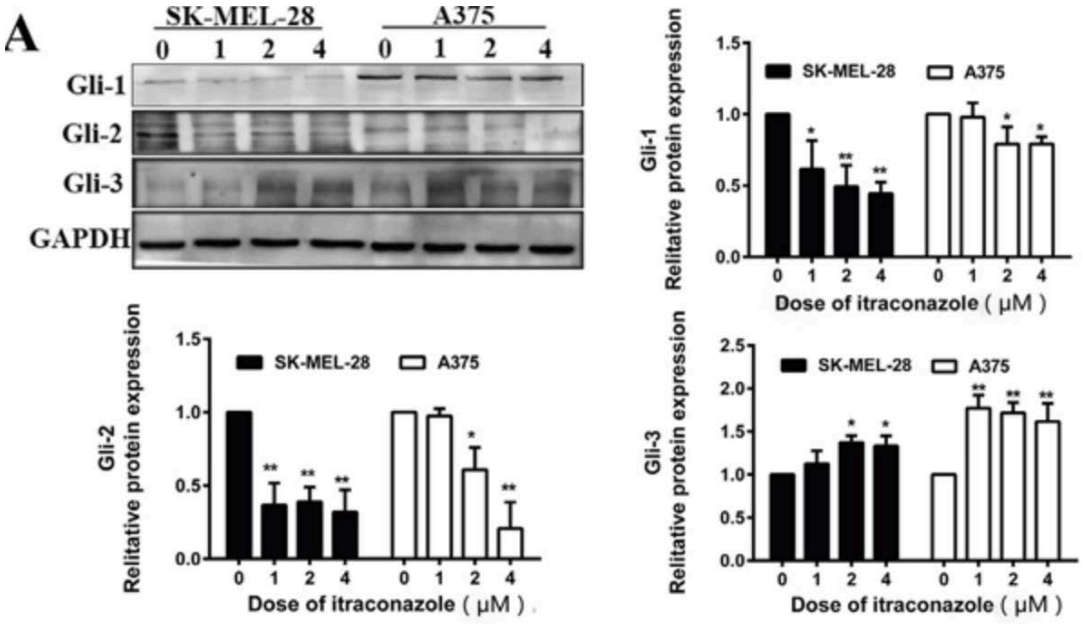

Figure 5A: Illustrates the experimental results of ITZ effect on A375 and SK-MEL-28 melanoma cells by effective the protein expression of Gli-1, Gli-2 and Gli-3 genes[12].

2.4.3. Induce autophagy: ITZ has a very important role in inducing autophagy in response to kill cancer cells[15]. There have been many studies which illustrates the effect of the ITZ on non-small cell lung cancer and medulloblastoma in some animal models[14,15,20]. These properties of ITZ are attributed to its inhibitory effects on endothelial cell proliferation, mTOR (mechanistic target of rapamycin) by disrupting the cholesterol trafficking and distribution and Matrigel-mediated angiogenesis by reducing the lanosterol-14 ademethylase and sterol biosynthesis[15,17,18]. All these factors help in endothelial cell arrest and thus inhibits tumor growth[15,20]. Autophagy is described as cell destruction process under cellular stress. During this process, the cellular material from cytoplasm and intracellular membranes leaks out into double- or multi- membrane autophagosomes which fuses to form lysosomes to form autolysosomes where final degradation of the cell takes place as shown in Figure 6[16].

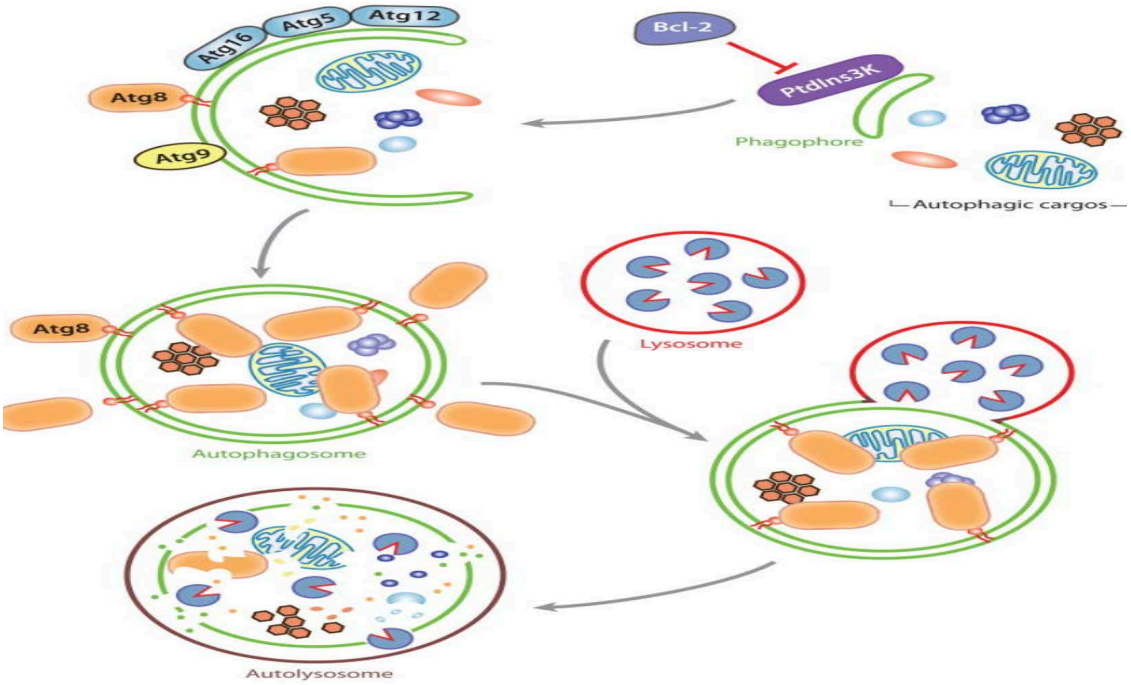

Figure 6: Schematic model of autophagy[16].

Dose-dependent effect of ITZ is seen in glioblastoma tumor cells in which two cell lines U87 and C6 were taken and MTT assay was performed to verify the effect of ITZ on cell viability[15,22]. It was seen that with increasing concentration of ITZ, there is significant reduction in cell viability as describe in Figure 7[15.21.22].

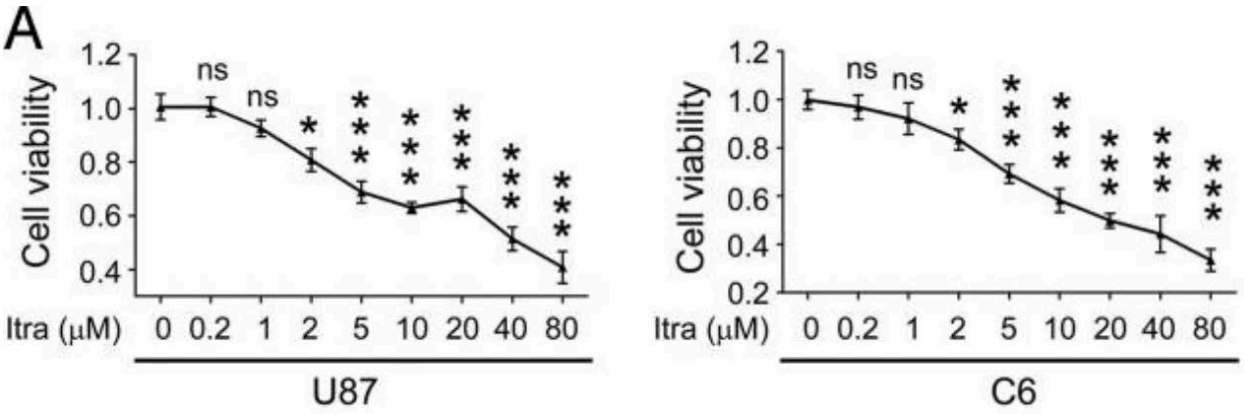

Figure 7: Dose-dependent effect of ITZ for 36h on U87 and C6 cell line which showed significant reduction in cell viability when measured by MTT assay[15].

2.4.4. Reversal of multi-drug resistance: MDR is common in cancer patients and it is mainly caused by activity of drug efflux proteins of ATP binding cassette (ABC)transporter family[25,26]. Most common transporter is P-glycoprotein (P-gp) which is widely associated with resistance due to antibiotics and anti-cancer agents[9]. It is seen that with IC50 of 2 μM ITZ can reduce P-gp function by 50%[9,25,26].

2.5. Conclusion: ITZ had been very promising board spectrum antifungal agent but with ongoing researches it can be repurposed for effective anti-cancer agent through different mechanisms. It has well-known pharmacokinetics profile with all possible toxicities which make it good candidate for anti-neoplastic agent which can be used along with other anti-neoplastic drugs in market or with other repurposed drugs. A large number of multi-drug combinations are being used in clinical trials to verify their efficacy in treating the cancer.

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