PyridoCarbazoles and Indolo[2,3-a]carbazoles as potential anticancer agents- A Review

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Abstract :

Cancer is characterized by an uncontrolled division of cells, which spread to distant sites of the body and cause severe health consequences. External factors like tobacco consumption, alcohol abuse, chemicals, infectious agents, and radiation along with internal factors like hormones, immune conditions, inherited mutations, and mutations occurring in metabolism can cause DNA mutation in normal cells, resulting in cancer initiation and development [1]. According to WHO data, Cancer is the second leading cause of death globally [2]. On 14th December 2020, IARC (International Agency for Research on Cancer) released the updated Globocan with new estimates on the global cancer burden, indicating that it rose to 19.3 million cases and 10 million cancer deaths in 2020 [3]. The most commonly diagnosed cancers worldwide are those of the lung (1.8 million, 12.6% of the total), breast (1.7 million, 11.9%), colorectal (1.4 million, 9.8%), and prostate (1.1 million, 7.7%) cancers [4]. Currently available cancer treatments are not permanent, but aid to contain the spread of the disease. Treatments such as chemotherapy, radiation and invasive operations are painful and come with significant side effects. It exerts tremendous physical, emotional and financial strain on individuals, families, communities, and health systems. With increasing cancer cases and resistance to anti-cancer drug regimens are emerging, research and development of new powerful cancer treatments have become extremely crucial for the next decades. This paper aims to review Pyrido-carbazole and Indolo[2,3-a]carbazole derivatives which exhibit promising anti-cancer properties these hold the potential to change the façade of the cancer treatment and cancer drug industry

Keywords: Topoisomerase II, antineoplastic, carbazole, Ellipticine, Pyrido- carbazole and Indolo[2,3-a]carbazole

1. Introduction

Carbazole is a polycyclic aromatic hydrocarbon consisting of two six-membered benzene rings fused on either side of a five-membered nitrogen-containing ring, with a large, aromatic system and a central nitrogen atom showing extensive electron delocalization. The compound's structure is based on the indole structure, but in which a second benzene ring is fused onto the five-membered ring at the 2–3 position of the indole [5]. Carbazole appears in wide variety of forms, such as white crystals, plates, leaflets, or light tan powder and sublimes readily [6]. It has shown to exhibit strong fluorescence and long phosphorescence on exposure to ultraviolet light. Carbazoles acts as an important class of hetero-cycles that is discovered from a large variety of organisms, including bacteria, fungi, plants, and animals exhibiting diverse biological activities such as anti-tumor properties in its natural and synthetic products. Carbazoles are classified according to their structure, starting from the tri-cyclic carbazole to fused tetracyclic, pentacyclic, hexa-cyclic and hepta-cyclic carbazoles. In past decades, several scientific groups have managed to isolate many alkaloids that belong to the carbazole class of compounds. The continuous increase of isolable natural products, as well as pharmacological action of these derivatives, have generated significant synthetic interest, consequently, the synthesis of carbazoles has been an interesting area of study [7]. Ever since, there has been a strong interest in this area by chemists due to the intriguing structural features and promising biological activities exhibited by many carbazole alkaloids. The explosive growth of carbazole chemistry is emphasized by numerous monographs, accounts, and reviews [8]. Based on the emerging importance of the carbazole derivatives as indicated above, hence it is worthwhile to review of hetero-annulated carbazoles and their biological aspects along with the X-ray studies to analyze the geometry which will have a greater impact on the design of drug targets. Several natural or synthetic poly-cyclic molecules with carbazole nucleus, which show attractive

drug-like properties, were identified to increase their biological activities and their specificity, obtaining cytotoxic agents, effective in a panel of cancer cell lines. carbazole derivatives are well known for a range of pharmacological activities such as antioxidant, anti-inflammatory, anti-bacterial, anti-convulsant, antipsychotic and anti-diabetic. Numerous carbazoles with promising anticancer activity have undergone clinical trials.

2. Pyrido-carbazoles:

Pyrido-carbazoles or more specifically Pyrido[4,3-*b*] Carbazoles are a class of compounds of the carbazole family whose heteroaromatic skeleton constitutes the scaffolds of many pharmacologically interesting alkaloids. They were the earliest discovered carbazole derivatives and have received a lot of attention from the international community for their antineoplastic properties. In this review we would be primarily concentrating on natural pyrido-carbazoles alkaloids such as ellipticine, olivacine and their derivatives. These natural products were predominantly isolated from species of the Apocyanaceae family. The first derivatives of this series 5,11-dimethyl substituted ellipticine and its isomer, the 1,5-dimethyl substituted olivacine were first isolated from Ochrosia elliptica in 1959 and from Aspidosperma olivaceum in 1958, respectively. The antineoplastic property of ellipticine and its derivative 9-methoxy ellipticine were first publishes in 1967. Both these compounds exhibited promising activities against experimental mouse leukemia L1210 cell line and olivacine followed suit. Pyrido-carbazoles and its analogues present a broad spectrum of antineoplastic activities against leukemias and solid tumors [9]. These alkaloids are also water soluble, have a limited toxic side effects and have no haematological toxicity.

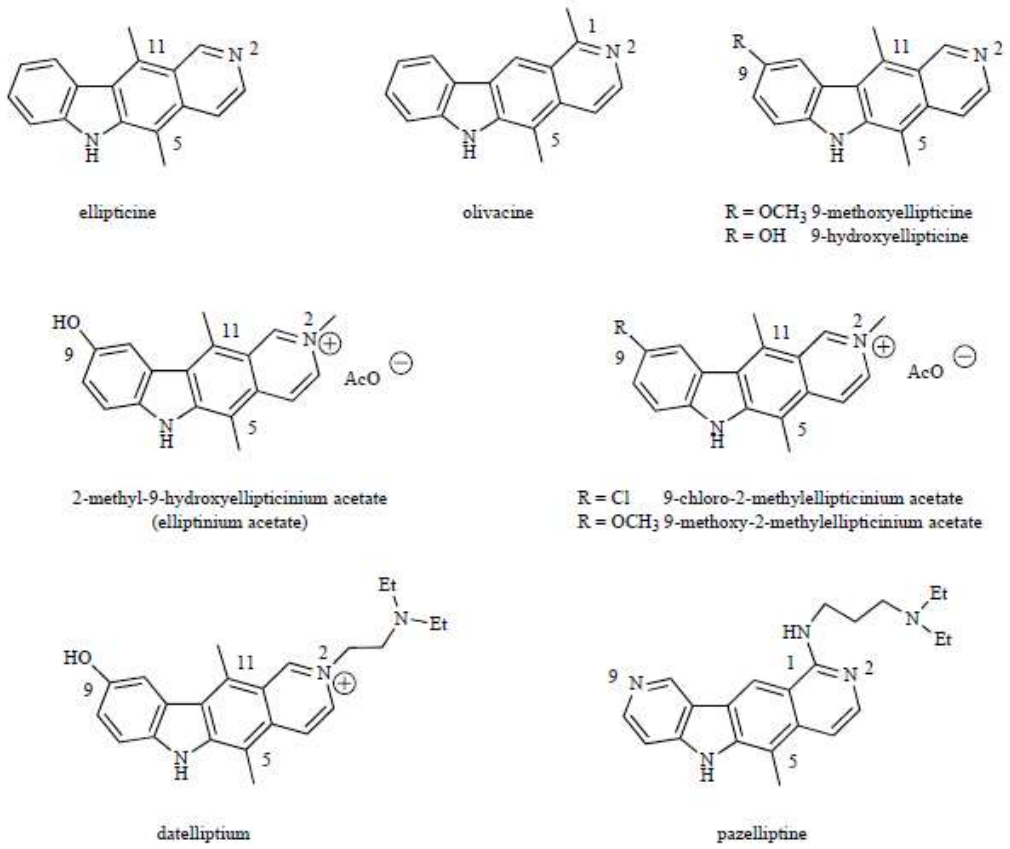

Figure 1 : Pyrido[4,3-*b*]carbazoles.

2.1. Ellipticine

Ellipticine or 5,11-dimethyl-6H-pyrido [4,3-b]carbazole (IUPAC name) is a natural product which was isolated from tropical evergreen tree (Ochrosia elliptica) by Goodwin et al in 1959. [10].It has been the heart of extensive chemical and pharmacological research since the discovery of its antitumor effects. Ellipticine was shown to be an antineoplastic agent that intercalates into DNA and alters topoisomerase II activity. Topoisomerase II is an enzyme essential for chromosome segregation, DNA replication and chromosome condensation. Inhibitors of topoisomerase II are considered important drugs as they are used in treatment of many neoplasms including breast cancer, lung cancer, testicular cancer, lymphomas and sarcoma [11]. The early studies only focused on this mechanism of action and in the later decades ellipticine's Ability of bio-oxidation and formation of cytotoxic adducts with DNA. Previous extensive biological reviews of ellipticine by Auclair and Graves have focused majorly on these modes of action. In our literature survey it was

concluded that the 9th position and the 11th position of the ellipticine ring are of importance and substitution with functional groups at these positions improve the antitumor property of ellipticine. Especially ellipticine analogues with a 9-oxygen function, such as a hydroxyl or methoxy group, have stronger anticancer efficacy than those without this function. For instance, replacing the 9th position with hydroxyl or methoxyl group gives 9-hyd9roxyellipticine and 9-methoxyellipticine respectively, the 9-hydroxyl variant especially showed promising results in selectively inhibiting p53 protein phosphorylation in Lewis lung carcinoma and also against SW480 colon cancer cell lines. 2-methyl-9-hydroxyellipticinium acetate (Celiptium) is another well-known derivative of ellipticine, it has been clinically used against advanced breast cancer with great success [9]. In the last decade, more evidence has come to limelight regarding the cell-cycle effects of ellipticine. Till today, it has been demonstrated that ellipticine is capable of interacting with Akt kinase, c-Kit kinase and p53 tumor suppressor protein. Its effects on other cellular proteins are still being explored. equivocally, ellipticine exhibits multimodal cytotoxic activity. Ellipticine is primarily used in therapy of metastatic Breast cancer.

2.2 Olivacine:

Olivacine, also known as 1,5-Dimethyl-6H-indolo[2,3-g]isoquinoline, is a pyridocarbazole structure that contains alkaloids. In 1958, it was discovered in Aspidosperma olivaceum. It was found to have potent anticancer properties against experimental mouse leukemia L1210 cell line. This paved the path for the development of novel olivacine-based pyridocarbazole derivatives, both semisynthetic and completely synthetic. In 1994, Jasztold-Horwoko et al succeeded in synthesising 9-hydroxy olivacine derivatives with distinct protonable N-dialkylaminoalkyl carboxamido side chains. A number of these chemicals were found to have anticancer properties against colon 38 adenocarcinoma and P388 leukaemia cells. Compared to ellipticine, 9-aza-olivacine, a derivative of pazelliptine, showed better cytotoxicity and anticancer activity. . In addition, we discovered in our literature review that a number of known olivacine derivatives have a high level of cytotoxinity against a variety of cancer cell lines. The majority of them were not authorized for use since their clinical trials did not generate encouraging results.

3 Indolo[2,3-a] carbazoles:

Indolocarbazoles belong to a family of heterocyclic compounds which contain indole and carbazole elements in their structure a planar ring system. The first indolocarbazoles were discovered in streptomycetes and were subsequently isolated from various species of flora and fauna. Till today, Scientist and researchers from thought the world have contributed to add numerous synthetic derivatives to this family of hetero cycles. The motivation behind the synthetic derivation of indolocarbazoles is because of their high pharmacological and biological activity. The indolocarbazole family has five subclasses of compounds that differ in the structure because of the orientation of flat aromatic ring system. These five isomers of the polycyclic system are: indolo[2,3-*a*] carbazole (**1**), indolo[2,3-*b*] carbazole (**2**), indolo[2,3-*c*] carbazole (**3**), indolo[3,2-*a*] carbazole (**4**), and indolo[3,2-*b*] carbazole (**5**) (Fig. 1). The subclass of derivatives of 11,12-dihydroindolo[2,3-*a*] carbazole is the most common, biologically significant, and studied in detail [12].

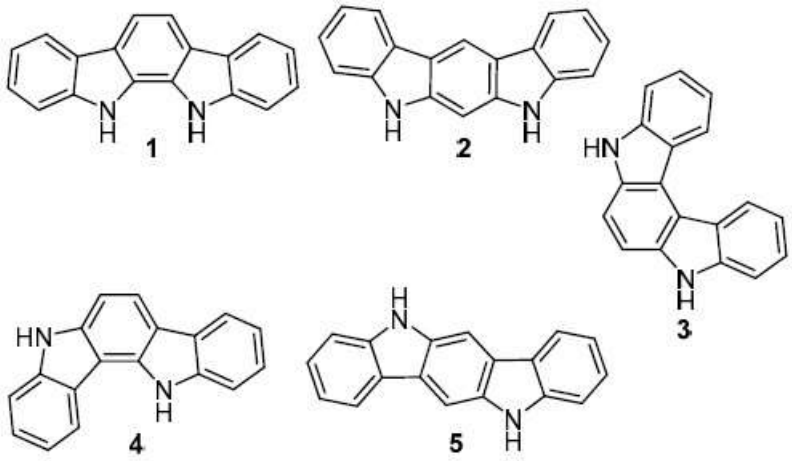

Figure 4 : Structures of the five isomers of indolocarbazoles: indolo[2,3-*a*]carbazole (**1**), indolo[2,3-*b*]carbazole (**2**), indolo[2,3-*c*]carbazole (**3**), indolo[3,2-*a*]carbazole (**4**), and indolo- [3,2-*b*]carbazole (**5**).

3.2Rebeccamycin & Becatecarin:

1,11-dichloro-12-(4-O-methyl-beta-Dglucopyranosyl)-12,13-dihydro-5H-indolo[2,3a]pyrrolo[3,4-c]carbazole-5,7(6H)-dione (IUPAC name) was first isolated in 1985.Rebeccamycin is naturally produced by the filamentous bacterium Lentzea aerocolonigenes (Nouioui et al. 2018). It is a hydrophobic substance with a yellow crystalline appearance. The structure of rebeccamycin consists of a indolocarbazole framework with two chlorine substituents and one methylglucose substituent. Rebeccamycin displays antibacterial properties against a few Gram-positive bacteria such as Staphylococcus aureus or Streptococcus faecalis and it also exhibits antitumor effects, which is why it has gathered interest for medical applications. However, Rebeccamycin is hydrophobic by nature and this is a major drawback as it is insoluble in water. Hence, is not suitable to be used as a drug in the human body.To over come this drawback and to exploit and explore the extent of its anti-tumour activity , a new analogue with higher hydrophilicity was derived. Becatecarin or 1,11-Dichloro-6-[2-(diethylamino)ethyl]-12,13-dihydro-12-(4-Omethyl-beta-D-glucopyranosyl)-5H indolo[2,3a]pyrrolo[3,4- c]carbazole-5,7(6H)-dione (IUPAC name) was synthesised by chemically adding an aminoalkyl group to rebeccamycin moiety. Despite the structural differences of Rebeccamycin & becatecarin, they both display the same extent of antitumor activity. rebeccamycin is particularly interesting in the field of cancer treatment because it is a DNA-intercalating substance that causes DNA double-strand breaks and acts as a topoisomerase I inhibitor(Bush et al. 1987; Facompré et al. 2002). Because of the hydrophobic nature of rebeccamycin, only its analogues have been further investigated with respect to cancer therapy. Becatecarin,on the other hand displays topoisomerase I and II (Long et al. 2002). Becatecarin, has been subjected to several phase I and II clinical trials,during which its effects on small cell lung cancer, breast cancer, metastatic colorectal cancer, and metastatic renal cell cancer were studied (Nock et al. 2011; Burstein et al. 2007; Hussain et al. 2003; Schwandt et al. 2012; Goel et al. 2003) [13].

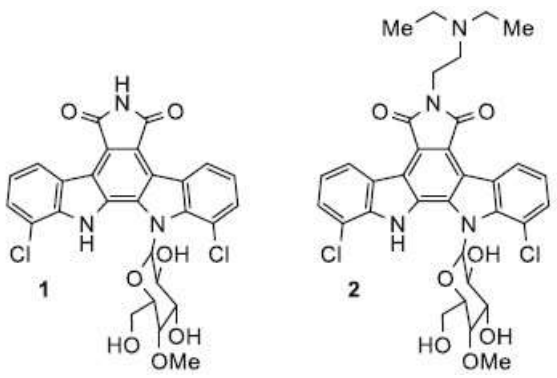

Figure 5: Chemical structure of rebeccamycin (1) and becatecarin (2)

3.3. Staurosporine

Staurosporine was initially a natural product isolated in 1977 from the bacterium Streptomyces staurosporeus. It was the first first representative of indolocarbazoles family of alkaloid [14]. This compound has been widely used in both traditional and modern medicine. It also sparked the interest for exploration of Indolo Carbazoles and revolutionised drug discovery. Soon after its discovery staurosporine was discovered to be a nanomolar inhibitor of protein kinases. Protein kinases are responsible in regulating all essential aspects of cells, such as metabolism, cell cycle progression, cytoskeletal arrangement and cellular replication. Staurosporine is an extremely strong and non-specific - inhibitor of protein kinases, specifically tyrosine kinases, and has a powerful cytotoxic effect on cancer cells. These findings encouraged the global pharmaceutical industry to look for exploitable protein kinase inhibitors through natural and synthetic routes. Today staurosporine, is widely recognized as precursors of many highly successful anticancer chemotherapeutic agents.Staurosporine is a bulky and highly hydrophobic molecule. Its indolocarbazole structure consist of two molecules of tryptophan and, the sugar moiety is derived from glucose and methionine. The cleavage at the domain interface is extremely hydrophobic, such that large free surface areas surround the bisindolylcarbazole ring system of attached staurosporine. Staurosporine overlaps very well with the adenosine group of ATP, the lactam ring mimicking the hydrogen-bonding interactions of adenine, while the glycosyl group mimics those of ribose so, not surprisingly, it acts as a non-selective protein kinase inhibitor [15]. Staurosporine was proven too be effective during clinical trials against leukaemias, lymphomas, advanced solid tumours, melanomas and small-cell lung cancer.

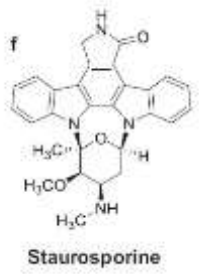

Figure 5: **Staurosporine**

3.4 Imatinib:

Imatinib or 4-[(4-methylpiperazin-1-yl) methyl]-*N*-[4-methyl-3-[(4-pyridin-3-ylpyrimidin-2-yl) amino] phenyl] benzamide (IUPAC name). It is also commercially known “Gleevec” or “Glivec”. It was invented by a biochemist named Nicholas Lyndon who was then working for Ciba-Geigy (today Novartis) in the late 1990’s [16]. Imatinib was tyrosine kinase inhibitor (TKI) and it forever revolutionised the treatment of chronic myeloid leukemia (CML) in the year 2001and hence was even termed as “a magical bullet”. Imatinib is a derivative of 2-phenyl amino pyrimidine which functions as a specific inhibitor of numerous of tyrosine kinase enzymes. It occupies the *TK* active site, leading to a decrease in activity. There are a large number of *TK* enzymes in the human body. Imatinib is designed specifically for the *TK* domain in the Abelson proto-oncogene. Each active sites of tyrosine kinases have a binding site for ATP. The enzymatic activity catalysed by a tyrosine kinase is the transfer of the terminal phosphate from ATP to tyrosine residues on its substrates, is a process known as protein tyrosinephosphorylation. Imatinib’s first clinical trial took place in 1998 and received its FDA approval in May of 2001.FDA also approved it for advanced GIST patients in 2002, imatinib was approved for use after the surgical removal of KIT-positive tumors to help prevent recurrence,on 1 February 2012.The FDA has approved imatinib for use in adult patients with relapsed or refractory Philadelphia chromosome-positive acute lymphoblastileukemia. Even today imatinib continues to be used in cancer therapy. More derivatives of the same are being synthesised and studied to improve and enhance its pharmacological activity.

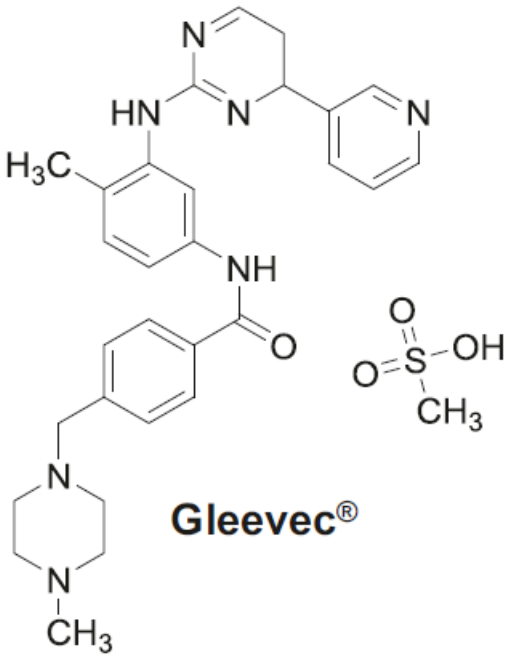

Figure 6: Imatinib

Conclusion:

The scope of this review is focused on the most commonly and widely studied pyrido- carbazoles and Indolo[2,3-a] Carbazoles. This study infers that alkaloid belonging to the carbazole family are highly pharmacologically active and diverse. These compounds are being widely researched across the world and have led to development of numerous synthetic successors for cancer treatment. The derived novel compounds have not yet been completely studied and are in the early stages of clinical trials. If successful, they will change the face and efficacy of cancer treatments. This would transpire new opportunities for the pharmaceutical sector and make it more cancer treatments bearable and affordable.

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